Original Article

A validated sensitive liquid chromatographic method for the estimation of Sumatriptan succinate in bulk drug and tablet dosage form

Abstract

Aim: The present study was undertaken to develop a validated, sensitive, rapid, simple and economic an isocratic HPLC method for estimating Sumatriptan succinate in tablet dosage form. **Materials and Methods:** Normal phase chromatographic analysis was performed on an Ascentis® Si HPLC Column (25cm×2.1mm, 5µm) with ammonium phosphate – acetonitrile (80:20, v/v, pH 3.5 adjusted with ortho-phosphoric acid) at a flow rate of 1 ml/min and detection wavelength of 230 nm. System suitability tests essential for the assurance of quality performance of the method were performed. The method was validated for accuracy, precision, reproducibility, specificity, and robustness, limit of detection (LOD), and limit of quantification (LOQ), as per International Conference on Harmonization (ICH) guidelines. **Results:** A single sharp peak was obtained for Sumatriptan succinate at retention time of 6.8±0.01 min. The polynomial regression data for the calibration plots exhibited good linear relationship (r=0.9999) over a concentration range of 50–1050 ng/ml and the linear regression equation was y=120.9x+33.56. Accuracy ranged from 99.96% to 101.49%. The LOD and LOQ values were 11 and 35 ng/ml, respectively. **Conclusion:** The proposed method gave good resolution of Sumatriptan succinate. System suitability tests and statistical analysis performed prove that the method is precise, accurate and reproducible, and hence can be employed for routine analysis of Sumatriptan succinate in bulk and commercial formulations.

Key words:
HPLC, sumatriptan succinate, sensitive, validated

Introduction

Sumatriptan succinate is a selective 5-hydroxytryptamine1 receptor subtype agonist and for the treatment of migrane headaches. Sumatriptan succinate is chemically designated as 3-[2-(dimethylamino) ethyl]-*N*-methyl-indole-5-methanesulfonamide succinate (1:1). Sumatriptan succinate is official in European Pharmacopoeia[1] and United Pharmacopoeia.[2]

Literature review reveals that few methods have been published for analysis of Sumatriptan succinate in the bulk form and in pharmaceutical preparations. Available methods including United Pharmacopoeia,[2] and European Pharmacopoeia,[1] which suggest chromatographic method for sumatriptan succinate, simultaneous RP HPLC,[3] simultaneous spectrophotometric,[4] HPTLC,[5] LC–MS fully automated solid phase extraction,[6] HPLC with fluorescence detection in plasma,[7] HPLC in plasma,[8] HPLC for transdermal diffusion study,[9] single spectrophotometric,[10] LC/Electrospray tandem mass spectrometry in human serum,[11] HPLC with electrochemical detection,[12] and densitometric TLC.[13] The disadvantages of other HPLC methods include low sensitivity, long analysis time, and mostly in plasma and tissue. The objective of this work was to develop and validate an isocratic HPLC–UV method for quantitative analysis of Sumatriptan succinate in a tablet

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dosage form. The validated method was also used for analysis of Sumatriptan succinate in commercially available tablets.

Materials and Methods

Chemicals

Sumatriptan succinate was received as a gift from Vesta Pharmachem Pvt. Ltd., Surat, India. Ammonium phosphate monobasic (HPLC grade), acetonitrile (HPLC grade) were purchased from Merck, India. Millipore purification system was used for high purity water. All other chemicals and reagents employed were of analytical grade and were purchased from CDH, India. MIGRATAN tab® Dabur brand of Sumatriptan succinate was procured form local pharmacy.

HPLC instrument and chromatographic conditions

HPLC was performed with a Shimadzu UV **(**Japan), UV–visible detector (190–1100 nm), and Operating Temperature range was 15–35^{0}C. Chromatographic response was measured in area under curve (AU). Chromatographic separation of Sumatriptan succinate was achieved at 27^{0}C using Ascentis® Si HPLC Column (25 cm×2.1 mm, 5 µm) column with a mobile phase containing mixture of ammonium phosphate (Monobasic) and acetonitrile (80:20, v/v) (pH 3.5 adjusted with ortho phosphoric acid) at a flow rate of 1.0 ml/min. Before use, the mobile phase was filtered through a 0.45 µm membrane filter (Whatman), under vacuum, and degassed. The detector was set at 230 nm and the injection volume was 50 µl.

Method development

Initial trial experiments were conducted, with a view to select a suitable solvent system for the accurate estimation of the drug and to achieve good resolution. The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, time required for the analysis, ease of preparation, and use of readily available cost-effective solvents. These included methanol– ammonium acetate (In 20:80, 70:30, 50:50, %v/v), acetonitrile–water, (In 20:80, 70:30, 50:50, %v/v), acetonitrile–ammonium phosphate (20:80, 70:30, 50:50, %v/v). A mobile phase system comprising of acetonitrile–ammonium Phosphate (20:80, %v/v) was found to be optimum and further optimized by adjusting pH 3–4 by adding ortho phosphoric acid. The Composition acetonitrile–ammonium Phosphate (20:80, %v/v) with pH 3.5 gave the best resolution, sensitivity and free from tailing. The same solvent mixture was used for the solubilization of the drug from the formulation containing excipients. The solvents were mixed, filtered through a membrane filter of 0.45µm pore and degassed before use.

Method validation

Linearity

A series of standard curves were prepared over a concentration range of 50–1050 ng/ml from a stock solution of Sumatriptan succinate (1000 ng/ml) in acetonitrile. Dilutions were prepared in the mobile phase: Acetonitrile–ammonium phosphate (20:80, %v/v). The data from peak area versus drug concentration plots were treated by linear least square regression analysis. The standard curves were evaluated for intra-day and inter-day reproducibility and are reported in Table 1 and Figure 1. The experiment was performed in triplicate.

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of Sumatriptan succinate (300 ng/ml) were spiked with 50, 100, and 150% extra Sumatriptan succinate standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate.

Precision

The three components of precision, i.e., repeatability, intermediate precision and reproducibility, in accordance with ICH recommendations, were determined as follows:

Injection repeatability

Five injections of 600 ng/ml solution of Sumatriptan succinate were analyzed and %RSD calculated for injection repeatability.

Analysis repeatability

It was obtained by determining the relative standard

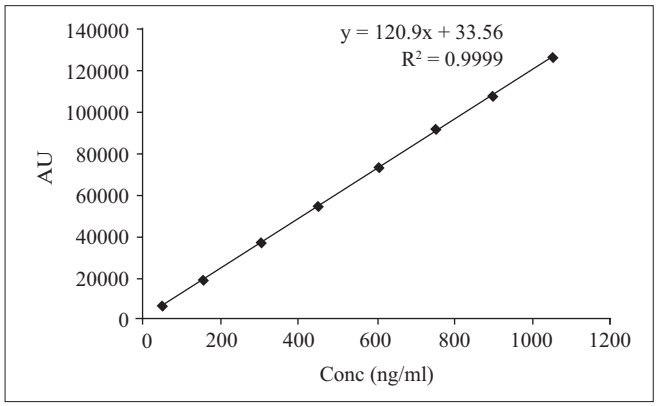

Figure 1: Calibration curve

Table 1: Validation parameters

Parameters	Results
Linearity range	50–1050 ng/ml
Correlation coefficient	0.9999
Slope	120.9
Intercept	33.56
Retention time	6.8
Theoretical Plates	7896
LOQ	11 ng/ml
LOD	35 ng/ml

deviation (RSD) of replicate samples of the intermediate precision and reproducibility study.

Intermediate precision (Interday variation)
Measurement of inter-day variation of Sumatriptan succinate solutions at 450ng/ml concentrations in triplicate on five consecutive days determine the intermediate precision.

Reproducibility
The reproducibility of the method was checked by determining precision on the same instrument, but by a different analyst. For both intra- and inter-day variation, solutions of Sumatriptan succinate at concentration (600 ng/ml) were analyzed in triplicate.

LOD and LOQ
In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ) values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level while ten times the noise value gave the LOQ.

Robustness
The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of Sumatriptan succinate. Robustness was determined by using reagents from two different lots and two different manufacturers.

Sample solution stability
The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution for 72 h under laboratory bench conditions (25±1°C) and under refrigeration (8±0.5°C). The solution was subjected to HPLC analysis immediately and after a period of 24, 48, and 72 h.

Specificity/selectivity
The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method. Better resolution was found for the drug peak with no interference proved that the method was found to be selective to the drug.

System suitability tests
The chromatographic systems used for analyses must pass the system suitability limits before sample analysis can commence. The injection repeatability, theoretical plate numbers (*N*) for the principal peak were the parameters tested on a 50 ng/ml sample of Sumatriptan succinate to assist the accuracy and precision of the developed HPLC system.

Analysis of sumatriptan succinate in marketed tablets
Ten tablets (strength: 50 mg/tablet) were crushed and triturated well in a mortar. A powder sample, equivalent to 0.5 mg of Sumatriptan succinate was accurately weighed and transferred to a 50 ml volumetric flask, extracted into acetonitrile and mixed thoroughly for 30 min using a sonicator. The solution was filtered through 0.45µm pore filter after making up the volume, This tablet stock solution (10000 ng/ml) was further 100 times adequately diluted with mobile phase to obtain approximately 300 ng/ml drug and analyzed by the proposed HPLC method and results are reported in Table 2. The possibility of interference of excipients with the analysis was studied.

Results

Method development
Acetonitrile-ammonium phosphate (20:80, %v/v) was selected as the optimum mobile phase. Under these conditions the retention time and tailing factor were 6.8 min and 0, respectively. A typical chromatogram is represented in Figure 2.

Method validation
Linearity
Peak area versus drug concentration was plotted to construct a standard curve for Sumatriptan succinate [Figure 1]. The polynomial regression for the calibration plots showed good linear relationship with coefficient of correlation, *r*=0.9999; slope=120.9 and intercept=33.56 over the concentration

Table 2: Analysis of tablet dosage form

S N.	Dilution used (ng/ml)	Brand	Amount per tablet	Area under curve	Amount found (ng/ml)	% Drug found
Run 1	300	MIGRATAN tab® Dabur	50 mg/ tab	36315	300.09	100.02
Run 2				36313	300.07	
Run 3				36315	300.09	
Mean					300.083	
%RSD					0.0038	

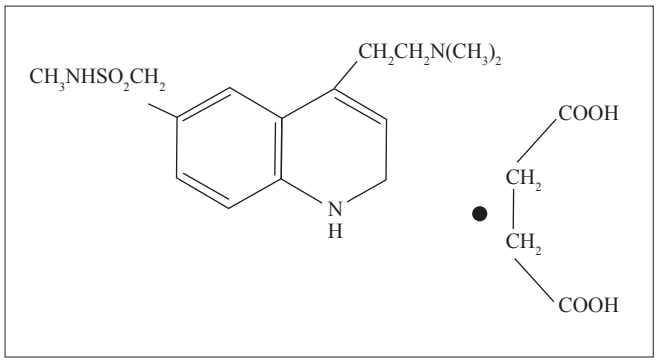

Figure 2: Chemical structure of Sumatriptan succinate

range studied. The range of reliable quantification was set at 50–1050 ng/ml as no significant difference was observed in the slopes of the standard curves in this range. The linear regression data for the calibration plot is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance.

Precision

Precision was measured in accordance with ICH recommendations. Five consecutive injections of 600 ng/ml solution of Sumatriptan succinate by the proposed method showed excellent injection repeatability with %RSD of only 0.0009 and are reported in Table 3.

Repeatability of sample injection was determined as intraday variation while intermediate precision was determined by measuring interday variation for triplicate determination of Sumatriptan succinate at three different concentrations. The results of the determination of repeatability, intermediate precision and reproducibility are listed in Table 4. Reproducibility was checked by measuring the precision of the proposed method with analysis being performed by another person and are reported in Table 5. The low RSD values indicate the repeatability and reproducibility of the method.

Recovery

The recovery of the method, determined by spiking a previously analyzed test solution with additional drug standard solution, was found to be in the range of 99.96–101.49%.

Table 3: Injection repeatability

S. no.	Area under curve	%RSD	SEM
Inj 1	72511	0.0009	0.3162
Inj 2	72511		
Inj 3	72512		
Inj 4	72510		
Inj 5	72511		

Conc.: 600 ng/ml, Volume injected: 50 μl, $n=5$

The values of recovery (%) listed in Table 6 indicate the method is accurate.

Detection and quantification limits

The limit of detection was found to be 11 ng/ml where the drug could be detected without any noise. The limit of quantification was 35 ng/ml. This indicated the method can be used for detection and quantification of Sumatriptan succinate over a very wide range of concentrations.

Robustness

There was no significant change in the retention time of Sumatriptan succinate when reagents (acetonitrile and ammonium phosphate) from different lots and different manufacturers were used.

Stability

There was no significant change in analyte composition (sample concentration=300 ng/ml) over a period of 72 h. The mean RSD between peak areas, for the samples stored under refrigeration (8±1°C) and at laboratory temperature (25±1°C) was found to be 0.990% and 0.771% respectively, suggesting that the drug solution can be stored without any degradation over the time interval studied.

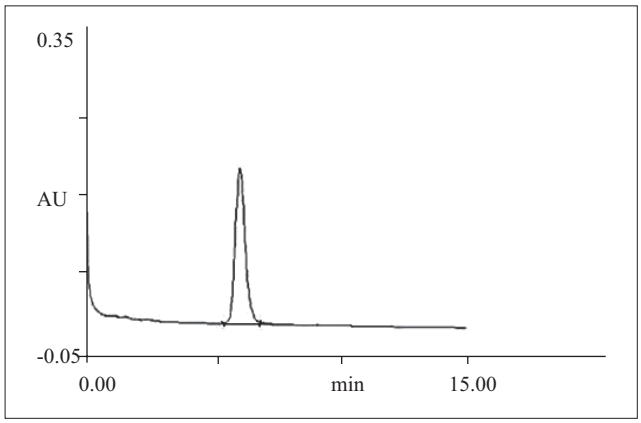

Figure 3: A chromatogram for Sumatriptan succinate

Table 4: Intermediate precision

	Interday						
Time gap	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	%RSD
Intraday							
0 Hour	54438	54440	54439	54438	54439	54438.8	0.0015
2 Hours	54439	54439	54436	54439	54439	54438.4	0.0024
6 Hours	54438	54437	54438	54438	54440	54438.2	0.0014
12 Hours	54440	54438	54439	54440	54437	54438.8	0.0024
24 Hours	54439	54441	54437	54439	54438	54438.8	0.0027
Mean	54438.8	54439	54437.8	54438.8	54438.6		
%RSD	0.0015	0.0022	0.0023	0.0015	0.0021		
Average							
Interday							0.0021
Intraday							0.0019

Table 5: Reproducibility

	Run 1	Run 2	Run 3	Run 4	Run 5	%RSD	SEM
Analyst 1	72512	72511	72511	72512	72511	0.002	0.0668
Analyst 2	72510	72511	72513	72512	72510		
Analyst 3	72512	72510	72512	72511	72512		

Conc.=600 ng/ml, n=5

Table 6: Recovery studies

Recovery level (%)	Amount in dilution (ng/ml)	Amount of Sumatriptan succinate added (ng/ml)	Total area under curve	Amount recovered (ng/ml)	% Recovery
50	300	150	54432	449.94	99.96
100		300	72517	599.53	99.84
150		450	91522	756.72	101.49

Specificity

A single peak was observed at the retention time of Sumatriptan succinate when a suitably diluted solution of the tablet formulation was chromatographed [Figure 3]. No interaction was observed between Sumatriptan succinate and excipients present in the tablets. The Sumatriptan succinate content was found to be 100.02% and the RSD was 0.0038%. The low RSD indicated the suitability of this method for routine analysis of Sumatriptan succinate in pharmaceutical dosage forms.

Discussion

The final decision on mobile phase composition and flow rate was made on the basis of peak shape, peak area, tailing factor, baseline drift and time required for analysis.

The solvent system selected acetonitrile–ammonium phosphate gave good resolution of drug peak. No internal standard was used because no extraction or separation step was involved. Other mobile phases tried resulted either in much lower sensitivity, delayed retention time or poor peak shapes, and so were not considered.

The proposed HPLC method of analysis was also found to be precise and accurate, as depicted by the statistical data of analysis. High values of correlation coefficients and small values of intercepts validated the linearity of the calibration plots and obedience to Beer's laws. The RSD values and the slopes and intercepts of the calibration graphs indicate the high reproducibility of the proposed method. The method was also found to be robust as there was no significant change in the peak area, peak shape and retention time of Sumatriptan succinate. Furthermore, the low values of LOD and LOQ indicate that the method can be employed over a wide concentration range for linearity.

Conclusion

The HPLC method developed is accurate, precise, reproducible, and specific. The method is linear over a wide range, economical and utilizes a mobile phase which can be easily prepared. All these factors make this method suitable for quantification of Sumatriptan succinate in bulk drugs and in pharmaceutical dosage forms. It can, therefore, be concluded that use of the method can save much time and money and it can be used even in small laboratories with very high accuracy and precision. The method can also be used for the routine analysis of Sumatriptan succinate in bulk preparations of the drug and in pharmaceutical dosage forms without interference.

References

1. The European Pharmacopoeia, 6th ed, Vol 2, Strasbourg: European Directorate for the Quality of Medicine; 2008. p. 3005.
2. The United States Pharmacopoeia. 31st Revision, Vol 3. Rockville MD: US Pharmacopoeial convention. Inc; 2008. p. 3310.
3. Havaldar FH, Vairal DL. Simultaneous estimation of sumatriptan succinate and ergotamine tartrate in the tablet dosage form. Int J of Chem and Anal Sci 2010;1:76-8.
4. Trinath M, Banerjee SK, Teja HD, Bonde CG. Development and validation of spectrophotometric method for simultaneous estimation of Sumatriptan and Naproxen sodium in tablet dosage form. Der Pharmacia Sinica 2010;1:36-41.
5. Shah R, Shah N, Suhagia B, Shah C. Development and validation of a hptlc method for the estimation of sumatriptan in tablet dosage forms. Ind J of Pharma Sci 2008;70:831-4.
6. Xu X, Bartlett MG, Stewart JT. Determination of degradation products of sumatriptan succinate using LC-MS and LC-MS-MS. J Pharm Biomed Anal 2001;26:367-77.
7. Ge Z, Tessier E, Neirinck L, Zhu Z. High performance liquid chromatographic method for the determination of sumatriptan with fluorescence detection in human plasma. J of Chromat B 2004;806:299-303.
8. Majithiya RJ, Majithiya JB, Umrethia ML, Ghosh PK, Murthy. HPLC method for the determination of sumatriptan in plasma and brain tissue. Ars Pharm 2006;47:199-210.
9. Femenía Font A, Merino V, Rodilla V, López-Castellano A. High-performance liquid chromatographic determination of sumatriptan after *in vitro* transdermal diffusion studies. J Pharm Biomed Anal 2005;37:621-6.
10. Prabahar EA, Kalaichelvi R, Thangabalan R, Karthikeyan B, Prabhakar Ch, Vijayaraj Kuma P. Validated spectroscopic method for estimation of sumatriptan succinate in pure and from tablet formulation. Res J Pharm Techn 2009;2:495-7.
11. Vishwanathan K, Bartlett MG, Stewart JT. Determination of antimigraine compounds rizatriptan, zolmitriptan, naratriptan and sumatriptan in human serum by liquid chromatography/electrospray tandem mass spectrometry. Rapid Commun Mass Spectrom 2000;3:168-72.
12. Dunne M, Andrew P. Fully automated assay for the determination of sumatriptan in human serum using solid-phase extraction and high-performance liquid chromatography with electrochemical detection. J Pharm Biomed Anal 1996;14:721-6.
13. Bebawy LI, Moustafa AA, Abo Talib NF. Stability-indicating methods for the determination of sumatriptan succinate. J Pharm Biomed Anal 2003;32:1123-33.

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