Area under Curve Method Development and Validation of Rizatriptan Benzoate

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Abstract

A simple, rapid, accurate and economical AUC method has been developed for estimation of Rizatriptan benzoate from bulk and pharmaceutical formulation. The λmax of rizatriptan benzoate in water was found to be 280 nm. The drug follows linearity in the concentration range 6-36 µg/ml with correlation coefficient value 0.999. The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 98.00% – 102.00% was found in good agreement with the label claim. 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 98.00% – 102.00%. The low values of % 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 % R.S.D. value less than 2 indicate that the method was precise. Ruggedness of the proposed method was studied with the help of two analysts. The above method was a rapid and cost-effective quality-control tool for routine analysis of Rizatriptan benzoate in bulk and in pharmaceutical dosage form.

Keywords: Area under Curve, Rizatriptan benzoate, Validation.

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Introduction

Rizatriptan benzoate is chemically 3-[2-(dimethylamino) ethyl]-5-(1H-1, 2, 4-triazol-1-yl methyl) indole monobenzoate][1] (Fig. 1). The mechanism action of Rizatriptan benzoate is through incentive of post synaptic 5-HT 1B receptors within cerebral and dural vessel walls creating vasoconstriction of peri-vascular nerve terminals[2]. Migraine is an extraordinary disorder symbolized by vibration headache, usually blocked to one side, which comes in outbreak lasting 4-48 hours and is often correlated with nausea, vomiting, sensitivity to light and sound, flashes of light, vertigo, loose motion and other symptoms[3]. It was already think that migraine headache was simply caused by vasodilatation that caused the freed of chemicals from nerve fibers that coil around the large arteries of the brain. While these blood vessels do certainly grow, advanced imaging studies of blood wind in the brain have shown that changes in blood flow cannot be totally responsible for all features of migraine. Instead, complex processes within the nervous system are believed to initiate the progress of migraine headache[4].

Analysis part is an important from formulation development of any drug molecule. A suitable and validated method should be vacant for the drug delivery system for analysis of bulk drug, for release dissolution studies and estimation of drug in biological samples. The literature survey acknowledges that various methods for the determination of Rizatriptan benzoate are noted. Among these processes, liquid chromatography was reported for the determination of rizatriptan benzoate in human plasma[5].

Mass spectrometry and NMR methods used for the description of metabolites excreted in urine, and use for the pharmacokinetic study[6]. All reported methods are time as well as uneconomical. Hence, our study reports a simple, precise and economical Area under Curve method for estimation of rizatriptan benzoate in tablet formulation. The method was validated according to ICH guidelines[7].

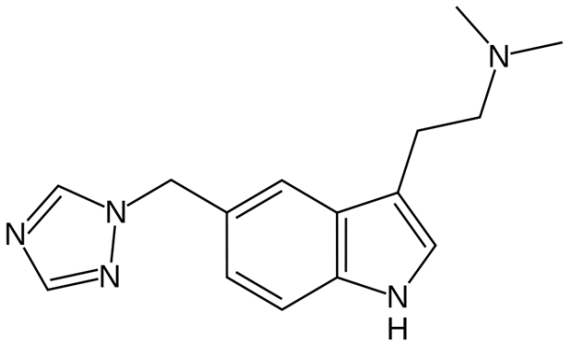

Fig. 1: Chemical structure of Rizatriptan Benzoate

Material and Method

Rizatriptan benzoate working standard was obtained as gift sample from Analytical solution. The drug was used without further purification. A tablet formulation containing 0.5 mg of Rizatriptan benzoate was purchased from local market. An analytical grade solvent was used for the experiment.

Instrument

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; derivative mode: 1D (first order derivative, dA/dλ); band width (Δλ):10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Preparation of standard stock and working standard solution

The standard stock solution of Rizatriptan benzoate was prepared by dissolving accurately weighed 10mg of the drug in water and diluted to 100 ml with same solvent to obtain 6µg/ml as a final concentration.

Method: Area under curve

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The spectrum obtained of first order derivative was used to calculate AUC. The calibration curve was constructed by plotting concentration (6-36µg/mL) versus AUC.

Validation of the Method

The method was validated in terms of linearity, accuracy, precision, and ruggedness.

Linearity study: Different aliquots of rizatriptan benzoate in range 0.6-3.6ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with water to get concentrations 6, 12, 18, 24, 30 and 36µg/ml, respectively. The solutions were scanned on spectrophotometer in the UV range 200-400 nm. The two wavelengths **263.5 and 291.5nm** was selected for the determination of Area Under Curve (Fig. 2). The calibration plot was constructed as Area Under Curve vs. concentration.

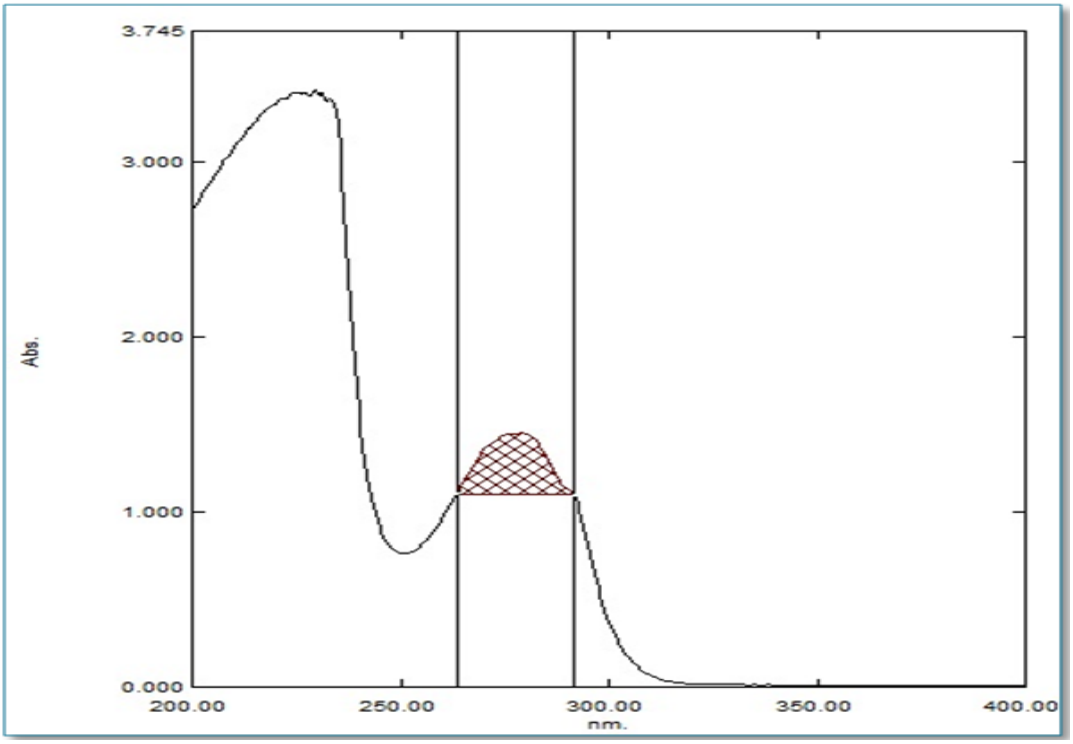

Fig. 2: Area under curve spectrum of Rizatriptan Benzoate in water

Accuracy (% Recovery): To the pre analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120%. The solutions were reanalyzed by proposed method.

Precision: Precision of the method was studied as intraday and inter-day variations. Intra-day precision was determined by analyzing the 12, 18 and 24µg/ml of rizatriptan benzoate solutions for three times in the same day. Inter-day precision was determined by analyzing the 12, 18 and 24µg/ml of rizatriptan benzoate solutions daily for three days over the period of week.

LOD and LOQ (Sensitivity): The sensitivity of measurements of rizatriptan benzoate by the use of the proposed method was estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n =3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability: Repeatability was determined by analyzing 24µg/ml concentration of rizatriptan benzoate solution for six times.

Ruggedness: Ruggedness of the proposed method is determined for 24µg/ml concentration of rizatriptan benzoate by analysis of dilution from homogenous slot by two analysts using same operational and environm-ental conditions.

Determination of Rizatriptan in Bulk: Accurately weighed 10mg of rizatriptan benzoate was transferred to a 100ml volumetric flask and 50ml water was added. After ultrasonic vibration for 15min, the mixture was diluted up to mark with water. The whole solution filtered using whatman filter paper no. 42. From filtrate correct dilution was taken in such a way that the final concentration is 24µg/ml. The concentrations of the drug were calculated from linear regression equations. The resulting solution was scanned on a spectrophotometer in the UV range 200-400nm. The spectrum was recorded at 280nm.

Application of proposed method for pharmaceutical formulation: For analysis of commercial formulation 10mg of rizatriptan benzoate tablet (Rizact 5mg, Cadila) was transferred to a 100ml volumetric flask and 50 ml water was added. After ultrasonic vibration for 15 min, the mixture was diluted up to mark with water. The whole solution filtered using what man filter paper no. 42. From filtrate correct dilution was taken in such a way that the final concentration is 24µg/ml. The concentrations of the drug were calculated from linear regression equations. The resulting solution was scanned on a spectrophotometer in the UV range 200-400nm. The spectrum was recorded at 280nm.

Results and Discussion

Method Validation: The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Linearity studies: The linear regression data for the calibration curves showed good linear relationship over the concentration range 6-36µg/ml for Rizatriptan Benzoate (Fig. 3). Linear regression equation was found to be Y=0.128x – 0.057 (r2 = 0.999). The result is expressed in Table 1.

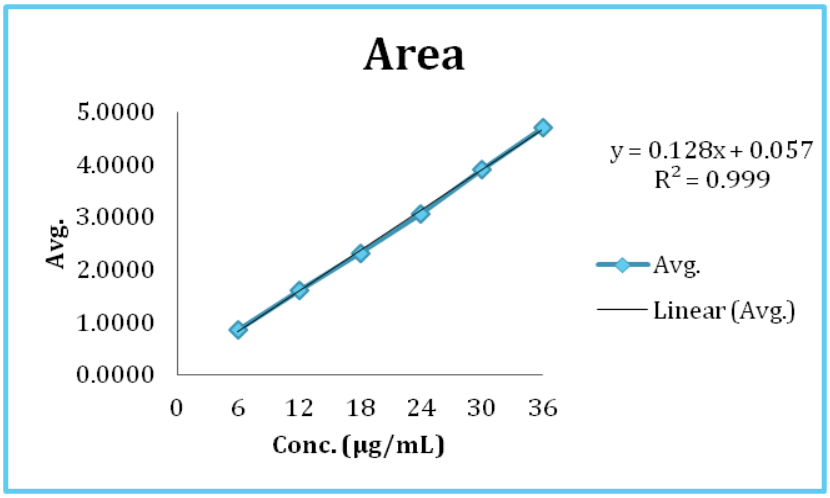

Fig. 3 Calibration curve of Rizatriptan Benzoate

Accuracy: The solutions were reanalyzed by proposed method; results of recovery studies are reported in Table 2 which showed that the % amount found was between 98.00% to 102.00% with % R.S.D. >2.

Precision: The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation (Table 3).
Sensitivity: The linearity equation was found to be Y=0.128x – 0.057 (r2 = 0.999). The LOQ and LOD for Rizatriptan Benzoate were found to be 1.2230 µg and 0.4036 µg, respectively (Table 4).
Repeatability: Repeatability was determined by analyzing 24 µg/ml concentration of Rizatriptan Benzoate solution for six times and the % amount found was between 98% to 102% with % R.S.D. less than 2 (Table 5).
Ruggedness: Peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The results are given in Table 6. The result showed that the % R.S.D. was less than 2%.
Determination of Rizatriptan Benzoate in bulk: The concentrations of the drug were calculated from linear regression equations. The % amount found was between 98.00% to 102.00% (Table 7).
Application of proposed method for pharmaceutical formulation: The spectrum was recorded at 280 nm. The concentrations of the drug were calculated from linear regression equation. The % amount was found between 98.00% to 102.00% (Table 8).

Table 1: Linearity study of rizatriptan benzoate

Concentration µg/mL	Area,[a] mean± SD (n=6)	% RSD
6	0.8634 ± 0.0148	1.7152
12	1.6104 ± 0.0101	0.6250
18	2.3144 ± 0.0458	1.9806
24	3.0628 ± 0.0438	1.4313
30	3.9068 ± 0.0622	1.5909
36	4.7112 ± 0.0590	1.2519

(n= no. of estimations**)**

Table 2: Recovery studies

Drug	Initial amount (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL, n=3)	% Recovered	% RSD
Rizatriptan benzoate	12	9.6	9.6807	100.8409	0.6057
	12	12	12.1833	101.5278	0.4855
	12	14.4	14.4181	100.1953	0.8406

(n= no. of estimations**)**

Table 3: Results of Precision studies

Component	Conc. (µg/mL)	Intra –day Precision[a] (n=3)		Inter –day Precision[a] (n=3)	
		Amt. found	% RSD	Amt. found	% RSD
Rizatriptan Benzoate	12	12.0461	0.4860	11.9351	0.6526
	18	18.0809	0.8570	18.1208	0.2526
	24	24.2127	.4192	24.1387	0.6109

[a]Average of three estimation

Table 4: Sensitivity studies

LOD(µg/mL)	LOQ(µg/mL)
0.4036	1.2230

Table 5: Repeatability Studies

Component	Amount taken (µg/mL) (n=6)	Amount found[a] (%)	% RSD
Rizatriptan benzoate	24	99.22 ± 0.24	0.25

[a]Average of six estimations

Table 6: Ruggedness study

Component	Amount taken (µg/mL) (n=6)	Amount found[a] (%)		% RSD	
		Analyst I ± SD	Analyst II ± SD	Analyst I	Analyst II
Rizatriptan benzoate	24	100.5577 ± 0.7413	100.7205 ± 0.5083	0.7372	0.5047

[a]Average of six estimations

Table 7: Analysis of rizatriptan benzoate in Bulk

Concentration (µg/mL)	Amount found (µg)	Amount found (%)
24	23.8578	99.4076
	23.7797	99.0820
	23.8109	99.2122
	23.8422	99.3424
	23.8344	99.3099
	23.8188	99.2448
Mean ± SD	23.8240 ± 0.1140	99.2665 ± 0.1140
% RSD	0.1149	0.1149

Table 8: Analysis of rizatriptan benzoate in Formulation (Rizact 5mg, Cipla)

Concentration (µg/mL)	Amount found (µg)	Amount found (%)
24	23.8578	99.4076
	23.5844	98.2682
	23.8891	99.5378
	23.7953	99.1471
	23.8656	99.4401
	23.7406	98.9193
Mean ± SD	23.7888 ± 0.4746	99.1200 ± 0.0.4746
% RSD	0.4788	0.4788

Conclusion

This UV Spectrophotometric method is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of rizatriptan benzoate in tablet formulation. The validation procedure confirms that this is an appropriate technique for their quantification in the formulation. It is also used in routine quality control of the formulations containing this entire compound.

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Conflict of Interest: None

Source of Support: Nil

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