(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 October 2009 (15.10.2009)

PCT

(10) International Publication Number
WO 2009/124906 A2

(51) **International Patent Classification**:
C07D 401/12 (2006.01)

(21) **International Application Number**:
PCT/EP2009/054085

(22) **International Filing Date**:
6 April 2009 (06.04.2009)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
PD2008A000106 7 April 2008 (07.04.2008) IT

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2009/124906 A2

(54) **Title**: METHOD FOR PREPARING ARGATROBAN MONOHYDRATE

(57) **Abstract**: A method is described for preparing argatroban monohydrate obtained from (2R,4R)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2- piperidine carboxylic acid by suitably treating crude argatroban. The method either comprises preparation of argatroban monohydrate in a continuous step or an intermediate step of isolating a purified argatroban. Also obtainable from argatroban monohydrate is anhydrous argatroban, shown to have new physico- chemical characteristics. The described argatroban synthesis and purification process hence enables three different forms of argatroban, not previously described, to be obtained, each with distinctive physico-chemical characteristics and in particular enables argatroban monohydrate to be obtained with high yield and with high purity, being therefore a product suitable for use as active principle in proprietary medicines.

METHOD FOR PREPARING ARGATROBAN MONOHYDRATE

Field of the invention

The present invention relates to a method for preparing argatroban monohydrate. Said method enables argatroban to be obtained in three different crystalline forms i.e. in the form of argatroban monohydrate, purified argatroban and argatroban anhydrous, each having specific and new physico-chemical characteristics. The present invention also relates to the three different isolated forms, namely argatroban monohydrate, purified argatroban and argatroban anhydrous.

State of the art

US patent 4,201,863 (6th May 1980) and EP 8746 (filed on 22nd August 1979 with priority based on the application for the cited US patent) describe a class of N^2-arylsulphonyl-L-argininamide drugs, with anti-thrombotic activity, and the processes for obtaining them. Of these, the compound 4-methyl-1-[N^2-(3-methyl-1,2,3,4-tetrahydro-8-quinolinesulphonyl)-L-arginyl]-2-piperidine carboxylic acid (argatroban, isomers mixture) is described. The described process comprises the synthesis of an intermediate N^G–substituted-N^2- quinolinesulphonyl-L-argininamide from which the desired compound is obtained by catalyzed hydrogenolysis or acidolysis and catalyzed hydrogenation. The general conditions provided for the hydrogenolysis and hydrogenation reaction are: i) inert solvents (methanol, ethanol, tetrahydrofuran or dioxane); ii) presence of a catalyst (Raney nickel, palladium, platinum, ruthenium, rhodium); iii) hydrogen atmosphere at a pressure between 1 and 100 kg/cm^2 and preferably between 5 and 50 kg/cm^2; iv) temperature between 0ºC and 200ºC and preferably between 50ºC and 150ºC; v) reaction temperature from 2 hours to 120 hours. The crude product obtained is then purified by trituration or by re-crystallization from diethyl ether-tetrahydrofuran, diethyl ether-methanol or from water-methanol or by chromatography. No example is given of this purification step. In particular, both US 4,210,863 and EP 8746 in example 1(E) describe the preparation of argatroban, isomers mixture. This compound is obtained in amorphous form by hydrogenation of [N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid in ethanol in the presence of Pd/C with hydrogen pressure of 10 kg/cm^2 at 100ºC for 8 hours. The catalyst is removed by filtration of

the ethanol solution which is then evaporated without further purification and/or re-crystallization steps. In the US patent at issue as indeed in patent application EP 8746, no mention is made of polymorphic forms of the compounds and, for the obtained compound, the following characteristics are reported: *Amorphous solid*, *I.R. (KBr) (cm^{-1})* 3400; 1620; 1460; 1380; *Molecular composition (%): theoretical* C 54.31; H 7.13; N 16.52; *found (%)* C 54.01; H 6.98; N 16.61.

US 4,258,192 (24th March 1981) (continuation-in-part of the aforesaid patent application US 4,201,863) and the same patent application EP 8746 describe the stereoisomers and the preparation thereof, including argatroban used as an active principle in medicaments, i.e. the stereoisomer (2*R*,4*R*)-4methyl-1-[N^2-(3-methyl-1,2,3,4-tetrahydro-8-quinolinesulphonyl)-L-arginyl]-2-piperidine carboxylic acid, with the following characteristics: *melting point* (*m.p.*). 188-191°C; *I.R. (KBr) (cm^{-1})* 3400, 1620, 1460, 1380; *Molecular composition (%): theoretical* C 54.31; H 7.13; N 16.52; *found (%)* C 54.05; H 6.94; N 16.65. The compound is prepared according to the description given in examples 1(E) in US 4,258,192 and 2(E) and 3 in EP 8746 respectively by hydrogenation of (2*R*,4*R*) 1-[N^G-nitro-N^2-(3-methyl-1,2,3,4-tetrahydro-8-quinolinesulphonyl)-L-arginyl]-2-piperidine carboxylic acid in ethanol in presence of acetic acid catalyzed by Pd/C. After filtering the mass to remove the catalyst, the solvent is evaporated and the residue suspended in chloroform, the solution treated with a saturated sodium bicarbonate solution or 1N sodium hydroxide solution and after washing, the solvent is evaporated. The compound is then re-crystallized from ethanol. Again in this case, no reference is made to the obtainment of monohydrate polymorphic forms.

Said polymorphic forms are described instead in the publication Biochem. Biophys. Res. Comm. 1981, 101, 440-446 in the context of stereoisomer preparation. The monohydrate polymorph of the (2*R*,4*R*) stereoisomer is prepared by re-crystallization from ethanol/water and the reported characteristics are: *m.p.* 176-180°C; $[\alpha]_D^{27}$ +76.1° (c 1, 0.2N HCl).

US 5,925,760 (20th July 1999) and EP 0823430 (filed 4th August 1997) subsequently describe a new method for preparing argatroban by means of a new intermediate N^2-(3-methyl-8-quinolinesulphonyl)-N^G–nitro-L-arginine. In particular the patent makes reference to the preparation of a crystalline monohydrate form of

argatroban, referring back to examples (D) and (E) of Japanese patent publication No. (Hei)-2-31055/1990 and generically to an I.R. spectrum identical to that of the commercially available argatroban compound. The relevant example in the cited patent publication is example (E), while example (D) concerns the preparation of (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid. This compound represents the starting compound for argatroban preparation by catalytic reduction in the presence of Pd/C. The crude argatroban obtained is then purified by extraction with chloroform, treatment with a saturated sodium bicarbonate solution and, after solvent evaporation, re-crystallization from ethanol or from 15% alcohol in water. It should be noted however that the Japanese patent makes no mention of the monohydrate form of argatroban being obtained and that for the compound the following characteristics are reported: *m.p.* 188-191°C; *molecular composition (theoretical/found) (%)*: C 54.31/54.01; H 7.13/6.98; N 16.52/16.61; *I.R. (KBr) (cm^{-1})* 3400; 1620; 1460; 1380. These analytical data, with the exception of the unreported melting point, are the same as those indicated in the cited patent documents describing a mixture of (2*R*,4*R*)-4methyl-1-[N^2-(3S-methyl-1,2,3,4-tetrahydro-8-quinolinesulphonyl)-L-arginyl]-2-piperidine carboxylic acid and (2*R*,4*R*)-4methyl-1-[N^2-(3R-methyl-1,2,3,4-tetrahydro-8-quinolinesulphonyl)-L-arginyl]-2-piperidine carboxylic acid isomers of argatroban, but do not correspond to the melting point given in the publication, being the only document that identifies the monohydrate form of argatroban.

More recently, patent application CN 1,951,937 (filing date 10th November 2006) described a method for preparing hydrated argatroban by treating argatroban with large quantities of water (more than 60 and up to 80 volumes of distilled water per gram of argatroban) at a temperature of 80-100ºC for a time of 0.5-1 hour and crystallization by cooling. The water content reported is comprised between 3.3 and 3.8% and the ratio of dextroisomer *R* to levoisomer *S* is *R*:*S* = 63-67 : 37-33.

Argatroban is a compound of wide therapeutic use, for which reason the need still exists to provide a compound of pharmaceutically acceptable quality obtained by easily industrialized and economically convenient methods. With regard to the monohydrate, this form is preferable for the applicative purpose since the

anhydrous form is unstable and tends to become hydrated and/or wet. Moreover it crystallizes only with difficulty at the correct ratio between the diastereoisomers.

A first main purpose of the present invention is therefore to provide a method for preparing argatroban monohydrate usable as active principle of pharmaceutical quality for the preparation of proprietary drugs. A further purpose is to obtain said active principle by a synthesis and purification process which enables it to be obtained with a good yield and very high purity.

Summary

For the previously indicated purposes the preparation method of argatroban monohydrate according to the invention consists of preparing crude argatroban, preferably starting from the compound (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid according to the known synthesis process of catalyzed hydrogenation and hydrogenolysis, then treating it to be purified and separated from the reaction mass into the monohydrate form by controlled crystallization in a methanol/water solvent. Preferably argatroban monohydrate is prepared by separating an intermediate consisting of purified argatroban which is then treated to be transformed into the monohydrate form by the previously mentioned process of crystallization from methanol/water. The preparation by separation of the purified intermediate is to be preferred, as this intermediate presents new and distinctive characteristics and allows argatroban monohydrate to be obtained with high purity and with the correct diastereoisomer ratio.

Furthermore, it has been surprisingly found that an argatroban anhydrous having new and distinctive physico-chemical characteristics can be subsequently obtained from argatroban monohydrate.

In a first aspect the invention hence relates to the preparation of argatroban monohydrate and optionally to argatroban anhydrous.

Therefore, the present invention provides a method for preparing argatroban monohydrate comprising at least the step of:

- separating argatroban monohydrate from a concentrated reaction mass containing crude argatroban or from a purified argatroban by crystallization from a medium solvent consisting of methanol and water by adding a

decolorizing carbon, heating the mass at the reflux temperature then subjecting it to carbon filtering and to controlled and gradual cooling from the reflux temperature to a temperature between 15-25ºC in a time comprised between 11-17 hours.

When the method of preparation is from a crude argatroban, according to a first embodiment of the invention, said method is substantially in one continuous step, but the argatroban monohydrate can be also prepared in two steps with isolation of a purified argatroban by crystallization from an organic solution of crude argatroban with a crystallization solvent selected from isopropanol and normal-propanol before separating the argatroban monohydrate by crystallization.

According to this second embodiment, the method of preparing argatroban monohydrate comprises at least the following steps of:

- concentrating a reaction mass containing crude argatroban to a stirrable residue;
- dissolving the residue containing crude argatroban with an organic solvent and separating a purified argatroban by crystallization by treating the organic solution with a crystallization medium solvent selected from isopropanol and normal-propanol;
- separating argatroban monohydrate by re-crystallizing the purified argatroban isolated in the preceding step from a methanol and water mixture solution by adding a decolorizing carbon, heating the mass at the reflux temperature then subjecting it to carbon filtering and to controlled and gradual cooling from the reflux temperature to a temperature between 15-25ºC in a time comprised between 11-17 hours.

The crystalline precipitate consisting of argatroban monohydrate, obtained either by the continuous mode or in two steps by separating the purified argatroban intermediate, can be dried under nitrogen flow or under vacuum at a temperature comprised from 50ºC and 80ºC for at least 8 hours.

By subjecting the obtained argatroban monohydrate to re-crystallization from water by quickly cooling to a temperature of 15ºC in a time not greater than 2 minutes, argatroban anhydrous can be obtained, shown to possess new physico-chemical characteristics.

In a second aspect, therefore, the invention relates to the obtained and isolated single forms of argatroban and hence to: argatroban monohydrate, purified argatroban and argatroban anhydrous having the physico-chemical characteristics described below and reported in the claims that follow.

The advantages attainable with the present invention will be clear to an expert of the art from the following detailed description of particular embodiments of the method and from the compounds obtainable therewith given as non-limiting examples, with reference to the following figures.

Brief description of the figures

Figure 1: the figure shows a representative I.R. spectrum of crystalline argatroban monohydrate obtained by crystallization from a solvent consisting of methanol and water according to the described method.

Figure 2: the figure shows the differential scanning calorimetry (DSC) for a representative sample of argatroban monohydrate obtained by crystallization from a solvent consisting of methanol and water according to the described method.

Figure 3: the figure shows a thermogravimetric analysis of a representative sample of argatroban monohydrate obtained by crystallization from a solvent consisting of methanol and water according to the described method.

Figure 4: the figure shows a diffractometry (XRPD) profile of argatroban monohydrate obtained by crystallization from a solvent consisting of methanol and water according to the described method.

Figure 5: the figure shows a representative I.R. spectrum of argatroban anhydrous obtained from argatroban monohydrate by the described method.

Figure 6: the figure shows the differential scanning calorimetry (DSC) for a representative sample of argatroban anhydrous obtained from argatroban monohydrate by the described method.

Figure 7: the figure shows a thermogravimetric analysis of a representative sample of argatroban anhydrous obtained from argatroban monohydrate by the described method.

Figure 8: the figure shows a diffractometry (XRPD) profile of argatroban anhydrous obtained from argatroban monohydrate by the described method.

Figure 9: the figure shows a representative I.R. spectrum of purified argatroban

obtained by the described method.

Figure 10: the figure shows the differential scanning calorimetry (DSC) for a representative sample of purified argatroban obtained by the described method.

Figure 11: the figure shows a thermogravimetric analysis of a representative sample of purified argatroban obtained by the described method.

Figure 12: the figure shows a diffractometry (XRPD) profile of purified argatroban.

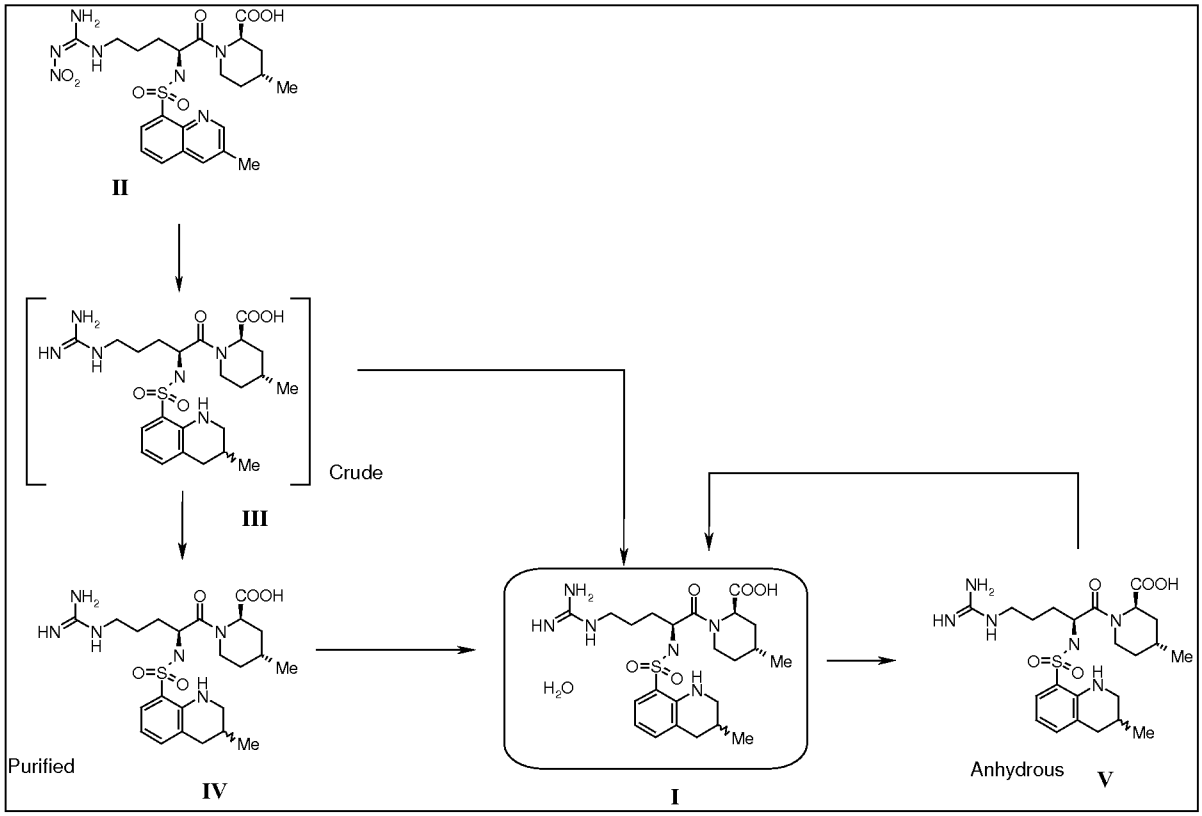
Figure 13: the figure shows a comparison between the DSC for argatroban anhydrous (2°peak) and the corresponding monohydrate (1°peak) form obtained by the described method.

Figure 14: the figure shows a comparison between the thermogravimetric analyses of argatroban anhydrous (upper curve) and monohydrate (lower curve) obtained by the described method.

Figure 15: the figure shows a comparison between the XRPD diffractograms of the monohydrate, purified and anhydrous species obtained by the described method.

Detailed description of the invention

The process for preparing argatroban monohydrate of the present invention derives from the following scheme:



In the argatroban monohydrate preparation method of the invention, comprising the steps reported in the Summary, the starting compound for preparing argatroban monohydrate is (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinosulphyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid (**II**) already described in US 4,258,192 and EP 8746.

For the purposes of the present invention said compound (**II**) is subjected to a hydrogenation and hydrogenolysis catalyzed by a catalyst consisting of palladium on carbon (Pd/C) in a solvent formed from methanol in the presence of acetic acid in which the v/v ratio between the two is comprised between 1 (acetic acid) and 4-16 (alcohol) and the ratio between the starting compound (**II**) and acetic acid in w/v is comprised between 0.5 and 2.5. The hydrogenation reaction is conducted under hydrogen atmosphere in the manner known to an expert skilled in the art and preferably at a pressure from 6 to 12 bar and at temperatures from 50ºC to 100ºC for a time comprised between 6 and 18 hours.

Continuous mode process

When the process is in the continuous mode, after cooling to room temperature, the Pd/C catalyst is removed from the reaction mass, said obtained mass then

being heated to temperatures between 40ºC and 80ºC and concentrated to at least half its volume.

The mixture thus obtained can be optionally treated with an aqueous solution of a base preferably chosen from sodium hydroxide, sodium bicarbonate and ammonia at a concentration between 10 and 30%, to bring the pH of the mixture itself to between 7.0 and 7.5.

The solution thus obtained is diluted with water to obtain a crystallization solvent for argatroban monohydrate (**I**) formed of a mixture of water and methyl alcohol in which the methyl alcohol has a concentration between 10 and 20%. The MetOH:water crystallization solvent is in a quantity up to 50 volumes per gram of argatroban (**III**) and preferably up to 40 volumes and more preferably comprised from 25 to 35 volumes, the MetOH:water ratio being preferably 1:7 v/v. Crystallization is subsequently achieved by: adding a decolorizing carbon, heating the mass at the reflux temperature and maintaining it under stirring for a time comprised between 1 and 3 hours followed by filtering the carbon and controlled cooling for a time comprised between 11-17 hours to bring the mixture from reflux temperature to 15-25ºC, but preferably to 20ºC. In particular the mass is subjected to the following temperature gradient: heating to 90-95ºC, maintenance at reflux temperature from 1 to 3 hours, preferably for 2 hours, cooling to 70-75ºC in at least one hour and maintenance at this temperature for at least one hour, cooling to 20ºC in a time comprised between 2 and 6 hours, preferably in 4 hours, and maintenance at this temperature for at least 6 hours. The crystalline precipitate which forms is argatroban monohydrate (**I**) with the physico-chemical characteristics described hereinafter.

Considering all the step hereinabove reported in this first embodiment the preparation method of argatroban monohydrate comprises the steps of:

- preparing the crude argatroban from (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid in methanol/acetic acid;
- concentrating the reaction mass up to at least half of the volume;
- optionally treating the reaction mass with an aqueous solution of a base to bring the pH of the mixture itself to between 7.0 and 7.5;

- crystallizing the argatroban monohydrate by forming a crystallization medium solvent consisting of a mixture of methanol:water, wherein the methyl alcohol has a concentration between 10 and 20%, and subjecting the mass to an addition of carbon and to a heating at the reflux temperature then subjecting it to carbon filtration and to controlled and gradual cooling from the reflux temperature to a temperature between 15-25ºC in a time comprised between 11-17 hours.

In particular, the controlled and gradual cooling is at the following temperature gradient: heating to 90-95ºC, maintenance at reflux temperature for a time between 1 and 3 hours, cooling to 70-75ºC in at least one hour and maintenance at this temperature for at least one hour, cooling to 20ºC in a time comprised between 2 and 6 hours and maintenance at this temperature for at least 6 hours.

A step of drying under nitrogen flow or under vacuum at a temperature comprised from 50ºC and 80ºC for at least 8 hours can be further comprise.

Process with isolation of the purified argatroban (**IV**) intermediate

The synthesis reaction is conduced as aforedescribed and on completion of hydrogenation, after cooling to room temperature, the Pd/C catalyst is removed from the obtained mass. Said mass is then heated to a temperature from 40ºC to 80ºC and concentrated to at least a stirrable residue.

The residue containing crude argatroban (**III**) is dissolved in an organic solvent, being preferably dichloromethane.

The mixture thus obtained can be optionally treated with an aqueous solution of a base preferably chosen from sodium hydroxide, sodium bicarbonate and ammonia at a concentration between 10 and 30%, to bring the pH of the mixture itself to between 7.0 and 7.5. Optionally in case of excess, the base is then removed either by washing the organic phase with water or under vacuum.

In this case a crystallization solvent selected from isopropyl alcohol and normal-propyl alcohol is added to the obtained mixture after treating the crude argatroban (**III**) with a base. Preferably for the purposes of the present invention the crystallization solvent is isopropyl alcohol; the mixture is heated to the solvent reflux temperature, the chlorinated solvent being removed by distillation, then cooled to 0-20ºC. In this step, purified argatroban (**IV**) is obtained which, from the

analyses carried out, proves to be solvated with the crystallization alcohol, in particular isopropanol, and has the physico-chemical characteristics described below.

This crystalline precipitate is then treated to obtain argatroban monohydrate (**I**) as previously described after solubilization of the purified argatroban (**IV**) in a methanol:water mixture as previously described.

Separation of the purified argatroban by crystallization from normal-propanol or isopropanol allows a more efficient preparation in terms of argatroban monohydrate yield and purity and moreover a better processability of the product and reliability of the process, as propanol alcohols are better for these aspects than ethanol. As a crystallization solvent, ethanol, in case of even slight volumetrical errors, can actually cause significant yield reductions with negative consequences on the diastereoisomer ratio. This would lead to greater difficulties in achieving the correct diastereoisomer ratio in the subsequent crystallization. With methanol, this effect is accentuated. The solvate obtained, indicated herein as purified argatroban, as well as being new, instead exhibits crystalline characteristics such as to greatly facilitate its purification and isolation without negatively affecting the diastereoisomer ratio.

Considering all the step hereinabove reported in this second embodiment the preparation method of argatroban monohydrate comprises the steps of:

- preparing the crude argatroban from (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid in methanol/acetic acid;
- optionally treating the reaction mass with an aqueous solution of a base to bring the pH of the mixture itself to between 7.0 and 7.5;
- concentrating the reaction mass containing crude argatroban to a stirrable residue;
- dissolving the residue containing crude argatroban with an organic solvent and separating the purified argatroban by crystallization by treating the organic solution with a crystallization solvent selected from isopropanol and normal-propanol;
- separating argatroban monohydrate by re-crystallizing the purified

argatroban isolated in the preceding step from a solvent medium consisting of a mixture of methanol:water, wherein the methyl alcohol has a concentration between 10 and 20%, and subjecting the mass to an addition of decolorizing carbon, a heating at the reflux temperature then subjecting it to carbon filtration and controlled and gradual cooling to a temperature between 15-25ºC in a time comprised between 11-17 hours.

In particular, the controlled and gradual cooling is at the following temperature gradient: heating to 90-95ºC, maintenance at reflux temperature for a time between 1 and 3 hours, cooling to 70-75ºC in at least one hour and maintenance at this temperature for at least one hour, cooling to 20ºC in a time comprised between 2 and 6 hours and maintenance at this temperature for at least 6 hours.

A step of drying under nitrogen flow or under vacuum at a temperature comprised from 50°C and 80ºC for at least 8 hours can be further comprise.

The preparation method of the argatroban monohydrate according to the invention proves to fulfill the purposes of the same.

In fact, the yields of argatroban monohydrate obtained, either in the case of a continuous step or a two step preparation, are between 65% and 70%; the obtained compound has shown a purity at least equal to or higher than 99%, a KF of at least 3.4% and an isomer ratio of 65:35 ± 2.

With regard to the purity, the argatroban monohydrate obtainable with the method according to the invention has an impurity profile where any single impurity is less than 0.1% and preferably below 0.03%. Accordingly the purity of the argatroban monohydrate is at least equal to or higher than 99.0% and preferably at least of or higher than 99.8%.

Optionally, argatroban anhydrous (**V**) can be obtained from argatroban monohydrate, this being obtained either in a continuous mode or in two distinct steps, by re-dissolving in water the obtained crystalline precipitate, consisting of argatroban monohydrate (**I**), heating the solution to a temperature between 75º and 100ºC, preferably 80ºC, removing the un-dissolved residues, then quickly cooling the solution to 10ºC-20ºC, preferably to 15ºC, in a time of 2 minutes and maintaining it at said temperature for a period of up to 1 hour.

The argatroban obtained in this manner is anhydrous and has the physico-

chemical characteristics described hereinafter.

Characterization of argatroban monohydrate

The analytical data of (2*R*,4*R*)-4-methyl-1-[N^2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolylsulphonyl]-L-arginyl]pipecolic acid monohydrate (**I**) are given below:

Empirical formula: $C_{23}H_{38}N_6O_6S$;

Molecular weight: 526.65;

Molecular composition:

	C	H	N	S
Calculated (%):	52.45	7.27	15.96	6.09
Found (%):	52.46	7.30	15.95	6.11;

Purity by HPLC: 99.0% - 99.8%;

I.R. (KBr): 3416, 1272, 1157 cm^{-1}. A representative I.R. spectrum of argatroban monohydrate obtained by the described method is shown in figure 1;

Optical rotation power: $[\alpha]_D^{27}$= +78 (c=1 mg/ml in 0.2 N HCl);

Melting point: 176-182ºC. Melting point was determined using glass capillary tubes;

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA): Differential scanning calorimetry was carried out using a perforated aluminium crucible. The argatroban monohydrate shows an endothermic event at about 150ºC. Figure 2 gives a typical DSC profile. The endothermic phenomenon represents water loss and subsequent melting of the compound. Water loss is clearly demonstrated by the thermogravimetric analysis profile in figure 3. Thermogravimetric analysis shows that the compound appears as a monohydrate, as the water loss is 3.68% in a temperature range from 85-177ºC, a value which totally fits the theoretical value of 3.42%. The high temperature range for weight loss, being greater than 175ºC, also indicates that the nature of the contained water is of crystalline type;

Analysis by X-ray diffractometry: argatroban monohydrate appears as a white crystalline solid. The peaks relating to argatroban monohydrate and shown in figure 4 are summarized in the table 1 below:

Table 1

Peak No.	*Angle (2θ)*	*Relative intensity (%)*
1	4.680	43
2	9.230	100
3	13.850	18
4	15.980	23
5	17.120	12
6	18.040	10
7	18.430	14
8	18.950	10
9	20.080	16
10	20.560	12
11	21.260	10
12	21.590	11
13	22.820	24
14	23.740	8
15	24.480	11
16	26.140	12
17	27.620	16

18	28.980	8
19	31.320	10
20	33.440	17
21	37.210	8

Characterization of argatroban anhydrous

The description of a representative sample of (2*R*,4*R*)-4-methyl-1-[N^2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulphonyl]-L-arginyl]pipecolic acid in anhydrous form (**V**), and a comparison with the corresponding monohydrate form are given below:

Molecular formula: $C_{23}H_{36}N_6O_5S$;

Molecular weight: 508.63;

Melting point: 220ºC with decomposition;

I.R. (KBr): 3432, 1265, 1164. A representative I.R. spectrum of the argatroban anhydrous obtained by the described method is shown in figure 5;

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA): Differential scanning calorimetry and thermogravimetric analysis were carried out under the same conditions adopted for the argatroban monohydrate analysis. Figure 6 gives a representative DSC analysis of an argatroban anhydrous sample. The argatroban anhydrous shows an endothermic event at about 215ºC. The endothermic phenomenon is associated with melting of the compound.

The thermogravimetric analysis is shown in figure 7. The loss of around 1% in weight within a temperature range of between 25º and 140ºC is ascribable to the presence of a small quantity of water of imbibition;

X-ray diffraction: the XRPD analysis was carried out by following the procedure described for argatroban monohydrate. The following table 2 gives a summary of the peaks shown in figure 8:

Table 2

Peak No.	*Angle (2θ)*	*Relative intensity (%)*
1	7.790	64
2	9.560	95
3	10.480	27
4	11.320	75
5	12.120	61
6	14.640	23
7	16.010	25
8	16.940	14
9	18.030	45
10	18.900	100
11	20.960	47
12	22.550	53
13	24.370	61
14	26.170	81
15	28.300	32
16	30.960	40
17	33.970	40
18	38.930	23

Characterization of argatroban solvated in isopropanol

The analytical data of purified (2*R*,4*R*)-4-methyl-1-[N^2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulphonyl]-L-arginyl]pipecolic acid (**IV**) solvated with isopropanol are given below:

I.R. (KBr): 3399, 1270, 1160 cm^{-1}. A representative I.R. spectrum of argatroban solvated with iPrOH obtained by the described method is given in figure 9;

Melting point: 183-185ºC. Melting point determination was carried out using glass capillary tubes;

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA): differential scanning calorimetry was carried out using a perforated aluminium crucible. The argatroban solvated with isopropanol has an endothermic peak at about 160ºC. Figure 10 gives a typical DSC profile. The endothermic event represents solvent loss and melting of the compound. Solvent loss is clearly demonstrated from the thermogravimetric analysis profile in figure 11. Thermogravimetric analysis shows that the compound appears as a solvate with isopropanol, as the solvent loss is 9.0%, a value which totally fits the theoretical value of 10.55% for a monosolvate form. The high temperature range for weight loss, being between 70ºC and 176ºC, also indicates that the nature of the contained solvent is of crystalline type;

Analysis by X-ray diffraction: the argatroban solvated with isopropanol appears as a white crystalline solid. The peaks obtained for argatroban solvated with isopropanol and shown in figure 12 are summarized in the table 3 below:

Table 3

Peak No.	*Angle (2θ)*	*Relative intensity (%)*
1	6.800	100
2	7.840	12
3	9.800	13
4	10.020	13
5	10.700	6
6	11.780	5
7	13.080	12
8	13.540	32
9	14.540	21
10	16.900	8
11	17.540	18
12	18.640	14
13	19.060	13
14	19.620	13
15	20.000	20

16	21.420	16
17	21.780	21
18	22.660	15
19	23.680	15
20	24.080	16
21	24.740	25
22	25.340	9
23	26.480	10
24	27.580	10
25	28.220	7
26	29.120	8
27	29.740	11
28	31.820	9
29	34.840	7
30	40.580	6

Comparison of argatroban monohydrate, argatroban anhydrous and purified argatroban

Differential scanning calorimetry (DSC): as can be seen by comparison, the DSC profile of argatroban monohydrate differs considerably from the profile noted for the anhydrous compound. In this respect argatroban monohydrate has an endothermic peak at about 160ºC whereas that of argatroban anhydrous is shown at 220ºC (figure 13).

Thermogravimetric analysis (TGA): with regard to the thermogravimetric analysis, a substantial difference between the curves of monohydrate and anhydrous argatroban can again be seen, indicating the fact that the respective weight losses are different in nature (fig. 14).

Figure 15 gives a comparison between the diffractometry profiles of argatroban anhydrous, argatroban monohydrate and purified argatroban. As is evident from the superimposition, the three crystalline structures are clearly different.

The examples given below for the purposes of non limiting illustration of the synthesis process of the invention, relate to the different steps that form the

method in its entirety and refer to the previously reported scheme.

Experimental part

Examples of the synthesis of purified argatroban (**IV**) from (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid (**II**)

Example 1: treatment of crude argatroban with NaOH and separation of the purified argatroban with isopropanol

A one-litre glass autoclave was fed with 60 g of (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid, 480 ml of methanol, 120 ml of acetic acid and 20 g of palladium on 5% carbon (60% wetted). The mixture thus obtained was treated, under vigorous stirring, at 85ºC in a 8.5 bar hydrogen atmosphere for 8.5 hours.

The mass was then cooled to room temperature and the catalyst removed by filtration. The obtained solution was concentrated to a residue under reduced pressure. 600 ml of methylene chloride, 300 ml of water and 75 ml of an aqueous solution of 30% sodium hydroxide were added to the oily residue obtained until the residual acetic acid was neutralized (pH=7.5).

30 ml of methanol were then added and the mixture maintained under agitation for 1 hour. The aqueous phase was removed and the organic phase washed twice with 300 ml of water adding each time the necessary quantity of methanol to achieve a good separation.

The solution in methylene chloride (a third of the total solution) thus obtained was then percolated into a 500 ml jacketed reactor containing 120 ml of 2-propanol. The mass was then heated and 200 ml of solvent were distilled off. The solution was then cooled to 0ºC over a period of 2 hours and maintained at this temperature for 3 hours.

The solid obtained by crystallization was filtered off and dried at 50ºC under vacuum for 16 hours, giving 16.0 g of purified (2*R*,4*R*)-4-methyl-1-[N^2-[(1,2,3,4-tetrahydro-3-methyl-8- quinolylsulphonyl]-L-arginyl]pipecolic acid (**IV**). Yield = 86%.

Example 2: treatment of the crude argatroban with NaOH and crystallization from normal propanol

A one-litre glass autoclave was fed with 50 g of (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid, 375 ml of

methanol, 95 ml of acetic acid and 16.4 g of palladium on 5% carbon (60% wetted).

The mixture thus obtained was treated, under vigorous stirring, at 85ºC in a 8.5 bar hydrogen atmosphere for 8 hours.

The mass was then cooled to room temperature and the catalyst removed by filtration. The obtained solution was concentrated to a residue under reduced pressure.

570 ml of methylene chloride was added to the oily residue obtained and the solution divided into two parts. Half of the obtained solution was fed into a jacketed 1-litre glass reactor and to it 120 ml of water were added. The acetic acid residue was then neutralized by addition of a 30% sodium hydroxide solution until pH=7.5 was achieved. In order to obtain a net separation between the organic phase and aqueous phase 12 ml of methanol were then added. The mixture was maintained under stirring for 1 hour, then the aqueous phase was removed and the organic phase washed twice with 120 ml of water, adding each time the necessary quantity of methanol to achieve a good separation.

The solution in methylene chloride thus obtained was then percolated into a 500 ml jacketed reactor containing 140 ml of 1-propanol. The mass was then heated to 55ºC to distil off the dichloromethane present. The solution was then cooled to 0ºC over a period of 2 hours and maintained at this temperature for 2 hours.

The solid obtained by crystallization was filtered off and dried at 50ºC under vacuum for 16 hours, giving 17.0 g of purified (2*R*,4*R*)-4-methyl-1-[N^2-[(1,2,3,4-tetrahydro-3-methyl-8- quinolylsulphonyl]-L-arginyl]pipecolic acid (**IV**). Yield = 73%.

Example 3: treatment of crude argatroban without neutralization and with crystallization from isopropanol

Half of the compound (**III**) solution in dichloromethane obtained in example 2 was extracted twice with a mixture of 120 ml of water and 12 ml of methanol.

The solution in methylene chloride thus obtained was then percolated into a 500 ml jacketed reactor containing 140 ml of 2-propanol, the mass obtained was concentrated by distillation.

When the distillation was complete the temperature was brought to 90ºC, the mass left under agitation for 1 hour, cooled to 20ºC and finally to 0ºC where it was

maintained for 2 hours.

The solid obtained by crystallization was filtered off and dried at 50ºC under vacuum for 16 hours to give 21.5 g of purified (2*R*,4*R*)-4-methyl-1-[N^2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolylsulphonyl]-L-arginyl]pipecolic acid (**IV**). Yield = 93%.

Examples of argatroban monohydrate (**I**) synthesis from (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid (**II**)

Example 4: treatment of crude argatroban with NH_3 to directly obtain the monohydrate form

A 250 ml glass autoclave was fed with 20 g of (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid (**II**), 160 ml of methanol, 10.4 ml of acetic acid and 5.6 g of palladium on 5% carbon (50% wetted).

The mixture thus obtained was treated, under vigorous stirring, at 85ºC in a 9 bar hydrogen atmosphere for 8 hours.

The mass was then cooled to room temperature and the catalyst removed by filtration. The obtained solution was concentrated at atmospheric pressure.

After having distilled 60 ml of solvent, the solution was cooled to 0ºC and neutralized with 12 ml of an aqueous solution of 30% ammonia.

Excess ammonia was removed under vacuum, then 700 ml of water were added to the reaction mixture. The solution obtained was heated at reflux (at about 95ºC), maintained under stirring at this temperature for 1 hour, then cooled to 20ºC over a period of 4 hours and maintained at this temperature for 8 hours.

The crystalline precipitate was filtered off and dried at 80ºC at atmospheric pressure and nitrogen flow for 8 hours to give 15.8 g of argatroban monohydrate (I) (HPLC purity = 99.8%; isomer ratio = 63.8:36.2; KF = 3.5%). Yield = 82.4%.

Example of crystallization for obtaining argatroban monohydrate (**I**) from purified argatroban (**IV**)

Example 5: crystallization with 35 volumes of water and 5.5 volumes of methanol

A jacketed steel reactor was fed with 2218 g of purified (2*R*,4*R*)-4-methyl-1-[N^2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolylsulphonyl]-L-arginyl]pipecolic acid (**IV**), 12192 ml of methanol and 77647 ml of demineralized water. The mixture obtained, to which were added 66.6 g of decolorizing carbon, was heated at reflux and

maintained at this temperature for 1 hour under nitrogen. The carbon was then removed under hot conditions by filtration.

The solution was then brought to reflux and maintained at this temperature for 2 hours, cooled to the temperature of initial crystallization (75ºC) over a period of 1 hour, maintained at this temperature for 1 hour, then cooled to 20ºC over 4.5 hours and left under stirring for 6 hours.

The solid obtained by crystallization was filtered off, washed with 2218 ml of demineralized water and dried at 55ºC under reduced pressure for 12 hours to give 1998 g of (2*R*,4*R*)-4-methyl-1-[N^2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolylsulphonyl]-L-arginyl]pipecolic acid (**I**) monohydrate. (HPLC purity = 99.9%; isomer ratio = 63.2:36.8; KF = 3.5%). Yield = 87%.

Example of crystallization to obtain argatroban anhydrous (**V**)

Example 6: Crystallization from water

A jacketed glass reactor was fed with 100 ml of water and brought to 80ºC; 0.8 g of argatroban monohydrate was added at this temperature. The mass was maintained under stirring for 10 minutes at this temperature then the un-dissolved residue was removed by filtration. The mother liquors were quickly cooled to 15ºC (in about 2 minutes) and maintained at this temperature for 1 hour. The obtained solid was separated by filtration and dried at 80ºC at atmospheric pressure for 10 hours in a nitrogen flow, giving 0.5 g of argatroban anhydrous (HPLC purity = 99.9%; isomer ratio = 60.6:39.4; KF = 0.26%). Yield 65%.

Claims

1. A method for preparing argatroban monohydrate comprising at least the step of:

- separating argatroban monohydrate from a concentrated reaction mass containing crude argatroban or from a purified argatroban by crystallization from a medium solvent consisting of methanol and water by adding a decolorizing carbon, heating the mass at the reflux temperature then subjecting it to carbon filtering and to controlled and gradual cooling from the reflux temperature to a temperature between 15-25ºC in a time comprised between 11-17 hours.

2. The method for preparing argatroban monohydrate according to claim 1, further comprising, before separating the argatroban monohydrate, the step of:

- separating a purified argatroban by crystallization by treating an organic solution of a crude argatroban with a crystallization medium solvent selected from isopropanol and normal-propanol.

3. A method for preparing argatroban monohydrate comprising at least the steps of:

- concentrating a reaction mass containing crude argatroban to a stirrable residue;
- dissolving the residue containing crude argatroban with an organic solvent and separating a purified argatroban by crystallization by treating the organic solution with a crystallization medium solvent selected from isopropanol and normal-propanol;
- separating argatroban monohydrate by re-crystallizing the purified argatroban isolated in the preceding step from a methanol and water mixture solution by adding a decolorizing carbon, heating at the reflux temperature followed by carbon filtering and to controlled cooling from the reflux temperature to a temperature between 15 and 25ºC in a time between 11-17 hours.

4. The method for preparing argatroban monohydrate according to one of the claims 1 and 3, further comprising the step of:

- preparing the crude argatroban from (2*R*,4*R*)-1-[N^G-nitro-N^2-(3-

methyl-8-quinolinesulphonyl)-L-arginyl]-4-methyl-2-piperidine carboxylic acid in methanol/acetic acid.

5. The method for preparing argatroban monohydrate according to one of the claims 1 and 3, wherein the reaction mass is treated with an aqueous solution of a base to bring the pH of the mixture itself to between 7.0 and 7.5.

6. The method for preparing argatroban monohydrate according to claim 5, wherein the base solution is selected from sodium hydroxide, sodium bicarbonate and ammonia at a concentration comprised between 10 and 30%.

7. The method for preparing argatroban monohydrate according to one of the claims 1 and 3, wherein the crystallization or re-crystallization from methanol and water is achieved by subjecting the mass to the following temperature gradient: heating to 90-95ºC, maintenance at reflux temperature for a time between 1 and 3 hours, cooling to 70-75ºC in at least one hour and maintenance at this temperature for at least one hour, cooling to 20ºC in a time comprised between 2 and 6 hours and maintenance at this temperature for at least 6 hours.

8. The method according to claims 1, 3 and 7, wherein the methyl alcohol and water mixture medium solvent has a concentration of between 10 and 20% of alcohol and is in a quantity up to 50 volumes per gram of crude or purified argatroban.

9. The method for preparing argatroban monohydrate according to one of the claims 1 and 3 further comprising the step of drying the obtained crystalline precipitate consisting of argatroban monohydrate.

10. A method for preparing argatroban monohydrate comprising the steps as defined in the claims 1 and 4-9.

11. A method for preparing argatroban monohydrate comprising the steps as defined in the claims from 3 to 9.

12. A method for preparing argatroban anhydrous comprising at least the steps of:

- preparing argatroban monohydrate according to a method as defined in one of the claims 1-9;
- re-crystallizing thereof after dissolution in water by quickly cooling to a temperature of 10-20ºC in a time not greater than 2 minutes.

13. Argatroban monohydrate characterized by having:
    - melting point of 176-182ºC;
    - purity at least of 99%;
    - I.R. spectrum (KBr) (cm^{-1}) of 3416, 1272, 1157;
    - differential scanning calorimetry (DSC) with an endothermic event at about 150ºC;
    - thermogravimetric analysis (TGA) with a water loss of 3.68% in a temperature range from 85ºC to 177ºC;
    - XRPD: *Peak No.* 1 *Angle (2θ)* 4.680, Relative intensity *(%)* 43; *Peak N°2 Angle (2θ)* 9.230, *Relative intensity (%)* 100; *Peak No.* 3 *Angle (2θ)* 13.850, *Relative intensity (%)* 18; *Peak No.* 4 *Angle (2θ)* 15.980, *Relative intensity (%)* 23; *Peak No.* 5 *Angle (2θ)* 17.120, *Relative intensity (%)* 12; *Peak No.* 6 *Angle (2θ)* 18.040, *Relative intensity (%)* 10; *Peak No.* 7 *Angle (2θ)* 18.430, *Relative intensity (%)* 14; *Peak No.* 8 *Angle (2θ)* 18.950, *Relative intensity (%)* 10; *Peak No.* 9 *Angle (2θ)* 20.080, *Relative intensity (%)* 16; *Peak No.* 10 *Angle (2θ)* 20.560, *Relative intensity (%)* 12; *Peak No.* 11 *Angle (2θ)* 21.260, *Relative intensity (%)* 10; *Peak No.* 12 *Angle (2θ)* 21.590, *Relative intensity (%)* 11; *Peak No.* 13 *Angle (2θ)* 22.820, *Relative intensity (%)* 24; *Peak No.* 14 *Angle (2θ)* 23.740, *Relative intensity (%)* 8; *Peak No.* 15 *Angle (2θ)* 24.480, *Relative intensity (%)* 11; *Peak No.* 16 *Angle (2θ)* 26.140, *Relative intensity (%)* 12; *Peak No.* 17 *Angle (2θ)* 27.620, *Relative intensity (%)* 16; *Peak No.* 18 *Angle (2θ)* 28.980, *Relative intensity (%)* 8; *Peak No.* 19 *Angle (2θ)* 31.320, *Relative intensity (%)* 10; *Peak No.* 20 *Angle (2θ)* 33.440, *Relative intensity (%)* 17; *Peak No.* 21 *Angle (2θ)* 37.210, *Relative intensity (%)* 8.

14. Argatroban monohydrate, wherein the purity is at least of 99.8%.

15. Argatroban anhydrous characterized by having:
    - melting point of 220ºC with decomposition;
    - I.R. spectrum (KBr) (cm^{-1}) of 3432, 1265, 1164;
    - differential scanning calorimetry with an endothermic event at about

215ºC;

- XRPD: *Peak N°1 Angle (2θ)* 7.790, *Relative intensity (%)* 64; *Peak N°2 Angle (2θ)* 9.560, *Relative intensity (%)* 95; *Peak N°3 Angle (2θ)* 10.480, *Relative intensity (%)* 27; *Peak No.* 4 *Angle (2θ)* 11.320, *Relative intensity (%)* 75; *Peak No.* 5 *Angle (2θ)* 12.120, *Relative intensity (%)* 61; *Peak No.* 6 *Angle (2θ)* 14.640, *Relative intensity (%)* 23; *Peak No.* 7 *Angle (2θ)* 16.010, *Relative intensity (%)* 25; *Peak No.* 8 *Angle (2θ)* 16.940, *Relative intensity (%)* 14; *Peak No.* 9 *Angle (2θ)* 18.030, *Relative intensity (%)* 45; *Peak No.* 10 *Angle (2θ)* 18.900, *Relative intensity (%)* 100; *Peak No.* 11 *Angle (2θ)* 20.960, *Relative intensity (%)* 47; *Peak No.* 12 *Angle (2θ)* 22.550, *Relative intensity (%)* 53; *Peak No.* 13 *Angle (2θ)* 24.370, *Relative intensity (%)* 61; *Peak No.* 14 *Angle (2θ)* 26.170, *Relative intensity (%)* 81; *Peak No.* 15 *Angle (2θ)* 28.300, *Relative intensity (%)* 32; *Peak No.* 16 *Angle (2θ)* 30.960, *Relative intensity (%)* 40; *Peak No.* 17 *Angle (2θ)* 33.970, *Relative intensity (%)* 40; *Peak No.* 18 *Angle (2θ)* 38.930, *Relative intensity (%)* 23.

16. Purified argatroban characterized by having:

- melting point of 183-185ºC;
- I.R. spectrum (KBr) (cm^{-1}) of 3399, 1270, 1160;
- differential scanning calorimetry with an endothermic event at about 160ºC;
- XRPD: *Peak No.* 1 *Angle (2θ) 6.800*, *Relative intensity (%) 100*; *Peak No. 2 Angle (2θ)* 7.840, *Relative intensity (%) 12*; *Peak No.* 3 *Angle (2θ)* 9.800, *Relative intensity (%) 13*; *Peak No.* 4 *Angle (2θ)* 10.020, *Relative intensity (%) 13*; *Peak No.* 5 *Angle (2θ)* 10.700, *Relative intensity (%)* 6; *Peak No. 6 Angle (2θ)* 11.780, *Relative intensity (%)* 5; *Peak No. 7 Angle (2θ)* 13.080, *Relative intensity (%)* 12; *Peak No. 8 Angle (2θ)* 13.540, *Relative intensity (%)* 32; *Peak No. 9 Angle (2θ)* 14.540, *Relative intensity (%)* 21; *Peak No. 10 Angle (2θ)* 16.900, *Relative intensity (%)* 8; *Peak No. 11 Angle (2θ)* 17.540, *Relative intensity (%)* 18; *Peak No. 12 Angle (2θ)* 18.640, *Relative intensity*

(%) 14; *Peak No. 13 Angle (2θ)* 19.060, *Relative intensity (%) 13*; *Peak No. 14 Angle (2θ)* 19.620, *Relative intensity (%)* 13; *Peak No. 15 Angle (2θ)* 20.000, *Relative intensity (%)* 20; *Peak No. 16 Angle (2θ)* 21.420, *Relative intensity (%)* 16; *Peak No. 17 Angle (2θ)* 21.780, *Relative intensity (%)* 21; *Peak No. 18 Angle (2θ)* 22.660, *Relative intensity (%)* 15; *Peak No. 19 Angle (2θ)* 23.680, *Relative intensity (%)* 15; *Peak No. 20 Angle (2θ)* 24.080, *Relative intensity (%)* 16; *Peak No. 21 Angle (2θ)* 24.740, *Relative intensity (%)* 25; *Peak No. 22 Angle (2θ)* 25.340, *Relative intensity (%)* 9; *Peak No. 23 Angle (2θ)* 26.480, *Relative intensity (%)* 10; *Peak No. 24 Angle (2θ)* 27.580, *Relative intensity (%)* 10; *Peak No. 25 Angle (2θ)* 28.220, *Relative intensity (%)* 7; *Peak No. 26 Angle (2θ)* 29.120, *Relative intensity (%)* 8; *Peak No. 27 Angle (2θ)* 29.740, *Relative intensity (%)* 11; *Peak No. 28 Angle (2θ)* 31.820, *Relative intensity (%)* 9; *Peak No. 29 Angle (2θ)* 34.840, *Relative intensity (%)* 7; *Peak No. 30 Angle (2θ)* 40.580, *Relative intensity (%)* 6.

3601 4000.00 400.00 3.17 85.95 1.00 %T 4 10.00
REF: 4000 77.11 2000 85.71 250

3415.00	8.98	3256.00	23.11	3096.00	17.40	2952.00	15.09
2912.00	18.28	2867.00	22.32	1684.00	7.28	1653.00	3.17
1600.00	11.65	1567.00	17.93	1516.00	21.85	1478.00	21.44
1462.00	16.39	1424.00	11.01	1319.00	13.01	1319.00	29.23
1296.00	18.18	1272.00	12.04	1240.00	42.35	1201.00	33.31
1181.00	39.90	1157.00	16.28	1133.00	16.72	1118.00	16.80
1095.00	31.13	1071.00	41.62	1023.00	43.73	985.00	43.61
967.00	52.61	927.00	34.71	855.00	51.62	812.00	31.01

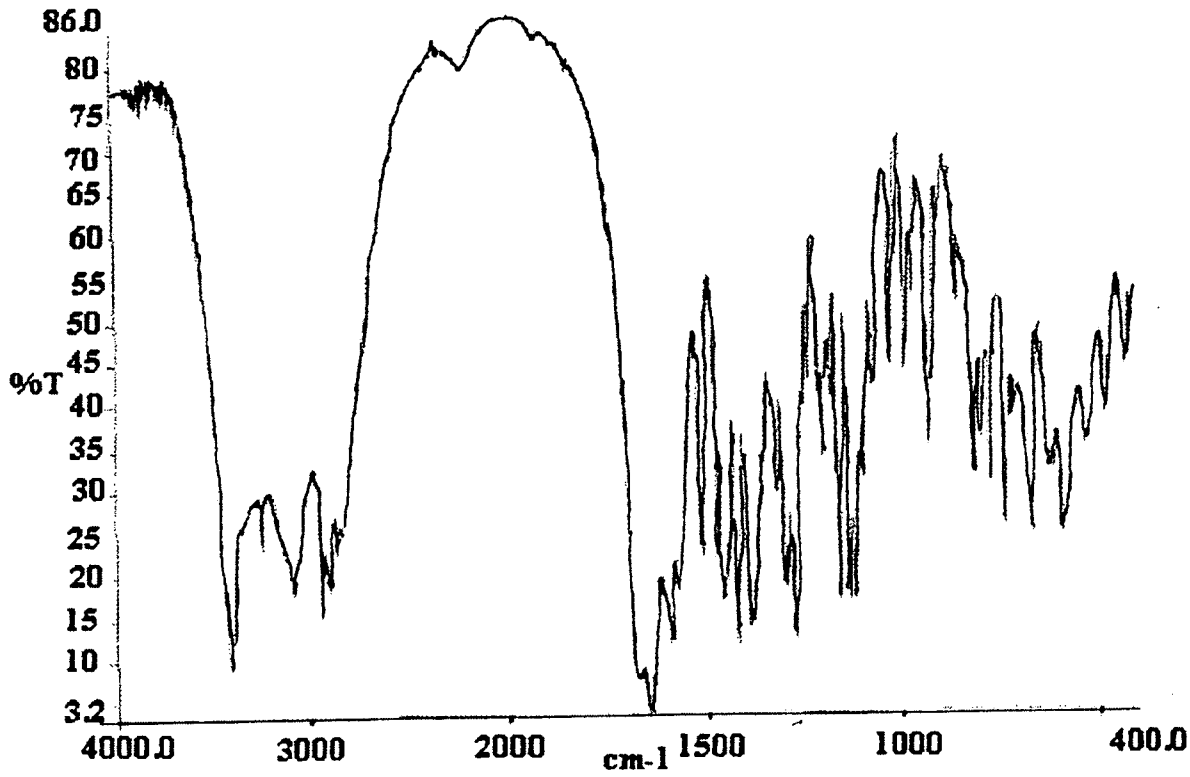


Figure 1

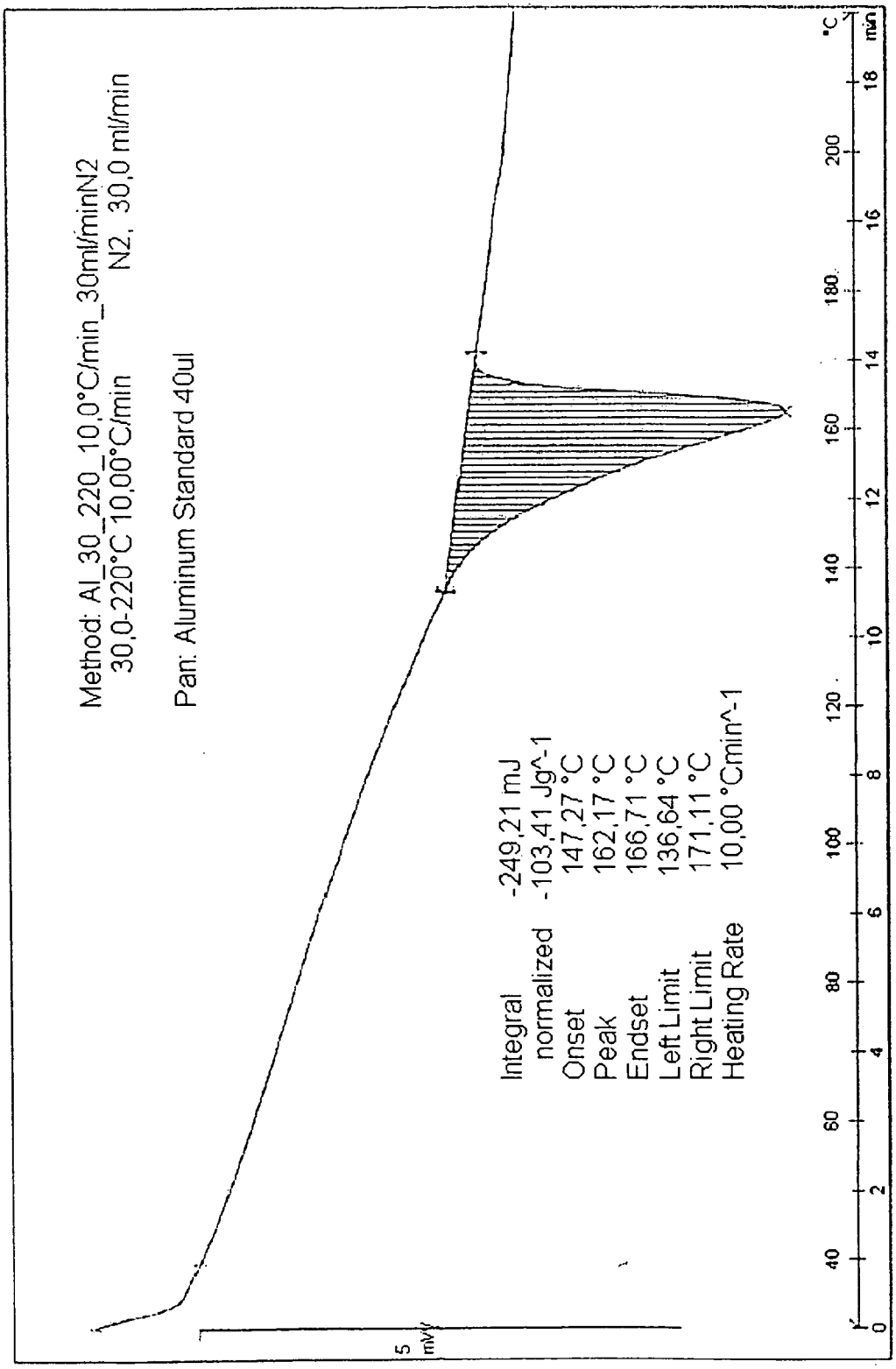

Method: Al_30_220_10,0°C/min_30ml/minN2
30,0-220°C 10,00°C/min N2, 30,0 ml/min
Pan: Aluminum Standard 40ul
Integral -249,21 mJ
normalized -103,41 Jg^-1
Onset 147,27 °C
Peak 162,17 °C
Endset 166,71 °C
Left Limit 136,64 °C
Right Limit 171,11 °C
Heating Rate 10,00 °Cmin^-1
5 mW
°C
min

Figure 2

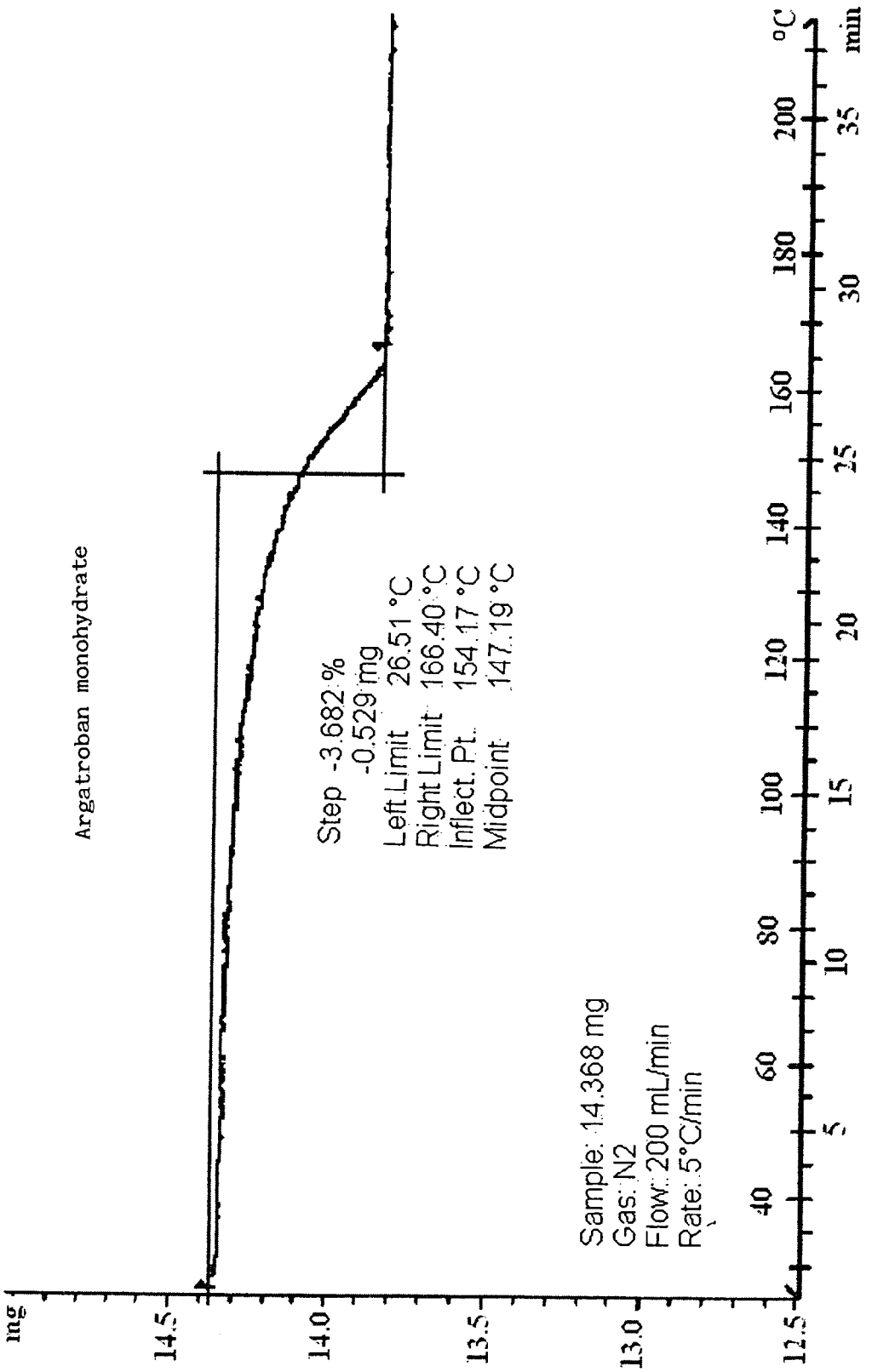

Argatroban monohydrate
Step -3.682 %
-0.529 mg
Left Limit 26.51 °C
Right Limit 166.40 °C
Inflect. Pt. 154.17 °C
Midpoint 147.19 °C
Sample: 14.368 mg
Gas: N2
Flow: 200 mL/min
Rate: 5°C/min
mg
14.5
14.0
13.5
13.0
12.5
40
60
80
100
120
140
160
180
200
°C
5
10
15
20
25
30
35
min

Figure 3

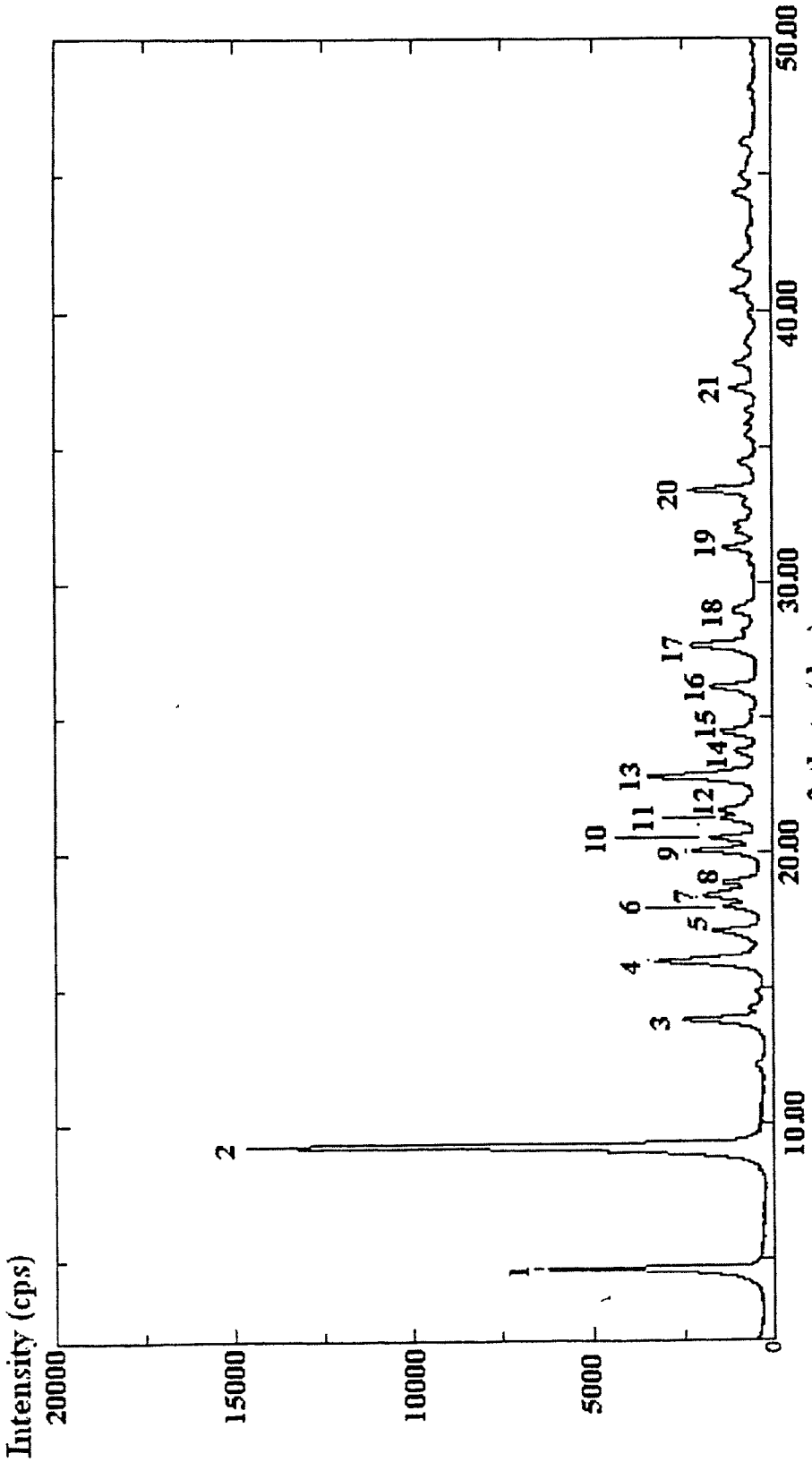

Intensity (cps)
20000
15000
10000
5000
0
10.00
20.00
30.00
40.00
50.00
2 theta (deg)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21

Figure 4

REF 4000 97.34 2000 103.77 600
3849.55 95.87 3432.50 39.14 3351.05 48.76 3227.93 50.90 2950.10 51.39
1687.79 53.20 1629.38 25.57 1609.61 20.05 1513.84 58.60 1480.17 57.44
1462.75 55.86 1427.28 48.48 1392.66 43.37 1363.05 54.83 1335.95 57.82
1302.63 47.31 1265.47 50.87 1200.40 71.23 1181.57 63.25 1164.10 51.76
1142.05 55.98 1084.28 74.85 1022.27 86.55 978.89 83.59 950.54 88.50
930.85 77.73 847.32 93.55 791.87 87.82 770.38 82.39 751.48 65.65
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482.01 88.98

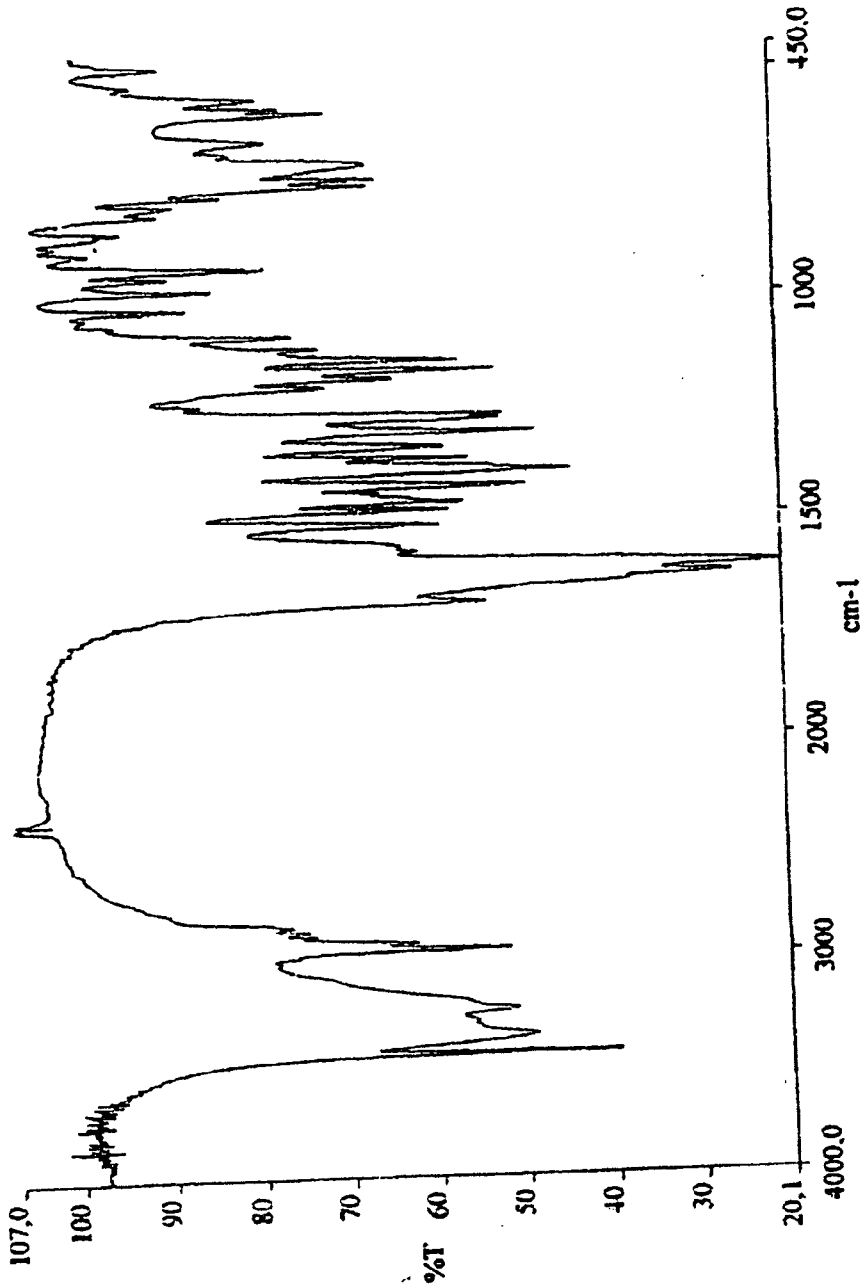


Figure 5

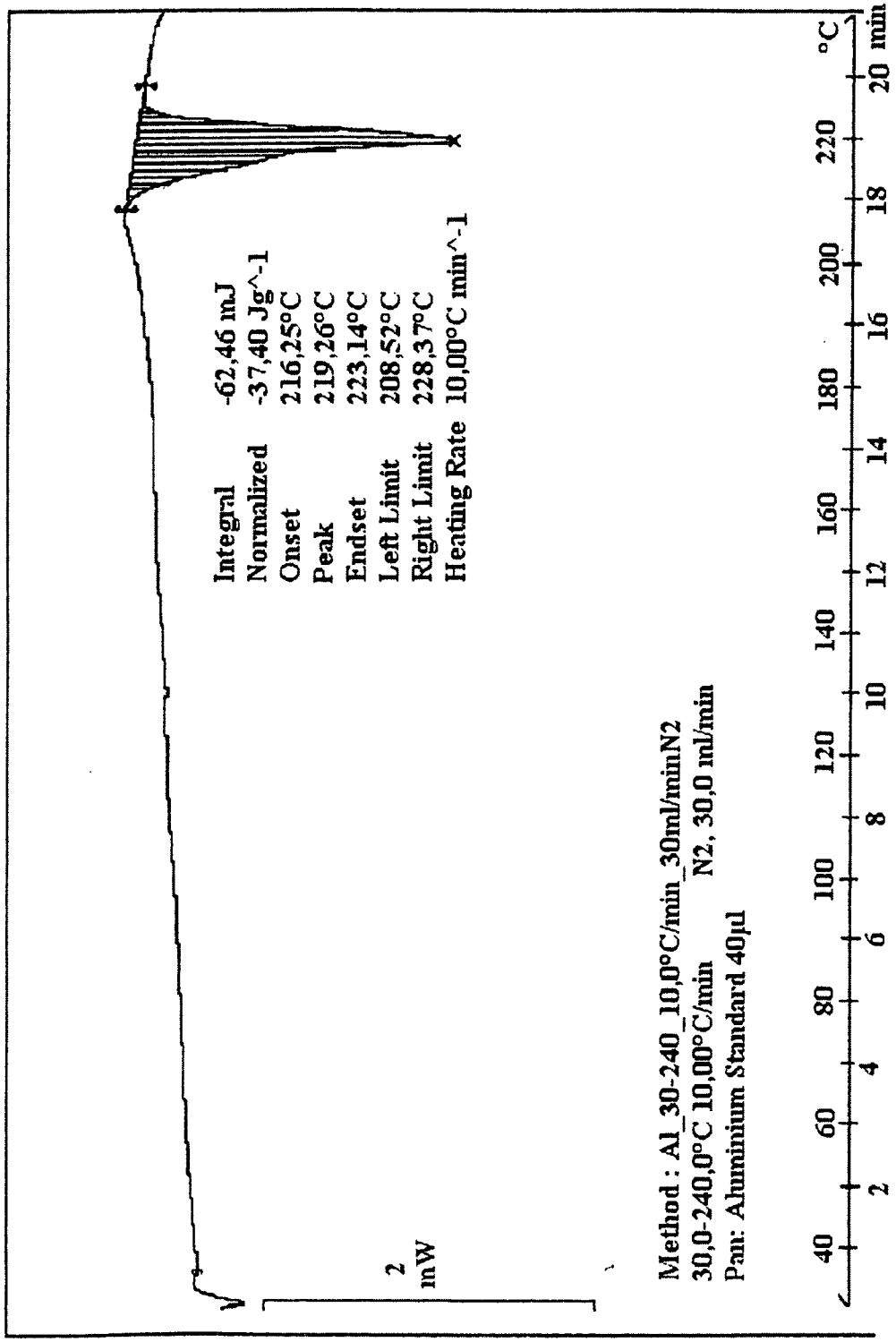

Integral -62,46 mJ
Normalized -37,40 Jg^-1
Onset 216,25°C
Peak 219,26°C
Endset 223,14°C
Left Limit 208,52°C
Right Limit 228,37°C
Heating Rate 10,00°C min^-1
2
mW
Method : Al_30-240_10,0°C/min_30ml/minN2
30,0-240,0°C 10,00°C/min N2, 30,0 ml/min
Pan: Aluminium Standard 40µl
40 60 80 100 120 140 160 180 200 220 °C
2 4 6 8 10 12 14 16 18 20 min

Figure 6

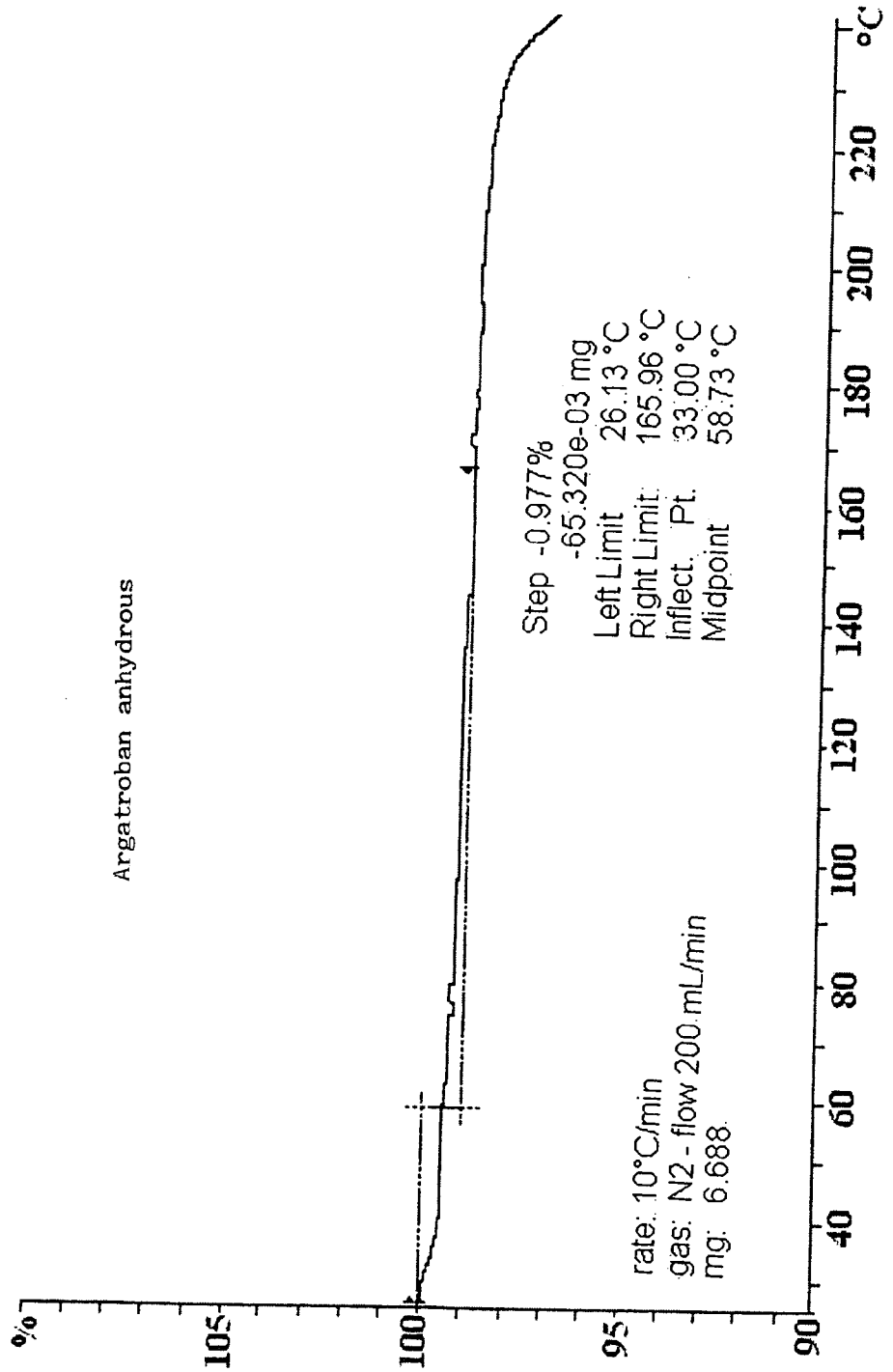

Argatroban anhydrous
Step -0.977%
-65.320e-03 mg
Left Limit 26.13 °C
Right Limit 165.96 °C
Inflect. Pt. 33.00 °C
Midpoint 58.73 °C
rate: 10°C/min
gas: N2 - flow 200 mL/min
mg: 6.688
%
105
100
95
90
40
60
80
100
120
140
160
180
200
220
°C

Figure 7

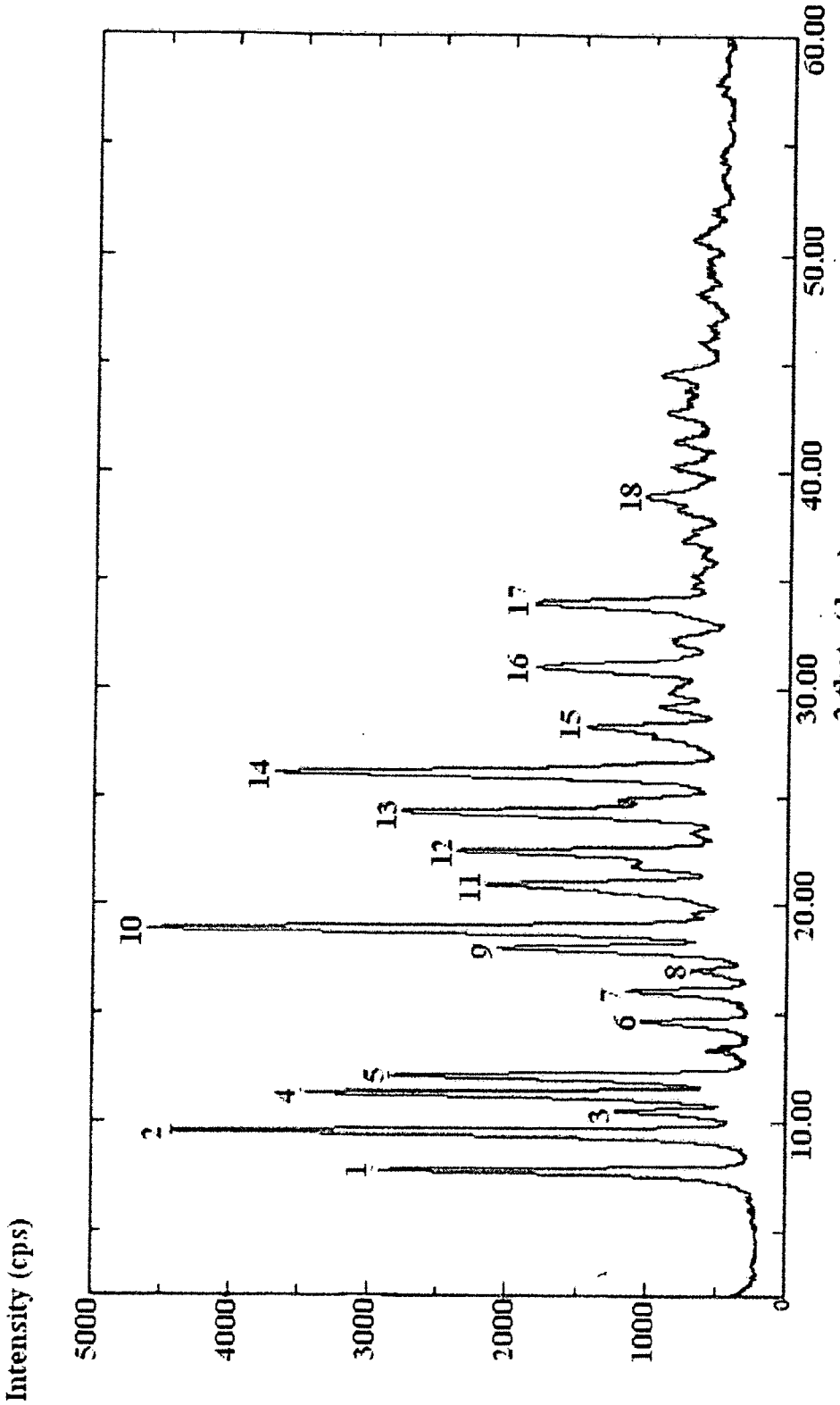

Intensity (cps)
5000
4000
3000
2000
1000
0
10.00
20.00
30.00
40.00
50.00
60.00
2 theta (deg.)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Figure 8

REF 4000 100.46 2000 106.90 600
3849.50 98.84 3399.25 47.07 2950.49 49.20 1633.49 28.54 1572.94 55.25
1511.16 60.06 1461.60 55.22 1425.91 57.51 1377.91 51.27 1304.62 56.24
1270.89 54.50 1194.55 74.91 1177.74 69.47 1160.24 57.10 1132.88 51.98
982.36 83.37 953.44 81.15 854.81 90,53 810.18 72.62 765.96 75.47
732.40 72.68 683.58 66.71 661.20 66.96 580.52 56.18

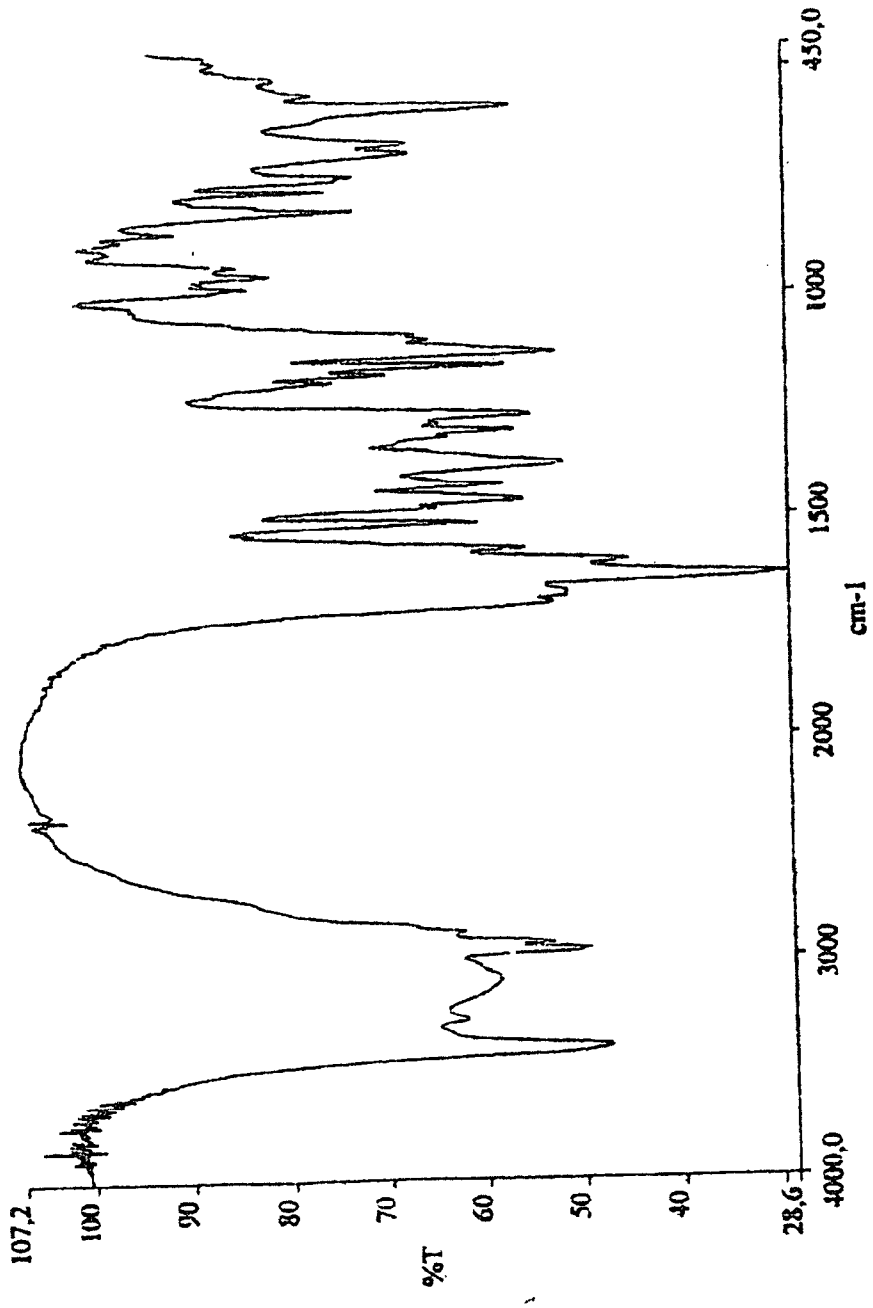


Figure 9

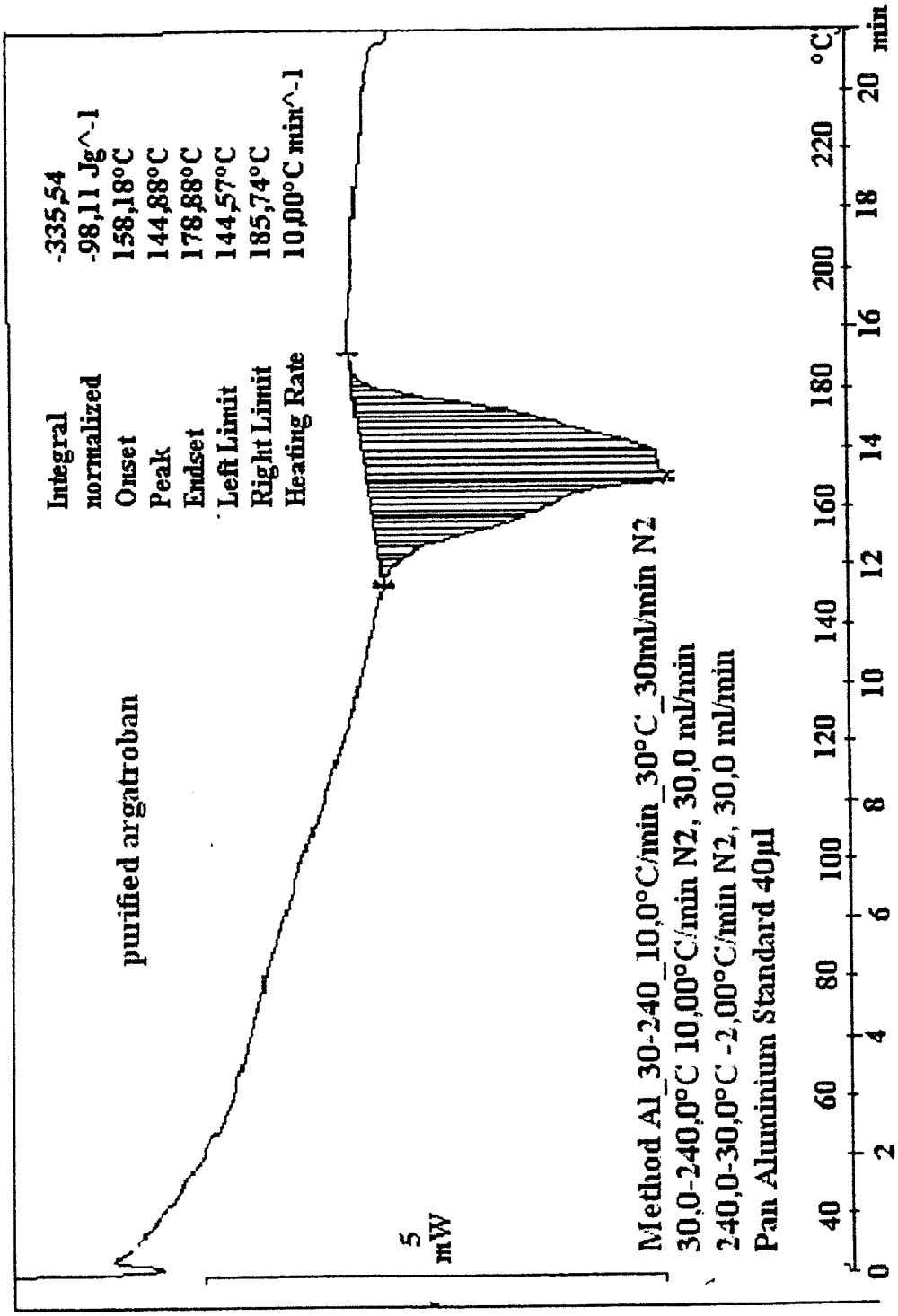

purified argatroban
Integral -335,54
normalized -98,11 Jg^-1
Onset 158,18°C
Peak 144,88°C
Endset 178,88°C
Left Limit 144,57°C
Right Limit 185,74°C
Heating Rate 10,00°C min^-1
5 mW
Method Al_30-240_10,0°C/min_30°C_30ml/min N2
30,0-240,0°C 10,00°C/min N2, 30,0 ml/min
240,0-30,0°C -2,00°C/min N2, 30,0 ml/min
Pan Aluminium Standard 40µl
°C
min

Figure 10

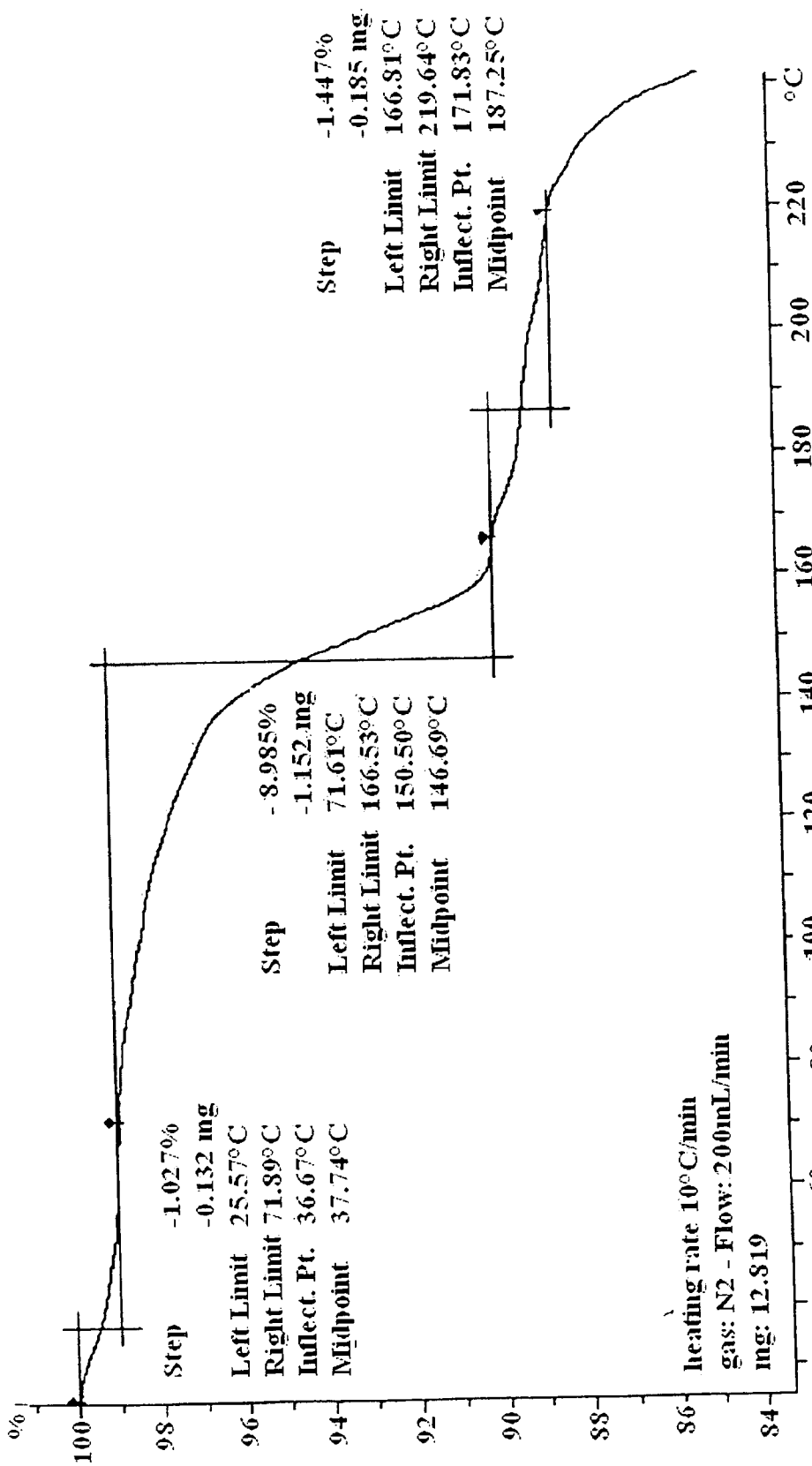

%
100
98
96
94
92
90
88
86
84
40
60
80
100
120
140
160
180
200
220
°C
Step -1.027%
-0.132 mg
Left Limit 25.57°C
Right Limit 71.89°C
Inflect. Pt. 36.67°C
Midpoint 37.74°C
Step -8.985%
-1.152 mg
Left Limit 71.61°C
Right Limit 166.53°C
Inflect. Pt. 150.50°C
Midpoint 146.69°C
Step -1.447%
-0.185 mg
Left Limit 166.81°C
Right Limit 219.64°C
Inflect. Pt. 171.83°C
Midpoint 187.25°C
heating rate 10°C/min
gas: N2 - Flow: 200mL/min
mg: 12.819

Figure 11

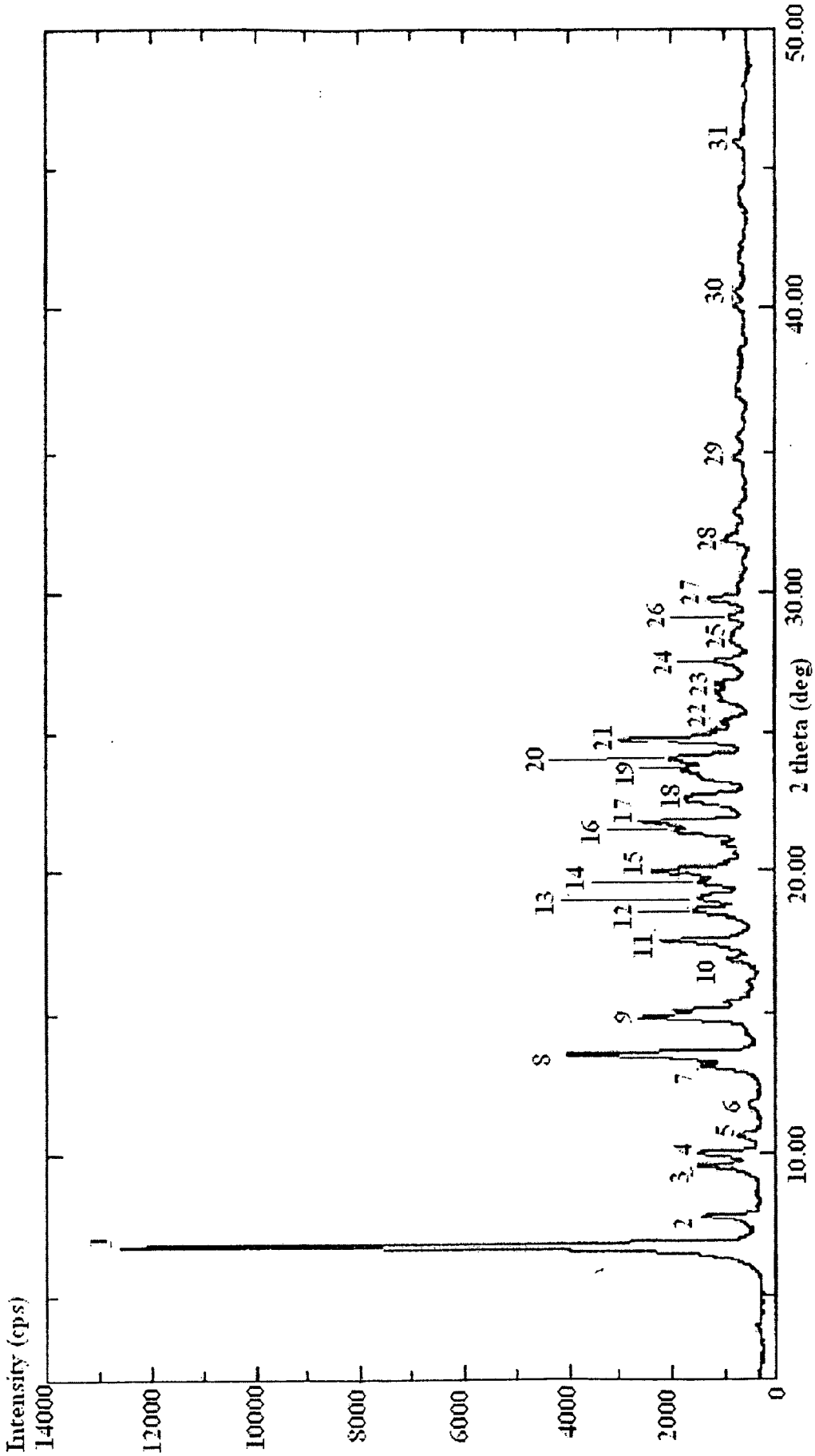

Intensity (cps)
14000
12000
10000
8000
6000
4000
2000
0
10.00
20.00
30.00
40.00
50.00
2 theta (deg)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

Figure 12

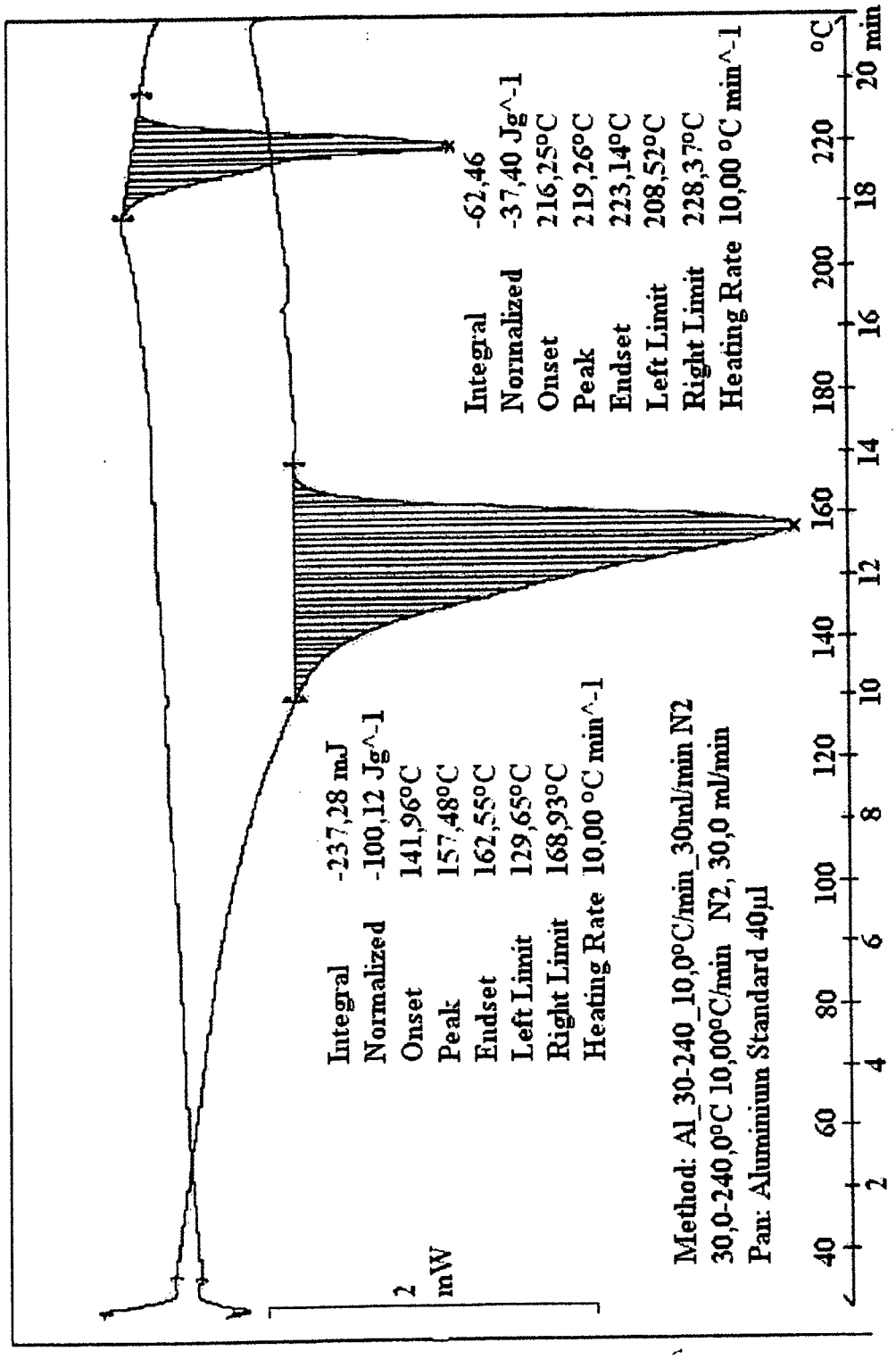

Integral -237,28 mJ
Normalized -100,12 Jg^-1
Onset 141,96°C
Peak 157,48°C
Endset 162,55°C
Left Limit 129,65°C
Right Limit 168,93°C
Heating Rate 10,00 °C min^-1
Integral -62,46
Normalized -37,40 Jg^-1
Onset 216,25°C
Peak 219,26°C
Endset 223,14°C
Left Limit 208,52°C
Right Limit 228,37°C
Heating Rate 10,00 °C min^-1
Method: Al_30-240_10,0°C/min_30ml/min N2
30,0-240,0°C 10,00°C/min N2, 30,0 ml/min
Pan: Aluminium Standard 40µl
2
mW
°C
20 min

Figure 13

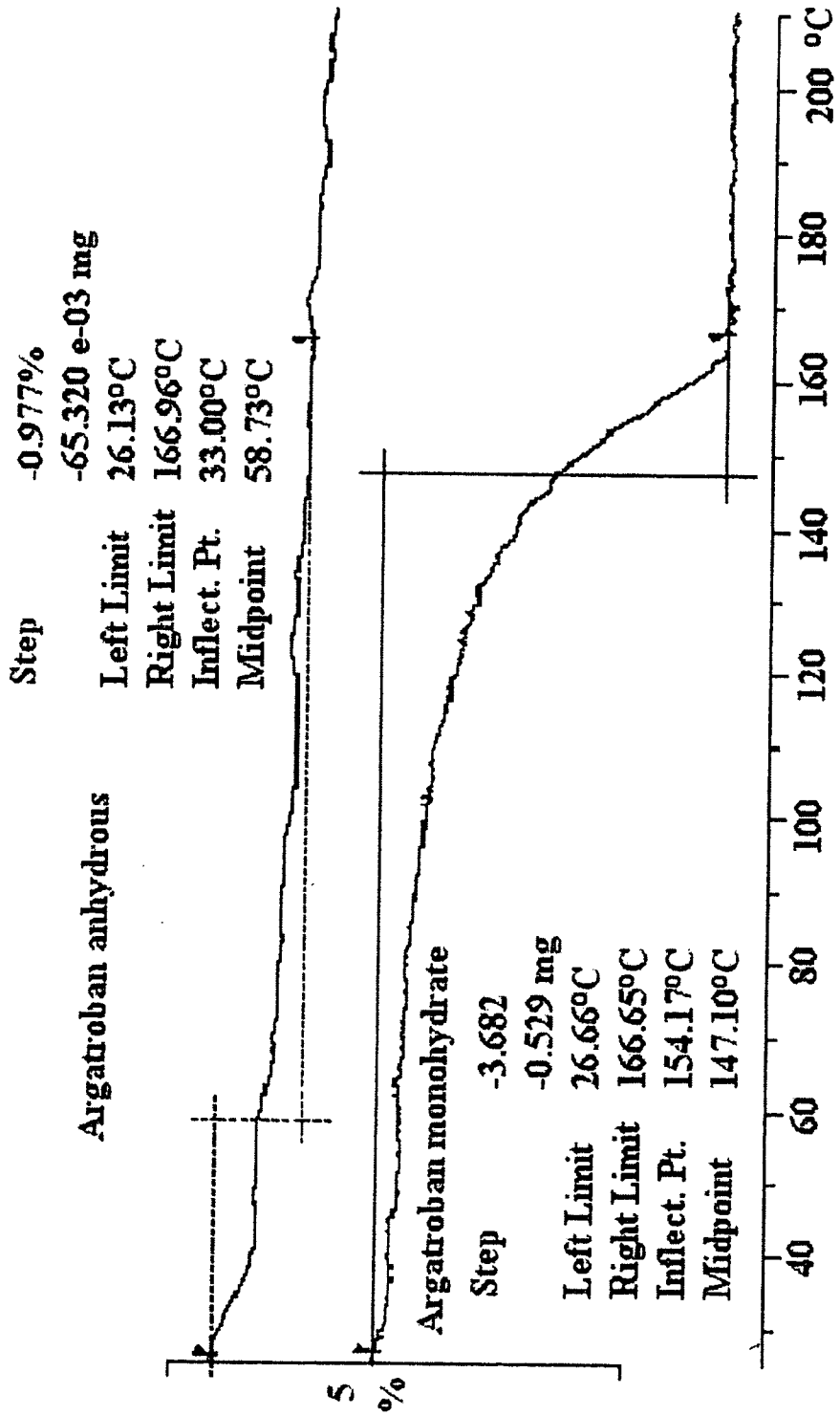

Argatroban anhydrous
Step -0.977%
-65.320 e-03 mg
Left Limit 26.13°C
Right Limit 166.96°C
Inflect. Pt. 33.00°C
Midpoint 58.73°C
Argatroban monohydrate
Step -3.682
-0.529 mg
Left Limit 26.66°C
Right Limit 166.65°C
Inflect. Pt. 154.17°C
Midpoint 147.10°C
5 %
40 60 80 100 120 140 160 180 200 °C

Figure 14

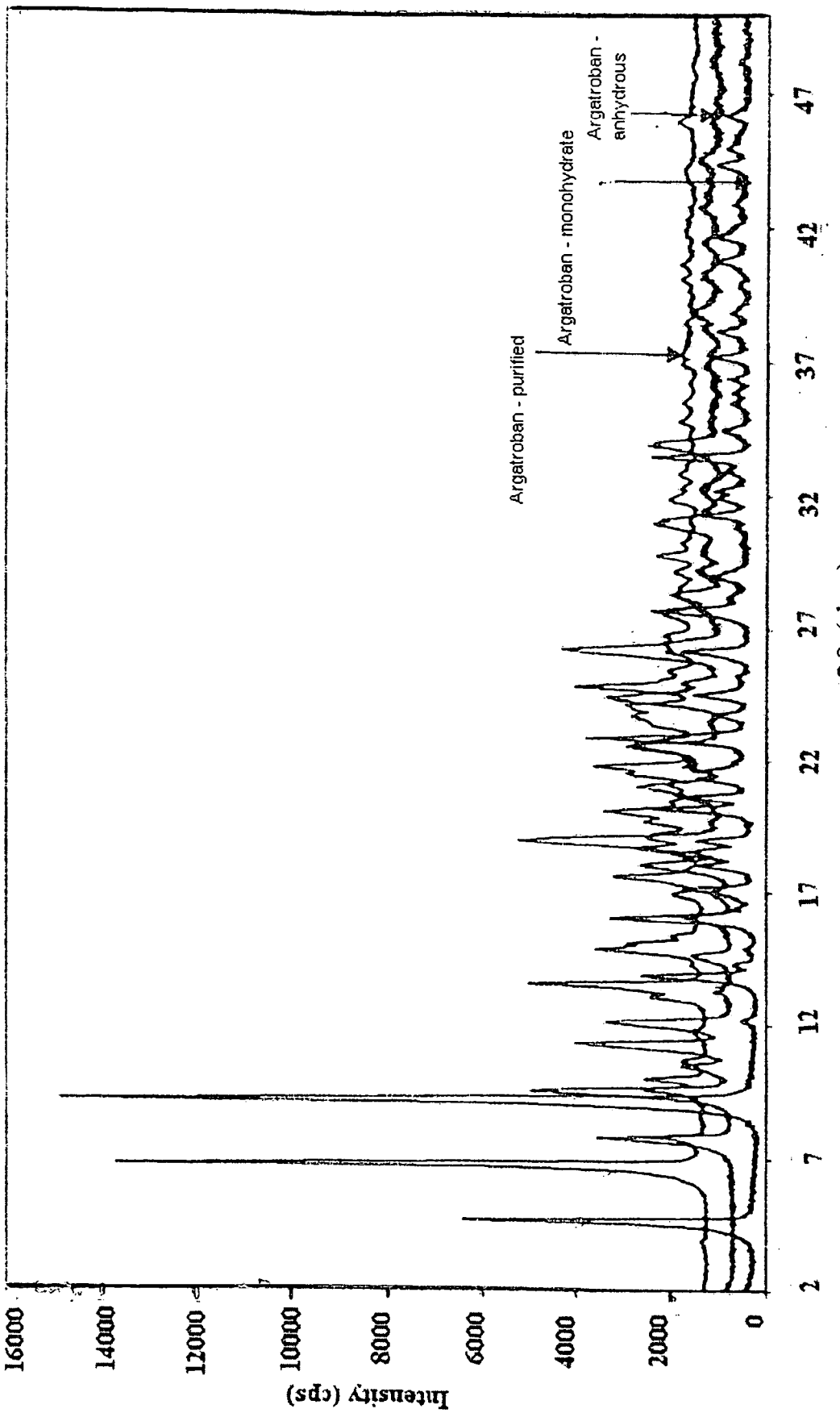

Argatroban - purified
Argatroban - monohydrate
Argatroban - anhydrous
Intensity (cps)
16000
14000
12000
10000
8000
6000
4000
2000
0
2
7
12
17
22
27
32
37
42
47
2θ (deg)

Figure 15