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VALIDATION OF ANALYTICAL PROCEDURES FOR DETERMINATION OF RIZATRIPTAN BENZOATE

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ABSTRACT

Two accurate, easy spectrophotometric methods for the determination of Rizatriptan benzoate were described. The first method was based on formation of ion-pair complexes with the acidic Methyl orange dye (method MO). The formed complex is extracted into trichloromethane, and absorbance was measured at 420nm. The second method was based on the reaction of the drug (RTB) in the presence of the ligand 2, 2'-Bipyridyl to form a highly stable orange colored chromogen having λ_{\max} at 490 nm (method BPL). Under the optimum reaction conditions, Beer's law was obeyed with a good correlation coefficient ($r = 0.9999$) for method MO and 0.9999 for method BPL in the concentration ranges of 10–50 and 4–20 $\mu\text{g/mL}$ for MO and BPL methods respectively. The parameters molar absorptivity, precision, accuracy and recovery were studied. The proposed methods were successfully applied for determination of the drug in tablets with good accuracy and precision. Statistical comparison of the results with those obtained by a reported method showed good agreement and indicated no significant difference in accuracy and precision.

Keywords: Rizatriptan Benzoate (RTB), Spectroscopy, Molar absorptivity, Beer's Law, Analysis validation

1. Introduction:

Rizatriptan Benzoate¹ is n, n-dimethyl-5-(1h-1,2,4-triazol-1-ylmethyl)-1h-indole-3-ethanamine, benzoate; which is used in the treatment of migraine headaches. Rizatriptan

Benzoate is a selective 5-hydroxytryptamine receptor subtype agonist indicated for the acute treatment of migraine attacks² with or without aura in adults. Rizatriptan is not intended for the prophylactic therapy of migraine or for use in

the management of hemiplegic or basilar migraine. Its chemical structure¹¹⁻¹³ is shown in Figure 1.

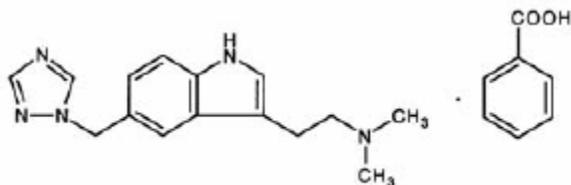


Fig: 1. structure of Rizatriptan Benzoate

Among the various methods that have been reported for the determination of RTB, include, high-performance column liquid chromatography RP-HPLC^{7,8} for its determination in plasma and pharmaceutical formulations, LC-MS [Sol] MS^{3,5}, Detection of impurities⁴, LC-MS/MS^{6,10, 14,15}, RP-LC-DAD⁹, Spectrophotometry^{16, 17}. The review revealed that only a few spectrophotometric methods are proposed for the determination of Rizatriptan Benzoate. This prompted us to develop accurate and inexpensive spectrophotometric methods that can be considered for routine determination of RTB in bulk and tablets. The present study describes two methods that are based on reaction of Rizatriptan benzoate with MO and BPL, that

are sensitive, and cost effective for the assay of the selected drug.

EXPERIMENTAL

Instrumentation:

After due calibration of the instrument, spectral and absorbance measurements are made using Genesys 10 UV Spectrophotometer procured from Thermo Scientific company marketed by Merck. All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. Reagents were prepared a fresh for every method.

Reagents:

Method – MO:

Aqueous solution of methyl orange dye solution (0.1%w/v in water) was prepared for analysis.

Method – BPL:

Aqueous solutions of reagents such as 2, 2'-BPL (0.01 M), Ferric chloride (0.003 M) and ortho phosphoric acid (0.2 M) were prepared for analysis.

Standard and Sample solution of Rizatriptan Benzoate:

About 100 mg of Rizatriptan Benzoate (Bulk sample) was accurately weighed on a digital single pan balance and dissolved in 100 ml of

water in a volumetric flask to prepare a solution that has a concentration equal to 1 mg/ml standard solution and further dilutions are made with the same solvent (100 µg/ml) for **Methods MO and BPL.**

Recommended Procedure for the determination of RTB:

Method MO: Aliquots of standard RTB (1ml=100 µg) solution ranging from 1.0-5.0 ml were transferred into a series of 250 ml separating funnels. To that 2 ml of MO (0.1%) was added and the total volume of the aqueous phase was made up to 10 ml with distilled water. About 10 ml of chloroform was added to each funnel and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the chloroform layer was measured at 420nm against the corresponding reagent blank. The amount of RTB present in the sample solution was computed from its calibration curve.

Method BPL:

Aliquots (0.4-2.0 ml, 100 µg/ml) of standard RTB were transferred into a series of 10 ml calibrated tubes and then solutions of FeCl₃ (1.0

ml) and 2, 2 Bipyridyl (1.0 ml) was added successively. The total volume in each test tube was brought up to 4.0 ml with distilled water and heated for 10 minutes in a boiling water bath at 90°C. After cooling to the room temperature, 2.0 ml of o-phosphoric acid was added in each test tube. The absorbance of the orange colored complex was measured after 5 minutes at 490 nm against reagent blank prepared similarly. In all the above methods, a calibration curve was prepared by plotting absorbance against concentration and the unknown was read from the calibration curve, or deduced using a regression equation, calculated from Beers law data.

RESULTS AND DISCUSSION

The results obtained in **method MO** were based on extractive spectrophotometry, and the final color developed is due to ion association complex between Methyl orange (MO) and RTB resulting in the formation of a yellow color solution that exhibited maximum absorption at a wavelength of 420 nm against the corresponding reagent blank. The mechanism of reaction is represented in Scheme -1.1

The results obtained in **method BPL** is based on oxidation followed by complex formation that involved the reaction of Rizatriptan benzoate with 2, 2'-bipyridine, ferric chloride and ortho phosphoric acid to form a **orange** colored chromogen that exhibited maximum absorption at 490 nm against the corresponding reagent blank. The mechanism of reaction is represented in Scheme -1. 2

Beer's law was obeyed over the concentration range of 10-50 $\mu\text{g}\cdot\text{mL}^{-1}$ for method MO and 4-20 $\mu\text{g}\cdot\text{mL}^{-1}$ for method BPL. The proposed

procedures are validated by determining various optical parameters, which are listed in Table 1. The linearity, intercepts and the slope have been calculated using regression equation $Y = ax + b$, where Y represents optical density, 'x', the concentration of the drug in $\mu\text{g}\cdot\text{mL}^{-1}$ and 'a' and 'b' represents slope and intercepts respectively. Precision and accuracy of the proposed methods were tested by carrying out the determination of six replicates of pure and dosage samples of the drug, whose concentration lie within beer's law range.

TABLE -1 Analytical parameters for the determination of RTB with MO and BPL

Parameter	Method MO	Method BPL
λ_{max} (nm)	420	490
Beer's law limit ($\mu\text{g}/\text{mL}$) ^a	10-50	4-20
Sandell's Sensitivity ($\mu\text{g}/\text{cm}^2/0.001 \text{ abs. unit}$)	0.03846	0.0391
Molar absorptivity ($\text{litre}\cdot\text{mole}^{-1}\cdot\text{cm}^{-1}$)	1.0178×10^4	1.0006×10^4
Correlation coefficient (r)	0.9999	0.9999
Regression Equation (Y) ^b Slope a	-0.088	-0.1142
Intercept b	0.00244	0.00358
% RSD ^c	0.23	0.60
%Range of errors (95%Confidence limit)		
0.05 level of Significance	± 0.1923	± 0.5016
0.01 level of Significance	± 0.2845	± 0.7422

^a Data obtained from 4 determinations ($n = 4$).

^b $Y = aX + b$, where Y is the absorbance of a 1 cm layer of solution, a is the slope, b is the intercept, and X is the concentration of the drug in $\mu\text{g}/\text{mL}$.

^c For six replicates

The values of standard deviation (% R.S.D) and percent range of error (0.05 level and 0.01 level confidence limits) were calculated for the above two methods are presented in Table 1. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparation and the mixtures were analyzed by the proposed methods.

Accuracy and Precision

The interday repeatability of the proposed methods was studied by performing 6

independent analyses of RTB in pure form at 3 different concentration levels on 5 consecutive days (Table 2). The intraday reproducibility of the proposed method was determined by measuring the drug at 3 concentration levels within one day 6 times (Table 2). The reagent solutions were prepared freshly and analyzed as described under General Procedures and Calibration Graphs. The analytical results obtained from these investigations revealed that standard deviation (SD), relative standard deviation (RSD), and recoveries were very satisfactory (Table 2).

Table 2. Interday and intraday assays of RTB by the proposed methods

Concentration, $\mu\text{g/mL}$				
Method	Taken	Found \pm SD ^a	Recovery, %	RSD, %
MO				
Interday	10	9.98 \pm 0.04	99.33	1.06
	20	19.85 \pm 0.08	99.06	0.55
	30	30.08 \pm 0.16	100.44	0.86
Intraday	10	10.99 \pm 0.06	99.67	1.47
	20	19.85 \pm 0.09	99.06	0.55
	30	30.09 \pm 0.16	100.50	0.86
BPL				
Interday	4	3.85 \pm 0.13	99.00	1.32
	8	7.32 \pm 0.12	98.76	0.24
	12	12.76 \pm 0.52	100.89	0.64
Intraday	4	3.79 \pm 0.14	98.60	1.40
	8	8.21 \pm 0.23	100.38	0.46
	12	11.59 \pm 0.46	99.52	0.58

^a n = 6.

Recovery

To study the accuracy of the proposed methods, the standard addition method was applied. For

this, known amounts of pure drug were added to a known amount of tablet solution, and the mixtures were analyzed by the proposed procedures in the 6 replicates. From the amount of drug found, the recovery was calculated from:

$$\text{Recovery, \%} = [(C_t - C_u)/C_a] \times 100$$

Where C_t = total concentration of the analyte found, C_u = concentration of the analyte present in the formulation, and C_a = concentration of the pure analyte added to the formulation. The results were reproducible with low SD and RSD values and accurate with high average recovery values, as shown in Table 3.

Table 3. Recovery of RTB in tablets by standard addition analyses

Concn, $\mu\text{g/mL}$					
Method	Given	Added	Found \pm SD ^a	Recovery, %	RSD, %
MO	10	10	19.89 \pm 0.08	99.26	0.55
	20	10	29.96 \pm 0.04	99.78	0.25
BPL	5	5	10.07 \pm 0.17	100.04	0.28
	8	4	11.94 \pm 0.10	99.54	0.80

^a n = 6

Percent recovery

The percent recoveries are given in Table 4. For method BPL, the reaction of colored species formation was slow at room temperature 25 °c and requires longer time for completion. Hence, efforts were made to accelerate by carrying out the reaction at higher temperatures. It was observed that the maximum color intensity was obtained by

heating the reaction mixture at 90 °c on a boiling water bath for 10 minutes. For method MO, room temperature 25 °c is considered suitable for the development of colored chromogens. The absorbencies remained constant at room temperature for more than 10 and 6 hours for method BPL and MO respectively.

TABLE-4 Percent Recovery of Rizatriptan Benzoate (RTB) in Pharmaceutical formulations

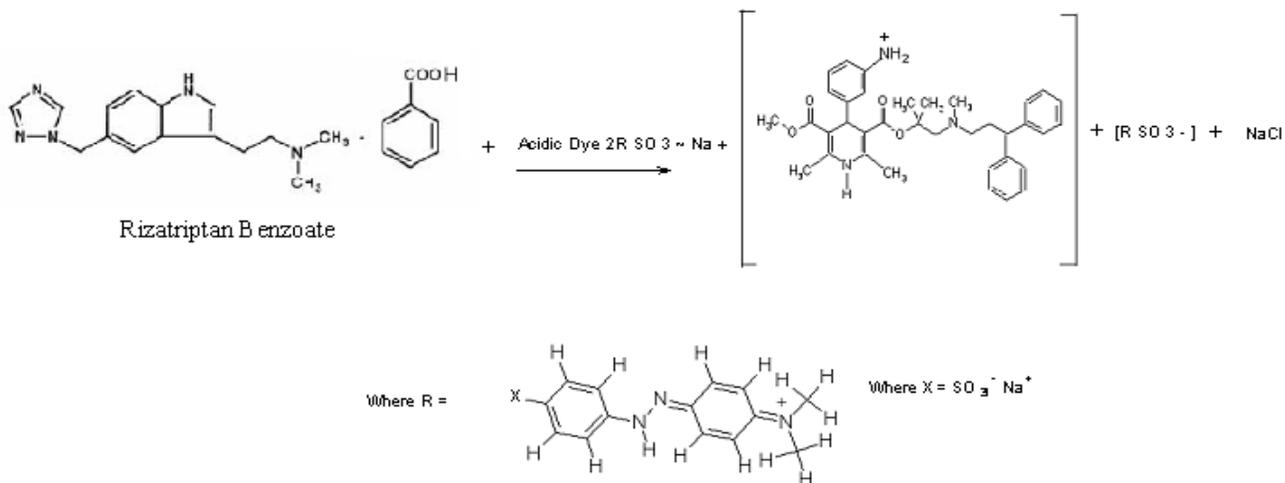
Formulations	Labeled amount Bulk /Tablet	% Recovery by proposed methods	
		Method A	Method B
Tablet 1	10 mg	99.4	98.38
Tablet 2	10 mg	99.7	98.85
Tablet 3	10 mg	99.8	99.15
Tablet 4	10 mg	100.2	100.1

Interferences

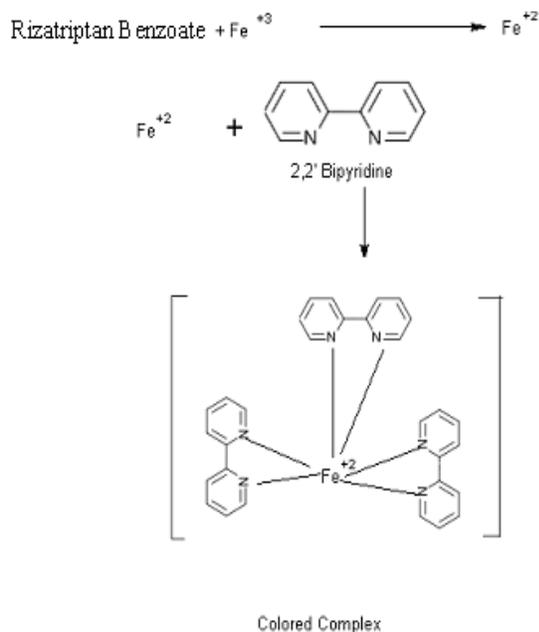
Each RTB tablet contained the following inactive ingredients, which are common in pharmaceutical formulations: lactose monohydrate, magnesium stearate,

microcrystalline cellulose, and Pregelatinized starch. The effect of these excipients was examined using the proposed methods, and it was found that the excipients did not interfere with the determination.

Scheme-1. 1 Reaction of MO with Rizatriptan Benzoate



Scheme-1. 2 Reaction of BPL with Rizatriptan Benzoate



CONCLUSIONS

It is evident from the data given above that the proposed methods are selective, stable for about 6–24 h, rapid, and economical, and have good precision and accuracy compared with other reported methods. They also have the advantage of a wide linear range. Because the absorbance arises from the colored products of the drug in the visible absorption region, the detection of the drug is more specific and accurate than in the UV absorption region. Moreover, the higher λ_{max} values of the proposed methods have a decisive advantage because interference caused

by the excipients expected to be present in tablets is generally far less at higher wavelengths than at UV wavelengths. The proposed methods are also rapid with respect to analysis time as compared to sophisticated techniques that may not be available in most analytical laboratories, such as HPLC, HPLC/MS, and HPLC/tandem MS. These kinds of complicated techniques may be more useful in determination of the drug in clinical samples and biological fluids, in which the drug concentration level will be low, rather than for assay of pharmaceutical formulations having a high drug concentration.

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