Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 67–73

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Detection, identification and quantification of a new de-fluorinated impurity in casopitant mesylate drug substance during late phase development: An analytical challenge involving a multidisciplinary approach

Lucilla Turco[a,*], Stefano Provera[a,1], Ornella Curcuruto[a,1], Elena Bernabè[b,2], Anna Nicoletti[b,1], Luca Martini[b,1], Damiano Castoldi[b,1], Zadeo Cimarosti[b,1], Damiano Papini[b,1], Carla Marchioro[a,1], Riet Dams[b,1]

[a] Analytical Chemistry, GlaxoSmithKline Medicines Research Centre, Via Fleming 4, 37135 Verona, Italy
[b] Chemical Development, GlaxoSmithKline Medicines Research Centre, Via Fleming 4, 37135 Verona, Italy

ARTICLE INFO

Article history:
Received 22 June 2010
Received in revised form 3 August 2010
Accepted 6 August 2010
Available online 18 August 2010

Keywords:
Casopitant mesylate
Orthogonal analytical control
NMR spectroscopy
LC–MS

ABSTRACT

During late phase development of the selective NK1 receptor antagonist casopitant mesylate, a de-fluorinated impurity was discovered and quantified by an orthogonal analytical approach, using NMR and LC–MS. A dedicated ^{19}F NMR method was initially developed for first line identification and semi-quantification of the impurity. Subsequently, a more accurate quantification was achieved by means of a selective normal-phase LC–MS method, which was fully validated. The results obtained on the development batches of the drug substance were used by the project team to set up a suitable control strategy and ultimately to ensure patient safety and the progression of the project.

1. Introduction

Neurokinin (NK) receptors have been of particular interest in the last decade as potential novel therapeutic targets for several disorders. In particular, a great deal of pre-clinical and clinical data was generated to support the efficacy of selective NK1 receptor antagonists in the treatment of anxiety and depressive disorders. Casopitant mesylate (**1**) was identified as part of a wider drug discovery program within GlaxoSmithKline and represents one of the most potent *in vitro* and *in vivo* NK1 receptor antagonists ever identified [1].

In the development of new medicines, impurity profiling [2] is a crucial step for assuring the quality of the drug substance. Understanding how impurities form and potentially react in the downstream chemistry is essential to establish critical control points in the synthetic process, where appropriate in process controls and specification limits have to be set [3–6]. Regulatory requirements for the identification and control of impurities in the pharmaceutical industry are subject to the International Conference on Harmonization (ICH) Guideline Q3A2, Impurities in New Drug Substances [7]. These aspects, which are fundamental to patient safety throughout the whole development process, become even more critical in the late stage of development, due to their impact on the success of registration and the transfer to commercial production.

Identification and control of impurities in drug substances are usually performed by means of a series of state-of-the-art analytical techniques. While Nuclear Magnetic Resonance (NMR) is often the easiest and most straightforward technique for the unambiguous identification of unknown impurities, High Performance Liquid Chromatography (HPLC), especially in combination with Mass Spectrometry (MS), offers a more sensitive and accurate technique for the detection of trace level impurities. In addition, quantitative NMR methods are also reported and may be successfully used [8,9]. However, due to the intrinsic limitation of each individual analytical technique, the concept of orthogonality is becoming increasingly popular to ensure the accuracy of the analytical results reported [10].

This work describes the analytical strategy, based on the orthogonal use of NMR and HPLC–MS techniques, adopted for the development of the most suitable analytical methodology to detect,

* Corresponding author. Present address: Medicines Research Centre, Aptuit Verona srl, Via Fleming 4, 37135 Verona, Italy. Tel.: +39 045 8219432; fax: +39 045 8218072.
E-mail addresses: lucilla.turco@aptuit.com, lucillaturco@yahoo.it (L. Turco).
[1] Present address: Medicines Research Centre, Aptuit Verona srl, Via Fleming 4, 37135 Verona, Italy.
[2] Present address: Manufacturing Technology, Infineum Italia srl, Strada Di Scorrimento, 2, Vado Ligure, 17047 Savona, Italy.

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.08.010

identify and quantify an impurity in casopitant mesylate drug substance batches.

2. Experimental

2.1. NMR

^{1}H NMR spectra were recorded in DMSO-d_6 at 25 °C using a Varian INOVA 600 Spectrometer operating at 599.7 MHz. The following parameters were used: 9 kHz sweep width, 32K time-domain points, acquisition time of 3.56 s, 60° pulse width, pre-acquisition delay 10 s, 16 scans.

^{19}F NMR spectra of casopitant mesylate batches were recorded at 25 °C using a Varian INOVA 400 Spectrometer operating at 376.1 MHz, using a number of scans of 512, after dissolution in DMSO-d_6. The reference standards of the test mix were prepared using a stock solution of 10% (v/v) methanesulfonic acid in DMSO-d_6, in order to achieve complete salt formation. For these samples 16 scans were used. The ^{19}F resonances were referenced automatically on the first spectrum (external reference trifluoroacetic acid −76.5 ppm) and then adjusted on the following spiked spectra by fixing the casopitant mesylate CF_3 line at −61.257 ppm.

For all the ^{19}F NMR spectra the acquisition parameters were: 85 kHz sweep width, 64K time-domain points, acquisition time 0.6 s, pulse width 30°, pre-acquisition delay 1 s. ^{19}F NMR data were zero-filled (fn = 128K) and processed using a Lorentzian–Gaussian apodization parameters (lb = −0.5, gf = 0.4).

The quantification was performed by integration of the relevant signal in the CF_3 ^{19}F NMR region against an isolated ^{13}C satellite of the casopitant mesylate CF_3 resonance. As all compounds involved in this study have in common the presence of two symmetric CF_3 groups, no correction for the number of fluorine nuclei was applied in the calculation.

2.2. Reverse phase LC–MS/MS method for intermediate (3)

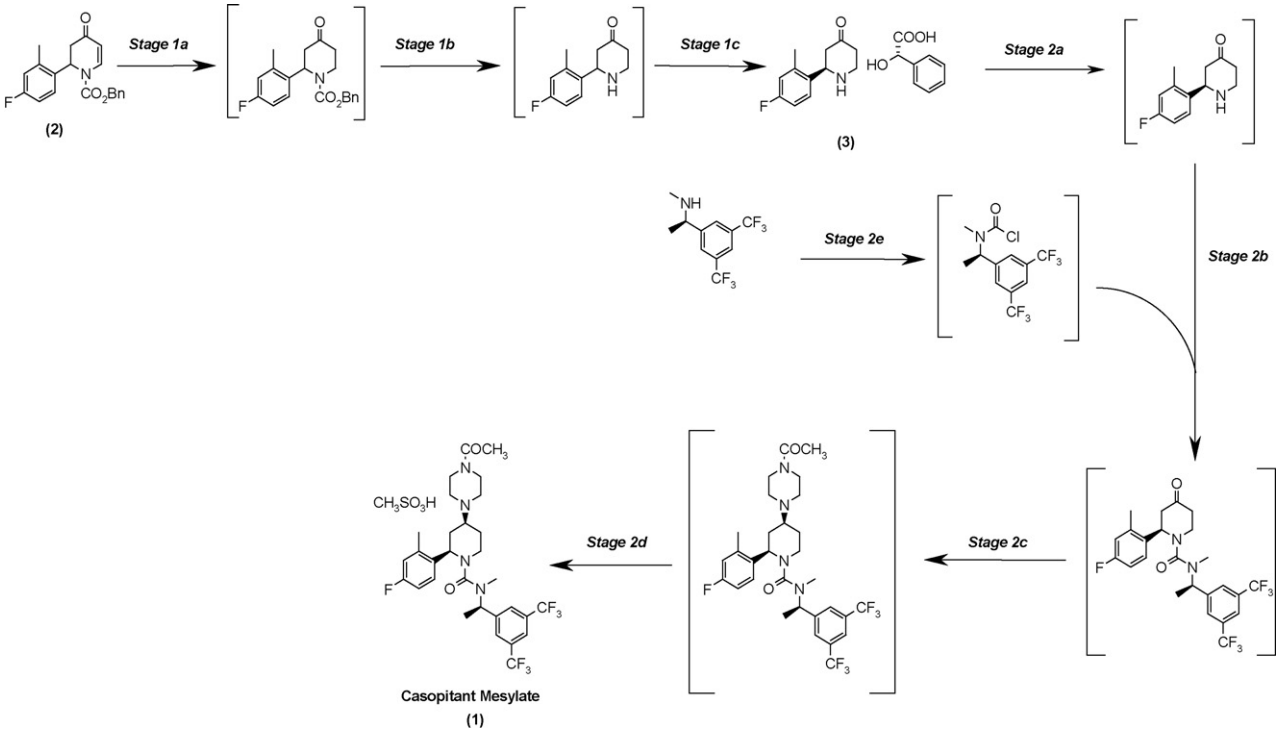
Reverse phase LC–MS/MS experiments have been performed on Agilent 1100 HPLC system (Palo Alto, CA, USA) coupled to an Agilent MSD XCT Trap Mass spectrometer (Agilent Technology, Santa Clara, CA, USA), operating in positive Electrospray ion mode. The separation conditions were: analytical column Waters X-Bridge C18 (4.6 mm × 150 mm, particle size 3.5 μm) (Waters Corporation, Milford, MA, USA), mobile phase A = water + 0.2% (v/v) ammonium hydroxide; mobile phase B = acetonitrile + 0.2% (v/v) ammonium hydroxide; gradient elution: from 25% B to 80% B in 15 min, column temperature: 40 °C, flow rate: 1 ml/min split to 200 μl prior the mass spectrometer. Data were acquired by Auto MS (2) mode, with MSMS fragment amplitude of 0.40 V.

2.3. Normal-phase LC–MS method

A normal-phase LC–MS method for accurate quantification of the de-fluorinated impurity in casopitant mesylate was previously developed and validated [11].

3. Results and discussion

The commercial process to synthesize casopitant mesylate (**1**) is a multi-stage convergent process (Scheme 1) [12]. In an advanced phase of development, an impurity was detected by HPLC during monitoring of the quality of the intermediate (**3**). This impurity had not been noticed before, due to the smaller scale of synthesis and the limited information available at earlier stages of development. Therefore, targeted studies of the process were undertaken to understand the origin and the fate of this impurity and to put in place a suitable analytical control strategy [13].



Scheme 1. Casopitant mesylate synthetic process.

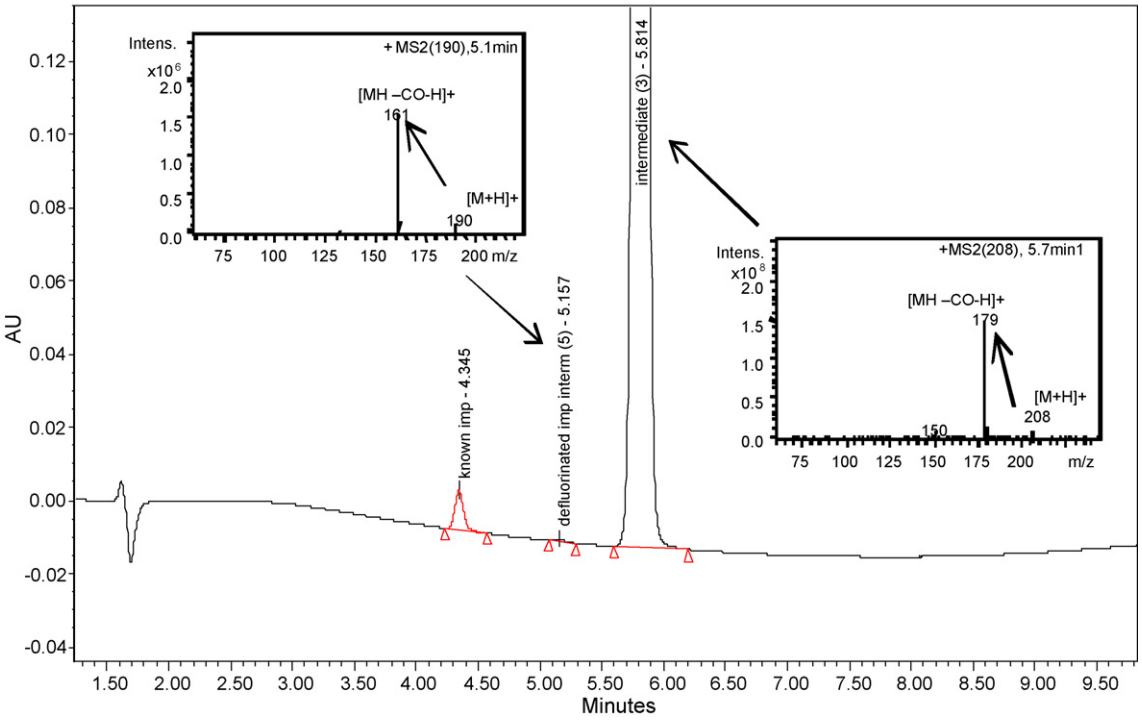


Fig. 1. HPLC chromatogram of the intermediate (**3**). The de-fluorinated impurity (**5**), Rt = 5.16 min, is detected at approximately 0.1% a/a.

The HPLC method for the intermediate (**3**), coupled with mass spectrometry detection suggested that the impurity at this stage could be the de-fluorinated analogue (**5**) (Fig. 1 and Scheme 2).

Considering the chemistry applied, it was immediately clear that this impurity could carry through to the next synthetic steps and lead to the de-fluorinated analogue of casopitant mesylate drug substance, as illustrated in Scheme 2.

Unfortunately, the reverse phase-HPLC method currently used for the drug substance was unsuitable in terms of detecting any new impurities associated with potential de-fluorination of drug substance (that is, four possible diastereoisomers). A preliminary investigation was performed by LC–MS and confirmed co-elution of casopitant mesylate with one of its de-fluorinated analogues [11]. Following these results, a strategy for the detection and quantification of this co-eluting impurity was put in place, by means of the evaluation of two new methods involving LC–MS and NMR spectroscopy, respectively.

3.1. Detection and first quantification method: NMR spectroscopy

Initially, the full NMR characterization of the de-fluorinated analogues of the drug substance was carried out with three main objectives: (a) the characterization of all potential diastereoisomers, (b) the identification of which diastereoisomer was actually present as a contaminant of casopitant mesylate batches and (c) the evaluation of an NMR-based approach for the detection and quantification of this new impurity.

The synthesis of all the four potential diastereoisomers of casopitant mesylate de-fluorinated analogues was carried out according to Scheme 2.

Starting from compound (**4**), the de-fluorinated analogue of (**2**), the racemic de-fluorinated analogue of (**3**) was obtained (**5**). After coupling with benzylamine (**7**), the two piperidone-ureas (**8**) and (**9**) were obtained and separated *via* chromatographic column. Each piperidone-urea was separately submitted to reductive amination with *N*-acetylpiperazine obtaining compounds *syn* 1 and *anti* 2 from piperidone-urea (**8**) and compounds *syn* 2 (**6**) and *anti* 1 from piperidone-urea (**9**). Each mixture was then treated with methanesulfonic acid using the same conditions as for casopitant mesylate, leading to precipitation of diastereoisomers *anti* 2 and *syn* 2 (**6**), respectively. This experiment allowed excluding the possibility that the diastereoisomers *syn* 1 and *anti* 1 could be contaminants of casopitant mesylate batches.

The two isolated de-fluorinated diastereoisomers *syn* 2 (**6**) and *anti* 2 were then fully characterized by NMR. The relative *syn* and *anti* configurations were determined by using information from both nOe's and ^{1}H–^{1}H coupling constants. Despite some representative resonances of these diastereoisomers were separated in ^{1}H NMR spectra, ^{1}H NMR could not be used for evaluating their presence in casopitant mesylate batches, due to the almost complete overlapping of their signals with those of the drug substance.

As a consequence, ^{19}F NMR was evaluated as it could provide a higher spectral simplification: the potentially diagnostic sharp CF_3 signals could be detected at an adequate distance from the other aryl-F resonance (which are obviously relevant for the drug substance identification, but not for its de-fluorinated analogues). ^{19}F NMR spectra were collected for both isolated *syn* 2 (**6**) and *anti* 2 diastereoisomers and also for the mixture of all the four diastereoisomers, as free bases. A stock solution of methanesulfonic acid in DMSO-d_6 (10%, v/v) was used to dissolve the mixture of the four free bases in order to achieve a complete salt formation and thus properly compare the ^{19}F chemical shifts of the CF_3 groups with those of the two precipitated *syn* 2 (**6**) and *anti* 2 diastereoisomers (Fig. 2).

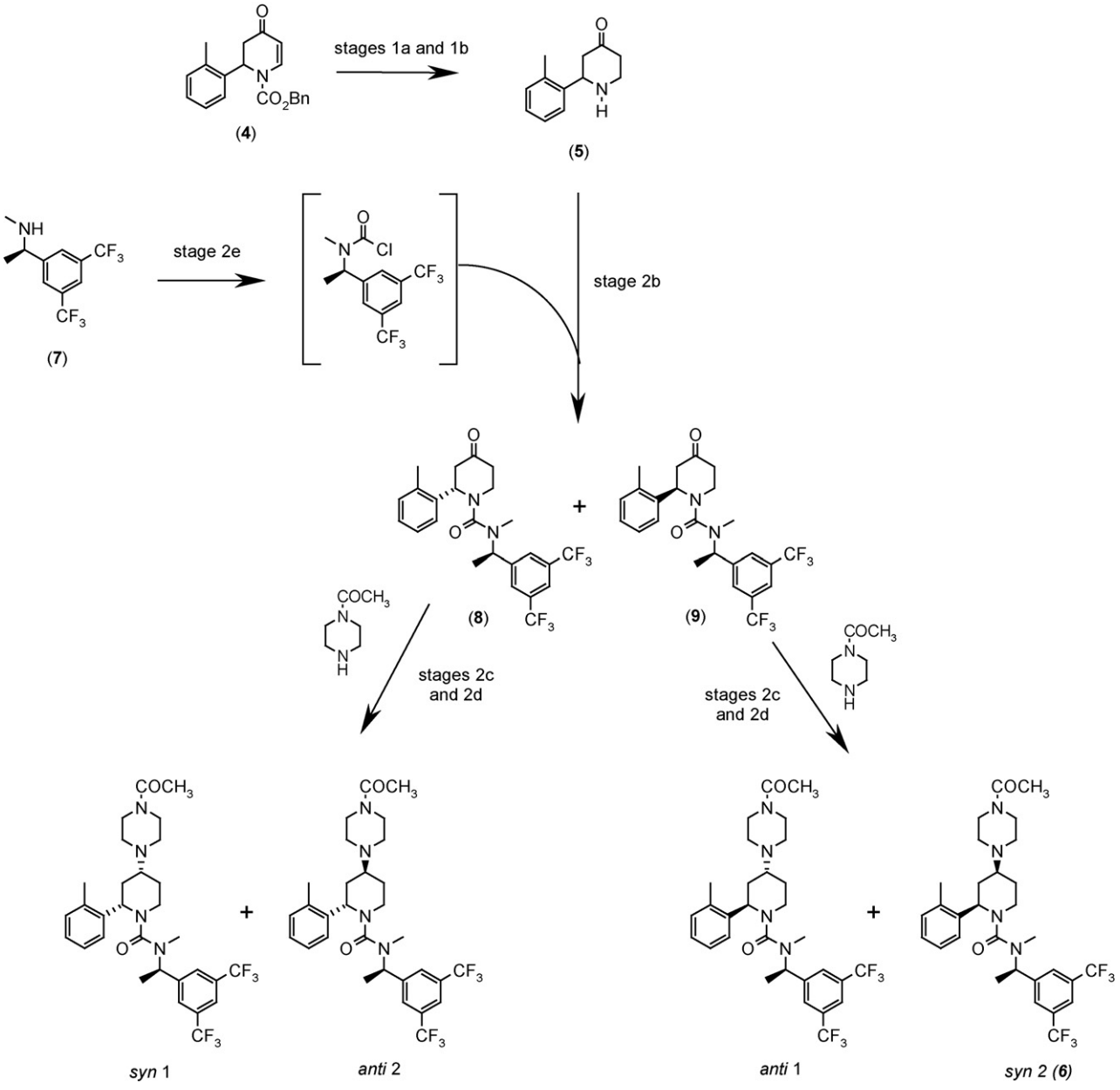
3.2. ^{19}F NMR method validation and specificity

Two batches of the intermediate (**3**) were opportunely spiked with 0.1% (w/w) and 0.3% (w/w) of the corresponding de-

fluorinated analogue (**5**), respectively, in order to prove that the de-fluorinated impurity carries through to the chemical process stoichiometrically. The synthetic process was carried out as shown in Scheme 1 and the two final batches of the drug substance were analyzed by ^{19}F NMR. Both samples showed the presence of an extra peak in the CF_3 region of ^{19}F NMR spectrum (compared to the ^{19}F NMR of casopitant mesylate), which corresponded to the previously reported *syn* 2 (**6**) de-fluorinated casopitant mesylate analogue. Moreover, the integration of this signal in both samples was consistent with the spiking percentage of the starting material used for their synthesis (ca. 0.1% and ca. 0.3% molar ratio by ^{19}F NMR in the two spiking experiments, respectively).

Further confirmation that this CF_3 NMR line was effectively due to the de-fluorinated analogue *syn* 2 (**6**) was demonstrated by spiking the sample enriched with the de-fluorinated analogue with the standard mixture of four de-fluorinated diastereoisomers, directly in the NMR tube (Fig. 3).

A validation of ^{19}F NMR method for the detection of all the potential impurities in casopitant mesylate batches was then performed with the aim to demonstrate specificity towards the de-fluorinated analogues. A progressive spiking of a representative batch of casopitant mesylate with all the components of the HPLC test mixture was carried out (Fig. 4). The NMR analysis of this sample demonstrated good selectivity of the method for the *syn* 2 (**6**), but not for the *anti* 2 diastereoisomer (as its resonance overlapped with that of casopitant mesylate). However this overlap of peaks was not a concern in terms of the quality of the drug substance. In fact the reductive amination reaction (Stage 2c on compound (**9**) in Scheme 2) occurs with a *syn*/*anti* ratio of approximately 2/1 and, since the specification limit is set at not more than



Scheme 2. Fate of the de-fluorinated impurity (**5**) of (**3**) impacting the quality of final casopitant mesylate drug substance.

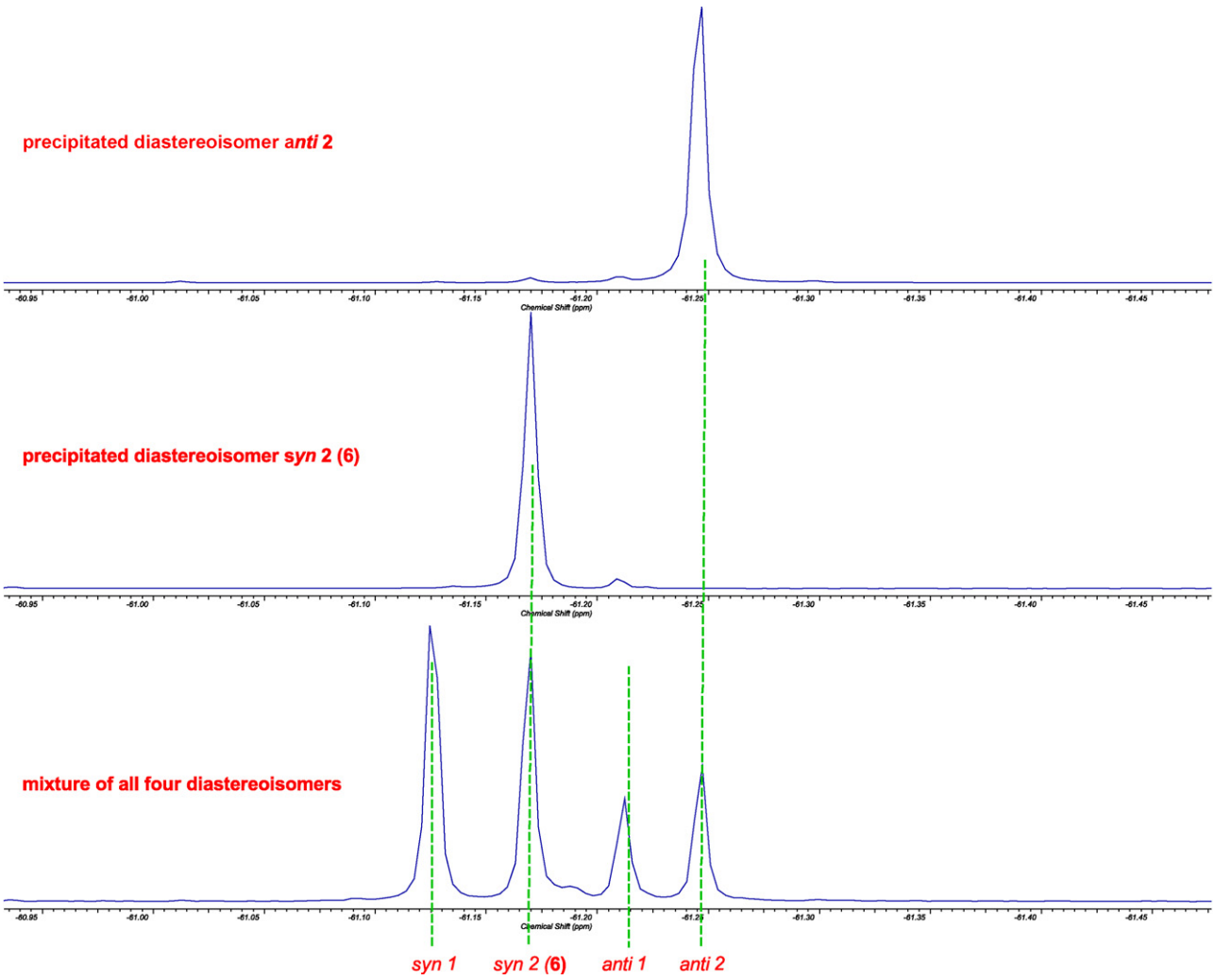


Fig. 2. Top and middle: ^{19}F NMR spectra expanded plot of the isolated diastereoisomers *anti* 2 and *syn* 2 compared with the ^{19}F NMR spectrum of the original mixture of four de-fluorinated analogues diastereoisomers of (**1**) (bottom). The other two ^{19}F resonances in the latter spectrum were assigned to *syn* 1 and *anti* 1 diastereoisomers from ^{1}H NMR data.

0.15% a/a for compound (**5**) in intermediate (**3**), full control of the *anti* 2 diastereoisomer in the drug substance is ensured within ICH limits [9].

3.3. Orthogonal quantification method: normal-phase LC–MS

In order to confirm the semi-quantitative NMR results, an alternative method for the accurate quantification of the *syn* 2 (**6**) de-fluorinated diastereoisomer in casopitant mesylate drug substance was successfully developed and validated [11]. The original reversed-phase liquid chromatography–ultraviolet detection method for routine testing of casopitant mesylate was initially considered for a possible revision. However, the number of potential impurities to be detected and their structure similarity did not lead to a unique robust method suitable for full separation of the all the new impurities. Therefore a change in separation mode was performed by the use of normal-phase LC separation coupled with UV detection. To increase the sensitivity of the method, mass spectrometric detection by single ion monitoring of the *syn* 2 (**6**) de-fluorinated diastereoisomer of casopitant mesylate was applied (Fig. 5).

The method was fully validated and fulfilled validation criteria. Quantification of the de-fluorinated analogue in casopitant mesylate was performed against calibration curves and expressed as % w/w.

3.4. Quality control of casopitant mesylate batches

Casopitant mesylate batches produced during the manufacturing campaigns were analyzed by means of both ^{19}F NMR and normal-phase LC–MS, in order to demonstrate the orthogonality of both techniques in verifying the presence and measuring the correct amount of its de-fluorinated analogue.

Table 1
Quantitative results obtained by means of ^{19}F NMR and normal-phase HPLC.

Batch number	% w/w of (**6**) by ^{19}F NMR	% w/w of (**6**) by NP HPLC
Batch 1	N.D.	0.01
Batch 2	N.D.	0.02
Batch 3	0.07	0.06
Batch 4	0.08	0.06
Batch 5	0.06	0.04
Batch 6	0.09	0.08

N.D. = not detected.

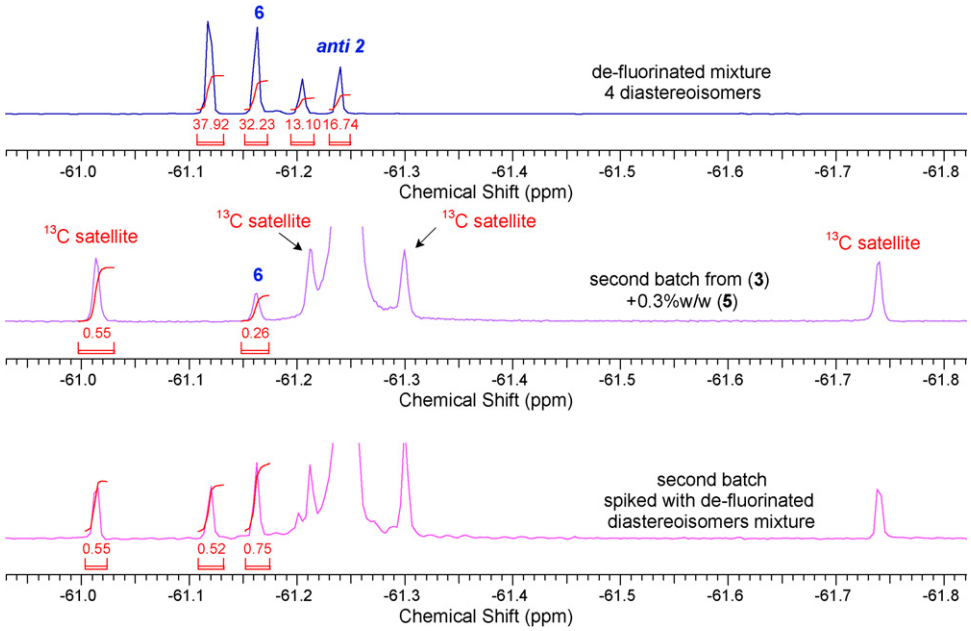


Fig. 3. ^{19}F NMR spectrum expanded plot of the four de-fluorinated diastereoisomers in mixture (top) compared with that of the sample deriving from (**3**) containing 0.3% (w/w) of its de-fluorinated analogue (**5**) (middle) and with the ^{19}F NMR spectrum obtained after spiking with the four de-fluorinated analogues diastereoisomers mixture (bottom).

Overall the quantitative results reported in Table 1 demonstrate a good correlation between NMR and LC–MS data. This consistency allowed the project team to put in place an orthogonal analytical approach to support future manufacturing of casopitant mesylate, in which NMR could be used as a fit-for-purpose tool for first line identification and quantification of the new impurity, while LC–MS could be dedicated to obtain accurate quantitative results as demanded by regulatory or quality control requirements.

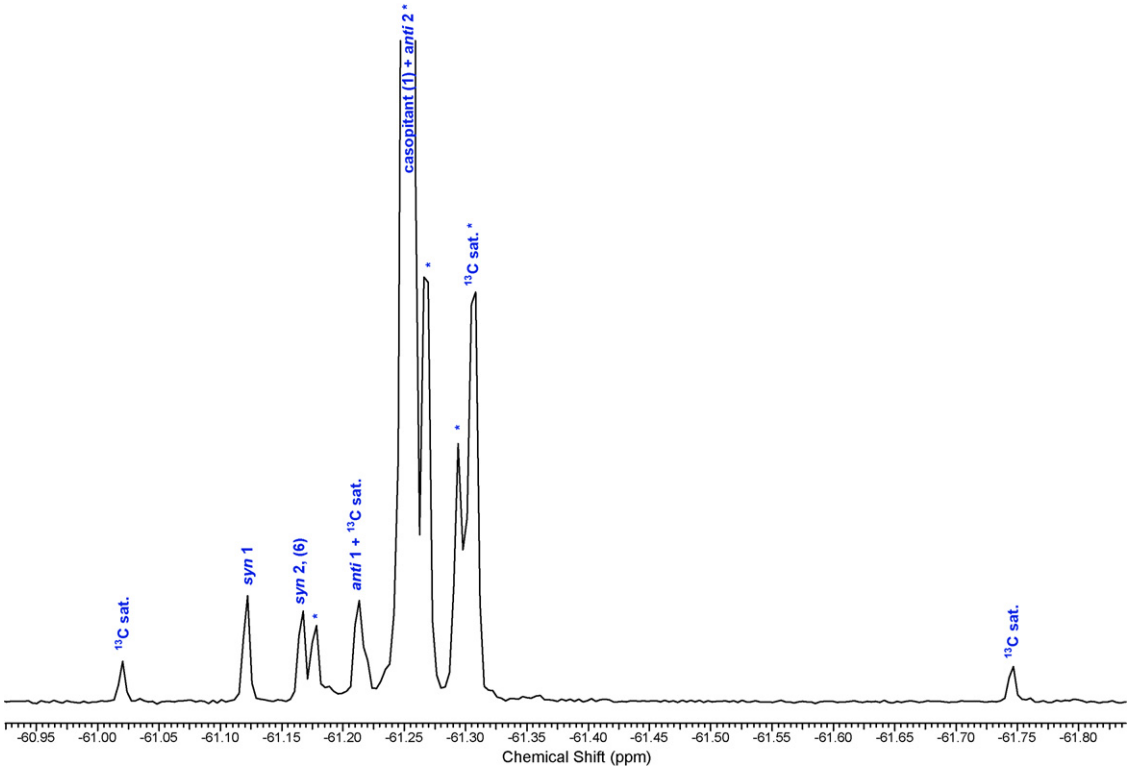


Fig. 4. Expanded CF_3 region of the ^{19}F NMR spectrum collected on a sample obtained after progressive spiking of a casopitant mesylate batch with its structure-related impurities (HPLC test mixture). The four de-fluorinated diastereoisomers are highlighted, as well as ^{13}C satellites associated to casopitant mesylate CF_3 resonance. The acceptable separation of de-fluorinated *syn* 1 and *syn* 2 (**6**) can be appreciated. The *anti* 1 diastereoisomer can also be monitored; however, its resonance is overlapped with one ^{13}C satellite of casopitant mesylate CF_3 resonance. Besides, the *anti* 2 diastereoisomer peak is completely hidden by the main casopitant mesylate CF_3 peak. The other CF_3 resonances, belonging to the qualified casopitant mesylate impurities, are highlighted with an asterisk (*).

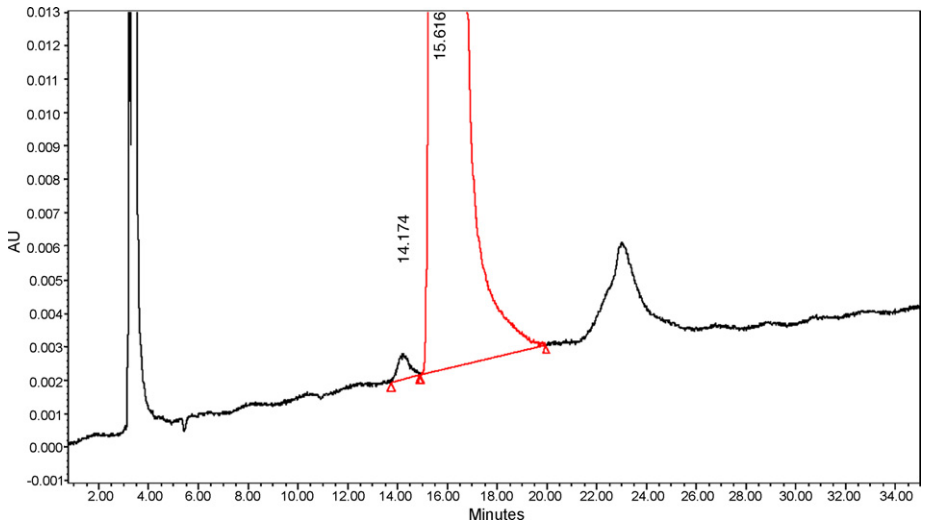


Fig. 5. Normal-phase LC–MS chromatogram of casopitant mesylate (Rt = 15.6 min) showing the peak of the de-fluorinated analogue of the drug substance *syn 2* (**6**) at Rt = 14.2 min.

4. Conclusions

This work describes the successful development of an analytical control strategy for a de-fluorinated impurity in casopitant mesylate during late phase development. Once both source and nature of the new impurity were confirmed by analyzing samples of casopitant mesylate spiked with markers of the potential impurities, ^{19}F NMR proved to be a selective and efficient first line detection technique to verify the presence of de-fluorinated analogue impurities in casopitant mesylate batches. In addition, these NMR results, although still semi-quantitative, showed a good correlation with the quantitative results obtained by analyzing casopitant mesylate batches using a more accurate normal-phase LC–MS.

The development and orthogonal use of these methodologies helped us to better understand the potential issues related to the presence of traces of the de-fluorinated impurity and therefore to take the correct decisions in order to progress the project at this late stage of drug development.

Acknowledgements

The authors would like to thank Fernando Bravo, Giulio Camurri and Jill Trewartha for the support they provided to this work.

References

[1] R. Di Fabio, C. Griffante, G. Alvaro, G. Pentassuglia, D.A. Pizzi, D. Donati, T. Rossi, G. Guercio, M. Mattioli, Z. Cimarosti, C. Marchioro, S. Provera, L. Zonzini, D. Montanari, S. Melotto, P.A. Gerrard, D.G. Trist, E. Ratti, M. Corsi, Discovery process and pharmacological characterization of 2-(S)-(4-fluoro-2-methylphenyl)piperazine-1-carboxylic acid [1-(R)-(3,5-bis-trifluoromethylphenyl)ethyl]methylamide (Vestipitant) as a potent, selective, and orally active NK1 receptor antagonist, J. Med. Chem. 52 (2009) 3238–3247.
[2] F. Qiu, D.L. Norwood, Identification of pharmaceutical impurities, J. Liq. Chromatogr. R. T. 30 (2007) 877–935.
[3] K.M. Alsante, T.D. Hatajik, L.L. Lohr, T.R. Sharp, Isolation and identification of process related impurities and degradation products from pharmaceutical drug candidates, part I, Am. Pharm. Rev. 4 (2001) 70–78.
[4] L.L. Lohr, T.R. Sharp, K.M. Alsante, T.D. Hatajik, Isolation and identification of process related impurities and degradation products from pharmaceutical drug candidates: part II—the roles of NMR and mass spectrometry, Am. Pharm. Rev. (2001) 104–113.
[5] D. Bartos, S. Görög, Recent advances in the impurity profiling of drugs, Curr. Pharm. Anal. 4 (2008) 215–230.
[6] S. Vasanti, S. Sulabha, Impurity profile—a review, Drug Invention Today 1 (2009) 81–88.
[7] Q3A (R2), Impurities in new drug substances, in: ICH Harmonized Tripartite Guideline, 2006.
[8] R.F. Evilia, Quantitative NMR spectroscopy, Anal. Lett. 34 (2001) 2227–2236.
[9] G. Maniara, K. Rajamoorthi, S. Rajan, G.W. Stockton, Method performance and validation for quantitative analysis by ^{1}H NMR and ^{31}P NMR spectroscopy. Applications to analytical standards and agricultural chemicals, Anal. Chem. 70 (1998) 4921–4928.
[10] M.D. Argentine, P.K. Owens, B.A. Olsen, Strategies for the investigation and control of process-related impurities in drug substances, Adv. Drug Deliv. Rev. 59 (2007) 12–28.
[11] R. Dams, E. Bernabe, A. Nicoletti, C. Loda, L. Martini, D. Papini, Quantitation of a de-fluorinated analogue of casopitant mesylate by normal phase liquid chromatography–mass spectrometry, Rapid Commun. Mass Spectrom. (2010), doi:10.1002/rcm.4688.
[12] Z. Cimarosti, F. Bravo, D. Castoldi, F. Tinazzi, S. Provera, A. Perboni, P. Westerduin, Application of the QbD principles in the development of the Casopitant Mesylate manufacturing process. Process research studies for the definition of the control strategy for Stages 2a, 2b and 2c, Org. Process Res. Dev. 14 (2010) 805–814.
[13] F. Bravo, Z. Cimarosti, F. Tinazzi, D. Castoldi, P. Stonestreet, A. Galgano, P. Westerduin, Development of a control strategy for the control of the manufacturing process of a de-fluorinated analogue of Casopitant Mesylate following the quality by design principles, Org. Process Res. Dev. 14 (2010) 832–839.