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Simultaneous estimation of rizatriptan, sumatriptan and zolmitriptan by RP-HPLC method in bulk

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ABSTRACT

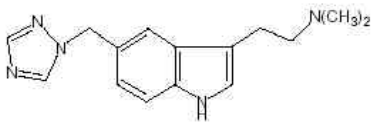
A simple reverse phase HPLC method was developed for the simultaneous estimation of Rizatriptan, Sumatriptan and Zolmitriptan in bulk form. Chromatography was performed by gradient reverse phase separation on a Stainless steel column 4.6 x 250mm, symmetry column packed with octa decyl silane bonded to porous silica (C18) with particle size 5 micron with mobile phase Acetonitrile: Sodium Phosphate buffer. The flow rate was 1.0ml/ min and effluent was monitored at 280 nm. The retention times were 7.215min, 8.432 and 9.185min for of Rizatriptan, Sumatriptan and Zolmitriptan respectively. The standard curve was linear over a working range of 1–10 µg/ml and gave an average correlation coefficient of 0.9996, 0.9992, and 0.9992 for Rizatriptan, Sumatriptan and Zolmitriptan respectively. The limit of quantitation (LOQ) of this method was 2µg/ml for rizatriptan Sumatriptan and Zolmitriptan. The absolute recovery was 101.84 for rizatriptan, 101.492 for sumatriptan and 101.44 for zolmitriptan. This method can be easily and conveniently adopted for routine analysis of Rizatriptan, Sumatriptan and Zolmitriptan in pure form and can also be used for dissolution or similar studies.

Key words: Rizatriptan, Sumatriptan, Zolmitriptan, Chromatography, Acetonitrile, Sodium Phosphate Buffer.

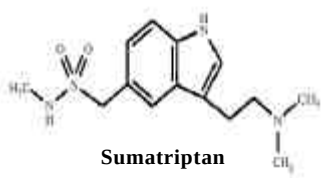
INTRODUCTION

Rizatriptan[1], *N*,*N*-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)- 1H-indole-3-ethanamine, is an orally active serotonin 5-HT(1) receptor agonist that potently and selectively binds to 5-HT(1B/1D) subtypes. Sumatriptan succinate[1], 3-[2-(dimethylamino)ethyl]-*n*-methyl- 1H-indole-5-methanesulphonamide succinate is a 5-hydroxytryptamine (5-HT1B/1D) receptor agonist, efficacious in the treatment of migraine. The mechanism of action of the (5-HT1B/1D) receptor agonist has been thoroughly studied and leads to two main theories. Sumatriptan acts as a vasoconstrictor of dilated intracranial blood vessels and, also as an inhibitor of the pro-inflammatory neuropeptide release which leads to headache relief. Zolmitriptan[1], (4*S*)-4-[[3-[2-(dimethylamino) ethyl] - 1H-indol-5-yl] methyl]-2-oxazolidinone, is a selective serotonin 5-HT (1B/1D) receptor agonist. The method was developed and then validated as per ICH guidelines[2]. Several analytical methods have been developed and published for the determination of rizatriptan, sumatriptan and Zolmitriptan in biological fluids[3, 7]. Literature survey indicates that stability indicating methods had been developed by using LC method[4, 8]. Among methods described in the literature, there are HPLC and RP-HPLC methods with UV detector [5, 6]. At the moment, there is no method available in the literature for the simultaneous determination of rizatriptan, sumatriptan and Zolmitriptan in bulk using a UV detection technique.

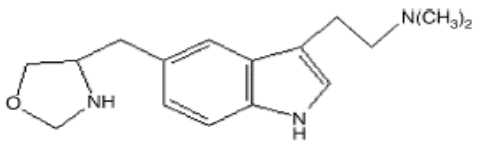
In this paper, we present a method for the simultaneous determination of rizatriptan, sumatriptan and Zolmitriptan in bulk using a different detection technique. The method uses an HPLC with UV detection.



Rizatriptan



Sumatriptan



Zolmitriptan

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MATERIALS AND METHODS:

Rizatriptan benzoate, sumatriptan succinate and Zolmitriptan bulk drugs are supplied by NATCO Pharma. Sodium Dihydrogen phosphate, 85% Ortho-phosphoric acid, Methanol, Acetonitrile, Dichloromethane, Hydrochloric Acid, Triethyl-Amine, Sodium Hydroxide are purchased from MERCK. HPLC used was Waters alliance 2695 HPLC system with Waters 2996 PDA detector.

Stock solutions and standards:

Stock solution of Rizatriptan, Sumatriptan and Zolmitriptan (1 mg/ml) was prepared by dissolving 25 mg of Rizatriptan, Sumatriptan and Zolmitriptan in 25 ml of volumetric flask containing 10 ml of Methanol. The solution was sonicated for about 10 min and then made up to volume with mobile phase. Daily working standard solutions of Rizatriptan, Sumatriptan and Zolmitriptan was prepared by suitable dilution of the stock solution with Distilled water. Working standard solutions of Rizatriptan, Sumatriptan and Zolmitriptan were prepared by taking suitable aliquots of drug solution from the standard stock solution 1000µg/ml, and the volume was made upto 10 ml with mobile phase (Acetonitrile: Sodium phosphate buffer, were used in different compositions as mobile phases).

Preparation of sample solution:

An accurately weighed sample of powdered drug containing 25 mg of Rizatriptan, Sumatriptan and Zolmitriptan was dissolved with Methanol in a 25ml volumetric flask using ultra sonicator. This solution was filtered through 0.45µm filter paper. The solution obtained was diluted with the distilled water so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in duplicate.

Apparatus and chromatographic conditions:

Quantitative HPLC was performed on Waters alliance 2695 HPLC system with Waters 2996 PDA detector. Empower software is used along with a Stainless steel column 4.6 x 250mm, symmetry column packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron. To develop a suitable and robust HPLC method for the determination of Rizatriptan, Sumatriptan and Zolmitriptan, different mobile phases Acetonitrile: Sodium phosphate buffer, were used in different compositions of mobile phases (30:70, 40:60, 50:50, 70:30, 80:20) at different flow rates (0.5,0.75,1.0, 1.2, 1.5, ml/ min). The mobile phase Acetonitrile: Sodium phosphate buffer with Gradient elution at a flow rate of 1.0 ml/ min gave peaks of good resolution and were eluted at retention times around 7.215min, 8.432 and 9.185min for of Rizatriptan, Sumatriptan And Zolmitriptan respectively with symmetric peak shape. The HPLC gradient program was set as: **time / % solution B**: 0/ 10, 4/12, 6/15, 10/10 and 15/10 with a post run time of 15 min. The detection is performed at the wavelength 280 nm.

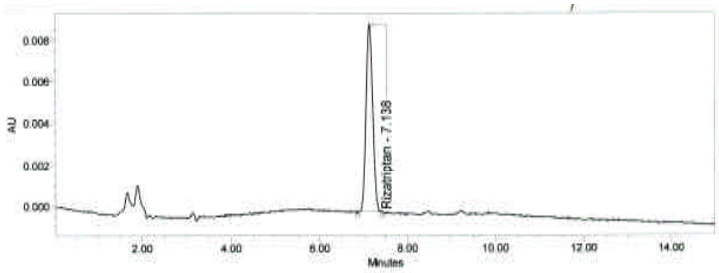


Fig. 1: A Typical Chromatogram of Rizatriptan in pure drug.

Table no. 1: A Typical Chromatogram of Rizatriptan in pure drug.

S.NO	Name of the Peaks	Retention time (min)
1.	Rizatriptan	7.138

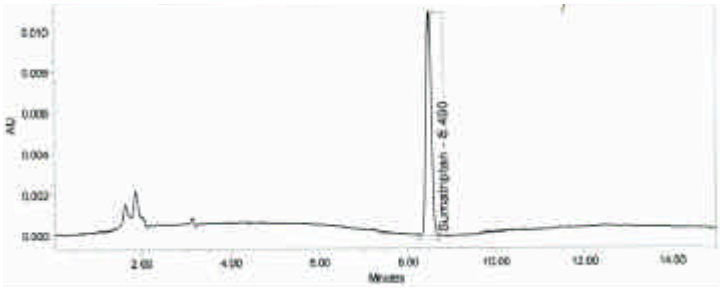


Fig.2: A Typical Chromatogram of Sumaitriptan in pure drug.

Table 2: A Typical Chromatogram of Sumatriptan in pure drug.

S.No	Name of the Peaks	Retention time (min)
1.	Sumatriptan	8.490

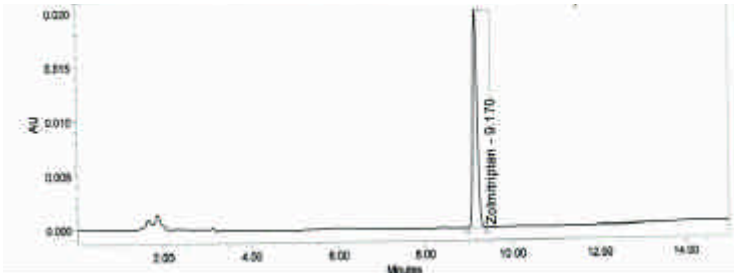


Fig. 3: A Typical Chromatogram of Zolmitriptan in pure drug

Table 3: A Typical Chromatogram of Zolmitriptan in pure drug

S.No	Name of the Peaks	Retention time (min)
1.	Zolmtriptan	9.170

Fig. 4: A Typical Chromatogram of Rizatriptan, Sumatriptan and Zolmitriptan in pure form

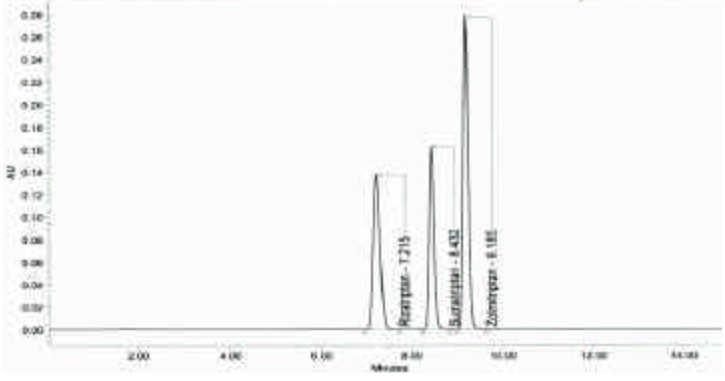


Table 4: A Typical Chromatogram of Rizatriptan, Sumatriptan and Zolmitriptan in pure form

S.No	Name of the Peaks	Retention time (min)
1.	Rizatriptan	7.215
2.	Sumatriptan	8.432
3.	Zolmitriptan	9.185

Validation of the assay method[2]:

Linearity:

Linearity test solutions for assay method were prepared from stock solution at four concentration levels from 50 to 150% of assay analyte concentration (100, 150, 200, 300µg/ml). The peak area versus concentration data was performed by least-squares linear regression analysis. The linear fit of the system was illustrated graphically. The linearity range was found to be 0 - 300 µg/ml. The samples were assayed using the method described above. The standard calibration curves for rizatriptan, sumatriptan and zolmitriptan were constructed using the peak-area versus the nominal concentrations of the analytes. Linear least-squares regression analysis was performed to assess the linearity it is given in table no. 6.

Recovery and accuracy:

The accuracy of the assay method was evaluated in triplicate at three concentration levels, i.e. 50, 75, 100 and 150µg/ml in bulk drug sample. The percentages of recoveries were calculated from the slope and *Y*-intercept of the calibration curve obtained. Accuracy/recovery experiments were performed in triplicate. Samples were prepared in at concentrations of 100- 300µg/ml, and assayed as described. From the results shown in accuracy Table, it was found that the percentage recovery values of pure drugs were in between 99.21-101.82 which indicates that the method was accurate it is given in table no.7

Precision:

The precision of method was ascertained from the peak area response obtained by actual determination of six replicates of a fixed amount of drug. The percent relative standard deviations were calculated for Rizatriptan, Sumatriptan and Zolmitriptan. The results shown in precision table no. 8, it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility.

LOD & LOQ: The LOD and LOQ for Rizatriptan, Sumatriptan and Zolmitriptan 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. Precision study was also carried at the LOQ level by injecting six individual preparations of Rizatriptan, Sumatriptan, Zolmitriptan and calculating the percentage of R.S.D. of the area. The parameters LOD and LOQ were determined on the basis of peak response and slope of the regression equation. The parameter LOD for Rizatriptan and Sumatriptan was found to be 0.2ppm and LOD for Zolmitriptan was found to be 0.1ppm.

The parameter LOQ for Rizatriptan, Sumatriptan and Zolmitriptan was found to be 2ppm. The Sample of this concentration was injected for repeatability and RSD is calculated. The concentration of 2ppm gives required precision.

Ruggedness: Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It is checked that the results are reproducible under differences in, analysts and experimental periods. Hence the proposed method was found to be rugged and it is given in table no.9.

Robustness: The percent recovery of Rizatriptan, Sumatriptan and Zolmitriptan was good under most conditions and didn't show any significant change when the critical parameters were modified. The tailing factor for Rizatriptan, Sumatriptan and Zolmitriptan was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

RESULTS AND DISCUSSION:

Method development and optimization:

The main target of the chromatographic method is to get the separation of critical closely eluting drugs, namely Rizatriptan, Sumatriptan and Zolmitriptan. Drugs were co-eluted by using different stationary phases like C18, C8 with varying lengths and different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2-7) 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 Rizatriptan, Sumatriptan and Zolmitriptan. The chromatographic separation was achieved on a stainless steel column, symmetry (4.6 x 250mm) column packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron, by using solutions A and B as mobile phase. The solution A contains sodium dihydrogen phosphate, pH adjusted to 2.5 using ortho phosphoric acid (buffer) and solution B contains 100% Acetonitrile. The flow rate of the mobile phase was 1.0 ml/min. The HPLC gradient program was set as: time/% solution B: 0/ 10, 4/12, 6/15, 10/10 and 15/10 with a post run time of 15 min. At 25 ^{0}C column temperature, the peak shape of Sumatriptan, Rizatriptan and zolmitriptan was found symmetrical at 10 min, at flow rate 1.2 ml/min with mobile phase 70:30 ratio. In the optimized conditions Rizatriptan, Sumatriptan, and zolmitriptan were well separated with a resolution of greater than 3 and the typical retention times of Sumatriptan, Rizatriptan and zolmitriptan were about 7.215min, 8.432, 9.185min, respectively. The system suitability results are given in table no.5 and the developed LC method was validated.

Table 5: System suitability parameters

Drug	RT	Peak Area	Peak Height	USP Plate count	USP Tailing	Resolution	RRT
Rizatriptan	7.215	1484161	138096	9999	1.15		0.79
Sumatriptan	8.432	1270560	162860	25606	1.14	4.97	0.92
Zolmitriptan	9.185	2024986	278398	35144	1.18	3.80	1.00

Results of method validation

Linearity:

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 0- 300 µg/ml and the correlation coefficient obtained was greater than 0.999. The results show that an excellent correlation existed between the peak area and concentration of the analyte which is given in table no.6.

Table 6: Linearity table of Rizatriptan, Sumatriptan and Zolmitriptan

Concentration	Peak areas		
	Rizatriptan	Sumatriptan	Zolmitriptan
0	0	0	0
50	781045	673711	1067619
75	1161948	998885	1587713
100	1499173	1287926	2046838
150	2074814	1777014	2824694

Fig. 5a : Calibration curve of Rizatriptan

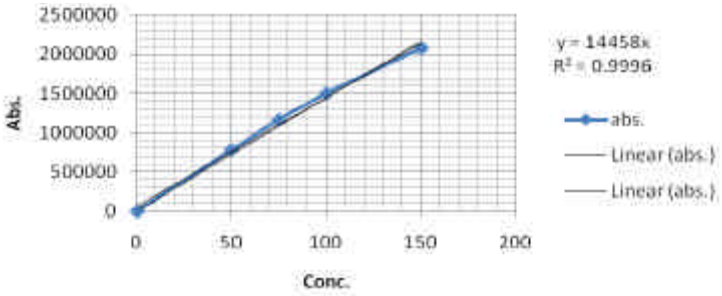


Fig. 5b : Calibration curve of Sumatriptan

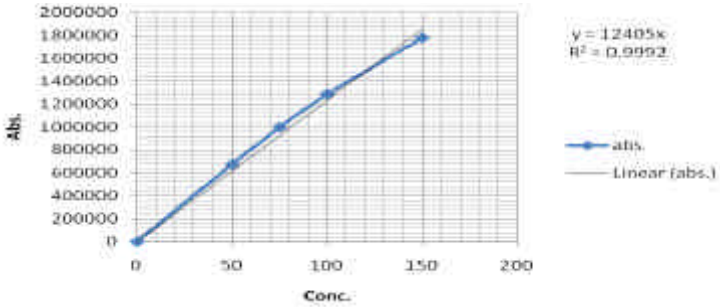
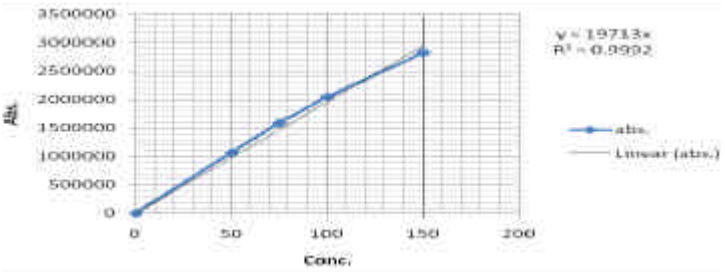


Fig.5c: Calibration curve of Zolmitriptan



Recovery and accuracy:

The percentage recovery of Rizatriptan, Sumatriptan and Zolmitriptan in bulk drugs samples was ranged from 99.21-101.82 which indicates that the method was accurate which is given in table no.7.

Table 7: % Recovery of Rizatriptan, Sumatriptan and Zolmitriptan

% Level	% Recovery		
	Rizatriptan	Sumatriptan	Zolmitriptan
50	101.04	101.492	101.18
75	101.84	101.44	101.44
100	99.86	100.06	99.86
150	99.469	99.213	99.21

Precision:

From the results shown in precision Table, it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility given in table no.8.

Table 8: Precision Readings for Rizatriptan, Sumatriptan and Zolmitriptan

Sl.No.	Rizatriptan peak area response	Sumatriptan peak area response	Zolmitriptan peak area response
1	1474819	1263504	2015367
2	1473676	1261558	2011445
3	1465342	1256945	2001159
4	1464053	1256672	2000781
5	1468128	1258525	2002170
6	1482564	1270026	2024167
Average area	1471430	1261205	2009181
%RSD	0.47	0.40	0.47

LOD & LOQ:

The parameter LOD for Rizatriptan and Sumatriptan was found to be 0.2ppm and LOD for Zolmitriptan was found to be 0.1ppm. The parameter LOQ for Rizatriptan, Sumatriptan and Zolmitriptan was found to be 2ppm. The Sample of this concentration was injected for repeatability and RSD is calculated. The concentration of 2ppm gives required precesion.

Ruggedness:

In the ruggedness study, the relative standard deviation of area of Rizatriptan, Sumatriptan and Zolmitriptan in bulk drugs samples was found to be 1.299, 0.97 and 1.063%, respectively. The results show that R.S.D. values were in the same order of magnitude than those obtained for repeatability. This confirms the ruggedness of the method given in table no.8.

Table 9: Ruggedness readings

	Date	%Rizatriptan	%Sumatriptan	%Zolmitriptan
Analyst-1	30.06.2008	100.756%	100.82%	100.705%
Analyst-2	01.07.2008	99.527%	99.851%	99.642%
% Deviation		1.299%	0.97%	1.063%

Robustness:

The percent recovery of Rizatriptan, Sumatriptan and Zolmitriptan was good under most conditions and didn't show any significant change when the critical parameters were modified. The tailing factor for Rizatriptan, Sumatriptan and Zolmitriptan was always less than 2.0 and the components were well separated

under all the changes carried out. Considering the modifications in the system suitability parameters, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

CONCLUSION:

The proposed method was found to be simple, precise, accurate and rapid for determination of Rizatriptan, Sumatriptan and Zolmitriptan in pure form. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement within the limit. Hence, this method can be easily and conveniently adopted for routine analysis of Rizatriptan, Sumatriptan and Zolmitriptan in pure form and can also be used for dissolution or similar studies.

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