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(54) Title: SALT OF (R)-3-(4-(7H-PYRROLO [2,3-D] PYRIMIDIN-4-YL)-LH-PYRAZOL-L-YL)-3-CYCLOPENTYLPROPANENITRILE WITH BENZENESULFONIC ACID

(57) **Abstract**: Provided herein are solid state forms of Ruxolitinib besylate, processes for preparing the solid state forms, as well as pharmaceutical compositions and formulations comprising said solid state forms.

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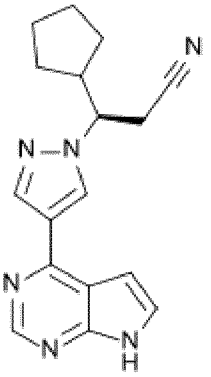
Salt of (R)-3-(4-(7H-pyrrolo [2,3-d] pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile with benzenesulfonic acid

FIELD OF THE INVENTION

The present invention relates to a salt of (R)-3-(4-(7H-pyrrolo [2,3-d] pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile with benzenesulfonic acid, to solid states forms thereof, processes for the preparation thereof and formulations comprising thereof.

BACKGROUND OF THE INVENTION

(R)-3-(4-(7H-pyrrolo [2,3-d] pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile has the chemical formula $C_{17}H_{18}N_6$ and is reported in WO2007/070514 as a selective inhibitor of protein kinases, such as the enzyme Janus Kinase in its subtypes 1 and 2 (hereinafter also referred to as "JAK1" and "JAK2") with IC_{50} values of 3.3 nM and 2.8 nM, respectively. The compound modulates JAK1/JAK2-mediated cell's signaling, which regulates a number of cytokines and growth factors that are important for hematopoiesis and immune function and as such it has been asserted that it is useful in the therapy of various diseases, e.g. cancer. The compound is also known under the INN Ruxolitinib and apparently has the following structure (I):



Ruxolitinib is marketed under the trade name Jakafi® in the US and Jakavi® in Europe for the treatment of patients with myelofibrosis, polycythemia vera, essential Thrombocythemia and hematological malignancies. Ruxolitinib may also be effective for treatment of autoimmune related inflammatory diseases, such as psoriasis.

WO2007/070514 discloses the trifluoroacetate salt of Ruxolitinib as a synthetic intermediate. Pharmaceutically acceptable salts of Ruxolitinib have been reported in WO2008/157208, namely Ruxolitinib phosphate, Ruxolitinib sulfate and Ruxolitinib maleate.

A pharmaceutically acceptable salt is not only defined by its chemical composition but also by the so-called polymorphism. Polymorphism, the occurrence of different crystal forms, is a property of some molecules and molecular complexes. A single molecule, like Ruxolitinib or salts thereof, may give rise to a variety of polymorphs having distinct crystal structures and physical properties like melting point, thermal behaviors (e.g. measured by thermogravimetric analysis – “TGA”, or differential scanning calorimetry – “DSC”), powder X-ray diffraction (PXRD) pattern, infrared absorption and solid state NMR spectrum. One or more of these techniques may be used to characterize a particular polymorph and to distinguish different polymorphic forms of a compound.

Different solid state forms (including solvated forms) of an active pharmaceutical ingredient may possess different properties. Such variations in the properties of different solid state forms and solvates may provide a basis for improving certain aspects of the API, such as its formulation, for example, by facilitating better processing or handling characteristics, changing the dissolution profile in a favorable direction, or improving stability (polymorph as well as chemical stability) and shelf-life. These variations in the properties of different solid state forms may also offer improvements to the final dosage form, for instance, if they serve to improve bioavailability. Different solid state forms and solvates of an active pharmaceutical ingredient may also give rise to a variety of polymorphs or crystalline forms, which may in turn provide additional opportunities to assess variations in the properties and characteristics of a solid active pharmaceutical ingredient.

Discovering new polymorphic forms and solvates of a pharmaceutical product can provide materials having, inter alia, desirable processing properties, such as ease of handling, ease of processing, chemical and polymorphic stability upon storage and processing, and ease of purification or are useful as advantageous intermediate crystal forms that facilitate conversion to other solid state forms (including other solvates) of said pharmaceutical product.

New polymorphic forms and solvates of a pharmaceutically useful compound can also provide an opportunity to improve the performance characteristics of a pharmaceutical product. It enlarges the repertoire of materials that a formulation scientist has available for formulation optimization, for example by providing a product with different properties, e.g., better processing or handling characteristics, improved dissolution profile, or improved shelf-life. Lastly, new polymorphic forms may be prepared with improved reliability and reproducibility compared to other forms, for example, in terms of crystallinity or polymorphic purity.

The intestinal tract obeys different pH ranges, which vary from pH 1 to 2 of the empty stomach, pH 3-5 for stomach filled with chyme, to pH 5-7.5 of the colon. A dissolution screening test regime for solid state forms of an active ingredient may be set up for example at pH 1.2, pH 4.5 and pH 6.8 in order to cover a broad range of the gastro-intestinal tract.

Solubility studies showed good solubility for Ruxolitinib phosphate at pH 1.2, but considerably less solubility at higher pH points. Ruxolitinib maleate exhibited less solubility at pH 1.2 in comparison with Ruxolitinib phosphate and in addition a distinct pH dependency at pH 4.5 and 6.8. Ruxolitinib sulfate gave a moderate solubility with less pH dependency.

In order to provide a modified release formulation, such as delayed release, prolonged release, sustained release, repeated action release, extended release, controlled release, it is of favor when the solid state forms of an active ingredient show moderate solubility. This may be of advantage, when a so-called matrix system is used. For such systems, the active ingredient is embedded in an appropriate polymer, for example hydroxy propyl methyl cellulose, and release of the active ingredient is diffusion controlled over a period of time.

It is of favor if salts of active ingredients show comparable solubilities through all pH ranges of the gastro-intestinal tract when used in a modified release formulation in order to be available for permeation into the body through the entire passage of the gastro-intestinal passage. This passage may take a few hours.

Object of the present invention is to find a Ruxolitinib salt, which gives moderate solubility for all pH ranges compared to the state of the art Ruxolitinib salts, while the salt is still pharmaceutically acceptable.

SUMMARY OF THE INVENTION

The present invention provides a solid state form of Ruxolitinib benzenesulfonate or Ruxolitinib besylate, processes for the preparation thereof, and pharmaceutical compositions and formulations comprising the solid state form of Ruxolitinib besylate, and processes for the preparation of the pharmaceutical compositions and formulations.

The present invention also provides the use of said solid state form of Ruxolitinib besylate for the manufacture of pharmaceutical compositions and formulations. Accordingly, the present invention further provides a pharmaceutical composition comprising said solid state form of Ruxolitinib besylate of the present invention. The pharmaceutical composition may additionally comprise at least one pharmaceutically acceptable excipient to form a pharmaceutical formulation that can, for example, be administered to patients in need of such treatment.

The present invention comprises a process for preparing the above-mentioned pharmaceutical formulations. The process comprises combining the solid state form of Ruxolitinib besylate with at least one pharmaceutically acceptable excipient.

The solid state form as defined herein as well as the pharmaceutical compositions and formulations of Ruxolitinib besylate can be used as medicaments, particularly for the treatment of cancer, myelofibrosis, polycythemia vera, essential Thrombocythemia, hematological malignancies and psoriasis. The present invention also provides a method of treating cancer, myelofibrosis, polycythemia vera, essential

Thrombocythemia hematological malignancies and psoriasis comprising administering a therapeutically effective amount of the solid state form of Ruxolitinib besylate of the present invention, or a therapeutically effective amount of at least one of the pharmaceutical compositions or formulations of the present invention comprising said solid state form of Ruxolitinib besylate of the present invention to a patient in need thereof.

The present invention also provides the use of said solid state form of Ruxolitinib and/or Ruxolitinib salt, particularly Ruxolitinib besylate, or at least one of the above pharmaceutical compositions and/or formulations for the manufacture of a medicament for treating cancer, myelofibrosis, polycythemia vera, essential Thrombocythemia, hematological malignancies and psoriasis.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a comparison of solubility of Ruxolitinib besylate, Ruxolitinib sulfate, Ruxolitinib maleate and Ruxolitinib phosphate at pH 1.2, pH 4.5 and pH 6.8

Figure 2a shows a powder X-ray diffraction pattern of Ruxolitinib besylate.

Figure 2b shows a ^1H NMR spectrum of Ruxolitinib besylate.

Figure 2c shows a DSC thermogram of Ruxolitinib besylate.

Figure 3a shows a DVS experiment with Ruxolitinib besylate.

Figure 3b shows a comparison of powder X-ray diffraction patterns of Ruxolitinib besylate before and after the 12 week stability experiment at 25°C and 60% humidity.

Figure 4 shows the IR spectra of Ruxolitinib besilat

Detailed Description of the Invention

The present invention provides a solid state form of Ruxolitinib besylate, processes for preparing the solid state form, as well as pharmaceutical compositions and formulations comprising said solid state form.

In accordance with WO 2008/157208, Ruxolitinib sulfate, phosphate and maleate have high crystallinity and give good handling properties. However, WO 2008/157208 is silent for solubility over all pH ranges in the gastro-intestinal tract.

Ruxolitinib sulfate has moderate solubility in comparison with Ruxolitinib phosphate. It still obeys a pH dependency, *i.e.* solubility increases at low pH.

Ruxolitinib maleate shows moderate solubility as well as pH dependency, but solubility turns to very poor at higher pH points.

The ratio of the solubility of Ruxolitinib phosphate at pH 1.2 to pH 4.5 is 7 and the ratio of pH 1.2 to pH 6.8 is 9. The ratio of the solubility of Ruxolitinib maleate at pH 1.2 to pH 4.5 is 4 and the ratio of pH 1.2 to pH 6.8 is 4 as well. The ratio of the solubility of Ruxolitinib sulfate at pH 1.2 to pH 4.5 is 1.7 and the ratio of pH 1.2 to pH 6.8 is 1.9.

The present invention offers crystalline Ruxolitinib besylate with improved ratio with the tendency to 1, *i.e.* equal at all pH points (Figure 1). Ratio solubility of pH 1.2 to 4.5, as well as pH 1.2 to 6.8 is 1.4 for both ratios for Ruxolitinib besylate.

Further, depending on which other solid state form it is compared with, the Ruxolitinib besylate form of the present invention may have advantageous properties selected from at least one of: chemical or polymorphic purity, increased crystallinity, flowability, solubility, dissolution rate, bioavailability, morphology or crystal habit, specific surface and pycnometric density, bulk/tap density, stability – such as chemical stability as well as thermal and mechanical stability with respect to polymorphic conversion, stability towards dehydration and/or storage stability, a lower degree of hygroscopicity, low content of residual solvents and advantageous processing and handling characteristics such as compressibility, and bulk density.

Solid state forms of Ruxolitinib besylate comprise crystal forms, or crystalline forms, or amorphous forms of Ruxolitinib besylate. As used herein, solid state forms, crystal forms, crystalline forms, polymorphs and polymorphic forms are used interchangeably.

A crystal form may be referred to herein as being characterized by graphical data "substantially as depicted" in a Figure. Such data include, for example, powder X-ray diffractograms, infrared spectra and DSC thermograms. The graphical data potentially provides additional technical information to further define the respective solid state form which can not necessarily or easily be described by reference to numerical values for peak positions and/or relative intensities. In any event, the skilled person will understand that such graphical representations of data may be subject to small variations, *e.g.*, in peak relative intensities and peak positions due to factors such as variations in instrument response and variations in sample concentration and purity, which are well known to the skilled person. Nonetheless, the skilled person would readily be capable of comparing the graphical data in the Figures herein with graphical data generated for an unknown solid state form and confirm whether the two sets of graphical data are characterizing the same solid state form or two different solid state forms.

A solid state form (or polymorph) may be referred to herein as substantially free of any other crystalline (or polymorphic) forms. As used herein in this context, the expression "substantially free of any other forms" will be understood to mean that the solid state form contains 20% or less, 10% or less, 5% or less, 2% or less, or 1% or less of any other forms of the subject compound as measured, for example, by PXRD. Thus, solid state forms of Ruxolitinib oxalate described herein as substantially free of any other polymorphic forms would be understood to contain greater than 80% (w/w), greater than 90% (w/w), greater than 95% (w/w), greater than 98% (w/w), or greater than 99% (w/w) of the subject solid state form of Ruxolitinib oxalate. Accordingly, in some embodiments of the invention, the described solid state forms of Ruxolitinib oxalate may contain from 1% to 20% (w/w), from 5% to 20% (w/w), or from 5% to 10% (w/w) of one or more other solid state forms of Ruxolitinib or salts thereof.

As used herein, the expression "room temperature" or "RT" refers to a temperature between about 20 °C and about 30 °C. Usually, room temperature ranges from about 20°C to about 25 °C.

As used herein, the term “overnight” refers to a period of between about 15 and about 20 hours, typically between about 16 to about 20 hours.

As used herein, the term “reduced pressure” refers to a pressure of about 10 mbar to about 50 mbar.

As used herein, the term "isolated" corresponds to product or solid state form thereof that is physically separated from the reaction mixture in which it is formed.

As used herein, unless stated otherwise, XRPD peaks reported herein are preferably measured using CuK radiation, λ = 1.5418 Å.

As used herein, the expression “dry crystalline form” refers to a polymorph that was dried using any conventional techniques to remove residual solvent. Such conventional techniques include, but are not limited to, evaporation, vacuum drying, oven drying, drying under nitrogen flow, etc.

As used herein, and unless stated otherwise, the term “anhydrous” in relation to crystalline Ruxolitinib besylate relates to a crystalline Ruxolitinib besylate which contains no more than 1% (w/w) of either water or organic solvents as measured by conventional methods, for example TGA, GC or KF. An anhydrous form of the solid states of Ruxolitinib besylate of the present invention refers to a form that does not contain crystalline water (or other solvents) in a defined, stoichiometric amount within the crystal.

As used herein, and unless indicated otherwise, the term "polymorphic stability" in relation to the crystalline forms of Ruxolitinib besylate means that there is less than 20%, 10%, 5%, 1%, 0.5% or 0.1% (w/w) conversion of crystalline Ruxolitinib besylate to any other solid state form of Ruxolitinib besylate under the specified conditions, as measured by PXRD.

The present invention encompasses a crystalline form of Ruxolitinib besylate.

Ruxolitinib besylate can be characterized by data selected from one or more of the following: a powder X-ray diffraction pattern having peaks at 22.9, 15.2, 18.8, 24.7, 20.6 and 22.3 new anyway degrees two theta ± 0.2 degrees two theta; it may be further characterized by the X-ray powder diffraction pattern having peaks at 22.9, 15.2, 18.8, 24.7, 20.6, 18.2, 21.0, 22.5 and 22.3 degrees two theta ± 0.2 degrees two theta and also having one, two, three, four, five, six, seven, eight or nine additional peaks selected from 22.0, 21.7, 17.8, 30.7, 11.9, 7.6, 9.1, 9.9 and 19.9 degrees two theta ± 0.2 degrees two theta; and combinations of these data; a powder X-ray diffraction pattern substantially as depicted in Figure 2a.

Ruxolitinib besylate can be further characterized by one or more of the following: a DSC thermogram substantially as depicted in Figure 2c; by two sharp endotherms with onset temperatures at 177 ± 2 °C and 267 ± 2°C

Ruxolitinib besylate can be characterized by any combinations of the above data. For example, by a powder X-ray diffraction pattern having peaks at degrees 22.9, 15.2, 18.8, 24.7, 20.6, 18.2, 21.0, 22.5 and 22.3 two theta ± 0.2 degrees two theta and also by a DSC thermogram substantially as depicted in Figure 2c.

The above Ruxolitinib besylate has advantageous properties selected from at least one of: chemical or polymorphic purity, flowability, solubility, dissolution rate, bioavailability, morphology or crystal habit, stability – such as such as chemical stability as well as thermal and mechanical stability with respect to polymorphic conversion, storage stability, stability to dehydration, low hygroscopicity, and low content of residual solvents and advantageous processing and handling characteristics such as compressibility, or bulk density.

Particularly, crystalline Ruxolitinib besylate has high chemical purity and excellent stability properties. Specifically, it is stable upon storage at 25°C and 60% relative humidity (RH) for up to at least 12 weeks. Furthermore, crystalline Ruxolitinib besylate has good solubility and it can be used to prepare an oral formulation, i.e. a tablet or a capsule.

The described solid state form Ruxolitinib besylate can be used to prepare Ruxolitinib base or other different salts of Ruxolitinib, as well as solid state forms thereof and/or pharmaceutical formulations comprising one or more of the salts and/or solid state forms thereof.

The present invention further encompasses 1) a pharmaceutical composition comprising said solid state form described herein; 2) a pharmaceutical formulation comprising said solid state forms or pharmaceutical compositions described herein, and at least one pharmaceutically acceptable excipient; 3) a process to prepare such formulations comprising combining the above-described solid state forms and at least one pharmaceutically acceptable excipient; 4) the use of the above-described solid state form in the manufacture of a pharmaceutical composition, and 5) a method of treating cancer comprising administering a therapeutically effective amount of the above-described solid state forms, optionally in the form of pharmaceutical compositions or formulations. The present invention also provides a crystalline form of Ruxolitinib besylate as described above for use as a medicament, preferably for the treatment of cancer. The pharmaceutical compositions can also be used for preparing said medicament. Preferably the pharmaceutical composition is a pharmaceutical composition for modified release, such as delayed release, prolonged release, sustained release, repeated action release, extended release, controlled release and/or targeted release. More preferred the pharmaceutical composition is a composition for extended release.

Delayed release usually indicates that the active pharmaceutical ingredient (i.e., Ruxolitinib besylate) is not being released immediately after administration but at a later time, preferably less than 10 % are released within two hours after administration. Prolonged release usually indicates that the active pharmaceutical ingredient (i.e., Ruxolitinib besylate) is provided for absorption over a longer period of time than immediate release forms, preferably for about 2 to 24 hours, in particular for 3 to 12 hours.

Sustained release usually indicates an initial release of the active pharmaceutical ingredient (i.e., Ruxolitinib besylate), sufficient to provide a therapeutic dose soon after administration, preferably within two hours after administration, and then a gradual release after an extended period of time, preferably for about 3 to 18 hours, in particular for 4 to 8 hours. Repeated action release is distinguished from a sustained release by the fact that the release of the second dose is released at a later time point separated from the initial dose in contrast to a gradual release.

Extended release usually indicates a slow release of the active pharmaceutical ingredient (i.e., Ruxolitinib besylate), so that plasma concentrations are maintained at a therapeutic level for a time period of between 6 and 36 hours, preferably between 8 and 24 hours. Controlled release dosage forms usually release the active pharmaceutical ingredient (i.e., Ruxolitinib besylate) at a constant rate and provide plasma concentrations that remain essentially invariant with time.

Having described the invention with reference to certain preferred embodiments, other embodiments will become apparent to one skilled in the art from consideration of the specification. The invention is further defined by reference to the following examples describing in detail the preparation of the composition and methods of use of the invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the invention.

ANALYTICAL METHODS

NMR Spectroscopy

Instrument: Varian Mercury 400 Plus NMR Spectrometer, Oxford AS, 400 MHz.

Differential Scanning Calorimetry (DSC)

Instrument:	Mettler Toledo DSC 822E coupled with a Mettler Toledo Gas-Flow-Controller TS0800GC1 (Mettler-Toledo GmbH, Gießen, Germany)
Aluminium crucible:	40 µL
Lid:	perforated
Temperature range:	30°C to 350°C
Heating rate:	10°C/ min
Nitrogen flush:	50 mL / min
Software:	STARe Version. 8.10
Interpretation:	Endothermic modus

X-Ray Powder Diffraction (PXRD)

The sample was analyzed on a D8 Advance X-ray powder diffractometer (Bruker-AXS, Karlsruhe, Germany). The sample holder was rotated in a plane parallel to its surface at 20 rpm during the measurement. Further conditions for the measurements are summarized in the table below. The raw data were analyzed with the program EVA (Bruker-AXS, Germany). The samples were layered onto a silicon specimen holder.

	standard measurement
radiation	Cu K_α ($\lambda = 1.5418$ Å)
source	38 kV / 40 mA
detector	Vantec
detector slit	variable
divergence slit	v6
antiscattering slit	v6
2θ range / °	$2 \leq 2\theta \leq 55$
step size / °	0.017

Hygroscopicity

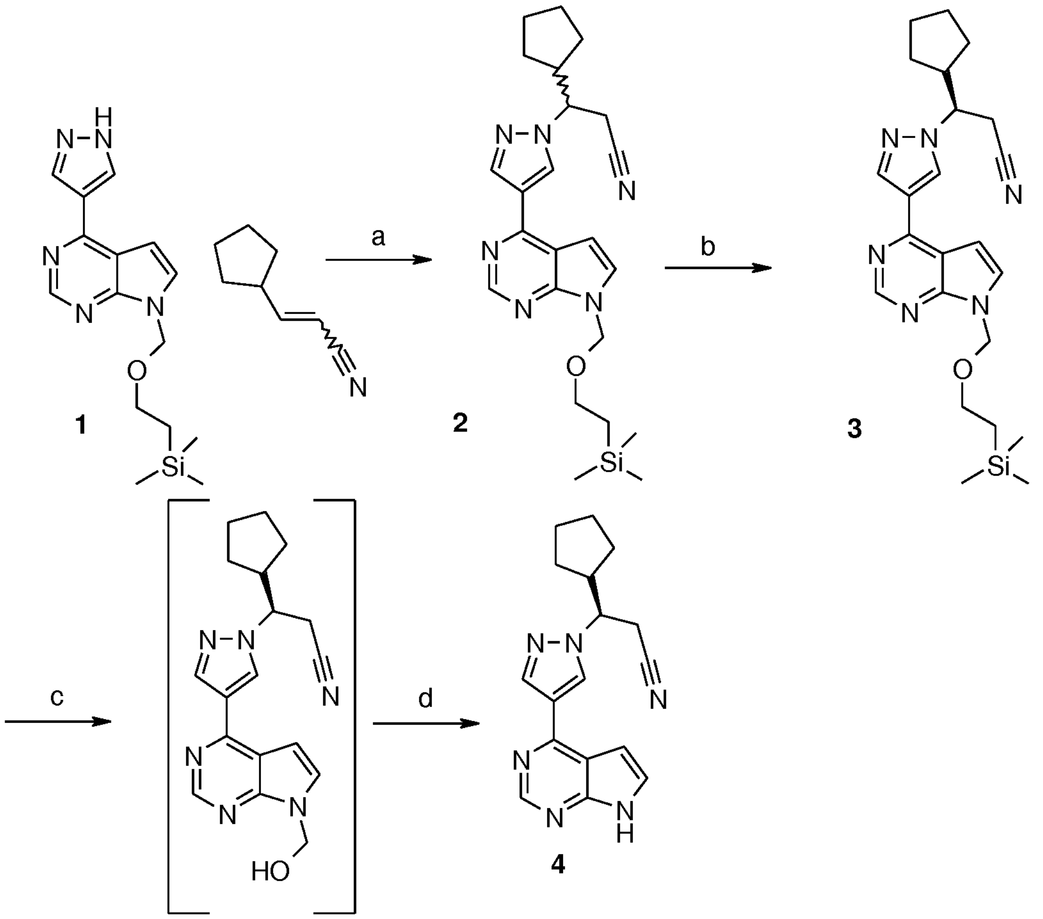
Vapour sorption experiments were performed in the instrument SPSx-1µ (Projekt Messtechnik, Ulm, Germany) at temperature of 25°C and the humidity cycles as shown below.

Cycle no.	rel. humidity (% RH) start value	end value	Number of steps	Time (h)
1	40	0	4	
2	5	65	6	
3	75	75	1	24 h
4	85	95	1	
5	90	0	9	
6	5	35	3	

EXAMPLES

Example 1: Preparation of Ruxolitinib Base.

Ruxolitinib base was prepared in four steps. The route of synthesis is given in the following scheme. The final three steps are described in detail.



Intermediate 2 (*rac*-3-Cyclopentyl-3-{4-[7-(2-trimethylsilanyl-ethoxymethyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-pyrazol-1-yl}-propionitrile)

231.67 g (1.90 mol) 3-Cyclopentyl-acrylonitrile and 23.70 ml (0.16 mol) 1,8-Diazabicyclo[5.4.0]undec-7-en (DBU) were added to a suspension of the starting material (**1**) (500 g, 1.56 mol) in 4000 ml acetonitrile (MeCN) at room temperature under stirring and nitrogen atmosphere. The reaction mixture was heated to 60°C for ~22 hours, then to 80°C for 4 hours. The reaction mixture was cooled to room temperature. The cooled reaction mixture was evaporated under reduced pressure at 42°C to yield a viscous black oil. The oil was dissolved in 1300 ml dichloromethane (DCM) and 650 g silica (63-200 µm) were added. The suspension was evaporated under reduced pressure at 44°C. The obtained crude product on silica was dry loaded on a silica column (1.25 kg (2.5 l) silica 63-200 µm, diameter of column 7 cm length 92 cm). The whole separation was accomplished via flash system (companion "combi flash") with a solvent flow of 100 ml/min. (pressure 5-15 bar). Elution was accomplished with EtOAc (ethylacetate)/n-hexane 1/2. The solvent was evaporated at 50°C to yield the product as highly viscous yellow oil.

Intermediate 3 ((R)-3-Cyclopentyl-3-{4-[7-(2-trimethylsilanyl-ethoxymethyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-pyrazol-1-yl}-propionitrile)

The racemic starting material (1159 g; 2. 7 mol) was dissolved in 2.000 ml MeCN at 50°C. The obtained solution was transferred into a 25 l reactor. 9560 ml of MeCN, 1960 ml THF and 1960 ml acetone were added, resulting in a yellow solution. The heating jacket was adjusted to 55°C (Ti = 51°C). (+)-Dibenzoyl tartaric acid monohydrate 2137 g (2.14 eq) was added resulting in a slightly orange solution. After ~30 minutes, the solution became turbid and the heating jacket was adjusted to 80°C, after 25 minutes a clear orange solution resulted and the heating jacket was adjusted to 55°C. At 56 °C (Ti) enantiomeric pure crystals were added to initiate the crystallization and the heating jacket was adjusted to 50°C. After 1.5 h heating was turned off and the mixture was allowed to cool to room temperature and stirred overnight (Ti after 1 h 45 min. 37°C, final temperature overnight: 22°C). The precipitate was filtered off and washed with 2x100 ml of a mixture of MeCN : THF: acetone (5.8:1:1) yielding 1197 g of the tartrate. (85 % of theory, 52.4% ee).

This and another batch with an enantiomeric purity of 52.0% ee were combined.

2000 g of the combined batches were charged into a reactor and 14.48 l of a mixture of MeCN, THF and acetone (10:1.69:1.69) were added. The reactor was heated to 80°C (Tj) (Ti = 71°C), the product was almost completely dissolved. Further 200 ml of the above solvent mixture were added, resulting in a clear solution. The reactor was cooled to 52°C (cooling rate: after 1.5 h 52°C). A colorless suspension was formed after ~1.5 h. After ~3.5 h the heating was turned off and the suspension was stirred overnight. (Ti final = 20°C). The product was filtered off and washed with 2 x 600 ml of the above described solvent mixture. The obtained product showed an enantiomeric purity of 80.2% ee and a yield of 1528 g (76% of theory).

1903 g of crystallized material with an optical purity of 80% ee were charged in a reactor and 13.38 l of a mixture of MeCN/THF and acetone (10:1.69:1.69) were added. The mixture was heated to 80°C (Tj) (Ti=72.7°C), after addition of further 200 ml of the above solvent mixture, the suspension became a clear solution. The temperature was adjusted to 55°C and after 2.5 h at 52.8°C a colorless suspension was formed, the heating was turned off and stirring was continued overnight. The precipitated product was filtered off (T = 21°C) (1356 g, 92.0% ee).

1356 g of this material were charged in a reactor and 11.99 l of a mixture of MeCN/THF and acetone (10:1.69:1.69) were added. The mixture was heated to 80°C (Tj) (Ti = 73.0°C) (clear solution). The temperature was adjusted to 55°C and after 2.5 h at 52.8°C, a colorless suspension was formed. The temperature was adjusted to 50°C. After 2 hours, heating was turned off and stirring was continued overnight. The product was filtered off (Ti = 20.3°C) and washed with 2 x 600 ml MeCN/THF/acetone (10:1.69:1.69) to yield 1063 g of the product with an enantiomeric purity of 96.6% ee.

1063 g of this material were charged in a reactor and 9390 ml MeCN/THF/acetone (10:1.69:1.69) were added. The mixture was heated to 80°C (Tj) (Ti=73.0°C) (clear solution). The temperature was adjusted to 55°C and after 1 h at 52.8°C a colorless suspension was formed. The temperature was adjusted to 50°C. After 2 hours, heating was turned off and stirring was continued overnight. The product was filtered off (Ti=20.3°C) and washed with 2 x 600 ml MeCN/THF/acetone (10:1.69:1.69) to yield 813 g of the product with an enantiomeric purity of 98.6% ee.

Procedure to liberate the free base:

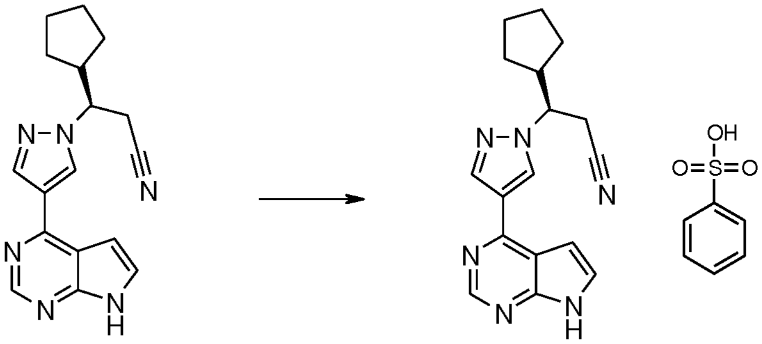
To 100 g of the tartaric acid salt were added 2000 ml ethylacetate (white suspension) and afterwards 600 ml water. The mixture was cooled to 0°C and 2N NaOH was added. The water layer was extracted with 2x 400 ml ethylacetate. The organic layers were combined and washed with 0.01M NaOH (700 ml). The organic layer was evaporated to yield 54.5 g of the product as yellow oil. chemical purity: 99.45 %; HPLC/UV (λ= 266 nm)).

Intermediate 4

(R)-3-Cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-pyrazol-1-yl]-propionitrile

The starting material (244 g; 0.559 mol) was dissolved in 2370 ml MeCN, followed by the addition of 210 ml water and lithium tetrafluoroborate ($LiBF_4$, 520.48 g; 5.55 mol) at room temperature (rt). The reaction temperature decreased by addition of water to 14°C (Ti= 22°C) and increased by addition of $LiBF_4$ to 27°C. The reaction mixture was heated to reflux (~80°C) for 11 h. The reaction was monitored by HPLC. After complete consumption of the starting material the reaction mixture was cooled to 5°C, followed by dropwise addition of 20% aqueous ammonium hydroxide (274 ml) to adjust to pH 9. The ice bath was removed and the reaction mixture was gradually warmed to rt. After complete deprotection, which was monitored by HPLC/TLC, the reaction mixture was filtered and the solids were washed with MeCN (530 ml). The combined filtrates were evaporated under reduced pressure at 46°C and to the residue 3160 ml EtOAc and 1580 ml half saturated brine were added. The two layers were separated and the aqueous layer was extracted with EtOAc (1050 ml). The combined organic layers were washed with half saturated $NaHCO_3$ (1600 ml) and brine (1600 ml), dried over Na_2SO_4 and concentrated under reduced pressure to yield the crude product as orange oil. The crude product was purified by flash chromatography on silica (eluent: EtOAc). Purification procedure: the crude product was dissolved in 250 ml ethylacetate and was divided in two nearly equal portions; each portion was purified separately by a silica column (prepacked puriflash column/puriflash/1600 g/50 μm) on a Armen spot flash system flow: 80 ml/min. The obtained colorless oil was evaporated, resulting in a foam, which was triturated with 200 ml n-pentane (each portion) resulting in a colorless solid which was filtrated off and dried under vacuum at rt. The obtained product was stored under argon atmosphere in the fridge.

Example 2: Preparation of Ruxolitinib besylate.



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To a solution of Ruxolitinib free base (2.5 g; 8.2mmol) in isopropanol (35 ml) at 60°C was added benzenesulfonic acid (1.42 g; 9.0 mmol) in isopropanol (7 ml). The reaction mixture was stirred overnight. The precipitated product was filtrated off, washed with isopropanol (15 ml) and dried for 3 days at 45°C and 20 mbar in a vacuum oven to yield 3.07 g (81% of theory) of the product as colourless crystalline solid.

^{1}H NMR (400 MHz, DMSO-d_6) d ppm 1.10 - 1.23 (m, 1 H) 1.23 - 1.38 (m, 2 H) 1.39 - 1.68 (m, 5 H) 1.77 - 1.85 (m, 1 H) 2.42 (m, J=8.60 Hz, 1 H) 3.14 - 3.34 (m, 2 H) 3.70 - 3.81 (m, 1 H) 4.60 - 4.67 (m, 1 H) 7.28 - 7.33 (m, 3 H) 7.36 - 7.39 (m, 1 H) 7.59 - 7.63 (m, 2 H) 7.99 - 8.02 (m, 1 H) 8.64 (s, 1 H) 9.01 (s, 1 H) 9.14 (s, 1 H) 13.32 (br. s., 1 H) IR (ATR) [cm^{-1}]: 3174, 3109, 2949, 2866, 2814, 2247, 1618, 1597, 1554, 1444, 1427, 1387, 1346, 1313, 1265, 1227, 1167, 1122, 1068, 1036, 1016, 997, 976, 926, 893, 872, 816, 756, 725, 694, 652, 609 HPLC/UV at λ= and 230 nm: > 99.9 %

Example 3: Dynamic vapor sorption experiment with Ruxolitinib besylate

A dynamic vapor sorption experiment was performed with Ruxolitinib besylate. At a constant temperature of 25°C, the substance was exposed to two cycles of varying relative humidity (rh) (40% → 0% →95% (75% r.h. kept constant for 48h) → 0% →40%). Figure 3a presents the relative weight change as a function of time and relative humidity and in Figure 3a the weight changes are illustrated as a function of humidity.

For Ruxolitinib besylate, a relative weight change of 0.55% (by the end of the 75% r.h. step) and 3.7 % (by the end of the 95% r.h. step) was observed. Reducing humidity to 0% and increasing to 40% resulted in the initial starting weight.

Time [h]	RH Set [%]	Relative weight change (dm [%])
0,00	40,0	0.0
16.7	0,0	-0.00
27.7	45	+0.03
95.1	75	+0.55
125.3	95	+3.71
180.7	40	+3.33
218.1	0	-0.40
227.1	35	-0.01

Table 1 relative weight change as a function of time and relative humidity

Example 4: Stability.

Samples were stored in closed and open containers at 25°C/60% rh and at 40°C/75% rh and analyzed after 4, 8 and 12 weeks.

The results of determination of the chemical purity during storage at 40°C/75% rh are summarized. The salt remained chemically stable when stored under these conditions. Therefore, the analysis of samples, which were stored at 25°C/60% rh, was omitted.

Compound	Conditions	Chemical purity ([area-%] at λ=230 nm)			
		t = 00 (= initial)	4 weeks	8 weeks	12 weeks
Ruxolitinib besylate	closed	98.16	98.14%	98.27%	98.06%
	open		98.16%	98.21%	98.22%

The solid-state stability was investigated by XRPD analysis for 25°C/60% rh. The results of evaluation are summarized. Ruxolitinib besylate remained unchanged throughout the observation period.

Compound	Conditions		Evaluation of XRPD analysis		
			4 weeks	8 weeks	12 weeks
Ruxolitinib besylate	25°C/ 60% rh	closed	unchanged	unchanged	unchanged
		open	unchanged	unchanged	unchanged

Example 5: pH-dependencies of solubility (mg/ml) at 37°C

solvent	phosphate			besilate			sulfate			maleate		
	0.5 h	1 h	24 h	0.5 h	1h	24 h	0.5 h	1 h	24 h	0.5 h	1 h	24 h
0.1M HCl pH1.2	N/D	43.2	44.0	9.6	9.6	9.3	18.5	18.5	16.7	12.5	12.0	12.2
20mM (NaOAc / NaAc) –pH 4.5	N/D	6.0	6.8	6.7	7.1	6.1	10.4	10.6	9.5	2.8	3.2	2.4
50mM KH_2PO_4 pH 6.8	N/D	4.7	5.7	6.8	7.0	5.8	9.6	9.8	8.3	2.9	2.9	2.5

<u>**What is claimed is**</u>:

1. Salt of Ruxolitinib with benzene sulfonic acid.

2. Ruxolitinib salt of claim 1, characterized by a powder X-ray diffraction pattern having peaks at 22.9, 15.2, 18.8, 24.7, 20.6, 18.2, 21.0, 22.5 and 22.3 ± 0.2 degrees two theta.

3. The Ruxolitinib salt of any of the preceding claims 1-2 , further characterized by an additional one, two, three, four, five, six, seven, eight or nine PXRD peaks selected from 22.0, 21.7, 17.8, 30.7, 11.9, 7.6, 9.1, 9.9 and 19.9degrees two theta ± 0.2 degrees two theta.

4. The Ruxolitinib salt of any of the preceding claims 1-3, further characterized by one or more of the following: a DSC thermogram substantially as depicted in Figure 2c; by two sharp endotherms with onset temperatures at 177 ± 2 °C and 267 ± 2°C

5. A pharmaceutical composition comprising the Ruxolitinib besylate according to any of the preceding claims 1-4.

6. A pharmaceutical formulation comprising Ruxolitinib besylate according to any of the preceding claims 1-5, and at least one pharmaceutically acceptable excipient.

7. Use of Ruxolitinib besylate according to any of the preceding claims 1-4 in the manufacture of a pharmaceutical composition or formulation.

8. A process for preparing the pharmaceutical formulation according to claim 6, comprising combining Ruxolitinib besylate according to any of the preceding claims 1-4, with at least one pharmaceutically acceptable excipient.

9. Ruxolitinib besylate according to any of the preceding claims 1-4, the pharmaceutical composition according to claim 5, or the pharmaceutical formulation according to claim 6 for use as a medicament.

10. Ruxolitinib besylate according to any of the preceding claims 1-4, the pharmaceutical composition according to claim 5, or the pharmaceutical formulation according to claim 6 for use in treating a subject suffering from cancer, myelofibrosis, polycythemia vera, essential Thrombocythemia, hematological malignancies and psoriasis.

11. A method of treating a subject suffering from cancer, comprising administering a therapeutically effective amount of Ruxolitinib besylate according to any of

the preceding claims 1-4, the pharmaceutical composition according to claim 5, or the pharmaceutical formulation according to claim 6.

12. Use of Ruxolitinib besylate according to any of the preceding claims 1-4 for the preparation of Ruxolitinib, other Ruxolitinib salts; solid state forms thereof; and pharmaceutical formulations thereof.

13. Use of Ruxolitinib besylate according to claim 13 for the prepration of a modified release formulation.

Figure 1: comparison of solubility Ruxolitinib besylate, Ruxolitinib sulfate, Ruxolitinib maleate and Ruxolitinib phosphate at pH 1.2, pH 4.5 and pH 6.8 after 1 h

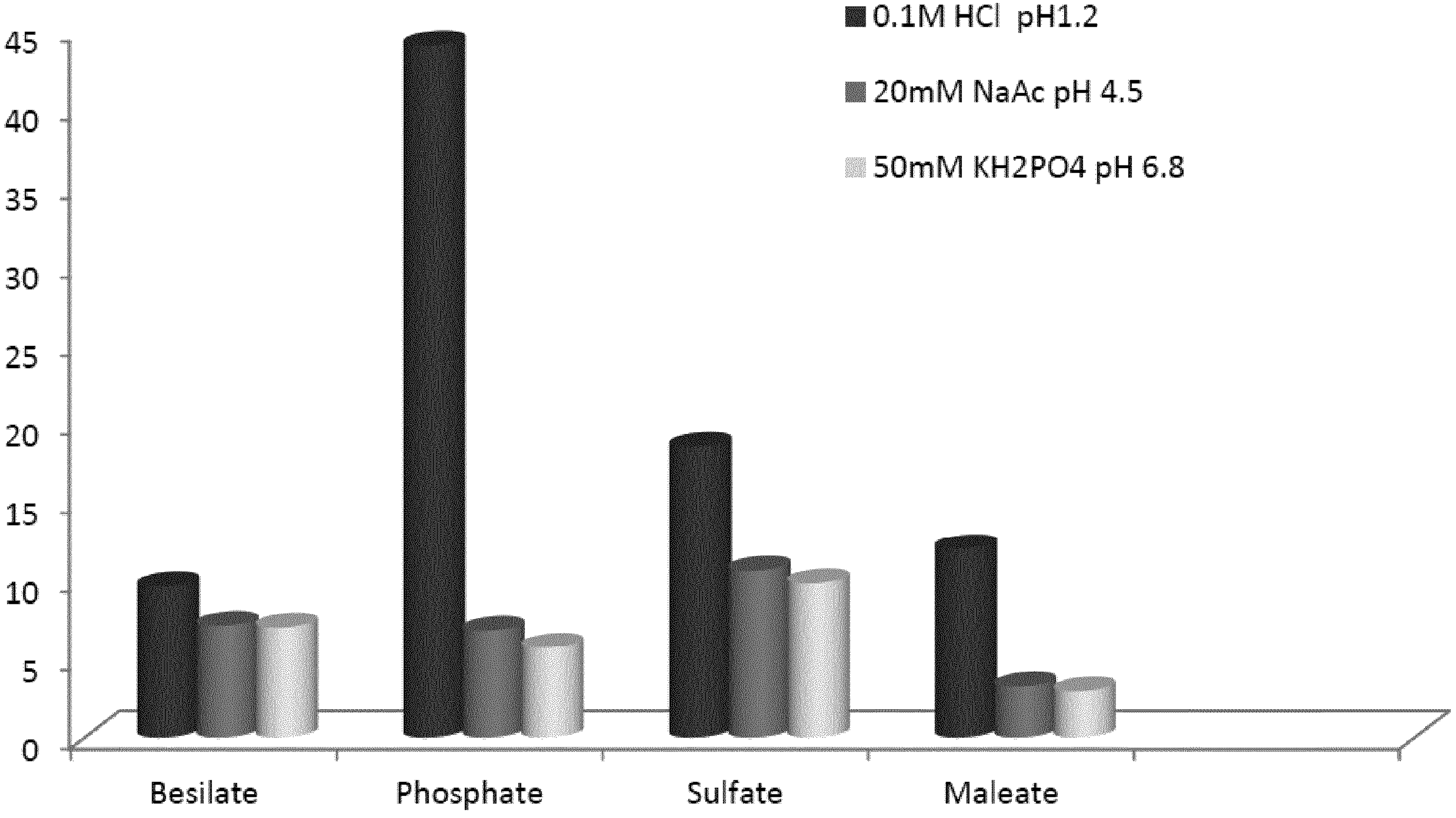


Figure 2a: powder X-ray diffraction pattern of Ruxolitinib besylate

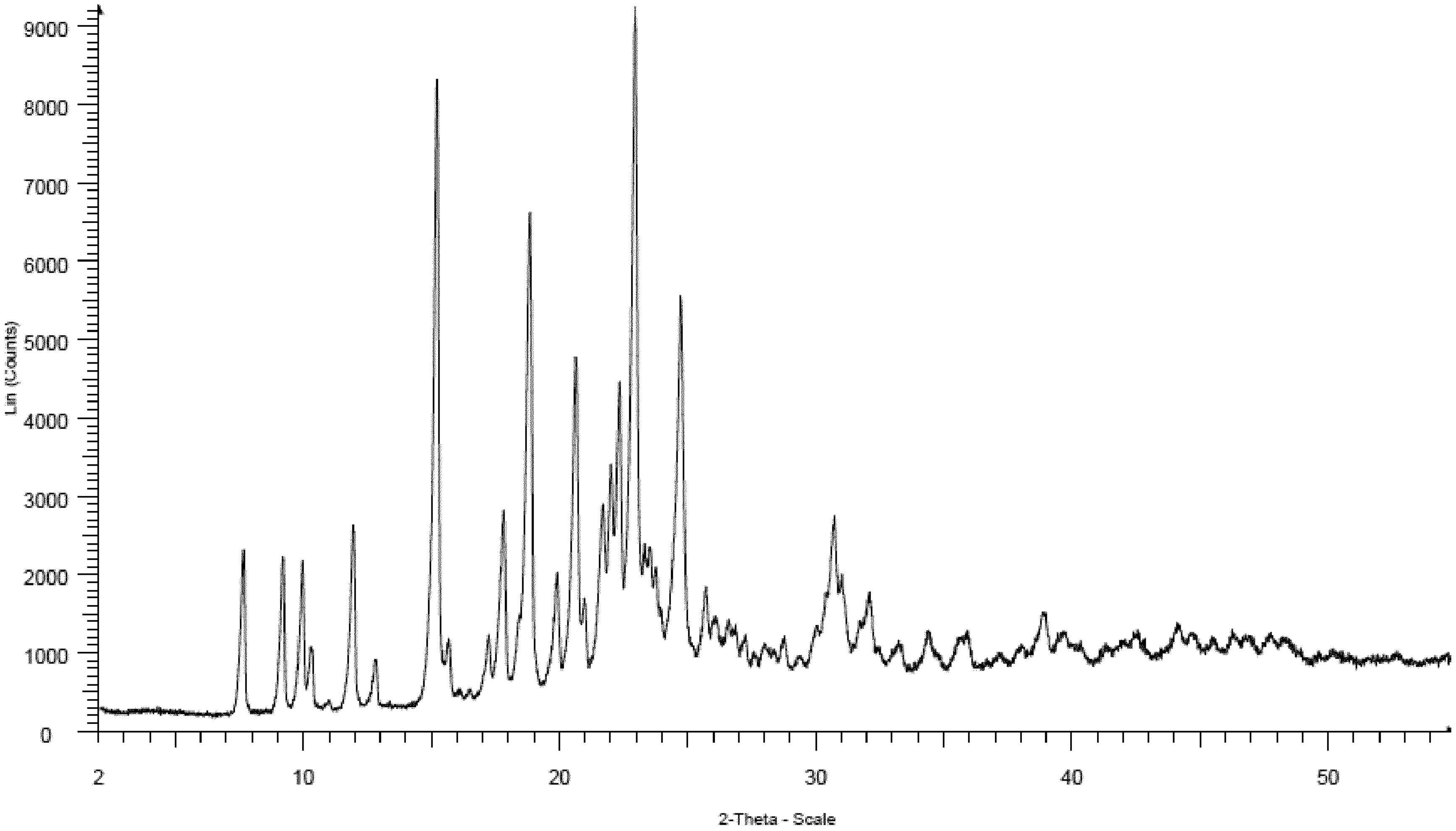
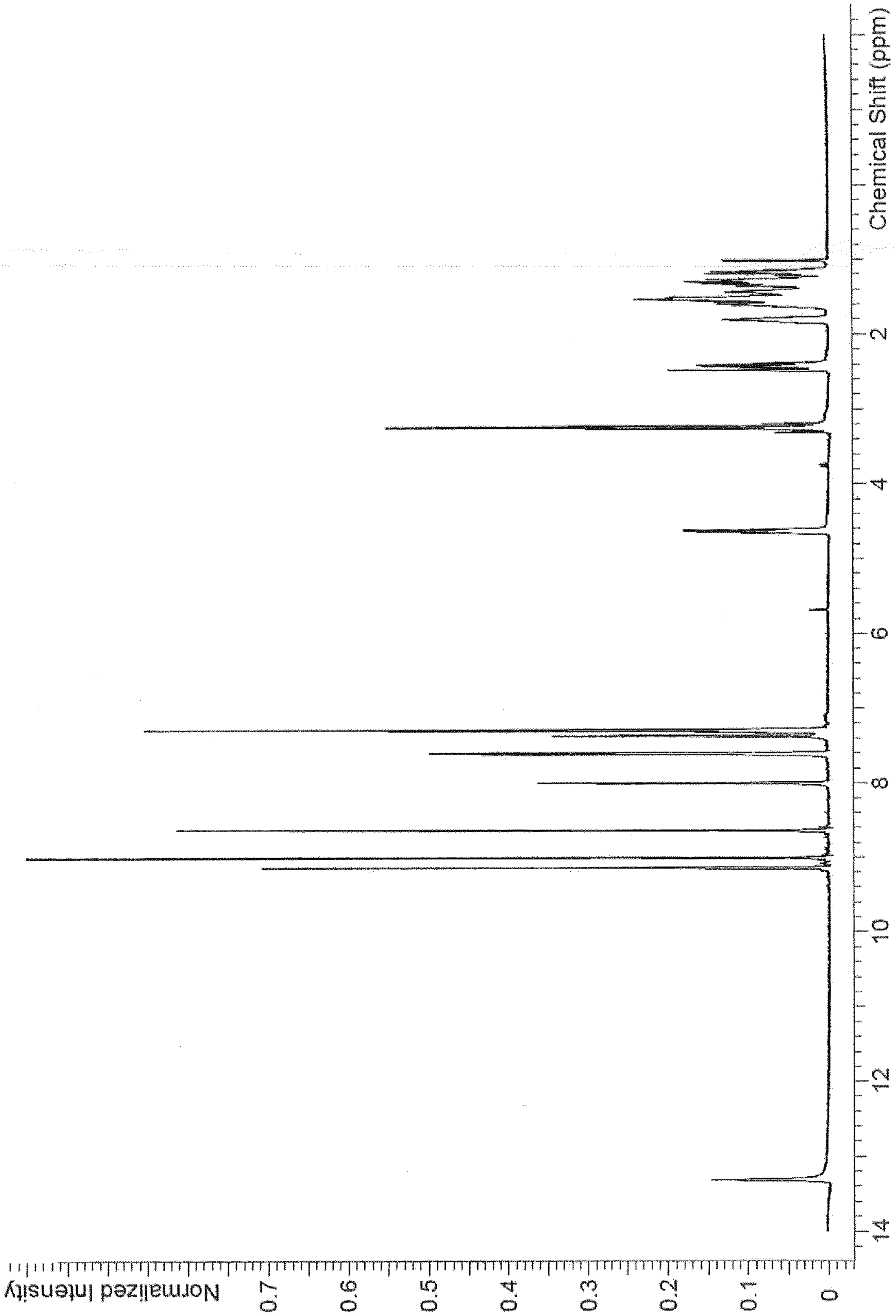


Figure 2 b: ^{1}H-NMR of Ruxolitinib besylate



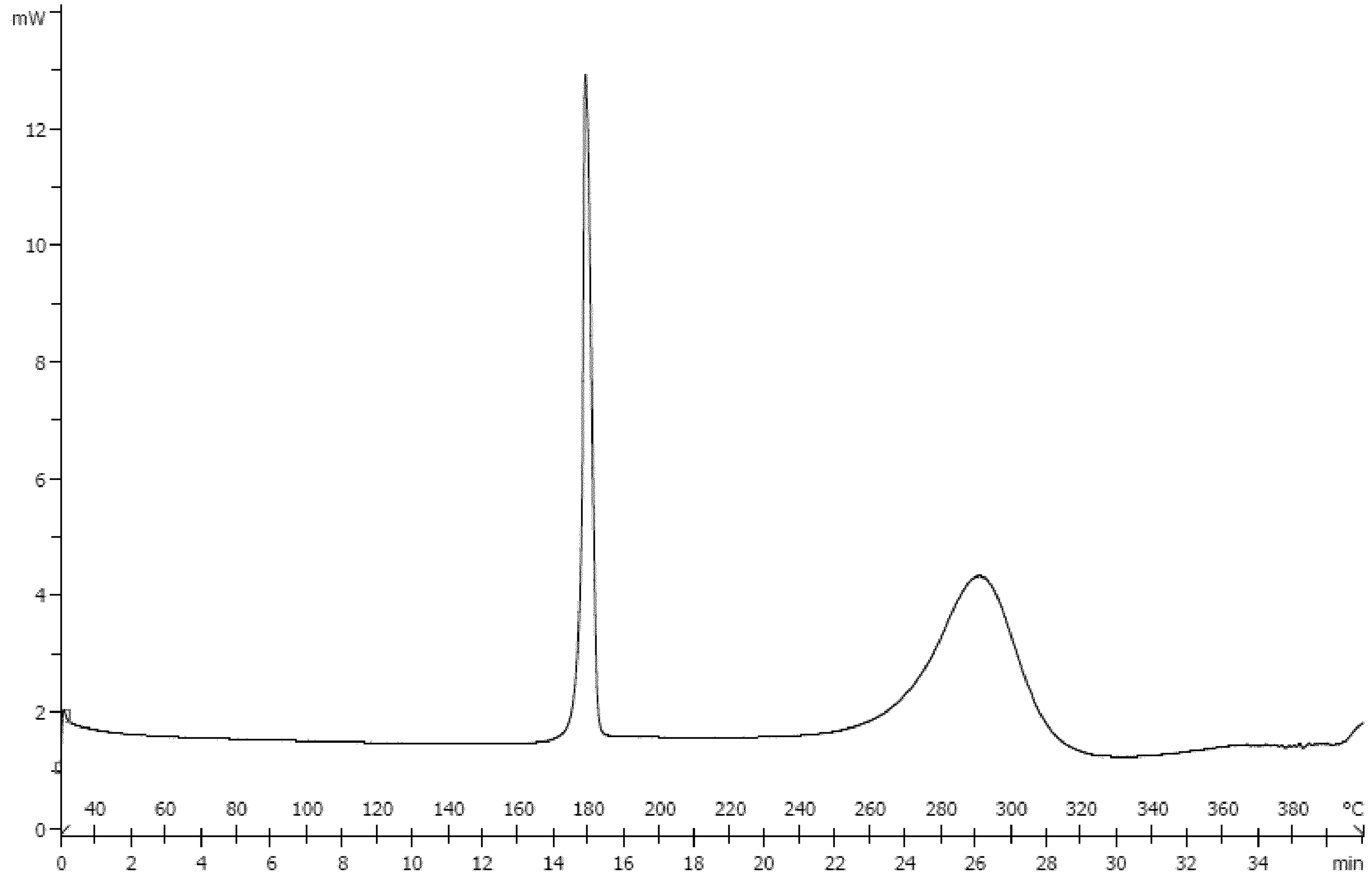


Figure 2c: DSC thermogram of Ruxolitinib besylate

Figure 3a: a DVS experiment with Ruxolitinib besylate

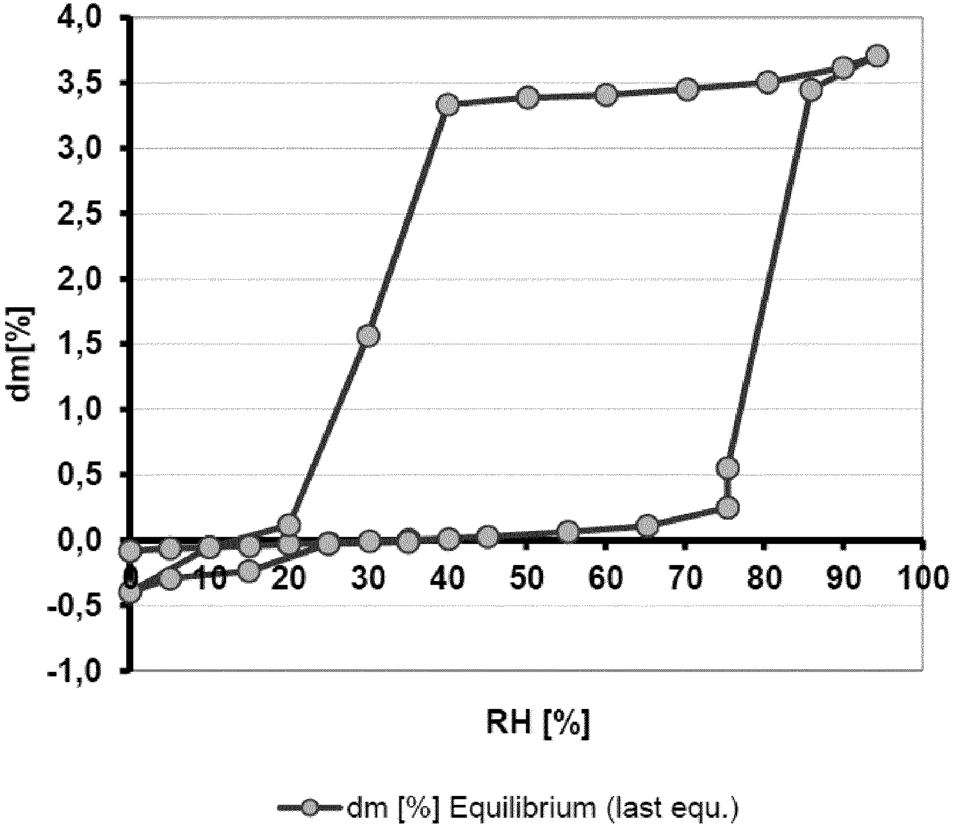


Figure 3b: comparison of powder X-ray diffraction patterns of Ruxolitinib besylate before and after 12 week stability experiment at 25°C and 60% humidity (below: prior stability experiment, top: after 12 weeks stability experiment)

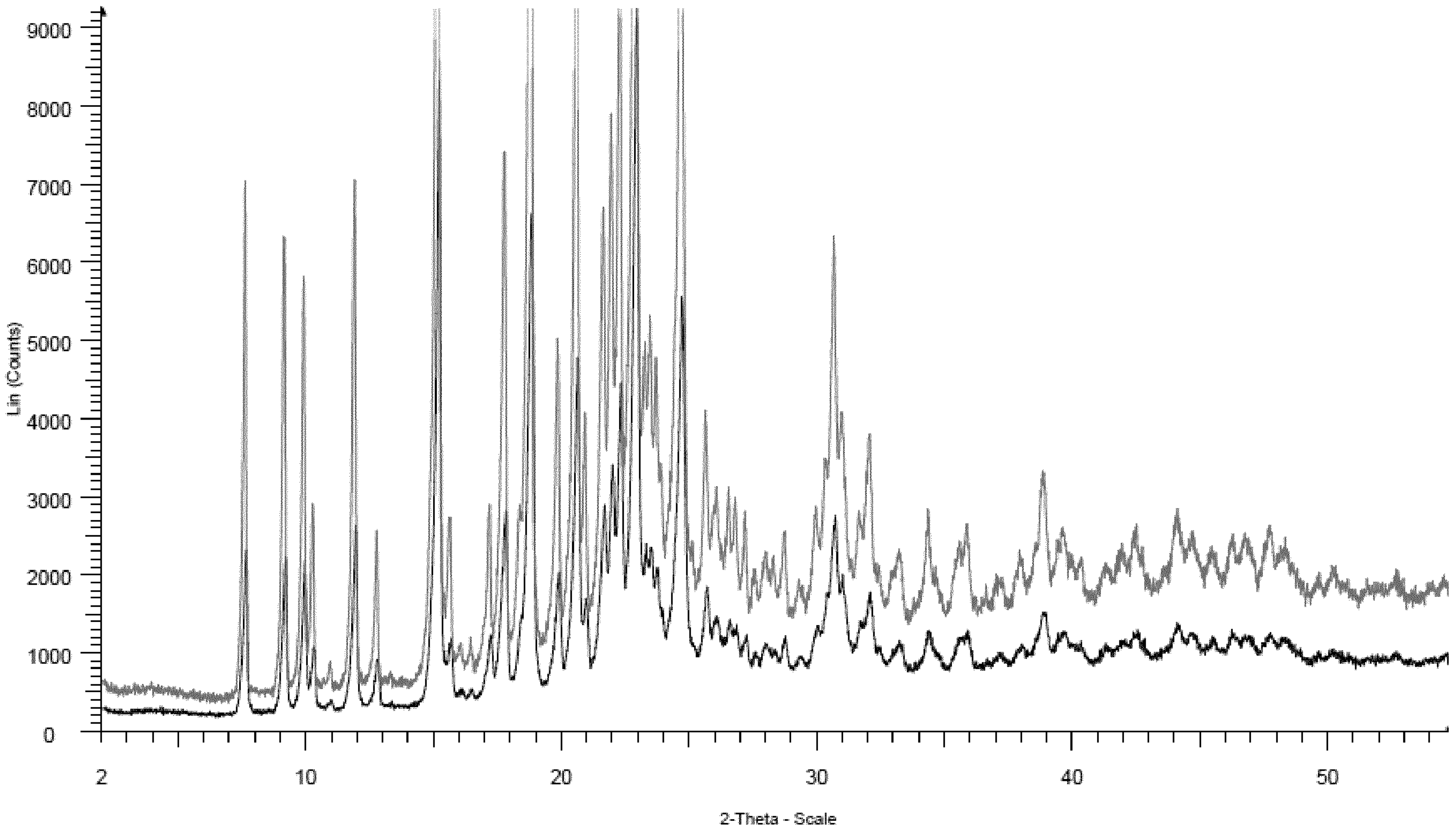
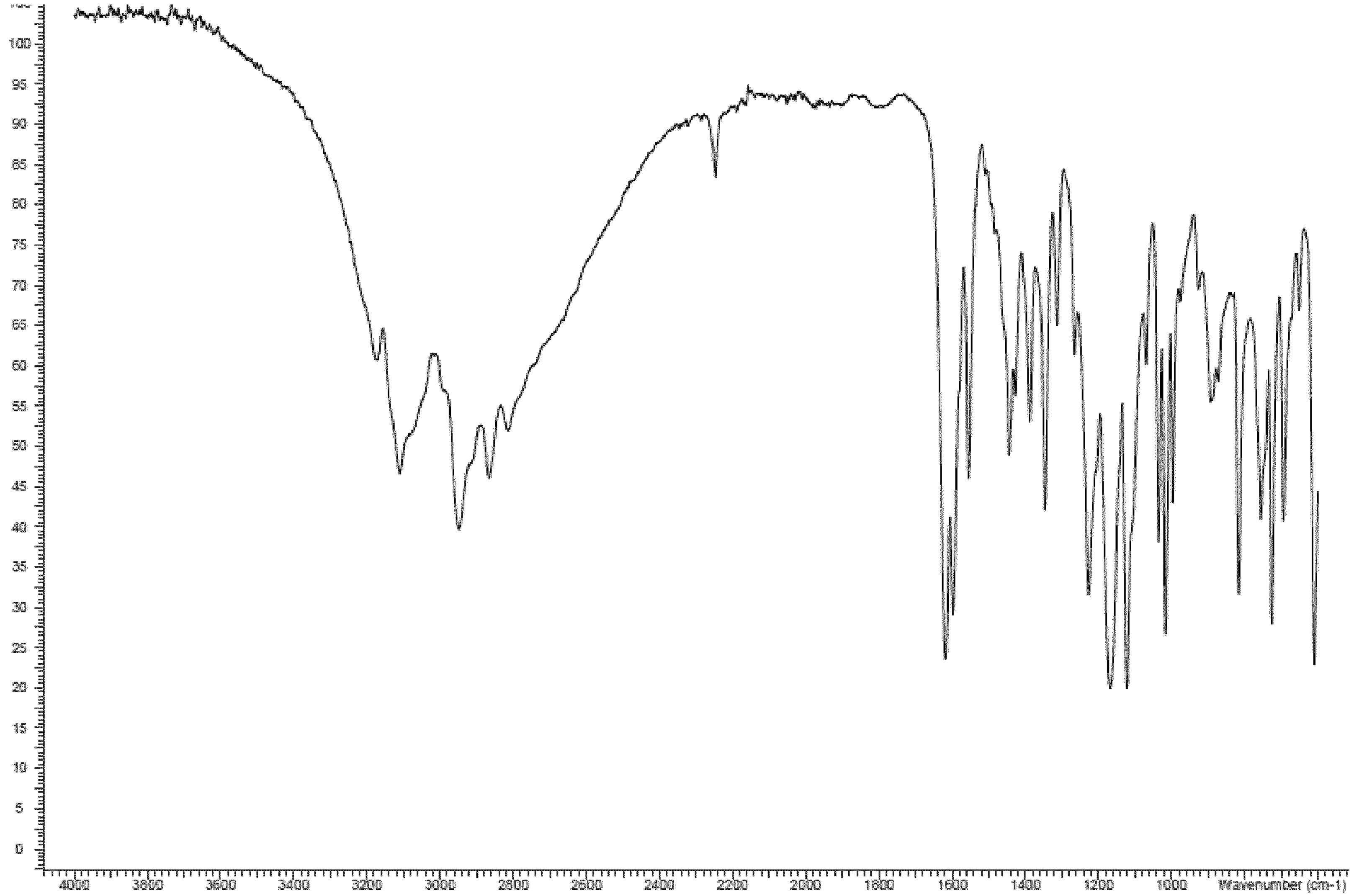


Figure 4: IR spectra of Ruxolitinib Besylate



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/069297

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D487/04 A61K31/519 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

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

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

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/157208 A2 (INCYTE CORP [US]; RODGERS JAMES D [US]; LI HUI-YIN [US]) 24 December 2008 (2008-12-24) cited in the application page 3, lines 28-30 claims 1-6 -----	1-13
A	WO 2014/016396 A1 (RATIOPHARM GMBH [DE]) 30 January 2014 (2014-01-30) page 1, line 22 - page 2, line 13 page 5, line 27 - page 6, line 17 -----	1-13

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search 11 September 2015	Date of mailing of the international search report 23/09/2015
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Samsam Bakhtiary, M

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/EP2015/069297

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