Research Article

Stability Indicating RP-HPLC Method for the Quantification of Sumatriptan Succinate in Bulk and Tablet Dosage Form

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ABSTRACT

A rapid, simple, precise, accurate and robust HPLC method has been established and authorised for quantification of Sumatriptan Succinate in tablet dosage form. Chromatographic separation was conducted on Waters HPLC equipped with photo diode array detector using Kromasil C18 column (150mm x 4.6 mm i.d., 5 µm particle size) as stationary phase. Mobile phase consisted of Phosphate buffer pH 6.5: Acetonitrile in the ratio of 75:25% v/v, set at a flow rate of 1 mL/minute and detection wavelength was set at 234 nm. Sumatriptan Succinate follows linearity in the concentration range of 0.2-1.2 µg /mL with good correlation coefficient value of 0.9995. The % RSD value for intra-day and inter-day precision is less than two indicates that the method is precise. The % recovery was found to be in the range of 99.49 - 100.04%. Percentage assay of Sumatriptan Succinate tablets (Sumitrex 100mg) was found to be 100.34%. The developed RP-HPLC method was validated as per the ICH Q2 (R1) guidelines and was found to be specific, sensitive, accurate, precise and robust.

Keywords: Sumatriptan succinate, RP-HPLC, Stability, Validation.

INTRODUCTION

Sumatriptan Succinate [fig.1] is a Serotonin 5HT-1 receptor agonist, used as an Anti-Migraine drug with a pKa of 9.54 and log P is 1.17. It is freely soluble in distilled water, sparingly soluble in methanol and practically insoluble in methylene chloride[1]. Migraine is a neurologoical disorder, characterized by multiple phases: premonitory, aura, headache, postdrome and interictal and is manifested by nausea, vomiting, phonophobia and photophobia[2]. Literature survey revealed that several RP-HPLC based methods have been reported for the estimation of Sumatriptan which were time consuming and expensive[3-11]. The aim of the present work was to develop simple, rapid, sensitive, specific, accurate, precise, economic and reliable RP-HPLC method for the quantification of Sumatriptan succinate in bulk and tablet dosage form suitable for routine quality control analysis.

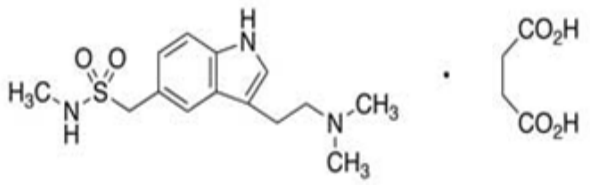

Figure 1: Structure of Sumatriptan Succinate

MATERIALS AND METHODS

Chemicals

Sumatriptan succinate reference standard was received as gift sample from Dr.Reddy's Laboratories, Hyderabad and sample tablets (Label claim: 100mg; Sumitrex tablets, manufactured by Sun Pharmaceuticals) were procured from a local medical shop. HPLC grade Acetonitrile and water, OrthoPhosphoric acid (AR grade were purchased from Merck Specialities Private Ltd., Mumbai and Potassium dihydrogen phosphate from Sd Fine Chem. Limited, Mumbai.

Chromatographic Conditions

HPLC-Waters chromatographic system equipped with PDA detector was used for the method development. The output signal was monitored and processed using Empower 2 software. Chromatographic separation was carried out on Kromasil C_{18} column (150 x 4.6mm, 5µ) column at 30°C. The mobile phase containing phosphate buffer pH 6.5 and acetonitrile in the ratio of 75:25 (%v/v) was pumped at 1.0 ml/min and detection was carried out at 234 nm. The injection volume for standard and sample was 10µl (fixed loop) and the total run time was 5 min [Figure 2, table 1].

Table 1: Optimized Chromatographic Conditions for Sumatriptan Succinate

Parameter	Optimized Conditions
Column	Kromasil C18 (150mm x 4.6mm, 5µm)
Mobile phase	Phosphate buffer pH 6.5: Acetonitrile (75:25% v/v)
Elution	Isocratic
Flow rate	1.0 ml/min
Detector and λ_{max}	PDA and 234 nm
Column temperature	30^0C
Injection volume	10 µl
Diluent	HPLC grade water : ACN [1:1]
Run time	5.0 min

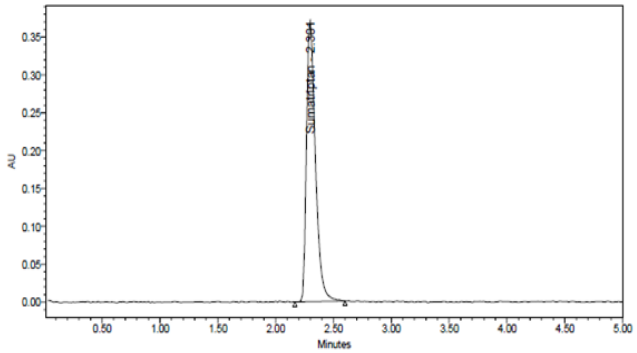

Figure 2: Optimized Chromatogram of Sumatriptan Succinate

Preparation of Phosphate buffer pH 6.5:

Phosphate buffer of pH 6.5 was prepared by accurately weighing and transferring 1.36g of potassium dihydrogen orthophosphate to a 1000ml calibrated volumetric flask. About 900ml of milli-Q water was added and the solution was then degassed with an ultrasonicator for 20 minutes. Final volume was made up to the mark with milli-Q water and then the pH was adjusted to 6.5 with dilute orthophosphoric acid solution using a calibrated pH meter. The solution was then filtered through 0.22µ nylon filter using a vacuum filtration apparatus.

Preparation of standard solution of Sumatriptan Succinate:

Stock Solution-I (1000µg/ml)

Approximately 10 mg of Sumatriptan Succinate API was carefully weighed and placed into a 10 ml calibrated volumetric flask. 5 ml of diluent was added, which was then sonicated for 10 minutes to thoroughly dissolve the drug. The diluent was added to the make up the final volume and then filtered using a 0.45 PTFE filter.

Stock Solution-II (10µg/ml)

About 0.1 ml of stock solution-I was transferred into a 10ml calibrated volumetric flask and the volume was then made up to the mark with the diluent.

Working standard Solution (0.8µg/ml)

Working standard solution was prepared by transferring 0.8 ml of standard stock solution-II into a 10 ml calibrated volumetric flask and making the volume up to the mark with the diluent. Accurately 10 µl was injected into the HPLC system and chromatogram was recorded.

Preparation of Sample solution of Sumatriptan Succinate:

Sample Stock Solution-I (1000µg/ml)

Twenty tablets (Sumitrex-100mg) were weighed and the average tablet weight was determined. The tablets were then crushed to fine powder and the tablet powder equivalent to 100 mg of Sumatriptan Succinate was accurately weighed and transferred to a 10 ml calibrated volumetric flask, about 5ml of diluent was added, sonicated for 10min to completely dissolve the drug and finally the volume was made up to the mark with the diluent. The solution was then filtered through 0.45 µ PTFE filter.

Sample Stock Solution-II (10µg/ml)

About 0.1ml of sample solution-I was pipetted out and transferred to a 10ml calibrated volumetric flask and then the volume was made up to the mark with the diluent.

Working sample (0.8µg/ml)

Working sample solution was prepared by diluting about 0.8 ml of sample stock solution-II to 10ml in a 10ml calibrated volumetric flask. Accurately 20 µl was injected into the HPLC system and chromatogram was recorded and the peak area was recorded at 234nm.

VALIDATION OF THE DEVELOPED METHOD

The method developed was validated as per ICH guidelines for Specificity, Stability testing, Accuracy, Precision [Intra-day and Inter-day Precision], Linearity, Limit of Detection [LOD], Limit of Quantitation [LOQ], Stability of standard solution, Robustness and System suitability parameters[12].

Specificity

Specificity of the developed method was confirmed by injecting about 10 µl of the blank, standard and sample working solutions into the HPLC column six times to check the interference of other peaks with the main drug peak.

Forced degradation studies[13]

Specificity and stability of the drug were assessed by performing the forced degradation studies under different stress conditions.

Table 2: Degradation of Sumatriptan Succinate

Stress Condition	Sumatriptan Succinate			
	Purity angle	Purity threshold	% Assay	% degraded
Acid	0.244	1.527	93.03	6.97
Alkali	1.040	1.431	95.22	4.78
Oxidative	0.822	1.033	95.87	4.13
Thermal	0.899	1.080	97.74	2.26
Photolytic	0.809	1.051	98.23	1.77
Neutral	0.874	1.131	99.02	0.98

Table 3: Accuracy [% Recovery] of Sumatriptan Succinate

% Spiked level	Fixed sample concentration (µg/ml)	Amount Spiked (µg/ml)	Total Amount recovered (µg/ml)	% Recovery	Statistical Analysis	
					Mean ± SD	% RSD
80	0.8	0.64	1.427	99.10	99.49±0.357	0.356
			1.434	99.58		
			1.437	99.79		
100	0.8	0.8	1.601	100.06	100.04±0.156	0.156
			1.599	99.88		
			1.603	100.19		
120	0.8	0.96	1.763	100.18	100.04±0.447	0.447
			1.752	99.54		
			1.767	100.40		

Table 4: Robustness of Sumatriptan Succinate

Parameter	Variation	% RSD of Peak Area [NMT 2.0 %]	Theoretical plates* [N>2000]	Tailing factor* <2.0
Flow rate (ml/min)	0.9	0.9	5093	1.38
	1.0	0.6	4947	1.38
	1.1	1.0	4686	1.35
Mobilephase (%v/v) [Phosphate buffer pH 6.5 : ACN]	80:20	0.3	4990	1.36
	75 :25	0.6	4947	1.38
	70 :30	0.5	5039	1.36
Temperature (^{0}C)	25	0.3	4706	1.38
	30	0.6	4947	1.38
	35	0.8	4893	1.36

* All the values were expressed as mean of six determinations

Table 5: Summary of Validation Parameters of Sumatriptan Succinate

Parameter		Values	ICH Limits
System Suitability Parameters		% RSD of peak area – 0.6	NMT 2.0
		Theoretical plates- 4947	MT 2000
		Tailing factor-1.38	NMT 2.0
Range [µg/ml]		0.2-1.2	-
Correlation coefficient [R^2]		0.9995	NLT 0.999
% Recovery		99.49-100.04	98-102
% RSD	Intra-day Precision	0.5	NMT 2.0
	Inter-day Precision	0.2	NMT 2.0
LOD [µg/ml]		0.0024	-
LOQ [µg/ml]		0.0071	-
Solution stability, % Assay difference at 24 hrs		1.64	NMT 2.0
% Assay		100.34 ± 0.82	90-110

Acid Degradation

Acid degradation was done by taking 1ml of standard stock solution – II, 1ml of 2N HCl and refluxed for 30min at 60±2^{0}C. 0.8ml of the acid refluxed solution was taken into a 10ml calibrated volumetric flask and the volume was made upto the mark with diluent.

Alkali Degradation

Alkaline or basic degradation study was done by adding 1ml of standard stock solution – II and 1ml of 2N NaOH which was then refluxed for 30min at 60±2^{0}C. After 30min, about 0.8ml of this solution was transferred into a 10 calibrated volumetric flask and the volume was made upto the mark with the diluent.

Oxidative Degradation

About 1ml of standard stock solution-II and 1ml of 20%v/v hydrogen peroxide solution were kept for 30min at 60±2^{0}C. After 30min, about 0.8 ml of this solution of Sumatriptan Succinate was pipetted out and made to 10ml with diluent in a 10ml calibrated volumetric flask.

Thermal Degradation

Thermal degradation of Sumatriptan Succinate was done by exposing standard stock solution-II to a temperature of 105±2^{0}C in hot air oven for 6 hrs. After 6hrs, about 0.8 ml of thermally exposed solution of Sumatriptan Succinate was diluted to 10ml with the diluent.

Photolytic degradation

Photolytic degradation of Sumatriptan Succinate was estimated by exposing standard solution-II to UV light in a UV Cabinet for a time period of 7 days. 0.8µg/ml concentration was prepared by diluting 0.8ml of the solution to 10ml with the diluent in a 10ml calibrated volumetric flask.

Neutral Degradation

Standard stock solution-II was refluxed with HPLC grade water for 6 hrs at 60±2^{0}C. The solution was diluted to 10 ml with the diluent to prepare 0.8µg/ml.

Approximately 10 µl of each solution exposed to various stress conditions were injected separately into the column and the chromatograms were recorded. Peak area of the working standard solution and the peak area of stressed condition were compared to determine the % degradation which helps to evaluate the stability of the drug [Table.2].

Accuracy

Recovery studies at 80, 100, and 120 percent levels were performed by standard addition method to determine the accuracy of the developed method. To 0.8ml of pre-analysed sample concentration, 0.64ml (80%), 0.8ml (100%) and 0.96ml (120%) of standard solution – II was spiked in a 10ml calibrated volumetric flask and made upto the volume with the diluent. About 10µl volume of spiked solutions were injected into the HPLC column and the chromatograms were recorded. Percent recovery was calculated by comparing the peak area obtained with the peak area of standard concentration in the linearity curve [Table.3].

Precision

Intra-day precision (on the same day) and inter-day Precision (on consecutive days) were done to evaluate precision studies. Working standard solution (0.8µg/ml) was prepared and injected into the column six times on the same day and on consecutive days to assess intra-day and inter-day precision the developed method was demonstrated by intra-day and inter-dayprecision. % RSD of the peak areas were calculated [Table.5].

Linearity

Linearity for the developed method was done for six different concentrations. Exactly 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2ml of standard stock solution - II were transferred to six individual 10ml calibrated volumetric flasks and the volume was made upto the mark with diluent to get concentrations of 0.2-1.2 µg/ml. About 10µl of the resultant solutions were injected three times into the column and the chromatograms were recorded. A graph was plot by taking concentration of drug on x-axis and peak area of the respective concentration on y-axis. R^2 [Fig.3] was calculated by using the method of least squares in MS Office Excel 2007 [Table.5].

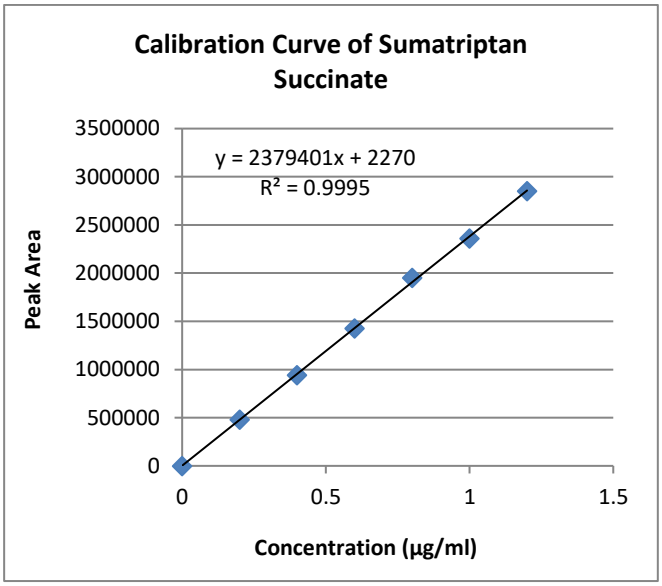

Figure 3: Calibration Curve of Sumatriptan Succinate

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of Sumatriptan Succinate were calculated by using the formulae,

$$\boldsymbol{LOD = 3.3\, x\, \frac{\sigma}{s}}$$

$$LOQ = 10\, x\, \frac{\sigma}{s}$$

where σ – standard deviation of y-intercepts of calibration curve

s – slope of the calibration curve

The calculated concentration solutions were prepared and about 10µl were injected into the column and checked for the signal to noise (s/n) ratio [Table.5].

Stability of standard solution

Working standard solution of Sumatriptan Succinate was freshly prepared and kept for about 24hrs at temperature of 30 ± 2°C. About 10 µl of the solution of time intervals were injected six times into the column immediately after preparation of solution and after 24hrs. % assay difference was calculated [Table.5].

Robustness

Minute variations in the chromatographic conditions such as flow rate (± 0.1ml/min), organic phase composition (± 5%v/v) in the mobile phase, temperature (± 5°C) of the optimised method were done to evaluate method robustness. Changes in the present method were flow rate 0.9 to 1.1 ml/min, ACN ratio in the mobile phase 20 – 30%v/v and temperature 25^0C to 35^0C. About 10 µl of standard working solution of Sumatriptan Succinate was injected into the column and chromatograms were recorded at the varied chromatographic conditions. System suitability parameters i.e., %RSD of peak area, theoretical plates and tailing factor were evaluated [Table.4].

System suitability testing

Working standard solution (0.8µg/ml) of Sumatriptan Succinate was prepared and about 10 µl of the solution was injected into the column. % Relative Standard Deviation of peak areas, theoretical plates, tailing factor were assessed from the recorded chromatograms [Table.5].

Assay

Working sample solution of Sumatriptan Succinate was injected six times into the column. Peak areas were compared with the mean of standard peak area for calculating the % assay by using the formula [Table.5].

$$\% \, Assay = \frac{Sample\ area}{Standard\ area} \times \frac{dilution\ of\ standard}{dilution\ of\ sample} \times \frac{P}{100} \times \frac{Avg.wt}{LC} \times 100$$

Where P - Potency of the standard drug

Avg. wt = average weight of each tablet

LC= Label Claim

RESULTS AND DISCUSSION

Based on the solubility studies, Diluent chosen was Water [HPLC grade]: Acetonitrile in equal volume, as this combination showed better results when compared to HPLC water alone.

Method Optimisation

Trial runs were conducted to determine the optimum conditions by varying stationary phase and mobile phase, its combination and composition, pH and temperature. Mobile Phase used was Phosphate buffer (pH 6.5) and Acetonitrile in the ratio of 75:25 %v/v. pH of the buffer should be ±2 of the pKa value of the drug. So, the pH of the buffer 6.5 was selected in order to get a good peak shape satisfying all the system suitability parameters. Stationary phase selected was C18 (ODS) column as it is tough and highly retentive.

The optimized condition for the quantitation of Sumatriptan Succinate was Kromasil C18 (150 mm x 4.6mm, 5 µm) column, Phosphate buffer (pH 6.5): ACN [75:25 %v/v] as mobile phase at a flow rate of 1.0 ml/min, column temperature maintained at 30 ± 2^0C and detection wavelength of 234nm. Sumatriptan Succinate was eluted at 2.30 ± 0.2 minutes with a run time of 5.0 minutes.

Method Validation

The method was validated in accordance to ICH guidelines. In the specificity study, no extra peaks were found in the chromatograms of the blank, placebo, standard, and sample when the respective solutions were injected into HPLC, indicating that no excipients interfered with the main drug peak confirming that the method was specific.

Stability indicating assay method confirms the stability of the drug in various exposed stress conditions. The drug was considered stable, when the purity threshold was larger than the purity angle, and the percent degradation should be less than 20. When Sumatriptan Succinate was subjected to various stress conditions of 2N HCl, 2N NaOH, 20 % v/v H_2O_2, HPLC grade water at 60 ± 2 °C for 30 min, in the oven at 105 ± 2 °C for 6 h and in the UV chamber for 7 hours, the observed purity angle (0.244 – 1.040) was lesser than the purity threshold (1.033 – 1.527), indicating that the degradant peaks did not co-elute with the main drug. The percent degradation was 0.98 – 6.97 showing that the developed method was stable.

Accuracy was measured in percent recovery and should be within the limits 98 and 102. The accuracy of the developed method was verified at 80, 100, and 120 percent levels by standard addition method, and the percent recovery of the drug was found to be 99.49 to 100.04 percent, indicating that there was no interference with the excipients and that the developed method was accurate.

Precision is given in terms of percent RSD. The ICH limitation for percent RSD for intra-day and inter-day precision is less than 2.0. The percent RSD of intra-day precision was 0.5, while the inter-day precision was 0.2, implying that variation was low and the established method was precise.

To express linearity, the correlation coefficient [R^2] should be NLT 0.999. A linear relationship was seen between the peak area and the drug concentration from 0.2 to 1.2 µg/ml, with a correlation value of 0.9995, indicating that there was a good correlation between the peak size and the drug concentration. The LOD and LOQ for Sumatriptan Succinate were 0.0024 µg/ml (s/n -3:1) and 0.0071 (s/n-9.7:1), respectively, reflecting that the developed method was sensitive for quantification of drug even in nanogram range. The difference in percent assay values in a solution stability studies should be NMT 2.0. The percent assay difference of Sumatriptan Succinate was 1.64 for 24 hours in a solution stability analysis, showing that the standard drug solution was stable for 24 hours at 30°±2°C.

System suitability parameters are used to evaluate the method robustness. The system suitability parameter limits include percent RSD not more than 2.0, theoretical plates not less than 2000 and tailing factor less than 2.0. The method robustness was tested by varying the flow rate [1.0±0.1ml/min], ACN ratio in the mobile phase [75±5

percent v/v], and temperature [30±5°C]. The observed system suitability parameters were percent RSD of peak area 0.3 - 1.0, theoretical plates 4686 - 5093, and tailing factor 1.35 - 1.38, indicating that variations in flow rate, ACN ratio in the mobile phase, and temperature had no effect on the developed method and that the method was robust. The chromatograms revealed the system suitability parameters such as percent RSD of peak area 0.6, theoretical plates of 4947, and tailing factor of 1.38, indicating that the system was appropriate for drug estimation.

Assay confirms that the average amount of Sumatriptan Succinate present in the tablets was 100.34 ± 0.82 mg for labeled amount of 100 mg indicating that the amount of drug is equivalent to the amount specified on the label.

CONCLUSION

An improved stability indicating RP-HPLC method was developed and validated for estimation of Sumatriptan Succinate in bulk and tablets. The present method developed was simple, more sensitive and cost effective with change in the mobile phase, less retention time, decrease in run time, reduced linearity concentration range, LOD and LOQ when compared to the best method reported. The developed method could be suitable for routine analysis of the Sumatriptan Succinate in bulk and tablet formulation.

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