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Research Article

Development and Validation of a New RP-HPLC Method for the Determination of Aprepitant in Solid Dosage Forms

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Abstract

***Purpose*:** To develop and validate a new, simple, sensitive, rapid, cost-effective and accurate reverse phase-high performance liquid chromatography (RP-HPLC) method for the determination of aprepitant (APT) in capsule dosage form.

Methods: The method developed for the determination of APT in capsule formulation involved using RP-HPLC which incorporated a C_{18} column 250 x 2.5 mm i.d, 5 µm particle size, in isocratic mode, with mobile phase comprising of methanol: water in the ratio of 90:10 (v/v). The flow rate was 1 mL/min and the detection was monitored at 220 nm. The total run time was 10 min and the column was maintained at ambient temperature.

Results: APT was eluted in the given mobile phase with a retention time (t_r) of 4.473 min. The linearity for the quantification of APT was 0.1 - 10 µg/mL (R^2 =0.989, Y= 33032x + 71501) with coefficients of variation (based on mean peak area for six replicate injections) in the range 0.04 to 0.132. The limits of detection and of quantification were 0.028 and 0.094 µg/mL, respectively. Recovery of the method was 99.56 - 100.5 % while the relative standard deviation (RSD) of intra-day and inter-day precision was 0.85 and 1.05, respectively. System precision and method precision were 0.013 and 1.400 %, respectively. The specificity data of the proposed method indicated that excipients in the capsules did not interfere with the drug peak. Furthermore, the well-shaped peaks buttressed the specificity of the method.

***Conclusion*:** The proposed RP-HPLC method is simple, sensitive, rapid, cost-effective and accurate for the determination of APT in both bulk materials and pharmaceutical dosage forms.

Keywords: Aprepitant, RP-HPLC, Isocratic, Pharmaceutical dosage forms, Analysis, Validation

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INTRODUCTION

Aprepitant (APT) is substance P/neurokinin 1 (NK1) receptor antagonist, and chemically, it is 5-[[(2R,3S)-2-[(1R)-1-[3,5-bis (trifluoro-methyl) phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2dihydro-3H-1,2,4-triazol-3-one (Fig 1).

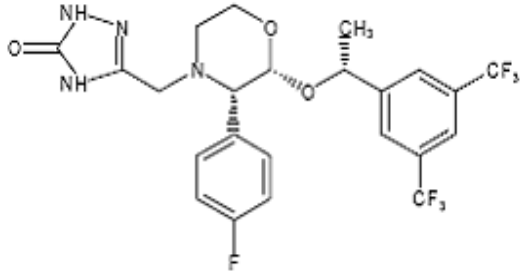


Fig 1: Chemical structure of aprepitant

It is a white to off-white crystalline solid, with a molecular weight of 534.43 and empirical formula $C_{23}H_{21}F_7N_4O_3$. It is a selective high-affinity antagonist of human substance P/neurokinin 1 (NK1) receptors and has little or no affinity for serotonin (5-HT3), dopamine, and corticosteroid receptors, which are the targets of existing therapies for chemotherapy-induced nausea and vomiting (CINV) as well as postoperative nausea and vomiting (PONV) [1]. APT has been shown in animal models to inhibit emesis induced by cytotoxic chemotherapeutic agents, such as cisplatin, via central actions.

Animal and human positron emission tomography (PET) studies with APT have shown that it crosses the blood brain barrier and occupies brain NK 1 receptors [2]. Animal and human studies show that APT augments the antiemetic activity of the 5-HT_3 receptor antagonist, ondansetron, and the corticosteroid, dexamethasone, and inhibits both the acute and delayed phases of cisplatin-induced emesis.

It has recently been demonstrated that substance P (SP) and neuro kinin -1 (NK-1) receptor antagonists induce cell proliferation and cell inhibition in human melanoma cells. Literature review reveals that very few analytical methods have been established for the determination of APT and its metabolites in human plasma [3,4]. They include HPLC chromatographic reactor approach for investigating the hydrolytic stability of a pharmaceutical compound [5], estimation of APT in rhesus macaque plasma [6], characterization and quantitation of APT drug substance polymorphs by attenuated total reflectance Fourier transform infrared spectroscopy [7], and estimation of APT by RP-HPLC [8]. To the best of our knowledge, only one RP-HPLC method has been reported in the literature [8].

This work is an attempt to develop a simple, sensitive, rapid, accurate and low-cost RP-HPLC method for the determination of APT in solid dosage forms with the advantage of shorter retention time, run-time and low-cost mobile phase.

EXPERIMENTAL

Materials

A pure sample of APT (99.96 %) was received as a gift from Dr Reddys Laboratories Pvt Ltd, Hyderabad, India. HPLC grade water and methanol (Qualigens Fine Chemicals, Mumbai India), electronic analytical balance (Dhona, Delhi, India), micropipette (Inlabs, Mumbai, India,10 – 100 µl) and Aprecap® capsules (80 mg, Glenmark Pharmaceuticals Ltd, India) were employed in the study. All the glassware employed in the work were cleaned with hot water, then acetic anhydride and finally acetone, and dried in a hot air oven until used. The working environment was maintained at 18-22 °C. The chemical structure and purity of the sample obtained were confirmed by TLC, IR, melting point, DSC studies.

Instrumentation and chromatographic conditions

The HPLC system consisted of an Agilent LC 1200 HPLC pump, solvent degasser, a

variable wavelength detector with deuterium lamp, a rheodyne injector and EZ chromelite software. Isocratic elution of the mobile phase, comprising of methanol and water in the ratio of 90: 10(v/v), at a flow rate of 1mL/min was performed on a C_{18} column (250x 4.6 mm i.d, 5µm particle size). The run time was set at 10 min and column temperature was maintained at ambient. The volume of injection was 20 µL; prior to injection of analyte, the column was equilibrated for 30 - 40 min with mobile phase. The eluent was detected at 220 nm.

Method Validation

The analytical method was validated for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity, robustness, and ruggedness, in accordance with International Conference on Harmonization (ICH) guidelines.

Linearity

Linearity was studied by preparing standard solutions at different concentrations from 0.1 to 10 µg/mL, plotting a graph of concentration against peak area, and determining the linearity by least-squares regression.

Accuracy/Recovery

Accuracy was evaluated in triplicate, at three different concentrations equivalent to 80, 100, and 120% of the active ingredient, by adding a known amount of APT standard to a sample of known concentration and calculating the recovery of APT, RSD (%), and % recovery for each concentration.

Precision

The precision of the procedure was determined by repeatability and intermediate precision studies. Repeatability was evaluated by performing six determinations at the same concentration, during the same day, under the same experimental conditions. Intermediate precision was evaluated by comparing the assays on 3 different days using different analysts.

Robustness

The robustness of the method was investigated by making small deliberate changes in the chromatographic conditions at three different levels. The chromatographic conditions selected were flow rate (1, 1.1, and 0.9 mL/ min), varying column temperature (28, 30 and 32 ^{0}C), and amount of methanol in the mobile phase (88, 90, and 92%). Deliberate variations in method parameters had no significant effect on the determination of the drug in the pharmaceutical dosage form, demonstrating that measurements are not susceptible to variations in analytical conditions.

Ruggedness

The ruggedness of the method was assessed by making six replicate injections of the APT, as per the proposed method and the assay of APT performed by two analysts in the same laboratory, two different columns on same system and two different systems.

System suitability

System suitability tests are an integral part of liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. A system suitability test was carried out to evaluate the reproducibility of the analytical system using five replicate injections of a reference solution for the parameters such as theoretical plates, tailing factor, limit of detection (LOD) and limit of quantification (LOQ) were calculated for the proposed method as follows

Limits of detection (LOD) and of quantification (LOQ)

The limits of detection and quantification were calculated by the method based on the standard deviation (σ) of responses for triplicate blank injections and the slope (S) of the calibration plot, using the formulae LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$.

Theoretical plates (n)

This is a measure of the sharpness of the peaks and therefore the efficiency of the Column. This was calculated using the USP method as in Eq 1.

$$n = 16 \left(t/_{wb}\right)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where wb is the peak width at the base.and t = retention time of peak

Tailing factor (T)

This is a measure of the asymmetry of the peak

$$T = W/2f \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where W = width at 5 % of the peak height, and f = distance between the maximum and the leading edge of the peak

Preparation of mobile phase

HPLC grade solvents of methanol and water were used for the preparation of the mobile phase in a ratio of 90:10 (v/v). The contents of the mobile phase were filtered before use through a 0.45µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 mL/min.

Preparation of standard solutions

A standard stock solution of APT was prepared by dissolving 10 mg of pure APT in 100 mL of volumetric flask containing 70 mL of methanol (HPLC grade) and sonicated for 15 min and then made up to volume with methanol to obtain a concentration of 100µg/mL. Daily working standards of APT were prepared by suitable dilution of the stock solution with mobile phase. Different sets of drug solution were prepared in the mobile phase containing APT in a concentration range of 0.1 - 10µg/mL. Each of the these dilutions (20 µL) was injected six times into the column set at a flow rate of 1.0 mL/min, and the peak area of each of the drug concentrations, and the retention times were recorded.

Construction of calibration curve

Different concentrations of analyte were prepared from the stock solution by taking suitable volumes of APT and diluting them to obtain the desired concentrations for linearity in the range of 0.1 - 10 µg/mL. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected six times into the column. The calibration curve for APT was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). It was found to be linear in the concentration range 0.1 - 10 µg/mL with good correlation between concentration and mean peak area.

Application of proposed method to solid dosage form

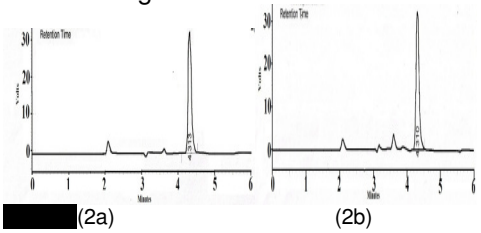
Twenty capsules were weighed and their contents were removed and mixed thoroughly. A sample of the powder equivalent to the label claim (10 mg of the active ingredient) was mixed with 70 mL of methanol and the mixture was allowed to stand for 30 min with intermittent sonication to ensure complete solubility of the drug. It was then filtered through a 0.45 µm membrane filter, followed by the addition of methanol to the 100 mL mark to obtain a stock solution of 100 µg/mL. An aliquot of this solution (1 mL) was transferred to a volumetric flask and made up to a sufficient volume with the mobile phase to give a concentration of 10 µg/mL. The prepared dilution was injected in triplicate into the column to obtain a chromatogram. The drug

content of the capsules was quantified from the peak area.

RESULTS

Method development

The drug was eluted in the given mobile phase at a short retention time (t_r) of 4.313 min. Typical chromatograms of APT are shown in Fig 2.



(2a) (2b)

Fig 2: Typical chromatograms of pure APT (2a) and APT in capsules (2b)

Method validation

The method was validated as per International Conference on Harmonization (ICH) guidelines for the different validation parameters. The method was validated for its accuracy, precision, robustness and ruggedness.

Linearity

The standard plot for APT was constructed by plotting the ratio of the peak area of APT to the peak area of the internal standard (Y) against concentration (X). It was found to be linear with a correlation coefficient (r^2) of 0.989 with the linear regression equation being, y = 33032x+71501 in the linear range of 0.1 - 10 µg/mL, the coefficients of variation (CV) based on the peak area ratios for six replicate injections, were between 0.12 - 0.67.

Statistical data of calibration curves

Regression characteristics, namely, standard deviation of slope (S_b), RSD of the slope and standard deviation of intercept (S_a) were 250.10, 0.753 and 393.8, respectively.

Precision of the method

The precision results are shown in Table. 1. The intraday precision of the method (mean, % ± RSD, %) was 100.07 ± 0.85 (n = 6). The intermediate precision was evaluated by comparing the results obtained on 3 different days and between analysts. The value was found to be 99.62 ± 1.05 (n = 18). The low variability of the experimental values obtained for the determination of APT in samples indicates that the precision of the method is acceptable.

Table 1: Intraday (n = 6) and interday (n = 18) precision of aprepitant

RLZ	% Recovery		
	Day 1	Day 2	Day 3[a]
1	100.15	99.00	99.97
2	100.44	98.43	100.2
3	101.54	100.40	97.5
4	100.19	99.98	98.3
5	99.08	98.39	99.2
6	99.04	100.00	101.4
Intraday[b]	***100.07±0.85***	***99.36±0.80***	***99.43±1.29***
Interday[b]		***99.62±1.05***	

[a]*Different analyst;* [b]*mean ± %RSD*

Accuracy

By spiking previously analysed test solution with additional standard drug the recovery of the method was found to be 99.56 and 100.50 %. Values of recovery (%) and RSD (%) are listed in the Table. 2. RSD was always < 1 %, which indicates that the method is accurate.

Content of APT in commercial capsule formulation

The assay of commercial APT capsules showed that the developed method, shown in Table 3, was accurate and reliable, with mean drug content of 99.97 % of the labeled claim. No interference peaks were found in the chromatogram, indicating that the

determination of the drug content was free from interference by excipients.

Table 2: Accuracy of the method

Sample no.	80% level	100% level	120% level
1	101.27	100.2	99.65
2	99.66	99.8	98.9
3	100.11	101.5	100.13
Mean	***100.35***	***100.50***	***99.56***
% RSD	***0.676***	***0.722***	***0.508***

Ruggedness

Ruggedness of the method was evaluated by comparing the results of assay of APT obtained from two analysts, two systems and two columns. RSD was always <2%, which indicates the method is rugged.

Table 3: Assay data for commercial aprepitant capsules (n = 3)

Branded product	Labeled claim	Drug content (mean±S.D)	Mean drug content (%)
Aprecap	80 mg	79.98±0.12 mg	99.97

The results of the evaluation of the ruggedness of the proposed method are shown in Table 4.

Robustness

System suitability parameters were established and found to be within acceptable limits as indicated in Table 5. The results indicate that the proposed method was sufficiently robust for normally expected variations in chromatographic conditions.

System suitability

To determine the reproducibility of the method, system suitability test was employed. The tailing factor was 1.223, theoretical plates 1188.87, limit of detection (LOD) 0.028 and limit of quantification (LOQ) 0.094.

Specificity of the method

The specificity of the proposed method demonstrated that excipients in the capsules did not interfere with the drug peak. Furthermore, the well-shaped peaks also indicate the specificity of the method.

Table 4: Ruggedness of the method

Sample no.	Analyst 1	Analyst 2	System 1	System 2	Column 1	Column 2
1	80.12	80.06	79.20	80.00	80.51	79.10
2	80.35	80.75	78.74	81.06	82.25	81.01
3	81.23	79.62	80.32	79.34	80.62	80.00
4	80.15	80.00	79.98	80.22	80.00	80.00
5	79.26	80.56	78.71	81.82	81.22	79.21
6	79.23	78.54	80.00	80.16	80.25	80.76
Mean ±SD	***80.06±0.68***	***79.92±0.72***	***79.49±0.64***	***80.43±0.80***	***80.81±0.75***	***80.01±0.71***
%RSD	***0.85***	***0.90***	***0.80***	***0.99***	***0.92***	***0.89***

Table 5: Robustness of the method

System suitability parameter	Variation in methanol content of mobile phase			Variation in flow rate (mL/min)			Variation in column temperature		
	88	90	92	0.9	1	1.1	28 ^{0}C	30 ^{0}C	32 ^{0}C
% RSD	0.2	0.1	0.3	0.2	0.1	0.2	0.1	0.4	0.3

DISCUSSION

The development of HPLC methods for the determination of drugs has received great attention in analytical research because of their importance in quality control. The technique is unique, versatile, universal, basic and well-utilized the researchers because of its ease of operation. The main objective of method development is to determine the drug content of formulations as well as purity. In analytical research, the time and cost of method development and validation are of great importance. The objective of this study was to develop and validate a simple, sensitive, rapid, economic and accurate RP-HPLC method for the estimation of APT in commercial capsule products.

The developed method, using simple HPLC grade solvents (methanol and water), had a short retention time and high peak symmetry. The method developed was validated successfully. The selected mobile phase system gave a single sharp peak for APT without interfering peaks. The retention time *(t_r)* of APT was 4.313 min with a tailing factor of 1.223. The method was linear in the concentration range of 0.2 - 10µg/ml, obeyed Beers' law and the calibration curves showed good correlation between concentration and peak area.

The method was validated as per ICH guidelines for parameters such as accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ), which indicates that the results obtained were reliable.

CONCLUSION

The results of the study showed that the proposed RP-HPLC method for the estimation of APT is simple, sensitive, rapid, cost-effective and accurate for the determination of APT in solid dosage forms.

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