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(54) **Titre** : CRISTAUX DE SODIUM DE LAQUINIMOD ET PROCEDE AMELIORE POUR LEUR FABRICATION
(54) **Title**: CRYSTALS OF LAQUINIMOD SODIUM AND IMPROVED PROCESS FOR THE MANUFACTURE THEREOF

(57) **Abrégé/Abstract:**

The present application provides subject-matter related to a mixture of crystalline laquinimod sodium particles, wherein (i) > 90% of the total amount by volume of the laquinimod sodium particles have a size of < 40 μιη or (ii) > 50% of the total amount by volume of the laquinimod sodium particles have a size of < 15 μιη and wherein one or more of further requirements are fulfilled with regard to bulk density, tapped density and the amount of certain impurities. The present application also provides a pharmaceutical composition comprising an amount of laquinimod and at least one of BH-3-HLAQ, MCQ, MCQCA, MCQME, NEA, and MCQEE. The subject invention also provides processes for preparing BH-3-HLAQ, MCQ, MCQCA, MCQME, MCQEE, and compounds prepared by said processes. Further provided is a process for testing whether a sample of laquinimod contains an undesirable impurity. Further provided are processes for (a) preparing a validated pharmaceutical composition comprising laquinimod, (b) distributing a validated batch of a pharmaceutical composition comprising laquinimod, (c) validating a batch of a pharmaceutical product containing laquinimod and (d) preparing a packaged pharmaceutical composition comprising laquinimod, each comprising determining the amount of at least one of BH-3-HLAQ, MCQ, MCQCA, MCQME, NEA, and MCQEE in a sample or batch. The subject invention further provides use of BH-3-HLAQ, MCQ, MCQCA, MCQME, MCQEE as a reference standard to detect trace amounts of the impurity in a pharmaceutical composition comprising laquinimod. Finally, the subject invention provides methods of determining the concentration of BH-3-HLAQ, MCQ, MCQCA, MCQME, MCQEE, 5-HLAQ, SPIRO-LAQ or 3 H-LAQ in a pharmaceutical composition comprising laquinimod.

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(57) Abstract: The present application provides subject-matter related to a mixture of crystalline laquinimod sodium particles, wherein (i) > 90% of the total amount by volume of the laquinimod sodium particles have a size of < 40 µιη or (ii) > 50% of the total amount by volume of the laquinimod sodium particles have a size of < 15 µιη and wherein one or more of further requirements are fulfilled with regard to bulk density, tapped density and the amount of certain impurities. The present application also provides a pharmaceutical composition comprising an amount of laquinimod and at least one of BH-3-HLAQ, MCQ, MCQCA, MCQME, NEA, and MCQEE. The subject invention also provides processes for preparing BH-3-HLAQ, MCQ, MCQCA, MCQME, MCQEE, and compounds prepared by said processes. Further provided is a process for testing whether a sample of laquinimod contains an undesirable impurity. Further provided are processes for (a) preparing a validated pharmaceutical composition comprising laquinimod, (b) distributing a validated batch of a pharmaceutical composition comprising laquinimod, (c) validating a batch of a pharmaceutical product containing laquinimod and (d) preparing a packaged pharmaceutical composition comprising laquinimod, each comprising determining the amount of at least one of BH-3-HLAQ, MCQ, MCQCA, MCQME, NEA, and MCQEE in a sample or batch. The subject invention further provides use of BH-3-HLAQ, MCQ, MCQCA, MCQME, MCQEE as a reference standard to detect trace amounts of the impurity in a pharmaceutical composition comprising laquinimod. Finally, the subject invention provides methods of determining the concentration of BH-3-HLAQ, MCQ, MCQCA, MCQME, MCQEE, 5-HLAQ, SPIRO-LAQ or 3 H-LAQ in a pharmaceutical composition comprising laquinimod.

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CRYSTALS OF LAQUINIMOD SODIUM AND IMPROVED PROCESS FOR THE MANUFACTURE THEREOF

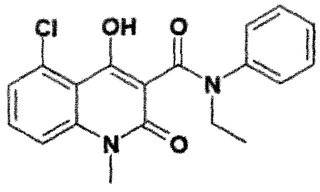
This application claims priority of U.S. Provisional Application No. 61/785,575, filed March 14, 2013, the entire content of which is hereby incorporated by reference herein.

Throughout this application, various publications are referred to by first author and year of publication. Full citations for these publications are presented in a References section immediately before the claims. Disclosures of the publications cited in the References section in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as of the date of the invention described herein.

Background of the Invention

Laquinimod is a compound which has been shown to be effective in the acute experimental autoimmune encephalomyelitis (aEAE) model (U.S. Patent No. 6,077,851). Its chemical name is N-ethyl-N-phenyl-1,2,-dihydro-4-hydroxy-5-chloro-1-methyl-2-oxoquinoline-3-carboxamide, and its Chemical Registry number is 248281-84-7.

Laquinimod is a small molecule having the following chemical structure:



laquinimod

Laquinimod sodium has high oral bioavailability and has been suggested as an oral formulation for the treatment of Multiple Sclerosis (MS). (Polman, C. et al., (2005) "Treatment with laquinimod reduces development of active MRI lesions in relapsing MS", Neurology. 64:987-991; Sandberg-Wollheim M, et al. (2005) "48-week open safety study with high-dose oral laquinimod in patients", *Mult Scler.* 11:S154). Studies have also shown that laquinimod can reduce development of active MRI lesions in relapsing MS. (Polman, C. et al., (2005) "Treatment with laquinimod reduces development of active MRI lesions in relapsing MS", Neurology. 64:987-991).

In order to prepare laquinimod as a pharmaceutical drug product, manufacturing processes need to take into consideration the possibility of the impurities disclosed herein being present in the product. Early syntheses of laquinimod produced product contaminated with impurities such as methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate and 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline (K. Jansson et al., (2006) "Synthesis and Reactivity of Laquinimod, a Quinoline-3-carboxamide: Intramolecular Transfer of the Enol Proton to a Nitrogen Atom as a Plausible Mechanism for Ketene Formation, *J. Org. Chem.*, 71, 1658-1667). Several other impurities have also been disclosed (U.S. Patent No. 6,077,851; U.S. Patent No. 6,875,869; and U.S. Patent No. 7,884,208). U.S. Patent Application Publication No. 2012/0010239, the contents of which are incorporated by reference into this application, discloses the impurity 5-chloro-4-hydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide. U.S. Patent Application Publication No. 2013/0217724, the contents of which are incorporated by reference into this application, discloses the impurity *N*-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide. U.S. Patent Application Publication No. 2013/00345256, the contents of which are incorporated by reference into this application, discloses the impurity N-ethyl-4-hydroxy-1-methyl-5-(methyl(2,3,4,5,6-pentahydroxyhexyl)amino)-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide. U.S. Patent No. 8,178,127, the contents of which are incorporated by reference into this application, discloses the impurities 2-chloro-6-(1-ethyl-N-methyl-2-oxoindoline-3-carboxamido)benzoic acid, 1H,3H-spiro[5-chloro-1-methylquinoline-2,4-dione-3,3'-[1]ethylindolin-[2]-one and 5-chloro-N-ethyl-3-hydroxy-1-methyl-2,4-dioxo-N-phenyl,1,2,3,4-tetrahydroquinoline-3-carboxamide.

The processes of synthesis of laquinimod and the preparation of its sodium salt are disclosed in U.S. Patent No. 6,077,851. An additional process of synthesis of laquinimod acid, but not laquinimod sodium, having low levels of certain impurities is disclosed in U.S. Patent No. 6,875,869. U.S. Patent No. 7,884,208 teaches a process for the preparation of laquinimod sodium which removes certain impurities present after the salt formation step, thus resulting in a crystalline mixture of higher purity as well as a crystalline mixture having large particles, and improved tapped and bulk density. Pharmaceutical compositions comprising laquinimod sodium are disclosed in PCT International Application Publication No. WO 2005/074899.

In the preparation of laquinimod sodium disclosed in U.S. Patent No. 6,077,851, laquinimod acid was suspended in ethanol, and 5M sodium hydroxide solution was added. After stirring, the resulting precipitate was filtered, washed with ethanol, and dried. The method used to make laquinimod sodium in U.S. Patent No. 6,077,851 is commonly referred to as a slurry-to-slurry salt formation.

In the slurry-to-slurry salt formation method of U.S. Patent No. 6,077,851, the laquinimod sodium is not dissolved in solution. Any solid impurities, if present in the laquinimod sodium suspension, are therefore not removed by filtration.

U.S. Patent No. 6,875,869 discloses a process of preparing the base compound laquinimod in high yield and low level of impurities. However, the process in U.S. Patent No. 6,875,869 is only for synthesis of the base compound (lauqinimod acid) and not the salt. As such, the slurry-to-slurry salt formation process would still be needed to form the sodium salt.

U.S. Patent No. 7,884,208 teaches an improved process for preparing laquinimod sodium resulting in crystals of higher purity as well as crystals having improved crystalline characteristics, e.g., comprising no more than 2 ppm of a heavy metal and having higher tapped density. In the processes disclosed in Examples 13-17 of U.S. Patent No. 7,884,208, laquinimod sodium is dissolved in water to form an aqueous solution; the aqueous solution is concentrated; and then a water-miscible anti-solvent is added to the concentrated solution to form laquinimod sodium crystals. The process of U.S. Patent No. 7,884,208 removes the impurities after salt formation, thus resulting in laquinimod sodium of higher purity than the laquinimod sodium produced directly from the "slurry to slurry" process of U.S. Patent No. 6,077,851.

Summary of the Invention

The subject invention provides a mixture of crystalline laquinimod sodium particles, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less or (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and wherein:

a) the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL;

b) the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL;

c) an amount of heavy metal which is present in the mixture is no more than 20 ppm of heavy metal relative to the amount by weight of laquinimod sodium;

d) an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

e) an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

f) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC,

g) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

h) an amount of N-ethylaniline (NEA) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC; or

i) an amount of *N*-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide (5-HLAQ) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC.

The subject invention provides a mixture of crystalline laquinimod sodium particles, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 40 microns, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 15 microns, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 5 microns and wherein:

a) the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL;

b) the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL;

c) an amount of heavy metal which is present in the mixture is no more than 20 ppm of heavy metal relative to the amount by weight of laquinimod sodium;

d) an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) which is presentin the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

e) an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

f) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC;

g) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

h) an amount of N-ethylaniline (NEA) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC; or

i) an amount of *N*-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide (5-HLAQ) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC.

The subject invention provides a pharmaceutical composition comprising the mixture as described herein and a pharmaceutically acceptable carrier.

The subject invention provides a process of recrystallization of laquinimod sodium comprising:

a) dissolving an amount of laquinimod sodium in water to form an aqueous solution;

b) concentrating the aqueous solution to form a concentrated solution comprising approximately 1.7-1.8 mL of water per gram of laquinimod sodium;

c) adding acetone to the concentrated solution of step b); and

d) isolating recrystallized laquinimod sodium.

The subject invention also provides for a mixture of crystalline laquinimod sodium particles prepared by the process as described herein, and a pharmaceutical composition comprising said mixture.

The subject invention provides a mixture of crystalline laquinimod sodium particles, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less or (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and wherein

a) an amount of aluminium in the mixture is less than 5 ppm relative to the amount by weight of laquinimod sodium;

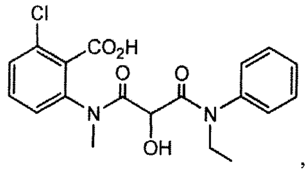
b) an amount of calcium in the mixture is less than 60 ppm relative to the amount by weight of laquinimod sodium;

c) an amount of copper in the mixture is less than 1 ppm relative to the amount by weight of laquinimod sodium; or

d) an amount of zinc in the mixture is less than 7 ppm relative to the amount by weight of laquinimod sodium.

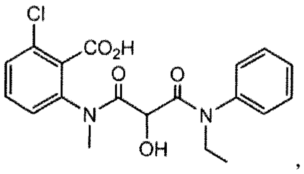
The subject invention also provides a pharmaceutical composition comprising the mixture as described herein and a pharmaceutically acceptable carrier.

The subject invention also provides an isolated compound having the structure:



, or a salt thereof.

The subject invention also provides aa composition comprising a compound having the structure:



or a salt thereof, wherein the composition is free of laquinimod or a salt thereof.

The subject invention also provides a pharmaceutical composition comprising an amount of laquinimod and at least one of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE), wherein a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or f) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

The subject invention also provides a process for preparing 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) comprising the steps of: a) adding sodium hydroxide solution to a suspension of 5-chloro-N-ethyl-3-hydroxy-1-methyl-2,4-dioxo-N-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide in water, b) stirring the mixture of step a) followed by addition of hydrochloric acid solution, c) extracting the aqueous solution with ethyl acetate, d) washing the organic phase with brine, e) drying the organic phase over sodium sulfate,

f) filtering the suspension, g) evaporating the filtrate, h) purifying the residue by crystallization from isopropyl alcohol, i) cooling the suspension followed by filtering and washing with isopropyl alcohol, and j) obtaining and drying the resulting white solid.

The subject invention also provides 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) prepared by the process described above.

The subject invention also provides a process for preparing 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) comprising the steps of: a) heating a mixture of 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) and dimethylsulfoxide, b) cooling the mixture of step a), and c) filtering the mixture of step b) and collecting the resulting filtrate.

The subject invention also provides 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) prepared by the process described above.

The subject invention also provides a process for preparing 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) comprising the steps of: a) heating a mixture of ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in a solution of hydrochloric acid in acetic acid, b) cooling the mixture of step a), c) diluting the mixture of step b) with 2-propanol and further cooling the diluted mixture, and d) filtering off the crystals resulting from step c).

The subject invention also provides 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) prepared by the process described above.

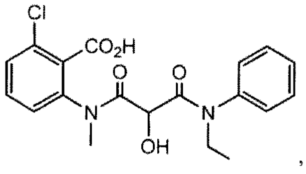
The subject invention also provides a process for preparing Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) comprising the steps of: a) forming sodium dimethylmalonate by reaction of dimethylmalonate in dimethlformamide with sodium methoxide solution, b) reacting the intermediate 5-chloro-1-methyl-1h-benzo[D][1,3] oxazine-2,4-dione with sodium dimethylmalonate to form methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) sodium salt, and c) acidifying methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) sodium salt to methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME).

The subject invention also provides Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) prepared by the process described above.

The subject invention also provides a process for preparing Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) comprising the steps of: a) adding sodium hydride to a solution of 5-chloro-1-methyl-1h-benzo[D][1,3] oxazine-2,4-dione and diethyl malonate in dimethylformamide, b) heating the mixture of step a) while stirring, c) cooling the solution of step b), d) quenching the reaction mixture of step c), e) acidifying the mixture of step d), f) filtering then drying the mixture of step e) , and g) crystallizing the crude product of step f) by dissolving in ethanol following by slow cooling.

The subject invention also provides Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) prepared by the process described above.

The subject invention also provides a process for testing whether a sample of laquinimod contains an undesirable impurity which comprises determining whether the sample contains a compound having the structure:



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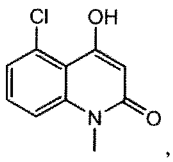

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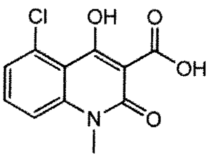

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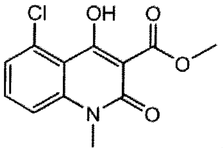

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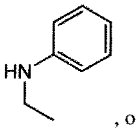

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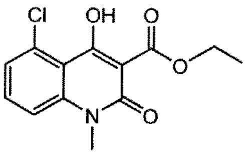

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The subject invention also provides a process for preparing a validated pharmaceutical composition comprising laquinimod comprising: a) obtaining a batch of laquinimod; b) determining the amount of at least one of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in the batch using by an HPLC method; and c) preparing the pharmaceutical composition from the batch only if

i) the batch is determined to have not more than 0.15% 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), relative to the concentration of laquinimod, or

ii) the batch is determined to have not more than 0.15% 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), relative to the concentration of laquinimod, or

iii) the batch is determined to have not more than 0.15%, 0.12% or 0.1% Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), relative to the concentration of laquinimod, or

iv) the batch is determined to have not more than 0.1% N-Ethylaniline (NEA), relative to the concentration of laquinimod, or

v) the batch is determined to have not more than 0.1% Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE), relative to the concentration of laquinimod.

The subject invention also provides a process for preparing a pharmaceutical composition comprising laquinimod, or for distributing a validated batch of a pharmaceutical composition comprising laquinimod, comprising a) obtaining a batch of laquinimod or of the pharmaceutical composition; b) performing stability testing with a sample of the batch; c) determining the total amount of at least one of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in the sample of the batch after stability testing by an HPLC method; and d) validating the batch for distribution or preparing the pharmaceutical composition from the batch only if the sample of the batch after stability testing contains

i) not more than a total of 0.15% relative to the concentration of laquinimod of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), or

ii) not more than a total of 0.15% relative to the concentration of laquinimod of 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), or

iii) not more than a total of 0.15%, 0.12% or 0.1% relative to the concentration of laquinimod of Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), or

iv) not more than a total of 0.1% relative to the concentration of laquinimod of N-Ethylaniline (NEA), or

v) not more than a total of 0.1% relative to the concentration of laquinimod of Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE).

The subject invention also provides a process for validating a batch of a pharmaceutical product containing laquinimod or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier for distribution comprising a) subjecting a sample of the batch to stability testing; b) determining the amount of at least one of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) in the sample of the batch after stability testing by an HPLC method; and c) validating the batch for distribution only if the sample of the batch after stability testing contains

i) not more than a total of 1.0% relative to the concentration of laquinimod of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), or

ii) not more than a total of 0.5% or 0.1% relative to the concentration of laquinimod of N-Ethylaniline (NEA), or

iii) not more than a total of 0.5% relative to the concentration of laquinimod of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) combined.

The subject invention also provides a process for preparing a packaged pharmaceutical composition comprising laquinimod or a pharmaceutically acceptable salt thereof comprising: a) obtaining a batch of pharmaceutical composition comprising laquinimod or a pharmaceutically acceptable salt thereof; b) performing stability testing with a sample from the batch; c) determining the amount of at least one of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in the sample by an HPLC method after stability testing; and d) packaging the pharmaceutical composition in only if

i) the content of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) in the sample is determined to be not more than 1.0% to the concentration of laquinimod, or

ii) the content of N-Ethylaniline (NEA) in the sample is determined to be not more than 0.5% or 0.1% to the concentration of laquinimod.

iii) the combined content of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the sample is determined to be not more than a total of 0.5% relative to the concentration of laquinimod.

The subject invention also provides an impurity or a salt thereof for use, as a reference standard to detect trace amounts of the impurity in a pharmaceutical composition comprising laquinimod or a pharmaceutically acceptable salt thereof, wherein the impurity is selected from the group consisting of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE).

The subject invention also provides a method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod, the method comprising, a) Preparing a sample solution from the pharmaceutical composition, b) Preparing a standard solution comprising the impurity, c) Preparing a resolution solution comprising laquinimod and the impurity, d) Preparing a buffer solution by dissolving ammonium acetate in water and adjusting to pH of 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid, e) Preparing a diluent solution comprising the buffer solution and acetonitrile, f) Preparing a blank solution comprising the diluent solution and aqueous acetonitrile, g) Injecting into the HPLC the resolution solution, the blank solution, the standard solution, and the sample solution, h) Running the HPLC using ultraviolet absorption at 240 nm and the diluent solution as the mobile phase, i) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and j) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3- carboxylate (MCQME), *N*-Ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2 dihydroquinoline-3-carboxamide (5-HLAQ) or Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE).

The subject invention also provides a method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod, the method comprising, a) Preparing a sample solution from the pharmaceutical composition, b) Preparing a standard solution comprising the impurity, c) Preparing a resolution solution comprising laquinimod and the impurity, d) Preparing a buffer solution by dissolving ammonium acetate in water, and adjusting to pH of 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid, e) Preparing a blank solution comprising the buffer solution and acetonitrile, f) Injecting into the HPLC the resolution solution, the blank solution, the standard solution, and the sample solution, g) Running the HPLC using ultraviolet absorption at 240 nm and a mobile phase of a mixture of the buffer solution, and acetonitrile, h) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and i) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is N-Ethylaniline (NEA), 1*H*,3*H*-spiro[5-chloro-1-methylquinoline-2,4-dione-3,3'-[1]ethylindolin-[2]-one] (SPIRO-LAQ), or 5-Chloro-*N*-ethyl-3-hydroxy-1-methyl-2,4-dioxo-*N*-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide (3-HLAQ).

The subject invention also provides a method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod and a pharmaceutically acceptable carrier, the method comprising, a) Preparing a sample solution from the pharmaceutical composition, b) Preparing a standard solution comprising the impurity, c) Preparing a Quantitation Limit (QL) solution comprising the impurity, d) Preparing a resolution solution comprising laquinimod and the impurity, e) Preparing a buffer solution by dissolving ammonium dihydrogen phosphate in water, and adjusting to pH of 7.0 ± 0.10 with aqueous ammonia or phosphoric acid, f) Preparing a blank solution comprising the buffer solution and acetonitrile, g) Injecting into the HPLC the resolution solution, the blank solution, the QL solution, the standard solution and the sample solution, h) Running the HPLC using a ultraviolet absorption at 212 nm and a mobile phase of a mixture of the buffer solution, acetonitrile, and methanol, i) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and j) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) or N-ethyl-4-hydroxy-1-methyl-5-(2,3,4,5,6-pentahydroxyhexylamino)-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide (MEG-LAQ).

The subject invention also provides a method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod and a pharmaceutically acceptable carrier, the method comprising, a) Preparing a sample solution from the pharmaceutical composition, b) Preparing a standard solution comprising the impurity, c) Preparing a resolution solution comprising laquinimod and the impurity, d) Preparing a buffer solution by dissolving ammonium acetate in water, and adjusting to PH or 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid, e) Preparing a blank solution comprising the buffer solution and acetonitrile, f) Injecting into the HPLC the resolution solution, the blank solution, the standard solution and the sample solution, g) Running the HPLC using a ultraviolet absorption at 242 nm and the blank solution as the mobile phase, h) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and i) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3- carboxylate (MCQME) or *N*-Ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2 dihydroquinoline-3-carboxamide (5-HLAQ).

The subject invention also provides a method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod and a pharmaceutically acceptable carrier, the method comprising, a) Preparing a sample solution from the pharmaceutical composition, b) Preparing a standard solution comprising the impurity, c) Preparing a resolution solution comprising laquinimod and the impurity, d) Preparing a blank solution comprising methanol and acetonitrile, e) Preparing a buffer solution by dissolving ammonium acetate in water, and adjusting to pH of 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid, f) Injecting into the HPLC the resolution solution, the blank solution, and the sample solution, g) Running the HPLC using a ultraviolet absorption at 240 nm and a mobile phase comprising acetonitrile and the buffer solution, h) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and i) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is N-Ethylaniline (NEA), 5-Chloro-*N*-ethyl-3-hydroxy-1-methyl-2,4-dioxo-*N*-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide (3-HLAQ) or 1*H*,3*H*-spiro[5-chloro-1-methylquinoline-2,4-dione-3,3'-[1]ethylindolin-[2]-one] (SPIRO-LAQ).

Brief Description of Figures

Figure 1: Fig. 1 from U.S. Patent No. 7,884,208.

Figure 2: Fig. 2 from U.S. Patent No. 7,884,208.

Figure 3: HPLC Data – Example chromatogram of mixture of laquinimod sodium.

Figure 4: HPLC Data – Pure chromatogram of Laquinimod Sodium.

Figure 5: Microscopic photograph of typical batch of crude Laquinimod Sodium at a first magnification level.

Figure 6: Microscopic photograph of typical batch of crude Laquinimod Sodium at a second magnification level.

Figure 7: Microscopic photograph of Batch C at a first magnification level.

Figure 8: Microscopic photograph of Batch C at a second magnification level.

Figure 9: 1H-NMR Spectrum of MCQ in DMSO.

Figure 10: 13C-NMR Spectrum of MCQ in DMSO.

Figure 11: Mass Spectrum of MCQ (ES+ mode).

Figure 12: Fourier Transform-Infrared Spectrum of MCQ in KBr.

Figure 13: 1H-NMR Spectrum of MCQCA in D_2O+KOH.

Figure 14: 13C-NMR Spectrum of MCQCA in D_2O +KOH.

Figure 15: Mass Spectrum of MCQCA (ES+ mode).

Figure 16: Fourier Transform Infrared Spectrum of MCQCA.

Figure 17: 1H-NMR Spectrum of MCQEE in $CDCl_3$.

Figure 18: 13C-NMR Spectrum of MCQEE in $CDCl_3$.

Figure 19: Mass Spectrum of MCQEE (ES^+ mode).

Figure 20: Fourier Transform Infrared Spectrum of MCQEE.

Figure 21: 1H-NMR Spectrum of 5-HLAQ in DMSO.

Figure 22: 13C-NMR Spectrum of 5-HLAQ in DMSO.

Figure 23: Mass Spectrum of 5-HLAQ (ES+ mode).

Figure 24: Fourier Transform Infrared Spectrum of 5-HLAQ.

Figure 25: 1H-NMR Spectrum of DELAQ in $CDCl_3$.

Figure 26: 13C-NMR Spectrum of DELAQ in $CDCl_3$.

Figure 27: Mass Spectrum of DELAQ (ES+ mode).

Figure 28: Fourier Transform Infrared Spectrum of DELAQ in KBr.

Figure 29: 1H-NMR Spectrum of 3-HLAQ in $CDCl_3$.

Figure 30: 13C-NMR Spectrum of 3-HLAQ in $CDCl_3$.

Figure 31: Mass Spectrum of 3-HLAQ (ES+ mode).

Figure 32: Fourier Transform Infrared Spectrum of 3-HLAQ.

Figure 33: 1H-NMR Spectrum of SPIRO-LAQ in DMSO.

Figure 34: 13C-NMR Spectrum of SPIRO-LAQ in DMSO.

Figure 35: Mass Spectrum of SPIRO-LAQ (ES+ mode).

Figure 36: Fourier Transform Infrared Spectrum of SPIRO-LAQ.

Figure 37: 1H-NMR of BH-3-HLAQ in DMSO.

Figure 38: 13C-NMR of BH-3-HLAQ in DMSO.

Figure 39: Mass Spectrum of BH-3-HLAQ (ES+ mode).

Figure 40: FT-IR Spectrum of BH-3-HLAQ.

Figure 41: Resolution test chromatogram (Laquinimod Sodium Before Milling (Cryst) and Drug Substance) – Identification By Retention Time And Determination Of Assay And Polar Impurities / Degradation Products (Polar IDD) By HPLC.

Figure 42: Diluent chromatogram (Laquinimod Sodium Before Milling (Cryst) and Drug Substance) – Identification By Retention Time And Determination Of Assay And Polar Impurities / Degradation Products (Polar IDD) By HPLC.

Figure 43: Typical chromatogram for assay (Laquinimod Sodium Before Milling (Cryst) and Drug Substance) – Identification By Retention Time And Determination Of Assay And Polar Impurities / Degradation Products (Polar IDD) By HPLC.

Figure 44: Typical chromatogram for Impurities testing (Laquinimod Sodium Before Milling (Cryst) and Drug Substance) – Identification By Retention Time And Determination Of Assay And Polar Impurities / Degradation Products (Polar IDD) By HPLC.

Figure 45: Resolution test chromatogram (Laquinimod Sodium Before Milling (Cryst) and Drug Substance) – Determination Of N-Ethylaniline And Nonpolar Impurities (Non-Polar IDD) By HPLC.

Figure 46: Diluent chromatogram (Laquinimod sodium before milling (Cryst) and Drug Substance) – Determination Of N-Ethylaniline And Nonpolar Impurities (Non-Polar IDD) By HPLC.

Figure 47: Typical sample chromatogram (Laquinimod sodium before milling (Cryst) and Drug Substance) – Determination Of N-Ethylaniline And Nonpolar Impurities (Non-Polar IDD) by HPLC.

Figure 48: Resolution Solution 1 (Resolution Test) Chromatogram (Laquinimod Capsules) Identification And Determination Of Assay And Polar Impurities / Degradation Products By HPLC.

Figure 49: Resolution Solution 2 Chromatogram (Laquinimod Capsules) Identification And Determination Of Assay And Polar Impurities / Degradation Products By HPLC.

Figure 50: Diluent (Blank) chromatogram (Laquinimod Capsules) Identification and Determination of Assay and Polar Impurities / Degradation Products By HPLC.

Figure 51: Sample chromatogram for Assay (Laquinimod Capsules) Identification and Determination of Assay and Polar Impurities / Degradation Products By HPLC.

Figure 52: Sample chromatogram for determination of Impurities / Degradation products – (Laquinimod Capsules) Identification and Determination of Assay and Polar Impurities / Degradation Products by HPLC.

Figure 53: Resolution test chromatogram (Laquinimod Capsules) Determination of Non-Polar

Impurities / Degradation Products.

Figure 54: Blank (Diluent) chromatogram (Laquinimod Capsules) Determination of Non-Polar Impurities / Degradation Products.

Figure 55: Sample chromatogram (Laquinimod Capsules) Determination of Non-Polar Impurities / Degradation Products.

Figure 56: Typical Chromatogram of Resolution Solution (Laquinimod Capsules) – Determination of MEG-LAQ and BH-3-HLAQ by HPLC.

Figure 57: Blank (Diluent 2) chromatogram (Laquinimod Capsules) – Determination of MEG-LAQ and BH-3-HLAQ by HPLC.

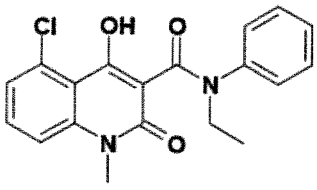
Figure 58: Chromatogram of QL Solution (Laquinimod Capsules) – Determination of MEG-LAQ and BH-3-HLAQ by HPLC.

Figure 59: Chromatogram of Standard Solution (Laquinimod Capsules) – Determination of MEG-LAQ and BH-3-HLAQ by HPLC.

Figure 60: Typical Sample Chromatogram (Laquinimod Capsules) – Determination of MEG-LAQ and BH-3-HLAQ by HPLC.

Detailed Description of the Invention

Laquinimod is a small molecule having the following chemical structure:



Laquinimod

It is an oral immunomodulator which has demonstrated therapeutic effect in various experimental inflammatory/autoimmune animal models, such as Experimental Autoimmune Encephalomyelitis (EAE), an animal model for Multiple Sclerosis (MS), Dextran Sodium Solphate (DSS) induced colitis for Inflammatory Bowel Disease, Non–Obese Diabetic (NOD) mice for Type I Diabetes (IDDM), Experimental Autoimmune Neuritis (EAN) for Guillain-Barre Syndrome, Systemic Lupus Erythematosus (SLE), lupus nephritis, lupus arthritis, Crohn's Disease and Rheumatoid arthritis. The therapeutic activity of laquinimod in these models results from a variety of mechanistic effects, including reduction of leukocyte infiltration into target tissues by modulation of chemokine-mediated T-cell adhesion, modulation of cytokine balance, down regulation of MHC class II resulting in alteration of antigen presentation, and effects on dendritic cells subpopulations (PCT International Application Publication No. WO2013/169746).

A pharmaceutically acceptable salt of laquinimod includes lithium, sodium, potassium, magnesium, calcium, manganese, copper, zinc, aluminum and iron. Salt formulations of laquinimod and the process for preparing the same are described, e.g., in U.S. Patent Application Publication No. 2005/0192315 and PCT International Application Publication No. WO 2005/074899, which are hereby incorporated by reference into this application.

The subject invention provides a mixture of crystalline laquinimod sodium particles, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less or (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and wherein:

a) the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL;

b) the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL;

c) an amount of heavy metal which is present in the mixture is no more than 20 ppm of heavy metal relative to the amount by weight of laquinimod sodium;

d) an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

e) an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

f) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC;

g) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

h) an amount of N-ethylaniline (NEA) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC; or

i) an amount of *N*-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide (5-HLAQ) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC.

In an embodiment of the mixture, (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 40 microns or (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 15 microns.

In an embodiment of the mixture, 10% or more of the total amount by volume of the laquinimod sodium particles have a size of 5 microns or less and wherein:

a) the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL; or

b) an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC.

The subject invention provides a mixture of crystalline laquinimod sodium particles, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 40 microns, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 15 microns, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 5 microns and wherein:

a) the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL;

b) the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL;

c) an amount of heavy metal which is present in the mixture is no more than 20 ppm of heavy metal relative to the amount by weight of laquinimod sodium;

d) an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

e) an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

f) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC;

g) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

h) an amount of N-ethylaniline (NEA) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC; or

i) an amount of *N*-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide (5-HLAQ) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC.

In an embodiment of the mixture, the mixture is prepared in a single batch comprising 2.5 kg or more of laquinimod sodium. In an embodiment of the mixture, the laquinimod sodium particles are determined based on an unmilled sample of the mixture. In an embodiment of the mixture, the size and amount by volume of laquinimod sodium particles are determined based on a milled sample of the mixture.

In an embodiment of the mixture, the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL. In an embodiment of the mixture, the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL.

In an embodiment of the mixture, an amount of aluminium in the mixture is less than 5 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of aluminium in the mixture is less than 2 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of calcium in the mixture is less than 60 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of calcium in the mixture is less than 25 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of copper in the mixture is less than 1 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of copper in the mixture is less than 0.6 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of zinc in the mixture is less than 7 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of zinc in the mixture is less than 4 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of heavy metal in the mixture is no more than 20 ppm relative to the amount by weight of laquinimod sodium.

In an embodiment of the mixture, a total amount of polar impurities in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC.

In an embodiment of the mixture, an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the mixture, an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the mixture, an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC. In another embodiment, the amount of MCQME in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the mixture, an amount of ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the mixture, a total amount of non-polar impurities in the mixture is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the mixture, an amount of N-ethylaniline (NEA) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the mixture, an amount of *N*-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide (5-HLAQ) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the mixture, an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide (DELAQ) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC.

In an embodiment of the mixture, an amount of laquinimod acid in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the mixture, an amount of dimethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the mixture, an amount of diethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the mixture, an amount of dimethyl sulfate in the mixture is no more than 1 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of water in the mixture is no more than 1.5% by weight relative to the amount of laquinimod sodium as measured by K.F. coulometric titration. In an embodiment of the mixture, an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium.

In an embodiment of the mixture, an amount of ethanol in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of n-heptane in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of n-octane in the mixture is no more than 2000 ppm relative to the amount by weight of laquinimod sodium. In another embodiment of the mixture, an amount of n-octane in the mixture is no more than 200 ppm relative to the amount by weight of laquinimod sodium. In another embodiment of the mixture, an amount of n-octane in the mixture is no more than 20 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of methanol in the mixture is no more than 3000 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of acetone in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of dioxane in the mixture is no more than 380 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the mixture, an amount of dimethyl formamide in the mixture is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

In an embodiment of the mixture, the recited compound is present in the mixture. In another embodiment, the recited compound is present in at least trace amounts.

The subject invention provides a pharmaceutical composition comprising the mixture of of the subject invention and a pharmaceutically acceptable carrier.

In an embodiment of the pharmaceutical composition, a total amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the pharmaceutical composition, a total amount of polar impurities in the pharmaceutical composition is no more than 2.00% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the pharmaceutical composition, an amount of N-ethylaniline (NEA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the pharmaceutical composition, an amount of 5-chloro-N-ethyl-3-hydroxy-1-methyl-5-2,4-dioxo-N-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide (3-HLAQ) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the pharmaceutical composition, a total amount of non-polar impurities in the pharmaceutical composition is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC. In an embodiment of the pharmaceutical composition, an amount of N-ethyl-4-hydroxy-1-methyl-5-(methyl(2,3,4,5,6-pentahydroxyhexyl)amino)-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide (MEG-LAQ) in the pharmaceutical composition is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC.

In an embodiment of the pharmaceutical composition, an amount of water in the pharmaceutical composition is no more than 1.50% relative to the amount of laquinimod sodium as measured by K.F. coulometric titration. In an embodiment of the pharmaceutical composition, an amount of water in the pharmaceutical composition is no more than 0.80% relative to the amount of laquinimod sodium as measured by K.F. coulometric titration. In an embodiment of the pharmaceutical composition, an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium.

In an embodiment of the pharmaceutical composition, an amount of ethanol in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the pharmaceutical composition, an amount of n-heptane in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the pharmaceutical composition, an amount of n-octane in the pharmaceutical composition is no more than 2000 ppm relative to the amount by weight of laquinimod sodium. In another embodiment of the pharmaceutical composition, an amount of n-octane in the pharmaceutical composition is no more than 200 ppm relative to the amount by weight of laquinimod sodium. In another embodiment of the pharmaceutical composition, an amount of n-octane in the pharmaceutical composition is no more than 20 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the pharmaceutical composition, an amount of methanol in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the pharmaceutical composition, an amount of acetone in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the pharmaceutical composition, an amount of dioxane in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium. In an embodiment of the pharmaceutical composition, an amount of dimethyl formamide in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

In an embodiment of the pharmaceutical composition, the recited compound is present in the pharmaceutical composition. In another embodiment, the recited compound is present in at least trace amounts.

The subject invention provides a method of treating a subject afflicted with a form of multiple sclerosis, lupus nephritis, lupus arthritis, rheumatoid arthritis, a BDNF-related disorder, Crohn's disease, a GABA-related disorder, a cannabinoid receptor type 1 (CB1) mediated disorder, or an ocular inflammatory disorder comprising administering to the subject the mixture of the subject invention or the pharmaceutical composition of the subject invention so as to thereby treat the subject.

The subject invention provides a method for alleviating a symptom of multiple sclerosis, lupus nephritis, lupus arthritis, rheumatoid arthritis, a BDNF-related disorder, Crohn's disease, a GABA-related disorder, a cannabinoid receptor type 1 (CB1) mediated disorder, or an ocular inflammatory disorder in a subject afflicted with a form of multiple sclerosis comprising administering to the subject the mixture of any one of the subject invention or the pharmaceutical composition of the subject invention thereby alleviating the symptom of multiple sclerosis in the subject.

In one embodiment, the mixture or the pharmaceutical composition for use in the treatment of, or alleviation of symptoms of, a form of multiple sclerosis, lupus nephritis, lupus arthritis, rheumatoid arthritis, a BDNF-related disorder, Crohn's disease, a GABA-related disorder, a cannabinoid receptor type 1 (CB1) mediated disorder, or an ocular inflammatory disorder.

The subject invention provides a use of the mixture or the pharmaceutical composition for the manufacture of a medicament for treating, or alleviating a symptom of, a form of multiple sclerosis, a GABA-related disorder, a cannabinoid receptor type 1 (CB1) mediated disorder, lupus nephritis, lupus arthritis, rheumatoid arthritis, a BDNF-related disorder, Crohn's disease, or an ocular inflammatory disorder.

The subject invention provides a process of recrystallization of laquinimod sodium comprising: a) dissolving an amount of laquinimod sodium in water to form an aqueous solution; b) concentrating the aqueous solution to form a concentrated solution comprising approximately 1.7-1.8 mL of water per gram of laquinimod sodium; c) adding acetone to the concentrated solution of step b); and d) isolating recrystallized laquinimod sodium.

In an embodiment of the process, the amount of laquinimod sodium in step a) is 2.5 kg or greater. In an embodiment of the process, step a) is performed with 10-12 mL of water per gram of laquinimod sodium. In an embodiment of the process, step a) is performed with approximately 11 mL of water per gram of laquinimod sodium. In an embodiment of the process, step a) is performed by heating the aqueous solution to a temperature of 58-75°C. In an embodiment of the process, step a) is performed by heating the aqueous solution to a temperature of 60-73°C.

In an embodiment of the process, crystallization occurs after step a) and before step c).

In an embodiment of the process, crystallization is induced by rapid stirring during or after the concentrating step b). In an embodiment of the process, crystallization is induced by addition of a seed crystal during or after the concentrating step b). In an embodiment of the process, crystallization occurs without addition of a seed crystal.

In an embodiment of the process, step b) is performed under conditions appropriate to induce crystallization at the concentration of 1.7-1.8 mL of water per gram of laquinimod sodium. In an embodiment of the process, step b) is performed at 28-45°C. In an embodiment of the process, step b) is performed at 30-40°C. In an embodiment of the process, step c) is performed with the concentrated solution at 40-55°C. In an embodiment of the process, step c) is performed with the concentrated solution at 45-50°C. In an embodiment of the process, step c) is performed with 6-12 mL of acetone per gram of laquinimod sodium. In an embodiment of the process, step c) is performed with approximately 10 mL of acetone per gram of laquinimod sodium. In an embodiment of the process, step c) is performed over a period of 1-4 hours. In an embodiment of the process, step c) is performed over a period of 1.2-2.5 hours. In an embodiment of the process, step c) is followed by cooling the solution to a temperature no less than -14°C and no more than 6°C. In an embodiment of the process, step c) is followed by cooling the solution to a temperature no less than -4°C and no more than 4°C.

In an embodiment of the process, the solution is cooled over a period of 3-5 hours. In an embodiment of the process, the solution is cooled over a period of 3.5-4.5 hours.

In an embodiment of the process, step d) further comprises washing the recrystallized laquinimod sodium with 1-4 mL of acetone per gram of crude laquinimod sodium used in step a). In an embodiment of the process, step d) further comprises washing the recrystallized laquinimod sodium with approximately 3 mL of acetone per gram of crude laquinimod sodium used in step a). In an embodiment of the process, step d) further comprises drying the recrystallized laquinimod sodium for no less than one hour at 30-40°C under a vacuum of no more than 50 mmHg.

In an embodiment of the process, the isolated recrystallized laquinimod sodium in step d) is a mixture of crystalline laquinimod sodium particles having a particle size distribution such that (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 5 microns or less.

The subject invention provides a mixture of crystalline laquinimod sodium particles prepared by the process of the subject invention.

In an embodiment of the mixture of crystalline laquinimod sodium particles prepared by the process of the subject invention, (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of 5 microns or less.

In an embodiment of the mixture,

a) the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL;

b) the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL;

c) an amount of aluminium in the mixture is less than 5 ppm relative to the amount by weight of laquinimod sodium;

d) an amount of calcium in the mixture is less than 60 ppm relative to the amount by weight of laquinimod sodium;

e) an amount of copper in the mixture is less than 1 ppm relative to the amount by weight of laquinimod sodium;

f) an amount of zinc in the mixture is less than 7 ppm relative to the amount by weight of laquinimod sodium;

g) an amount of heavy metal in the mixture is no more than 20 ppm relative to the amount by weight of laquinimod sodium;

h) a total amount of polar impurities in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

i) an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

j) an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

k) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC;

l) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

m) an amount of ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

n) a total amount of non-polar impurities in the mixture is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

o) an amount of N-ethylaniline (NEA) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

p) an amount of laquinimod acid in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

q) an amount of dimethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

r) an amount of diethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

s) an amount of dimethyl sulfate in the mixture is no more than 1 ppm relative to the amount by weight of laquinimod sodium;

t) an amount of water in the mixture is no more than 1.5% by weight relative to the amount of laquinimod sodium as measured by K.F. coulometric titration;

u) the mixture comprises an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium;

v) an amount of ethanol in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

w) an amount of n-heptane in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

x) an amount of n-octane in the mixture is no more than 2000 ppm relative to the amount by weight of laquinimod sodium;

y) an amount of n-octane in the mixture is no more than 200 ppm relative to the amount by weight of laquinimod sodium;

z) an amount of n-octane in the mixture is no more than 20 ppm relative to the amount by weight of laquinimod sodium;

aa) an amount of methanol in the mixture is no more than 3000 ppm relative to the amount by weight of laquinimod sodium;

bb) an amount of acetone in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

cc) an amount of dioxane in the mixture is no more than 380 ppm relative to the amount by weight of laquinimod sodium; or

dd) an amount of dimethyl formamide in the mixture is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

In an embodiment of the mixture,

a) the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL;

b) the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL;

c) an amount of aluminium in the mixture is less than 5 ppm relative to the amount by weight of laquinimod sodium;

d) an amount of calcium in the mixture is less than 60 ppm relative to the amount by weight of laquinimod sodium;

e) an amount of copper in the mixture is less than 1 ppm relative to the amount by weight of laquinimod sodium;

f) an amount of zinc in the mixture is less than 7 ppm relative to the amount by weight of laquinimod sodium;

g) an amount of heavy metal in the mixture is no more than 0.002% relative to the amount by weight of laquinimod sodium;

h) a total amount of polar impurities in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

i) an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

j) an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

k) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC;

l) an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

m) an amount of ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

n) a total amount of non-polar impurities in the mixture is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

o) an amount of N-ethylaniline (NEA) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

p) an amount of laquinimod acid in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

q) an amount of dimethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

r) an amount of diethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

s) an amount of dimethyl sulfate in the mixture is no more than 1 ppm relative to the amount by weight of laquinimod sodium;

t) an amount of water in the mixture is no more than 1.5% by weight relative to the amount of laquinimod sodium as measured by K.F. coulometric titration;

u) the mixture comprises an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium;

v) an amount of ethanol in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

w) an amount of n-heptane in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

x) an amount of n-octane in the mixture is no more than 2000 ppm relative to the amount by weight of laquinimod sodium;

y) an amount of methanol in the mixture is no more than 3000 ppm relative to the amount by weight of laquinimod sodium;

z) an amount of acetone in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

aa) an amount of dioxane in the mixture is no more than 380 ppm relative to the amount by weight of laquinimod sodium; and

bb) an amount of dimethyl formamide in the mixture is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

In one embodiment, the amount of n-octane in the mixture is no more than 200 ppm. In another embodiment, the amount of n-octane in the mixture is no more than 20 ppm.

In an embodiment of the mixture, the recited compound is present in the mixture. In another embodiment, the recited compound is present in at least trace amounts.

The subject invention provides a pharmaceutical composition comprising the mixture of crystalline laquinimod sodium particles prepared by the process of the subject invention, and a pharmaceutically acceptable carrier.

In an embodiment of the pharmaceutical composition,

a) a total amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

b) a total amount of polar impurities in the pharmaceutical composition is no more than 2.00% relative to the amount of laquinimod sodium as measured by HPLC;

c) an amount of N-ethylaniline (NEA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

d) a total amount of non-polar impurities in the pharmaceutical composition is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

e) an amount of water in the pharmaceutical composition is no more than 0.80% relative to the amount of laquinimod sodium as measured by K.F. coulometric titration;

f) the pharmaceutical composition comprises an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium;

g) an amount of ethanol in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

h) an amount of n-heptane in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

i) an amount of n-octane in the pharmaceutical composition is no more than 2000 ppm relative to the amount by weight of laquinimod sodium;

j) an amount of n-octane in the pharmaceutical composition is no more than 200 ppm relative to the amount by weight of laquinimod sodium;

k) an amount of n-octane in the pharmaceutical composition is no more than 20 ppm relative to the amount by weight of laquinimod sodium;

l) an amount of methanol in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium;

m) an amount of acetone in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium;

n) an amount of dioxane in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium; or

o) an amount of dimethyl formamide in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

In an embodiment of the pharmaceutical composition,

a) a total amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

b) a total amount of polar impurities in the pharmaceutical composition is no more than 2.00% relative to the amount of laquinimod sodium as measured by HPLC;

c) an amount of N-ethylaniline (NEA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

d) a total amount of non-polar impurities in the pharmaceutical composition is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

e) an amount of water in the pharmaceutical composition is no more than 0.80% relative to the amount of laquinimod sodium as measured by K.F. coulometric titration;

f) the pharmaceutical composition comprises an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium;

g) an amount of ethanol in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

h) an amount of n-heptane in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

i) an amount of n-octane in the pharmaceutical composition is no more than 2000 ppm relative to the amount by weight of laquinimod sodium;

j) an amount of methanol in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium;

k) an amount of acetone in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium;

l) an amount of dioxane in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium; and

m) an amount of dimethyl formamide in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

In one embodiment, the amount of n-octane in the pharmaceutical composition is no more than 200 ppm relative to the amount by weight of laquinimod sodium. In another embodiment, the amount of n-octane in the pharmaceutical composition is no more than 20 ppm relative to the amount by weight of laquinimod sodium.

The subject invention provides a mixture of crystalline laquinimod sodium particles, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less or (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and wherein a) an amount of aluminium in the mixture is less than 5 ppm relative to the amount by weight of laquinimod sodium; b) an amount of calcium in the mixture is less than 60 ppm relative to the amount by weight of laquinimod sodium; c) an amount of copper in the mixture is less than 1 ppm relative to the amount by weight of laquinimod sodium; or d) an amount of zinc in the mixture is less than 7 ppm relative to the amount by weight of laquinimod sodium.

In an embodiment of the pharmaceutical composition, the recited compound is present in the pharmaceutical composition. In another embodiment, the recited compound is present in at least trace amounts.

The subject invention provides a pharmaceutical composition comprising the mixture of the subject invention and a pharmaceutically acceptable carrier.

In an embodiment of the mixture, 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 1 micron or greater, 2 microns or greater, 3 microns or greater or 4 microns or greater. In an embodiment of the mixture, 90% of the total amount by volume of the laquinimod sodium particles have a size of greater than 1 micron, greater than 2 microns, greater than 3 microns, greater than 4 microns or greater than 5 microns.

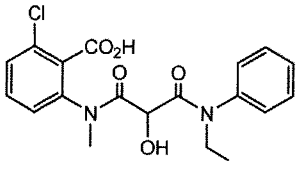
In a further embodiment of the mixture, the mixture has a tapped density of 0.4 g/mL to 0.7 g/mL, 0.44 g/mL to 0.7 g/mL, 0.45 g/mL to 0.7 g/mL, 0.46 g/mL to 0.7 g/mL or 0.5 g/mL to 0.7 g/mL. In a further embodiment of the mixture, the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL, 0.2 g/mL to 0.33 g/mL, 0.2 g/mL to 0.31 g/mL.

In a further embodiment of the pharmaceutical composition, the pharmaceutical composition comprises mannitol. In a further embodiment of the pharmaceutical composition, the pharmaceutical composition comprises meglumine. In a further embodiment of the pharmaceutical composition, the pharmaceutical composition comprises sodium stearyl fumarate.

In a further embodiment of the pharmaceutical composition, not less than 70% of the labeled amount of laquinimod is dissolved in 30 minutes. In a further embodiment of the pharmaceutical composition, capsules of the pharmaceutical composition contain 90-110% of the labeled amount of laquinimod. In a further embodiment of the pharmaceutical composition, capsules of the pharmaceutical composition contain 95-105% of the labeled amount of laquinimod. In a further embodiment of the pharmaceutical composition, capsules of the pharmaceutical composition contain 98.0-102.0% of the labeled amount of laquinimod.

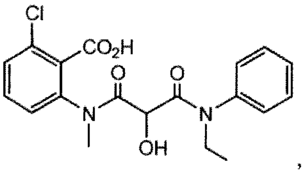
In a further embodiment of the pharmaceutical composition, the pharmaceutical composition has content uniformity conforming to the U.S. Pharmacopeia. In a further embodiment of the pharmaceutical composition, the pharmaceutical composition has content uniformity conforming to European Pharmacopeia.

The subject invention also provides an isolated compound having the structure:



, or a salt thereof.

The subject invention also provides a composition comprising a compound having the structure:



,

or a salt thereof, wherein the composition is free of laquinimod or a salt thereof.

The subject invention also provides a pharmaceutical composition comprising an amount of laquinimod and at least one of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE), wherein a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or f) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) is present in the pharmaceutical composition in an amount not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid

(MCQCA) is present in the pharmaceutical composition in an amount not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.12%, relative to the concentration of laquinimod, based on a determination by an HPLC method. In another embodiment, methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an less than 0.06%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) is present in the pharmaceutical composition in an amount greater than 0.02%, and not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.02%, and not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount greater than 0.02%, and not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.02%, and not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount greater than 0.02%, and not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than

0.12%, relative to the concentration of laquinimod, based on a determination by an HPLC method. In another embodiment, methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method. In yet another embodiment, 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) is present in the pharmaceutical composition in an amount greater than 0.05%, and not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.05%, or greater than 0.10%, and not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount greater than 0.05%, and less than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.06%, and not more than 0.10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount greater than 0.05%, and not more than 0.10%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.12%, relative to the concentration of laquinimod, based on a determination by an HPLC method. In another embodiment, methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.02%, or greater than 0.03%, and less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.02%, and less than 0.06%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment, 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, the composition further comprises at least one pharmaceutically acceptable carrier.

In an embodiment of the pharmaceutical composition as described herein, a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount not more than 1.0%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an less than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or f) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or g) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is less than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid

(MCQCA) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount greater than 0.02%, and not more than 1.0%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.03%, and not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is greater than 0.03%, and not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount greater than 0.05%, and not more than 1.0%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.1%, and not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is greater than 0.1%, and not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In an embodiment of the pharmaceutical composition as described herein, a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or b) 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or c) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or d) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or e) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.03%, and less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or f) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or g) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is greater than 0.03%, and less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method. In another embodiment, 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

In one embodiment, the pharmaceutical composition as described herein further comprises laquinimod sodium salt. In another embodiment, the pharmaceutical composition is in an oral unit dosage form. In another embodiments, it is in the form of a capsule, a tablet, or a liquid suspension. In another embodiment, the oral unit dosage form comprises more than 0.3 mg laquinimod. In another embodiments, the oral unit dosage form comprises more than 0.5 mg laquinimod. In yet another embodiment, the oral unit dosage form comprises more than 0.6 mg laquinimod.

The subject invention also provides a process for preparing 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) comprising the steps of: a) adding sodium hydroxide solution to a suspension of 5-chloro-N-ethyl-3-hydroxy-1-methyl-2,4-dioxo-N-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide in water, b) stirring the mixture of step a) followed by addition of hydrochloric acid solution, c) extracting the aqueous solution with ethyl acetate, d) washing the organic phase with brine, e) drying the organic phase over sodium sulfate, f) filtering the suspension, g) evaporating the filtrate, h) purifying the residue by crystallization from isopropyl alcohol, i) cooling the suspension followed by filtering and washing with isopropyl alcohol, and j) obtaining and drying the resulting white solid.

The subject invention also provides 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) prepared by the process described above.

The subject invention also provides a process for preparing 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) comprising the steps of: a) heating a mixture of 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) and dimethylsulfoxide, b) cooling the mixture of step a), and c) filtering the mixture of step b) and collecting the resulting filtrate.

The subject invention also provides 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) prepared by the process described above.

The subject invention also provides a process for preparing 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) comprising the steps of: a) heating a mixture of ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in a solution of hydrochloric acid in acetic acid, b) cooling the mixture of step a), c) diluting the mixture of step b) with 2-propanol and further cooling the diluted mixture, and d) filtering off the crystals resulting from step c).

The subject invention also provides 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) prepared by the process described above.

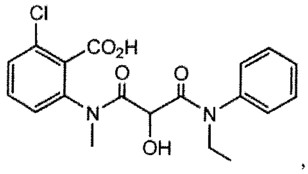
The subject invention also provides a process for preparing Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) comprising the steps of: a) forming sodium dimethylmalonate by reaction of dimethylmalonate in dimethlformamide with sodium methoxide solution, b) reacting the intermediate 5-chloro-1-methyl-1h-benzo[D][1,3] oxazine-2,4-dione with sodium dimethylmalonate to form methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) sodium salt, and c) acidifying methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) sodium salt to methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME).

The subject invention also provides Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) prepared by the process described above.

The subject invention also provides a process for preparing Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) comprising the steps of: a) adding sodium hydride to a solution of 5-chloro-1-methyl-1h-benzo[D][1,3] oxazine-2,4-dione and diethyl malonate in dimethylformamide, b) heating the mixture of step a) while stirring, c) cooling the solution of step b), d) quenching the reaction mixture of step c), e) acidifying the mixture of step d), f) filtering then drying the mixture of step e) , and g) crystallizing the crude product of step f) by dissolving in ethanol following by slow cooling.

The subject invention also provides Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) prepared by the process described above.

The subject invention also provides a process for testing whether a sample of laquinimod contains an undesirable impurity which comprises determining whether the sample contains a compound having the structure:



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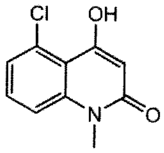

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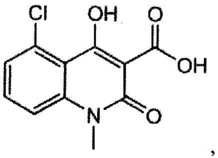

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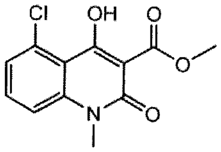

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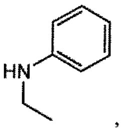

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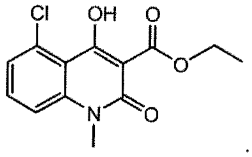

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The subject invention also provides a process for preparing a validated pharmaceutical composition comprising laquinimod comprising: a) obtaining a batch of laquinimod; b) determining the amount of at least one of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in the batch using by an HPLC method; and c) preparing the pharmaceutical composition from the batch only if

i) the batch is determined to have not more than 0.15% 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ), relative to the concentration of laquinimod, or

ii) the batch is determined to have not more than 0.15% 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), relative to the concentration of laquinimod, or

iii) the batch is determined to have not more than 0.15%, 0.12% or 0.1% Methyl 5-chloro-4-

hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), relative to the concentration of laquinimod, or

iv) the batch is determined to have not more than 0.1% N-Ethylaniline (NEA), relative to the concentration of laquinimod, or

v) the batch is determined to have not more than 0.1% Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE), relative to the concentration of laquinimod.

In one embodiment, in step c) the pharmaceutical composition is prepared from the batch only if the batch is determined to have not more than 0.12% Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), relative to the concentration of laquinimod. In another embodiment, in step c) the pharmaceutical composition is prepared from the batch only if the batch is determined to have not more than 0.1% Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), relative to the concentration of laquinimod.

The subject invention also provides a process for preparing a pharmaceutical composition comprising laquinimod, or for distributing a validated batch of a pharmaceutical composition comprising laquinimod, comprising a) obtaining a batch of laquinimod or of the pharmaceutical composition; b) performing stability testing with a sample of the batch; c) determining the total amount of at least one of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in the sample of the batch after stability testing by an HPLC method; and d) validating the batch for distribution or preparing the pharmaceutical composition from the batch only if the sample of the batch after stability testing contains

i) not more than a total of 0.15% relative to the concentration of laquinimod of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ), or

ii) not more than a total of 0.15% relative to the concentration of laquinimod of 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), or

iii) not more than a total of 0.15%, 0.12% or 0.1% relative to the concentration of laquinimod of Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), or

iv) not more than a total of 0.1% relative to the concentration of laquinimod of N-Ethylaniline (NEA), or

v) not more than a total of 0.1% relative to the concentration of laquinimod of Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE).

In one embodiment, the process further comprises step e) distributing the batch if in step d) the batch is validated for distribution. In another embodiments, in step d) the batch is validated for distribution or the pharmaceutical composition is prepared from the batch only if the sample of the batch after stability testing contains not more than 0.12% Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) relative to the concentration of laquinimod. In another embodiment, in step d) the batch is validated for distribution or the pharmaceutical composition is prepared from the batch only if the sample of the batch after stability testing contains not more than 0.1% Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), relative to the concentration of laquinimod.

The subject invention also provides a process for validating a batch of a pharmaceutical product containing laquinimod or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier for distribution comprising a) subjecting a sample of the batch to stability testing; b) determining the amount of at least one of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) in the sample of the batch after stability testing by an HPLC method; and c) validating the batch for distribution only if the sample of the batch after stability testing contains

i) not more than a total of 1.0% relative to the concentration of laquinimod of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), or

ii) not more than a total of 0.5% or 0.1% relative to the concentration of laquinimod of N-Ethylaniline (NEA), or

iii) not more than a total of 0.5% relative to the concentration of laquinimod of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) combined.

In an embodiment, in step c) the batch is validated for distribution only if the sample of the batch after stability testing not more than a total of the 0.1% of N-Ethylaniline (NEA) relative to the concentration of laquinimod.

The subject invention also provides a process for preparing a packaged pharmaceutical composition comprising laquinimod or a pharmaceutically acceptable salt thereof comprising: a) a) obtaining a batch of pharmaceutical composition comprising laquinimod or a pharmaceutically acceptable salt thereof; b) performing stability testing with a sample from the batch; c) determining the amount of at least one of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in the sample by an HPLC method after stability testing; and d) d) packaging the pharmaceutical composition in only if

i) the content of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) in the sample is determined to be not more than 1.0% to the concentration of laquinimod, or

ii) the content of N-Ethylaniline (NEA) in the sample is determined to be not more than 0.5% or 0.1% to the concentration of laquinimod.

iii) the combined content of 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the sample is determined to be not more than a total of 0.5% relative to the concentration of laquinimod.

In one embodiment, in step d) the pharmaceutical composition is packaged only if the content of N-Ethylaniline (NEA) in the sample is determined to be not more than 0.1% to the concentration of laquinimod.

In one embodiments, the laquinimod is laquinimod sodium salt.

The subject invention also provides an impurity or a salt thereof for use, as a reference standard to detect trace amounts of the impurity in a pharmaceutical composition comprising laquinimod or a pharmaceutically acceptable salt thereof, wherein the impurity is selected from the group consisting of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid

(BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE).

The subject invention also provides a method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod, the method comprising, a) Preparing a sample solution from the pharmaceutical composition, b) Preparing a standard solution comprising the impurity, c) Preparing a resolution solution comprising laquinimod and the impurity, d) Preparing a buffer solution by dissolving ammonium acetate in water and adjusting to pH of 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid, e) Preparing a diluent solution comprising the buffer solution and acetonitrile, f) Preparing a blank solution comprising the diluent solution and aqueous acetonitrile, g) Injecting into the HPLC the resolution solution, the blank solution, the standard solution, and the sample solution, h) Running the HPLC using ultraviolet absorption at 240 nm and the diluent solution as the mobile phase, i) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and j) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3- carboxylate (MCQME), N-Ethyl-4,5-dihydroxy-1-methyl-2-oxo-N-phenyl-1,2 dihydroquinoline-3-carboxamide (5-HLAQ) or Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE).

The subject invention also provides a method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod, the method comprising, a) Preparing a sample solution from the pharmaceutical composition, b) Preparing a standard solution comprising the impurity, c) Preparing a resolution solution comprising laquinimod and the impurity, d) Preparing a buffer solution by dissolving ammonium acetate in water, and adjusting to pH of 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid, e) Preparing a blank solution comprising the buffer solution and acetonitrile, f) Injecting into the HPLC the resolution solution, the blank solution, the standard solution, and the sample solution, g) Running the HPLC using ultraviolet absorption at 240 nm and a mobile phase of a mixture of the buffer solution, and acetonitrile, h) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and i) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions, wherein the impurity is N-Ethylaniline (NEA), 1H,3H-spiro[5-chloro-1-methylquinoline-2,4-dione-3,3'-[1]ethylindolin-[2]-one] (SPIRO-LAQ), or 5-Chloro-N-ethyl-3-hydroxy-1-methyl-2,4-dioxo-N-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide (3-HLAQ).

The subject invention also provides a method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod and a pharmaceutically acceptable carrier, the method comprising, a) Preparing a sample solution from the pharmaceutical composition, b) Preparing a standard solution comprising the impurity, c) Preparing a Quantitation Limit (QL) solution comprising the impurity, d) Preparing a resolution solution comprising laquinimod and the impurity, e) Preparing a buffer solution by dissolving ammonium dihydrogen phosphate in water, and adjusting to pH of 7.0 ± 0.10 with aqueous ammonia or phosphoric acid, f) Preparing a blank solution comprising the buffer solution and acetonitrile, g) Injecting into the HPLC the resolution solution, the blank solution, the QL solution, the standard solution and the sample solution, h) Running the HPLC using a ultraviolet absorption at 212 nm and a mobile phase of a mixture of the buffer solution, acetonitrile, and methanol, i) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and j) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) or N-ethyl-4-hydroxy-1-methyl-5-(2,3,4,5,6-pentahydroxyhexylamino)-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide (MEG-LAQ).

The subject invention also provides a method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod and a pharmaceutically acceptable carrier, the method comprising, a) Preparing a sample solution from the pharmaceutical composition, b) Preparing a standard solution comprising the impurity, c) Preparing a resolution solution comprising laquinimod and the impurity, d) Preparing a buffer solution by dissolving ammonium acetate in water, and adjusting to PH or 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid, e) Preparing a blank solution comprising the buffer solution and acetonitrile, f) Injecting into the HPLC the resolution solution, the blank solution, the standard solution and the sample solution, g) Running the HPLC using a ultraviolet absorption at 242 nm and the blank solution as the mobile phase, h) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and i) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is 5-Chloro-4-hydroxy-1-methylquinolin-2(1H)-one (MCQ), 5-Chloro-4-

hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3- carboxylate (MCQME) or N-Ethyl-4,5-dihydroxy-1-methyl-2-oxo-N-phenyl-1,2 dihydroquinoline-3-carboxamide (5-HLAQ).

The subject invention also provides a method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod and a pharmaceutically acceptable carrier, the method comprising, a) Preparing a sample solution from the pharmaceutical composition, b) Preparing a standard solution comprising the impurity, c) Preparing a resolution solution comprising laquinimod and the impurity, d) Preparing a blank solution comprising methanol and acetonitrile, e) Preparing a buffer solution by dissolving ammonium acetate in water, and adjusting to pH of 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid, f) Injecting into the HPLC the resolution solution, the blank solution, and the sample solution, g) Running the HPLC using a ultraviolet absorption at 240 nm and a mobile phase comprising acetonitrile and the buffer solution, h) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and i) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is N-Ethylaniline (NEA), 5-Chloro-N-ethyl-3-hydroxy-1-methyl-2,4-dioxo-N-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide (3-HLAQ) or 1H,3H-spiro[5-chloro-1-methylquinoline-2,4-dione-3,3'-[1]ethylindolin-[2]-one] (SPIRO-LAQ).

Each embodiment disclosed herein is contemplated as being applicable to each of the other disclosed embodiments. Thus, all combinations of the various elements described herein are within the scope of the invention. For example, any embodiment for an element of a mixture a contemplated as being applicable to a pharmaceutical composition. As another rexample, an embodiment to a tapped density of 0.46 g/mL to 0.7 g/mL is contemplated as being applicable to a mixture having an amount of aluminium in the mixture less than 5 ppm relative to the amount by weight of laquinimod sodium.

Comparison to the Prior Art

Applicants have unexpectedly achieved a mixture of crystalline laquinimod sodium that is improved over the laquinimod sodium mixture of the prior art.

U.S. Patent No. 7,884,208 teaches a process for recrystallizing laquinimod sodium which produces a mixture of crystalline laquinimod sodium having larger crystals, lowered impurity levels and certain improved crystalline characteristics as compared to the laquinimod sodium crystals known at the time.

Specifically, the process of U.S. Patent No. 7,884,208 achieves a mixture of crystalline laquinimod sodium having (i) a mixture wherein 10% or more of the total amount by volume of the laquinimod sodium particles has a size of greater than 40 microns and wherein 50% or more of the total amount by volume of the laquinimod sodium particles has a size of greater than 15 microns, (ii) high density (tapped and bulk), (iii) low heavy metal content, and (iv) low content of certain polar impurities.

However, the recrystallization process of U.S. Patent No. 7,884,208 (Examples 13-17, Tables 1-4) does not produce a mixture of recystallized laquinimod sodium particles wherein 90% or more of the total amount by volume of the laquinimod sodium have a size of 40 microns or less, or 50% or more of the total amount by volume of the laquinimod sodium has a size of 15 microns or less.

Likewise, the recrystallization process of U.S. Patent No. 7,884,208 does not produce a mixture of recystallized laquinimod sodium particles wherein 90% or more of the total amount by volume of the laquinimod sodium have a size of less than 40 microns, wherein 50% or more of the total amount by volume of the laquinimod sodium has a size of less than 15 microns, and wherein 10% or more of the total amount by volume of the laquinimod sodium has a size of less than 5 microns.

Example 14 of U.S. Patent No. 7,884,208 produced a mixture of recystallized laquinimod sodium particles wherein 10% or more of the total amount by volume of the laquinimod sodium have a size of less than 5 microns. However, this Example also shows reduced quality of crystalline characteristics, specifically Tapped Density. The mixture produced by Example 14 has an acceptable D(0.1) value but an undesired Tapped Density.

Conversely, Example 13 of U.S. Patent No. 7,884,208 produced a mixture having high Tapped Density, but did not produce crystals wherein 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less or 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less.

Importantly, U.S. Patent No. 7,884,208 is unable to achieve the advantages of recrystallization, i.e., better density and impurity profiles, while also producing laquinimod sodium crystals wherein 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less, or 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less.

U.S. Patent No. 7,884,208, by way of Example 1 (batches A, B and C) and Tables 1-3, also teaches that the process disclosed in U.S. Patent No. 6,077,851 results in a mixture of crystalline laquinimod sodium having all four of (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less, or 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, (ii) poor density (tapped and bulk), (iii) high heavy metal content, and (iv) high polar impurity content. Importantly, although U.S. Patent No. 6,077,851 achieves a mixture wherein 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less or, 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, but it does not achieve crystals having acceptable density or low levels of impurities.

The prior art contains no teaching of a process for preparing laquinimod sodium wherein 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less, or 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and having desirable density and purity profiles.

The present invention provides a process which is capable of producing a mixture of recystallized laquinimod sodium crystals wherein 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less or (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and having desirable density and purity profiles, specifically, (i) high density (tapped or bulk), (ii) low heavy metal content, or (iii) low polar impurity content.

The mixture of laquinimod sodium provided by the present invention is achieved by an improved recrystallization process.

Improved Recrystallization Process

The laquinimod sodium manufactured by the recrystallization processes of the present invention has improved purity and density profiles over the laquinimod sodium disclosed in U.S. Patent No. 6,077,851 and improved crystalline characteristics, especially smaller particles, over U.S. Patent No. 7,884,208.

The modified recrystallization process of the present invention unexpectedly results in different recrystallization conditions than achieved by the process disclosed in U.S. Patent No. 7,884,208 and, thusly, results in different products. Specifically, concentrating the aqueous solution to 1.7-1.8 unexpectedly results in crystalline laquinimod sodium particles having reduced levels of impurities, improved crystalline characteristics, and wherein 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less, 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and 10% or more of the total amount by volume of the laquinimod sodium particles have a size of 5 microns or less.

Without being limited to any one particular theory, an important factor affecting crystallization is initial concentration of crystallizing solution. Careful control of concentration of the solution can facilitate initiation of spontaneous crystallization prior to the end of the concentration step. The process of the present invention can initiate spontaneous crystallization prior to addition of acetone.

Concentrating the aqueous solution to form a concentrated solution comprising approximately 1.7-1.8 mL of water per gram of laquinimod sodium is an important aspect of the present invention.

Improved Mixture Of Crystalline Laquinimod Sodium

U.S. Patent No. 7,884,208 teaches advantages associated with large particles. Specifically, U.S. Patent No. 7,884,208 teaches that larger particles of laquinimod sodium are more "processable" when making pharmaceutical compositions and that smaller particles are often associated with dust-like properties which may interfere with processing, and sometimes associated with flowability problems which may interfere with manufacturing. Further, U.S. Patent No. 7,884,208 teaches that chemical stability has been shown to be decreased by the increase in surface area that results from smaller particle size. (Felmeister, A. Chpt 88, *Remington's Pharmaceutical Sciences*, 15th Edition, Mack Publishing Company, Easton, PA (1975)).

However, the subject invention has unexpectedly achieved an improved mixture of laquinimod sodium wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of 5 microns or less.

Laqinimod has been graded as a highly potent API, demanding special caution and avoiding material contact with workers and environment. Laquinimod has been graded as having the highest potency rate, corresponding to a recommended acceptable daily intake (ADI) during operations and manufacturing of less than 0.01 mg/day or <1 μg/m^3 as an 8-hour TWA. High potency compounds are associated with controls, whether engineering, administrative or procedure-related, that afford the desired level of worker protection. For example, high potency compounds may require no human intervention or manual operations. (Bruce D. Naumann, Control Banding In The Pharmaceutical Industry, http://www.aioh.org.au/downloads/documents/ControlBandingBNaumann.pdf)

Importantly, although the mixture of the subject invention may be milled or unmilled, the present invention is achieved without the need of a milling operation.

The mixture of laquinimod sodium of the present invention overcomes the potential problems associated with mixtures having large particles. With respect to processing and manufacturing, the small size of the laquinimod sodium particles of the present invention may obviate the need for milling and comminution steps. U.S. Patent No. 7,884,208 reflected the understanding in the art that there are problems associated with small particles in pharmaceutical compositions, but the laquinimod sodium of the present invention has no problems associated with stability, processing or manufacturing.

Comminution introduces its own set of problems to a drug substance beyond the disadvantages of introducing an additional drug processing step. For example, milling can introduce impurities, new polymorphs, amorphous sections in the crystalline structure of the API, other changes to particle morphology, differences in agglomeration, increased solubility, changes in moisture levels, and changes in compressability (Hausner, "The Role of Particle Size in the Development of Generic Products" 2003). As a result, comminution may affect the efficacy and safety of a drug substance. Some of the disadvantages of comminution are illustrated by the side effects resulting from microcrystalline Nitrofurantoin compared to macrocrystalline Nitrofurantoin. (Brumfitt, W. and J. M. T. Hamilton-Miller, *J. Antimicrobial Chemotherapy* 42:363:371 (1998)).

Accordingly, it is advantageous to produce a drug substance which does not contain large particles to avoid the inefficiencies of additional process steps such as milling or sieving. The laquinimod sodium of the present invention provides a mixture of laquinimod sodium particles having small particle sizes which avoids safety problems and additional problems related to milling.

Another concern during formulation processes is maintaining uniformity of content of the drug product. In the case of laquinimod, the unit dose of laquinimod is quite low relative to the total weight of the drug product, e.g., tablet or capsule. A typical formulation, for example, may comprise only a small amount of laquinimod, e.g., 0.3, 0.6 or 1.2 mg, in a capsule with total weight of over 200 mg. As such, small fluctuation in the amount of laquinimod due to problems of flowability, segregation, uniformity, or poor homogenous distribution could result in a large percent deviation from the desired amount, e.g., 0.3, 0.6 or 1.2 mg. The mixture of laquinimod sodium of the present invention provides high uniformity of content and minimal fluctuations in the amount of laquinimod in the capsules.

Table 1. Uniformity of Laquinimod Blend (UoB) and Uniformity of the content of filled capsules (UoC)

Batch	UoB AVG	UoB RSD	UoC	UoC AV
a	99.5	1.67	99.3	4.5

b	100.6	0.61	98.7	2.7
c	102.6	1.10	99.9	2.2
d	97.3	0.90	99.4	4.7
e	101.3	1.02	100.6	2.4
f	101.3	1.02	100.8	3.6
g	97.4	0.85	96.4	5.9
h	101.6	1.08	98.6	2.8
i	98.5	0.71	94.2	8.6
j (0.3mg)	101.2	0.66	96.9	8.7
k	101.2	0.66	94.6	7.1
l	97.1	1.35	96.8	5
m	95.3	0.88	98.6	3.5
n	98.6	0.91	101.4	5.2
o	97.1	0.59	96.6	5.7
p	98.8	0.58	98.4	5.3
q	98.2	0.54	98.5	4.3
r	97.2	0.96	100.7	2.4
s	102.2	1.20	100.2	4.6
t	102.9	1.82	98.4	3.3
u	103.6	1.01	99.7	4.4
v	97.9	0.5	98.5	1.4
w	98.7	0.5	99.2	1.6
xA	95.3	0.7	94.2	12.1
xB				
y	97.1	1.2	98	
z1 (0.6mg)	99.8	1.8	97.4	2.2
z2 (0.6mg)	99.8	1.8	97.4	2.2

UoB: Uniformity of Blend, RSD is the parameter to describe the uniformity before filling into capsules; UoC: Uniformity of the content of the filled capsule, AV is the acceptance value which is related to RSD to uniformity.

Uniformity of the shape of laquinimod particles is also an important concern during formulation as a lack of uniformity of shape can cause variation in density of drug substance and cause problems during drug product formation, e.g., capsule or tablet formation. Crystalline laquinimod sodium particles are rod-shaped particles. It is known that milling operations may result in changes to particle shape.

Decreased particle size is known to result in faster dissolution profiles. The rate of dissolution of small particles is usually faster than that of large particles because a greater surface area of the drug substance is in contact with the liquid medium. When formulating a drug with a low dissolution rate, it is desirable to decrease particle size in order to increase dissolution and thus facilitate rapid gastrointestinal or oral absorption.

In such cases where drug substances have no recognized problems associated with dissolution rate, particle size reduction may be inadvisable and even deleterious. Increasing surface area can increase degradation rates of the drug substance. As discussed, for example, in U.S. Patent Nos. 8,178,127 and 7,989,473, laquinimod sodium is susceptible to degradation.

Unexpectedly, in spite of known disadvantages associated with small particle sizes, it was found that an improved drug substance and drug product resulted from a mixture of crystalline laquinimod sodium particles wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of 5 microns or less.

Accordingly, an advantage of the recrystallization process of the present invention is that the resulting mixture of crystalline laquinimod sodium has particles having small particle sizes, which is associated with high uniformity and homogeneity with respect to distribution of the API into capsules, tablets and other drug products. Laquinimod sodium crystals having small particle sizes can obviate or reduce the need for additional milling steps. The small particle sizes of the laquinimod sodium of the present invention are achieved without sacrificing desirable purity or density profiles and without the need for prior milling operations.

Another advantage of the present invention is that laquinimod sodium crystals have a higher density than the laquinimod sodium crystals produced by the slurry-to-slurry process of U.S. Patent No. 6,077,851. Low tapped density is anathema to certain prized qualities in a drug substance or drug product such as compressibility, the ability of a powder to decrease in volume under pressure, and compactibility, the ability of a powder to be compressed into a tablet of certain strength or hardness. Crystals with low tapped density are also known to have poor flowability, which results in a lack of uniformity of content in finished dosage forms, especially in tablets. (Rudnic et al. Chpt. 45, *Remington's Pharmaceutical Sciences*, 20th Edition, Lippincott Williams & Wilkins, Baltimore, MD. (2000)) Uniformity of content is especially important for pharmaceutical compositions comprising a potent drug substance, e.g., Laquinimod sodium.

Compared to the the slurry-to-slurry process of U.S. Patent No. 6,077,851, the present invention also shows low aggregation of the particles and, additionally, provides particles with acceptable density and lower levels of impurities. As shown in Figures 5-8, the crude laquinimod resulting from the process described in U.S. Patent No. 6,077,851 has a high rate of aggregates (Figures 5 and 6), compared to a low rate of aggregates of the present invention (Figures 7 and 8).

Another advantage of the present invention is that the process of the present invention is environmentally friendly without sacrificing desirable crystalline characteristics. Specifically, by use of water as the primary solvent, the present invention achieves both environmental friendliness and improved crystalline characteristics, specifically with respect to particle size distribution over U.S. Patent No. 7,884,208.

Terms

As used herein, and unless stated otherwise, each of the following terms shall have the definition set forth below.

As used herein, "laquinimod" means laquinimod acid or a pharmaceutically acceptable salt thereof, including laquinimod sodium.

As used herein, "laquinimod acid" is N-ethyl-N-phenyl-1,2,-dihydro-4-hydroxy-5-chloro-1-methyl-2-oxoquinoline-3-carboxamide, and its Chemical Registry number is 248281-84-7. "Laquinimod sodium" is the sodium salt of laquinimod acid.

As used herein, D(0.1) is the particle size, in microns, below which 10% by volume distribution of the population is found.

As used herein, D(0.5) is the particle size, in microns, below which 50% by volume distribution of the population is found.

As used herein, D(0.9) is the particle size, in microns, below which 90% by volume distribution of the population is found.

As used herein, "crystalline characteristics" includes particle size distribution, bulk density and tapped density.

As used herein, "drug substance" refers to the active ingredient in a drug product or for use in a drug product, which provides pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of man or animals.

As used herein, "drug product" refers to the formulated or finished dosage form containing the drug substance as well as at least one pharmaceutically acceptable carrier.

As used herein, a composition that is "free" of a chemical entity means that the composition contains, if at all, an amount of the chemical entity which cannot be avoided following an affirmative act intended to purify the composition by separating the chemical entity from the composition.

For example, a composition which is "free" of an alkalizing agent means that the alkalizing agent, if present at all, is a minority component of the composition by weight. Preferably, when a composition is "free" of a component, the composition comprises less than 0.1 wt%, 0.05 wt%, 0.02 wt%, or 0.01 wt% of the component.

As used herein, an "isolated" compound is a compound isolated from the crude reaction mixture following an affirmative act of isolation. The act of isolation involves separating the compound from the other known components of the crude reaction mixture, with some impurities, unknown side products and residual amounts of the other known components of the crude reaction mixture permitted to remain. Purification is an example of an affirmative act of isolation.

As used herein, "stability testing" refers to tests conducted at specific time intervals and various environmental conditions (e.g., temperature and humidity) to see if and to what extent a drug product degrades over its designated shelf life time. The specific conditions and time of the tests are such that they accelerate the conditions the drug product is expected to encounter over its shelf life. For example, detailed requirements of stability testing for finished pharmaceuticals are codified in 21 C.F.R §211.166, the entire content of which is hereby incorporated by reference.

As used herein, "dissolution rate" is determined based on the amount of drug substance dissolved in 30 min. as indicated in the U.S. Pharmacopeia <711>.

As used herein, "atmospheric pressure" refers to a pressure of about 1 atm.

As used herein, "ambient temperature" refers to a temperature of about 20°C to about 30°C.

As used herein, "about" in the context of a numerical value or range means ±10% of the numerical value or range recited or claimed.

As used herein, "approximately" in the context of a numerical value or range means ±5% of the numerical value or range recited or claimed.

The term "stable pharmaceutical composition" as used herein in connection with the composition according to the invention denotes a composition, which preserves the physical stability/integrity and/or chemical stability/integrity of the active pharmaceutical ingredient during storage. Furthermore, "stable pharmaceutical composition" is characterized by its level of degradation products not exceeding 5% at 40oC/75%RH after 6 months or 3% at 55oC/75% RH after two weeks, compared to their level in time zero.

As used herein, "treating" and "treatment" encompasses, e.g., inducing inhibition, regression, or stasis of a disease, disorder or condition, or ameliorating or alleviating a symptom of a disease, disorder or condition. "Ameliorating" or "alleviating" a condition or state as used herein shall mean to relieve or lessen the symptoms of that condition or state. "Inhibition" of disease progression or disease complication in a subject as used herein means preventing or reducing the disease progression and/or disease complication in the subject.

"Administering to the subject" means the giving of, dispensing of, or application of medicines, drugs, or remedies to a subject to relieve, cure, or reduce the symptoms associated with a condition, e.g., a pathological condition.

The drug substance of the present invention, e.g., laquinimod sodium, may be administered in admixture with suitable pharmaceutical diluents, extenders, excipients, or carriers (collectively referred to herein as a pharmaceutically acceptable carrier) suitably selected with respect to the intended form of administration and as consistent with conventional pharmaceutical practices. The unit will be in a form suitable for oral, rectal, topical, intravenous or direct injection or parenteral administration. The compounds can be administered alone or mixed with a pharmaceutically acceptable carrier.

This carrier can be a solid or liquid, and the type of carrier is generally chosen based on the type of administration being used. The active agent can be co-administered in the form of a tablet or capsule, liposome, as an agglomerated powder or in a liquid form. Examples of suitable solid carriers include lactose, sucrose, gelatin and agar. Capsules or tablets can be easily formulated and can be made easy to swallow or chew; other solid forms include granules, and bulk powders.

Capsules or tablets may contain suitable binders, lubricants, disintegrating agents, diluents, coloring agents, flavoring agents, flow-inducing agents, and melting agents. For instance, for oral administration in the dosage unit form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, gelatin, agar, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like.

Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth, or sodium alginate, povidone, carboxymethylcellulose, polyethylene glycol, waxes, and the like.

Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, stearic acid, sodium stearyl fumarate, talc and the like.

Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum, croscarmellose sodium, sodium starch glycolate and the like.

Specific examples of the techniques, pharmaceutically acceptable carriers and excipients that may be used to formulate oral dosage forms of the present invention are described, e.g., in U.S. Patent Application Publication No. 2005/0192315, PCT International Application Publication Nos. WO 2005/074899, WO 2007/047863, and WO 2007/146248, and in U.S. Patent No. 7,589,208. For example, the oral dosage form of the present invention may comprise an alkaline-reacting component, said component preferably amounting from about 1 to 20% by weight of the formulation in order to keep the pH above 8.

Techniques and compositions for making dosage forms useful in the present invention are described in the following references: 7 Modern Pharmaceutics, Chapters 9 and 10 (Banker & Rhodes, Editors, 1979); Pharmaceutical Dosage Forms: Tablets (Lieberman et al., 1981); Ansel, Introduction to Pharmaceutical Dosage Forms 2nd Edition (1976); Remington's Pharmaceutical Sciences, 17th ed. (Mack Publishing Company, Easton, Pa., 1985); Advances in Pharmaceutical Sciences (David Ganderton, Trevor Jones, Eds., 1992); Advances in Pharmaceutical Sciences Vol. 7. (David Ganderton, Trevor Jones, James McGinity, Eds., 1995); Aqueous Polymeric Coatings for Pharmaceutical Dosage Forms (Drugs and the Pharmaceutical Sciences, Series 36 (James McGinity, Ed., 1989); Pharmaceutical Particulate Carriers: Therapeutic Applications: Drugs and the Pharmaceutical Sciences, Vol 61 (Alain Rolland, Ed., 1993); Drug Delivery to the Gastrointestinal Tract (Ellis Horwood Books in the Biological Sciences. Series in Pharmaceutical Technology; J. G. Hardy, S. S. Davis, Clive G. Wilson, Eds.); Modern Pharmaceutics Drugs and the Pharmaceutical Sciences, Vol 40 (Gilbert S. Banker, Christopher T. Rhodes, Eds.). All of the aforementioned publications are incorporated by reference herein. Examples of suitable liquid dosage forms include solutions or suspensions in water, pharmaceutically acceptable fats and oils, alcohols or other organic solvents, including esters, emulsions, syrups or elixirs, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules. Such liquid dosage forms may contain, for example, suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, thickeners, and melting agents.

Oral dosage forms optionally contain flavorants and coloring agents. Parenteral and intravenous forms may also include minerals and other materials to make them compatible with the type of injection or delivery system chosen.

The compounds used in the method of the present invention may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamallar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines. The compounds may be administered as components of tissue-targeted emulsions.

The compounds used in the method of the present invention may also be coupled to soluble polymers as targetable drug carriers or as a prodrug. Such polymers include polyvinylpyrrolidone, pyran copolymer, polyhydroxylpropylmethacrylamide-phenol, polyhydroxyethylasparta-midephenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the compounds may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacylates, and crosslinked or amphipathic block copolymers of hydrogels.

For oral administration in liquid dosage form, the oral drug components are combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Examples of suitable liquid dosage forms include solutions or suspensions in water, pharmaceutically acceptable fats and oils, alcohols or other organic solvents, including esters, emulsions, syrups or elixirs, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules. Such liquid dosage forms may contain, for example, suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, thickeners, and melting agents.

Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance. In general, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration preferably contain a water soluble salt of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field.

The drug substance of the present invention, e.g., laquinimod sodium, may be administered in various forms, including those detailed herein. The treatment with the compound may be a component of a combination therapy or an adjunct therapy, i.e. the subject or patient in need of the drug is treated or given another drug for the disease in conjunction with one or more of the instant compounds. This combination therapy can be sequential therapy where the patient is treated first with one drug and then the other or the two drugs are given simultaneously. These can be administered independently by the same route or by two or more different routes of administration depending on the dosage forms employed.

Gelatin capsules may contain the active ingredient compounds and powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as immediate release products or as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract.

The compounds used in the method of the present invention may also be administered in intranasal form via use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will generally be continuous rather than intermittent throughout the dosage regimen.

Parenteral and intravenous forms may also include minerals and other materials to make them compatible with the type of injection or delivery system chosen.

A dosage unit of the compounds used in the method of the present invention may comprise a single compound or mixtures thereof with additional antibacterial agents. The compounds can be administered in oral dosage forms as tablets, capsules, pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. The compounds may also be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular form, or introduced directly, e.g. by injection, topical application, or other methods, into or onto a site of infection, all using dosage forms well known to those of ordinary skill in the pharmaceutical arts.

A "dose" or "dosage unit" of laquinimod as measured in milligrams refers to the milligrams of laquinimod acid present in a preparation, regardless of the form of the preparation. A dosage unit may comprise a single compound or mixtures of compounds thereof. A dosage unit can be prepared for oral dosage forms, such as tablets, capsules, pills, powders, and granules. For example, the “dose” or “dosage unit” of laquinimod may be 0.3, 0.6, or 1.2 mg.

As used herein, a "pharmaceutically acceptable" component is one that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio.

As used herein, “pharmaceutically acceptable carrier” refers to a carrier or excipient that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio. It can be a pharmaceutically acceptable solvent, suspending agent or vehicle, for delivering the instant compounds to the subject. The carrier may be liquid or solid and is selected with the planned manner of administration in mind. “Pharmaceutically acceptable carrier” includes “fillers”, which fill out the size of a tablet or capsule, making it practical to produce and convenient for the consumer to use. By increasing the bulk volume, the fillers make it possible for the final product to have the proper volume for patient handling. “Pharmaceutically acceptable carrier” also includes “lubricants”, which prevent ingredients from clumping together and from sticking to the tablet punches or capsule filling machine. Lubricants also ensure that tablet formation and ejection can occur with low friction between the solid and die wall. “Pharmaceutically acceptable carrier” also includes inert carriers such as lactose, gelatin, agar, starch, sucrose, glucose, methyl cellulose, dicalcium phosphate, calcium sulfate, mannitol, sorbitol, microcrystalline cellulose and the like. Liposomes are also a pharmaceutically acceptable carrier.

It is understood that where a parameter range is provided, all integers within that range, and tenths and hundredth thereof, are also provided by the invention. For example, “0.15-0.35%” includes 0.15%, 0.16%, 0.17% etc. up to 0.35%.

The subject invention is also intended to include all isotopes of atoms occurring on the compounds disclosed herein, including impurities. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include tritium and deuterium. Isotopes of carbon including C-13 and C-14.

As used herein, “detection limit” for an analytical method used in screening or testing for the presence of a compound in a sample is a threshold under which the compound in a sample cannot be detected by the analytical method used. The detection limits of a given HPLC method for detecting an impurity in a sample containing laquinimod may vary based on the method and the impurity or impurities being detected. For example, the detection limit of the typical HPLC method for detecting 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is 0.03% and the detecting limit of a given method for detecting methyl 5-chloro-4-

hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline (MCQ), 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) and unknown impurities is 0.02%.

As used herein, “quantitation limit” for an analytical method used in screening or testing for the presence of a compound in a sample is a threshold under which the compound in a sample cannot be quantified by the analytical method used. The quantitation limits of a given HPLC method for detecting an impurity in a sample containing laquinimod may vary based on the impurity or impurities being detected.

As used herein, "density" is a measurement defined as the mass of a substance per unit volume.

As used herein, "bulk density" or “BD” refers to a density measurement of a loose, uncompacted substance, wherein the volume of the substance includes the air trapped between particles.

As used herein, "tapped density" or “TD” refers to a density measurement of a substance that has been tapped or vibrated, thus minimizing the volume of the substance by eliminating or minimizing the air trapped between particles.

As used herein, “rapid stirring” refers to stirring which splashes solvent onto the walls of the vessel.

As used herein, “blend uniformity” refers to the homogeneity of blend or granulate including laquinimod sodium particles prior to encapsulation, tableting or otherwise finalizing the drug product beyond formation of the final blend, and can represent either one sample or the average of more than one sample. Blend uniformity may be measured, for example, by taking 10 samples that represent the upper, middle and lower layer of each batch of the final blend, performing an HPLC assay to measure the amount of active ingredient in the samples, and comparing the amount of active ingredient in each sample to the labeled amount of active ingredient. The standard deviation and relative standard deviation can be determined based on the individual amounts of the tested samples expressed as percentages of the labeled amount of drug substance in each sample.

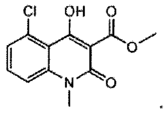
As used herein, “content uniformity” refers to the homogeneity of the laquinimod sodium content among dosage forms, e.g., capsules or tablets, after formulation. The uniformity of dosage units by content uniformity of the pharmaceutical composition described herein meets the U.S. Pharmacopeia <905> Acceptance Value and range (as specified); L1 = 15.0 and L2 = 25.0. Content uniformity may be measured, for example, as indicated by the United States Pharmacopoeia which includes 1) assaying ten tablets (or other dosage form of the drug product) to ensure that the relative standard deviation (RSD) of active content is less than or equal to 6.0% and no value is outside 85-

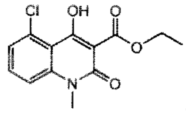
115%; and 2) assaying twenty more tablets (or other dosage form of the drug product) to ensure that the RSD for all thirty is less than or equal to 7.8%, no more than one value is outside 85-115% and no value is outside 75-125% of stated content.

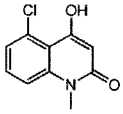
As used herein, "residual solvents" include ethanol, n-heptane, n-octane, methanol, acetone, dioxane, and dimethyl formamide. Residual solvents may be determined, for example, based on the manufacturer's statements of residual solvent levels in the active ingredients/excipients and calculation as per U.S. pharmacopeia <467> Option 2, product meets the USP <467> Residual Solvents limit criteria. Testing is not necessarily required.

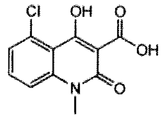
As used herein, "NMT" means no more than.

As used herein, "LT" means less than.

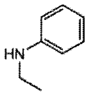
As used herein, "MCQME" means methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate. MCQME is disclosed in U.S. Patent No. 7,560,557 and J. Org. Chem., 2006, 71, 1658-1667, the contents of which are incorporated by reference into this application. MCQME has the structure:  .

As used herein, "MCQEE" means ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate. MCQEE is disclosed in U.S. Patent No. 7,560,557, the contents of which are incorporated by reference into this application. MCQEE has the structure:  .

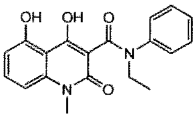
As used herein, "MCQ" means 5-chloro-4-hydroxy-1-methylquinolin-2(1H)-one. MCQ is disclosed in U.S. Patent No. 7,560,557 and J. Org. Chem., 2006, 71, 1658-1667, the contents of which are incorporated by reference into this application. MCQ has the structure:  .

As used herein, "MCQCA" means 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid. MCQCA is disclosed in U.S. Patent No. 7,560,557 and J. Org. Chem., 2006, 71, 1658-1667, the contents of which are incorporated by reference into this application. MCQCA has the structure:  .

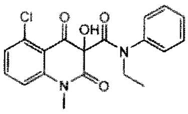
As used herein, "NEA" means N-ethyl aniline. NEA is disclosed in U.S. Patent No. 7,560,557, the contents of which are incorporated by reference into this application,. NEA has the structure:

 .

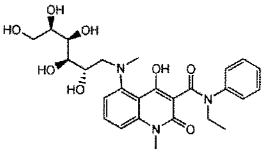
As used herein, "5-HLAQ" means N-ethyl-4,5-dihydroxy-1-methyl-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide. 5-HLAQ is disclosed in PCT International Application No. PCT/US13/26476 and U.S. Application Publication No. US 2013/0217724 A1, the contents of which are incorporated by reference into this application. 5-HLAQ has the structure:

 .

As used herein, "3-HLAQ" means 5-chloro-N-ethyl-3-hydroxy-1-methyl-2,4-dioxo-N-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide. 3-HLAQ is disclosed in PCT International Application No. PCT/US2008/013890 and U.S. Patent No 8,178,127 B2, the contents of which are incorporated by reference into this application. 3-HLAQ has the structure:

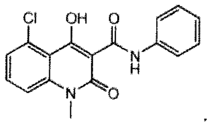
 .

As used herein, "MEG-LAQ" means N-ethyl-4-hydroxy-1-methyl-5-(methyl(2,3,4,5,6-pentahydroxyhexyl)amino)-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide. MEG-LAQ is disclosed in U.S. Application Publication No. US 2013/0345256 A1, the contents of which are incorporated by reference into this application. MEG-LAQ has the structure:

 .

As used herein, "DELAQ" means 5-chloro-4-hydroxy-1-methyl-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide. DELAQ is disclosed in PCT International Application No.

PCT/US2011/043391 and U.S. Application Publication No. US 2012/0010239 A1, the contents of which are incorporated by reference into this application. DELAQ has the structure:



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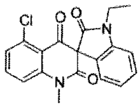
As used herein, "SPIRO-LAQ" means 1H,3H-spiro[5-chloro-1-methylquinoline-2,4-dione-3,3'-[1]ethylindolin-[2]-one]. SPIRO-LAQ is disclosed in PCT International Application No. PCT/US2008/013890 and U.S. Patent No 8,178,127 B2, the contents of which are incorporated by reference into this application. SPIRO-LAQ has the structure:



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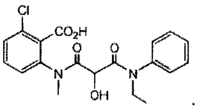
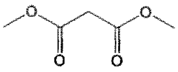
As used herein, "BH-3-HLAQ" means 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid. BH-3-HLAQ has the structure:



.

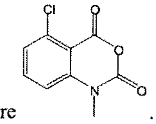
As used herein, "DMM" means dimethylmalonate. DMM is a synthetic reagent, and has the structure:



.

As used herein, "DMS" means Dimethyl sulfate. DMS is a synthetic reagent.

As used herein, "MCIA" means 5-chloro-1-methyl-1H-benzo[d][1,3]oxazine-2,4-dione. MCIA has the structure



.

As used herein, "DMF" means N,N-dimethylformamide.

As used herein, "LOD" means loss on drying.

The following commercially available solvents of appropriate quality are also used as analytical standards for testing of drug substance: Ethanol, n-heptane, n-octane, methanol, acetone, 1,4-dioxane and N,N-dimethylformamide (DMF). The ID number and source of analytical standards for dimethyl malonate is S4695599 (Merck), sodium methyl sulfate is A0294777 (Arcos), and 13C2-dimethyl sulfate is P-5052 (Holland Moran).

By any range disclosed herein, it is meant that all hundredth, tenth and integer unit amounts within the range are specifically disclosed as part of the invention. Thus, for example, 0.01 mg to 50 mg means that 0.02, 0.03 ... 0.09; 0.1, 0.2 ... 0.9; and 1, 2 ... 49 mg unit amounts are included as embodiments of this invention.

Impurities are measured by common pharmacopeial methods unless otherwise specified.

As used herein, an "anti-solvent" is a solvent in which laquinimod sodium is slightly soluble, very slightly soluble, practically insoluble, or insoluble at room temperature (20-25°C). The solubility terms are defined below, in accordance with the United States Pharmacopoeia XXV.

Term	Parts of solvent required for 1 part solute
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble	10,000 and over
Insoluble	10,000 and over

The purification of impure crystalline compounds is usually attained by recrystallization from a suitable solvent or mixture of solvents. (Vogel's Textbook of Practical Organic Chemistry. 5th edition. Longman Scientific & Technical, 1989.) The recrystallization process generally comprises the following steps: a) dissolving the impure crystalline substance in a suitable solvent near the boiling point; b) filtering the hot solution from particles of insoluble material and dust; c) allowing the hot solution to cool to cause the dissolved substance to crystallize out; and d) separating the crystals from the supernatant solution. (*Id.*) However, standard recrystallization techniques were accompanied by low or no yields when applied to laquinimod sodium as taught in U.S. Patent No. 7,884,208. As shown in Examples 2-7 of U.S. Patent No. 7,884,208, attempts to recrystallize laquinimod sodium using standard recrystallization procedures resulted in poor yields, if any. The process of U.S. Patent No. 7,884,208 overcomes the difficulties associated with recrystallizing laquinimod sodium by use of an anti-solvent in which laquinimod sodium is practically insoluble. In addition, the process of U.S. Patent No. 7,884,208 concentrates the laquinimod sodium aqueous solution before the addition of the anti-solvent. The process of the present invention is an improvement over the process of U.S. Patent No. 7,884,208.

This invention will be better understood by reference to the Experimental Details and Examples which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Determination of Powder Density

Bulk Density

1. Mix powder;
2. Tare a 50 ml empty cylinder on a 0.01 g sensitivity balance;
3. Transfer the powder, without compacting, to the cylinder being held at approximately a 45 degree angle to achieve an untapped apparent volume of 40 to 50 ml.
4. Bring the cylinder containing the sample to a vertical position by a sharp move in order to level the volume for reading.
5. Read the apparent volume (Va) to the nearest graduated unit;
6. Weigh the cylinder with sample (the balance gives sample weight M);
7. Calculate bulk density in g/ml according to the following equation: BD = M/Va;
8. Perform steps 1-7 again and report the average data of duplicates.

Tapped Density

1. Put the same cylinder used to calculate Bulk Density in a Quantachrome Dual Autotap instrument;
2. Perform 1250 taps;
3. Read the tapped volume (Vf) to the nearest graduated unit;
4. Calculate the tapped density in g/ml according to the following equation: TD = M/Vf;

5. Perform steps 1-4 again and report the average data of duplicates.

Determination of Particle Size

The particle size distributions were measured by Malvern Laser Diffraction, using the Mastersizer S model. Laser diffraction relies on the fact that diffraction angle of light is inversely proportional to particle size. Properties of particles are measured and interpreted as measurements of a sphere (a sphere being the only shape that can be described by one unique number). In addition, laser diffraction calculates a particle size distribution based around volume terms, thus eliminating particle count from the determination of particle size. The Mastersizer S model measures particles using a single technique and a single range setting.

D(0.1) is the particle size, in microns, below which 10% by volume distribution of the population is found. D(0.5) is the particle size, in microns, below which 50% by volume distribution of the population is found. D(0.9) is the particle size, in microns, below which 90% by volume distribution of the population is found.

Determination of Heavy Metals

Metal content was measured using inductively coupled plasma atomic emission spectrometry using an inductively coupled plasma atomic emission spectrometry ("ICP-AES") system manufactured by Spectro (Kleve, Germany). Sample digestion was performed in 65% nitric acid, and the internal standard used was scandium.

Note: In the following examples the volumes of solvents used are calculated relative to starting weight of laquinimod sodium. The yields are calculated in weight percent.

Determination of Purity – An Exemplary HPLC Procedure For Identification And Determination Of Assay And Polar Impurities/Degradation Products In Laquinimod Capsules

Laquinimod sodium and polar impurity/degradation products were determined by isocratic reversed phase high performance liquid chromatography (RP-HPLC), using an ODS-3V column and a mobile phase comprised of a mixture of ammonium acetate buffer at pH 7.0 (80%) and acetonitrile (20%). The detection technique was ultraviolet absorption at 240nm.

Specific HPLC Conditions:

- Column & Packing : Inertsil ODS-3V, 5 μm, 4.6 x 250mm, GL Sciences Inc.
- Guard column : Opti-Guard C 18, 1x10mm

- Mobile phase : Acetonitrile:Buffer pH 7.0 – 20:80 (v/v). Mix and degas
- Buffer pH 7.0 preparation : Dissolve 7.7g Ammonium acetate in 2000 mL water and adjust to pH 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid. Filter through a 0.45 μm membrane filter.
- Flow rate : 1.5 mL/min
- Detection : UV at 240nm
- Injection volume : 50 μL
- Diluent A : Acetonitrile / Water – 50:50 (v/v)
- Diluent B (and blank) : Mobile Phase
- Column temperature : 40°C
- Autosampler temperature : 5°C
- Run time : 40 minutes

Typical HPLC Procedure:

1. Standard Solutions Preparation

1.1 Laquinimod Standard Stock Solution (Solution S)

Weigh accurately in duplicate about 15 mg of laquinimod sodium standard into a 50 mL volumetric flask. Dilute with diluents A up to 2/3 of the volume, sonicate for 2 minutes in a cold sonication bath and dilute to volume with diluents A.

Concentration of standard stock solution is about 300 µg/mL laquinimod sodium. Standard stock solution may be used for one month when stored in a refrigerator 2°C-8°C.

1.2 Laquinimod Standard Working Solution For Assay (Solution A)

Dilute 3mL of the Standard Stock Solution to 10mL with diluents B (dilution factor 3.33).

Concentration of Laquinimod sodium is about 90 µg/mL. Concentration expressed as laquinimod (acid) is about 85 µg/mL.

Standard working solution A may be used for 7 days when stored in a refrigerator (2°C-8°C).

1.3 MCQCA Standard Stock Solution

Weigh accurately about 18mg of MCQCA standard into a 100 mL volumetric flask. Dilute to volume with acetonitrile, sonicate (in a cold sonication bath) until the substance is completely dissolved – stock MCQCA solution.

Concentration of MCQCA is about 180 µg/mL.

MCQCA Stock standard solution should be freshly prepared.

1.4 Standard Solution for Determination of Impurities (Solution I)

Prepare a solution in diluents B, containing Laquinimod in a concentration of 0.2% and MCQCA – in a concentration of 0.1%, with respect to the working concentration of Laquinimod in Standard solution A. As an example, apply the following procedure.

Transfer 4.0mL of laquinimod sodium standard solution for assay (Solution A) and 1.0mL of MCQCA stock standard solution to a 100mL volumetric flask and dilute to volume with the diluents B (intermediate dilution).

Place 2.5mL of this intermediate dilution into a 50 mL volumetric flask and make up to volume with diluents B.

Total dilution factor for laquinimod standard is 1666.67, for MCQCA 2000.

Concentration of laquinimod sodium is about 0.18 μg/mL (0.2%)

Concentration of MCQCA is about 0.09 μg/mL (0.1%, QL level).

Standard solution I may be used for 24 hours when stored in a refrigerator.

2. Resolution Solutions Preparation

2.1 Mixed Solution

Prepare solution containing the following potential impurities standards (markers) using the Diluent A as a solvent:

Mixed Solutition:

MCQ: 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one

MCQCA: 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid

MCQMA: Methyl 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate

5-HLAQ: *N*-Ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide

The Mixed Solution may be prepared as follows:

Weigh about 3mg of each impurity standard / marker into a 100mL volumetric flask, dissolve (sonication is acceptable) and dilute to volume with the Diluent A.

Concentration of each impurity in the Mixed Solution is about 30 μg/mL. Mixed Solution may be used for up to 4 months when stored frozen at about -20°C. For this purpose, the freshly prepared Mixed Solution should be divided into aliquots, immediately frozen and stored at -20°C. After thawing, the aliquots should be mixed well and should not be refrozen.

2.2 Stock Solutions of Additional Impurities

Weigh about 3mg of MCQEE (Ethyl 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate) into a 100mL volumetric flask, dissolve (sonication is acceptable) and dilute to volume with the Diluent A. This is MCQEE Stock solution.

This solution may be used for up to 4 months when stored frozen at about -20°C.

For this purpose, the freshly prepared MCQEE Stock Solution should be divided into aliquot, immediately frozen and stored at -20°C. After thawing, the aliquots should be mixed well and should not be refrozen.

Weigh about 3mg of MEG-LAQ (Meglumine Adduct of Laquinimod) into a 100mL volumetric flask, dissolve (sonication is acceptable) and dilute to volume with the Diluent A. This is MEG-LAQ Stock solution.

This solution may be used for one week when stored in refrigerator (2°C-8°C).

2.3 Resolution Solutions

Prepare two Resolution Solutions separately as follows, using the Mixed Solution.

2.3.1 Resolution Solution 1

Transfer 3mL of laquinimod standard stock solution (solution S), 0.3 mL of the Mixed Solution and 0.3 mL of the MCQEE Stock solution to a 10 mL volumetric flask and dilute to volume with the Diluent B. This is Resolution Solution 1.

Concentration of laquinimod sodium in it is about 90 μg/mL. Concentration of each impurity – is about 0.9 μg/mL (about 1% with respect to the working concentration of laquinimod).

Resolution Solution 1 is used for resolution test (for system suitability) and for determination of retention times (RT) / relative retention times (RRT) of five impurities: MCQ, MCQCA, MCQME, MCQEE, and 5-HLAQ.

Resolution Solution 1 may be used for 9 days if stored in a refrigerator 2°C-8°C).

2.3.2 Resolution Solution 2

Transfer 3mL of laquinimod standard stock solution (solution S), 0.3 mL of the Mixed Solution and 0.3 mL of the MEG-LAQ Stock solution to a 10 mL volumetric flask and dilute to volume with the Diluent B. This is Resolution Solution 2.

Concentration of laquinimod sodium in it is about 90 μg/mL. Concentration of each impurity – is about 0.9 μg/mL (about 1% with respect to the working concentration of laquinimod).

Resolution Solution 2 is used for determination of retention time of MEG-LAQ.

Resolution Solution 2 may be used for 9 days if stored in a refrigerator 2°C-8°C).

3. Sample Solution Preparation

Weigh accurately 20 capsules and completely empty their contents into a mortar. Pay attention to complete emptying of capsule contents into the mortar, using spatula when necessary. Weigh the empty capsules. Calculate the average weight of the capsule contents. Mix and grind the capsule contents in a mortar and keep the powder in a tightly closed container protected from light.

Weigh accurately, in duplicate, the amount of powder corresponding to 7 capsules, into a 50mL volumetric flask.

Add diluents B up to 2/3 of the volume, shake for 30 minutes at 200 mot/min. Dilute to volume with Diluent B. Mix well. Filter before use through a 0.45 μm GHP ACRODISC GF filter or equivalent, discarding the first 0.5-1mL.

Working concentration of laquinimod (acid) is about 84ug/mL. Immediately after preparation place sample solutions into a refrigerator or in a cooled to 5°C autosampler. The sample solutions may be used for 24 hours when kept at the temperature 2°C-8°C.

4. Procedure

Inject the Resolution Solutions, Diluent B (Blank), Standard Solutions for assay and IDD and Sample solutions, according to standard operating procedures.

Determine the retention time (RT) and the area of the laquinimod peak in the chromatograms of Sample and Standard Solutions for identification and assay.

Determine the RT, the relative retention time (RRT) and peak areas of all known impurities and any other impurities in the chromatograms of Sample Solutions, for calculation of the content of impurities / degradation products.

Ignore early elution peaks of excipients and system peaks (See chromatogram for determination of impurities / degradation products). For example. Use integration inhibition between 0 and RRT 0.15 (about 2.5 minutes).

Adjust integration parameters to reject peaks with area less than 10% of the average response of Laquinimod peak in the injections of Standard Solution I (for determination of impurities /

degradation products).

Disregard peak of MEG-LAQ in sample injections (identified following Resolution Solution 2). The content of MEG-LAQ is tested by Example 23.

5. System Suitability Test

5.1 Resolution Test

Typical retention time of laquinimod peak is 15.5 ± 2.0 minutes.

Tailing factor (USP) for laquinimod peak should be not more than 2.0.

Resolution factor for all the pairs of peaks should be not less than (NLT) 2.

RRT of the peaks of known impurities / degradation products should be as follows:

MCQME: 0.33-0.38

MCQ: 0.49-0.58

MCQEE: 0.56-0.65

MCQCA: 0.71-0.85

5-HLAQ: 1.2-1.4 (Should not be more than 23 minutes)

MEG-LAQ peak is substantially broadened in comparison with neighboring peaks. Retention time of MEG-LAQ is variable, being very sensitive to slightest changes in chromatographic conditions (pH, % acetonitrile, Temperature, etc.) and therefore should be defined using its peak in the chromatogram of the Resolution Solution 2. Typically, its RRT is about 0.66.

5.2 System Precision Test

Evaluate laquinimod standards for assay and IDD in order to test the system precision according to the standard operating procedures.

MCQCA in Solution I is used to test the sensitivity of the system. RSD of the area of six injections of Std 1 as well as the difference between Std 1 and Std 2 should be no more than (NMT) 20%.

5.3 Blank

Injection diluents B to detect system peaks.

6. Identification By Retention Time (RT)

The RT of the main peak obtained in the sample chromatogram should correspond to that obtained for the laquinimod peak in the injection of Standard Solution.

7. Calculation and Report

7.1 Assay Calculation

$$\% \text{ Assay (to Label Claim)} = \frac{Area_{Smp} \times Conc_{Std} \times 0.94 \times V_{Smp} \times AvgWt_{CapsContent}}{Area_{Std} \times W_{Smp} \times Label\ Claim}$$

Where 0.94 is the conversion factor of laquinimod sodium salt to laquinimod (acid).

7.2 Calculation and Evaluation of Impurities / Degradation Products

7.2.1 Calculation of Relative Retention Time (RRT)

$$\text{RRT Impurity} = \frac{RT\ Impurity}{RT\ Laquinimod}$$

7.2.2 Calculation of Content of Impurities / Degradation Products

$$\% \text{ Impurity} = \frac{Area_{Impurity} \times Conc_{Std} \times V_{Smp} \times AvgWt_{CapsContent} \times 0.94 \times RRF}{Area_{Std} \times W_{Smp} \times Label\ Claim}$$

$Area_{impurity}$ is the area of an impurity/degradation product (known or unknown) peak in the Sample Solution.

$Area_{std}$ is the laquinimod peak in chromatogram of Standard Solution I.

0.94 is the conversion factor of laquinimod sodium salt to laquinimod (acid).

RRF is the relative response factors of impurities / degradation products calculated as the following ratio: slope of Laquinimod regression line / slope of impurity regression line.

The values for relative response factors with respect to laquinimod are: MCQME: 0.74; MCQ: 0.65; MCQEE: 0.85; MCQCA: 0.62; and 5-HLAQ: 1.0.

RRF for unknown impurities/degradation products is taken as 1.0.

7.2.3 Evaluation and Report of Impurities / Degradation products

Quantitation level (QL) MCQME, MCQ, MCQEE, 5-HLAQ, and unknown impurities is 0.05%. Detection level (DL) of MCQME, MCQ, MCQEE, 5-HLAQ and unknown impurities is 0.02%. QL of MCQCA is 0.1%. Detection level DL of MCQCA is 0.03%.

Correlate all the peaks in sample chromatogram with those in the system suitability chromatogram, with ±5% of the actual corresponding retention times. Report data as shown in Table 2.

Table 2. Reporting Guidelines for HPLC data

	Result	Report
Specified impurities		
5-HLAQ	≥ 0.05% < 0.05% < 0.02% (or ND)	The calculated result < 0.05% < 0.02%
MCQ and MCQCA sum	≥ 0.01% < 0.01% < 0.03% (or ND)	The calculated result < 0.01% < 0.03%
Other impurities		
MCQME, MCQEE	≥ 0.05% < 0.05% < 0.02% (or ND)	The calculated result < 0.05% < 0.02%
Unknown peaks (by RRT to laquinimod)	≥ 0.05% < 0.05% < 0.02% (or ND)	The calculated result < 0.05% Not to be reported*
Total	≥ 0.05% < 0.05% (or < 0.02% or ND)	The sum of calculated results < 0.05%

* If no impurities were detected, report: any other < 0.2%.

Table 3. Example System Suitability Results 1

	Name	RT	RT Ratio	Area	USP Resolution	USP Trailing	Int Type
1	MCQME	5.460	0.36	35801		1.07	BB
2	MCQ	8.260	0.54	60687	8.2	1.16	BV
3	MCQEE	9.249	0.6	24029	2.6	1.05	VB
4	MCQCA	12.031	0.78	18609	5.7	1.34	BB
5	Laquinimod	15.332		12332469	5.2	1.84	BB
6	5-HLAQ	20.451	1.33	89463	7.3	1.04	BB

Example 1 - Modified Recrystallization of Laquinimod sodium – Pilot production, 100 fold scale-up, (Pilot Scale Batches A and B)

Recrystallization of laquinimod sodium was performed on pilot scale (Batches A and B) as follows.

Re-crystallization of Laquinimod Na on pilot scale is performed in two glass-lined reactors (Reactor A, 30 liter volume and Reactor B 60 liter). Solid product is filtered and dried in Hastelloy C agitated filter-dryer with 20 micron mesh.

Batch size is 2.5 kg of starting crude Laquinimod Na.

Batch of crude Laquinimod Na (2.5kg) is introduced to Reactor A with 10 volumes of process water. The batch is heated to 60-73°C at stirring until complete dissolution of solid.

The hot solution in Reactor A is transferred to Reactor B through 0.2μm filtration system. Reactor A and filters washed with 1.2 volumes of process water and the wash is transferred to the Reactor B.

Vacuum is built-up and the solution in the Reactor B is evaporated at P< 45 mmHg and jacket temperature T< 65°C until volume of the residue reaches 5.4 liters (2.16 volumes). Then atmospheric pressure is built-up and jacket temperature 40-50°C is adjusted. The batch is stirred for not less than 10 minutes and then seeded with Laquinimod Na crystals to initiate crystallization.

The batch is stirred at 45°C for additional 90 minutes and 7.9 volumes of acetone are added to the reactor in 1.5 – 2.5 hrs. Reactor temperature during the addition maintained between 40 and 50°C.

Resulting slurry is cooled to 0±4°C during 3.5-4.5hrs and stirred at this temperature for 10-15hrs. Then the slurry is transferred to filter-dryer and solid is filtered under pressure of nitrogen.

The cake is washed twice (2 · 2kg) with acetone, purged with nitrogen and then dried under vacuum (P< 50 mmHg) and elevated temperature (T=40°C) at agitation.

Dry product is discharged, sampled for analysis and packed.

Discussion of Example 1:

Example 1The pilot scale process of recrystallization of laquinimod sodium was based on Example 15 of U.S. Patent No. 7,884,208. The starting material was crude laquinimod sodium having low particle size (d(0.1) = 1-2μ, d(0.5) = 6-11μ; d(0.9) = 20-35μ) and appears as aggregated solid. Example 15 of U.S. Patent No. 7,884,208 involves 25.0 g of laquinimod sodium (laboratory scale) prepared according to the method disclosed in U.S. Patent No. 6,875,869. In Example 15, the 25.0 g of laquinimod sodium is dissolved in an aqueous solution of laquinimod sodium and then evaporated under vacuum at stirring to a concentrated solution having a volume ratio of 2.14 v/w, the resulting residue is seeded to induce crystallization then treated with an anti-solvent (acetone).

The modified pilot scale process was performed with 2.5 kg of laquinimod sodium which is a 100-fold scale up from Example 15. In addition, the modified pilot scale process had significant differences from the laboratory scale process of Example 15 of U.S. Patent No. 7,884,208. Specifically, evaporation on the laboratory scale was performed in a round-bottom flask in a rotary evaporator without stirring, while evaporation on the pilot scale was performed in a reactor with stirring. On the pilot scale, the evaporation residue is stirred aggressively, liquid splashes on the reactor walls, solid depositions form, and crystallization was spontaneous. On the laboratory scale, a metastable solution could be concentrated to a volume ratio of 2.1-2.2 v/w at which point crystallization did not take place and nucleation was controlled by seeding. On the pilot scale, conditions and concentration were such that spontaneous crystallization took place, i.e., crystallization was induced without seeding.

Surprisingly, the pilot batches did not result in laquinimod sodium particles having a particle size distribution expected based on Example 15 of U.S. Patent No. 7,884,208. Instead, applicants unexpected found that the pilot batches resulted in a mixture of recrystallized laquinimod sodium particles wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 40 microns, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 15 microns, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 5 microns. A comparison of particle size distributions resulting from the two different processes is shown in Table 4.

Table 4.

PSD by Malvern	**Laboratory Scale**	**Modified Pilot Scale**	
	25 g batch	2.5 kg batch	
	Example 15 from US 7,884,208	Pilot Scale Batch A	Pilot Scale Batch B
d(0.1) um	6.1	3.3	4.1
d(0.5) um	21.2	12.9	13.5
D(0.9) um	51.8	33.8	33.9
Appearance	White free-flowing powder	White free-flowing powder	White free-flowing powder

The process of U.S. Patent No. 7,884,208 and the process of Example 1, above, each produce different products and are not equivalent processes. Applicants' pilot scale version of the process of U.S. Patent No. 7,884,208 resulted in substantially different conditions from the prior art and resulted in a substantially different product having smaller particle sizes as shown in Table 4.

Since laquinimod Na is a potent drug substance, small particle size is advantageous for this API. Formation of non-aggregated laquinimod sodium crystals with reduced particle size could provide better uniformity of drug product and avoid milling or de-lumping operations. The starting material, crude laquinimod sodium, appears as aggregated solid. The re-crystallized product is free flowing powder. Powders with smaller particles have a stronger trend to aggregate. Crude laquinimod sodium prepared by slurry-to-slurry recrystallization (i.e., the process of U.S. Patent No. 6,077,851) produces particles having a low particle size and are highly aggregated. The modified process produces particles having a low particle size and are free flowing.

The aim was scalable crystallization procedure giving smaller crystal size, PSD and low aggregation on laboratory, pilot and commercial scale. The desirable PSD profile was the following: $d(0.1) < 5\mu m$, $d(0.5) < 15\mu m$ and $d(0.9) < 40\mu m$. The method is based on spontaneous crystallization initiated in aqueous phase prior to acetone addition. The important factor affecting crystallization is initial concentration of crystallizing solution. In the new crystallization procedure, reduced water volume ratio in the end of evaporation from 2.14 v/w to 1.7-1.8 v/w. Higher concentration of the solution ensures initiation of spontaneous crystallization in the end of evaporation operation and provides higher supersaturation level and lower crystal size.

Example 2 - Recrystallization of Laquinimod sodium – Laboratory Scale (Laboratory Scale Batch A)

Recrystallization of laquinimod sodium was performed on laboratory scale (Batch A) as follows.

All operations of Laquinimod Na re-crystallization step including evaporation were performed on laboratory scale in transparent agitated glass reactors equipped with stirrer, thermometers and circulating bath for heating and cooling.

25g crude Na Laquinimod and 275 ml deionized water introduced into 250 ml stirred jacketed glass reactor. The mixture is stirred and heated to 70°C, after complete dissolution of the solid the solution is filtered through paper filter. Resulting clear filtrate introduced to 250ml jacketed glass reactor equipped with circulating bath, stirrer, thermometer and vacuum distillation system.

Vacuum is applied and water is distilled at stirring, pressure during the evaporation is 38-40 mbar and jacket temperature is 55°C.

After distillation of ca. 2/3 volume spontaneous crystallization on the reactor wall above liquid level is observed.

The distillation is continued until the residue volume reaches 45 ml then atmospheric pressure is build up and the batch is stirred at 50°C for one hour. On this step intensive crystallization takes place.

200ml acetone is added to the resulting slurry in one hour and the batch is stirred for one additional hour at 50°C.

The batch is cooled to 0-5°C during one hour and filtered on Büchner filter. The solid cake is washed with 75ml of acetone.

Collected wet product (28.0g) is dried in oven under vacuum at 50°C to constant weight.

Dry product – 23.8g; Crystallization yield – 95.2%

Analysis:

Microscopic observation – rod-shape particles

Particle Size Distribution by Malvern:

D(0.1) = 2.3μm; D(0.5) = 10.8μm; D(0.9) = 32.7μm

Example 3 - Recrystallization of Laquinimod sodium – Laboratory Scale (Laboratory Scale Batch B)

Recrystallization of laquinimod sodium was performed on laboratory scale (Batch B) as follows.

All operations of Laquinimod Na re-crystallization step including evaporation were performed on laboratory scale in transparent agitated glass reactors equipped with stirrer, thermometers and circulating bath for heating and cooling.

25g Na Laquinimod crude and 275 ml deionized water introduced into 250 ml stirred jacketed glass reactor. The mixture is stirred and heated to 70°C, after complete dissolution of the solid the solution is filtered through paper filter. Resulting clear filtrate introduced to 250ml jacketed glass reactor equipped with circulating bath, stirrer, thermometer and vacuum distillation system. Vacuum is applied and water is distilled at stirring, pressure during the evaporation is 38-40 mbar and jacket temperature is 55°C. During the distillation spontaneous crystallization on the reactor wall is observed when the residue volume reached ca. 120ml. The distillation is continued until the residue volume reaches 45 ml then atmospheric pressure is build up and the batch is stirred at 50°C for one hour. On this step intensive crystallization takes place.

200ml acetone is added to the resulting slurry in one hour and the batch is stirred for one additional hour at 50°C.

The batch is cooled to 0-5°C during one hour, stirred at this temperature for one additional hour and filtered on Büchner filter. The solid cake is washed with 75ml of acetone.

Collected wet product (27.5g) is dried in oven under vacuum at 50°C to constant weight.

Dry product – 23.65g; Crystallization yield – 94.6%

Analysis:

Microscopic observation – rod-shape particles

Particle Size Distribution by Malvern:

D(0.1) = 2.6μm; D(0.5) = 12.4μm; D(0.9) = 34.3μm

Table 5. Laboratory scale Laquinimod Na crystallization results

Laboratory Scale Batch No.		**Laboratory Scale Batch A**	**Laboratory Scale Batch B**
Starting material, crude Laquinimod Na, g		25 g	25 g
Dry product, g		23.8 g	23.65 g
Crystallization yield, %		95.2%	94.6%
PSD	D(0.1)μm	2.3	2.6
	D(0.5)μm	10.8	12.4
	D(0.9)μm	32.7	34.3

Discussion of Example 2 and Example 3:

The results of Example 2 and Example 3 are summarized in Table 5. Table 5 shows that the process reliably produced a mixture of laquinimod sodium crystals a mixture of recrystallized laquinimod sodium particles wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 40 microns, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 15 microns, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 5 microns.

Example 4 – Recrystallization of Laquinimod sodium – Production Scale (Production Scale Batches C, D and E)

Recrystallization of laquinimod sodium was performed on a production scale (Batches C, D and E) as follows.

Re-crystallization of Laquinimiod Na on the production scale is performed in two 250 liter glass-lined reactors (Reactor I and Reactor II). Solid product is filtered and dried in Hastelloy C-22 agitated filter-dryer with 20 micron mesh.

Batch size is 6.5-7.5 kg of dry API.

Batch of crude Laquinimod Na is introduced to Reactor I with 11 volumes of process water. The batch is heated to 60-73°C at stirring until complete dissolution of solid.

The hot solution in Reactor I is circulated through 0.2μm filtration system at heating and stirring during 15-20 minutes. After the circulation completion filtered solution is transferred to Reactor II through 0.2μm filter. Reactor I and filters washed with 1.75 volumes of process water and the wash is transferred to the Reactor II.

Vacuum is build-up and the solution in the Reactor II is evaporated at $P < 45$ mmHg and jacket temperature $T < 65$°C until volume of the residue reaches 14-16 liter (ca. 1.7-1.8 v/w water/weight crude laquinimod Na starting material). On this step spontaneous crystallization is initiated on the reactor walls. Then atmospheric pressure is build-up and jacket temperature 40-50°C is adjusted. The batch is stirred for not less than 10 minutes.

The batch is stirred at 45°C for additional 90 minutes and 7.9 volumes of acetone are added to the reactor in 1.5 – 2.5 hrs. Reactor temperature during the addition maintained between 40 and 50°C.

Resulting slurry is cooled to 0±4°C during 2-5hrs and stirred at this temperature for 10-15hrs. Then the slurry is transferred to filter-dryer and solid is filtered under pressure of nitrogen.

The cake is washed twice (2 x 10 liter) with acetone, purged with nitrogen and then dried under vacuum ($P< 50$ mmHg) and elevated temperature ($T=35\pm5$°C) at agitation.

Dry product is discharged, sampled for analysis and packed.

Data for 3 typical production GMP batches is summarized in Table 6. The PSD values presented in Table 6 are in a good accordance to the results of laboratory scale experiments presented in Example 2 and Example 3.

Table 6. Production scale Laquinimod Na crystallization results

Production Scale Batch No.		**Batch C**	**Batch D**	**Batch E**
Starting material, crude Laquinimod Na, kg		8.50	8.55	9.45
Dry product, kg		7.40	7.00	6.80
Crystallization yield, %		87	82	72
Product Apperance		White free-flowing powder	White free-flowing powder	White free-flowing powder
Bulk Density, g/ml		0.308	0.288	0.245
Tapped Density, g/ml		0.609	0.609	0.460
PSD	D(0.1)μm	4.1	4.1	2.5
	D(0.5)μm	14.0	14.7	9.5
	D(0.9)μm	32.0	33.6	21.0

Batch C and D had reduced levels of impurities (Table 7) and good bulk and tapped density (Table 6).

Analysis of powder density of laquinimod sodium produced on the production scale (22 crystallization batches) shows that the bulk density varies in range between 0.237 and 0.364 g/ml. Tapped density is with 0.432 and 0.609 g/ml.

The results of analyzing Batches C and D are shown in Table 7 and Table 8.

Table 7. Analytical Results

Test	**Specifications**		**Batch C**	**Batch D**
Assay (by HPLC)	98.0-102.0%		99.3%	99.9%
Related Polar	MCQ	NMT 0.15%	LT 0.02%	LT 0.02%

Substances (by HPLC)	MCQCA	NMT 0.15%	LT 0.03%	LT 0.03%
	MCQEE	NMT 0.10%	LT 0.02%	LT 0.02%
	MCQME	NMT 0.12%	LT 0.02%	LT 0.02%
	5-HLAQ	NMT 0.10%	LT 0.02%	LT 0.02%
	Any other impurities	NMT 0.10%	LT 0.02%	LT 0.02%
	Total impurities	NMT 1.00%	LT 0.05%	LT 0.05%
Related Non-Polar Substances (by HPLC)	N-Ethyl aniline	NMT 0.10%	LT 0.02%	LT 0.02%
	Any other impurities	NMT 0.10%	LT 0.02%	LT 0.02%
	Total impurities	NMT 0.50%	LT 0.02%	LT 0.02%
Des-ethyl laquinimod (DELAQ) content (by HPLC)	NMT 0.10%		LT 0.1%	LT 0.1%
LAQ content by HPLC)	NMT 1.00%		LT 0. 2%	LT 0. 2%
Dimethylmalonate content (by HPLC)	NMT 0.10%		LT 0.05%	LT 0.05%
Dimethyl sulfate content (by LC-MS)	NMT 1 ppm		LT 1 ppm	LT 1 ppm
Water (by K.F coulometric)	NMT 1.5% (w/w		0.3% (w/w)	0.2% (w/w)
Heavy metals (by IPC-AES)	NMT 20 ppm		LT 20 ppm	LT 20 ppm
Sodium content	5.8-6.4%		6.1%	6.1%
Residual solvents	Ethanol	NMT 5000 ppm	LT 5 ppm	LT 5 ppm
	n-Heptane	NMT 5000 ppm	LT 10 ppm	LT 10 ppm
	n-octane	NMT 2000 ppm	LT 10 ppm	LT 10 ppm
	Methanol	NMT 3000 ppm	LT 30 ppm	LT 30 ppm
	Acetone	NMT 5000 ppm	LT 250 ppm	LT 250 ppm
	Dioxane	NMT 380 ppm	LT 10 ppm	LT 10 ppm
	DMF	NMT 880 ppm	LT 40 ppm	LT 40 ppm
Microbiological tests	Total viable aerobic count	NMT 1000 CFU/g	LT 10CFU/g	LT 10CFU/g
	Fungi /yeasts and moulds	NMT 100 CFU/g	LT 10CFU/g	LT 10CFU/g
	Escherica Coli	Absence of E-Coli	Absence	Absence

Table 8. Metal impurities in PPM of Laquinimod Sodium

Impurity	Example 1, (Batch D) of U.S. Patent 7,884,208	Example 17 of U.S. Patent 7,884,208	Example 4 New Batch C	Example 4 New Batch D
Al	14.0	5.6	1.63	1.13
Ca	165	65	6.3	22
Cr	2.6	<0.5	0.55	<0.26
Cu	2.8	1.3	0.325	<0.26
Fe	31.5	5.8	3.44	3.55
Ni	5.5	<0.5	0.79	0.61
S	466	<1	6.7	3.9
Zn	20.5	7.5	<1	3.15

Table 9. Purification of Laquinimod Sodium from MCQME

	Starting material, Crude	Re-crystallized product
Batch No.	Crude	C
Appearance	Aggregated white solid	Free-flowing white powder
MCQME by HPLC, %	0.10	N.D.*

*N.D. – Not Detected (<0.02%)

Microscopic photographs of typical batches of Crude and re-crystallized Laquinimod Na at different magnification are presented on **Figures 5-8**.

Discussion of Example 4:

The modified crystallization procedure demonstrated good reproducibility of particle size distribution on the production scale. Reduction of the evaporation residue volume to a ratio of 1.7-1.8 v/w and initiation of spontaneous crystallization provided desirable crystal size. Improved crystallization procedure demonstrates reduction in crystal size to a level of d(0.9)<40μm and good reproducibility of Particle Size Distribution on the production scale.

The product with reduced crystal size has no trend to aggregation and does not need milling or de-lumping for homogenization. The product with similar PSD prepared by slurry-to-slurry procedure is aggregated and thus problematic in formulation.

The modified crystallization procedure also results in laquinimod sodium having desirable density and purity profiles.

The improved crystallization procedure also provides effective purification from organic impurities, e.g., MCQME.

The data shown in Table 9 demonstrates complete removal of MCQME impurity by re-crystallization of commercial scale batch of Laquinimod sodium. Since this intermediate has genotoxic potential it should be purified to undetectable level. The crystallization process also provides purification of all other known organic impurities to the level below the limit of detection.

Microscopic photographs of typical batches of crude and re-crystallized Laquinimod sodium at different magnification are presented on Figures 5-8 and show rod-shape morphology of the both products. At the same time, the Crude presented on Figures 5 and 6 are much more aggregated than the re-crystallized "Cryst" product of Figures 7 and 8.

Example 5 – Laquinimod Capsules of Pharmaceutical composition of Laquinimod Sodium

Laquinimod capsules are manufactured according to the method as described in Example 2 of PCT International Application Publication No. WO 2007/146248, the entire content of which is hereby incorporated by reference. Steps of Example 2 of WO 2007/146248 are performed. Each capsule contains 0.64 mg of laquinimod sodium equivalent to 0.6 mg laquinimod.

The capsules had quantities of impurities within specification based on HPLC relative to the amount of laquinimod.

The capsules have a water content of no more than 1.5%.

The dissolution profiles, content uniformity, and residual solvents of the encapsulated pharmaceutical composition conforms to U.S. Pharmacopeia <711> (dissolution), U.S. Pharmacopeia <905> (uniformity), and U.S. Pharmacopeia <467>.

Each capsule contains 90.0 – 110.0 % of the labeled amount.

The capsules contain a total aerobic microbial count (TAMC) of NMT 10^3 cfu/g, a total combined yeasts/moulds count (TYMC) of NMT 10^2 cfu/g, and an absence of Escherichia Coli in 1 g.

Discussion of Example 5:

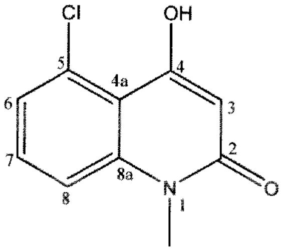
Example 5 demonstrates that, in a commercial-scale production, pharmaceutical compositions of laquinimod can be prepared with non-detectable levels or a low level of polar impurities and non-polar impurities.

Example 6 - Preparation Of Laquinimod Sodium Reference Standard

Laquinimod sodium primary reference standard batch was prepared by crystallization of laquinimod sodium batch, followed by purification by crystallization from a water/acetone mixture (1:4.5 w/w). The crystallization was performed by dissolving the drug substance batch in water while heating. The clear solution was filtered and concentrated to a known residue volume under reduced pressure. Acetone was added and the solution was cooled. The obtained crystals were filtered, washed and dried. The chromatographic purity of this batch was found to be 100.0%. The total of both polar and non-polar impurities was LT 0.05%, content of DELAQ was LT 0.1%, and laquinimod acid content was LT 0.2%.

Example 7 - Preparation Of MCQ

MCQ is a white solid. The molecular structure, chemical formula and molecular weight of MCQ are provided below.



5-chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one
Chemical Formula: $C_{10}H_8ClNO_2$
Molecular Weight: 209.629

MCQ was prepared in the following manner: MCQCA and DMSO were heated at 75°C for 2 hours and were then cooled to room temperature. Water was added and the precipitate was collected by filtration, washed with water and dried in a vacuum oven at 50°C. Material of sufficient purity to be suitable for use as a reference standard was obtained.

MCQ was characterized by NMR, MS, elemental analysis and FT-IR as detailed hereafter.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^1H-NMR and ^{13}C-NMR characterization of MCQ was performed in DMSO on a Bruker 300MHz instrument. The peak assignments are summarized in Table 10. The ^1H-NMR and ^{13}C-NMR spectra are presented in Figure 9 and Figure 10, respectively.

Table 10. **^{13}C and ^1H-NMR Peak Assignments for MCQ**

	^{13}C (ppm)	^1H (ppm)	Multiplicity (Hz) *
N-CH_3	29.30	3H 3.55	brs
C # 2	161.53	--	--
C # 3	99.57	1H 5.94	brs
C # 4	161.53	--	--
C # 4a	ca. 113.2	--	--
C # 5	ca. 130.5	--	--
C # 6	124.78	1H 7.47	d (8.0)
C # 7	130.93	1H 7.53	t (8.0)
C # 8	114.22	1H 7.29	d (8.0)
C # 8a	142.41	--	--

*brs = broad singlet, d = doublet, t = triplet

Mass Spectroscopy (MS)

The mass spectrum of MCQ was obtained on a Q-TOF Micro-TM-MICROMASS (TOF) mass spectrometer, using electrospray ionization in positive ion mode (ES^+). The ratio between MS signals containing ^{35}Cl and ^{37}Cl corresponds to the natural abundances of these isotopes (about 3:1). The spectrum shown in Figure 11 is in agreement with the molecular weight of MCQ. The attribution of the main signals in ES^+ mass spectrum of MCQ is presented in Table 11.

Table 11. **Attribution of Main Peaks of ES^+ Mass Spectrum of MCQ**

m/z	Attribution
210	$[M(^{35}Cl)+H]^+$
212	$[M(^{37}Cl)+H]^+$
232	$[M(^{35}Cl)+Na]^+$
234	$[M(^{37}Cl)+Na]^+$

Elemental Analysis

The test for elemental analysis was performed on a Perkin-Elmer 2400 Series II C H N Analyzer. The results for MCQ are presented in Table 12. C, H and N weight percentages are accurate and reproducible to within ±0.3%. The accuracy for halogens is ± 0.5%.

Table 12. **Elemental Analysis Results for MCQ**

Element	%C	%H	%N	%Cl
Theoretical	57.30	3.85	6.68	16.91
Experimental	57.31	3.64	6.54	17.41

Infrared Spectroscopy

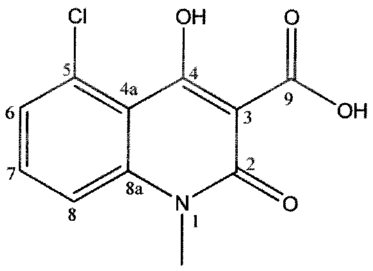
The FT-IR spectrum of MCQ was measured with a Nicolet 6700 "Thermo Scientific" FT-IR apparatus. A representative spectrum is presented in Figure 12. A summary of the band assignments is provided in Table 13.

Table 13. Summary of IR Band Assignments of MCQ in KBr

Transition Energy (cm^{-1})	Band Assignment
702-1280	Aromatic C-H deformation vibration
1093 and 1135	Aromatic C–Cl stretching
1164-1280	Aromatic C–OH stretching
1164 -1413	C–N stretching
1554 – 1613	Aromatic C – C stretching
1449-1613	Aromatic C=C stretching
1637	C=O stretching
2890	N-CH_3 stretching
3450	OH stretching

Example 8 - Preparation Of MCQCA

MCQCA is a white solid. The molecular structure, chemical formula and molecular weight of MCQCA are provided below.



5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid
Chemical Formula: $C_{11}H_8ClNO_4$
Molecular Weight: 253.639

MCQCA was prepared in the following manner:

A mixture of MCQEE in a 2.8M solution of HCl in acetic acid was heated for 6 hours at 65°C using a reflux condenser. The mixture was cooled to room temperature, diluted with 2-propanol and was further cooled to 8°C. The crystals were filtered off, washed with 2-propanol and dried in a vacuum oven at 50°C.

Material of sufficient purity to be suitable for use as a reference standard was obtained.

MCQCA was characterized by NMR, MS, elemental analysis and FT-IR as detailed below.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The 1H-NMR and 13C-NMR characterization of MCQCA was performed in D2O+KOH on a Bruker 300 MHz instrument. The peak assignments are summarized in Table 14. The 1H-NMR and 13C-NMR spectra are presented in Figure 13 and Figure 14, respectively.

Table 14. ^{13}C and ^{1}H-NMR Peak Assignments for MCQCA

	^{13}C (ppm)	^{1}H (ppm)	Multiplicity (Hz) *
N-CH_3	29.78	3H 3.43	s
C # 2	162.31	--	--
C # 3	113.46	--	--
C # 4	176.93	--	--
C # 4a	119.28	--	--
C # 5	131.56	--	--
C # 6	124.88	1H 7.05	d (8.0)
C # 7	129.38	1H 7.21	t (8.0)
C # 8	114.03	1H 7.14	d (8.0)
C # 8a	141.32	--	--
C # 9	169.62	--	--

*d = doublet, t = triplet, s = singlet

Mass Spectroscopy (MS)

The mass spectrum of MCQCA was obtained on a Q-TOF Micro-TM-MICROMASS (TOF) mass spectrometer, using electrospray ionization in positive ion mode (ES$^+$). The ratio between MS signals containing ^{35}Cl and ^{37}Cl corresponds to the natural abundances of these isotopes (about 3:1). The spectrum shown in Figure 15 is in agreement with the molecular weight of MCQCA. The attribution of the main signals in ES$^+$ mass spectrum of MCQCA is presented in Table 15.

Table 15. Attribution of Main Peaks of ES$^+$ Mass Spectrum of MCQCA

m/z	Attribution
236	$[M(^{35}Cl)+H-H_2O]^+$
238	$[M(^{37}Cl)+H-H_2O]^+$
254	$[M(^{35}Cl)+H]^+$
256	$[M(^{37}Cl)+H]^+$
276	$[M(^{35}Cl)+Na]^+$
278	$[M(^{37}Cl)+Na]^+$

Elemental Analysis

The test for elemental analysis was performed on a Perkin-Elmer 2400 Series II C H N Analyzer. C, H and N weight percentages are accurate and reproducible to within ± 0.3%. The accuracy for halogens is ± 0.5%.

The results for MCQCA are presented in Table 16.

Table 16. Elemental Analysis Results for MCQCA

Element	%C	%H	%N	%Cl
Theoretical	52.09	3.18	5.52	13.98
Experimental	52.11	3.16	5.56	13.68

Infrared Spectroscopy

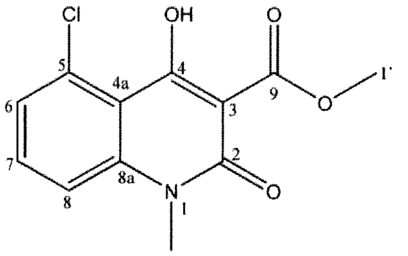
The attenuated total reflectance (ATR) FT-IR spectrum of MCQCA was measured with a Nicolet 6700 "Thermo Scientific" FT-IR apparatus. A representative spectrum is presented in Figure 16. A summary of the band assignments is provided in Table 17.

Table 17. Summary of IR Band Assignments of MCQCA

Transition Energy (cm^{-1})	Band Assignment
706-1206	Aromatic C–H deformation vibration
1008-1102	Aromatic C–Cl stretching
1148-1284	Aromatic C–OH stretching
1008-1434	C–N stretching
1571-1678	Aromatic C–C stretching
1571-1678	Aromatic C=C stretching
1678	C=O stretching
2520	COO-H stretching
3090	Aromatic C–H stretching

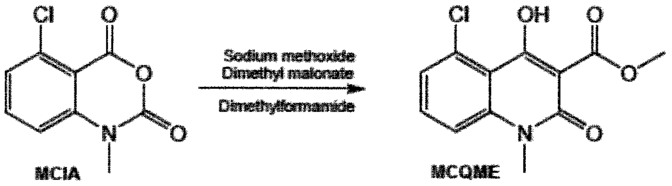
Example 9 - Preparation Of MCQME

MCQME is a white to yellowish solid. The molecular structure, chemical formula and molecular weight of MCQME are provided below.



methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate
Chemical Formula: $C_{12}H_{10}ClNO_4$
Molecular Weight: 267.665

The preparation of MCQME started with the formation of sodium dimethylmalonate by reaction of dimethylmalonate in DMF with sodium methoxide solution. Then, the intermediate MCIA was reacted with sodium dimethylmalonate to form the MCQME sodium salt. Finally the MCQME sodium salt was acidified to MCQME, which was washed with water and dried. The following is a detailed description of the preparation of MCQME.



DMF (66.5 kg [70.4 liter]), dimethyl malonate (9.6 kg [72.7 mole, 8.3 liter]) and a 30% methanolic solution of sodium methoxide (11.5 kg [63.9 mole]) were charged to a reactor . The reaction mixture was stirred and heated to 82°C to 88°C and kept at this temperature for 1½ to 2½ hours in order to form sodium dimethyl malonate. Then the mixture was cooled to 30-40°C. Vacuum was applied and the reactor jacket temperature was heated gradually in order to evaporate out methanol. After evaporation of methanol (not less than 12 kg [14.5 liter] distillate) the vacuum was broken with nitrogen and the reaction mixture was heated to 82°C to 88°C C. Then MCIA (8.5 kg [40.1 mole]) was added gradually to the reaction mixture and washed with DMF (10 kg [10.6 liter]). The reactor content was cooled to less than 75 °C, vacuum was applied, the reactor jacket temperature was gradually raised and methanol was evaporated (12 kg [15 liter] distillate). The vacuum was broken with nitrogen and the reaction mixture was cooled to 12°C to 35°C. Process water (119Kg) was added gradually, keeping the reaction mixture temperature at 12°C to 35°C, then the reaction mixture was heated to 42-48°C. 32% Aqueous HCl solution (11.1 kg [97.3 mole]) was added during 2 to 3 hours and remains of hydrochloric solution in feeding line were washed out with DMF. The formed slurry is cooled to 20°C to 30°C and was maintained at that temperature for not less than 30 minutes.

The slurry was transferred gradually to a filter-dryer. Mother liquor was removed by pressing the slurry. Process water (29.8 kg for each cycle) was added to the filter-dryer and pressed out. Washing of the filter-dryer content with water was performed until the pH of outflow washing liquor was NLT 4. The product was dried under vacuum at 40°C to 50°C (jacket temperature of filterdryer).

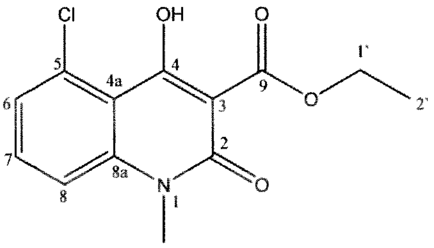
Vacuum drying was performed until the LOD of a sample from the filter-dryer was less than 2.0% and the water content (by Karl-Fischer) was not more than 0.5%. Then the jacket was cooled to 20°C to 30°C and dry MCQME was discharged.

The yield is 62% to 87%.

The dry MCQME was delumped, analyzed and released.

Example 10 - Preparation Of MCQEE

MCQEE is a light yellow solid. The molecular structure, chemical formula and molecular weight of MCQEE are provided below.



ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate
Chemical Formula: $C_{13}H_{12}ClNO_4$
Molecular Weight: 281.692

MCQEE was prepared in the following manner:

Sodium hydride was added to a solution of MCIA and diethyl malonate in DMF. The mixture was gradually heated to 95°C and stirred for 3.5 hours, then cooled to 35°C. The reaction mixture was quenched with water and then acidified with 37% HCl. After 1 hour at 8°C the mixture was filtered and washed with water (until the pH of the mother liquor was 4.5) and dried in a vacuum oven at 40°C. The crude product was crystallized by dissolving in ethanol at 85°C followed by slow cooling (in an ice water bath). The obtained crystals were filtered, washed with ethanol and dried in vacuum oven at 50°C.

Material of sufficient purity to be suitable for use as a reference standard was obtained.

MCQEE was characterized by NMR, MS, elemental analysis and FT-IR as detailed below.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^{1}H-NMR and ^{13}C-NMR characterization of MCQEE was performed in CDCl$_3$ on a Bruker 300 MHz instrument. The peak assignments are summarized in Table 18. The ^{1}H-NMR and ^{13}C-NMR spectra are presented in Figure 17 and Figure 18, respectively.

Table 18. **^{13}C and ^{1}H-NMR Peak Assignments for MCQEE**

	^{13}C (ppm)	**^{1}H (ppm)**	**Multiplicity (Hz) ***
N-CH$_3$	30.08	3H 3.63	s
C # 2	158.76	--	--
C # 3	98.18	--	--
C # 4	172.99	--	--
C # 4a	112.48	--	--
C # 5	134.50	--	--
C # 6	125.96	1H 7.23	d (8.0)
C # 7	133.28	1H 7.48	t (8.0)
C # 8	113.29	1H 7.24	d (8.0)
C # 8a	143.53	--	--
C # 9	172.67	--	--
C # 1'	62.56	2H 4.51	q (7.0)
C # 2'	14.20	3H 1.48	t (7.0)

* d = doublet, t = triplet, s = singlet, q = quartet

Mass Spectroscopy (MS)

The mass spectrum of MCQEE was obtained on a Q-TOF Micro-TM-MICROMASS (TOF) mass spectrometer, using electrospray ionization in positive ion mode (ES$^+$). The ratio between MS signals containing ^{35}Cl and ^{37}Cl corresponds to the natural abundances of these isotopes (about 3:1). The spectrum shown in Figure 19 is in agreement with the molecular weight of MCQEE. The attribution of the main signals in ES$^+$ mass spectrum of MCQEE is presented in Table 19.

Table 19. **Attribution of Main Peaks of ES$^+$ Mass Spectrum of MCQEE**

m/z	**Attribution**
236	$[M(^{35}Cl)+H-(CH_3-CH_2-OH)]^+$
238	$[M(^{37}Cl)+H-(CH_3-CH_2-OH)]^+$
282	$[M(^{35}Cl)+H]^+$
284	$[M(^{37}Cl)+H]^+$
304	$[M(^{35}Cl)+Na]^+$
306	$[M(^{37}Cl)+Na]^+$

Elemental Analysis

The test for elemental analysis was performed on a Perkin-Elmer 2400 Series II C H N Analyzer. C, H and N weight percentages are accurate and reproducible to within ± 0.3%. The accuracy for halogens is ± 0.5%. The results for MCQEE are presented in Table 20.

Table 20. Elemental Analysis Results for MCQEE

Element	%C	%H	%N	%Cl
Theoretical	55.43	4.29	4.97	12.59
Experimental	55.44	4.25	4.87	12.57

Infrared Spectroscopy

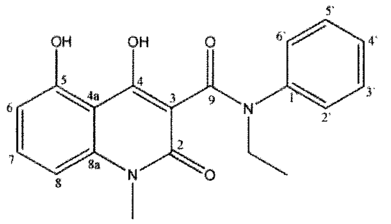
The attenuated total reflectance (ATR) FT-IR spectrum of MCQEE was measured with a Nicolet 6700 "Thermo Scientific" FT-IR apparatus. A representative spectrum is presented in Figure 20. A summary of the band assignments is provided in Table 21.

Table 21. Summary of IR Band Assignments of MCQEE

Transition Energy (cm^{-1})	Band Assignment
676-1213	Aromatic C–H deformation vibration
1019-1092	C–Cl stretching
1145-1438	C–N stretching
1145-1270	Aromatic C–OH stretching
1438-1603	Aromatic C–C stretching, Aromatic C=C stretching
1656	C=O stretching

Example 11 - Preparation Of 5-HLAQ

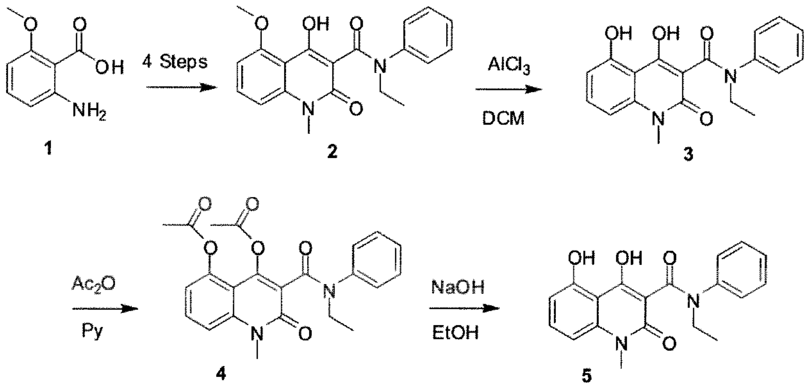
5-HLAQ is an off-white solid. The molecular structure, chemical formula and molecular weight of 5-HLAQ are provided below.



N-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide
Chemical Formula: $C_{19}H_{18}N_2O_4$
Molecular Weight: 338.357

5-HLAQ is unstable in most organic solvents as well as in aqueous acidic medium. Therefore purification by the commonly used methods is extremely difficult. Consequently, purification of the material was performed by formation of the di-acetate derivative followed by basic hydrolysis, acidic precipitation and rapid filtration.

5-HLAQ was prepared by a multi-step procedure, depicted below.



The synthesis involved: 1) preparation of 5-MeO-laquinimod (compound **2**) from 2-amino-6-methoxybenzoic acid (compound **1**); 2) preparation of 5-HLAQ crude (compound **3**); 3) acetylation of the phenolic groups to produce the di-acetate-laquinimod derivative (compound **4**); 4) hydrolysis of the di-acetate derivative to produce the purified 5-HLAQ (compound **5**).

The procedure for the preparation of 5-HLAQ is further described step-wise in the following paragraphs:

5-MeO-laquinimod (compound **2**)

5-MeO-laquinimod was obtained by a four-step synthetic procedure starting from 2-amino-6-methoxybenzoic acid (compound **1**). These four steps are identical to the manufacturing process of laquinimod starting from ACBA.

5-HLAQ Crude (compound **3**)

5-MeO-laquinimod was dissolved in dichloromethane and the solution was cooled to 0-5°C. $AlCl_3$ was added and the reaction mixture was stirred for 0.5 hour at 0-5°C followed by 7 hours' stirring at ambient temperature. The solution was evaporated to dryness at 30°C (in a water bath) and water was added. The obtained grey solid was filtered, washed with 1N HCl and dried at 30°C in a vacuum oven.

Di-acetate-laquinimod (compound **4**)

Acetic anhydride was added to a solution of 5-HLAQ crude in pyridine and the reaction mixture was stirred for 1 hour at room temperature. The pyridine was evaporated to dryness and the oily residue was dissolved in dichloromethane. The organic solution was washed with 1N HCl followed by aqueous washings. The crude **2** was purified by flash chromatography on silica gel (mobile phase: 1% methanol in dichloromethane).

5-HLAQ (compound **5**)

1N NaOH solution was added to a suspension of **4** in ethanol. The reaction mixture was stirred for 40 min and the ethanol was evaporated. The residue was acidified with 5N HCl down to pH 1-2 and the white solid was filtered, washed with water and dried. 5-HLAQ was obtained as a white solid with a purity of >99% by HPLC.

Material of sufficient purity to be suitable for use as a reference standard was obtained.

5-HLAQ was characterized by NMR, MS, elemental analysis and FT-IR as detailed below.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^{1}H-NMR and ^{13}C-NMR characterization of 5-HLAQ was performed in DMSO on a Bruker 300 MHz instrument. The peak assignments are summarized in Table 22. The ^{1}H-NMR and ^{13}C-NMR spectra are presented in Figure 21 and Figure 22, respectively.

Table 22. ^{13}C and ^{1}H-NMR Peak Assignments for 5-HLAQ

	^{13}C (ppm)	^{1}H (ppm)	Multiplicity (Hz) *
N-CH_3	29.09	3H 3.39	brs

C # 2	157.95	--	--
C # 3	102.85	--	--
C # 4	159.65	--	--
C # 4a	--	--	--
C # 5	155.20	--	--
C # 6	107.79	1H 6.84	d (8.0)
C # 7	131.91	1H 7.36	t (8.0)
C # 8	106.35	1H 6.61	d (8.0)
C # 8a	140.47	--	--
C # 9	158.79	--	--
N-**CH$_2$**-CH$_3$	42.81	2H 3.76	dq (7)
N-CH$_2$-**CH$_3$**	12.91	3H 1.08	t (7)
C # 1'	141.59	--	--
C # 2'/6'	126.84	1H 7.3	m
C # 3'/5'	128.46	1H 7.3	m
C # 4'	127.31	1H 7.18	m

* brs = broad singlet, d = doublet, t = triplet, s = singlet, m = multiplet, dq = double quartet

Mass Spectroscopy (MS)

The mass spectrum of 5-HLAQ was obtained on a Q-TOF Micro-TM-MICROMASS (TOF) mass spectrometer, using electrospray ionization in positive ion mode (ES$^+$).

The spectrum shown in Figure 23 is in agreement with the molecular weight of 5-HLAQ. The attribution of the main signals in ES$^+$ mass spectrum of 5-HLAQ is presented in Table 23 below.

Table 23. Attribution of Main Peaks of ES$^+$ Mass Spectrum of 5-HLAQ

m/z	Attribution
339	[M+H]$^+$
361	[M+Na]$^+$

Elemental Analysis

The test for elemental analysis was performed on a Perkin-Elmer 2400 Series II C H N Analyzer. C, H and N weight percentages are accurate and reproducible to within ± 0.3%. The accuracy for halogens is ± 0.5%. The results for 5-HLAQ are presented in Table 24.

Table 24. Elemental Analysis Results for 5-HLAQ

Element	%C	%H	%N
Theoretical	67.44	5.36	8.28
Experimental	67.05	5.06	8.07

Infrared Spectroscopy

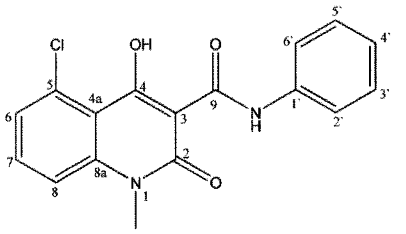
The attenuated total reflectance (ATR) FT-IR spectrum of 5-HLAQ was measured with a Nicolet 6700 "Thermo Scientific" FT-IR apparatus. A representative spectrum is presented in Figure 24. A summary of the band assignments is provided in Table 25.

Table 25 Summary of IR Band Assignments of 5-HLAQ

Transition Energy (cm^{-1})	Band Assignment
686-1257	Aromatic C–H deformation vibration
1019-1416	C–N stretching
1157-1249	Aromatic C–OH stretching
1586-1631	Aromatic C–C stretching, Aromatic C=C stretching
1659	C=O stretching

Example 12 - Preparation Of DELAQ

DELAQ is an off-white to pale yellow solid. The molecular structure, chemical formula and molecular weight of DELAQ are provided below.



5-chloro-4-hydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide
Chemical Formula: $C_{17}H_{13}ClN_2O_3$
Molecular Weight: 328.750

DELAQ was prepared in the following manner:

A mixture of MCQME, n-heptane, n-octane and aniline was heated in a jacketed reactor to Tj=135°C and the volatiles were distilled for 3 hours. The distillation was stopped and additional portions of n-heptane and aniline were added to the mixture. The distillation was continued for an additional 2 hours at Tj=135°C. The reaction mixture was cooled to room temperature and n-heptane was added. The crystalline solid formed was collected by filtration and washed with n-heptane. The wet material was dried in a vacuum oven at 50°C.

Material of sufficient purity to be suitable for use as a reference standard was obtained.

DELAQ was characterized by NMR, MS, elemental analysis and FT-IR, as detailed below.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^{1}H-NMR and ^{13}C-NMR characterization of DELAQ was performed in $CDCl_3$ on a Bruker 300 MHz instrument. The peak assignments are summarized in Table 26. The ^{1}H-NMR and ^{13}C-NMR spectra are presented in Figure 25 and Figure 26, respectively.

Table 26. **^{13}C and ^{1}H-NMR Peak Assignments for DELAQ**

	^{13}C (ppm)	**^{1}H (ppm)**	**Multiplicity (Hz) ***
N-CH_3	30.23	3H 3.68	s
NH	--	12.57	brs
C # 2	162.09	--	--
C # 3	96.84	--	--
C # 4	173.81	--	--
C # 4a	113.50	--	--
C # 5	134.51	--	--
C # 6	126.61	1H 7.27	m
C # 7	132.89	1H 7.47	m
C # 8	113.81	1H 7.27	m
C # 8a	142.08	--	--
C # 9	169.61	--	--
C # 1'	137.01	--	--
C # 2'/6'	121.29	1H 7.65	m
C # 3'/5'	129.02	1H 7.36	m
C # 4'	124.95	1H 7.17	m

*brs = broad singlet, s = singlet, m = multiplet,

Mass Spectroscopy (MS)

The mass spectrum of DELAQ was obtained on a Q-TOF Micro-TM-MICROMASS (TOF) mass spectrometer, using electrospray ionization in positive ion mode (ES^+). The ratio between MS signals containing ^{35}Cl and ^{37}Cl corresponds to the natural abundances of these isotopes (about 3:1). The spectrum shown in Figure 27 is in agreement with the molecular weight of DELAQ. The attribution of the main signals in ES^+ mass spectrum of DELAQ is presented in Table 27.

Table 27. **Attribution of Main Peaks of ES^+ Mass Spectrum of DELAQ**

m/z	**Attribution**
236	$[M(^{35}Cl)+H-(CH_3)-(Ph)]^+$
238	$[M(^{37}Cl)+H-(CH_3)-(Ph)]^+$
329	$[M(^{35}Cl)+H]^+$
331	$[M(^{37}Cl)+H]^+$

Elemental Analysis

The test for elemental analysis was performed on a Perkin-Elmer 2400 Series II C H N Analyzer. C, H and N weight percentages are accurate and reproducible to within ± 0.3%. The accuracy for halogens is ± 0.5%. The results for DELAQ are presented in Table 28.

Table 28. **Elemental Analysis Results for DELAQ**

Element	**%C**	**%H**	**%N**	**%Cl**
Theoretical	62.11	3.99	8.52	10.78

Experimental	61.90	3.83	8.38	10.96

Infrared Spectroscopy

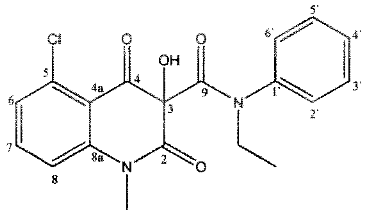
The FT-IR spectrum of DELAQ was measured with a Nicolet 6700 "Thermo Scientific" FT-IR apparatus. A representative spectrum is presented in Figure 28. A summary of the band assignments is provided in Table 29.

Table 29. Summary of IR Band Assignments of DELAQ in KBr

Transition Energy (cm^{-1})	**Band Assignment**
686-1203	Aromatic C–H deformation vibration
735-833	N-H deformation vibration
1077-1089	C–Cl stretching
1203-1449	C–N stretching
1203	Aromatic C–OH stretching
1489-1620	Aromatic C–C stretching, Aromatic C=C stretching
1659	C=O stretching
2951	COO-H stretching

Example 13 - Preparation Of 3-HLAQ

3-HLAQ is a yellow solid. The molecular structure, chemical formula and molecular weight of 3-HLAQ are provided below.



5-chloro-*N*-ethyl-3-hydroxy-1-methyl-2,4-dioxo-*N*-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide
Chemical Formula: $C_{19}H_{17}ClN_2O_4$
Molecular Weight: 372.802

3-HLAQ was prepared in the following manner:

A solution of laquinimod sodium in water was added drop-wise to a solution of disodium hydrogenphosphate dihydrate and oxone ($2KHSO_5\ KHSO_4\ K_2SO_4$) in water. 10% NaOH was added until the pH was 8.0. The solution was mixed for 30 minutes at room temperature. The obtained solid was filtered, washed with water and dried in a vacuum oven at 50°C. The crude product was recrystallized twice from ethyl acetate and n-heptane mixture. Each time the product was isolated by cooling the mixture to 10°C and stirring for 1 hour. The obtained crystals were filtered off, washed with n-heptane and dried in a vacuum oven at 50°C.

Material of sufficient purity to be suitable for use as a reference standard was obtained.

3-HLAQ was characterized by NMR, MS, elemental analysis and FT-IR, as detailed below.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^{1}H-NMR and ^{13}C-NMR characterization of 3-HLAQ was performed in $CDCl_3$ on a Bruker 300 MHz instrument. The peak assignments are summarized in Table 30. The ^{1}H-NMR and ^{13}C-NMR spectra are presented in Figure 29 and Figure 30, respectively.

Table 30. ^{1}H and ^{13}C -NMR Peak Assignments for 3-HLAQ

	^{13}C (ppm)	**^{1}H (ppm)**	**Multiplicity (Hz) ***
N-CH_3	30.56	3H 3.21	s
C # 2	165.45	--	--
C # 3	77.92	--	--
C # 4	188.90	--	--
C # 4a	136.16	--	--
C # 5	ca. 131.6	--	--
C # 6	126.75	1H 7.14	d (8.0)
C # 7	134.90	1H 7.35	t (8.0)
C # 8	113.78	1H 6.84	d (8.0)
C # 8a	144.51	--	--
C # 9	167.87	--	--
N-$\mathbf{CH_2}$-CH_3	46.96	2H 3.79	q (6.0)
N-CH_2-$\mathbf{CH_3}$	12.55	3H 1.16	bt (6.0)
C # 1`	137.19	--	--
C # 2`/6`	131.62	1H 7.07-7.15	m
C # 3`/5`	128.91	1H 7.07-7.15	m
C # 4`	129.11	1H 7.07-7.15	m

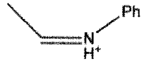
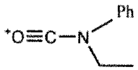
*d = doublet, t = triplet, s = singlet, m = multiplet, q = quartet, bt = broad triplet

Mass Spectroscopy (MS)

The mass spectrum of 3-HLAQ was obtained on a Q-TOF Micro-TM-MICROMASS (TOF) mass spectrometer, using electrospray ionization in positive ion mode (ES$^+$). The ratio between MS signals containing ^{35}Cl and ^{37}Cl corresponds to the natural abundances of these isotopes (about 3:1). The spectrum shown in Figure 31 is in agreement with the molecular weight of 3-HLAQ. The attribution of the main signals in ES$^+$ mass spectrum of 3-HLAQ is presented in Table 31.

Table 31. Attribution of Main Peaks of ES$^+$ Mass Spectrum of 3-HLAQ

m/z	**Attribution**

120	
148	
373	[M(^{35}Cl)+H]$^+$
375	[M(^{37}Cl)+H]$^+$
395	[M(^{35}Cl)+Na]$^+$
397	[M(^{37}Cl)+Na]$^+$

Elemental Analysis

The test for elemental analysis was performed on a Perkin-Elmer 2400 Series II C H N Analyzer. C, H and N weight percentages are accurate and reproducible to within ± 0.3%. The accuracy for halogens is ± 0.5%. The results for 3-HLAQ are presented in Table 32.

Table 32. Elemental Analysis Results for 3-HLAQ

Element	%C	%H	%N	%Cl
Theoretical	61.21	4.60	7.51	9.51
Experimental	61.09	4.68	7.38	9.30

Infrared Spectroscopy

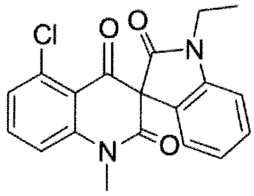
The attenuated total reflectance (ATR) FT-IR spectrum of 3-HLAQ was measured with a Nicolet 6700 "Thermo Scientific" FT-IR apparatus. A representative spectrum is presented in Figure 32. A summary of the band assignments is provided in Table 33.

Table 33. Summary of IR Band Assignments of 3-HLAQ

Transition Energy (cm^{-1})	Band Assignment
794-1244	Aromatic C–H deformation vibration
1035-1116	C–Cl stretching
1061-1417	C–N stretching
1458-1480	CH_2 deformation vibration
1583	Aromatic C–C stretching, Aromatic C=C stretching
1663-1693	C=O stretching
2979	C-H stretching in N-CH_2

Example 14 - Preparation Of SPIRO-LAQ

SPIRO-LAQ is a white powder. The molecular structure, chemical formula and molecular weight of SPIRO-LAQ are provided below.



1H,3H-spiro[5-chloro-1-methylquinoline-2,4-dione-3,3’-[1]ethylindolin-[2]-one]
Chemical Formula: $C_{19}H_{15}ClN_2O_3$
Molecular Weight: 354.787

SPIRO-LAQ was prepared in the following manner:

A mixture of laquinimod (free acid), ammonium cerium (IV) nitrate, ethanol and acetic acid in water was mixed for 1 hour. Then the precipitate was washed and filtered with water followed by ethanol, and dried in a vacuum oven at 50°C.

Material of sufficient purity to be suitable for use as a reference standard was obtained.

SPIRO-LAQ was characterized by NMR, MS, elemental analysis and FT-IR as detailed below.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^{1}H-NMR and ^{13}C-NMR characterization of SPIRO-LAQ was performed in DMSO on a Bruker 300 MHz instrument. The peak assignments are summarized in Table 34. The ^{1}H-NMR and ^{13}C-NMR spectra are presented in Figure 33 and Figure 34, respectively.

Table 34. ^{13}C and ^{1}H-NMR Peak Assignments for SPIRO-LAQ

	^{13}C (ppm)	**^{1}H (ppm)**	**Multiplicity (Hz) ***
N-CH_3	30.95	3H 3.44	brs
C # 2	164.10	--	--
C # 3	71.76	--	--
C # 4	185.60	--	--
C # 4a	124.66	--	--
C # 5	133.49	--	--
C # 6	126.13	1H 7.54	d (8.0)
C # 7	136.03	1H 7.74	t (8.0)
C # 8	115.77	1H 7.20	d (8.0)
C # 8a	145.62	--	--
N-**CH_2**-CH_3	34.90	2H 3.75	q (8.0)
N-CH_2-**CH_3**	12.40	3H 1.16	t (8.0)
C # 2’	167.40	--	--
C # 3’a	117.83	--	--
C # 4’	124.87	1H 7.20	d (8.0)
C # 5’	122.84	1H 7.38	ddd (8.0,6.5,2.5)
C # 6’	129.84	1H 7.0-7.1	m

C # 7'	109.64	1H 7.0-7.1	m
C # 7'a	143.17	--	--

* brs = broad singlet, d = doublet, t = triplet, s = singlet, m = multiplet, dd = double doublet, ddd = double double doublet, q = quartet

Mass Spectroscopy (MS)

The mass spectrum of SPIRO-LAQ was obtained on a Q-TOF Micro-TM-MICROMASS (TOF) mass spectrometer, using electrospray ionization in positive ion mode (ES^+). The ratio between MS signals containing ^{35}Cl and ^{37}Cl corresponds to the natural abundances of these isotopes (about 3:1). The spectrum shown in Figure 35 is in agreement with the molecular weight of SPIRO-LAQ. The attribution of the main signals in ES^+ mass spectrum of SPIRO-LAQ is presented in Table 35.

Table 35. Attribution of Main Peaks of ES^+ Mass Spectrum of SPIRO-LAQ

m/z	Attribution
311	$[M(^{35}Cl)+H-(CH_2CH_3)-(CH_3)]^+$
313	$[M(^{37}Cl)+H-(CH_2CH_3)-(CH_3)]^+$
355	$[M(^{35}Cl)+H]^+$
357	$[M(^{37}Cl)+H]^+$

Elemental Analysis

The test for elemental analysis was performed on a Perkin-Elmer 2400 Series II C H N Analyzer. C, H and N weight percentages are accurate and reproducible to within ± 0.3%. The accuracy for halogens is ± 0.5%. The results for SPIRO-LAQ are presented in Table 36.

Table 36. Elemental Analysis Results for SPIRO-LAQ

Element	%C	%H	%N	%Cl
Theoretical	64.32	4.26	7.90	9.99
Experimental	64.08	4.22	7.75	10.12

Infrared Spectroscopy

The attenuated total reflectance (ATR) FT-IR spectrum of SPIRO-LAQ was measured with a Nicolet 6700 "Thermo Scientific" FT-IR apparatus. A representative spectrum is presented in Figure 36. A summary of the band assignments is provided in Table 37.

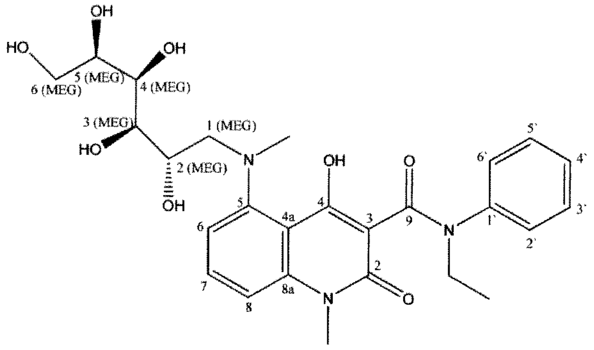
Table 37. Summary of IR Band Assignments of SPIRO-LAQ

Transition Energy (cm^{-1})	Band Assignment
687-1244	Aromatic C–H deformation vibration
1020-1091	C–Cl stretching
1020-1454	C–N stretching
1153-1278	Aromatic C–OH stretching

1582	Aromatic C–C stretching, Aromatic C=C stretching
1667-1738	C=O stretching

Example 15 - Preparation Of MEG-LAQ

The molecular structure, chemical formula and molecular weight of MEG-LAQ is presented below.



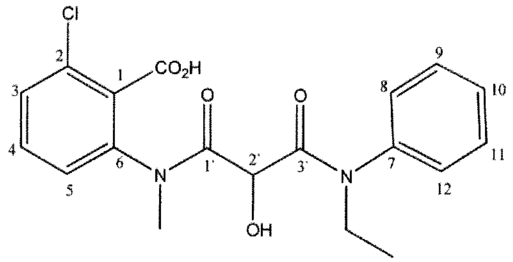
N-ethyl-4-hydroxy-1-methyl-5-(2,3,4,5,6-pentahydroxyhexylamino)-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide
Chemical Formula: $C_{26}H_{33}N_3O_8$
Molecular Weight: 515.556

An aqueous solution of laquinimod sodium and meglumine was refluxed and bubbled with air for about 1 month. The obtained solution was diluted twice with water and acidified with concentrated hydrochloric acid to pH 1-2. The aqueous solution was filtered followed by extraction with chloroform. Then, a concentrated ammonium hydroxide solution was added to the aqueous solution, up to neutralization. The solution was evaporated and the obtained brown syrup was washed with methanol. Meglumine was solidified and filtered followed by silica gel addition to the methanolic solution. The solvent was evaporated and the obtained mixture was purified by silica-gel column chromatography (mobile phase: 20% methanol in dichloromethane).

This synthesis and purification affords a material of sufficient purity to be suitable for use as a standard.

Example 16 - Preparation Of BH-3-HLAQ

The molecular structure, chemical formula and molecular weight of BH-3-HLAQ is presented below.



2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-*N*-methyl-3-oxopropanamido)benzoic acid
Chemical Formula: $C_{19}H_{19}ClN_2O_5$
Molecular Weight: 390.818

A 1N NaOH solution was added to a suspension of 3-HLAQ in water. The yellow solution was stirred for 0.5 hr followed by the addition of 1N HCl solution. The aqueous solution that contained white solid was extracted with ethyl acetate. The organic phase was washed with brine and dried over sodium sulfate. The suspension was filtered, evaporated to dryness and the solid residue was purified by crystallization from IPA: water mixture (1:3.75 v/v). The suspension was cooled to 0-5°C and was kept for 1 hr, filtered and washed with IPA: water mixture (1:10 v/v). The obtained white solid was dried in a vacuum oven at 50°C.

At the time of initial qualification, the BH-3-HLAQ standard was demonstrated to conform to the molecular structure by IR, elemental analysis, MS, and NMR. Additional testing included chromatographic purity and loss on drying.

This synthesis and purification affords a material of sufficient purity to be suitable for use as a standard.

For structure elucidation purposes, a sample of BH-3-HLAQ was characterized by NMR, MS, elemental analysis and FT-IR as depicted below.

NMR Spectroscopy

The ^{1}H-NMR and ^{13}C-NMR characterization of BH-3-HLAQ was performed in DMSO on a Bruker 300MHz instrument. The peak assignments are summarized in Table 38. The ^{1}H-NMR and ^{13}C-NMR spectra are presented in Figure 37 and Figure 38, respectively.

Table 38. ^{13}C and ^{1}H-NMR Peak Assignments for BH-3-HLAQ

	^{13}C (ppm)	^{1}H (ppm)	Multiplicity (Hz) *
CO_2H	165.62	--	--
C # 1	130.08	--	--

C # 2	135.13	--	--
C # 3	130.20	1H 7.77	d (8.0)
C # 4	131.66	1H 7.66	t (8.0)
C # 5	129.38	1H 7.19	d (8.0)
C # 6	140.13	--	--
$N\text{-}CH_3$	37.43	3H 3.09	--
C # 1'	167.55	--	--
C # 2'	68.67	1H 4.25	s
C # 3'	168.93	--	--
$N\mathbf{CH_2}CH_3$	43.43	2H 3.43, 3.38	ABq of q (14.0, 7.0, 2.0)
$NCH_2\mathbf{CH_3}$	12.24	3H 0.86	t (7.0)
C # 7	137.34	--	--
C # 8/12	127.86	2H 6.46	br
C # 9/11	128.93	2H 7.27	m
C # 10	127.80	1H 7.27	m

* s = singlet, br = broad, m = multiplet, d = doublet, t = triplet, q = quartet

Mass Spectrometry

The mass spectrum of BH-3-HLAQ was obtained on a Q-TOF Micro-TM-MICROMASS (TOF) mass spectrometer, using electrospray ionization in positive ion mode (ES^+). The spectrum shown in Figure 39 is in agreement with the molecular weight of BH-3-HLAQ. The attribution of the main signals in ES^+ mass spectrum of BH-3-HLAQ is presented in Table 39.

Table 39. Attribution of Main Peaks of ES^+ Mass Spectrum of BH-3-HLAQ

m/z	Attribution
391	$[M+H]^+$
413	$[M+Na]^+$

FT-IR

The attenuated total reflectance (ATR) FT-IR spectrum of BH-3-HLAQ was measured with a Nicolet 6700 "Thermo Scientific" FT-IR apparatus. Figure 40 shows a typical spectrum. A summary of the band assignments is shown in Table 40.

Table 40. Summary of IR Band Assignments of BH-3-HLAQ

Transition Energy (cm^{-1})	Band Assignment
703, 737, 769	Aromatic C–H deformation vibration
1090	Aromatic C–Cl stretching
1492	Aromatic C=C stretching
1586	C–N stretching
1652, 1728	C=O stretching
3081	Aromatic C–H stretching
3442	O-H stretching

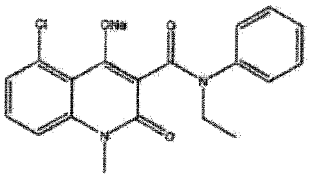
Elemental Analysis

The test for elemental analysis was performed on a Perkin-Elmer 2400 Series II C H N Analyzer. C, H and N weight percentages are accurate and reproducible to within ± 0.3%. The accuracy for halogens is ± 0.5%. The results for BH-3-HLAQ are presented in Table 41.

Table 41. Elemental Analysis Results for BH-3-HLAQ

Element	%C	%H	%N	%Cl
Theoretical	58.39	4.90	7.17	9.07
Experimental	58.31	4.79	7.15	9.56

Example 17 - Specification of Laquinimod Sodium Drug Substance



Chemical Formula: $C_{19}H_{16}ClN_2NaO_3$
Exact Mass: 378.07
Molecular Weight: 378.78
m/z: 378.07 (100.0%), 380.07 (32.0%), 379.08 (20.8%), 381.08 (6.9%), 380.08 (2.8%)
Elemental Analysis: C, 60.25; H, 4.26; Cl, 9.36; N, 7.40; Na, 6.07; O, 12.67

Laquinimod sodium is a white to off-white powder, freely soluble in water which should be stored in a well-closed container, protected from light, at room temperature.

The specifications of Laquinimod sodium are as follows:

Table 42.

TEST	SPECIFICATIONS
Description	White to off-white powder
Identification: a) HPLC (refer to Example 19)	The retention time of the main peak in the sample chromatogram corresponds to that of the Standard. (See, Example 19)
b) Infrared Spectrum	The transmission spectrum exhibits minima at the same wavenumbers as the reference standard.

c) Ultraviolet spectrum	The absorption spectrum exhibits maxima at the same wavelengths as the reference standard.
d) sodium identification	Conforms.
Assay of LAQ-Na (by HPLC, refer to Example 19)	98.0-102.0%
Related Substances a) Polar impurities (refer to Table 43)	MCQ – NMT 0.15% MCQCA – NMT 0.15% MCQME – NMT 0.12% 5-HLAQ – NMT 0.10% MCQEE – NMT 0.10% Any other impurity – NMT 0.10% Total – NMT 1.00%
b) N-ethylaniline and non-polar impurities (refer to Table 43)	N-ethylaniline – NMT 0.10% Any other impurity – NMT 0.10% Total – NMT 0.50%
c) DELAQ content (by HPLC)	DELAQ – NMT 0.1%
LAQ content (by HPLC)	NMT 1.0%
Dimethyl Malonate content (by HPLC)	NMT 0.1%
Dimethyl sulfate content (by LC-MS)	NMT 1ppm
Water content (by KF coulometric)	NMT 1.5% (w/w)
Heavy metals	NMT 20 ppm
Sodium Content	5.8-6.4%
Color of solution	Not more intensely colored than reference solution BY_7
Free Chloride	NMT 0.1%
Residual solvents (by GC)	Ethanol – NMT 5000 ppm n-Heptane – NMT 5000 ppm n-Octane – NMT 2000 ppm Methanol – NMT 5000 ppm Acetone – NMT 5000 ppm Dioxane – NMT 380 ppm
Residual solvent – DMF (by HS GC)	NMT 880 ppm
Microbial purity test	Total viable aerobic count – NMT 10^3 cfu/g Fungi/yeast and mold – NMT 10^2 cfu/g Escherichia Coli – Absence/g

For MCQME, alternative specifications are NMT 10% and LT 10%.

Table 43 lists the non-pharmacopoeial analytical methods currently in use for the determination of impurity levels in the drug substance at the time of release and during stability testing. The method numbers and the detection and quantitation limits for each impurity are also listed.

Table 43. Analytical Methods for Determination of Impurities

Parameter	Method[1]	Method Type	Quantitation Limit	Detection Limit
MCQ	Example 19	RP-HPLC	0.05%	0.02%
MCQCA	Example 19	RP-HPLC	0.05%[2]	0.02%
MCQME	Example 19	RP-HPLC	0.05%	0.02%
5-HLAQ	Example 19	RP-HPLC	0.05%	0.02%
MCQEE	Example 19	RP-HPLC	0.05%	0.02%
LAQ	Example 19	RP-HPLC	0.2%	0.03%
NEA	Example 20	RP-HPLC	0.06%	0.02%

Example 18 - Specification of Laquinimod Sodium Drug Product

Laquinimod drug product was prepared as 0.6 mg capsules of laquinimod sodium. The specifications of laquinimod sodium (0.6 mg) capsules are as follows:

Table 44. Release and Shelf Life Specifications

Test	Use[1]	Acceptance Criteria
Description	R	Off-white opaque cap and body, hard gelatin capsule filed with white to off-white granulate. Imprinted "0.6mg" on the body, and "LAQ" on the cap.
Appearance	S	Off-white opaque cap and body, hard gelatin capsule filed with white to off-white granulate. Imprinted "0.6mg" on the body, and "LAQ" on the cap. No cracks, discoloration and other appearance defects are Observed
Identification	R	By HPLC (Example 21) : The retention time of the major peak in the chromatogram of sample preparation corresponds to that in the chromatogram of the standard preparation.
	R	By UV Spectrum: The sample spectrum exhibits the same maxima as that of the standard
Dissolution	R+S	Not less than 70% (Q) of the labeled amount is dissolved in 30 min. Conforms to current Ph.Eur. (2.9.3)
Uniformity of Dosage Units by Content Uniformity	R	Conforms to current Ph.Eur. (2.9.40)
Assay	R+S	95.0 – 105.0 % of the labeled amount (By HPLC, Example 21)
Impurities / Degradation Product Determination	R+S	Sum of MCQ[2] and MCQCA[3]: NMT 0.5% 5-HLAQ[4]: NMT 0.5% Any Other: NMT 0.5%

(See, Table 46 for methods)		MEG - LAQ[5]: NMT 1.0%
		BH-3-HLAQ[6]: NMT 1.0%
Water Content	R	NMT 0.8%
	S	NMT 1.5%
Identification of Color[7]	R	Positive for the correct color
Microbiological Examination of Non-Sterile Products[8]	R+S	Total aerobic microbial count (TAMC): NMT 10^3 cfu/g Total combined yeasts/moulds count (TYMC): NMT 10^2 cfu/g Absence of Escherichia Coli in 1 g

[1] R = Release; S = Stability

[2] MCQ: 5-chloro-4hydroxy-1-methylquinolin-2(1*H*)-one

[3] MCQCA: 5-chloro-4-hydroxy-1methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid

[4] 5-HLAQ: *N*-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2- dihydroquinoline-3-carboxamide

[5] MEG-LAQ: N-ethyl-4-hydroxy-1 methyl-5-(methyl(2,3,4,5,6-pentahydroxyhexyl)amino)-2-oxo-N-phenyl 1,2-dihydroquinoline-3-Carboxamide.

[6] BH-3-HLAQ: 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid

[7] Frequency: Pilot batches, first three production batches and occasional test upon request thereafter.

[8] Frequency: For Release: Pilot batches, first 3 production batches and every fifth batch thereafter, or at least one batch per year if less than 5 batches per year are manufactured. For Stability: a) For long term testing at zero time and annually and/or expiry date. b) For intermediate testing at zero time and 12 months. c) For accelerated testing at zero time and 6 months.

Finished product stability protocol package sizes were the largest and smallest of each marketing container/closure and /or unidose configuration.

Table 45.

Storage Conditions	Test Intervals	Stability Batch Frequency
Long-term testing 25±2°C/60±5%RH	0,3,6,9,12,18,24 and 36 months and/or at expiry date[9]	Pilot batches, first three production batches, an annual batch thereafter and variations as requested.
Accelerated testing 40±2°C/75±5%RH	0,1,2,3, and 6 months	Pilot batches and variations as requested.
Accelerated testing 40±2°C/75±5%RH	0,3, and 6 months	First three production batches.
Intermediate testing[10] 30±2°C/65±5%RH	0, 3,6,9 and 12 months	Pilot batches, first three production batches, and variations as requested.

[9] For validation batches and commercial batches only.

[10] Only if accelerated testing fails.

The following examples describe non-pharmacopoeial analytical methods for the determination of impurity levels in the drug product at the time of release and during stability testing. The method numbers and the detection and quantitation limits for each impurity are also listed, as well as the corresponding validation numbers.

Table 46. Analytical Methods for Determination of Impurities in the Drug Product

Parameter	Example Number	Method Type	Quantitation Limit	Detection Limit
MCQ & MCQCA	Example 21	RP-HPLC	0.1%	0.03%
5-HLAQ	Example 21	RP-HPLC	0.05%	0.02%
NEA[1]	Example 22	RP-HPLC	0.1%	0.03%
3-HLAQ[1]	Example 22	RP-HPLC	0.1%	0.03%
MEG-LAQ	Example 23	RP-HPLC	0.2%	0.1%
BH-3-HLAQ	Example 23	RP-HPLC	0.05%	0.02%

[1] NEA and 3-HLAQ were omitted from the commercial specifications since they were never observed. The monitoring of these impurities in the primary stability batches will continue until completion of the stability programs, using the listed method.

Example 19 - Method Of Determining Amount Of MCQ, MCQCA, 5-HLAQ, MCQME and MCQEE In A Sample Of Laquinimod Drug Substance.

Laquinimod sodium drug substance was analyzed by HPLC. The amount of MCQ, MCQCA, MCQME, MCQEE and 5-HLAQ was determined using the following HPLC method.

1. HPLC CONDITIONS

Column & Packing	Inertsil ODS-3V, 5μm, 4.6×250mm, GL Sciences
Guard column	Opti-Guard C 18, 1mm
Detection	UV at 240nm
Flow rate	1.5mL/min
Injection volume	50μL
Column temperature	40°C
Autosampler temperature	5°C
Run time	30 minutes (Run time for standards may be shortened to at least 25 minutes.)
Mobile phase	800mL of Ammonium acetate buffer 1 solution, pH 7.0; and 200mL Acetonitrile. Mix well and degas.
Buffer pH 7.0	Dissolve 7.7g of Ammonium acetate in 2000mL water and adjust to pH 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid. Filter using 0.45μm membrane filter.
Diluent A	Acetonitrile / Water (1:1)
Diluent B	Mobile phase
Blank solution	Diluent A / Diluent B (3:7)
Note: Adjust flow rate and mobile phase composition to achieve the required system suitability parameters.	

All preparations are performed in amber flasks with non transparent caps. Immediately after preparation place the solutions in a refrigerator or in a cooled to 5°C autosampler rack.

2. STANDARD SOLUTIONS PREPARATION

2.1 STOCK STANDARD SOLUTION (SOLUTION S)

Weigh accurately about 15mg of Laquinimod sodium standard into a 50mL volumetric flask. Dilute with diluent A up to 2/3 of the volume, sonicate for about 2 minutes in cold sonication bath and dilute to volume with the Diluent A. Concentration of Stock standard solution is about 300µg/mL. Stock standard solution may be stored for one month in refrigerator.

2.2 STANDARD SOLUTION FOR ASSAY (SOLUTION A)

Dilute 3 mL of the Stock standard solution to 10 mL with diluent B (dilution factor 3.33). Concentration of Laquinimod sodium is about 90 µg/mL. Standard solution A may be used for 7 days when stored in a refrigerator.

2.3 MCQCA STOCK STANDARD SOLUTION

Weigh accurately about 18mg of MCQCA standard into a 100mL volumetric flask. Dilute to volume with acetonitrile, sonicate until the substance is completely dissolved – stock MCQCA solution.

Concentration of MCQCA is about 180 µg/mL.

MCQCA Stock standard solution should be freshly prepared.

2.4 STANDARD SOLUTION FOR IMPURITIES DETERMINATION (SOLUTION I)

Prepare a solution in diluent B, containing Laquinimod in a concentration of 0.2% and MCQCA – in a concentration of 0.1%, with respect to the working concentration of Laquinimod in Standard solution A. As an example, apply the following procedure.

Transfer 4.0mL of Laquinimod sodium standard solution for assay (Solution A) and 1.0mL of MCQCA stock standard solution to a 100mL volumetric flask and dilute to volume with the diluent B (Intermediate dilution solution).

Place 2.5mL of this intermediate dilution into a 50 mL volumetric flask and make up to volume with the diluent B.

Total dilution factor for Laquinimod standard is 1666.67, for MCQCA 2000.

Concentration of Laquinimod sodium is about 0.18 μg/mL (0.2%).

Concentration of MCQCA is about 0.09μg/mL (0.1%, QL level).

Standard solution I may be used for 24 hours when stored in a refrigerator.

3. RESOLUTION SOLUTION PREPARATION

3.1 MIXED SOLUTION

Prepare a solution of the following impurities in the Diluent A:

Abbreviation	Full name
MCQ	5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one
MCQCA	5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid
MCQME	Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate
5-HLAQ	*N*-Ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide
MCQEE	Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate

Mixed solution may be prepared as follows:

Weigh about 3mg of each impurity to a 100mL volumetric flask, dissolve and dilute to volume with the Diluent A – Mixed solution. Concentration of each impurity in the Mixed solution is about 30μg/mL. The Mixed solution is used only for the determination of the exact retention times of the known impurities and may be used up to four months when stored at about –20°C. For this purpose, the freshly prepared Mixed solution is divided into aliquots and stored immediately at about –20°C. After thawing, the aliquots should not be refrozen.

3.2 RESOLUTION SOLUTION

Transfer 3mL of the stock standard solution (Solution S) and 0.3mL of the Mixed solution to a 10mL volumetric flask and dilute to volume with the Diluent B. Concentration of Laquinimod sodium form is about 90μg/mL. Concentration of each impurity is about 0.9μg/mL (1% with respect to Laquinimod sodium). Resolution solution may be used for 9 days if stored in refrigerator.

4. SAMPLE PREPARATION

Weigh accurately about 15mg of Laquinimod sodium Cryst or DS into a 50mL volumetric flask. Dilute with the Diluent A up to 2/3 of the volume, sonicate for about 2 minutes in cold sonication bath and dilute to volume with the Diluent A – Stock sample solution.

Dilute 3mL of the Stock sample solution to 10mL with the Diluent B – Working sample solution (dilution factor 3.33).

Concentration of Laquinimod sodium is about 90μg/mL.

The sample must be analyzed not later than within 24 hours after preparation.

5. PROCEDURE

Inject the Resolution solution, Blank, Standard solutions for assay and IDD, Laquinimod sample solutions according to the relevant SOP's.

6. SYSTEM SUITABILITY TEST

6.1 RESOLUTION TEST

1. The typical retention time for the Laquinimod peak is 15.5±2.0 minutes.

2. The tailing factor for the Laquinimod peak should be not more than 2.0.

3. Resolution factor of NLT 2 between the peaks should be achieved.

4. The typical RRT of the specified impurity peaks should be as the following:

IMPURITY	Typical RRT
MCQME	0.33 – 0.38
MCQ	0.49 – 0.58
MCQEE	0.56 – 0.65
MCQCA	0.72 – 0.85
5-HLAQ	1.2 – 1.4*

* Retention time of 5-HLAQ should be not more than 23 minutes.

6.2 SYSTEM PRECISION TEST

Evaluate Laquinimod standards for assay and IDD in order to test the system precision according to the relevant SOP's.

MCQCA in Solution I is used to test the sensitivity of the system. The RSD of the area of the six injections of Std 1 as well as the difference between Std1 and Std2 should be NMT 20%.

7. IDENTIFICATION BY RETENTION TIME (RT)

The RT of the main (Laquinimod) peak obtained in the sample chromatogram should correspond to that obtained for the Standard solution.

8. CALCULATION AND REPORT

8.1 ASSAY CALCULATION FOR LAQUINIMOD SODIUM

$$\frac{Area\ of\ smp \times Conc.\ of\ std \times 100}{Area\ of\ std\ (sol\ A) \times Conc.\ of\ smp} = \%\ Assay\ of\ Laquinimod\ \ sodium$$

Report the calculated result of the Assay on dry basis after subtraction of Laquinimod acid content.

8.2 EVALUATION AND CALCULATION OF IMPURITIES

Calculations are performed with respect to the diluted Laquinimod sodium standard (Solution I).

Quantitation level of MCQME, MCQ, MCQEE, 5-HLAQ and unknown impurities is 0.05%.

Detection level of MCQME, MCQ, MCQEE, 5-HLAQ and unknown impurities is 0.02%.

Reporting level of MCQCA is 0.10%.

Detection level of MCQCA is 0.03%

Adjust integration parameters to reject peaks with the area less than 10% of Laquinimod sodium standard (solution I) average area.

Correlate the known impurity peaks in the sample chromatogram with those in the system suitability chromatogram within ± 5% of the actual corresponding retention times.

Calculation of impurities in Laquinimod sodium:

$$\frac{Area of\ imp \times RRF \times Conc of\ std \times 100}{Area of\ Laq\ Na\ std\ (Sol\ I) \times Conc of\ smp} = \%\ Impurity of\ Laquinimod Sodium$$

RRF – relative response factors of impurities calculated as the following ratio: slope of Laquinimod sodium regression line / slope of impurity regression line

Values for Relative Response Factors (RRF) with respect to Laquinimod sodium are:

Impurity	MCQME	MCQ	MCQEE	MCQCA	Unknown impurities / degradation products
RRF	0.70	0.60	0.80	0.58	1.0

9. REPORTING OF IMPURITIES

Report impurities as follows:

	Result	**Report**
MCQME, MCQ, MCQEE, 5-HLAQ	≥ 0.05% < 0.05% < 0.02% (or ND)	The calculated result < 0.05% < 0.02%
MCQCA	≥ 0.10% < 0.10% < 0.03% (or ND)	The calculated result < 0.10% < 0.03%
Unknown impurities	≥ 0.05% < 0.05% < 0.02% (or ND)	The calculated result < 0.05% Not to be reported*
Total	Variant 1 Variant 2 Variant 3 Variant 4	The sum of calculated results < 0.10% < 0.05% < 0.03%

*If no unknown impurities were detected, report: Any other < 0.02%.

Variant 1: At least one of the impurities ≥ 0.05% or MCQCA ≥ 0.10%

Variant 2: All impurities < 0.05% and MCQCA < 0.10%

Variant 3: All impurities < 0.05% and MCQCA < 0.03%

Variant 4: All impurities < 0.02% and MCQCA < 0.03%

Figure 41 presents a resolution test chromatogram according to the above method.

System Suitability Results For Figure 41.

	Name	RT	RRT	Area	USP Resolution	USP Tailing	Int Type
1	MCQME	5.682	0.37	167799		1.1	BB
2	MCQ	8.863	0.57	199156	8.8	1.1	BB
3	MCQEE	9.710	0.63	158206	2.1	1.1	BB
4	MCQCA	11.572	0.75	228929	3.8	1.6	BB
5	Laq	15.481		13270800	6.1	1.6	BB
6	5-HLAQ	20.482	1.32	129109	6.6	1.0	BB

Figure 42 presents a diluent chromatogram according to the above method.

Figure 43 presents a typical chromatogram for assay according to the above method.

Peak Results For Figure 43.

	Name	RT	Area	%Area	Int Type

1	Laq	15.598	12787417	100.00	BB

Figure 44 presents a typical chromatogram for Impurities testing according to the above method.

Peak Results For Figure 44.

	Name	RT	Area	%Area	Int Type
1	Laq	15.598	12790697	100.00	BB

Example 20 - Method Of Determining Amount Of NEA, 3-HLAQ And SPIRO-LAQ In A Sample Of Laquinimod Drug Substance.

Laquinimod sodium drug substance was analyzed by HPLC. The amount of NEA, 3-HLAQ And SPIRO-LAQ was determined using the following HPLC method.

1. HPLC CONDITIONS

Column & Packing	Inertsil ODS-3V, 5μm, 4.6x250mm, GL Sciences
Guard column	Opti-Guard C 18, 1mm (recommended)
Detection	UV at 240nm
Flow rate	1.5mL/min
Injection volume	50μL
Column temperature	40°C
Autosampler temperature	5°C
Run time	30 minutes (Run time for standards may be reduced to at least 18 minutes.)
Mobile phase	50% Solution A – 50% Solution B
Solution A	800mL of Ammonium acetate buffer solution, pH 7.0; and 200mL Acetonitrile, degas
Solution B	350mL of Ammonium acetate buffer solution, pH 7.0; and 650mL Acetonitrile. Mix Solution B on a magnetic stirrer for about 0.5 hour and then degas.
Buffer pH 7.0	Dissolve 7.7g of Ammonium acetate in 2000mL water and adjust to pH 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid. Filter through a 0.45μm membrane filter.
Diluent A	Acetonitrile / Water (1:1)
Diluent B	Solution B
Note: Adjust flow rate and mobile phase composition to achieve the required system suitability parameters.	

All the preparations should be performed in amber flasks. It is recommended to wash volumetric flasks and pipettes with acetonitrile before use. The use of plastic Pasteur pipettes should be excluded.

2. STANDARD SOLUTION PREPARATION

2.1 NEA STANDARD STOCK SOLUTION

Weigh accurately about 25μL (about 25mg) of N-Ethylaniline (NEA) standard into a 50mL volumetric flask. Dilute to volume with Diluent A.

Concentration of stock standard solution is about 500μg/mL.

NEA stock standard solution may be used for three weeks when stored in a refrigerator.

2.2 NEA STANDARD PRIMARY DILUTION SOLUTION

Dilute 0.5 mL of NEA standard stock solution to 50mL with Diluent B.

Concentration of N-Ethylaniline is about 5μg/mL.

2.3 NEA WORKING STANDARD SOLUTION

Dilute 0.5 mL of NEA standard primary dilution solution to 25mL with Diluent B (total dilution factor – 5000).

Concentration of N-Ethylaniline is about 0.1μg/mL (about 0.1% of the working concentration).

NEA working standard solution may be used for four days when stored in a refrigerator.

3 RESOLUTION SOLUTION PREPARATION

3.1 LAQUINIMOD SODIUM STOCK SOLUTION

Weigh accurately about 15mg of Laquinimod sodium standard into a 50mL volumetric flask. Dilute with Diluent A up to 2/3 of the volume, sonicate for about 2 minutes in cold sonication bath and dilute to volume with Diluent A.

Laquinimod stock solution may be used for one month when stored in refrigerator.

3.2 SPIRO-LAQ STOCK SOLUTION

Weight about 2.5mg of SPIRO-LAQ into a 50mL volumetric flask. Dissolve and dilute to volume with a mixture of 95% acetonitrile – 5% water.

3.3 3-HLAQ STOCK SOLUTION

Weight about 2.5mg of 3-HLAQ into a 50mL volumetric flask. Dissolve and dilute to volume with 95% acetonitrile – 5% water.

3.4 RESOLUTION SOLUTION

Transfer the following aliquots to 50mL volumetric flask and dilute to volume with Diluent B:

15mL of Laquinimod sodium stock solution, 1.0mL of SPIRO-LAQ stock solution, 1.0mL of 3-HLAQ stock solution and 0.1mL of NEA standard stock solution.

Concentration of Laquinimod sodium is about 90μg/mL.

Concentration of NEA, SPIRO-LAQ and 3-HLAQ is about 1μg/mL (1%).

The Resolution Solution is used only for the determination of the retention times of Laquinimod, NEA, 3-HLAQ and SPIRO-LAQ and may be used for 38 days when stored at -20°C. For this purpose, the freshly prepared resolution solution should be divided into aliquots and stored immediately at -20°C. After thawing , the solution aliquots should not be refrozen.

Mix vigorously resolution solution after thawing before injection.

After thawing, the Resolution Solution may be used for 4 days when stored in refrigerator.

4. SAMPLE SOLUTION PREPARATION

Weigh accurately about 15mg of Laquinimod sodium Cryst or DS into a 50mL volumetric flask. Dilute with Diluent B up to 2/3 of the volume, sonicate until the substance is completely dissolved and dilute to volume with Diluent B – sample stock solution.

Dilute 3mL of sample stock solution to 10mL with Diluent B (dilution factor 3.33).

Concentration of Laquinimod sodium is about 90μg/mL.

Note: Immediately after preparation put the sample solution to refrigerator or at cooled to 5°C auto sampler. The sample must be analyzed not later than 12 hours after preparation.

5. PROCEDURE

Inject resolution solution, Diluent B (two or three times), Standard and Sample solutions according to relevant SOP.

6. SYSTEM SUITABILITY TEST

6.1 RESOLUTION TEST

The typical retention time is 2.2 ± 0.5 minutes for Laquinimod peak.

The typical retention time is 12.2 ± 1.5 minutes for NEA peak.

The chromatographic mapping of the specified impurity peaks should be in the following order: 3-HLAQ, NEA and SPIRO-LAQ.

Resolution of NLT 2 for both pairs of peaks should be achieved.

6.2 SYSTEM PRECISION TEST

Evaluate standards in order to test system precision according to the relevant SOP.

6.3 BLANK

Inject Diluent B to detect system peaks.

7. EVALUATION AND CALCULATION

Quantitation limit for NEA and any other impurities is 0.06%

Detection limit for NEA and any other impurities is 0.02%.

Adjust integration parameters to reject peaks with the area less than 15% of standard area.

Disregard all the impurity peaks eluting between 0 and the RT of Laquinimod plus one minute.

Calculate the content of non-polar impurities following the equation:

$$\frac{\textit{Area of imp in smp} \times \textit{Conc. of std} \times 100 \times \textit{RRF}}{\textit{Area of std} \times \textit{Conc. of smp}} = \%\ \textit{Impurity}$$

Where RRF is the Relative Response Factor of NEA to impurities as per the Table:

Impurity	NEA	3-HLAQ	SPIRO-LAQ	Unknown impurities
RRF	**1.00**	**1.37**	**0.92**	**1.23**

8. REPORT MODE

	Result	**Report**
NEA, 3-HLAQ or SPIRO-LAQ	≥ 0.06% < 0.06% < 0.02% (or ND)	The calculated result < 0.06% < 0.02%
Unknown impurities (by RRT to Laquinimod)	≥ 0.06% < 0.06% < 0.02% (or ND)	The calculated result < 0.06% Not to be reported*
Total	≥ 0.06% < 0.06% <0.02, or ND	The sum of calculated results < 0.06% < 0.02%

*If no unknown impurities were detected, report: Any other < 0.02%.

Figure 45 presents a resolution test chromatogram according to the above method.

System Suitability Separation Results For Figure 45.

	Name	Int Type	RT	Height (μV)	Area	USP Resolution
1	Laq	bb	2.156	1098982	12220719	
2	System peak	BB	3.442	173	1293	5.2
3	3-HLAQ	BB	10.067	7917	119088	21.9
4	NEA	BB	11.034	12544	165542	2.5
5	Spiro-LAQ	BB	12.496	8797	146324	3.6

Figure 46 presents a diluent chromatogram in according to the above method.

Peak Results For Figure 46.

	Name	Int Type	RT	Height (μV)	Area	%Area
1	System peak	BB	3.423	313	2191	100.00

Figure 47 presents a typical sample chromatogram according to the above method.

Peak Results For Figure 47.

	Name	Int Type	RT	Height (μV)	Area	%Area
1	Laquinimod	bb	2.145	96369	11564891	99.99
2	System peak	BB	3.421	166	1216	0.01

Example 21 - Method Of Determining Amount Of MCQ, MCQCA, 5-HLAQ, MCQME and MCQEE In A Sample Of Laquinimod Drug Product.

Laquinimod capsules containing 0.6 mg Laquinimod were analyzed by HPLC. The amount of

MCQ, MCQCA, 5-HLAQ, MCQME and MCQEE was determined using the following HPLC method.

1. HPLC CONDITIONS

Column & Packing	Inertsil ODS-3V, 5μm, 4.6×250mm, GL Sciences Inc.
Guard column	Opti-Guard C 18, 1×10mm
Mobile phase	Acetonitrile : Buffer pH 7.0 – 20:80 (v/v). Mix and degas
Buffer pH 7.0 preparation	Dissolve 7.7g Ammonium acetate in 2000mL water and adjust to pH 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid. Filter through a 0.45μm membrane filter.
Flow rate	1.5mL/min
Detection	UV at 240nm
Injection volume	50μL
Diluent A	Acetonitrile / Water – 50:50 (v/v)
Diluent B (and Blank)	Mobile phase
Column temperature	40°C
Autosampler temperature	5°C
Run time	40 minutes
Run time for standards	May be shortened to 23 minutes
Note: Flow rate, mobile phase composition and column temperature may be varied in order to achieve the required system suitability parameters.	

All the preparations should be protected from light. For liquid preparations use amber flasks with non transparent caps. Use only glass Pasteur pipettes. Standard and sample solutions should be kept refrigerated at 2 – 8°C after preparation.

2. STANDARD SOLUTIONs PREPARATION

2.1 LAQUINIMOD STANDARD STOCK SOLUTION (SOLUTION S)

Weigh accurately in duplicate about 15mg of Laquinimod sodium standard into a 50mL volumetric flask. Dilute with diluent A up to 2/3 of the volume, sonicate for 2 minutes in a cold sonication bath and dilute to volume with diluent A.

Concentration of standard stock solution is about 300μg/mL Laquinimod sodium.

Standard stock solution may be used for one month when stored in a refrigerator (2°C-8°C).

2.2 LAQUINIMOD STANDARD WORKING SOLUTION FOR ASSAY (SOLUTION A)

Dilute 3mL of the Standard Stock Solution to 10mL with diluent B (Dilution factor 3.33).

Concentration of Laquinimod sodium is about 90μg/mL. Concentration expressed as Laquinimod (acid) is about 85μg/mL.

Standard working solution A may be used for 7 days when stored in a refrigerator (2°C-8°C).

2.3 MCQCA STANDARD STOCK SOLUTION

Weigh accurately about 18mg of MCQCA standard into a 100mL volumetric flask. Dilute to volume with acetonitrile, sonicate (in a cold sonication bath) until the substance is completely dissolved – stock MCQCA solution.

Concentration of MCQCA is about 180 μg/mL.

MCQCA Stock standard solution should be freshly prepared.

2.4 STANDARD SOLUTION FOR DETERMINATION OF IMPURITIES (SOLUTION I)

Prepare a solution in diluent B, containing Laquinimod in a concentration of 0.2% and MCQCA – in a concentration of 0.1%, with respect to the working concentration of Laquinimod in Standard solution A. As an example, apply the following procedure.

Transfer 4.0mL of Laquinimod sodium standard solution for assay (Solution A) and 1.0mL of MCQCA stock standard solution to a 100mL volumetric flask and dilute to volume with the diluent B (Intermediate dilution).

Place 2.5mL of this intermediate dilution into a 50 mL volumetric flask and make up to volume with the diluent B.

Total dilution factor for Laquinimod standard is 1666.67, for MCQCA 2000.

Concentration of Laquinimod sodium is about 0.18 μg/mL (0.2%).

Concentration of MCQCA is about 0.09μg/mL (0.1%, QL level).

Standard solution I may be used for 24 hours when stored in a refrigerator.

3. RESOLUTION SOLUTIONs PREPARATION

3.1 MIXED SOLUTION

Prepare solution containing the following potential impurities standards (markers) using the Diluent A as a solvent:

Mixed Solution:

Impurity	
Abbreviation	**Full name**
MCQ	5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one
MCQCA	5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid
MCQME	Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate
5-HLAQ	*N*-Ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide

The Mixed Solution may be prepared as follows:

Weigh about 3mg of each impurity standard / marker into a 100mL volumetric flask, dissolve (sonication is acceptable) and dilute to volume with the Diluent A.

Concentration of each impurity in the Mixed Solutions is about 30μg/mL.

Mixed Solution may be used for up to 4 months when stored frozen at about -20°C.

For this purpose, the freshly prepared Mixed Solution should be divided into aliquots, immediately frozen and stored at -20°C. After thawing, the aliquots should be mixed well and should not be refrozen.

3.2 STOCK SOLUTIONS OF ADDITIONAL IMPURITIES

Weigh about 3mg of MCQEE (Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate) into a 100mL volumetric flask, dissolve (sonication is acceptable) and dilute to volume with the Diluent A. This is MCQEE Stock solution.

This Solution may be used for up to 4 months when stored frozen at about -20°C.

For this purpose, the freshly prepared MCQEE Stock Solution should be divided into aliquots, immediately frozen and stored at -20°C. After thawing, the aliquots should be mixed well and should not be refrozen.

Weigh about 3mg of MEG-LAQ (Meglumine Adduct of Laquinimod) into a 100mL volumetric flask, dissolve (sonication is acceptable) and dilute to volume with the Diluent A. This is MEG-LAQ Stock solution.

This Solution may be used for one week when stored in a refrigerator (2°C – 8°C).

3.3 RESOLUTION SOLUTIONS

Prepare two Resolution Solutions separately as follows, using the Mixed solution.

3.3.1 RESOLUTION SOLUTION 1

Transfer 3mL of Laquinimod standard stock solution (Solution S), 0.3mL of the Mixed solution and 0.3mL of the MCQEE Stock solution to a 10mL volumetric flask and dilute to volume with the Diluent B. This is Resolution Solution 1.

Concentration of Laquinimod sodium in it is about 90μg/mL. Concentration of each impurity – is about 0.9μg/mL (about 1% with respect to the working concentration of Laquinimod).

Resolution Solution 1 is used for resolution test (for system suitability) and for determination of retention times (RT) / relative retention times (RRT) of five known impurities: MCQ, MCQCA, MCQME, MCQEE and 5-HLAQ.

Resolution Solution 1 may be used for 9 days if stored in a refrigerator (2°C – 8°C).

Note: Instead of Resolution Solution 1, a Resolution Solution prepared as per the Method of Example 19 may be used.

3.3.2 RESOLUTION SOLUTION 2

Transfer 3mL of Laquinimod standard stock solution (Solution S), 0.3mL of the Mixed solution and 0.3mL of the MEG-LAQ Stock solution to a 10mL volumetric flask and dilute to volume with the Diluent B. This is Resolution Solution 2.

Concentration of Laquinimod sodium in it is about 90μg/mL. Concentration of each impurity – is about 0.9μg/mL (about 1% with respect to the working concentration of Laquinimod).

Resolution Solution 2 is used for determination of retention time of MEG-LAQ.

Resolution Solution 2 may be used for one week if stored in a refrigerator (2°C – 8°C).

4. SAMPLE SOLUTION PREPARATION

Weigh accurately 20 capsules and completely empty their contents into a mortar. Pay attention to complete emptying of capsule contents into the mortar, using spatula when necessary. Weigh the empty capsules. Calculate the average weight of the capsule contents.

Mix and grind the capsule contents in a mortar and keep the powder in a tightly closed container protected from light.

Weigh accurately, in duplicate, the amount of powder corresponding to 7 capsules, into a 50mL volumetric flask.

Add diluent B up to 2/3 of the volume, shake for 30 minutes at 200 mot/min. Dilute to volume with diluent B. Mix well. Filter before use through a 0.45μm GHP ACRODISC GF filter or equivalent, discarding the first 0.5-1mL.

Working concentration of Laquinimod (acid) is about 84μg/mL.

Note: Immediately after preparation place sample solutions into a refrigerator or in a cooled to 5°C autosampler. The sample solutions may be used for 24 hours when kept at the temperature 2°C – 8°C.

5. PROCEDURE

Inject the Resolution Solutions, Diluent B (Blank), Standard Solutions for assay and IDD and Sample solutions, according to the relevant SOP's.

Determine the retention time (RT) and the area of the Laquinimod peak in the chromatograms of Sample and Standard Solutions for identification and assay.

Determine the RT, the relative retention time (RRT) and peak areas of all known impurities and any other impurities in the chromatograms of Sample Solutions, for calculation of the content of impurities / degradation products.

Ignore early eluting peaks of excipients and system peaks (See chromatogram for determination of impurities / degradation products). For example, use integration inhibition between 0 and RRT 0.15 (about 2.5 minutes).

Adjust integration parameters to reject peaks with area less than 10% of the average response of Laquinimod peak in the injections of **Standard Solution I** (for determination of impurities / degradation products).

Disregard peak of MEG-LAQ in sample injections (identified following Resolution Solution 2). The content of MEG-LAQ will be tested by a different method.

6. SYSTEM SUITABILITY TEST

6.1 RESOLUTION TEST

Typical retention time of Laquinimod peak is 15.5 ± 2.0 minutes.

Tailing factor (USP) for Laquinimod peak should be not more than 2.0.

Resolution factor for all the pairs of peaks should be NLT 2.

RRT of the peaks of known impurities / degradation products should be as follows:

Impurity	RRT
MCQME	0.33 – 0.38
MCQ	0.49 – 0.58
MCQEE	0.56 – 0.65
MCQCA	0.71 – 0.85
5-HLAQ	1.2 – 1.4*

* Retention time of 5-HLAQ should not be more than 23 minutes.

MEG-LAQ peak is substantially broadened in comparison with neighboring peaks. Retention time of MEG-LAQ is variable, being very sensitive to slightest changes in chromatographic conditions (pH, % Acetonitrile, Temperature, etc.) and therefore should be defined using its peak in the chromatogram of the Resolution Solution 2. Typically, its RRT is about 0.66.

6.2 SYSTEM PRECISION TEST

Evaluate Laquinimod standards for assay and IDD in order to test the system precision according to the relevant SOP's.

MCQCA in Solution I is used to test the sensitivity of the system. RSD of the area of six injections of Std 1 as well as the difference between Std 1 and Std 2 should be NMT 20%.

6.3 BLANK

Inject diluent B to detect system peaks.

7. IDENTIFICATION BY RETENTION TIME (RT)

The RT of the main peak obtained in the sample chromatogram should correspond to that obtained for the Laquinimod peak in the injection of Standard Solution.

8. CALCULATION AND REPORT

8.1 ASSAY CALCULATION

$$\textit{\% Assay (to Label Claim)} = \frac{Area_{Smp} \times Conc_{Std} \times 0.94 \times V_{Smp} \times AvgWt_{CapsContent}}{Area_{Std} \times W_{Smp} \times \textbf{Label Claim}} \times 100$$

Where: ***0.94*** : Conversion factor of Laquinimod sodium salt to Laquinimod (acid).

8.2 CALCULATION AND EVALUATION OF IMPURITIES / DEGRADATION PRODUCTS

8.2.1 CALCULATION OF RELATIVE RETENTION TIME (RRT)

$$\textbf{RRT Impurity} = \frac{\textbf{RT Impurity}}{\textbf{RT Laquinimod}}$$

8.2.2 CALCULATION OF CONTENT OF IMPURITIES / DEGRADATION PRODUCTS

$$\textit{\% Impurity} = \frac{Area_{Impurity} \times Conc_{Std} \times V_{Smp} \times AvgWt_{CapsContent} \times 0.94 \times RRF}{Area_{Std\ I} \times W_{Smp} \times \textbf{Label Claim}} \times 100$$

$Area_{Impurity}$: Area of an impurity/ degradation product (known or unknown) peak in Sample Solution.

***$Area_{std}$* :** Area of Laquinimod peak in chromatogram of Standard **Solution I**.

0.94 : Conversion factor of Laquinimod sodium salt to Laquinimod (acid).

RRF **:** Relative response factors of impurities / degradation products calculated as the following ratio: slope of Laquinimod regression line / slope of impurity regression line.

The values for relative response factors with respect to Laquinimod are:

Impurity	MCQME	MCQ	MCQEE	MCQCA	5-HLAQ
RRF	0.74	0.65	0.85	0.62	1.0

RRF for unknown impurities / degradation products is taken as 1.0.

8.2.3 EVALUATION AND REPORT OF IMPURITIES / DEGRADATION PRODUCTS

Quantitation level (QL) of MCQME, MCQ, MCQEE, 5-HLAQ and unknown impurities is 0.05%. Detection level (DL) of MCQME, MCQ, MCQEE, 5-HLAQ and unknown impurities is 0.02%.

QL of MCQCA is 0.1%. Detection level DL of MCQCA is 0.03%.

Correlate all the peaks in sample chromatogram with those in the system suitability chromatogram, within ±5% of the actual corresponding retention times.

Report as follows:

	Result	Report
Specified impurities:		
5-HLAQ	≥ 0.05% < 0.05% < 0.02% (or ND)	The calculated result < 0.05% < 0.02%
MCQ and MCQCA sum	≥ 0.1% < 0.1% < 0.03% (or ND)	The calculated result < 0.1% < 0.03%
Other impurities:		
MCQME, MCQEE	≥ 0.05% < 0.05% < 0.02% (or ND)	The calculated result < 0.05% < 0.02%
Unknown peaks (by RRT to Laquinimod)	≥ 0.05% < 0.05% < 0.02% (or ND)	The calculated result < 0.05% Not to be reported*
Total	≥ 0.05% < 0.05% (or<0.02 or ND)	The sum of calculated results < 0.05%

*If no impurities were detected, report: any other < 0.02%.

Figure 48 presents a resolution test (resolution solution 1) chromatogram according to the above method.

System Suitability Separation Results For Figure 48.

	Name	RT	RT Ratio	Area	USP Resolution	USP Tailing	Int Type
1	MCQME	5.460	0.36	35801		1.07	BB
2	MCQ	8.260	0.54	60897	8.2	1.16	BV
3	MCQEE	9.249	0.60	24029	2.6	1.05	VB
4	MCQCA	12.031	0.78	18609	5.7	1.34	BB
5	Laq	15.332		12332469	5.2	1.84	BB
6	5-HLAQ	20.451	1.33	89463	7.3	1.04	BB

Figure 49 presents a resolution test (resolution solution 2) chromatogram according to the above method.

System Suitability Separation Results For Figure 49.

	Name	RT	RT Ratio	Area	USP Resolution	USP Tailing	Int Type
1	MCQME	5.457	0.36	151440		1.06	BB
2	MCQ	8.260	0.54	16608	8.1	1.17	BV
3	MCQEE	10.081	0.66	86529	1.7		VB
4	MCQCA	11.856	0.77	183439	1.5	1.9	BB
5	Laq	15.330		12314140	5.8	1.82	BB
6	5-HLAQ	20.446	1.33	120400	7.2	1.04	BB

Figure 50 presents a diluent blank chromatogram according to the above method.

Figure 51 presents a sample chromatogram for assay according to the above method.

Figure 52 presents a sample chromatogram for determination of impurities/degradation products according to the above method.

Example 22 - Method Of Determining Amount Of NEA, 3-HLAQ And SPIRO-LAQ In A Sample Of Laquinimod Drug Product.

Laquinimod capsules containing 0.6 mg Laquinimod were analyzed by HPLC. The amount of NEA, 3-HLAQ And SPIRO-LAQ was determined using the following HPLC method.

1. ABBREVIATIONS

Abbreviation	Full name
NEA	N-Ethylaniline
3-HLAQ	5-chloro-N-ethyl-3-hydroxy-1-methyl-2,4-dioxo-N-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide
SPIRO-LAQ	1H,3H-spiro[5-chloro-1-methylquinoline-2,4-dione-3,3'-[1]-ethylindolin-[2]-one]

2. HPLC CONDITIONS

Column & Packing	Inertsil ODS-3V, 5µm, 4.6×250mm, GL Sciences
Guard column	Opti-Guard C 18, 1×10mm
UV detection	240nm
Mobile phase:	50% Solution A – 50% Solution B (v/v)
Solution A:	Acetonitrile: Buffer pH 7.0 – 20 : 80 (v/v)
Solution B:	Acetonitrile: Buffer pH 7.0 – 65 : 35 (v/v). Mix well by magnetic stirring for about 0.5 hour.
Buffer Solution pH 7.0	Dissolve 7.7g Ammonium acetate in 2000mL water and adjust to pH 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid. Filter through a 0.45µm membrane filter.
Flow rate	1.5mL/min
Injection volume	20µL
Diluent A	Methanol / Acetonitrile – 50:50 (v/v)
Blank (Final diluent)	Methanol / Acetonitrile – 10:90 (v/v)
Column temperature	40°C
Autosampler temperature	5°C
Run time	20 minutes
Needle and seal wash	Acetonitrile / water – 50:50 (v/v)
Note: Flow rate, mobile phase composition and column temperature may be varied to achieve the required system suitability parameters.	

All the preparations should be protected from light. For liquid preparations use amber flasks with non transparent caps.

Use only glass Pasteur pipettes.

Standard and sample solutions should be kept refrigerated at 2 – 8°C after preparation.

3. STANDARD SOLUTION PREPARATION

3.1 NEA STANDARD STOCK SOLUTION

Weigh accurately in duplicate about 18μL (about 18mg) of N-Ethylaniline (NEA) standard into a 50mL volumetric flask. Dilute to volume with acetonitrile.

Concentration of standard stock solution is about 360μg/mL.

NEA standard stock solution may be used for three weeks when stored in a refrigerator (2°C – 8°C).

3.2 NEA STANDARD PRIMARY DILUTION SOLUTION

Dilute 0.5mL NEA standard stock solution to 50mL with acetonitrile.

Concentration of standard primary dilution solution is about 3.6μg/mL.

3.3 NEA Standard Working Solution

Transfer 0.5mL NEA standard primary dilution solution to a 20mL volumetric flask. Add 4mL diluent A and dilute to volume with acetonitrile.

Concentration of NEA is about 0.09μg/mL (about 0.1% of the Laquinimod working concentration).

NEA working standard solution may be used for one week when stored in a refrigerator (2°C – 8°C).

4. RESOLUTION SOLUTION PREPARATION

Resolution solution from Example 20 may be used.

4.1 LAQUINIMOD STOCK SOLUTION FOR RESOLUTION TEST

Weigh accurately about 15mg of Laquinimod sodium standard into a 50mL volumetric flask. Dilute with acetonitrile: water [50:50 (v/v)] up to 2/3 of the volume, sonicate for 2 minutes in a cold sonication bath and dilute to volume with acetonitrile: water [50:50 (v/v)].

Concentration of Laquinimod stock solution is about 300µg/mL.

Laquinimod stock solution may be used for one month when stored in a refrigerator (2°C-8°C).

4.2 NEA STOCK SOLUTION FOR RESOLUTION TEST

Weigh accurately about 25µL (about 25mg) of N-Ethylaniline (NEA) standard into a 50mL volumetric flask. Dilute to volume with acetonitrile: water [50:50 (v/v)].

Concentration of NEA stock solution is about 500µg/mL.

NEA stock solution may be used for 3 weeks if stored in a refrigerator (2°C – 8°C).

4.3 SPIRO-LAQ STOCK SOLUTION FOR RESOLUTION TEST

Weigh about 2.5mg of SPIRO-LAQ into a 50mL volumetric flask. Dissolve and dilute to volume with acetonitrile: water [95:5 (v/v)].

Concentration of SPIRO-LAQ stock solution is about 50µg/mL.

4.4 3-HLAQ STOCK SOLUTION FOR RESOLUTION TEST

Weigh about 2.5mg of 3-HLAQ into a 50mL volumetric flask. Dissolve and dilute to volume with acetonitrile: water [95:5 (v/v)].

Concentration of 3-HLAQ stock solution is about 50µg/mL.

4.5 RESOLUTION SOLUTION

Transfer the following aliquots to a 50mL volumetric flask and dilute to volume with mobile phase solution B [acetonitrile: buffer pH 7.0 (65: 35 v/v)]:

15mL of Laquinimod stock solution for resolution test,

1.0mL of SPIRO-LAQ stock solution for resolution test,

1.0mL of 3-HLAQ stock solution for resolution test,

0.1mL of NEA stock solution for resolution test.

Concentration of Laquinimod sodium is about 90µg/mL.

Concentration of NEA, SPIRO-LAQ and 3-HLAQ is about 1μg/mL (about 1% with respect to Laquinimod working concentration).

The resolution solution is used only for the determination of the retention times of the laquinimod and known potential impurities/ degradation products and may be used for 38 days when stored at -20°C. For this purpose, the freshly prepared resolution solution should be divided into aliquots and stored immediately at -20°C. After thawing, the solution aliquots should be mixed vigorously and should not be refrozen.

The resolution solution may be used for 4 days when stored in a refrigerator (2°C – 8°C).

5. SAMPLE SOLUTION PREPARATION

Weigh accurately at least 10 capsules and empty the contents in a mortar. Weigh the empty capsules. Calculate the average weight of the capsule contents.

Grind the sample in a mortar and keep the powder in a tightly closed protected from light container.

Weigh accurately, in duplicate, the amount of powder corresponding to 3 Laquinimod capsules into a 20 mL volumetric flask.

Add 4 to 10 mL of Diluent A and shake for 15 minutes at 200 mot/min to dissolve. Dilute to volume with acetonitrile. Mix well. Filter before use through a 0.45μm GHP Acrodisc GF filter or equivalent, discarding the first 2mL.

Concentration of Laquinimod is about 90μg/mL.

Note: Immediately after preparation place the sample solution into a refrigerator or in a cooled to 5°C autosampler. The sample should be analyzed not later than within 24 hours after preparation.

6. PROCEDURE

Inject resolution solution, blank, standard and sample solutions according to the relevant SOP's for impurities/ degradation products determination.

Determine the peak area of NEA in standard working solutions.

Determine the retention time (RT) of the Laquinimod peak in the chromatograms from resolution and sample solutions

Determine the RT, the relative retention time (RRT) to Laquinimod peak and the peak area of all known impurities and unknown impurities in the chromatograms from Sample solutions.

Apply integration inhibition between 0 and the 1.6 × RT of Laquinimod peak (RT of system peak).

Adjust integration parameters to reject peaks with the area less than 20% of NEA standard area.

7. SYSTEM SUITABILITY TEST

7.1 RESOLUTION TEST

The typical retention time for Laquinimod is 2.2 ± 0.5 minutes.

The typical retention time for NEA is 12.2 ± 1.5 minutes.

The RRT of the specified impurity/degradation product peaks relative to NEA should be as follows:

Impurity / Degradation product	RRT to NEA
3-HLAQ	0.85 – 0.95
SPIRO-LAQ	1.05 – 1.20

Resolution factor of NLT 2 for both known impurities/ degradation products peaks from NEA peak should be achieved.

7.2 SYSTEM PRECISION TEST

Evaluate standards in order to test the system precision according to the relevant SOP for impurities/ degradation products determination.

7.3 BLANK

Inject blank to detect system peaks.

8 EVALUATION, CALCULATION AND REPORT

8.1 RRT OF IMPURITIES / DEGRADATION PRODUCTS

$$RRT_{Imp} = \frac{RT_{Imp}}{RT_{Laq}}$$

8.2 CALCULATION OF CONTENT OF IMPURITIES / DEGRADATION PRODUCTS

Calculate % IDD in Laquinimod capsules as follows:

$$\%\,\boldsymbol{Impurity} = \frac{Area_{Impurity} \times Conc_{Std\,NEA} \times V_{Smp} \times AvgWt_{CapsContent} \times RRF}{Area_{Std\,NEA} \times W_{Smp} \times \boldsymbol{LabelClaim}} \times 100$$

$Area_{Imp}$: Area of the NEA, 3-HLAQ or unknown impurity / degradation product peak in the sample solution.

See note in 8.3.

$Area_{Std\,NEA}$: Area of the NEA in standard working solution.

RRF : Relative response factors of impurities calculated as the following ratio:

Slope of NEA regression line / slope of impurity regression line.

Note: For Unknown impurities/ degradation product, the slope of Laquinimod regression line was used.

The values for relative response factors with respect to NEA are:

Impurity	NEA	3-HLAQ	Unknown
RRF	1.0	1.39	1.15

8.4 EVALUATION AND REPORT

Quantitation limit (QL) for NEA, 3-HLAQ and any other unknown impurity / degradation product is 0.1% with respect to the Laquinimod working concentration.

Detection limit (DL) for NEA, 3-HLAQ and any other unknown impurity / degradation product is 0.03% with respect to the Laquinimod working concentration.

Report content of Impurities / Degradation products as follows:

	Result	**Report**
NEA or 3-HLAQ	≥ 0.1% < 0.1% < 0.03% or ND	The calculated result < 0.1% < 0.03%
Any other impurity peak (by RRT to Laquinimod)	≥ 0.1% < 0.1% < 0.03% or ND	The calculated result < 0.1% Not to be reported**
Total	If peak/s ≥ 0.1% If all peaks < 0.1% or <0.03%	The sum of calculate results < 0.1%

* Correlate the NEA and 3-HLAQ peaks in the in the sample chromatogram with those in the system suitability chromatogram within ± 5% of the actual retention times.

** If no unknown impurities are present, report: "Any other <0.03%"

Note: Peaks eluting at RT of the SPIRO-LAQ (± 5% of the actual retention time in system suitability test) report as "any other impurity" peak.

Figure 53 presents a resolution test chromatogram according to the above method.

System Suitability Separation Results For Figure 53.

	Name	RT	RRT	Area	Height (µV)	USP Tailing	USP Resolution
1	LAQ	2.166		5587169	964422	1.22	
2	3-HLAQ	11.228	0.92	81831	4436	1.35	28.23
3	NEA	12.226		63991	4744	1.06	2.34
4	Spiro-LAQ	1.14	78255	78255	4725	1.02	4.26

Figure 54 presents a blank (diluents) chromatogram according to the above method.

Peak Results For Figure 54.

	Name	Int Type	RT	Height (µV)	Area	%Area
1	System peak	BB	3.508	116	858	52.53
2	Sys. peak	BB	5.740	41	775	47.47

Figure 55 presents a sample chromatogram according to the above method.

Peak Results For Figure 55.

	Name	Int Type	RT	Height (µV)	Area	%Area
1	Laquinimod	BB	2.1459	923247	4717424	99.97
2	System peak	BB	3.499	71	666	0.01
3	Sys. peak	BB	5.736	38	642	0.01

Example 23 - Method Of Determining Amount Of MEG-LAQ And BH-3-HLAQ In A Sample Of Laquinimod Drug Product.

Parameter	**Example Number**	**Method Type**	**Quantitation Limit**	**Detection Limit**
MEG-LAQ	Example 23	RP-HPLC	0.2%	0.1%
BH-3-HLAQ	Example 23	RP-HPLC	0.05%	0.02%

Laquinimod capsules containing 0.6 mg Laquinimod were analyzed by HPLC. The amount of

MEG-LAQ and BH-3-HLAQ was determined using the following HPLC method.

1. CHROMATOGRAPHIC SYSTEM

Column & Packing	Inertsil ODS-3 V, 5μm, 3.0×150mm, GL Sciences Inc.
Guard column (recommended)	Opti-Guard C 18, 1 mm, Optimize Technologies
Column Temperature	43°C ± 4°C
Buffer Solution Preparation	Dissolve 5.75 g of Ammonium Dihydrogen Phosphate in 300mL water. Filter through a 0.45μm membrane filter. Dilute to 2000mL with water and adjust to pH 7.0 ± 0.10 with aqueous Ammonia or Phosphoric acid. Apply proportional adjustments for preparation of a different volume.
Solvent A	Mixture of 80% (v/v) Buffer Solution and 20% (v/v) Acetonitrile. Degas
Solvent B	Mixture of 70% (v/v) Buffer Solution and 30% (v/v) Methanol. Degas.
Mobile Phase	Option 1: Work from 2 reservoirs: 60% (v/v) Solvent A and 40% (v/v) Solvent B; Adjust to achieve system suitability requirements. Option 2: Work from 1 reservoir: Mix Solvent A and Solvent B in the ratio 60:40 (v/v); degas after mixing.
Flow Rate:	1.0 mL/min
Detector	UV at 212 nm, 10 mm flow cell path length
Autosampler Temperature	5°C
Injection Volume	35 μL
Diluent 1	Acetonitrile / Water – 50:50 (v/v)
Diluent 2/ Blank Solution	Solvent A
Injector Wash Solution	Acetonitrile / Water – 50:50 (v/v)
Run Time	30 minutes

Mobile phase parameters, flow rate and column temperature may be altered in order to achieve the system suitability requirements. The Mobile phase parameters may be altered in the range: 55% - 62% (v/v) Solvent A and 45% -38% (v/v) Solvent B. All the solvents must be of HPLC grade or equivalent. All the preparations should be kept refrigerated at 2 – 8°C and be protected from light. Use amber flasks (low actinic glassware) with non transparent caps. Avoid heating of sonication bath.

2. LIST OF ABBREVIATIONS

Abbeviation	Full chemical name
MEG-LAQ	N-ethyl-4-hydroxy-1-methyl-5-(methyl(2,3,4,5,6-pentahydroxyhexyl)amino)-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide
BH-3-HLAQ	2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl 3-oxopropanamido)benzoic acid

3. PREPARATION OF STANDARD SOLUTIONS

3.1 STOCK SOLUTION OF BH-3-HLAQ

Prepare in duplicate. Accurately weigh about 11.5 mg of BH-3-HLAQ into a 50 mL volumetric flask, sonicate to dissolve and dilute to volume with the Diluent 1.

Concentration of BH-3-HLAQ stock solution is about 230 μ g/mL.

BH-3-HLAQ stock solution may be used for 1 week if stored refrigerated (2°C – 8°C).

3.2 STOCK SOLUTION OF MEG-LAQ

Prepare in duplicate. Accurately weigh about 11.5 mg of MEG-LAQ into a 50 mL volumetric flask, sonicate to dissolve and dilute to volume with the Diluent 1. Concentration of MEG-LAQ stock solution is about 230 μg/mL.

MEG-LAQ stock solution may be used for 1 week if stored refrigerated (2°C – 8°C).

Note: Due to high hygroscopicity of MEG-LAQ, its exposure to air should be minimal: close the vial with standard immediately after weighing; it is recommended to weigh MEG-LAQ under controlled low humidity conditions.

3.3 PRIMARY DILUTED SOLUTION OF BH-3-HLAQ

Transfer 2.0 mL of BH-3-HLAQ Stock solution to a 50 mL volumetric flask and dilute to volume with Diluent 2. Concentration of BH-3-HLAQ is about 9.2 μ g/mL.

3.4 PRIMARY DILUTED SOLUTION OF MEG-LAQ

Transfer 2.0 mL of MEG-LAQ Stock solution to a 50 mL volumetric flask and dilute to volume with Diluent 2. Concentration of MEG-LAQ is about 9.2 μg/mL.

3.5 STANDARD SOLUTION

Transfer 1.0 mL of the Primary Diluted Solution of BH-3-HLAQ and 2.5 mL of the Primary Diluted Solution of MEG LAQ to a 50 mL volumetric flask and dilute to volume with Diluent 2.

Total dilution factor in this standard solution is: 1250 for BH-3-HLAQ and 500 for MEG-LAQ (from the stock solution).

Concentration of BH-3-HLAQ is about 0.18 µg/mL (0.2% of Laquinimod working concentration), and of MEG-LAQ is about 0.45 µg/mL (0.5% of Laquinimod working concentration).

Standard solution may be used for 1 week if stored refrigerated (2°C – 8°C).

3.6 QL SOLUTION

Prepare once. Transfer 0.5 mL of Primary Diluted Solution of BH-3-HLAQ and 2.0 mL of the Primary Diluted Solution of MEG LAQ (one of the duplicates each) to a 100 mL volumetric flask and dilute to volume with Diluent 2.

Concentration of BH-3-HLAQ is about 0.045 µg/mL (0.05% of Laquinimod working concentration), and of MEG-LAQ is about 0.18 µg/mL (0.2% of Laquinimod working concentration).

4. PREPARATION OF RESOLUTION SOLUTION

4.1 LAQUINIMOD STOCK SOLUTION

Prepare once. Weigh about 15 mg of Laquinimod sodium standard into a 50 mL volumetric flask. Dilute with the Diluent 1 up to 2/3 of the volume, sonicate for 2 minutes in a sonication bath and dilute to volume with the Diluent 1. Concentration of Laquinimod stock solution is about 300 µg/mL Laquinimod sodium.

Laquinimod Stock solution may be used for 1 month if stored refrigerated (2°C – 8°C).

4.2 RESOLUTION SOLUTION

Transfer 3 mL of Laquinimod stock solution and 1 mL of each of the Primary Diluted solutions (one of the duplicates of each) to a 10 mL volumetric flask. Dilute to volume with Diluent 2. Mix well.

Concentration of Laquinimod Sodium in the Resolution Solution is about 90 µg/mL (about 85 µg/mL if expressed as Laquinimod - free acid). Concentration of each impurity is about 0.9 µg/mL (about 1% with respect to the working concentration of Laquinimod).

Resolution Solution may be used for one week if stored refrigerated (2°C – 8°C).

5. PREPARATION OF SAMPLE SOLUTIONS

Weigh accurately 20 capsules and completely empty their contents into a mortar. Pay attention to complete emptying of capsule contents into the mortar, using spatula when necessary. Weigh the empty capsules. Calculate the average weight of the capsule contents.

Mix and grind the capsule contents in a mortar and keep the powder in a tightly closed container protected from light.

Weigh accurately, in duplicate, the amount of powder corresponding to 7 capsules, each into a separate 50 mL volumetric flask.

Add diluent 2 up to 2/3 of the volume, shake for 30 minutes at 200 mot/min. Dilute to volume with diluent 2. Mix well. Filter before use through a 0.45 μ m ACRODISC GHP GF filter or equivalent, discarding the first 0.5 - 1mL.

Immediately after preparation place sample solutions into a refrigerator or in a cooled to 5°C autosampler.

Working concentration of Laquinimod (acid) is about 84 μ g/mL.

Sample solutions may be used for 24 hours when kept at the temperature 2°C – 8°C.

6. SYSTEM SUITABILITY TEST

6.1 RESOLUTION TEST

Inject the Resolution solution once.

Typical retention time of Laquinimod peak* is within the range 12 – 18 minutes.

Order of elution of peaks in the injection of Resolution Solution and typical RRT of the impurities peaks, with respect to Laquinimod peak, are as follows:

Peak	MEG-LAQ	BH-3-HLAQ	Laquinimod
Typical RRT*	0.55 – 0.75	0.80 – 0.95	1.0

* For information only, may differ for various lots of columns or as a result of variations in actual

mobile phase composition (Solvent A / Solvent B ratio) and pH.

Acceptance criteria:

- Tailing factor (USP) for MEG-LAQ and BH-3-HLAQ peaks should be within 0.8 – 1.5.
- Resolution factor between all the three peaks (MEG-LAQ, BH-3-HLAQ and Laquinimod) should be not less than 1.5.

Flow rate, temperature and mobile phase composition (ratio of Solvent A and Solvent B) may be adjusted to achieve the required system suitability parameters.

6.2 BLANK INJECTIONS

Inject Blank (Diluent 2) twice to detect system peaks. Disregard system peaks.

6.3 SENSITIVITY TEST

From 6 replicate injections of the QL Solution, evaluate areas of BH-3-HLAQ and MEG-LAQ peaks.

RSD for each of the peaks, in 6 replicates, should be not more than 20%.

6.4 SYSTEM PRECISION TEST

Evaluate areas of BH-3-HLAQ and MEG-LAQ peaks in the chromatograms of the standard injections in order to test the system precision according to relevant SOP's.

7. PROCEDURE

7.1 INJECTIONS SEQUENCE

Inject Resolution Solution, Blank – twice, QL solution (6 replicates), Standard solutions and Sample solutions, according to relevant SOP's.

7.2 PROCESSING OF CHROMATOGRAMS

Disregard all the peaks other than MEG-LAQ and BH-3-HLAQ in the injections of standard and sample solutions.

Determine the retention time (RT) and the areas of the peaks of MEG-LAQ and BH-3-HLAQ in the chromatograms of Sample solutions, for identification and quantitation.

Adjust integration parameters to reject peaks with area less than 10% of the average response of BH-3-HLAQ peak in the chromatograms of six successive injections of the first preparation of Standard Solution.

This corresponds to 0.02% (DL of BH-3-HLAQ) with respect to working concentration of Laquinimod.

8. CALCULATION AND REPORTING

8.1 CALCULATION

Quantitation of each of the impurities (MEG-LAQ and BH-3-HLAQ) is performed with respect to the corresponding peaks in the chromatograms of the Standard solutions.

Use the following equation to calculate the content of MEG-LAQ and BH-3-HLAQ:

$$\%\,\mathrm{BH\text{-}3\text{-}HLAQ/MEG\text{-}LAQ} = \frac{\mathrm{Area}_{Smp} \times Conc_{Std} \times V_{Smp} \times AvgWt_{CapsContent}}{Area_{Std} \times W_{Smp} \times \mathrm{Label\ Claim}} \times 100$$

AreaSmp : Area of MEG-LAQ or BH-3-HLAQ peak in the chromatograms of the Sample solutions

***AreaStd* :** Area of peak MEG-LAQ (average) or BH-3-HLAQ peak (average), respectively, in the chromatograms of Standard solutions

Label Claim : 0.6 mg

8.2 EVALUATION AND REPORTING

QL (Quantitation limit) of BH-3-HLAQ is 0.05%; QL of MEG-LAQ is 0.2%

DL (Detection limit) of BH-3-HLAQ is 0.02%; DL of MEG-LAQ is 0.1%

For the values equal or above QL, report the calculated result.

For the values below QL but above DL, report: "Less than 0.05% (QL)" for BH-3-HLAQ and "Less than 0.2% (QL)" for MEG-LAQ.

For the values below DL, report: "Less than 0.02% (DL)" for BH-3-HLAQ and "Less than 0.1% (DL)" for MEG-LAQ.

Figure 56 presents a typical chromatogram of resolution solution according to the above method.

Peak Results For Figure 56.

	Name	RT	RTT	Area	%Area	Int. Type	USP Resolution	Width (50%)	USP Tailing	Height (μV)
1	MEG-LAQ	9.650	0.66	63177	0.66	BB		1.01	0.56	1322
2	BH-3HLAQ	13.203	0.88	145888	1.16	BB	2.61	0.49	0.98	4661
3	Laq	14.962		12369065	86.18	BB	1.76	0.64	2.44	306644

Figure 57 presents a blank (diluents 2) chromatogram according to the above method.

Peak Results For Figure 57.

	Name	RT	Area	%Area	Int. Type
1	System peak	2.944	12.128	100.00	BB

Figure 58 presents a chromatogram of QL solution according to the above method.

Peak Results For Figure 58.

	Name	RT	Int. Type	Height (μV)	Area	%Area
1	Meg-LAQ	10.080	BB	303	15402	75.11
2	BH-3HLAQ	15.313	BB	201	5777	23.89

Figure 59 presents a chromatogram of standard solution according to the above method.

Peak Results For Figure 59.

	Name	RT	Int. Type	Height (μV)	Area	%Area
1	Meg-LAQ	10.177	BB	760	45319	54.89
2	BH-3HLAQ	15.293	BB	814	25059	35.11

Figure 60 presents a typical sample chromatogram according to the above method.

Peak Results For Figure 60.

	Name	RT	Int. Type	Area	%Area
1	Meg-LAQ	10.503	BB	20741	68.17
2	BH-3HLAQ	13.952	BB	9683	31.83

Example 24 – Computational Toxicology Evaluation of Potentially Genotoxic Impurities in Laquinimod Sodium Drug Substance and Intermediates

DEREK prediction software (DEREK Nexus, version 2.0.0, 2011i, LHASA Ltd, Leeds, UK) was used to evaluate the genotoxic potential of MCQ and MCQME. Computational toxicology evaluation is summarized in Table 47.

Table 47. Computational Toxicology Evaluation of MCQ and MCQME

Potential Impurity	Origin	Toxicophore	Endpoint	Probability[1]
MCQ	By-product and potential degradation product	Quinoline	Mutagenicity	Plausible
		AMES data negative in the pre-incubation method (CRL study No. 962723)		
MCQME	Intermediate in the synthesis and process related impurity in the DS	No structural alert		
		Weak genotoxic activity in the *in vitro* AMES test (CRL study No. 963892, but negative in the *in vivo* MN (Covance study No. 8246631)		

The DEREK definition of the likelihood level of a structure being toxic is as follows:

Plausible: The weight of evidence supports the proposition.

Equivocal: There is an equal qeight of evidence for and against the proposition.

Example 25 - Batch Analysis Of Laquinimod Sodium Drug Substance

A number of batches of laquinimod sodium drug substance were manufactured at various manufacturing facilities and subsequently analyzed. During manufacture and analysis, sufficient light protection of sample solution was provided.

All batches of drug substance were within specification as provided in

Example 17.

Table 48 and Table 49 shows batch analyses of laquinimod sodium drug substance batches. Table 50 shows batch analyses of laquinimod acid.

Table 48. **Analysis of Laquinimod Sodium Drug Substance Batches 1-12**

Batch No.	**Polar Impurities**							**NEA and Non-Polar Imp.**		
	MCQ	**MCQCA**	**Sum of MCQ & MCQCA**	**MCQME**	**MCQEE**	**Any Other Impurity**	**Total**	**NEA**	**Any Other Impurity**	**Total**
Analytical Method	Example 19							Example 20		
Specifications	**NMT 0.15**	**NMT 0.15**	NA	**NMT 0.12**	**NMT 0.10**	**NMT 0.10**	**NMT 1.00**	**NMT 0.10**	**NMT 0.10**	**NMT 0.50**
Batch 1	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05
Batch 2	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05	LT 0.05
Batch 3	LT 0.02	LT 0.03	NA	LT 0.02	LT 0.02	LT 0.02	LT 0.05	LT 0.02	LT 0.02	LT 0.05
Batch 4	LT 0.02	LT 0.03	NA	LT 0.02	LT 0.02	LT 0.02	LT 0.05	LT 0.02	LT 0.02	LT 0.05
Batch 5	LT 0.02	LT 0.03	NA	LT 0.02	LT 0.02	LT 0.02	LT 0.05	LT 0.02	LT 0.02	LT 0.06
Batch 6	LT 0.02	LT 0.03	NA	LT 0.02	LT 0.02	LT 0.02	LT 0.05	LT 0.02	LT 0.02	LT 0.06
Batch 7	LT 0.02	LT 0.03	NA	LT 0.02	LT 0.02	LT 0.02	LT 0.05	LT 0.02	LT 0.02	LT 0.06
Batch 8	LT 0.02	LT 0.03	NA	LT 0.02	LT 0.02	LT 0.02	LT 0.05	LT 0.02	LT 0.02	LT 0.06
Batch 9	LT 0.02	LT 0.03	NA	LT 0.02	LT 0.02	LT 0.02	LT 0.05	LT 0.02	LT 0.02	LT 0.02
Batch 10	LT 0.02	LT 0.03	NA	LT 0.02	LT 0.02	LT 0.02	LT 0.05	LT 0.02	LT 0.02	LT 0.02
Batch 11	LT 0.02	LT 0.03	NA	LT 0.02	LT 0.02	LT 0.02	LT 0.05	LT 0.02	LT 0.02	LT 0.02
Batch 12	LT 0.02	LT 0.03	NA	LT 0.02	LT 0.02	LT 0.02	LT 0.05	LT 0.02	LT 0.02	LT 0.02

QL And DL Values Of Impurities Listed In Table 48:

	Quantitation Limit	Detection Limit
MCQ	0.05%	0.02%
MCQCA	0.05%[1]	0.02%
MCQME	0.05%	0.02%
5-HLAQ	0.05%	0.02%
MCQEE	0.05%	0.02%
LAQ	0.2%	0.03%
NEA	0.06%	0.02%
DELAQ	Not applicable (limit test)	0.03%
DMM	Not applicable (limit test)	0.03%
DMS	Not applicable (limit test)	0.5 ppm
Acetone	250 ppm	25 ppm
Dioxane	20 ppm	10 ppm
Ethanol	25 ppm	5 ppm
n-Heptane	250 ppm	10 ppm
Methanol	150 ppm	30 ppm
n-Octane	100 ppm	10 ppm
DMF	190 ppm	50 ppm

[1] Reporting limit 0.10%.

Table 49. Analysis of Additional Laquinimod Sodium Drug Substance Batches 13-18

Batch No.	Related Substances [%]			
	Any individual impurity			Sum of imp.
Specifications	≤ 1.0			≤ 2.0
	MCQ	MCQCA	MCQME	
13	Any single impurity <0.05			
14	Any single impurity <0.05			<0.05
15	0.03	0.03	0.03	0.13
16	-	-	0.04	0.17
17	0.01	-	0.01	0.13
18	-	-	0.05	0.14

Table 50. Analyses of Laquinimod (Acid Form) Drug Substance Batches (Batches 19-22)

Batch No.	NEA [%]	Related Substances [%]				
		MCQ	MCQCA	MCQME	Any Unknown	Total
19	-	0.75	-	-	1.2	1.95
20	-	-	-	0.24[4]	0.05[4] 0.05[4] 0.07[4] 0.14[4]	0.55
21	-	-	-	0.26[4]	0.05[4] 0.05[4] 0.07[4] 0.13[4]	0.56
22	<0.05	0.19				<0.5

[4] Deviation from method. Different mobile phase and wavelength used.

Example 26 - Observed Impurities At Release And After Long-Term And Accerated Storage Of Laquinimod Drug Substance

Impurities were observed in laquinimod sodium batches at release and after long-term and accelerated storage.

The ranges of impurity levels observed in the laquinimod sodium batches, including the primary stability and validation batches, are summarized in Table 51.

Table 51. Ranges of Impurity Levels Observed in Laquinimod Drug Substance Batches upon Release and after Long-Term and Accelerated Storage

Parameter	Current Specification	Range of Observed Impurity Levels
MCQ	NMT 0.15%	LT 0.02-LT 0.05%
MCQCA	NMT 0.15%	LT 0.03-LT 0.05%
MCQME	NMT 0.12%	LT 0.02-LT 0.05%
5-HLAQ	NMT 0.10%	LT 0.02-LT 0.05%
MCQEE	NMT 0.10%	LT 0.02-LT 0.05%
LAQ	NMT 1.0%	LT 0.025-LT 0.2%
NEA	NMT 0.10%	LT 0.02-LT 0.05%
DELAQ	NMT 0.1%	LT 0.05-LT 0.1%
DMM	NMT 0.1%	LT 0.05-LT 0.1%
DMS	NMT 1 ppm	LT 1 ppm
Ethanol	NMT 5000 ppm	LT 5-LT 25 ppm
n-Heptane	NMT 5000 ppm	LT 10-LT 250 ppm
Methanol	NMT 3000 ppm	LT 30-LT 150 ppm
Dioxane	NMT 380 ppm	LT 10 ppm
DMF	NMT 880 ppm	LT 40-93 ppm
Acetone	NMT 5000 ppm	LT 250 ppm
n-Octane	NMT 2000 ppm	LT 10-LT 20 ppm
Heavy Metals	NMT 20 ppm	NMT 20 ppm
Free chloride	NMT 0.1%	LT 0.1%

Example 27 - Batch Analysis Of Laquinimod Sodium Drug Product

A number of batches of laquinimod sodium drug product were manufactured at various manufacturing facilities and subsequently analyzed. During manufacture and analysis, sufficient light protection of sample solution was provided.

All batches of drug product were within specification.

Evaluation of the release data shows that all batches have been within the specifications in effect at the time of their release. Furthermore it can be seen that all manufactured batches of the product proposed for commercialization, e.g., laquinimod 0.6 mg capsules, have shown compliance with the proposed commercial specifications. Batch-to-batch consistency over time was found in the results for all the measured parameters. The batch analysis results demonstrate that the quality of all batches does not differ significantly from each other and that production at different manufacturing sites affords material of similar quality.

Table 52, Table 53, Table 54, Table 55, Table 55, Table 56 and Table 57 relate to batch analyses of laquinimiod sodium drug product batches.

Table 52. Batch Analysis for Laquinimod 0.6 mg Capsules Batches 23-31

Batch Number	Polar Impurities/Degradation Products [%]			Non Polar Impurities/Degradation Products [%]			BH-3-HLAQ [%]
	Sum of MCQ & MCQCA	Any Other	Total Imp.	NEA	Any Other	Total Imp.	
Current Method	Example 21			Example 22			Example 23
Current Specifications	**NMT 0.5**	**NMT 0.5**	**NMT 2.0**	**NMT 0.5**	**NMT 0.5**	**NMT 1.0**	**NMT 1.0**
Batch 23	<0.03	<0.02	<0.05	<0.03	<0.03	<0.1	<0.02[5]
Batch 24	<0.03	<0.02	<0.05	<0.03	<0.03	<0.1	<0.02[5]
Batch 25	<0.03	<0.02	<0.05	<0.03	<0.03	<0.1	<0.02[5]
Batch 26	<0.03	<0.02	<0.05	<0.03	<0.03	<0.1	<0.02[5]
Batch 27	<0.03	<0.02	<0.05	<0.03	<0.03	<0.1	<0.02[5] 0.06[5,6]
Batch 28	<0.03	<0.05	<0.05	<0.03	<0.03	<0.1	<0.02[5]
Batch 29	<0.03	<0.02	<0.05	<0.03	<0.03	<0.1	<0.02[5]
Batch 30	<0.03	<0.05	<0.05	<0.03	<0.03	<0.1	<0.02[5]
Batch 31	<0.03	<0.05	<0.05	<0.03	<0.03	<0.1	<0.02[5]

[4] The analysis was performed using the previous method for determination of MEG-LAQ.

[5] The test for BH-3-HLAQ was introduced after the release of these batches. The listed results were obtained for BH-3-HLAQ at the last available stability timepoint at 25°C/60%RH (18 months for batch 23 and batches 27-30, 9 months for batches 24-26 and 3 months for batch 31) for all packaging configurations, with one exception (see footnote 6).

[6] For batch 27 in Alu/Alu blisters a result of 0.06% was obtained.

Current QL and DL values for Table 52:

	Quantitation Limit	Detection Limit
Sum of MCQ and MCQCA	0.1%	0.03%
5-HLAQ	0.05%	0.02%
NEA[1]	0.1%	0.03%
3-HLAQ[1]	0.1%	0.03%
MEG-LAQ	0.2%	0.1%
BH-3-HLAQ	0.05%	0.02%

[1] NEA and 3-HLAQ were omitted from the commercial specifications since they were never observed upon batch release and stability testing completed to date at all tested storage conditions. The monitoring of these impurities in the primary stability batches will continue until the end of the stability program, using the listed method.

Table 53. Batch Analyses for Laquinimod 0.6 mg Capsules (Batches 32-38)

Batch Number	Polar Impurities/Degradation Products [%]			Non Polar Impurities/Degradation Products [%]			BH-3-HLAQ [%]
	Sum of MCQ & MCQCA	Any Other	Total Impurities	NEA	Any Other	Total Imp.	
Current Specifications	**NMT 0.5**	**NMT 0.5**	**NMT 2.0**	**NMT 0.5**	**NMT 0.5**	**NMT 1.0**	**NMT 1.0**
Batch 32	<0.03	MCQME: <0.02 MCQEE: <0.02	<0.05	<0.03	<0.03	<0.1	NA[6]
Batch 33	<0.03	MCQME: <0.02 MCQEE: <0.02	<0.05	<0.03	<0.03	<0.1	NA[6]
Batch 34	<0.03	MCQME: <0.02 MCQEE: <0.02	<0.05	<0.03	<0.03	<0.1	NA[6]
Batch 35	<0.03	MCQME: <0.02 MCQEE: <0.02	<0.05	<0.03	<0.03	<0.1	NA[6]
Batch 36	<0.03	MCQME: <0.02 MCQEE: <0.02	<0.05	<0.03	<0.03	<0.1	NA[6]
Batch 37	<0.03	MCQME: <0.02 MCQEE: <0.02 RRT 0.23: 0.05	0.05	<0.03	<0.03	<0.1	NA[6]
Batch 38	<0.03	MCQME: <0.02 MCQEE: <0.02	<0.05	<0.03	<0.03	<0.1	NA[6]

[6] Determination of BH-3-HLAQ and water content were introduced in the specifications after the release of this batch.

Current QL and DL values of Table 53:

	Quantitation Limit	**Detection Limit**
Sum of MCQ and MCQCA	0.1%	0.03%
5-HLAQ	0.05%	0.02%
NEA[1]	0.1%	0.03%
3-HLAQ[1]	0.1%	0.03%
MEG-LAQ	0.2%	0.1%
BH-3-HLAQ	0.05%	0.02%

[1] NEA and 3-HLAQ were omitted from the commercial specifications since they were never observed upon release and during the stability testing completed to date at all tested storage conditions. The monitoring of these impurities in the primary stability batches will continue until the end of the stability program, using the listed method.

Table 54. Batch Analyses for Laquinimod Capsules 0.3 mg (Batches 39-42)

Batch Number	Polar Impurities/Degradation Products [%]				Non Polar Impurities/Degradation Products [%]		
	Sum of MCQ & MCQCA	MCQEE	Any Other	Total Impurities	NEA	Any Other	Total Impurities
Current Specifications	**NMT 0.5**	NA	**NMT 0.5**	**NMT 2.0**	**NMT 0.5**	**NMT 0.5**	**NMT 1.0**
Batch 39	MCQ: <0.02 MCQCA: <0.03	<0.02	RRT 0.35: <0.05	<0.05	<0.02	<0.02	<0.05
Batch 40	<0.03	NA	MCQME: <0.02 MCQEE: <0.02	<0.05	<0.03	<0.03	<0.1
Batch 41	<0.03	NA	MCQME: <0.02 MCQEE: <0.02	<0.05	<0.03	<0.03	<0.1
Batch 42	<0.03	NA	MCQME: <0.02 MCQEE: <0.02	<0.05	<0.03	<0.03	<0.1

Table 55. Batch Analyses for Laquinimod Capsules 0.3 mg (Batch 43)

Batch Number	Strength (Formula)	Polar Impurities/Degradation Products [%]					Non Polar Impurities/Degradation Products [%]		
		MCQ	MCQEE	MCQCA	Any Other	Total Impurities	NEA	Any Other	Total Impurities
Specifications		**NMT 0.5**	**NMT 0.5**	**NMT 0.5**	**NMT 0.5**	**NMT 2.0**	**NMT 0.5**	**NMT 0.5**	**NMT 1.0**
Batch 43	`0.3 mg (III)`	<0.02	<0.02	<0.03	RRT 0.35: <0.05 RRT 0.18: 0.09 RRT 0.24: 0.08	0.3	<0.02	RRT 2.30: 0.09	0.1

Table 56. Batch Analyses for Laquinimod Tablets 0.05 mg, 0.25 mg and 0.3 mg (Batches 44-49)

Batch Number	Strength	Related Substances [%]				
		NEA	MCQ & MCQCA	MCQE E	Any unknown	Sum of Imp.
Last Effective Specifications		**NMT 0.1**	**NMT 0.5**	**NMT 0.1**	**NMT 0.2**	**NMT 1.5**
Batch 44	0.05 mg	<0.0080	Each <1.0	Each <1.0	Each <1.0	<0.10
Batch 45	0.25 mg	0.009	Each <1.0	Each <1.0	Each <1.0	<0.10
Batch 46	0.3 mg	0.031	0.22	<0.02	Largest <0.03	0.22[4]
Batch 47	0.3 mg	0.04	0.05	<0.020	Conforms	0.05
Batch 48	0.3 mg	0.03	0.05	<0.020	Conforms	0.05
Batch 49	0.3 mg	0.016	0.17	<0.020	<0.030	0.20

[4] An additional specified impurities were reported: 0.03% of MCQME.

Table 57. Batch Analysis for Laquinimod Oral Solution 1 mg/mL (Batch 50)

Batch Number	Strength	Related Substances [%]	N-Ethylaniline [%]
Specifications		**NMT 4.0**	**NMT 0.1**
Batch 50	1 mg/mL	<4.0	<0.01

References

1. U.S. Patent No. 6,077,851;
2. U.S. Patent No. 6,875,869;
3. U.S. Patent No. 7,560,557;
4. U.S. Patent No. 7,589,208;
5. U.S. Patent No. 7,884,208;
6. U.S. Patent No. 7,989,473;
7. U.S. Patent No. 8,178,127;
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What is Claimed:

1. A mixture of crystalline laquinimod sodium particles, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less or (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and wherein:

   a. the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL;

   b. the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL;

   c. an amount of heavy metal which is present in the mixture is no more than 20 ppm of heavy metal relative to the amount by weight of laquinimod sodium;

   d. an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

   e. an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

   f. an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC;

   g. an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

   h. an amount of N-ethylaniline (NEA) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC; or

   i. an amount of *N*-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide (5-HLAQ) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC.

2. The mixture of claim 1, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 40 microns or (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 15 microns.

3. The mixture of claim 1 or 2, wherein 10% or more of the total amount by volume of the laquinimod sodium particles have a size of 5 microns or less and wherein:

   a. the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL; or

   b. an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC.

4. A mixture of crystalline laquinimod sodium particles, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 40 microns, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 15 microns, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 5 microns and wherein:

   a. the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL;

   b. the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL;

   c. an amount of heavy metal which is present in the mixture is no more than 20 ppm of heavy metal relative to the amount by weight of laquinimod sodium;

   d. an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

   e. an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) which is present in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

   f. an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC;

   g. an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

   h. an amount of N-ethylaniline (NEA) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC; or

   i. an amount of *N*-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide (5-HLAQ) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC.

5. The mixture of any one of claims 1-4 prepared in a single batch comprising 2.5 kg or more of laquinimod sodium.

6. The mixture of any one of claims 1-5, wherein the laquinimod sodium particles are determined based on an unmilled sample of the mixture or wherein the size and amount by volume of laquinimod sodium particles are determined based on a milled sample of the mixture.

7. The mixture of any one of claims 1-6, having a bulk density of 0.2 g/mL to 0.4 g/mL, and/or having a tapped density of 0.40 g/mL to 0.7 g/mL.

8. The mixture of any one of claims 1-7, wherein:

   a. an amount of aluminum in the mixture is less than 5 ppm or less than 2 ppm relative to the amount by weight of laquinimod sodium;

   b. an amount of calcium in the mixture is less than 60 ppm or less than 25 ppm relative to the amount by weight of laquinimod sodium;

   c. an amount of calcium in the mixture is less than 25 ppm relative to the amount by weight of laquinimod sodium;

   d. an amount of copper in the mixture is less than 1 ppm or less than 0.6 ppmrelative to the amount by weight of laquinimod sodium; and/or

   e. an amount of zinc in the mixture is less than 7 ppm or less than 4 ppm relative to the amount by weight of laquinimod sodium.

9. The mixture of any one of claims 1-8, wherein an amount of heavy metal in the mixture is no more than 20 ppm relative to the amount by weight of laquinimod sodium.

10. The mixture of any one of claims 1-9, wherein a total amount of polar impurities in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC.

11. The mixture of any one of claims 1-10, wherein:

a. an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

b. an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

c. an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC;

d. an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) which is present in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC; and/or

e. an amount of ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC.

12. The mixture of any one of claims 1-11, wherein a total amount of non-polar impurities in the mixture is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC.

13. The mixture of any one of claims 1-12, wherein:

a. an amount of N-ethylaniline (NEA) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

b. an amount of *N*-ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide (5-HLAQ) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC; and/or

c. an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide (DELAQ) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC.

14. The mixture of any one of claims 1-13, wherein:

a. an amount of laquinimod acid in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

b. an amount of dimethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

c. an amount of diethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

d. an amount of dimethyl sulfate in the mixture is no more than 1 ppm relative to the amount by weight of laquinimod sodium;

e. an amount of water in the mixture is no more than 1.5% by weight relative to the amount of laquinimod sodium as measured by K.F. coulometric titration; and/or

f. an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium.

15. The mixture of any one of claims 1-14, wherein:

a. an amount of ethanol in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

b. an amount of n-heptane in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

c. an amount of n-octane in the mixture is no more than 2000 ppm relative to the amount by weight of laquinimod sodium;

d. an amount of n-octane in the mixture is no more than 200 ppm relative to the amount by weight of laquinimod sodium;

e. an amount of n-octane in the mixture is no more than 20 ppm relative to the amount by weight of laquinimod sodium;

f. an amount of methanol in the mixture is no more than 3000 ppm relative to the amount by weight of laquinimod sodium;

g. an amount of acetone in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

h. an amount of dioxane in the mixture is no more than 380 ppm relative to the amount by weight of laquinimod sodium; and/or

i. an amount of dimethyl formamide in the mixture is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

16. A pharmaceutical composition comprising the mixture of any one of claims 1-15 and a pharmaceutically acceptable carrier.

17. The pharmaceutical composition of claim 16, wherein;

a. a total amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

b. a total amount of polar impurities in the pharmaceutical composition is no more than 2.00% relative to the amount of laquinimod sodium as measured by HPLC;

c. an amount of N-ethylaniline (NEA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

d. an amount of 5-chloro-*N*-ethyl-3-hydroxy-1-methyl-2,4-dioxo-*N*-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide (3-HLAQ) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

e. a total amount of non-polar impurities in the pharmaceutical composition is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC; and/or

f. an amount of N-ethyl-4-hydroxy-1-methyl-5-(methyl(2,3,4,5,6-pentahydroxyhexyl)amino)-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide (MEG-LAQ) in the pharmaceutical composition is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC.

18. The pharmaceutical composition of claims 16 or 17, wherein:

a. an amount of water in the pharmaceutical composition is no more than 1.50% relative to the amount of laquinimod sodium as measured by K.F. coulometric titration;

b. an amount of water in the pharmaceutical composition is no more than 0.80% relative to the amount of laquinimod sodium as measured by K.F. coulometric titration; and/or

c. the pharmaceutical composition comprises an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium.

19. The pharmaceutical composition of any one of claims 16-18, wherein:

a. an amount of ethanol in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

b. an amount of n-heptane in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

c. an amount of n-octane in the pharmaceutical composition is no more than 2000 ppm relative to the amount by weight of laquinimod sodium;

d. an amount of n-octane in the pharmaceutical composition is no more than 200 ppm relative to the amount by weight of laquinimod sodium;

e. an amount of n-octane in the pharmaceutical composition is no more than 20 ppm relative to the amount by weight of laquinimod sodium;

f. an amount of methanol in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium;

g. an amount of acetone in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium;

h. an amount of dioxane in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium; and/or

i. an amount of dimethyl formamide in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

20. A method of treating a subject afflicted with, or allevaitaing a symptom of, a form of multiple sclerosis, lupus nephritis, lupus arthritis, rheumatoid arthritis, a BDNF-related disorder, Crohn's disease, a GABA-related disorder, a cannabinoid receptor type 1 (CB1) mediated disorder, or an ocular inflammatory disorder comprising administering to the subject the

mixture of any one of claims 1-15 or the pharmaceutical composition of any one of claims 16-20 so as to thereby treat the subject or alleviate the symptom in the subject.

21. The mixture of any one of claims 1-15 or the pharmaceutical composition of any one of claims 16-19 for use in the treatment of, or alleviation of symptoms of, a form of multiple sclerosis, lupus nephritis, lupus arthritis, rheumatoid arthritis, a BDNF-related disorder, Crohn's disease, a GABA-related disorder, a cannabinoid receptor type 1 (CB1) mediated disorder, or an ocular inflammatory disorder.

22. Use of the mixture of any one of claims 1-15 or the pharmaceutical composition of any one of claims 16-19 for the manufacture of a medicament for treating, or alleviating a symptom of, a form of multiple sclerosis, a GABA-related disorder, a cannabinoid receptor type 1 (CB1) mediated disorder, lupus nephritis, lupus arthritis, rheumatoid arthritis, a BDNF-related disorder, Crohn's disease, or an ocular inflammatory disorder.

23. A process of recrystallization of laquinimod sodium comprising:

    a. dissolving an amount of laquinimod sodium in water to form an aqueous solution;

    b. concentrating the aqueous solution to form a concentrated solution comprising approximately 1.7-1.8 mL of water per gram of laquinimod sodium;

    c. adding acetone to the concentrated solution of step b); and

    d. isolating recrystallized laquinimod sodium.

24. The process of claim 23, wherein:

    a. the amount of laquinimod sodium in step a) is 2.5 kg or greater;

    b. step a) is performed with 10-12 mL of water per gram of laquinimod sodium, preferably step a) is performed with approximately 11 mL of water per gram of laquinimod sodium; and/or

    c. step a) is performed by heating the aqueous solution to a temperature of 58-75°C, or wherein step a) is performed by heating the aqueous solution to a temperature of 60-73°C.

25. The process of claims 23 or 24, wherein crystallization occurs after step a) and before step c), preferably wherein crystaliztion:

a. is induced by rapid stirring during or after the concentrating step b);

b. is induced by addition of a seed crystal during or after the concentrating step b); or

c. occurs without addition of a seed crystal.

26. The process of any one of claims 23-25, wherein:

a. step b) is performed under conditions appropriate to induce crystallization at the concentration of 1.7-1.8 mL of water per gram of laquinimod sodium; and/or

b. step b) is performed at 28-45°C or at 30-40°C.

27. The process of any one of claims 23-26, wherein:

a. step c) is performed with the concentrated solution at 40-55°C or at 45-50°C;

b. step c) is performed with 6-12 mL of acetone per gram of laquinimod sodium, preferably step c) is performed with approximately 10 mL of acetone per gram of laquinimod sodium;

c. step c) is performed over a period of 1-4 hours or over a period of 1.2-2.5 hours; and/or

d. step c) is followed by cooling the solution to a temperature no less than -14°C and no more than 6°C or wherein step c) is followed by cooling the solution to a temperature no less than -4°C and no more than 4°C.

28. The process of any one of claims 23-27, wherein the solution is cooled over a period of 3-5 hours or over a period of 3.5-4.5 hours.

29. The process of any one of claims 23-28, wherein

a. step d) further comprises washing the recrystallized laquinimod sodium with 1-4 mL of acetone per gram of crude laquinimod sodium used in step a), preferably step d) further comprises washing the recrystallized laquinimod sodium with approximately 3 mL of acetone per gram of crude laquinimod sodium used in step a);

b. step d) further comprises drying the recrystallized laquinimod sodium for no less than one hour at 30-40°C under a vacuum of no more than 50 mmHg; and/or

c. the isolated recrystallized laquinimod sodium in step d) is a mixture of crystalline laquinimod sodium particles having a particle size distribution such that (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of less than 5 microns or less.

30. A mixture of crystalline laquinimod sodium particles prepared by any one of claims 23-29.

31. The mixture of claim 30, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less, (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and (iii) 10% or more of the total amount by volume of the laquinimod sodium particles have a size of 5 microns or less.

32. The mixture of claim 30 or 31, wherein:

a. the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL;

b. the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL;

c. an amount of aluminum in the mixture is less than 5 ppm relative to the amount by weight of laquinimod sodium;

d. an amount of calcium in the mixture is less than 60 ppm relative to the amount by weight of laquinimod sodium;

e. an amount of copper in the mixture is less than 1 ppm relative to the amount by weight of laquinimod sodium;

f. an amount of zinc in the mixture is less than 7 ppm relative to the amount by weight of laquinimod sodium;

g. an amount of heavy metal in the mixture is no more than 20 ppm relative to the amount by weight of laquinimod sodium;

h. a total amount of polar impurities in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

i. an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

j. an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

k. an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC;

l. an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

m. an amount of ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

n. a total amount of non-polar impurities in the mixture is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

o. an amount of N-ethylaniline (NEA) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

p. an amount of laquinimod acid in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

q. an amount of dimethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

r. an amount of diethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

s. an amount of dimethyl sulfate in the mixture is no more than 1 ppm relative to the amount by weight of laquinimod sodium;

t. an amount of water in the mixture is no more than 1.5% by weight relative to the amount of laquinimod sodium as measured by K.F. coulometric titration;

u. the mixture comprises an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium;

v. an amount of ethanol in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

w. an amount of n-heptane in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

x. an amount of n-octane in the mixture is no more than 2000 ppm relative to the amount by weight of laquinimod sodium;

y. an amount of n-octane in the mixture is no more than 200 ppm relative to the amount by weight of laquinimod sodium;

z. an amount of n-octane in the mixture is no more than 20 ppm relative to the amount by weight of laquinimod sodium;

aa. an amount of methanol in the mixture is no more than 3000 ppm relative to the amount by weight of laquinimod sodium;

bb. an amount of acetone in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

cc. an amount of dioxane in the mixture is no more than 380 ppm relative to the amount by weight of laquinimod sodium; or

dd. an amount of dimethyl formamide in the mixture is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

33. The mixture of claim 30 or 31, wherein:

a. the mixture has a bulk density of 0.2 g/mL to 0.4 g/mL;

b. the mixture has a tapped density of 0.40 g/mL to 0.7 g/mL;

c. an amount of aluminum in the mixture is less than 5 ppm relative to the amount by weight of laquinimod sodium;

d. an amount of calcium in the mixture is less than 60 ppm relative to the amount by weight of laquinimod sodium;

e. an amount of copper in the mixture is less than 1 ppm relative to the amount by weight of laquinimod sodium;

f. an amount of zinc in the mixture is less than 7 ppm relative to the amount by weight of laquinimod sodium;

g. an amount of heavy metal in the mixture is no more than 20 ppm relative to the amount by weight of laquinimod sodium;

h. a total amount of polar impurities in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

i. an amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

j. an amount of 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the mixture is no more than 0.15% relative to the amount of laquinimod sodium as measured by HPLC;

k. an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.12% relative to the amount of laquinimod sodium as measured by HPLC;

l. an amount of methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

m. an amount of ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

n. a total amount of non-polar impurities in the mixture is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

o. an amount of N-ethylaniline (NEA) in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

p. an amount of laquinimod acid in the mixture is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

q. an amount of dimethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

r. an amount of diethyl malonate in the mixture is no more than 0.10% relative to the amount of laquinimod sodium as measured by HPLC;

s. an amount of dimethyl sulfate in the mixture is no more than 1 ppm relative to the amount by weight of laquinimod sodium;

t. an amount of water in the mixture is no more than 1.5% by weight relative to the amount of laquinimod sodium as measured by K.F. coulometric titration;

u. the mixture comprises an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium;

v. an amount of ethanol in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

w. an amount of n-heptane in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

x. an amount of n-octane in the mixture is no more than 2000 ppm, 200 ppm or 20 ppm relative to the amount by weight of laquinimod sodium;

y. an amount of methanol in the mixture is no more than 3000 ppm relative to the amount by weight of laquinimod sodium;

z. an amount of acetone in the mixture is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

aa. an amount of dioxane in the mixture is no more than 380 ppm relative to the amount by weight of laquinimod sodium; and

bb. an amount of dimethyl formamide in the mixture is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

34. A pharmaceutical composition comprising the mixture of any one of claims 30-33 and a pharmaceutically acceptable carrier.

35. The pharmaceutical composition of claim 34, wherein:

a. a total amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

b. a total amount of polar impurities in the pharmaceutical composition is no more than 2.00% relative to the amount of laquinimod sodium as measured by HPLC;

c. an amount of N-ethylaniline (NEA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

d. a total amount of non-polar impurities in the pharmaceutical composition is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

e. an amount of water in the pharmaceutical composition is no more than 0.80% relative to the amount of laquinimod sodium as measured by K.F. coulometric titration;

f. the pharmaceutical composition comprises an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium;

g. an amount of ethanol in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

h. an amount of n-heptane in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

i. an amount of n-octane in the pharmaceutical composition is no more than 2000 ppm relative to the amount by weight of laquinimod sodium;

j. an amount of n-octane in the pharmaceutical composition is no more than 200 ppm relative to the amount by weight of laquinimod sodium;

k. an amount of n-octane in the pharmaceutical composition is no more than 20 ppm relative to the amount by weight of laquinimod sodium;

l. an amount of methanol in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium;

m. an amount of acetone in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium;

n. an amount of dioxane in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium; or

o. an amount of dimethyl formamide in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

36. The pharmaceutical composition of claim 34, comprising:

a. a total amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

b. a total amount of polar impurities in the pharmaceutical composition is no more than 2.00% relative to the amount of laquinimod sodium as measured by HPLC;

c. an amount of N-ethylaniline (NEA) in the pharmaceutical composition is no more than 0.50% relative to the amount of laquinimod sodium as measured by HPLC;

d. a total amount of non-polar impurities in the pharmaceutical composition is no more than 1.00% relative to the amount of laquinimod sodium as measured by HPLC;

e. an amount of water in the pharmaceutical composition is no more than 0.80% relative to the amount of laquinimod sodium as measured by K.F. coulometric titration;

f. the pharmaceutical composition comprises an amount of sodium from 5.8% to 6.4% relative to the amount by weight of laquinimod sodium;

g. an amount of ethanol in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

h. an amount of n-heptane in the pharmaceutical composition is no more than 5000 ppm relative to the amount by weight of laquinimod sodium;

i. an amount of n-octane in the pharmaceutical composition is no more than 2000 ppm, 200 ppm or 20 ppm relative to the amount by weight of laquinimod sodium;an amount of methanol in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium;

j. an amount of acetone in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium;

k. an amount of dioxane in the pharmaceutical composition is no more than 380 ppm relative to the amount by weight of laquinimod sodium; and

l. an amount of dimethyl formamide in the pharmaceutical composition is no more than 880 ppm relative to the amount by weight of laquinimod sodium.

37. A mixture of crystalline laquinimod sodium particles, wherein (i) 90% or more of the total amount by volume of the laquinimod sodium particles have a size of 40 microns or less or (ii) 50% or more of the total amount by volume of the laquinimod sodium particles have a size of 15 microns or less, and wherein

a. an amount of aluminium in the mixture is less than 5 ppm relative to the amount by weight of laquinimod sodium;

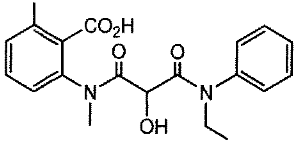
b. an amount of calcium in the mixture is less than 60 ppm relative to the amount by weight of laquinimod sodium;

c. an amount of copper in the mixture is less than 1 ppm relative to the amount by weight of laquinimod sodium; or

d. an amount of zinc in the mixture is less than 7 ppm relative to the amount by weight of laquinimod sodium.

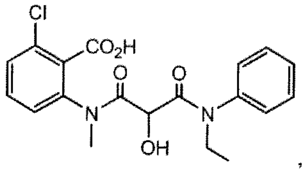
38. A pharmaceutical composition comprising the mixture of claim 37 and a pharmaceutically acceptable carrier.

39. An isolated compound having the structure:



, or a salt thereof.

40. A composition comprising a compound having the structure:



or a salt thereof, wherein the composition is free of laquinimod or a salt thereof.

41. A pharmaceutical composition comprising an amount of laquinimod and at least one of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE), wherein

a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

d) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

e) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

f) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount not more than 10%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

42. The pharmaceutical composition of claim 41, wherein

a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

43. The pharmaceutical composition of claim 42, wherein methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.12%, relative to the concentration of laquinimod, based on a determination by an HPLC method; and/or wherein methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

44. The pharmaceutical composition of any one of claims 41-43, wherein

a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an less than 0.06%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

45. The pharmaceutical composition of claim 44, wherein 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

46. The pharmaceutical composition of claims 44 or 45, wherein

a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

47. The pharmaceutical composition of claim 46, wherein 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

48. The pharmaceutical composition of claim 42, wherein

a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount greater than 0.02%, and not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.02%, and not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount greater than 0.02%, and not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.02%, and not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount greater than 0.02%, and not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

49. The pharmaceutical composition of claim 48, wherein

a. methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.12%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or wherein methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, and/or

b. 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

50. The pharmaceutical composition of of claims 48 or 49, wherein

a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount greater than 0.05%, and not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.05%, or greater than 0.10%, and not more than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount greater than 0.05%, and less than 0.15%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.06%, and not more than 0.10%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount greater than 0.05%, and not more than 0.10%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

51. The pharmaceutical composition of claim 50, wherein methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount not more than 0.12%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

52. The pharmaceutical composition of claims 48 or 49, wherein

a) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.02%, or greater than 0.03%, and less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.02%, and less than 0.06%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

e) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount greater than

0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

53. The pharmaceutical composition of claim 52, wherein 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

54. The pharmaceutical composition of claim 41, further comprising at least one pharmaceutically acceptable carrier.

55. The pharmaceutical composition of claim 54, wherein

a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount not more than 1.0%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

56. The pharmaceutical composition of claim 55, wherein N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

57. The pharmaceutical composition of claims 55 or 56, wherein

a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

d) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

e) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

58. The pharmaceutical composition of claim 57, wherein 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

59. The pharmaceutical composition of claims 57 or 58, wherein

a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

d) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

e) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an less than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

f) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

g) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is less than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

60. The pharmaceutical composition of claim 59, wherein 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount less than 0.02%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

61. The pharmaceutical composition of claim 54, wherein

a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount greater than 0.02%, and not more than 1.0%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.03%, and not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is greater than 0.03%, and not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

62. The pharmaceutical composition of claim 61, wherein N-Ethylaniline (NEA) is present in the pharmaceutical composition in an amount not more than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

63. The pharmaceutical composition of claim 54, wherein

a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount greater than 0.05%, and not more than 1.0%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.1%, and not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is greater than 0.1%, and not more than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

64. The pharmaceutical composition of claim 54, wherein

a) 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

b) 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

c) 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than

0.02%, and less than 0.5%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

d) Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

e) N-Ethylaniline (NEA) is present in the pharmaceutical composition in an greater than 0.03%, and less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

f) Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) is present in the pharmaceutical composition in an amount greater than 0.02%, and less than 0.05%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or

g) the combined amount of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) present in the pharmaceutical composition is greater than 0.03%, and less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

65. The pharmaceutical composition of claim 64, wherein 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) is present in the pharmaceutical composition in an amount greater than 0.03%, relative to the concentration of laquinimod, based on a determination by an HPLC method, or is present in the pharmaceutical composition in an amount less than 0.1%, relative to the concentration of laquinimod, based on a determination by an HPLC method.

66. The pharmaceutical composition of any one of claims 41-65, comprising laquinimod sodium salt.

67. The pharmaceutical composition of any one of claims 54-66, in a oral unit dosage form, or in the form of a capsule, a tablet, or a liquid suspension.

68. The pharmaceutical composition of claims 67, wherein the oral unit dosage form comprises more than 0.3 mg laquinimod, more than 0.5 mg laquinimod or more than 0.6 mg laquinimod.

69. A process for preparing 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) comprising the steps of:

    a) adding sodium hydroxide solution to a suspension of 5-chloro-N-ethyl-3-hydroxy-1-methyl-2,4-dioxo-N-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide in water,

    b) stirring the mixture of step a) followed by addition of hydrochloric acid solution,

    c) extracting the aqueous solution with ethyl acetate,

    d) washing the organic phase with brine,

    e) drying the organic phase over sodium sulfate,

    f) filtering the suspension,

    g) evaporating the filtrate,

    h) purifying the residue by crystallization from isopropyl alcohol,

    i) cooling the suspension followed by filtering and washing with isopropyl alcohol, and

    j) obtaining and drying the resulting white solid.

70. 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) prepared by the process of claim 69.

71. A process for preparing 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) comprising the steps of:

    a) heating a mixture of 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) and dimethylsulfoxide,

    b) cooling the mixture of step a), and

    c) filtering the mixture of step b) and collecting the resulting filtrate.

72. 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) prepared by the process of claim 71.

73. A process for preparing 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) comprising the steps of:

    a) heating a mixture of ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) in a solution of hydrochloric acid in acetic acid,

    b) cooling the mixture of step a),

    c) diluting the mixture of step b) with 2-propanol and further cooling the diluted mixture, and

    d) filtering off the crystals resulting from step c).

74. 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) prepared by the process of claim 73.

75. A process for preparing Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) comprising the steps of:

    a) forming sodium dimethylmalonate by reaction of dimethylmalonate in dimethlformamide with sodium methoxide solution,

    b) reacting the intermediate 5-chloro-1-methyl-1h-benzo[D][1,3] oxazine-2,4-dione with sodium dimethylmalonate to form methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) sodium salt, and

    c) acidifying methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) sodium salt to methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME).

76. Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) prepared by the process of claim 75.

77. A process for preparing Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) comprising the steps of:

    a) adding sodium hydride to a solution of 5-chloro-1-methyl-1h-benzo[D][1,3] oxazine-2,4-dione and diethyl malonate in dimethylformamide,

    b) heating the mixture of step a) while stirring,

c) cooling the solution of step b),

d) quenching the reaction mixture of step c),

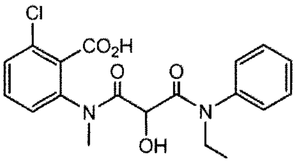
e) acidifying the mixture of step d),

f) filtering then drying the mixture of step e) , and

g) crystallizing the crude product of step f) by dissolving in ethanol following by slow cooling.

78. Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) prepared by the process of claim 77.

79. A process for testing whether a sample of laquinimod contains an undesirable impurity which comprises determining whether the sample contains a compound having the structure:



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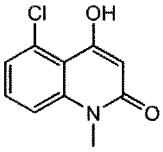

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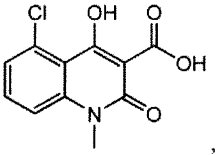

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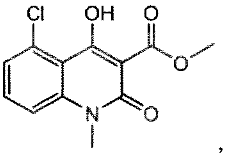

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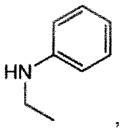

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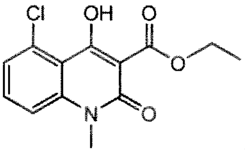

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80. A process for preparing a validated pharmaceutical composition comprising laquinimod comprising:

a) obtaining a batch of laquinimod;

b) determining the amount of at least one of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in the batch using by an HPLC method; and

c) preparing the pharmaceutical composition from the batch only if

i) the batch is determined to have not more than 0.15% 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), relative to the concentration of laquinimod, or

ii) the batch is determined to have not more than 0.15% 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), relative to the concentration of laquinimod, or

iii) the batch is determined to have not more than 0.15%, 0.12% or 0.1% Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), relative to the concentration of laquinimod, or

iv) the batch is determined to have not more than 0.1% N-Ethylaniline (NEA), relative to the concentration of laquinimod, or

v) the batch is determined to have not more than 0.1% Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE), relative to the concentration of laquinimod.

81. The process of claim 80, wherein in step c) the pharmaceutical composition is prepared from the batch only if the batch is determined to have not more than 0.12% Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), relative to the concentration of laquinimod, or wherein in step c) the pharmaceutical composition is prepared from the batch only if the batch is determined to have not more than 0.1% Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), relative to the concentration of laquinimod.

82. A process for preparing a pharmaceutical composition comprising laquinimod, or for distributing a validated batch of a pharmaceutical composition comprising laquinimod, comprising

a) obtaining a batch of laquinimod or of the pharmaceutical composition;

b) performing stability testing with a sample of the batch;

c) determining the total amount of at least one of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-

chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in the sample of the batch after stability testing by an HPLC method; and

d) validating the batch for distribution or preparing the pharmaceutical composition from the batch only if the sample of the batch after stability testing contains

   i) not more than a total of 0.15% relative to the concentration of laquinimod of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), or

   ii) not more than a total of 0.15% relative to the concentration of laquinimod of 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), or

   iii) not more than a total of 0.15%, 0.12% or 0.1% relative to the concentration of laquinimod of Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), or

   iv) not more than a total of 0.1% relative to the concentration of laquinimod of N-Ethylaniline (NEA), or

   v) not more than a total of 0.1% relative to the concentration of laquinimod of Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE).

83. The process of claim 82, further comprising step e) distributing the batch if in step d) the batch is validated for distribution.

84. The process of claims 82 or 83, wherein in step d) the batch is validated for distribution or the pharmaceutical composition is prepared from the batch only if the sample of the batch after stability testing contains not more than 0.12% Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME) relative to the concentration of laquinimod, or wherein in step d) the batch is validated for distribution or the pharmaceutical composition is prepared from the batch only if the sample of the batch after stability testing contains not more than 0.1% Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), relative to the concentration of laquinimod.

85. A process for validating a batch of a pharmaceutical product containing laquinimod or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier for distribution comprising

a) subjecting a sample of the batch to stability testing;

b) determining the amount of at least one of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE) in the sample of the batch after stability testing by an HPLC method; and

c) validating the batch for distribution only if the sample of the batch after stability testing contains

  i) not more than a total of 1.0% relative to the concentration of laquinimod of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), or

  ii) not more than a total of 0.5% or 0.1% relative to the concentration of laquinimod of N-Ethylaniline (NEA), or

  iii) not more than a total of 0.5% relative to the concentration of laquinimod of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) combined.

86. The process of claim 85 wherein in step c) the batch is validated for distribution only if the sample of the batch after stability testing not more than a total of the 0.1% of N-Ethylaniline (NEA) relative to the concentration of laquinimod.

87. A process for preparing a packaged pharmaceutical composition comprising laquinimod or a pharmaceutically acceptable salt thereof comprising:

a) obtaining a batch of pharmaceutical composition comprising laquinimod or a pharmaceutically acceptable salt thereof;

b) performing stability testing with a sample from the batch;

c) determining the amount of at least one of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-

hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE) in the sample by an HPLC method after stability testing; and

d) packaging the pharmaceutical composition in only if

   i) the content of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) in the sample is determined to be not more than 1.0% to the concentration of laquinimod, or

   ii) the content of N-Ethylaniline (NEA) in the sample is determined to be not more than 0.5% or 0.1% to the concentration of laquinimod.

   iii) the combined content of 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ) and 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA) in the sample is determined to be not more than a total of 0.5% relative to the concentration of laquinimod.

88. The process of claim 87, wherein in step d) the pharmaceutical composition is packaged only if the content of N-Ethylaniline (NEA) in the sample is determined to be not more than 0.1% to the concentration of laquinimod.

89. The process of any one of claims 69-88, wherein the laquinimod is laquinimod sodium salt.

90. An impurity or a salt thereof for use, as a reference standard to detect trace amounts of the impurity in a pharmaceutical composition comprising laquinimod or a pharmaceutically acceptable salt thereof, wherein the impurity is selected from the group consisting of 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ), 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQME), N-Ethylaniline (NEA), and Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2- dihydroquinoline-3-carboxylate (MCQEE).

91. A method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod, the method comprising,

a) Preparing a sample solution from the pharmaceutical composition,

b) Preparing a standard solution comprising the impurity

c) Preparing a resolution solution comprising laquinimod and the impurity,

d) Preparing a buffer solution by dissolving ammonium acetate in water and adjusting to pH of 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid,

e) Preparing a diluent solution comprising the buffer solution and acetonitrile,

f) Preparing a blank solution comprising the diluent solution and aqueous acetonitrile,

g) Injecting into the HPLC the resolution solution, the blank solution, the standard solution, and the sample solution,

h) Running the HPLC using ultraviolet absorption at 240 nm and the diluent solution as the mobile phase,

i) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and

j) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3- carboxylate (MCQME), *N*-Ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2 dihydroquinoline-3-carboxamide (5-HLAQ) or Ethyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate (MCQEE).

92. A method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod, the method comprising,

a) Preparing a sample solution from the pharmaceutical composition,

b) Preparing a standard solution comprising the impurity

c) Preparing a resolution solution comprising laquinimod and the impurity,

d) Preparing a buffer solution by dissolving ammonium acetate in water, and adjusting to pH of 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid,

e) Preparing a blank solution comprising the buffer solution and acetonitrile,

f) Injecting into the HPLC the resolution solution, the blank solution, the standard solution, and the sample solution,

g) Running the HPLC using ultraviolet absorption at 240 nm and a mobile phase of a mixture of the buffer solution, and acetonitrile.

h) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution,

i) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is N-Ethylaniline (NEA), 1*H*,3*H*-spiro[5-chloro-1-methylquinoline-2,4-dione-3,3'-[1]ethylindolin-[2]-one] (SPIRO-LAQ), or 5-Chloro-*N*-ethyl-3-hydroxy-1-methyl-2,4-dioxo-*N*-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide (3-HLAQ).

93. A method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod and a pharmaceutically acceptable carrier, the method comprising,

a) Preparing a sample solution from the pharmaceutical composition,

b) Preparing a standard solution comprising the impurity

c) Preparing a Quantitation Limit (QL) solution comprising the impurity,

d) Preparing a resolution solution comprising laquinimod and the impurity,

e) Preparing a buffer solution by dissolving ammonium dihydrogen phosphate in water, and adjusting to pH of 7.0 ± 0.10 with aqueous ammonia or phosphoric acid,

f) Preparing a blank solution comprising the buffer solution and acetonitrile,

g) Injecting into the HPLC the resolution solution, the blank solution, the QL solution, the standard solution and the sample solution,

h) Running the HPLC using a ultraviolet absorption at 212 nm and a mobile phase of a mixture of the buffer solution, acetonitrile, and methanol,

i) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and

j) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is 2-chloro-6-(3-(ethyl(phenyl)amino)-2-hydroxy-N-methyl-3-oxopropanamido)benzoic acid (BH-3-HLAQ) or N-ethyl-4-hydroxy-1-methyl-5-(2,3,4,5,6-pentahydroxyhexylamino)-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide (MEG-LAQ).

94. A method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod and a pharmaceutically acceptable carrier, the method comprising,

a) Preparing a sample solution from the pharmaceutical composition,

b) Preparing a standard solution comprising the impurity

c) Preparing a resolution solution comprising laquinimod and the impurity,

d) Preparing a buffer solution by dissolving ammonium acetate in water, and adjusting to PH or 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid,

e) Preparing a blank solution comprising the buffer solution and acetonitrile,

f) Injecting into the HPLC the resolution solution, the blank solution, the standard solution and the sample solution,

g) Running the HPLC using a ultraviolet absorption at 242 nm and the blank solution as the mobile phase,

h) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and

i) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is 5-Chloro-4-hydroxy-1-methylquinolin-2(1*H*)-one (MCQ), 5-Chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (MCQCA), Methyl 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3- carboxylate (MCQME) or *N*-Ethyl-4,5-dihydroxy-1-methyl-2-oxo-*N*-phenyl-1,2 dihydroquinoline-3-

carboxamide (5-HLAQ).

95. A method of determining the concentration of an impurity in a pharmaceutical composition comprising laquinimod and a pharmaceutically acceptable carrier, the method comprising,

a) Preparing a sample solution from the pharmaceutical composition,

b) Preparing a standard solution comprising the impurity

c) Preparing a resolution solution comprising laquinimod and the impurity,

d) Preparing a blank solution comprising methanol and acetonitrile,

e) Preparing a buffer solution by dissolving ammonium acetate in water, and adjusting to pH of 7.0 ± 0.05 with aqueous ammonia or glacial acetic acid,

f) Injecting into the HPLC the resolution solution, the blank solution, and the sample solution,

g) Running the HPLC using a ultraviolet absorption at 240 nm and a mobile phase comprising acetonitrile and the buffer solution,

h) Determining the retention time (RT) and the areas of the peaks of the impurity in the chromatograms of the sample solution, and

i) Performing quantitation of the impurity with respect to the corresponding peaks in the chromatograms of the standard solutions,

wherein the impurity is N-Ethylaniline (NEA), 5-Chloro-*N*-ethyl-3-hydroxy-1-methyl-2,4-dioxo-*N*-phenyl-1,2,3,4-tetrahydroquinoline-3-carboxamide (3-HLAQ) or 1*H*,3*H*-spiro[5-chloro-1-methylquinoline-2,4-dione-3,3'-[1]ethylindolin-[2]-one] (SPIRO-LAQ).

Figure 1.

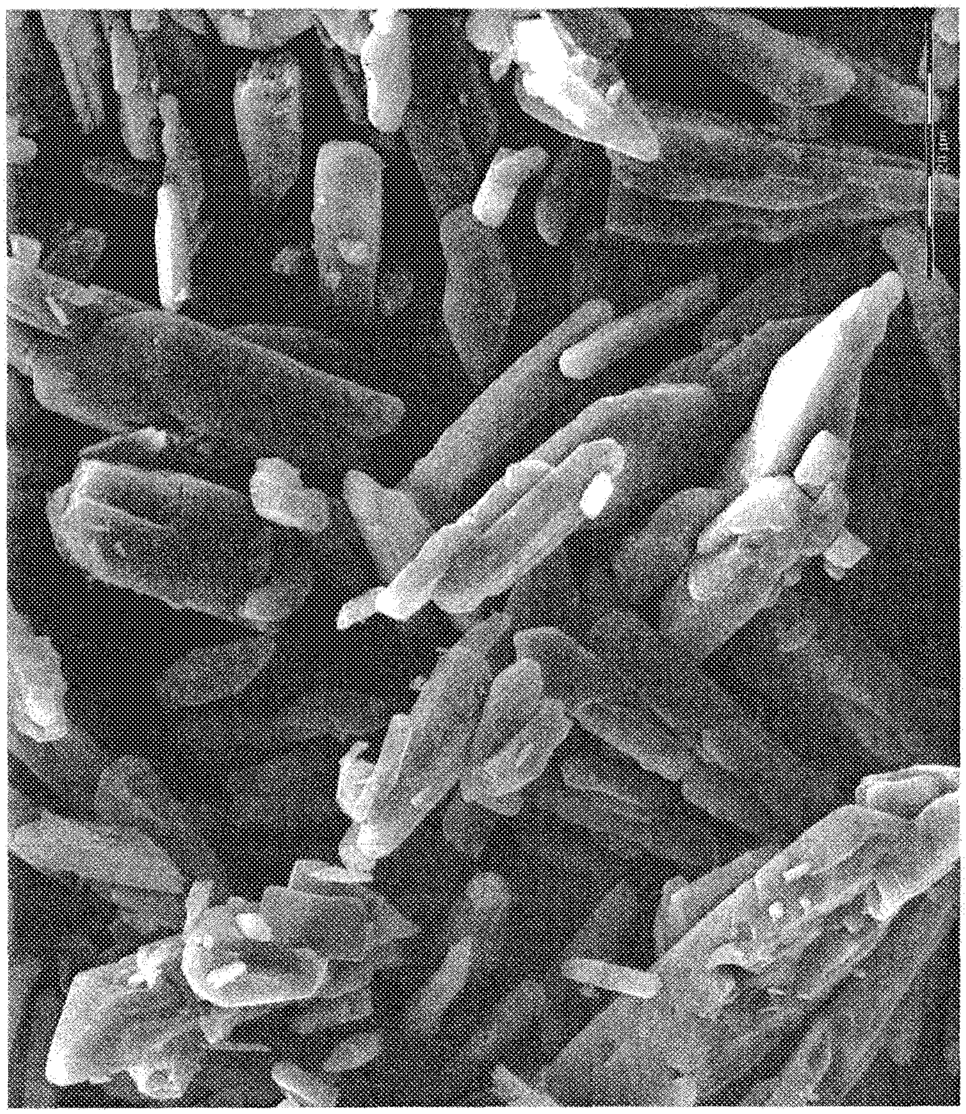
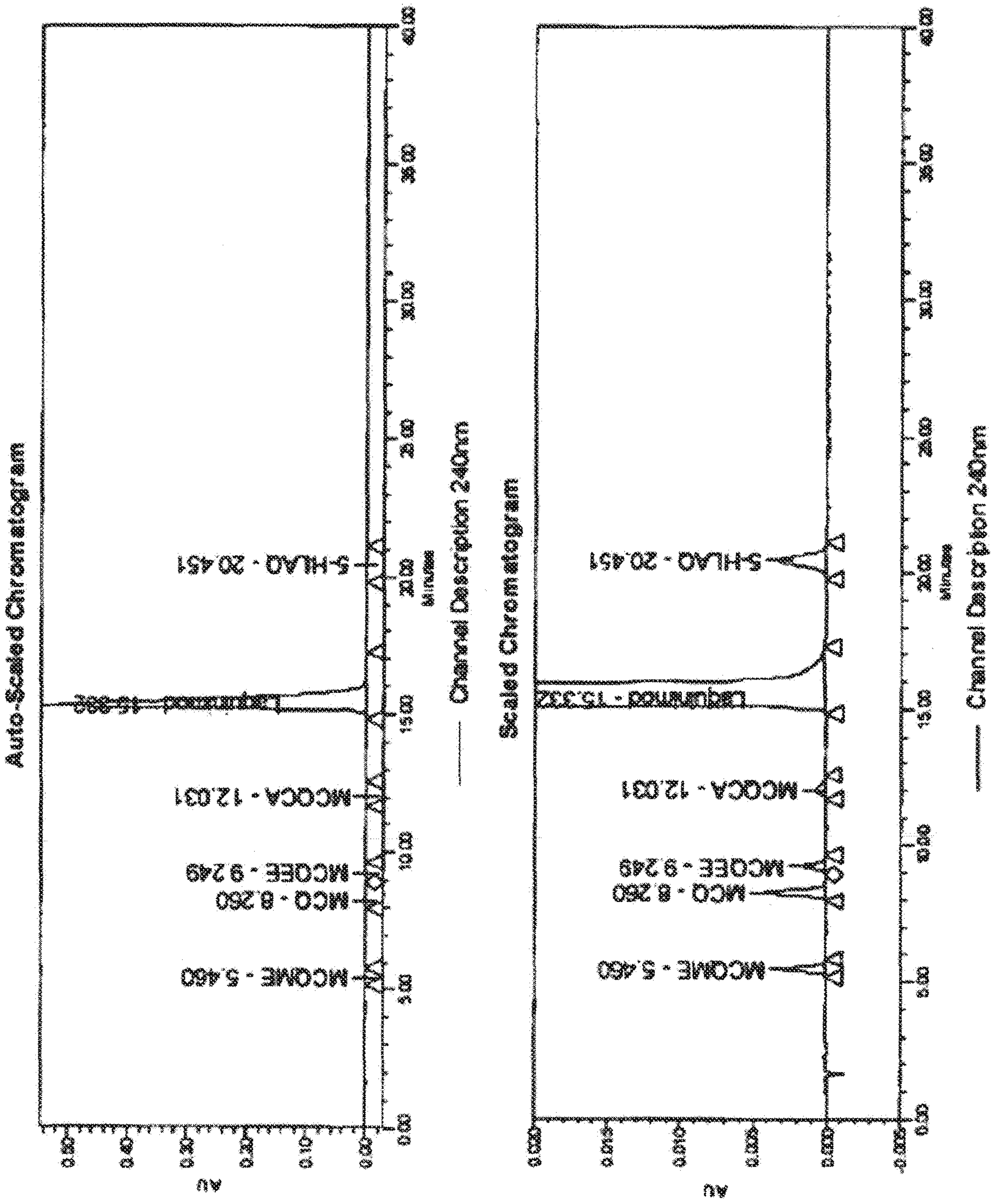


Figure 2.

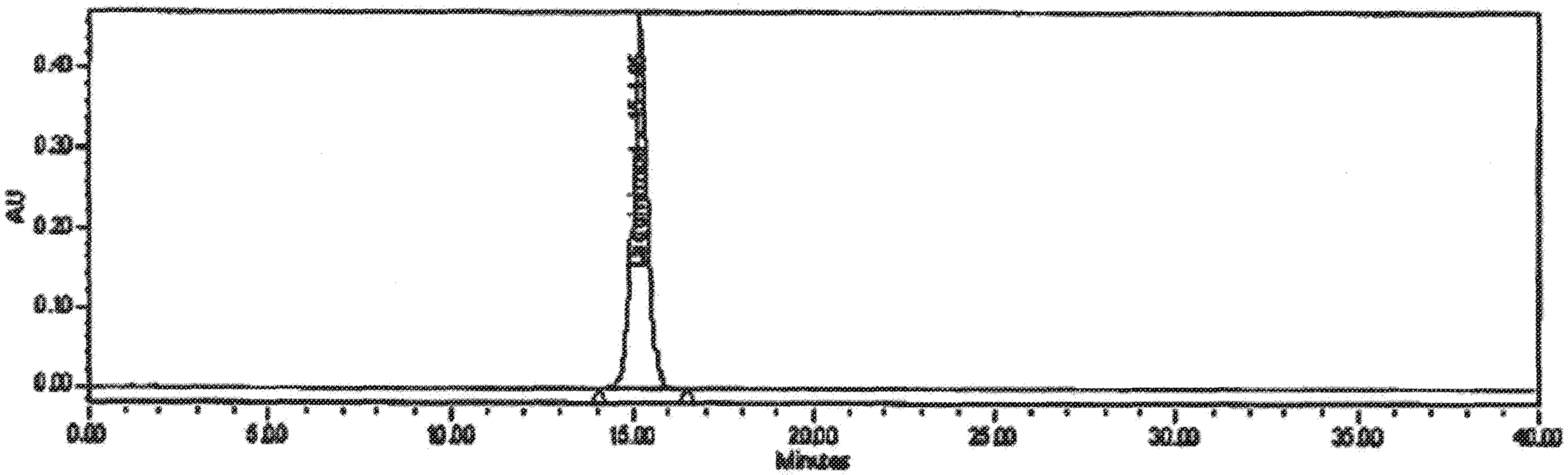
Figure 3.



Auto-Scaled Chromatogram

Scaled Chromatogram

Figure 4.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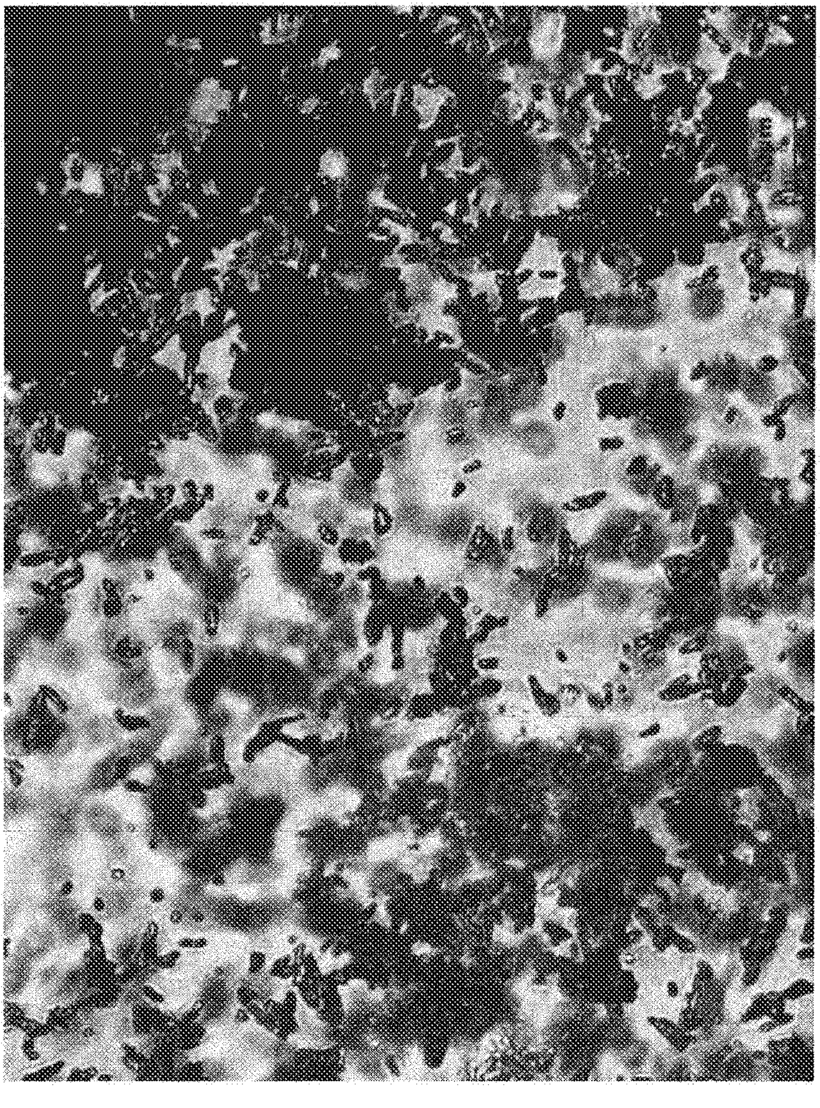
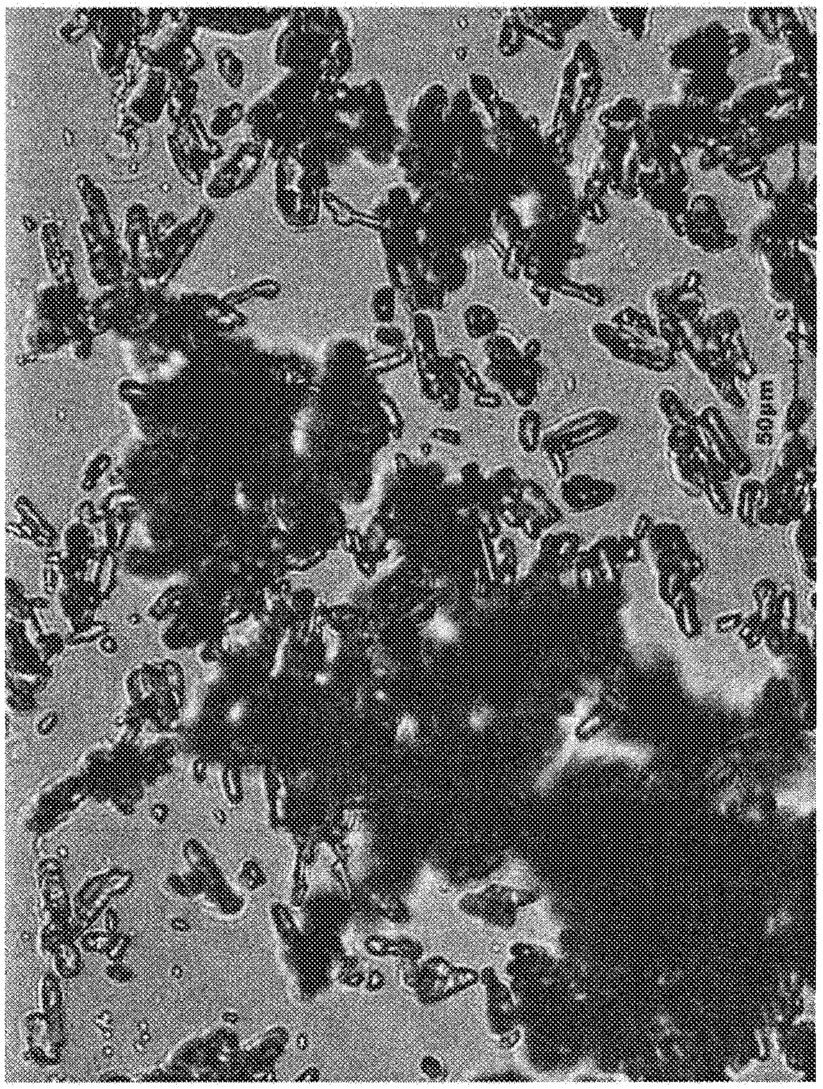


Figure 5.

Figure 6.



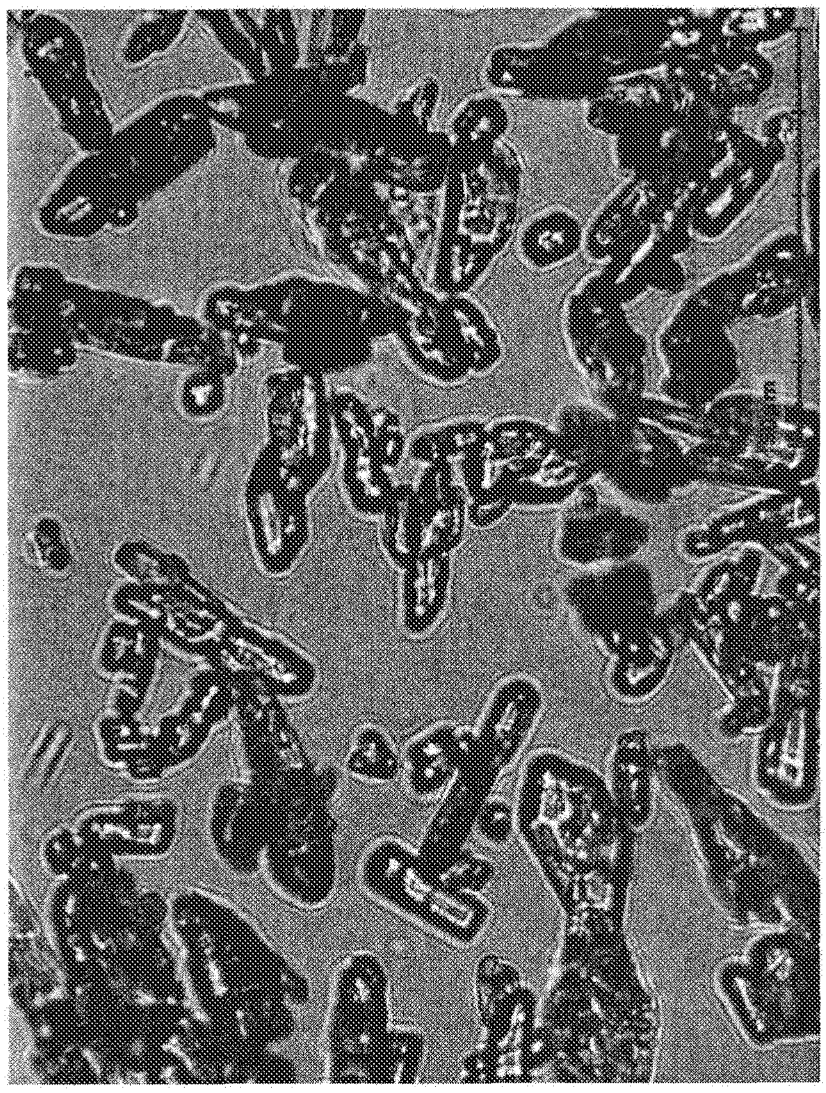


Figure 7.

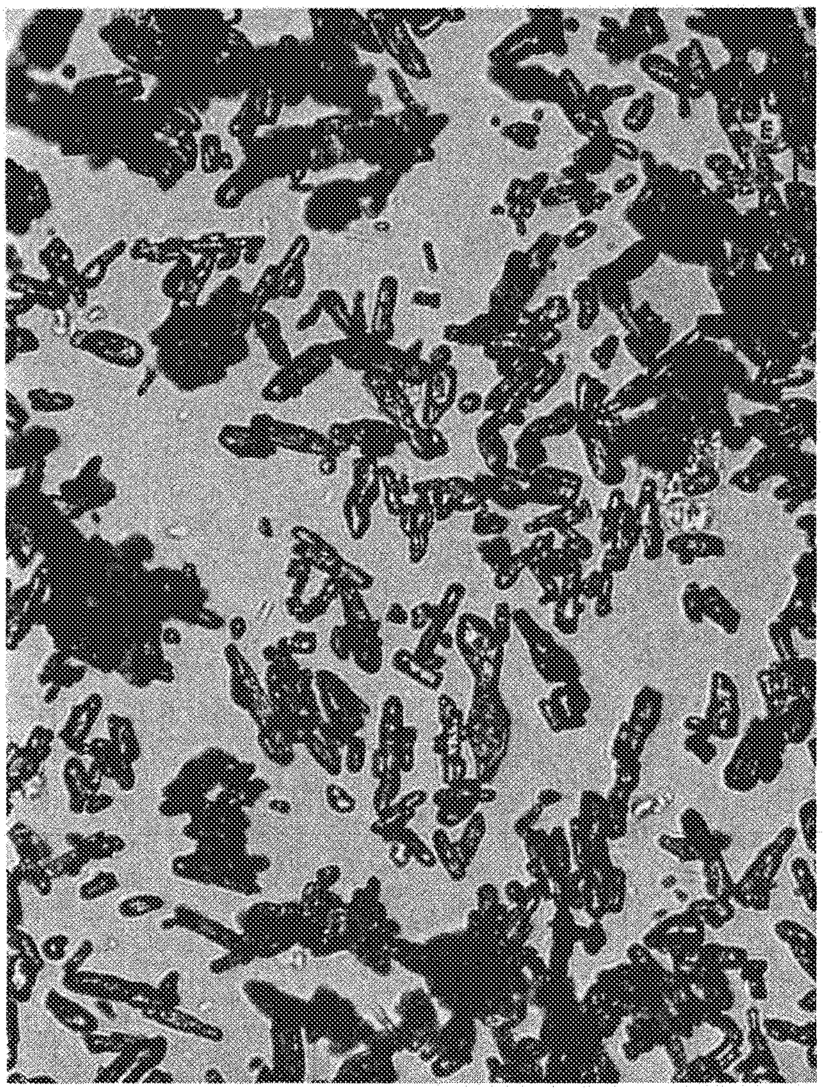


Figure 8.

Figure 9.

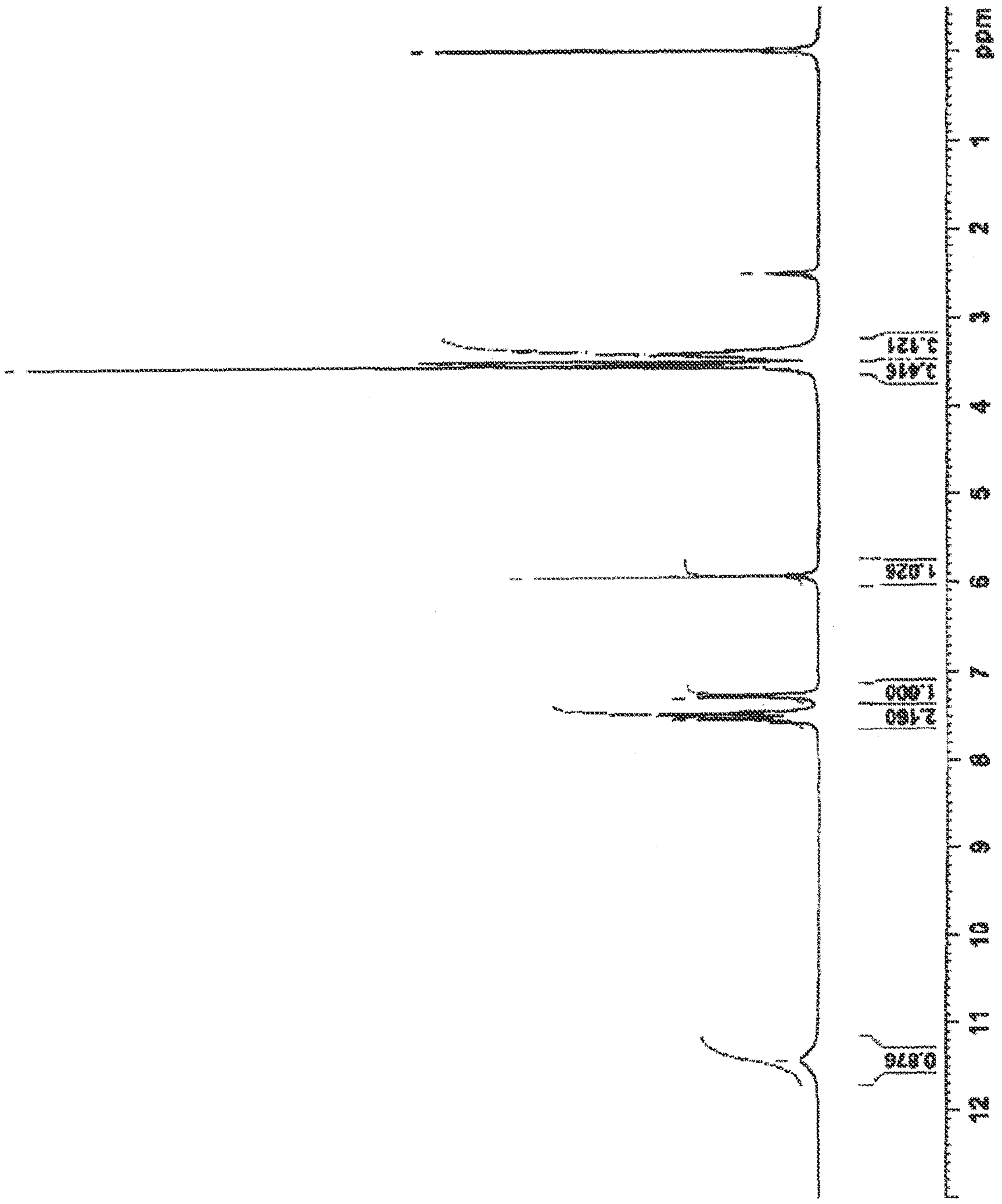


Figure 10.

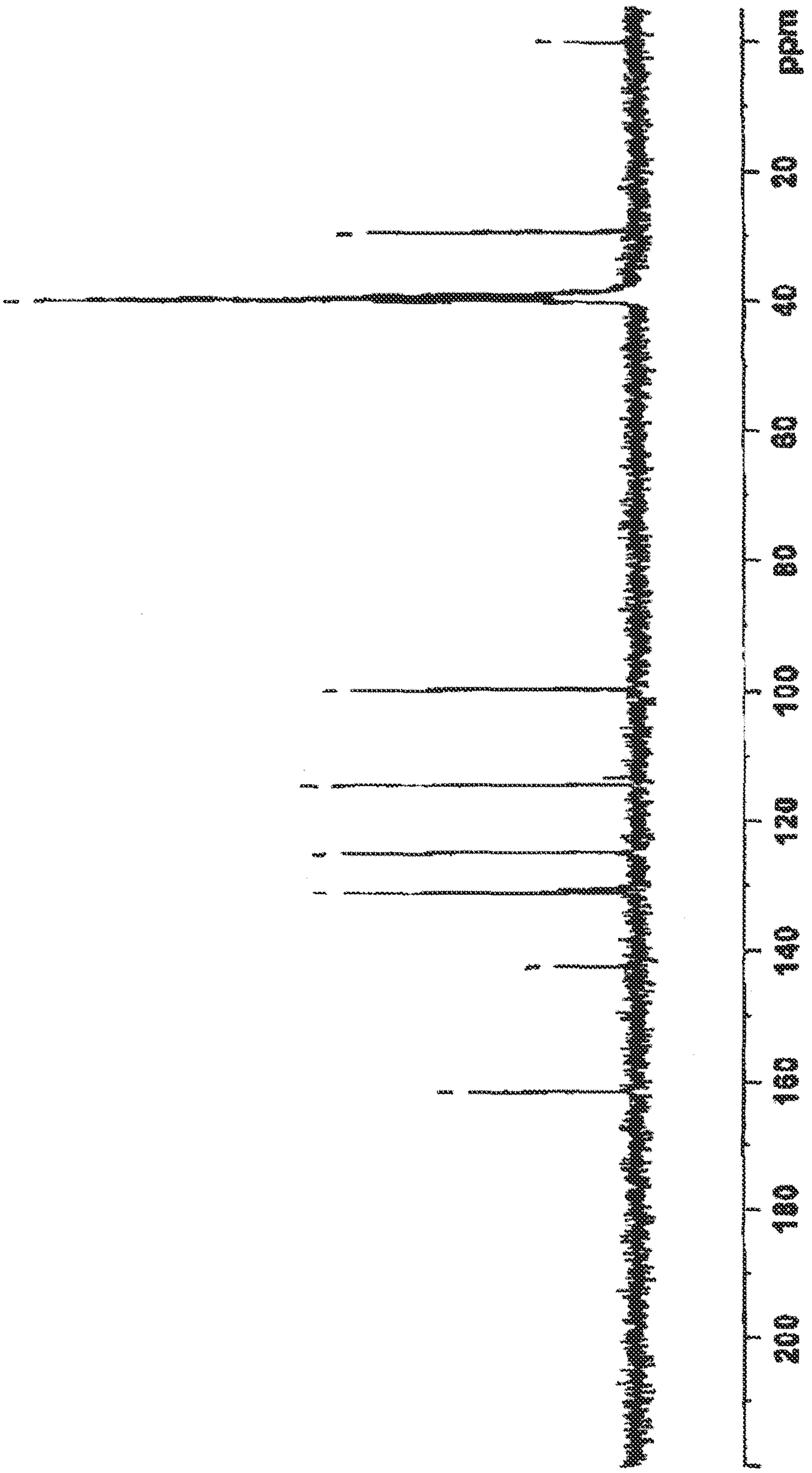
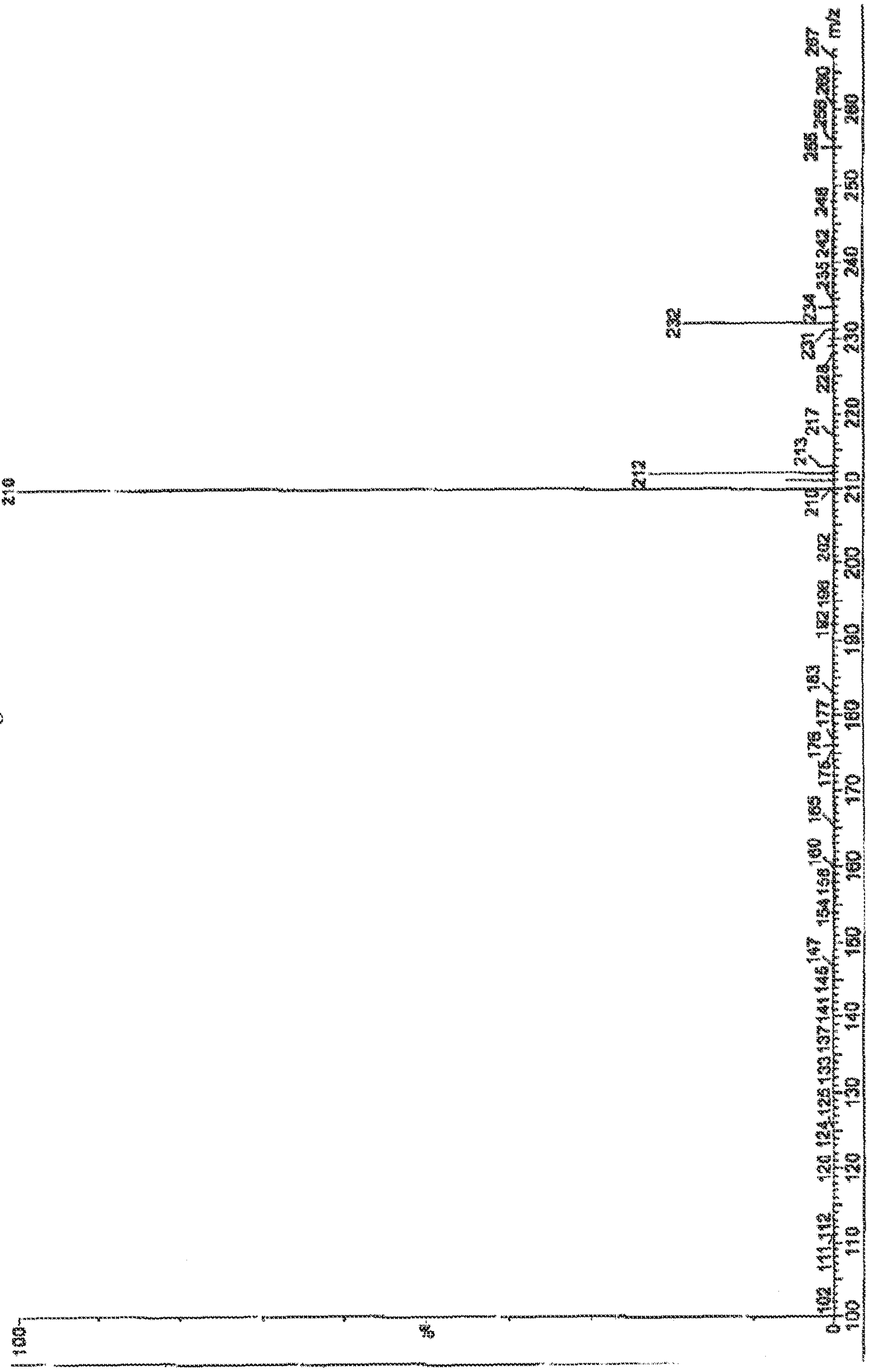


Figure 11.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260

Figure 12.

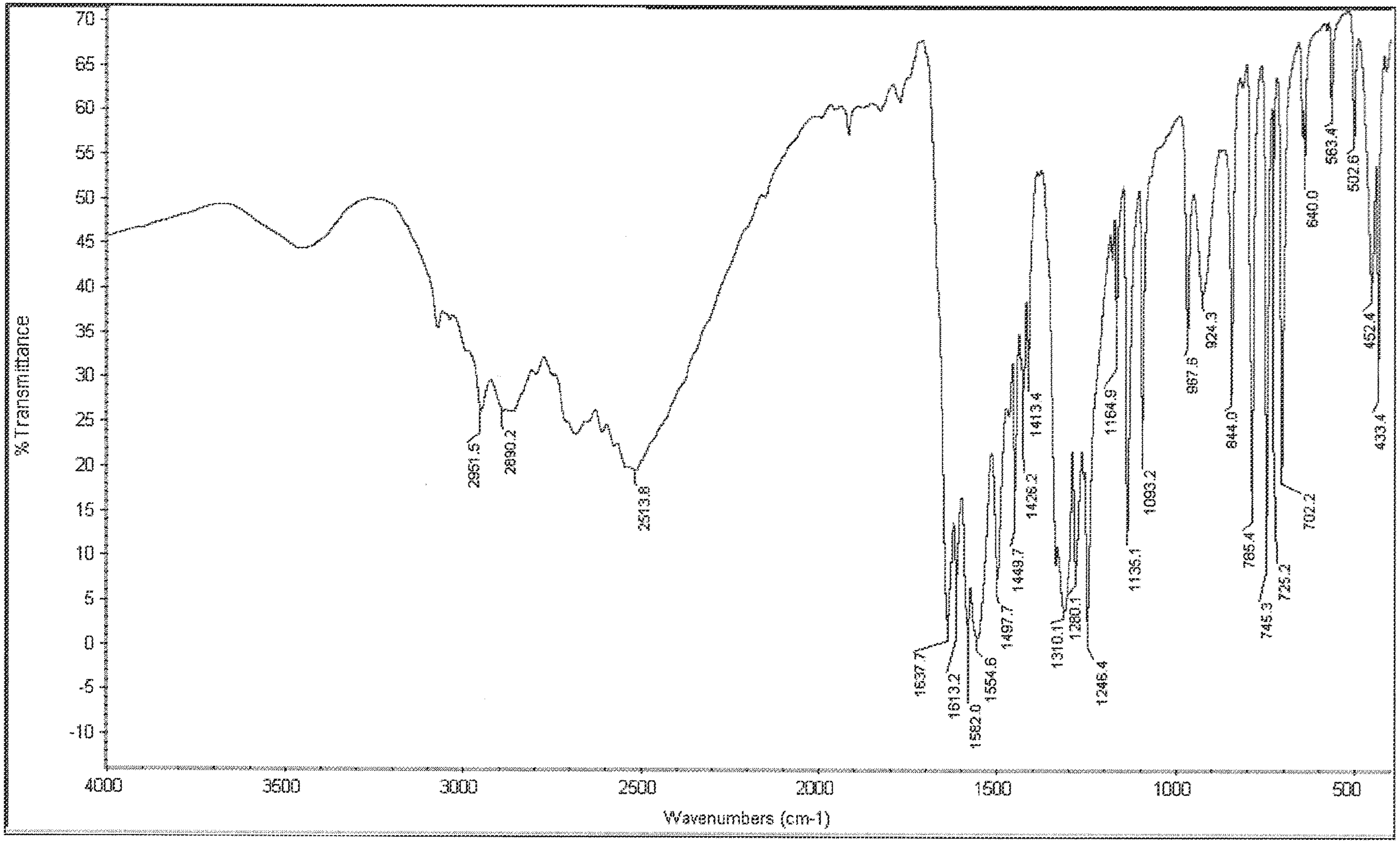

% Transmittance
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
-5
-10
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)
2951.5
2890.2
2513.8
1637.7
1613.2
1582.0
1554.6
1497.7
1449.7
1426.2
1413.4
1310.1
1280.1
1246.4
1164.9
1135.1
1093.2
967.5
924.3
844.0
785.4
745.3
725.2
702.2
640.0
563.4
502.6
452.4
433.4

Figure 13.

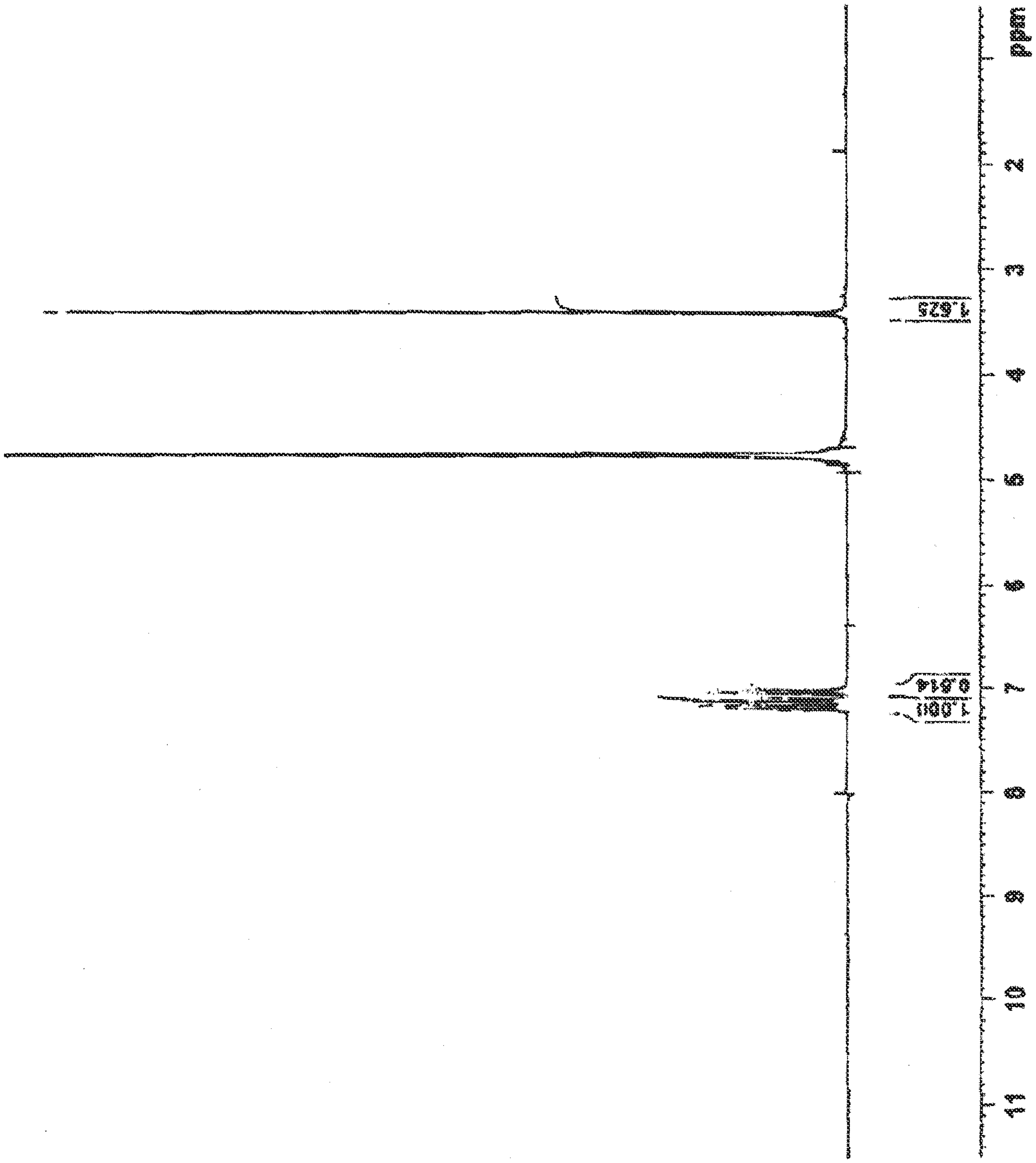


Figure 14.

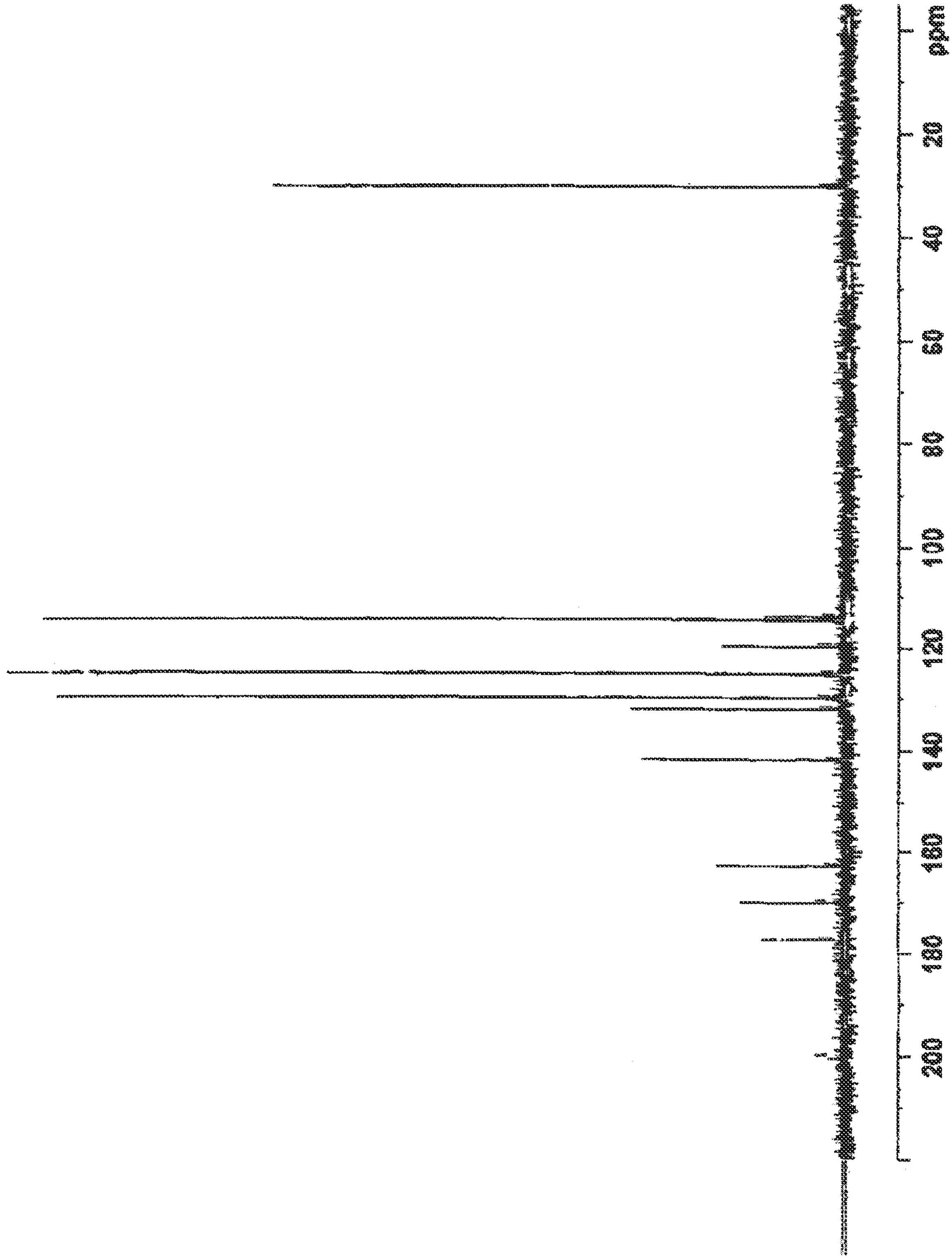


Figure 15

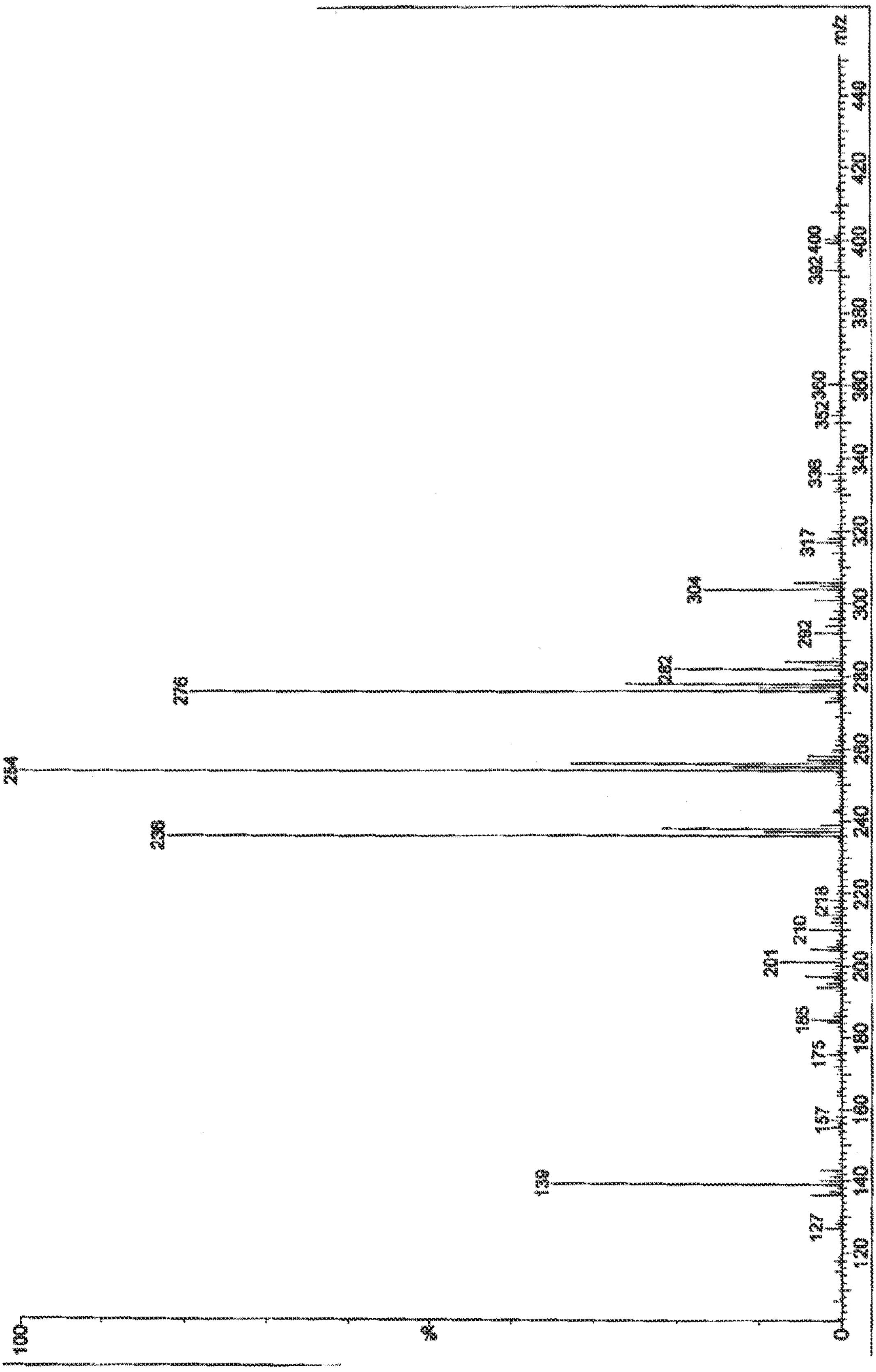

100
%
0
127
139
157
175
195
201
210
218
238
254
276
282
292
304
317
335
352
360
392
400
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
m/z

Figure 16.

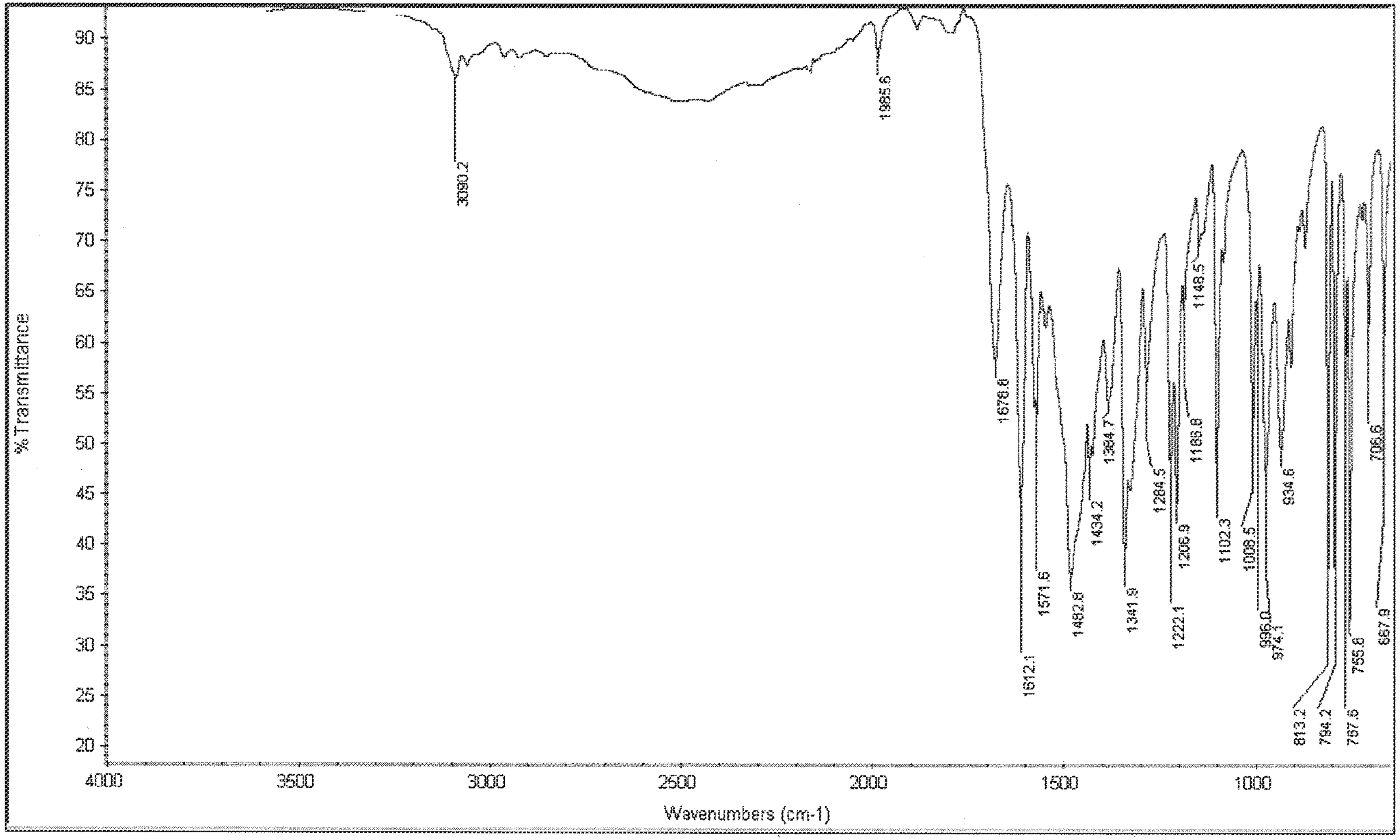

% Transmittance
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
4000
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)
3090.2
1985.6
1676.8
1612.1
1571.6
1482.8
1434.2
1384.7
1341.9
1284.5
1222.1
1206.9
1186.8
1148.5
1102.3
1008.5
996.0
974.1
934.6
813.2
794.2
767.6
755.8
706.6
667.9

Figure 17.

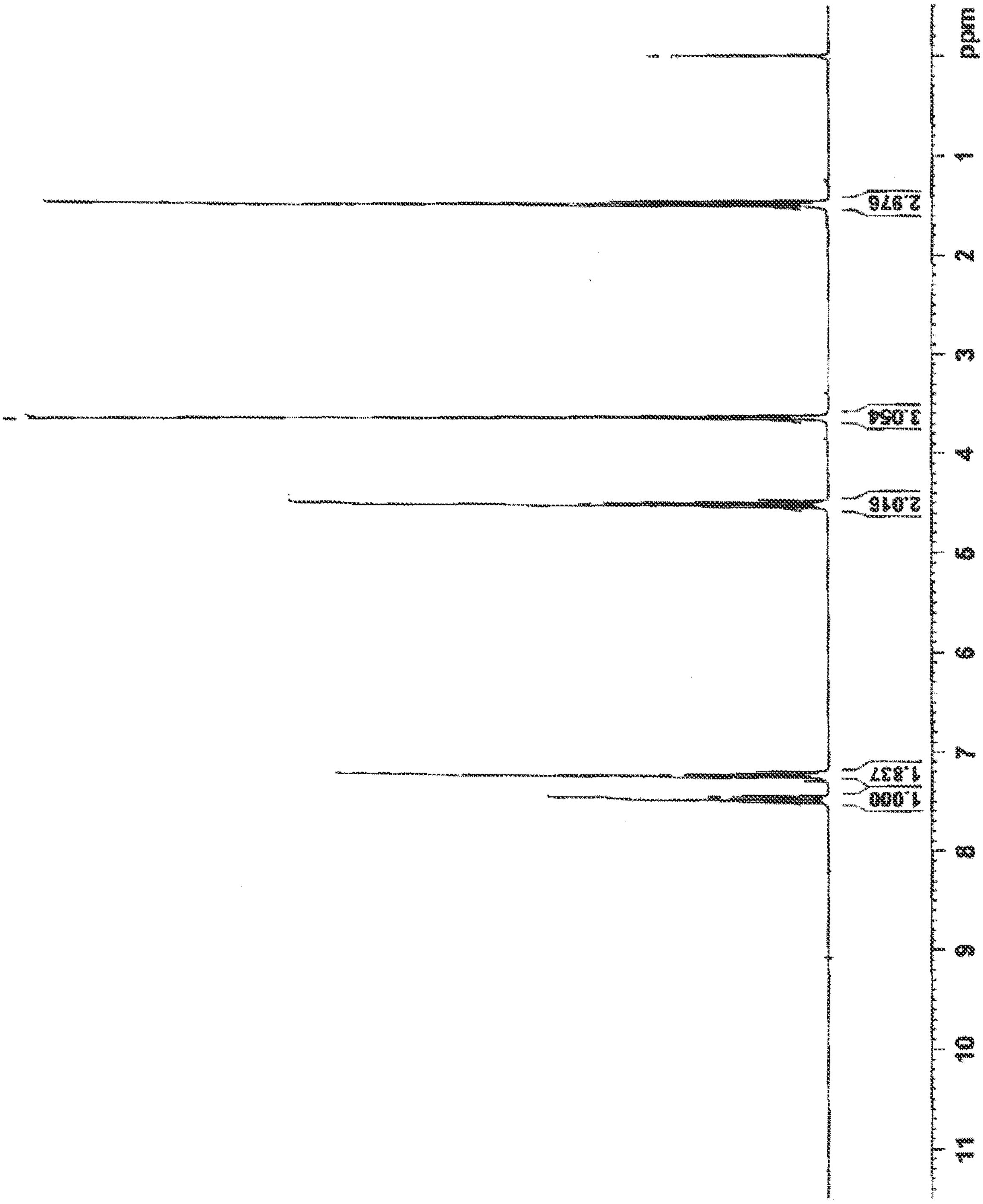


Figure 18.

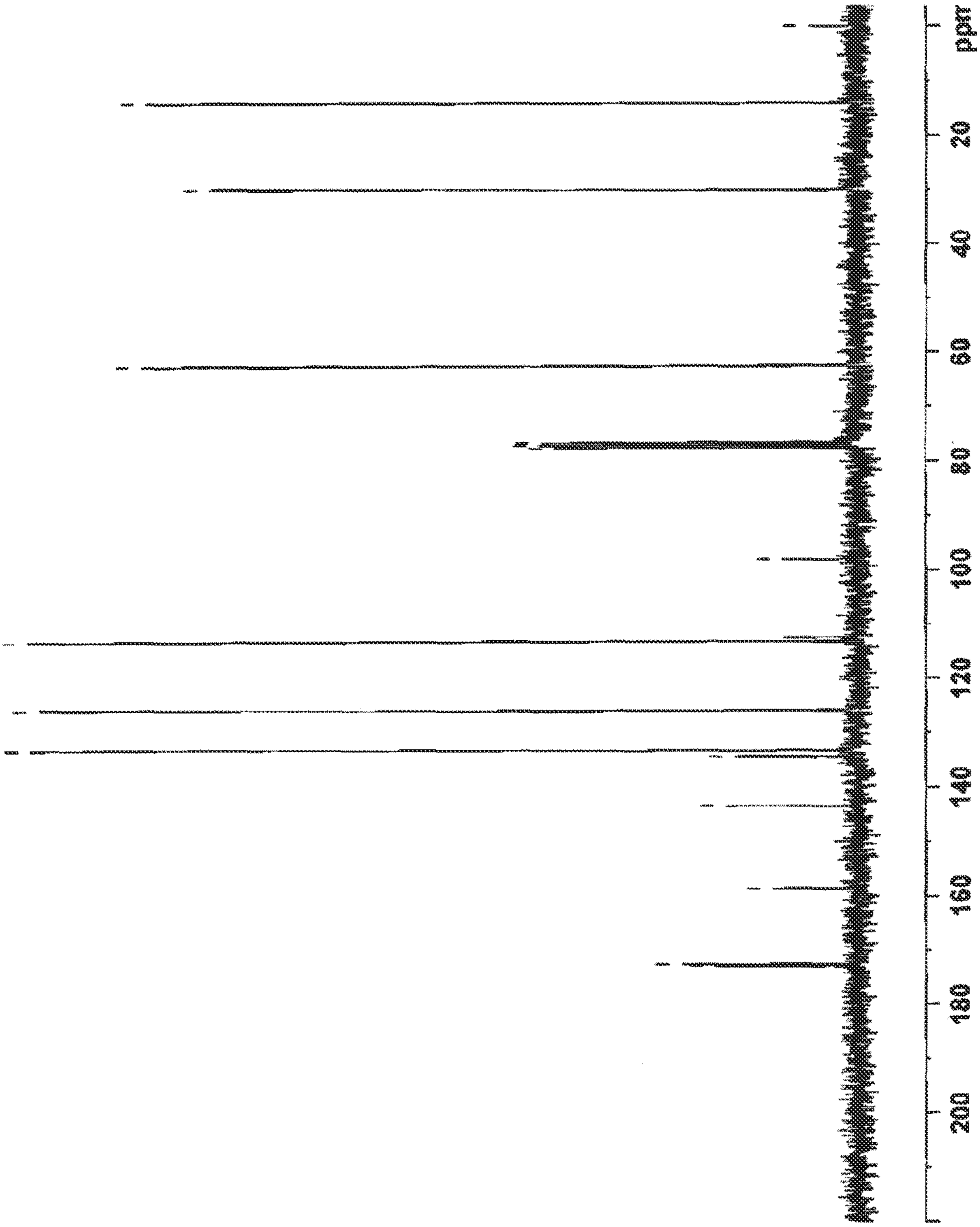


Figure 19.

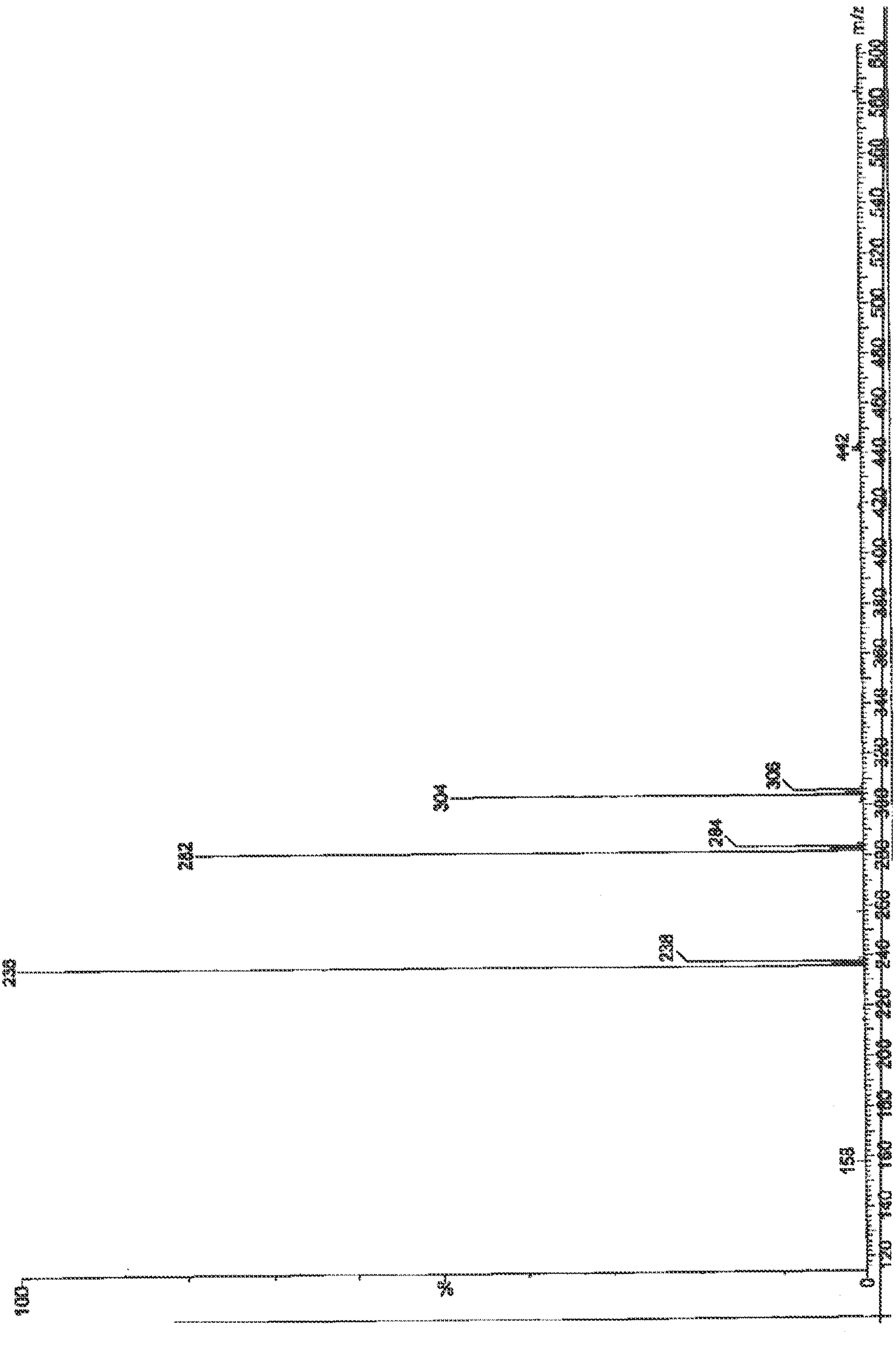

100
%
0
238
238
282
284
304
306
155
442
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
560
580
600
m/z

Figure 20.

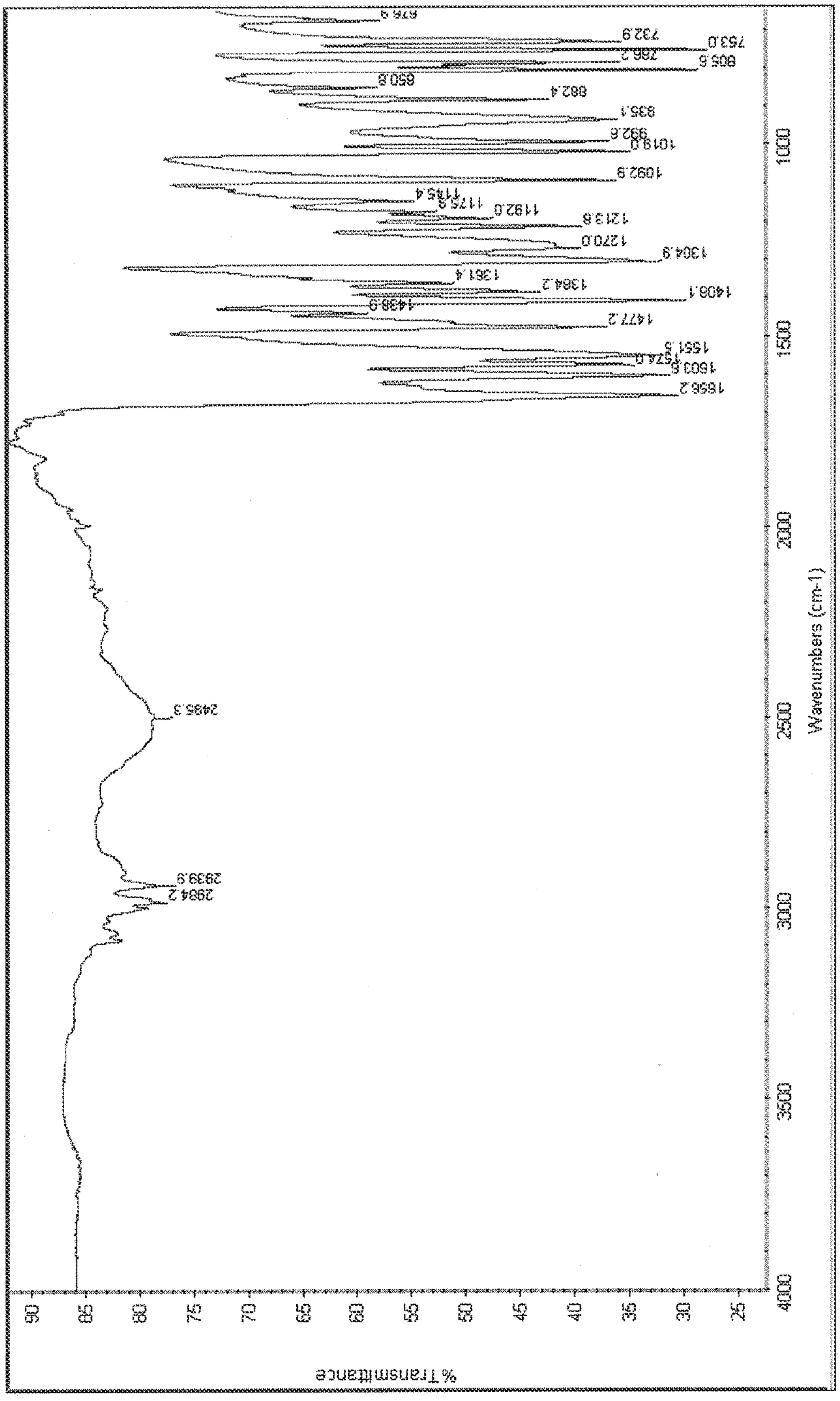

%Transmittance
90
85
80
75
70
65
60
55
50
45
40
35
30
25
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
2984.2
2939.9
2495.3
1656.2
1603.6
1574.0
1551.5
1477.2
1438.9
1408.1
1384.2
1361.4
1304.9
1270.0
1213.8
1192.0
1175.8
1145.4
1092.9
1019.0
992.6
935.1
882.4
850.8
805.6
786.2
753.0
732.9
678.9

Figure 21.

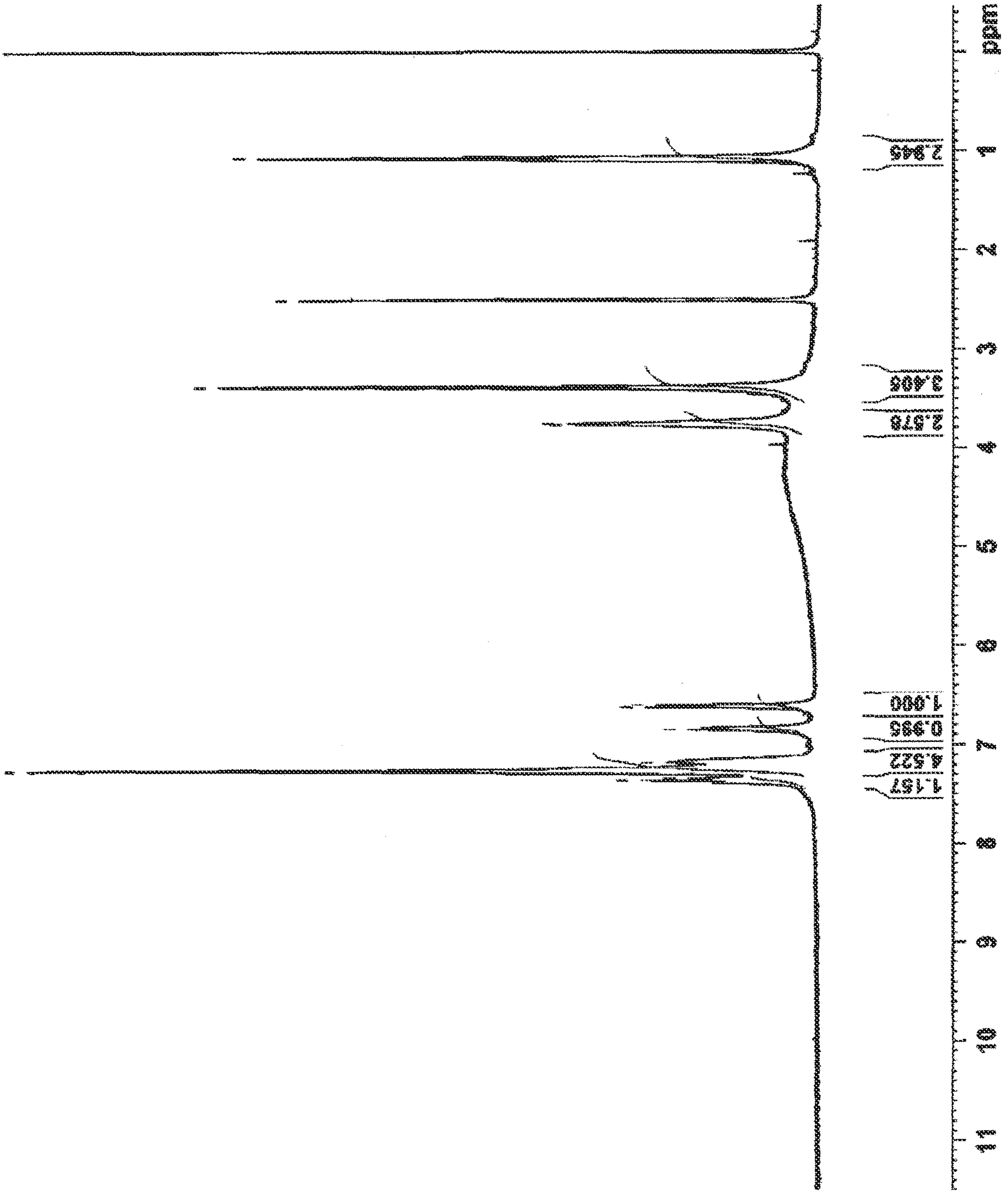


Figure 22.

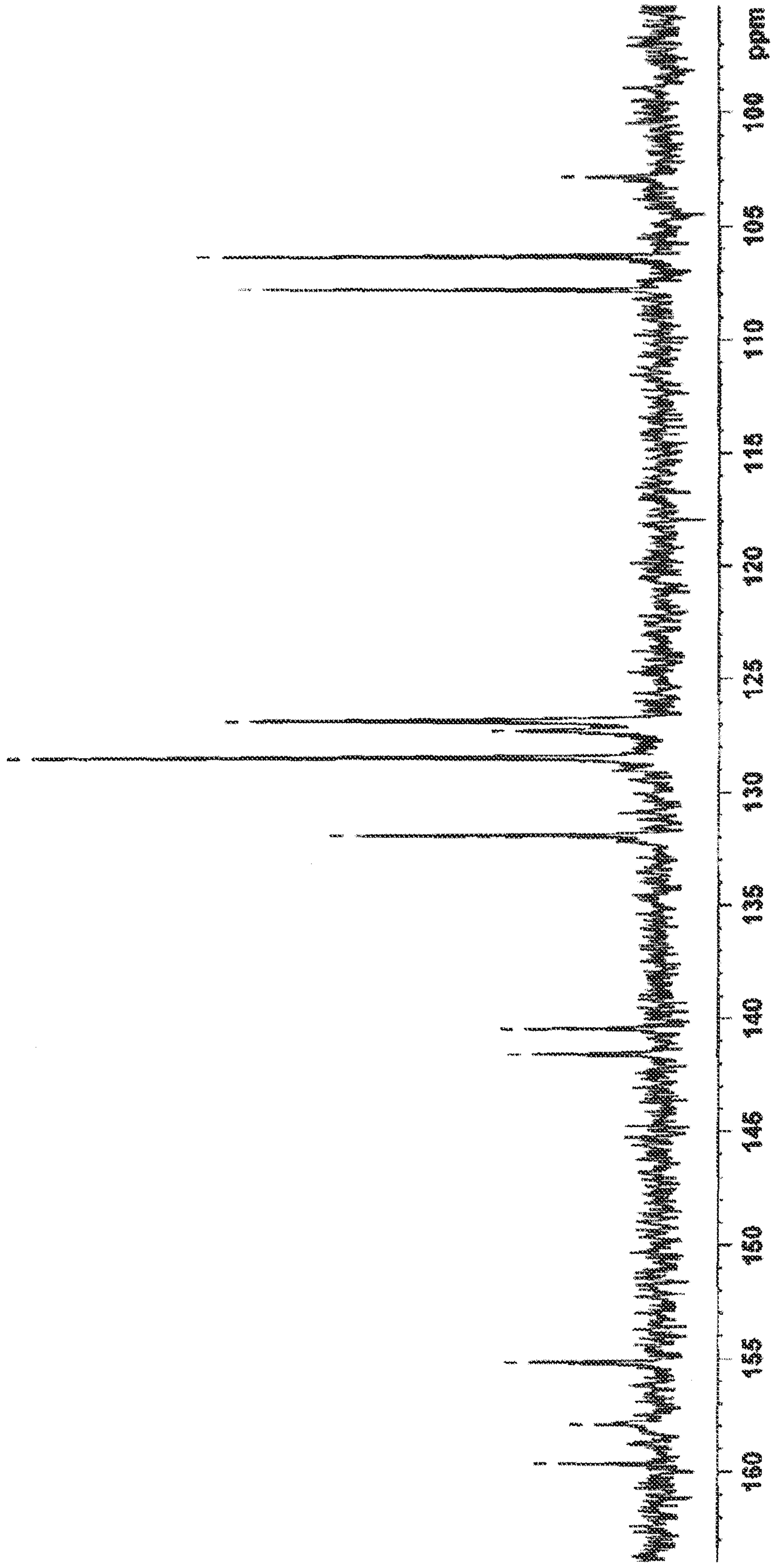


Figure 23.

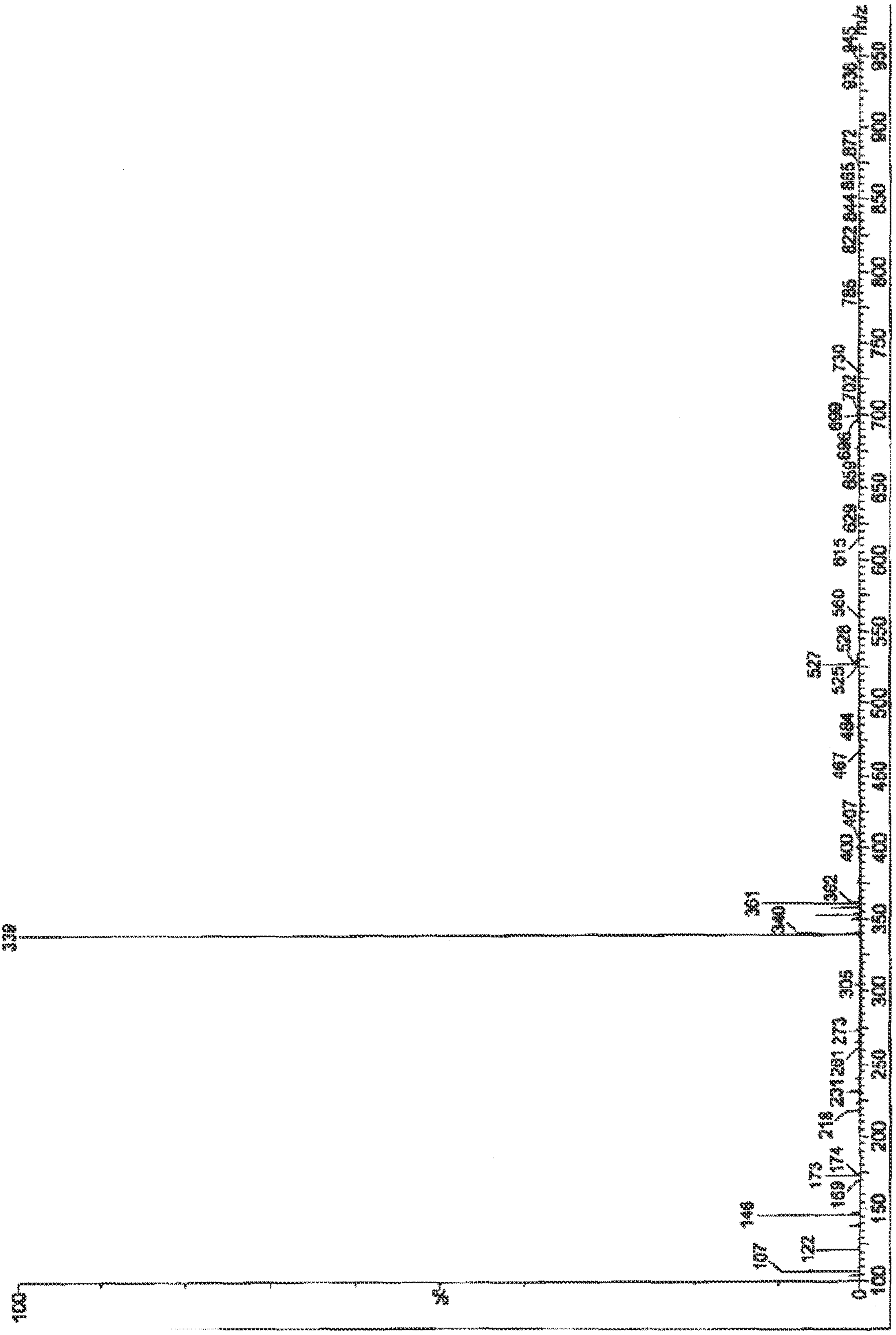

100
%
0
339
361
340
362
107
122
148
169
173
174
218
231
251
273
305
400
407
467
494
525
527
528
560
615
629
659
696
699
702
730
785
822
844
855
872
936
945
m/z
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950

Figure 24.

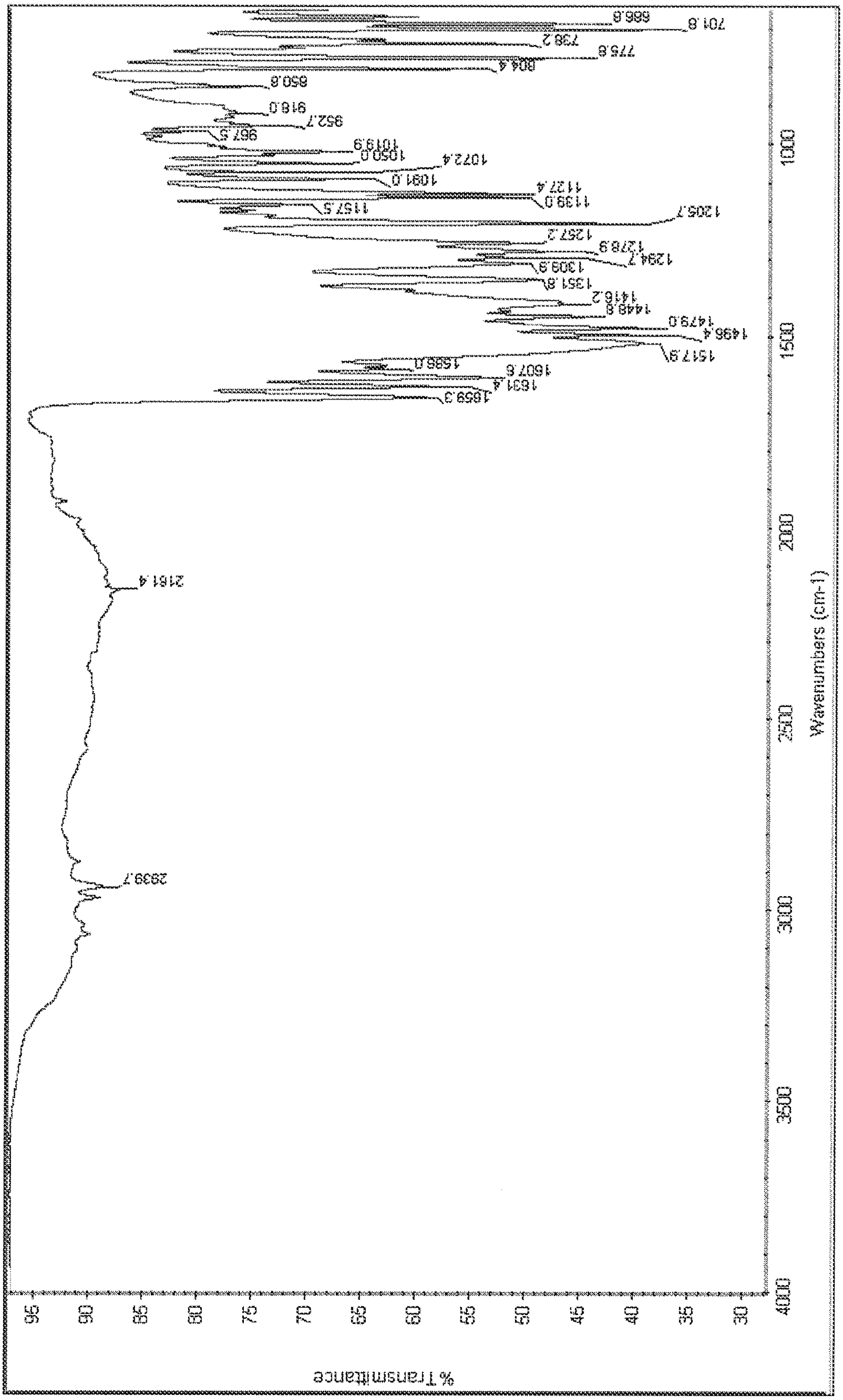

% Transmittance
95
90
85
80
75
70
65
60
55
50
45
40
35
30
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
2939.7
2161.4
1659.3
1631.4
1607.6
1586.0
1517.9
1496.4
1479.0
1448.8
1416.2
1351.8
1309.9
1294.7
1278.9
1257.2
1205.7
1157.5
1139.0
1127.4
1091.0
1072.4
1050.0
1019.9
967.5
952.7
918.0
850.8
804.4
775.8
738.2
701.8
666.8

Figure 25.

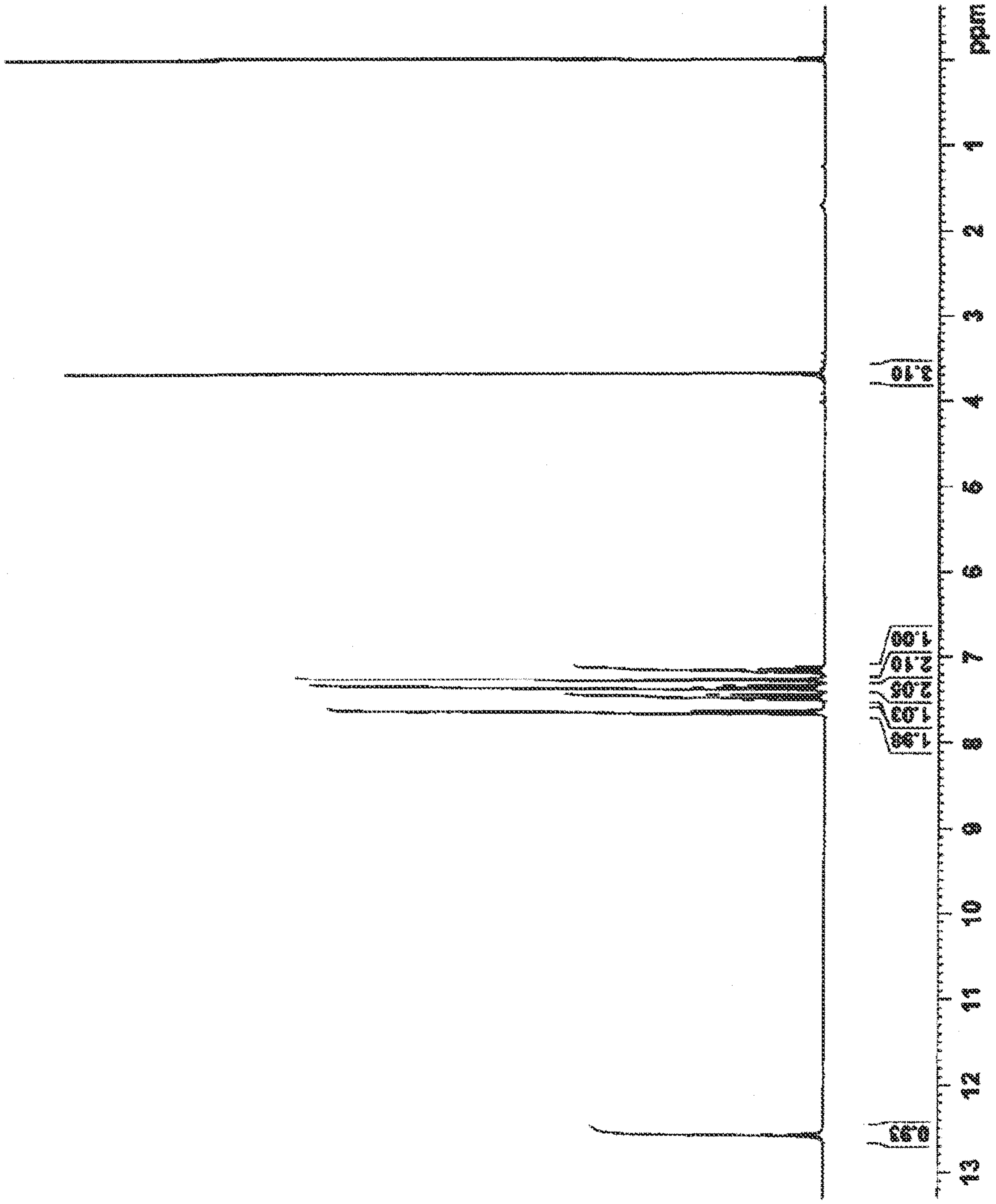


Figure 26.

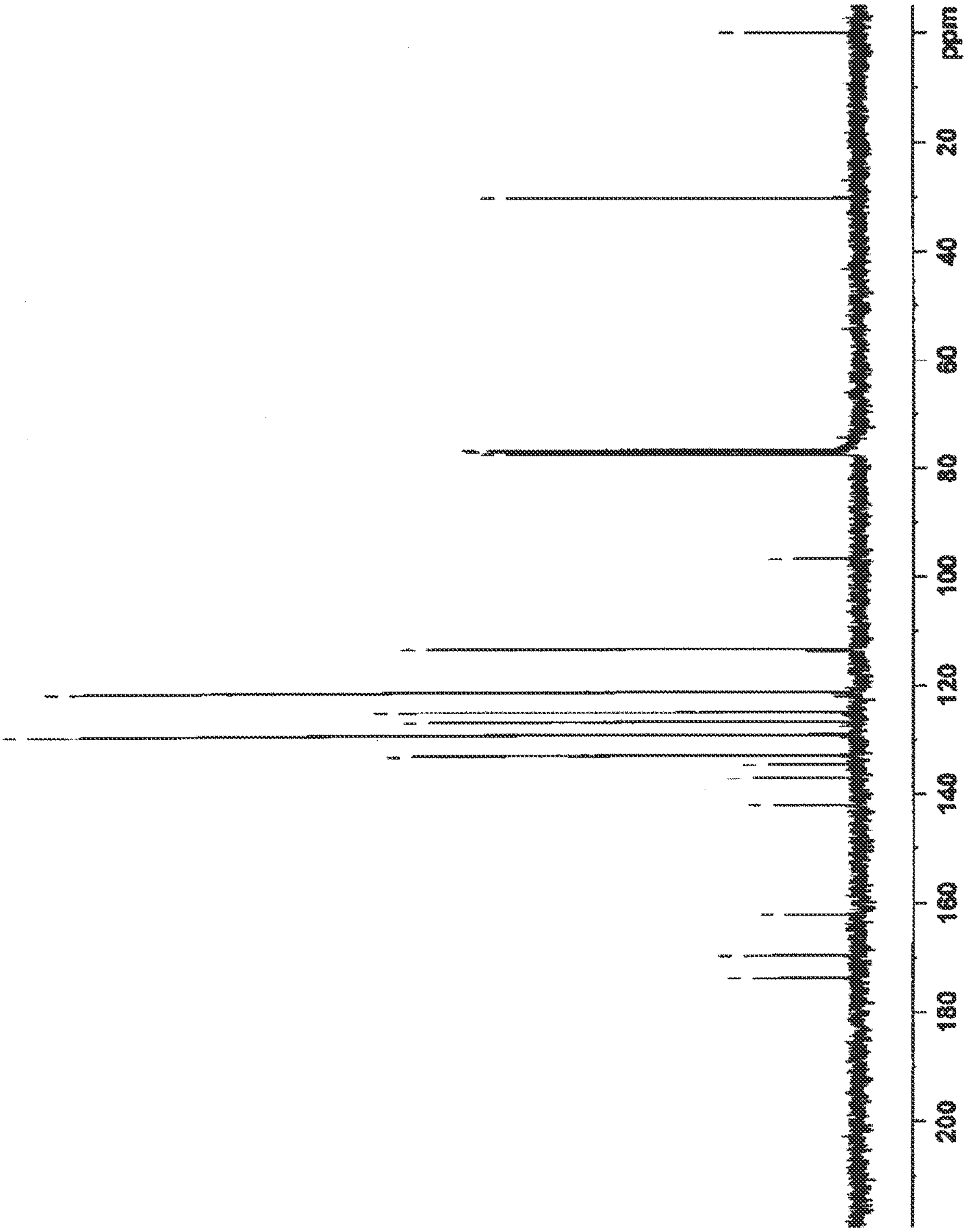


Figure 27.

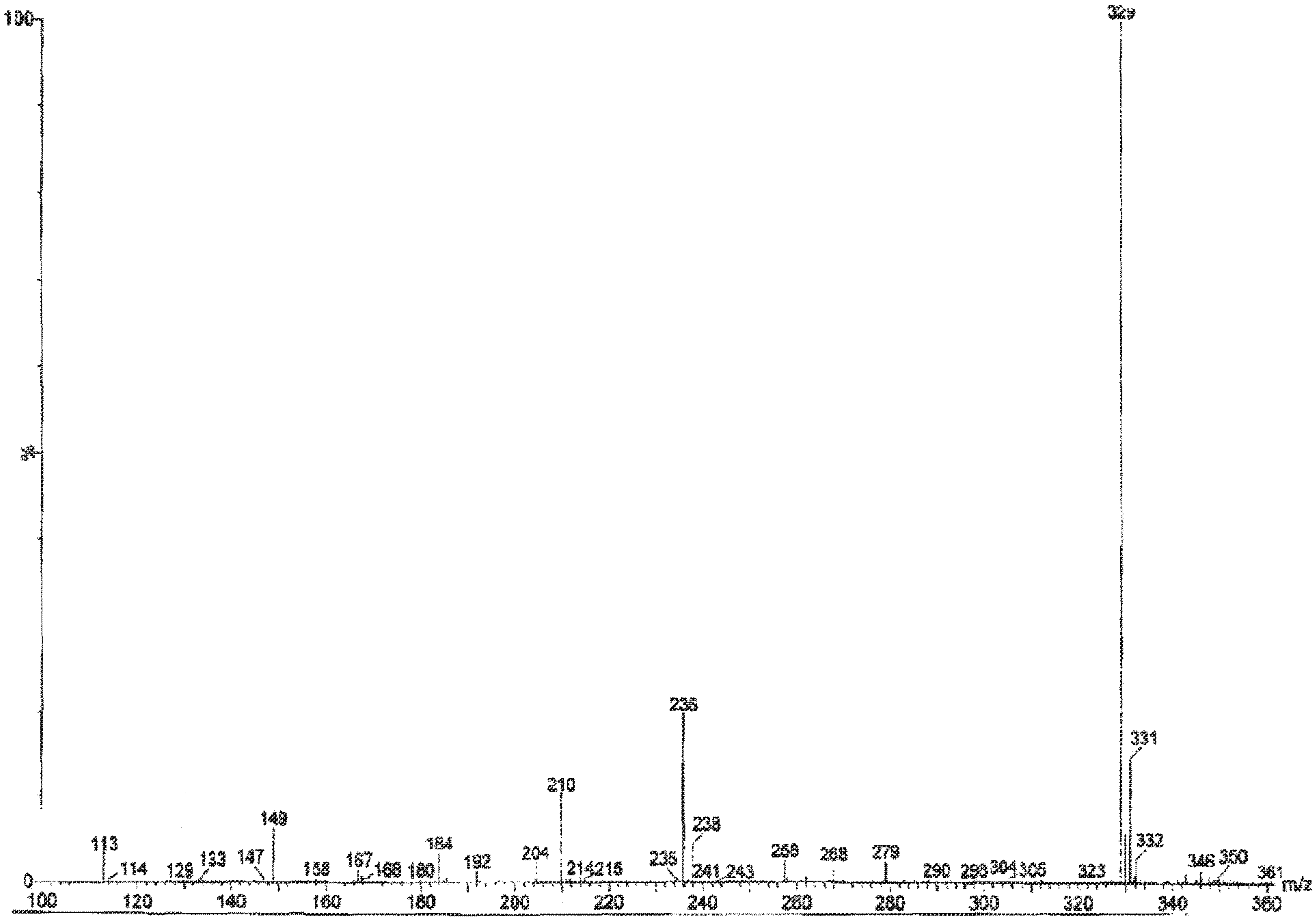

100
%
0
329
331
332
236
238
210
149
113
114
129
133
147
158
167
168
180
184
192
204
214
215
235
241
243
258
268
279
290
298
304
305
323
346
350
361
m/z
100
120
140
160
180
200
220
240
260
280
300
320
340
360

Figure 28.

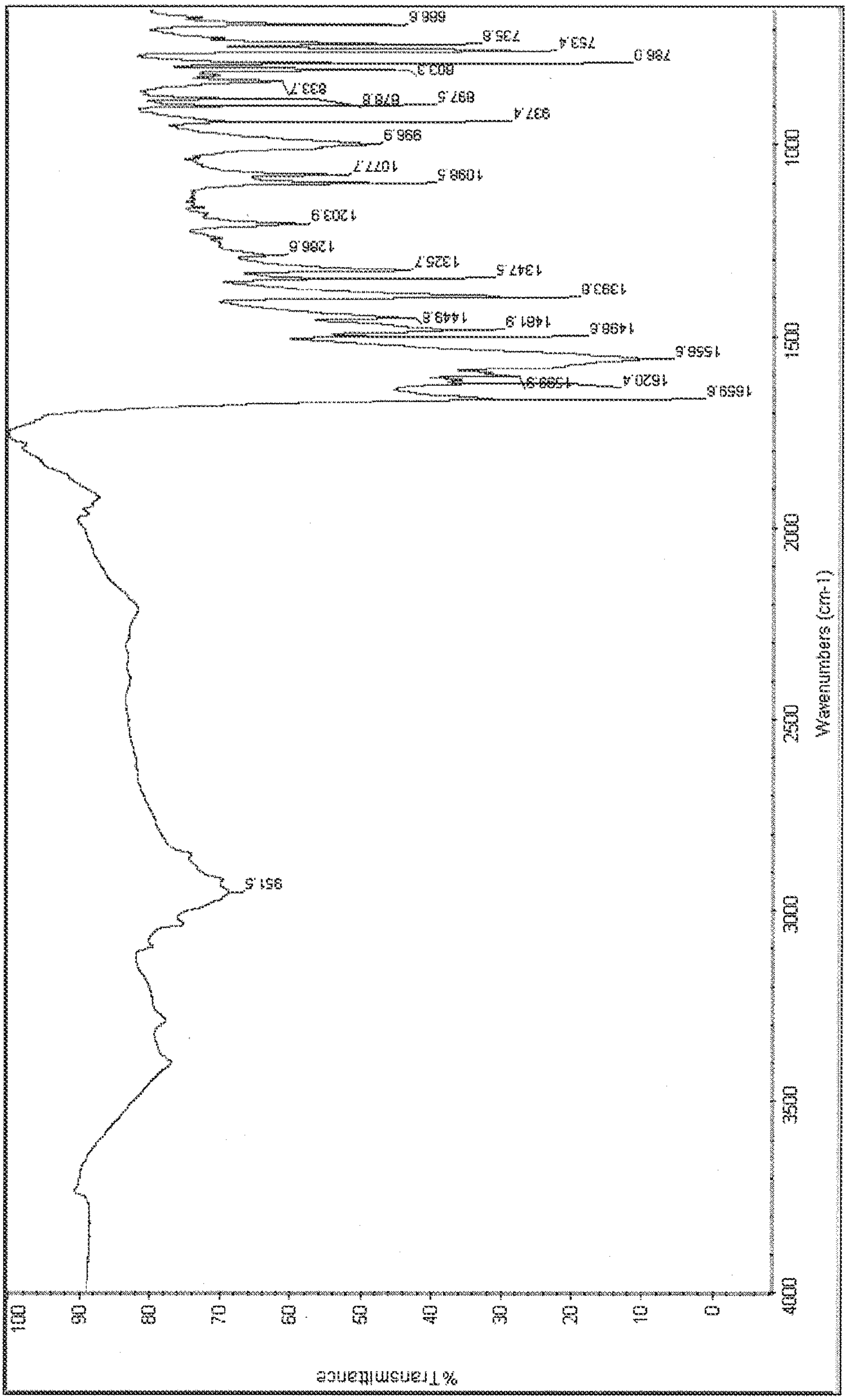

%Transmittance
100
90
80
70
60
50
40
30
20
10
0
4000
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)
2951.5
1659.6
1620.4
1589.9
1558.5
1498.6
1481.9
1449.6
1393.6
1347.5
1325.7
1286.6
1203.9
1098.5
1077.7
996.9
937.4
897.5
878.8
833.7
803.3
786.0
753.4
735.6
666.6

Figure 29.

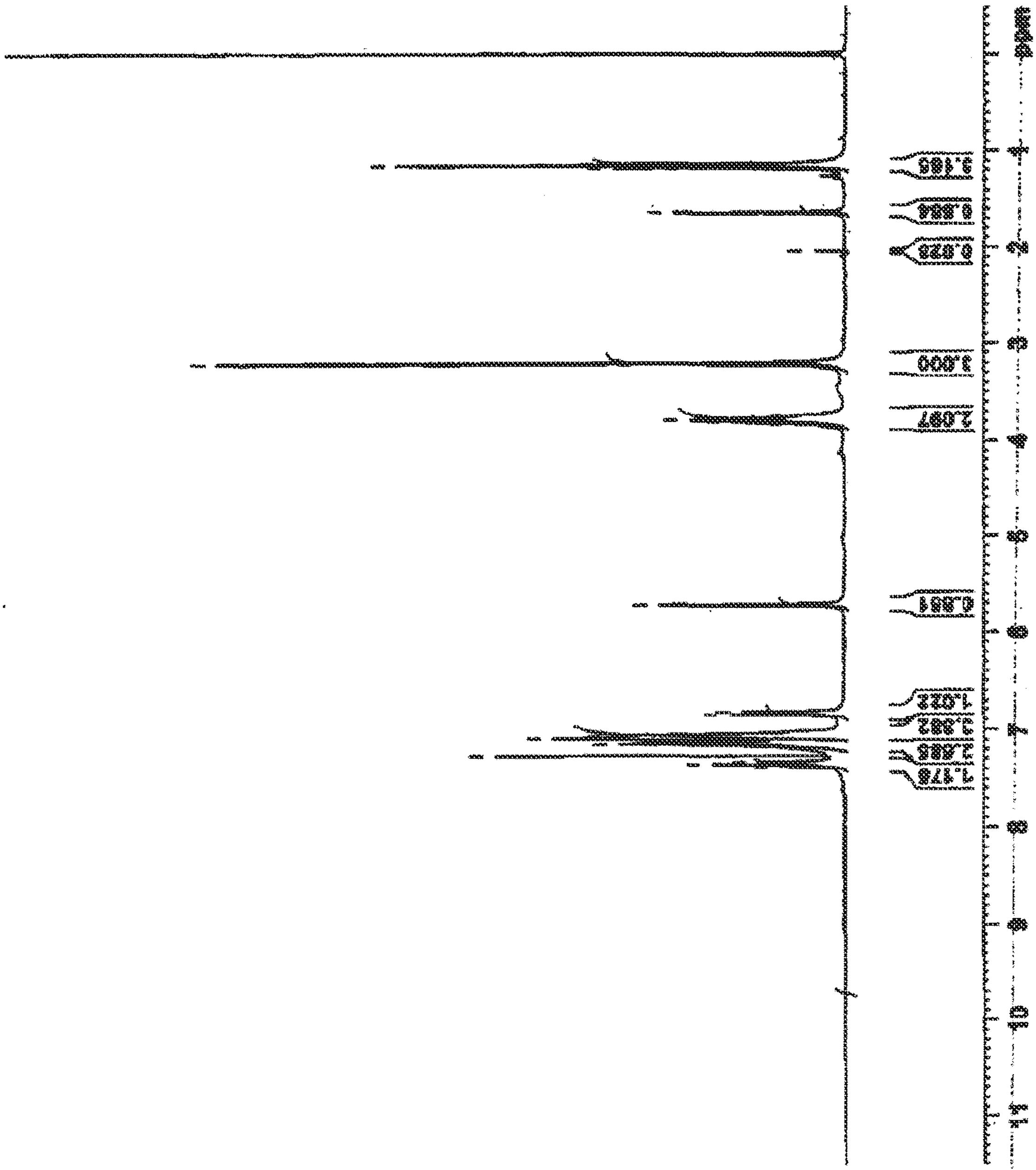

1.978
2.888
3.382
1.022
0.881
2.097
3.000
0.928
0.884
3.165
11
10
9
8
7
6
5
4
3
2
1
ppm

Figure 30.

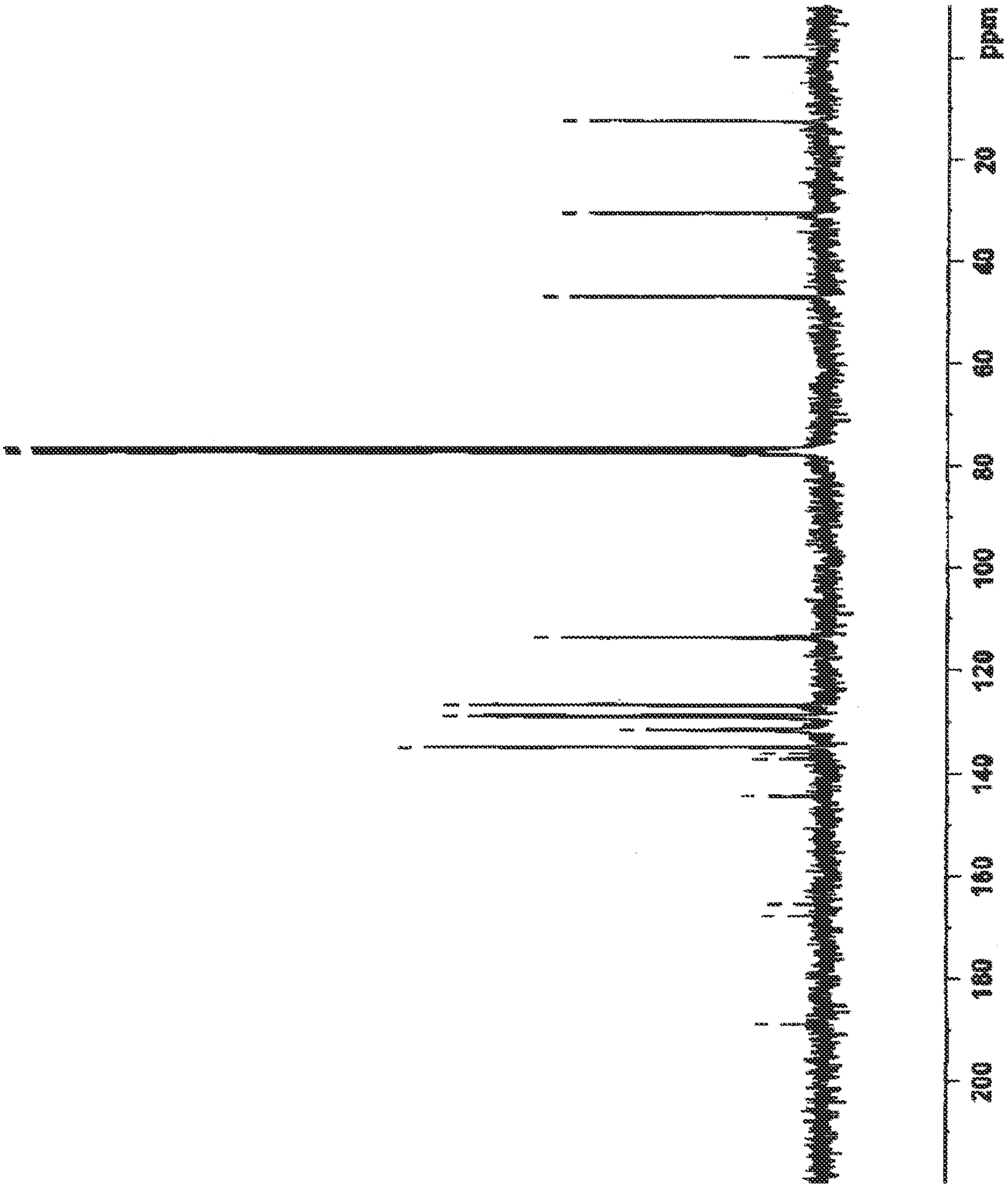


Figure 31.

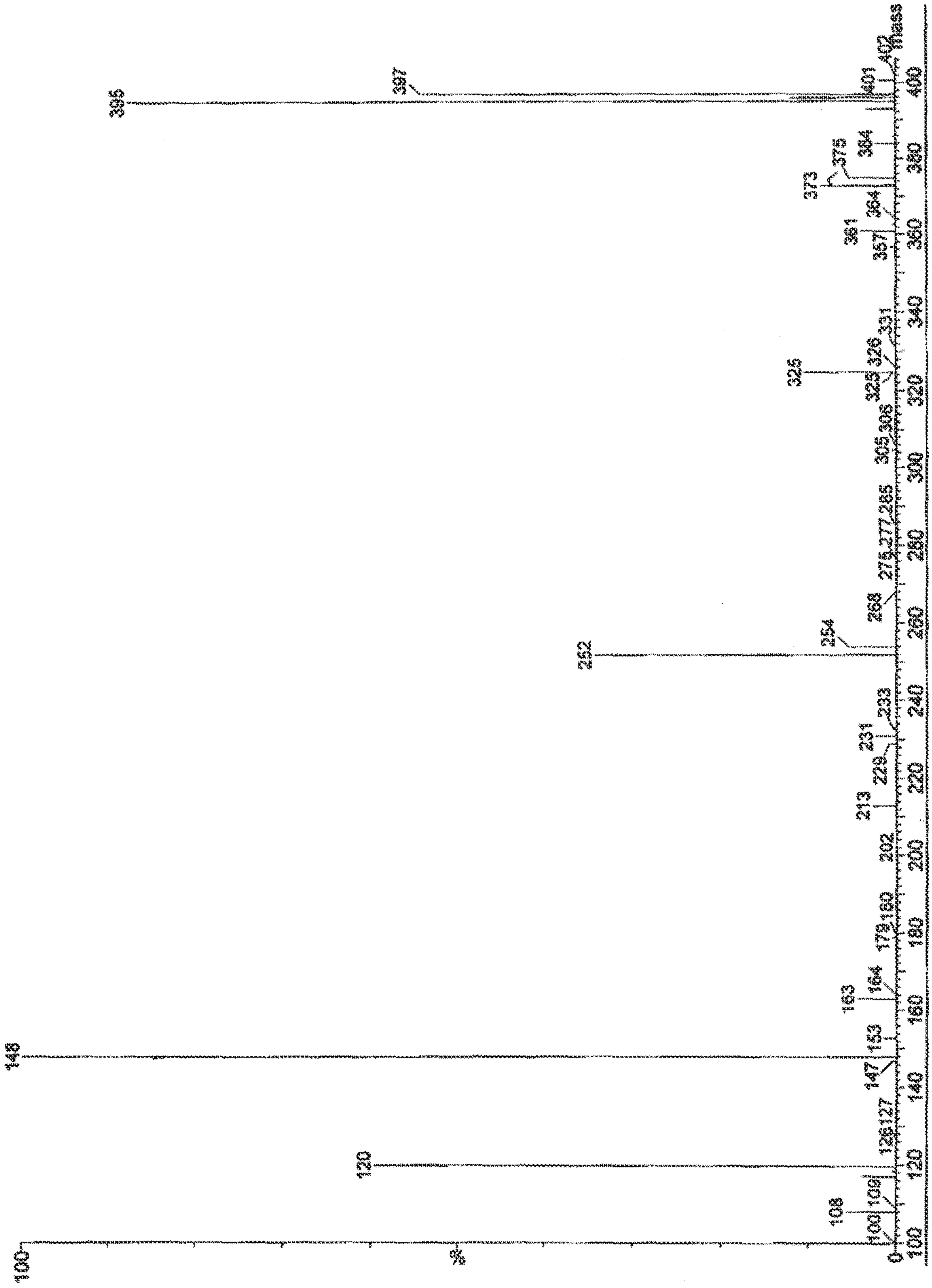

100
%
0
100
108
109
120
126
127
147
148
153
163
164
179
180
202
213
229
231
233
252
254
268
275
277
285
305
306
325
325
326
331
357
361
364
373
375
384
395
397
401
402
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
mass

Figure 32.

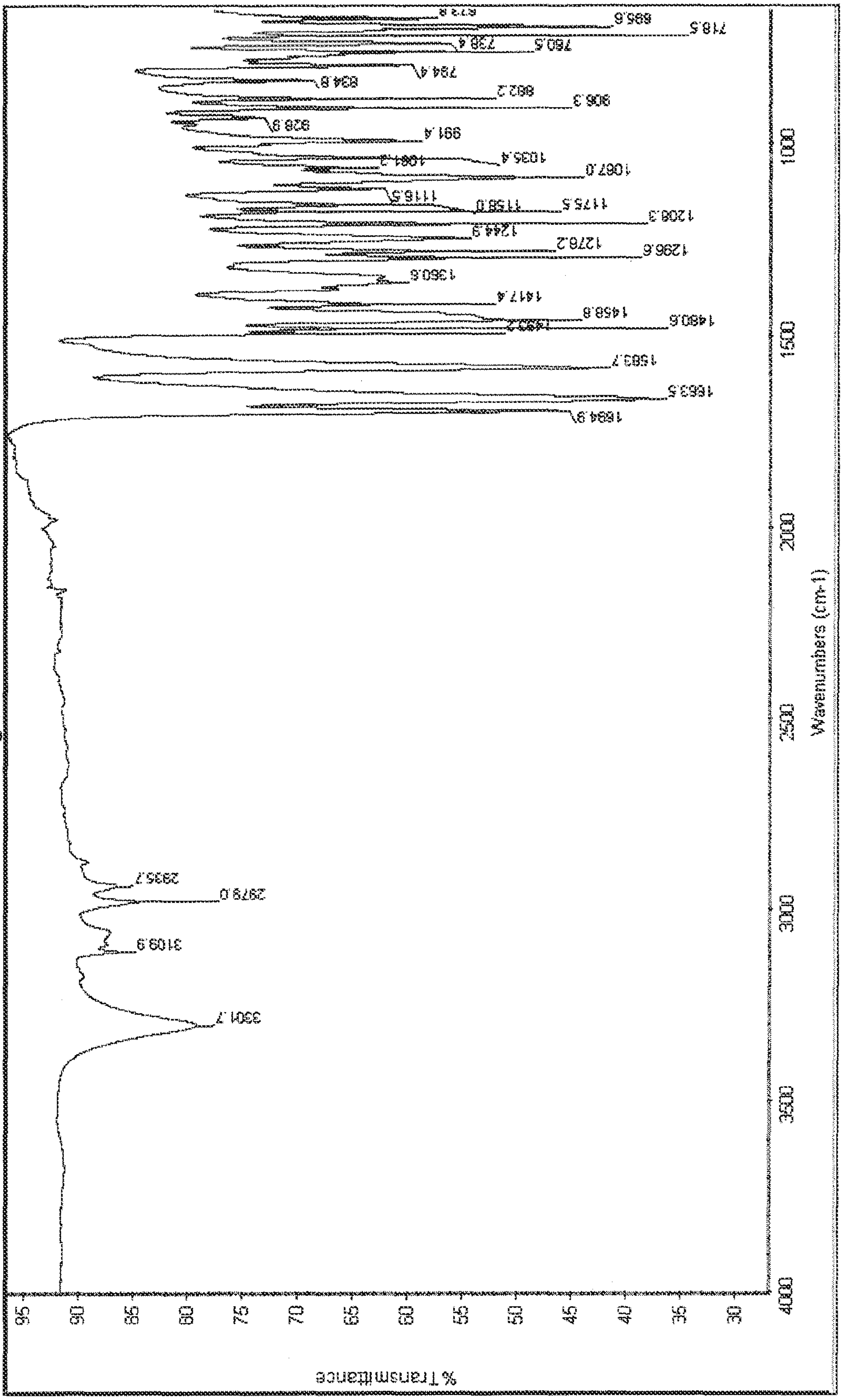

% Transmittance
95
90
85
80
75
70
65
60
55
50
45
40
35
30
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
3301.7
3109.9
2979.0
2935.7
1694.9
1663.5
1583.7
1493.2
1480.6
1458.8
1417.4
1360.6
1296.6
1278.2
1244.9
1208.3
1175.5
1158.0
1116.5
1087.0
1081.2
1035.4
991.4
928.9
906.3
882.2
834.8
794.4
760.5
738.4
718.5
685.6
672.8

Figure 33.

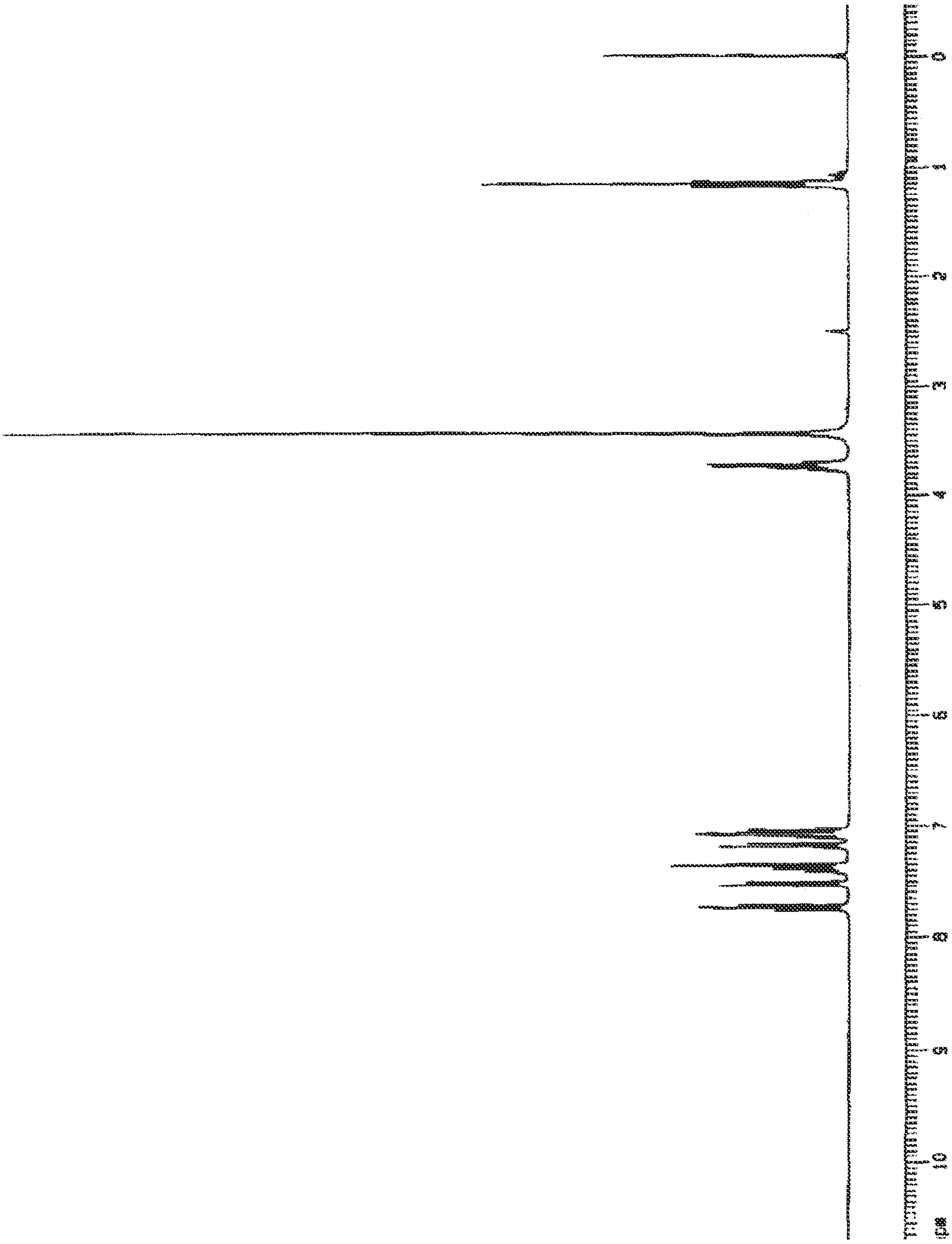


Figure 34.

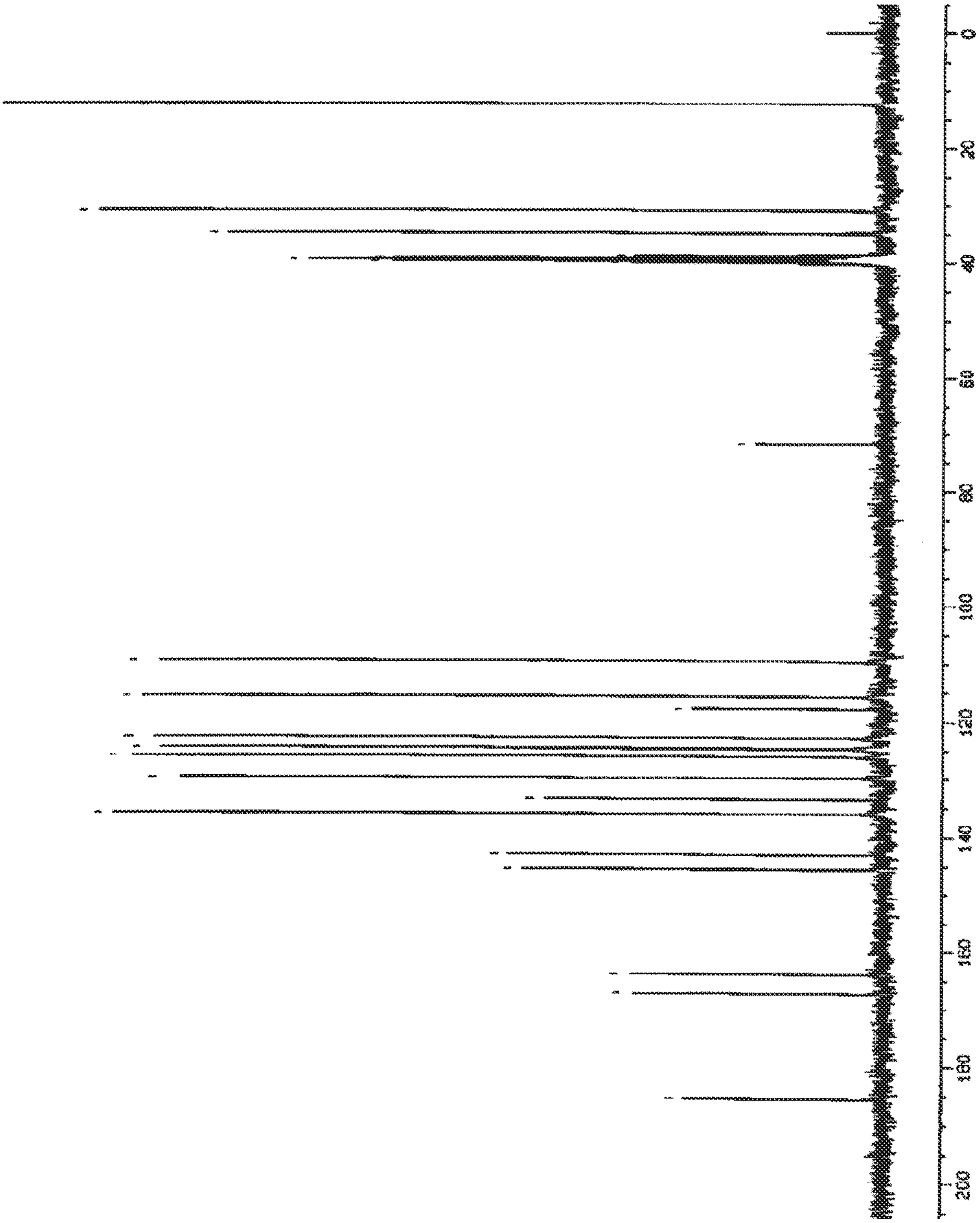


Figure 35.

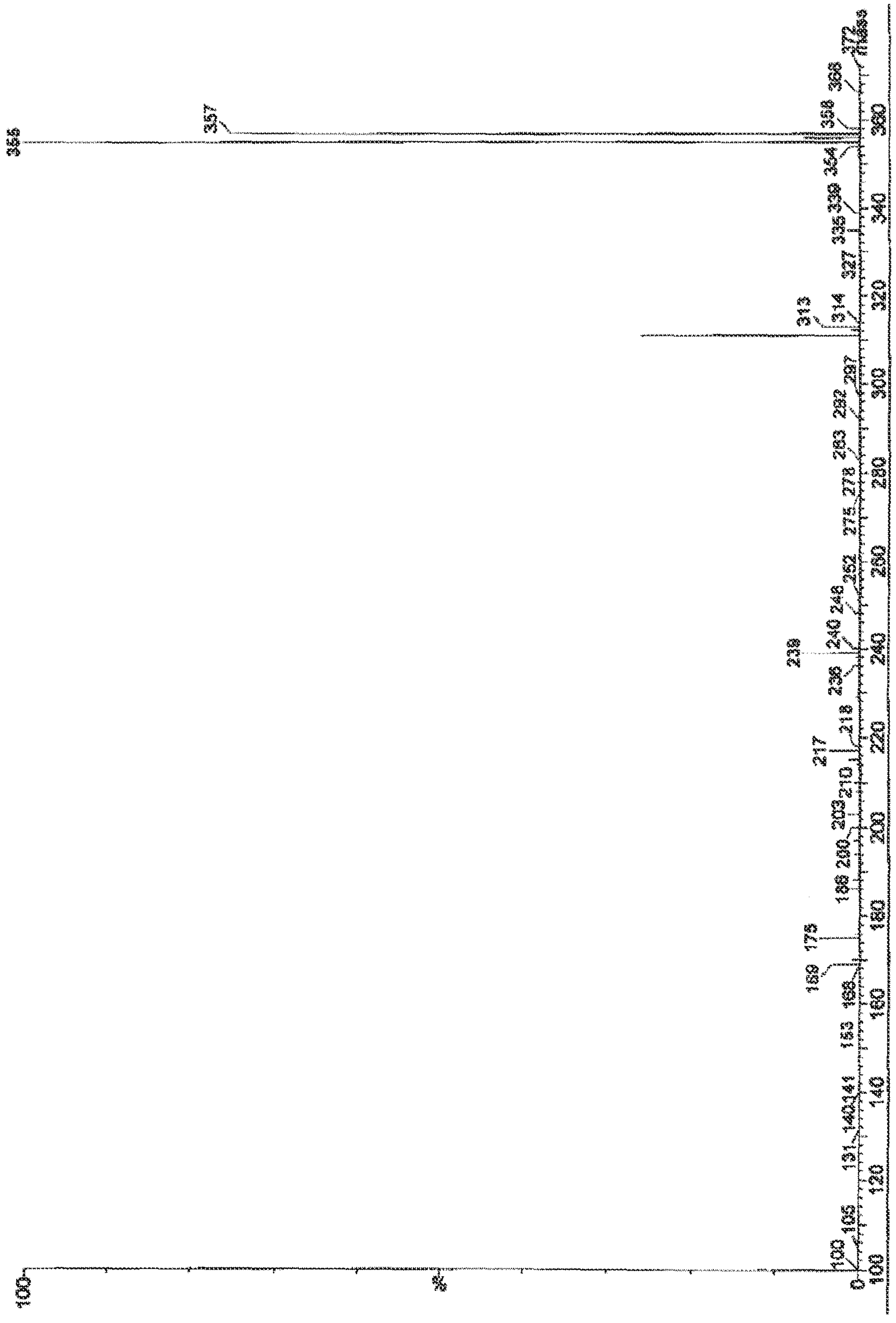

100
%
0
355
357
313
238
217
175
169
100
105
131
140
141
153
168
188
200
203
210
218
236
240
248
252
275
278
283
292
297
314
327
335
339
354
358
368
372
mass
100
120
140
160
180
200
220
240
260
280
300
320
340
360

Figure 36.

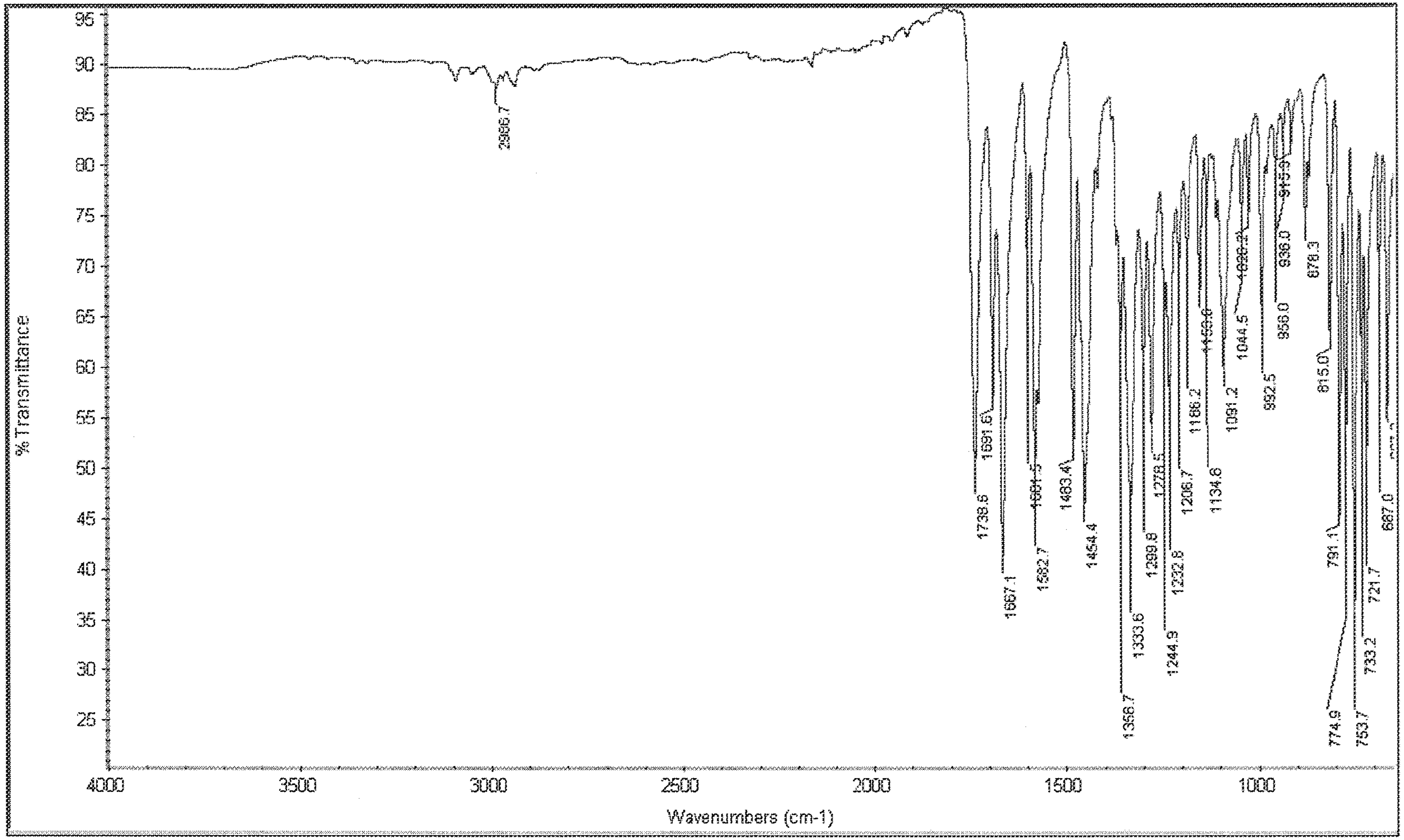

% Transmittance
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
4000
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)
2986.7
1738.5
1691.6
1667.1
1601.5
1582.7
1483.4
1454.4
1358.7
1333.6
1299.8
1278.5
1244.9
1232.8
1206.7
1186.2
1160.6
1134.6
1091.2
1044.5
1028.2
992.5
956.0
936.0
915.9
878.3
815.0
791.1
774.9
753.7
733.2
721.7
687.0

Figure 37.

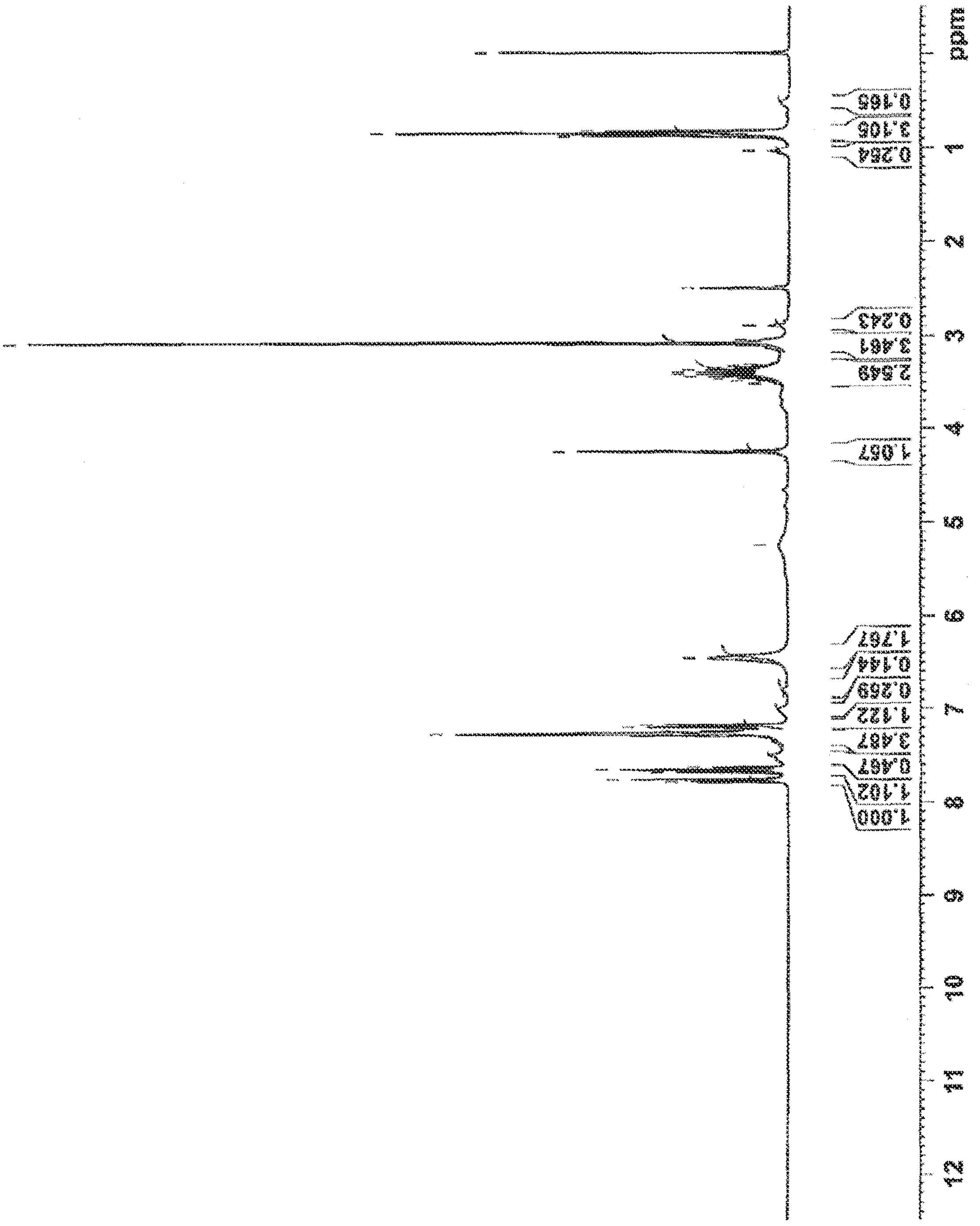

0.165
3.106
0.264
0.243
3.461
2.549
1.067
1.767
0.144
0.269
1.122
3.467
0.467
1.102
1.000
ppm
1
2
3
4
5
6
7
8
9
10
11
12

Figure 38.

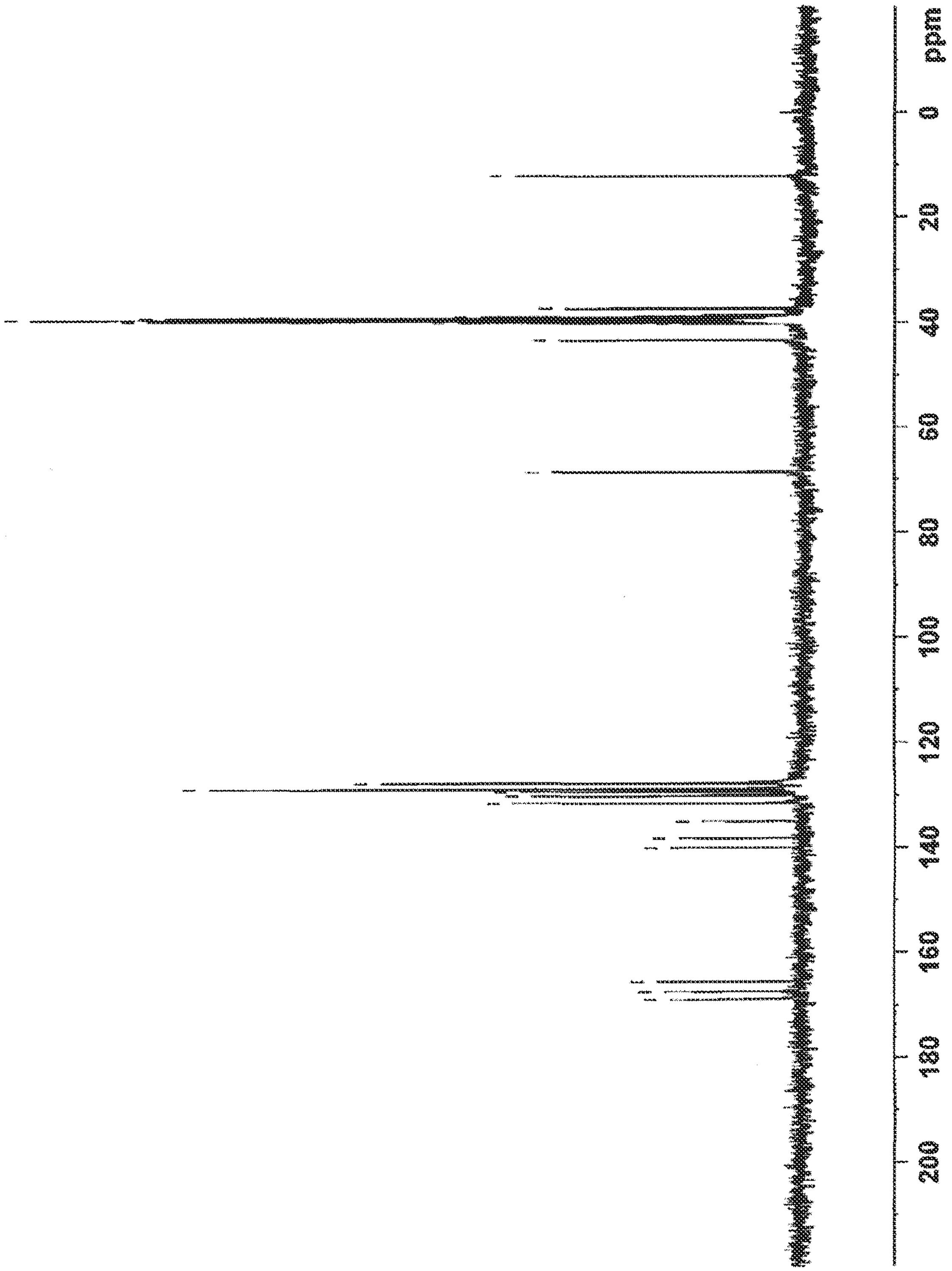


Figure 39.

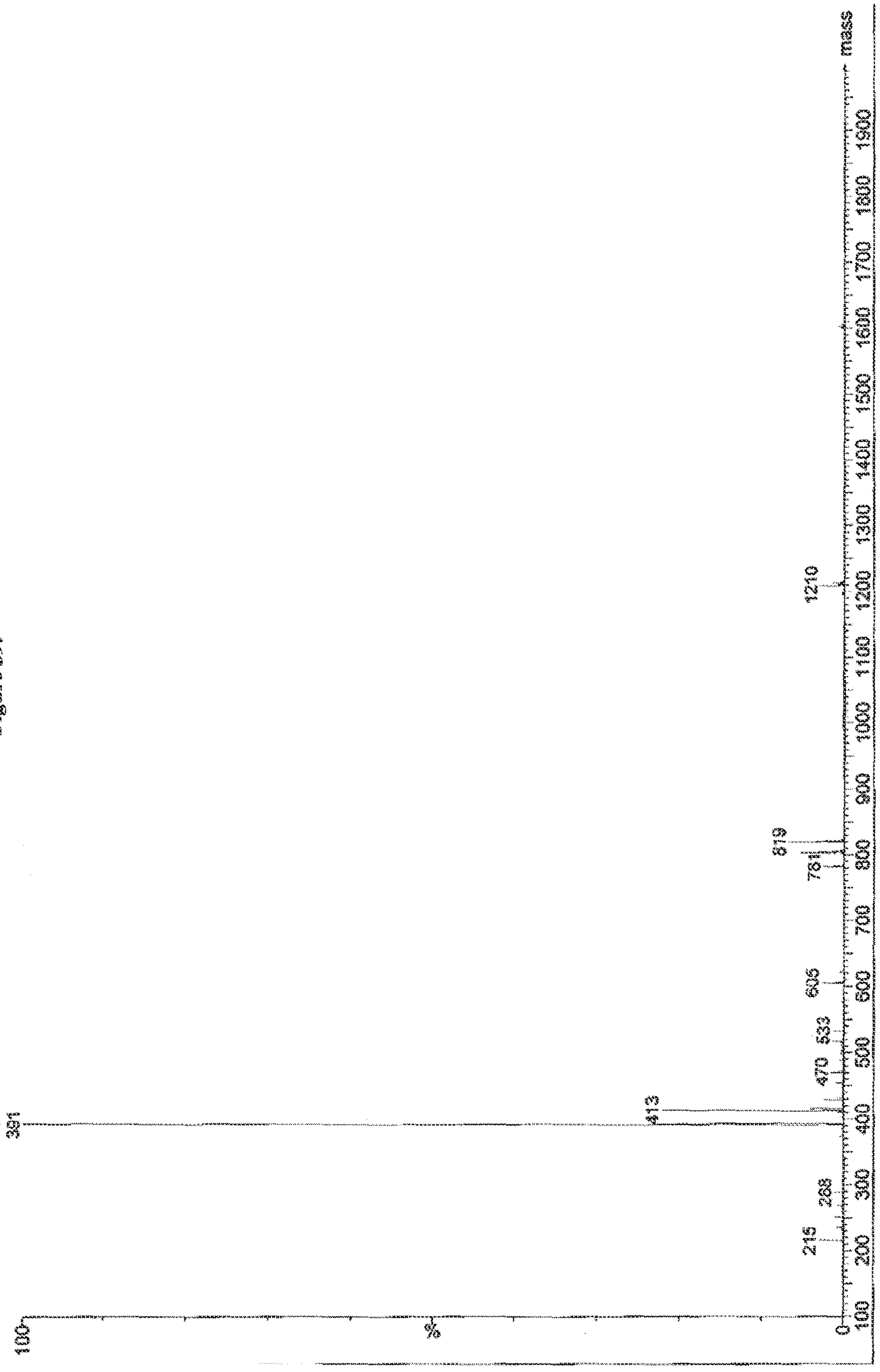

100
%
0
391
413
215
268
470
533
605
761
819
1210
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
mass

Figure 40.

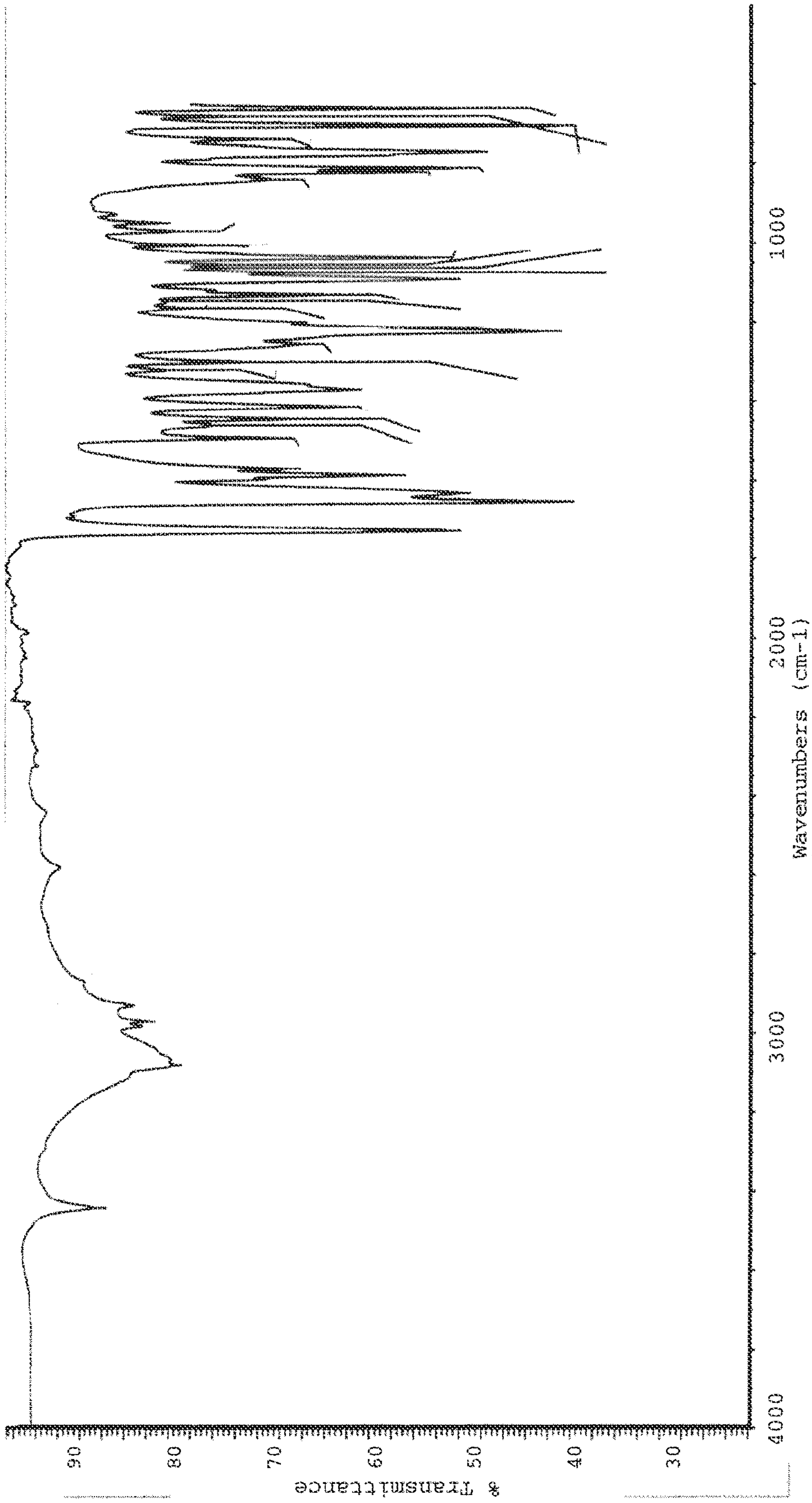
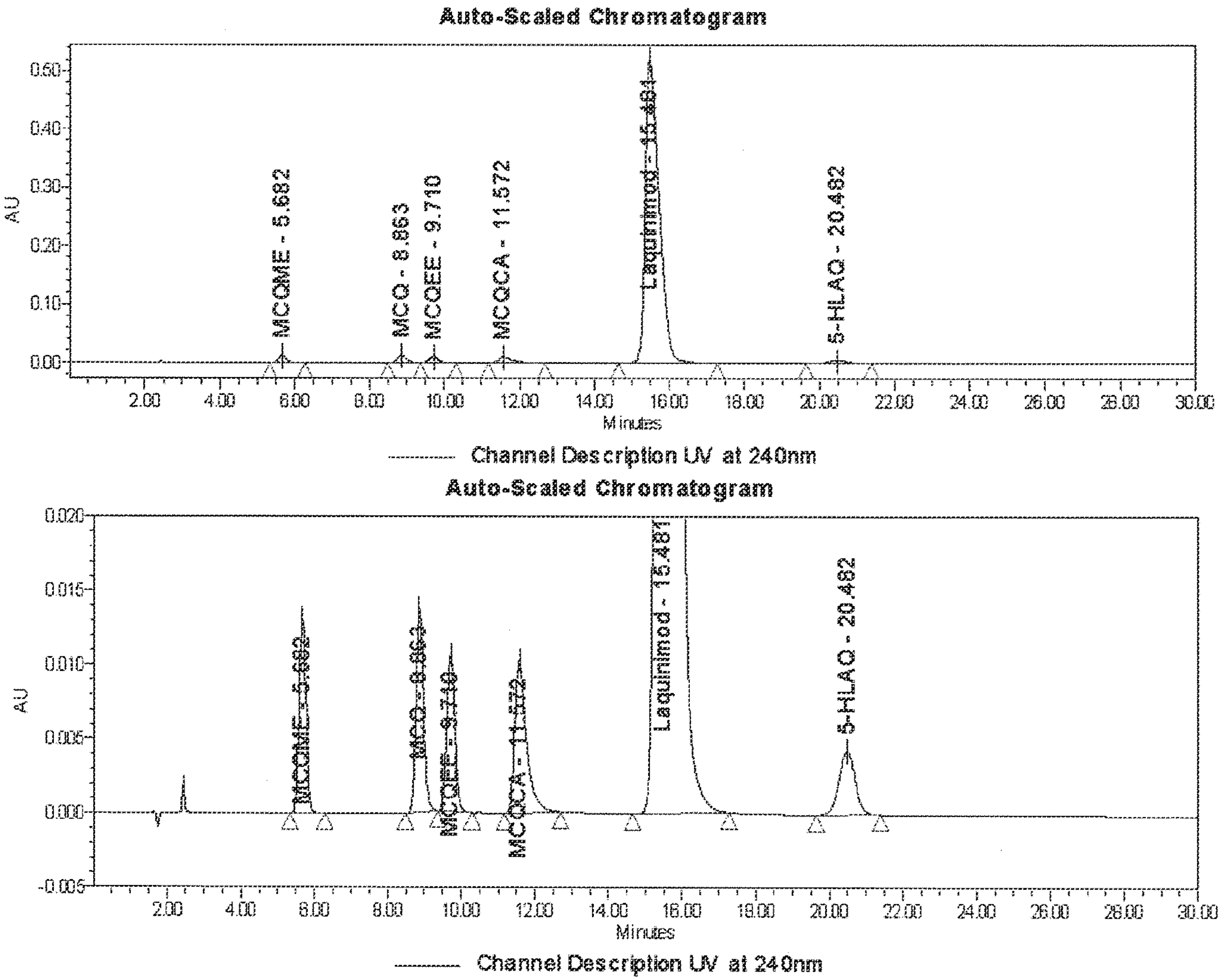

% Transmittance
90
80
70
60
50
40
30
Wavenumbers (cm-1)
4000
3000
2000
1000

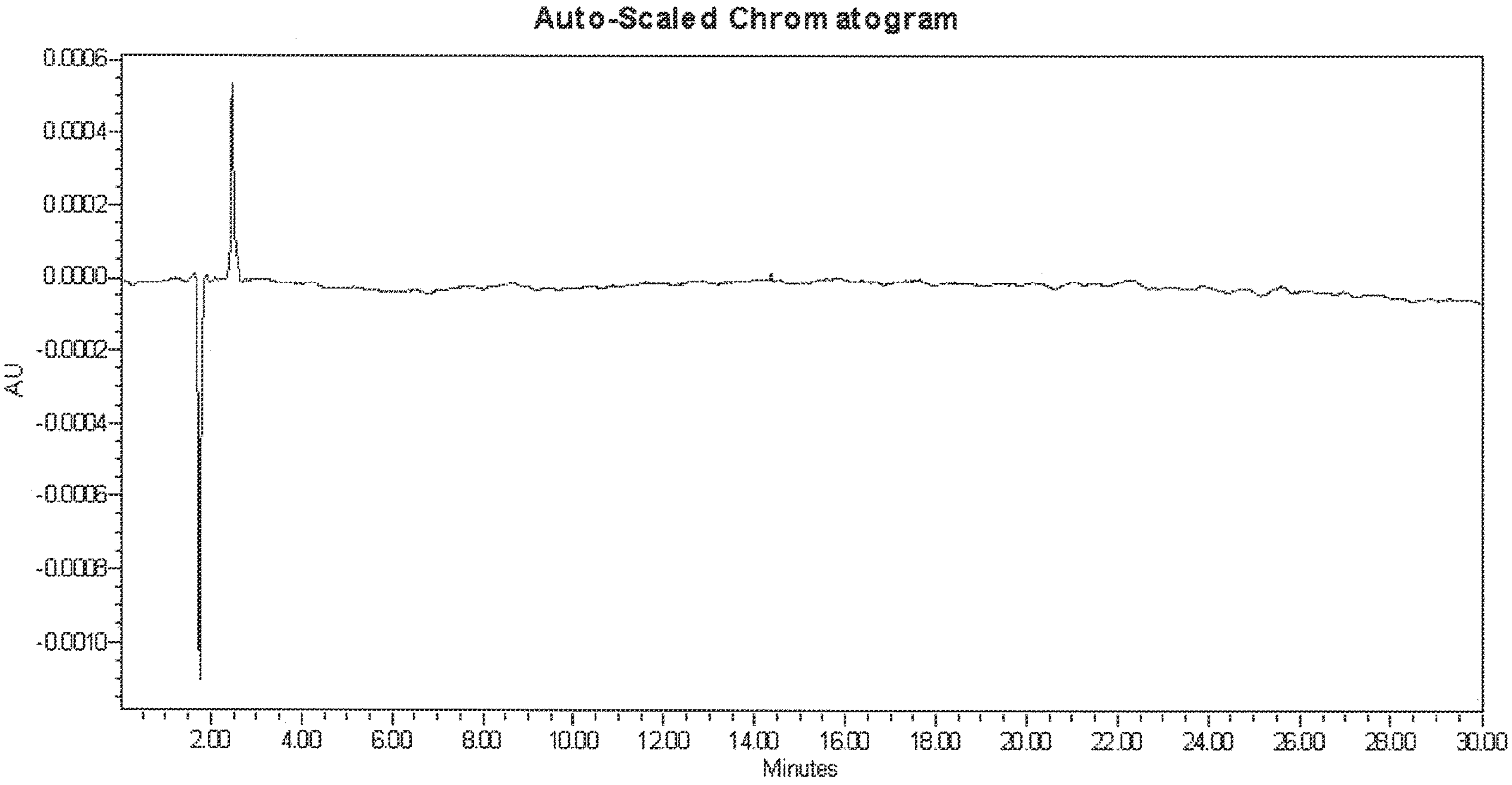
Figure 41.



Auto-Scaled Chromatogram

Auto-Scaled Chromatogram

Figure 42.



Auto-Scaled Chromatogram

Figure 43.

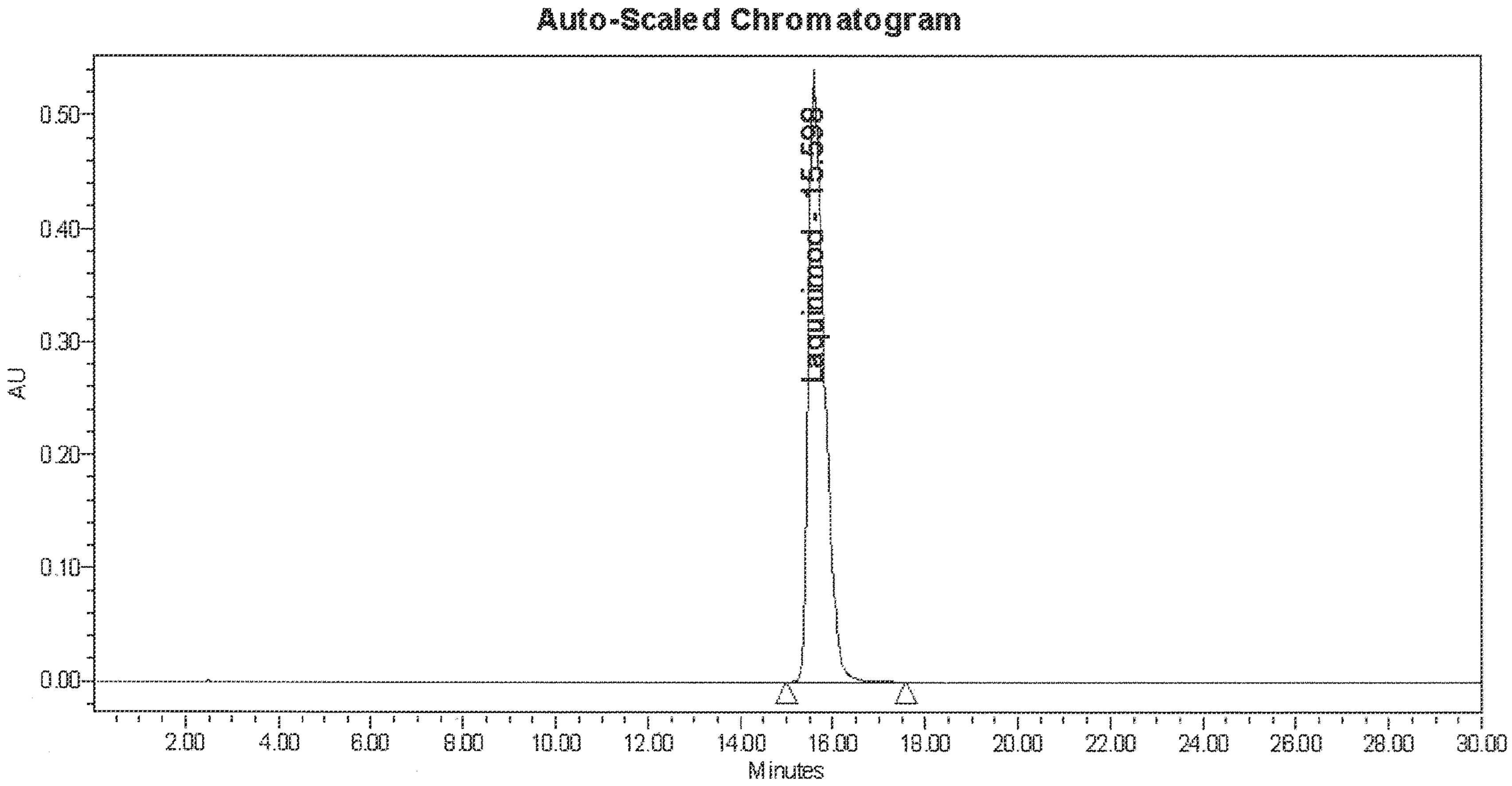
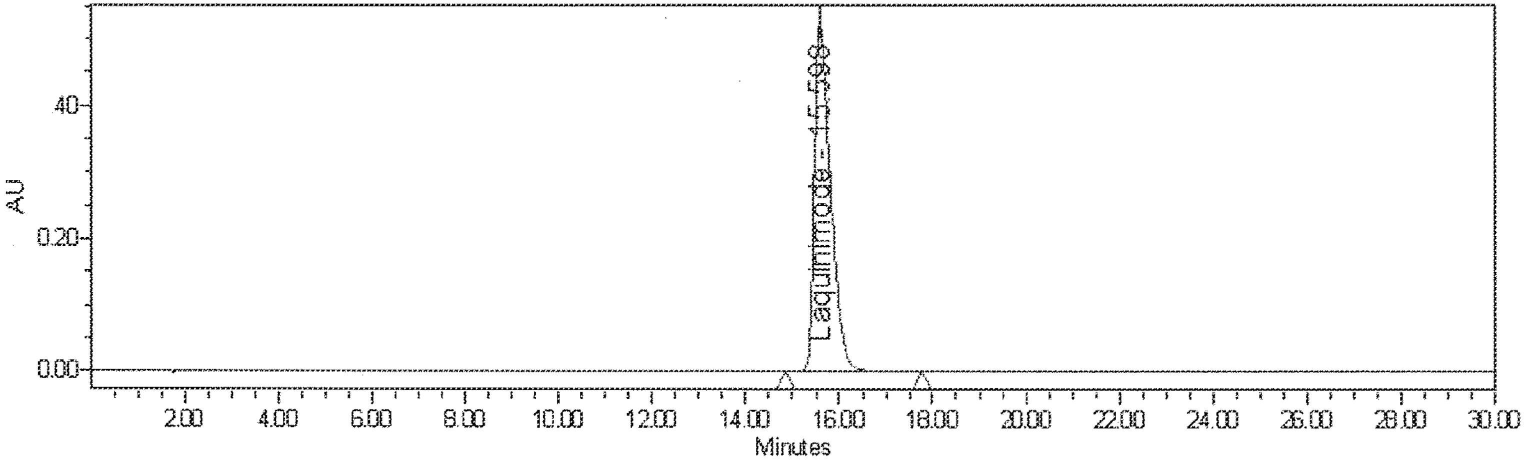
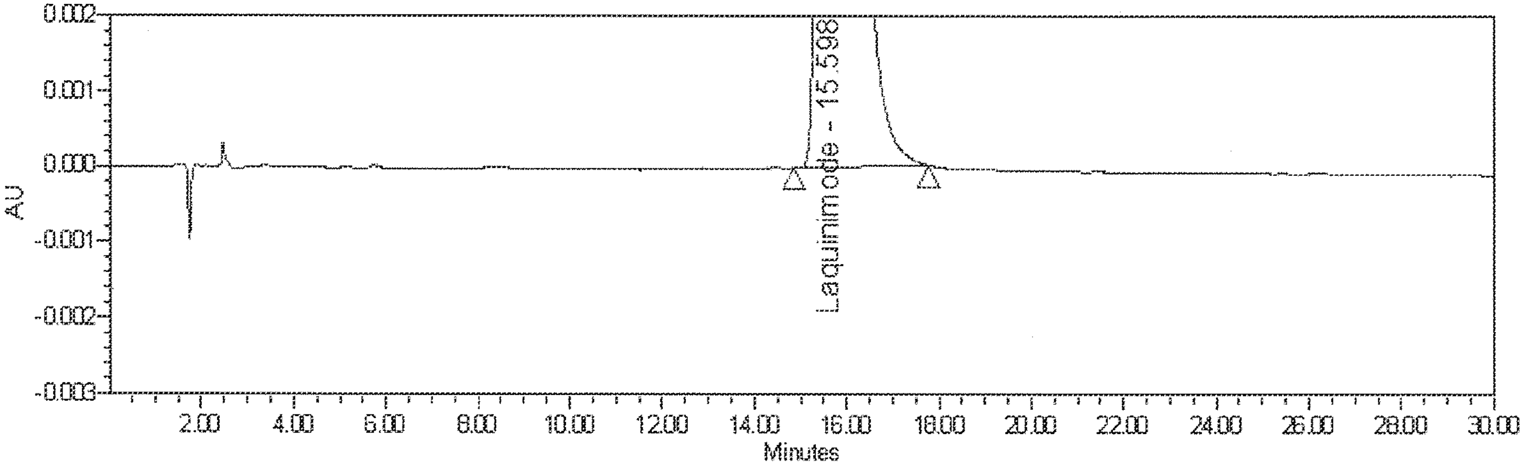

Auto-Scaled Chromatogram
AU
0.50
0.40
0.30
0.20
0.10
0.00
Laquinimod - 15.598
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Minutes

Figure 44.

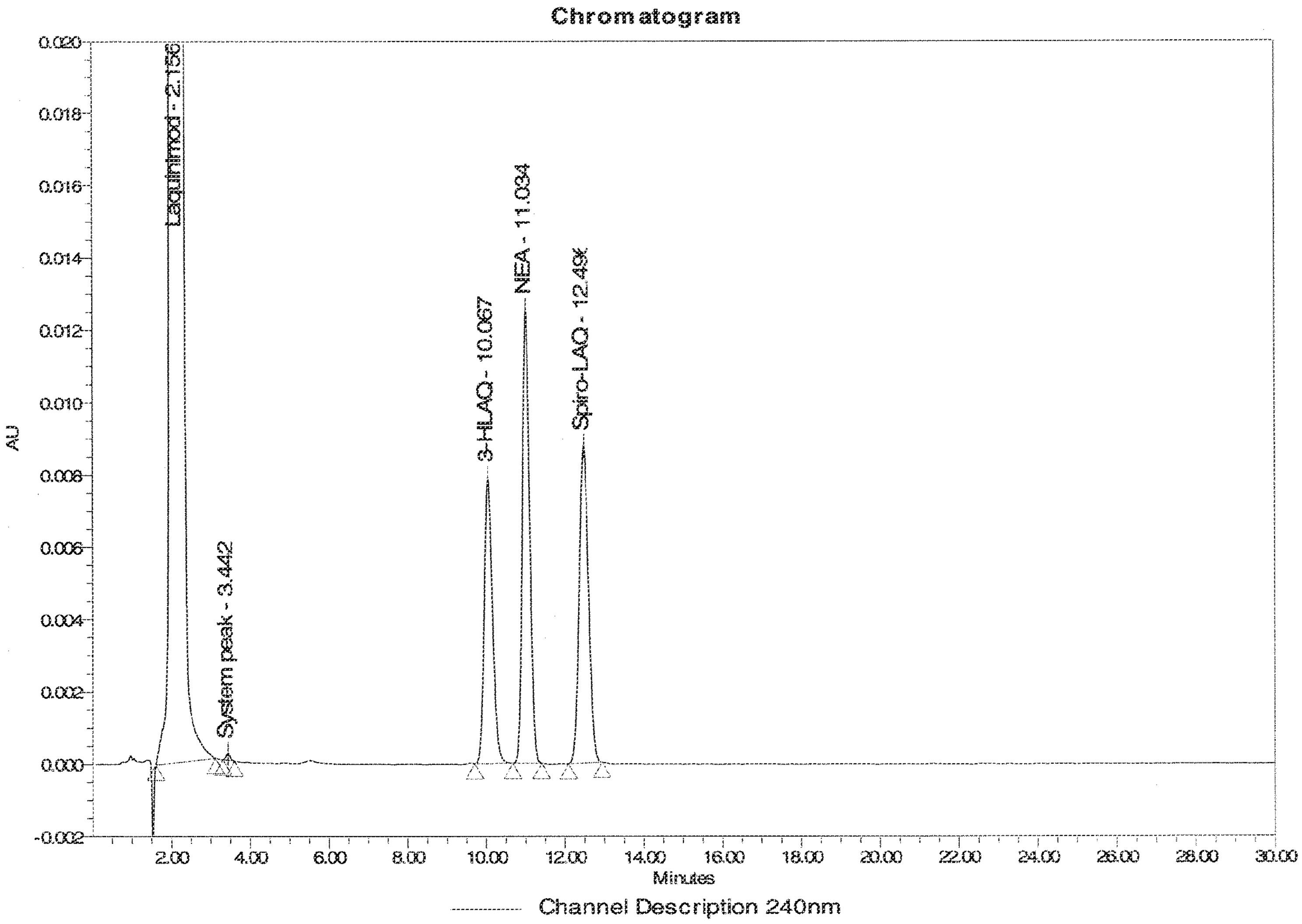


Auto-Scaled Chromatogram



Auto-Scaled Chromatogram

Figure 45.



Chromatogram

Figure 46.

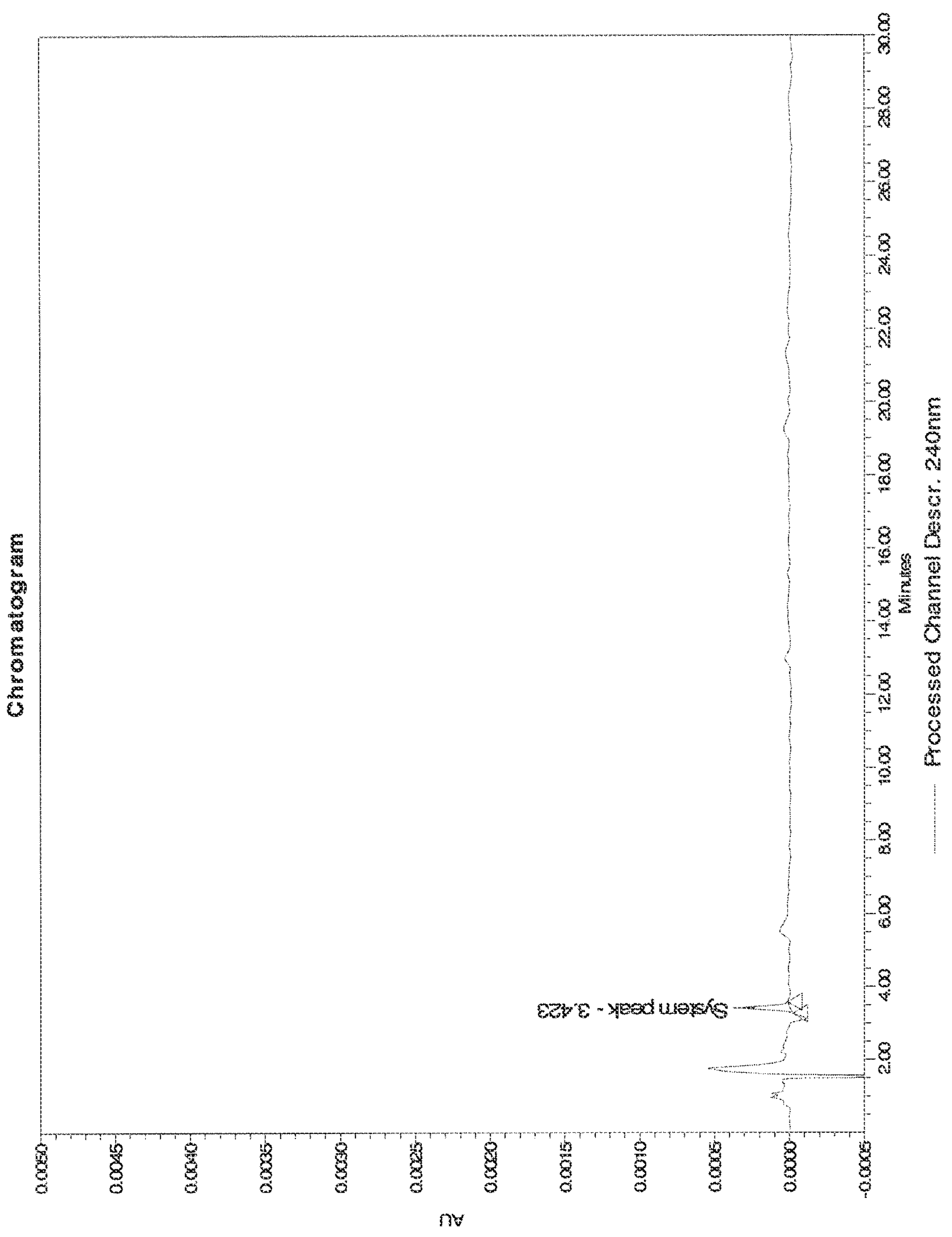

Chromatogram
AU
0.0050
0.0045
0.0040
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
-0.0005
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Minutes
System peak - 3.423
Processed Channel Descr. 240nm

Figure 47.

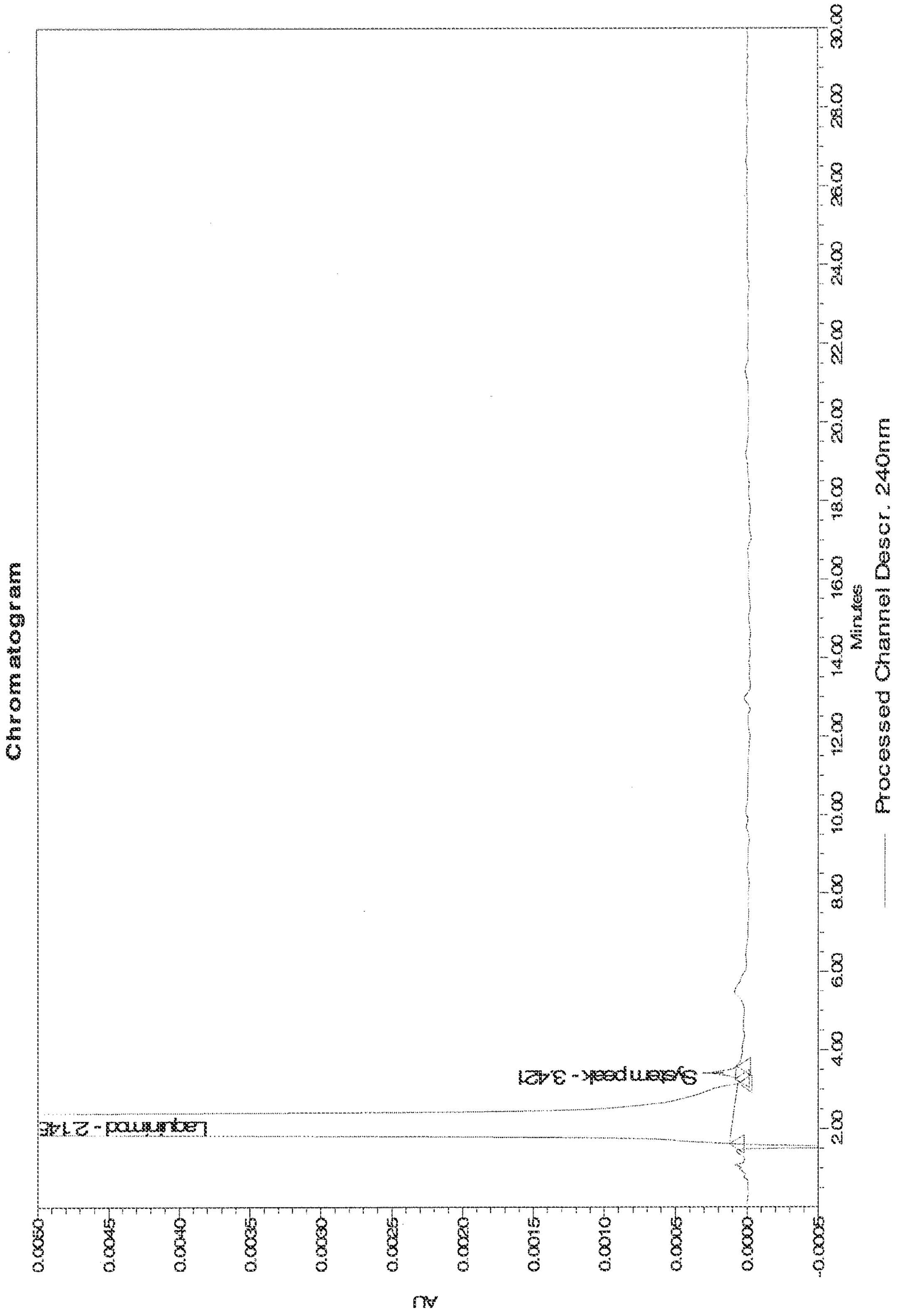

Chromatogram
AU
0.0050
0.0045
0.0040
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
-0.0005
Laquinimod - 2.14
System peak - 3.421
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Minutes
Processed Channel Descr. 240nm

Figure 48.

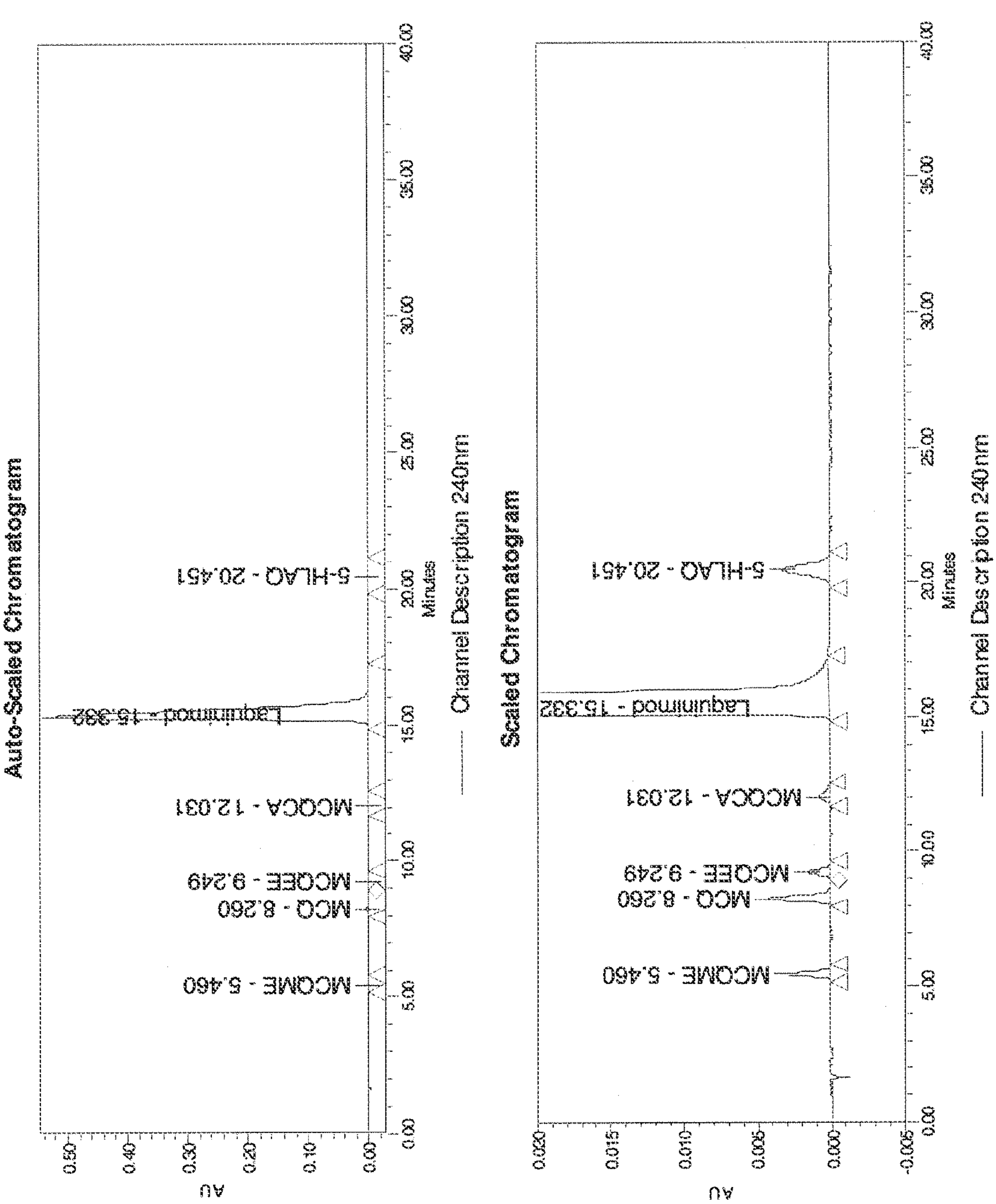
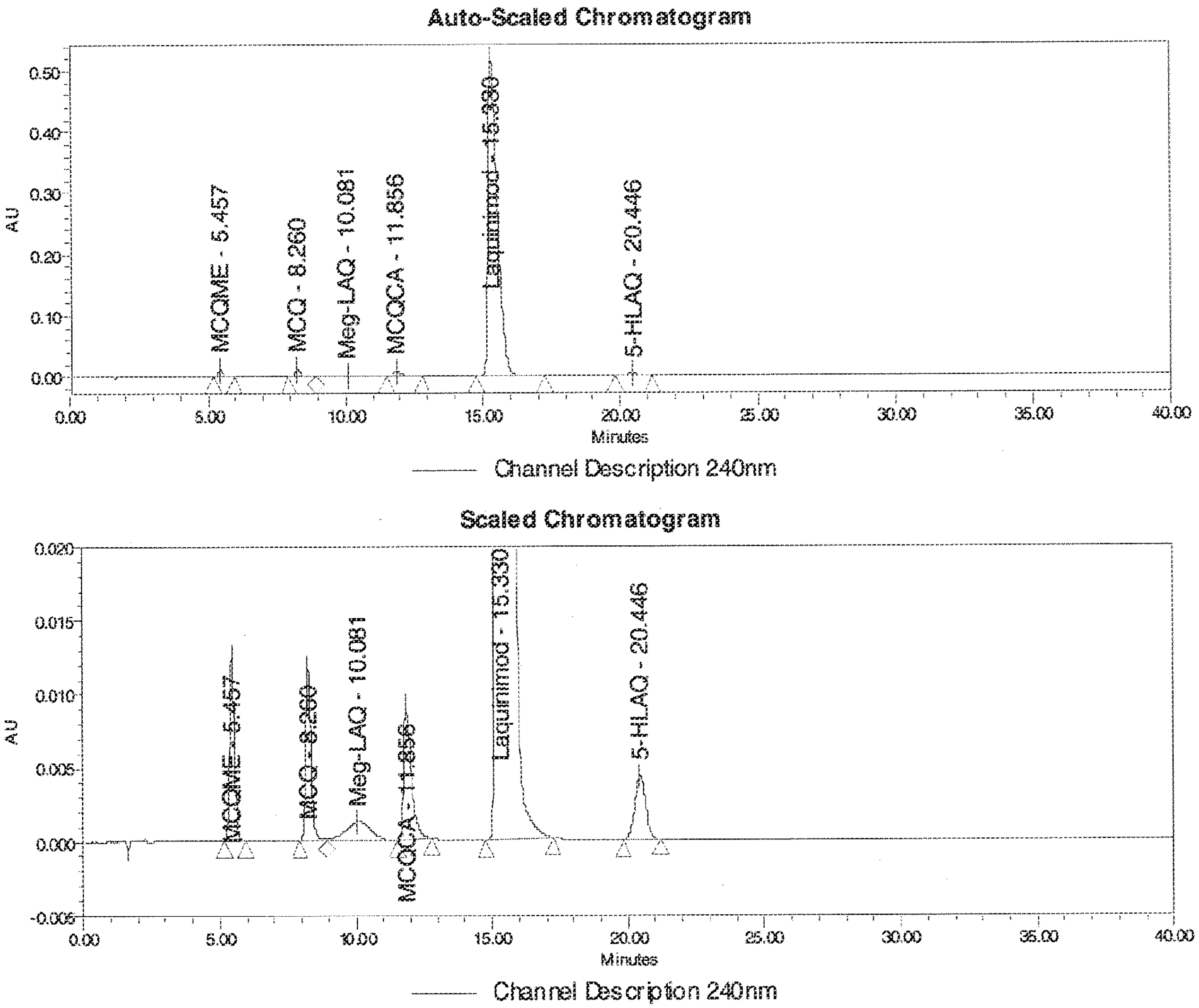

Auto-Scaled Chromatogram
MCQME - 5.460
MCQ - 8.260
MCQEE - 9.249
MCQCA - 12.031
Laquinimod - 15.332
5-HLAQ - 20.451
AU
0.50
0.40
0.30
0.20
0.10
0.00
Minutes
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
Channel Description 240nm
Scaled Chromatogram
MCQME - 5.460
MCQ - 8.260
MCQEE - 9.249
MCQCA - 12.031
Laquinimod - 15.332
5-HLAQ - 20.451
AU
0.020
0.015
0.010
0.005
0.000
-0.005
Minutes
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
Channel Description 240nm

Figure 49.



Auto-Scaled Chromatogram

Scaled Chromatogram

Figure 50

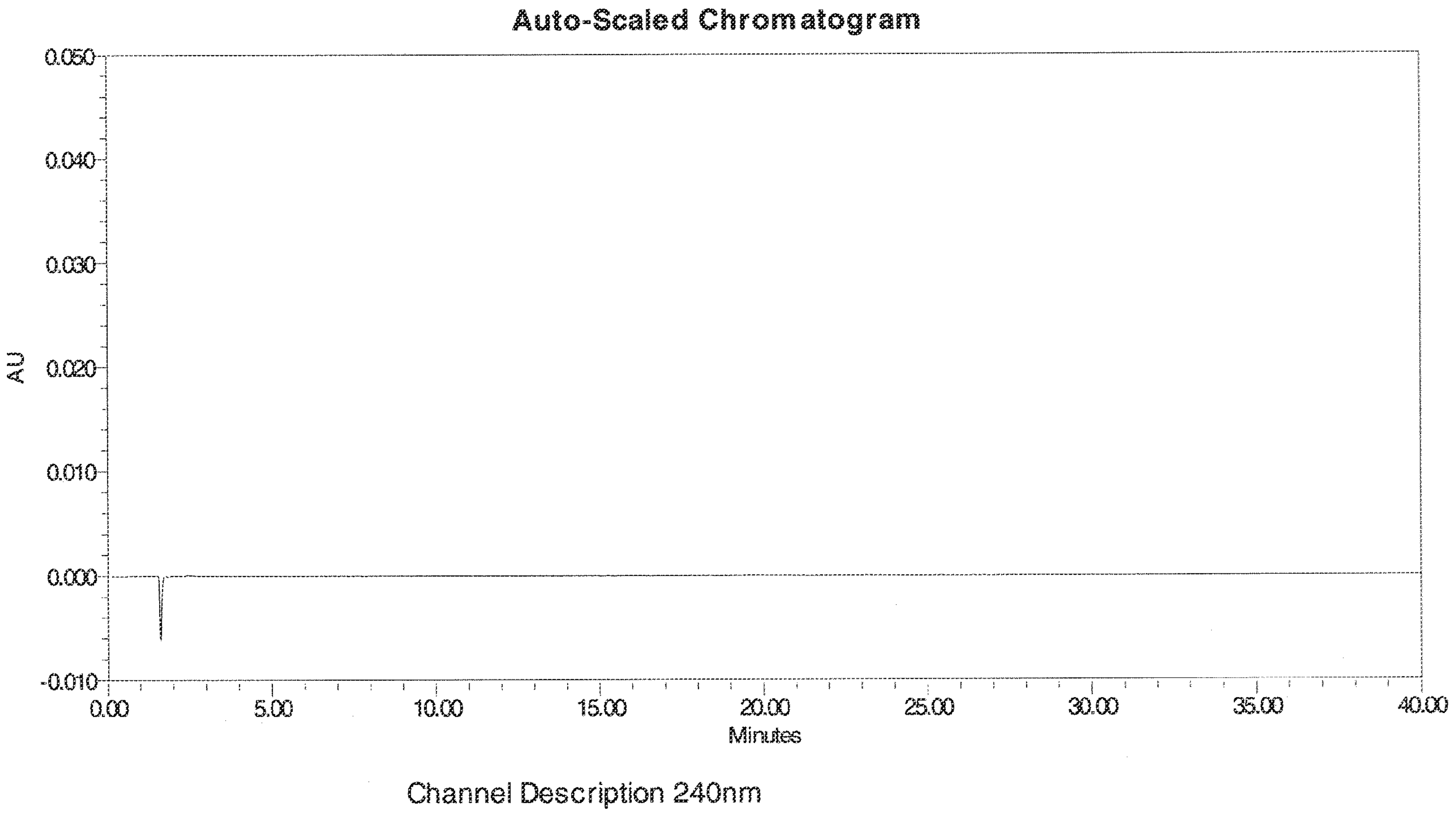

Auto-Scaled Chromatogram
AU
0.050
0.040
0.030
0.020
0.010
0.000
-0.010
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
Minutes
Channel Description 240nm

Figure 51.

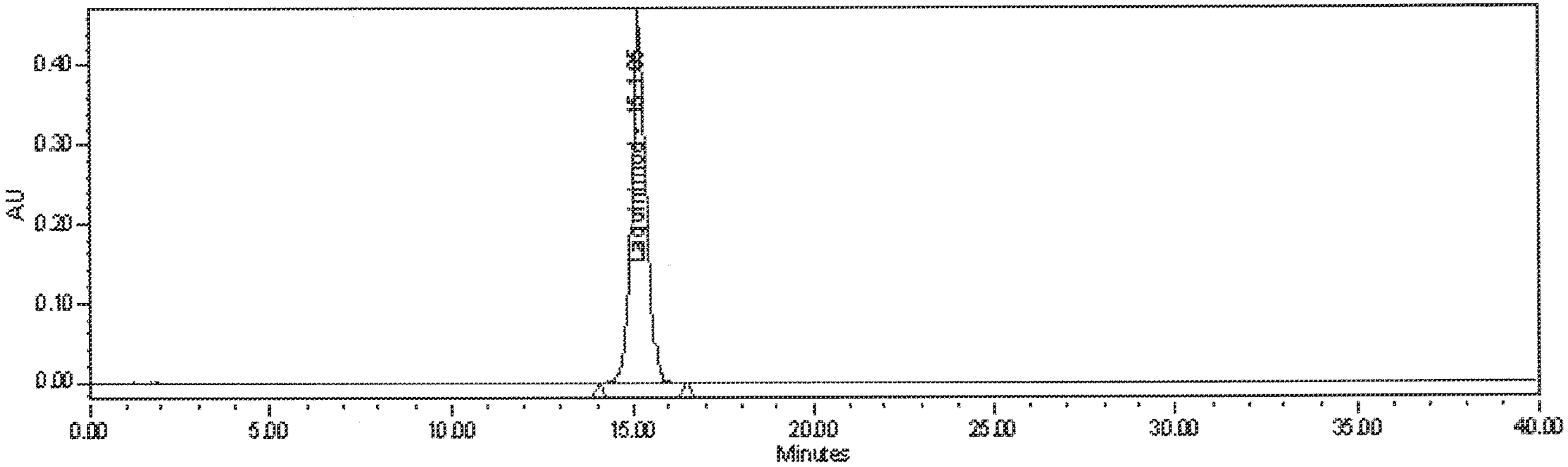

AU
0.40
0.30
0.20
0.10
0.00
Compound - 15.186
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
Minutes

Figure 52.

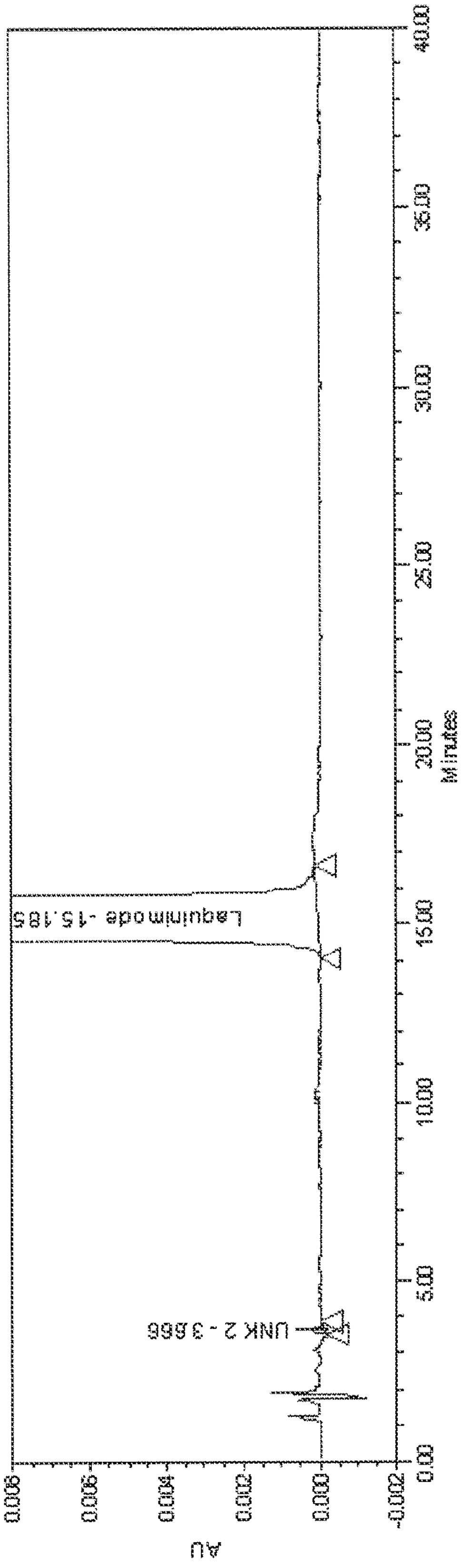

AU
0.008
0.006
0.004
0.002
0.000
-0.002
Minutes
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
UNK 2 - 3.666
Laquinimode -15.185

Figure 53.

Auto-Scaled Chromatogram

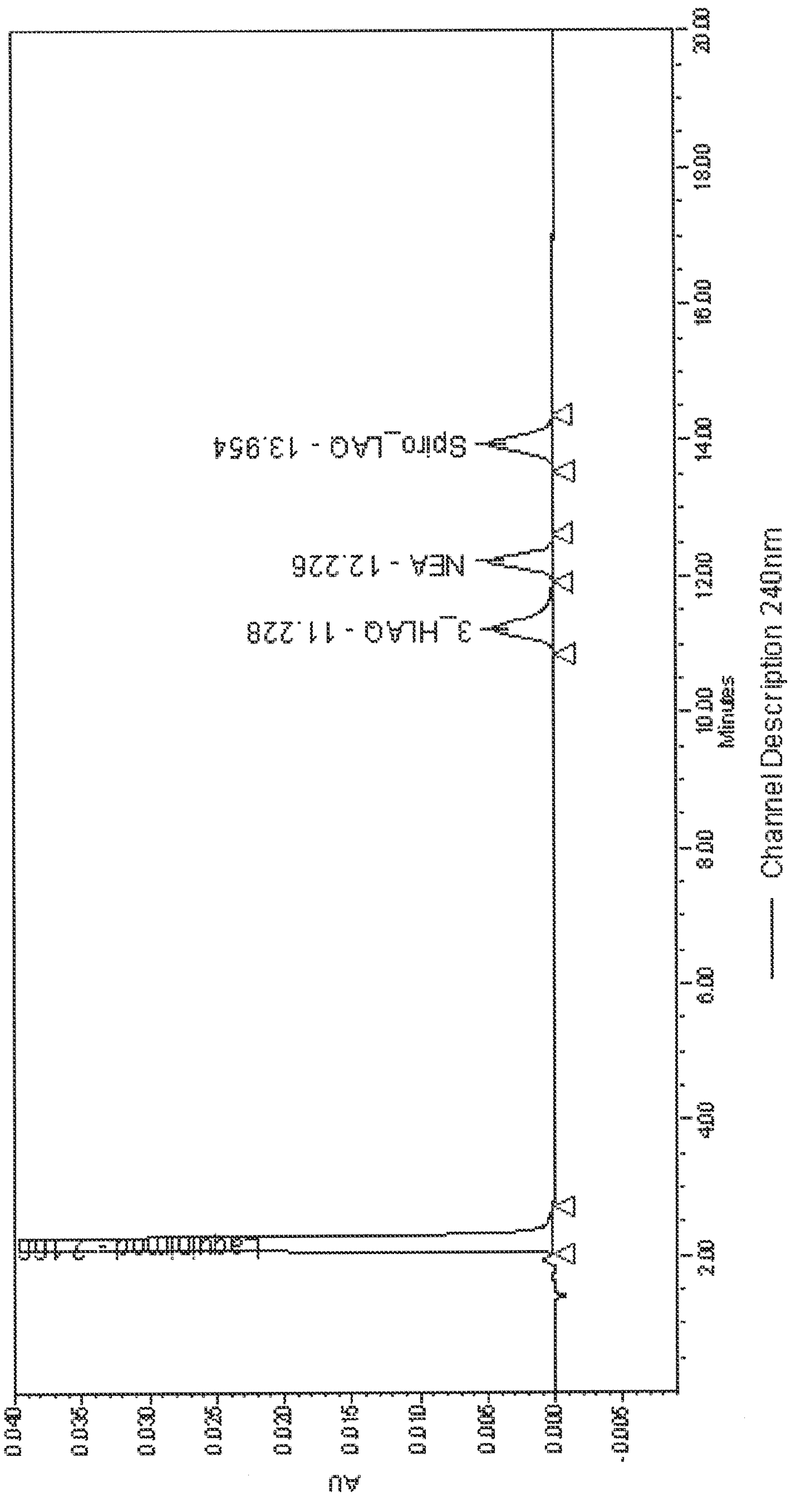


Figure 54.

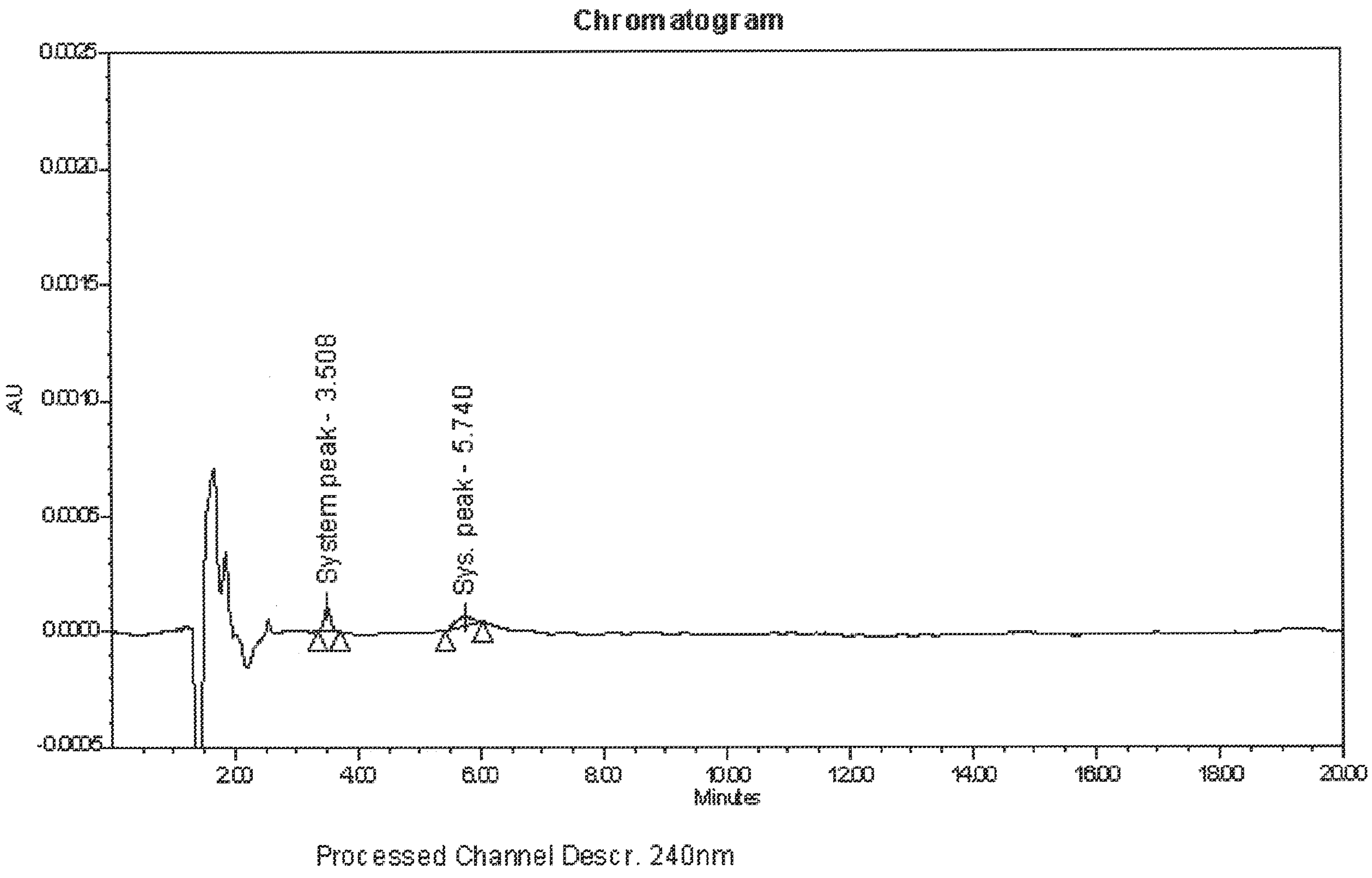

Chromatogram
AU
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
-0.0005
System peak - 3.508
Sys. peak - 5.740
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes
Processed Channel Descr. 240nm

Figure 55.

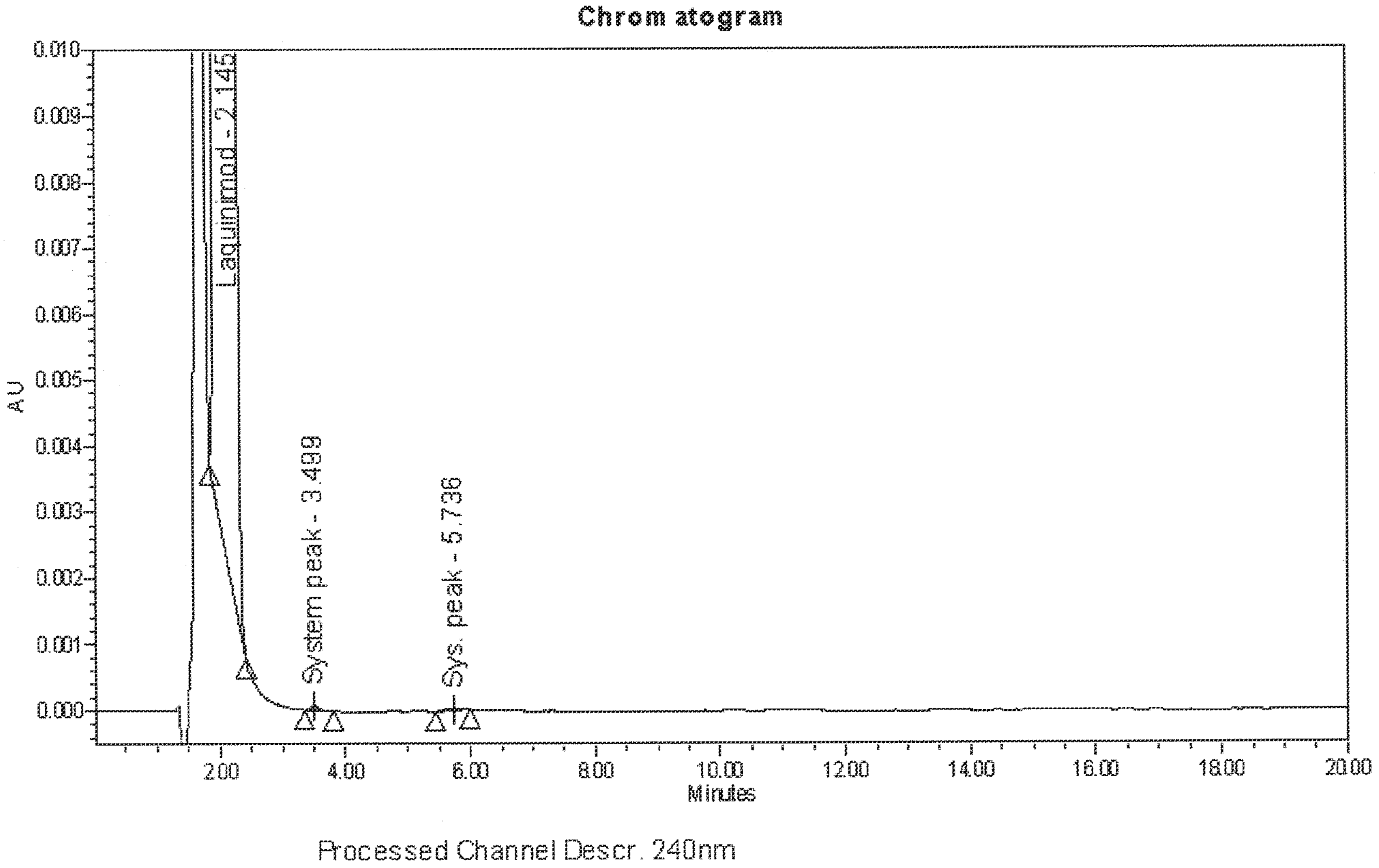

Chrom atogram
AU
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
Laquinimod - 2.145
System peak - 3.499
Sys. peak - 5.736
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes
Processed Channel Descr. 240nm

Figure 56.

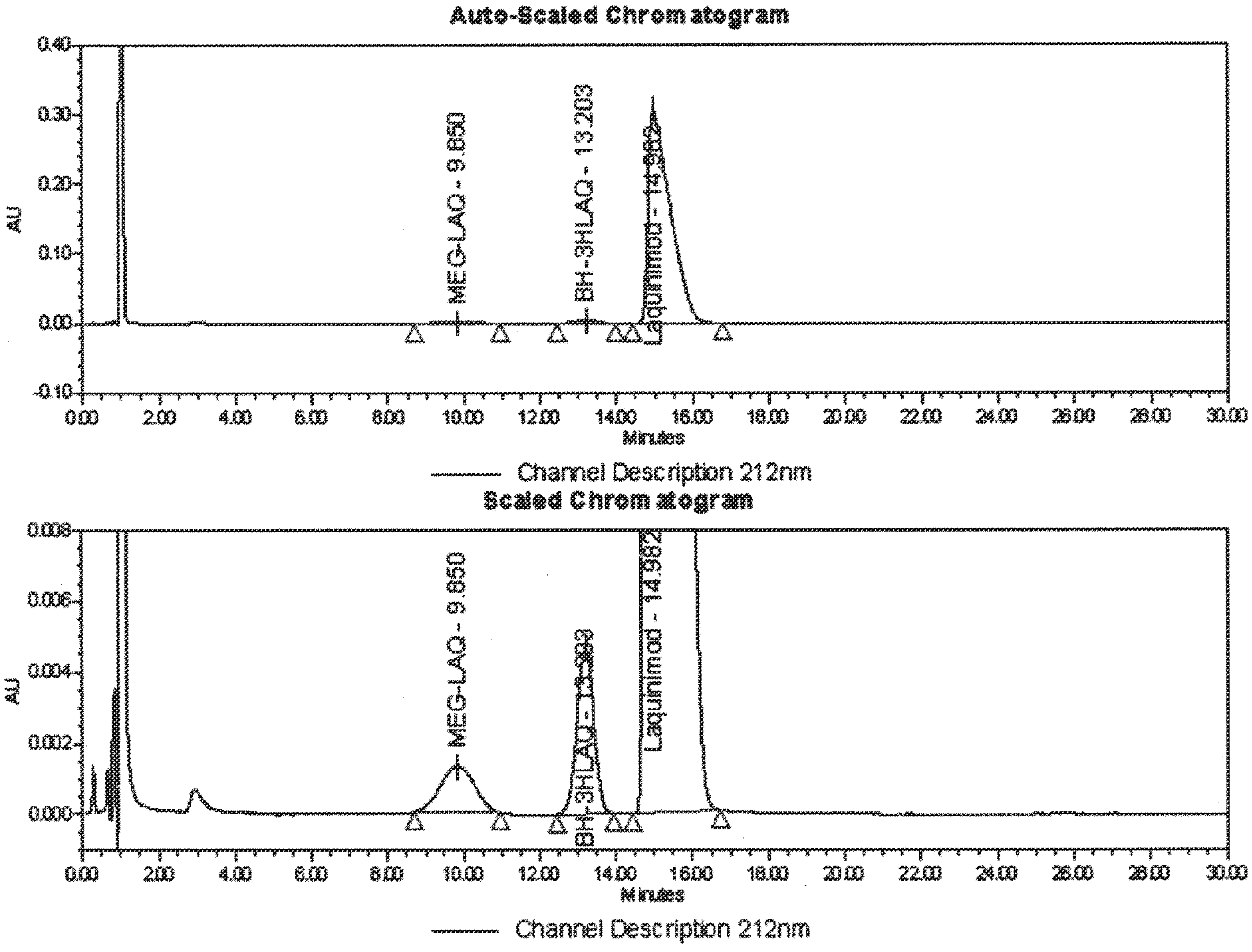

Auto-Scaled Chromatogram
AU
0.40
0.30
0.20
0.10
0.00
-0.10
MEG-LAQ - 9.850
BH-3HLAQ - 13.203
Laquinimod - 14.982
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Minutes
Channel Description 212nm
Scaled Chromatogram
AU
0.008
0.006
0.004
0.002
0.000
MEG-LAQ - 9.850
BH-3HLAQ
Laquinimod - 14.982
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Minutes
Channel Description 212nm

Figure 57.

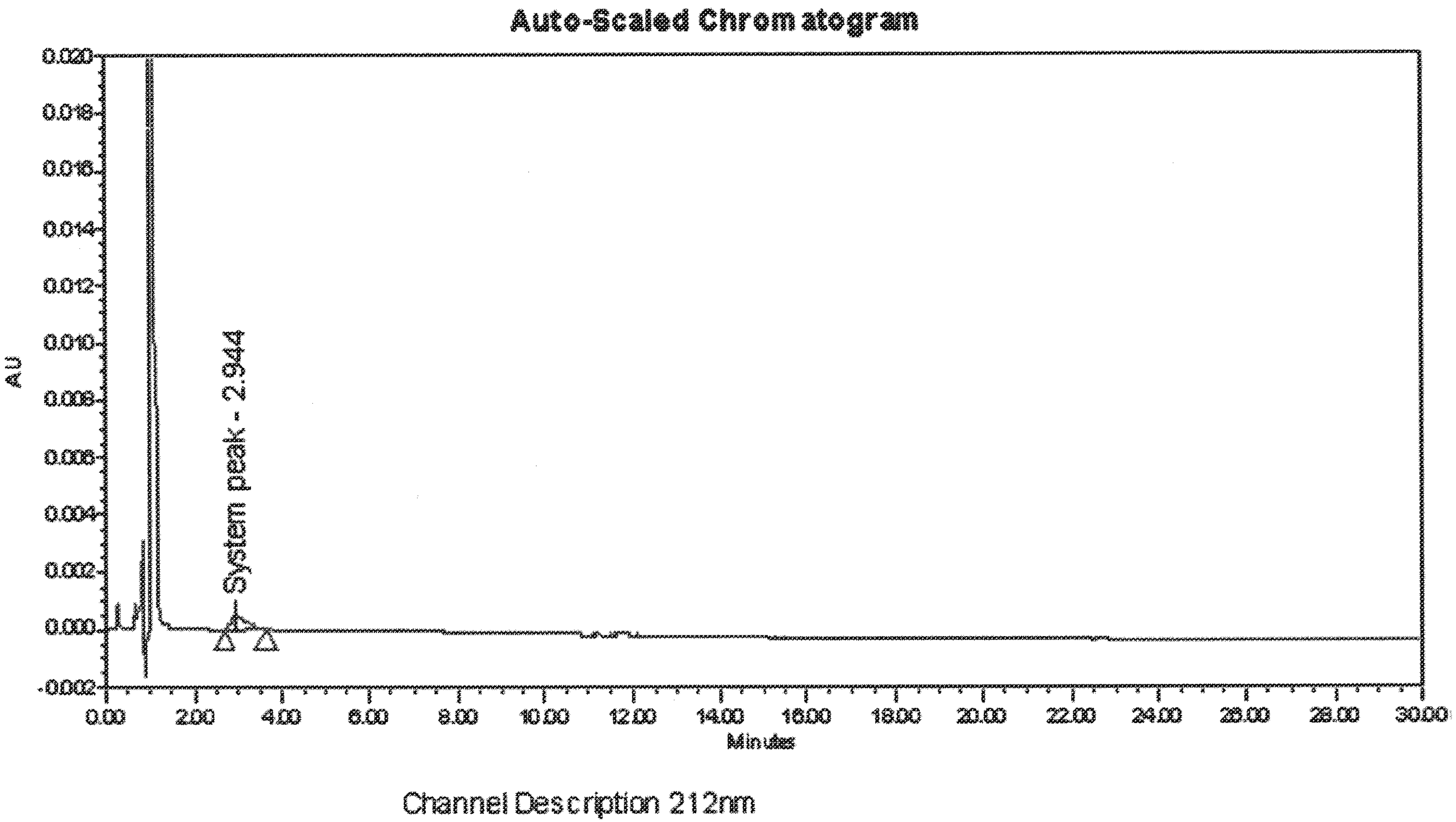

Auto-Scaled Chromatogram
AU
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
-0.002
System peak - 2.944
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Minutes
Channel Description 212nm

Figure 58.

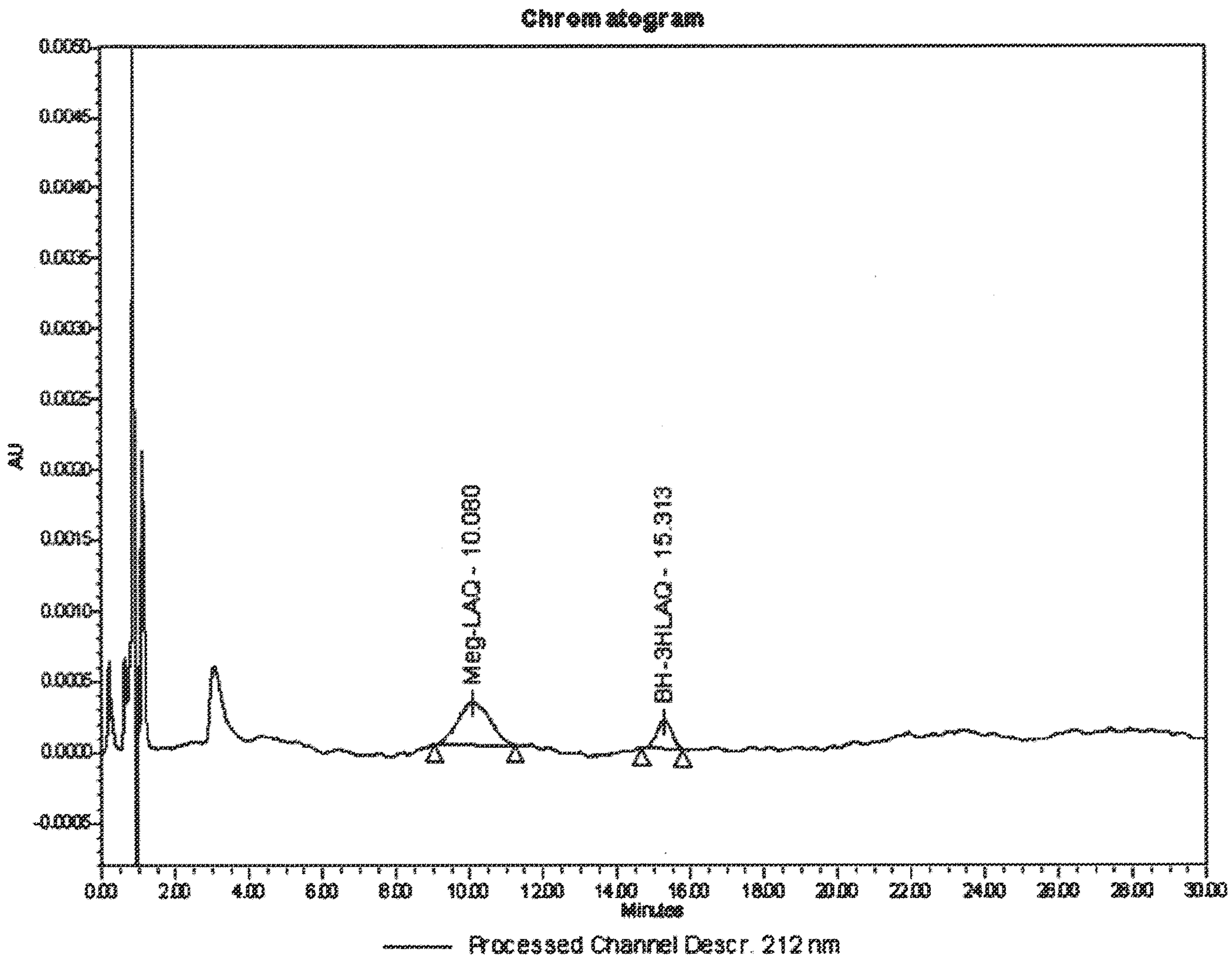

Chromatogram
AU
0.0050
0.0045
0.0040
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
-0.0005
Meg-LAQ - 10.080
BH-3HLAQ - 15.313
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Minutes
Processed Channel Descr. 212 nm

Figure 59.

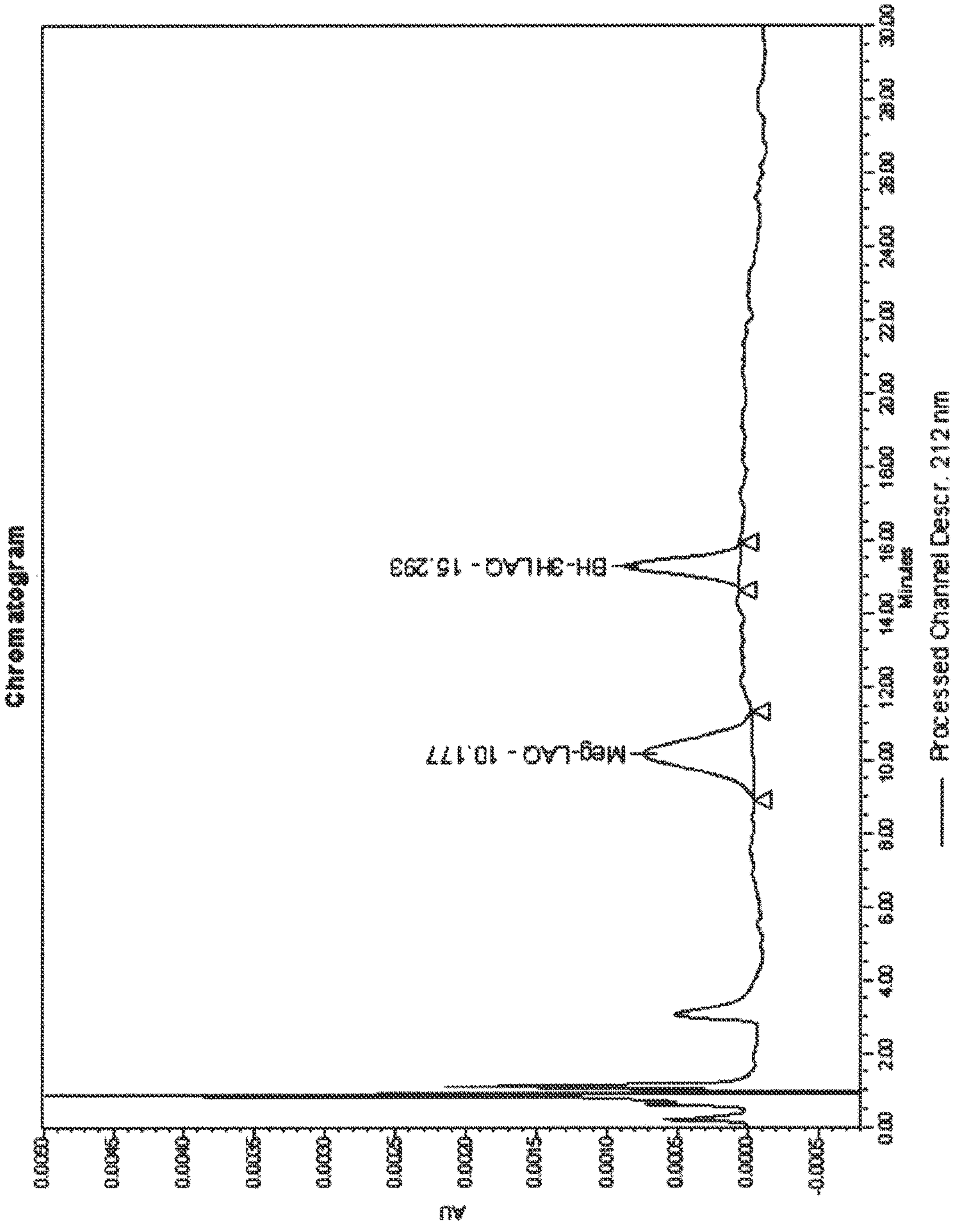
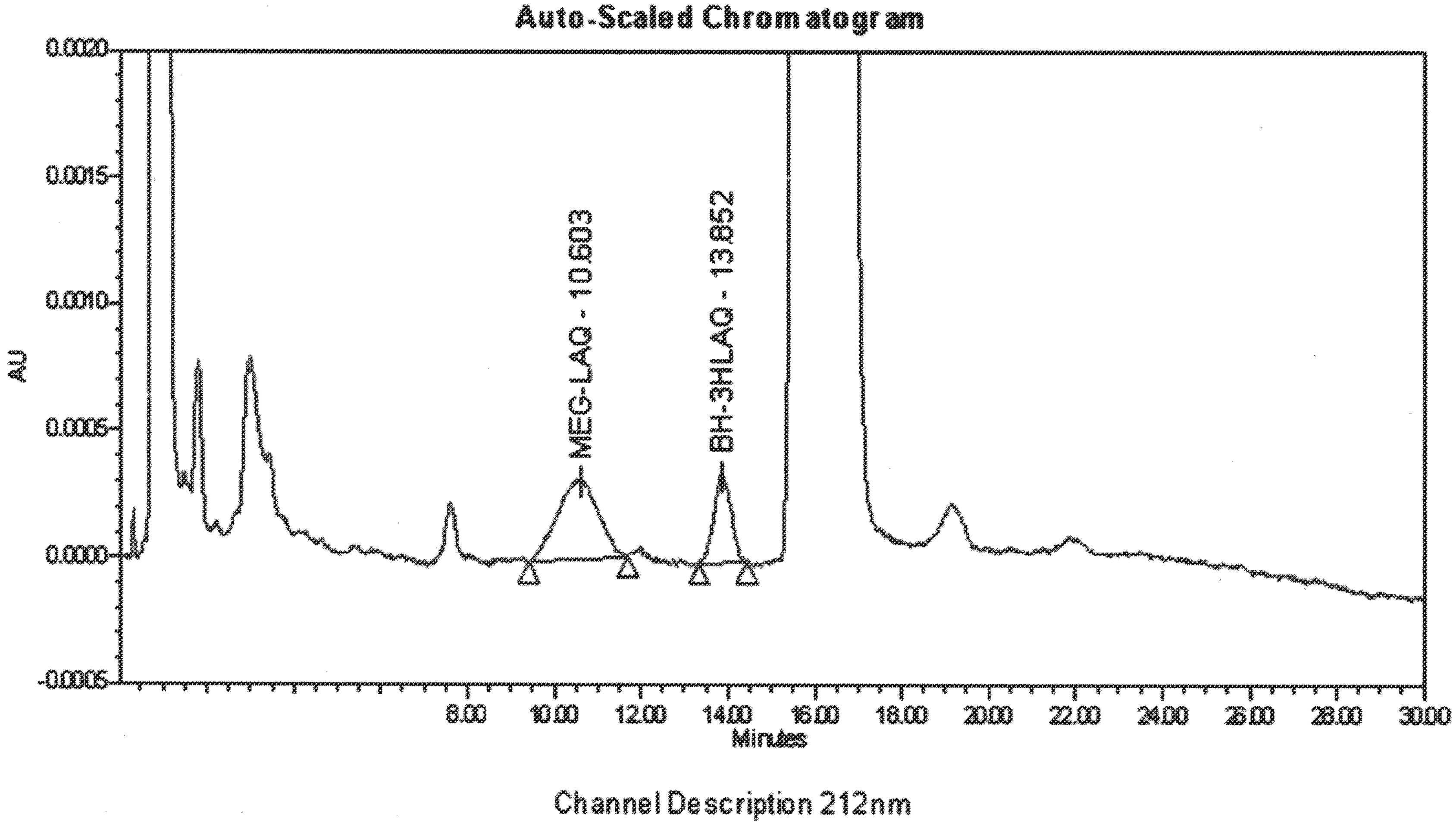

Chromatogram
Meg-LAQ - 10.177
BH-3HLAQ - 15.293
AU
0.0050
0.0045
0.0040
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
-0.0005
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Minutes
Processed Channel Descr. 212 nm

Figure 60.


Auto-Scaled Chromatogram
AU
0.0020
0.0015
0.0010
0.0005
0.0000
-0.0005
MEG-LAQ - 10.603
BH-3HLAQ - 13.852
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Minutes
Channel Description 212nm