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Determination of eight isomers and related substance of Aprepitant using normal-phase and reverse-phase HPLC methods with mass spectrophotometric detection

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ABSTRACT

Introduction: Aprepitant is a chiral prodrug possessing three stereogenic centers and exists in eight stereochemical forms. It was aimed to estimate and validate Aprepitant using reverse phase HPLC.
Materials and methods: Chiral liquid chromatography with an ion-trap mass spectrophotometer was used to isolate all the isomers. The separation of the eight isomers was achieved on an amylase-based chiral column (Chiralpak ADH, 250 mm × 4.6 mm) using *n*-hexane/isopropyl alcohol/methanol/trifluoroacetic acid (970/40/4/0.5, vol/vol/vol/vol) as the mobile phase at a flow rate of 0.5 ml/min. A reverse-phase HPLC method was used to analyze Aprepitant and its related substances. The determination of Aprepitant and its related substances was developed by using Xterra RP – 18, 250 mm × 4.6 mm, 5 μm column.
Results and conclusion: These methods were validated according to the International Conference on Harmonization (ICH) guidelines. The limit of detection (LOD) and limit of quantitation (LOQ) for Aprepitant were found to be 0.14 μg/ml and 0.41 μg/ml, respectively, for chiral substance and 0.07 μg/ml and 0.21 μg/ml, respectively, for the related substance.

Aprepitant (APT) is a substance P (SP)/neurokinin 1 (NK_1) receptor antagonist and is chemically described as 5-[[(2R, 3S)-2-[(1R)-1-[3, 5-Bis (trifluoromethyl) phenyl] ethoxy] -3-(4-fluorophenyl)-4-morpholinyl] methyl]-1, 2-dihydro-3H-1,2,4-triazol-3-one (Fig. 1). It is a white to off-white crystalline solid with a molecular weight of 534.43 and an empirical formula of $C_{23}H_{21}F_7N_4O_3$. APT has recently been approved in combination with other agents for use as an effective treatment for preventing acute and delayed chemotherapy-induced nausea and vomiting (CINV).[1]This is the first FDA-approved drug for the treatment of CINV that persists for more than 24 h after chemotherapy treatment. It is a drug known to elicit activity against the human neurokinin-1 (NK_1) receptor.[2] This receptor is located at the brain stem nuclei of the dorsal vagal complex and is a crucial part of the regulation of emesis (vomiting), which is due to the receptor binding with substance P, a peptide neurotransmitter.[3–5]

Chiral separations can be achieved with HPLC through the following approaches: (a) direct separation of racemates to their corresponding enantiomers using chiral stationary phases (CSPs)[6]; (b) indirect separation of diastereoisomers, formed by the reaction of the enantiomers with a chiral derivatizing agent, using achiral stationary phases[7]; or (c) separation of chiral derivatives, formed by reaction with non-chiral derivatizating agents, using CSPs.[8–13] Direct methods based on CSPs are the preferred separation approaches because they are simple and rapid to apply at both the analytical and preparative scales.

Literature reviews reveal that very few analytical methods have been established for the estimation of Aprepitant in human plasma.[14–17] The following have been reported: an HPLC chromatographic reactor approach for investigating the hydrolytic stability of a pharmaceutical compound,[18] an estimation of APT in rhesus macaque plasma,[19] the characterization and quantization of APT drug substance polymorphs by attenuated total reflectance Fourier infrared spectroscopy,[20] an investigation into the stability of an extemporaneous oral liquid APT formulation,[21,22] and an estimation of APT capsules by RP-HPLC.[23]

Enantioseparation of chiral compounds containing multiple stereogenic centers poses some analytical challenges.[24] A complete resolution of all the potential stereoisomers of these compounds

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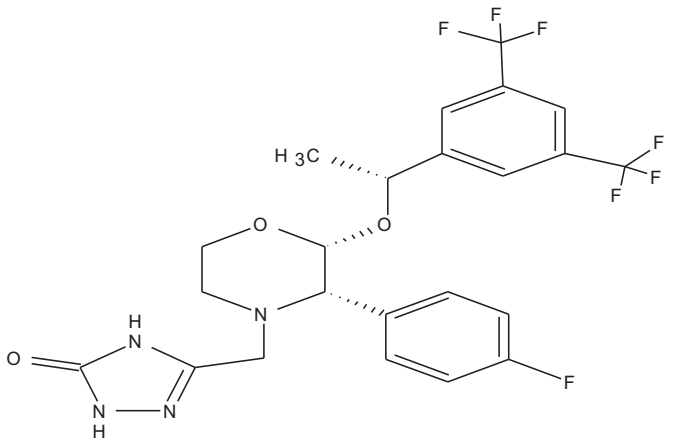

Fig. 1. The chemical structure of aprepitant.

can only be achieved if the CSP used is highly discriminating for both enantiomers and diastereomers. This paper will highlight the challenges we faced in developing a chiral HPLC separation for APT, a novel chiral drug possessing three stereogenic centers that allow for eight stereoisomers (Table 1). The development of a comprehensive HPLC method was the proposed strategy for separating all eight isomers of Aprepitant. This strategy was based on the fact that CSP is highly effective in resolving enantiomers with complementary intermolecular interaction sites.[25] When the method development strategy was executed, several different CSPs, including an amylase-based CSP, were identified that possessed enantioselectivity for both enantiometric pairs (SR/RS and RR/SS) of APT. Furthermore, amylase-based CSPs showed the promise of simultaneously separating all eight isomers with the help of ion-trap mass spectrophotometry.

A relative response factor for all these impurities with respect to Aprepitant was determined for the quantitative determination of the known impurities, and other unknown impurities were determined using the diluted standard method. The determination of the related substances and degradation products in an API at a lower level of 0.10% or below often leads to a misleading interpretation of the results in the absence of a relative response factor. It has been observed in many cases that the related substance or the degradation product generated during the manufacturing of an API may not have the same UV response at the wavelength of determination in these tests. Therefore, there is a need to use either impurity standards or a relative response factor (RRF) for the quantitative determination of low levels of impurities and degradation products. Reverse-phase-HPLC methods were used for the qualitative and quantitative determination of Aprepitant. The column selection and the mobile-phase composition were found to play a vital role in the separation and sensitivity of the developed method. The proposed RP-HPLC method with UV detection has been validated using the ICH and USP[26,27] guidelines as a reference.

1. Experimental

1.1. Standards and solvents

Aprepitant, 5-[[2(R)-[1(S)-[3,5-bis(trifluoromethyl)phenyl] ethoxy]-3(S)-(4-fluorophenyl)-4 morpholinyl]methyl]-1,2-dihydro-3H-1,2,4-triazol-3-one (Impurity A), hydrazinecarboxylic acid, 2-[2-[(2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]-1-iminoethyl]-, methyl ester (Impurity B) and 5-[[2(R)-[1(R)-[3,5- bis(trifluoromethyl)phenyl] ethoxy]-3(S)- phenyl-4- morpholinyl]methyl]-1,2-dihydro-3H-1,2,4-triazol-3-one (Impurity C) were used as inhouse reference standards (Glenmark Generics Ltd., Glenmark R & D Centre, MIDC, Mahape, Navi Mumbai, India). HPLC grade *n*-hexane, isopropyl alcohol (IPA), methanol, ethanol, and acetonitrile (ACN) were purchased from Rankem and GR-grade trifluoroacetic acid (TFA) and ortho-phosphoric acid (OPA) were purchased from Merck Specialties Chemicals Private Limited (Mumbai, India). Deionized water was purified with a TKA Ultrapure water system (Germany).

Table 1
The stereochemistry of all the possible isomers of Aprepitant.

Diastereomeric pair	Compound	Absolute configuration
I	I_a (Aprepitant)	$R_1R_2S_3$
	I_b (The enantiomer of Aprepitant)	$S_1S_2R_3$
II	II_a	$R_1S_2R_3$
	II_b	$S_1R_2S_3$
III	III_a	$R_1R_2R_3$
	III_b	$S_1S_2S_3$
IV	IV_a	$S_1R_2R_3$
	IV_b	$R_1S_2S_3$

1.2. Analytical mode high-performance liquid chromatography

A Waters (Milford, MA, USA) 2695 series HPLC system equipped with a 2695 series quaternary gradient pump, an autosampler with a cooler and a PDA system and a Shimadzu LC 2010 CHT (Japan) HPLC module equipped with a quaternary gradient pump, a column oven, an autosampler and a DAD system were used for the analysis and validation of the proposed method. The data were recording using Empower 2 and LC solution software for the Waters and Shimadzu systems, respectively.

1.3. Chromatographic conditions

1.3.1. Stereochemical purity

The analysis was conducted on an amylase-based Chiralpak AD-H (250 mm × 4.6 mm, 5 μm, Daicel Chemical Industries Limited, Japan) using a mobile phase consisting of *n*-hexane/isopropyl alcohol/methanol/trifluoroacetic acid (970/40/4/0.5, vol/vol/vol/vol) with a UV detector at a wavelength of 210 nm and a flow rate of 0.5 ml/min. The column was maintained at 35 °C throughout the analysis. A 20 μl sample of the concentrate was injected, and the chromatogram was recorded for 70 min.

1.3.2. The related substances

The mobile phase consisted of 0.1% OPA in water and acetonitrile. Analyses were performed with a gradient elution starting with 58/42 (vol/vol) for 44 min, then modified to 30/70 (vol/vol) for 10 min, and returned to 58/42 (vol/vol) after 55 min from the time of injection. The flow rate was 1.0 ml/min, and the total duration of the chromatographic run was 65 min. The Xterra RP – 18, 250 mm × 4.6 mm, 5 μm column was used for the determination of the related substances of Aprepitant.

1.4. Preparation of the reference, the test solutions and the system suitability solutions

1.4.1. Stereochemical purity

A diluent was prepared by mixing *n*-hexane and ethanol in a 95:05 ratio (vol/vol). Approximately 10.0 mg of the standard mixture of isomers was transferred into a 10 ml volumetric flask, and 5 ml of the diluent was added. After the isomers were dissolved, the flask was filled to the mark with the diluent. This