Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2015, Volume 3, Issue 6
ISSN: 2321–3647(online)

Development and Validation of Common RP-HPLC Method for Estimation of Selected Triptans

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ABSTRACT

This paper describes the common analytical method suitable for the estimation of selected triptans (Naratriptan, Sumatriptan succinate, Zolmitriptan) by reversed phase high performance liquid chromatography (RP-HPLC). Chromatographic separations were conducted on Phenomenex Luna, C_{18} 250 X 4.6 mm, 5 microns column at room temperature using 6.8 pH phosphate buffer: acetonitrile (60: 40) as a mobile phase at a flow rate of 1.0ml min^{-1}, while UV detection was performed at 225nm. The retention time was found to be 3.130, 3.153, 2.143min respectively for the selected triptans. The method was found to be linear in the range of 2-10µg ml^{-1} for all the drugs. The proposed method is having good sensitivity due to low LOD and LOQ values. Analytical recovery was >99.3%. The method was validated statistically and applied for the quantitative analysis of triptans in bulk and formulations.

Keywords: Triptans, RP-HPLC, analytical method, validation.

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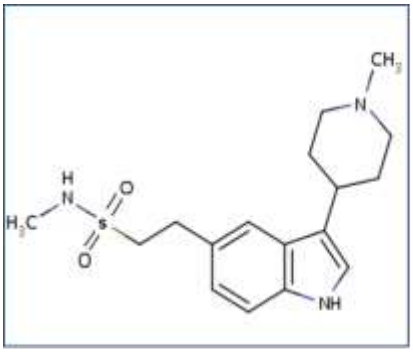
Received 01 June 2015, Accepted 07 June 2015

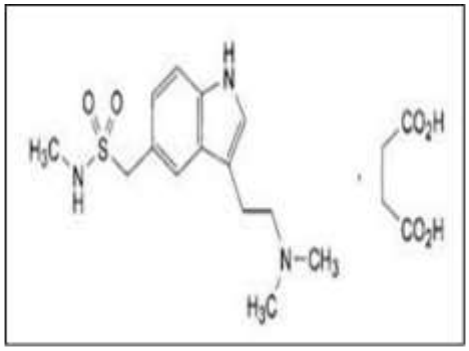
Please cite this article as: Veditha K *et al*., Development and Validation of Common RP-HPLC Method for Estimation of Selected Triptans. American Journal of Pharmacy & Health Research 2015.

INTRODUCTION

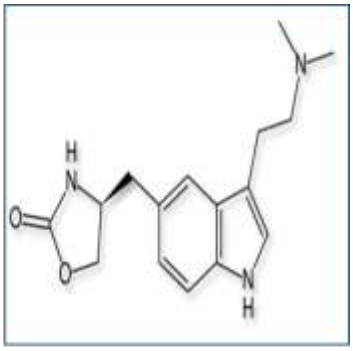
Triptans are used as anti-migraine drugs[1]. All triptans have an indole structure identical to the neurotransmitter 5-HT. Classic triptan structure contain side chain on the indole ring, and a basic nitrogen in a similar distance from the indole structure. The main structural difference of the triptans is the position of the sulfonamide and the side chain attached to it. Sumatriptan is having sulfonamide and nitrogen alkyl chain, in naratriptan nitrogen-alkyl chain is replaced with a 1-methyl-piperidine ring, zolmitriptan have triazole and 2-oxazolidoneinstead of a sulfonamide [2-4]. Very few analytical methods are reported for the estimation of these triptans. There is only few reported analytical methods for the estimation of these drugs from formulations. No reports are available on common analytical method suitable for estimation of triptans. So there is a need to develop validated common analytical method for formulations containing these drugs .Whenever the formulations undergone modifications in composition, there is a need to validate the existing method to test its suitability as the excipients may interfere in the estimation of the drug. If a common method is developed for the existing triptans, then this method can be tested for estimation of newer triptan series. In the present work, an attempt was made to develop newer, simple, accurate and low cost common RP-HPLC method for the quantitative estimation of naratriptan, sumatriptan succinate, zolmitriptan from pharmaceutical preparations. The selected triptan chemical structures are furnished below.



Naratriptan



Sumatriptan succiante



Zolmitriptan

MATERIALS AND METHODS

Naratriptan, Sumatriptan succinate and Zolmitriptan bulk drugs are supplied by NATCO Pharma, Acetonitile, Methanol, Triethyl amine, Glacial acetic acid, Potassium di-hydrogen phosphate, Sodium di-hydrogen orthophosphate, Ammonium di-hydrogen ortho phosphate and Water are purchased from MERCK. HPLC used was Shimadzu HPLC system, Phenomenex Luna, C_{18} 250 X 4.6 mm, 5 microns column with Shimadzu UV detector.

Preparation of standards solutions

10mg of Naratriptan, Sumatriptan and Zolmitriptan were accurately weighed separately in each case and transferred into different volumetric flasks of 10ml capacity. The drugs were dissolved with diluents [acetonitrile: 6.8 pH phosphate buffers (40:60)] and the volume were made up to the mark with the same solvents. The stock solution was suitably diluted to get a concentration range of 2- 10 µg/ ml.

Preparation of sample solutions

A required number of tablets containing Naratriptan, Sumatriptan and Zolmitriptan were collected separately for each drug, weighed and crushed to a fine powder in each case. A quantity equivalent to 100 mg of drug was weighed and transferred to 100ml standard flask. The powder was dissolved by sonication using sufficient amount of diluent and then made up to the mark with the same. The solutions were filtered and the stock solutions were suitably diluted to get a concentration range of 2-10 µg/ml.

Chromatographic conditions

To develop a suitable and robust HPLC method for the determination of Naratriptan, Sumatriptan and Zolmitriptan, different mobile phases like Acetonitrile: Water (10:90); Methanol: Water (10:90); Acetonitrile: Methanol: 6.8pH phosphate buffer (20:10:70); Acetonitrile: Methanol: 6.8pH phosphate buffer (10:20:70); Acetonitrile: 6.8pH phosphate buffer (10:90,20:80,30:70), were used at flow rate of 1ml/min. The detection is performed at the wave length 225nm.

Optimization of the HPLC method

The mobile phase Acetonitrile: 6.8pH phosphate buffer (40:60) with Isocratic elution at a flow rate of 1 ml/min gave peaks of good resolution and were eluted at retention times at 3.130, 3.153, 2.143min respectively for Naratriptan, Sumatriptan and Zolmitriptan with symmetric peak shape. The developed HPLC technique was optimized by varying the composition of the mobile phase. The composition ratio of 6.8Phosphate buffer: acetonitrile used as mobile phase include 65:35 and 55:45. The other chromatographic conditions were not changed during the optimization studies.

Validation of the Optimized HPLC Technique [5-9]

The optimized HPLC technique was subjected to validation by observing the following parameters.

Linearity and range

The chromatogram peak areas were observed by injecting different concentrations of

Naratriptan, Sumatriptan and Zolmitriptan (2-10 µg/ml). The linear graphs were constructed with the observed peak area vs. concentration.

Recovery and Accuracy

Twenty tablets were weighed and crushed to a fine powder. A quantity of powder equivalent to 10 mg of Naratriptan, Sumatriptan and Zolmitriptan were weighed and transferred to 10ml standard flasks. The drug was extracted with 5ml of the selected diluent 6.8 phosphate buffer: acetonitrile (60:40) then diluted up to the mark with the diluent. Then it was filtered .Appropriate volume of these aliquot was collected and transferred in to 100 ml volumetric flask and the final volume was made up to the mark with the diluent to obtain 12 (120%), 10 (100%) and 8 (80%) µg/ml concentrations .The resulting solutions were analyzed. The recovery studies were conducted in triplicate.

Precision

The following precision analysis was carried out.

1) System precision

The peak area was observed repeatedly for six times by injecting the standard solutions (10µg/ml) of Naratriptan, Sumatriptan and Zolmitriptan.

2) Intermediate precision/ ruggedness

To determine the intra-day precision, the sample solution of Naratriptan (6µg/ml), Sumatriptan (6µg/ml) and Zolmitriptan (10µg/ml) were prepared with the selected diluent separately for three times in same day and subjected to HPLC analysis. The corresponding peak areas were noted down. The intermediate precision (inter-day precision) of the method was determined by analysing the fresh sample solutions of Naratriptan (6µg/ml), Sumatriptan (6µg/ml) and Zolmitriptan (10µg/ml) continuously for three successive days. The average area, standard deviation and % RSD were calculated from the observed area.

3) Method precision

Sample solution of Naratriptan (6µg/ml), Sumatriptan (6µg/ml) and Zolmitriptan (10µg/ml) were prepared with the selected diluent for six times and subjected to chromatographic analysis. % assay and % RSD were calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of Naratriptan, Sumatriptan and Zolmitriptan were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines the LOD and LOQ are calculated by using following formulas

$$LOD = 3\sigma / S$$

$$LOQ = 10\sigma / S$$

Where σ- the standard deviation of the y-intercept and S- the slope of regression line

Robustness

The optimized chromatographic conditions were modified by changing the flow rate of the mobile phase (0.8 and 1.2ml/min), pH (6.6 and 7.0) and detector wavelength (223 and 227nm). The resulting retention time, peak area and other system suitability parameters were observed.

RESULTS AND DISCUSSIONS

Method development and optimization

The main objective of the present investigation is to develop a common HPLC method for the estimation of Naratriptan, Sumatriptan and Zolmitriptan from bulk and pharmaceutical dosage forms. The chromatographic studies were conducted on Phenomenex Luna C_{18} column. The composition of mobile phases included for elution of the selected triptans were ACN: pH6.8 phosphate buffer in the ratio 90:10, 80:20, 70:30, ACN: water (90:10), methanol: pH 6.8 phosphate buffer (10: 90), ACN: methanol: water in the ratio 10:20:70, 20:10:70. A sharp chromatographic peak with good number of theoretical plates was observed with the mobile phase acetonitrile: pH 6.8 phosphate buffer (30:70). The mobile phase was optimized by conducting HPLC analysis with the mobile phase having the composition ACN: pH 6.8 phosphate buffer in the ratios of 35:65, 40:60 and 45:55. Various system suitability parameters such as theoretical plates and tailing factor were observed. Among these mobile phases, the mobile phase containing 60:40 ratio of 6.8 pH phosphate buffer: ACN was found to be more suitable at the flow rate 1ml/min. The chromatogram observed with this mobile phase offered more theoretical plates, high peak area, less tailing factor and low retention time. In the optimized conditions the retention times of Naratriptan, Sumatriptan, and zolmitriptan were about 3.130, 3.153, 2.143min, respectively. The system suitability compliance observed with the optimized mobile phase is given in table 2 and the developed LC method was validated.

Linearity and range

The chromatograms observed with the injection of different concentrations of Naratriptan, Sumatriptan and Zolmitriptan are also showed in overlay mode (figures 4 -6). Good linearity was observed in between chromatographic peak area and concentration in table 3 .The linear regression equation observed between these two parameters for Naratriptan, Sumatriptan and Zolmitriptan were, y=948.9599x+ 364.56, y=814.339x+ 265.086, y=837.414x+ 99.1689 with a

correlation coefficient 0.999.

Recovery and Accuracy

Accuracy of the method was established by subjecting the tablets prepared in our laboratory to recovery studies. The recovery studies were carried out in triplicate at three different concentration levels 80%, 100% and 120%. The % recovery was found to be in between 98.6 to 99.1 % and the corresponding data is shown in table 4. The experimental findings clearly indicated that the developed method is having good accuracy for the estimation of drug content from the pharmaceutical dosage forms.

Precision

The optimized method was analyzed for system precision, intermediate precision and method precision as per standard procedure. The method satisfied the precision requirement as the observed % RSD of peak area was found to be less than 2 (table 5a, 5b, 5c).

Limit of detection and limit of quantification

LOD and LOQ values were calculated based on the slope of calibration graph and standard deviation whose values are given in table 6. Lower LOD and LOQ values indicated better sensitivity of the method.

Robustness

Robustness of the method was performed to determine the capability of the intended method to withstand the small variations in the parameters like flow rate, wavelength variation, pH variation. The corresponding data was provided in table 7. The results obtained were unaffected by small variations in these parameters indicating the robustness of the method.

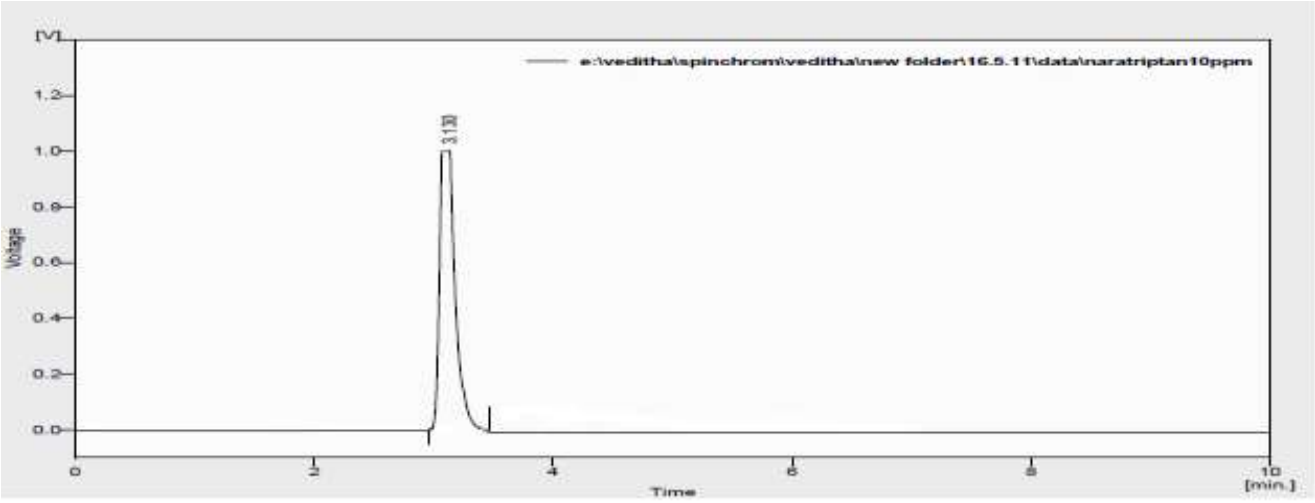


Figure 1 Typical chromatogram of Naratriptan

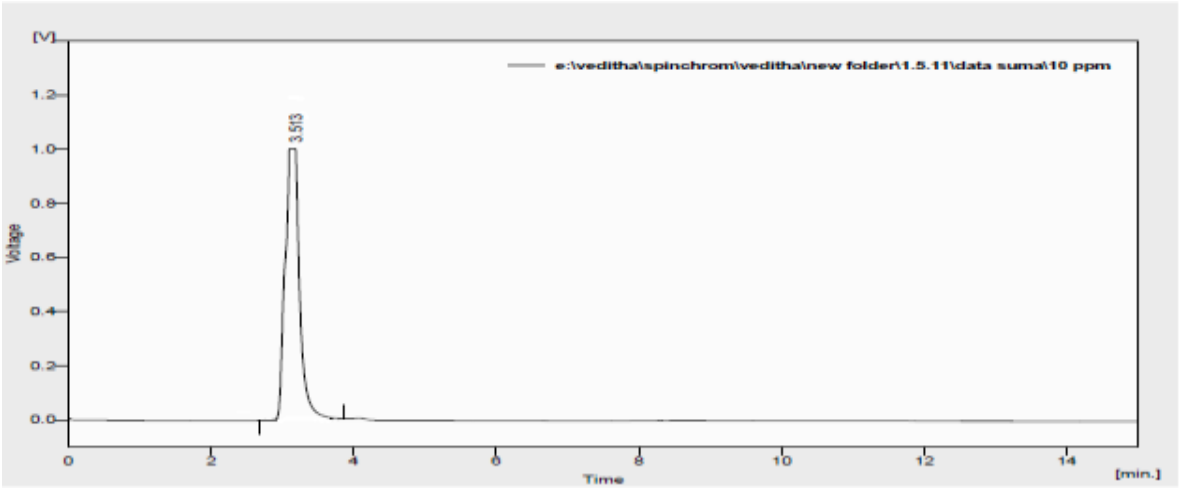


Figure 2 Typical chromatogram of Sumatriptan

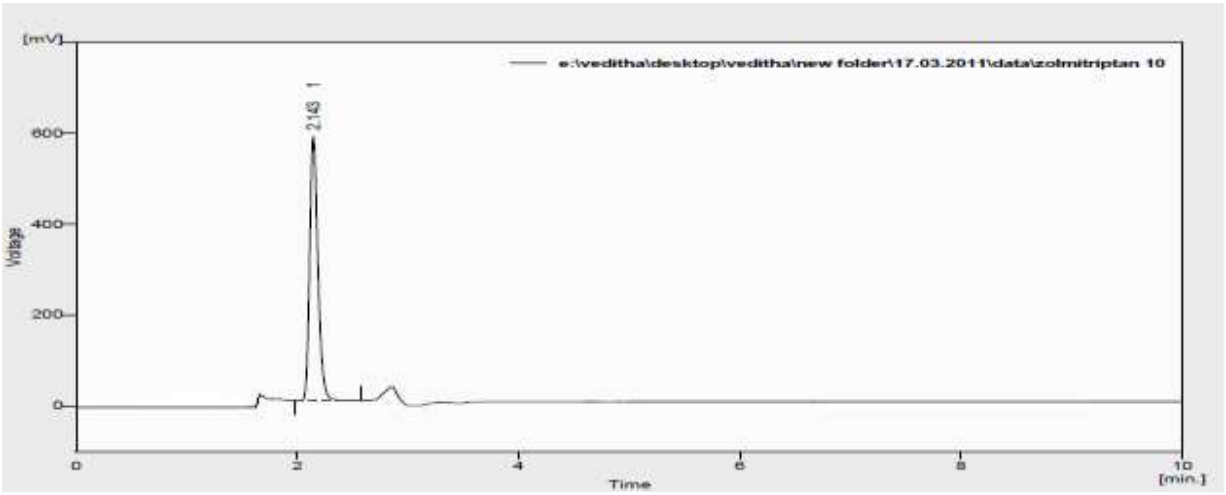


Figure 3 Typical chromatogram of Zolmitriptan

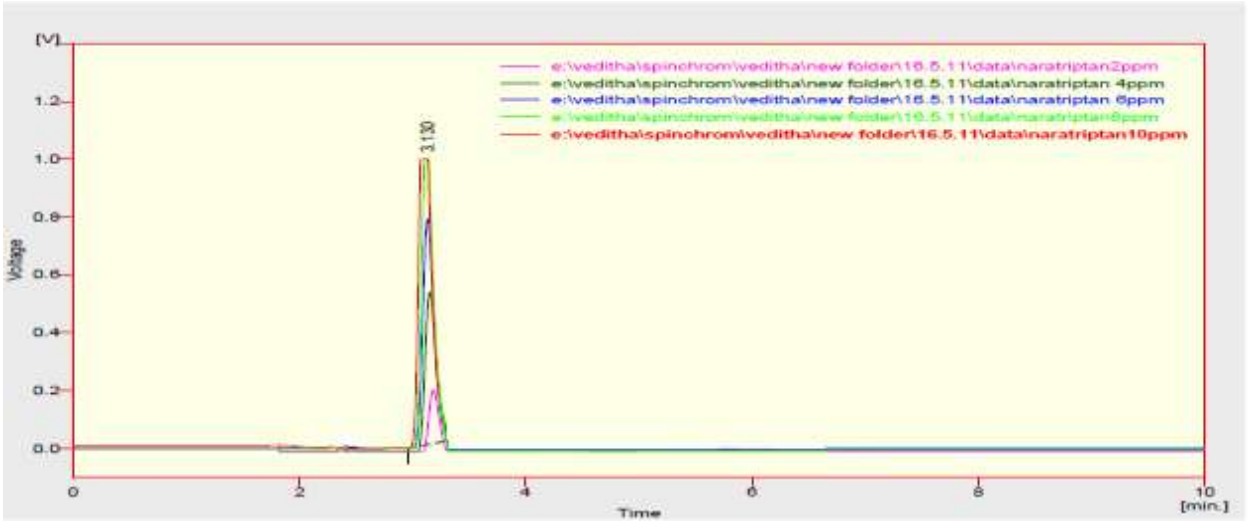


Figure 4: Chromatogram of Naratriptan indicating linearity (overlay mode)

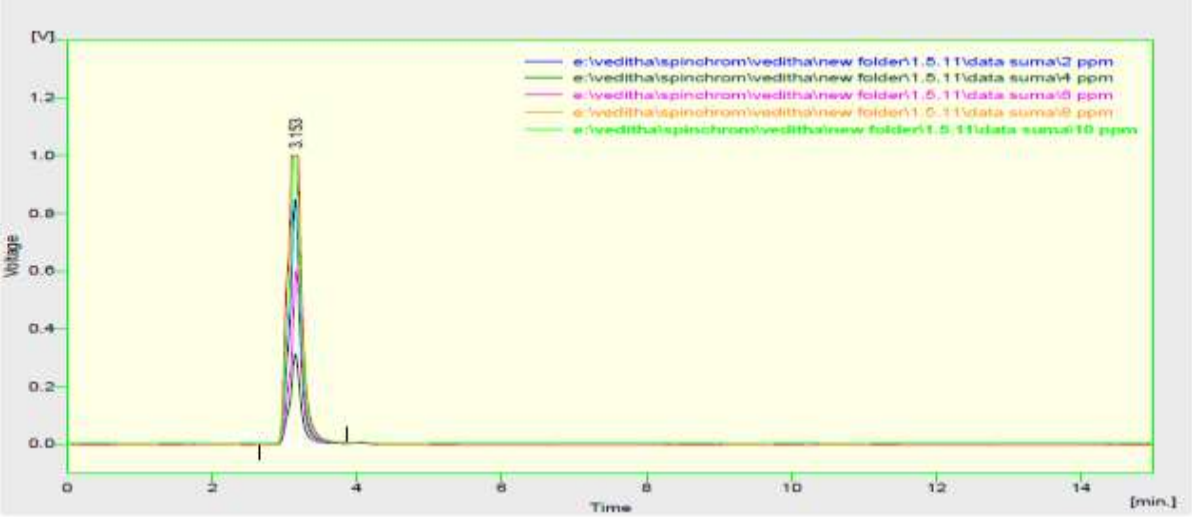


Figure 5: Chromatogram of Sumatriptan indicating linearity (overlay mode)

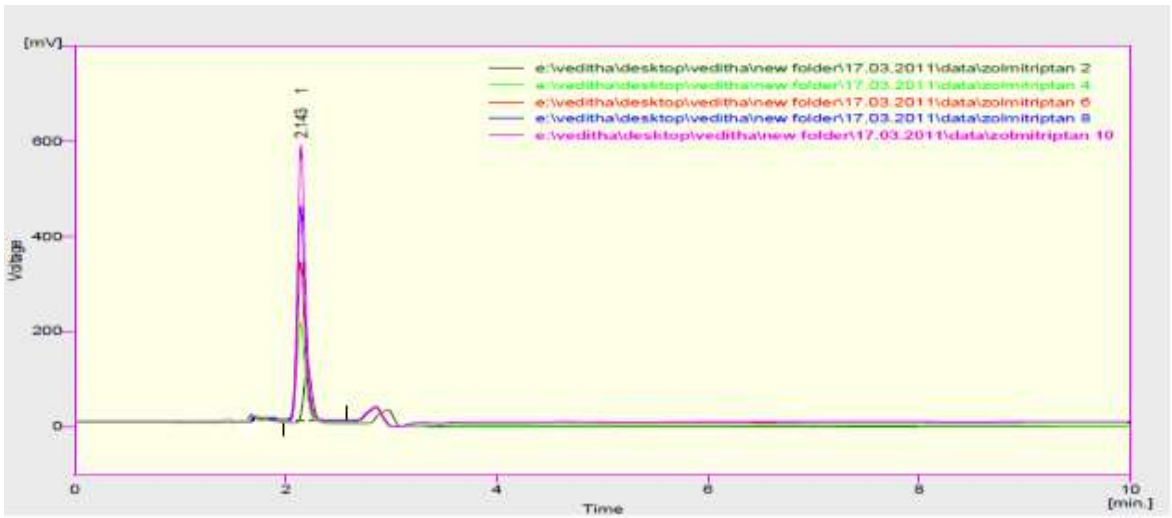


Figure 6: Chromatogram of Zolmitriptan indicating linearity (overlay mode)

Table1: Typical chromatogram of Naratriptan, Sumatriptan and Zolmitriptan

S. No	Concentration	Name of the peak	Retention time (min)
1	10µg/ml	Naratriptan	3.130
2		Sumatriptan	3.153
3		Zolmitriptan	2.143

Table 2: System suitability compliance observed with the optimized mobile phase

Parameter	Acceptance criteria	Observed values
Theoretical plates	NLT 3000	3054
Tailing factor	NMT 2.0	1.0
% RSD 0f peak area	NMT 2.0	0.40
% RSD 0f retention time	NMT 2.0	0.083

Table 3: Linearity Data of Naratriptan, Sumatriptan and Zolmitriptan

Concentration (mcg/ml)	Peak area of Naratriptan	Peak area of Sumatriptan	Peak area of Zolmitriptan
2	1954.821	2009.119	1761.721
4	3690.939	3786.692	3487.6
6	5632.312	5170.961	5362.988
8	7856.356	6811.722	6748.448
10	9356.243	8242.313	8356.687
Correlation Coefficient	0.999	0.999	0.999
Intercept	364.56	265.086	99.1689
Slope	948.959	814.339	837.414

Table 4: % Recovery data of Naratriptan, Sumatriptan and Zolmitriptan

% Level added	% Recovery of Naratriptan	% Recovery of Sumatriptan	% Recovery of Zolmitriptan
80	99.3	99.2	99.4
100	99.4	99.4	99.7
120	99.5	99.3	99.5

Table 5a: System precision readings of Naratriptan, Sumatriptan and Zolmitriptan

Concentration	Trials/ Injections	Peak area of Naratriptan	Peak area of Sumatriptan	Peak area of Zolmitriptan
6 µg/ml for Naratriptan, Sumatriptan& 10 µg/ml for Zolmitriptan	1	5632.312	5201.465	8356.687
	2	5655.857	5224.731	8350.378
	3	5698.232	5170.961	8423.647
	4	5621.238	5194.184	8387.387
	5	5598.938	5251.756	8427.644
	6	5589.233	5157.208	8362.356
Mean		5632.635	5194.050	8384.683
Standard deviation		39.978	25.914	34.147
% RSD		0.70	0.49	0.40

Table 5b: Intermediate precision readings of Naratriptan, Sumatriptan and Zolmitriptan

Concentration	Trials/ Injections	Peak area of Naratriptan		Peak area of Sumatriptan		Peak area of Zolmitriptan	
		Analyst I	Analyst II	Analyst I	Analyst II	Analyst I	Analyst II
6 µg/ml for Naratriptan, Sumatriptan& 10 µg/ml for Zolmitriptan	1	5628.912	5655.857	5189.657	5190.726	8389.183	8358.837
	2	5613.185	5598.938	5219.198	5177.366	8432.471	8345.274
	3	5614.587	5589.233	5234.019	5214.135	8442.372	8373.198
	4	5598.447	5632.312	5247.784	5218.387	8356.687	8389.827
	5	5632.312	5698.232	5178.467	5185.736	8378.387	8369.012
	6	5646.278	5621.238	5176.961	5167.384	8362.983	8339.372
Mean		5622.286	5632.635	5207.681	5192.289	8393.680	8262.586
Standard deviation		16.914	39.978	30.188	20.232	35.890	18.700
% RSD		0.30	0.70	0.57	0.38	0.42	0.22

Table 5c: Method precision readings of Naratriptan, Sumatriptan and Zolmitriptan

Concentration	Trials/ Injections	Peak area of Naratriptan	Peak area of Sumatriptan	Peak area of Zolmitriptan
6 µg/ml for Naratriptan, Sumatriptan& 10 µg/ml for Zolmitriptan	1	5632.312	5248.784	8356.687
	2	5614.587	5189.657	8327.984
	3	5598.447	5221.019	8233.764
	4	5613.185	5184.467	8438.373
	5	5642.978	5170.961	8347.112
	6	5589.817	5209.198	8421.897
Mean		5615.221	5204.014	8354.302
Standard deviation		19.969	28.288	73.371
% RSD		0.35	0.54	0.87

Table 6: Values of LOD&LOQ ofNaratriptan, Sumatriptan and Zolmitriptan

Parameter	Naratriptan	Sumatriptan	Zolmitriptan
LOD (ng/ml)	0.0194	0.0346	0.022
LOQ (ng/ml)	0.0647	0.1154	0.073

Table 7:Observations of chromatograms in Robustness study

Parameter		Flow rate			pH			Wave length		
		0.8	1	1.2	6.6	6.8	7.0	223	225	227
Retention time	Naratriptan	4.993	3.150	3.183	3.133	3.153	3.183	3.130	3.150	3.153
	Sumatriptan	4.193	3.510	1.933	3.511	3.510	3.517	3.505	3.510	3.507
	Zolmitriptan	3.180	2.143	1.180	2.143	2.143	2.147	2.163	2.163	2.173
Theoretical plates	Naratriptan	3019	3018	3016	3014	3016	3018	3077	3079	3074
	Sumatriptan	2928	2934	2937	3049	3054	3050	3122	3127	3125
	Zolmitriptan	3056	3054	3055	3026	3023	3025	3080	3083	3082
Tailing factor	Naratriptan	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Sumatriptan	1.0	0.9	1.0	1.0	1.0	1.0	1.0	0.9	1.0
	Zolmitriptan	1.1	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
RSD of peak area	Naratriptan	0.50	0.50	0.50	0.50	0.51	0.52	0.41	0.40	0.40
	Sumatriptan	0.40	0.40	0.41	0.37	0.37	0.37	0.41	0.42	0.42
	Zolmitriptan	0.41	0.40	0.40	0.37	0.37	0.36	0.37	0.37	0.37

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Naratriptan, Sumatriptan and Zolmitriptan in pure form and formulations. 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 Naratriptan, Sumatriptan and Zolmitriptan from bulk and formulations.

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