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Stability Indicating RP-HPLC Method Development and Validation for the Estimation of Sumatriptan in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, highly sensitive stability indicating reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of sumatriptan succinate in bulk and tablet dosage form. The analysis was performed on reverse phase C_{18} ODS Inertsil (250×4.6mm, 5µm) column, with a mobile phase containing buffer: aetonitrile: methanol (80:10:10 v/v/v), pH was adjusted to 2.5 with orthophosphoric acid (OPA) at 221nm, by an isocratic elution mode with 1ml/min flow rate using photo diode array (PDA) detector at ambient temperature. The injection volume and retention time was found 20 µl and 4.4 minutes respectively. The method produced linear responses in the concentration range of 5-150 µg/ml, with a correlation coefficient of 0.999. The limit of detection (LOD) and limit of quantification (LOQ) values for HPLC method were found to be 1.967 and 5.961 µg/ml respectively. The recovery of the method was 98% of the labelled value. This method was validated for accuracy, precision, linearity and robustness. Sumatriptan subjected to different ICH prescribed stress conditions of acid, alkali, peroxide, reduction, thermal, photolytic and humidity degradation. This method can easily and conveniently take up for routine quantitative analysis of sumatriptan in bulk and pharmaceutical dosage form by easily available materials with low cost.

INTRODUCTION

Sumatriptan succinate was the first triptan drug used as anti-migraine, launched by Glaxo in the Netherlands in 1991. Sumatriptan (Fig. 1) is used mainly for the treatment of migraine attacks with or without aura. The 5-HT_{1B} and 5-HT_{1D} receptors function as auto receptors, which inhibit the firing of serotonin neurons and a reduction in the synthesis and release of serotonin upon activation. After sumatriptan binds to these receptors, adenylate cyclase (AC) activity is inhibited via regulatory G proteins, intracellular calcium levels increase and other intracellular events are affects. This results in vasoconstriction and inhibition of sensory nociceptive (trigeminal) nerve firing and vasoactive neuropeptide release (Öztürk *et al.,* 2013). Few derivative UV methods were reported for sumatriptan in combination (Rajesh Kumar *et al.,* 2011; Gondalia and Dharamsi, 2010; Sagar *et al.,* 2011; Trinath *et al.,* 2010), visible (Kalyanaramu and Raghubabu, 2011, Kalyanaramu and Raghubabu, 2010; Lokesh *et al.,* 2011) spectrophotometric methods were also reported for sumatriptan succinate in bulk and pharmaceutical dosage forms.

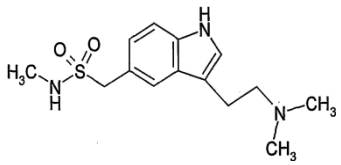

Fig. 1: Structure of sumatriptan.

HPLC methods for quantitative determination of sumatriptan succinate in combination were reported in literature (Majithiya *et al.,* 2006; Ravi *et al.,* 2012; Shirsat *et al.,* 2008; Singh and Jain 1997).

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HPTLC and UPLC methods were also reported for the estimation of sumatriptan succinate in single and combination dosage form (Badwe *et al.,* 1997; Shah *et al.,* 2006; Amruta *et al.,* 2013). The reported methods were not rapid, used expensive solvents and high solvent consumption. There was no stability indicating method available. In this regard, we felt it necessary to develop and validate a new rapid, economic and stability indicating RP-HPLC method for estimating sumatriptan in bulk and tablet dosage forms (Ramakoti *et al.,* 2011; Sagar *et al.,* 2010; Swapna *et al.,* 2013; Handrakant and Sadhana, 2012; Gondalia and Abhay, 2013).

MATERIALS AND METHODS

Materials and reagents

Sumatriptan succinate was kindly provided as a gift sample by NATCO Pharma limited, Hyderabad, India. Other reagents such as tri ethyl amine (TEA), acetonitrile (ACN), methanol and orthophosphoric acid [HPLC grade] were purchased from the Merck [INDIA]. All other reagents used for the analysis were analytical grade. Distilled water was used throughout the investigation.

Instrumentation

The HPLC system consisted of Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters 2695 solvent delivery module in a quaternary gradient mode and a waters 2669 PDA detector. Data acquisition was performed by the Empower 2 software. Analysis was carried out at 221 nm with reversed phase C_{18} ODS Inertsil (250×4.6mm, 5µm) column using buffer (tri ethyl amine): acetonitrile: methanol in 80:10:10 v/v/v ratios as the mobile phase by an isocratic elution mode with flow rate at 1ml/min. The mobile phase was degassed and filtered through 0.45 µm membrane filter before pumping into HPLC system.

Preparation of Solutions

Preparation of Buffer Solution

Accurately measured a 1000 ml of HPLC grade Water, 1 ml of tri ethyl amine was dissolved in it and adjust the pH 2.5 ± 0.5 with orthophosphoric acid (OPA).

Preparation of mobile phase

A mobile phase containing buffer: acetonitrile: methanol 80:10:10 v/v/v was prepared, filtered and degassed. It was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for sumatriptan.

Preparation of standard solution

Accurately weighed 25 mg of the pure drug, transferred into a 100 ml volumetric flask and dissolved in 70 ml diluent (mobile phase) and sonicated to dissolve it completely, the volume was made up to mark with the diluent to get standard (primary stock) solution (250 µg/ml). From this 5 ml of solution was pipette out and transferred into separate 50 ml volumetric flask and made up the volume up to the mark with mobile phase to get the concentration of secondary stock solution (25µg/ml). This solution was filtered through 0.45µm pore size nylon syringe filter.

Preparation of sample solution

20 tablets were accurately weighed and crushed. The powder equivalent to one tablet (50 mg) was taken into a 100 ml volumetric flask. 70 ml of diluents was added, sonicated and the volume was made up to mark with the diluent to get standard (primary stock) solution (500µg/ml). From this 5 ml of solution was pipette out and transferred into separate 50 ml volumetric flask and make up the volume up to the mark with diluent to get the concentration of secondary stock solution (50µg/ml). Further dilution was carried out to get the concentration of 25µg/ml then filtered through the 0.45 µm nylon syringe filter.

Chromatographic parameters

Equipment	:	HPLC equipped with Auto Sampler
Column	:	C_{18} ODS Inertsil (250×4.6mm, 5µm)
Flow rate	:	1ml/min.
Detector	:	PDA
Wavelength	:	221 nm
Injection volume	:	20 µl
Column oven	:	Ambient
Run time	:	10 min.

Method Development

Many trials have been performed by various mobile phase, flow rate and stationary phase. After observing the theoretical plates, stability factor the above chromatography parameter were chosen.

System suitability

Secondary Stock solution (25 µg/ml) of sumatriptan standard (20 µl) was injected six times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the percentage of related standard deviation (%RSD) of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Assay

Assay was performed by taking 8 sets of the drug solutions were prepared in diluents containing sumatriptan at a concentration range of 5-150 µg/ml. Then 20 µl of each standard and sample solution were injected for 4 times separately. The retention time of sumatriptan in drug and pharmaceutical dosage form were found to be 4.4 min. The peak areas of the drug concentration were calculated. The regression of the drug concentration over the peak areas was obtained. This regression equation was used to estimate the amount of sumatriptan in tablet dosage form.

Linearity

Preparation of stock solution

The linearity of the method was demonstrated over the concentration range of 5-150 µg/ml. Aliquots of 5, 10, 25, 50, 75, 100, 125, 150 µg/ml were prepared from secondary stock solution and labelled as solution 1, 2, 3, 4, 5, 6, 7 and 8 respectively. Volume of 20 µl of sample was injected for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration.

Accuracy

Accuracy was performed in triplicate for various concentrations of Sample solutions, prepared by spiking at about 50%, 100% and 150% of specification limit to Placebo and analyzed by the proposed HPLC method.

a. System Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

b. Method precision

Six sample solutions were prepared and injected into the HPLC system as per test procedure.

c. Intermediate precision

25 µg/ml of Standard solution was prepared and injected 6 times into HPLC system on the next day as per test procedure.

Robustness

The robustness was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, wavelength and mobile phase composition which may differ but the responses were still within the specified limits of the assay.

Forced Degradation

Acid degradation (5% HCl)

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask and add 2 ml of 5% HCl, A little amount of diluent were added and heated at 80°C for 20 min. Cooled the solution and neutralize the solution by adding 2 ml of 5% NaOH and made up to the volume with diluent.

Alkali degradation (5% NaOH)

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask and added 2 ml of 5% NaOH, A little amount of diluent were added and heated at 80 ^{0}C for 20 min. Cooled the solution and neutralize the solution by adding 2 ml of 5% HCl and made up to the volume with diluents.

Peroxide degradation

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask and added 2 ml of H_2O_2. A little amount of diluent were added & heated at 80 °C for 20 min. Cooled and made up to the volume with diluent.

Reduction degradation

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask and added 2 ml of Sodium bisulphate, little amount of diluents were added and heated at 80 °C for 20 min. Cooled and made up to the volume with diluent

Thermal degradation

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask, add diluents and heated at 105^{o}C for 24 hrs.

Photolytic degradation

From the sample stock solutions 5 ml of sample was transferred into 20 ml volumetric flask and added diluent, subjected to sun light for 24 hrs.

Humidity degradation

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask and added little amount of diluent, subjected to 90% relative humidity at 25 ^{0}C for 24 hrs. Then the volume was made up with diluent.

All above prepared solutions used for forced degradation studies, filter the solution through 0.45 µm nylon syringe filter by discarding little amount of solution. And prepare the blank in the same way without sample.

Solution stability

The solution stability of sumatriptan in diluents was determined by leaving 25 µg/ml sample solution in a tightly capped volumetric flask at room temperature for 24 hrs and measuring the amount at 0, 4, 8, 12, 24 hrs and compared the results with those obtained from freshly prepared solution. The mobile phase was prepared at the beginning of the study period and not changed during the experiment.

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

Calibration curve was repeated for 3 times and the standard deviation (SD) of the intercepts was calculated. The LOD and LOQ were determined by the following formulas:

$$LOD = 3.3\ \sigma/S$$

$$LOQ = 10\ \sigma/S$$

Where σ = Standard deviation of Intercepts of calibration curves;
S = Mean of slopes of the calibration curves.
The slope 'S' may be estimated from the calibration curve of the analyte.

RESULTS AND DISCUSSION

In this study, the determination of sumatriptan succinate and their degraded products was described. Several proportions of

buffer and solvents were evaluated in order to obtain suitable composition of the mobile phase. Various experiments were performed by changing the concentration and pH of mobile phase, stationary phase selection etc., to optimize the chromatographic conditions to achieve better efficiency of the chromatographic system. The composition of mobile phase buffer: acetonitrile: methanol (80:10:10 v/v/v) of pH 2.5 with flow rate of 1ml/min. and runtime of 4.4 min. at 221nm perfect chromatogram was eluted. Peaks were eluted properly and retention time of peak is less than 10 min.; Fig. 2

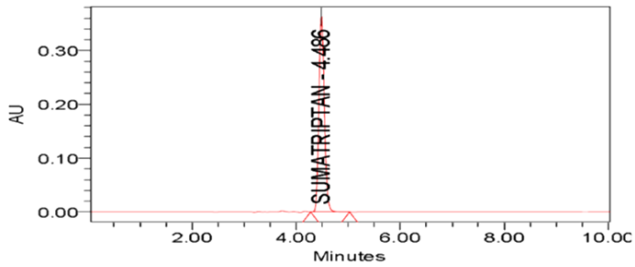

Fig. 2: Standard chromatogram for system suitability

All individual assays of should be 98% - 102%. Relative standard deviation (RSD) of % assay results should not be more than 2.0%. Assay of standard and sample chromatograms for sumatriptan shown in Fig. 3.

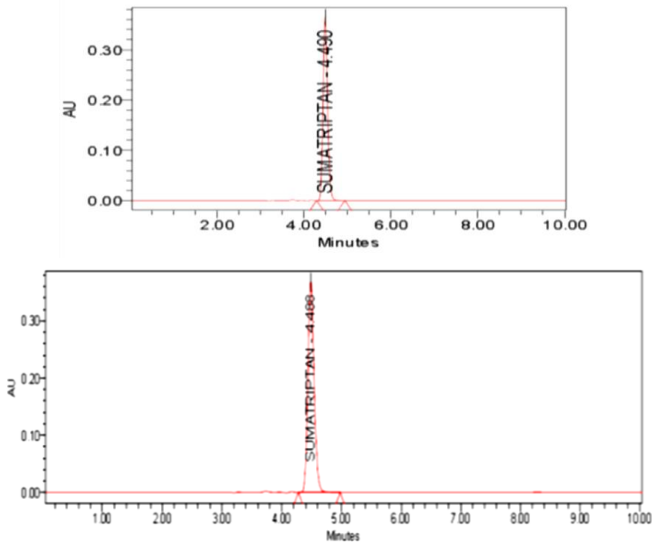

Fig. 3: Assay of standard and sample chromatograms for sumatriptan.

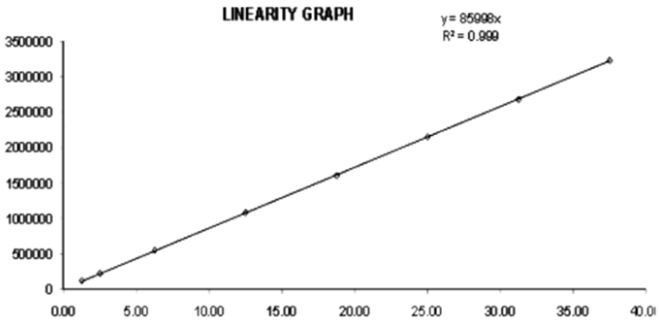

Fig. 4: Calibration curve of sumatriptan.

The procedure for the determination of linearity and range was same as mentioned in the selection of analytical concentration range and preparation of calibration curve for sumatripatan. The calibration curve and chromatograms of linearity results were shown in Fig. 4 and Table 1 respectively. The average % recovery of sumatriptan was calculated and the accuracy results were given in Table 2. The mean % recovery at each spike level should be not less than 98.0% and not more than 102.0%

Table 1: Linearity results.

Sl. No.	Concentration (µg/ml)	Rt (min.)	Avg. Area	USP tailing
1	5	4.481	223644	1.144
2	10	4.487	549968	1.076
3	25	4.492	1080045	1.155
4	50	4.498	1601982	1.159
5	75	4.499	2150780	1.173
6	100	4.508	2678422	1.174
7	125	4.508	3232317	1.178
8	150	4.468	4149922	1.170

R_t: retention time.

Table 2: Accuracy results of sumatriptan.

% Accuracy	Mean	SD	%RSD
50	100.5	0.68	0.670
100	100.3	0.40	0.390
150	100.5	0.26	0.260

SD: standard deviation, RSD: relative standard deviation

Changing with the flow rate and column over temperature and % RSD calculated. The robustness results were found within the limits shown in Table 3.

Table 3: Robustness values of sumatriptan.

Proposed variations		USP Plate Count	USP tailing
Variation in mobile phase composition	2% less	6231	1.194
	*Actual	8563	1.156
	2% more	8346	1.143
Variation in flow rate	0.8 ml/min	9642	1.175
	*1 ml/min	8563	1.156
	1.2 ml/min	7899	1.127
Variation in wavelength	216 nm	8463	1.152
	*221 nm	8563	1.156
	226 nm	8477	1.152

During the study it was observed that upon treatment of sumatriptan with acid (HCL), base (NaOH), hydrogen peroxide (H_2O_2), thermal, reduction, photolytic and humidity degradation was observed. And it was found to be the purity angle is less than purity threshold and peak purity test was passed. The forced degradation study values were shown in Table 4.

Table 4: Forced degradation study values of sumatriptan.

Stress type	% Degradation	Purity angle	Purity threshold	Pass/Fail
Acid	9.9	0.081	1.032	Pass
Alkali	17.5	0.097	1.043	Pass
Peroxide	25.7	0.08	1.027	Pass
Reduction	17	0.078	1.035	Pass
Thermal	9.3	0.09	1.031	Pass
Photolytic	6.2	0.078	1.033	Pass
Humidity	6.8	0.076	1.036	Pass

The % assay result should not differ from the initial value by more than ±2.0. The mobile phase was prepared at beginning, measured the amount at initial, 4, 8, 12 and 24 hrs, compared the results with those obtained from freshly prepared solution and system stability shown in Table 5.

Table 5: Stability of sumatiptan.

Solution stability (hr's)	% Label claim	% Deviation
Initial	99.5	-
4 hrs	100.3	0.80
8hrs	101.2	1.71
12 hrs	102.3	2.81
24 hrs	101.8	2.31

The limit of Detection (LOD) and limit of Quantification (LOQ) were determined according to the ICH guidelines, and found to be 1.967 and 5.967 respectively. The above motioned results was performed based on ICH guidelines, the method was validated with regard specificity, system suitability, linearity, accuracy, precision, forced degradation, LOD and LOQ. All the results were summarized in the Table 6.

Table 6: Results obtained by RP-HPLC method.

Sl. No.	Parameter	Acceptance criteria		Results obtained	Inference
1	System suitability	% RSD - NMT 2		0.260	Complies
2	Linearity	Correlation coefficient NLT-0.999		0.9998	Complies
3	LOD	-		1.967	Complies
4	LOQ	-		5.961	Complies
5	Precision	% RSD - NMT 2	System	0.260	Complies
			Method	0.164	
			Inter Mediate	0.263	
6	Accuracy	Recovery of the spiked drug (98-102%)	50%	0.670	Complies
			100%	0.390	
			150%	0.260	
7	Specificity	**1.** No interference of blank, Placebo and other degradation products with the main peak.		No interference	Complies
		2. Purity angle < Threshold		Peak pure	
8	Solution stability	> 12 hour		Stable up to 24 hour	Complies

CONCLUSION

A sensitive, specific and validated stability indicating assay was described for estimation of sumatriptan, used in anti-migraine therapy. The short retention times allows the analysis of a large number of samples in a short period of time with effective cost. Hence, this method is used for the routine analysis in the pharmaceutical industries. The method was accurately determined the amounts of API in the presence of impurities and excipients.

The method can be used to determine the purity of the drug obtained from different sources by detecting related impurities. Because the method separates the drug from its degradation products, it can be used as stability indicating. It was an accurate, precise, liner, robust, highly sensitive, stability indicating method. As per my knowledge this method was rapid, very simple method for determination of sumatriptan succinate in both bulk and pharmaceutical dosage form.

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