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# Development of Drug Substances as Mixture of Polymorphs: Studies to Control Form 3 in Casopitant Mesylate

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**Abstract:**

**Polymorphism is characterized as the ability of a drug substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice and this can impact the physical properties of a drug substance. In this paper the studies carried out on casopitant mesylate, a $NK_1$ antagonist developed in GlaxoSmithKline (GSK), are reported.**

**During process development studies it was discovered that what was initially considered a single crystalline phase, Form 1, was actually a mixture of two different forms, Form 1 and Form 3. A retrospective analysis of all the key drug substance batches clearly indicated that Form 3 was always present as minor component in mixture with Form 1. Furthermore any attempt to generate either pure Form 1 or pure Form 3 failed. As a result of this, the project team explored the opportunity to develop the drug substance as a mixture of polymorphs. The studies performed to assess the ability of the manufacturing process to control the amount of Form 3 in the drug substance, according to the Quality by Design principle and the assessment of the impact of this finding on the drug product performance are reported in this paper. Collectively, the data demonstrated that the level of Form 3 in the drug substance (up to a level of 27% w/w) is not a drug substance critical quality attribute (drug substance-CQA) for casopitant mesylate.**

## 1. Introduction

Many pharmaceutical solids can exist in different physical forms. Polymorphism is characterized as the ability of a drug substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice. Polymorphic forms of a drug substance can have different chemical and physical properties, including melting point, apparent solubility, dissolution rate, optical and mechanical properties, vapour pressure, and density. These properties can have a direct effect on the ability to process and/or manufacture the drug substance and the drug product, as well as on drug product stability, dissolution, and bioavailability. Polymorphism can affect the quality, safety, and efficacy of the drug product.[1] This explains why it has to be considered an important attribute for a drug substance and its control has to be achieved.[2]

Casopitant was identified as a potent $NK_1$ antagonist by GlaxoSmithKline (GSK). It was selected as part of a wide drug discovery programme within GSK for its potential activity in a number of therapeutic areas for the treatment of inflammatory bowel disease, overactive bladder, CNS disorders, and emesis.

A Quality by Design approach has been applied to the development of the manufacturing process for casopitant mesylate. This approach, where the quality is built-in rather than tested in the product, is described in published guidelines (ICH Q8, ICH Q9, and ICH Q10).[3] For example, ICH Q8 describes an enhanced approach by the use of process understanding. ICH Q9 describes the risk management tools that can be used to successfully manage the risk, and ICH Q10 introduces the concept of a control strategy, defined as a set of controls, derived from current product and process understanding to assure process performance and to obtain drug substance that meets the critical quality attributes (CQA, the measurable properties that are critical to ensuring patient safety and efficacy).

Casopitant mesylate Form 1 was initially selected for full development as a result of its physicochemical properties. Among the attributes of casopitant mesylate, crystalline form was defined as a drug substance-CQA. Extensive polymorph screening on the molecule was carried out according to due diligence strategies: Form 1 has always appeared as the most thermodynamically stable crystalline polymorph. Late in the development, before fixing the commercial process, it was

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(1) Guidance for Industry: ANDAs: Pharmaceutical Solid Polymorphism Chemistry, Manufacturing, and Controls Information; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, July 2007.

(2) ICH Q6a Specifications: Test Procedures and Acceptance Criteria for New Drug Substances andNew Drug Products: Chemical Substances.

(3) (a) *ICH Q8 Pharmaceutical Development*, (R2); U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, Aug 2009. (b) *ICH Q9 Quality Risk Management*; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, June 2006. (c) *ICH Q10 Pharmaceutical Quality System*; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, April 2009.

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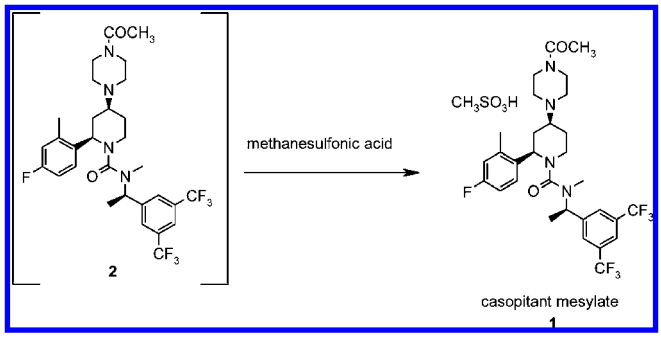
***Scheme 1.*** **Final crystallization to casopitant mesylate as a mixture of Form 1 and Form 3**




discovered that the crystalline Form 1 of the drug substance was actually a combination of two polymorphs (Form 1 and Form 3).[4]

This report describes the studies carried out to characterize these forms and to assess the ability of the manufacturing process to control the resulting crystalline phase and the impact of these findings on the drug product performance.

For the reader's benefit, a glossary section with the definitions of the terms used within this text is included in the Appendix.

## 2. Final Crystallization Process

The commercial process to synthesise casopitant mesylate is described in a previous contribution.[5] In the final crystallization step, casopitant mesylate **1** is obtained (see Scheme 1) via a seeded reactive crystallization, where methanesulfonic acid is added to casopitant **2** in a mixture of ethyl acetate, acetone, and isooctane. The seeding temperature is in the range of 34−44 °C, and crystals are isolated at ∼20 °C. This procedure is also described in detail in the Experimental Section (see section 12).

To be able to deliver the drug substance with consistent properties, an extensive optimisation process was undertaken. As a result, the experimental conditions evolved during the course of the development of the process. In particular, during the preclinical and clinical studies, the particle-forming-step solvent was changed twice. Initially ethyl acetate was selected as the crystallization solvent. This was later changed to the aforementioned mixture. The relative ratio of these three solvents was finally optimized before setting the commercial process.

After Phase II clinical studies, before fixing the commercial process, it was discovered that, independently from the crystallization conditions, the drug substance crystalline phase was always a combination of two polymorphs (Form 1 and Form 3). As a result of this, an extensive investigation was undertaken to assess the effectiveness and the robustness of the crystallization process in the control of the amount of Form 3 present in the drug substance.

## 3. Characterisation of the Polymorphs

Despite many attempts, pure Form 1 and Form 3 have never been obtained; therefore, samples of predominantly crystalline Form 1 (11% w/w Form 3) or predominantly crystalline Form 3 (78% w/w Form 3)[6] were used for the characterisation of the two forms.[7]

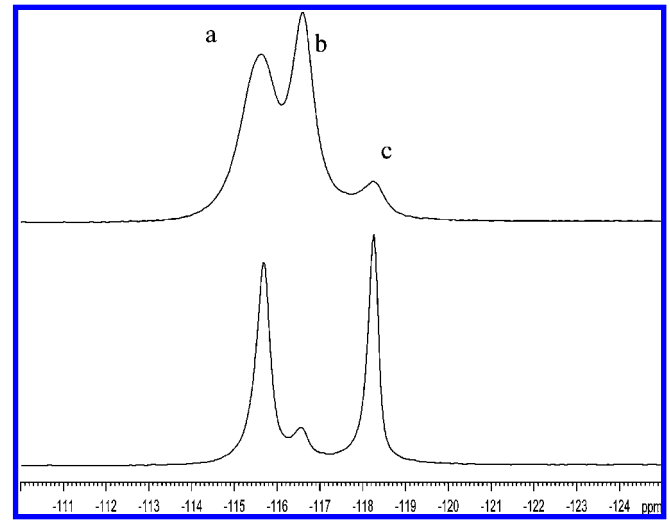



***Figure 1.*** **Solid-state NMR aryl fluoride region for Form 1 and Form 3.**

**3.1. Solid-State NMR spectra of Form 1 and Form 3.** Solid-state NMR (ssNMR) was the first analytical technique capable of detecting and quantifying the two crystalline phases. While the detailed description of the quantitative method will be given in a forthcoming paper, in this paragraph the differences in ssNMR spectra of the two forms are reported.

The aryl fluoride region (∼−110 to −125 ppm) of the single pulse 338.8 MHz $^{19}F\{^{1}H\}$ solid-state NMR spectra of Form 1 (bottom-14% w/w Form 3) and Form 3 (top-78% w/w Form 3) of casopitant mesylate is shown in Figure 1. The $CF_3$ region (∼−63 ppm) does not show resolution of the polymorphs and is not shown. Both forms show two resonances in the aryl fluoride region, reflecting the crystallographically distinct molecules in the unit cells of these forms. Only three signals are observed due to the complete overlap of one of the resonances of each form, i.e. peak "a" (−115.7 ppm) consists of one resonance each of Form 1 and Form 3. The distinct resonance from Form 3 (peak "b", −116.6 ppm) is partially overlapped; thus, deconvolution procedures (rather than simple integration) were employed to quantify each form. Full details of the procedures and methods will be presented elsewhere.

**3.2. X-ray Powder Diffraction (XRPD).** An overlay of the XRPD patterns (reflectance mode) for a Form 1 batch, the working reference standard (11% w/w Form 3, by solid-state NMR), and a Form 3 batch (78% w/w Form 3, by solid-state NMR) is presented in Figure 2. Routine XRPD (reflectance mode) has been shown to qualitatively distinguish Form 1 and Form 3. However, given the similarity of the two forms in most regions of the diffraction patterns, this method has not been used for quantitation of Form 3 in batches of casopitant mesylate.

(4) Form 2 is not discussed in this contribution as it is not relevant. Form 2 is a metastable form only obtained from tetrahydrofuran; it is not stable on storage and quickly converts to Form 1.

(5) Cimarosti, Z.; Bravo, F.; Castoldi, D.; Tinazzi, F.; Provera, S.; Perboni, A.; Papini, D.; Westerduin, P. *Org. Process Res. Dev.* **2010**, *14*, 805.

(6) The Form 3 sample of casopitant mesylate was obtained by a combination of annealing and slurry experiments and the experimental conditions will be reported in 7.1.2 section and in the Supporting Information.

(7) The microscope images of Form 1 and Form 3 were very similar, the pictures are reported in the Supporting Information.

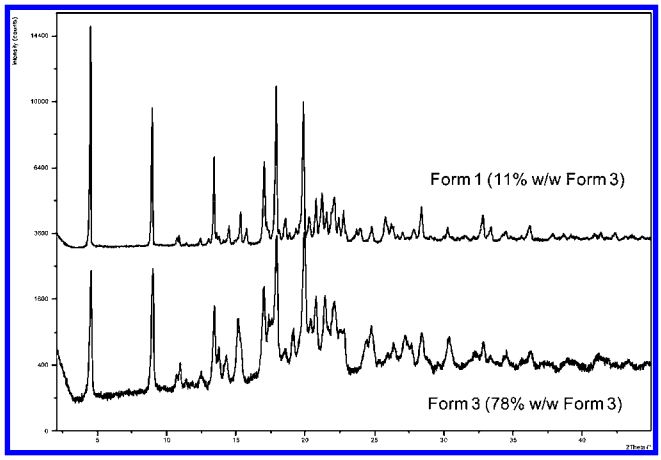



***Figure 2.*** **XRPD pattern comparison of Form 1 (11% w/w Form 3) and Form 3 (78% w/w Form 3).**

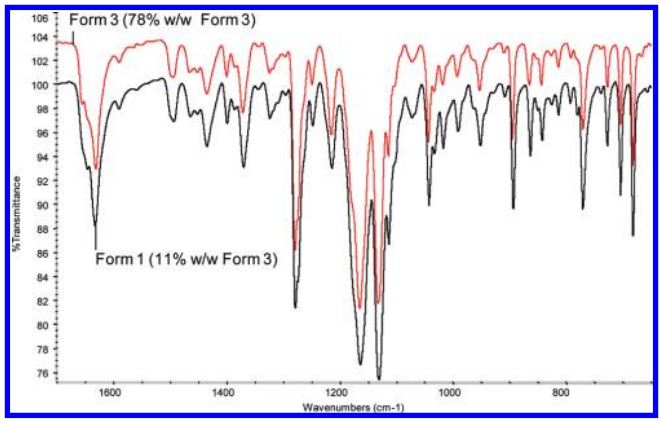



***Figure 3.*** **ATR-IR comparison of Form 3 (78% w/w Form 3) and casopitant mesylate (11% w/w Form 3) - transmittance mode, expanded region 1700−650 cm$^{-1}$.**

**3.3. Infrared and Raman Spectroscopy.** Analysis of a Form 3 reference sample (78% w/w Form 3 by solid-state NMR) by attenuated total reflectance infrared spectra (ATR-IR) shows that the spectrum collected in ATR is very similar to that of the Form 1 (working reference standard of casopitant mesylate) containing 11% w/w Form 3 by solid-state NMR. A very subtle difference can be observed at 780 cm$^{-1}$ in standard processing, and no further relevant differences are noticed in second derivative processing mode. Some differences are noticed in terms of relative intensity of some of the bands as well. The unavailability of pure Form 1 and Form 3 samples does prohibit a clear evaluation of the significance of the difference in bands intensities.

Figure 3 and Figure 4 show the comparison between the Form 3 batch and standard casopitant mesylate (11% w/w Form 3 by solid-state NMR) in the regions of interest.

Raman spectroscopy of a Form 3 sample (78% w/w Form 3 by solid-state NMR) shows that the spectrum is very similar to that of the Form 1 (working reference standard of casopitant mesylate, 11% w/w Form 3 by solid-state NMR). This is consistent with the small differences observed by FT-IR and supports the similarity between the two crystalline forms. Figure 5 shows the spectra comparison of Form 3 and Form 1.

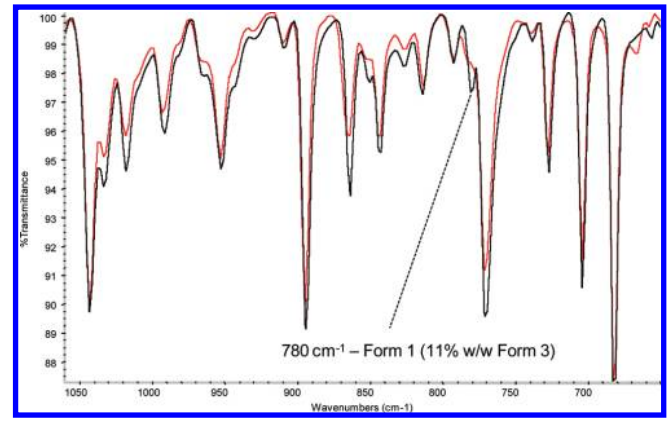



***Figure 4.*** **ATR-IR comparison of Form 3 (78% w/w Form 3) and casopitant mesylate (11% w/w Form 3) - transmittance mode, expanded region 1060−650 cm$^{-1}$.**

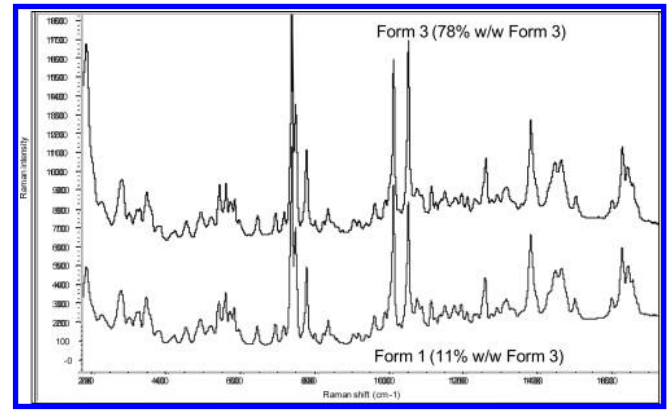



***Figure 5.*** **Comparison of the Raman spectra of Form 3 (78% w/w Form 3) and Form 1 (11% w/w Form 3).**

## 4. Development of a Quantitative Method for the Detection of Form 3 in the Drug Substance

As discussed in section 3.2 the reflectance XRPD spectra of the two forms were too similar to allow for quantitation. Among the analytical techniques evaluated, only solid-state NMR and transmission XRPD could distinguish Form 1 and Form 3 sufficiently to be used for quantitation. The development and validation of these methods will be reported elsewhere; all the quantitative levels of Form 3 reported in this paper were obtained with solid-state NMR method.

## 5. Summary of the Characterization Studies

Numerous properties of Form 1 and Form 3 are similar as previously mentioned in section 3, in particular no differences between Form 1 and Form 3 were observed in:

- the solubility experiments in water and different biorelevant media (SGF, FaSSIF, FeSSIF[8]) at 25 °C.
- the Intrinsic Dissolution Rate in SGF at pH 1.6 and 25 °C.
- the gravimetric vapour sorption (GVS) analysis.
- the thermal analysis (DSC and TGA[9]).

(8) SGF is the acronym of Simulated Gastric Fluid, FaSSIF of Fasted State Simulated Intestinal Fluid, FeSSIF of Fed State Simulated Intestinal Fluid.

(9) DSC is the acronym of Differential Scanning Calorimetry, TGA of Thermogravimetric Analysis.

The similarity in the spectroscopic features, in the thermal analysis and in the solubility of the two forms, highlighted in this section might suggest close similarities in the crystal lattice of the two polymorphic phases.

## 6. Historical Data on the Development Batches

Despite some changes in the particle forming step, introduced during the route development, a mixture of Form 1 and Form 3 has always been obtained (see Table 1). The full details of the studies related to solubility, intrinsic dissolution, moisture effect and the thermal properties will be reported in a dedicated paper.

Retrospectively, the available pre-clinical and clinical data (see Table 1) qualify the level of Form 3 in the final active pharmaceutical ingredient (API) by providing toxicological and clinical safety coverage, the limit (27% w/w Form 3) is set by the maximum amount of Form 3 present in the preclinical and clinical batches.

***Table 1.*** **Level of Form 3 in Form 1 obtained in different crystallization processes**

| crystallization process | phase | typical form 3 level (% w/w by solid-state NMR) |
|---|---|---|
| ethyl acetate | preclinical/clinical | 27 |
| ethyl acetate/acetone/ isoctane (1/7.5/5) | clinical | 11−21 |
| ethyl acetate/acetone/ isooctane (2.4/4.5/3) | clinical/commercial | 7−11 |

Therefore the commercial crystallization process has to consistently deliver a drug substance having a level of Form 3 below 27% w/w. Besides that, the impact of Form 3 on the drug product performance has to be assessed.

## 7. Process Understanding and Control Studies

In this section, the studies carried out to understand the relative stability of the two forms and to develop a process capable of controlling the level of Form 3 in the drug substance are reported.

**7.1. Relative Stability Studies.** To complete the physicochemical characterization and to understand the nature of the two polymorphic forms, relative stability studies were undertaken.

*7.1.1. Solubility in Organic Systems.* A recommended approach to understand the relative stability between Form 1 and Form 3 is based on the solubility measurement of the two forms in a suitable solvent system, other than water or biorelevant media (already presented in section 5). The thermodynamically stable form is characterized by a lower solubility.

Not having access to pure Form 1 or Form 3 samples, the solubility measurements were made using enriched Form 1 (containing 7% w/w Form 3) and Form 3 (containing 76.6% w/w Form 3) samples. The samples were equilibrated for 20−24 h at the set-point temperature in acetone and ethyl acetate/acetone (36/64). This solvent system is particularly relevant, being the one used in the particle-forming step. The solubility curves for the two enriched samples have been shown to be comparable.

*7.1.2. Competitive Slurry Experiments.* To understand the relative thermodynamic stability of Form 1 and Form 3, competitive slurries were performed in acetone and ethyl acetate/acetone (36/64) at temperatures from −20 to 50 °C. The ethyl acetate/acetone system was selected as it was representative of the composition at the crystallization seeding point in the synthetic process for casopitant mesylate drug substance. None of the experiments show complete conversion of one form into the other. The competitive slurries were monitored over time, and the data show that the ratios of Form 1/Form 3 depend on the temperature.

Slurrying performed at 40 and 50 °C show that the amount of Form 3 does not increase beyond ∼80% w/w by solid-state NMR, and at 0 and −20 °C does not decrease below the limit of detection. In summary, the casopitant mesylate slurries reach ratios that favor Form 1 below 20 °C and favor Form 3 at 30 °C and above.

Thermodynamics dictate that, if Forms 1 and 3 are distinct polymorphs and the system is allowed to reach equilibrium, the substance will convert completely to the more stable polymorph at that temperature because it will have the lowest energy and, hence, solubility. This is independent of the solvent. However, we did not observe complete conversion between the forms at any temperature studied.[10] Hence, the relationship between Form 1 and Form 3 is summarised as showed in Figure 6.

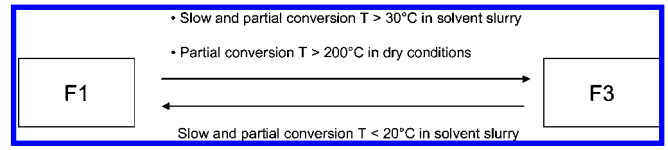



***Figure 6.*** **Relationships between Form 1 and Form 3.**

The conversion of Form 1 to Form 3 and vice versa is very slow in the evaluated temperature range (data obtained at 0 and 40 °C are shown in Table 2). The formation of Form 3 in the current crystallisation process appears to be disfavored as a consequence of the seeding-point temperature which is very close to the likely transition temperature. Kinetics as well may play a role in disfavoring any complete form conversion.[11]

The amount of Form 3 decreases in competitive slurries of casopitant mesylate at temperatures below 20 °C. Therefore, the conclusion is that there will be insignificant conversion of Form 1 to Form 3 while the crystallisation is cooled and the product isolated.

The results from these competitive slurry experiments are consistent with the observed difficulty in crystallising pure Form 1.

A full discussion on the impact of Form 3 in the crystallisation process is made in the next paragraph.

**7.2. Multivariate Experimental Studies on Crystallisation Process with Respect to Form 3.** The definition of the design space (see Glossary in the Appendix for the definition) of the casopitant mesylate particle-forming step at commercial scale is based on the Quality by Design principle where process understanding and risk assessment are combined to optimise a

(10) Irrespectively from the chemical purity of the sample used as input in the slurry, the conversion to Form 3 at 50 °C in acetone reaches a plateau value and does not proceed to completion. Nevertheless some differences in the kinetic of conversion were observed in the first 14 days. All the details are available in the Supporting Information.

***Table 2.*** **Competitive slurry data**

| | | % w/w by solid-state NMR | | | |
|---|---|---|---|---|---|
| | temperature (°C) | initial Form 3 level | Form 3 after 14 days | Form 3 after 28 days | Form 3 after 42 days |
| ethyl acetate/ acetone 36/64 | 40 | 45 | 51 | 77 | 80 |
| acetone | 40 | 38 | 65 | 80 | 80 |
| ethyl acetate/ acetone 36/64 | 0 | 41 | 23 | 27 | 20 |
| acetone | 0 | 40 | 20 | 20 | 14 |

robust manufacturing process. In this case the experimental studies were performed using a DoE approach. These studies were performed in 2-L laboratory-scale equipment configured to mimic the equipment at the site of commercial manufacture. This was achieved by scale-down maintaining geometrical similarity and operating under conditions scaled according to accepted chemical engineering principles, e.g. using the constant power-per-unit volume (*P*/*V*) principle for scaling agitation speed.

When the presence of Form 3 was discovered in the batches of drug substance, the particle-forming step process was close to finalisation. For this reason, additional crystallization studies were initiated with the objective to obtain pure Form 1. Several attempts were made by changing solvent, temperature, addition order, and the other main parameters impacting the crystallization without success.[12]

This finding was a confirmation of the close relation between the two forms and supported the decision to develop the drug substance as a mixture of Form 1 and Form 3. Therefore, it was necessary to demonstrate that the selected process was able to control the level of Form 3 in the drug substance at the levels present in the preclinical and clinical studies (no more than 27% w/w, see Table 1).

A risk assessment was carried out to understand which process parameters could potentially affect the level of Form 3 in the drug substance. The output of this analysis showed that all the potentially relevant process parameters were part of the aforementioned multivariate studies (see Tables 3 and 4). On the basis of this rationale, the considered process parameters and their relative ranges were evaluated against the Form 3 level in the drug substance and, in particular, against the maximum level of 27% w/w.

In the first set of experiments (35 in total), a multivariate approach was used to study the main process parameters (see Table 3), using relatively wide experimental ranges. In the second set of experiments (10 in total), the most critical process parameters (described in Table 4)[13] were examined with respect to the level of Form 3 in the drug substance in the narrower experimental ranges resulting from the previous set of experiments. This second set is used to demonstrate the robustness of the particle-forming step and to define the PARs (the design space) for the commercial process.

The impact of the impurity profile was assessed by using different purity batches and was included in the design as a categorical factor. Three batches of different purity were used in these studies, denoted standard, modified and low purity. These were prepared by using starting materials, key reagents, and intermediates that contained different levels of impurities. The modified purity batch was prepared by artificially doping high levels of impurities[14] into a solution of casopitant free base at or above the specification limits. The Form 3 content of the seed was 9% w/w.

The levels of Form 3 for each DoE run are plotted graphically in Figure 7. All runs gave drug substance containing levels of Form 3 less than 14% w/w (using the solid-state NMR method) well below the maximum 27% w/w level.

A statistical model was created by combining the data obtained from the first and second sets of multivariate experi-

***Table 3.*** **Process parameters and ranges used in the first set of multivariate experiments[15]**

| | process parameter | unit | levels studied low/middle/high |
|---|---|---|---|
| 1 | impurity profile of casopitant | categorical (batches used) | low, modified, standard purity |
| 2 | volume of ethyl acetate[a,b] | vol | 3.0/4.0/5.0 |
| 3 | volume of acetone[a] | vol | 3.5/4.5/5.5 |
| 4 | stirring rate | *P*/*V* (W/m$^{-3}$) | 40/180/320 |
| 5 | seed amount | wt % | 0.25/0.5/0.75 |
| 6 | seeding temperature | °C | 30/40/50 |
| 7 | aging time before isooctane addition | h | 0.5/1.0/2.5 |
| 8 | volume of isooctane | vol | 2/3/4 |
| 9 | isooctane addition time | h | 0.5/1.0/1.5 |

[a] Volumes and weights are referred to 0.86 wt of casopitant free base. [b] Refers to the total volume of casopitant in ethyl acetate after distillation plus volume of additional ethyl acetate added.

***Table 4.*** **Process parameters and ranges used in the second set of multivariate experiments**

| | process parameter | unit | levels studied low/middle/high |
|---|---|---|---|
| 1 | impurity profile of casopitant | categorical (batches used) | low, modified, standard purity |
| 2 | volume of ethyl acetate[a,b] | vol | 3.0/4.0/5.0 |
| 3 | volume of acetone[a] | vol | 4.2/4.5/4.8 |
| 4 | seeding temperature | °C | 34/39/44 |
| 5 | aging time before isooctane addition | h | 0.5/1/2.5 |

[a] Volumes and weights are referred to 0.86 wt of casopitant free base. [b] Refers to the total volume of casopitant in ethyl acetate after distillation plus volume of additional ethyl acetate added.

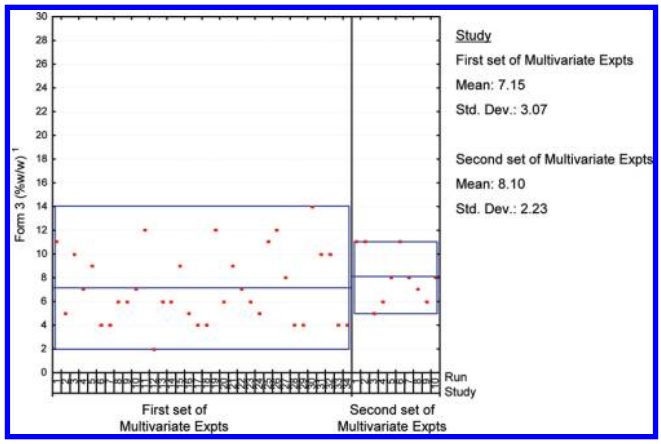



***Figure 7.*** **Combined data from the first and second sets of multivariate experiments. Note: data were generated with the solid-state NMR method.**

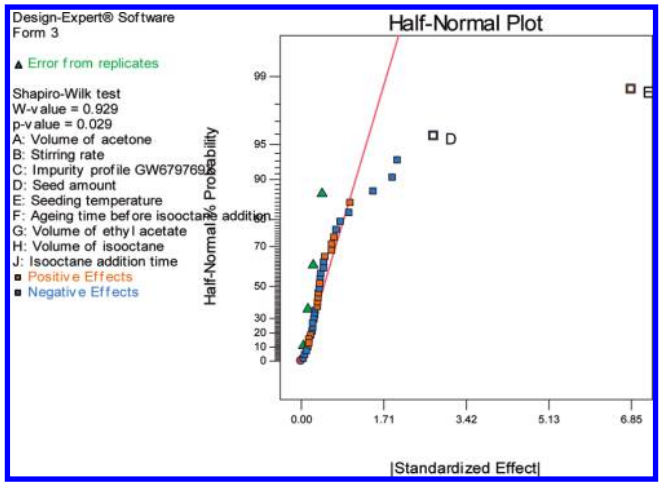



***Figure 8.*** **Half-normal plot for Form 3 using combined data from the first and second set of multivariate experiments.**

ments. The half-normal plot for this is shown in Figure 8. The statistical model shows that the most important parameters affecting the level of Form 3 are the seeding temperature (E in Figure 8) and the seed amount (D in Figure 8). The remaining parameters have a minor or negligible effect on the level of Form 3. It is worth noting that the analysis of variance, ANOVA, suggests that the factor interactions are not significant. Higher seeding temperature and lower seed amount result in increased levels of Form 3, but even when the current commercial process has been stretched at forcing conditions or outside the process ranges identified in Table 4, the levels are significantly lower (not greater than 14% w/w) than the level found in batches used for preclinical and clinical studies (27% w/w).

***Table 5.*** **Impact of seed on Form 3 content of casopitant mesylate**

| Form 3 level in the seed[a] | Form 3 level in the casopitant mesylate (solid-state NMR % w/w) |
|---|---|
| none | 7 |
| 78% w/w Form 3 | 12 |
| control, 9% w/w Form 3 | 8 |

[a] It is worth noting that the temperature for all these experiment was the typical process temperature (40 °C).

The effect of the seeding temperature on the Form 3 level can be explained by the fact that Form 1 and Form 3 are enantiotropically related, as shown in the competitive slurry experiments (see section 7.1.2) with a transition temperature between Form 1 and 3 in the evaluated experimental range as resulting from the competitive slurries (see section 7.1.2). The level of Form 3 in the final drug substance can be affected by the parameters influencing the crystallization kinetic, i.e. the available seed surface area when crystallization starts. In particular faster crystallization kinetic can be induced by seed amount, seed PSD attributes, or stirring rate (influencing secondary nucleation). A faster nucleation rate seems to be associated with a higher Form 3 level in the final API. In conclusion, to minimize the Form 3 level in the API, the temperature has to be kept low, and the seed surface area has to be maximized. Higher seeding temperature and lower seed amount result in increased levels of Form 3, but even when the current commercial process has been stretched at forcing conditions or outside the current design space, the levels of Form 3 are low.

These data show that a large number of parameters can be varied over a relatively wide range with respect to the commercial process, delivering casopitant mesylate batches with acceptable Form 3 levels.

**7.3. Seeding Studies with Respect to Form 3.** The use of a proper seeding strategy to control the polymorphic modification in batch-wise crystallisation processes is widely discussed in literature. Seeding with the desired polymorph when crystallization conditions are well understood usually represents the best way to control the drug substance crystalline phase outcome.[16] For this reason, additional univariate experiments were performed to understand the effect of the seeding on the Form 3 level in crystallised casopitant mesylate (Table 5). These results showed that, when the particle-forming step is not seeded or when it is seeded with essentially Form 3 casopitant mesylate

(11) For the purpose of the regulatory submission we mainly focused the investigation on experimental conditions close to the commercial process both in terms of solvent systems and main process parameters (i.e. temperature, seed attributes).

(12) Experiments were performed to get pure Form 1 by seeding the commercial process at 20 °C or by performing slow evaporative crystallizations at room temperature. Moreover a DoE study explored wider process parameter ranges (temperature 0−50 °C, seed amount 0.2−2% w/w, seed PSD X90 from 4 to 13 $\mu$m). In all these trials the lowest level of Form 3 in the drug substance obtained was 2% w/w. At-line form monitoring of the crystal formation was not feasible as the only techniques capable of discriminating the two forms are ssNMR and transmission XRPD. However, when solid samples at different stages of the crystallization process were isolated, no difference in the Form 1/Form 3 ratio was observed.

(13) As said earlier in this section, Form 3 was discovered at a late stage of the process development. For this reason the selection of the most critical process parameters to be used in the second set of multivariate experiments was done with respect to the casopitant impurities and the casopitant particle size.

(14) The impurities added to casopitant are drug substance-CQA, their formation and impact on the process are discussed in Cimarosti, Z.; Bravo, F.; Castoldi, D.; Tinazzi, F.; Provera, S.; Perboni, A.; Papini, D.; Westerduin, P. *Org. Process Res. Dev.* **2010**, *14*, 805.

(15) It is worth noting that the addition rate of methanesulfonic acid was not included amongst the crystallization process parameters as particle formation is controlled by seed addition. When methanesulphonic acid is added, the degree of supersaturation is such that spontaneous nucleation is disfavoured.

(16) Beckmann, W. *Org. Process Res. Dev.* **2000**, *4*, 372.

*Table 6.* **Effect of slurrying casopitant mesylate in ethyl acetate (60 °C for 24 h)[17]**

| input level of Form 3 in drug substance (by solid-state NMR % w/w) | output level of Form 3 in drug substance (by solid-state NMR % w/w) |
|---|---|
| 10% | 11% |

(sample containing 78% w/w of Form 3), this does not significantly impact the resulting solid-state form, producing casopitant mesylate having a Form 3 level comparable to the one resulting from the use of Form 1 seed.

**7.4. Drying Studies with Respect to Form 3.** As previously shown in section 7.1.2, the kinetics of transformation of Form 1 to Form 3 are slow. Studies were performed in which casopitant mesylate was suspended in ethyl acetate at 60 °C for 24 h (Table 6). In the commercial process, casopitant mesylate is washed with ethyl acetate and dried at not more than 60 °C under vacuum; hence, this study mimicked an artificially long contact time with solvent in the drying step. The typical contact time at commercial scale is less than 13 h.

These data show no substantial increase in the Form 3 level under the conditions that might be experienced under drying, and hence the drying step has no significant impact on the level of Form 3.

**7.5. Summary of the Findings.** As a result of the presented data it can be concluded that the proposed commercial manufacturing process is robust and able to ensure the control of the formation of drug substance with the appropriate level of Form 3. In particular

- The process performed in the ranges of the particle-forming step as described in Table 4 delivers low levels of Form 3 in the drug substance (not greater than 14% w/w).
- The seeding temperature is set in the range 34−44 °C, where Form 3 is favored with respect to Form 1; this is acceptable because the kinetics of interconversion is slow (section 7.1.2).
- The level of Form 3 in the seed does not appear to significantly impact the level of Form 3 in the resulting drug substance (section 7.3).
- Drying steps performed in stressed conditions (longer time and higher temperature) did not increase the level of Form 3 in the drug substance (section 7.4).

## 8. Stability

Stability studies on drug substance support the good physicochemical stability of casopitant mesylate. Routine XRPD (reflectance mode) data generated during the primary stability studies showed no significant change in solid-state form at the long-term, accelerated, and stress conditions up to the 24-month time point. Although, as demonstrated in section 3.2, the XRPD reflectance method is not able to quantitatively determine levels of Form 3, the method provides a qualitative assessment that there has been no significant interconversion of forms on storage and casopitant mesylate remains predominantly Form 1.

(17) The solubility of casopitant mesylate in ethyl acetate is very low. The full dataset generated for ethyl acetate and acetone is reported in the Supporting Information.

Quantitative testing of the level of Form 3 for information has been introduced in the stability studies of the drug substance produced at the commercial site. The available data are fully disclosed as Supporting Information and summarized below.

Data for Form 3 content after 3 months storage at 50 °C/ambient (two batches), up to 8 months storage at 40 °C/75% RH (either protected or not protected by the primary pack) and up to 9 months storage at 30 °C/65% RH have been collected. Available samples on two batches have been also been tested after 3, 8, and 9 months storage at 5 °C/ambient, providing a reference for the higher-temperature storage data. No difference that could be considered significant was detected for Form 3 content at any of the test points or storage conditions and in comparison with the 5 °C/ambient reference data, by either of the two quantitative methods that could support an increase of Form 3 in casopitant mesylate on storage.

## 9. Impact on Drug Product

Two formulations were developed for casopitant mesylate a tablet (with two strengths, 50 and 150 mg) and an injectable solution (prepared from freeze-dried casopitant mesylate). Quality by Design principles were applied to drug product development, in particular the attributes of the drug substance were studied as factors potentially impacting on the final drug product quality. In this section the assessment of Form 3 impact on the drug product performance and manufacturing processes is described.

**9.1. Freeze-Dried Injection Product and Manufacturing Process.** There is considered to be no impact of Form 3 levels in casopitant mesylate drug substance on the freeze-dried injection product performance and manufacturing process, as the first step for the manufacturing process is the dissolution in water of casopitant mesylate (the compound is in solution) followed by water elimination to form the freeze-dried compound as amorphous. Casopitant mesylate is present and maintained in the amorphous state in the freeze-dried injection product. It is quite common that amorphous material is obtained from a lyophilization process where a solution is first frozen and then dried via ice sublimation. Experiments on casopitant mesylate solutions that were frozen using different cooling rates always yielded amorphous casopitant mesylate.

**9.2. Tablet Product Performance: In Vitro Dissolution.** Due to the great similarity of solubility and intrinsic dissolution rates between Form 3 and Form 1, no differences in the in vitro dissolution behaviour and ultimately the in vivo pharmacokinetics can be observed (consideration of the impact of Form 3 levels on clinical pharmacokinetics is provided in section 10). Tablet product in vitro dissolution behaviour is also consistent over time as demonstrated by stability data collected on the tablets to date.

Analysis of variance (ANOVA) conducted on the dissolution data acquired at each time point (15 min, 30 min, 45 min) for three casopitant tablet batches (150 mg) manufactured using input drug substance containing different levels of Form 3 was performed. The tablet batches selected for the analysis and the

***Table 7.*** **Tablet batches analysed by ANOVA**

| tablet batch[18] | Form 3 level in the input drug substance (% w/w by solid-state NMR) |
|---|---|
| batch 1 | 7 |
| batch 2 | 15−20 |
| batch 3 | 9 |

corresponding input drug substance batch/Form 3 level are reported in Table 7.

The results of ANOVA confirmed that there is no significant difference in the dissolution data generated at each time-point across the three batches ($P > 0.05$). Dissolution profiles with confidence interval bars are reported in Figure 9.

This finding indicates that there is no statistically significant impact of Form 3 level on in vitro dissolution. The observed variability of dissolution data at the 15-min time point is known to be related to the disintegration characteristics of the 150 mg tablet strength in the dissolution vessel and cannot be ascribed to input drug substance characteristics; hence, the level of Form 3 in the drug substance has no impact on tablet product performance.

**9.3. Tablet Product Manufacturing Process.** Form 1 containing varying amounts of Form 3 observed to date across nonclinical, clinical stability and commercial drug substance batches was successfully processed into tablets, indicating that there is no adverse impact of Form 3 content on the various process unit operations. This is consistent with the similarity in physical/chemical properties between the two forms (Form 1 and Form 3) of the mixture as described in the relevant sections of this document. In particular, the similarity in solubility and GVS profile (section 5) indicates that wet granulation, drying, and film-coating operations during tablet manufacture are unlikely to be affected by the variation of the Form 3 amounts present in the Form 1 mixture. Also, the similarity in thermal properties (TGA and DSC traces, section 5) shows that Form 3 content does not impact thermal behaviour of casopitant mesylate, confirming that melting and associated decomposition is not a risk during the relatively moderate processing conditions used in tablet manufacture.

## 10. Impact of Form 3 in Clinical Studies: Pharmacokinetics

The potential impact of Form 3 on the clinical exposure of casopitant in human subjects was evaluated from pharmacokinetic data. All studies presented were conducted in healthy subjects, with single-dose oral administration of solution or tablet formulations of casopitant mesylate in the fasted state. As casopitant generally

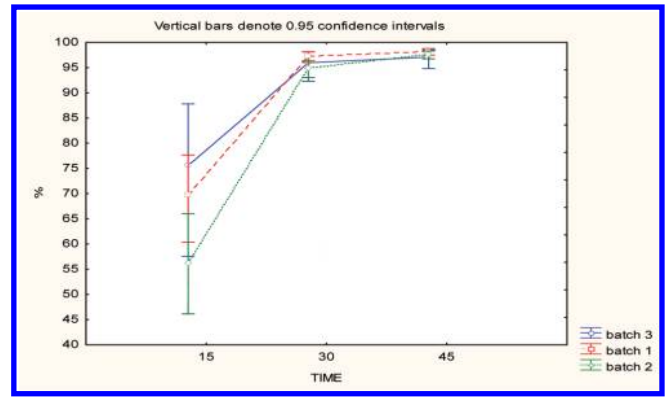



***Figure 9.*** **Dissolution profiles of 150 mg tablets.[19]**

exhibits dose-proportional pharmacokinetics, pharmacokinetic parameters were dose-normalized for ease of data presentation. Pharmacokinetic parameters across a range of Form 3 levels in casopitant mesylate (% w/w by solid-state NMR) are summarized in Figure 10 ($C_{max}$ represents the maximum plasma concentration, and AUC represents the area under the plasma concentration−time curve, either to the last sampling time or extrapolated to infinity). Pharmacokinetic data were also available where casopitant was administered as an oral solution formulation (no crystal form). While there is modest variability between studies, there is no apparent pattern between the level of Form 3 and the exposure parameters $C_{max}$ and AUC. Although more rapid absorption (and therefore higher dose-normalized $C_{max}$) is evident with the oral solution formulation, the extent of absorption (AUC) is similar between the oral solution formulation (where no crystal form considerations apply) and oral tablets containing a range of Form 3 levels.

## 11. Conclusion

Casopitant mesylate is a $NK_1$ antagonist in development in GlaxoSmithKline. This drug substance is not produced as a single polymorph; it is a combination of forms, Form 1 and Form 3, that have very similar properties. In particular:

- Form 3 and Form 1 have very similar spectroscopics features, they can only be qualitatively distinguished by powder XRPD, IR, and Raman spectroscopy; only transmission XRPD and solid-state NMR allow a quantitative detection of the two forms (see section 3).
- The solubility of Form 3 is very similar to that of Form 1 in water, biorelevant, and organic media (see sections 5 and 7.1.1).
- The GVS profile of Form 3 is similar to that obtained for Form 1 (see section 5).
- The profiles for the thermal analysis of Forms 3 and 1 are very similar (see section 5).
- Competitive slurry experiments indicate that Form 3 and Form 1 are enantiotropically related and a transition temperature has been found in the range 30−40 °C. Nevertheless, the kinetic of interconversion is very slow, and both Form 3 and Form 1 have never been obtained as pure crystalline phases (see section 7.1.2).
- Tablets prepared with drug substance having different levels of Form 3 (from 9 to 20% w/w) did not show

(18) It is worth noting that PSD of the three batches of drug substance used to prepare the drug product batches submitted to dissolution was not the same, but this was not considered an issue as extensive data collected during development demonstrated that drug substance PSD has no significant impact on the dissolution rate of the drug product at the Q point (30 minutes). These considerations are based on what is observed for all the ranges on PSD of the drug substance produced (where no differences were seen when the D90 was ranging from 13 to 70 $\mu$m).

(19) Batches 1 and 3 ranges were obtained as averages of 12 replications, while batch 2 range was an average of 6 replications.

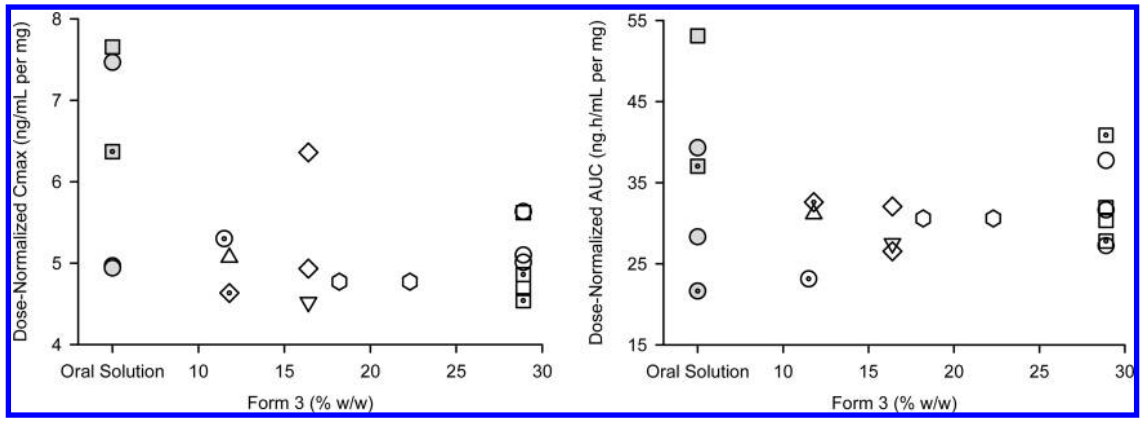



***Figure 10.*** **Dose-normalized geometric mean pharmacokinetic parameters versus levels of Form 3. Note: Each unique symbol type represents an individual clinical study, although multiple dose levels may have been administered within a study.**

any different behavior in the in vitro dissolution test (section 9.2).
- In clinical studies, the extent of absorption (AUC) is similar between the oral solution formulation (where no crystal form considerations apply) and oral tablets containing a range of Form 3 levels (11 to 27% w/w).

Moreover the following considerations apply for the drug substance and drug product manufacturing processes:

- The appropriate control strategy for the drug substance manufacturing process is in place. The manufacturing process has the ability to control the level of Form 3 in the drug substance (not greater than 14% w/w) well below the limit used during the preclinical and clinical development (27% w/w) as reported in sections 7.2, 7.3, and 7.4.
- Once the drug substance is isolated, it is stable in a range of conditions (up to 9 months at 30 °C/65% RH for example) as reported in section 8.
- The unit operations of the tablet manufacturing process (wet granulation, drying, and film-coating) are unlikely to be affected by the Form 3 level in the drug substance, and this is confirmed by the fact that the performance of the tablet manufacturing process does not change if drug substance with different levels of Form 3 (up to 27% w/w) is used (see section 9.3).

All of these experimental findings demonstrate that the level of Form 3 (up to 27% w/w) in the drug substance is not a drug substance-CQA.

In addition, the control strategy defined for the drug substance manufacturing process by following the Quality by Design principles allows adequate control of the level of Form 3 in the drug substance, ensuring the drug product performance. The studies presented in this paper to demonstrate that the level of Form 3 (up to 27% w/w) in the drug substance is not a drug substance-CQA were preliminarily discussed with regulatory agencies with positive feedback.

## 12. Experimental Section

**(2*R*,4*S*)-4-(4-Acetyl-1-piperazinyl)-*N*-{(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethyl}-2-(4-fluoro-2-methylphenyl)-*N*-methyl-1-piperidinecarboxamide Methanesulfonate Salt (Casopitant Mesylate 1).** The solution of casopitant **2** (0.86 kg) was diluted with EtOAc (overall solution of **2** in EtOAc was 4 L) and acetone (4.5 L), and heated to the required temperature (from 39 °C). Then neat methanesulfonic acid (0.12 L, 1.64 mol) was added, followed by a slurry of **2** (0.005 kg) in EtOAc (0.05 L) as seed. The obtained suspension was stirred for 1 h. Then isooctane (3 L) was added in the required time (1 h), and the slurry was cooled to 20 °C in 2 h and aged 3 h.

The suspension was filtered, and the solid was washed with EtOAc (3 × 4 L). The white solid was dried overnight under vacuum at 40 °C to give the desired casopitant mesylate **1** (0.94 kg).

$^1$H NMR (600 MHz, DMSO-$d_6$): 9.57 (br s, 1H), 7.99 (br s, 1H), 7.68 (br s, 2H), 7.23 (m, 1H), 6.95 (dd, 1H), 6.82 (m, 1H), 5.31 (q, 1H), 4.45 (m, 1H), 4.20 (dd, 1H), 3.99 (m, 1H), 3.56 (m, 1H), 3.47 (m, 3H), 3.37 (m, 1H), 3.15 (m, 1H), 2.96 (m, 1H), 2.87 (m, 1H), 2.80 (t, 1H), 2.74 (s, 3H), 2.36 (s, 3H), 2.30 (s, 3H), 2.13 (m, 1H), 2.08 (m, 1H), 2.10 (s, 3H), 1.87 (m, 1H), 1.73 (m, 1H), 1.46 (d, 3H), MS: *m*/*z* 617 $[MH]^+$, as free base.

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## Glossary

**Drug product critical quality attributes or drug substance critical quality attributes** are the measurable properties of drug product or API that are critical to ensuring patient safety and efficacy. The property must be within a predetermined range to ensure product quality. A property which is measured outside the range indicates a batch failure.

**Critical quality attributes in the unit operation or stage inputs, stage outputs, device, etc.** are the measurable properties of inputs and outputs that (as determined by risk assessment) present a **high risk** to the process falling outside the design space or proven acceptable ranges.

**Quality attribute in the unit operation or stage inputs, stage outputs, device etc** is the measurable property of inputs

and outputs that (as determined by risk assessment) present a low risk to the process falling outside the Design Space or proven acceptable ranges.

**Quality critical process parameters** are process parameters that influence a critical quality attribute and (as determined by risk assessment) present a high risk to the process falling outside the Design Space or proven acceptable ranges.

**Quality process parameters** are process parameters that influence a critical quality attribute but (following a risk assessment) present a low risk of the process falling outside the design space or proven acceptable ranges.

**Control strategy** is a (planned) set of controls, derived from (current) product and process understanding that assures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control. (ICH Q10 definition - words in brackets are felt unnecessary).

**Proven acceptable ranges (PAR)** are the upper and/or lower limits for process parameter or attribute values between which the parameter or attribute is known to produce a process output (e.g., intermediate, API or DP) that meets the CQAs. The PAR may or may not represent the point of failure. The PAR for a given process parameter or attribute may be dependent upon the PAR values for one or more other process parameters or attributes (e.g., multivariate).

**Design Space** is the multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval.

## Supporting Information Available

ssNMR and transmission XRPD spectra for Forms 1 and 3, microscopes images of Forms 1 and 3, moisture sorption profiles for Form 1 and Form 3, slurry on purified casopitant mesylate, solubility of Forms 1 and 3 in ethyl acetate and acetone, stability data of Form 3. This material is available free of charge via the Internet at http://pubs.acs.org.

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