Title: PROCESS FOR THE PREPARATION OF HIGHLY PURE BEXAROTENE

The present invention provides an improved process for the preparation of highly pure bexarotene of formula (I). The present invention also provides impurities of bexarotene, method of isolation and identification of these impurities, and use of these impurities as reference marker as well as reference standard.

Formula (I):

\[ \text{H} \quad \text{H} \quad \text{O} \]

\[ \text{O} \]

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"PROCESS FOR THE PREPARATION OF HIGHLY PURE BEXAROTENE"

FIELD OF THE INVENTION
The present invention provides an improved process for the preparation of highly pure bexarotene of formula I.

![Chemical structure of bexarotene](image)

The present invention also provides isolated impurities of bexarotene, process for their removal and use of impurities as reference standard as well as reference marker.

BACKGROUND OF THE INVENTION
Bexarotene of formula I, is a retinoid specifically selective for retinoid X receptors, as opposed to the retinoic acid receptors and is chemically known as 4-[(1-(3,5,5,8,8-pentamethyltetralin-2-yl)ethenyl]benzoic acid.

RXRs (retinoid X receptors) are located primarily in visceral organs such as the liver and kidney. Activated RXRs form homodimers or heterodimers with RAR (retinoic acid receptors), vitamin D receptors, thyroid receptors or peroxisome proliferator activator receptors. Retinoid agonists can activate the expression of retinoid regulated genes by removing negative transcription control or by facilitating positive transcriptional activity. They exert anticancer action by interfering with the growth of cells of the tumor.

Bexarotene and related compounds are first disclosed in US patent 6,320,074. The patent discloses synthesis of bexarotene via the reaction of 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene with monomethyl terphtalate in presence of phosphorus pentachloride and aluminium chloride to give keto ester intermediate which is crystallized from methanol. The above keto ester intermediate is then reacted with methyl triphenylphosphonium bromide-sodium amide in dry tetrahydrofuran to form olefinic ester intermediate, which is purified using silica chromatography followed by crystallization from methanol. The olefinic ester intermediate thus obtained is hydrolyzed using aqueous potassium hydroxide in methanol to give bexarotene, which is recrystallized from a mixture of ethyl acetate and hexane. The main disadvantage of the process is use of silica gel chromatography for the purification of the intermediate which is time consuming and cumbersome technique. The patent is also silent about the purity of the intermediate as well as of bexarotene.
US patent 5,466,861 describes a process for the preparation of bexarotene by reaction of 1,2,3,4,5-tetrahydro-1,4,4,6-pentamethylnaphthalene with 4-carbomethoxybenzoyl chloride in presence of aluminium chloride in dichloromethane to form keto ester intermediate which was purified by flash chromatography. The keto ester intermediate is then reacted with methyl triphenylphosphonium bromide in the presence of potassium hexamethyldisilazide to form olefinic ester intermediate which is purified using flash chromatography. The olefinic ester intermediate is then hydrolyzed using potassium hydroxide in methanol to form bexarotene. The main disadvantage is use of flash chromatography for the purification of intermediate which suffers from disadvantages in convenience, safety and reliability.

Chinese patent CN 1429807 describes the synthesis of bexarotene by reaction of 4-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthenylcarbonyl)benzoic acid with Grignard reagent in a solvent followed by dehydration using p-toluenesulfonic acid to give bexarotene. The process as shown in the scheme below:

A publication namely, Journal of Medicinal Chemistry 1994, 37, 2930-2941 discloses a process for preparation of bexarotene by reaction of 1,2,3,4,5-tetrahydro-1,4,4,6-pentamethylnaphthalene with 4-carbomethoxybenzoyl chloride in presence of aluminium chloride to form keto ester intermediate which is crystallized from ethyl acetate followed by the addition of methanol. Keto ester intermediate is then reacted with methyl triphenylphosphonium bromide in the presence of sodium amide to form olefinic ester intermediate which is crystallized from a mixture of ethyl acetate and methanol. Olefinic ester intermediate is then hydrolyzed using potassium hydroxide in methanol to form bexarotene, which is then crystallized from a mixture of ethyl acetate and hexane. The process involve the use of sodium amide as a base for the Wittig reaction which is highly flammable, reacts violently with water producing very toxic fumes.

It is well in the art that direct product of a chemical reaction is rarely a single compound with sufficient purity to comply with pharmaceutical standards. The impurities that can be present in pharmaceutical compounds are starting materials, by-products of the reaction, products of side reactions, or degradation products. Similarly, synthetic strategy employed for the preparation of bexarotene is complex, therefore may results in the formation of several undesired by products due to competing side
reactions. Impurities in bexarotene or any other active pharmaceutical ingredient are undesirable and in extreme cases, might even be harmful to a patient being treated with a dosage form containing the API. According to ICH guidelines, process impurities should be maintained below set limits by specifying the quality of raw materials, their stoichiometric ratios, controlling process parameters, such as temperature, pressure, time and including purification steps, such as crystallization, distillation and liquid-liquid extraction, in the manufacturing process. Typically, these limits are less than about 0.15 % by weight of each identified impurity. Limits for unidentified and/or uncharacterized impurities are obviously lower, typically less than 0.1 % by weight. Therefore, in the manufacture of a drug substance, the purity of the products, such as bexarotene is required before commercialization.

Therefore, pharmaceutical active compounds must be either free from these impurities or contain impurities in acceptable limits. In addition to this, regulatory authorities worldwide require that drug manufacturers should isolate, identify and characterize the impurities in their products.

In the view of above, there is a need to develop a process for the synthesis of bexarotene that will overcome prior art disadvantages and avoid the need for chromatographic purification, hazardous base such as sodium amide.

Thus, present invention fulfills the need in the art and provides an improved, simple and commercially viable process for the preparation of highly pure bexarotene having impurities in acceptable amounts or free from impurities. The present invention also provides novel impurities, their preparation, isolation and use as reference standard as well as reference marker. The present invention provides a process for the removal of these impurities along with other unidentified from bexarotene.

Even though crystallization is known in prior art and also considered a simplest process that can be used for purification of organic compounds, many of the impurities are difficult to remove as they either have same functional group and/or similar structure as that of desired compounds. The right choice of solvents for crystallization plays a major role in removing selected impurities from the compound and therefore purifying it. The solvent of choice should effectively remove the impurity without sacrificing the yield. Therefore, present invention describes a selective method for purification of the intermediate as well as bexarotene to minimize presence of impurities.

**OBJECTIVE OF THE INVENTION**

It is a foremost objective of the present invention to provide highly pure bexarotene containing any individual identified impurity less than 0.15% and unidentified impurity less than 0.10 % by HPLC. Another objective of the invention is to provide a process for the preparation of bexarotene free from process related impurities.

Another objective of the invention is to provide a process for removal of impurities.
Still another objective of the invention is to identify, isolate and characterized various impurities of bexarotene.

Yet another objective of the present invention is to provide a method for determining identification and quantification of impurities in a sample of bexarotene.

5 SUMMARY OF THE INVENTION

Accordingly, the present invention provides an efficient and industrially advantageous process for the preparation of highly pure bexarotene containing any individual identified impurity less than 0.15\% and unidentified impurity less than 0.10 % by HPLC.

According to one embodiment, present invention provides a process for the preparation of highly pure bexarotene, comprising the steps of:

a). activating the mono alkyl ester of terphthalic acid using a suitable activating agent to form reactive derivative of formula II;

\[
\text{Formula II}
\]

wherein \(X\) is selected from halo such as chloro, bromo and the like; and \(R\) is alkyl selected from methyl, ethyl, \(n\)-propyl, isopropyl, \(n\)-butyl, isobutyl and the like

b). reacting the reactive derivative of formula II with 1,2,3,4,-tetrahydro-1,1,4,4,6-pentamethylnaphthalene of formula III,

\[
\text{Formula III}
\]

in the presence of catalyst to form keto ester intermediate of formula IV;

\[
\text{Formula IV}
\]

wherein \(R\) is as defined above

c). optionally, purifying the keto ester intermediate of formula IV with a suitable solvent;

d). reacting the keto ester intermediate of formula IV with methyl triphenylphosphonium halide in the presence of a suitable base in inert solvent to form olefinic ester intermediate of formula V;

\[
\text{Formula V}
\]

wherein \(R\) is as defined above

e). optionally, purifying olefinic ester intermediate of formula V with a solvent;
f). hydrolyzing olefinic ester intermediate of formula V;
g). washing the reaction mixture with a suitable solvent or mixture thereof;
h). isolating bexarotene of formula I there from; and
i). optionally, purifying bexarotene of formula I.

According to another embodiment, present invention provides a process for the removal of impurities from bexarotene, comprising the steps of:

a). washing the reaction mixture containing bexarotene or salts thereof with a suitable solvent or solvent mixture; and

b). isolating pure bexarotene of formula I there from.

According to another embodiment, the present invention provides a process for the identification of an impurity in a sample of bexarotene, selected from one or more of the following compounds:

\[
\text{Impurity A} \quad \begin{array}{c} \text{Impurity B} \\
\text{Impurity C} \quad \begin{array}{c} \text{Impurity D} \\
\text{Impurity E} \quad \begin{array}{c} \text{Impurity F} \\
wherein } R \text{ is as defined above}
\]

the process comprising performing steps (a) and (b) in either order:

a). carrying out a chromatographic analysis on the reference sample, containing one or more of impurities amongst A, B, C, D, E or F (reference marker) and bexarotene, to determine the relative retention time of the reference marker compared to bexarotene;

b). carrying out a chromatographic analysis on the sample of bexarotene to determine the relative retention time of impurities present in the sample compared to bexarotene;
and comparing the relative retention-times determined in step (a) and (b); where if the relative retention times determined are substantially same, the impurity of bexarotene in the sample is identified as being the same as reference marker.

According to another embodiment, the present invention provides a method of determining the amount of an impurity, selected from one or more of impurity among A, B, C, D, E and F, the method comprising performing steps (a) and (b) in either order:

a), carrying out a chromatographic analysis on the reference sample, containing known amount of one or more impurities amongst A, B, C, D, E or F (reference standard) and bexarotene, to determine the relative retention time of impurities compared to bexarotene; and measuring the area under a peak corresponding to the reference standard;

b). carrying out a chromatographic analysis on the sample of bexarotene to determine area under HPLC peak corresponding to each and every impurity by reference to the relative retention times thus determined compared to bexarotene;

and calculating the amount of one or more of impurity among A, B, C, D, E and F in bexarotene, by reference to area of the HPLC peak in the sample against the area of HPLC peak associates with the same impurity in step (a).

According to another embodiment, present invention provides novel impurity A, B, C, D, E and F.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is an X-ray powder diffraction pattern of bexarotene

Figure 2 is an infrared spectrum of bexarotene

Figure 3 is DSC thermogram of bexarotene

Figure 4 is HPLC chromatogram of a highly pure bexarotene sample

Figure 5 is HPLC chromatogram of a bexarotene sample containing impurities

**DETAILED DESCRIPTION OF THE INVENTION**

As used herein term "highly pure bexarotene" refers to bexarotene containing any individual identified impurity less than 0.15% and unidentified impurity less than 0.10 % by HPLC.

As used herein term "relative retention time (RRT)" refers to a relation of amount of time a compound elutes from a column relative to bexarotene.

The present invention provides an improved and industrially advantageous process for the preparation of highly pure bexarotene.

According to one embodiment, process involves the synthesis of highly pure bexarotene starting from mono alkyl ester of terephthalic acid.
Generally, process involves conversion of mono alkyl ester of terphthalic acid into reactive derivative by reaction with a suitable activating agent at a temperature -20 to 150 °C for few minutes to several hours, preferably till the completion of the reaction. Suitable activating agent employed for the reaction includes phosphorus halides such as phosphorus pentachloride; phosphorus tribromide, phosphorus oxy halide such as phosphorus oxy chloride; oxalyl halide such as oxalyl chloride, oxalyl bromide; thionyl halide such as thionyl chloride and the like. The reaction can be carried out in the presence of aprotic solvent selected from halogenated solvent such as dichloromethane, 1,2-dichloroethane, chloroform and the like. The progress of the reaction can be monitored by suitable chromatographic techniques such as high-pressure liquid chromatography (HPLC), thin layer chromatography (TLC), ultra pressure liquid chromatography (UPLC), gas chromatography (GC) and the like. After completion of the reaction, reactive derivative of formula II can be isolated or reacted further in situ. However, it is advantageous to proceed further without isolation, as compound of formula II is highly sensitive to moisture.

The compound of formula II is then in situ made to react with 1,2,3,4,5-tetrahydro-1,1,4,4,6-pentamethylnaphthalene of formula III in presence of suitable catalyst in suitable solvent to form keto ester intermediate of formula IV. Generally, reaction is carried out at temperature of -50 to 100 °C for few minutes to several hours, preferably for 5 minutes to 24 hours, more preferably till completion of the reaction. Catalyst employed for the reaction can be Lewis acids that include aluminum chloride, titanium tetrachloride, boron trifluoride and the like. Suitable solvent can be selected from halogenated solvents such as dichloromethane, chloroform, 1,2-dichloroethane; ethers such as tetrahydrofuran, 1,2-dimethoxyethane, 1,2-dioxyethane and the like. After completion of reaction, keto ester intermediate of formula IV can be isolated from reaction mixture using suitable techniques. Preferably, after completion of reaction, the reaction mixture is quenched with suitable quenching agent such as water, hydrochloric acid, dilute sulfuric acid and the like. Thereafter layers are separated. Optionally, organic layer can be charcoaled and/or washed with water. Keto ester intermediate of formula IV can be recovered from the resulting organic layer after removal of solvent.

Keto ester intermediate of formula IV, when isolated, may contain certain identified and unidentified impurities. It is found that samples of keto ester intermediate of formula IV may contain certain impurities out of which two major impurities, which have been identified as impurity A and impurity B. The percentage of impurity A found to be directly proportional to amount of terphthalic acid present as an impurity in starting material i.e. mono alkyl ester of terphthalic acid.
Keto ester intermediate of formula IV, if desired, can be purified to enhance the purity of the intermediate and/or to minimize the presence of identified and unidentified impurities or to make the intermediate free from these impurities.

Specifically, keto ester intermediate of formula IV in a suitable solvent may be stirred at a temperature -25 to 50°C for few minutes to several hours. Preferably, the reaction mixture is stirred at a temperature of 5 to 25 °C for 5 minutes to 3 hours. Suitable solvents include aliphatic hydrocarbons such as n-pentane, n-heptane, n-hexane, cyclohexane, pentane, hexane, heptane; aliphatic ethers such as isopropyl ether, methyl tert-butyl ether, diethyl ether; C₅₋₄ alcohols such as methanol, ethanol, propanol, isopropanol and the like or mixture thereof. Purified keto ester intermediate of formula IV can be isolated from the reaction mixture using a suitable techniques such as filtration, centrifugation and the like.

Keto ester intermediate of formula IV, thus purified, may have purity more than 95.0 %, preferably more than 99.0% by HPLC, more preferably 99.5% by HPLC and may have impurities (identified and unidentified) less than 0.5 %, preferably less than 0.1 % by HPLC. Specific purification method as described by the present invention is highly efficient in removing non-polar impurities along with some identified impurities such as impurity A and keto acid impurity B from the product.

As is known in the art, the management of process impurities is greatly enhanced by understanding their chemical structures and by identifying the parameters that influence the amount of impurities in the final product. It has been found by present inventors that some process related impurities like impurity A and keto acid impurity B may be present in keto ester intermediate of formula IV. The possible cause of formation of these impurities is the presence of terphthalic acid as an impurity in mono alkyl ester of terphthalic acid, which reacts with reagents used for the usual reaction sequence, which is shown as in following scheme:

*wherein* $X$ *is hallo selected from chloro, bromo, iodo and the like*
Terphthalic acid, if present in mono alkyl ester of terphthalic acid, even in traces will undergo reaction with activating agent to form a reactive derivative of the terphthalic acid, wherein both carboxylic groups are activated. The activated diacid derivative then may reacts with 1,2,3,4,-tetrahydro- 1,1,4,4,6-pentamethylnaphthalene of formula III under usual reaction conditions to give an halo impurity and/or impurity A as described above, depending upon the molar ratio of the compound of formula III present in the reaction medium. Hydrolysis of halo impurity will result in another keto acid impurity namely impurity B as shown in the above scheme. Thus desired product i.e. keto ester intermediate of formula IV may be contaminated with the process related impurities such as impurities A and B along with some other unidentified impurities. As the main source of the above impurities is presence of terphthalic acid in starting material. So, higher contamination of teiphthalic acid in mono alkyl ester of terphthalic acid, will result in formation of said impurities during reaction in higher amounts. Therefore, formation of above impurities in keto ester intermediate of formula IV can be controlled by reducing the amount of terphthalic acid in the starting material. Further, impurities can be removed by purifying keto ester intermediate of formula IV using suitable solvents as described above.

Keto ester intermediate of formula IV, with or without purification, is then made to react with methyl triphenylphosphonium halide in the presence of a suitable base to form olefinic ester intermediate of formula V. Specifically, the process involves the reaction of compound of formula IV with a methyl triphenylphosphonium halide in the presence of suitable base in an inert solvent at a temperature of -15 to 60°C for few minutes to few hours, preferably till the completion of the reaction. Suitable bases employed for the reaction include alkali or alkaline metal hexamethyldisilazanes, C14 alkoxide, hydrides thereof such as potassium hexamethyldisilazane, sodium hydride (in paraffin oil), potassium hydride, sodium methoxide, sodium tert-butoxide, potassium tert-butoxide and the like. Solvents used for the reaction include aliphatic or aromatic hydrocarbons such as toluene, 1,2-xylene, 1,4-xylene; ethers such as tetrahydrofuran, 2-methyl tetrahydrofuran, methyl tert-butyl ether, 1,2-dimethoxy ethane, 1,2-diethoxy ethane and the like or mixture thereof. Usually, reaction completes in approximately in 2-3 hours. After completion of the reaction, olefinic ester intermediate of formula V can be isolated from the reaction mixture using suitable techniques. Preferably, after the completion of the reaction, reaction mixture can be quenched with suitable quenching agent that includes organic acid such as carboxylic acid like acetic acid, formic acid, tartaric acid; or inorganic acid such as hydrochloric acid, dilute sulfuric acid and the like. Layers are separated by the addition of aqueous acid. Optionally, the organic layer can be charcoalized and/or washed with water and olefinic ester
intermediate of formula V can be recovered from the resulting organic layer by removal of solvent by distillation or evaporation and the like.

Olefinic ester intermediate of formula V thus obtained can be optionally purified to enhance the purity as well to minimize the presence of impurities. Specifically, olefinic ester intermediate of formula V in a suitable solvent can be stirred at a temperature of -25 to 100 °C for few minutes to several hours, preferably at a temperature of 10 to 60 °C for 30 minutes to 12 hours. Suitable solvents include C$_{14}$ alcohols such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol; alkyl nitriles such as acetonitrile, propionitrile; C$_{5-8}$ aliphatic alkanes such as n-pentane, n-heptane, n-octane, cyclohexane, cyloheptane, pentane, hexane, heptane; aliphatic ethers such as diethyl ether, isopropyl ether, methyl tert-butyl ether, 1,2-dimethoxyethane, 1,2-diethoxyethane; cyclic ethers such as tetrahydrofuran and the like or mixture thereof or in mixture with water. Olefinic ester intermediate of formula V may be isolated from the reaction mixture using suitable techniques such as filtration, centrifugation and the like. Above described process for the purification of olefinic ester intermediate of formula V is highly efficient in removing triphenylphosphine oxide, which is formed as by product in the reaction, along with some other impurities.

It has been found that some process related impurities that may form during the reaction sequence are mainly due to the reaction of the methyl triphenylphosphonium halide present in the reaction mixture with olefinic ester intermediate. The excess amount of methyl triphenylphosphonium halide present in the reaction mixture under goes reaction with the generated product i.e. compound of formula V and results in the formation of keto olefinic impurity C that again may react with methyl triphenylphosphonium halide to form impurity D as shown in the following scheme:

\[
\text{Impurity C} \xrightarrow{(\text{C}_3\text{H}_5)_2\text{PCH}_3\text{X}} \text{Impurity D}
\]

*wherein R and X are as defined above*

Other potential impurities that may be present in the intermediate of formula V can be formed due to presence of impurity A, which may be carried forward from keto ester intermediate of formula IV. The presence of impurity A in a sample of keto ester intermediate of formula IV results in the formation of impurities E and F by the sequence of reaction sequence as shown below:
wherein $R$ and $X$ are as defined above

It is observed that if the reaction is performed by using less amounts of methyl triphenylphosphonium halide and base, then less percentage of above impurities (impurity C, D, E and F) are formed but at the same time reaction does not undergo completion which is checked by the presence of unreacted intermediate of formula IV in the reaction mixture. It is very difficult to remove unreacted keto intermediate of formula IV from olefinic ester intermediate of formula V. As a result, unreacted keto intermediate of formula IV gets converted to impurity B during base assisted hydrolysis in next stage. Thus, molar ratio of methyl triphenylphosphonium halide as well as base is crucial for the successful completion of the reaction as well as for impurities control. Therefore, formation of impurities can be controlled by employing optimum amount of methyl triphenylphosphonium halide and base. Preferably, reaction is carried out using 1.5 to 2.0 mol equivalent of methyl triphenylphosphonium halide and 1.6 to 1.8 mol equivalent of base which will result in olefinic ester intermediate of formula V having fewer amounts of impurities C, D, E and F.

These non-polar impurities, if present, remain intact with compound of formula V and are carried forward due to similar non-polar nature of olefinic ester intermediate of formula V. In our hands, it is observed that purification of intermediate of formula V to minimize the amount of impurities result in extensive loss of yield, which makes the process commercially unviable. Removal of such non-polar impurities from intermediate of formula V require tedious purification method such as column chromatography or flash chromatography which is not amenable for the industrial synthesis. Therefore, the present invention avoids extensive purification as well as chromatographic purification at this stage and result in the development of a method for the removal of such impurities at final stage.

Olefinic ester intermediate of formula V is then hydrolysed to form bexarotene of formula I.
Specifically, the reaction involves reaction of compound of formula V in the presence of suitable hydrolyzing agent at a temperature of 10 to 90°C for few minutes to several hours, preferably till the completion of the reaction. Preferably, hydrolysis reaction can be carried out using basic conditions. Base employed for hydrolysis includes alkali or alkaline earth metal hydroxide, such as lithium hydroxide, sodium hydroxide, potassium hydroxide and the like. The reaction can be carried out in the presence of a suitable solvent that includes C_{1-4} alcohols such as methanol, ethanol, n-propanol, isopropanol, butanol, iso-butanol; aliphatic ketone such as acetone; ethers such as diethyl ether, ethyl methyl ether, tetrahydrofuran, 1,2-dimethoxy ethane, 1,2-diethoxy ethane and the like or mixture thereof. Preferably, reaction can be carried out in methanol, acetone or tetrahydrofuran. After completion of the reaction, it is highly advantageous to wash the reaction mixture containing alkali or alkaline metal salt of bexarotene with a suitable solvent or mixture of solvents. The suitable solvents for washing include aliphatic esters such as ethyl acetate, n-propyl acetate, n-butyl acetate; C_{5-9} aliphatic alkanes such as n-pentane, n-hexane, n-heptane, n-octane, pentane, hexane, heptane, octane, cyclopentane, cyclohexane; aromatic hydrocarbons such as benzene, toluene, 1,2-xylene, 1,4-xylene; halogenated solvents such as dichloromethane, chloroform, 1,2-dichloroethane, C_{5-8} alkyl ethers such as diethyl ether, diisopropyl ether, methyl tert-butyl ether, and the like or mixture thereof. It is preferable to use mixture of aliphatic ester and aliphatic alkane for washing of reaction mixture as salt of bexarotene formed during reaction has very less or no solubility in the solvent mixture and most of impurities have high solubility in solvent mixture. Thereafter, upon removal of impurities, aqueous reaction mixture is acidified to precipitate the desired product from the reaction mixture. The product, thus precipitated, may be isolated from the reaction mixture by suitable techniques such as filtration, centrifugation and the like.

During hydrolysis of intermediate of formula V, a number of known impurities as described above and certain unknown impurities, that may be present, are carried forward as such or in form of their derivatives to final stage i.e. bexarotene. Another impurity keto acid impurity B that may form during hydrolysis is due to presence of keto ester intermediate as an impurity in bexarotene.

The present inventors have developed a selective purification method for the removal of polar and non-polar impurities from the reaction mixture containing bexarotene or salts thereof that are carried forward from olefinic intermediate of formula V along with other impurities to obtain highly pure bexarotene which complies with all the regulatory needs of process development and ICH guidelines.

Process of removal of impurities involves washing of the reaction mixture containing bexarotene or salts thereof with a suitable solvent as specified above. The process of present invention is highly advantageous to remove non-polar impurities from the reaction mixture.
Bexarotene of formula I, thus prepared can be optionally further purified using a suitable solvent to enhance the purity by crystallization.

Specifically, process involves dissolution of bexarotene in a suitable solvent; which can be accomplished by optionally heating to a temperature of 25 to 100 °C for 5 minutes to 6 hours, preferably till clear solution is obtained. Suitable solvents include C1-4 alcohols such as methanol, ethanol, propanol, isopropanol; aliphatic C3-5 ketones such as acetone, ethyl methyl ketone, diethyl ketone; C4-8 aliphatic esters such as methyl acetate, ethyl acetate, n-propyl acetate, n-butyl acetate; C5-8 aliphatic ethers such as diethyl ether, ethyl methyl ether, isopropyl ether, methyl tert-butyl ether; cyclic ether such as tetrahydrofuran; halogenated solvent such as dichloromethane, dichloroethane, chloroform and the like or mixture thereof. The resulting mixture can be optionally charcoalized to improve colour of product. Crystallization of bexarotene can be induced by cooling reaction mixture or by adding suitable anti solvent to reaction mixture. Anti-solvents employed include solvents in which bexarotene has less solubility or no solubility, preferably selected from water, aliphatic hydrocarbons such as n-pentane, n-hexane, n-heptane, n-octane, pentane, hexane, heptane, octane, and the like or mixture thereof. Purified bexarotene can be isolated from the mixture using a suitable technique such as filtration, centrifugation and the like.

In another embodiment, bexarotene of formula I can be dissolved in a suitable solvent as described above followed by removal of solvent to give a residue. Purified bexarotene can be isolated from the residue by addition of suitable anti solvent. The reaction mixture can be stirred for few minutes to few hours, preferably for 2 to 4 hours and filtered or centrifuged to give pure bexarotene.

In an alternate way, bexarotene can be purified by washing with a suitable solvent.

Specifically, bexarotene is slurried in a suitable solvent at temperature of -20 to 25 °C for few minutes to few hours. Suitable solvent employed include water, C1-4 alcohols such as methanol, ethanol; C3-5 aliphatic ketones such as acetone, ethyl methyl ketone, diethyl ketone; C4-8 aliphatic ester such as methyl acetate, ethyl acetate, n-propyl acetate, n-butyl acetate; C5-8 ethers such as diethyl ether, ethyl methyl ether, isopropyl ether, methyl tert-butyl ether and the like or mixture thereof. Purified bexarotene can be isolated from the mixture using a suitable technique such as filtration, centrifugation and the like.

Purification process as described by the present invention has several advantageous effects; mainly it improves the purity of compound and increases assay of bexarotene by reducing inorganic impurities present in bexarotene. Purification processes described are also efficient in removing non-polar as well as polar impurities present in crude bexarotene.
Crystallization process can be repeated or can be used along with other purification processes till the product of desired purity is obtained. It is advantageous to involve purification of bexarotene with water miscible organic solvent followed by crystallization from water immiscible organic solvent to get rid of impurities and to yield highly pure bexarotene. Bexarotene thus prepared have displays purity of more than 99.0% by HPLC, preferably 99.5% by HPLC, more preferably 99.9%. Bexarotene prepared by the present invention contain identified and unidentified impurities less than 0.15%, preferably less than 0.10 %, respectively more preferably free from impurities.

Bexarotene, thus synthesized can be characterized by X-Ray diffraction (XRD), Infrared spectroscopy (IR) or differential scanning calorimetry (DSC). Crystalline nature of bexarotene is characterized by X-ray diffractogram, which shows the unique characteristic peaks as shown in Figure 1. X-ray diffraction patterns of bexarotene is measured on a PANalytical X'Pert Pro diffractometer with Cu radiation and expressed in terms of two-theta, d-spacings and relative intensities. One ordinarily skilled in the art understands that experimental differences may arise due to differences in instrumentation, sample preparation or other factors, which can alter the two-theta values, d-spacings and relative intensities slightly.

Bexarotene is also characterized by Infrared spectrum (IR) and differential scanning calorimetry (DSC) as shown in Figures 2 and 3 respectively. In DSC, thermogram shows an endothermic peak at around 224 °C. Highly pure bexarotene obtained by the process of present invention is also characterized by chromatographic analysis, preferably by HPLC as shown by Figure 4.

According to another embodiment, present invention provides novel impurities such as A, B, C, D, E and F.

The impurities as described in the present invention can be either isolated from reaction mixture or can be synthesized so that they can be used as reference standard as well as reference marker. The isolation as well as synthesis of each of impurities is described herein below.

Isolation of impurities A and/or B is accomplished by concentrating the filtrate, obtained during purification of keto ester intermediate of formula IV, followed by separation of impurities using various purification techniques such as column chromatography, preparative column chromatography, preparative thin layer chromatography and the like.

Alternatively, impurity A and/or B can be prepared by following same reaction sequence as described earlier for the preparation of keto ester intermediate of formula IV using terphthalic acid in place of mono alkyl ester of terphthalic acid. Keto acid impurity B can also be prepared by the hydrolysis of the keto ester intermediate of formula IV. The hydrolysis can be carried out under acidic or basic condition. Thus, keto ester intermediate of formula IV, if present, unreacted in olefinic ester
intermediate of formula V then keto ester intermediate present as an impurity gets hydrolyzed in the last stage of base assisted hydrolysis and result in the formation of impurity B in the final product i.e. bexarotene.

Isolation of impurities C, D, E and F is accomplished from residue obtained by concentrating the organic layer obtained after washing of aqueous reaction mixture containing bexarotene salt. Preferably, these impurities may be eluted from the column using mixture of solvents such as ethyl acetate: hexane or ethyl acetate: heptane. The impurities are eluted in different fractions based upon the polarity of the solvent used for elution. Impurity F is eluted first followed by impurities E, D and C. Alternatively, impurities C and D can be synthesized by reacting olefinic ester intermediate of formula V with methyl triphenylphosphonium halide using the reaction sequence as described in the present invention. Further impurities E and F can be synthesized by reacting impurity A with methyl triphenylphosphonium halide using the reaction sequence as described in the present invention.

All the identified impurities as described in the present invention thus isolated or synthesized can be characterized by various spectroscopic techniques like $^1$H Nuclear magnetic resonance ($^1$H-NMR) and $^{13}$C Nuclear magnetic resonance ($^{13}$C-NMR), Ultraviolet spectroscopy (UV), Mass spectrometry (MS), Infrared spectroscopy (IR).

The isolated impurities of the present invention are characterized by following spectral data:

<table>
<thead>
<tr>
<th>Identification / Structure</th>
<th>RRT (+0.02)</th>
<th>$^1$H-NMR (CDCl$_3$) δ</th>
<th>Mass (APCI) $\text{M}^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurity A</td>
<td>1.46</td>
<td>1.20 (s, 6H, CH$_3$); 1.25 (s, 6H, CH$_3$); 1.69 (s, 4H, CH$_2$); 2.37 (s, 3H, CH$_3$); 7.21 (s, 1H, Ar-H); 7.20-7.31 (m, 6H, Ar-H)</td>
<td>535.0</td>
</tr>
<tr>
<td>Impurity B</td>
<td>0.72</td>
<td>1.16 (s, 6H, CH$_3$); 1.27 (s, 6H, CH$_3$) 1.66 (s, 4H, CH$_2$), 2.22 (s, 3H, CH$_3$), 7.23 (s, 1H, Ar-H); 7.31 (s, 1H, Ar-H); 7.75 -8.10 (dd, 4H, Ar-H)</td>
<td>351.0</td>
</tr>
<tr>
<td>Impurity C</td>
<td>1.13</td>
<td>1.27 (s, 6H, 2 CH$_3$); 1.30 (s, 6H, 2 CH$_3$); 1.70 (s, 4H, 2 CH$_2$); 1.94 (s, 3H, CH$_3$); 2.58 (s, 3H, CH$_3$); 5.33 (d, 1H, olefinic H), 5.82 (d, 1H, olefinic H); 7.08-7.9 (m, 6H, Ar-H)</td>
<td>347.0</td>
</tr>
</tbody>
</table>
In addition to characterization, the presence or absence of an impurity in a product should be identified in any quality control process in order to ensure that process complies with the required standards set down in the regulatory approval of that product prior to it being released for commercial sale.

Therefore, presence of the above impurities present at intermediate stage or bexarotene may be identified by chromatographic techniques like thin layer chromatography (TLC) or high-pressure liquid chromatography (HPLC) and preferably using HPLC.

According to another embodiment, present invention provides a HPLC method for identification as well as quantification of the impurities present in a sample of bexarotene.

<table>
<thead>
<tr>
<th>Instrumentation</th>
<th>HPLC instrument with UV detector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Cosmosil, PAQ (250 x 4.6 mm), 5 μιη</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>A: Buffer: tetrahydrofuran = 95: 5 (%v/v)</td>
</tr>
<tr>
<td></td>
<td>B: Acetonitrile</td>
</tr>
<tr>
<td></td>
<td>Buffer: 0.1%v/v Diethylamine in water (pH = 3.10)</td>
</tr>
<tr>
<td>Elution</td>
<td>Gradient type</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Flow (mL/minute)</th>
<th>% A</th>
<th>% B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.30</td>
<td>1.25 (s, 6H, 2CH₃); 1.27 (s, 6H, 2 CH₃); 1.69 (s, 4H, 2 CH₂); 1.98 (s, 3H, CH₃); 2.10 (s, 3H, CH₃); 5.06 (s, 1H, olefinic H), 5.18 (d, 1H olefinic H); 5.38 (d, 1H, olefinic H); 5.70 (d, 1H, olefinic H); 7.07-7.41 (m, 6H, Ar-H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.69</td>
<td>1.27 (s, 12H, 4 CH₃); 1.29 (s, 12 H, 4 CH₃); 1.68 (s, 8H, 4 CH₂); 1.97 (s, 3H, CH₃); 2.29 (s, 3H, CH₃); 5.33 (d, 1H, olefinic H); 5.85 (d, 1H, olefinic H); 7.0-7.9 (m, 7H, Ar-H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.29</td>
<td>1.32 (s, 12H, 4CH₃); 134 (s, 12H, 4CH₂); 1.74 (s, 8H, 4CH₂); 2.01 (s, 6H, CH₃); 5.23-5.24(d, 2H olefinic H); 5.78-5.79 (d, 2H, olefinic H); 7.10-7.32 (m, 4H Ar-H)</td>
<td>345.0</td>
<td>533.0</td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>----</td>
<td>-----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>20</td>
<td>1.5</td>
<td>32</td>
<td>68</td>
</tr>
<tr>
<td>25</td>
<td>1.8</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>55</td>
<td>1.8</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>58</td>
<td>1.0</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>65</td>
<td>1.0</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>

Diluent : Mobile phase A : Mobile phase B in the ratio of 30:70
Detector : UV: Wavelength: 220 nm
Column temperature : 40°C
Injection volume : 20 µL
Run time : 65 minutes
Reagents : Acetonitrile, Tetrahydrofuran, diethyl amine, ortho phosphoric acid (for chromatography); water (purified)

A reference sample of bexarotene containing all the six impurities along with the intermediate of formula V, as an impurity is prepared by dissolving bexarotene (containing impurities) in diluent as specified above and then analyzed chromatographic HPLC method of the present invention to identify the impurities by calculating their RRT. Specifically, 1 mg sample of bexarotene is dissolved in 1 ml of diluent (mixture of mobile phase A and B in ration of 30:70). Bexarotene containing impurities displays HPLC chromatogram as shown in Figure 5.

According to another embodiment, the present invention provides a process for identification of an impurity in a sample of bexarotene, selected from one or more of impurities amongst A, B, C, D, E or F.

The process for identification of impurities involves the chromatographic analysis of reference sample containing one or more of impurities amongst A, B, C, D, E or F (reference marker) and bexarotene. The same analysis is also carried out on the sample of bexarotene. Thereafter, relative retention times of the impurities present in reference sample and sample of bexarotene is carried out. Then by comparing the relative retention times calculated in the two samples, presence of impurity can be determined. The reference sample can be prepared by adding any one or more of the identified impurities of present invention to bexarotene sample. If the relative retention times thus determined in the two samples are substantially same, the impurity of bexarotene in the sample is identified as being
the same as reference marker. The chromatographic analysis can be carried out by HPLC or TLC. Preferably, HPLC analysis is carried out by the method as described above.

According to another embodiment, the present invention provides a method of determining the amount of an impurity, selected from one or more of impurity among A, B, C, D, E and F.

The process for quantification of impurities involves the chromatographic analysis of the reference sample containing known amount of one or more of impurities amongst A, B, C, D, E or F (reference marker) and bexarotene. Same analysis is also carried out on sample of bexarotene. Thereafter, relative retention times of the impurities present in reference sample and sample of bexarotene is calculated and also area under the peak with the specific impurity or impurities of present invention are calculated.

Then by comparing the area under the peaks (referring to RRT of the specific impurity) amount of one or more of impurity among A, B, C, D, E and F in bexarotene can be calculated. The reference sample can be prepared by adding known concentration of one or more of the identified impurities of present invention to bexarotene sample. The chromatographic analysis can be carried out by HPLC. Preferably, HPLC analysis is carried out by the method as described above.

Major advantages realized in the present invention are that process can be easily and conveniently scaled-up for industrial large-scale production and that the process is simple, economical, high throughput and provides highly pure bexarotene. The other advantage of the present invention is that it circumvents the need for chromatographic purification and use of hazardous reagent such as sodium amide. The present invention provides method for the selective removal of the impurities. The present invention also provides novel impurities that may be present in bexarotene and their isolation, characterization so that their presence in final product can be easily identified and quantified.

Although, the following examples illustrate the present invention in more detail, but should not be construed as limiting the scope of the invention.

Example 1: Preparation of methyl [4-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]benzoate

To a solution of 4-carboxy benzoic acid (100 g) in dichloromethane (800 ml) under nitrogen gas atmosphere, phosphorous pentachloride (200 g) was added at 25-30 °C and temperature was slowly raised to 38-44 °C. After completion of the reaction (monitored by GC), the reaction mass was cooled to -5 to -10 °C. Thereafter aluminium chloride (340g) and 1,2,3,4,-tetrahydro-1,4,4,6-pentamethyl naphthalene (120 g) were successively added to the reaction mixture and heated slowly to 38-44°C and refluxed for 1 hour. After completion of reaction (monitored by TLC), the reaction mass was cooled to 15-20 °C and poured into 5N hydrochloric acid (1L). Layers were separated and aqueous layer was extracted with dichloromethane (600 ml). The combined organic layer was washed successively with
demineralized water (2 L) and sodium bicarbonate solution (8 % solution, 1600 ml). The resulting organic layer was distilled off at atmospheric pressure to give residue which was dried to give title compound having purity 98.58 %, impurity A: 0.52 % and keto acid impurity B: 0.22 % by HPLC.

**Purification:**

n-Heptane (400 ml) was added to the resulting product and stirred for 30 minutes. The mixture is then cooled to 5-10 °C, stirred for 1 hour and filtered. The solid, thus obtained, is slurry washed with cold n-heptane (200 ml) and dried at 65°C to give 173 g of title compound as white to off white crystalline powder having purity 99.75%, impurity A: 0.14 % and keto acid impurity B: 0.02% by HPLC.

**Example 2: Preparation of methyl 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-1-ethenyl]benzoate**

To a suspension of methyl triphenylphosphonium bromide (167 g) in toluene (1000 ml) under nitrogen gas atmosphere at 25-30°C, potassium hexamethyldisilazide in toluene (0.5 M, 760 ml) was added. The solution was heated to 45-50°C, stirred for 30-45 minutes and then cooled to 0-5°C. A solution of methyl [4-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]benzoate (100 g), dissolved in toluene (600 ml) was slowly added to the reaction mixture and stirred for 2 hours. After completion of the reaction (monitored by HPLC), reaction mixture was poured into 2N hydrochloric acid (1 L). Layers were separated and aqueous layer was extracted with toluene (300 ml). The combined organic layer was washed with water (2 L). Activated carbon (0.05 g) was added to the resulting organic layer and stirred for 15 minutes at 25-30 °C. Resulting reaction mass was filtered through hyflo. The filtrate was distilled off under vacuum at 50-55°C to give title compound which was stirred in methanol (1 L) to remove triphenylphosphate oxide. The resulting product was filtered and dried to give 80 g of title compound having purity 95.65%, impurity C: 0.56 %, Impurity D: 1.90 %; Impurity E: 0.31%; impurity F: 0.22% by HPLC.

**Example 3: Preparation of bexarotene**

To suspension of methyl 4-[1-(5,6,7,8-tetrahydro-3, 5,5,8,8-pentamethyl-2-naphthalenyl)-1-ethenyl]benzoate (100 g) in methanol (1000 ml), 5N aqueous potassium hydroxide (170 ml) was added at 25-30°C and the reaction mixture was refluxed for 8 hours. After completion of reaction (monitored by HPLC), reaction mass was cooled to 35-40 °C and washed with 50% ethyl acetate: n-heptane mixture (2 x 500 ml). The organic layer was discarded and the aqueous layer was cooled to 10-15 °C. Thereafter pH of aqueous layer was adjusted to 1-2 by addition of concentrated hydrochloric acid (100 ml). The resulting mixture was then stirred at 20-25°C, stirred for 1 hour and precipitated solid was filtered. The resulting solid was slurry washed with demineralized water (100 ml), centrifuged and
dried to give 84.6 g of title compound having purity 98.89 %, none of the impurities C, D, E, F were detected by HPLC.

**Example 4: Purification of bexarotene**

**Method A**: Bexarotene (100g) was dissolved in tetrahydrofuran (400 ml) and activated carbon (0.04 g) was added to the solution. The reaction mixture was stirred for 30 minutes, filtered through hyflow and bed was washed with tetrahydrofuran (50 ml). Demineralized water (500 ml) was added to resulting filtrate at 15-20°C and stirred for 60 minutes. The precipitated solid was filtered, slurry washed with demineralized water (2 x 250 ml) and dried under vacuum at 55-60°C to give 75.6 g of title compound having purity 99.87% by HPLC.

**Method B**: Bexarotene (100 g) in ethyl acetate (1500 ml) was heated to 60-65°C to dissolve the solid and filtered hot to remove the suspended particles. n-Heptane (1500 ml) was added to the resulting mixture and stirred for 30 minutes. Thereafter, the reaction mixture was cooled to 5-10 °C and stirred for 1 hour. The precipitated solid was filtered, slurry washed with n-heptane (500 ml), dried under vacuum at 60-65°C to give 69.8 g of the title compound having purity 99.95 % by HPLC.

**Method C**: Bexarotene (100 g) in ethyl acetate (1500 ml) was heated to 60-65°C to dissolve the solid and filtered hot to remove the suspended particles. The resulting solution is distilled off under vacuum at 40-45 °C to give white to off white powder. n-Heptane (500 ml) was added to the resulting product at 25-30 °C and stirred. The reaction mixture was heated to 60-65°C and stirred for 30 minutes. Thereafter, reaction mixture was cooled to 5-10°C and stirred for 1 hour. The precipitated solid was filtered, slurry washed with n-heptane (100 ml) and then dried under vacuum at 60-65°C to give 90.2 g of the title compound having purity 99.99 % by HPLC.

**Method D**: Bexarotene (10 g) in methanol (500 ml) was stirred at room temperature and heated to 65-68°C to obtain a clear solution. The reaction mixture was stirred for 25 minute at 65-68°C, followed by addition of activated carbon (0.5g) at 65-68°C. Thereafter, the reaction mixture was filtered hot through hyflo at 60-65°C and the bed was washed with methanol (150 ml). The resulting solution was cooled to 10-15°C, stirred for 1 hour and filtered. The solid thus obtained was washed with cooled methanol (30 ml) and dried under vacuum at 55-60°C to give 5.82 g of title compound having purity 99.81 % by HPLC.

**Method E**: Bexarotene (10 g) in ethyl acetate (150 ml) was stirred at room temperature and heated to 65-68°C to obtain a clear solution. The reaction mixture was stirred for 25 minute at 65-68°C, followed by addition of activated carbon (0.5g) at 65-68°C. Reaction mixture was then filtered hot through hyflo bed, cooled to 10-15°C, stirred for 1 hour and filtered. The solid thus obtained was washed with cold
ethyl acetate (20 ml) and dried under vacuum at 55-60°C to give 5.01 g of title compound having purity 99.81 % by HPLC.

**Method F:** Bexarotene (10 g) in acetone (400 ml) was stirred at room temperature and heated to 55-58°C to obtain a clear solution. The reaction mixture was stirred for 25 minute at 55-58°C, followed by addition of activated carbon (0.5g) at 55-58°C. Thereafter, reaction mixture was filtered through hyflo bed at 50-55°C, cooled to 0-5°C, stirred for 1 hour and filtered. The solid thus obtained was washed with cold acetone (20 ml) and dried under vacuum at 55-60°C. to give 4.82 g of title compound having purity 99.81 % by HPLC.

**Method G:** Bexarotene (50 g) was dissolved in tetrahydrofuran (200 ml) and activated carbon (5.0 g) was added to the solution. The reaction mixture was stirred for 30 minutes, filtered through hyflow and bed was washed with tetrahydrofuran (50- ml). Demineralized water (200ml) was slowly added to resulting filtrate at 15-20°C and stirred for 60 minutes. Solid thus precipitated was filtered and dissolved in ethyl acetate 600 ml) at 60-65°C and was filtered to remove the suspended particles, n-Heptane (720 ml) was added to the resulting mixture and stirred for 30 minutes. Thereafter, reaction mixture was cooled to 5-10 °C stirred for 1 hour, slurry washed with n-heptane (100 ml). The resulting solid was dried under vacuum at 60-65°C to give 28 g of the title compound having purity 99.72 % by HPLC.

**Method H:** Bexarotene (10 g) in dichloromethane (400 ml) was stirred at room temperature and heated to 35°C to obtain a clear solution. n-Hexane (600 ml) was added to the resulting mixture and stirred for 1 hour at 15-20 °C. The precipitated solid was filtered and washed with a mixture of dichloromethane and n-hexane. The resulting solid was dried under vacuum at 65°C to give 6.35 g of the title compound having purity 99.91 % by HPLC.

**Method I:** Bexarotene (10 g) in ethyl acetate (150 ml) was stirred at room temperature and heated to 65-70°C to obtain a clear solution. n-Hexane (400 ml) was added to the resulting mixture and stirred for 1 hour at 15-20 °C. The precipitated solid was filtered and washed with ethyl acetate and n-hexane (1:2, 100 ml). The resulting solid was dried under vacuum at 65°C to give 5.21 g of the title compound having purity 99.51 % by HPLC.

**Method J:** Bexarotene (10 g) in dichloromethane (400 ml) was stirred at room temperature and heated to 35-38°C to obtain a clear solution. The reaction mixture was stirred for 25 minute at 35-38°C, followed by addition of activated carbon (0.5g) at 35-38°C. Thereafter, reaction mixture was filtered through hyflo bed at 35-38°C and the bed was washed with dichloromethane (50 ml). The resulting solution was cooled to 0-5°C, stirred for 1 hour and filtered. The solid thus obtained was washed with
cold dichloromethane (20 ml) and dried under vacuum at 55-60°C to give 4.25 g of title compound having purity 99.85 % by HPLC.

**Example 5: Preparation of impurity A**

To a solution of terephthalic acid (20 g) in dichloromethane (160 ml) under nitrogen gas atmosphere, phosphorous pentachloride (60g) was added at 25-30 °C and reaction mixture was heated to 38-44°C. After completion of reaction (monitored by TLC), the reaction mass was cooled to -5 to -10 °C. Thereafter, aluminum chloride (113 g) and 1,2,3,4-tetrahydro-1,4,4,6-pentamethylnaphthalene (40 g) were successively added to the reaction mixture and refluxed for 2 hours at 35 to 40 °C. After completion of the reaction (monitored by TLC), the mixture was cooled to 15-20 °C, poured into 5N hydrochloric acid (300 ml) followed by layer separation. Aqueous layer was extracted with dichloromethane (120 ml), the combined organic layer was washed with demineralized water (300 ml) followed by 8 % sodium bicarbonate solution (260 ml). Dichloromethane was distilled off at atmospheric pressure and the residue thus obtained was cooled to 15-20 °C. n-Heptane (80 ml) was added to the resulting reaction mass and stirred for 30 minutes. The mixture was further cooled to 5-10 °C, stirred for 1 hour and filtered. The solid thus obtained was slurry washed with cold n-heptane (60 ml) and dried at 65 °C to give 22 g of title compound having purity 92.90% by HPLC. The resulting product was subjected to column chromatography on silicagel (ethyl acetate: Hexane) to give 10.30 g of the title compound having purity 98.57% by HPLC.

**Example 6: Preparation of Impurity B**

To a suspension of methyl [4-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]benzoate (5 g) in methanol (200 ml), 5N potassium hydroxide solution (20 ml) was added at 25-30 °C and heated to reflux. After completion of reaction (monitored by TLC), the reaction mixture was cooled to 20-25 °C and acidified with 5N hydrochloric acid (20 ml). The solid, thus precipitated out, was stirred at 20-25 °C for 1 hour, filtered, washed with water (50 ml) and dried at 60 °C to give 3.1 g of the title compound as white solid having purity 99.52 % by HPLC.

**Example 7: Preparation of impurities C and D (wherein in R is methyl)**

The combined extract containing mixture of ethyl and heptane (obtained in example 3 after washing the reaction mixture) was concentrated and was chromatographed over silica gel using mixture of ethyl acetate and heptane. The first fraction eluted with mixture of ethyl acetate and heptane was collected and the solvent was distilled to give 1.2 g of impurity D. The second fraction eluted with mixture of ethyl acetate and heptane was collected and concentrated to give 2.0 g of impurity C.
Example 8: Preparation of impurities E and F

To a suspension of methyl triphenylphosphonium bromide (15g) in toluene (150ml) under nitrogen atmosphere at 25-30 °C, potassium hexamethyldisilazide in toluene (0.5 M, 25 ml) was added and solution was heated at 65-70°C with stirring for 30 minutes. The suspension was cooled to 5-10°C and a solution of impurity A (6 g) dissolved in toluene (90 ml) was added drop wise to the reaction mixture and stirred for 2 hours. After the completion of the reaction (monitored by TLC), reaction mixture was poured into 2N hydrochloric acid (100 ml). Layers were separated and aqueous layer was extracted with toluene (30ml). The combined toluene layer was washed with water (50 ml), filtered through hyflbed and solvent was completely distilled off under vacuum at 50-55°C to afford a residue.

Methanol (35 ml) was added to the resulting residue and stirred for 1 hour at 25-30 °C. The solid thus formed was filtered and dried at 65 °C to yield 3.2 g of the solid which contain mixture of impurity E and impurity F. The resulting solid was subjected to column chromatography on silcagel eluted with a mixture of ethyl acetate and heptane. The first fraction eluted with mixture of ethyl acetate and heptane was collected and the solvent was distilled to give 1.08 g of impurity F. The second fraction eluted with mixture of ethyl acetate and heptane was collected and concentrated to give 1.8 g of impurity E.
We Claim:

1). A process for the preparation of highly pure bexarotene, comprises the steps of:

a) activating mono alkyl ester of terphthalic acid using a suitable activating agent to form a reactive derivative of formula II;

\[ \text{Formula II} \]

wherein \( X \) is selected from halo such as chloro, bromo and the like; and \( R \) is alkyl selected from methyl, ethyl, \( n \)-propyl, isopropyl, \( n \)-butyl, isobutyl and the like

b) reacting the reactive derivative of formula II with 1, 2, 3, 4-tetrahydro- 1, 1, 4, 4, 6-pentamethylnaphthalene of formula III,

\[ \text{Formula III} \]

in the presence of a catalyst to form keto ester intermediate of formula IV;

\[ \text{Formula IV} \]

wherein \( R \) is as defined above

c). optionally, purifying keto ester intermediate of formula IV with a suitable solvent;

d). reacting keto ester intermediate of formula IV with methyl triphenylphosphonium halide in the presence of a suitable base in inert solvent to form olefinic ester intermediate of formula V;

\[ \text{Formula V} \]

wherein \( R \) is as defined above

e). optionally, purifying olefinic ester intermediate of formula V with a suitable solvent;

f). hydrolyzing olefinic ester intermediate of formula V;

g). washing the reaction mixture with a suitable solvent;

h). isolating bexarotene of formula I there from; and

i). optionally, purifying bexarotene of formula I.

2). The process according to claim 1, wherein in step a) activating agent includes phosphorus halides such as phosphorus pentachloride; phosphorus tribromide, phosphorus oxy halide such as
phosphorus oxy chloride; oxalyl halide such as oxalyl chloride, oxalyl bromide; thionyl halide such as thionyl chloride and the like; and in step b) catalyst is Lewis acids that include aluminum chloride, titanium tetrachloride, boron trifluoride and the like.

3). The process according to claim 1, wherein in step c) suitable solvent includes aliphatic hydrocarbons such as n-pentane, n-heptane, n-hexane, cyclohexane, pentane, hexane, heptane; aliphatic ethers such as isopropyl ether, methyl tert-butyl ether, diethyl ether; C_{1-4} alcohols such as methanol, ethanol, propanol, isopropanol and the like or mixture thereof.

4). The process according to claim 1, wherein in step d) suitable base includes alkali or alkaline metal hexamethyldisilazanes, C_{1-4} alkoxide, hydrides thereof such as potassium hexamethyldisilazane, sodium hydride (in paraffin oil), potassium hydride, sodium methoxide, sodium tert-butoxide, potassium tert-butoxide and the like; and suitable solvent includes aliphatic or aromatic hydrocarbons such as toluene, 1,2-xylene, 1,4-xylene; ethers such as tetrahydrofuran, 2-methyl tetrahydrofuran, methyl tert-butyl ether, 1,2-dimethoxy ethane, 1,2-dimethoxy methane and the like or mixture thereof.

5). The process according to claim 1, wherein in step e) suitable solvents include C_{1-4} alcohols such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol; alkyl nitriles such as acetonitrile, propionitrile; C_{5-8} aliphatic alkanes such as n-pentane, n-heptane, n-octane, cyclohexane, cyloheptane, pentane, hexane, heptane; aliphatic ethers such as diethyl ether, isopropyl ether, methyl tert-butyl ether, 1,2-dimethoxyethane, 1,2-diethoxyethane; cyclic ethers such as tetrahydrofuran and the like or mixture thereof or in mixture with water.

6). The process according to claim 1, wherein in step f) hydrolysis is carried out using base which include alkali or alkaline earth metal hydroxides, such as lithium hydroxide, sodium hydroxide, potassium hydroxide and the like; and in step g) suitable solvent includes aliphatic esters such as ethyl acetate, n-propyl acetate, n-butyl acetate; C_{5-9} aliphatic alkanes such as n-pentane, n-hexane, n-heptane, n-octane, pentane, hexane, heptane, octane, cyclopentane, cyclohexane; aromatic hydrocarbons such as benzene, toluene, 1,2-xylene, 1,4-xylene; halogenated solvents such as dichloromethane, chloroform, 1,2-dichloroethane, C_{5-8} alkyl ethers such as diethyl ether, diisopropyl ether, methyl tert-butyl ether, and the like or mixture thereof.

7). The process according to claim 1, wherein in step i) bexarotene is purified by washing or crystallization with a suitable solvent selected from C_{1-4} alcohols such as methanol, ethanol, propanol, isopropanol; aliphatic C_{3-5} ketones such as acetone, ethyl methyl ketone, diethyl ketone; C_{4-8} aliphatic esters such as methyl acetate, ethyl acetate, n-propyl acetate, n-butyl acetate; C_{5-8} aliphatic ethers such as diethyl ether, ethyl methyl ether, isopropyl ether, methyl tert-butyl ether;
cyclic ether such as tetrahydrofuran; halogenated solvent such as dichloromethane, dichloroethane, chloroform and the like or mixture thereof.

8). A process for removal of impurities from bexarotene comprising the steps of:
   a). washing the reaction mixture containing bexarotene or salts thereof with a suitable solvent or solvent mixture; and
   b). isolating pure bexarotene of formula I there from.

9). The process according to claim 8, wherein suitable solvent includes aliphatic esters, C₅₋₉ aliphatic alkanes, aromatic hydrocarbons, halogenated solvents, C₅₋₈ alkyl ethers, and the like or mixture thereof.

10). The process according to claim 9, wherein suitable solvent is selected from ethyl acetate, n-propyl acetate, n-butyl acetate, n-pentane, n-hexane, n-heptane, n-octane, pentane, hexane, heptane, octane, cyclopentane, cyclohexane, benzene, toluene, 1,2-xylene, 1,4-xylene, dichloromethane, chloroform, 1,2-dichloroethane, diethyl ether, diisopropyl ether, methyl tert-butyl ether and the like or mixture thereof.

11). Bexarotene containing less than 0.15% of impurity of formula, 

\[ \text{Impurity A or} \]

\[ \text{Impurity B or} \]

\[ \text{Impurity C or} \]

\[ \text{Impurity D or} \]

\[ \text{Impurity E or} \]

\[ \text{Impurity F} \]
wherein \( R \) is alkyl selected from methyl, ethyl, \( n \)-propyl, isopropyl, \( n \)-butyl, isobutyl and the like

12). Highly pure bexarotene containing any individual identified impurity less than 0.15\% and unidentifed impurity less than 0.10\% by HPLC.

13). A process for identification of an impurity in a sample of bexarotene, selected from one or more of the impurities amongst A, B, C, D, E or F, the process comprising performing steps (a) and (b) in either order

a). carrying out a chromatographic analysis on the reference sample, containing one or more of impurities amongst A, B, C, D, E or F (reference marker) and bexarotene, to determine the relative retention time of the reference marker compared to bexarotene;

b). carrying out a chromatographic analysis on the sample of bexarotene to determine the relative retention time of impurities present in the sample compared to bexarotene; and comparing the relative retention times determined in step (a) and (b); where if the relative retention times determined are substantially same, the impurity of bexarotene in the sample is identified as being the same as reference marker.

14). A method of determining the amount of an impurity, selected from one or more of impurities among A, B, C, D, E and F, the method comprising performing steps (a) and (b) in either order:

a), carrying out a chromatographic analysis on the reference sample, containing known amount of one or more impurities amongst A, B, C, D, E or F (reference standard) and bexarotene, to determine the relative retention time of impurities compared to bexarotene; and measuring the area under a peak corresponding to the reference standard;

b). carrying out a chromatographic analysis on the sample of bexarotene to determine the area under HPLC peak corresponding to each and every impurity by reference to the relative retention times thus determined compared to bexarotene; and calculating the amount of one or more of impurity among A, B, C, D, E and F in bexarotene, by reference to area of the HPLC peak in the sample against the area of the HPLC peak associates with the same impurity in step (a).

15). A compound of formula;

![Impurity A](image)

16). The compound according to claim 15, is in isolated form.
17). A compound of formula:

\[
\begin{array}{c}
\text{Impurity C} \\
\end{array}
\]

\textit{wherein } R \textit{ is alkyl selected from methyl, ethyl, n-propyl, isopropyl, } n\text{-butyl, isobutyl } \textit{and the like}

18). The compound according to claim 17, is in isolated form.

19). A compound of formula:

\[
\begin{array}{c}
\text{Impurity D} \\
\end{array}
\]

\textit{wherein } R \textit{ is alkyl selected from methyl, ethyl, n-propyl, isopropyl, } n\text{-butyl, isobutyl } \textit{and the like}

20). The compound according to claim 19, is in isolated form.

21). A compound of formula:

\[
\begin{array}{c}
\text{Impurity E} \\
\end{array}
\]

22). The compound according to claim 21, is in isolated form.

23). A compound of formula:

\[
\begin{array}{c}
\text{Impurity F} \\
\end{array}
\]

24). The compound according to claim 23, is in isolated form.
Figure 5
INTERNATIONAL SEARCH REPORT

International application No. PCT/IN2011/000322

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: C07C 63/00; C07C 49/00; C07C 69/00; C07C13/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI; EPDOC; CPRS; CNKI; CAplus; STN registry

Bexarotene, pentamethyl, tetrahydro, naphthyl, benzoic acid, retinoid, RXR, antitumour, retinoid x receptor, CAS No.: 153559-19-0

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<tr>
<td>P.A</td>
<td>pages 187-189</td>
<td>13-24</td>
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<tr>
<td>X</td>
<td>US5466861 A (SRI INTERNATIONAL, et al.) 14 Nov. 1995(14.11.95) examples 10,11</td>
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<td>Y</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search: 10 Oct. 2011 (10.10.2011)

Date of mailing of the international search report: 27 Oct. 2011 (27.10.2011)

Name and mailing address of the ISA/CN
The State Intellectual Property Office, the P.R.China
6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088.
Facsimile No. 86-10-62019451

Authorized officer: WANQYing
Telephone No. (86-10) 62084578

Form PCT/ISA /210 (second sheet) (July 2009)
**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/IN2011/000322

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>1.☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:</td>
</tr>
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<td>2.☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:</td>
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<td>3.☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).</td>
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<td>1.☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</td>
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<td>2.☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of additional fee.</td>
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<td>3.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:</td>
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<td>4.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:</td>
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**Remark on protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.
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<td>01.06.2005</td>
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Form PCT/ISA /210 (patent family annex) (July 2009)
INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2011/000322

Continuation of:

Box No. III

This International Searching Authority found multiple inventions in this international application, as follows:

1) Claims 1-7 directed to a process for the preparation of bexarotene comprising the steps of a) to i);
2) claims 8-10 directed to a process for removal of impurities from bexarotene comprising the steps of washing the reaction mixture and isolating pure bexarotene of formula I there from;
3) Claims 11-12 directed to compound of bexarotene containing certain impurities;
4) Claims 13-14 directed to a process for identification an impurity or determining the amount of an impurity in a sample of bexarotene, comprising steps of chromatographic analysis on the reference sample and the sample of bexarotene in either order;
5) Claims 15-16 directed to compound of impurity A;
6) Claims 17-18 directed to compound of impurity C;
7) Claims 19-20 directed to compound of impurity D;
8) Claims 21-22 directed to compound of impurity E;
9) Claims 23-24 directed to compound of impurity F.

Since bexarotene is a known compound, and the subject-matter of independent claim 1 and the subject-matters of independent claims 8, 11, 12, 13, 14, 15, 17, 19, 21, 23 are not linked by common or corresponding special technical features and define multiple inventions not linked by a single general inventive concept.

The application, hence does not meet the requirement of unity of invention as defined in PCT Rule 13.1.

A. CLASSIFICATION OF SUBJECT MATTER

C07C 63/44 (2006.01) i
C07C 49/788 (2006.01) i
C07C 69/76 (2006.01) i
C07C 13/38 (2006.01) i