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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPY AND RP-HPLC METHODS FOR THE QUANTIFICATION OF APREPITANT IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Racha Spandana[1*], Puli Ramya Krishna[1], K Chandrasekhar Rangaiah[1], Sravanthi Chada[1], Shakapuram Shailaja[1], Suchitra Duddagi[2]

[1]Siddhartha Institute of Pharmacy, Narapally, Ghatkesar, Hyderabad, Telangana, India.
[2]Vision College of Pharmaceutical Sciences and Research, RNS Colony, Boduppal, Hyderabad, Telangana, India.

*Corresponding author E- mail: rachaspandana_xf@siddhartha.co.in

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ABSTRACT

The purpose of the investigation was to develop a new RP-HPLC method for the estimation of aprepitant in API and pharmaceutical dosage forms. Chromatography was performed using a 2487 HPLC system and a Kromosil C_{18} column (4.6 x 250 mm, 5 µm particle size) with an isocratic mobile phase composed of 0.1% perchloric acid and acetonitrile (80:20 v/v) at a flow rate of 1.0 mL/min. The column temperature was maintained at 30°C, and detection was carried out using a 2996 PDA detector at 210 nm with Empower-3 software. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), standard stock solutions, and robustness were evaluated according to International Conference on Harmonisation (ICH) guidelines. The retention time for aprepitant was 3.277 min. The percentage recovery of aprepitant was 100.03%. The relative standard deviation for the assay of capsules was found to be less than 2%. The method was fast, accurate, precise, and sensitive, making it suitable for routine quality control of capsules containing aprepitant in quality control laboratories and pharmaceutical industries.

INTRODUCTION:

Aprepitant is a neurokinin-1 (NK1) receptor antagonist, an antiemetic agent. Chemically described as 5-[[(2R,3S)-2-[(1R)-1-[3,5bis(trifluoromethyl)phenyl]-3-(4-fluorophenyl)-4-morpholinyl)-4-morpholinyl] methyl]-1,2-dihydro3H-1,2,4-triazol-3one. Its empirical formula is $C_{23}H_{21}F_7N_4O_3$. Aprepitant with a molecular weight of 534.43 g/mol [1,2]. It is a crystalline solid that ranges from white to off-white. It is slightly soluble in water. Aprepitant has a weak solubility in acetonitrile and a sparing solubility in Ethanol and isopropyl acetate. Because it inhibits the signals that NK1 receptors Produce, aprepitant is categorized as an NK1 antagonist. As a result, patients are less likely to throw up as a result. Emend is typically taken as a prophylactic measure against nausea and vomiting brought on by chemotherapy, a major side effect that affects more than 80% of patients [3,4] The literature provides reports on a number of HPLC assay techniques for aprepitant estimation. A literature review indicates that an official method exists for estimating

aprepitant in capsule dose forms using RP-HPLC. As a result, efforts have been made to provide an improved technique for estimating aprepitant in formulation in compliance with ICH recommendations [5-7].

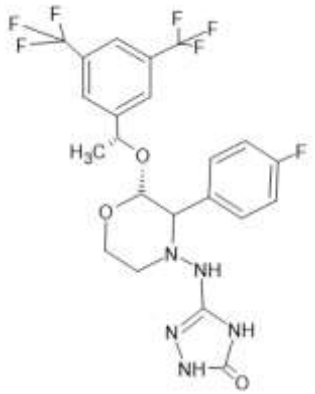

Figure 1: Chemical structure of aprepitant

MATERIALS AND METHODS

Instrumentation: Chromatography was performed with Alliance waters 2487 HPLC provided with highspeed auto sampler, column oven, degasser and 2996 PDA detector to provide a compact and with class Empower-3 software.

Reagents and chemicals: The reference sample of aprepitant was provided as gift samples from Zaint Health Care, Hyderabad, Telangana. HPLC grade acetonitrile, HPLC grade methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Qwater purification system was used throughout the study. Commercial formulation (Emend; Dosage: Aprepitant-40 mg capsule) were purchased from the Local Pharmacy, Hyderabad, Telangana.

Chromatographic conditions [8] The chromatographic separation was carried under the isocratic conditions. Chromatographic separation was achieved by injecting a volume of 10µl of standard into Kromosil (250 x 4.6mm,5µ) column. The mobile phase composed of 0.1% perchloric acid, acetonitrile (80:20v/v) was allowed to flow through the column at a flow rate of 1.0ml per min for a period of 7 min at 30°C column temperature. Detection of the component was carried out at a wavelength of 210nm. The retention time of the component was found to be 3.277 min for aprepitant.

Preparation of diluent solution: Dilution solution was prepared by mixing 500ml of HPLC grade water with 500ml of acetonitrile, in a 1000 ml beaker and sonicated for 15 min.

Preparation of standard stock solution [9,10] Accurately weighed and transferred 4mg of aprepitant working standards into 10ml clean dry volumetric flasks, add 3/4th volume of diluent, sonicated for 30 min and make up to the final volume with diluents. From the above stock solution, 1 ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent and taken the overline spectrum of aprepitant.

Preparation of working standard solution: Aliquot of 0.25, 0.50, 0.75, 1, 1.25 and 1.5mL were pipette out from stock solution into 10mL volumetric flask separately for both aprepitant and volume was made up to 10mL with diluent. This gives the solutions of 10, 20, 30, 40, 50 and 60µg/mL for aprepitant respectively.

Sample preparation: One capsule was weighed and powered and it was taken into a 100ml volumetric flask and made up with diluents and labelled as sample stock solution. Sample stock solution was filtered by HPLC filters. 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluents.

Method validation [10-13]

System suitability test: To ensure the resolution and reproducibility of HPLC system was adequate for the analysis, a system suitability test was established. Data from six injections of 10µl of the working standard solutions of aprepitant was used for the evaluation of the system suitability

parameters like tailing factor, the number of theoretical plates, retention time.

Linearity: By appropriate aliquots of the standard aprepitant solution with the mobile phase, six working solutions ranging between 10-60 µg/mL was prepared. Each experiment linearity point was performed in triplicate according to optimized chromatographic conditions. The peak area of the chromatograms was plotted against the concentration of aprepitant to obtain the calibration curve.

Accuracy: Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analysed samples of aprepitant to which known amounts of standard aprepitant corresponding to 50%, 100% and 150% of target concentration were added. The accuracy was expressed as the percentage of analyte recovered by the percentages of analyte recovered by the proposed method.

Precision: Precision was determined as repeatability and intermediate precision (ruggedness), in accordance with ICH guidelines. The intraday and interday precision were determined by analysing the samples of aprepitant. Determinations were performed on the same day as well as on consequent days.

Limit of Detection and Limit of Quantification: Limit of detection (LOD) and limit of quantification (LOQ) of aprepitant was determined by calibration curve method. Solution of aprepitant was prepared in linearity range and injected in triplicate. Average peak area of three analyses was potted against concentration.

$$\mathbf{LOD = (3.3 \times Syx)/b}$$

$$\mathbf{LOQ = (10.0x\ Syx)/b}$$

Where, Syx is residual varieties due to regression; b is slope.

Robustness: The robustness of aprepitant was performed by deliberately changing the chromatographic conditions. The organic strength was varied by ±5%, column temperature was varied by ±5 °C and flow rate varied by ±0.1mL.

Statistical analysis: Wherever available, results were expressed as the Mean ± SD, %RSD and data were analysed statistically by using t-test with aid of Microsoft Excel-2016 software and data were considered not significantly different at 5% significance level of probability $p \leq 0.05$.

RESULTS AND DISCUSSION

Method development: Initially, different ratios of methanol and water, as well as acetonitrile and water, were used as mobile phases in reverse phase liquid chromatography separation. Still, the medications did not respond appropriately in these circumstances, and the resolution was also subpar. In order to maximize the separation of both pharmaceuticals, the organic content of the mobile phase was also looked into. The pH of the mobile phase becomes a crucial component in improving the tailing factor. Following that, 1.0 mL/min of 0.1% perchloric acid:acetonitrile was used at an isocratic ratio of 80:20. To boost resolution, a 4.6 x 250 mm Kromosil column with a 5µ particle size was chosen as the stationary phase. This resulted in a significant reduction of both peaks' tailing, bringing them very close to 1. At 210 nm wavelengths, attempts were made to analyze drug detection.

Method validation

System suitability and Specificity: System suitability parameters such as number of theoretical plates, peak tailing, retention time was determined. The total run time required for the method is only 6 min for eluting aprepitant.

Linearity: Aprepitant showed a linearity of response between 10-60 µg/mL. These were represented by a linear regression equation as follows: y (aprepitant) = 1330.2x+0.006 (r^2=0.9983) and regression line was established by least squares method.

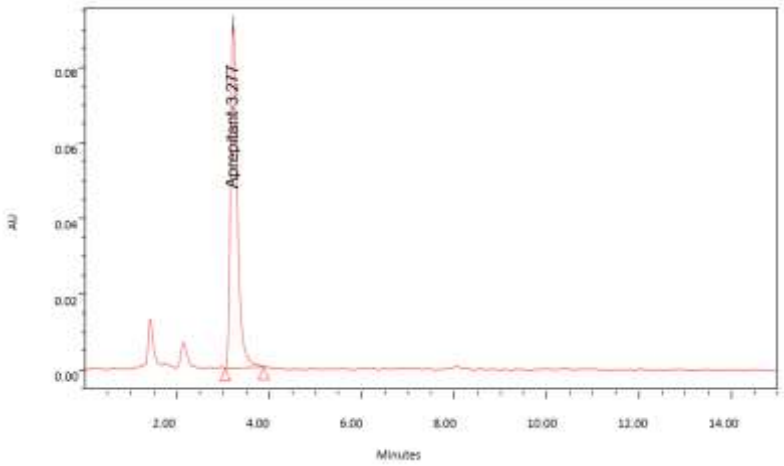

Figure 2: Typical chromatogram of aprepitant

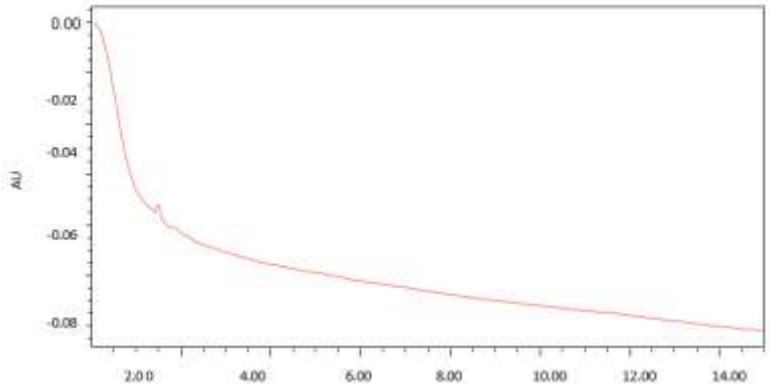

Figure 3: Chromatogram of blank

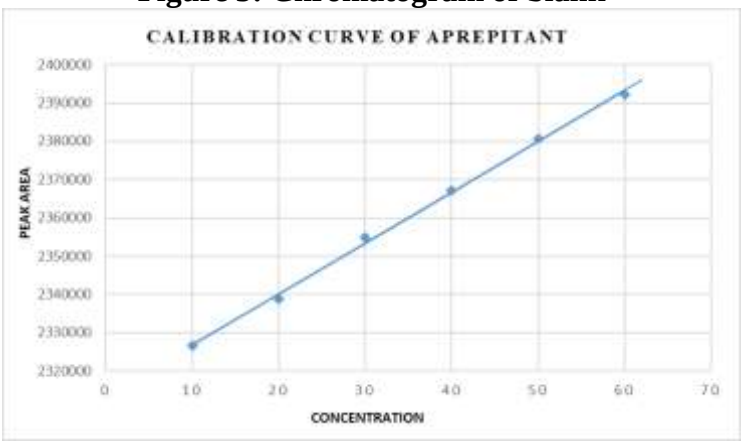

Figure 4: Overline sprectrum of aprepitant

Table 1: System suitability of aprepitant

System suitability parameters	Aprepitant
No. of theoretical plates	2551.27
Tailing factor	1.42
RT	3.277
Mean area	2392282
%RSD	0.96

Table 2: Accuracy of aprepitant

Sample	Amount taken (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	%RSD
Aprepitant	20	19.98	99.98	0.35
	40	40.01	100.01	0.49
	60	60.05	100.02	0.51

Table 3: Precision of aprepitant

Repeatability data		Interday precision	
S. No.	Aprepitant	S. No.	Aprepitant
1	2348836	1	2326617
2	2352868	2	2332868
3	2356060	3	2342886
4	2392282	4	2355988
5	2376525	5	2362121
6	2326617	6	2319988
Mean	2358865	Mean	2340078
Std. Dev.	22860.09	Std. Dev.	16635.49
%RSD	0.96	%RSD	0.71

Table 4: Robustness

Analytical conditions	Flow rate (ml/min)		Column temperature (°C)		Mobile phase composition	
Evaluation parameters	1.1	1.0	35	30	+5%	-5%
Mean RT	3.485	3.275	3.492	3.281	3.495	3.277
Mean area	2329628	2358865	2328733	2340078	2343181	2353081
SD	17732.5	22860.1	18758.47	16635.5	17502.29	18558.72
RSD	0.76	0.96	0.80	0.71	0.75	0.81
Tailing factor	1.59	1.42	1.62	1.50	1.51	1.44
No. of theoretical plates	2138.12	2551.27	2382.56	2558.99	2321.94	2546.95

Table 5: HPLC analysis of capsule for aprepitant

Label amount (mg)	Amount found (mg) n=6	%Assay (Mean±SD)	RSD
40	4.003	100.03 ± 1.099	0.60

Correlation coefficient (r^2) for aprepitant is found to be greater than 0.98, hence the curve established were linear.

Accuracy: To pre analysed sample solution, a definite concentration of standard drug (50%, 100% & 150% level) was added and recovery was studied. The % Mean recovery is 100.06% and these results are within acceptable limit of 98-102. The % RSD aprepitant is within limit of <2.

Precision

Repeatability: Six replicates' injections in same concentration (40 µg/ml of aprepitant) were analysed in the same day for repeatability and the %RSD for aprepitant found to be 0.96 and %RSD for aprepitant found to be within acceptable limit of <2.

Intermediate precision: Six replicates' injections in same concentration were analysed on two different days with different analyst and column for verifying the variation in the precision and the %RSD for Aprepitant found to be 0.71 and it is within acceptable limit of <2. Hence The method is reproducible on different days with different analyst and column, and this method is precise.

Robustness

The robustness was established by changing the flow rate, column temperature and composition of the mobile phase within allowable limits from actual chromatographic conditions. It was observed that there was no marked change in mean R_t and RSD is within limit of ≤2. The tailing factor, resolution factor and number of theoretical plates are found to be acceptable limits for aprepitant. Hence the method is reliable with variation in analytical conditions.

Limit of Detection and Limit of Quantification (LOD and LOQ)

LOD and LOQ for aprepitant were 1.9µg/mL and 5.9µg/mL respectively. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive.

Capsule analysis: The content of aprepitant in the capsules was found by the proposed method. %RSD values for both aprepitant was within limit of ≤2.

CONCLUSION: A new, precise, accurate, and simple HPLC method was developed and validated for the estimation of Aprepitant in its pharmaceutical dosage form. The method is fast, accurate, precise, and sensitive, making it suitable for routine quality control of capsules containing the drug in QC laboratories and industries. The method's robustness was demonstrated through various tests, confirming its reliability under different conditions. Specificity studies showed that the method effectively separates aprepitant from other components and impurities, ensuring accurate results. The linearity of the method was established over a wide concentration range, indicating its suitability for different dosage levels. Additionally, the method's high sensitivity allows for the detection of even trace amounts of the drug, enhancing its utility in quality control processes. Reproducibility was verified through repeated trials, showing consistent and reliable performance. The method also demonstrated excellent recovery rates, indicating minimal loss during the analysis process. Stability studies confirmed that aprepitant remains stable under various conditions, further validating the method's accuracy. This HPLC method, with its combination of speed, precision, and sensitivity, represents a significant advancement in the analytical techniques used for aprepitant estimation. It can significantly streamline quality control processes in both QC laboratories and pharmaceutical industries.

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Conflicts of interest: None

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