Research Article

# Determination of Rizatriptan Benzoate in Bulk and Tablets by Spectrophotometry

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**ABSTRACT**
This paper describes a visible spectrophotometric method for quantitative analysis of rizatriptan benzoate (RTB) using vanillin. The absorbance measurements were performed analyzing the colored complex ($\lambda_{max}$=579 nm) produced from the reaction between RTB and vanillin in acid medium. The experimental variables were optimized in order to obtain the best reaction conditions. These variables included concentration of sulphuric acid and vanillin. The linearity was studied in the range of 50-250 μg/ml with a regression coefficient of 0.9997. The limit of detection and limit of quantification was estimated to be 0.156 and 0.472 μg/ml, respectively. The RTB content in commercial samples were analyzed using the proposed method and the results were favorably compared with those of the reported UV spectrophotometric method, showing that quantitative analysis by visible spectrophotometric method using vanillin could be effectively used to determine rizatriptan benzoate in tablet dosage forms.

## INTRODUCTION

Rizatriptan benzoate (RTB) is an antimigraine drug used to treat migraines[1-3]. Chemically RTB is known as *N,N*-dimethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indole-3-ethanamine monobenzoate (Figure 1). RTB belongs to a class of drugs known as serotonin 5-hydroxytryptamine (5-$HT_1$) receptor agonist. RTB act as agonist at specific 5-$HT_1$ receptor sites in intracranial vessels, causing vasoconstriction. RBT also act on sensory trigeminal nerves, reducing transmission along pain pathways.

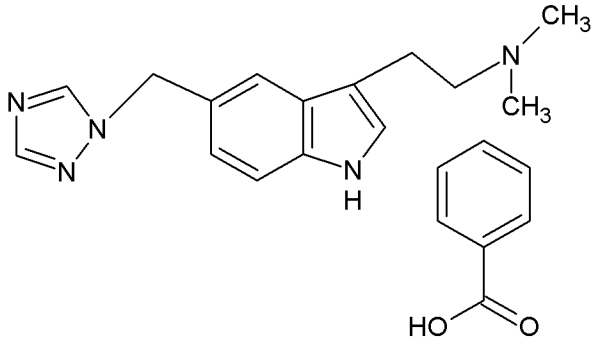


**Fig. 1: Chemical Structure of Rizatriptan Benzoate**

Rizatriptan benzoate was determined using liquid chromatography-electrospray tandem mass spectrometric[4-6], high performance liquid chromatographic[7-11], spectrofluorimetric[12] and micro emulsion electro kinetic chromatographic[13] methods. The aforesaid methods are complicated, costly and time consuming.

Spectrophotometry has been widely used in pharmaceutical analysis. This is mainly due to simple, low cost, adequate selectivity, low detection limit, high accuracy and availability in most quality control and clinical laboratories. A survey of literature revealed that there are very few UV spectrophotometric[12,14-17] and visible spectrophotometric[18-21] methods are available for the determination of RTB. The reported UV spectrophotometric methods are less selective. They involve measurements at shorter wavelength where the interference from tablet excipients is more. The earlier reported visible spectrophotometric methods suffer from disadvantages like extraction, rigid pH control, careful control of experimental

variables, long time for the reaction to complete, heating and lack of sensitivity.
So, an attempt was made to develop a simple, precise, accurate, specific, and robust visible spectrophotometric method for the quantitative determination of RTB in tablets and bulk form. The method is based on the reaction between RBT and vanillin, in the presence of sulphuric acid, producing a colored compound having absorption maximum at 579 nm. The proposed method was applied for the quantitative analysis of RBT in pharmaceutical raw and tablet dosage forms.

## EXPERIMENTAL

### Instrumentation

A calibrated shimadzu UV-VIS spectrophotometer model UV-1800 (Shimadzu Corporation, Tokyo, Japan) and a 1-cm matched quartz cells, was used to record Absoption spectra and measure absorbance. Samples were weighed by Shimazdu electronic weighing balance (Tokyo, Japan) BL 220 H model.

### Chemicals

The rizatriptan benzoate reference substance was obtained gift sample from Matrix laboratories (Hyderabad, India). Commercial tablet dosage forms of rizatriptan benzoate, Rizact (Cipa Pharmaceuticals Limited, Hyderabad, India) with a stated content of 5 and 10 mg, were purchased from a local pharmacy store. Concentrated sulphuric acid, methanol and vanillin were obtained from Merck specialities private limited, Mumbai, India. Vanillin was prepared as 4% in methanol. Vanillin reagent was prepared afresh daily.

### Preparation of working standard solution

The working standard solution was prepared by accurately weighing 100 mg of RTB reference substance, which was transferred to a 100 ml volumetric flask, dissolved and diluted to volume with water, to obtain a concentration of 1000 µg/ml.

### General procedure for the assay of RTB

Into a series of 10 ml volumetric flasks, volumes (0.5-2.5 ml) of RBT working standard solution equivalent to 50-250 µg/ml were transferred. The volume in each flask was brought up to the volume with water. To each flask, 1 ml of 4% vanillin and 2 ml of sulphuric acid was added and mixed well. The absorbance of the chromogenic enamine formed at room temperature (25 ± 1 °C) was measured at 579 nm against the reagent blank prepared similarly omitting the drug. The analytical curve was constructed by plotting the absorbance *versus* final concentration of RTB. The amount of the drug in the unknown sample was computed either from analytical curve or from regression equation.

### Procedure for the assay of RTB in tablet dosage forms

Weigh and grind twenty tablets containing RTB. Dissolve the powdered sample, equivalent to 100 mg of the RTB, in water. Filter the solution using Whatmann filter paper No. 1 and wash the residue with water. Dilute the filtrate and washings to 100 ml with the same solvent. Apply the proposed method for the determination of RTB.

## RESULTS AND DISCUSSION

### Chemistry of the colored complex

Enamines are formed by a condensation reaction between a secondary amine and an aldehyde or ketone in the presence of an acid catalyst[22,23]. The formation of enamine forms the basis for the spectrophotometric determination of compounds of pharmaceutical significance.

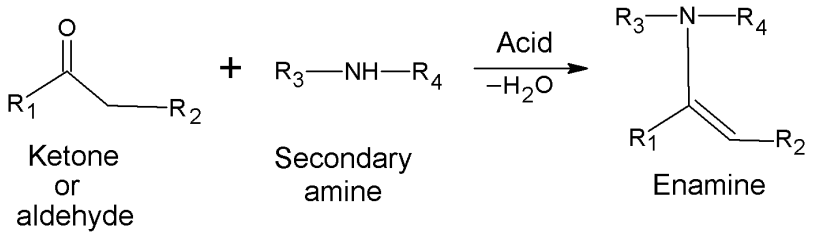


**Fig. 2: Formation of Enamine**

Vanillin, an aromatic aldehyde, has been applied to the quantification of drugs with primary or secondary amine in acidic medium using spectrophotometry[24-26]. The proposed method is based on the formation of chromogenic enamine between the secondary amino group of RTB and aldehyde group of vanillin. The probable reaction mechanism is given in figure 3. The produced chromogen was scanned in the range 400–800 nm. The chromogen exhibits $\lambda_{max}$ at 579 nm (Figure 4). The reagent blank showed negligible absorbance at 579 nm. The stability of the colored complex was evaluated and stable absorbance readings were obtained for 2 hours of standing at room temperature without any change in color intensity.

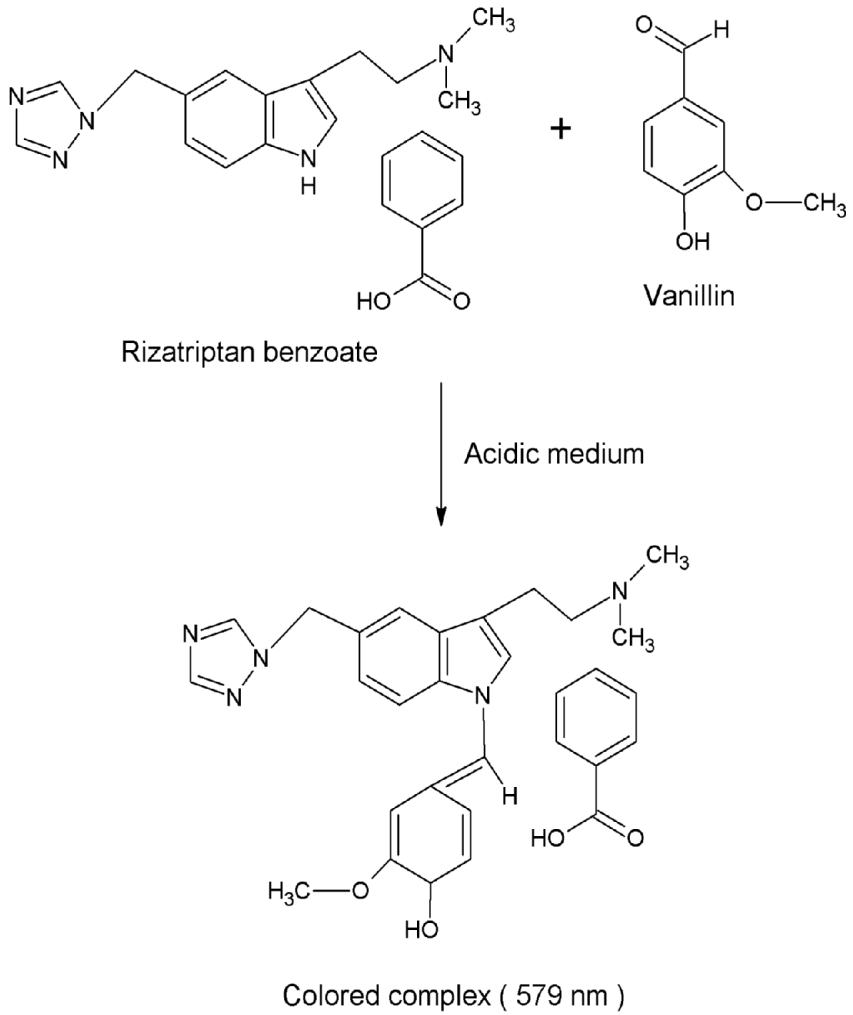


**Fig. 3: Formation of colored complex by condensation of RTB with vanillin**

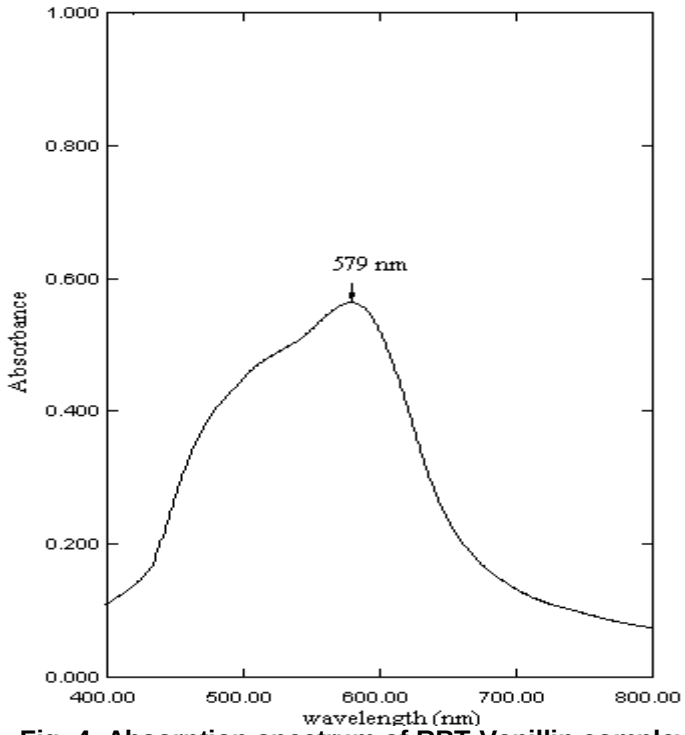


**Fig. 4: Absorption spectrum of RBT-Vanillin complex**

**Optimization of the experimental variables**

**Effect of concentration of vanillin**

The effect of the concentration of vanillin was studied by treating 100 µg/ml RTB with 2 ml of sulphuric acid and varying volumes (0.5–3.0 ml) of 4 % vanillin. The absorbance of the colored complex at 579 nm was increased with increasing volume of 4 % vanillin and became constant at 1.0 ml; above this volume, the absorbance remained unchanged (Figure 5). Thus a volume of 1 ml of 4% vanillin was chosen for the quantification process.

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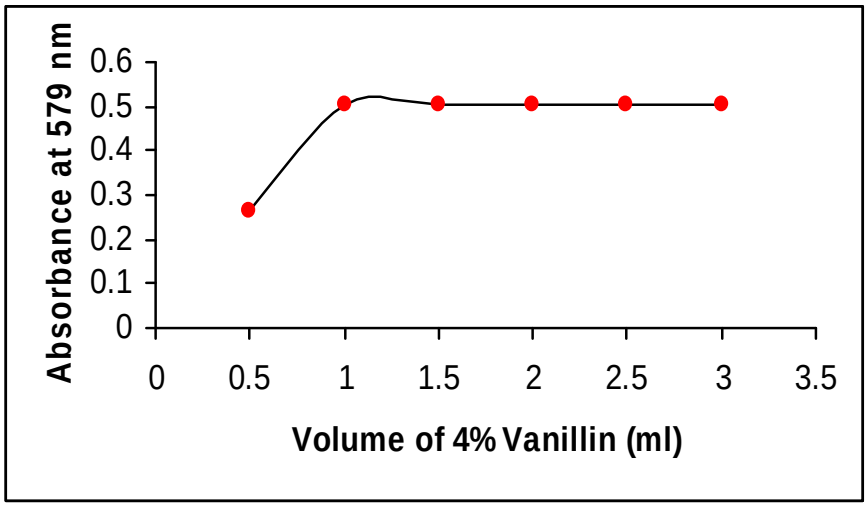


**Fig. 5: Effect of vanillin concentration**

**Effect of concentration of sulphuric acid**

The effect of sulphuric acid concentration on the absorbance of colored complex at 579 nm was studied by adding varying volumes (0.5–4.0 ml) of sulphuric acid and 1 ml of 4% vanillin to 1.0 ml of RTB (100 µg/ml). As shown in Figure 6, the absorbance of the colored complex was found to increase with increasing sulphuric acid concentration and became constant at 2 ml. Beyond this volume, the absorbance remained constant. Therefore all subsequent measurements were made using 2 ml of sulphuric acid.

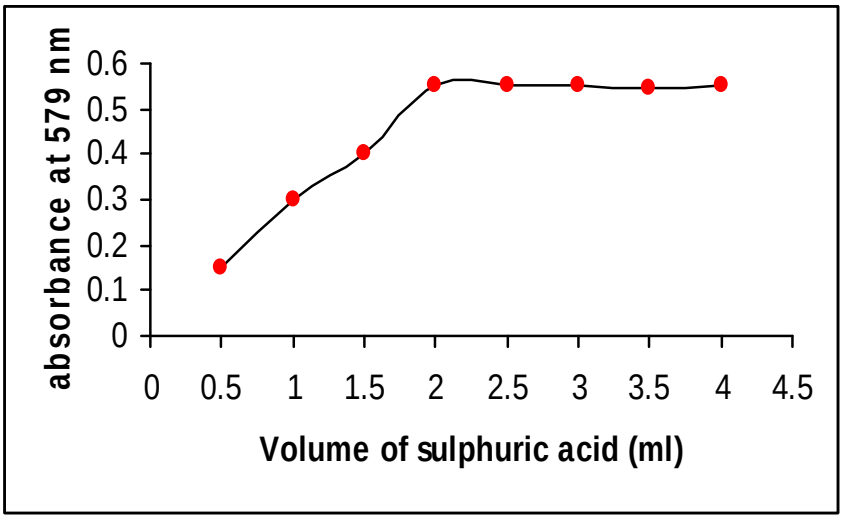


**Fig. 6: Effect of sulphuric acid concentration**

**Linearity**

Linearity was determined by constructing analytical curve with five calibration points for RTB, with the concentrations 50, 100, 150, 200 and 250 µg/ml. The absorbance values were plotted against the respective concentrations of RBT 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 ($R^2$). The results are presented in figure 7.

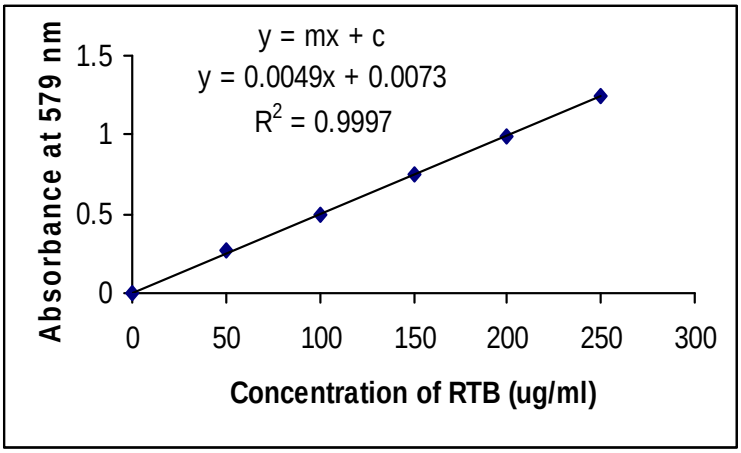


**Fig. 7: Linearity of the proposed method**

**Sensitivity**

The sensitivity of the proposed method was evaluated by calculating molar absorptivity, Sandell's sensitivity, limit of detection (LOD) and limit of quantification (LOQ). The results are summarized in table 1. The high value of molar absorptivity and low values of Sandell's

sensitivity, LOD and LOQ confirmed the sensitivity of the proposed method.

**Table 1: Sensitivity parameters**

| Parameters | Values |
|---|---|
| Molar Absorbtivity (L mole$^{-1}$ cm$^{-1}$) | 1.33 x10$^{4}$ |
| Sandell's sensitivity (µg cm$^{-2}$/0.001 Absorbance unit) | 0.202 |
| LOD (µg/ml) | 0.156 |
| LOQ (µg/ml) | 0.472 |

## Precision and Accuracy

The precision and accuracy of the proposed method was assessed by determining the concentration of RTB in pure form at three different concentration levels (50, 150 and 250 µg/ml). The intra day precision and accuracy of the proposed method was performed by carrying out five independent analyses at each concentration level within one day. The inter day precision and accuracy was evaluated by measuring the RTB content at each concentration level on three consecutive days. The results of standard deviation, relative standard deviation, recoveries and percent error by proposed method were calculated and are presented in Table 2. The results can be considered to be acceptable. Thus the proposed method is very effective for the assay of RTB.

**Table 2: Precision and accuracy of the proposed method**

| Concentration of RTB (µg/ml) | | % RSD | % Recovery | % Error |
|---|---|---|---|---|
| Taken | Found@ ± SD | | | |
| **Intra day** | | | | |
| 50 | 49.60 ± 0.039 | 0.792 | 99.20 | 0.80 |
| 150 | 149.80 ± 0.050 | 0.345 | 99.86 | 0.14 |
| 250 | 249.20 ± 0.062 | 0.243 | 99.68 | 0.32 |
| **Inter day** | | | | |
| 50 | 49.98 ± 0.033 | 0.608 | 99.96 | 0.04 |
| 150 | 149.92 ± 0.017 | 0.136 | 99.94 | 0.06 |
| 250 | 249.83 ± 0.060 | 0.242 | 99.93 | 0.07 |

@ Average of three determinations

## Robustness

The robustness of the proposed method was assessed by analyzing the RTB at two different concentrations levels (50 and 150 µg/ml) by altering the experimental parameters such as, volume of 4% vanillin (0.8 to 1.2 ml) and volume of concentrated sulphuric acid (1.8 to 2.2 ml). The results are summarized in Table 3. The percent recovery and relative standard deviation of the method were found to be appreciable, indicating that the proposed method is robust.

**Table 3: Robustness of the proposed method**

| Experimental variable | Concentration of RTB (µg/ml) | % Recovery@ | % RSD |
|---|---|---|---|
| 4% vanillin* | 50 | 99.96 | 0.608 |
| | 100 | 99.94 | 0.136 |
| Sulphuric acid** | 50 | 99.92 | 0.801 |
| | 100 | 99.96 | 0.128 |

@ Average of three determinations
* Volume of 4% vanillin: 0.8, 1.0 and 1.2 ml
** Volume of sulphuric acid: 1.8, 2.0 and 2.2 ml

## Recovery studies

Recovery experiments were carried out by standard addition method. For this, pre-analyzed tablet was spiked with pure RTB at three different concentration levels (50, 100 and 100%). The total was found by the proposed method. The recoveries and relative standard deviation of the added pure RTB were in the range 98.00-101.53 % and 0.160-0.360%, respectively (Table

4) indicating that co-formulated did not interfere in the assay.

**Table 4: Recovery studies by standard addition method**

| Formulation | Concentration of RBT (mg) | | Found@ ± SD | % RSD | % Recovery |
|---|---|---|---|---|---|
| | In tablet | Spiked value | | | |
| Rizact | 10 | 5 | 15.08 ± 0.001 | 0.248 | 101.53 |
| | | 10 | 19.60 ± 0.001 | 0.160 | 98.00 |
| | | 15 | 24.95 ± 0.021 | 0.360 | 99.80 |

@ Average of five determinations

**Assay in tablet dosage forms**

The proposed method was applied to the determination of RTB in tablets containing RTB in two doses (5 and 10 mg RTB per tablet). The results obtained are summarized in table 5. The RTB content of the same batch tablets was determined by the reference method[17]. The results show (Table 5) that there is close agreement between the results obtained by the proposed and the reference methods. The results were also compared statistically by means of a Student's t-test and a variance ratio F-test for accuracy and precision, respectively with those of the reference method at 95% confidence level. The calculated t and F-values (Table 5) did not surpass the tabulated values (t = 2.306 and F=6.390) for four degrees of freedom. The results indicate that there was no significant difference among the proposed and the reference methods in terms of accuracy and precision.

**Table 5: Assay of tablet dosage forms**

| Formulation | Proposed method | Reference method |
|---|---|---|
| Rizact 5 mg/tablet | % Recovery@: 99.60<br>% RSD: 0.186 | % Recovery@: 99.80<br>% RSD: 0.165 |
| | t value: 1. 268<br>F value: 3.486 | |
| Rizact 10 mg/tablet | % Recovery@: 99.80<br>% RSD: 0.248 | % Recovery@: 100.50<br>% RSD: 0.366 |
| | t value: 0.937<br>F value: 2.647 | |

@ Average of five determinations

## CONCLUSION

The visible spectrophotometric method developed herein is simple, rapid and allows the determination of rizatriptan benzoate in bulk and tablet dosage forms using vanillin as analytical reagent. The proposed method provides good results to determine the rizatriptan benzoate in terms of linearity, sensitivity, accuracy, precision, recoveries and robustness.

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## REFERENCES

1. Vyas KP, Halpin RA, Geer LA, Ellis JD, Liu L, Cheng H, Chavez-Eng C, Matuszewski BK, Varga SL, Guiblin AR and Rogers JD. Disposition and pharmacokinetics of the antimigraine drug, rizatriptan, in humans. Drug Metabolism and Disposition. 2000;28(1):89-95.
2. Dahlof CG, Rapoport AM, Sheftell FD and Lines CR. Rizatriptan in the treatment of migraine. Clinical Therapeutics. 1999;21(11):1823-1836.
3. Dooley M and Faulds D. Rizatriptan: a review of its efficacy in the management of migraine. Drugs. 1999;58(4):699-723.
4. Guo J, Zhang A, Zhao L, Sun X, Zhao Y, Gao H, Liu Z and Qiao S. Determination of rizatriptan in human plasma by liquid

chromatographic–eletrospray tandem mass spectrometry: application to a pharmacokinetic study. Biomedical Chromatography. 2006;20(1):61-66.
5. Chen Y, Miao H, Lin M, Fan G, Hong Z, Wu H and Wu Y. Development and validation of a selective and robust LC–MS/MS method for high-throughput quantifying rizatriptan in small plasma samples: Application to a clinical pharmacokinetic study. Journal Chromatography B. 2006;844(2):268-277.
6. Vishwanathan K, Bartlett MG and Stewart JT. Determination of antimigraine compounds rizatriptan, zolmitriptan, naratriptan and sumatriptan in human serum by liquid chromatography/electrospray tandem mass spectrometry. Rapid Communications in Mass Spectrometry. 2000;14(3):168-172.
7. Qin YP, Zou YG, Liang MZ and Yu Q. Determination of rizatriptan in human plasma by RP-HPLC with fluorescence detection. Yaowu Fenxi Zazhi. 2006;26(1):7-9.
8. Chen J, Jiang X, Jiang W, Mei N, Gao X and Zhang Q. Liquid chromatographic method for the determination of rizatriptan in human plasma. Journal Chromatography B. 2004;805(1):169-173.
9. Zecevic M, Jocic B, Zivanovic L and Protic A. Application of multicriteria methodology in the development of improved RP-LC-DAD for determination of rizatriptan and its degradation products. Chromatographia. 2008;68(11-12): 911-918.
10. Jocic B, Zecevic M, Zivanovic L and Licanski A. A chemometrical approach to optimization and validation of an HPLC assay for rizatriptan and its impurities in tablets. Analytical Letters. 2007;40(12): 2301-2316.
11. Sagar PV, Kumar D, Suddhasattya D and Samal HB. Simultaneous estimation of rizatriptan, sumatriptan and zolmitriptan by RP-HPLC method in bulk. Journal of Pharmacy Research. 2010;3(12):2930-2933.
12. Altinoz S, Ucar G and Yıldız E. Determination of rizatriptan in its tablet dosage forms by UV spectrophotometric and spectrofluorimetric methods. Analytical Letters. 2002;35(15):2471-2485.
13. Mahuzier PE, Clark BJ, Crumpton AJ and Kevin DA. Quantitative microemulsion electrokinetic capillary chromatography analysis of formulated drug products. Journal of Separation Sciences. 2001; 24(9):784-788.
14. Amol K, Vivek R, Alpana K, Hassan DM, Maria S and Swaroop L. Spectrophotometric method for analysis of rizatriptan benzoate. International Journal of Pharmaceutical Sciences. 2009;1(2):307-309.
15. Kumari AS, Subhasish S, Kaushik DK and Annapurna MM. UV-spectroscopic methods for estimation of rizatriptan benzoate in pharmaceutical preparations. International Journal of ChemTech Research. 2010;2(1): 653-659.
16. Vivek R, Amol K, Alpana K, Hassan DM, Zaria S and Swaroop L. Spectrophotometric estimation of rizatriptan benzoate. Asian Journal of Research in Chemistry. 2010;3(1):175-177.
17. Devprakash, Senthilkumar GP, Prithviraj SY and Mani TT. Determination of rizatriptan in bulk and its tablet dosage forms by uv spectroscopic method. International Journal of Pharmaceutical Sciences and Research. 2011;2(8):2041-2044.
18. Shanmukha kumar JV, Ramachandran D, Settaluri VS, Lakshmi LA and Shechinah FC. Validation of analytical procedures for determination of rizatriptan

benzoate. The Pharma Research. 2010;4(1):28-37.
19. Gowry SD and Vamsi KM. Visible spectrophotometric methods for the determination of rizatriptan in pure form and in pharmaceutical formulations. Analytical Chemistry. An Indian journal. 2007; 3(4-6):4-6.
20. Shanmukha kumar JV, Prasad KRS, Ramachandran D and Settaluri VS. Development and validation of spectrophotometric methods for determination of maxalt (rizatriptan benzoate) in pure and pharmaceutical formulation. Journal of Analytical Chemistry. 2011;1 (5):1-5.
21. Ramzia IE, Nashwah GM and Heba A. Fluorimetric and colorimetric methods for the determination of some antimigraine drugs. Journal of Chemical and Pharmaceutical Research. 2011;3(4):304-314.
22. Joseph BL and Wallace RB. Condensations of secondary amines with aldehydes and naphthols. Journal of American Chemical Society. 1930;52(4):1655–1659.
23. Youngman MA and Dax SL. Solid-phase Mannich condensation of amines, aldehydes, and alkynes: investigation of diverse aldehyde inputs. Journal Combinatorial Chemistry. 2001;3(5):469-472.
24. Manikya Shastry T and Ramakrishna K. New spectrophotometric methods for the estimation of lercanidipine hydrochloride. Indian Journal of Chemical Technology. 2009;16:431-436.
25. Siddappa K, Mahesh T, Mallikarjun M and Mallikarjun K. Spectrophotometric methods for the determination of sulfamethaxazole by a Schiff's Base reactions in pure and pharmaceutical dosage forms. Journal of Pharmacy Research. 2011;4(2): 308-311.
26. Enoche FO. Spectrophotometric determination of isoniazid in pure and pharmaceutical formulations using vanillin. International Journal of Pharmacy and Pharmaceutical Sciences. 2010;2(suppl 1) 55-58.