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Stability indicating reversed-phase high-performance liquid chromatographic method for the determination of rizatriptan benzoate in bulk powder and in pharmaceutical formulations

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ABSTRACT

Our objective was to study the degradation behavior of rizatriptan benzoate under different ICH recommended stress conditions by LC-UV, and to establish a validated stability indicating LC assay method. Rizatriptan benzoate was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal decomposition. Extensive degradation was found to occur in alkaline medium. Mild degradation was observed in acidic and oxidative conditions. Rizatriptan benzoate was stable to photolytic and thermal stress conditions. Successful separation of drug from degradation products formed under stress conditions was achieved on a Perfectsil ($C_{18,}$ 250 mm × 4.6 mm, 5.0 µ) and 0.01 M Sodium dihydrogen Phosphate buffer : methanol (80:20 v/v) as the mobile phase at a flow rate of 1.0 mL /min at ambient temperature and detected at 225 nm. pH of buffer is adjusted 3.5 with 85 % of otho phosphoric acid. The method was validated according to ICH guidelines. The retention time (R_T) of rizatriptan benzoate was found to be 5.882 ± 0.01 and. The linearity of rizatriptan benzoate was in the range of 1000-7000 µg/L with mean percentage recovery of 100.77 ± 1.214 the limit of detection and limit of quantification were found to be 20 µg/L and 70 µg/L respectively.

Keywords: rizatriptan benzoate; RP-HPLC, Validation, purity evaluation; degradation products.

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INTRODUCTION

Rizatriptan benzoate is chemically described as: N, N-dimethyl-5-(1H-1, 2, 4-triazol-1-ylmethyl)-1H-indole-3-ethanamine mono benzoate. It is a selective 5- hydroxyl triptamine -1B/1D receptor agonist [1] to relive migraine headaches. Its empirical formula is $C_{15}H_{19}N_5.C_7H_6O_2$ and its molecular weight is 391.47. Current theories on the etiology of headache suggest that symptoms are due to local cranial vasodilatation and/or to the release of vasoactive and pro-inflammatory peptides from sensory nerve ending in an activated trigeminal system [2-4].

The literature reveals that various methods for the determination of rizatriptan benzoate and pharmaceutical validations among these methods are LC-MS and LC-MS/MS [5-7], HPLC method for rizatriptan benzoate [8], Application and Development of Improved RP-LC-DAD for Rizatriptan and its degradation products [9], a method based on LC/MS/MS [10] using the UV detector were reported.

EXPERIMENTAL

Material and methods

Rizatriptan benzoate (percent purity was 99.8% on dried basis) working standards were produced as a gift sample from Cipla pharmaceutical Ltd., Kurkumbh, Pune, India. HPLC-grade acetonitrile, methanol and all other analytical grade chemicals were purchased from Merck (Mumbai, India). High-purity water was prepared using a Millipore 0.45 µm, white nylon HNWP 47 mm filter. A commercially available combined tablet formulation of rizatriptan benzoate was procured from a local market.

Instrumentation

The HPLC system consisted of a pump (model Jasco PU 1580, intelligent HPLC pump) with auto injecting facility (AS-1555 sampler) programmed at 20µl capacity. The detector used was ultra-violate (UV) and operated at a wavelength of 225 nm [Figure 1]. The software used was Jasco Borwin version 1.5. The column used was Perfectsil C_{18} (250 mm × 4.6 mm, 5 µm particle size).

HPLC analysis in pharmaceutical formulation

Chromatographic condition

A prepacked column Perfectsil ($C_{18,}$ 250 mm × 4.6 mm, 5.0 µ) and 0.01 M Sodium dihydrogen Phosphate buffer: methanol (80:20 v/v) as the mobile phase at a flow rate of 1.0 mL/min at ambient temperature and detected at 225 nm. pH of buffer is adjusted 3.5 with 85 % of otho phosphoric acid. Buffer was filtered through a Millipore filter 0.45 m, white nylon HNWP 47 mm and Mobile phase was degassed before use. The injection volume was 20 µL.

Forced degradation studies

A stock solution containing 100 mg rizatriptan benzoate in 100 mL methanol was prepared. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of proposed method. In all degradation studies the average peak area of rizatriptan benzoate [Figure 2] after application (5000 µg/L) of six replicates was obtained.

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Acid and base induced degradation

Acid decomposition studies were performed by refluxing the solution of drug in 2 M hydrochloric acid at 80°C for 1 h. The studies in alkaline conditions were carried out in 0.2 M sodium hydroxide and the solution was refluxed for 0.5 h at 60°C. The resultant solutions were diluted to obtain 5000 µg/L solutions and 20 µL were injected into the system.

Hydrogen peroxide induced degradation

To study hydrogen peroxide induced degradation, initial studies were performed in 3% hydrogen peroxide at room temperature for 6 h. Subsequently drug was exposed to 30% hydrogen peroxide at room temperature for a period of 24 h, Considerable degradation observed when solution of the drug was refluxed with 3% hydrogen peroxide for 2 h at 60 °C . The resultant solutions were diluted to obtain 5000 µg/L solutions and 20 µL were injected into the system.

Dry heat and wet heat degradation

The standard drug in solid form was placed in oven at 50°C for 30 days to study dry heat degradation and for wet heat degradation drug was kept in humidity chamber at 50°C, 75% relative humidity (RH) for 3 months.

Photochemical degradation

The photochemical stability of the drug was studied by exposing the stock solution (1 mg/mL) as well as solid drug to direct sunlight for 30 days on a wooden plank and kept on terrace. The solution was diluted with methanol to obtain a solution of 5000 µg/L and then 20 µL of the solution was injected into system.

Optimization of stability indicating HPLC method

The LC procedure was optimized with a view to develop stability indicating assay method. Pure drug alongwith its degraded products were injected and run in different solvent systems. Initially methanol and water in different ratios were tried. It was found that when methanol concentration was increased in the mobile phase, the degradation product started to elute in dead volume. Hence concentration of methanol was decreased and there was improvement in resolution. It was found that methanol: 0.01 M sodium dihydrogen phosphate buffer pH 3.5 adjusted with ortho phosphoric acid (20:80) as a mobile phase at flow rate 1.0 mL/min gives acceptable retention time (t_R), theoretical plates and good resolution of drug and degradation products [Figure 3, 4, 5].

Validation of the method

Validation of optimized LC method was done with respect to following parameters.

Linearity

Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in the range of 1000-7000 µg/L in triplicate into the LC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

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Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of three different concentrations (2000, 4000, 6000 µg/L) of the drug in hexaplicate on the same day. Intermediate precision of the method was checked by repeating studies on three different days. Additionally, the developed LC method was checked through separation studies on the mixture of reaction solutions on a different chromatographic system on a different day.

Limit of detection and limit of quantitaiton

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), the signal to noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. The LOD and LOQ were experimentally verified by diluting known concentrations of standard solution of rizatriptan benzoate until the average responses were approximately 3 and 10 times respectively the standard deviation of the responses for six replicate determinations.

Robustness of the method

To evaluate robustness of the LC method, few parameters were deliberately varied. The parameters included variation of flow rate, percentage of methanol in the mobile phase, pH of mobile phase. The resolution of drug in a mixture of stressed samples was studied by performing the analyses on a different chromatographic system.

Specificity

The specificity of the LC method was determined by the complete separation of rizatriptan benzoate in presence of its degradation products along with other parameters like retention time, capacity factor, tailing or asymmetrical factor etc.

Accuracy

Accuracy of the developed method was tested by fortifying a mixture of decomposed reaction solutions with three concentrations of drug corresponding to 80%, 100% and 120% and determining the recovery of added drug. At each level of the amount six determinations were performed.

Analysis of marketed formulation

To determine the content of rizatriptan benzoate in conventional tablets (Brand name: Rizact-5, label claim: 5 mg rizatriptan benzoate per tablet), twenty tablets were weighed, their mean weight determined and they were finely powdered and powder equivalent to 100 mg rizatriptan benzoate was weighed. This was transferred into a 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min and diluted to 100 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and after suitable dilution the sample solution was filtered using 0.45 µ filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution concentrations of 5000 µg/L. A 20 µL volume of each sample solution was injected into LC, six times, under the conditions described above. The peak areas were measured at 225 nm and concentrations in the samples were determined using multilevel calibration developed on the same LC system under the same conditions using linear regression equation.

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RESULTS AND DISCUSSION

Stability indicating property

LC-UV studies of samples obtained on stress testing of rizatriptan benzoate under different conditions using methanol: 0.01 M sodium dihydrogen phosphate buffer pH 3.5 adjusted with ortho-phosphoric acid (20:80) as a mobile phase suggested the following degradation behavior.

Hydrolysis

The rate of degradation in acid was slower as compared to that of alkali. Initially 0.1 M and 1 M hydrochloric acid used at 80°C for 24 h but no degradation was observed hence the strength of acid was increased, 10.50% degradation was observed by heating drug solution with 2 M hydrochloric acid at 80°C for 1 h forming degradation product at retention time 9.932 min in LC-UV [Figure 3].

The drug was found to be highly liable to alkaline condition. Initially 0.5 M sodium hydroxide used at room temperature for 24 h extensive degradation was observed hence the severity of stress condition decreased, 16.96% degradation was observed by refluxing drug solution with 0.2M sodium hydroxide at 60°C for 0.5 h, associated with rise in a major degradation product at retention time 9.925 min [Figure 4] in LC-UV. Complete degradation of the drug was observed in 4 h when refluxed with 0.5M sodium hydroxide at 60°C.

In both acid as well as base hydrolysis, the degradation products formed were same, which was confirmed by similar retention time as well as retardation factor was observed by LC and TLC for major degradation product formed under acid and base hydrolysis.

Oxidation

In 3% hydrogen peroxide for 6 h drug was found to be very stable at room temperature showing no degradation. 18.00% degradation was observed by refluxing drug solution with 3% hydrogen peroxide at 60 °C for 2.0 h. Degradation peaks were found at 6.205 min. and 9.320 min. [Figure 5]

Photochemical degradation

Rizatriptan benzoate was found to be stable to photochemical degradation as negligible (less than 2%) degradation was seen after exposing drug to sunlight for 30 days. [Figure 6]

Dry and wet heat degradation

Drug was also found to be stable to these conditions showing negligible degradation when subjected for dry and wet heat degradation. [Figure 7]

Validation of stability indicating method

The results of validation studies on the stability indicating method developed for rizatriptan benzoate in the current study involving methanol: 0.01 M sodium dihydrogen phosphate buffer pH 3.5 adjusted with orth-ophosphoric acid (20:80) as a mobile phase are given below.

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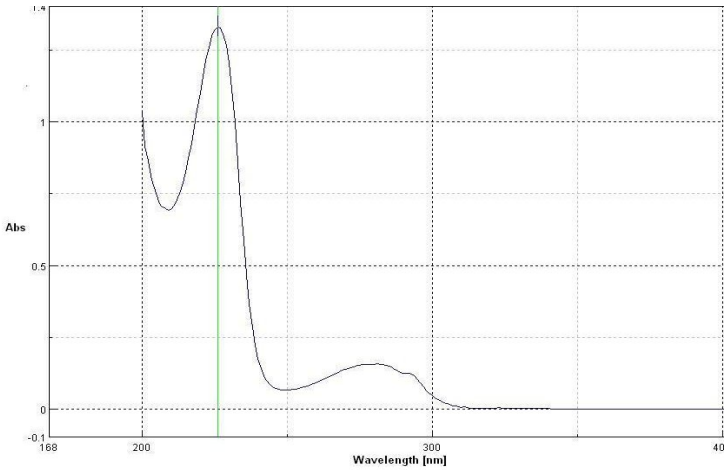

λ_{max} =225 Absorbance =1.3313

Figure 1: UV spectra of Rizatriptan benzoate

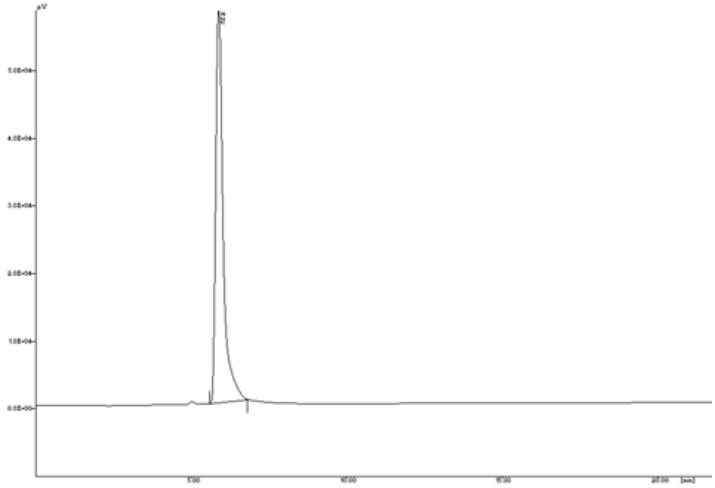

Figure 2: Chromatogram of rizatriptan benzoate [5000 µg/L, Rt 5.882 min].

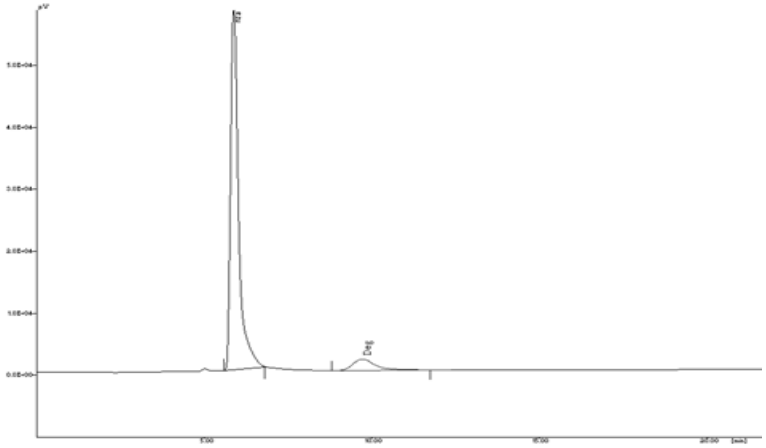

Figure 3: Chromatogram of acid degraded sample showing additional peak at R_t 9.932 min

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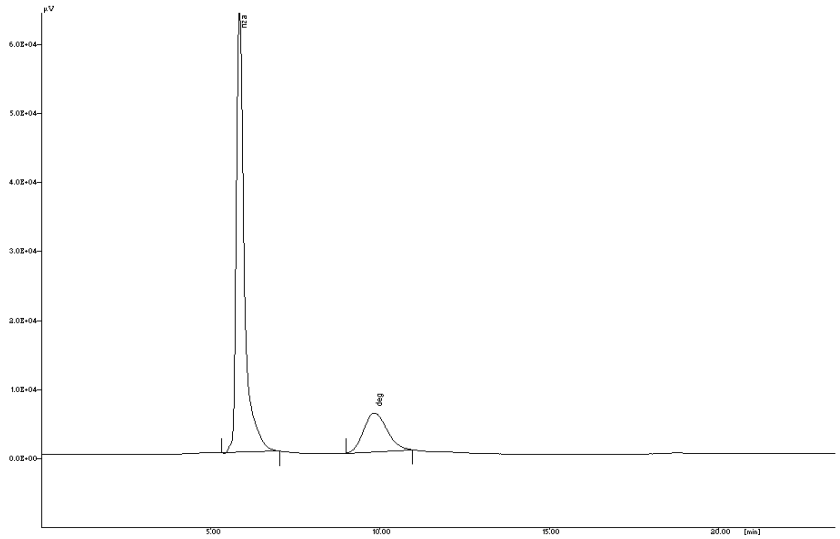

Figure 4: Chromatogram of base degraded sample showing additional peak at R_t 9.925 min.

Linearity

The response for the drug was linear (0.9993) in the concentration range between 1000-7000 µg/L. The mean (±RSD) values of slope, intercept and correlation coefficient were 123977 (±1.30), 7594 (±1.57) and 0.9996 (±0.03), respectively.

Precision

The results of the repeatability and intermediate precision experiments are shown in [Table 1]. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guideline. Separation of the drug and different degradation products in stressed samples was found to be similar when analyses were performed on different chromatographic system on different days.

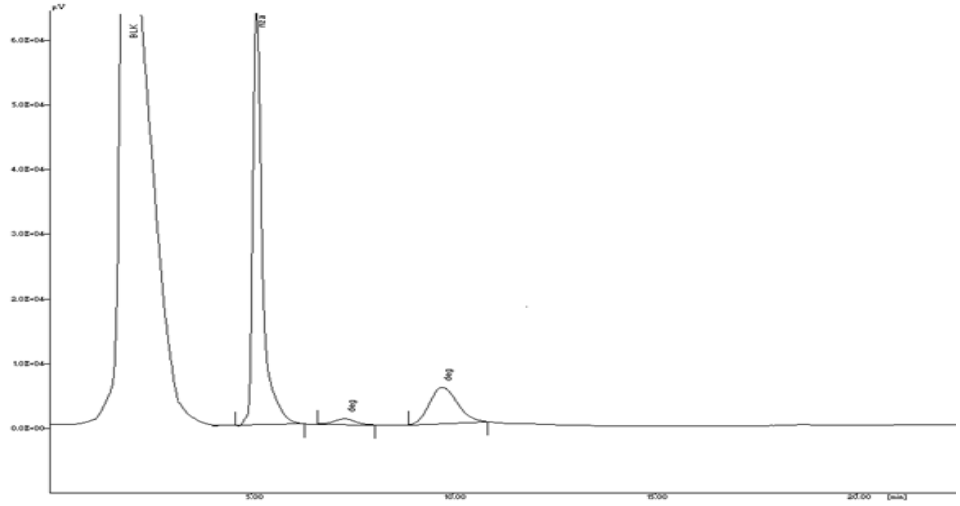

Figure 5: Chromatogram of oxidative degraded sample showing additional peak at R_t 6.205 and 9.320 min.

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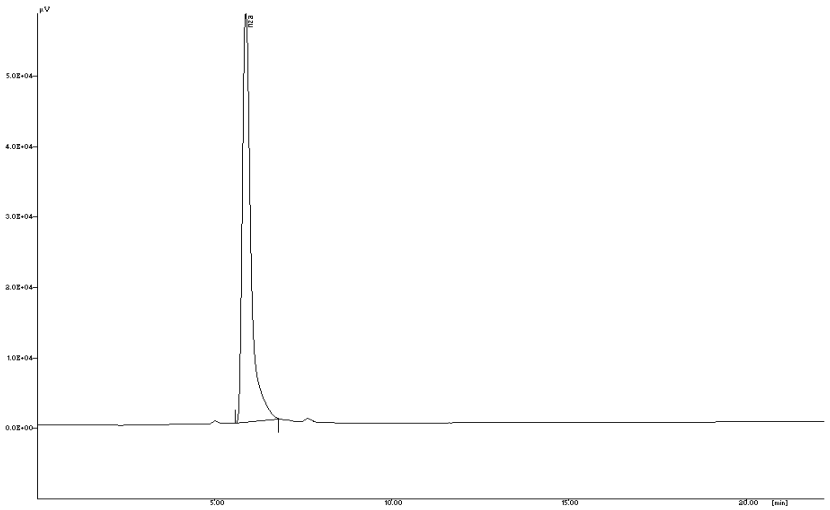

Figure 6: Chromatogram of photolytic degradation

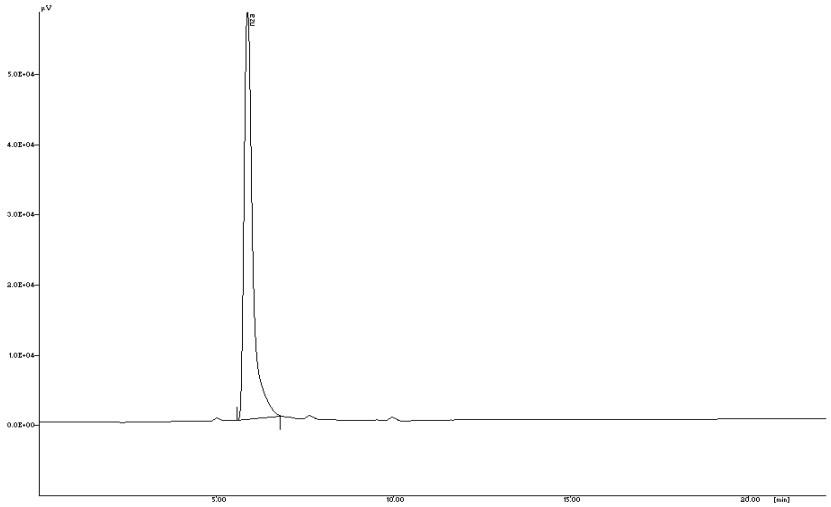

Figure 7: Chromatogram of thermal degradation

LOD and LOQ

The signal: noise ratios of 3:1 and 10:1 were considered as LOD and LOQ respectively. The LOD and LOQ were found to be 20 µg/L and 70 µg/L respectively.

Robustness of the method

The factors varied includes, flow rate, mobile phase composition, pH of mobile phase and column from different manufacturers (Perfectsil and Thermo columns). Each factor selected was changed at three levels (-1, 0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 3) of standard solution 5000 µg/L were performed under small changes of four chromatographic parameters (factors) mention below. Insignificant differences in peak areas, asymmetry, and resolution were observed and changes in retention times were within the limits. The data is given in [Table 2]. The resolution of drug in the mixture of stressed sample was found to be similar when studies were performed on different chromatographic system on different days indicating that the method has sufficient ruggedness.

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Table 1: Precision studies

Concentration (µg/L)	Measured concentration ± RSD (%)	
	Repeatability (n = 6)	Intermediate precision (n = 6)
2000	2.01 ± 0.635	2.02 ± 0.706
4000	4.04 ± 0.607	4.03 ± 0.861
6000	5.94 ± 0.691	6.00 ± 1.231

Table 2: Robustness testing (n = 6)

Parameter	Level	RT
Flow rate (mL/min.)		
0.9	-1	6.050
1.0	1	5.882
1.1	+1	5.830
Mean ± SD, % RSD (n = 3)		5.921 ± 0.115, 1.942
% methanol in mobile phase		
38	-1	6.010
40	1	5.885
42	+1	5.950
Mean ± SD, % RSD (n = 3)		5.948 ± 0.063, 1.051
pH of mobile phase		
3.4	-1	5.850
3.5	1	5.882
3.6	+1	5.891
Mean ± SD, % RSD (n = 3)		5.874 ± 0.022, 0.367
Different columns		
Perfectsil ODS		5.882
Thermo ODS		6.015
Mean ± SD, % RSD (n = 3)		5.949 ± 0.094, 1.581

Table 3: Recovery studies (n = 6)

Actual Concentration (mg)	Measured concentration (mg)	Recovery (%) ± R.S.D.
4	4.08	100.86 ± 0.413
5	5.11	101.13 ± 1.260
6	6.04	100.34 ± 1.971

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Table 4: Summary of validation parameters

Parameter	Data
Linearity range	1000-7000 µg/L
Correlation coefficient	0.9993
Limit of detection	20 µg/L
Limit of quantitation	70 µg/L
% Recovery (n=6)	100.77 ± 1.214
Precision (%RSD)	
Repeatability (n=6)	0.644
Intermediate (n=6)	0.932
Robustness	Robust
Specificity	Specific

Table 5: Analysis of commercial formulation

Commercial formulation Rixact-5 (5 mg)	Assay
	Mean ± RSD (n= 6)
B. No.K60747	101.28 ± 1.526

Specificity

The specificity of the LC method is illustrated in [Figure 2, 3, 4] where complete separation of rizatriptan benzoate in presence of its degradation products was noticed. The peaks obtained were sharp and have clear baseline separation. The resolution factor for drug from nearest resolving was > 3 [Figure. 3, 4, 5]. The photodiode array detector scanned all the components present in mixture in whole wavelength range from 200 to 400 nm and it indicated that there is no degradation peak (hiding) under or unresolved from the analyte peak (pure drug), which also reflected the specificity of the method.

Recovery studies

As shown from the data in [Table 3] good recoveries of the drug in the range from 100.34% to 100.86% were made at various added concentrations, despite the fact that the drug was fortified to a mixture that contained drug as well as degradation product formed at various reaction conditions. Summary of the validation parameter is given in [Table 4].

Analysis of marketed formulation

The drug content was found to be 101.28 ± 1.526. Two different lots of commercially available rizatriptan benzoate tablet were analyzed using the proposed procedures and the results are summarized in [Table 5].

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CONCLUSION

Stability indicating LC method was developed for rizatriptan benzoate and validated as per ICH guidelines. UV detection allowed an accurate quantitation of chromophoric compounds. In this study, intrinsic stability of rizatriptan benzoate was established using various ICH recommended stress conditions. The drug as such was very stable in solid form and in methanolic solution. In the latter case, unknown decomposition products were formed under stress condition. The drug was found to degrade extensively in alkaline condition than in acidic condition. Mild degradation was also seen in oxidative stress conditions but the drug was stable to thermal stress and photolytic degradation. Peak purity testing of degradation products were done by LC-PDA. The method was validated for parameters like linearity, precision, accuracy, specificity, ruggedness etc. and was also applied to real marketed samples. Thus, the method can be employed for analysis of drug during stability studies.

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