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Research Article

PRELIMINARY VALIDATION OF UV SPECTROPHOTOMETRIC FOR DETERMINATION OF ANTIEMETIC DRUG APREPITANT IN BULK FORM

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ABSTRACT

Objective: The main objective of this work was to put forth the assorted strategies to develop and validate a novel, specific, precise and reliable method for estimation of aprepitant in bulk using UV-visible spectroscopy method. Method: The validation of Aprepitant was done by using UV-visible spectrophotometric method by using double beam systronics UV-visible spectrometer, model UV-2201 (India). The validation method involves various parameters like linearity, precision, accuracy, robustness, ruggedness, detection, quantification limits of formulation analysis according to International Conference on Harmonization (ICH) guidelines. Results: UV-spectroscopic determination was carried out at maximum absorption 263.6nm using pH 6.8 buffer & 1.1% tween 80 and 263.8nm using methanol and distilled water. The method obeyed Beer Lambert's Law in the concentration range of 8-48µg/ml and R^2 was found to be 0.999. Conclusion: As per the results were concerned, the %RSD was found to be less than 2% which is compliance with the acceptance criteria of Q1 (R1) and According to results, the currently developed method shows compliance with acceptance criteria with Q1 (R1) and international conference on harmonization (2005) guidelines. Thus, the developed method was found to be simple accurate and précised.

Keywords: Aprepitant, UV-Visible spectrophotometer, Correlation Coefficient, λmax

INTRODUCTION

Aprepitant is a substance P receptor antagonist used for the treatment of chemotherapy induced nausea and vomiting. It is made up of a morpholine core with two substituents attached to adjacent ring carbons[1]. These substitute groups are trifluoromethylated phenyl ethanol and fluorophenyl group as shown in fig. 1. The drug is a white to off white crystalline powder having two crystalline forms but only one form, which is thermodynamically stable polymorph, is produced and used in the drug product[2].

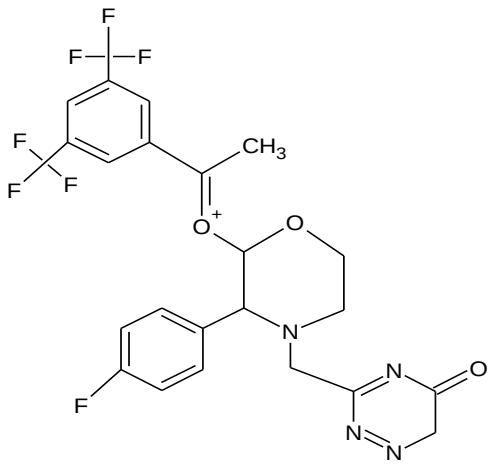

Figure 1: Structure of Aprepitant

Aprepitant is used as an antiemetic agent; blocking the neurokinin 1 receptor thus effectively prevents chemotherapy-induced nausea and vomiting, used to prevent upset stomach having half-life (9-13) hours[3].

A suitable and validated method has to be developed for the analysis of drug in bulk, in drug delivery systems, in dissolution studies (in vitro), and in biological samples (in vivo)[4]. If such a suitable method for a specific need is not available, then it becomes essential to develop a economic or accurate method for the estimation of drug samples. By the extensive literature survey, we found that there are numerous methods, such as high-performance liquid chromatography (RP-HPLC)[5], liquid chromatography with mass detector (LC-MS)[6], UPLC-MS/MS[7], have been used to measure the Aprepitant (Apr) in formulations as well as in biological samples. However, these methods are involved with sophistication skills, extraction, and more expensive than proposed method. Thus, the present study was undertaken to develop and validate a cost effective, simple, sensitive, accurate, precise, and reproducible UV validation method for aprepitant.

MATERIALS AND METHODS

Chemical and reagents

Approximately 5g was purchased by Swapanroop Drugs and Pharmaceuticals Maharashtra, India, Sodium chloride, potassium dihydrogen orthophosphate, Sodium hydroxide, methanol, disodium hydrogen phosphate from CDH laboratories. All chemicals and reagents used in the study were of analytical grade.

Instrumentation

A double beam systronics UV-visible spectrophotometer, model UV-2201(India) with a spectral bandwidth of 1nm, wavelength accuracy of ±0.5nm and a pair of 1cm quartz cells were used to measure the absorbance of the resulting solutions.

Preparation of solvent system for analysis studies

For the spectroscopic analysis of drug, two solvents were selected.

Phosphate Buffer (pH 6.8)

Dissolve 2.72gm of potassium dihydrogen phosphate in 100ml of water and 0.4gm of sodium hydroxide in 50ml of water. From prepared potassium dihydrogen phosphate take 62.5ml and 28ml of sodium hydroxide and then make up the volume up to 250ml[8].

Preparation of standard stock solution and working solution

The 10mg of Aprepitant was weighed accurately and transferred into 10ml of volumetric flask and dissolved. Then, the solution was diluted up to the mark with an appropriate solvent (phosphate buffer pH7.4, pH6.8 and distilled water). The clear solution was obtained having the strength of 1000µg/ml (standard stock solution). From this solution, 1ml was taken into a 10ml volumetric flask, diluted up to 10ml to get the solution of 10µg/ml concentration and filtered through Whatman filter before analyzing (working solution)[9].

Preparation of working solution in distilled water

Aprepitant is poorly water-soluble lipophilic drug (log P at pH 7 = 4.8), weakly basic with a pKa value of 9.78 and belongs to BCS Class II drug & easily soluble in methanol. Furthermore, prepare stock solution with distilled water& methanol (6:4) to dissolve the Aprepitant. Firstly, dissolve the Aprepitant in 4 ml methanol after then add 6 ml distilled water in it to make the clear solution. Further dilutions lead to conversion of clear solution into turbid & this problem was overcome by using tween 80 (1%) as solvent for dilution. The same problem exists for phosphate buffer pH 6.8 & pH 7.4, so tween 80 (1%) again can be used as dilution solvent[10].

Procedure for calibration curve

The standard solutions were prepared by the proper dilution of the primary stock solution with phosphate buffer pH 7.4, pH 6.8 and distilled water& methanol to obtain working standard. All the measurements were performed at room temperature. The stock solutions scanned in the UV range 200-800 nm by using an appropriate blank. For linearity study, dilutions were made for the drug in the range of 8-48 µg/ml concentrations were prepared by diluting the stock solution with all the three working solvents[11].

VALIDATION OF PROPOSED METHOD

Linearity

The aliquots of concentration ranging 4-24µg/ml was analyzed in triplicate. The results obtained were used to calculate the equation of line by using linear regression by the least squares regression method[12].

Accuracy

The accuracy of the method was performed by calculating recovery of Aprepitant by the standard addition method. In this method, known number of standard solutions of Aprepitant were prepared at level 75%, 100% and 125% of the test solution of taken absorbance at each solution in triplicate[13].

Precision

The intra-day and inter-day precisions of the prepared spectrophotometric methods were determined by estimating the corresponding response thrice on the same day and on three different days over a period of one week and the results were reported in terms of relative standard deviation[14].

Repeatability

The repeatability was determined by analyzing six samples of same concentrations of drug (20µg/ml). From the resulting absorbance, the standard deviation and relative standard deviation were calculated[15].

Limit of detection (LOD) and limit of qualification (LOQ)

It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The LOD and LOQ were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. LOD and LOQ were calculated using the relation,

$$LOD = 3* \sigma/s$$

The lowest concentration or amount of analyte that can be determined quantitatively with an acceptable level of repeatability precision and trueness

$$LOQ = 10*\sigma/s$$

Where σ is the standard deviation [n=3] of reagent blank determination and s is the slope of the calibration curve[18].

Ruggedness and Robustness

Ruggedness test was determined between two columns or two analysts or two instruments. Robustness of the proposed method was determined by small deliberate changes in flow rate, change in composition of mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of RSD indicating that the method was rugged and robust. On evaluation of these results, it can be concluded that the variation of flow rate and variation of org. composition in mobile phase do not affect the method significantly. Hence it indicates that the method is robust even by change in flow rate slightly[17].

RESULT AND DISCUSSION

Determination of absorption maxima (λmax)

The standard stock solution of drug having the concentration 1000µg/ml was further diluted to 100µg/ml with methanol& water (6:4), pH 6.8 buffer& tween 80. The calibration curve was linear in concentration range of 8-48µg/ml. The linearity ranges were found to be 8-48µg/ml for all the methods.

Linearity

The linearity studies of the drug were performed by plotting different concentrations of standard solution against their respective absorbance as shown in table 1. The drug was found to be linear in the concentration range of 8-48µg/ml and R^2 value was found to be 0.999. The correlation coefficient values was not be less than 0.99 and the calibration curve shows that the drug obeys beer's law limit within the concentration range.

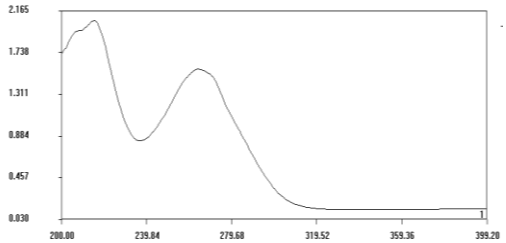

Figure 2: Absorption spectrum of Aprepitant showing maximum absorption in 263.6nm

Table 1: Comparison of absorbance of Aprepitant in different solvent

Conc. (µg/ml)	Group 1	Group 2
8	0.017	0.032
16	0.083	0.061
24	0.164	0.101
32	0.221	0.133
40	0.291	0.163
48	0.356	0.198

Precision (Intraday and Interday Study)

Intraday precision

The intraday precision was determined by analyzing the drug at particular concentration for three times on the same day taking the time intervals of 3h at 9:30am, 12:30pm, 3:30pm respectively. The acceptable limit for intraday variation should be within 1%[18].

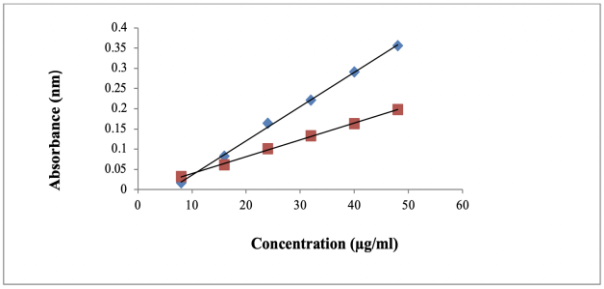

Figure 3: Calibration plot of Aprepitant in phosphate buffer 6.8 & tween 80(in blue dots) and distilled water, methanol & tween 80 in (red dots)

Interday Precision

The Interday precision was determined by analyzing the samples daily, for three consecutive days. The values of relative standard deviation (%RSD) were in the range of 0.089-0.651% respectively. This indicates the reproducibility of the method. The precision results indicate that the current method was reliable and repeatable. The acceptable limit for interday variation should be within 2% [16].

Table 2: Interday and intraday precision data and statistical results

Solvent	Absorbance (intraday) (µg/ml)	Absorbance (interday) (µg/ml)	Intraday Precision (%) ±SD	Interday precision (%) ±SD	Intraday precision (%RSD)	Interday precision (%RSD)
Group 1	0.164	0.163	98±0.005	97±0.002	0.390	0.508
Group 2	0.101	0.102	99±0.004	98.9±0.005	0.192	0.570

*Each value is the average of the three determinations.

Accuracy

The recovery experiment was carried out by spiking the already analyzed samples and percentage recovery values were calculated [19]. Recovery experiment indicated the absence of interferences from the commonly encountered pharmaceutical additives and excipients. The results shown that the best recoveries (99.58%,98.11%,99.55%) indicating that the method was accurate.

Table 3: Results of recovery studies at three levels and statistical analysis

Solvent	80%(10+5µg/ml) *±SD	100%(10+10µg/ml) * ±SD	120%(10+15µg/ml) *±SD
Group 1	99.58 ±0.1	98.11± 0.46	99.55 ±0.09
Group 2	100.12± 0.12	100.7±0.32	101.39± 0.03

*Each value is the average of the three determinations

Repeatability

The repeatability of the instrument was validated by taking the absorbance of six samples of the same concentration (20µg/ml) in different working solvents. The SD and %RSD were in the given limits. The repeatability of methodology is very important for routine result analysis of drug in bulk as well as in formulations[16]. Moreover, the current results proved that there was no significant change in results on repetition of methodology.

Table 4: Results of repeatability studies in different working solvents

Conc, (µg/ml)	Group 1*	Group 2*
24	0.164	0.101
24	0.165	0.101
24	0.164	0.100
24	0.165	0.101
24	0.164	0.101
24	0.164	0.100
Mean	0.164	0.101
SD	0.000488	0.000489
%RSD	0.707	0.205

*is the mean of three values

Robustness study

Robustness studies were done to prove that small variations in any variable show no significant difference in results[17]. The robustness study shows the liability of the validated method during routine analysis and results showed that by the change of instrument no change in results was observed.

Ruggedness study

Ruggedness of the method was determined by selecting the different analyst and for that purpose, the selected concentration was 20µg/ml. Furthermore, the %RSD was found to be less than 2 which show that the results were repeatable, and no significant difference was found while changing the analyst.

Table 5: Results of robustness studies and statistical analysis

Conc. (µg/ml)	Group 1*	Group 2*
24	0.164	0.101
24	0.165	0.101
24	0.163	0.102
24	0.164	0.100
24	0.164	0.104
24	0.165	0.101
Mean	164	0.102
SD	0.0005	0.0012
%RSD	0.169	0.212

*is the mean of three values

Table 6: Results of ruggedness studies (by two analysts) in different working solvents

Concentration (µg/ml)	Group 1*		Group 2*	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2
24	0.164	0.164	0.101	0.100
24	0.162	0.160	0.102	0.101
24	0.164	0.162	0.100	0.102
24	0.163	0.163	0.101	0.100
24	0.160	0.162	0.099	0.101
24	0.160	0.162	0.101	0.101
Mean	0.162	0.162	0.100	0.100
SD	0.001	0.0008	0.001	0.0007
%RSD	0.353	0.282	0.091	0.212

*is the mean of three values

Table 7: Summary of all the validation parameters

Validation parameter	Group 1	Group 2
Absorption maxima (nm)	263.6nm	263.8nm
Linearity Range	8-48	8-48
Standard Regression Equation	y= 0.008x-0.048	y=0.004x-0.002
Intercept	0.048	0.002
Slope	0.008	0.004
Correlation Co-efficient	0.998	0.998
%RSD for Intra-day (n=3) Precision	0.390	0.192
%RSD for Inter-day (n=3) Precision	0.508	0.570
Repeatability (% RSD)	0.707	0.205
LOD	0.280	0.560
LOQ	0.915	0.997

CONCLUSION

The developed UV spectrophometric method was simple précised and rapid to estimate the Aprepitant in any developed formulation. Thus, this validated method can be used for routine analysis like analysis of drug in pharmaceutical industry as well as laboratories. Moreover, as compared to other analysis techniques like HPLC, LC/MS, HPTLC or other chromatographic technique, the UV instrument was found to be user friendly, economical as well as calculations of data is quite, and statistical analysis was found to be quite easy as compared to other techniques.

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