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Derivative spectrophotometric methods for determination of aprepitant in bulk and pharmaceutical formulation

Benjamin.T[1*], Rajyalakshmi. Ch[2] and Rambabu. C[3]

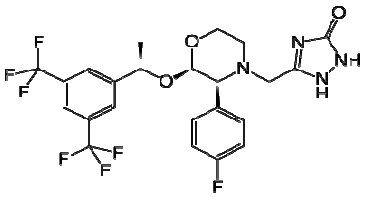
[1]*Dept.of Chemistry, Noble College, Machiliptnam, A.P, India*
[2]*Dept. of Chemistry, Vishnu Institute of Technology, Bhimavaram, A.P, India*
[3]*Dept. of Chemistry, Acharya Nagarjuna University, Guntur, A.P, India*

ABSTRACT

Simple, fast and reliable derivative spectrophotometric methods were developed for determination of Aprepitant in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in methanol. The quantitative determination of the drug was carried out using the zero order derivative values measured at 210 nm and the first order derivative values measured at 269 nm (n=6). Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Aprepitant using 6-14 μg.mL^{-1} (r^2 = 0.998 and r^2 = 0.998) for zero order and first order derivative spectrophotometric method. All the proposed methods have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. Developed spectrophotometric methods in this study are simple, accurate, precise and sensitive to assay of Aprepitant in tablets.

Keywords: Aprepitant, Derivative spectrophotometric, Zero order derivative spectrum, First order derivative spectrum.

INTRODUCTION



Aprepitant is a novel antiemetic agent used in cancer chemotherapy; with a chemical name-5-[[(2R,3S)-2-((R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy)-3-(4-fluorophenyl) morpholino] methyl)-1H-1,2,4-triazol-3(2H)-one. It's molecular weight is 534.427 g/mol with molecular formula $C_{23}H_{21}F_7N_4O_3$. It mediates its effect by blocking the neurokinin receptor. For the quantitative analysis of dosage forms mostly HPLC and LC-MS methods are used. Literature survey reveals that the drug can be estimated by RP-HPLC in Capsules [1], [2], stability indicating RP-HPLC [3], rapid liquid chromatography-tandem mass spectrometry method [4],[5], quantification of process related impurities by RP-LC method [6] and UPLC [7]. Present study aims to develop simple, rapid, accurate, precise and validated Derivative spectrophotometric method for the determination of Aprepitant in Capsules. Literature survey reveals there is no derivative spectrophotometric validation method for Aprepitant. 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 UV Derivative spectrophotometric method for the estimation of Aprepitant in commercial tablet products.

MATERIALS AND METHODS

INSTRUMENTATION

A Lab India model 1885 double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV win system software (UV win version 3000). A Sartorius CP 224 S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India)

REAGENTS AND MATERIALS

Aprepitant was supplied by Pharmatrain, analytical testing center, Hyderabad, India as a gift sample. The commercially available tablets of Aprepitnat were procured from local market labeled to contain 40 mg Aprepitant. Methanol (AR Grade, S. D. Fine Chemicals LtD., Mumbai, India) and Whatmann filter paper no. 41 (Whatmann International Ltd., England) were used in the study.

PREPARATION OF STANDARD AND SAMPLE SOLUTION

Aprepitant was accurately weighed (equivalent to 10 mg Aprepitant) in 100 ml volumetric flask and diluent (70 ml) was added, shaken and sonicate till dissolved and volume was made up to the mark with diluent and mixed well (100 mg/ml stock solution) Aprepitant working standard solution was prepared by diluting standard stock solution (1 ml) to 10ml with diluent to produce required concentration (10 µg/ml). Twenty tablets were weighed and powdered. The quantity of the powder (equivalent to 10 mg of Aprepitant) was transferred to a 100 ml volumetric flask, ultrasonicated for 30 minutes with methanol (70 ml) to dissolve the drug as completely as possible. Further diluted and make up the volume using diluent. The solution was filtered through a Whatmann filter paper No. 41. The resulting solution (1 ml) was diluted to 10 ml with diluents.
Diluent: Methanol is used as diluent.

METHOD DEVELOPMENT

METHOD A: ZERO ORDER SPECTROSCOPIC METHOD

The solutions were scanned in the range from 400-200 nm, and the peak was observed and gives maximum absorbance at 210 nm. So, the wavelength selected for the analysis of the drug was 210 nm. The drug followed the Beer's-Lamberts law in the range of 6-14 µg/ml.

METHOD B: FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD

The standard drug solution was diluted so as to get the final concentration in the range of 6-14µg/ml and scanned in the first order derivative spectra. The first order derivative spectra at n = 1, showed a maxima and minima at 269 and 273 nm respectively. The amplitude of absorbance was measured at 269 nm (peak maxima) and at 273 nm (peak minima) and was plotted against concentration to give calibration curve, and regression equation was calculated. The amplitude was linear in the concentration range of 6-14 µg/ml.

VALIDATION OF THE PROPOSED METHOD

The proposed method is validated according to the International Conference on Harmonization ICH guide lines.

LINEARITY

Calibration curves for Aprepitant were plotted over a concentration range of 6 – 14 µg/ml for all the methods. Accurately measured standard working solutions of Aprepitant (0.6, 0.8, 1.0, 1.2, and 1.4 ml) were transferred to a series of 10 ml volumetric flasks and diluted up to the mark by diluent. Absorbance was measured at a wavelength 210 nm and was plotted absorbance versus concentration to give calibration curve for method A and from this curve regression equation was calculated.First derivative curves of different concentration solutions were obtained, which shows maxima at 269 nm and minima at 273 nm. The calibration curve of amplitude against concentration of the drug showed linearity for method B.

ACCURACY (% RECOVERY)

The accuracy of the method was performed by calculating recovery of Aprepitant by the standard addition method. Known amounts of standard solutions of Aprepitant were added at 50, 100 and 150% levels to prequantified sample solutions of Aprepitant. At each level of the amount 3 determinations were performed. The amount of Aprepitant was estimated by applying obtained values to regression equation. Calculate the amount found and amount added for Aprepitant and calculate the individual recovery and mean recovery values.

METHOD PRECISION (% REPEATABILITY)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 5) of Aprepitant without changing the parameters for the methods. The repeatability was expressed in terms of relative standard deviation (% RSD). The % RSD for the five replicates absorbance was found to be within the specified limits.

INTERMEDIATE PRECISION (REPRODUCIBILITY)

The intraday and interday precision of the proposed method was performed by analyzing the corresponding responses 3 times on the same day and on 3 different day with same dimensions. The results were reported in terms of relative standard deviation (% RSD).

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by visually or by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guidelines. LOD = 3.3 σ /S & LOQ = 10 σ /S
Where σ = the standard deviation of the response , S = slope of the calibration curve.

ESTIMATION OF APREPITANT IN PHARMACEUTICAL FORMULATION

Pharmaceutical formulation of Aprepitant was purchased from local pharmacy. Sample solutions were prepared as described earlier. Then this solution was analyzed by two methods. The nominal content of the tablets was determined either from the calibration curve or using the regression equation.

RESULTS AND DISCUSSION

Method A is simple UV spectrophotometric method. In this method the simple UV spectrum of Aprepitant in methanol was obtained which exhibits absorption maxima at 210 nm (Figure 1). The calibration curve was linear in concentration range of 6 - 14µg/ml. Method B is the first derivative spectrophotometric method. Maxima occur at 269 nm and minima at 273 nm (Figure 2). The calibration curve was linear in concentration range of 6 – 14 µg/ml. The proposed methods were found to be simple, sensitive, rapid, accurate, precise and economic for the routine analysis of Aprepitant in pharmaceutical formulations. The linearity ranges was found to be 6-14 µg/ml for all the methods. Accuracy was determined by calculating the recovery and the mean was determined. Precision was calculated as repeatability (relative standard deviation) and intra and inter day variation (% RSD) for Aprepitant.The % RSD values for precision studies of Aprepitant were found to be less than 2 indicate methods are precise. LOD values for Aprepitant were found to be 0.105 and 0.025 µg/ml for methods A and B respectively. LOQ values for Aprepitant were found to be 0.356, and 0.083 µg/ml for methods A and B respectively , indicates sensitivity of the proposed methods. The methods were successfully used to determine the amounts of Aprepitant present in tablets. The results obtained are in good agreement with the corresponding labeled amount. Characteristic parameters and summary of validation parameters for the two methods are given in Table 4. By observing the validation parameters, the methods were found to be sensitive, accurate and precise. Hence the methods can be employed for the routine analysis of Aprepitant in tablet formulations.

Acknowledgement

The authors are gratefully acknowledging Pharmatrain, analytical testing center, Hyderabad, India for providing the gift samples of Aprepitant and also providing necessary facilities for the research work.

TABLE 1 : RESULTS OF INTRADAY AND INTER DAY PRECISION

parameters	Intraday precision		Interday precision	
	S.D	%RSD	S.D	%RSD
Zero derivative	0.004658	1.16	0.00114	0.28
First derivative	0.00001	0.01	0.00002	0.11

TABLE 2 : ASSAY RESULTS FOR DETERMINATION OF FEBUXOSTAT IN PHARMACEUTICAL FORMULATION

Parameters	Amount of Tablet label claim	Drug content %
Zero order	40 mg	98.4%
First order	40 mg	99.8%

TABLE 3 : RESULTS OF ACCURACY

Zero order derivative method					
Accuracy level	**Absorbance**	**Amount added**	**Amount found**	**% recovery**	**Mean recovery**
50%	0.202	5.0	5.0	100%	99.6%
100%	0.407	10.0	10.0	100.7%	
150%	0.595	15.0	14.7	98.2%	
First order derivative method					
50%	0.001	5.0	4.99	99.8%	99.8%
100%	0.002	10.0	9.98	99.8%	
150%	0.003	15.0	14.9	99.8%	

TABLE 4 : REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHODS

Parameter	Zero order	First order
Absorption maxima and minima (nm)	210	269 & 273
Beer's-Lamberts range (µg/ml)	6-14	6-14
Regression equation y=mx+c	Y=0.032x +0.072	Y= 0.0024x-0.00042
Slope(m)	0.032	0.0024
Intercept(c)	0.072	-0.00042
Correlation coefficient (r^2)	0.998	0.9988
Mean Recovery %	99.6	99.8
Precision (% RSD)	1.16	0.00
Intermediate precision	0.28	0.11
LOD (µ g/ml)	0.117	0.275
LOQ (µ g/ml)	0.356	0.083

Figure : 1

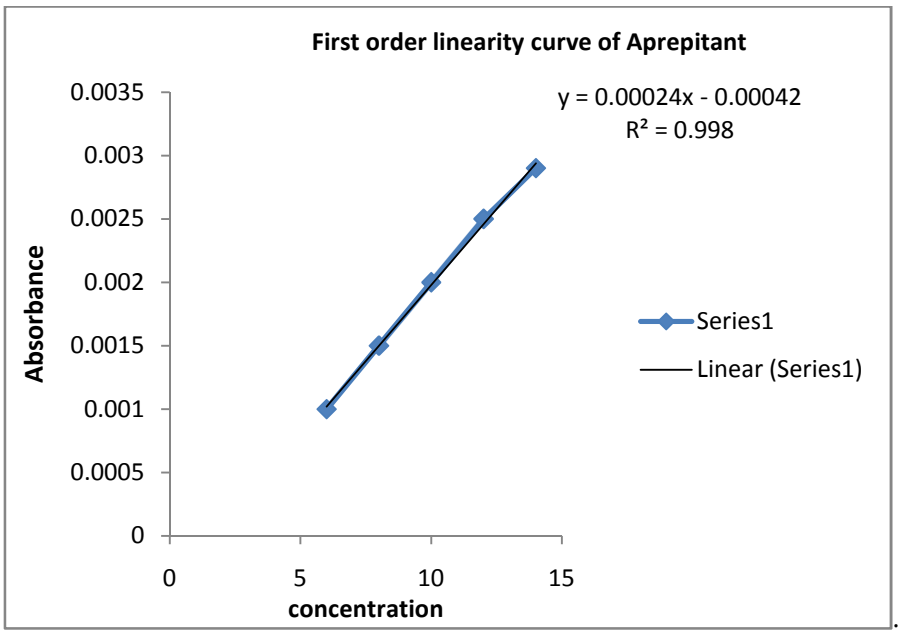


Figure : 2

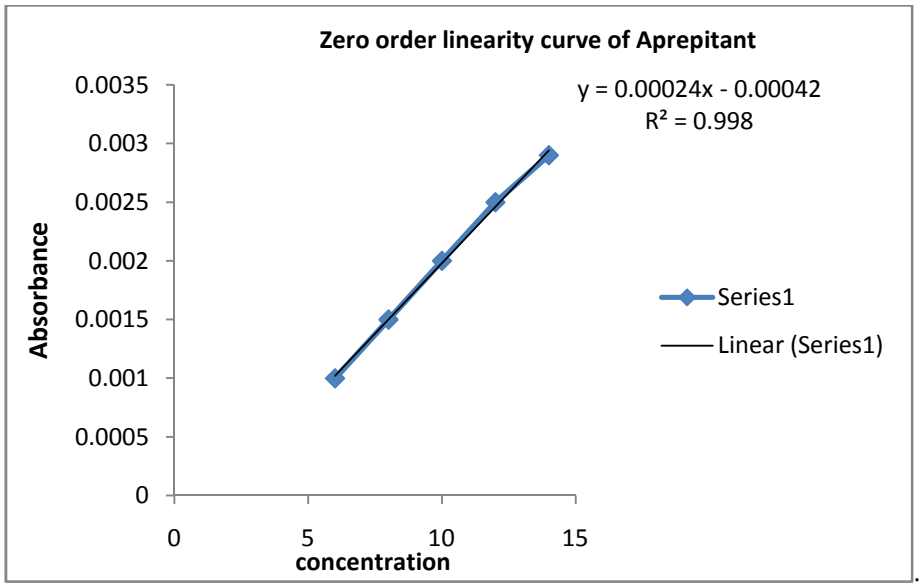


Figure : 3

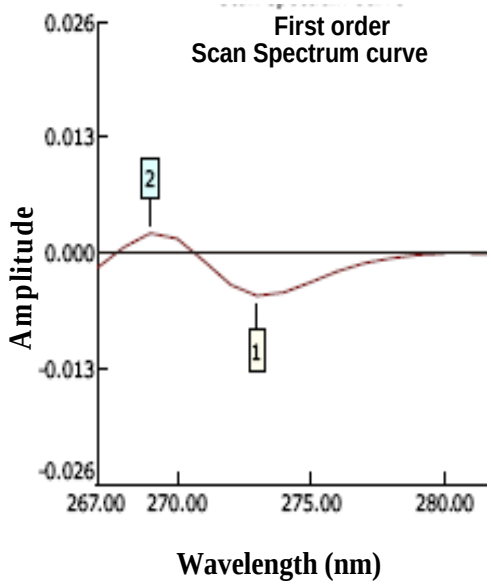
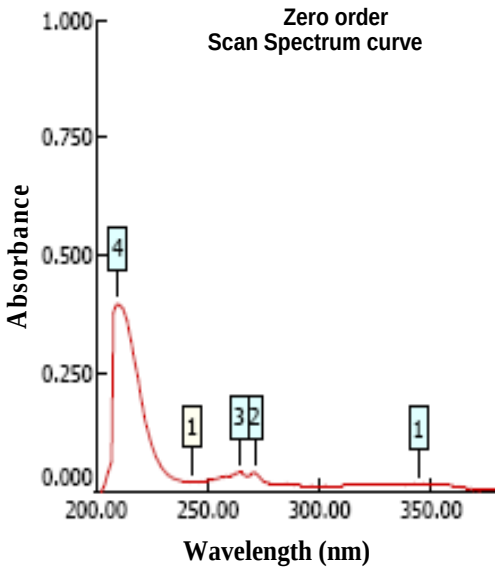


Figure : 4



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