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Development and validation of a specific stability indicating high performance liquid chromatographic method for rizatriptan benzoate

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Abstract

A gradient, reversed-phase liquid chromatographic (RP-LC) method was developed for the quantitative determination of rizatriptan benzoate, used to treat relieves migraine headache symptoms. The developed method can be also employed for the related substance determination in bulk samples. Forced degradation studies were performed on bulk sample of rizatriptan benzoate using acid (0.5N hydrochloric acid), base (0.1N sodium hydroxide), oxidation (3.0% hydrogen peroxide), water hydrolysis, heat (60 °C) and photolytic degradation. Mild degradation of the drug substance was observed in base hydrolysis and considerable degradation observed during oxidative stress. The chromatographic method was fine tuned using the samples generated from forced degradation studies. Good resolution between the peaks corresponds to degradation products and the analyte was achieved on Agilent Zorbax SB-CN (250 mm × 4.6 mm, 5 μm) column. The mobile phase consists of a mixture of aqueous potassium di hydrogen ortho phosphate (pH 3.4), acetonitrile and methanol. The stress sample solutions were assayed against the qualified reference standard of rizatriptan benzoate and the mass balance in each case was close to 99.7% indicating that the developed method is stability indicating. Validation of the developed method was carried out as per ICH requirements.

Keywords: RP-LC; Forced degradation; R.S.D. and validation

1. Introduction

Rizatriptan benzoate is described chemically as: *N*,*N*-dimethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indole-3-ethanamine mono benzoate (Fig. 1). It is used in the treatment of migraines. Its empirical formula is $C_{15}H_{19}N_5 \cdot C_7H_6O_2$ and its molecular weight is 391.47.

Rizatriptan benzoate belongs to a group of medicines known as serotonin (or 5HT) agonists. It is used in the treatment of migraines. Rizatriptan binds to serotonin receptors in the brain, which causes the blood vessels to narrow. By decreasing the width of blood vessels in the brain rizatriptan relieves the pain. Rizatriptan is available as orally disintegrating tablets with a brand name Maxalt.

A liquid chromatography–atmospheric pressure chemical ionization mass spectrometry method for the quantitative determination of rizatriptan and sumatriptan from plasma samples was reported in the literature [1]. A liquid chromatographic method for the determination of rizatriptan from human plasma using fluorescence detection has been developed and reported in the literature [2]. Two more liquid chromatographic methods were reported in literature for the determination of rizatriptan from human plasma and serum [3,4]. As on date no stability indicating liquid chromatographic method was reported in the literature.

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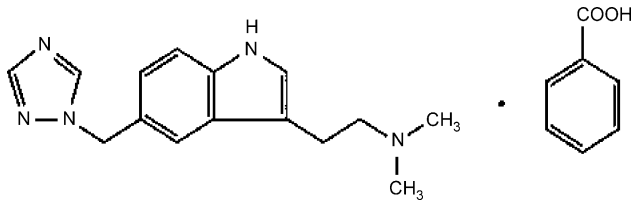


Fig. 1. Chemical structure of Rizatriptan benzoate.

Attempts were made to develop a suitable single stability indicating LC method that can be used to determine the related substances and also the assay of bulk samples of rizatriptan benzoate. This paper deals with the development of stability indicating analytical method using the samples generated from forced degradation studies. The developed method was validated to ensure the compliance in accordance with ICH guidelines.

2. Experimental

2.1. Chemicals

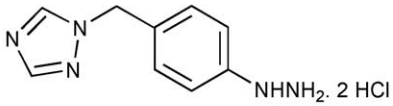
Samples of rizatriptan benzoate and its three impurities (Fig. 2) were received from Process Research Department of Custom Pharmaceutical Services of Dr. Reddy's Laboratories Limited, Hyderabad, India. HPLC grade acetonitrile and methanol were purchased from Rankem, India. Analytical reagent grade potassium di hydrogen ortho phosphate were purchased from S.D. Fine chem. limited, India and ortho phosphoric acid were purchased from Qualigens Fine chemicals, India. High pure water was prepared by using Millipore Milli Q plus purification system.

2.2. Equipment

The LC system, used for method development, forced degradation studies and method validation was Agilent 1100 series

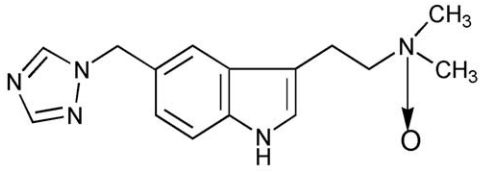
Imp-1

1-(4-Hydrazinophenyl) methyl-1, 2,4-triazole. dihydrochloride



Imp-2

N, N-Dimethyl -5-(1H-1,2,4-triazol-1-yl - methyl)-1H-indole-3-ethanamine.N-oxide



Imp-3

N1, N1-dimethyl-2-(2-(3-(2-dimethyl aminoethyl)-1H-4-indolyl methyl)-5-(1H-1,2,4-triazol-1-yl methyl)-1H-3-indolyl)-1-ethanamine

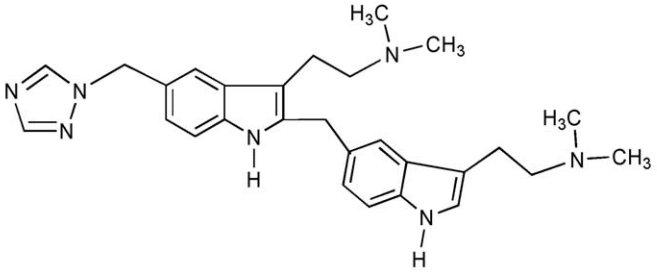


Fig. 2. Chemical structures of Imp-1, Imp-2 and Imp-3.

(manufactured by Agilent Technologies, Waldbronn, Germany) LC system with a diode array detector. The out put signal was monitored and processed using Chemstation software (designed by Agilent Technologies, Waldbronn, Germany) on Pentium computer (Digital Equipment Co).

2.3. Chromatographic conditions

The chromatographic column used was an Agilent Zorbax SB-CN 250 mm × 4.6 mm column with 5 μm particles. The mobile phase consists of a mixture of 10 mM potassium di hydrogen ortho phosphate, pH adjusted to 3.4 using diluted orthophosphoric acid (solvent A), acetonitrile (solvent B) and methanol (solvent C). The flow rate of the mobile phase was kept at 1.0 ml/min. The HPLC gradient was set as: T/%B: 0/8, 10/9, 16/9, 19/15, 23/32, 28/50, 34/50 and 35/8 and solvent C was kept as T/%C: 0/0, 10/3, 16/3, 19/3, 23/3, 28/3, 34/3 and 35/0 with a post-run time of 5 min. The column temperature was maintained at 25 °C and the wavelength was monitored at a wavelength of 225 nm. The injection volume was 10 μl for related substances determination and 5 μl for assay determination. Solvent A was used as diluent during the standard and test samples preparation.

2.4. Preparation of standard solutions

A working solution of 500 μg/ml was prepared for related substances determination and assay determination analysis. A stock solution of Impurity (mixture of Imp-1, Imp-2 and Imp-3) at 500 μg/ml was also prepared in diluent.

2.5. Method validation

2.5.1. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products [5]. The specificity of the developed LC method for rizatriptan benzoate was carried out in the presence of its impurities namely Imp-1, Imp-2 and Imp-3.

Forced degradation studies were also performed for bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of photolytic degradation (as per ICH recommended condition), thermal degradation (drug substance exposed at 60 °C), acid hydrolysis (using 0.5N HCl), base hydrolysis (using 0.1N NaOH), water hydrolysis and oxidative degradation (using 3.0% H_2O_2) to evaluate the ability of the proposed method to separate rizatriptan from its degradation products. For heat and light studies, study period was 10 days where as for acid, base, water hydrolysis and oxidative degradation it was 48 h. To check and ensure the homogeneity and purity of rizatriptan peak in the stressed sample solutions, diode array detector was employed. Assessment of mass balance in the degraded samples checked to see whether the amount of impurities detected in a stressed sample matches the amount present before the stress was applied. Assay studies were carried out on the stressed samples against rizatriptan benzoate qualified reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degradants) was tabulated. Assay was also calculated for bulk sample by spiking all three impurities (Imp-1, Imp-2 and Imp-3) at the specification level (i.e. at 0.15%).

2.5.2. Precision

Assay method precision was evaluated by carrying out six independent assays of test sample of rizatriptan benzoate against qualified reference standard and calculated the % R.S.D.

The precision of the related substance method was checked by injecting six individual preparations of (0.5 mg/ml) rizatriptan benzoate spiked with 0.15% of Imp-1, Imp-2 and Imp-3 with respect to analyte concentration. % R.S.D. of area for each Imp-1, Imp-2 and Imp-3 was calculated.

The intermediate precision of the method was also evaluated using different analyst, different day and different make instrument in the same laboratory.

2.5.3. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for Imp-1, Imp-2 and Imp-3 were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively by injecting a series of diluted solutions with known concentration [6]. Precision study was also carried at the LOQ level by injecting six individual preparations of Imp-1, Imp-2 and Imp-3 and calculating the % R.S.D. of the area.

2.5.4. Linearity

Linearity test solutions for assay method were prepared from stock solution at six concentration levels from 25% to 150% of assay analyte concentration (125, 250, 375, 500, 625 and 750 μg/ml). The peak area versus concentration data was performed by least-squares linear regression analysis.

Linearity test solutions for related substance method were prepared by diluting the Impurity stock solution (2.4) to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% with respect to the impurities specification level of 0.15% (i.e. LOQ, 0.075%, 0.15%, 0.1875%, 0.225% and 0.3%). The calibration curve was drawn by plotting the peak areas of Imp-1, Imp-2 and Imp-3 versus its corresponding concentration.

Linearity test was performed for two consecutive days in the same concentration range for both assay and related substance method. The % R.S.D. value of the slope and *Y*-intercept of the calibration curve was calculated.

2.5.5. Accuracy

The accuracy of the assay method was evaluated in triplicate at three-concentration levels, i.e. 250, 500 and 750 μg/ml in bulk drug sample. The % recoveries were calculated from the slope and *Y*-intercept of the calibration curve obtained in Section 2.5.4.

The bulk sample, provided by Process Research Department of Custom Pharmaceutical Services, does not show the presence of Imp-1 and Imp-3. Some of the bulk samples received from process research department of Dr. Reddy's Laboratories show the presence of Imp-2 in between 0.03% and 0.05% lev-

els. Standard addition and recovery experiments were conducted to determine accuracy of the related substance method for the quantification of all three impurities in bulk drug samples.

The study was carried out in triplicate at 0.075%, 0.15% and 0.225% of the analyte concentration (500 μg/ml). The % recoveries for Imp-1, Imp-2 and Imp-3 were calculated from the slope and *Y*-intercept of the calibration curve obtained in Section 2.5.4.

2.5.6. Robustness

To determine the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between rizatriptan, Imp-1, Imp-2 and Imp-3 was evaluated.

The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, the same was altered by 0.2 units, i.e. from 0.8 to 1.2 ml/min. The effect of pH on resolution of impurities was studied by varying ±0.1 pH units (at 3.30 and 3.50 buffer pH). The effect of column temperature on resolution was studied at 20 and 30 °C instead of 25 °C. All the other mobile phase components were held constant as stated in Section 2.3.

3. Results and discussion

3.1. Optimization of chromatographic conditions

Imp-2 was the potential impurity present in bulk samples produced by Dr. Reddy's laboratories. The main target of the chromatographic method is to get the separation of benzoic acid, Imp-2 and degradants generated from analyte peak. Impurities were co-eluted by using different stationary phases like C18, C8 and Phenyl and different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (7–10) and using organic modifiers like acetonitrile, methanol and ethanol in the mobile phase. Apart from the co-elution of impurities, we have also observed poor peak shapes for some impurities and degradants. pH of the buffer found to be critical in achieving the separation between rizatriptan and benzoic acid peak. At lower pH (<3.0) the co-elution of the above peaks were observed. Satisfactory chromatographic separation was achieved using a solvent A is 10 mM potassium di hydrogen ortho phosphate (pH 3.40 adjusted with diluted orthophosphoric acid), solvent B is acetonitrile and solvent C is methanol. The HPLC gradient of solvent B was kept as T/%B: 0/8, 10/9, 16/9, 19/15, 23/32, 28/50, 34/50 and 35/8, solvent C was kept as T/%C: 0/0, 10/3, 16/3, 19/3, 23/3, 28/3, 34/3 and 35/0 with a post-run time of 5 min. In the optimized conditions the rizatriptan, Imp-1, Imp-2 and Imp-3 were well separated with a resolution of greater than 4 and the typical retention times of Imp-1, Imp-2, Imp-3 and rizatriptan were about 4.0, 19.3, 23.3 and 12.7 min, respectively (benzoic acid retention time at about 14.8 min) (Fig. 3). The system suitability results were given in Table 1 and the developed LC method was found to be specific for rizatriptan and its three impurities, namely Imp-1, Imp-2 and Imp-3.

Table 1
System suitability report

Compound ($n = 3$)	USP resolution	USP tailing factor (R_S)	Number of theoretical plates (N) USP tangent method
Rizatriptan	–	1.3	15984
Benzoic acid	5.4	1.1	21138
Imp-2	8.3	1.6	12124

n: number of determinations.

3.2. Results of forced degradation studies

No considerable degradation observed in rizatriptan benzoate bulk samples, under stress conditions such as photolytic stress, thermal stress, acid, base and water hydrolysis. To achieve some level of degradation the test solutions in acid, base, 3% hydrogen peroxide and water were heated for 2 h at 60 °C.

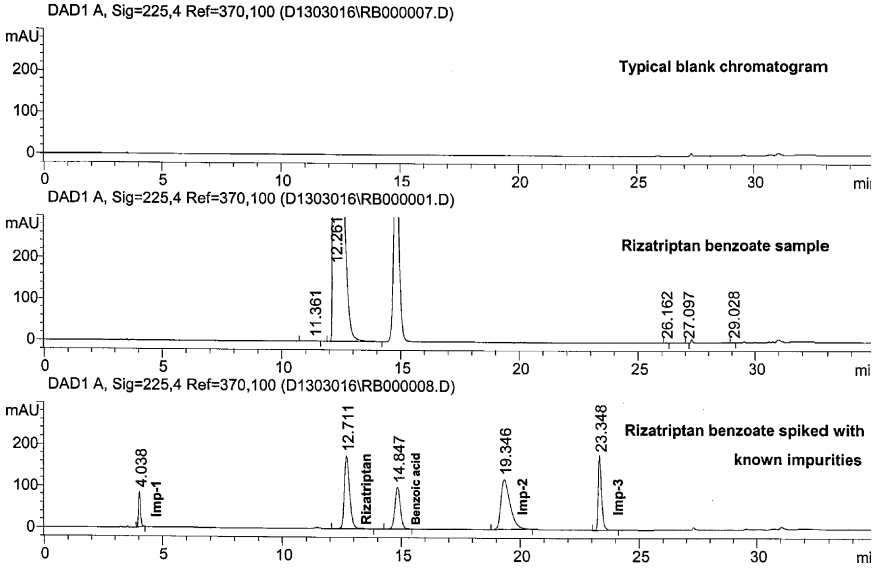


Fig. 3. HPLC chromatograms of blank unspiked and spiked (Imp-1, Imp-2 and Imp-3 were spiked in pure rizatriptan) samples.

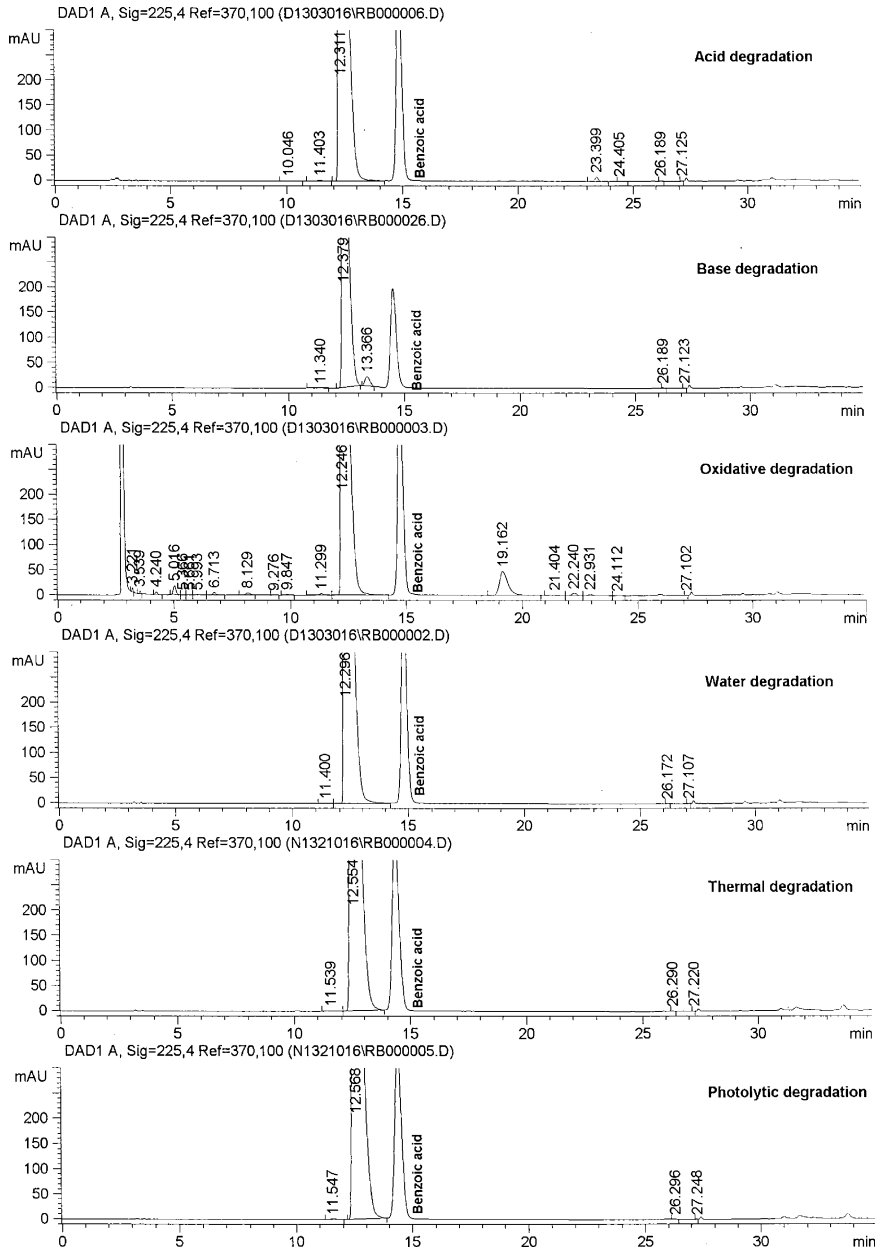


Fig. 4. Typical HPLC chromatograms of stressed test samples of rizatriptan benzoate.

Under these conditions the degradation of drug substance was observed during oxidative stress and base hydrolysis (Fig. 4). Rizatriptan benzoate was degraded into Imp-2 under oxidation conditions (treated with 3% hydrogen peroxide at 60 °C for 2 h) and it was confirmed by co-injection with a qualified Imp-2 standard. Mild degradation of the drug substance was observed under base hydrolysis conditions (treated with 0.1N NaOH at 60 °C for 2 h) leads to the formation some un known degradation peaks. Peak purity test results obtained from PDA confirm that the rizatriptan peak is homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 99.7% (Table 2). The assay of rizatriptan is unaffected in the presence of Imp-1, Imp-2 and Imp-3, which confirms the stability indicating power of the developed method.

3.3. Results of method validation experiments

3.3.1. Precision

The % R.S.D. of assay of rizatriptan during assay method precision study was well within 0.8% and the % R.S.D. of area of Imp-1, Imp-2 and Imp-3 in related substance method precision study was within 10%. The % R.S.D. of assay results obtained in intermediate precision study was within 1.0% and the % R.S.D. of area of Imp-1, Imp-2 and Imp-3 were within 9%, confirming good precision of the method.

3.3.2. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) of Imp-1, Imp-2 and Imp-3 were 0.04%, 0.03% and 0.02% (of analyte concentration, i.e.

Table 2
Summary of forced degradation results

Stress condition	Time	% Assay of active substance	Mass balance (% assay + % sum of impurities + % sum of all degradants)	Remarks
Acid hydrolysis (0.5N HCl at 60 °C)	48 h at RT and 2 h at 60 °C	99.6	99.8	No degradation products formed
Base hydrolysis (0.1N NaOH at 60 °C)	48 h at RT and 2 h at 60 °C	97.5	99.6	Unknown degradation products formed
Oxidation (3% H_2O_2 at 60 °C)	48 h at RT and 2 h at 60 °C	92.8	99.5	Degraded into Imp-2 and other unknown degradants formed
Water hydrolysis at 60 °C	48 h at RT and 2 h at 60 °C	99.8	99.8	No degradation products formed
Photolytic degradation	10 days	99.8	99.8	No degradation products formed
Thermal degradation	10 days	99.7	99.7	No degradation products formed

Table 3
Recovery results of in bulk drug sample

Added (μg) ($n = 3$)	Recovered (μg)	% Recovery	% R.S.D.
252	254	100.8	0.9
501	501.5	100.1	0.7
751	746.1	99.3	0.8

n: number of determinations.

500 μg/ml) for 10 μl injection volume. The limit of quantification (LOQ) of Imp-1, Imp-2 and Imp-3 were 0.13%, 0.09% and 0.06% (of analyte concentration, i.e. 500 μg/ml) for 10 μl injection volume. The method precision for Imp-1, Imp-2 and Imp-3 at LOQ level was below 10% R.S.D.

3.3.3. Linearity

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 125–750 μg/ml and the correlation coefficient obtained was greater than 0.999. Linearity was checked for assay method over the same concentration range for three consecutive days. The % R.S.D. values of the slope and *Y*-intercept of the calibration curves were 2.3 and 6, respectively. The results show that an excellent correlation existed between the peak area and concentration of the analyte.

Linear calibration plot for related substance method was obtained over the calibration ranges tested, i.e. LOQ to 0.3% for Imp-1, Imp-2 and Imp-3. The correlation coefficient obtained was greater than 0.991. Linearity was checked for related substance method over the same concentration range for three consecutive days. The % R.S.D. values of the slope and *Y*-intercept of the calibration curves were 4.2 and 11, respectively. The results show that an excellent correlation existed between the peak area and concentration of Imp-1, Imp-2 and Imp-3.

3.3.4. Accuracy

The percentage recovery of rizatriptan in bulk drug samples was ranged from 99.3 to 100.8 (Table 3). The percentage recovery of Imp-1, Imp-2 and Imp-3 in bulk drugs samples was ranged from 90.5 to 107.3. HPLC chromatograms of blank, pure sample and all three impurities spiked in rizatriptan bulk drug sample were shown in Fig. 3.

3.3.5. Robustness

In all the deliberate varied chromatographic conditions (flow rate, pH and column temperature) the resolution between rizatriptan, Imp-1, Imp-2 and Imp-3 was greater than 4.0, illustrating the robustness of the method.

4. Conclusions

In this paper, the simple, accurate and well-defined stability indicating HPLC method for the determination of rizatriptan benzoate in the presence of degradation products was described for the first time. The behaviour of rizatriptan benzoate under various stress conditions were studied and presented. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies.

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