DEVELOPMENT AND VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF FIMASARTAN IN PURE AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, precise, accurate, cost effective stability indicating UV- spectrophotometric method has been developed for the estimation of Fimasartan in bulk and tablet dosage form. Fimasartan shows highest λmax at 261 nm. Beer's law (linearity response) was found over a concentration range of 10-50 µg /mL with good correlation coefficient (r^2 = 0.999). The detection limit (DL) & quantitation limit (QL) were found to be 0.002 mcg/mL and 0.001 mcg/mL respectively. The results of the Fimasartan recovery analysis were found to be 96.80±0.001 to 98.28±0.002. Percentage assay of Fimasartan tablets (Fimanta) got more than 99.96 %. The proposed spectrophotometric method was validated as per the ICH Q1A (R2) guidelines. While estimating the Fimasartan in tablet formulation, there was no interference of additives & excipients. Hence this method can be safely being employed for the routine quality control analysis of Fimasartan in bulk and tablet dosage form.

Key words: Fimasartan, Method development, UV spectroscopy.

INTRODUCTION

Fimasartan is a non-peptide angiotensin II receptor antagonist used for the treatment of hypertension and heart failure. Fimasartan 2-[2-butyl -4-methyl-6-oxo-1-[[4-2-(2H- tetrazol-5yl)phenyl]phenyl]Methyl]Pyrimidin-5-yl]-N,N-dimethylethanoethioamide. It was found mostly in unmetabolized form in the plasma and in bile excretions. Urinary elimination of the drug was low, at less than 3% 24 hours after administration, entsiling that the fimasartan does not renal excretion.

Figure 1: Structure of Fimasartan

Literature review revealed that only pharmacological and clinical studies have been reported for the determination of Fimasartan. It has been not reported in UV/ method of Fimasartan in Bulk and pharmaceutical dosage forms. Hence the main objective of the present research is to develop and validate a simple, precise, sensitive liquid method for fimasartan in Bulk and tablet dosage forms.

MATERIALS AND METHODS

Instruments used ELICO Double beam SL 210 UV-VIS spectrophotometer consisting two matched quartz cells with one cm light path was used for recording and measuring of spectra and absorbance of Fimasartan. Essaevibra AJ (0.001g) analytical balance was used for weighing. Ultra sonicator bath Model no - 91250, PCI Ltd., Mumbai were used in this study.

Chemicals and Reagents

Fimasartan pure drug was supplied as gift sample by Hetero Drugs Ltd., Hyderabad, Telangana, India. The marketed formulation Karanb tablets containing 50mg of Fimasartan tablets were obtained from local market. Analytical grade Methanol was procured from E. Merck specialties private Ltd., Mumbai, India.

Selection of solvent:

Copious trails were done to find out the right solvent system for dissolving the drug. The solvents like acetonitrile, methanol, double distilled water and dimethyl sulfoxide [DMSO] were tried depending on the solubility of the Fimasartan. Fimasartan is soluble in organic solvents such as water, methanol, acetonitrile and DMSO. Based on the solubility methanol were selected all the way through the experiment.

Preparation of solutions

Preparation of stock standard solution:

Standard drug solution of Fimasartan was prepared by dissolving 10 mg of standard drug in 5 mL methanol in 10 mL volumetric flask. It was sonicated for 5 minutes for the complete solubility of the drug. After dissolving the drug the final volume was brought up to 10 mL by adding methanol to obtain eventual concentration of 1000 µg/mL.

Preparation of working standard solutions and construction of standard graph:

The prepared stock solution was further diluted with methanol to get working standard solution of 100 µg/mL of Fimasartan. From the working standard solution of 100 µg/mL, serial dilutions 1.0 µg/mL, 2.0 µg/mL, 3.0 µg/mL, 0.4 µg/mL & 0.5µg/mL were prepared by using same solvent. Then the sample was scanned in UV-VIS Spectrophotometer in the range 200 - 400 nm using methanol as a blank and the Wavelength corresponding to maximum absorbance (λmax) was found to be 261 nm

Selection of detection wavelength

To determine the optimum λmax, Fimasartan 10 mcg /mL of the working standard solution was prepared and scanned in the UV wavelength range of 200 - 400 nm utilizing as a blank. It was observed water that the drug showed maximum absorbance at 230 nm which was chosen as the detection wavelength for the estimation of Fimasartan.

Calibration curve for Fimasartan:

A calibration curve was plotted over a concentration range of 10-50 µg/mL for Fimasartan. Precisely measured standard solution of Fimasartan (10, 20, 30, 40 and 50 mL) was shifted to a series of 10 milliliter volumetric flasks and the volume was filled up to 10 mL with methanol. Calibration curve was plotted taking the absorbance on Y-axis and concentration on the X-axis and its calibration curve is represented in figure 7.2. The calibration data are presented in table 7.1.

Procedure for assay of pharmaceutical formulations:

Twenty Fimasartan (Fimanta) marketed tablets were accurately weighed, finely powdered and average weight of each tablet was determined and the tablet fine powder equivalent to 50 mg of Fimasartan was taken into hundred milliliter graduated flask and dissolved in methanol to get 100 µg/mL concentrations. The solution was then sonicated for 20 min and filtered & further dilutions were done with water to get eventual concentration (10µg/mL) within the linearity range and measured λmax at 261 nm. Finally the drug content in each tablet and also bulk drug were founded by utilizing the standard graph.

For analysis of bulk drug:

10 mg of bulk drug accurately weighed in 10 mL of measuring flask, 3 mL of water was added to get the drug solubility and the eventually the volume was filled up to 10 mL by utilizing water and required concentration

((10 µg/mL) was prepared and determined the absorbance. Table 10 shows the assay results of pharmaceutical formulation (Fimanta) and bulk drug.

Determination of precision:

Precision can be defined as "The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample". The precision of the method was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as % RSD. For this 10 µg /mL concentration solution was prepared from the working standard solution by taking 3.0 mL of the solution into a 10 mL volumetric flask and diluted with methanol. It was measured six times in the same day for intraday precision and on three different days for interday precision.

Limit of detection:

Limit of detection was determined based on the standard deviation of y intercepts of the regression line. The standard deviation of y intercepts obtained from the six measurements (n=6) was substituted for σ in the equation 3.3 σ/S, and S is the mean slope of the three calibration curves.

Limit of quantitation:

Limit of quantitation was determined based on the standard deviation of y intercepts of the regression line. The standard deviation of y intercepts obtained from the six measurements (n=6) was substituted for σ in the equation 10 σ/S, and S is the mean slope of the three calibration curves.

Ruggedness:

Ruggedness testing was determined between two columns or two analysts or two instruments.

Procedure:

Both the standard as well as the sample of the same concentration (5.0 µg/ml of Fimasartan) was prepared and analyzed by two analysts and between two instruments.

Robustness:

The prominent part of robustness is to develop methods that allow for predictable variations in the separation parameters. For the estimation of method robustness, parameters such as variation in detector wavelength vary within an accurate range and the quantitative influence of the variables is determined. The analysis showed % RSD less than two which indicates that the method established is robust.

RESULTS AND DISCUSSION

METHOD VALIDATION

The developed method has been validated as per ICH Q2 (R1) guidelines by means of the following parameters:

Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in the sample within a given range.

Appropriate aliquots solutions were pipetted out from the standard stock solution into a series of 10 mL volumetric flasks. The volume was made up to the mark with methanol to obtain a concentration ranging from 1.0-5.0 µg/mL. Absorbance of the above solutions was measured at 261 nm. A calibration graph of concentration vs. absorbance was established. Linearity determinations were carried and the data obtained was analyzed statistically. A calibration graph of concentration vs. absorbance was established.

The drug follows the Beer Lamberts law in the concentration range of 1.0-5.0 µg/mL. Regression equation was established and the correlation coefficient was determined. The results are shown in Table 1.

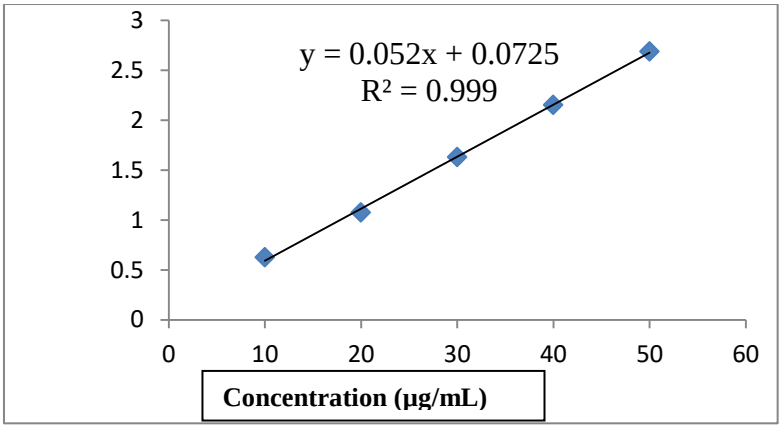

Figure 2: Calibration curve of Fimasartan

Table 1: Linearity Data of Fimasartan

S.No	Concentration (µg/mL)	Absorbances
1	1.0	0.6238
2	2.0	1.0756
3	3.0	1.6298
4	4.0	2.1504
5	5.0	2.6882

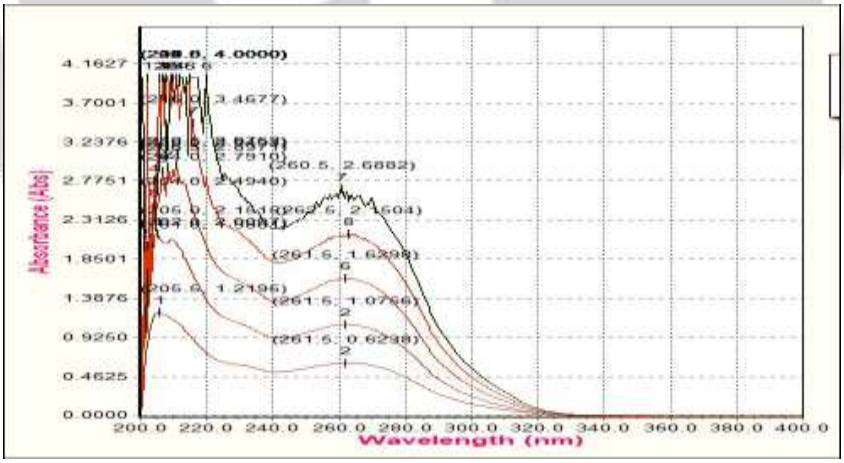

Fig 2: Overlay Spectrum of Fimasartan

Precision:

Precision can be defined as "The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample". The precision of the method was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as % RSD. For this 10 µg /mL concentration solution was prepared from the working standard solution by taking 3.0 mL of the solution into a 10 mL volumetric flask and diluted with methanol. It was measured six times in the same day for intraday precision and on three different days for interday precision.The results of statistical analysis are given in Table 2.

Table 2: Results of method precision for intra - day precision

Concentration (µg)	Sample absorbance	Mean absorbance ± S.D	% RSD (n=3)
20	1.0756 1.0759 1.0752	1.0755 ± 0.0003	0.0326
30	1.6296 1.6298 1.6295	1.6296 ± 0.0001	0.0093
40	2.1502 2.1505 2.1507	2.1504 ± 0.0002	0.0117

Table 3: Results of method precision for inter - day precision

Concentration (µg)	Sample absorbance	Mean absorbance ± S.D	% RSD (n=3)
20	1.0756 1.0753 1.0758	1.0755 ± 0.0002	0.0233
30	1.6294 1.6291 1.6297	1.6294± 0.0003	0.0184
40	2.1505 2.1503 2.1506	2.1504± 0.0001	0.0071

***Mean of six determinations**

Accuracy:

Recovery studies were performed by applying the standard addition method. To a known amount of the pre-analyzed drug sample an 80 %, 100 %, and 120 % of standard drug substance was added and suitably diluted. The absorbances of the resultant solutions were measured at 261 nm. The amount recovered was determined by fitting the absorbance values in the calibration graph.The results of accuracy studies are shown in Table 4

Table 4: Accuracy Studies of Fimasartan

Recovery level %	Absorbance	% Recovery	Mean % recovery	% RSD (n=3)
80	1.0751	97.40	96.80	1.81
80	1.0754	94.75		
80	1.0756	98.26		
100	1.6293	101.4	97.68	1.45
100	1.6297	97.63		
100	1.6296	94.01		
120	2.1502	101.1	98.28	1.73
120	2.1504	97.17		
120	2.1506	97.26		

*RSD= Relative standard deviation *= Average of 3 determinations

Limit of detection and quantification:

Limit of detection and quantification was determined based on the standard deviation of y intercepts of the regression line. The standard deviation of y intercepts obtained from the six measurements (n=6) was substituted for σ in the detection of equation 3.3 σ/S, and Quantification of equation 10 σ/S, and S is the mean slope of the three calibration curves. The results were given in table 5.

Table 5: LO D & LOQ of Fimasartan

Standard	LOD (µg/mL)	LOQ (µg/mL)
Fimasartan	0.002	0.001

Ruggedness:
Ruggedness testing was determined between two columns or two analysts or two instruments.
Procedure:
Both the standard as well as the sample of the same concentration (5.0 µg/ml of Fimasartan) was prepared and analyzed by two analysts and between two instruments. The results are shown in table 6

Table 6: Ruggedness of Fimasartan

Parameter	Absorbance for 1.0 µg/mL			
	Analyst-1	**Analyst-2**	**Instrument-1**	**Instrument-2**
SD	0.0003	0.0002	0.0001	0.0003
Mean	1.0755	1.0758	1.6296	1.6293
%RSD	0.0326	0.0233	0.0093	0.0184

*= Average of 3 determinations

Robustness:

The prominent part of robustness is to develop methods that allow for predictable variations in the separation parameters. For the estimation of method robustness, parameters such as variation in detector wavelength vary within an accurate range and the quantitative influence of the variables is determined. The analysis showed % RSD less than two which indicates that the method established is robust. The results are shown in table 7

Table 7: Robustness of Fimasartan

Parameter	λ max 1	λ max 2
Mean	1.6294	1.6296
SD	0.0003	0.0001
% RSD (n=6)	0.0184	0.0093

Optical Characteristics:

The optical characteristics such as Beer's law limit, Molar absorptivity, Sandell's sensitivity, Correlation coefficient, slope and intercept and % Relative Standard Deviation (Precision) were calculated and are summarized in Table 8.

Table 8: Optical characteristics, regression data of the proposed method.

Parameter	Results
Detection wavelength (λ_{max})	261 nm
Beer's law limits(µg/mL)	1.0-5.0
Molar absorptivity (L. mole^{-1} cm^{-1})	189.117
Sandell's sensitivity (µg /cm^2/0.001 absorbance unit)	0.00529
Regression equation (Y = mx+ c): Slope (b)	0.052x + 0.0725
Standard deviation of slope (S_b)	0.003873611

Intercept (a)	0.02546667
Standard error of intercept (S_a)	0.023455879
Standard error of estimation (S_e)	0.03240895
Correlation coefficient (r^2)	0.999

In this proposed method, the spectral absorbances of Fimasartan were measured against Methanol as a solvent blank at 261nm. Fimasartan obeyed Beer-Lambert's law in the concentration range of 1.0-5.0µg/ml with correlation coefficient (r^2) of 0.999. The accuracy of the method was confirmed by the recovery studies, by adding a known amount of the pure drug to the formulation and the percentage recovery was found to be between 96.80 to 98.28 % w/w, indicating that the developed method is accurate which indicates a good accuracy of the method and it shows that the method was free from the interference of excipients used in the formulation. The precision of the method was reported in terms of the relative standard deviation and it should be evaluated by using a minimum of 6 determinations over 100 % concentration which shows % RSD less than 2 indicates that the method was precise. The percentage purity of the marketed formulation was found to be 99.96 % w/w. All the results were found to be within the limits and therefore the proposed method was found to be free from interferences due to excipients in the tablet dosage form.

CONCLUSION

A simple, sensitive, rapid and economic UV Spectrophotometric method was developed and validated for the assay of Fimasartan in pure and pharmaceutical dosage forms. This method produced high recoveries with good linearity and precision. Thus, the developed method for Fimasartan was found to be simple, precise, accurate and cost effective and in actual fact feasible for routine sample analysis of Fimasartan in pharmaceutical dosage forms.

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