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Ministerio de Salud y Protección Social

Atención Dr. Javier Humberto Guzmán Cruz

Director de Medicamentos y Tecnologías en Salud

Secretario Técnico

Comité para la Declaratoria de Interés Público

E. S. D.

Re: Solicitud para la Declaratoria de Interés Público para Imatinib, presentada por MISIÓN SALUD, IFARMA y CENTRO DE INFORMACIÓN DE MEDICAMENTOS DE LA UNIVERSIDAD NACIONAL – CIMUN

Rad. 201524000237131

N/Ref.: L2012-000011

MEMORIAL DANDO RESPUESTA PARCIAL AL AUTO DE 18 DE JUNIO DE 2015

DECRETO DE PRUEBAS

CARLOS R. OLARTE, mayor de edad, domiciliado en Bogotá D.C., identificado como aparece al pie de mi firma, como apoderado de NOVARTIS A.G. en Colombia, de acuerdo con el poder que obra en el expediente, me dirijo respetuosamente a su Despacho en atención al requerimiento hecho en el Auto de 18 de junio de 2015, que abrió a pruebas el proceso administrativo de la referencia, y al requerimiento hecho el 19 de junio de 2015 a NOVARTIS DE COLOMBIA S.A, con el fin de aportar uno de los dos informes solicitados por su Despacho a mi representada en los documentos mencionados anteriormente.

1. Según el auto de 18 de junio de 2015, el Despacho le solicitó a NOVARTIS remitir dos informes que debían contener lo siguiente:

1. "Explique sus planes para abastecer la totalidad del mercado del medicamento IMATINIB en el evento en el que no se declare el interés público con fines de licencia obligatoria y los demás competidores terminen de salir del mercado. Este documento debe especificar los esquemas de distribución y cobertura del territorio nacional, los

precios que proyectan para esta cobertura en el corto y mediano plazo para el Glivec® en sus distintas presentaciones; y todos los demás aspectos que el titular de la patente considere pertinentes para garantizar el acceso y la satisfacción de la demanda del IMATINIB.

2. Presente toda la información que conozca o a la que tenga acceso sobre la posibilidad de que existan trazas del polimorfo β en otros estados polimórficos del IMATINIB por ejemplo el polimorfo α-, derivados de sus procesos productivos y la posibilidad de que este fenómeno infrinja la patente 29270 que tiene Novartis sobre la forma beta. Igualmente, especificar si es posible la existencia pura de cada polimorfo de manera independiente en una formulación farmacéutica"[2].

2. Con relación al punto 1, sobre los planes de Novartis para abastecer al mercado colombiano de IMATINIB, este será aportado por Novartis de Colombia S.A directamente.

3. Con relación al punto 2, por favor encuentre adjunta la declaración jurada en inglés de Michael Mutz y la traducción oficial al español de la misma. Michael Mutz es Senior Fellow en química del estado sólido de Novartis AG en Basilea, Suiza. Ha dedicado el ejercicio de su carrera al estudio del polimorfismo y la caracterización de los estados sólidos de los compuestos químicos en el desarrollo de fármacos.

4. La declaración jurada de Michael Mutz presenta un recuento sobre (i) el fenómeno de las estructuras cristalinas y el polimorfismo de manera general; (ii) los métodos de identificación de polimorfos; (iii) los polimorfos conocidos de mesilato de Imatinib y los procesos para su preparación; (iv) los posibles métodos para la producción del polimorfo α , o una formulación farmacéutica que contenga el polimorfo α, que además produzca el polimorfo β u otro polimorfo; y (v) la posibilidad de que el polimorfo α se convierta en el polimorfo β. En virtud de lo anterior, el contenido de la declaración responde a la solicitud de presentar toda la información que tiene mi representada sobre la posibilidad de que existan trazas del polimorfo β en otros estados polimórficos del Imatinib, incluyendo a la forma α; así como también la posibilidad de que exista cada polimorfo de manera independiente, tanto en la producción de la sustancia activa como en la producción de formulaciones farmacéuticas.

[1] Comité Técnico para la Declaratoria de Interés Público del Ministerio de Salud y Protección Social, Auto de 18 de junio de 2015, Artículo 3, Pág. 2.
[2] Comité Técnico para la Declaratoria de Interés Público del Ministerio de Salud y Protección Social, Comunicación dirigida a Novartis de Colombia S.A, 19 de junio de 2016. Pág. 1.

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5. La declaración jurada de Michael Mutz no responde al requerimiento de su Despacho sobre si constituye o no una infracción a la patente 29270 el hecho que existan trazas del polimorfo β en otros estados polimórficos del Imatinib por ejemplo en el polimorfo α. Lo anterior se debe a que las calificaciones del doctor Mutz no le permiten hacer esta afirmación jurídica en uno u otro sentido con relación a ese particular aspecto.

6. No obstante, con el ánimo de cumplir a satisfacción con lo requerido, y tratándose de una respuesta de contenido jurídico relacionada al derecho de patentes, como apoderado de Novartis AG para asuntos relacionados con patentes, me permito manifestar que Novartis considera que existirá una infracción a su Patente 29270 cuando se identifique la presencia de trazas del polimorfo β del mesilato de Imatinib en otras formas polimórficas del mesilato de Imatinib dentro del límite de detección (LOD) del método de identificación usado para el análisis.

7. El doctor Mutz indica en su declaración que los métodos de identificación de polimorfos, en general permiten determinar también la cantidad de éstos en una muestra, siempre que la cantidad presente sea mayor que el límite de detección (LOD) de la técnica utilizada. Cada técnica analítica tiene un LOD, que es la cantidad más baja de una substancia distinguible en la muestra. Por ejemplo, si una técnica particular tiene un LOD 5%, esta técnica podría inequívocamente identificar el polimorfo en una muestra que contiene 50%, 20% o 5% de ese polimorfo.

Atentamente,

CARLOS R. OLARTE
C.C No. 79.782.747 de Bogotá
T.P. 74.295 del C.S.J

Adjunto: lo mencionado

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I, Michael Mutz, aged 57 years, of the address, Freiburg i.B., Federal Republic of Germany, do hereby solemnly affirm and state as follows:

A. Experience

1. I am presently a Principal Fellow (senior expert in solid-state chemistry) at Novartis Pharma AG in Basel, Switzerland.

2. I was awarded a Master's Degree (Diploma) from the University of Freiburg i.B., Federal Republic of Germany (FRG) in 1984 and a PhD (Drscr.nat.) in physical and biophysical chemistry from the Free University of Berlin, FRG, in 1989.

3. From 1990 to 1991, I carried out postdoctoral research work at the Ecole Normale Superieure, Paris, France in biophysical chemistry.

4. I have been continuously employed since 1991, first at Ciba-Geigy AG, and subsequently at Novartis, following its formation in 1996 from the merger of Ciba-Geigy and Sandoz AG. I began at Ciba-Geigy as a laboratory head in the Scientific Services department, specialising in physical chemistry. The main focus of my work at Ciba-Geigy and Novartis has been polymorphism and solid-state characterisation of pharmaceutical solids in drug development, including analytical characterisation using crystallisation, X-ray powder diffraction (*XRPD*), microscopy, moisture sorption analysis, thermal analysis (differential scanning calorimetry (*DSC*), TGA and TMA[1]), microcalorimetry and spectroscopy (FTIR[2], Raman and Solid State NMR[3]).

5. I am the author or co-author of over twenty publications in the field of my work.

6. By virtue of the above, I consider myself to be an expert in the field of polymorphism and solid-state characterisation of pharmaceutical solids in drug development.

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[1] Thermogravimetric analysis and thermomechanical analysis.

[2] Fourier transform infrared.

[3] Nuclear magnetic resonance.

B. Instructions

7. I have been asked to prepare an affidavit on behalf of Novartis for this action in Colombia relating to a public interest declaration regarding Novartis' Colombian patent number 29270 (*CO 29270*) claiming the β polymorphic form of imatinib methanesulfonate.[4] In this affidavit, I have been asked to address the following points:

   - Crystal structures and polymorphism in general – see section C of this affidavit (paragraphs 8 to 11 below).
   - Methods of identifying polymorphs in a sample – see section D (paragraphs 12 to 17 below).
   - Known polymorphs of imatinib mesylate and processes for their preparation – see section E (paragraphs 18 to 39 below).
   - The possibility of methods for the production of the α polymorph, or a pharmaceutical formulation containing the α polymorph, also producing the β polymorph or other polymorphs – see section F (paragraphs 40 to 48 below).
   - The possibility of the α polymorph converting into the β polymorph – see section G (paragraphs 49 to 55 below).

C. Crystal structures and polymorphism in general

8. Most organic compounds form crystal structures. Imatinib mesylate is one such compound. Crystal structures are formed by a process known as crystallisation, whereby the molecules of a substance come together and align themselves into a regular repeating pattern of a crystal lattice.

9. Crystallisation is an important way of separating a product in a substantially pure form from an impure solution. This process is often utilised in the pharmaceutical industry because purity is very important when producing a pharmaceutical for human use.

[4] Which is also known by an abbreviated name, imatinib mesylate. I use the two interchangeably in this affidavit.

10. Some organic compounds, including imatinib mesylate, exhibit 'polymorphism', which means that the compound can exist in more than one crystal structure or form. Different polymorphs have different physicochemical properties, such as solubility, chemical stability, bioavailability, morphology, melting point, hygroscopicity and density. The selection of an appropriate polymorph of a compound is therefore an important aspect in the development of a pharmaceutical because of its potential influence on the properties of the pharmaceutical. A number of methods exist for identifying and characterising polymorphs of an organic compound. Many of these techniques are mentioned in paragraph 4 above and I explain how several of them work in paragraphs 12 to 17 below.

11. Imatinib mesylate may be crystallised into a number of polymorphic forms. Pharmaceutical companies around the world have discovered and documented many different forms of imatinib mesylate over the past two decades. I describe a number of these polymorphs in paragraphs 18 to 39 below.

D. Methods of identifying polymorphs in a sample

12. It is possible to analyse a sample to determine which of several known polymorphs are present. Tests commonly employed for this purpose include XRPD, microscopy, moisture sorption analysis and DSC. These techniques are able to detect differences in the properties of distinct polymorphs which arise due to differences in their crystal structures. Such properties are characteristic of each polymorph in that they can be used to identify the presence of the polymorph in a sample.

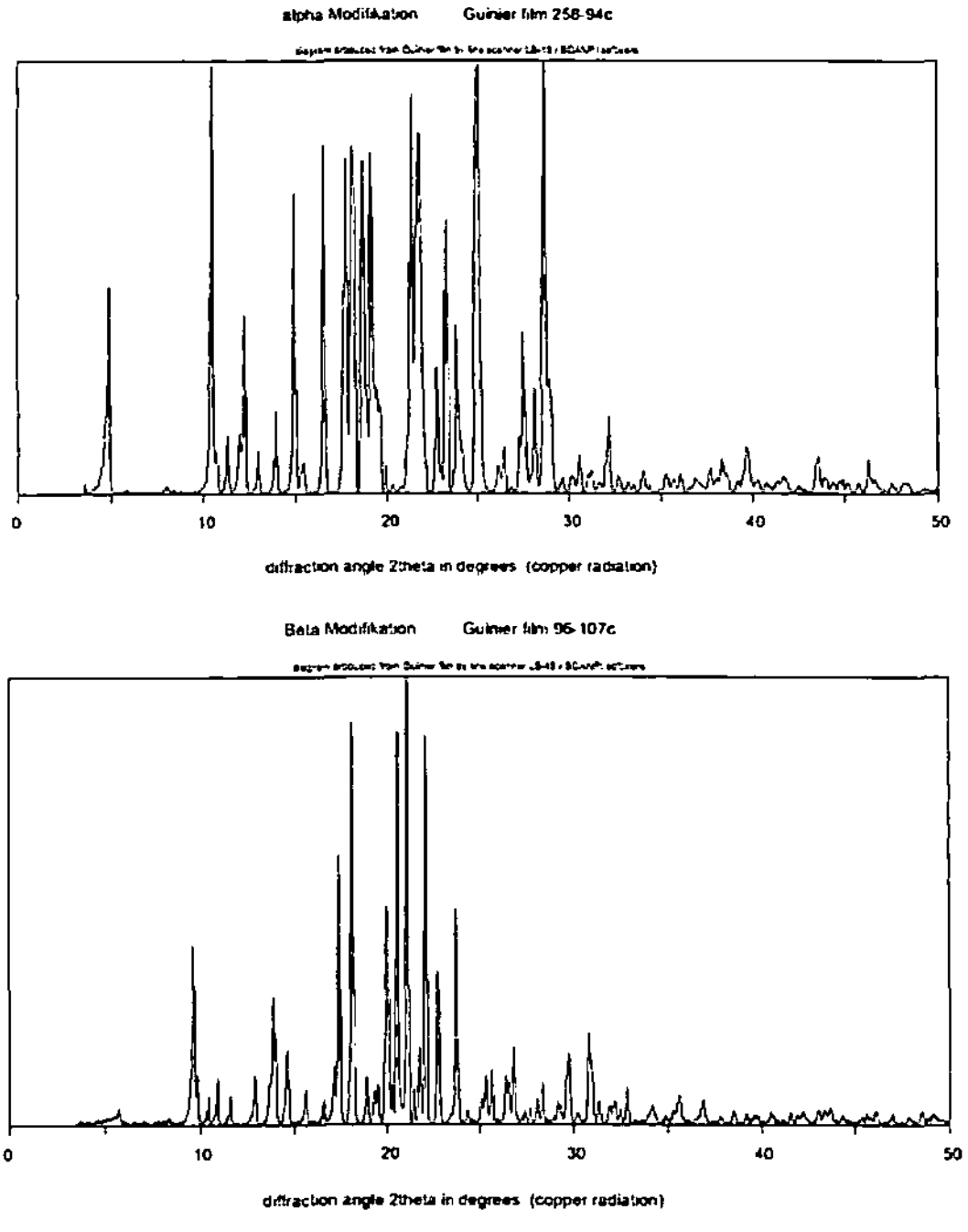
13. In the department in which I work at Novartis in Basel, we routinely use XRPD and DSC to determine whether samples contain particular polymorphic forms of an active substance. These techniques can reliably identify the presence of a particular polymorph in a sample, provided that the amount present in the sample is greater than the limit of detection (*LOD*) of the technique used. Every analytical technique has a LOD, which is the lowest quantity of a substance that can be distinguished from the absence of that substance. By way of example, if

a particular technique had a LOD of 5%, that technique could unequivocally identify the presence of a polymorph in a sample containing 50%, 20% or 5% of that polymorph. However, the technique would be unsuitable for identifying the presence of the polymorph in a sample containing only 2% of the polymorph. In such cases, a more sensitive method of detection would be required.

XRPD

14. XPRD is often used to identify polymorphs in a sample, as will become apparent from the example XRPD data referred to in subsequent paragraphs of this affidavit. It is generally considered to be a sensitive, reliable method of detecting polymorphs. XRPD involves projecting a beam of X-rays towards a powdered substance under analysis. When the beam reaches the sample, interactions between the X-rays and the atoms of the crystal structure(s) in the sample cause the beam to be scattered. Scattering of X-rays by atoms in different planes of the crystal causes interference between the scattered beams at some angles. The angles at which interference occurs are detected relative to the angle 2θ. The intensities of the signals are also detected. As the atoms in each distinct polymorph are arranged differently, interference occurs at a particular set of angles, and with particular intensities, which are characteristic of each polymorph. This data may be plotted on an XRPD spectrum as a pattern of peaks. In simple terms, this pattern can be regarded as a 'fingerprint' or 'signature' of the polymorph. Example XRPD spectra are shown in figure A below.

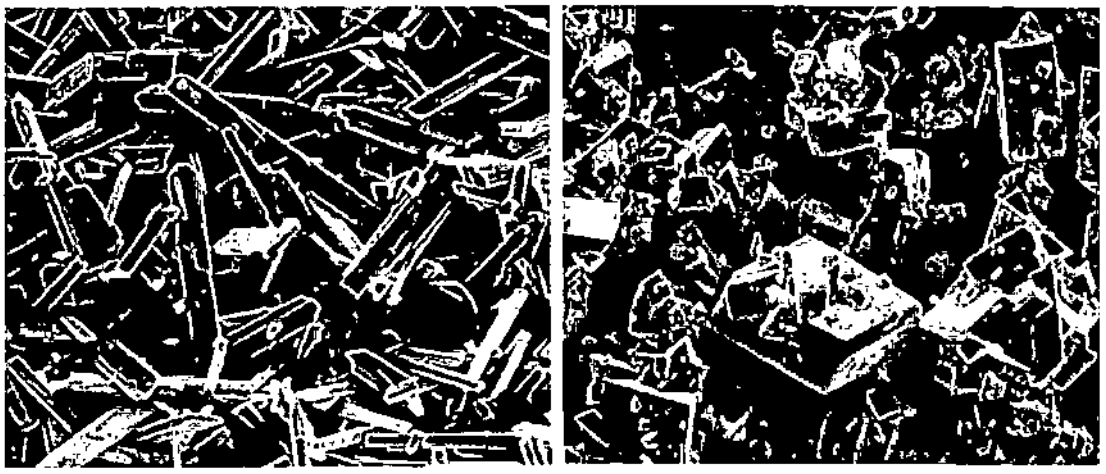
Figure A:



Microscopy

15. The crystal structure of a polymorph may be viewed using a scanning electron microscope (***SEM***), a powerful microscope which focuses a beam of electrons on to a solid sample and detects signals at its surface. These signals reveal information about the sample, including its external morphology. Figure B below shows SEM images of two crystal structures.

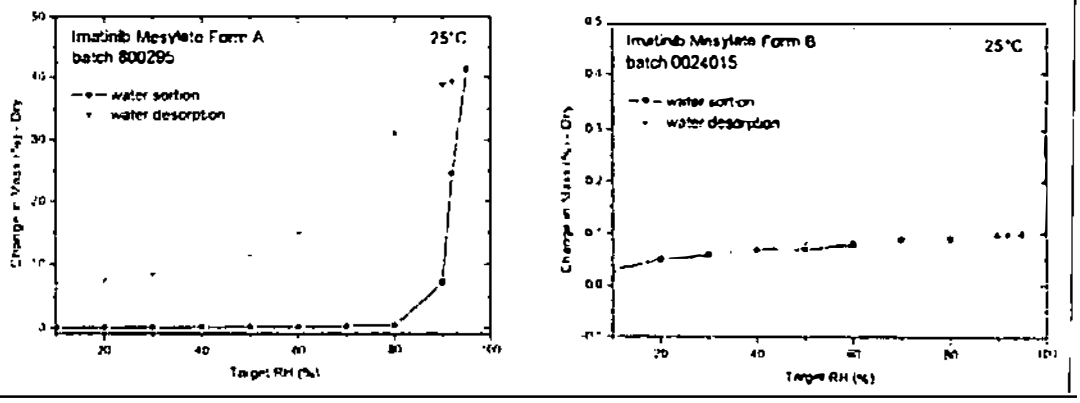
Figure B:



Moisture sorption analysis

16. Almost all substances interact to some degree with water vapour in their surroundings. Moisture sorption analysis measures the extent to which a material attracts and retains water molecules. The moisture sorption, or 'hygroscopicity', of materials generally varies depending on environmental conditions, so it is common when characterising a substance to determine its moisture content at a specified temperature and relative humidity. As distinct polymorphs have different crystal structures, they can interact with water molecules to varying extents, which makes them more or less hygroscopic under particular conditions. Figure C below depicts the results of moisture sorption analysis of two substances.

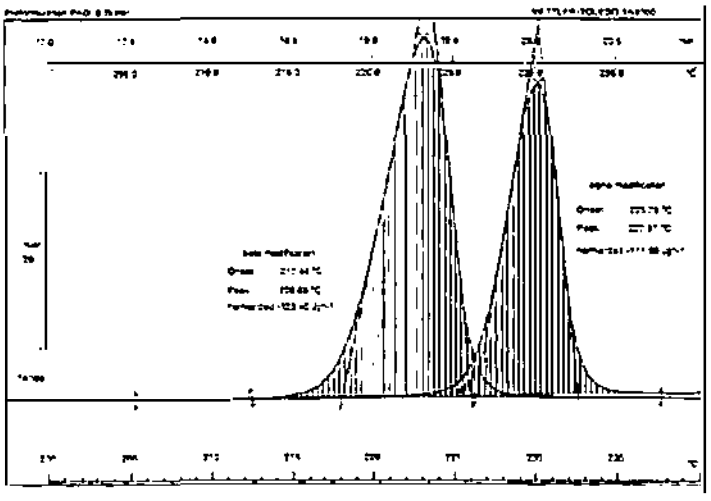
Figure C:



DSC

17. DSC is a technique used to analyse what happens to a substance, such as a crystal structure, when it is heated. As the substance is heated, it undergoes thermal transitions affecting its structure. One such transition is the melting of the substance, which occurs when the molecules making up the substance begin to come apart. In the case of a crystal structure, the regular, repeated structure breaks down leaving the constituent molecules. Melting is an endothermic reaction, meaning it takes energy to make it happen. Whereas heating a substance generally causes an increase in its temperature, this stops when it begins to melt and does not resume until the structure of the substance has completely broken down. DSC measures how much more heat must be applied to the substance for this to happen, allowing determination of the temperature at which this occurs, the melting point of the substance. As the crystal structures of polymorphs differ, so do their melting points. Example DSC thermograms are shown in figure D below.

Figure D:



E. Polymorphs of imatinib mesylate and processes for their preparation

18. I note in paragraph 11 above that pharmaceutical companies have documented many different polymorphs of imatinib mesylate. Not all of these polymorphs have crystal structures; in some arrangements (referred to as amorphous forms), there is no discernible crystal structure present. Further, different arrangements of an active substance need not be identical in terms of

chemical composition to be regarded as distinct polymorphs from a drug regulatory perspective. Molecules of water or another solvent may be incorporated into a crystal arrangement to give alternative polymorphs, referred to as hydrates and solvates respectively. In the interests of brevity, I discuss below only the α and β polymorphs and other polymorphs having a crystal structure and similar chemical composition. Table 1 below contains a full list of the different forms of which I am aware, each of which has been reported to be a distinct polymorph.

Table 1:

Form	Disclosure	Source
Forms discussed below		
α and β	WO 99/03854	Novartis
H1	WO 2004/106326	Hetero
I and II	WO 2006/054314	Natco
δ and ε	WO 2007/023182	Novartis
F, G, H, I and K	WO 2007/059963	Novartis
Y and Z	WO 2011/100282	Dr Reddy's
M, Y , B and P	EP 2 604 596 A1	Deva
Amorphous forms		
Amorphous	IN 2003/MU01208	Sun
Amorphous	WO 2008/112722	Dr Reddy's
Amorphous	US 2008/0234286	Chemagis
Amorphous	WO 2008/154262	Novartis
Amorphous, anhydrous	US 2009/0181977	Actavis
Amorphous, anhydrous	RU 2365587	Pharm-Sintez
Hydrates and solvates		
IV, V, VI, VII, VIII, IX, X, XI, XIII, XIV, XV and XVI (all solvates)	WO 2007/136510	Teva/Ivax
Z1 and Z2 (both hydrates)	WO 2011/023146	Zentiva

α and β forms

19. The first polymorphs of imatinib mesylate were disclosed by Novartis AG in 2000 in a patent application numbered WO 99/03854. This application identifies and characterises two polymorphs, known as the α crystal form and the β crystal form. I understand that WO 99/03854 is the published version of the patent application which was granted as CO 29270. CO 29270 only claims the β form of imatinib mesylate; Novartis does not claim the α

form in Colombia. As the description and drawings in these two patent documents are the same except for the difference in language, I generally refer to WO 99/03854 in preference to CO 29270 in the remaining paragraphs of this affidavit.

20. Figures 1 and 2 of WO 99/03854 show the XRPD spectra of the α form and the β form of imatinib mesylate. These spectra are copied in figure A above, in which the spectrum above is the α form and the spectrum below is the β form. The pattern of peaks in each spectrum is characteristic of that particular form of imatinib mesylate. The β form (lower part of Figure A) has peaks of significant intensity at 2θ values of 9.7, 13.9, 14.7, 17.5, 18.2, 20.0, 20.6, 21.1, 22.1, 22.7, 23.7, 29.8 and 30.8 degrees. Each of these peaks has a relative intensity of 20% or more in comparison to the most significant peak shown in the XRPD spectrum (which is the peak at 21.1°). The α form (upper part of Figure A) has peaks of significant intensity in particular at 2θ values of 4.9, 10.4, 14.9, 17.7, 18.0, 18.6, 19.0, 21.2, 21.6, 24.9 and 28.7 degrees.

21. The last paragraph on page 2 of WO 99/03854 notes that the α form is characterised by needle-shaped crystals. The third paragraph on page 3 explains that the invention relates to the β form, which has non-needle shaped crystals. The penultimate paragraph on page 5 adds that the β form is characterised by the presence of crystals displaying the form shown in the lower part of figure 3. The needle-shaped crystals of form α are shown in the upper part of figure 3. The SEM images shown in figure 3 of WO 99/03854 have been poorly copied. Higher resolution versions are shown in figure B above, in which the morphology of α form crystals is shown on the left and the morphology of β form crystals on the right.

22. The last paragraph on page 2 also notes that the α form of imatinib mesylate is hygroscopic, whereas the second paragraph on page 3 explains that the β form is less hygroscopic. Additional detail is given from the third paragraph on page 8 to the second paragraph on page 9. The first paragraph on page 9 concludes that, at 25°C and a relative humidity of 93%,

the β form remains dry whereas the α form rapidly takes up water to the extent that there is partial conversion to an amorphous (i.e. non-crystal) form.

23. Finally, the penultimate paragraph of page 2 explains that the melting points of the α and β crystal forms may be determined from a DSC thermogram, and the first paragraph on page 5 states that the onset of melting point of the β form is around 217 °C and that of the α form is around 226°C.

24. As the XRPD spectra, crystal morphology, hygroscopicity and melting points of the α and β polymorphs differ in a way that is characteristic of each polymorph, one can tell the α and β polymorphs apart using standard laboratory techniques.

25. The second paragraph of Example 1 on page 19 of WO 99/03854 describes a process for preparing the α polymorph from imatinib free base. The process involves adding imatinib free base to ethanol before adding methanesulfonic acid to the suspension. The resulting solution is heated under reflux before filtering and evaporation. The residue is suspended in ethanol and dissolved under reflux. Cooling, filtration and drying results in the α crystal form.

26. The α polymorph may be used to generate the β polymorph under specific reaction conditions, for example those described in the first paragraph of Example 1 on pages 18 to 19. A suspension of the α form is digested in methanol and crystals of the β form are isolated by filtration and dried. Alternatively, crystals of the β form may be used to 'seed' the generation of further β crystals in a solution containing imatinib methanesulfonate. Seeding provides a template crystal structure on which further molecules may assemble. It works because less energy is required to add crystals to an existing structure than to establish a new crystal structure. WO 99/03854 describes two methods of generating the β form with the assistance of seeding. Example 2 on page 19 explains that imatinib free base is suspended in methanol before methanesulfonic acid, methanol and activated carbon are added. The mixture is boiled under reflux, filtered and the water evaporated. The residue is dissolved in methanol and

inoculated with a small amount of β crystals. Drying at high temperature and pressure results in more significant quantities of the β crystal form. Example 3 on page 20 describes how heating the α polymorph in methanol and inoculating the solution with β crystals leads to further crystallisation. Drying the residue at high pressure and temperature yields more significant quantities of the β polymorph.

H1 form

27. A polymorph known as the H1 form was disclosed by Hetero Drugs Ltd in 2004 in a patent application published as WO 2004/106326. The application explains that this novel crystalline form is characterised by an XRPD spectrum having peaks at 2θ values of about 9.9, 11.1, 16.3, 17.3, 18.1, 19.1, 19.6, 20.3, 21.1, 21.9, 23.2, 23.6, 24.2, 24.9, 25.6, 26.0, 27.3, 27.9, 28.9, 29.4, 30.4 and 30.5 degrees.

28. WO 2004/106326 provides examples of the preparation of the H1 form, which is said to involve dissolving imatinib free base in a chlorinated solvent before adding methanesulfonic acid, then stirring, filtering and drying.

Forms I and II

29. WO 2006/054314, a patent application filed by Natco Pharma Ltd, disclosed two more polymorphs named forms I and II. The application lists the XRPD spectrum peaks of form I, the most significant of which are at 2θ values of about 9.7, 10.0, 16.0, 17.1, 17.9, 18.8, 19.3, 20.0, 21.7, 23.0, 23.9, 24.7, 25.1, 25.8 and 29.2 degrees. It also lists the peaks of form II, the most significant of which are at 2θ values of about 2.8, 4.4, 8.9, 9.6, 12.1, 14.1, 14.7, 16.1, 17.0, 17.6, 18.6, 19.4, 19.6, 20.3, 20.9, 21.4, 22.0, 23.5, 24.0, 24.6, 25.2, 25.7, 26.9, 27.7, 28.1, 28.6, 29.1, 29.5 and 30.1 degrees.

30. WO 2006/054314 states that form I may be prepared by slurrying the α2 or β polymorphs in chloroform and water with heating and distilling followed by filtration. It also states that form

II may be prepared by lyophilizing (freeze-drying) an aqueous solution of the α2 or β polymorphs. The α2 form referred to was the subject of an earlier patent application filed by Natco Pharma, WO 2005/077933.

Forms δ and ε

31. The δ and ε crystal forms were first disclosed in a patent application filed by Novartis AG first published in 2007 as WO 2007/023182. I was the inventor of the δ and ε forms. The application explains that the δ form produces an XRPD spectrum with peaks at 2θ values of 2.2, 13.0, 14.4, 16.0, 16.5, 16.8, 19.2, 19.4, 19.8, 20.3, 20.7, 20.9, 21.1, 21.5, 22.7, 23.7, 24.4, 24.7, 25.3, 25.6, 26.3 and 28.1 degrees. It also identifies the XRPD spectrum peaks of the ε form at 2θ values of 9.4, 11.9, 12.7, 13.3, 13.9, 15.0, 15.3, 17.0, 17.9, 18.5, 19.0, 19.6, 20.7, 21.4, 23.6, 24.1 and 28.2 degrees.

32. WO 2007/023182 explains that the δ form may be prepared by suspending a dry precipitate of imatinib mesylate in acetone and methanol before evaporating the solution under nitrogen. The ε form is prepared by a similar process except that the dry precipitate of imatinib mesylate is suspended in ethyl acetate and ethanol.

Forms F, G, H, I and K

33. Polymorphs F, G, H, I and K were disclosed in a patent application filed by Novartis first published as WO 2007/059963. I was again the inventor of the F, G, H, I and K polymorphs of imatinib mesylate.

34. The application explains that the five polymorphs have XRPD spectra with peaks at 2θ values of:

    a. in the case of the F polymorph, 8.4, 8.6, 10.4, 13.3, 14.7, 16.2, 16.8, 17.1, 19.5, 20.9, 22.2, 23.1, 23.6, 24.5, 25.1, 26.0, 26.9, 28.5, 29.1 and 30.3 degrees;

b. in the case of the G polymorph, 10.5, 12.9, 13.9, 14.1, 15.0, 16.6, 17.2, 17.5, 18.1, 18.7, 19.2, 19.8, 20.6, 21.1, 21.3, 21.7, 22.1, 22.8, 23.9, 24.3, 25.1 and 28.6 degrees;

c. in the case of the H polymorph, 10.5, 13.8, 15.7, 18.1, 21.0, 22.8, 24.3, 25.1, 26.3, 29.7 and 32.9 degrees;

d. in the case of the I polymorph, 9.6, 12.9, 14.1, 15.2, 15.6, 17.1, 18.0, 18.7, 19.1, 19.8, 20.9, 23.4, 23.9, 24.3, 25.2 and 28.4 degrees; and

e. in the case of the K polymorph, 12.1, 12.9, 13.6, 14.1, 15.2, 17.2, 18.2, 18.4, 19.8, 21.0, 22.4, 23.4, 24.3, 25.2, 28.4, 29.2 and 37.9 degrees.

35. Examples in WO 2007/059963 explain how to generate forms F, G, H, I and K. Examples 1 to 4 describe methods of generating form F. Each method involves dissolving imatinib mesylate in water and dispensing the solution into a well block. The solution in each well is dried by flushing it with nitrogen, leaving a dry precipitate. The precipitate is resuspended in different reagents in each of examples 1 to 4. These are benzyl alcohol (example 1); a mixture of benzyl alcohol and ethyl acetate (example 2); a mixture of benzyl alcohol and either 1,4-dioxane, 3-pentanone or di-isopropyl ether (example 3); or a mixture of benzyl alcohol and either acetonitrile or dimethyl formamide (example 4). In each case, the suspension is agitated and allowed to evaporate at around 50°C under nitrogen to generate crystal form F. The methods for producing crystal forms G, H, I and K are similar except that different reagents are used in the suspension step, as follows:

a. To generate form G, the precipitate is resuspended in a mixture of 3-pentanone and cyclohexane.

b. To generate form H, the precipitate is resuspended in a mixture of 3-pentanone and N,N-dimethylformamide.

c. To generate form I, the precipitate is resuspended in a mixture of ethyl acetate and diethyl ether.

d. To generate form K, the precipitate is resuspended in a mixture of 3-ethyl acetate and N,N-dimethylformamide.

Forms Y and Z

36. Crystal forms Y and Z were disclosed in a patent application filed by Dr Reddy's Laboratories Ltd published as WO 2011/100282. The application explains that these polymorphs have XRPD spectra with peaks at the following 20 values:

a. 6.1 , 8.7, 11.5, 14.5, 18.0, 18.7, 20.1 , 21.9 and 22.5 degrees in the case of form Y; and

b. 6.6, 8.0, 13.9, 16.6, 17.0, 17.3, 18.4, 19.0, 20.2, 22.2 and 24.2 degrees in the case of form Z.

37. WO 2011/100282 explains that the Y form may be prepared by suspending imatinib free base in methanol, cooling and adding methanesulfonic acid with stirring. Methyl tertiary-butyl ether is added with stirring and the suspension is filtered under nitrogen, washed with methyl tertiary-butyl ether and dried according to one of two methods. The application describes the preparation of form Z by mixing imatinib mesylate and dimethyl sulfoxide and heating for dissolution and filtering. The solution is added to pre-cooled dichloromethane and stirred at around 0°C. The solid is collected by filtration, washed with dichloromethane and dried.

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Forms M, Y[5]*, B and P*

38. Crystal forms M, Y, B and P were disclosed in a patent application filed by Deva Holdings AS published as EP 2 604 596 A1. The application explains that these polymorphs have XRPD spectra with peaks at the following 2θ values

a. in the case of form M, about 6.0, 9.6, 10.4, 10.9, 11.6, 12.9, 13.9, 14.6, 15.6, 17.4, 18.1, 18.8, 19.9, 20.5, 21.0, 21.8, 22.0, 22.6, 23.7, 24.2, 26.2, 26.8, 28.3, 29.1, 29.6, 30.1, 30.8, 31.8, 32.8, 34.0, 35.5, 36.8, 40.5, 42.2 and 43.5 degrees;

b. in the case of form Y, about 6.0, 9.7, 10.4, 10.9, 11.6, 12.9, 13.9, 14.6, 17.1, 17.4, 18.1, 20.0, 20.5, 21.0, 22.0, 23.7, 24.2, 26.2, 29.1, 29.6, 30.8, 31.7, 32.7, 34.0, 35.5, 36.8, 38.4, 39.0, 40.5, 43.5, 45.3 and 48.9 degrees;

c. in the case of form B, about 4.7, 9.7, 10.4, 10.9, 13.0, 13.9, 14.6, 17.4, 18.1, 20.0, 20.5, 21.0, 22.0, 22.6, 23.7, 25.1, 26.3, 26.8, 28.3, 29.6, 30.7, 31.7, 32.8, 34.0, 35.6 and 36.8 degrees; and

d. in the case of form P, about 4.9, 9.7, 10.4, 12.0, 12.9, 13.9, 14.7, 17.4, 18.1, 18.5, 19.1, 19.9, 20.5, 21.0, 21.7, 22.0, 24.8, 26.2, 28.4, 28.6, 30.8, 32.1, 32.8, 34.1, 35.5, 36.7, 38.5, 39.6, 43.4 and 45.3 degrees.

39. EP 2 604 596 A1 describes processes for the preparation of forms M, Y, B and P, as follows:

a. To generate form M, imatinib free base is suspended in a ketone and the mixture heated under reflux. Methanesulfonic acid is added, the mixture is cooled and crystals are isolated (e.g. by filtration, drying and suspension in a solvent) and dried.

[5] This appears to be a different polymorph than form Y referred to above.

b. To generate form Y, imatinib free base is suspended in an alcohol, the mixture stirred and methanesulfonic acid added. The mixture is stirred and crystals are isolated and dried.

c. To generate form B, imatinib free base is suspended in an ether, the mixture stirred and methanesulfonic acid added. The mixture is stirred and heated to induce crystal formation. The mixture is stirred and crystals are isolated and dried.

d. To generate form P, imatinib free base is suspended in an ether, the mixture stirred and heated under reflux. Crystals of the α form of imatinib mesylate are added, followed by methanesulfonic acid. The mixture is cooled and crystals are isolated and dried.

F. Possibility of methods for the production of the α polymorph also producing the β polymorph or other polymorphs

Production of active substance

40. I explain in paragraph 25 above a process for producing the α polymorph of imatinib mesylate. This is the process generally used by Novartis to produce the α polymorph, when that polymorph is required as a reference (comparison) material.

41. I am aware that a sample of the active substance in the pharmaceutical formulation marketed by Recalcine in Chile as 'Zeite'[6] has been analysed by the Universidad de Chile and found to contain only the α polymorph of imatinib mesylate. I exhibit as MM-1 to this affidavit a report on the results of the XRPD analysis performed by the Universidad de Chile, including XRPD spectra which identify the sample as containing pure α crystal form (down to the LOD). I have no reason to doubt the accuracy of these spectra. Therefore, the process used by the

[6] I understand that the Zeite product marketed by Recalcine in Chile is a different formulation to the Zeite marketed in Colombia.

manufacturer that supplies Recalcine produces α crystal form with no t acc of the β crystal form as an impurity (down to the LOD).

42. I exhibit as **MM-2** to this affidavit an article written by two individuals employed by Deva in Turkey dated 12 June 2015. Deva is a well-known generic pharmaceutical company. This article relates to the α and β polymorphs and considers (amongst other things) the accuracy of XRPD and DSC as methods of identifying polymorphs and analysing the stability of the α polymorph in pharmaceutical formulations. Section 2.1.2 describes a method for synthesising the α form which involves the use of di-isopropyl ether and isopropyl alcohol solvents. As the abstract of the article explains:

> *"[XRPD] was used to monitor the polymorphic purity of α form drug substance and corresponding drug products during the quality control analyses and stability studies, and the results indicated that α form was stable and not converted to β form during the manufacturing process and stability period."*

Therefore, Deva has a process for manufacturing imatinib mesylate which results in an active substance that is in pure α form (down to the LOD).

43. I describe in paragraphs 44 to 47 below a number of examples of generic tablet and capsule formulations containing the α polymo ph of imatinib mesylate and without trace of the β polymorph (insofar as we can detect given the LOD). Clearly the manufacturers of the active substance in these formulations have processes to produce imatinib mesylate in pure α form.

Production of pharmaceutical formulations

44. The analysis in the department in which I work of samples of pharmaceutical formulations marketed by generic pharmaceutical companies around the world has identified a number of formulations containing only the α polymorph of imatinib mesylate. I therefore know that

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formulators are able to manufacture formulations containing pure α crystal form. I provide some examples in the following paragraphs.

45. I understand that several generic tablet formulations were launched in Japan in or around June 2014. These comprised products marketed by Meiji Seika Pharma Co Ltd, Nipro Pharma Corporation and Daiichi Sankyo Espha Co Ltd. XRPD analysis performed in the department in which I work showed that samples of each of these tablets contained only the α polymorph (to the LOD).

46. In New Zealand, AFT Pharmaceuticals Ltd has marketed a generic capsule formulation. Samples of this capsule formulation were analysed by XRPD in the department in which I work and were found to contain only the α polymorph (to the LOD).

47. Rider Laboratories Ltd has marketed a generic tablet formulation in Chile as 'Kadir'. Samples of this formulation were again analysed by XRPD in the department in which I work and found to contain only the α polymorph (to the LOD).

48. Further, it is apparent from the article exhibited at MM-2 that Deva's method of formulating tablets containing only the α polymorph of imatinib mesylate does not generate the β form as an impurity (to the LOD). The conclusion of the article comments as follows (with emphasis added):

> *"The quantitative PXRD method reported herein represents a useful tool for monitoring the polymorphic content and crystal form stability of α form of imatinib mesylate in tablet formulations. Here, it was shown that tablets produced by α crystal form of API were highly stabilized by the excipients used in the formulation and no detectable polymorphic transformation to β form was occurred during manufacturing process or stability periods."*

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G. Possibility of the α polymorph converting into the β polymorph

49. In paragraphs 40 to 48 above, I gave a number of examples of the manufacture of imatinib mesylate in pure α form (to the LOD) and the preparation of a tablet or capsule formulation containing the active substance in pure α form (to the LOD). The following paragraphs explain that the α polymorph does not thereafter convert into the β polymorph unless exposed to particular conditions favouring that conversion or if left for a long period of time.

Conditions relevant to conversion

50. The second paragraph on page 8 of WO 99/03854 states that the α crystal form of imatinib mesylate is metastable at room temperature. This means that the stability of the α form at room temperature depends on the conditions to which it is exposed Conditions that may influence the stability of the α polymorph include:

a. polymeric purity of the α form, e.g. whether seed crystals of the β polymorph are present;
b. chemical purity of imatinib mesylate, e.g. whether degradation products, heavy metals, etc. are present;
c. temperature;
d. use of particular solvents;
e. relative humidity; and
f. pressure.

As the β polymorph is thermodynamically more stable at temperatures of around 140°C and below, some solid-state conversion of the α form into the β form is inevitable over an extended period of time. But by controlling the above conditions when manufacturing and storing the pharmaceutical formulation, the rate of conversion can be kept so slow as to be irrelevant from a pharmaceutical perspective. This is important if it is desired to maintain the active substance in the α form.

51. I explained in paragraph 26 above that the α polymorph may be used to generate the β polymorph under specific reaction conditions. Such conditions include those described in the first paragraph of Example 1 on pages 18 to 19 of WO 99/03854, namely digestion of the α polymorph in methanol followed by filtration and drying. They also include the use of crystals of the β polymorph to seed a solution of the α polymorph heated in methanol, as described in Example 3 on page 20.

52. Table 2 below is taken from an internal Novartis document which specifies certain physicochemical data relating to the α and β polymorphs of imatinib mesylate. The table summarises the effect of adding crystals of the α form, the β form or a mixture of the α and β forms to a number of different solvents at specified temperatures in equilibration or recrystallisation experiments.[7] It is a good illustration of the stability of the α and β polymorphs. The following trends are apparent:

a. The α form converts into the β form under certain conditions, specifically when added to acetone or methanol, but not under other conditions, such as if added to dichloromethane, ethyl acetate, MTBE or toluene.[8]

b. The β form remains in the same form in the two solvents to which it was added at the specified temperatures. It does not convert into the α form.

c. If both the α form and the β form are present, the α form always converts into the β form regardless of the solvent to which they are added. This is the effect of seeding, which is particularly conducive to conversion for the reasons noted in paragraph 26 above.

[7] Equilibration involves suspending crystals of an active substance in a solvent to form a slurry and allowing the crystals to adopt their most stable form under those conditions. Recrystallisation involves dissolving crystals of an active substance in a solvent before changing the conditions so that one or more polymorphs crystallise out of solution.

[8] E.g. see the row for dichloromethane in Table 2, which indicates that the α polymorph remained in that form following an equilibration experiment performed at 25°C, as determined by DSC.

Table 2:

1.1.3 Equilibration or recrystallisation in solvents[3]

Conditions			Observations	Characterisation method
Solvent	Experiment	Temp. range (°C)		
Water	equil.	-	-	-
	recrys.	-	-	-
Acetone	equil.	10 - 20	α + β ===> β	DSC
		45 - 20	α + β ===> β	DSC
		4	α + β ===> β	DSC
	equil.	25	β ===> β	DSC
		25	α ===> β	
	recrys.	45 - 20	α ===> β	DSC
Dichloromethane	equil.	25	α ===> α	DSC
	recrys.	-	-	-
Ethanol 96%	equil.	25	α + β ===> β	DSC
	recrys.	-	-	-
Ethyl acetate	equil.	25	α ===> α	DSC
		25	α + β ===> β	DSC
	recrys.	-	-	
Isopropanol	equil.	25	α + β ===> β	DSC
	recrys.	-	-	-
Methanol	equil.	25	α ===> β	DSC, Raman, XRPD
	recrys.	-	α ===> β	DSC
Methanol + water (9+1)	equil.	25	α + β ===> β	DSC
Methanol + water (8+2)	equil.	25	α + β ===> β	DSC
Methyl t-butyl ether	equil.	25	α ===> α	DSC
		25	α + β ===> β	DSC
	recrys.	-	-	-
Toluene	equil.	25	α ===> α	DSC
		25	β ===> β	DSC
		25	α + β ===> β	DSC
	recrys.	-	-	-
Sesame oil	equil.	25	α + β ===> α	DSC

Remarks:

Summary:
Two enantiotropic crystal forms named alpha and beta have been detected. The calculated transition temperature between the two forms is about 140°C. The alpha modification which was first isolated is thermodynamically metastable at room temperature but its solid state transformation into the beta polymorph is slow enough not to be detected.
No solvates or hydrates have been detected so far.

53. I note in paragraph 30 above that WO 2006/054314 describes the preparation of polymorphic forms I and II from the α polymorph, involving, in the case of form I, slurrying in chloroform, heating and distillation, and in the case of form II, freeze-drying an aqueous solution. Again, it can be seen that conversion of the α polymorph into forms I or II only takes place under specific reaction conditions; it does not occur spontaneously.

Pharmaceutical formulations

54. I refer in paragraphs 45 to 47 above to the analysis of generic product samples done in the department in which I work. In each case, XRPD spectra were prepared after the samples had been subjected to stability testing conditions. The 'intermediate' conditions used to test the samples from Japan, New Zealand and Chile were 25°C at 60% relative humidity for 6 months. The results – that no conversion occurred (down to the LOD) – demonstrate the stability of the α polymorph used in these generic products. A sample of the AFT capsule formulation from New Zealand was also tested under 'accelerated' conditions, which were 40°C and 75% relative humidity for 6 months. The XRPD spectrum showed that the active substance in the capsule remained in the α crystal form even under those accelerated conditions.

55. At this point, it is worth referring again to the article exhibited at MM-2 as the stability of Dova's tablet formulation containing the α polymorph is a focus of the article. The following quote, taken from section 3.5 of the article, summarises the results of Dova's analysis in relation to the stability of its active substance and tablet formulation (with emphasis added):

> *"Samples of α form containing tablets from different manufacturing batches were subjected to various stability testing conditions and analyzed according to the validated PXRD method to configure the stability of α form in the tablet formulation. The results indicated that α form was not converted to β form during the tablet manufacturing process, and also under routine (25 ± 2°C, 60 ± 5% humidity), intermediate (30 ± 2°C, 65 ± 5% humidity) and accelerated stability testing (40 ± 2°C, 75 ± 5% humidity) conditions in the original tablet form (film coated) and package (LDPE blister) (Supplementary Figs. S7 and S8).*
>
> *Additional studies were carried out under accelerated stability testing conditions by subjecting the tablets directly without any package and also by peeling their film coating. No polymorphic conversion to β form was observed on X-ray diffractograms of the samples taken from different parts of the tablets (surface and middle parts*

measured separately) which confirmed that α polymorph is stable in our tablet formulation.

Similar study was carried out by subjecting pure α form API in its original package (double LDPE bag) and in open flasks to the same conditions. In both case, α form API was found stable and not affected from the outside temperature and humidity, and no conversion was detected to β polymorph (Supplementary Fig. S9)."

H. VERIFICATION

56. I, Michael Mutz, do hereby verify and declare that the statements made hereinabove are true and correct to my knowledge and belief.

M. Mutz

Signed

13-Aug-2015

Dated

Attachments: Exhibit MM-1
Exhibit MM-2

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UR 2157 / 2015

Unterschriftsbeglaubigung

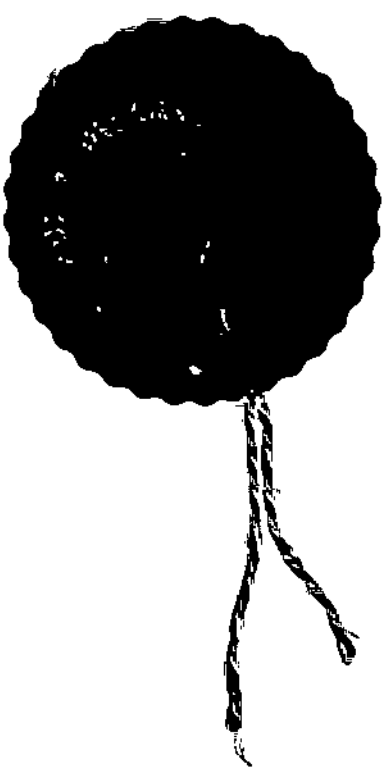
Vorstehende, vor mir als eigenhändig vollzogen anerkannte Unterschrift von

Herr Dr. Michael Mutz, geb. am 04.05.1958, geschäftsansässig bei der Novartis Pharma AG mit Sitz in CH-4002 Basel (Schweiz), Virchow-6.3.231

- ausgewiesen durch amtlichen Lichtbildausweis -

beglaubige ich. Der Notar ist der vorstehenden fremden Sprache nicht mächtig und konnte deshalb die rechtliche Zulässigkeit des vorstehenden fremden Textes nicht prüfen.

79098 Freiburg, den 13. August 2015



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MICHAEL KÜMMERLE
NOTAR IN FREIBURG

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EXHIBIT MM-1

65 (1)

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Informe de Análisis por Difracción de Rayos X.

Srs. Corporación Farmacéutica Recalcine
Atención Sr. Cristóbal Vallejos R.
PRESENTE.

Santiago, 12 de abril de 2005

Conforme a lo solicitado, se ha realizado el análisis de la muestra rotulada como **Imatinib GL-289** mediante Difracción de Rayos-X y posterior comparación con la documentación adjunta: "Canadian Intellectual Property Office, CA 2,296,604, Cristal Modification of a N-Phenil-2-Pyrimidineamine Derivate", proces for its manufacture and its use", que contiene los difractogramas Fig. 1/3 (alpha form) y Fig. 2/3 (beta form).

Especificaciones de la medida y tratamiento de datos.

- Instrumento: Difractometro Siemens D5000, para muestras policristalinas.
- Longitud de onda utilizada: $\lambda = 1.54$ Å, correspondiente a un ánodo de Cu.
- Potencia utilizada: 40 KV / 30mA.
- Rango de medida: 2° – 80° en 2θ.
- Paso: 0.02° por segundo.
- Software para análisis de datos: Difract plus

Se adjunta copia del difractograma obtenido, 2θ vs Counts , (Fig. 1) Imatinib GL-289 gráfica tratada, (Fig. 2) gráfica sin tratar y (Fig. 3) acercamiento a la zona de interés 2° a 50° en 2θ, (Tabla I) con los máximos de mayor importancia dentro del difractograma.

La comparación realizada para la muestra en cuestión con la información adjunta, Canadian Intellectual Property Office, CA 2,296,604, permite concluir que los difractogramas Fig 2/3 (beta form) e Imatinib GL-289 no coinciden, por ende, se trata de dos formas cristalinas diferentes. **No correspondiendo Imatinib GL-289 a la forma beta (Fig. 2/3).**

Atte.

Andrés Alejandro Ibáñez Muñoz
Ingeniero Químico, Responsable de la Difractometría de Polvo.
Laboratorio de Cristalografía, Departamento de Física.
UNIVERSIDAD DE CHILE

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Tabla 1.- Angulo 2Θ, Distancia interplanar e intensidad relativa para los principales máximos de difra ión de la mue tra **Imatinib GL-289, Laboratorio Recalcine.**

Angulo de difracción 2-Theta (2Θ)	Distancia Interplanar Ángstrom (Å)	Intensidad %
18,59	4,768	100
19,10	4,643	78
10,46	8,451	76
18,09	4,900	71
21,62	4,106	69
24,91	3,572	64
21,27	4,174	62
17,68	5,011	61
14,90	5,939	59
28,55	3,124	47
12,13	7,293	42
16,47	5,377	37
23,75	3,744	32
4,901	18,02	31
23,19	3,832	30
22,58	3,934	24
27,38	3,254	22
27,99	3,185	20
11,92	7,416	19
11,27	7,847	17
13,84	6,391	16
26,27	3,389	15
15,36	5,764	11
32,03	2,792	11
12,88	6,865	10

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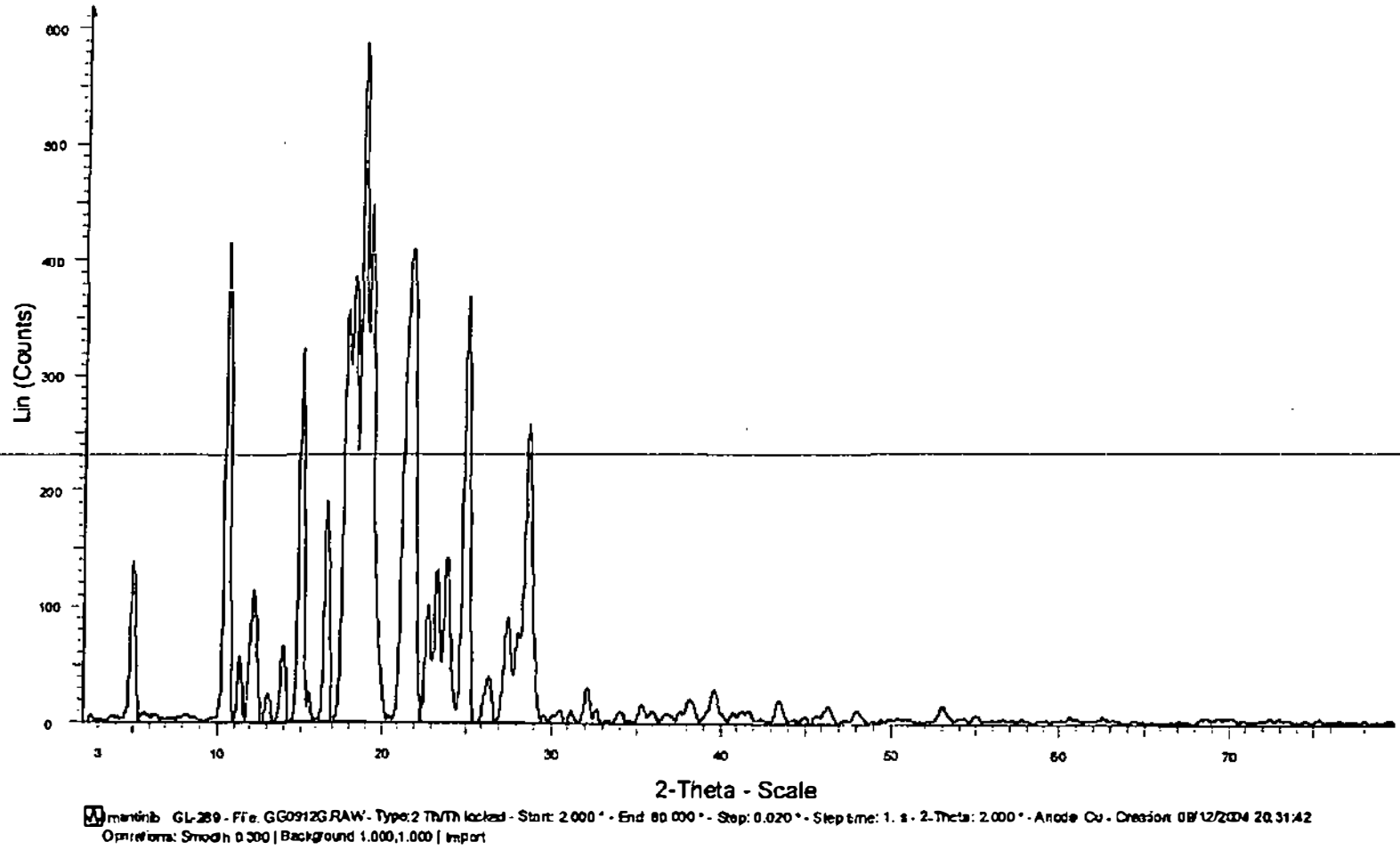
Imatinib GL-289

Fig. 1



Imatinib GL-289 (Dif RX sin tratar)

Fig. 2

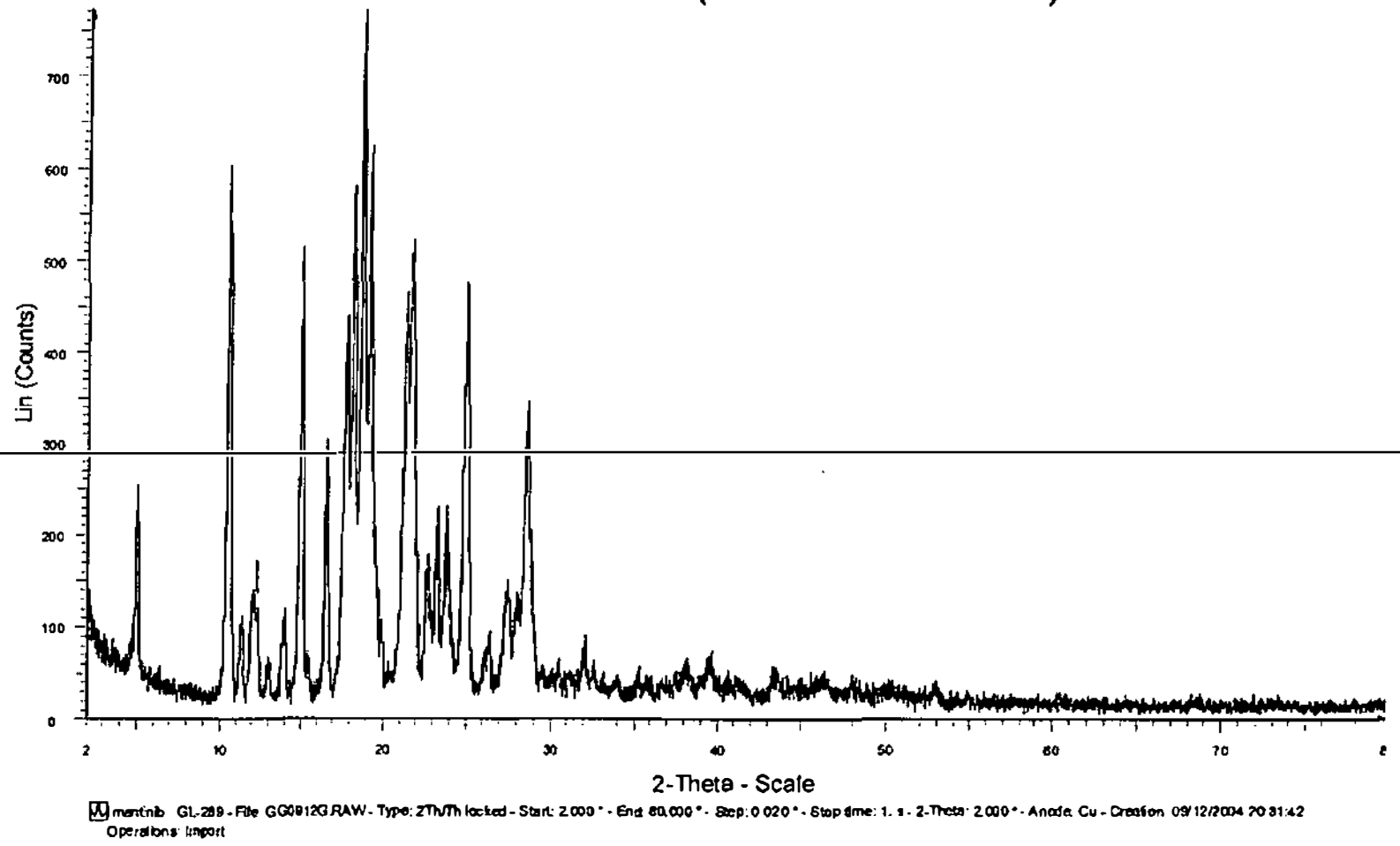
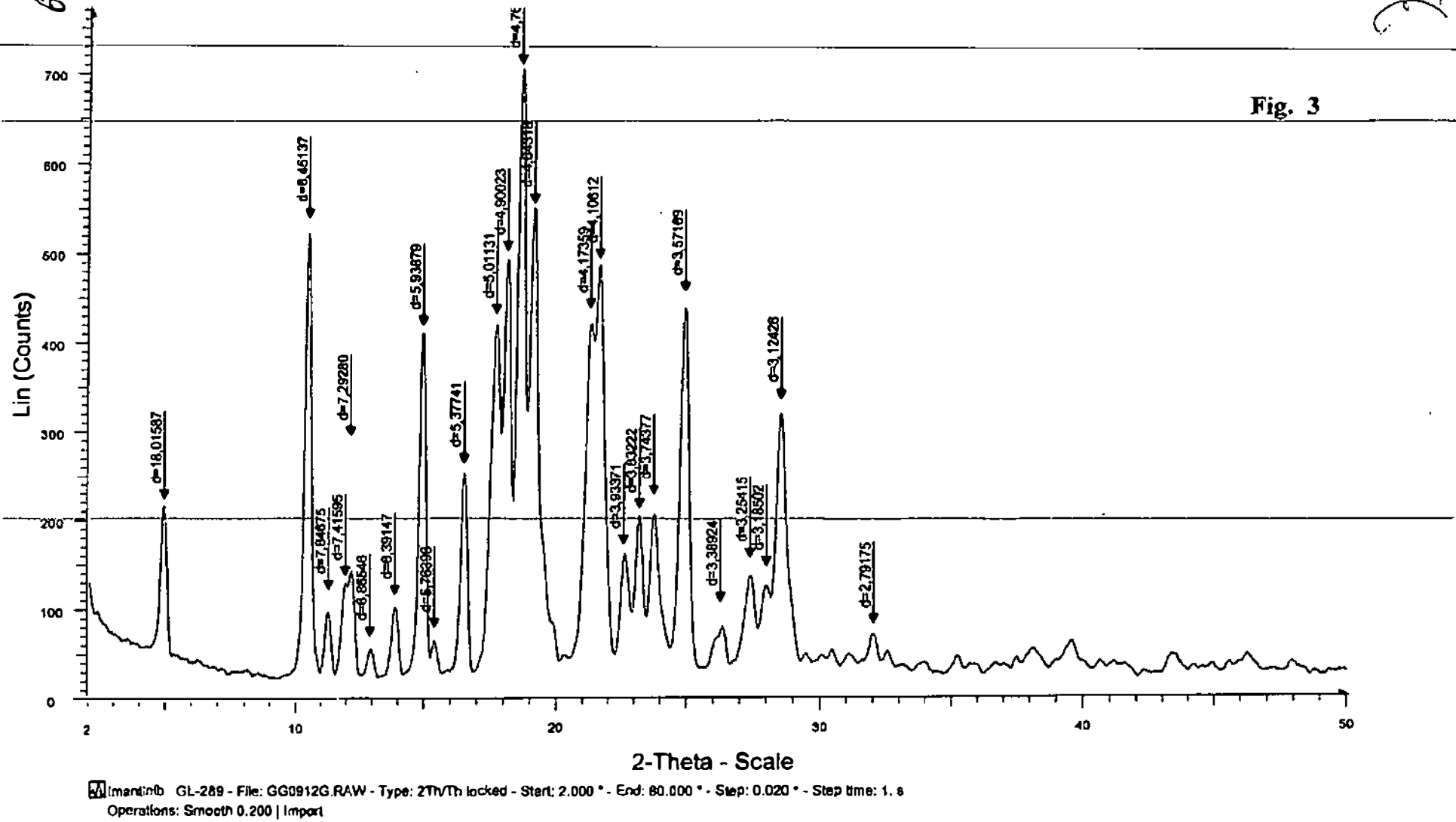


Fig. 3



Imatinib alfa Laboratorio Recalcine

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Quantitative determination of two polymorphic forms of imatinib mesylate in a drug substance and tablet formulation by X-ray powder diffraction, differential scanning calorimetry and attenuated total reflectance Fourier transform infrared spectroscopy

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ABSTRACT

Imatinib has been identified as a tyrosine kinase inhibitor that selectively inhibits the Abl tyrosine kinases, including Bcr-Abl. The active substance used in drug product is the mesylate salt form of imatinib, a phenylaminupyrimidine derivative and chemically named as *N*-(3-(4-(pyridin-3-yl) pyrimidin-2-ylamino)-4-methylphenyl)-4-((4-methylpiperazin-1-yl) methyl)-benzamide methanesulfonic acid salt. It exhibits many polymorphic forms and most stable and commercialized polymorphs are known as α and β forms. Molecules in α and β polymorphic forms exhibit significant conformational differences due to their different intra- and intermolecular interactions, which stabilize their molecular conformations and affect their physicochemical properties such as bulk density, melting point, solubility, stability, and processability. The manufacturing process of a drug tablet included granulation, compression, coating, and drying may cause polymorphic conversions. Therefore, polymorphic content of the drug substance should be controlled during quality control and stability testing. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, differential scanning calorimetry (DSC), and powder X-ray diffraction (PXRD) methods were evaluated for determination of the polymorphic content of the drug substance and drug product; and PXRD was the most accurate technique and selected as preferred method and validated. Prior to development of a quantification method, pure α and β polymorphs were characterized and used throughout the method development and validation studies. Mixtures with different ratios of α and β forms were scanned using X-ray diffractometer with a scan rate of 0.250°/min over an angular range of 19.5–21.0° 2θ and the peak heights for characteristic peak of β form at 20.5 ± 0.2° 2θ diffraction angle were used to generate a calibration curve. The detection limit of β polymorph in α form imatinib mesylate tablets was found as 4% and the linear regression analysis data for the calibration plots showed good linear relationship with correlation coefficient of 0.992 with respect to relative peak height in the concentration range of 12–75 wt% β form containing tablet mixtures. The obtained results at each stage of the validation study proved that the method is specific, repeatable, precise and accurate, and could be used for determination of β polymorph content in tablets produced by using α polymorph of imatinib mesylate. The developed PXRD quantification method was used to monitor the polymorphic purity of α form drug substance and corresponding drug products during the quality control analyses and stability studies, and the results indicated that α form was stable and not converted to β form during the manufacturing process and stability period.

1. Introduction

Polymorphism is defined in a different way depending on the scientific field of use, such as computer science, biology, and chemistry or material science. For the latter, polymorphism is the ability of a solid material to exist in more than one form or crystal structure. A recent study estimated that 80–90% of organic compounds can exist in multiple crystalline forms (polymorphs, hydrates, solvates), and more than half of the pharmaceutical drug compounds exhibit solid-state polymorphism [1]. Crystalline forms have different arrangements and/or conformations of the molecules in the crystal lattice. Amorphous forms consist of disordered arrangements of molecules that do not possess a distinguishable crystal

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0731-7085/

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lattice. Solvates are crystal forms containing either stoichiometric or nonstoichiometric amounts of a solvent [2–4].

Due to the differences in their intra- and intermolecular solid-state structures, polymorphic forms of a drug substance can have different chemical and physical properties, including melting point, solubility, mechanical properties, and bulk density. These properties can have a direct effect on the ability to process and/or manufacture the drug substance and the drug product, as well as on drug product stability, dissolution, and bioavailability. Thus, polymorphism can affect the quality, safety, and efficacy of the drug product [5]. Therefore, the investigation of polymorphism in raw materials and also in solid dosage forms is becoming a common practice in industrial quality control routines. A range of analytical techniques, such as PXRD, DSC, ATR-FTIR, near-infrared spectroscopy (NIR), diffuse reflectance infrared spectroscopy (DRIFTS), Raman spectroscopy, solid state ^{13}C NMR spectroscopy, and terahertz (THz) spectroscopy, have proven suitable for the analysis and quantification of polymorphic mixtures [6–14].

Imatinib is a drug used to treat certain types of cancer and marketed as its mesylate salt (Scheme 1). It is a tyrosine kinase inhibitor and one of the first molecularly targeted therapies used in the clinic. Its efficacy was proven in the treatment of chronic myeloid leukemia (CML), gastrointestinal stromal tumors, and also in other malignancies that involve expression of a tyrosine kinase [15]. Imatinib mesylate is prepared using a fully synthetic process and shows polymorphism. Due to its novel structure and potent biological activity, several synthetic methods were developed by organic and pharmaceutical chemists for imatinib, its salts, adsorbates, and polymorphs including amorphous form [16–21]. Among these crystalline forms α and β polymorphs were commercialized and used as a drug substance in pharmaceutical industry. Complete solid-state characterization of these two common forms was studied together with their thermal behavior and grinding effects on their crystalline form by PXRD and DSC techniques [22]. Also, a detailed vibrational spectroscopic investigation of β form was done by using FTIR and FT-Raman spectra supported by quantum mechanical calculations [23]. In particular, α form was described as metastable at room temperature and was then initially indicated as not useful for the preparation of pharmaceutical preparations [24]. Depending on the applied process and modification of thermodynamic variables like pressure or temperature, either a polymorphic conversion or formation of an amorphous substance may occur during production of solid dosage drug products [25]. The aim of the present work is to provide a quantification method for β form content in α form of imatinib mesylate in a tablet formulation. This method allows us to test the polymorphic purity of the drug substance in tablet and used for quality control and stability testing of the drug product. In spite of many studies reported, there is no report about polymorphic quantification of imatinib mesylate polymorphs. At first, we focused our work on the study of quantification of β form in α form of active pharmaceutical ingredient (API) by using PXRD, DSC, and ATR-FTIR. Subsequently, we have applied the corresponding methods to tablet formulations, and finally the selected quantification method (PXRD) was validated for determination of β form content in tablets produced by using α form of imatinib mesylate. The validated PXRD method was used for stability testing of tablets and results showed that α form of imatinib mesylate is stable in tablet formulation used during these studies and no conversion was observed to β crystalline form.

2. Materials and methods

2.1. Materials and preparation of polymorphs

Imatinib mesylate samples were taken from commercial batches produced by Deva Holding A.Ş. (Tekirdağ, Turkey). Imatinib mesylate standards were supplied by a specialized team on standardization of reference standards for analytical use in Deva. Imatinib base used for synthesis of imatinib mesylate was supplied from Cdymax Pharma (India). Synthetic and analytical reagents and solvents were supplied from different commercial chemical sources such as: Merck KGaA (Darmstadt, Germany), J.T. Baker (Phillipsburg, USA), Sigma-Aldrich (St. Louis, MO, USA), Lab-Scan (Gliwice, Poland), and Acros Organics (Geel, Belgium). Deionized water was prepared using MilliQ plus purification system (Millipore, USA). Deuterated solvents (dimethylsulfoxide-d6 and D_2O) were purchased from Merck (Darmstadt, Germany). Evidence of the chemical structures has been provided by an examination of ^1H NMR, ^{13}C NMR, and mass spectral data, and of polymorphic forms by PXRD, DSC and ATR-FTIR spectroscopy. Purity of each polymorph was tested by validated HPLC and GC methods and determined to contain <0.5% of other process related impurities. Both polymorphs were anhydrous and their water content was found below 0.5%. The analysis was carried out by Karl-Fischer (KF) titration of 1.0 g sample using pyridine-free iodosulphurous reagent (CombiTitrant 5 one-component reagent for volumetric KF titration 1 mL equal to ca. 5 mg H_2O, Merck, Germany) on Metrohm 795 KFT Titrino instrument (Switzerland). Tablet formulations were containing the following excipients besides the active drug substance: microcrystalline cellulose (Type 302) (JRS Pharma, Germany), hydroxypropylmethyl cellulose (HPMC E3) (Dow, USA), crospovidone (BASF, Germany), colloidal silicon dioxide (Evonik Industries, Germany), and magnesium stearate (Peter Greven GmbH, Germany).

2.1.1. Synthesis of imatinib mesylate β form

A solution of methanesulfonic acid in methanol was added slowly (in ca. 10 min) into a suspension of imatinib base in methanol at room temperature. After complete dissolution active carbon was added and the mixture was heated and stirred at reflux temperature (65–70 °C) for 30 min. Then, it was filtered through celite and the filtrate was concentrated to dryness. The foamy residue was dissolved in methanol and allowed to crystallize at ambient temperature. After crystallization, the mixture is stirred for 2 h at room temperature and the crystals were collected by filtration, and dried for 3–5 h at 60 °C in vacuo to afford off-white crystalline product (β form).

2.1.2. Synthesis of imatinib mesylate α form

Half amount of methanesulfonic acid solution in diisopropyl ether was added slowly (in ca. 30 min) into a suspension of imatinib base in isopropyl alcohol at reflux temperature (80–85 °C). After addition of hot isopropyl alcohol, second half of the methanesulfonic acid solution in diisopropyl ether was added slowly (in ca. 30 min) and the mixture was stirred at reflux temperature for 30 min. Then, it was cooled to 30–35 °C, filtered, and the crystals were dried for 6–8 h at 90–95 °C in vacuo to afford off-white crystalline product (α form).

2.2. Preparation of samples

Samples were prepared in fine powder form and sieved for uniformity of the particles. Sample preparation and measurements were carried out under controlled relative humidity (60 ± 5%) and temperature (25 ± 2 °C) conditions. Samples/standards were prepared by mixing them in a mortar and sieving to get homogeneous mixtures. Tablet samples were prepared by peeling the coating material and then the seed tablet was grinded in a mortar with a pestle and sieved to obtain a homogeneous powder. Standard α-β polymorphic API mixtures were prepared at different ratios

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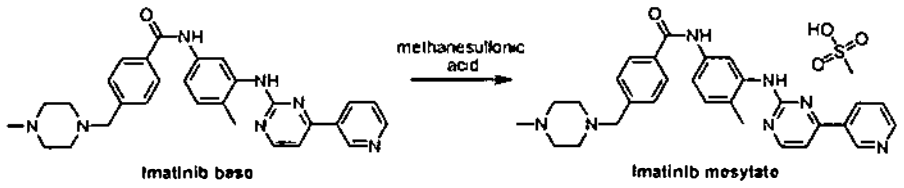

Scheme 1. Synthesis of imatinib mesylate.

and tablet samples were prepared by mixing 922 mg placebo and 478 mg corresponding standard API mixture.

2.3. Microscopes

High performance scanning electron microscope (SEM) images were taken on JEOL JSM-7001F (Thermal Field Emission Scanning Electron Microscope, Japan) with 10.00 kV accelerating voltage. The microscope images of crystals were taken on Swift Phase-Master II (USA) phase contrast microscope.

2.4. Powder X-ray diffractometry (PXRD)

PXRD patterns were obtained using a Shimadzu LabX XRD-6100 X-ray diffractometer (Shimadzu Corporation, Japan) by using the following instrument parameters: X-ray source: Cu (1.5406 Å), current: 40 mA, voltage: 45 kV, filter for Kβ: nickel, and measurement parameters: scan axis: $2\theta/\theta$, start angle (2θ): 3.0° (19.5° for quantitative β form analysis), end angle (2θ): 40.0° (21.0° for quantitative β form analysis), step size: 0.028°, and scan speed: 0.250 (°/min). A peak corresponding to imatinib mesylate β form of occurring at about 2θ value of 20.5 ± 0.2° is considered as characteristic peak for the determination of the amount of β form in α form imatinib mesylate. Samples were placed into the sample holder and the sample surface was smoothed with a glass slide. Measurements were done by scanning the linearity samples and plotting a calibration curve. Then two samples were scanned and intensity of the peak occurring at 2θ values of 20.5 ± 0.2° (related to β form) was determined. Amount of imatinib mesylate β form was calculated according to the calibration curve using the following formula $X(\%) = (a \pm y)/m$, where "a" is peak intensity of β form in the diffractogram of sample, "y" is y-intercept derived from linearity plot, and "m" is slope derived from linearity plot.

2.5. FTIR spectroscopy

Samples were measured as neat by using ATR on Shimadzu FTIR Spectrometer IR Prestige-21 (Shimadzu Corporation, Japan) in the range of 600–4000 cm^{-1} with 20 scans and 2 cm^{-1} resolution.

2.6. Differential scanning calorimetry (DSC)

DSC measurements were carried out using a Shimadzu DSC-60 instrument (Shimadzu Corporation, Japan). The instrument was calibrated for temperature and heat flow using indium and zinc standards. The samples (2–3 mg) were placed in sealed aluminum pans under nitrogen purge at flow rate of 30 mL/min. The samples were heated from 100 °C to 250 °C at a heating rate of 10 °C/min. Data acquisition and analysis were performed by using Shimadzu TA-60WS software. Start and end points for the integration of the thermal peak were identified by visual inspection. The amounts of the samples were weighed in a Sartorius ME23SS analytical balance (Sartorius AG, Gottingen, Germany) with a resolution of 0.01 mg.

2.7. Validation of analytical method

The analytical method developed for quantification of imatinib mesylate β form in α form tablets was checked for validation parameters like specificity, limit of detection (LOD), limit of quantitation (LOQ), linearity, range, accuracy and precision.

Specificity (selectivity) of the method was determined by scanning placebo, β form, α form, β form with placebo and α form with placebo samples. Diffraction peak of β form (20.5 ± 0.2° 2θ) on the diffractogram of β form with placebo should be clearly observed and no diffraction peak of β form should be observed on diffractograms of placebo and α form with placebo.

The LOD of the quantitative method was determined by analyzing α form tablet samples spiked with 1%, 2%, 3%, 4%, 5%, 6%, 8%, and 10% β form. Detection limit was set as the concentration at first selected diffraction peak appeared and quantitation limit was set as three times of LOD value.

Linearity of the method was determined by scanning five standards in the range from LOQ to 150% of specification limit of β form (LOQ, 50%, 100%, 125%, and 150%). Slope of regression line, correlation coefficient (R), and residual sum of squares (RSS) were reported. A linear relation between amount of β form and relative peak intensities was achieved.

Samples at level 1 (LOQ) and level 5 (75% β) were scanned six times for range study. Relative standard deviations (RSD) of intensities at minimum (LOQ) and maximum levels were calculated.

For the determination of accuracy of the method, recovery study was carried out by analyzing four different concentration (12, 25, 50, and 75% β) samples in triplicate. The percentage of the average recoveries was calculated.

Precision (repeatability) of the method was ascertained by analyzing six different samples containing 50% β form under the same working conditions. For inter-day precision, six different samples (50% β) were prepared and analyzed on a different day by a different analyst. RSD of intensities and difference between mean of recoveries of two studies were calculated.

2.8. Stability studies

Stability studies were conducted under accelerated (40 ± 2 °C, 75 ± 5% humidity), intermediate (30 ± 2 °C, 65 ± 5% humidity) and long-term (25 ± 2 °C, 60 ± 5% humidity) stability conditions [26]. The samples were analyzed to determine the polymorphic purity by the developed PXRD method reported herein. Different drug substance and drug product samples were subjected to various stability conditions and samples were analyzed by using DSC and PXRD methods.

3. Results and discussion

3.1. Characterization of imatinib mesylate polymorphs (α and β)

Microscopic and SEM images of imatinib mesylate α and β form crystals are shown in Fig. 1. Where, crystals of α form are needle shaped while β form crystals are plate-like shaped. This difference could be explained by their conformational differences in

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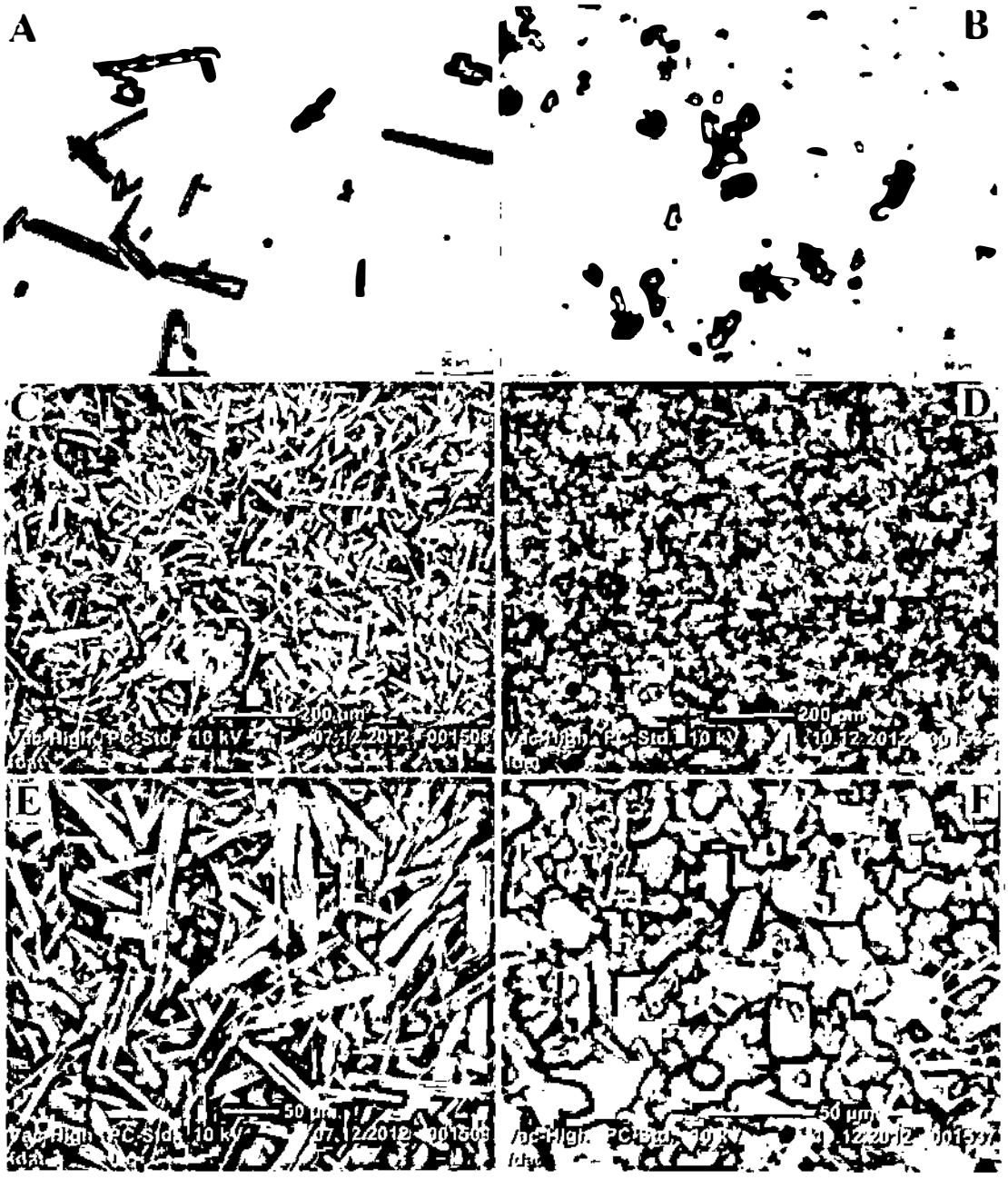

Fig. 1. Imatinib mesylate microscopic images (A) α form and (B) β form, and SEM images (C) α form (200 µm), (D) β form (200 µm), (E) α form (50 µm), and (F) β form (50 µm).

the crystal lattice and packing arrangement, caused by the differences in their intra- and intermolecular interactions. Single X-ray crystal diffraction studies [22] showed that each crystal form was subjected to different intramolecular interactions to stabilize their molecular conformation (Supplementary Figs. S1 and S2). In both forms weak intramolecular hydrogen bonds were determined, but at different positions. Both crystal structures presented a dimer-chain arrangement and neighboring chains were linked by very weak interactions and again differently from each other. Crystal shapes observed on microscopic images (Fig. 1) and dimer-chain arrangements shown on the packing diagrams (Supplementary Figs. S1 and S2) of polymorphs are complementary. Crystal shape of a drug substance is important especially for tablet products since it may influence the compaction behavior, flow-ability and the tendency to stick to the punches [27]. Of course, this effect could be minimized by addition of proper excipients as in the case of imatinib mesylate α form, which has worse flow properties due to its morphology (needle-like shaped) with respect to flow properties compared to β form (plate-like shaped).

Polymorphic purity of synthesized α and β polymorphs were confirmed by PXRD, FTIR, and DSC analyses (Supplementary Figs. S3 and S4), and they agreed well with the literature results [22,23]. Both polymorphs are thermodynamically stable and show similar aqueous solubility characteristics, therefore both could be used for the manufacturing of the drug product. In-vitro dissolution profile of the tablets manufactured by using α polymorph was similar to

the inventor's drug product manufactured by using β polymorph (Supplementary Fig. S5). α form is more hygroscopic compared to β form, but this does not affect its polymorphic stability when it is in pure α form. If the drug substance contains even small amount of β form and subjected to humidity, there is a polymorphic conversion tendency toward β form. This tendency was not observed in our tablet formulation and it was monitored by keeping the drug product samples at various stability conditions for several months.

3.2. Evaluation of PXRD, FTIR, and DSC methods for determination of β polymorphic impurity

PXRD, FTIR, and DSC methods were evaluated for determination of β form impurity in α form of imatinib mesylate both for drug substance and tablet product. First evaluation was done by comparison of pure drug substance polymorphs and then drug product was evaluated.

PXRD patterns (Supplementary Fig. S3A) were evaluated for determination of characteristic β form peaks compared to α form and a very distinct and strong diffraction peak at 20.5 ± 0.2° 2θ was selected to build the calibration model. X-ray diffractograms were acquired for the α form drug substance samples spiked with 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 8%, and 10% β polymorph for LOD study (Fig. 2A) and 3% (LOQ), 12%, 25%, 50%, 62.5%, and 75% for linearity studies (Fig. 2B) in the range of 19.5–21.0° 2θ; from which a linear calibration plot was obtained with a correlation coefficient of 0.992, y-intercept 411, and slope 570. Results for the mentioned α–β mixtures showed that PXRD is a suitable method for determination of β form content in α form of the drug substance and the detection limit was about 1% using the 20.5 ± 0.2° 2θ diffraction angle relative peak height. On the basis of these results, PXRD method was evaluated for determination of β polymorph in tablets where α polymorph of imatinib mesylate used. For this purpose, X-ray diffractograms for samples of pure α and β form containing tablets and placebo were compared and no diffraction peak was observed for α form drug product and placebo at the selected diffraction peak (20.5 ± 0.2° 2θ) of β form (Fig. 3A). All preliminary studies supported that PXRD method is also suitable for determination of β form content in tablets.

To investigate the potential of the ATR-FTIR technique for quantitative polymorph analysis, FTIR spectra of α–β form pure drug substances (Supplementary Fig. S3B) and drug products (Fig. 3B) were evaluated. The α form spectrum exhibits an additional vibrational band at 1446 cm^{-1} that clearly distinguishes it from β form, indicating that ATR-FTIR can provide definitive identification of the two polymorphs. Several standards containing 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 25, 50, 62.5, 75, 85, 90, 95, and 100 wt% of β form in mixtures of α–β form drug substances and drug products were analyzed. The ATR-FTIR spectra showed that intensity of the band at 1446 cm^{-1} increased by the increase of the weight percent of α form from 0 to 100% in the polymorphic mixture (Supplementary Fig. S6). By plotting the absorbance at 1446 cm^{-1} against the weight percent of β form in the polymorph mixture, a linear calibration plot was obtained with a correlation coefficient of 0.977 ($y = 0.2052x + 65.313$) and 0.959 ($y = 0.2762x + 67.605$) for drug substance and drug product, respectively. Better correlation was achieved by peak ratio approach and the band at 1630 cm^{-1} was selected as reference to eliminate baseline effects, any variation caused by sample particle size, or intensity of the FTIR source. This approach yielded better calibration curves with correlation coefficient of 0.994 ($y = 0.0022x + 0.651$) and 0.977 ($y = 0.0027x + 0.7231$) for drug substance and drug product, respectively. FTIR method by using peak ratio approach seemed to be useful especially for determination of polymorph content of imatinib mesylate drug substances rather than tablets, since it showed better correlation coefficient.

The potential of DSC technique was investigated for quantitative polymorph analysis of α-β form pure drug substances and drug products. The melting event for β form drug substance starts at 216 °C (onset temp.) and ends at 222 °C (endset temp.) and for α form starts at 225 °C (onset temp.) and ends at 230 °C (endset temp.) (Supplementary Fig. S4). There was no overlap between melting endotherms of α and β polymorphs, and they seemed useful to be used for evaluation of quantitative analysis. For this purpose, several standards containing 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 25, 50, 62.5, 75, 85, and 90 wt% of β form in mixtures of α–β form drug substances and drug products were analyzed. Pure α form showed lower melting enthalpy (98.29 J/g) compared to β form (110.85 J/g) as an API (Supplementary Fig. S4) and similar behavior was observed in drug formulation by melting enthalpies of 22.28 J/g and 31.38 J/g, respectively (Fig. 4A and B). Interestingly, melting enthalpy of β form decreased on DSC thermograms of α–β polymorph mixtures and for α form a distinct increase was observed. Fig. 4B and C shows DSC thermograms of 50% α–β mixtures of API and drug product where this enthalpy changes could be seen clearly as following: 72.23 J/g for α and 38.62 J/g for β in API mixtures; 9.65 J/g for α and 6.37 J/g for β in tablet formulation. This substantial change indicates an enantiotropic relationship between both forms and could be explained by conversion of the β form to α form at the melting temperature by seeding effect of the α form, since there is no additional endotherm in case of pure β polymorph. The converted β form then melts at the melting temperature of α polymorph which increases the melting enthalpy of α form. Luckily, this enantiotropic relationship did not affect the linearity of calibration curves drawn by plotting the heat (J/g) absorbed by β polymorph against the weight percent of β form in the selected levels of α–β polymorph mixtures of drug substances and drug products. Linear calibration plots were obtained with correlation coefficients of 0.998 ($y = 0.8504x - 3.3478$) and 0.997 ($y = 0.1728x - 1.947$) for drug substance and drug product, respectively. LOD and LOQ of the DSC method developed for quantitative analysis of β form in drug substances were found 1% and 3%; and in drug products 4% and 12%, respectively.

3.3. Comparison of PXRD, DSC, and ATR-FTIR techniques

Based on the comparison of their LOD values, PXRD and DSC methods were found equivalent and both could detect 1% of β polymorph in drug substance and 4% in tablet formulation. But, sensitivity of ATR-FTIR method was not so good and LOD for β form was found as 12% in drug substance and product.

The poor performance by ATR-FTIR, which is the easiest and fastest method among the polymorph quantification methods studied in this report, was mainly due to the selection of transmittance band. Unfortunately, there was no strong and distinct transmittance band for β polymorph on FTIR spectrum compared to α polymorph. Thus, a strong and distinct transmittance band (1446 cm^{-1}) of α form was selected to build a calibration curve but this did not give enough sensitivity for determination of β polymorphic content. For better results, different approaches may have to be applied, such as taking derivatives of the spectra, finding the different absorbance band (s) and developing a quantification method accordingly [28].

PXRD and DSC methods gave very good calibration with similar sensitivities and capable of providing proper determination of the polymorphic content in drug substance and tablet formulations of imatinib mesylate α and β forms. However, PXRD requires careful sample preparation compared to DSC, where no special sample preparation is required. PXRD was found more convenient and advantageous due to being non-destructive so the sample can still be used for further analysis after the measurement. Also, PXRD technique is more time saving by using automated sample holder

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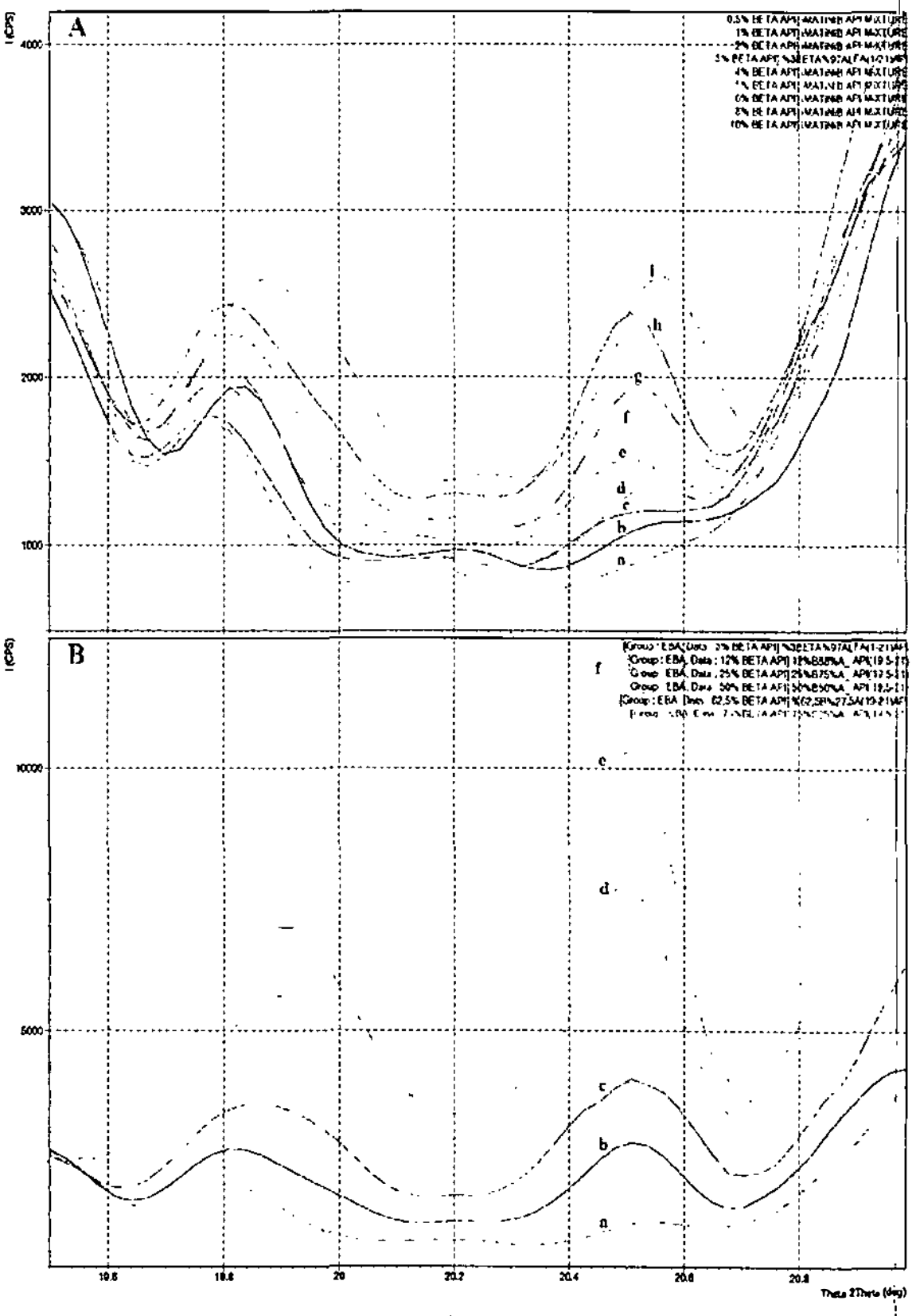

Fig. 2. X-ray diffractograms of (A) LOD study: (a) 0.5%, (b) 1%, (c) 2%, (d) 3%, (e) 4%, (f) 5%, (g) 6%, (h) 8%, and (i) 10% β form content; (B) linearity study: (a) 3% (LOQ), (b) 12%, (c) 25%, (d) 50%, (e) 62.5%, and (f) 75% β form content (2θ range: 19.5–21.0°) for drug substance.

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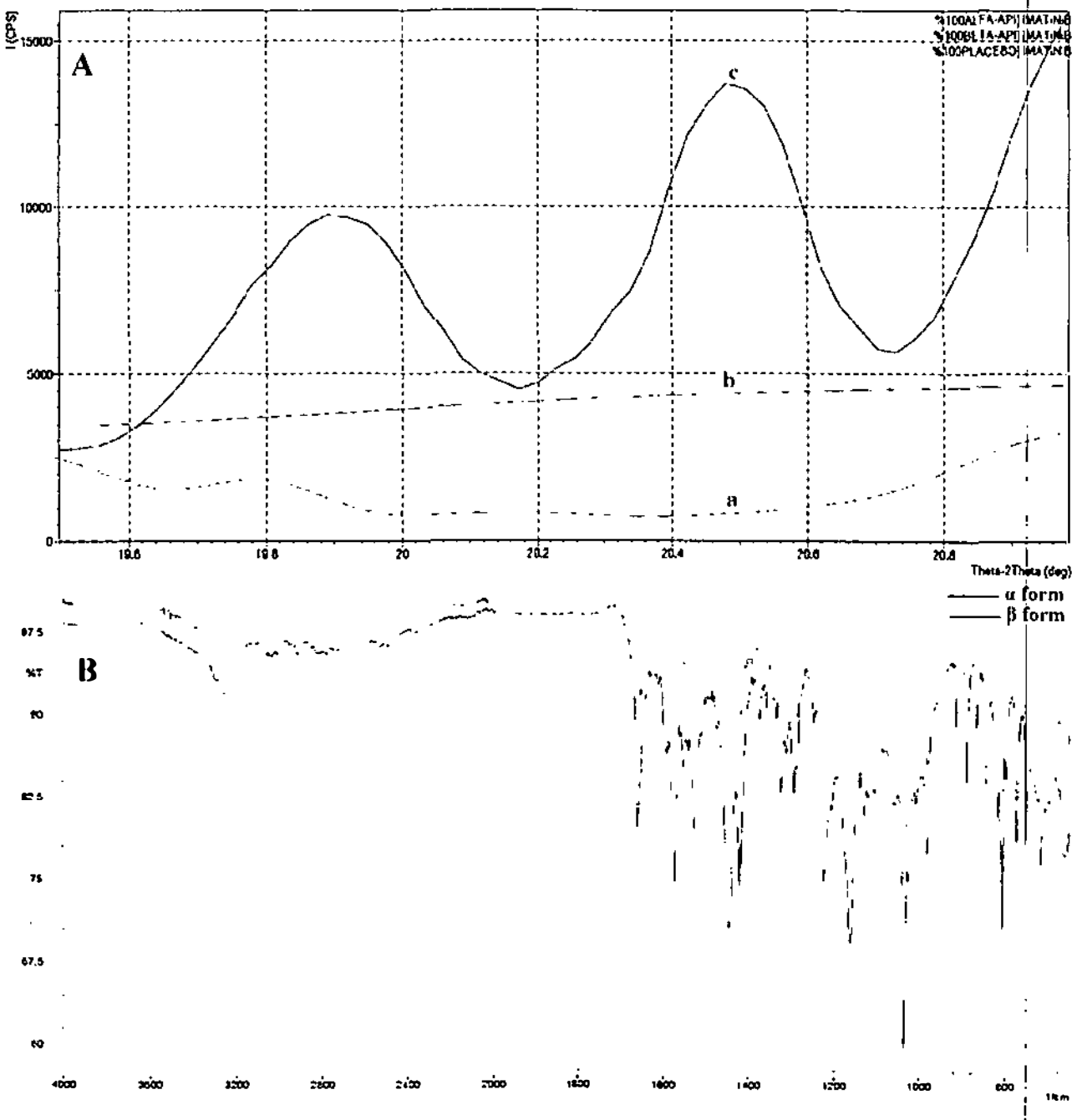

Fig. 3. (A) X-ray diffractograms of (a) α form tablet, (b) placebo, and (c) β form tablet (2θ range: 19.5–21.0°), and (B) FTIR spectra of α and β form tablets.

which is not applicable on DSC systems, where samples should be placed and run by the analyst one by one after each other. Despite the simplicity of the DSC method, there is a possibility of drug-excipients interactions, which may produce unpredictable change to the thermal events that interfere or overlap with the melting endotherms, thus resulting in unreliable quantification of the polymorphic content. According to our studies with imatinib mesylate tablet formulations, none of such effects and overlaps was detected. So, DSC could be an alternative convenient way for ensuring the polymorphic content of API and tablet samples. The peak interference of the API and the other contents (such as excipients) on the X-ray diffraction pattern should be minimal. Since each compound has its own set of characteristic peaks, PXRD is suitable for the analysis of formulated samples such as tablets (or other solid samples) besides the APIs. In both methods, other ingredients contained in the tablet formulation lowered the sensitivity, and detectable amount of the imatinib mesylate β polymorph was found as 4% for drug product, which was 1% for the drug substance.

To compare the accuracy of PXRD and DSC techniques for both α–β form binary mixtures in API and tablet formulation, six standard API samples containing different amounts of β form were prepared and four of these binary API mixtures where β form content was above 4%, were used for tablet formulation. All binary API and tablet mixtures were analyzed by the developed PXRD and DSC methods and results are summarized in Table 1. Among both techniques, PXRD provided more accurate results for determination of the β form amount for API and tablets.

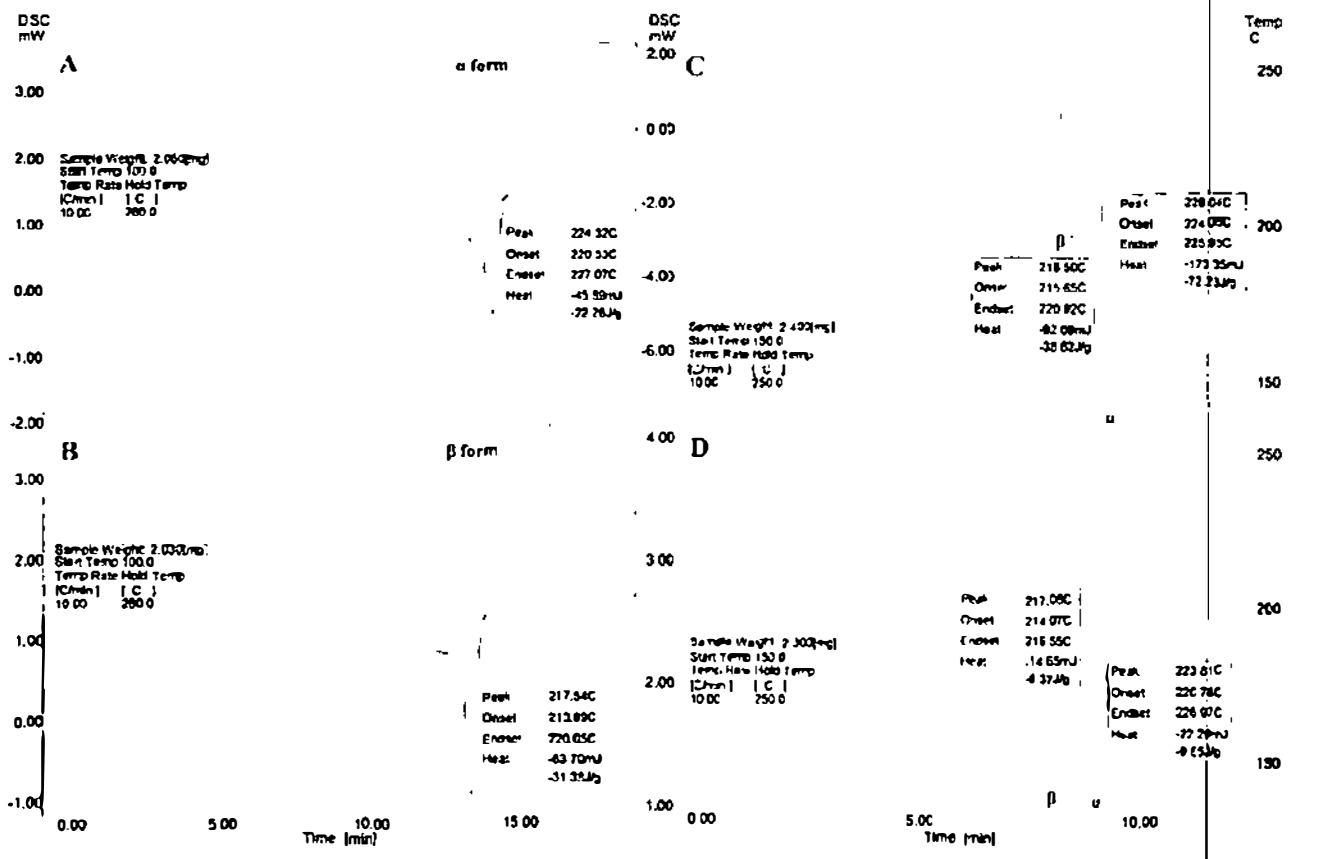

Fig. 4. DSC thermograms of (A) α and (B) β form in tablet formulation, (C) 50% α and β form API mixture, and (D) 50% α and β form API mixture in tablet formulation.

3.4. PXRD method validation

All preliminary studies supported that PXRD method is suitable for determination of β form content in drug product and the method was validated for the selected diffraction peak, in the 19.5–21.0° 2θ range with 50% specification limit.

3.4.1. Method validation

3.4.1.1. Specificity. Placebo, β form, α form, β form with placebo, and α form with placebo samples were scanned by using the specified measurement parameters given in Section 2.4. Method was found selective since diffraction peak of β form (occurring at about 2θ value of 20.5 ± 0.2°) in the diffractogram of β form/placebo mixture could be clearly observed and no diffraction peak was observed in the diffractograms of placebo and α form/placebo mixture (Fig. 3A).

Table 1
Comparison of PXRD and DSC techniques for the quantification of β form content in binary API and tablet mixtures.

Actual β form content (%)	β form content (%) determined for/by			
	API		Tablet	
	PXRD	DSC	PXRD	DSC
1.91	2.69	4.84	–	–
3.81	3.78	5.21	–	–
8.41	8.10	8.93	6.46	14.43
18.03	16.66	16.30	16.25	17.33
26.98	21.50	23.40	25.79	23.11
44.00	44.31	35.69	42.75	56.79

3.4.1.2. Limit of detection and limit of quantification. Placebo samples spiked with 1%, 2%, 3%, 4%, 5%, 6%, 8%, and 10% β form containing API standards were analyzed (Fig. 5A). First diffraction peak appeared at 4% β form containing sample and detection limit was determined as 4%. LOQ value was three times of the LOD value and set as 12%.

3.4.1.3. Linearity and range. Five standards in the range from LOQ to 150% of specification limit (LOQ, 25%, 50%, 62.5%, and 75%) were scanned (Fig. 5B, Supplementary Table 51). Calibration curve showed a linear relation between amount of β form and diffraction peak (20.5 ± 0.2°) intensity measured on X-ray diffractograms and correlation coefficient was found as 0.992, where y-intercept and slope was found as 261 and 194, respectively. Residual sum of squares (RSS) was found as 1586294. Samples at LOQ (12% β) and maximum concentration level (75% β) were used for range study. RSD of intensities of six replicate injections was found as 3.5% at LOQ level and 5.2% at maximum concentration level, and this working range was found appropriate.

3.4.1.4. Accuracy and precision. The accuracy of the method was evaluated on samples spiked with β polymorph in triplicate at four levels of 12% (LOQ), 25%, 50%, and 75% (LOQ, 50, 100, and 150% of the specification limit, respectively). Satisfactory recoveries were achieved at each level in the range of 87–112% for high concentrations and 83–92% for LOQ level (Table 2). The precision of the method was investigated by scanning six individual samples spiked with β polymorph at the specification limit (50%). The same procedure was applied for the inter-day precision by a different analyst on a different day. Recoveries for the intra-day and inter-day precision studies were obtained in the range of 108–112% and 100–112%.

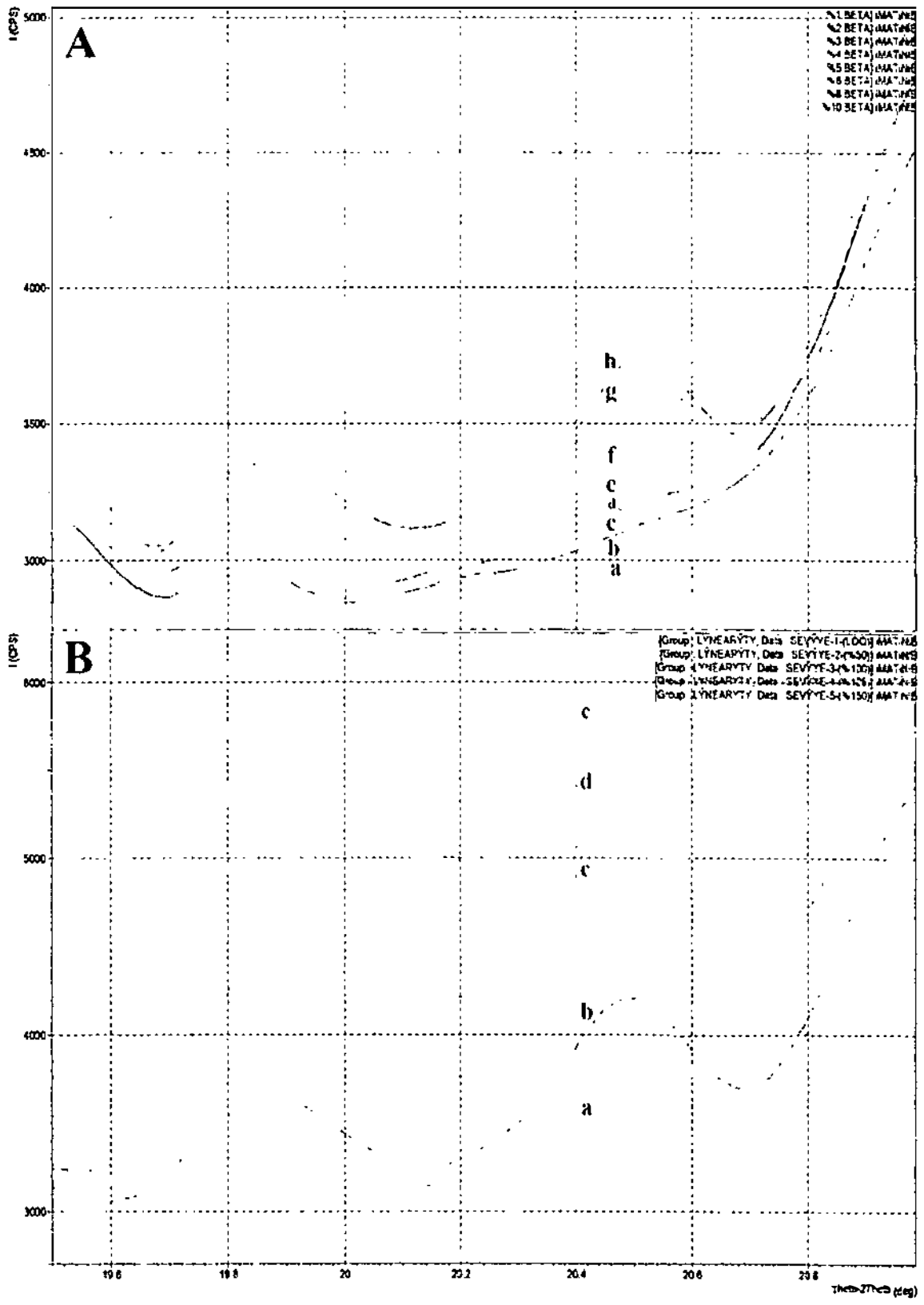

Fig. 5. X-ray diffractograms of (A) LOD study: (a) 1%, (b) 2%, (c) 3%, (d) 4%, (e) 5%, (f) 6%, (g) 8%, and (h) 10% β form content and (B) linearity study: (a) 12% (LOQ), (b) 25%, (c) 50%, (d) 62.5%, and (e) 75% β form content (2θ range: 19.5–21.0°) for drug product.

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Table 2
Results of the PXRD method validation accuracy and precision studies.

Accuracy				Precision				
Theo. β form (%)	Intensity	Exp. β form (%)	Recovery (%)		Theo. β form (%)	Intensity	Exp. β form (%)	Recovery (%)
12	2298	11	92	Intra-day	50	11111	56	112
	2158	10	83			11082	56	112
	2297	11	92			10895	55	110
25	4949	24	96			10849	55	110
	5750	28	112			10675	54	108
	4882	24	96			11121	56	112
50	10812	54	108	Inter-day	50	10668	54	108
	10013	50	100			11101	56	112
	10686	54	108			11114	56	112
75	12944	65	87			10702	54	108
	13292	67	89			10259	52	104
	14019	71	95			10026	50	100

Table 3
Stability results of binary tablet mixtures.

Condition	Actual β form content (%)	β form content (%) determined by PXRD				
		Initial	3 months	6 months	12 months	18 months
25 ± 2 °C, 60 ± 5% RH	10–15	14.5	13.7	14.1	13.5	13.9
40 ± 2 °C, 75 ± 5% RH			13.9	14.6	NA	NA
25 ± 2 °C, 60 ± 5% RH	35–40	39.3	37.1	36.9	37.3	NA
30 ± 2 °C, 65 ± 5% RH			39.1	38.7	39.5	NA
40 ± 2 °C, 75 ± 5% RH			38.2	37.1	NA	NA

RH: relative humidity; NA: not applicable.

respectively (Table 2). RSD of recoveries were found as 1.68% and 4.23%, respectively. Difference of the mean recoveries between two studies for intra-day and inter-day precision was found as 3% and the method was found to be sufficiently precise since no significant variation in the found results was observed on any day.

Requirements for each stage of the validation study were fulfilled. Thus, our developed PXRD method was specific, linear, selective, precise and accurate, and could be used for determination of β polymorph content in tablets produced by using α polymorph of imatinib mesylate. This method is used to control β form content in manufactured imatinib mesylate tablets (100 mg, 200 mg, and 400 mg α form) and to monitor the polymorphic stability of α form API in drug product during stability studies. Ten commercial size batches and their stability samples were analyzed by using this method and in none of them β polymorph was detected.

3.5. Polymorphic stability

Samples of α form containing tablets from different manufacturing batches were subjected to various stability testing conditions and analyzed according to the validated PXRD method to configure the stability of α form in the tablet formulation. The results indicated that α form was not converted to β form during the tablet manufacturing process, and also under routine (25 ± 2 °C, 60 ± 5% humidity), intermediate (30 ± 2 °C, 65 ± 5% humidity) and accelerated stability testing (40 ± 2 °C, 75 ± 5% humidity) conditions in the original tablet form (film coated) and package (LDPE blister) (Supplementary Figs. S7 and S8). Additional studies were carried out under accelerated stability testing conditions by subjecting the tablets directly without any package and also by peeling their film coating. No polymorphic conversion to β form was observed on X-ray diffractograms of the samples taken from different parts of the tablets (surface and middle parts measured separately) which confirmed that α polymorph is stable in our tablet formulation. Similar study was carried out by subjecting pure α form API in its original package (double LDPE bag) and in open flasks to the same conditions. In both case, α form API was found stable and not affected from the outside temperature and humidity, and no conversion was detected to β polymorph (Supplementary Fig. S9). Another study was carried out by subjecting α form API containing small amount of β polymorph (ca. 1%) in package and in open flasks to 40 ± 2 °C, 75 ± 5% humidity conditions. α-β polymorphic content of the packed sample did not change in time. But, in the case of open drug substance samples, complete conversion to β form was observed in time (approximately in two months) (Supplementary Fig. S10). The polymorphic conversion of the β form containing α form API samples subjected to high humidity directly could be explained by seeding effect of the more favorable crystalline β form. Fortunately, our tablet formulation was able to prevent such a polymorphic conversion and this was proved by subjecting two different tablet samples (400 mg API) containing 10–15% and 35–40% β polymorph to normal, intermediate and accelerated stability conditions and followed for 18 and 12 months, respectively. X-ray diffractograms of the samples (Supplementary Figs. S11 and S12) were analyzed and no significant difference was observed on polymorphic content of the tablets during this time period (Table 3). Both polymorphs were also subjected to high temperature (200 °C) in open flasks for one week and monitored by PXRD and DSC studies, where no change was observed on their crystal structure (Supplementary Figs. S13 and S14).

4. Conclusion

Mixtures of polymorphs may occur and the extent of their presence in pharmaceutical ingredients and tablets can be of considerable importance and lead to difficulties associated with the product development and registration phases. Quantification of imatinib mesylate α and β polymorphs in solid binary mixtures was studied using PXRD, DSC, and ATR-FTIR spectroscopic methods for determination of polymorphic purity of the API and tablets. Among these three techniques, PXRD provided the most accurate determination for the amount of β form followed by DSC, while ATR-FTIR spectroscopy was the least accurate. Thus, a quantitative method employing PXRD to determine the polymorphic content of

tablet samples was developed and validated. Careful and consistent sample preparation with particle size control was vital in developing a robust calibration curve for quantitation, and the method was able to quantify the β polymorph impurity down to 4% in tablets. The quantitative PXRD method reported herein represents a useful tool for monitoring the polymorphic content and crystal form stability of α form of imatinib mesylate in tablet formulations. Here, it was shown that tablets produced by α crystal form of API were highly stabilized by the excipients used in the formulation and no detectable polymorphic transformation to β form was occurred during manufacturing process or stability periods.

Acknowledgments

The authors are thankful to the management of Deva Holding A.Ş., Istanbul/Turkey, for supporting this work and the Scientific and Technological Research Council of Turkey (TUBITAK-TEYDEB Project No: 3090682) for the financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2015.06.011

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Supplementary information for

Quantitative determination of two polymorphic forms of imatinib mesylate in a drug substance and tablet formulation by X-ray powder diffraction, differential scanning calorimetry and attenuated total reflectance Fourier transform infrared spectroscopy

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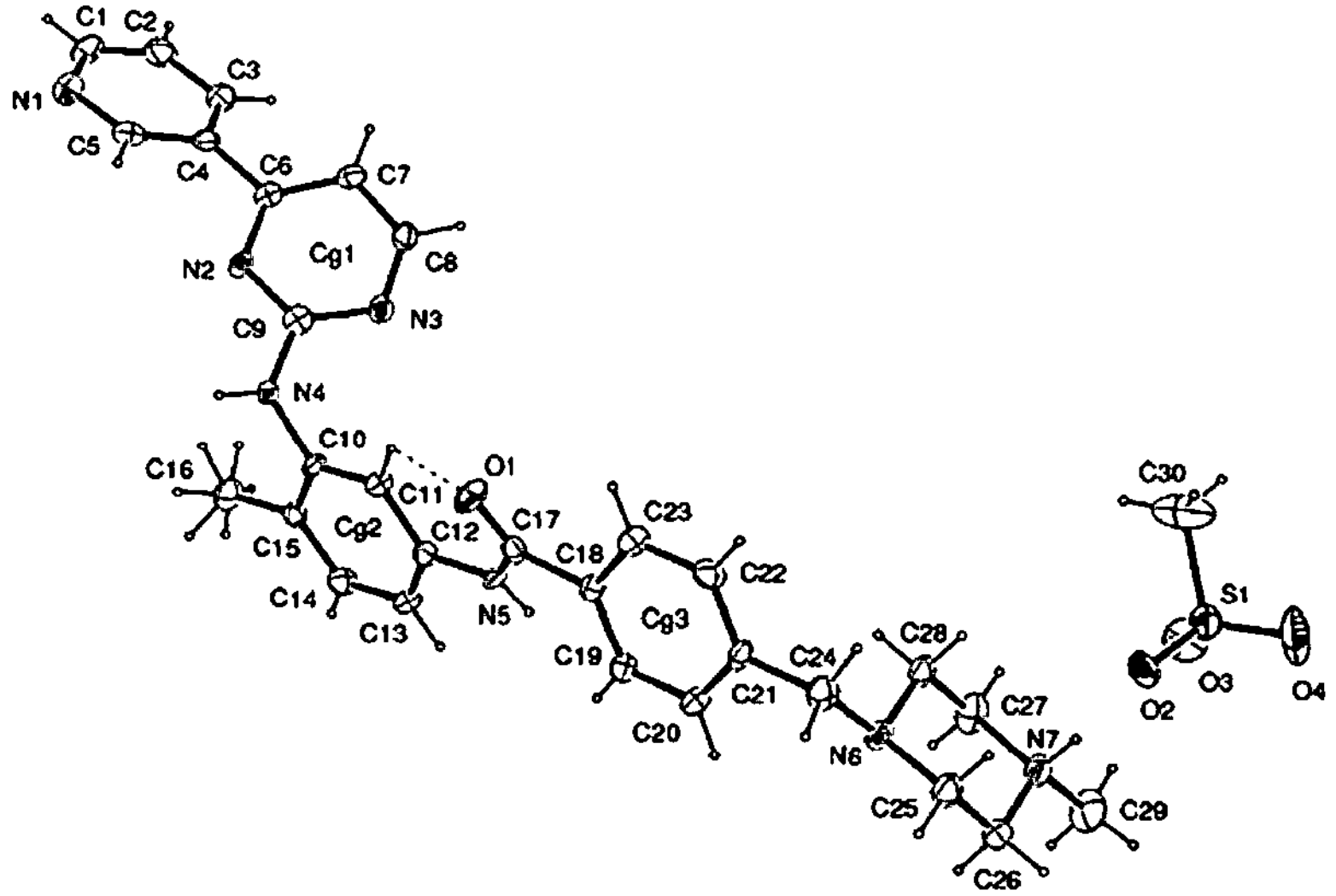
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C11
O1
C15
Cg2
C12
C17
C18
C23
C22
C14
C13
N5
Cg3
C19
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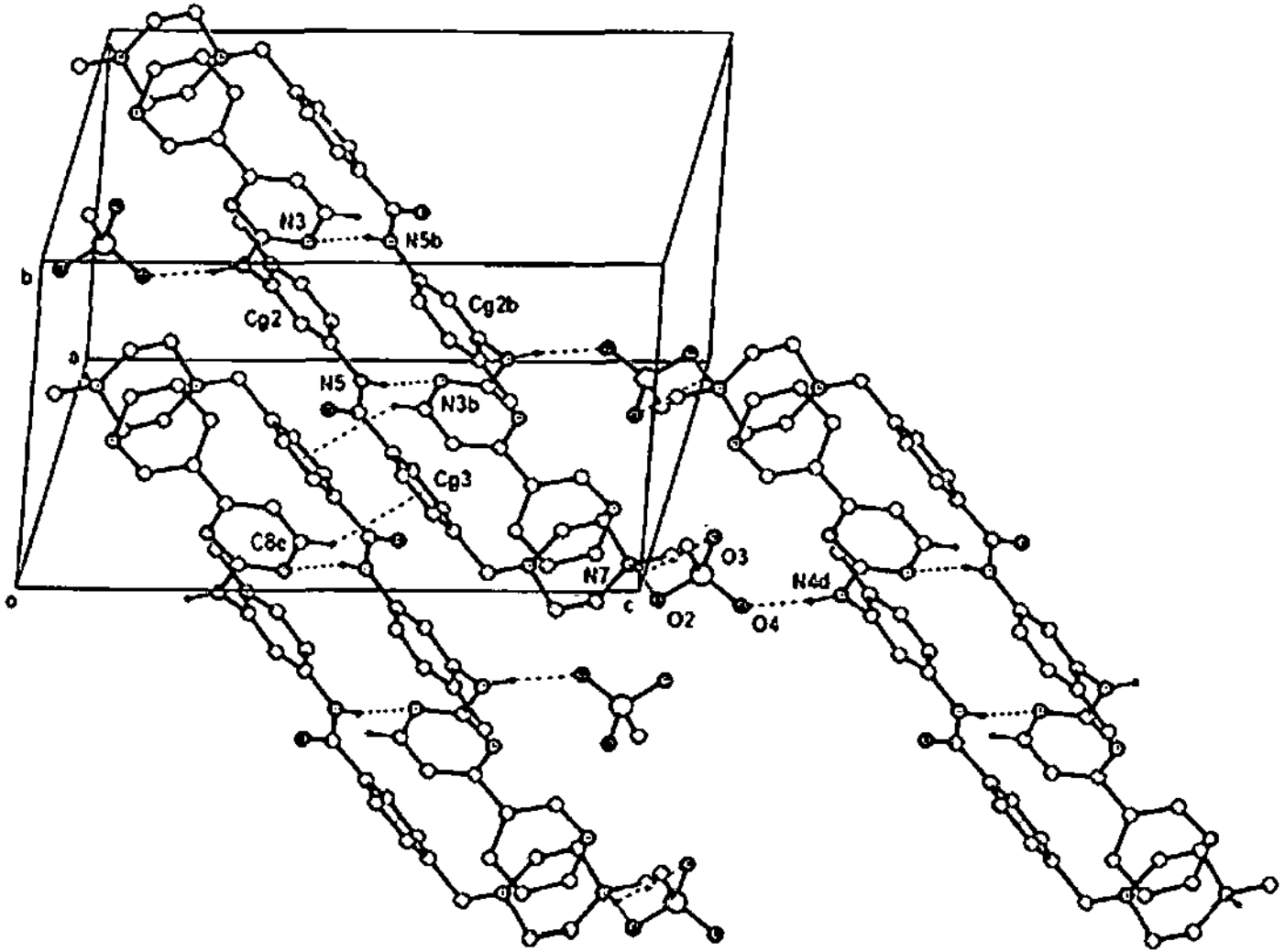
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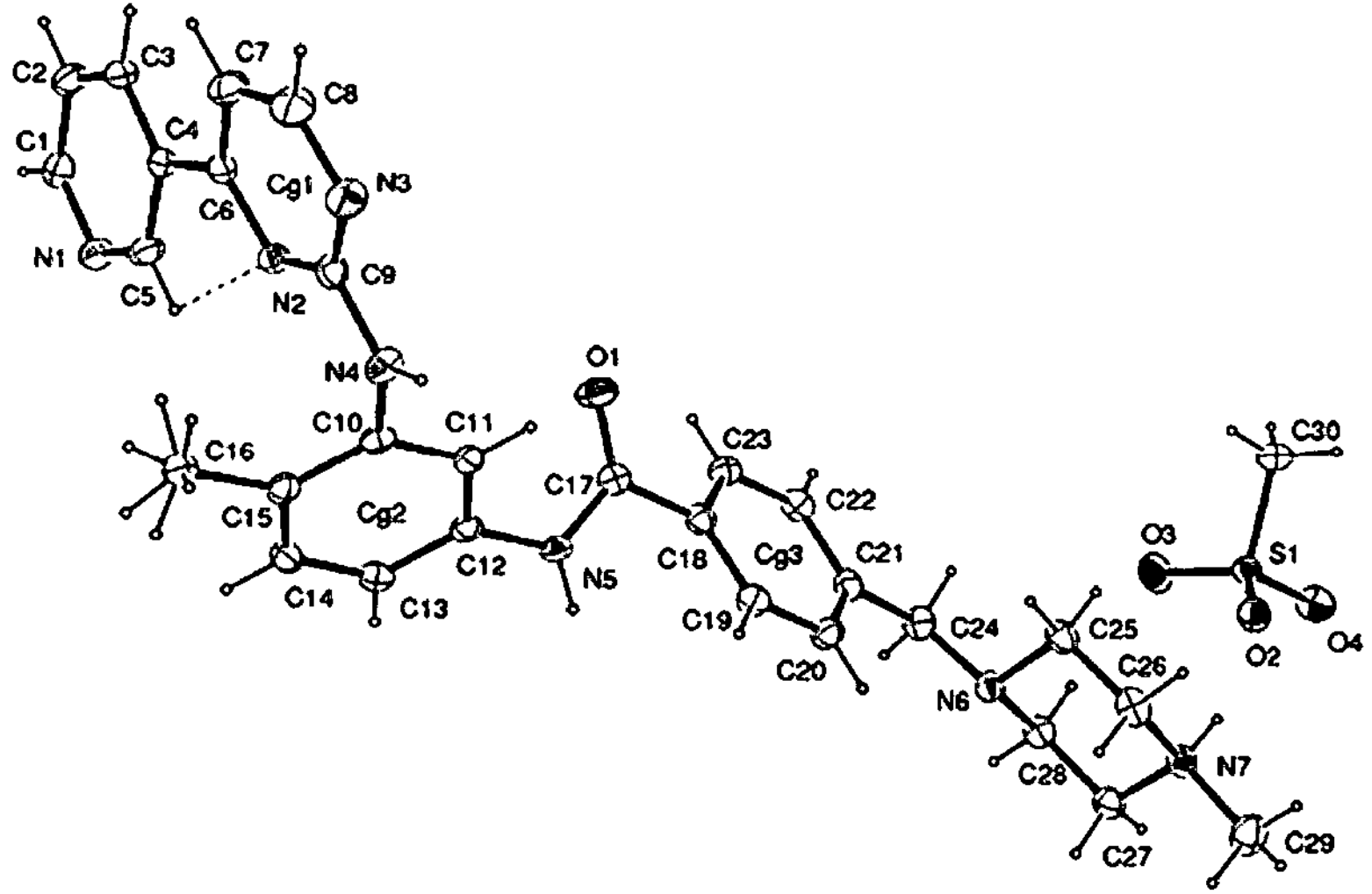
Fig. S1. Crystal structure and packing diagram of imatinib mesylate α form displacement ellipsoids drawn at 50% probability level, intra- and intermolecular interactions shown as dashed lines [22].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C11
C23
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C17
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Cg2
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Cg3
C21
C12
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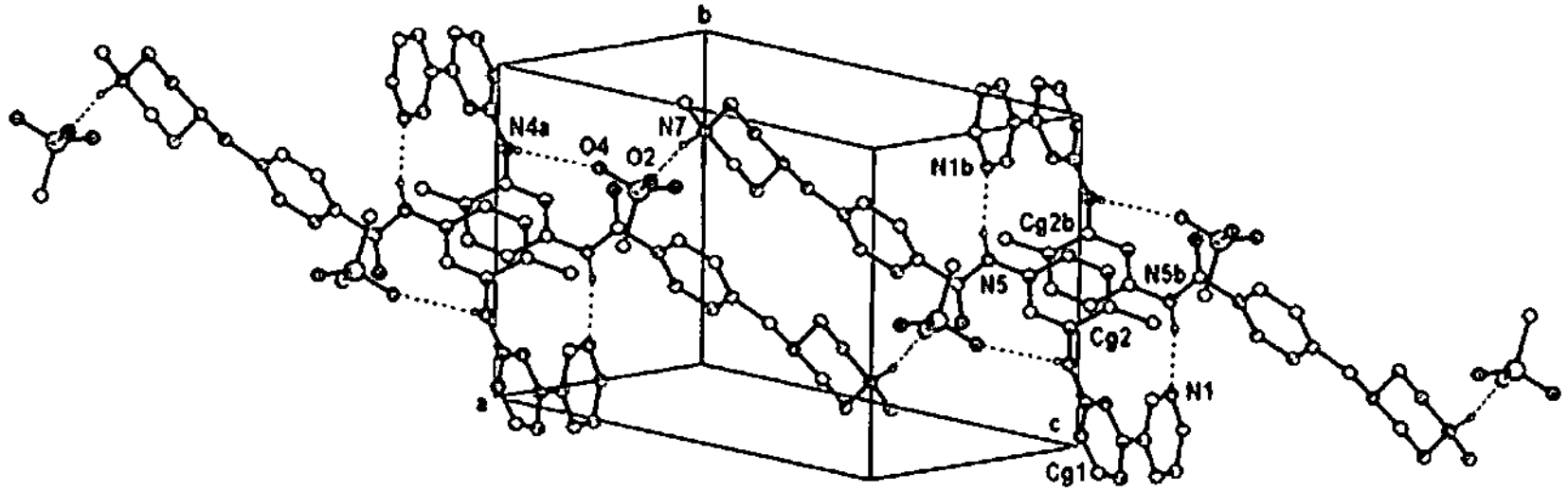


Fig. S2. Crystal structure and packing diagram of imatinib mesylate β form – displacement ellipsoids drawn at 50% probability level, intra- and intermolecular interactions shown as dashed lines [22].

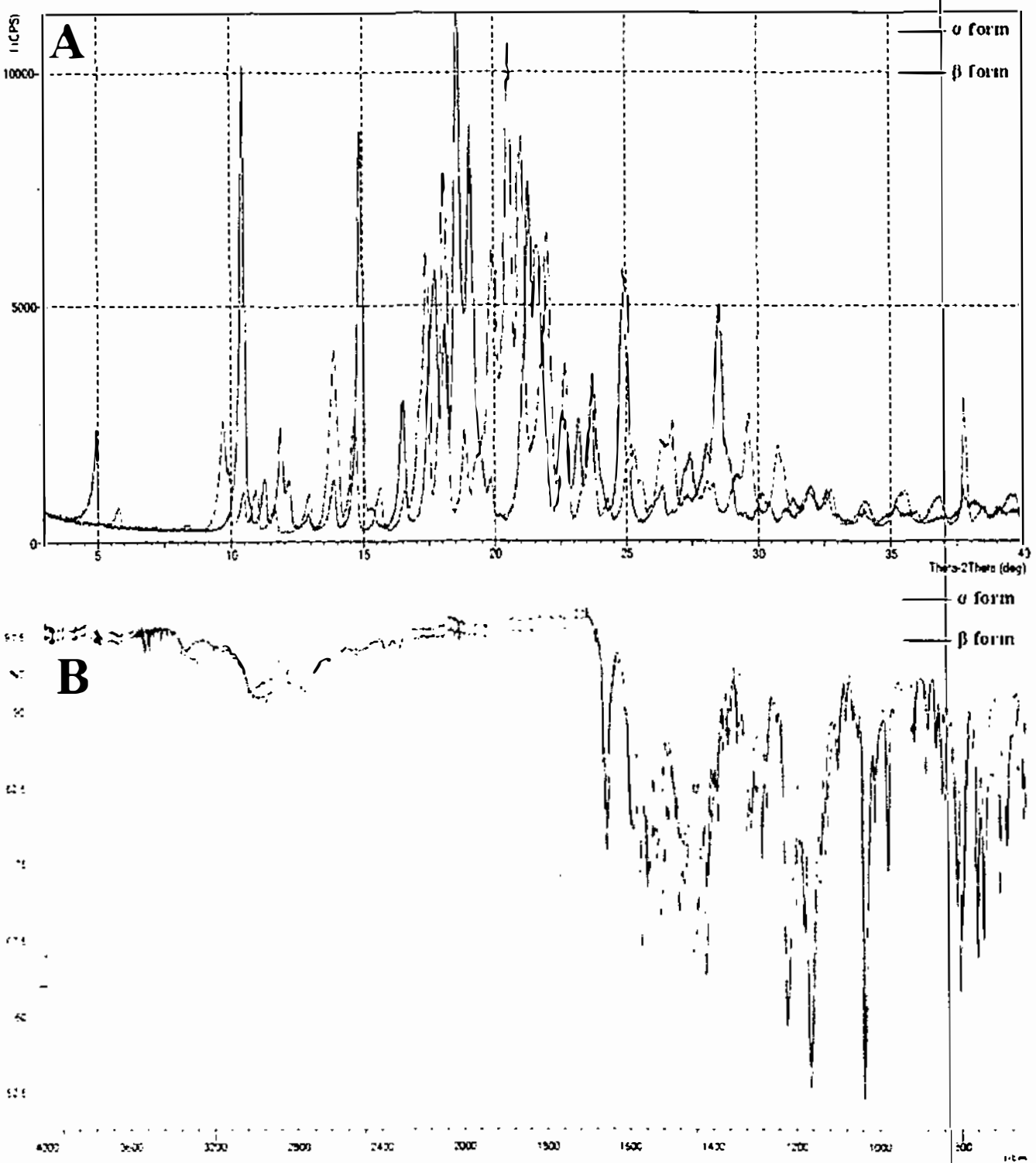


Fig. S3. (A) X-ray diffractograms and (B) FTIR spectra of imatinib mesylate α and β forms.

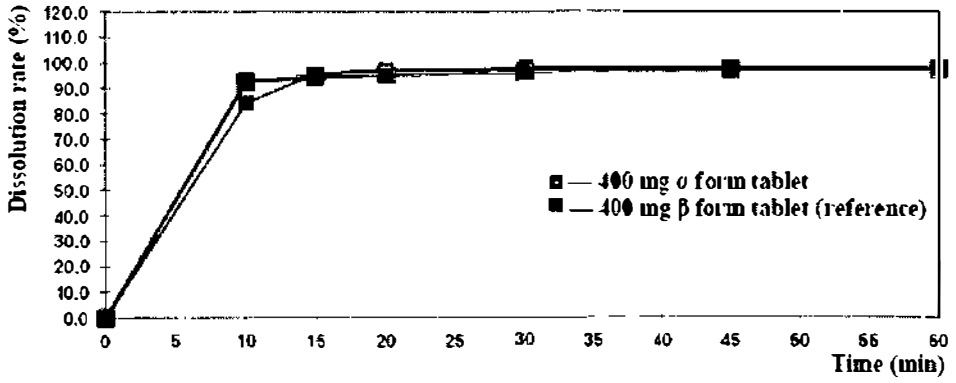


Fig. S5. Dissolution rate of imatinib mesylate α and β form tablets (in 1000 mL of 0.01 M aq. HCl solution at 37.0 ± 0.5 °C).

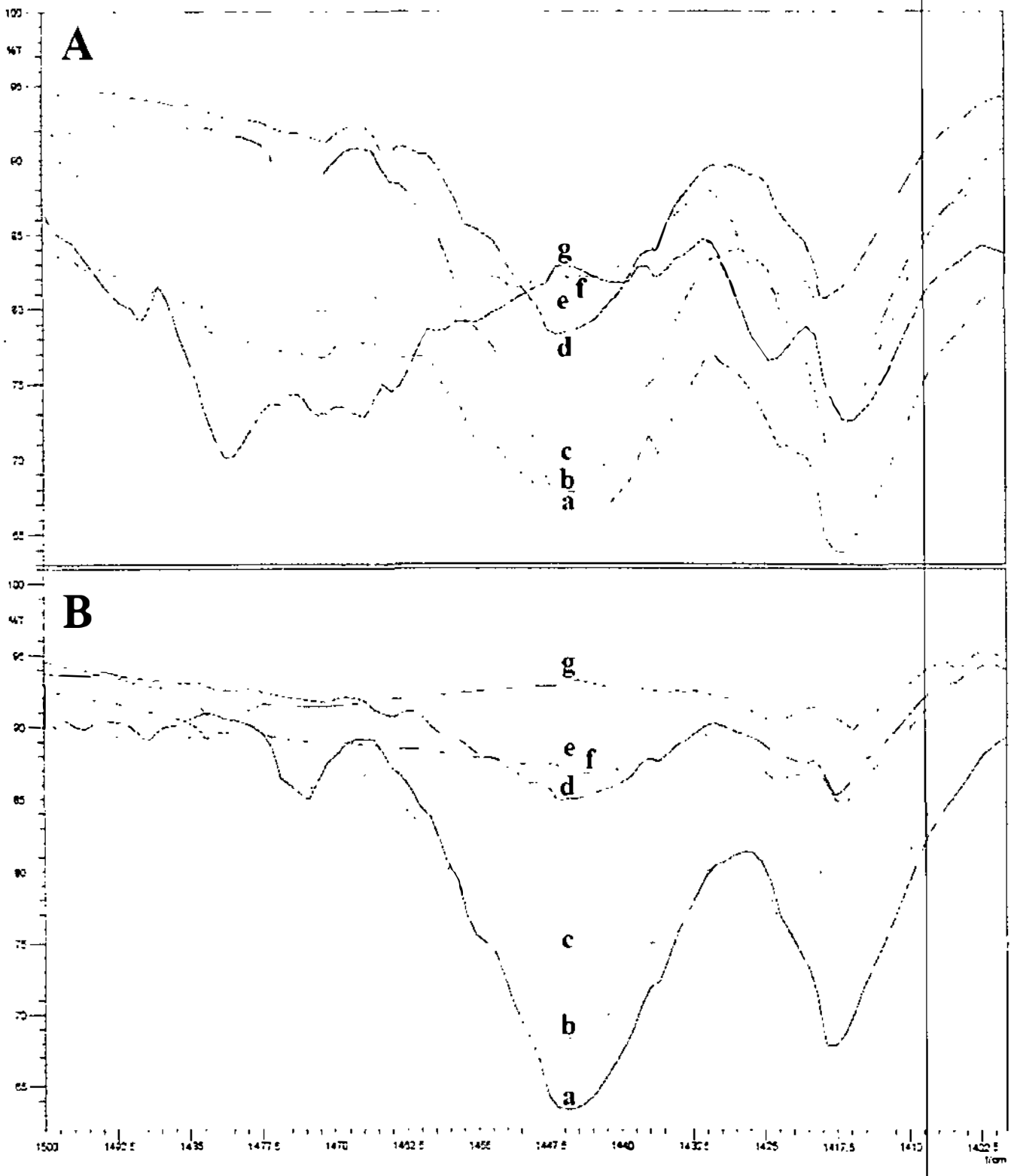


Fig. S6. FTIR spectra of (A) drug substance and (B) drug product containing (a) 0, (b) 12, (c) 25, (d) 50, (e) 62.5, (f) 85 and 75, respectively, and (g) 100 wt % of β form (range: 1400 – 1500 cm^{-1}).

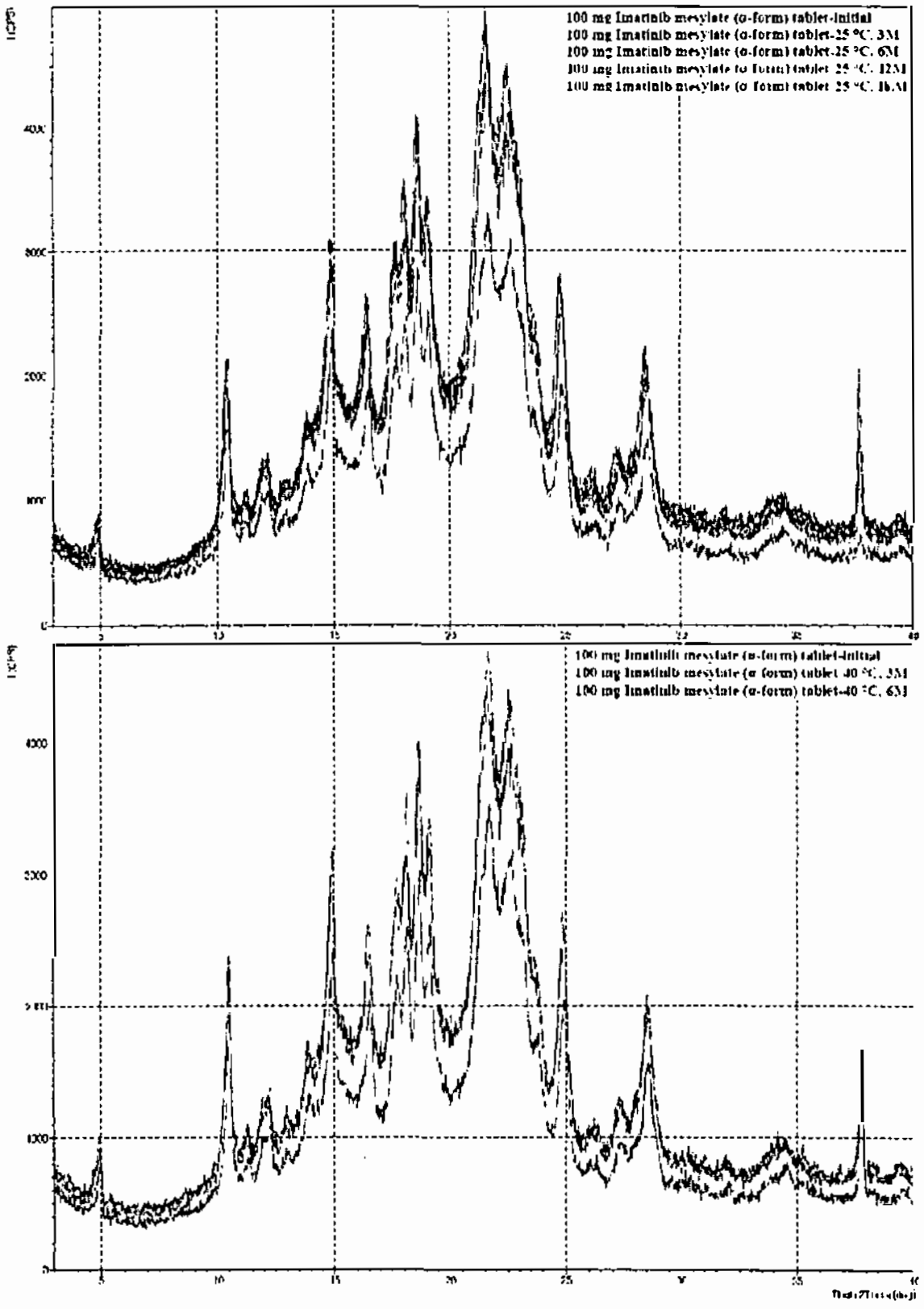


Fig. S7. Stability studies: X-ray diffractograms of 100 mg imatinib mesylate (α form) tablet (25 ± 2 °C, 60 ± 5% RH and 40 ± 2 °C, 75 ± 5% RH).

76

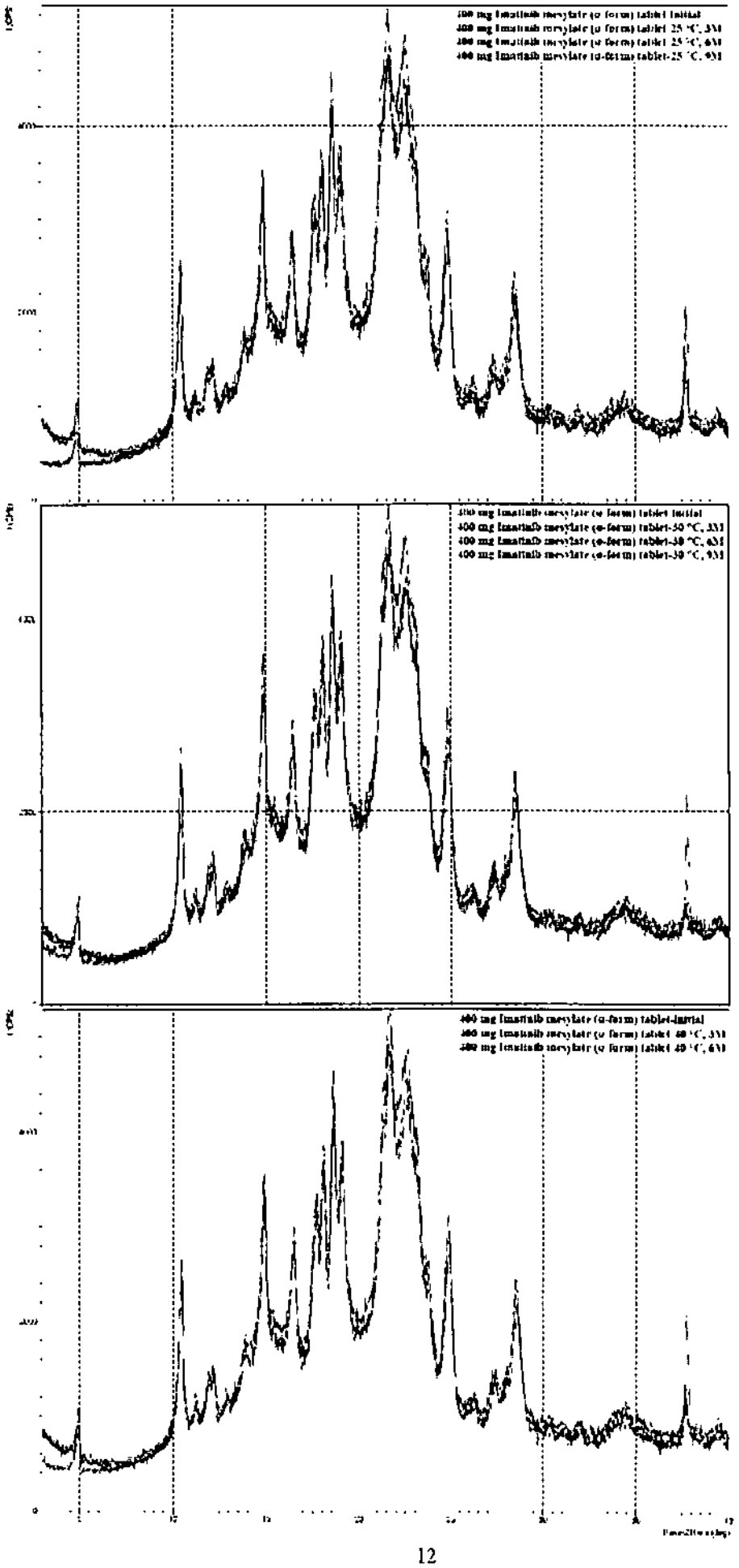


Fig. S8. Stability studies: X-ray diffractograms of 400 mg imatinib mesylate (α form) tablet (25 ± 2 °C, 60 ± 5% RH, 30 ± 2 °C, 65 ± 5% RH and 40 ± 2 °C, 75 ± 5% RH).

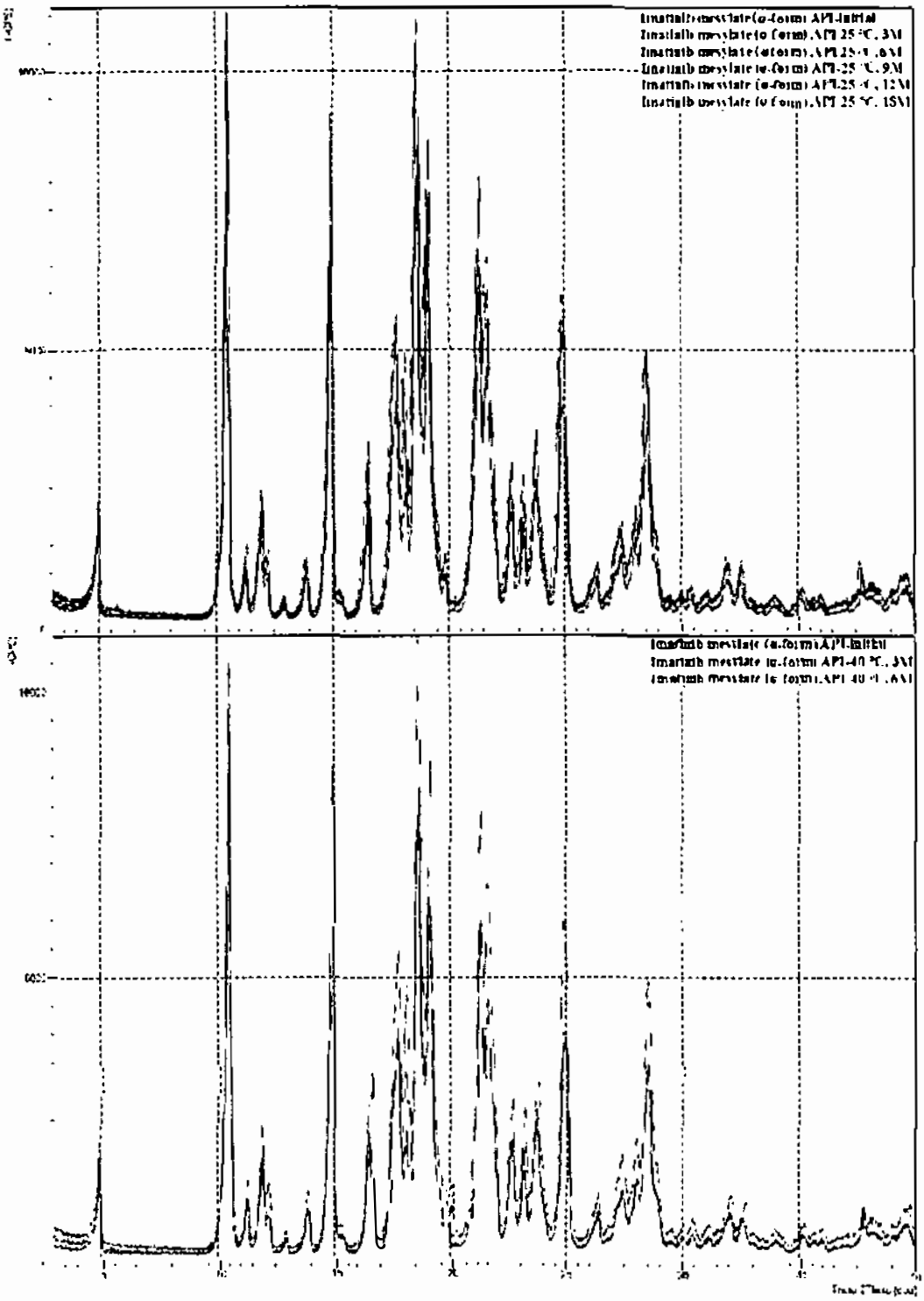
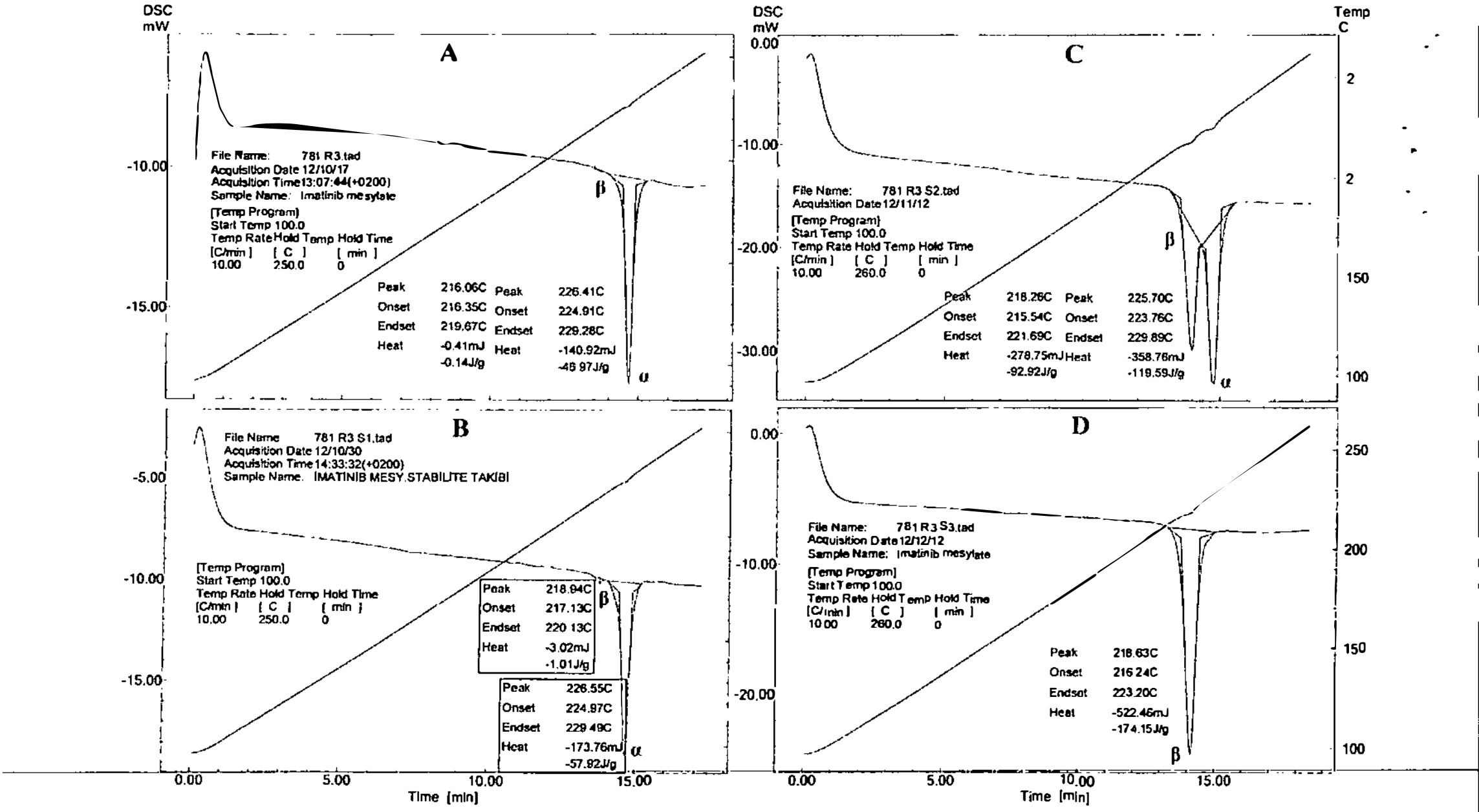


Fig. S9. Stability studies: X-ray diffractograms of imatinib mesylate (α form) API (25 ± 2 °C, 60 ± 5% RH and 40 ± 2 °C, 75 ± 5% RH).

18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β
α
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β
α
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DSC
mW
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-119.59J/g
β
α
Temp
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Heat -522.46mJ
-174.15J/g
β
250
200
150
100
0.00
5.00
10.00
15.00
Time [min]

Fig. S10. Stability studies: DSC thermograms of imatinib mesylate API α form (containing β form) conversion to β form in open flasks at 40 ± 2 °C, 75 ± 5% RH: (A) initial, (B) after two weeks, (C) after one month, and (D) after two months.

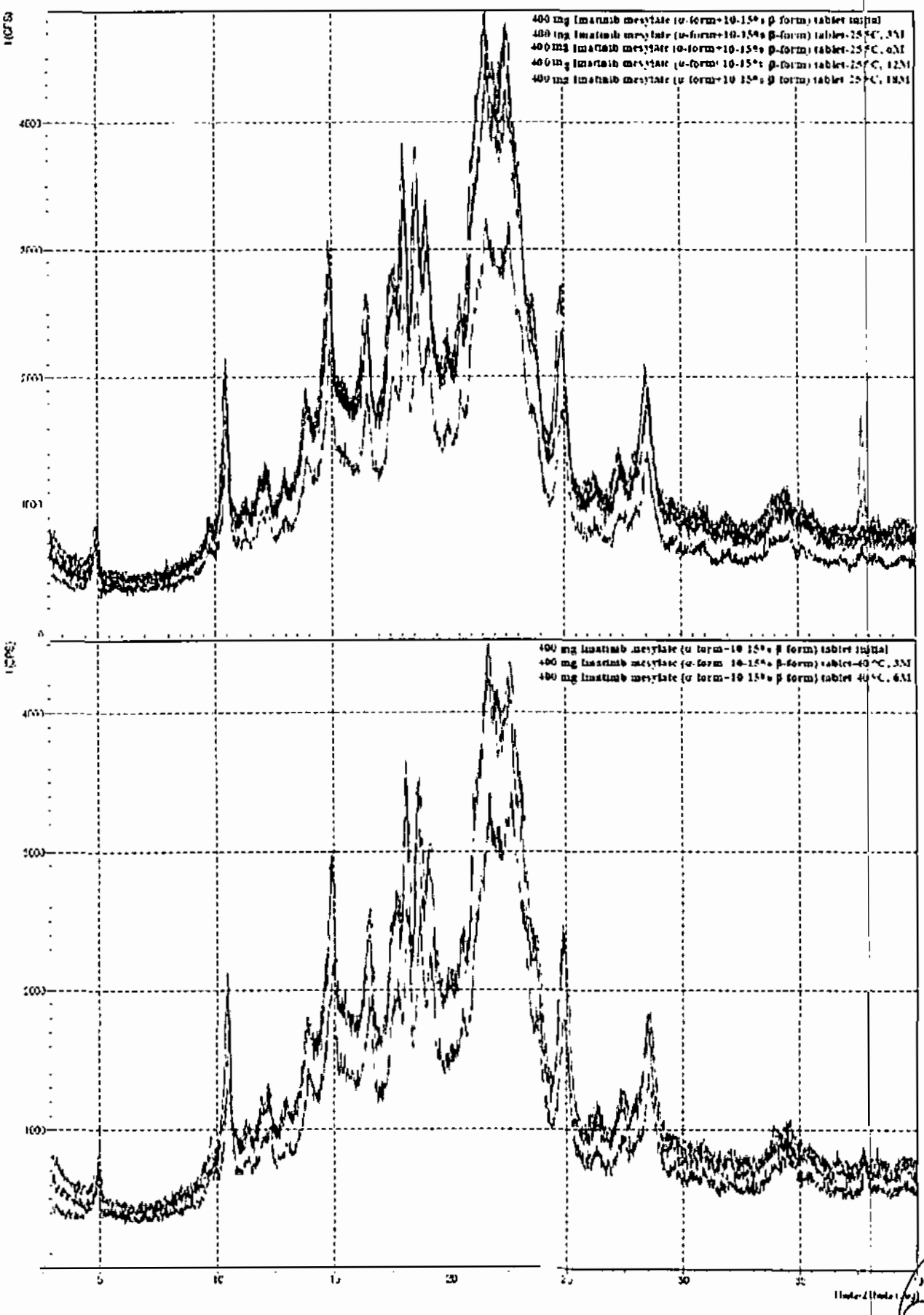
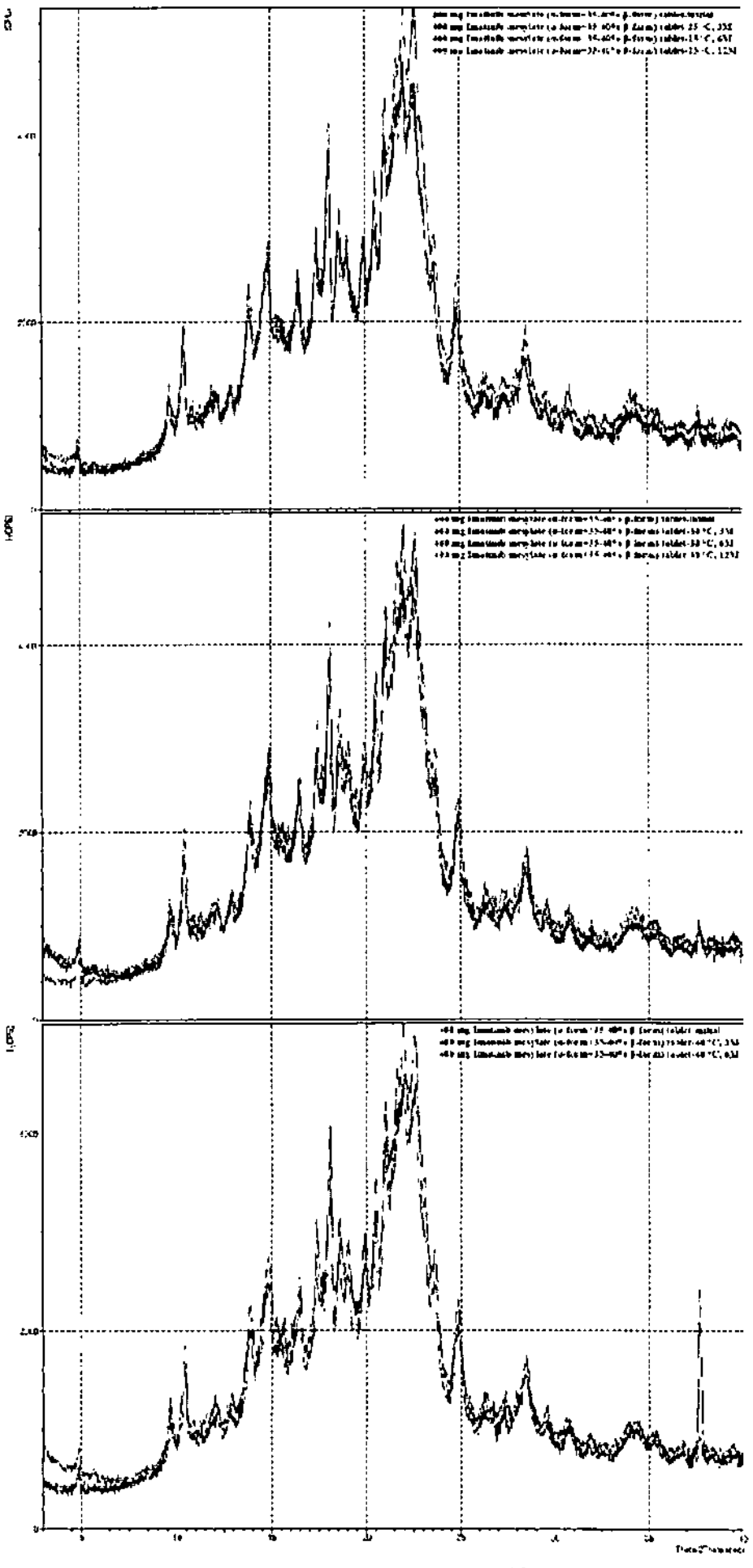

400 mg Imatinib mesylate (α-form+10-15% β-form) tablet initial
400 mg Imatinib mesylate (α-form+10-15% β-form) tablet-25°C, 3M
400 mg Imatinib mesylate (α-form+10-15% β-form) tablet-25°C, 6M
400 mg Imatinib mesylate (α-form+10-15% β-form) tablet-25°C, 12M
400 mg Imatinib mesylate (α-form+10-15% β-form) tablet-25°C, 18M
400 mg Imatinib mesylate (α-form+10-15% β-form) tablet initial
400 mg Imatinib mesylate (α-form+10-15% β-form) tablet-40°C, 3M
400 mg Imatinib mesylate (α-form+10-15% β-form) tablet-40°C, 6M

Fig. S11. Stability studies: X-ray diffractograms of 400 mg imatinib mesylate (α form containing 10-15% β form) tablet (25 ± 2 °C, 60 ± 5% RH and 40 ± 2 °C, 75 ± 5% RH).

62



63

Fig. S12. Stability studies: X-ray diffractograms of imatinib mesylate (α form containing 35-40% β form) API (25 ± 2 °C, 60 ± 5% RH, 30 ± 2 °C, 65 ± 5% RH and 40 ± 2 °C, 75 ± 5% RH).

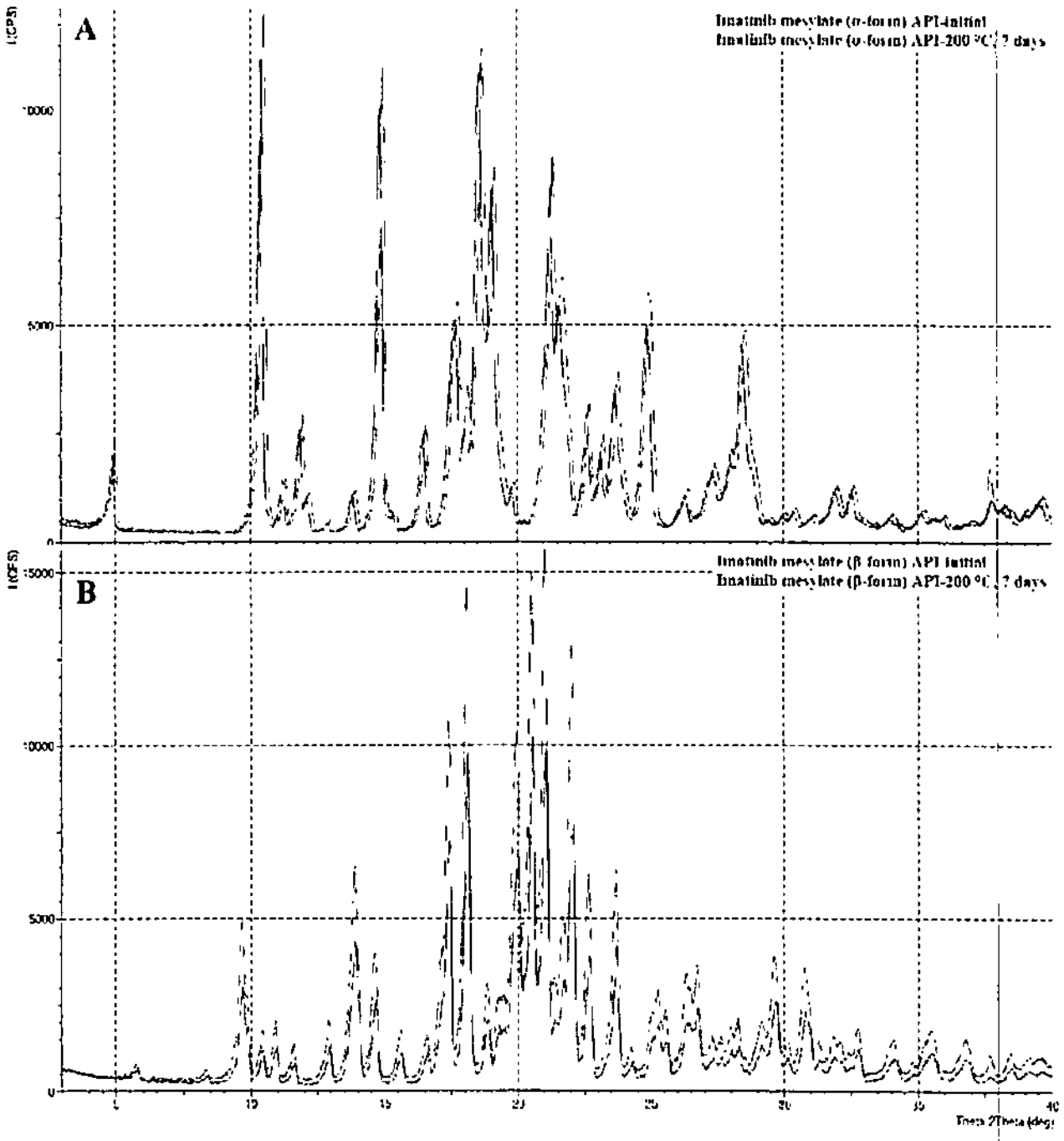


Fig. S13. Stability studies: X-ray diffractograms of imatinib mesylate (A) α form and (B) β form APIs before and after thermal stress testing (200 °C, 7 days).

64

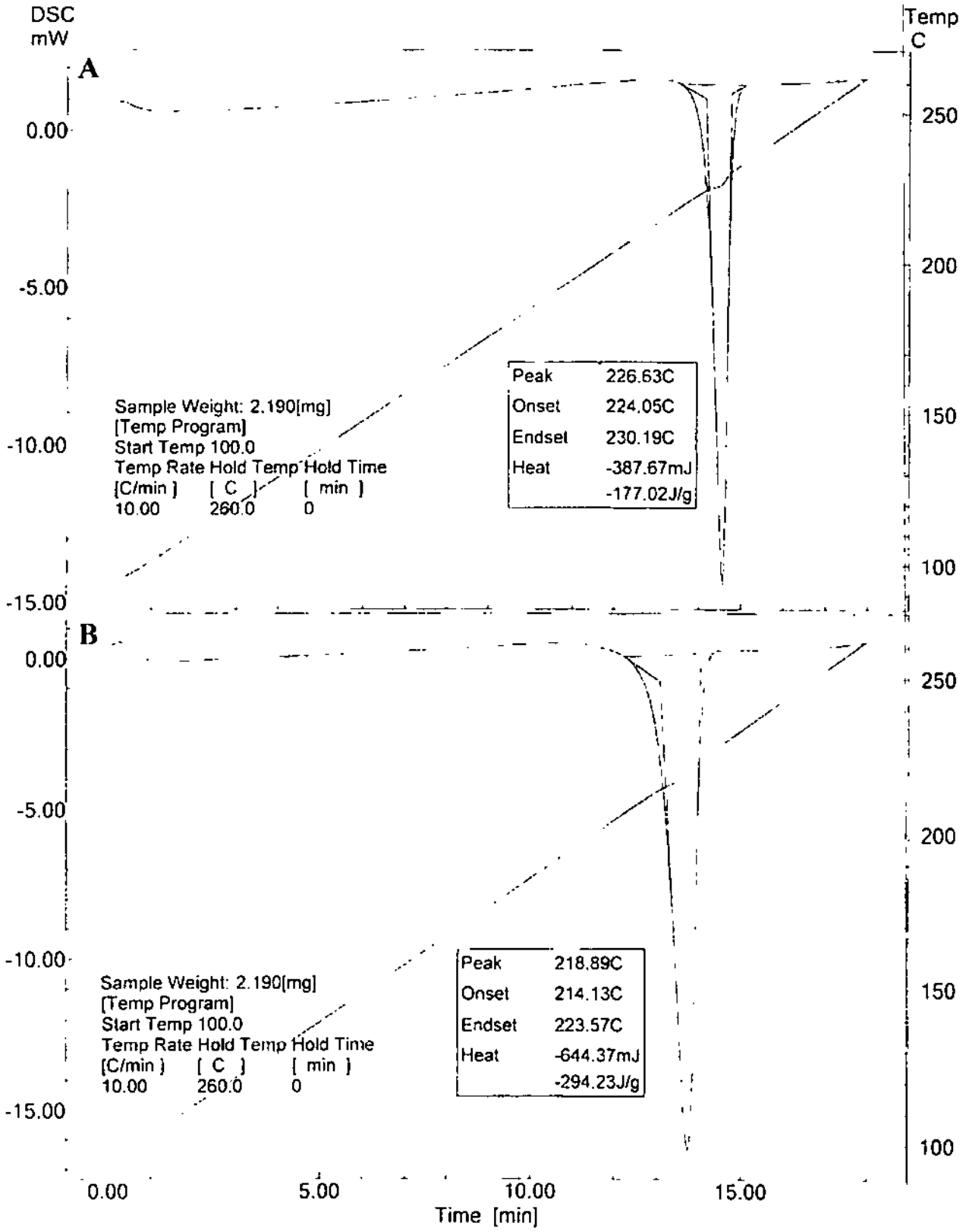


Fig. S14. Stability studies: DSC thermograms of imatinib mesylate (A) α form and (B) β form APIs after thermal stress testing (200 °C, 7 days).

65

Table S1. Results of the PXRD method validation linearity study.

β form (%)	Intensity found (Y1)	Intensity calcd. (Y2)	Square of residual $(Y1-Y2)^2$
12	2298	2589	84681
25	4949	5111	26244
50	10812	9961	724201
62.5	12738	12386	123904
75	14019	14811	627264

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Yo, Michael Mutz, edad 57 años, con domicilio en Freiburg i.B., República Federal de Alemania, afirmo y declaro lo siguiente:

A. Experiencia

1. Soy actualmente Fellow Principal (Experto senior en química del estado sólido) en Novartis Pharma AG en Basilea, Suiza.

2. Obtuve el Título de Magíster (diploma) de la Universidad de Friburgo i.B., República Federal Alemana(RFA) en 1984 y un Doctorado (Drser.nat.) en química física y biofísica de la Universidad Libre de Berlín, RFA, en 1989.

3. Entre 1990 y 1991, realicé trabajos de investigación postdoctoral en la Escuela Normal Superior, París, Francia en química biofísica.

4. He sido empleado de forma continua desde 1991, primero en Ciba-Geigy AG, y posteriormente en Novartis, tras su formación en 1996 de la fusión de Ciba-Geigy y Sandoz AG. Empecé en Ciba-Geigy como jefe de laboratorio en el departamento de Servicios Científicos, con especialidad en química física. El foco principal de mi trabajo en Ciba-Geigy y Novartis ha sido el polimorfismo y la caracterización de los estados sólidos de sólidos farmacéuticos en el desarrollo de fármacos, incluyendo la caracterización analítica usando cristalización, la difracción de polvo de rayos X *(XRPD)*, microscopía, análisis de sorción de humedad, análisis térmico (calorimetría diferencial de barrido *(DSC)*, TGA y TMA[1], microcalorimetría y espectroscopia (FTIR[2], Raman y RMN[3] de Estado Sólido).

[1] Análisis termogravimétrico y análisis termomecánico.

[2] Espectrometría infrarroja con transformada de Fourier

[3] Resonancia magnética nuclear.

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5. Soy autor o co-autor de más de veinte publicaciones en mi campo de trabajo.

6. En virtud de lo anterior, me considero un experto en el campo del polimorfismo y la caracterización del estado sólido de sólidos farmacéuticos en el desarrollo de fármacos.

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B. Instrucciones

7. Se me solicitó preparar una declaración juramentada en nombre de Novartis para esta acción en Colombia en relación con una declaración de interés público relacionada con la patente colombiana a nombre de Novartis número 29270 (CO *29270*) que reivindica la forma polimórfica β del metanosulfonato de imatinib.[4] En esta declaración juramentada, se me ha solicitado tratar los siguientes puntos:

- Estructuras cristalinas y polimorfismo en general - véase la sección C de esta declaración juramentada (párrafos 8 a 11 a continuación).
- Métodos de identificación de polimorfos en una muestra - véase la sección D (párrafos 12 a 17 a continuación).
- Polimorfos conocidos de mesilato de imatinib y los procesos para su preparación - véase la sección E (párrafos 18 a 39 a continuación).
- Posibles métodos para la producción del polimorfo α , o una formulación farmacéutica que contenga el polimorfo α, que además produzca el polimorfo β u otro polimorfo - véase la sección F (párrafos 40 a 48 a continuación).
- La posibilidad de que el polimorfo α se convierta en el polimorfo β - véase la sección G (párrafos 49 a 55 a continuación).

C. Estructuras cristalinas y polimorfismo en general

8. La mayoría de los compuestos orgánicos forman estructuras cristalinas. El mesilato de imatinib es uno de estos compuestos.
Las estructuras cristalinas se forman por un proceso conocido como cristalización, por el cual las moléculas de una sustancia se agrupan y se alinean en un patrón de repetición regular de una retícula cristalina.

9. La cristalización es una manera importante de separar un producto en una forma sustancialmente pura a partir de una solución impura. Este proceso se utiliza a menudo en la industria farmacéutica, pues la pureza es muy importante en la producción de un fármaco para uso humano.

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[4] Lo que también se conoce por su nombre abreviado mesilato de imatinib. Uso ambos términos de forma intercambiable en esta declaración juramentada.

10. Algunos compuestos orgánicos, incluyendo mesilato de imatinib, exhiben 'polimorfismo', lo que significa que el compuesto puede existir en más de una estructura cristalina o forma. Diferentes polimorfos tienen diferentes propiedades físico-químicas, tales como solubilidad, estabilidad química, biodisponibilidad, morfología, punto de fusión, higroscopicidad y densidad. La selección de un polimorfo apropiado es, por tanto, un aspecto importante en el desarrollo de un fármaco, dada su potencial influencia en las propiedades del fármaco. Existen varios métodos para identificar y caracterizar los polimorfos de un compuesto orgánico. Muchas de estas técnicas se mencionaron anteriormente en el párrafo 4, y yo explico cómo varios de ellos funcionan en los párrafos 12 a 17, a continuación.

11. El mesilato de imatinib se puede cristalizar en varias formas polimórficas. Compañías farmacéuticas de todo el mundo han descubierto y documentado muchas formas diferentes del mesilato de imatinib en las últimas dos décadas. Yo describo algunos de estos polimorfos en los párrafos 18 a 39, a continuación.

D. Métodos de identificación de polimorfos en una muestra

12. Es posible analizar una muestra para determinar cuáles de los múltiples polimorfos conocidos están presentes.

    Algunas pruebas comúnmente empleadas para este propósito incluyen XRPD, microscopia, análisis de sorción de humedad y DSC. Estas técnicas son capaces de detectar diferencias en las propiedades de distintos polimorfos, que surgen debido a las diferencias en sus estructuras cristalinas. Tales propiedades son características de cada polimorfo, de manera que se pueden utilizar para identificar la presencia del polimorfo en una muestra.

13. En el departamento donde trabajo en Novartis en Basilea, utilizamos rutinariamente XRPD y DSC para determinar si las muestras contienen formas polimórficas particulares de una substancia activa. Estas técnicas pueden identificar de forma fiable la presencia de un polimorfo particular en una muestra, siempre que la cantidad presente en la muestra sea mayor que el límite de detección *(LOD)* de la técnica utilizada. Cada técnica analítica tiene un LOD, que es la cantidad más baja de una substancia distinguible de la ausencia de dicha substancia. Por ejemplo, si

un técnica particular tiene un LOD de 5%, esta técnica podria inequivocamente identificar el polimorfo en una muestra que contiene 50%, 20% o 5% de ese polimorfo. Sin embargo, la técnica seria inadecuada para identificar la presencia del polimorfo en una muestra que contiene solamente 2% del polimorfo. En tales casos, un método más sensible de detección seria necesario.

XRPD

14. La XPRD se utiliza a menudo para identificar polimorfos en una muestra, como resulta evidente a partir del ejemplo de datos de XRPD al que se refieren los párrafos posteriores de esta declaración juramentada. Generalmente se considera que es un método sensible y fiable para detectar polimorfos. La XRPD consiste en proyectar un haz de rayos X hacia una substancia en polvo que está siendo analizada. Cuando el haz alcanza la muestra, las interacciones entre los rayos X y los átomos de la(s) estructura(s) cristalina(s) en la muestra causan que el haz de luz se disperse. La dispersión de rayos X por átomos en diferentes planos del cristal provoca interferencia entre los haces dispersos a algunos ángulos. Los ángulos a los que se produce la interferencia se detectan con relación al ángulo 2θ. Las intensidades de las señales también se detectan. Como los átomos en cada polimorfo diferente están dispuestos de manera distinta, la interferencia se produce en un conjunto particular de ángulos, y con intensidades particulares, que son característicos de cada polimorfo. Estos datos pueden ser trazados en un espectro XRPD como un patrón de picos. En términos sencillos, este patrón puede considerarse una 'huella digital' o 'firma' del polimorfo. Algunos ejemplos de espectros XRPD se muestran en la Figura A, a continuación.

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Microscopía

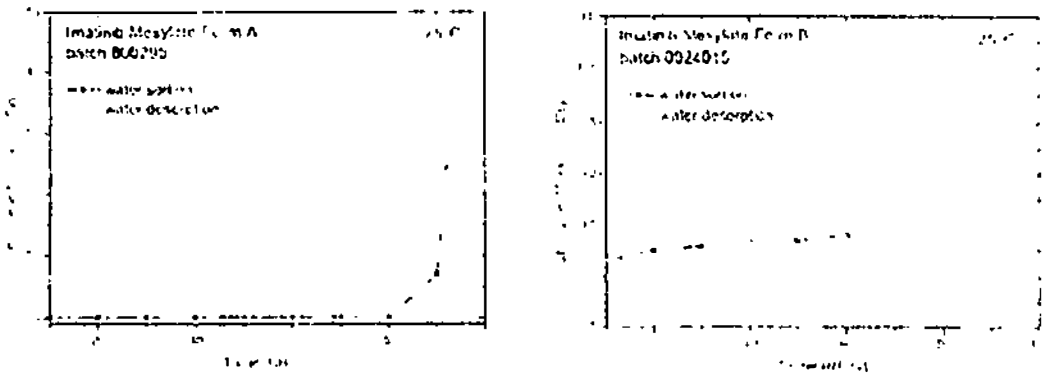
15. La estructura cristalina de un polimorfo puede verse utilizando un microscopio electrónico de barrido *(SEM)*, un poderoso microscopio que dirige un haz de electrones hacia la muestra sólida y detecta señales en su superficie. Estas señales revelan información sobre la muestra, incluyendo su morfología externa. La figura B muestra imágenes de SEM de dos estructuras cristalinas.

Figura B

[illegible]

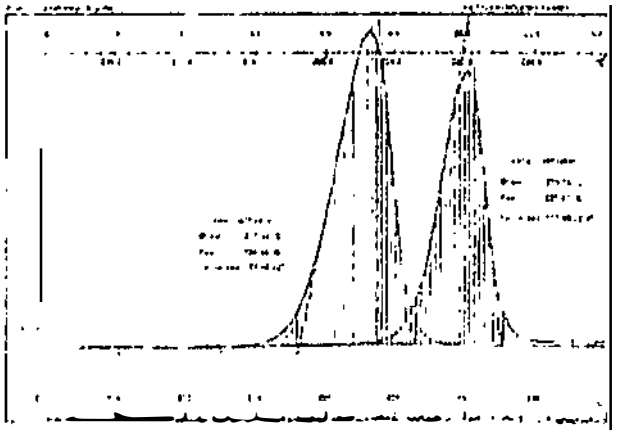
16. Casi todas las sustancias interactúan en algún grado con el vapor de agua en su entorno. El análisis de sorción de humedad mide el grado en el cual un material absorbe y retiene moléculas de agua. La sorción de humedad, o higroscopicidad, de los materiales, generalmente depende de las condiciones ambientales, por lo que es común caracterizar una sustancia en cuanto a su contenido de humedad a temperatura y humedad relativa específicas. Como los polimorfos tienen diferentes estructuras cristalinas, pueden interactuar con moléculas de agua en distintos grados, lo que los hace más o menos higroscópicos en condiciones específicas. La figura C a continuación muestra los resultados del análisis de sorción de humedad de dos sustancias.

Figura C:



17. La DSC es una técnica utilizada para analizar qué sucede con una substancia, tal como una estructura cristalina, cuando es climatizada. Al calentar la substancia, esta sufre transiciones térmicas que afectan su estructura. Una de estas es la fusión de la substancia, que se produce cuando las moléculas que componen la substancia comienzan a separarse. En el caso de una estructura cristalina, la estructura regular y repetida se descompone dejando las moléculas constituyentes. La fusión es una reacción endotérmica, lo que significa que toma energía para que suceda. Mientras que el calentamiento de una sustancia generalmente causa un aumento en su temperatura, este se detiene cuando comienza la fusión y no se reanuda hasta que la estructura de la sustancia se ha desglosado completamente. La DSC mide cuánto más calor debe ser aplicado a la sustancia para que esto suceda, lo que permite la determinación de la temperatura a la que ocurre esto, el punto de fusión de la sustancia. Como las estructuras cristalinas de los polimorfos son diferentes, así también lo son sus puntos de fusión. Algunos ejemplos de termogramas DSC se muestran en la figura D a continuación.

Figura D:



E. Polimorfos del mesilato de imatinib y procesos para su preparación

18. En el párrafo 11 anoto que las compañías farmacéuticas han documentado muchos polimorfos diferentes del mesilato de imatinib. No todos estos polimorfos tienen estructuras cristalinas: en algunas disposiciones (denominadas formas amorfas), no hay ninguna estructura cristalina discernible presente. Adicionalmente, diferentes disposiciones de una substancia activa no tienen que ser idénticas en términos de su composición química para ser consideradas polimorfos distintos desde una perspectiva de reglamentación farmacéutica. Las moléculas de agua u otro disolvente pueden incorporarse en una disposición cristalina para dar polimorfos alternativos, llamados hidratos y solvatos, respectivamente. Con miras a la brevedad, más adelante discutiré únicamente los polimorfos α y β, y otros polimorfos que tienen una estructura cristalina y una composición química similar. La Tabla 1 a continuación contiene una lista completa de las diferentes formas de las que yo tengo conocimiento, cada una de los cuales ha sido reportada como un polimorfo distinto.

Tabla 1:

Forma	Descripción	Fuente
Formas discutidas a		
α y β	WO 99/03854	Novartis
H1	WO 2004/106326	Hetero
I y II	WO 2006/054314	Natco
δ y ε	WO 2007/023182	Novartis
F, G, H, I y K	WO 2007/059963	Novartis
Y y Z	WO 2011/100282	DrReddy's
M, Y, B y P	EP 2 604 596 A1	Deva
Formas amorfas		
Amorfo	IN 2003/MU01208	Sun
Amorfo	WO 2008/112722	Dr Reddy's
Amorfo	US 2008/0234286	Chemagis
Amorfo	WO 2008/154262	Novartis

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22. El último párrafo de la página 2 también señala que la forma α del mesilato de imatinib es higroscópica, mientras que el segundo párrafo de la página 3 explica que la forma β es menos higroscópica. Detalles adicionales se proporcionan a partir del tercer párrafo de la página 8 y hasta el segundo párrafo de la página 9. El primer párrafo de la página 9 concluye que, a 25°C y con una humedad relativa de 93%,la forma β permanece seca mientras que la forma α toma rápidamente agua, al punto de haber una conversión parcial a una forma amorfa (es decir, no cristalina).

23. Finalmente, el penúltimo párrafo de la página 2 explica que los puntos de fusión de las formas cristalinas α y β pueden determinarse a partir de un termograma DSC, y el primer párrafo de la página 5 afirma que la aparición del punto de fusión de la forma β está alrededor de 217°C y del de la forma α está alrededor de 226°C.

24. Dado que los espectros XRPD, la morfología de los cristales, la higroscopicidad y los puntos de fusión de los polimorfos α y β difieren de maneras características de cada polimorfo, es posible distinguir los polimorfos α y β usando técnicas de laboratorio estándar.

25. El segundo párrafo del Ejemplo 1 en la página 19 de WO 99/03854 describe un proceso para la preparación del polimorfo α a partir de una base libre de imatinib. El proceso implica añadir la base libre de imatinib a etanol antes de añadir ácido metanosulfónico a la suspensión. La solución resultante se calienta bajo reflujo antes de la filtración y la evaporación. El residuo se suspende en etanol y se disuelve bajo reflujo. El enfriamiento, la filtración y el secado tienen como resultado la forma α del cristal.

26. El polimorfo α puede utilizarse para generar el polimorfo β bajo condiciones específicas de reacción, por ejemplo, aquellas descritas en el primer párrafo del Ejemplo 1 en las páginas 18 a 19. Una suspensión de la forma α se digiere en metanol y los cristales de la forma β se aíslan por filtración y se secan. Alternativamente, los cristales de la forma β pueden ser usados para 'sembrar' la generación de futuros cristales β en una solución que contiene metasulfonato de imatinib. El sembrado provee una estructura de cristal como plantilla, sobre la cual futuras moléculas se pueden montar. Ello funciona porque se requiere menos energía para añadir cristales a una estructura existente que para establecer una nueva estructura cristalina. WO 99/03854 describe dos métodos para generar la forma β con la ayuda del sembrado. El Ejemplo 2 en la página 19 explica que una base libre de imatinib se suspende en metanol antes de que se añadan el ácido metanosulfónico, el metanol y el carbón activado. La mezcla se hierve bajo reflujo, se filtra y el agua se evapora. El residuo se disuelve en metanol y es inoculado con una pequeña cantidad de cristales β. El secado a alta temperatura y alta presión lleva a cantidades más significativas de la forma β del cristal. El Ejemplo 3 en la página 20 describe cómo calentar

el polimorfo α en metanol e inocular la solución con cristales β conduce a más cristalización. Secar el residuo a alta presión y alta temperatura produce cantidades más significativas del polimorfo β.

Forma III

27. Un polimorfo conocido como la forma III fue descrito por Hetera Drugs Ltd en el año 2004 en una solicitud de patente publicada como WO 2004/106326. La solicitud explica que esta forma cristalina novedosa se caracteriza por un espectro XRPD que tiene picos en 2θ con valores de aproximadamente 9.9, 11.1, 16.3, 17.3, 18.1, 19.1, 19.6, 20.3, 21.1, 21.9, 23.2, 23.6, 24.2, 24.9, 25.6, 26.0, 27.3, 27.9, 28.9, 29.4, 30.4 y 30.5 grados.

28. WO 2004/106326 proporciona ejemplos de la preparación de la forma III, sobre la que se afirma que implica disolver la base libre de imatinib en un disolvente clorinado antes de agregar ácido metanosulfónico , para después agitar, filtrar y secar.

Formas I y II

29. WO 2006/054314, una solicitud de patente presentada por Natco Pharroa Ltd, divulga dos polimorfos más, llamados formas I y II. La solicitud hace una lista de los picos del espectro XRPD de la forma I, de los que los más significativos están en 2θ con valores de aproximadamente 9.7, 10.0, 16.0, 17.1, 17.9, 18.8, 19.3, 20.0, 21.7, 23.0, 23.9, 24.7, 25.1, 25.8 y 29.2 grados. También hace una lista de los picos de la forma II, de los que los más significativos están en 2θ con valores de aproximadamente 2.8, 4.4, 8.9, 9.6, 12.1, 14.1, 14.7, 16.1, 17.0, 17.6, 18.6, 19.4, 19.6, 20.3, 20.9, 21.4, 22.0, 23.5, 24.0, 24.6, 25.2, 25.7, 26.9, 27.7, 28.1, 28.6, 29.1, 29.5 y 30.1 grados.

30). WO 2006/054314 afirma que la forma I se puede preparar mediante suspensión de los polimorfos α2 o β en cloroformo y agua con calentamiento y destilación seguida de filtración. También establece que la forma II se puede preparar por liofilización (secado por congelación) de una solución acuosa de los polimorfos α o β. La forma α2 a la que se hizo referencia fue el objeto de una solicitud de patente anterior, presentada por Natco Pharma, WO 2005/077933.

Formas δ y ε:

31. Las formas cristalinas δ y ε se dieron a conocer por primera vez en una solicitud de patente presentada por Novartis AG, publicada inicialmente en 2007 como WO 2007/023182. Yo fui el inventor de las formas δ y ε. La solicitud explica que la forma δ produce un espectro XRPD con picos en 2θ con valores de 2.2, 13.0, 14.4, 16.0, 16.5, 16.8, 19.2, 19.4, 19.8, 20.3, 20.7, 20.9, 21.1, 21.5, 22.7, 23.7, 24.4, 24.7, 25.3, 25.6, 26.3 y 28.1 grados. También identifica los picos del espectro XRPD de la forma ε en 2θ con valores de 9.4, 11.9, 12.7, 13.3, 13.9, 15.0, 15.3, 17.0, 17.9, 18.5, 19.0, 19.6, 20.7, 21.4, 23.6, 24.1 y 28.2 grados.

32. WO 2007/023182 explica que la forma δ se puede preparar suspendiendo un precipitado seco de mesilato de imatinib en acetona y metanol antes de evaporar la solución bajo nitrógeno. La forma ε se prepara mediante un proceso similar, excepto por que el precipitado seco de mesilato de imatinib se suspende en acetato de etilo y etanol.

Formas F, G, H, I y K

33. Los polimorfos F, G, H, I y K se dieron a conocer por primera vez en una solicitud de patente presentada por Novartis, publicada inicialmente como WO 2007/059963. Yo fui, nuevamente, el inventor de los polimorfos F, G, H, I y K del mesilato de imatinib.

34. La solicitud explica que los cinco polimorfos tienen espectros XRPD con picos en 2θ con valores de:

a. En el caso del polimorfo F, 8.4, 8.6, 10.4, 13.3, 14.7, 16.2, 16.8, 17.1, 19.5, 20.9, 22.2, 23.1, 23.6, 24.5, 25.1, 26.0, 26.9, 28.5, 29.1 y 30.3 grados;

b. En el caso del polimorfo G, 10.5, 12.9, 13.9, 14.1, 15.0, 16.6, 17.2, 17.5, 18.1, 18.7, 19.2, 19.8, 20.6, 21.1, 21.3, 21.7, 22.1, 22.8, 23.9, 24.3, 25.1 y 28.6 grados;

c. En el caso del polimorfo H, 10.5, 13.8, 15.7, 18.1, 21.0, 22.8, 24.3, 25.1, 26.3, 29.7 y 32.9 grados;

d. En el caso del polimorfo I, 9.6, 12.9, 14.1, 15.2, 15.6, 17.1, 18.0, 18.7, 19.1, 19.8, 20.9, 23.4, 23.9, 24.3, 25.2 y 28.4 grados; y

e. En el caso del polimorfo K, 12.1, 12.9, 13.6, 14.1, 15.2, 17.2, 18.2, 18.4, 19.8, 21.0, 22.4, 23.4, 24.3, 25.2, 28.4, 29.2 y 37.9 grados.

35. Los Ejemplos en WO 2007/059963 explican cómo generar formas F, G, H, I y K. Los Ejemplos

1 a 4 describen los métodos para generar la forma F. Cada método implica disolver mesilato de imatinib en agua y dispensar la solución en un bloque de pocillos. La solución en cada pocillo se seca mediante lavado con nitrógeno, dejando un precipitado seco. El precipitado se re-suspende en diferentes reactivos en cada uno de los Ejemplos 1 a 4. Estos son alcohol bencílico (Ejemplo 1); una mezcla de alcohol bencílico y acetato de etilo (Ejemplo 2); una mezcla de alcohol bencílico y, o bien 1,4-dioxano, 3- pentanona o éter de diisopropilo (Ejemplo 3); o la mezcla de alcohol bencílico y, o bien acetonitrilo o dimetil formamida (Ejemplo 4). En cada caso, la suspensión se agita y se deja evaporar a alrededor de 50°C bajo nitrógeno para generar la forma F del cristal. Los métodos para producir las formas G, H, I y K del cristal son similares, excepto por que los reactivos utilizados en la etapa de suspensión son diferentes, como se ve a continuación:

a. Para generar la forma G, el precipitado se re-suspende en una mezcla de 3-pentanona y ciclohexano.

b. Para generar la forma H, el precipitado se re-suspende en una mezcla de 3-pentanona y N, N-dimetilformamida.

c. Para generar la forma I, el precipitado se re-suspende en una mezcla de acetato de etilo y éter dietílico.

d. Para generar la forma K, el precipitado se re-suspende en una mezcla 3-etil acetato y N. N-dimetilformamida.

Formas Y y Z

36. Las formas Y y Z del cristal se dieron a conocer en una solicitud de patente presentada por DrReddy's Laboratories Ltd. publicada como WO 2011100282. La solicitud explica que los cinco polimorfos tienen espectros XRPD con picos en 2θ con los valores a continuación:

a. 6.1 , 8.7, 11.5, 14.5, 18.0, 18.7, 20.1 , 21.9 y 22.5 grados en el caso de la forma Y: y

b. 6.6, 8.0, 13.9, 16.6, 17.0, 17.3, 18.4, 19.0, 20.2, 22.2 y 24.2 grados en el caso de la forma Z.

37. WO 2011/100282 explica que la forma Y se puede preparar mediante la suspensión de la base libre de imatinib en metanol, enfriamiento y la adición de ácido metanosulfónico con agitación. Se añade éter metil tert-butílico con agitación y la suspensión se filtra bajo nitrógeno, se lava con éter metil tert-butílico y se seca conforme a uno de los dos métodos. La solicitud describe la preparación de la forma Z mediante la mezcla de mesilato de imatinib y sulfóxido de dimetilo y calentamiento durante la disolución y el filtrado. Se añade la solución al diclorometano pre-enfriado y se agita a alrededor de 0°C. El sólido se recoge por filtración, se lava con

Tabla 2:

Equilibrio o recristalización en solventes (*)				
Condiciones			Observaciones	Método de caracterización
Solvente	Experimento	Rango de temperatura (°C)		
Agua	equil	-	-	-
	recris.	-	-	-
Acetona	equil.	10-20 15-20 rt	α + β ===> β α + β ===> β α + β ===> β	DSC DSC DSC
	equil.	25 25	β ===> β α ===> β	DSC
	recris.	15-20	α ===> β	DSC
Diclorometano	equil.	25	α ===> α	DSC
	recris.	-	-	-
Etanol 96%	equil.	25	α + β ===> β	DSC
	recris.	-	-	-
Acetato de etilo	equil.	25 25	α ===> α α + β ===> β	DSC DSC
	recris.	-	-	-
Isopropanol	equil.	25	α + β ===> β	DSC
	recris.	-	-	-
Metanol	equil.	25	α ===> β	DSC,RAMAN, XRPD
	recris.	-	α ===> β	DSC
Metanol + Agua (9+1)	equil.	25	α + β ===> β	DSC
Metanol + Agua (8+2)	equil.	25	α + β ===> β	DSC
Metyl t-butil éter	equil.	25 25	α ===> α α + β ===> β	DSC DSC
	recris.	-	-	-
Tolueno	equil.	25 25 25	α ===> α β ===> β α + β ===> β	DSC DSC DSC
	recris.	-	-	-
Aceite de sésamo	equil.	25	α + β ===> α	DSC

Observaciones:

Resumen:
Las dos formas cristalinas ** llamadas alfa y beta han sido detectadas. La temperatura de transición calculada es de alrededor de 140°C. La modificación alfa, que fue aislada por primera vez es termodinámicamente metaestable a temperatura ambiente, pero su transformación de estado sólido al polimorfo beta es suficientemente lenta para no detectarse.
Hasta el momento no se han detectado hidratos o solvatos.

35. Señalé en el párrafo 30 que WO 2006/054314 describe la preparación de las formas polimórficas I y II a partir del polimorfo α, involucrando, en el caso de la forma I, la suspensión en cloroformo, calentamiento y destilación, y en el caso de la forma II, congelación de una solución acuosa. De nuevo, puede verse que la conversión del polimorfo α en las formas I o II únicamente se lleva acabo bajo condiciones de reacción específicas; no ocurre de manera espontánea.

54. En los párrafos 45 a 47 hice referencia al análisis de muestras de productos genéricos hecho en el departamento donde trabajo. En cada caso, los espectros XRPD se prepararon después de que las muestras fueron sometidas a condiciones para probar la estabilidad. Las condiciones 'intermedias' usadas para probar las muestras procedemes de Japón, Nueva Zelanda y Chile fueron 25°C a una humedad relativa del 60% durante 6 meses. Los resultados -que ninguna conversión ocurrió (hasta el LOD)- demuestran la estabilidad del polimorfo α usado en estos productos genéricos. Una muestra de la formulación en cápsula AFT de Nueva Zelanda fue también sometida a prueba bajo condiciones 'aceleradas' , que fueron 40°C y 75% de humedad relativa durante 6 meses. El espectro XRPD mostró que la substancia activa en la cápsula permaneció en la forma α del cristal incluso bajo esas condiciones aceleradas.

55. En este punto, vale la pena referirse nuevamente al artículo expuesto en el anexo MM-2, dado que la estabilidad de la formulación en comprimidos de Deva que contiene el polimorfo α es un enfoque del artículo. La siguiente cita, tomada de la sección 3.5 del artículo, resume los resultados del análisis llevado a cabo por Deva en relación con la estabilidad de su substancia activa y la formulación en comprimido (con énfasis añadido):

> *"Muestras de comprimidos que contienen la forma α de diferentes lotes de fabricación fueron sometidas a diversas condiciones para probar la estabilidad y fueron analizadas de acuerdo al método PXRD para configurar la estabilidad de la forma α en la formulación en comprimido. Los resultados indicaron que la forma α no se convirtió en la forma β durante el proceso de fabricación, ni durante las condiciones para probar la estabilidad rutinaria (25±2°C, 60 ±5%humedad), intermedia (30±2°C, 65±5%humedad) y acelerado (40±2°C, 75 ±5% humedad) para la forma original en comprimido (recubierto de película) y empacado (ampolleta LDPE) (Figuras complementarias S7 y S8).*
>
> *Estudios adicionales se llevaron a cabo bajo condiciones aceleradas para probar la estabilidad, sometiendo los comprimidos directamente, sin ningún tipo de empaque, e igualmente pelando su recubrimiento de película. Ninguna conversión polimórfica a la forma β se observó en los difractogramas de rayos x de las muestras tomadas de diferentes partes de los comprimidos (superficie y parte central*

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medidas por separado), lo que confirmó que el polimorfo a es estable en nuestra formulación en comprimidos.

Un estudio similar se llevó a cabo al someter una forma a pura API en su empaque original (doble bolsa LDPE) y en contenedores abiertos a la mismas condiciones. En ambos casos, la forma a API se encontró estable y no afectada por la temperatura externa ni la humedad, y no se detectó conversión al polimorfo β (Figura complementaria S9)."

II. VERIFICACIÓN

56. Yo, Michael Mutz, por medio de la presente, verifico y declaro que las declaraciones hechas por mí arriba mencionadas son verdaderas y correctas a mi leal saber y entender.

Firmado
Firma ilegible

Fechado
13 de Agosto 2015

Archivos adjuntos: Anexo MM-1
Anexo MM-2

UR 2157/2015

ROBERTO M. OLARTE GARCIA
Traductor e Intérprete Oficial
Resolución No. 0041
Minijusticia 1996

Certificación de Firma

Lo anterior, ante mi funcionario certificado y facultado para reconocer la firma de

Dr. Michael Mutz, nacido el 04/05/1958, con domicilio en Novartis Pharma AG, con sede en CH-4002 Basilea (Suiza), Virchow 6.3.231

-Quien presentó un documento Oficial con foto –

Certifico, el notario no conoce en detalle el idioma en el que está redactado el anterior documento, por lo tanto no puede certificar el contenido del mismo.

Dado en 79098 Friburgo, el 13 de agosto de 2015

Kümmerle, Notar
79098 Freiburg, Bertoldstr. 65
(Tel.: 0761/217814 0; Fax: 0761/217814 29)

MICHAEL KÜMMERLE
NOTAR IN FREIBURG