

CRYSTALLINE BETA-LACTAMASE INHIBITOR

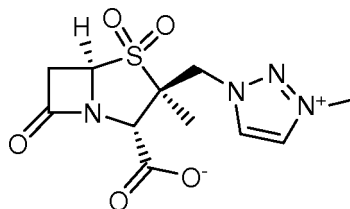
Field of the invention

The present invention relates to crystalline (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide, processes for the preparation thereof, pharmaceutical compositions comprising (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide and uses of the compound, including uses of compositions containing the compound, in particular use with an antibacterial agent in treatment or prevention of bacterial infection.

Background of the invention

Emergence and dissemination of resistance is an inevitable consequence of the evolutionary dynamic set in motion by the introduction of antibiotics, irrespective of structural class or mode of action (Shapiro S. 2013. Speculative strategies for new antibacterials: all roads should not lead to Rome. *J. Antibiot.* **66**: 371-386). Spread of resistance amongst clinically relevant pathogens has had an especially strong impact on the value of β -lactam antibiotics, heretofore regarded as very safe and efficacious therapies for serious bacterial infections. The appearance of new and aggressive β -lactamases, particularly extended spectrum β -lactamases (ESBLs) and other class A enzymes, has compromised the ability of β -lactams to combat infections, highlighting the need for development of new products (Fisher JF, Meroueh SO, Mobashery S. 2005. Bacterial resistance to β -lactam antibiotics: compelling opportunism, compelling opportunity. *Chem. Rev.* **105**: 395-424). Whilst several β -lactamase inhibitors, which protect β -lactam antibiotics from hydrolysis, have been used in combination with some β -lactams, the capability of these β -lactamase inhibitors to preserve the antibacterial activity of β -lactams has eroded severely during the past decade, necessitating the search for new, more potent β -lactamase inhibitors to restore therapeutic utility of their β -lactam partners (Watkins RR, Papp-Wallace KM, Drawz SM, Bonomo RA. 2013. Novel β -lactamase inhibitors: a therapeutic hope against the scourge of multidrug resistance. *Front. Microbiol.* **4**: 392).

WO 2008/010048 discloses the β -lactamase inhibitor (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (formula I):



(Formula I)

WO 2008/010048 discloses formation of an amorphous compound of Formula (I) which is isolated by filtering and lyophilisation.

The present inventors have found that the compound of formula (I) as prepared by the process of WO 2008/010048 is hygroscopic, and has limited stability when stored at room temperature.

It is an object of the invention to provide a more stable form of the compound of formula (I).

It is a further object of the invention to provide a form of the compound of formula (I) that is easy to purify.

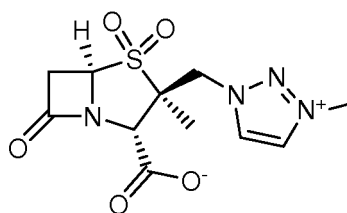
It is a further object of the invention to provide a form of the compound of formula (I) that is easy to handle.

Summary of the invention

The present inventors have developed crystalline compounds of formula (I). The present inventors have surprisingly found that crystalline compounds of formula (I) have improved

thermal stability, are less hygroscopic and easier to purify and handle than the compound of formula (I) in amorphous form.

In a first aspect the invention provides a crystalline compound of formula (I):



Formula (I).

In a first embodiment of the first aspect there is provided a crystalline compound of formula (I), hereinafter “Form A”, characterised by an XRPD spectrum comprising four or more (preferably five or more, preferably six or more, preferably seven or more, preferably eight or more, preferably nine or more, preferably all ten) peaks selected from peaks with 2θ angles of: 8.82, 12.07, 14.43, 14.92, 16.26, 18.25, 19.06, 19.78, 20.82 and 23.51 ± 0.1 degrees 2θ , optionally ± 0.05 degrees 2θ .

Preferably, the XRPD spectrum of Form A has one, two, three, four or all five peaks selected from peaks with 2θ angles of: 8.82, 12.07, 14.43, 18.25 and 19.78 ± 0.1 degrees 2θ .

Preferably, the XRPD spectrum of Form A has all ten peaks with 2θ angles of: 8.82, 12.07, 14.43, 14.92, 16.26, 18.25, 19.06, 19.78, 20.82 and 23.51 ± 0.1 degrees 2θ , optionally ± 0.05 degrees 2θ .

Preferably, Form A has a XRPD spectrum substantially as shown in Figure 1.

Form A may be further characterised by its Thermo Gravimetric Analysis (TGA) curve showing an endothermic event at about $163^\circ\text{C} \pm 2^\circ\text{C}$. The TGA curve may show a weight loss of about 6% up to $130^\circ\text{C} \pm 2^\circ\text{C}$ due to water loss.

Preferably, Form A has a TGA curve substantially as shown in Figure 9.

Form A may be further characterized by its differential scanning calorimetry (DSC) curve showing an endothermic event with a maximum at about $163^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The DSC curve may show an endothermic event starting at about $45^{\circ}\text{C} \pm 2^{\circ}\text{C}$ due to water loss.

Preferably, Form A has a DSC curve substantially as shown in Figure 5.

In a second embodiment of the first aspect there is provided a crystalline compound of formula (I), hereinafter “Form B”, characterised by an XRPD spectrum comprising four or more (preferably five or more, preferably six or more, preferably seven or more, preferably eight or more, preferably nine or more, preferably all ten) peaks selected from peaks with 2θ angles of: 9.37, 10.34, 12.59, 13.17, 15.00, 15.63, 18.51, 19.10, 20.79, 23.93 ± 0.1 degrees 2θ , optionally ± 0.05 degrees 2θ .

Preferably, the XRPD spectrum of Form B has one, two, three, four or all five peaks selected from peaks with 2θ angles of: 10.34, 15.00, 15.63, 18.51 and 23.93 ± 0.1 degrees 2θ .

Preferably, the XRPD spectrum of Form B has all ten peaks with 2θ angles of: 9.37, 10.34, 12.59, 13.17, 15.00, 15.63, 18.51, 19.10, 20.79 and 23.93 ± 0.1 degrees 2θ , optionally ± 0.05 degrees 2θ .

Preferably, Form B has a XRPD spectrum substantially as shown in Figure 2.

Form B may be further characterised by its Thermo Gravimetric Analysis (TGA) curve showing an endothermic event at about $155^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

The TGA curve may show a weight loss of about 8% up to $120^{\circ}\text{C} \pm 2^{\circ}\text{C}$ correlated with water desorption.

Preferably, Form B has a TGA curve substantially as shown in Figure 10.

Form B may be further characterized by its differential scanning calorimetry (DSC) curve showing an endothermic event with a maximum at about $180^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The DSC curve may show an endothermic event starting at about $50^{\circ}\text{C} \pm 2^{\circ}\text{C}$ due to water loss.

Preferably, Form B has a DSC curve substantially as shown in Figure 6.

In a third embodiment of the first aspect there is provided a crystalline compound of formula (I), hereinafter "Form C", characterised by an XRPD spectrum comprising four or more (preferably five or more, preferably six or more, preferably seven or more, preferably eight or more, preferably nine or more, preferably all ten) peaks selected from peaks with 2θ angles of: 9.33, 10.73, 14.85, 15.29, 15.77, 16.16, 18.60, 20.12, 21.00 and 23.22 ± 0.1 degrees 2θ , optionally ± 0.05 degrees 2θ .

Preferably, the XRPD spectrum of Form C has one, two, three, four or all five peaks selected from peaks with 2θ angles of: 10.73, 14.85, 15.29, 20.12 and 23.22 ± 0.1 degrees 2θ .

Preferably, the XRPD spectrum of Form C has all ten peaks with 2θ angles of: 9.33, 10.73, 14.85, 15.29, 15.77, 16.16, 18.60, 20.12, 21.00 and 23.22 ± 0.1 degrees 2θ , optionally ± 0.05 degrees 2θ .

Preferably, Form C has a XRPD spectrum substantially as shown in Figure 3 or Figure 20.

Form C may be further characterised by its Thermo Gravimetric Analysis (TGA) curve showing an endothermic event at about 149°C .

The TGA curve may show a weight loss of about 3% up to $120^{\circ}\text{C} \pm 2^{\circ}\text{C}$ correlated with water desorption.

Preferably, Form C has a TGA curve substantially as shown in Figure 11.

Form C may be further characterized by its differential scanning calorimetry (DSC) curve showing an endothermic event with a maximum at about $185^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Preferably, Form C has a DSC curve substantially as shown in Figure 7.

In a fourth embodiment of the first aspect there is provided a crystalline compound of formula (I), hereinafter “Form D”, characterised by an XRPD spectrum comprising four or more peaks (preferably five or more, preferably six or more, preferably seven or more, preferably eight or more, preferably nine or more, preferably all ten peaks) selected from peaks with 2θ angles of: 6.78, 15.45, 16.39, 17.10, 20.06, 20.63, 23.23, 23.68, 26.18 and 32.47 ± 0.05 degrees 2θ .

Preferably, the XRPD spectrum of Form D has one, two, three, four or all five peaks selected from peaks with 2θ angles of: 6.78, 16.39, 17.10, 20.63 and 23.23, ± 0.05 degrees 2θ .

Preferably, the XRPD spectrum of Form D has all ten peaks with 2θ angles of 6.78, 15.45, 16.39, 17.10, 20.06, 20.63, 23.23, 23.68, 26.18 and 32.47 ± 0.05 degrees 2θ .

Preferably, Form D has an XRPD spectrum substantially as shown in Figure 25.

In a fifth embodiment of the first aspect there is provided a crystalline compound of formula (I), hereinafter “Form E”, characterised by an XRPD spectrum comprising four or more peaks (preferably five or more, preferably six or more, preferably seven or more, preferably eight or more, preferably nine or more, preferably all ten peaks) selected from peaks with 2θ angles of: 6.82, 15.04, 15.68, 16.47, 17.17, 18.44, 20.69, 23.34, 23.88 and 25.38 ± 0.05 degrees 2θ .

Preferably, the XRPD spectrum of Form E has one, two, three, four or all five peaks selected from peaks with 2θ angles of: 15.04, 15.68, 16.47, 20.69 and 23.88 ± 0.05 degrees 2θ .

Preferably, the XRPD spectrum of Form E has all ten peaks with 2θ angles of: 6.82, 15.04, 15.68, 16.47, 17.17, 18.44, 20.69, 23.34, 23.88 and 25.38 ± 0.05 degrees 2θ .

Preferably, Form E has an XRPD spectrum substantially as shown in Figure 27.

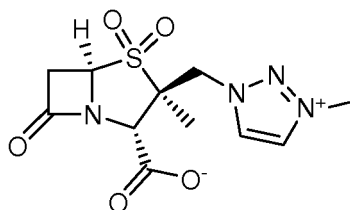
In a sixth embodiment of the first aspect there is provided a crystalline compound of formula (I), hereinafter “Form F”, characterised by an XRPD spectrum comprising four or more peaks (preferably five or more, preferably six or more, preferably seven or more, preferably eight or more, preferably nine or more, preferably ten or more, preferably all eleven peaks) selected from peaks with 2θ angles of: 12.73, 15.36, 15.95, 16.42, 18.12, 20.48, 22.85, 23.22, 27.04, 27.69 and 32.47 ± 0.05 degrees 2θ .

Preferably, the XRPD spectrum of Form F has one, two, three, four or all five peaks selected from peaks with 2θ angles of: 12.73, 15.36, 15.95, 16.42 and 20.48 ± 0.5 degrees 2θ .

Preferably, the XRPD spectrum of Form F has all eleven peaks with 2θ angles of: 12.73, 15.36, 15.95, 16.42, 18.12, 20.48, 22.85, 23.22, 27.04, 27.69 and 32.47 ± 0.05 degrees 2θ .

Preferably, Form F has an XRPD spectrum substantially as shown in Figure 29.

In a second aspect the invention provides a process for preparing crystalline compound of formula (I):



Formula (I)

the process comprising the steps of:

forming a formulation by dissolving or suspending an amorphous compound of formula (I) in a solvent or solvent mixture; and
crystallising the compound of formula (I) from the formulation.

The amorphous compound of formula (I) in the formulation may substantially all be dissolved in the formulation; may substantially all be dispersed in the formulation; or may partly be dissolved and partly dispersed in the formulation.

The quantity of the amorphous compound of formula (I) used in the process of the second aspect of the invention may be below a solubility limit of the amorphous compound in the solvent or solvent mixture, in which case the formulation is a solution, or may be above the solubility limit, in which case the formulation is a suspension.

Solvents for dissolving the amorphous compound of formula (I) may be selected from solvents in which the amorphous compound of formula (I) has a solubility at 20°C of greater than 200 mg/ml, optionally greater than 400 mg/ml. Solvents may be polar, protic or dipolar aprotic solvents. Exemplary polar, protic solvents are water; primary alcohols, preferably methanol, ethanol and 1-propanol. Further exemplary dipolar aprotic solvents are dimethylsulfoxide and N,N-dimethylformamide, N-methylpyrrolidone and the like. Primary alcohols are preferred. Methanol and ethanol are particularly preferred. Water content of a primary alcohol solvent is preferably less than 4 wt %, more preferably less than 2 wt %. When the primary alcohol is methanol the water content is preferably less than 1%.

Crystallisation of a crystalline compound of formula (I) may be induced by adding an antisolvent to a formulation containing dissolved amorphous compound of formula (I). Antisolvents may be solvents in which the amorphous compound of formula (I) has a solubility at 20°C of less than 50 mg/ml, optionally less than 30 mg/ml.

Antisolvents may be aprotic materials. Exemplary antisolvents are acetone, ethyl acetate, methyl-tert-butyl ether, heptane, 2-propanol, isopropyl acetate, diisopropyl ether, methylethyl ketone, tetrahydrofuran, anisole, and tert-butyl acetate.

In another embodiment of the second aspect, the amorphous compound of formula (I) may have little or no solubility in the solvent or solvent mixture used to form the formulation, in which case the formulation is a suspension.

A nucleating agent may be added to the formulation. The nucleating agent may be a crystalline seed of a compound of formula (I).

The purity of the solvent may affect solubility of the compound of formula (I) in the solvent, either in its amorphous form or in one or more of its crystalline forms.

The temperature of the formulation may be lowered following formation of the formulation. The solvent or solvent mixture may be heated during formation of the formulation, and may be cooled following formation of the formulation.

In a third aspect the invention provides crystalline compounds of formula (I) prepared by a process according to the second aspect of the invention.

The invention further provides crystalline compounds of formula (I) preparable by a process according to the second aspect of the invention.

For pharmaceuticals in which the active ingredient can exist in more than one polymorphic form, problems in dissolution and / or bioavailability of pharmaceutical compositions containing the compound can result if the manufacturing process leads to a polymorph with varying degrees of polymorphic purity and/or where the process does not control polymorphic interconversion.

If crystalline forms are made with polymorphic impurities, this may cause instability and it can accelerate significant interconversion to another polymorphic form. Therefore it is advantageous to produce crystalline forms with high polymorphic purity.

Preferably the crystalline compound of formula (I) according to the first or third aspects of the invention comprises more than 90 % of a single crystalline polymorph of the compound,

preferably more than 95 %, more preferably more than 99 %, even more preferably more than 99.5 % and most preferably more than 99.8 % as measured by XRPD or DSC, preferably as measured by XRPD. Preferably, the single polymorph is one of Form A, Form B, Form C, Form D, Form E, and Form F.

Preferably, the crystalline compound of formula (I) according to the first or third aspects of the present invention has a chemical purity of at least 95 wt %, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.5%, even more preferably at least 99.8%, and most preferably at least 99.9%, preferably as measured by HPLC.

The crystalline compound of formula (I) may be suitable for reconstitution with a pharmaceutically acceptable vehicle for administration.

In a fourth aspect of the present invention there is provided a pharmaceutical composition comprising an antibiotic and the crystalline compound of formula (I) according to the first or third aspects of the present invention. Preferably, the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

In a fifth aspect the invention provides a pharmaceutical composition according to the fourth aspect for treatment of bacterial infection.

In a sixth aspect the invention provides a method of treating a bacterial infection comprising administering to a patient in need thereof a therapeutically effective amount of the pharmaceutical composition according to the fourth aspect of the present invention.

In a seventh aspect the invention provides a method of forming a pharmaceutical composition comprising a compound of formula (I), the method comprising the step of dissolving or dispersing the crystalline compound of formula (I) in a carrier liquid. Optionally the carrier liquid is a pharmaceutically acceptable vehicle for intravenous injections such as Dextrose, Sodium chloride & Dextrose 5 mixture, Sodium chloride, Sodium lactate, etc. Optionally, the carrier liquid is an aqueous saline solution.

The concentration of a compound of formula (I) in the pharmaceutical composition range from 1mg/ml to 700mg/ml, preferably from 100 to 500mg/ml, more preferably from 150 to 250 mg/ml..

Description of the Drawings

The invention will now be described in more detail with reference to the Figures in which:

Figure 1 is a X-ray powder diffraction pattern of Form A of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 2 is a X-ray powder diffraction pattern of Form B of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 3 is a X-ray powder diffraction pattern of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 4 is a X-ray powder diffraction pattern of amorphous form of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 5 is a differential scanning calorimetric thermogram of Form A of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 6 is a differential scanning calorimetric thermogram of Form B of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 7 is a differential scanning calorimetric thermogram of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 8 is a differential scanning calorimetric thermogram of amorphous form of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 9 is a thermogravimetric curve of Form A of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 10 is a thermogravimetric curve of Form B of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 11 is a thermogravimetric curve of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 12 is a plot of HPLC response area vs. concentration for solutions or suspensions of amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 13 is a 25x magnified optical microscope image of Form A of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 14 is a 25x magnified optical microscope image of Form B of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 15 is a 25x magnified optical microscope image of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Figure 16 is a Raman spectrum of Form A of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Figure 17 is a FT-RT spectrum of Form A of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Figure 18 is a Raman spectrum of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Figure 19 is a FT-RT spectrum of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Figure 20 is a X-ray powder diffraction pattern of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide, obtained according to Example 13;

Figure 21 is a thermogravimetric curve of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide, obtained according to Example 13;

Figure 22 is a 25x magnified optical microscope image of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide, obtained according to Example 13;

Figure 23 is an ¹H-NMR spectrum of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Figure 24 shows particle size distribution curves of Form C of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide, obtained according to Example 13;

Figure 25 is a X-ray powder diffraction pattern of Form D of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 26 is a Raman spectrum of Form D of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Figure 27 is a X-ray powder diffraction pattern of Form E of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 28 is a Raman spectrum of Form E of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Figure 29 is a X-ray powder diffraction pattern of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figures 30 and 31 are Raman spectra of three batches of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Figures 32-39 are scanning electron microscopy images of samples of a first batch of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figures 40-46 are scanning electron microscopy images of samples of a second batch of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figures 47-50 are scanning electron microscopy images of samples of a third batch of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 51 is a FT-RT spectrum of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Figure 52 is a differential scanning calorimetric thermogram of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 53 is a thermogravimetric curve of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 54 is a gas evolution image of Evolved Gas Analysis (EGA) of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide;

Figure 55 is a plot of Dynamic Vapor Sorption (DVS) change in mass of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide; and

Figure 56 shows Dynamic Vapor Sorption (DVS) isotherm plots of Form F of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide.

Detailed Description of the Invention

The present invention provides crystalline (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide which is non-hygroscopic, thermally stable and has beneficial properties that avoid problems associated with the prior art forms.

The present invention further provides a process for forming crystalline (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide. The process allows formation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide in high polymorphic purity.

Suitable crystallization techniques for forming crystalline compounds of formula (I) include, without limitation, precipitation and re-crystallization (including antisolvent crystallization) processes, with or without seeding with nucleating agents. In a preferred embodiment, antisolvent crystallization processes are used.

Diluted, saturated or super-saturated solutions may be used for crystallization.

A solution of an amorphous compound of formula (I) may be cooled to promote crystallization of crystalline compounds of formula (I).

An amorphous compound of formula (I) may be dissolved at a temperature in the range of 20-50°C. The solution may be cooled down to about 0°C or about 10°C to promote the crystallization.

Methods of preparing crystalline forms of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide, include, without limitation, the following methods:

Form A Method 1:

- stirring a solution of amorphous compound of formula (I) in ethanol 96% at 20°C,
- collecting the solid by filtration.

Form A Method 2:

- stirring a saturated solution of amorphous compound of formula (I) in ethanol 96% at 20°C,
- adding methyl tert-butyl ether as antisolvent,
- stirring the mixture at room temperature overnight,
- collecting the solid by filtration.

Form A Method 3:

- stirring a saturated solution of amorphous compound of formula (I) in ethanol 96% at 20°C,
- seeding with nucleating agent,
- adding heptane as antisolvent,
- stirring the mixture at room temperature overnight,
- collecting the solid by filtration.
-

Form A Method 4

- stirring a saturated solution of amorphous compound of formula (I) in ethanol 96% at 20°C,
- seeding with nucleating agent,
- adding 2-propanol as antisolvent,
- stirring the mixture at room temperature overnight,
- collecting the solid by filtration.

Form A Method 5

- dissolving amorphous compound of formula (I) in ethanol 96% by heating to 35°C
- slowly adding (time: about 1 hour) methyl tert-butyl ether as antisolvent,
- cooling the mixture to 10°C
- stirring the mixture at 10°C overnight,

- collecting the solid by filtration.

Form A Method 6

- stirring a saturated solution of amorphous compound of formula (I) in ethanol 96% at 35°C,
- seeding the solution with nucleating agent,
- slowly adding (time: about 20 min.) methyl tert-butyl ether as antisolvent at 20°C,
- cooling the mixture to 20°C overnight,
- collecting the solid by filtration
-

Form A Method 7

- stirring a saturated solution of amorphous compound of formula (I) in ethanol 96% at 40°C,
- seeding the solution with nucleating agent,
- cooling the mixture to 20°C over about 5 hours,
- stirring the mixture at 20°C,
- collecting the solid by filtration

Form B Method 1

- stirring a saturated solution of amorphous compound of formula (I) in acetone at 40°C,
- collecting the solid by filtration.

Form C Method 1

- stirring a solution of amorphous compound of formula (I) in ethanol 99.8% at 40°C,
- seeding the solution with nucleating agent at 36°C
- cooling the solution at 15°C,
- stirring the mixture overnight

Forms D, E and F may be formed by crystallization from dimethylformamide solution. The present inventors have found that Forms D and E may crystallize initially from DMF solution but do not form once form F has formed. Without wishing to be bound by any theory, this may be due to Form F having greater stability than either Form D or Form E.

Surprisingly, the present inventors have found that one crystal form of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide can be used to form another crystal form of this compound. One of crystal forms A, B and C may be used as a seed in crystallisation of another of forms A, B and C.

A pharmaceutical composition as described herein may be in an injectable form for intravenous injection. The composition may contain stabilizing agents. The composition may be in suitable sterile solid form ready for reconstitution to form an injectable solution.

A pharmaceutical composition containing a crystalline compound of formula (I) as described herein may be administered either alone or may be co-administered with therapeutically effective amount of an antibiotic.

A pharmaceutical composition as described herein may comprise an antibiotic and may comprise one or more conventional pharmaceutically acceptable excipient(s).

Exemplary antibiotics are β -lactam antibiotics, in particular penicillins and cephalosporins and may be selected from Amoxicillin, Ampicillin, Apalcillin, Azlocillin, Bacampicillin, Carbenacillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Lenampicillin, Mecillinam, Methacillin, Mezlocillin, Nafcillin, Oxacillin, Penicillin G, Penicillin V, Piperacillin, Temocillin, Ticarcillin, Aztreonam, BAL30072, Carumonam, PTX2416, Tigemonam, Cefaclor, Cefadroxil, Cefalexin, Cefalotin, Cefamandole, Cefapirin, Cefazolin, Cefbuperazone, Cefdinir, Cefepime, Cefetamet, Cefixime, Cefmenoxime, Cefmetazole, Cefrnirox, Cefonicid, Cefoperazone, Cefotaxime, Cefotetan, Cefotiam, Ceftiofur, Cefovecin, Cefoxitin, Cefpodoxime, Cefprozil, Cefquinome, Cefradine, Cefminox, Cefsulodin, Ceftaroline, Ceftazidime, Ceftazole, Ceftibuten, Ceftizoxime, Ceftobiprole, Ceftolozane,

Ceftriaxone, Cefuroxime, Cefuzoname, Cephalexin, Cephalotin, Flomoxef, Latamoxef, Loracarbef Imipenem, Meropenem, Doripenem, Ertapenem, Biapenem, Panipenem, Faropenem or derivatives thereof.

The antibiotic may be selected from aminoglycosides: Amikacin, Arbekacin, Apramycin, Dibekacin, Gentamicin, Isepamicin, Kanamycin, Neomycin, Netilmicin, Plazomicin, Sisomicin, Spectinomycin, Streptomycin, Tobramycin or derivatives thereof.

The antibiotic may be selected from quinolones: Cinoxacin, Ciprofloxacin, Enofloxacin, Gatifloxacin, Gemifloxacin, Levofloxacin, Moxifloxacin, Nalidixic acid, Norfloxacin, Oxafloxacin, or derivatives thereof.

The antibiotic may be selected from antimicrobial peptides, for example Colistin, Polymyxin B or derivatives thereof.

A pharmaceutical composition as described herein may comprise only one or more than one antibiotic.

A pharmaceutical composition containing a crystalline compound of formula (I) may contain or be co-administered with bactericidal or permeability-increasing-g protein product (BPI) or efflux pump inhibitors to improve activity against gram negative bacteria and bacteria resistant to antimicrobial agents. Antiviral, antiparasitic, antifungal agents may also be administered in combination with the inhibitor compounds.

The pharmaceutical composition may contain complexing agents or anticoagulants, antioxidants, stabilizers, aminoglycosides, pharmaceutically acceptable salts or the like or mixtures thereof.

In particular the pharmaceutical composition may contain β -lactam antibiotics, preferably penicillins, cephalosporins, carbapenem, monobactams, more preferably piperacillin, cefepime; ceftriaxone; meropenem, aztreonam.

The pharmaceutical composition may contain buffers, for example sodium citrate, sodium acetate, sodium tartrate, sodium carbonate, sodium bicarbonate, morpholinopropanesulfonic acid, other phosphate buffers and the like and chelating agents like ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid, hydroxyethylenediaminetriacetic acid, nitrilotriacetic acid, 1,2-diaminocyclohexanetetraacetic acid, bis(2-aminoethyl)ethyleneglycoltetraacetic acid, 1,6-hexamethylenediaminetetraacetic acid and the like or pharmaceutically acceptable salts thereof.

A pharmaceutical composition as described herein may be administered to a human or warm-blooded animal by any suitable method, and preferably by intravenous injection.

Examples

All XRPD data described herein were acquired in transmission mode on an X'pert Pro instrument with X'celerator detector. The data were evaluated using the Highscore Plus software using copper as radiation source at a wavelength of 1.54Å.

DSC analyses were run on a TA Q2000 MDSC instrument.

TGA analyses were run on a TA Q5000 instrument. The data were evaluated using Universal Analysis software.

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide, was prepared according to example 1 of WO 2008010048, the contents of which are incorporated herein by reference.

Example 1

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form A

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (200 mg) was dissolved in ethanol 96% (0.5 mL). The solution was stirred at 20°C, after 30 minutes a solid was formed. The mixture was stirred for 4 hours at 20°C and the solid was isolated by filtration and dried overnight at room temperature in a vacuum oven. The obtained product (30 mg) was crystalline Form A which was characterized by an XRPD pattern as shown in Figure 1 and summarized in Table 1.

Angle [°2 θ]	d-spacing [Å]
8.8223	10.01516
12.0725	7.32517
14.4346	6.13137
14.9183	5.93364
16.2594	5.44711
18.2478	4.85778
19.0618	4.65213
19.7798	4.48485
20.8191	4.26326
23.5119	3.78074

DSC (Figure 5) showed the sample to have a melting endotherm with a maximum at 163°C. TGA thermal curve is shown in Figure 9.

An optical microscope image of Form A is shown in Figure 13.

Example 2

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form A

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (1 g) was suspended in ethanol 96% (3 mL). The resulting mixture was filtered through a syringe filter. The saturated

solution was treated with methyl tert-butyl ether (0.5 mL) as antisolvent. The antisolvent addition results in a solid precipitation. The mixture was stirred at room temperature overnight and the solid was isolated by filtration and dried overnight at room temperature in a vacuum oven. The solid recovered was crystalline Form A characterized by XRPD concordant with XRPD pattern given in Example 1.

Example 3

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form A

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (1 g) was suspended in ethanol 96% (5 mL). The resulting mixture was filtered through a syringe filter. A pinch of Form A material was added to the solution as seed. The seed was not dissolved and the saturated solution was treated with heptane (0.5 mL) as antisolvent. The antisolvent addition results in a solid precipitation. The mixture was stirred at room temperature overnight and the solid was isolated by filtration and dried overnight at room temperature in a vacuum oven. The solid recovered was crystalline Form A characterized by XRPD concordant with XRPD pattern given in Example 1.

Example 4

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form A

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (1 g) was suspended in ethanol 96% (5 mL). The resulting mixture was filtered through a syringe filter. A pinch of Form A material was added to the solution as seed. The seed was not dissolved and the saturated solution was treated with 2-propanol (0.5 mL) as antisolvent. The antisolvent addition results in a solid precipitation. The mixture was stirred at room temperature overnight and the solid

was isolated by filtration and dried overnight at room temperature in a vacuum oven. The solid recovered was crystalline Form A characterized by XRPD concordant with XRPD pattern given in Example 1.

Example 5

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form A

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (4 g) was weighed in a multimax vessel equipped with an impeller stirrer. The solid was suspended in ethanol 96% (32 mL). The mixture was heated to 35°C and stirred at 800 RPM. At 35°C the starting material seemed to be dissolved but the solution appeared slightly opaque. Methyl tert-butyl ether (8 mL) as antisolvent was added to the opaque solution over 1 hour. The addition of the antisolvent resulted in a solid formation. The mixture was cooled down to 10°C over 1 hour. During the cooling ramp the material became sticky and the majority of the material adhered to the vessel walls. The mixture was stirred overnight and the solid obtained was discharged from the vessel by mechanical removal of the sticky solid from the vessel wall. The obtained mixture was filtered under vacuum; the cake was dried at room temperature in a vacuum oven for 60 hours to afford 2.75 g of a white solid. The solid recovered was crystalline Form A characterized by XRPD concordant with XRPD pattern given in Example 1.

Example 6

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form A

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (5 g) was weighed in a multimax vessel equipped with an impeller stirrer. The solid was suspended in ethanol 96% (30 mL). The mixture was heated to 35°C and stirred at 800 RPM. At 35°C the starting material

seemed to be dissolved but the solution appeared slightly opaque. The opaque solution was filtered through a syringe filter to obtain a clear solution. A pinch of Form A material was added to the solution as seed; the seed was not dissolved and the mixture was cooled to 20°C over 45 minutes. At this temperature methyl tert-butyl ether (10 mL) was added as antisolvent over 20 minutes. The addition of the antisolvent resulted in a sticky solid formation, the majority of the material adhered to the vessel walls. The mixture was stirred overnight and the solid obtained was discharged from the vessel by mechanical removal of the sticky solid from the vessel wall. The obtained mixture was filtered under vacuum; the cake was dried at room temperature in a vacuum oven for 60 hours to afford 3.61 g of a white solid. The solid recovered was crystalline Form A characterized by XRPD concordant with XRPD pattern given in Example 1.

Example 7

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form A

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (7 g) was weighed in a multimax vessel equipped with an impeller stirrer. The solid was suspended in ethanol 96% (21 mL). The mixture was heated to 40°C and stirred at 400 RPM. At 40°C the starting material seemed to be dissolved but the solution appeared slightly opaque. The opaque solution was filtered through a syringe filter to obtain a clear solution. A pinch of Form A material was added to the solution as seed; the seed was not dissolved and the mixture was stirred at 40°C for 1 hour. The mixture is then cooled to 10°C over 5 hours and stirred for 60 hours. The obtained material adhered to the vessel walls and was discharged by mechanical removal of the sticky solid from the vessel wall. The obtained mixture was filtered under vacuum; the cake was dried at room temperature in a vacuum oven for 18 hours to afford 5.54 g of a white solid. The solid recovered was crystalline Form A characterized by XRPD concordant with XRPD pattern given in Example 1.

Example 8

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form B

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (200 mg) was suspended in acetone (0.5 mL) and the slurry was stirred for 4 hours at 40°C. The solid was isolated by filtration and dried overnight at room temperature in a vacuum oven. The obtained product (150 mg) was crystalline Form B which was characterized by an XRPD pattern as shown in Figure 2 and summarized in Table 2.

Angle [°2 θ]	d-spacing [Å]
9.3736	9.42739
10.343	8.54587
12.5922	7.024
13.172	6.71609
14.998	5.90227
15.636	5.66284
18.5083	4.79001
19.1049	4.64175
20.7935	4.26845
23.9264	3.71616

DSC (Figure 6) showed the sample to have a melting endotherm with a maximum at 180°C. TGA thermal curve is shown in Figure 10.

An optical microscope image of Form B is shown in Figure 14.

Example 9

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form C.

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (5 g) was weighed in a multimax vessel equipped with an impeller stirrer. The solid was suspended in ethanol HPLC grade 99.8% (20 mL). The mixture was heated to 40°C and stirred at 500 RPM. At 40°C the starting material seemed to be dissolved but the solution appeared slightly opaque. The opaque solution was filtered through a syringe filter to obtain a clear solution. The solution was cooled to 36°C over 15 minutes and Form B material (30 mg) was added to the solution as seed; the seed was not dissolved and promoted the product crystallization. The mixture was stirred at 36°C for 30 minutes and is then cooled to 15°C over 3.5 hours. The slurry was aged overnight and then was filtered under vacuum; the cake was dried at room temperature in a vacuum oven for 18 hours to afford 3.7 g of a white solid. The obtained product was crystalline Form C which was characterized by an XRPD pattern as shown in Figure 3 and summarized in Table 3.

Angle [°2 θ]	d-spacing [Å]
9.331	9.47026
10.7259	8.24161
14.8509	5.96039
15.2924	5.7893
15.7717	5.61443
16.1565	5.48158
18.6025	4.76595
20.1156	4.41074
20.9959	4.22776
23.2215	3.82734

DSC (Figure 7) showed the sample to have a melting endotherm with a maximum at 185°C.

TGA thermal curve is shown in Figure 11.

An optical microscope image of Form C is shown in Figure 15.

Comparative Example

The XRPD spectrum of amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide prepared as described in WO 2008/010048 is shown in Figure 4. No crystalline character is detectable in this spectrum.

Solubility evaluation

Solubility values of solvents were calculated with respect to the HPLC response factor, set out in Figure 12.

HPLC response factor was calculated for the amorphous compound of formula (I) using samples dissolved in acetonitrile/water 9/1 with the following method:

Column: ZORBAX Eclipse XDB-C18 (150x4.6mm, 5μm)

Temperature: 25 °C

Mobile phase: A: 0.05M Sodium ortophosphate/water, B: Acetonitrile

Gradient: from 5% of B to 95% of B in 10 min

Detector: UV λ= 220nm

Sample	Concentration (mg/ml)	HPLC area
1	0.62	333.445
2	1.24	660.935
3	1.68	1219.92
4	2.25	1643.32
5	2.30	1940.44
6	3.10	2830.31

Slurries of the amorphous compound of formula (I) in the selected solvents were prepared and stirred at 20 °C and 40 °C for 4 hours.

Samples of each slurry were filtered and the mother liquors injected in HPLC.

The solid residual were isolated and analyzed by XRPD.

The results are summarized in the following Table 4.

Table 4. Solubility of amorphous compound of formula (I)

Solvent	Solubility (mg/ml)	
	20°C	40°C
Acetone	0	0
Ethanol	420	>420
Ethyl acetate	0	0
Methyl tert-butyl ether	0	0
Heptane	0	0
Water	>400	>400
2-propanol	23	28
Iso-propyl acetate	0	0
Di-isopropyl ether	0	0
Methanol	>400	>400
Methylethyl ketone	0	0
Tetrahydrofuran	0	0
Anisole	0	0
Tert-butyl acetate	0	0
Dimethylsulfoxide	>400	>400
1-propanol	295	>400
1-butanol	97	167
Acetonitrile	6	n.a.
Chlorobenzene	0	n.a.

Dichloromethane	0	n.a.
1,4-dioxane	0	n.a.
Ethanol/methyl tert-butyl ether 20%	52	n.a.
Ethanol/methyl tert-butyl ether 40%	16	n.a.
Ethanol/acetone 20%	>300	n.a.
Ethanol/acetone 40%	>300	n.a.

Form A characterization by Raman spectrum and Fourier transform infrared spectroscopy (FT-IR)

The Raman spectrum of Form A is shown in Figure 16 with the related peak bands list in Table 5.

Peak list:

Position	Intensity
247.89	4066.127
268.70	4076.600
285.77	5666.532
297.80	7186.507
322.04	4385.802
411.78	3861.458
436.26	2433.529
499.66	2023.949
521.68	4054.372
560.04	2419.952
588.92	1163.452
629.52	6647.466
640.58	4792.760
687.14	1836.374
718.78	1714.527
758.37	1345.186
794.58	2302.231
836.54	1806.043
872.19	5315.287
932.18	1889.917
949.44	2637.407
962.31	2419.830
985.74	2736.112
1049.63	5534.104
1074.79	2056.236
1097.28	4171.412
1135.89	5311.271

1148.59	3581.329
1178.28	2121.957
1215.25	2643.923
1239.16	3338.948
1266.18	3677.753
1325.12	8522.793
1368.61	5404.136
1394.52	6973.028
1425.05	4802.836
1457.84	5583.813
1534.20	4855.332
1648.81	3369.165
1773.12	4261.622
2890.60	6428.710
2962.11	19794.615
2986.55	7243.053
3015.84	7382.472
3049.43	4000.206

Figure 17 shows the FT-IR spectrum of Form A with the related peak bands list in Table 6.

Peak list	
Position	Intensity
673.84	0.0846
686.51	0.118
718.98	0.111
756.67	0.0942
781.58	0.0916
797.38	0.100
834.89	0.0756
871.89	0.0672
932.05	0.0646
948.44	0.0932
1025.16	0.0712
1050.31	0.0580
1075.14	0.0752
1094.42	0.113
1134.65	0.124
1148.93	0.106
1204.60	0.0957
1240.06	0.0661
1235.85	0.0661
1309.76	0.147
1363.83	0.0819
1392.60	0.0512
1425.57	0.0468

1452.48	0.0538
1533.83	0.0601
1622.97	0.119
1766.49	0.109
2890.12	0.0390
2964.73	0.0446
3013.48	0.0440
3049.64	0.0414
3089.32	0.0425
3343.53	0.0427
3530.97	0.0395

Form C characterization by Raman spectrum and FT-IR

The Raman spectrum of Form C is shown in Figure 18 with the related peak bands list in Table 7.

Peak list:

Position	Intensity
240.20	4128.340
278.20	10739.558
299.77	10722.921
316.97	8908.874
389.49	3492.405
403.91	5676.352
419.31	6378.482
438.01	3159.695
514.23	9161.536
540.24	2881.736
560.59	5050.867
624.85	13700.852
640.80	5770.215
692.53	7222.112
715.48	2197.299
753.71	2920.133
800.11	2731.873
839.41	3232.516
868.99	6613.900
938.91	4443.281
967.79	3605.101
985.96	4480.407
1033.35	5823.568
1049.82	6638.105
1096.10	10022.146
1141.01	9717.918

1180.11	4361.805
1197.40	3267.057
1235.20	3502.896
1317.60	10464.665
1362.32	6745.435
1395.94	9937.875
1457.27	6235.580
1535.79	4771.901
1640.00	4841.217
1775.78	7336.955
2879.07	5109.468
2909.71	11865.885
2947.89	19208.596
2958.72	17883.816
2983.99	21848.400
2999.93	12395.464
3014.33	15550.745
3084.97	4124.013
3169.92	8548.841

The FT-IR spectrum of Form C is shown in Figure 19 with the related peak bands list in Table 8.

Peak list:

Position	Intensity
671.04	0.103
691.08	0.151
715.10	0.126
752.68	0.145
780.33	0.117
790.47	0.149
799.40	0.136
838.87	0.0751
868.41	0.0772
939.45	0.111
956.58	0.106
985.83	0.0629
1023.40	0.101
1089.49	0.135
1098.28	0.145
1138.00	0.213
1195.45	0.166
1233.08	0.0960
1269.19	0.142
1309.02	0.208

1361.03	0.114
1387.96	0.0675
1456.72	0.0694
1536.21	0.0645
1637.71	0.174
1770.33	0.174
2972.20	0.0498
3015.04	0.0553
3083.68	0.0481
3168.20	0.0456
3375.47	0.0422

The analyses performed on Form A and Form C, including the information collected on the influence of the water content during the crystallization, has supported the hypothesis that Form A is a hydrate form with a rapid water exchange with the ambient and Form C is a more stable anhydrous form. Therefore, Form C was selected for further optimisation and scale-up of the crystallization process, and assessments as described below.

Optimization of Form C crystallization

Example 10 - Crystallization procedure using a Form C seed

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form C

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (5 g) was weighed in a 50 ml multimax vessel equipped with an impeller stirrer. The solid was suspended in 20 ml of ethanol HPLC grade 99.8%. The mixture was heated to 40 °C and stirred at 700 RPM. At 40 °C the starting material was dissolved. The solution was cooled to 36 °C over 15 minutes and Form C material (27 mg) was added to the solution as seed; the seed was not dissolved and promoted the product crystallization. The mixture was cooled to 15 °C over 3.5 hours. The slurry was aged overnight and then was filtered under vacuum; the cake was dried at 30°C in a vacuum oven for 40 hours to afford 3.7 g of a white solid. The solid showed an XRPD pattern for Form C.

The quality of the ethanol system was also investigated in the production of Form C material using 96% ethanol instead of ethanol HPLC grade 99.8% as described in Example 11.

Example 11

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form A

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (5 g) was weighed in a 50 ml multimax vessel equipped with an impeller stirrer. The solid was suspended in 20 ml of ethanol 96%. The mixture was heated to 40 °C and stirred at 700 RPM. At 40 °C the starting material seemed to be dissolved but the solution appeared slightly opaque. The opaque solution was filtered through a syringe filter to obtain a clear solution. The solution was cooled to 35 °C over 15 minutes and Form C material (28 mg) was added to the solution as seed. After 10 minutes at 35 °C was dissolved. The temperature was lowered to 30 °C over 15 minutes and more Form C material (27 mg) was added as seed. The seed was dissolved after 15 minutes. The solution was heated up to 35 °C and a pinch of Form B material was added to the solution but was dissolved after few minutes. A pinch of Form A material was added as seed; this time the seed did not dissolve and promoted the product crystallization. The mixture was cooled to 15 °C over 3.5 hours. The slurry was aged overnight and then was filtered under vacuum; the cake was dried at 30°C in a vacuum oven for 18 hours to afford 3.1 g of a white solid. The solid showed an XRPD pattern concordant to Form A.

Examples 10 and 11 procedures demonstrate that the water content in the ethanol system can affect production of Forms A and C by a seeded approach. The formation of Form A material is possible in ethanol 96%, whereas the formation of Form C from a Form C crystal required use of ethanol HPLC grade 99.8%.

Example 12

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form C

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (8 g) was weighed in a 50 ml Multimax vessel equipped with an impeller stirrer. The solid was suspended in 20 ml of ethanol HPLC grade 99.8%. The mixture was heated to 40 °C and stirred at 800 RPM. At 40 °C the starting material was dissolved. The solution was cooled to 36 °C over 10 minutes and Form C material (24 mg) was added to the solution as seed; the seed was not dissolved and promoted the product crystallization. After 15 minutes stirring at 36 °C the mixture was cooled to 15 °C over 3.5 hours. The slurry was aged overnight and then was filtered under vacuum in nitrogen atmosphere (a funnel connected to a nitrogen flux was put over the filter). The cake was washed with 8 ml of ethanol HPLC grade 99.8%. The cake was dried inside the filter at 30 °C in a vacuum oven for 2 hours, after this time the product was transferred to a crystallizer and dried for further 16 hours. The product was analyzed by ¹H-NMR to check the solvent content and showed the presence of ~1.3% w/w of ethanol. The cake was further dried at 35 °C in the vacuum oven for 6 hours. A new sample was taken and analyzed by ¹H-NMR for solvent content. The ethanol residual was comparable to the first sample. The product was stored at -20 °C for the week-end and then put in the vacuum oven at 40 °C for 24 hours to yield 6 g of the product. The solid showed an XRPD pattern concordant with Form C. ¹H-NMR confirmed the presence of ~1.3% w/w of ethanol residual in the cake.

The decrease of the seed loading did not have any negative impact on the product crystallization and was implemented in the scaled-up procedure as described in Example 13.

Example 13

Preparation scale up of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form C at 36 g scale.

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (36.45 g) was weighed in a 250 ml multimax vessel equipped with an impeller stirrer. The solid was suspended in 146 ml of ethanol HPLC grade 99.8%. The mixture was heated to 40 °C over 20 minutes. After 15

minutes at 40 °C the starting material was completely dissolved and the solution was cooled to 36 °C over 10 minutes and Form C material (110 mg) was added to the solution as seed; the seed was not dissolved and promoted the product crystallization. After 10 minutes stirring at 36 °C the mixture was cooled to 15 °C over 3.5 hours. The obtained mixture was aged overnight and then was filtered under vacuum. The cake was washed with 40 ml of ethanol HPLC grade 99.8% and three times with 40 ml of methyl tert-butyl ether to remove residual ethanol from the cake. The cake was deliquored in nitrogen atmosphere (a funnel connected to a nitrogen flux was put over the filter) under vacuum. The cake was dried in a vacuum oven for 24 hours to yield 26.8 g of the final product as a white solid.

The solid was analyzed by XRPD, TGA, optical microscopy (OM) and ¹H-NMR.

The XRPD analysis of the product showed crystalline material with a pattern consistent with Form C (Figure 20).

The TGA analysis for the product (Figure 21) shows a weight loss of circa 2% up to 120°C probably due to adsorbed water and solvent residual.

The OM analysis in Figure 22 shows Form C crystals. Birifrangent particles using polarized light could be seen.

The ¹H-NMR spectrum (Figure 23) is consistent with the structure of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide. The ethanol residue was calculated comparing the ethanol signal at 1.06 ppm and the API signal at 1.40 ppm. Considering integrals values, number of protons and the molecular weight of the reference signals the estimated ethanol residue is equal to 0.4 % w/w respect to the API.

Solubility assessment in saline physiological solution

The Form C solubility was calculated by HPLC employing a dedicated walk-up method. The product obtained by the scaled up procedure described in Example 13 was used to perform the experiments.

1.9 g of the product was suspended in 1 ml of commercial physiologic solution (0.9% of NaCl) at ambient temperature (~20 °C). The suspension resulted slightly opaque and quite

viscous after 30 min. After this time the suspension was sampled and the sample injected in HPLC to determine its concentration. After 2 hrs stirring the solid residue was completely dissolved. The addition of more solid was not performed to avoid the gelatinisation of the viscous solution. A sample was taken and injected in HPLC to determine its concentration. The solution was stirred other 3 hrs and sampled again. The 5 hrs sample was also injected in HPLC to determine its concentration. The HPLC traces did not show the formation of significant impurities. Table 9 shows the solubility results for the time-points selected.

Physiological solution	Timepoint	Solubility (mg/ml) at ambient temperature
1	30 mins	772
2	2 hours	>883
3	5 hours	>812

Particle Size Distribution

The particle size analysis was performed on the product obtained by the scaled up procedure described in Example 13 using the procedure described below. Three measurements for each suspension were recorded and the results are shown in Figure 24 and in Table 10.

Sample Name	d (0.1)	d (0.5)	d (0.9)
Suspension 1, Measurement 1	29.08	129.34	249.92
Suspension 1, Measurement 2	28.94	128.69	246.31
Suspension 1, Measurement 3	28.90	128.42	247.37
Suspension 2, Measurement 1	28.26	130.37	251.74
Suspension 2, Measurement 2	26.80	125.95	248.77
Suspension 2, Measurement 3	25.40	119.25	239.11
Suspension 3, Measurement 1	28.54	133.06	256.35
Suspension 3, Measurement 2	26.85	128.64	249.66
Suspension 3, Measurement 3	26.09	126.42	244.15
Average	27.65	127.79	248.15

Example 14

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form D

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (30 g) was suspended in 200 mL of *N,N*-dimethylformamide, pre-heated to +20/25°C. After 5 minutes stirring a solution is obtained and after few minutes of stirring crystallization takes place. The suspension is stirred for about 2 hours. Then the suspension is cooled down to 0/+5°C and stirred for about 2 hours.

The obtained solid is filtered and washed with 50 mL of *N,N*-dimethylformamide pre-cooled to 0/+5°C. The wet product is then suspended in 300 mL of dichloromethane and the temperature is adjusted to +30/32°C. The suspension is stirred for 45 minutes then the solid is filtered and washed with 100 mL of dichloromethane pre-heated to +30/32°C. The product is dried under vacuum at +40°C until constant weight is achieved. The obtained product (19,3 g) was crystalline form D which was characterized by an XRPD pattern as shown in Figure 25 and summarized in the following Table 11.

No.	Angle [°2θ]	d-spacing [Å]	Height(cps)	FWHM(deg)	Int.	deg	Int.
1	6.7824	13.02204	369.45	0.2491	119.22	0.3227	333.64
2	9.5032	9.29904	105.18	0.2064	26.05	0.2477	403.40
3	10.4510	8.45774	31.17	0.2565	10.15	0.3256	324.89
4	11.6074	7.61762	109.02	0.2733	31.72	0.2910	305.11
5	12.7850	6.91847	41.58	0.2692	11.91	0.2865	310.18
6	13.4325	6.58642	64.83	0.2025	13.98	0.2156	412.47
7	14.2560	6.20776	275.43	0.2923	86.51	0.3141	286.05
8	15.4567	5.72810	77.92	1.8085	152.28	1.9543	46.30
9	16.3961	5.40199	835.69	0.4340	388.58	0.4650	193.15
10	17.1082	5.17871	522.62	0.3370	188.77	0.3612	249.00
11	18.2742	4.85081	148.14	0.3388	53.91	0.3639	248.02
12	20.0651	4.42173	194.88	0.5228	109.82	0.5635	161.19
13	20.6373	4.30040	624.11	0.3160	211.90	0.3395	266.91
14	22.7520	3.90524	167.10	0.2473	44.02	0.2635	342.21

15	23.2376	3.82472	236.56	0.6238	157.13	0.6642	135.79
16	23.6811	3.75409	198.42	0.5077	107.27	0.5406	167.00
17	25.6817	3.46600	163.48	0.4133	71.93	0.4400	205.89
18	26.1802	3.40112	205.89	0.5004	109.66	0.5326	170.25
19	26.9957	3.30020	138.32	0.4481	65.98	0.4770	190.41
20	27.7606	3.21098	221.24	0.3671	86.44	0.3907	232.85
21	28.7686	3.10073	75.61	0.2697	21.70	0.2870	317.65
22	30.4020	2.93775	125.52	0.3451	46.72	0.3722	249.17
23	31.4633	2.84104	102.42	0.6496	72.49	0.7078	132.70
24	32.4753	2.75478	268.97	0.4635	134.78	0.5011	186.43
25	34.4252	2.60307	21.60	0.6492	14.92	0.6911	133.80
26	34.9492	2.56524	12.83	0.4399	6.01	0.4682	197.76
27	36.0489	2.48946	80.97	0.3230	27.84	0.3438	270.15
28	38.4794	2.33762	119.18	0.6568	83.33	0.6992	133.80
29	40.2292	2.23989	27.09	0.9554	27.55	1.0170	92.49
30	42.6703	2.11723	63.61	0.5331	36.10	0.5675	167.09
31	43.9731	2.05748	22.45	0.4622	11.29	0.5030	193.59
32	53.8897	1.69994	16.28	0.5850	11.95	0.7339	159.10

The Raman spectrum of Form D is shown in Figure 26 with the related peak band list in the following Table 12 (using Raman Jasco RFT-600 instrument, light source Nd-YAG, 1064 nm: exciting wavelength).

Peak	Wave number	Y value	Peak	Wave number	Y value
1	3157.83	0.0281958	18	993.13	0.0292598
2	3009.34	0.072899	19	947.811	0.0372661
3	2974.63	0.100304	20	874.529	0.0711543
4	2904.24	0.0444922	21	838.853	0.028534
5	1772.23	0.0339617	22	783.892	0.0241906
6	1663.27	0.0258104	23	688.432	0.0231856
7	1474.28	0.0302334	24	661.434	0.0387182
8	1458.85	0.0264177	25	624.793	0.134281
9	1437.64	0.0373852	26	556.332	0.0499649
10	1400.04	0.0571817	27	514.87	0.0831978
11	1352.79	0.0302512	28	433.875	0.0597285
12	1302.65	0.0862235	29	414.59	0.0366139
13	1195.62	0.0247634	30	340.344	0.0295258
14	1175.37	0.0330307	31	324.916	0.058052
15	1138.73	0.0701386	32	287.311	0.0680197
16	1092.45	0.11397	33	249.706	0.0476452
17	1031.7	0.0433419			

Example 15

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form E

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (5 g) was suspended in 30 mL of *N,N*-dimethylformamide, pre-heated to +20/25°C. After 5 minutes of stirring, a solution is obtained and after few minutes a crystallization takes place. The suspension is stirred for about 2 hours.

The obtained solid is filtered and washed with 12,5 mL of *N,N*-dimethylformamide. The wet product is then suspended in 100 mL of ethyl acetate and the temperature is adjusted to +40/45°C. The suspension is stirred for 60 minutes then the solid is filtered and washed with 50 mL of ethyl acetate pre-heated to +40/45°C.

Finally the product is dried under vacuum at +40°C till constant weight is achieved.

The obtained product (2,4 g) was crystalline form E which was characterized by an XRPD pattern as shown in Figure 27 and summarized in the following Table 13.

No.	Angle [°2 θ]	d-spacing [Å]	Height(cps)	FWHM(deg)	Int.	deg)	Int.
1	6.8269	12.93732	260.98	0.2205	68.42	0.2622	376.99
2	9.5377	9.26545	102.12	0.2256	29.95	0.2933	369.14
3	10.4196	8.48314	117.20	0.2867	38.29	0.3267	290.56
4	11.6525	7.58825	82.26	0.2299	20.13	0.2447	362.76
5	12.6274	7.00451	83.34	0.3681	32.66	0.3919	226.76
6	13.3413	6.63125	95.57	0.3884	39.52	0.4135	215.07
7	14.2802	6.19726	104.47	0.2212	26.92	0.2577	378.06
8	15.0475	5.88296	494.41	0.3508	199.68	0.4039	238.55
9	15.6848	5.64531	378.40	0.3968	173.35	0.4581	211.11
10	16.4735	5.37678	557.04	0.3770	234.97	0.4218	222.38
11	17.1773	5.15801	229.03	0.3175	81.31	0.3550	264.28
12	18.4488	4.80530	297.04	0.3867	122.28	0.4117	217.36
13	19.0164	4.66312	93.86	0.2902	28.99	0.3089	289.90
14	20.0808	4.41830	143.91	0.4955	75.93	0.5276	170.05
15	20.6999	4.28752	421.62	0.3301	148.17	0.3514	255.52
16	22.2167	3.99811	90.66	0.6225	62.16	0.6857	135.85
17	22.7863	3.89944	130.42	0.4242	61.57	0.4721	199.52
18	23.3436	3.80760	273.25	0.4007	120.48	0.4409	211.43
19	23.8843	3.72261	447.75	0.4942	242.53	0.5417	171.61
20	25.3818	3.50627	95.40	1.0693	109.22	1.1449	79.54
21	26.2231	3.39566	113.17	0.5204	63.45	0.5606	163.70
22	27.8574	3.20005	112.17	0.2916	35.41	0.3157	293.14
23	29.9383	2.98219	52.64	0.4091	38.32	0.7279	209.96
24	31.3100	2.85459	70.72	0.3247	40.45	0.5720	265.41
25	33.3041	2.68809	41.89	0.3114	17.99	0.4295	278.15

26	38.5117	2.33574	15.66	1.2693	21.16	1.3512	69.24
27	41.1953	2.18957	21.01	1.1036	24.69	1.1748	80.32
28	49.2559	1.84846	16.52	0.9722	17.90	1.0835	93.88

The Raman spectrum of Form E is shown in Figure 28 with the related peak band list in the following Table 14 (using Raman Jasco RFT-600 instrument, light source Nd-YAG, 1064 nm: exciting wavelength).

Peak	Wave number	Y value	Peak	Wave number	Y value
1	3158.8	0.0221892	18	989.273	0.0474664
2	3051.77	0.0289691	19	949.74	0.0622083
3	3011.27	0.0722239	20	873.565	0.0993489
4	2973.67	0.168653	21	836.924	0.0338838
5	2900.38	0.0707581	22	782.927	0.0376849
6	1772.23	0.0829712	23	715.431	0.0287148
7	1482	0.036189	24	589.397	0.028656
8	1438.61	0.0318463	25	646.006	0.0458421
9	1397.14	0.0563706	26	624.793	0.177092
10	1352.79	0.0498639	27	556.332	0.0561384
11	1302.65	0.120509	28	613.906	0.109643
12	1266.01	0.0335002	29	433.875	0.0568177
13	1202.37	0.0466914	30	407.84	0.0759362
14	1185.01	0.0332323	31	325.881	0.68886
15	1139.69	0.0742465	32	288.276	0.0859623
16	1092.45	0.128341	33	254.527	0.050626
17	1031.7	0.0532132	34	216.922	0.0240766

Example 16

Preparation of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form F

Amorphous (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide (130 g) was suspended in 800 mL of *N,N*-dimethylformamide, pre-heated to +20/25°C. 100 mL of *N,N*-dimethylformamide was added to wash the walls of the flask. After 5 minutes stirring a solution is obtained and after few minutes of stirring crystallization takes place. The suspension is stirred for about 3 hours. Then the suspension is cooled down to 0/+5°C and stirred for about 3 hours.

The obtained solid is filtered and washed with 300 mL of *N,N*-dimethylformamide pre-cooled to 0/+5°C. The wet product is then suspended in 700 mL of ethyl acetate and the temperature is adjusted to +40/45°C. The suspension is stirred for 30 minutes then the solid is filtered and washed with 150 mL of ethyl acetate pre-heated to +40/45°C. The procedure

with the suspension in Ethyl acetate is repeated twice. Finally the product is dried under vacuum at +40°C till constant weight is achieved.

The obtained product (65-66 g, molar yield about 76%, with an assay of 98-99% was crystalline form F, which was characterized by an XRPD pattern as shown in Figure 29 and summarized in the following Table 15.

No.	Angle [°2 θ]	d-spacing [Å]	Height(cps)	FWHM(deg)	Int.	deg)	Int.
1	8.5718	10.30725	116.72	0.1981	26.19	0.2244	419.96
2	10.3165	8.56773	182.16	0.2142	42.67	0.2343	388.97
3	12.7398	6.94292	420.49	0.2216	103.83	0.2469	376.75
4	15.3615	5.76339	870.60	0.2471	241.26	0.2771	338.84
5	15.9547	5.55042	1374.98	0.2605	400.47	0.2913	321.60
6	16.4290	5.39123	1343.96	0.2344	352.88	0.2626	357.69
7	17.1990	5.15158	477.25	0.2281	118.86	0.2490	367.89
8	18.1207	4.89155	531.20	0.2398	146.12	0.2751	350.36
9	20.4870	4.33160	915.19	0.2443	275.10	0.3006	345.16
10	21.4040	4.14805	37.20	0.1769	7.01	0.1884	477.23
11	22.8548	3.88791	528.69	0.2904	164.14	0.3105	291.53
12	23.2204	3.82751	502.41	0.3500	188.64	0.3755	242.00
13	23.4688	3.78756	292.42	0.1501	47.04	0.1609	564.73
14	24.4199	3.64215	132.35	0.2404	34.95	0.2641	353.09
15	25.6394	3.47163	359.02	0.2563	104.03	0.2897	331.96
16	25.9983	3.42450	94.56	0.2531	27.13	0.2869	336.47
17	26.2914	3.38699	134.69	0.2951	45.04	0.3344	288.79
18	27.0457	3.29421	387.38	0.3463	151.47	0.3910	246.47
19	27.6934	3.21862	412.53	0.2941	136.95	0.3320	290.62
20	28.7394	3.10381	190.86	0.2739	56.91	0.2982	312.74
21	29.7603	2.99962	32.77	0.2736	9.54	0.2913	313.76
22	30.3078	2.94667	222.03	0.2854	67.46	0.3038	301.19
23	31.4660	2.84080	125.87	0.5371	71.97	0.5717	160.49
24	32.3054	2.76888	98.55	0.2002	21.00	0.2131	431.51
25	32.4785	2.75451	363.46	0.4069	157.43	0.4331	212.38
26	33.1981	2.69643	37.54	0.2403	9.60	0.2558	360.31
27	33.7446	2.65401	15.05	0.5057	8.10	0.5383	171.46
28	34.3283	2.61020	55.64	0.1955	11.58	0.2081	444.20
29	35.0200	2.56021	21.77	0.6046	14.01	0.6435	143.92
30	35.9880	2.49354	133.13	0.2751	38.98	0.2928	317.16
31	38.4256	2.34077	142.45	0.6826	103.50	0.7266	128.73
32	40.2911	2.23659	56.34	0.4183	25.09	0.4453	211.28
33	40.8969	2.20485	33.86	0.3473	12.52	0.3697	254.95
34	42.6047	2.12034	130.78	0.2718	59.44	0.4545	327.66
35	43.7327	2.06823	39.36	0.5339	22.37	0.5684	167.46
36	44.8088	2.02103	29.53	0.2009	6.31	0.2138	446.84
37	53.9562	1.69800	23.47	0.6255	15.68	0.6680	148.86

Raman spectra for three batches of Form F are shown in Figures 30 and 31.

Both XRPD and Raman spectra acquired for different batches of Form F product are overlapping.

Scanning electron microscopy images of samples of the three batches of Form F are shown in Figures 32-50. The SEM images of the samples were obtained using a JEOL JSM 5500 LV scanning electron microscope, operating at 30 kV in low vacuum (30 Pa) with the backscattered electron technique.

Form F characterization by FT-IR, DSC, TGA, EGA

Figure 51 shows the FT-IR spectrum of Form F with the related peak bands list in Table 16.

Peak list:

Position	Intensity
502	50.668
514	59.193
538	66.311
554	48.279
586	76.021
623	60.523
635	58.506
675	73.819
688	65.213
711	63.330
752	53.517
783	68.207
808	55.605
826	52.413
872	72.360
908	81.158
928	78.947
948	62.908
953	63.041
989	78.973
1020	62.785
1067	55.907
1088	52.453
1102	46.426
1136	35.517
1186	50.232
1199	50.943
1228	75.847
1266	64.974
1300	44.572

1307	44.644
1351	62.003
1396	78.685
1472	75.504
1525	78.318
1637	36.877
1735	80.927
1771	48.478
1783	51.962
2898	88.274
2972	84.793
3017	86.781
3051	88.751
3156	84.061

The DSC profile of form F is presented in Figure 52. The DSC profile shows an exothermic peak at approximately 184°C (Onset 175°C) associated with the degradation of the sample. The Thermo Gravimetric Analysis (TGA) profile of Form F presented on Figure 53 shows a significant weight loss after approximately 160°C associated with the degradation of the sample. That is further confirmed by an Evolved Gas Analysis (EGA) shown in Figure 54. The EGA evidences that the event observed in TGA analysis is caused by the loss of degradation products (e.g. carbon dioxide, sulphur dioxide, etc).

Form F characterization by Dynamic Vapor Sorption (DVS)

Kinetic moisture sorption measurements were performed at 25°C and at relative humidity (RH% target as follows:

- From 40%RH to 90%RH
- From 90%RH to 0%RH
- From 0%RH to 90%RH
- From 90%RH to 0%RH

The obtained results are presented in Figure 55, wherein the red line traces the percentage changes in mass as function of the time, while the blue line traces the relative humidity changes as function of the time.

DVS isotherms plots are reported in Figure 56, wherein the red line depicts the first sorption phase, the blue line depicts the first desorption phase, the green line depicts the second sorption phase and the pink line depicts the second desorption phase.

The DVS analyses show that Form F is stable at up to approximately 50% RH and that at 90% RH, the sample showed a weight increase that is greater than 50%w/w. After this event the sample releases and takes water reversibly.

Stability of Form F

The sample becomes a viscous liquid after a day at 25°C and 60%RH and after a day at 60°C and 75%RH.

Hygroscopicity of Form F

The hygroscopicity was calculated using the following equation:

$$\% \text{ Weight Change} = [(W2-W1)/W1]*100$$

wherein,

W1 is weight of sample at the start of the experiment; and

W2 is weight of sample at 25°C and 80%RH in the first absorption cycle.

Obtained results show that the sample is very hygroscopic, with a mass increase that is greater than 15%, and becomes a viscous liquid at high humidity.

The analytical methods used for the product assessment are performed as described below.

Analytical methods

HPLC method

Column: ZORBAX Eclipse XDB-C18 (150x4.6mm, 5µm); column temperature 25 °C

Mobile phase: A: Sodium dihydrogen orthophosphate dihydrate 0.05 M; B: Acetonitrile

Gradient:

Time (min)	% A	% B
0	95	5
10	5	95
10.2	95	5
12	95	5

Flow: 1.0 mL/min

Detector: UV DAD@220nm

The obtained crystalline products of (2*S*,3*S*,5*R*)-3-methyl-3-((3-methyl-1*H*-1,2,3-triazol-3-ium-1-yl)methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide Form A, B, C, D, E and F have an HPLC purity of at least 98%, preferably at least 99%, preferably at least 99.5%, preferably at least 99.6%, preferably at least 99.7%, preferably at least 99.8%, preferably at least 99.9%.

NMR

The samples for NMR analysis were prepared by complete dissolution of an appropriate amount of material in approximately 0.75ml of NMR solvent (DMSO-*d*6)

¹H NMR spectra were recorded at 25 °C using an either a Varian INOVA 400MHz NMR Spectrometer equipped with a Varian ATB probe.

Variable number of scans (16-256) was applied, using standard acquisition parameters. The pre-acquisition delay was set to 10 sec whenever NMR quantification was carried out. Appropriate phasing and baseline corrections were applied in processing the spectra.

XRPD

The XRPD spectra were collected in transmission mode on an analytical X'pert Pro instrument with X'celerator detector using a standard Aptuit method. The data were evaluated using the HighScore Plus software. The instrumental parameters used are listed below.

Instrumental parameter	Value
2-theta range	2-45
Step size [°2-theta]	0.0170
Time per step [sec]	60.7285 sec
Wavelength [nm]	0.154060 (Cu K-Alpha1)
Rotation [Yes/No]	Yes
Slits divergence/antiscatter.	Incident Mask fixed 10mm ; Divergence slits 1/2, Antiscat.slits 1/2 on incident beam ; 1/32 on diffracted

X-ray Mirror	Inc. Beam Cu W/Si focusing MPD, Acceptance Angle 0.8°, Length 55.3mm
Temperature	Room temperature
Humidity values [%RH]	Ambient
Fixed Slits	0.02 rad fixed Soler slits on incident and diffracted beam
Monochromator	None
Detector type	X'celerator (active length 2.122 2theta degree), scanning mode
Sample holder	Transmission sample holder. Use Insert to keep thickness at 1mm, 5mm diameter
Configuration	Transmission
Generator voltage/current	40KV / 40mA

Optical microscopy

Optical microscopy analyses were run on the Leica DM microscope equipped with a double polarizer and digital camera. The method parameters are listed below.

	Value
Polarized light [Y/N]	Yes
Magnification [eyepiece]	10x
Objective	Typically 5x, 10x, 20x, 40x
Filter slider	Use the best filter to optimize the image

TGA and DSC

The TGA analyses were run on a TA Q5000 instrument or on Mettler Toledo Star System (Form F analysis). The DSC analyses were run on the TA Q2000 MDSC or on the DSC 200 F3 Maia (Form F analysis) instruments. DSC and TGA method details are listed below:

TGA	
Instrumental parameter	Value
Balance purge gas [mL/min]	10

Sample purge gas [mL/min]	25
Gas	Nitrogen
Temperature-Time-Rate	Typically from room temperature to 250/350°C at 10°C/min (TA Q5000 instrument); or to 450°C at 10°K/min (Mettler Toledo Star System)
Typical sample amount [mg]	Usually from 2 mg to 20 mg
Pan [Pt/Al]	Hermetically sealed Al (punched)
DSC	
Instrumental parameter	Value
Cooling [ON/OFF]	ON
Gas	Nitrogen
Temperature-Time-Rate	From 0°C to ~160°C. Ramp at 10°C/min (TA Q2000 MDSC); or from 25°C to ~350°C. Ramp at 10°K/min (DSC 200 F3 Maia).
Typical sample amount [mg]	Usually from 0.5 mg to 2.5 mg
Pan	Not hermetic Al (TA Q2000 MDSC); or hermetically sealed Al ((DSC 200 F3 Maia)

Raman

Raman analyses were performed with a Keiser Optical Systems RXN1 MicroRaman with Leica Microscope and digital camera

Instrumental Parameter	Value
Probe	N
Objective	50x, 50x LWD, 10x
Exposure [sec]	Typically 0.5 - 1
Laser Power [mW]	50 - 400
Autofocus [Y/N]	Typically N
Accumulation	Typically 10
Cosmic ray filter [Y/N]	Y

Intensity calibration [Y/N]	Y
Dark subtract [Y/N]	Y

FT-IR

FT-IR analyses were performed with a Thermo Nicolet Nexus 470 FT-IR or with a Thermo Nicolet 6700 FT-IR (Form F analyses).

Instrumental Parameter	Value
Accessory	Attenuated Total Reflectance (ATR) – ZnSe Crystal
# of scans	64
Resolution [cm^{-1}]	4
Gain	Autogain
Detector	DTGS KBr
Spectral Range [cm^{-1}]	4000 - 650

Particle Size Distribution

Particle Size Distribution by laser light scattering was performed after developing a wet dispersion method using Malvern Mastersizer 2000 instrument. The method parameters are listed below.

Instrument	Malvern Mastersizer 2000
Accessory	Hydro2000S+
Parameter	Value
Stirring speed	1750rpm
Dispersant	0.1% w/v Span85/Cyclohexane
Sample Quantity	Around 100mg suspended in 5mL of dispersant
Calculation Model	General Purpose - Irregular
Optical Model	Fraunhofer with 1.426 refractive index for the dispersant
Sweeps number	15000 background/ 15000 sample
Laser Obscuration [%]	between 5 and 20% (typically 8-12%)

The experiments were conducted using the following sample preparation:

- (i) 100 mg of material were weighted in a 10 ml vial and they were suspended in 5mL of dispersant;
- (ii) once the material was all wetted the suspension was added into the cell and the vial was washed using additional 5 mL of the dispersant;
- (iii) the suspension was measured immediately.

EGA

The EGA analysis was carried out on the gas produced during the TGA analysis.

DVS Analyses

Instrument Details

Temperature range: 20–40°C (standard)

Maximum sample mass: (low/high mass instrument) 1g/4g

Mass change: +/- 150mg

Stability (24 hours @ 25°C and 0%RH) <5µg

Mass resolution: +/- 0.1µg

Humidity Range: 0-98%RH

RH Accuracy: +/- 1%RH

Temperature stability: +/- 0.1°C

Typical gas flow rate: 100/200sccm

Sample chamber: 40mm wide x 50mm deep x 50mm high

Reservoir volume: 100ml reservoir capacity

Heating system: Peltier + Cartridges

The kinetic moisture sorption measurement was performed at 25°C and in a RH% range described in the following:

From 40%RH to 90%RH

Form 90%RH to 0%RH

From 0%RH to 90%RH

From 90%RH to 0%RH

The experiment is performed on 10-15 mg of sample and the equilibrium criterion is set as $dm/dt < 0.002\%$ w/w in 10 min with a maximum step time of 240 min.

Stability tests

The sample was positioned on the sample holder and stored in the following conditions:

25°C and 60%RH for 7 days

60°C and 75%RH for 3 days

The samples were analyzed after the test by XRPD.

Hygroscopicity

The hygroscopicity of the sample was determined using the method reported in the academic article “*Efficient throughput method for hygroscopicity classification of an active and inactive pharmaceutical ingredients by water vapor sorption analysis*” V. Murikipudi et al., *Pharmaceutical Development and Technology*, 2013, 18(2): 348-358.

The hygroscopicity was calculated using the following equation:

% Weight Change = $[(W2-W1)/W1]*100$; wherein

W1 is a weight of sample at the start of the experiment; and

W2 is a weight of sample at 25°C and 80%RH in the first absorption cycle.

Classification Criteria

Non hygroscopic: increase in mass is less than 0.2%;

Slightly hygroscopic: increase in mass is less than 2% and equal to or greater than 0.2%;

Hygroscopic: increase in mass is less than 15% and equal to or greater than 2%;

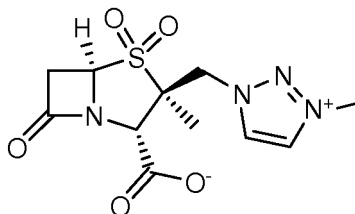
Very Hygroscopic: increase in mass is equal to or greater than 15%; and

Deliquescent: sufficient water is absorbed to form a liquid.

Although the present invention has been described in terms of specific exemplary embodiments, it will be appreciated that various modifications, alterations and/or combinations of features disclosed herein will be apparent to those skilled in the art without departing from the scope of the invention as set forth in the following claims.

Claims

1. A crystalline compound of formula (I):

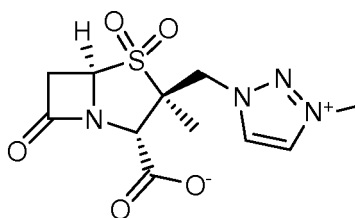


Formula (I).

2. A crystalline compound according to claim 1 characterised by an XRPD spectrum comprising four or more peaks selected from peaks with 2θ angles of: 8.82, 12.07, 14.43, 14.92, 16.26, 18.25, 19.06, 19.78, 20.82 and 23.51 ± 0.1 degrees 2θ .
3. A crystalline compound according to claim 1, with an XRPD spectrum substantially as shown in Figure 1.
4. A crystalline compound according to any one of claims 2 or 3 characterised by a Thermo Gravimetric Analysis (TGA) curve showing an endothermic event at about $163^\circ\text{C} \pm 2^\circ\text{C}$.
5. A crystalline compound according to any one of claims 2 or 3 characterised by a Differential Scanning Calorimetry (DSC) curve showing an endothermic event at about $163^\circ\text{C} \pm 2^\circ\text{C}$.
6. A crystalline compound according to claim 1 characterised by an XRPD spectrum comprising four or more peaks selected from peaks with 2θ angles of: 9.37, 10.34, 12.59, 13.17, 15.00, 15.63, 18.51, 19.10, 20.79 and 23.93 ± 0.10 degrees 2θ .
7. A crystalline compound according to claim 1, with an XRPD spectrum substantially as shown in Figure 2.

8. A crystalline compound according to any one of claims 6 or 7 characterised by a Thermo Gravimetric Analysis (TGA) curve showing an endothermic event at about $155^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
9. A crystalline compound according to any one of claims 6 or 7 characterised by a Differential Scanning Calorimetry (DSC) curve showing an endothermic event at about $180^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
10. A crystalline compound according to claim 1 characterised by an XRPD spectrum comprising four or more peaks selected from peaks with 2θ angles of: 9.33, 10.73, 14.85, 15.29, 15.77, 16.16, 18.60, 20.12, 21.00 and 23.22 ± 0.1 degrees 2θ .
11. A crystalline compound according to claim 1, with an XRPD spectrum substantially as shown in Figure 3 or in Figure 20.
12. A crystalline compound according to any one of claims 10 or 11 characterised by a Thermo Gravimetric Analysis (TGA) curve showing an endothermic event at about $149^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
13. A crystalline compound according to any one of claims 10 or 11 characterised by a Differential Scanning Calorimetry (DSC) curve showing an endothermic event at about $185^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
14. A crystalline compound according to claim 1 characterised by an XRPD spectrum comprising four or more peaks selected from peaks with 2θ angles of: 6.7824, 15.4567, 16.3961, 17.1082, 20.0651, 20.6373, 23.2376, 23.6811, 26.1802 and 32.4753 ± 0.05 degrees 2θ .
15. A crystalline compound according to claim 1, with an XRPD spectrum substantially as shown in Figure 25.

16. A crystalline compound according to claim 1 characterised by an XRPD spectrum comprising four or more peaks selected from peaks with 2θ angles of: 6.8269, 15.0475, 15.6848, 16.4735, 17.1773, 18.4488, 20.6999, 23.3436, 23.8843 and 25.3818 ± 0.05 degrees 2θ .
17. A crystalline compound according to claim 1, with an XRPD spectrum substantially as shown in Figure 27.
18. A crystalline compound according to claim 1 characterised by an XRPD spectrum comprising four or more peaks selected from peaks with 2θ angles of: 12.7398, 15.3615, 15.9547, 16.4290, 18.1207, 20.4870, 22.8548, 23.2204, 27.0457, 27.6934 and 32.4785 ± 0.05 degrees 2θ .
19. A crystalline compound according to claim 1, with an XRPD spectrum substantially as shown in Figure 29.
20. A crystalline compound according to any one of claims 18 or 19 characterised by a Thermo Gravimetric Analysis (TGA) curve showing an endothermic event at about 160°C .
21. A crystalline compound according to any one of claims 18 or 19 characterised by a DSC profile showing an exothermic peak at approximately 184°C .
22. A process for preparing a crystalline compound of formula (I):



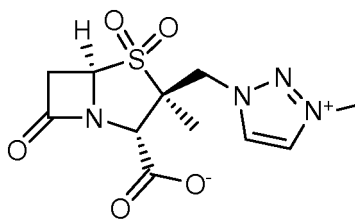
Formula (I)

the process comprising the steps of:

forming a formulation by dissolving or suspending an amorphous compound of formula (I) in a solvent or solvent mixture; and

crystallising the compound of formula (I) from the formulation.

23. A process according to claim 22 wherein the amorphous compound of formula (I) is dissolved in a solvent or solvent mixture in which the amorphous compound of formula (I) has a solubility at 20°C of greater than 200 mg/ml.
24. A process according to claim 23 wherein solvent or solvent mixture is selected from water, methanol, ethanol, 1-propanol, dimethylsulfoxide, N,N-dimethylformamide and mixtures thereof.
25. A process according to claim 23 or 24 comprising the step of adding to the formulation an antisolvent in which the amorphous compound of formula (I) has a solubility at 20°C of less than 50 mg/ml.
26. A process according to claim 25 wherein the antisolvent is selected from acetone, ethyl acetate, methyl-tert-butyl ether, heptane, 2-propanol, isopropyl acetate, diisopropyl ether, methylethyl ketone, tetrahydrofuran, anisole, tert-butyl acetate and mixtures thereof.
27. A process according to claim 22 wherein the amorphous compound of formula (I) is suspended in a solvent or solvent mixture.
28. A process according to any of claims 22-27 wherein a nucleating agent is added to the formulation.
29. A process according to claim 28 wherein the nucleating agent is a crystalline seed of a compound of formula (I).
30. A process according to any of claims 22-29 wherein the temperature of the formulation is lowered following formation of the formulation.
31. A crystalline compound of formula (I)



Formula (I)

wherein the compound is prepared by a process according to any of claims 22-30.

32. A crystalline compound of formula (I) according to any of claims 1-21 and 31 wherein the compound comprises more than 90 % of a single crystalline polymorph of the compound.
33. A crystalline compound of formula (I) according to any of claims 1-21 and 31 having a chemical purity of at least 95 wt %.
34. A pharmaceutical composition comprising a crystalline compound of formula (I) according to any of claims 1-21 and 31-33.
35. A pharmaceutical composition according to claim 34 further comprising an antibiotic.
36. A pharmaceutical composition according to claim 34 or 35 for use in treatment of bacterial infection.
37. A method of treating a bacterial infection comprising administering to a patient in need thereof a therapeutically effective amount of the pharmaceutical composition according to claim 34 or 35.
38. A method of treating a bacterial infection comprising co-administering to a patient in need thereof a therapeutically effective amount of the pharmaceutical composition according to claim 34 with therapeutically effective amount of an antibiotic.