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Research Article

DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROSCOPIC METHOD FOR ESTIMATION OF RIZATRIPTAN BENZOATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: Development of a simple, precise, accurate, specific, and robust UV-visible spectrophotometric method for the quantitative determination of rizatriptan benzoate in tablets and bulk form.
Method: The method is developed using phosphate buffer pH 6.8 as a solvent. The stock solution of rizatriptan benzoate was prepared in phosphate buffer pH 6.8 and subsequent dilutions were done in phosphate buffer pH 6.8. The standard solution of rizatriptan benzoate showed absorption maxima at 226 nm. The sample was prepared by simple extraction method. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity, robustness, ruggedness and solution stability.
Results: The drug solutions obeyed Beer–Lambert's law and linearity was studied in the concentration range of 1–8 µg/ml with correlation coefficient 0.9956 at 226 nm. The limit of detection and limit of quantification were found to be 0.00368µg/ml and 0.01126µg/ml respectively. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120 %. The % recovery was found to be in the range 98.75-100.52%. The low values of % relative standard deviation (RSD) are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % RSD value less than 2 indicate that the method is precise.
Conclusion: The above method was a cost-effective quality-control tool for routine analysis of rizatriptan benzoate in bulk and in tablet dosage form.

Keywords: Absorption, Beer–Lambert's law, Percent RSD, Rizatriptan benzoate, UV visible spectrophotometric.

INTRODUCTION

Spectrophotometric methods are a large group of analytical methods that are based on atomic and molecular spectroscopy. Spectroscopy is a branch of science dealing with the study of interaction of electromagnetic radiation with matter. The kind and amount of radiation absorbed by the molecule depends on the number of molecule interacting with the radiation.[1,2]

Rizatriptan benzoate (RB) is an antimigraine drug used to treat migraines. Chemically RB is known as N, N dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indole-3-ethanamine monobenzoate (Figure 1). RB belongs to a class of drugs known as serotonin 5-hydroxytryptamine (5-HT1) receptor agonist. RB acts as agonist at specific 5-HT1 receptor sites in intracranial vessels, causing vasoconstriction.[3]

The literature survey reveals that various methods for the determination of rizatriptan benzoate are reported. Among these methods liquid chromatography, LC-MS/MS, HPLC method for rizatriptan benzoate. However less suitable derivative spectrophotometric method is reported till date for the estimation of rizatriptan benzoate. [4,5,6,7,8,]

So, an attempt was made to develop a simple, precise, accurate, specific, and robust UV-visible spectrophotometric method for the quantitative determination of RB in tablets and bulk form using phosphate buffer pH 6.8 as a solvent. The current research work deals with development of spectrophotometric method and its validation as per ICH guidelines.[9] The devised method was found to be selective, reliable, faster and more straight forward than other reported methods.

MATERIALS AND METHODS

Instruments and Reference standard

An UV-visible double beam spectrophotometer (Shimadzu, 1800, Japan) with matched quartz cells corresponding to 1 cm path length. AUX – 220 single pan electronic balance was used for weighing the materials. Pure sample of rizatriptan benzoate was obtained from Alkem Lab. ltd, Mumbai, India. Potassium dihydrogen phosphate and sodium hydroxide were purchased from Loba Cheime, Mumbai, India.

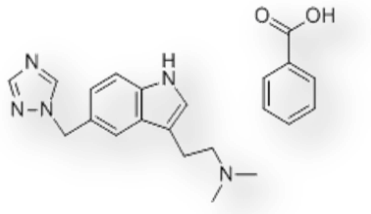

Fig. 1: Structure of Rizatriptan benzoate

Marketed tablet preparation was-

Trade Name	Company Name	Dose	Batch Number	Manufactured Date	Expiry
RIZACT-5	Cipla ltd.	5 mg	D22361	Oct. 2012	Sep. 2014

Procedure

Preparation of phosphate buffer pH 6.8[10]

Phosphate buffer pH 6.8 was prepared by dissolving 50ml of 0.2M potassium dihydrogen phosphate in 200 ml volumetric flask and then 22.4ml of 0.2M sodium hydroxide solution was added in it then volume was make up by distilled water upto 200 ml.

Preparation of 0.2M potassium dihydrogen phosphate: Accurately weighed 27.21 g of potassium dihydrogen phosphate was dissolved in distilled water; add distilled water upto 1000 ml.

Preparation of 0.2M sodium hydroxide: Accurately weighed 0.8g of sodium hydroxide was dissolved in 100ml of distilled water.

Preparation of standard stock Solution

Standard stock solution was prepared by dissolving drug equivalent to 10 mg of drug in small volume of phosphate buffer pH 6.8 into 100 ml volumetric flask and then volume was adjusted to 100 ml with phosphate buffer pH 6.8.

Scanning of stock solution for determination of λ_{max}

The stock solution prepared as above procedure then scanned for maximum absorption wavelength (λ_{max}). First in both cuvettes phosphate buffer pH 6.8 was added then spectrum was obtained between 400-200 nm. Then baseline correction was carried out. Further the stock solution was added into a cuvette and again spectrum was scanned from 400-200 nm and the wavelength at which maximum absorption observed was noted.

Beer's law range

The stock solution was suitably diluted with phosphate buffer pH 6.8 to get concentration range from 1 to8 µg/ml. The solutions were scanned in UV regions between 400 to 200nm then absorption was measured at maximum λ_{max}. Calibration curve was plotted by using absorbance and concentrations.

Analysis of tablet formulations

Twenty tablets were finely powdered. An accurately weighed quantity of powder equivalent to about 10mg of rizatriptan benzoate was transferred to a 100ml standard flask. The contents of the flask were mixed with phosphate buffer pH 6.8 and shaken to dissolve the active ingredients and then made up to the volume with the same solvent. The solution was filtered and the filtrate was further diluted with phosphate buffer pH 6.8 to give a final drug concentration of 1 to 8µg/ml. Absorbance values of sample solution were recorded at 226nm.

The proposed method was validated for the following parameters.

Linearity

Linearity was determined by constructing analytical curve with eight calibration points for drug, with the concentrations 1-8 µg/ml. The absorbance values were plotted against the respective concentrations of drug to get the analytical curve. The results were subjected to regression analysis by the least squares method to calculate the slope (m), intercept (c) and regression coefficient (R^2).

Sensitivity

The sensitivity of measurements of RB by the use of the proposed method was estimated in terms of the limit of quantification (LOQ) and limit of detection (LOD). The LOD and LOQ were calculated using formula LOD= 3.3×M/S and LOQ= 10×M/S

Where, M is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions.

Repeatability

Repeatability of the method was determined by analyzing six samples of 4 µg/ml concentrations of drug and the % RSD were calculated.

Intraday and interday precision

Intraday precision was determined by analyzing the drug at three different concentrations 2.5, 5 and 7.5 µg/ml and each concentration for three times, on the same day. Interday precision was determined similarly, but the analysis being carried out daily, for three consecutive days.

Ruggedness

The solutions were prepared and then analyzed with change in the analytical condition like different analyst.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ±2 nm. For changes of conditions, the sample was determined in triplicate. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values.

Accuracy (Recovery)

The accuracy of the methods was determined by performing recovery studies on tablet formulation and for prepared solutions containing known amount of drug by standard addition method in which preanalyzed samples were taken and standard drug was added at three different levels (80%, 100% and 120%) and constant was kept 2µg/ml.

Solution stability

The stability of the test solution was tested at intervals of 1, 4, and 10 hrs.

RESULTS

A simple, economic, precise, accurate method for estimation of rizatriptan benzoate was developed. This developed method was validated according to ICH guidelines.

Table 1: Linearity data of RB by UV spectroscopy

Concentration (µg/ml)	Abs 1	Abs 2	Abs 3	Mean (n=3)	±SD	%RSD
1	0.153	0.161	0.157	0.157	±0.004	2.547771
2	0.296	0.295	0.303	0.298	±0.004359	1.462718
3	0.408	0.405	0.414	0.409	±0.004583	1.120434
4	0.5	0.496	0.502	0.499	±0.003055	0.612235
5	0.642	0.648	0.645	0.645	±0.003	0.465116
6	0.805	0.803	0.798	0.802	±0.003606	0.44957
7	0.879	0.892	0.884	0.885	±0.006557	0.740954
8	1.076	1.071	1.039	1.062	±0.020075	1.890288

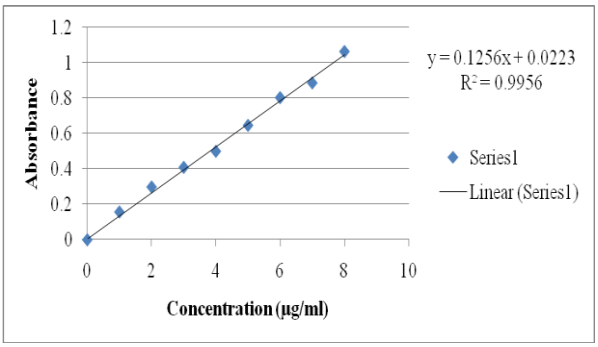

Fig. 2: Calibration curve of RB in pH 6.8 phosphate buffer

Table 2: Calibration parameters

Sr. no.	Parameters	Method wavelength (nm)
1)	Absorption maxima (nm)	226
2)	Beer's law limit (µg/ml)	1-8
3)	Correlation coefficient	0.9956
4)	Regression equation (y = mx + c)	y=0.1256x + 0.0223
5)	Slope (m)	0.1256
6)	Intercept (c)	0.0223

Table 3: Observations for intraday and inter-day precision

Condition	Concentration (µg/ml)	Abs 1	Abs 2	Abs 3	Mean (n=3)	±SD	%RSD
Intraday	2.5	0.334	0.331	0.33	0.331667	±0.002082	0.627638
	5	0.65	0.6456	0.64	0.6452	±0.005012	0.776811
	7.5	1.003	0.9956	0.97567	0.991423	±0.014136	1.42579
Inter-day	2.5	0.256	0.257	0.25	0.254333	±0.003786	1.488574
	5	0.527	0.512	0.523	0.520667	±0.007767	1.491828
	7.5	0.84	0.82	0.83	0.83	±0.01	1.204819

Table 4: Results of repeatability

Concentration (µg/ml)	Mean (n=6)	±SD	%RSD
4	0.506	±0.00992	1.960411

Table 5: Data of %recovery

Concentration level (%)	Test	Marketed	% Recovery	Mean (n=3)	±SD	%RSD
80	0.359	0.362	99.17127	99.07929	±0.125618	0.126785
	0.342	0.345	99.13043			
	0.372	0.376	98.93617			
80	0.322	0.328	98.17073	98.75666	±0.599616	0.607165
	0.311	0.315	98.73016			
	0.315	0.317	99.36909			
80	0.299	0.299	100	99.16326	±0.917687	0.925431
	0.287	0.289	99.30796			
	0.27	0.275	98.18182			
100	0.671	0.672	99.85119	99.37471	±0.499874	0.503019
	0.604	0.611	98.85434			
	0.684	0.688	99.4186			
100	0.545	0.538	101.3011	100.0081	±1.152802	1.152708
	0.547	0.549	99.6357			
	0.543	0.548	99.08759			
100	0.582	0.576	101.0417	100.5256	±1.057477	1.051948
	0.578	0.571	101.2259			
	0.575	0.579	99.30915			
120	0.99	0.991	99.89909	99.36247	±0.615339	0.619288
	0.98	0.993	98.69084			
	0.99	0.995	99.49749			
120	0.911	0.915	99.56284	99.81883	±0.439302	0.440099
	0.923	0.92	100.3261			
	0.921	0.925	99.56757			
120	0.891	0.883	100.906	99.92806	±0.854167	0.854781
	0.887	0.893	99.32811			
	0.885	0.889	99.55006			

Table 6: Results of robustness

Concentration (µg/ml)	Method wavelength (nm)	Condition (nm)	Mean (n=3)	±SD	%RSD
4		224 nm	0.493667	±0.005033	1.019559
4	226 nm	228 nm	0.481	±0.002646	0.550052

Table 7: Results of Ruggedness

Concentration (µg/ml)	Mean (n=3)	±SD	%RSD
	Analyst 1		
6	0.808333	±0.003512	0.43446
	Analyst 2		
6	0.806333	±0.005508	0.683039

Table 8: Observations for solution stability

Concentration (µg/ml)	Time (Hrs)	Mean (n=3)	±SD	%RSD
3	1	0.395667	0.004163	1.052232
	4	0.385667	0.004041	1.047913
	10	0.376667	0.001528	0.405538

DISCUSSION

Linearity studies

Various concentrations of drug were prepared in phosphate buffer pH 6.8 solvent. The linearity of RB was found to be in the range of 1-8µg/ml with correlation coefficient of 0.9956. Linear regression equation was found to be y=0.1256x + 0.0223. The linearity data were expressed in table 1 and calibration parameters in table 2, calibration curve is shown in fig. 2.

Sensitivity

The linearity equation was found to be y=0.1256x + 0.0223. The LOD and LOQ for RB were found to be 0.00368µg/ml and 0.01126µg/ml respectively.

Precision

The precision of the method was expressed in terms of % relative standard deviation (% RSD). The %RSD values found to be less than 2 for intra-day and inter-day precision, which indicate that the proposed method was precise for analysis. The results were expressed in table 3.

Repeatability

Repeatability was determined by analyzing 4µg/ml concentration of RB solution for six times and %RSD was found to be 1.9604, which is less than 2. The results were expressed in table 4.

Accuracy (Recovery)

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The % recovery for RB was found to be 98.75-100.52%. The recovery studies were reported in table 5.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered. The effect of detection wavelength was studied at ±2 nm. For changes of conditions, the sample was assayed in triplicate. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. The %RSD values found to be less than 2 for both condition of robustness, which indicate that the proposed method was precise for analysis. The results were shown in table 6.

Ruggedness

Two different analysts performed for same concentration solutions of the drug in similar operational and environmental conditions using developed method. The %RSD values found to be less than 2. The results were summarized in Table 7.

Solution stability

The stability of the drug solution was tested at intervals of 1, 4, and 10 h. The stability of solutions was determined by comparing absorbance of RB. The %RSD values found to be less than 2 after 10 hrs. These results indicate the solution was stable for 10 h at ambient temperature. The results were shown in table 8.

CONCLUSION

The developed UV-visible spectrophotometric method for the determination of RB has the advantage of being fast, simple, inexpensive, and applicable over a wide concentration range with high precision and accuracy. The developed UV-visible spectrophotometric method is cheaper, simpler and faster than HPLC and GC methods for analysis of RB in the pharmaceutical preparations. The method was validated as per the guidelines laid by ICH. The results of the validation tests were found to be satisfactory and therefore this method can be applied successfully to analyze drug formulations.

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