A stability indicating LC method for zolmitriptan

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Abstract

A gradient, reversed-phase liquid chromatographic (RP-LC) assay method was developed for the quantitative determination of zolmitriptan, used to treat severe migraine headaches. The developed method is also applicable for the related substances determination in bulk drugs. The chromatographic separation was achieved on a Waters X Terra RP18, 250 mm × 4.6 mm, 5 μm column. The gradient LC method employs solutions A and B as mobile phase. The solution A contains a mixture of phosphate buffer pH 9.85:methanol:acetonitrile (70:20:10, v/v/v) and solution B contains a mixture of phosphate buffer, pH 9.85:acetonitrile (30:70). The flow rate was 1.0 ml/min and the detection wavelength was 225 nm. In the developed HPLC method, the resolution between zolmitriptan and its potential impurities, namely Imp-1, Imp-2 and Imp-3 was found to be greater than 3. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in alkaline medium and oxidative stress conditions. Degradation product formed during base hydrolysis was found to be Imp-3. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.5%. The developed RP-LC method was validated with respect to linearity, accuracy, precision and robustness.
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1. Introduction

Zolmitriptan, 4(S)-4-[3-(2-dimethyl aminoethyl)-1H-5-indolyl-methyl]-1,3-oxazolan-2-one belongs to a group of medicines known as Serotonin 5-HT1D receptor agonist (Fig. 1). It works by stimulating serotonin receptors in the brain. Serotonin is a natural substance in the brain that, among other things, causes blood vessels in the brain to narrow. Zolmitriptan mimics this action of serotonin by directly stimulating the serotonin receptors in the brain. This causes the blood vessels to narrow.

Zolmitriptan is used to treat severe migraine headaches. Zolmitriptan is available in market as conventional tablets (Zomig), or as nasal spray (Zomig nasal spray).

Few HPLC methods were reported in the literature for the quantitative determination of Zolmitriptan in human plasma using fluorescent detection and also with coulometric detection [1,2].

An isocratic LC method was developed and reported [3] for the determination of zolmitriptan and its related impurities in quality control samples. Recently, a validated chiral LC method for the determination of zolmitriptan and its potential impurities was reported [4]. So far, to our present knowledge, no stability indicating analytical method for zolmitriptan was available in literature. It was felt necessary to develop a stability indicating LC method for the related substance determination and quantitative estimation of zolmitriptan. This paper

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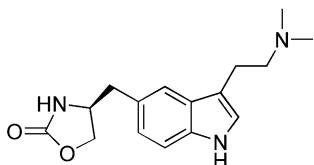 *E-mail addresses:* bmrao@drreddys.com, bmrao67@rediffmail.com
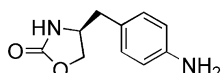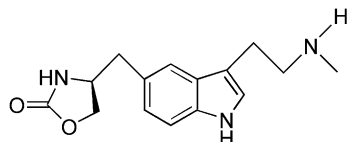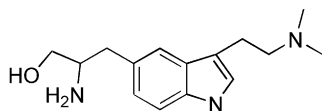(B.M. Rao).

4(S)-4 -[3-(2-dimethyl aminoethyl)-1H-5-indolyl-methyl]-1,3-oxazolan-2-one (zolmitriptan)

(4S)-4-(4-aminobenzyl)-1,3-oxazolan-2-one (Imp-1)

4(S)-4 -[3-(2-methyl aminoethyl)-1H-5-indolyl-methyl]-1,3-oxazolan-2-one (Imp-2)

(2S)-2-Amino-3- [3-(2-dimethyl-aminoethyl)-1H-5-indolyl]-propan-1-ol (Imp-3)

Fig. 1. Chemical structures of zolmitriptan, Imp-1, Imp-2 and Imp-3. 4(*S*)-4-[3-(2-dimethyl aminoethyl)-1H-5-indolyl-methyl]-1,3-oxazolan-2-one (zolmitriptan). (4*S*)-4-(4-aminobenzyl)-1,3-oxazolan-2-one (Imp-1). 4(*S*)-4-[3-(2-methyl aminoethyl)-1H-5-indolyl-methyl]-1,3-oxazolan-2-one (Imp-2). (2*S*)-2-amino-3-[3-(2-dimethyl-aminoethyl)-1H-5-indolyl]-propan-1-ol (Imp-3).

describes the assay and related substances method validation for accurate quantification of zolmitriptan and all three impurities in bulk samples, respectively.

2. Experimental

2.1. Chemicals

Samples of zolmitriptan and its three impurities 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 Merck, Darmstadt, Germany. Analytical reagent grade ammonium dihydrogen ortho phosphate and ammonia solution were purchased from Rankem, Mumbai, 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 LC system with a diode array detector. The output signal was monitored and processed using Chemstation software (Agilent) on Pentium computer (Digital Equipment Co.).

2.3. Chromatographic conditions

The chromatographic column used was a Waters 250 mm × 4.6 mm X Terra RP18 column with 5 μm particles. The gradient LC method employs solutions A and B as mobile phase. The solution A contains a mixture of 10 mM ammonium di hydrogen ortho phosphate, pH adjusted to 9.85 using ammonia solution (buffer):methanol:acetonitrile (70:20:10, v/v/v) and solution B contains a mixture of

buffer:acetonitrile (30:70, v/v). The flow rate of the mobile phase was 1.0 ml/min. The HPLC gradient program was set as: time/% solution B: 0/0, 10/0, 30/55, 35/55 and 36/0 with a post run time of 10 min.

The column temperature was maintained at 30 °C and the detection was monitored at a wavelength of 225 nm. The injection volume was 10 μl. A mixture of water:acetonitrile (1:1) was used as a diluent.

2.4. Preparation of standard solutions

A stock solution of zolmitriptan (5.0 mg/ml) was prepared by dissolving appropriate amount in the diluent. Working solutions of 500 and 100 μg/ml were prepared from above stock solution for related substances determination and assay determination, respectively. A stock solution of impurity (mixture of Imp-1, Imp-2 and Imp-3) at 0.5 mg/ml was also prepared in diluent.

2.5. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [5]. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used.

The specificity of the developed LC method for zolmitriptan was determined in the presence of its impurities, namely Imp-1, Imp-2, Imp-3 and degradation products. Forced degradation studies were also performed on zolmitriptan to provide an indication of the stability indicating property and specificity of the proposed method. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (60 °C), acid hydrolysis (0.1N HCl), base hydrolysis (0.1N NaOH), water hydrolysis and oxidation (0.01% H_2O_2). For heat and light studies, study period was 10 days whereas for acid, base, water hydrolysis and oxidation, it was 48 h. Peak purity of stressed samples of zolmitriptan was checked by using Agilent 1100 Diode array detector (DAD). The purity factor is within the threshold limit obtained in all stressed samples demonstrates the analyte peak homogeneity. Assay studies were carried out for stress samples against qualified reference standard and the mass balance (% assay + % impurities + % degradation products) was calculated. 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. 0.15% of analyte concentration which is 500 μg/ml).

2.6. Method validation

2.6.1. Precision

Assay method precision was evaluated by carrying out six independent assays of test sample of zolmitriptan against qualified reference standard. The percentage of R.S.D. of six assay values obtained was calculated.

The precision of the related substance method was checked by injecting six individual preparations of (0.5 mg/ml) zolmitriptan spiked with 0.15% each of Imp-1, Imp-2 and Imp-3 with respect to analyte concentration. The percentage of 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 and a different instrument in the same laboratory.

2.6.2. 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 dilute 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 percentage of R.S.D. of the area.

2.6.3. Linearity

Linearity test solutions for assay method were prepared from stock solution at six concentration levels from 25 to 150% of assay analyte concentration (25, 50, 75, 100, 125 and 150 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% (1.5 μg/ml) of the specification level (LOQ, 0.075, 0.15, 0.1875, 0.225 and 0.3%).

Linearity test was performed for 3 consecutive days in the same concentration range for both assay and related substance method. The percentage of R.S.D. value of the slope and Y-intercept of the calibration curve was calculated.

2.6.4. Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels, i.e. 50, 100 and 150 μg/ml in bulk drug sample. The percentage of recoveries were calculated from the slope and Y-intercept of the calibration curve obtained in Section 2.6.3. Accuracy/recovery experiments were performed in triplicate.

The bulk sample, provided by Process Research Department of Custom Pharmaceutical Services, does not show the presence of Imp-1 and Imp-2. Some of the bulk samples received from Process Research Department of Dr. Reddy's Laboratories shows the presence of Imp-2 in between 0.03 and 0.05% levels. 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 percentage of recoveries for Imp-1, Imp-2 and Imp-3 were calculated from the slope and *Y*-intercept of the calibration curve obtained in Section 2.6.3.

2.6.5. Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered and the resolution between zolmitriptan, 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, it was changed by 0.2 units 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 9.75 and 9.95 buffer pH). The effect of column temperature on resolution was studied at 25 and 35 °C instead of 30 °C. In the all above varied conditions, the components of the mobile phase were held constant as stated in Section 2.3.

2.6.6. Solution stability and mobile phase stability

The solution stability of zolmitriptan in the assay method was carried out by leaving both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed for 6 h interval up to the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions for 6 h interval up to 48 h. Mobile phase prepared was kept constant during the study period. The percentage of R.S.D. of assay of zolmitriptan was calculated for the study period during mobile phase and solution stability experiments.

The solution stability of zolmitriptan and its impurities in the related substance method was carried out by leaving spiked sample solution in tightly capped volumetric flask at room temperature for 48 h. Content of Imp-1, Imp-2 and Imp-3 were determined for every 6 h interval up to the study period. Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions for every 6 h interval. Content of Imp-1, Imp-2 and Imp-3 were checked in the test solutions. Mobile phase prepared was kept constant during the study period.

Table 1
System suitability report

Compound (n = 3)	USP resolution	USP tailing factor (R_s)	No. of theoretical plates (N) USP tangent method
Imp-1	–	1.1	5330
Imp-2	7.02	1.2	5108
Imp-3	4.95	1.5	4197
Zolmitriptan	10.0	1.2	7698

n, Number of determinations.

3. Results and discussion

3.1. Method development and optimization

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 critical closely eluting impurities, namely Imp-2 and Imp-3. Impurities were co-eluted by using different stationary phases like C18, C8 and cyano 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. pH of the buffer has played a significant role in achieving the separation between Imp-2, Imp-3 and zolmitriptan.

The chromatographic separation was achieved on a Waters X Terra RP18, 250 mm × 4.6 mm, 5 µm column, by using solutions A and B as mobile phase. The solution A contains a mixture of 10 mM ammonium di hydrogen ortho phosphate, pH adjusted to 9.85 using ammonia solution (buffer):methanol:acetonitrile (70:20:10, v/v/v) and solution B contains a mixture of buffer:acetonitrile (30:70, v/v). The flow rate of the mobile phase was 1.0 ml/min. The HPLC gradient program was set as: time/% solution B: 0/0, 10/0, 30/55, 35/55 and 36/0 with a post run time of 10 min. At 30 °C column temperature, the peak shape of zolmitriptan was found symmetrical. In the optimized conditions zolmitriptan, Imp-1, Imp-2 and Imp-3 were well separated with a resolution of greater than 3 and the typical retention times of Imp-1, Imp-2, Imp-3 and zolmitriptan were about 4.1, 6.1, 8.2 and 14.0 min, respectively. The system suitability results are given in Table 1 and the developed LC method was found to be specific for zolmitriptan and its three impurities, namely Imp-1, Imp-2 and Imp-3 (Table 2).

Table 2
Summary of forced degradation results

Stress condition	Time	% Assay of active substance	Mass balance (%assay + %impurities + %degradation products)	Remarks
Acid hydrolysis (0.1N HCl)	48 h	99.3	99.6	No degradation products formed
Base hydrolysis (0.1N NaOH)	48 h	87.0	99.5	Degraded into Imp-3
Oxidation (0.01% H_2O_2)	48 h	95.2	99.8	Degradation products formed
Thermal (60 °C)	10 days	99.9	99.5	No degradation products formed
Light (photolytic degradation)	10 days	99.8	99.7	No degradation products formed

3.2. Method validation

3.2.1. Precision

The R.S.D. of assay of zolmitriptan during assay method precision study was within 1% and the R.S.D. of area of Imp-1, Imp-2 and Imp-3 in related substance method precision study were within 7%. The R.S.D. of assay results obtained in intermediate precision study was within 0.7% and the R.S.D. of area of Imp-1, Imp-2 and Imp-

3 were well within 9%, confirming good precision of the method.

3.2.2. Limit of detection and limit of quantification

The limit of detection of Imp-1, Imp-2 and Imp-3 were 0.02, 0.015 and 0.02% (of analyte concentration, i.e. 500 µg/ml) for 10 µl injection volume. The limit of quantification of Imp-1, Imp-2 and Imp-3 were 0.06, 0.05 and 0.06% (of analyte concentration, i.e. 500 µg/ml) for 10 µl injection
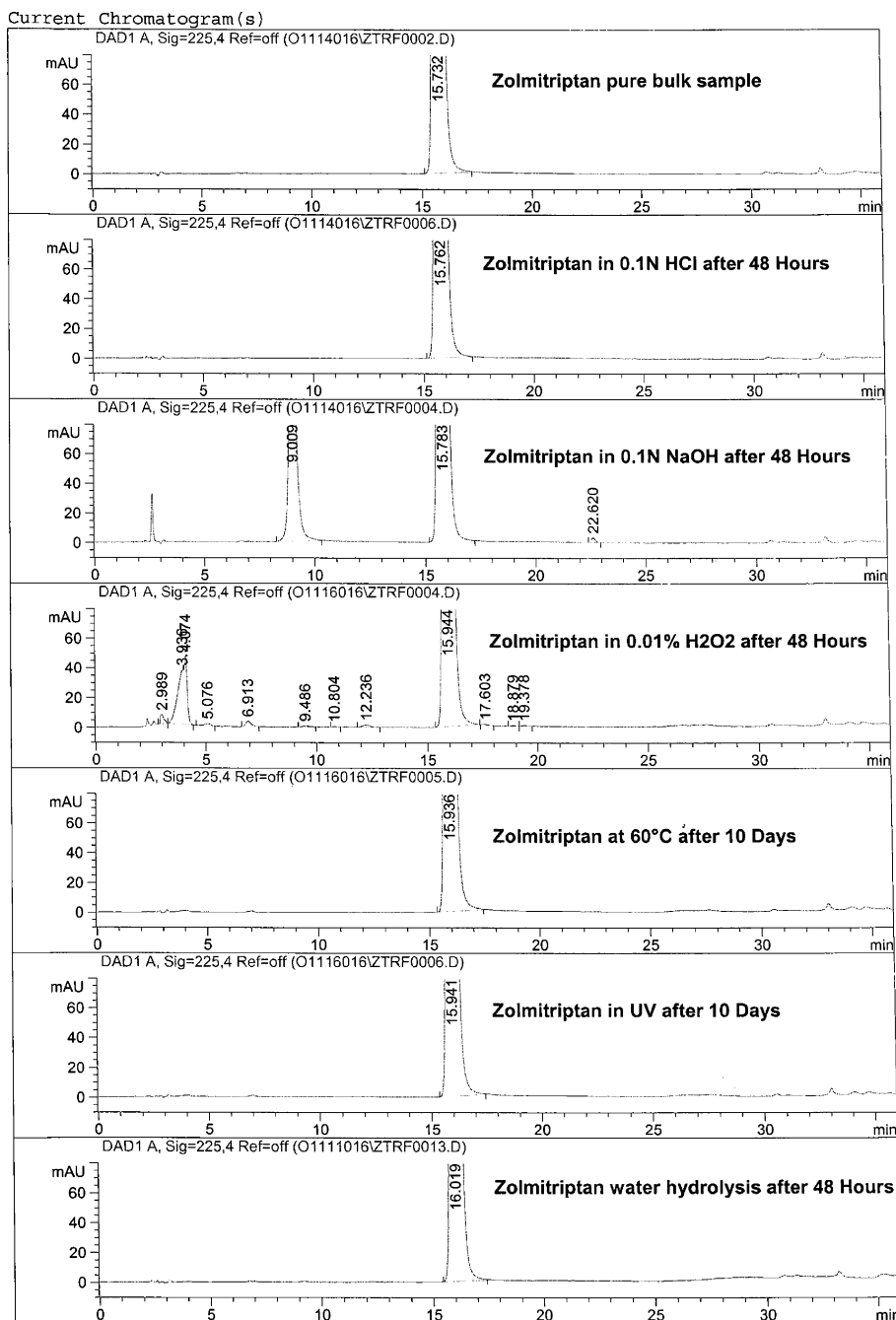

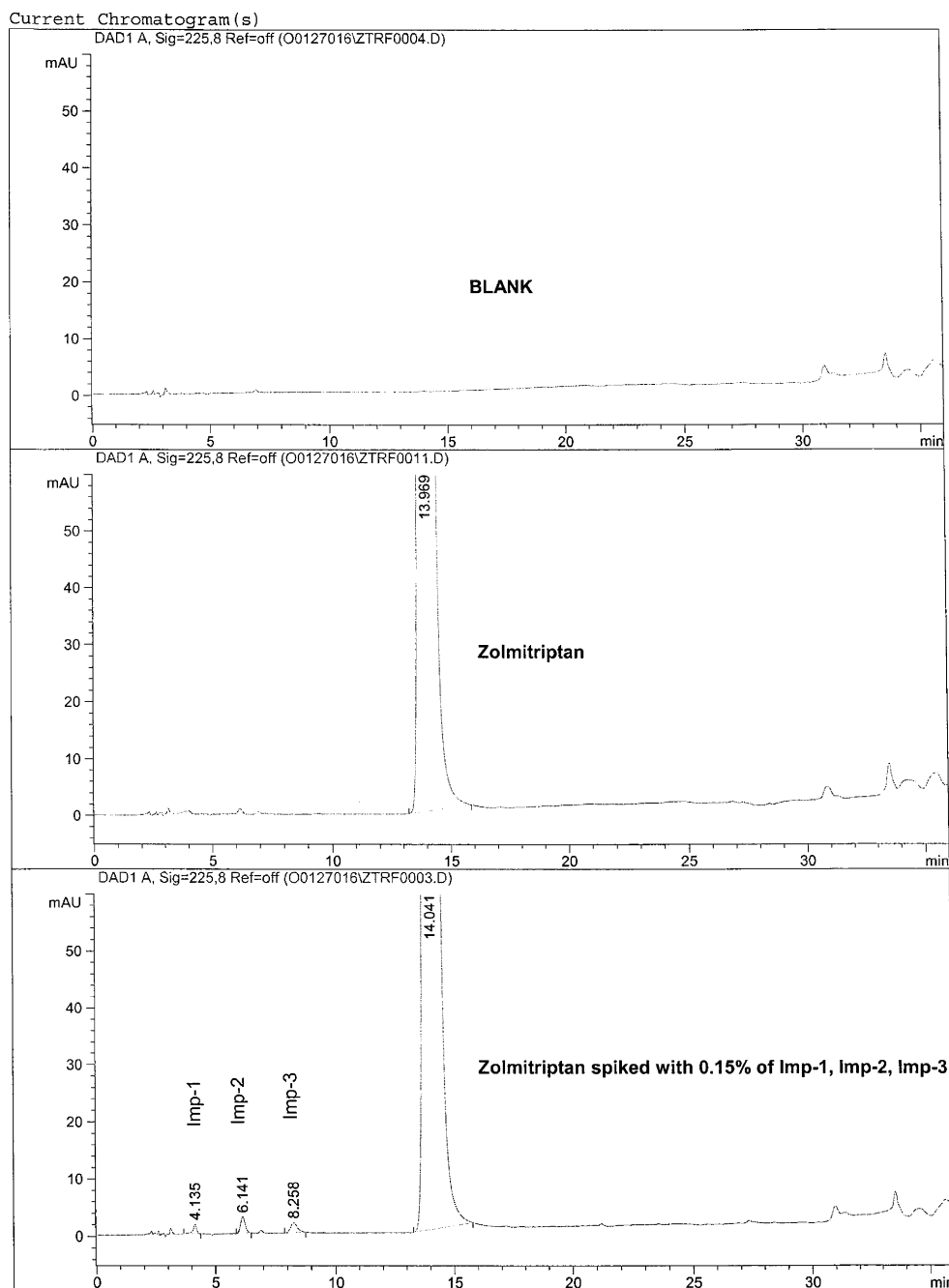Fig. 2. Typical HPLC chromatograms of pure bulk sample and stressed samples.

Fig. 3. HPLC chromatograms of blank, pure (unspiked) and spiked (Imp-1, Imp-2 and Imp-3 were spiked at 0.15% level in pure zolmitriptan) samples.

volume. The precision at LOQ concentration for Imp-1, Imp-2 and Imp-3 were below 10%.

3.2.3. Linearity

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 25–150 µg/ml and the correlation coefficient obtained was greater than 0.999. Linearity was checked for assay method over the same concentration range for 3 consecutive days. The percentage of 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 (0.06%) to 0.3% for Imp-1, Imp-2 and Imp-3. The correlation coefficient obtained was greater than 0.995. Linearity was checked for related substance method over the same concentration range for 3 consecutive days. The percentage of R.S.D. values of the slope and Y-intercept of the calibration curves were

Table 3
Recovery results of in bulk drug sample

Added (μg) (n = 3)	Recovered (μg)	% Recovery	% R.S.D.
51	50.5	99.0	0.9
102	102.6	100.5	0.6
155	156.5	101.0	0.8

n = 3 determinations.

Table 4
Results of robustness study

S. no.	Parameter	Variation	Resolution between Imp-2 and Imp-3
1	Temperature (±5 °C of set temperature)	(a) At 20 °C	5.0
		(b) At 30 °C	4.5
2	Flow rate (±20% of the set flow)	(a) At 0.8 ml/min	5.5
		(b) At 1.2 ml/min	4.6
3	pH (±0.1 units of set pH)	(a) At 9.75	4.8
		(b) At 9.95	5.2

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.

3.2.4. Accuracy

The percentage recovery of zolmitriptan in bulk drug samples ranged from 99.0 to 101.0 (Table 3). The percentage recovery of Imp-1, Imp-2 and Imp-3 in bulk drugs samples ranged from 92.3 to 106.5. HPLC chromatograms of blank, pure (unspiked) and spiked samples at 0.15% level of all three impurities in zolmitriptan bulk drug sample are shown in Fig. 3.

3.2.5. Robustness

In all the deliberate varied chromatographic conditions carried out as per Section 2.6.5 (flow rate, pH and column temperature), the resolution between closely eluting impurities, namely Imp-2 and Imp-3 was greater than 3.0, illustrating the robustness of the method (Table 4).

3.2.6. Solution stability and mobile phase stability

The R.S.D. of assay of zolmitriptan during solution stability and mobile phase stability experiments was within 1%. No significant changes were observed in the content of Imp-1, Imp-2 and Imp-3 during solution stability and mobile phase experiments when performed using related substances method. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay and related substance determination were stable up to 48 h.

4. Results of forced degradation studies

Degradation was not observed in zolmitriptan stressed samples that were subjected to light, heat, acid and water

hydrolysis. The degradation of drug substance was observed under oxidative conditions and base hydrolysis (Fig. 2). Zolmitriptan was degraded into Imp-3 during base hydrolysis (in 0.1N NaOH after 48 h treatment). The same was confirmed by co-injection with a qualified Imp-3 standard. The drug substance zolmitriptan under oxidative conditions (in 0.01% hydrogen peroxide after 48 h stress) leads to the formation of some unknown degradation peaks. Peak purity test results derived from DAD, confirmed that the zolmitriptan peak is homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 99.5%. The assay of zolmitriptan is unaffected in the presence of Imp-1, Imp-2, Imp-3 and its degradation products confirm the stability indicating power of the developed method.

5. Conclusions

The gradient RP-LC method developed for quantitative and related substance determination of zolmitriptan is precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of bulk samples of zolmitriptan.

Acknowledgements

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