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An analytical method development and validation for simultaneous estimation of Sumatriptan and Naproxen in bulk samples as well as in tablet dosage forms by using RP-HPLC

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ABSTRACT

The present work was undertaken with the aim to develop and validate a rapid and consistent RP-HPLC method in which the peaks will be appear with short period of time as per ICH Guidelines. The HPLC separation was achieved on a Kromosil -ODS C_{18} (250 X 4.6 mm; 5 µ) column in an Isocratic Mode. The mobile phase composed of Water [HPLC Grade] (45 %) [pH 2.5 adjusted with OPA] and Methanol (55 %). The flow rate was monitored at 1.0 mL/min. The wavelength was selected for the detection was 277 nm. The retention times found for Sumatriptan and Naproxen was 2.790 and 3.481 min respectively. The % recovery was 99.02- 100.75 for Sumatriptan and 99.85 - 100.22 for Naproxen. The linearity was established in the range of 20-80 µg/mL for both Sumatriptan and Naproxen. The LOD for Sumatriptan and Naproxen were 0.56 and 0.57 µg/mL respectively. The LOQ for Sumatriptan and Naproxen were 1.69 and 1.74 µg/mL respectively. The proposed method was adequate sensitive, reproducible, and specific for the determination of Sumatriptan and Naproxen in bulk as well as in tablet dosage forms.

Keywords: Sumatriptan, Naproxen, ICH Guideline, RP-HPLC, LOD, LOQ.

INTRODUCTION

Migraine is a mysterious disorder characterized by pulsating headache, usually restricted to one side which comes in attacks lasting 4-48 hours and is often associated with nausea, vomiting, sensitivity to light and sound and other symptoms. Migraine is of two types-with aura (classical) in which headache is preceded by visual or neurological symptoms and migraine without aura (common migraine). Drug therapy of migraine has to be individualized: severity and frequency of attacks and response of individual patient to various drugs determine the choice. Sumatriptan succinate, domperidone and naproxen are widely used for the treatment of migraine with aura either as monotherapy or as multiple drug therapy for better efficacy. Sumatriptan succinate (Fig. no. 1) is chemically 3-[2-(dimethylamino) ethyl]-N-methyl-indole-5-methanesulfonamide succinate [1-3]. Sumatriptan succinate is official in British pharmacopoeia [4], European Pharmacopoeia [5] and United States Pharmacopoeia [6]. It is a selective 5-

hydroxytryptamine receptor subtype agonist and used as anti migraine drug. Naproxen sodium is (Fig. 2) is chemically (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid, sodium salt. Naproxen [7-9] is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. Analytical methods available for determination of Sumatriptan succinate include RP-HPLC detection [10], HPTLC [11], UV [12]. Analytical journals report the estimation of naproxen through liquid chromatography [13-16]. Among these methods, HPLC is an accurate and precise method for estimation of drugs in bulk and their formulations. Various workers have reported HPLC methods for individualistic estimation of Sumatriptan and Naproxen with few reports of simultaneous estimation of two drugs. Simultaneous estimation of two or more drugs by reverse phase high performance liquid chromatography (RP-HPLC) is also common in literature.

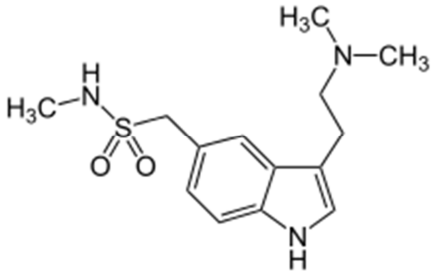

Fig. No. 1 Chemical Structure of Sumatriptan

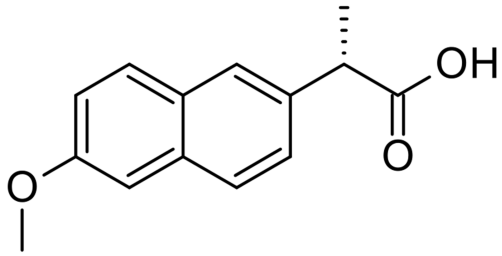

Fig. No. 2 Chemical Structure of Naproxen

MATERIALS AND METHODS

Chemicals and Reagents Used: The following chemicals were used for the process: Water [HPLC Grade], Sumatriptan and Naproxen [working standards], methanol [HPLC Grade] and orthophosphoric acid. All the chemicals were procured from Standard Solutions, Hyderabad, Andhra Pradesh.

0.45 µ membrane filters (Advanced Micro Devices Pvt. Ltd., Chandigarh, India) were used for filtration of various solvents and solutions intended for injection into the column.

Apparatus and Chromatographic Conditions: The equipment used was High Performance Liquid Chromatography Equipped with Auto Sampler and DAD or UV Detector. The column Kromosil -ODS C_{18} (250 X 4.6 mm; 5 µ) was selected. The flow rate was monitored at 1.0 mL/min. The detection was carried out at 277 nm. The injection volume selected 20 µL, the temperature of the column oven was maintained at 25 °C, the detector used was Photo diode array and the run time was 8.0 min.

The ultra violet spectra of the drugs used for the investigation were taken on a Lab India UV 3000 spectrophotometer for finding out their λ_{max} values.

Solubility of the compounds was enhanced by sonication on an ultra sonicator (Power Sonic 510, (Hwashin Technology).

All the weighings in the experiments were done with an Afcoset electronic balance. The Hermle microlitre centrifuge Z100 (model no 292 P01) was used for the centrifugation process and Remi equipments (model no-CM101DX) Cyclomixer was used.

Glassware: All the volumetric glassware used in the study was of Grade A quality Borosil.

Preparation of buffer [17]: The buffer solution was prepared by 1 X 10^{-5} M of ortho Phosphoric Acid in a 1000 mL beaker with water [HPLC grade]. Then the pH was adjusted to 2.5 with ortho phosphoric acid.

Preparation of mobile phase: The mobile phase was prepared by mixing a mixture of above buffer 450 mL (45 %) and 550 mL of methanol HPLC (55 %) and degas in ultrasonic water bath for 5 minutes. Then, the solution was filtered through a 0.45 µ filter under vacuum.

Preparation of standard solution of Sumatriptan and Naproxen: About 10 mg Sumatriptan was weighed accurately and transferred into a 10 mL clean and dry volumetric flask. Initially, the drug was mixed with 7 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up to the mark with the same solvent. Similarly, about 10 mg naproxen was weighed accurately and transferred into a 10 mL clean and dry volumetric flask. Initially, the drug was mixed with 7 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up to the mark with the same solvent to get a concentration of 1000 µg/mL.

From the above prepared stock solutions 0.4 mL of Sumatriptan and Naproxen were pipetted out into a 10 mL clean and dry volumetric flask and it was diluted up to the mark with diluent. This mixed stock solution contains 40.0 µg/mL of Sumatriptan and 40.0 µg/mL of Naproxen.

Preparation of sample solution of Sumatriptan and Naproxen: Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 20 mg of Sumatriptan and 20 mg of Naproxen were weighed and dissolved in the 70 mL mobile phase with the aid of ultra sonication for 20 min. The content was diluted with 100 mL mobile phase to furnish the preparation of stock solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric flask to get the desired concentration of 40.0 µg/mL of Sumatriptan and 40.0 µg/mL of Naproxen.

System Suitability: The tailing factor for the peaks due to Sumatriptan and Naproxen in Standard solution should not be more than 2.0. The Theoretical plates for the Sumatriptan and Naproxen peaks in Standard solution should not be less than 2000. The system suitability of the method was checked by injecting five different preparations of the Sumatriptan and Naproxen. The parameters of system suitability were checked.

VALIDATION DEVELOPMENT [18-25]

1. System Suitability: A Standard solution was prepared by using Sumatriptan succinate and Naproxen sodium working standards as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Sumatriptan succinate and Naproxen sodium, retention times and peak areas. The data are represented in table no. 1 and 2.

Acceptance Criteria: The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0 %. The number of theoretical plates (N) for the Sumatriptan succinate and Naproxen sodium peaks is NLT 3000. The Tailing factor (T) for the Sumatriptan succinate and Naproxen sodium peaks is NMT 2.0.

Table no. 1: System Suitability data for Sumatriptan

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	2.789	2748977	9478.317159	1.021108
2	2.790	2748357	9452.196217	1.080574
3	2.789	2748360	9569.928335	1.090824
4	2.780	2748206	9619.633847	1.089932
5	2.789	2748407	9749.907462	1.108610
Mean	2.788	2748461	9573.997	1.07821
SD	0.002345	297.998	-------	-------
% RSD	0.084118	0.0108	-------	-------

Table no. 2: System Suitability data for Naproxen

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.480	729374	10953.609752	1.604407
2	3.481	729587	10951.014286	1.604878
3	3.481	729020	10003.278630	1.590957
4	3.477	729174	10986.906427	1.584354
5	3.478	729744	10946.878423	1.566451
Mean	3.478	729379.8	10768.34	1.590209
SD	0.003317	294.7104	-------	-------
% RSD	0.9536	0.040	-------	-------

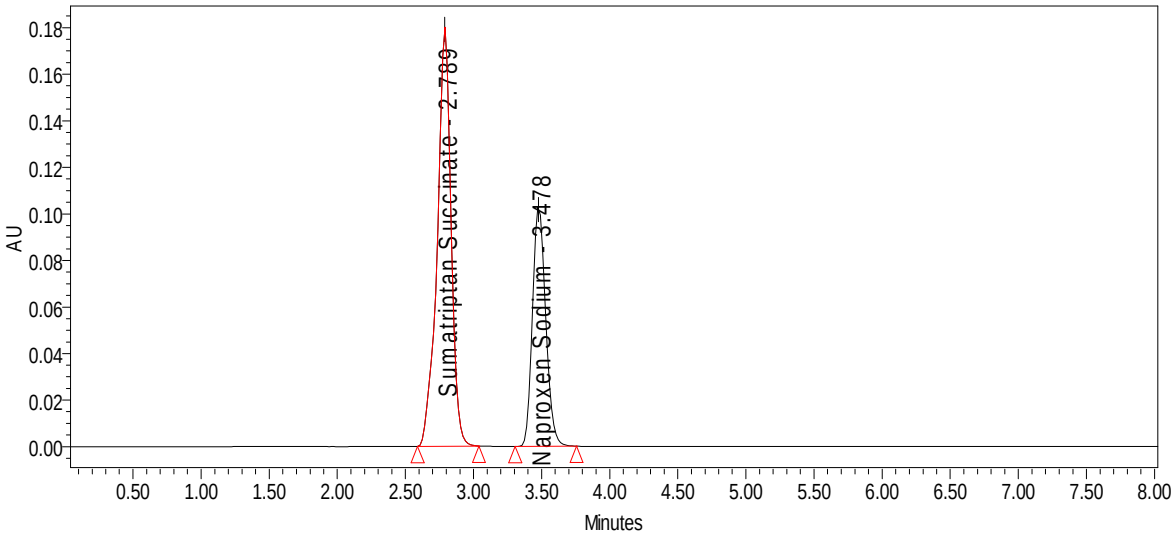

Fig. No. 3 A typical chromatogram for standard drugs

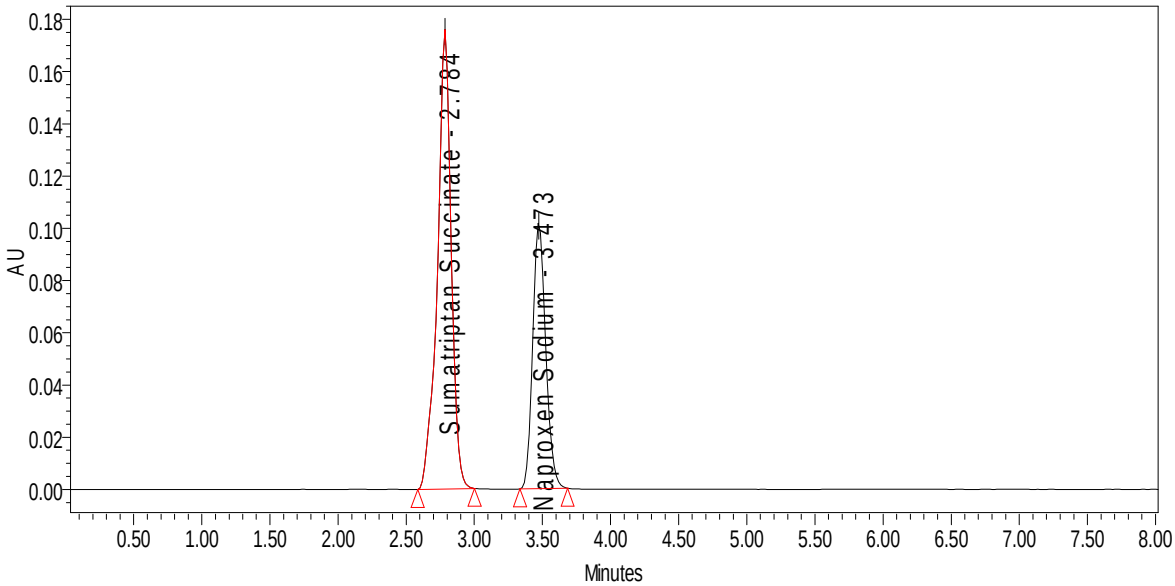

Fig. No. 4 A typical chromatogram for sample drugs

2. Specificity: Solutions of standard and sample were prepared as per the test method are injected into chromatographic system. The chromatograms of standard and sample should be identical with near retention time. The specificity are represented in fig.no.3 and 4.

3. Precision: It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. The data are represented in table no. 3 and 4.

Table no. 3: Precision results for Sumatriptan

Injection	Peak Areas of Sumatriptan	% Assay
1	205625	99.95
2	206225	100.24
3	205840	100.06
4	204283	99.30
5	205735	100.00
Mean	205541.6	99.91
SD	739.0046	0.35819
% RSD	0.35	0.35

Table no. 4: Precision results for Naproxen

Injection	Peak Areas of Naproxen	% Assay
1	734360	98.66
2	739098	99.30
3	755696	101.53
4	748289	100.53
5	744147	99.98
Mean	744318	100.00
SD	8241.164	1.107678
% RSD	1.1	1.10

Acceptance Criteria: The %RSD for the area of all the five injections should not be more than 2%.

4. Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, precision was performed on different day by using different make column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. The data are represented in table no. 5 and 6.

Table no. 5: Ruggedness results for Sumatriptan

Injection	Peak Areas of Sumatriptan	% Assay
1	205267	99.78
2	205625	99.95
3	205840	100.00
4	202735	98.55
5	208991	101.50
6	208543	101.37
Mean	206333.5	100.19
SD	2572.599	1.100898
% RSD	1.24	1.09

Table no. 6: Ruggedness results for Naproxen

Injection	Peak Areas of Naproxen	% Assay
1	736792	99.99
2	734360	99.66
3	755696	101.53
4	744147	99.98
5	744127	99.97
6	752525	101.10
Mean	744607.8	100.37
SD	8392.59	0.753536
% RSD	1.1	0.75

Acceptance Criteria: The %RSD for the area of all the five injections should not be more than 2%.

5. Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. The standard solution with Accuracy -50 %, Accuracy -100 % and Accuracy -150 % were injected into chromatographic system and calculated the amount found and amount added for Sumatriptan and Naproxen and further calculated the individual recovery and mean recovery values. The data are represented in table no. 7 and 8.

Table No. 7: Accuracy results for Sumatriptan

Concentration % of spiked level	Amount added (mg)	Amount found (mg)	% Recovery	Statistical Analysis of % Recovery	
50 % Injection 1	20	20.15	100.75	**MEAN**	99.69333
50 % Injection 2	20	19.86	99.31		
50 % Injection 3	20	19.80	99.02	**% RSD**	0.92
100 % Injection 1	40	39.88	99.70	**MEAN**	99.83333
100 % Injection 2	40	40.12	100.30		
100 % Injection 3	40	39.80	99.50	**% RSD**	0.41
150 % Injection 1	60	60.12	100.21	**MEAN**	99.97333
150 % Injection 2	60	59.76	99.61		
150 % Injection 3	60	60.06	100.10	**% RSD**	0.31

Table No. 8: Accuracy results for Naproxen

Concentration % of spiked level	Amount added (mg)	Amount found (mg)	% Recovery	Statistical Analysis of % Recovery	
50 % Injection 1	20	20.04	100.22	**MEAN**	100.06
50 % Injection 2	20	19.97	99.85		
50 % Injection 3	20	20.02	100.11	**% RSD**	0.18
100 % Injection 1	40	40.01	100.02	**MEAN**	100.04
100 % Injection 2	40	40.05	100.14		
100 % Injection 3	40	39.98	99.96	**% RSD**	0.091
150 % Injection 1	60	60.08	100.14	**MEAN**	100.02
150 % Injection 2	60	59.97	99.96		
150 % Injection 3	60	59.98	99.98	**% RSD**	0.09

Acceptance Criteria: The %Recovery for each level should be between 98.0 to 102.0 %.

6. Linearity: It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope or regression line. It is determined by series of three to six injections of five of more standards. Different levels of solution were prepared and injected to the chromatographic system and the peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The calibration curve was represented in fig. no. 3 and 4. The data are represented in table no. 5 and 6.

Table no. 9: Linearity results for Sumatriptan

Concentration (µg/mL)	Average Area	Statistical Analysis	
20	102965		
30	154371		
40	205856	Slope	5140
50	257167	y-Intercept	114.7
60	308577	Correlation Coefficient	1
70	359903		
80	411306		

Table no. 10: Linearity results for Naproxen

Concentration (µg/mL)	Average Area	Statistical Analysis	
20	372546		
30	558296		
40	744400	Slope	18600
50	930308	y-Intercept	276.2
60	1116282	Correlation Coefficient	1
70	1302046		
80	1488277		

Acceptance Criteria: The correlation coefficient should not be less than 0.999.

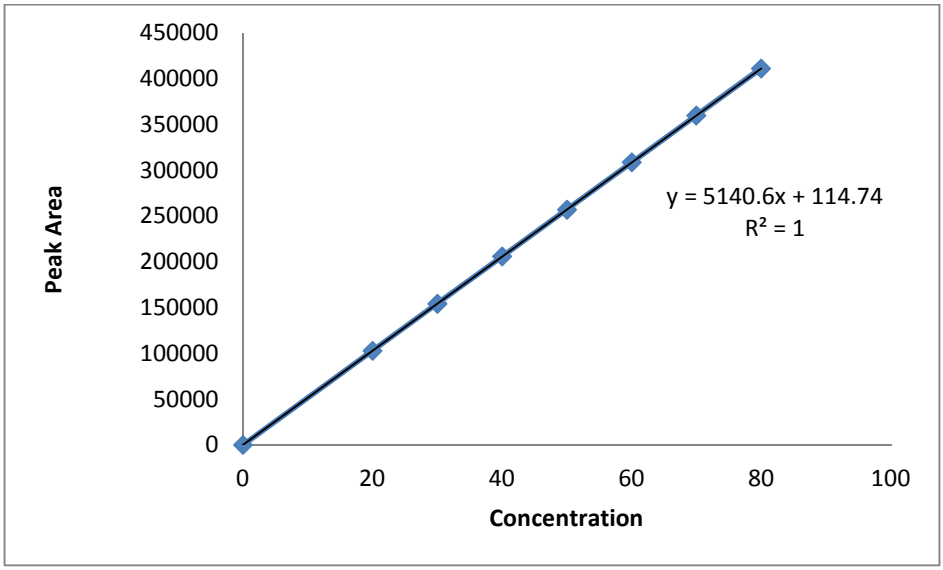

Fig. no. 5 Calibration curve for Sumatriptan

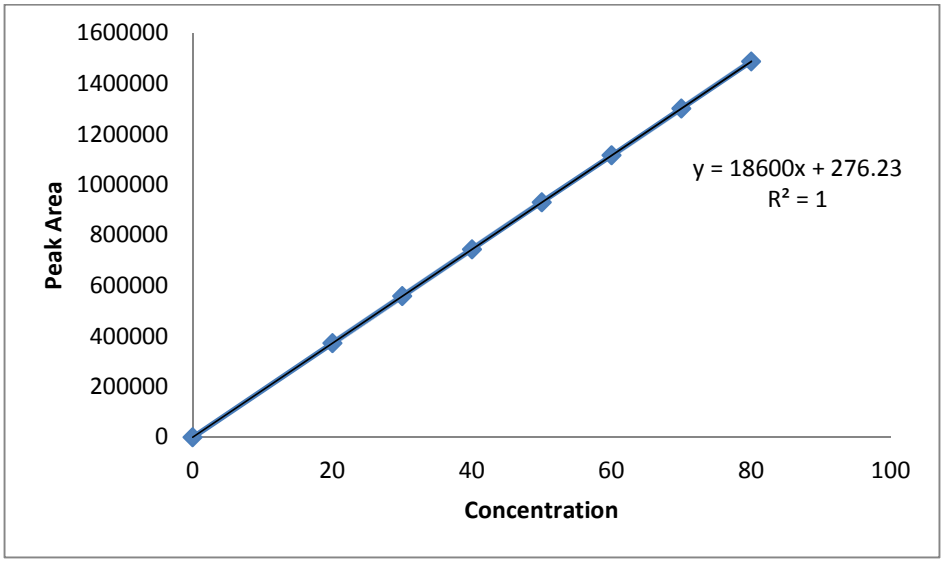

Fig. No. 6 Calibration curve for Naproxen

7. Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.

Limit of Detection for Sumatriptan and Naproxen: The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. Limit of detection is the lowest concentration of the substance that can be detected, not necessarily quantified by the method. (Regression statistics) The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the following formula.

Limit of detection (LOD) = $\frac{\sigma}{s} \times 3.3$

Where S – slope of the calibration curve

σ – Residual standard deviation

$$LOD = \frac{3.3\,\sigma}{S}$$

$$= \frac{3.3 \text{ X } 867.0705}{5140} = 0.56 \quad \text{for Sumatriptan}$$

$$LOD = \frac{3.3\,\sigma}{S}$$

$$= \frac{3.3 \times 3244.904}{18600} = 0.57 \text{ for Naproxen}$$

8. Limit of Quantification: It is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio.

9.

Limit of Quantification for Sumatriptan and Naproxen: The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. Limit of Quantification is the lowest concentration of the substance that can be estimated quantitatively. It can be determined from linearity curve by applying the following formula

Limit of Quantification (LOQ) = $\frac{\sigma}{s} \times 10$

Where S – slope of the calibration curve
σ – Residual standard deviation

$$LOQ = \frac{10\,\sigma}{S}$$

$$= \frac{10 \text{ X } 867.0705}{5140} = 1.69 \text{ for Sumatriptan}$$

$$LOQ = \frac{10\,\sigma}{S}$$

$$= \frac{10 \text{ X } 3244.904}{18600} = 1.74 \text{ for Naproxen}$$

10. Robustness: As part of the Robustness, deliberate change in the flow rate, mobile phase composition, temperature variation was made to evaluate the impact on the method. The standard and samples of Sumatriptan and Naproxen were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The data are represented in table no. 11 and 12 and fig. no. 7, 8 and 9.

Table No. 11: System Suitability Results for Sumatriptan (Change in Flow Rate)

Flow 0.8 mL/ min.	Std. Area	Tailing factor	Flow 1.0 mL/min.	Std. Area	Tailing factor	Flow 1.2 mL/min.	Std. Area	Tailing factor
	273707	1.362089		206349	1.280574		166195	1.285372
	273211	1.352617		205267	1.279932		165885	1.299385
	273948	1.376926		205625	1.261721		166303	1.308063
	273465	1.345752		205840	1.276089		167243	1.274662
	273862	1.374925		205735	1.250640		165762	1.267630
Avg	273638.6	1.362462	**Avg**	205763.2	1.269791	**Avg**	166277.6	1.287022
SD	301.369	0.013609	**SD**	392.1635	0.01314	**SD**	582.9758	0.016786
% RSD	0.11	0.99	**% RSD**	0.19	1.03	**% RSD**	0.35	1.3

Table No. 12: System Suitability Results for Naproxen (Change in Flow Rate)

Flow 0.8 mL/ min.	Std. Area	Tailing factor	Flow 1.0 mL/min.	Std. Area	Tailing factor	Flow 1.2 mL/min.	Std Area	Tailing factor
	1120286	1.322089		734322	1.604878		602077	1.285372
	1119282	1.331920		735792	1.584354		601854	1.319385
	1121337	1.296438		734360	1.543805		602403	1.292055
	1120456	1.315454		735696	1.568590		603421	1.304561
	1120765	1.326551		733147	1.559986		602465	1.294621
Avg.	1120425	1.31849	**Avg.**	734663.4	1.572323	**Avg.**	602444	1.299199
SD	754.0018	0.013728	**SD**	1100.917	0.023367	**SD**	599.8833	0.013223
%RSD	0.06	1.04	**% RSD**	0.14	1.48	**% RSD**	0.09	1.01

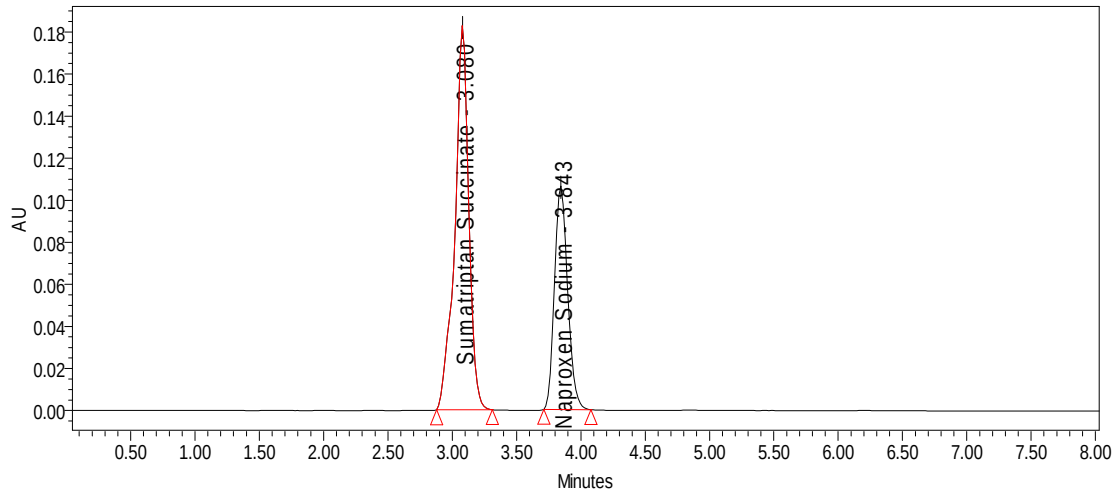

Fig. no. 7: A typical chromatogram for robustness with flow rate (for 0.8 mL/min flow)

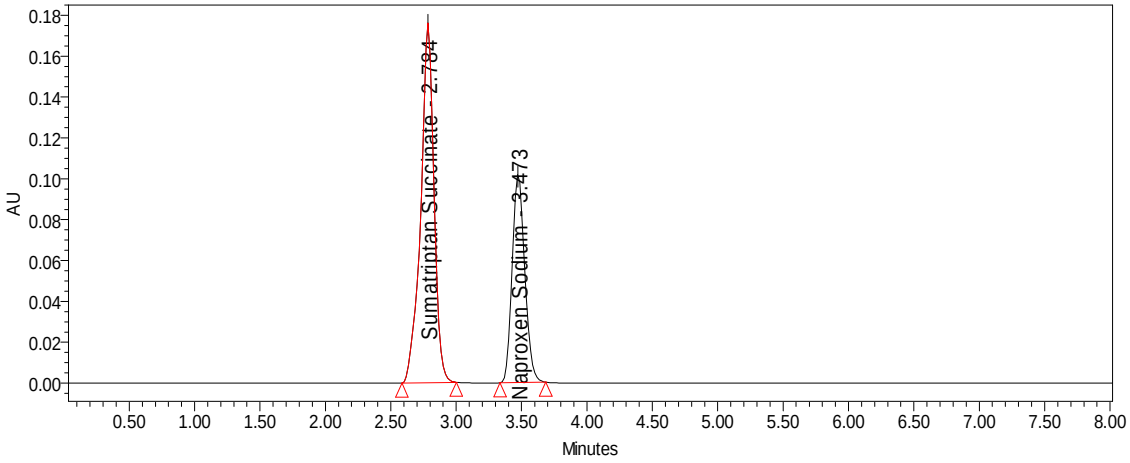

Fig. no. 8: A typical chromatogram for robustness with flow rate (for 1.0 mL/min flow)

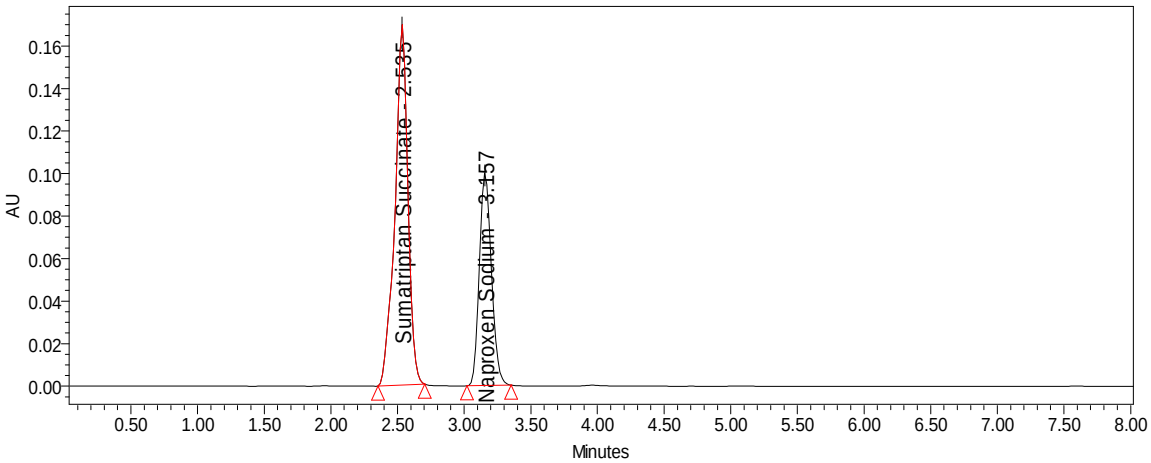

Fig. no. 9: A typical chromatogram for robustness with flow rate (for 1.2 mL/min flow)

RESULTS AND DISCUSSION

To optimize the mobile phase, various proportions of water [HPLC grade] (pH 2.5) with methanol [HPLC Grade] were tested. The use of water [HPLC grade] (pH 2.5) and methanol [HPLC Grade] in the ratio of 45:55 (v/v) resulted in peak with good shapes and resolution. A flow rate of 1.0 mL /min was found to be optimum in the 0.4-1.5 mL/min range resulting in short retention time, baseline stability and minimum noise.

By applying the proposed method, the retention times of Sumatriptan and Naproxen were observed at 2.790 and 3.481 min at 277 nm respectively. A typical chromatogram is represented in fig. no. 10.

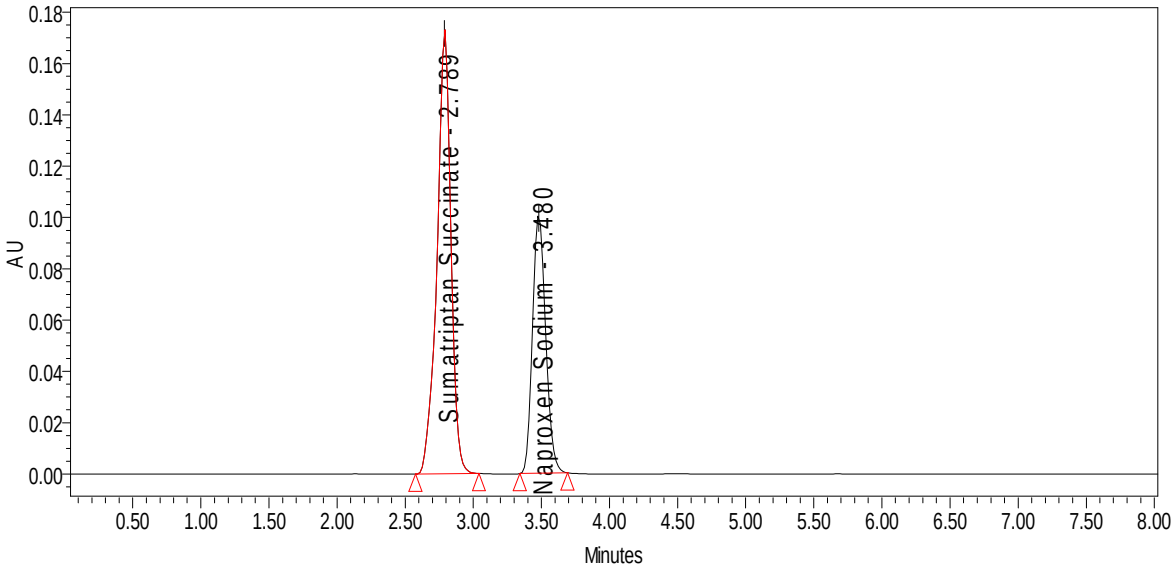

Fig. No. 10 A Typical chromatogram for Sumatriptan and Naproxen

Quantitative linearity was obeyed in the concentration ranges of 20-80 µg/mL for both Sumatriptan and Naproxen. The relevant regression equations were y = 5140.x + 114.7 for Sumatriptan (r^2= 1) and y = 18600x + 276.2 for Naproxen (r^2= 1) (where y is the peak area ratio and x is the concentration of Sumatriptan and Naproxen (µg/mL)). The intra-day and inter-day drugs variations by the proposed method showed an RSD less than 2 %, indicating that the method is precise. The corresponding mean recoveries of the drugs were 99.02- 100.75 % for Sumatriptan and 99.85 - 100.22 % for Naproxen. This reveals that the method is quite accurate. The tailing factor (1.27 and 1.57 for Sumatriptan and Naproxen); obtained were within the acceptance limits. The limits of detection for Sumatriptan and Naproxen obtained by the proposed method were 0.56 and 0.57 µg/mL respectively, and limits of quantification for atorvastatin and ezetimibe obtained by the proposed method were 1.69 and 1.74 µg /mL respectively, which indicate the sensitivity of the method. The method tolerated minor variations in optimized chromatographic conditions indicating good robustness, which indicate the efficient performance of the column.

No interfering peaks were found in the chromatograms indicating that the excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous determination of Sumatriptan and Naproxen. The method was validated as per ICH guidelines and all the parameters met within the acceptance criteria. Applicability of this method for simultaneous estimation of Sumatriptan and Naproxen from tablet dosage forms was confirmed. Hence, this method is specific and can be successfully used for the simultaneous estimation of Sumatriptan and Naproxen in bulk drug samples, pharmaceutical dosage forms. Hence, this method can be easily and conveniently adopted for routine quality control analysis of the above drugs.

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