ISSN : 0974-7419 Volume 15 Issue 5

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 15(5) 2015 [151-159]

A rapid novel RP- HPLC stability indicating assay method development and validation of simultaneous determination of Sumatriptan Succinate and Naproxen Sodium

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ABSTRACT

A simple, precise, specific, accurate and linear reversed phase liquid chromatographic method was developed for the simultaneous determination of Sumatriptan Succinate and Naproxen Sodium in Pharmaceutical dosage forms. The method was precise, linear and accurate over a range of 0.4-6.4 mg/ml for Sumatriptan Succinate and 0.076-1.2 mg/ml for Naproxen Sodium, at a detection wavelength of 280 nm and a gradient flow program. The method was also found to be stability indicating with all the known impurities of both Sumatriptan Succinate and Naproxen Sodium and also the degradents of the drug product well separated from the two analyte peaks. The method is validated for specificity, accuracy, precision, linearity and robustness in accordance with ICH guidelines.

KEYWORDS

Sumatriptan Succinate;
Naproxen Sodium;
Stability indicating assay method.

INTRODUCTION

Sumatriptan Succinate, (3-[2-(dimethylamino) ethyl]-N-methyl-indole-5-methanesulfonamide succinate (1:1)) is a serotonin subtype agonist drug for treatment of migraine headaches. The selective agonist for the vascular 5-hydroxytryptamine (5-HT_1) (serotonin) receptor in cranial arteries causes vasodilation with little or no effect on peripheral pressure.

Naproxen Sodium, (2-naphthaleneacetic acid, 6-methoxy-2-methyl-sodium salt, (S)) is a non-steroidal anti-inflammatory drug (NSAID) that is most often used to treat pain, inflammation, menstrual cramps and fever. It is also a favorite medication used to treat the stiffness associated with arthritis, osteoarthritis and rheumatoid arthritis and other conditions affecting joints.

The validation analysis of binary mixture of Naproxen sodium and Sumatriptan succinate has been made by a few spectrophotometric methods in pharmaceutical preparation[1-3] and few methods were also reported for the validation of simultaneous estimation of Sumatriptan Succinate and Naproxen sodium in bulk drug and pharmaceutical dosage form by UPLC, HPTLC and RP-HPLC[4-6].

So far few liquid chromatography procedures have been described for the determination of Sumatriptan succinate and Naproxen Sodium[7-29]. These procedures were developed to estimate either Sumatriptan succinate or Naproxen Sodium individually either from for-

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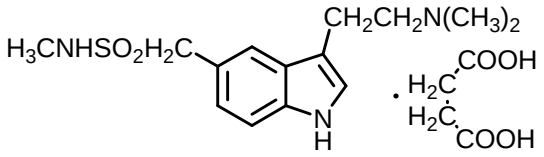
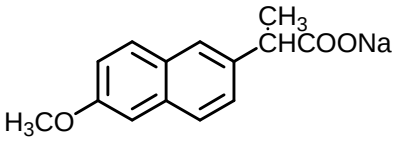
mulations or from biological matrices like plasma and serum. In the current paper we describe the procedures of stability indicating and validation of a rapid, accurate and specific method for the determination of Sumatriptan succinate and Naproxen Sodium from combined dosage form.



3-[2-(dimethylamino) ethyl]-N-methyl-indole-5-methanesulfonamide succinate



2-naphthaleneacetic acid, 6-methoxy-2-methyl-sodium salt, (S)

Figure 1: Structure of Sumatriptan Succinate and Naproxen Sodium

EXPERIMENTAL

Reference substances, samples, reagents and chemicals

Sumatriptan Succinate and Naproxen Sodium working Standards and drug product were supplied as a gift sample by regional Pharmaceutical Company, Hyderabad, India. Acetonitrile for HPLC, Methanol for HPLC, Triethylamine LR grade, Hexane-1-sulphonic acid sodium salt GR grade, ortho phosphoric acid GR grade were purchased from Merck, Mumbai, India. High purity deionised water for HPLC was obtained from Milli-Q, Millipore (Bedford, MA, USA) purification system. In-house Impurity working standards were used for method development and validations

Instrumentation and chromatographic conditions

HPLC system, Waters Alliance 2695 equipped with quaternary pump, auto sampler, column oven and PDA detector (2996) was employed for experimentation. Chromatographic data was analysed by using Waters Empower software.

A reverse Phase, Phenomenex Luna C8 column (250 mm x 4.6 mm, 5µm) was used as stationary phase. Mobile phase A containing buffer (0.05mm of 1-hexane sulphonate sodium salt and 3 ml of tri ethyl amine for 1000 ml of HPLC water, pH 6.7), acetonitrile and methanol in the ratio of 65:30:5 v/v/v and Mobile phase-B containing HPLC grade Water-Acetonitrile (10:90 v/v) were used for gradient elution. Gradient program was as shown in the table below. Detector was monitored at 280 nm and column oven temperature was maintained at 40^0 C. Injection volume was 10 µL.

Gradient program is (Time/ Flow rate/ % of Mobile Phase-B) 0/1.0/0, 6/1.0/0, 8/2.0/100, 11/2.0/100, 12/2.0/0, 14/2.0/0, 15/1.0/0, 20/1.0/0.

Standard solutions

A standard solution containing 5720 µg/ml of Sumatriptan succinate (equivalent to 85 mg) and 1100 µg/ml of Naproxen Sodium were prepared by dissolving Sumatriptan succinate and Naproxen Sodium in diluent of 50:50 Mille-Q-water and Acetonitrile.

Test solution preparation

Ten tablets were weighed and transferred to a 500 ml standard flask. Both the drugs were finally extracted in 350 ml of diluent (Acetonitrile-water, 50:50 v/v) by sonication for about 10 min. The solution was filtered through 0.45 µm Millipore PVDF filter. Representative chromatograms are shown in Figure 2 and Figure 3. The retention times of Sumatriptan succinate and Naproxen Sodium were found to be 3.55 and 4.44 min, respectively (Figure 2 and Figure 3).

Validation procedure

Method validation was performed as per ICH guidelines[30-32].

Not more than 2.0 % of relative standard deviation for peak areas of Sumatriptan succinate and Naproxen from five injections of standard and tailing factor of not more than 2.0 for both Sumatriptan succinate and Naproxen sodium peaks was established as system suitability.

Precision of the method was verified by calculating percentage assay for both the components in the six simultaneous assay preparations. Intermediate precision was verified by repeating the precision performed by the second analyst on a different day using different chromatographic system.

Specificity of the method was established by verifying non-interference of placebo, known impurities and

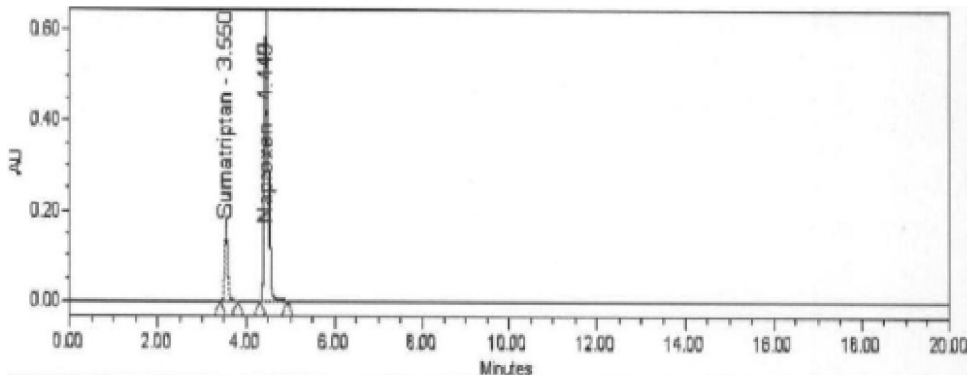


Figure 2 : A typical HPLC chromatogram of Sumatriptan Succinate and Naproxen Sodium standard

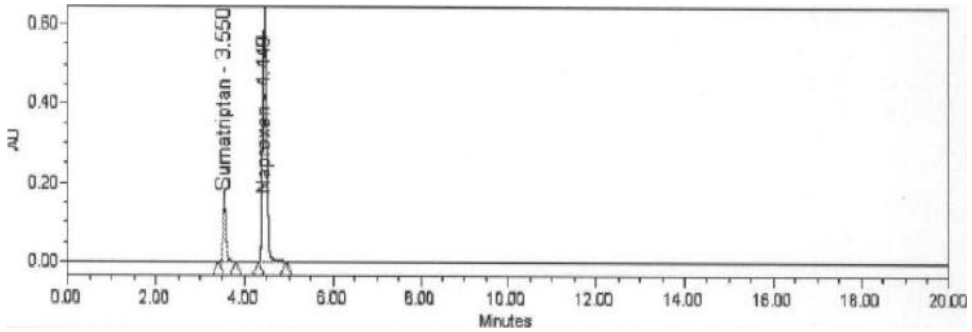


Figure 3 : A typical HPLC chromatogram of Sumatriptan Succinate and Naproxen Sodium tablets

degradation products. Placebo solution was prepared at the same concentrations as present in the sample solution.

Non-interference of all known impurities of both the drugs was checked by injecting the individual impurities of known concentrations and determining their retention times. Also both the standard and sample solutions spiked with all the known impurities at the levels of 0.5 % concentrations of the respective drug molecules were injected and checked for non interference.

Forced degradation studies were performed to prove non-interference of degradation products. The stress conditions employed for degradation study of Sumatriptan succinate and Naproxen Sodium includes UV light and florescent light exposure, heat (105°C) exposure, exposure to humidity (95% RH), acid hydrolysis, base hydrolysis, water hydrolysis and oxidation. For UV light and fluorescent light studies, the monitoring period was 10 days. Acid hydrolysis stress was performed by refluxing for about one hour at 80^0C. Base hydrolysis stress was achieved by refluxing for about 3 hours with 1 N NaOH. Oxidation was carried out on bench top for 1 hour with 1% hydrogen peroxide. Humidity stress study was conducted for about 7 days at the RH of 90%. Peak purity of the principal peaks in the chromatogram of stressed samples were checked using photo diode array detector.

Linearity of the response against concentration for Sumatriptan succinate and Naproxen Sodium was carried out at six concentration levels. Correlation co-efficient was established for both components.

A study of accuracy was performed by preparing sample solutions at 10%, 50%, 100%, 120%, and 150% of the target test concentration of both the components along with the equivalent amounts of placebo.

Robustness of the method was checked by deliberately changing the chromatographic conditions. Flow rate was changed by ± 0.2 ml/min, organic strength was changed by 10% for both acetonitrile and methanol, column oven temperature was changed by $\pm 5^0$C and pH was changed by ± 0.5 units. System suitability was checked for each change.

Solution stability for both components in test and standard solution was established by comparing the change in percentage assay values of initial and stability testing periods.

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TABLE 1 : Results of forced degradation

Stress Conditions	%Degradation of Sumatriptan Succinate	Purity angle	Purity threshold	% Degradation of Naproxen Sodium	Purity angle	Purity threshold
Refluxed with 1N HCl solution for about 1 hour at 80ºC	2.4	0.177	0.298	2.4	7.551	51.140
Refluxed with 1 N NaOH solution for about 3hours at 80ºC.	Nil	0.172	0.352	Nil	9.048	46.451
Exposed to 1% Hydrogen peroxide (H_2O_2) for about 1 hour at Bench top	0.77	0.280	0.289	0.77	6.363	63.958
Refluxed with purified water for about 6 hrs at 100ºC.	Nil	0.398	0.427	Nil	7.659	43.727
Exposed to Sunlight for about 140 watt Hours/square meter.	3.0	0.151	0.315	3.0	7.742	28.313
Exposed to UV light for about 1.2 Million Lux hours.	3.1	0.146	0.321	3.1	9.248	56.287
Dry heating done at 105°C for about 12 hrs.	Nil	0.226	0.391	Nil	10.533	62.502
Exposed to humidity at 25°C/ 90% RH for about 7 days.	Nil	0.186	0.391	Nil	8.387	31.911

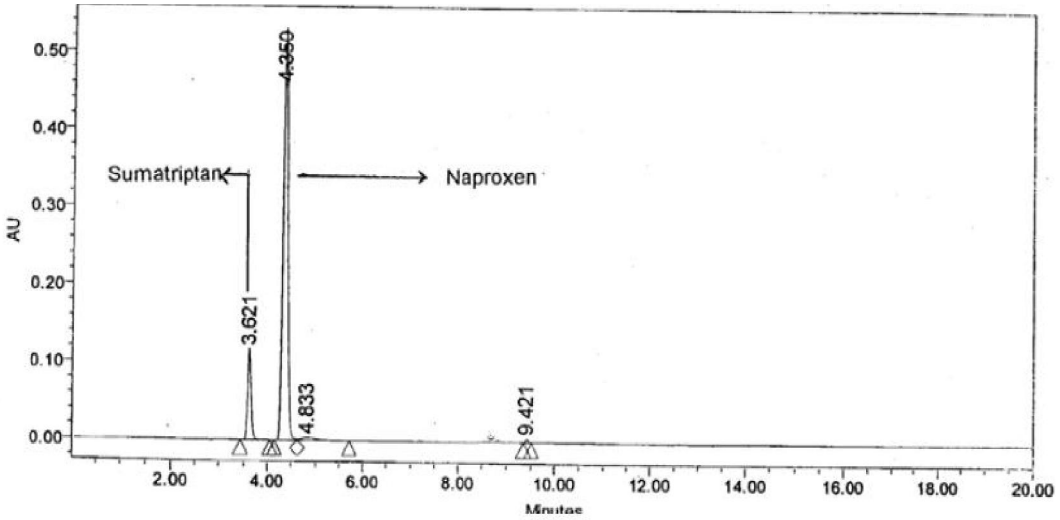

Figure 4 : Chromatogram of acid stress sample

RESULTS AND DISCUSSION

System suitability

The system suitability for the method was evaluated with relative standard deviation for replicate injections of standard solution and the tailing factor for each component**.** The results are summarized in the TABLE 2.

Precision

All the six percentage assay values were with in the acceptance limit of ± 3% (97% to 103%) and also with in a relative standard deviation of 2%. The mean assay value for six preparations of Sumatriptan Succinate was 101.1 with a range of 99.9% to 102.6%. Corresponding values of Naproxen Sodium were 98.9% and 97.8% to 99.6%. Intermediate precision values were also within the acceptance values.

Specificity

Chromatograms for placebo preparations did not show any peak at the RT's of Sumatriptan and Naproxen. Purity angle was less than purity threshold in the Chromatograms for solutions spiked with impurities. This proves non-interference of placebo and impurities.

Degradation was not observed in Sumatriptan succinate and Naproxen Sodium stressed samples that were subjected to light, heat, water, and Humidity. However, degradation was observed under oxidative conditions, base hydrolysis, acid hydrolysis. The peak purity test results derived from PDA (Photo Diode Array detector) confirmed that the Sumatriptan succinate and Naproxen Sodium peaks were pure and homogeneous in all the analyzed stress tests. This indicates that the method is specific and stability indicating. Results of specificity stud-

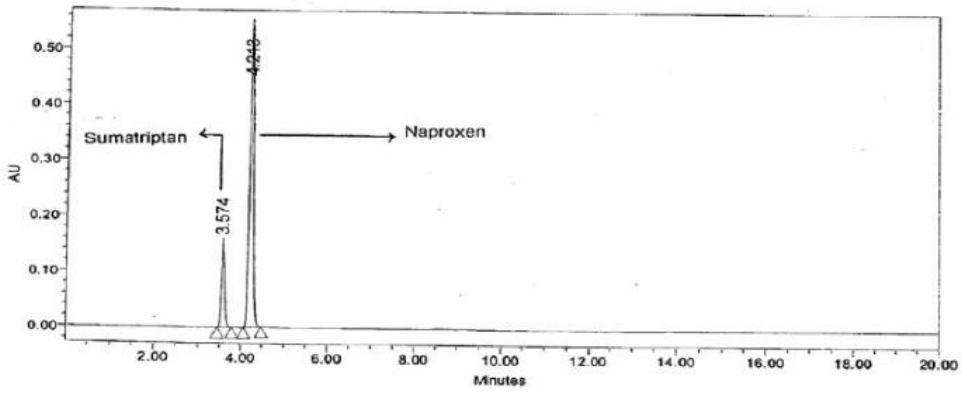

Figure 5 : Chromatogram of base stress sample

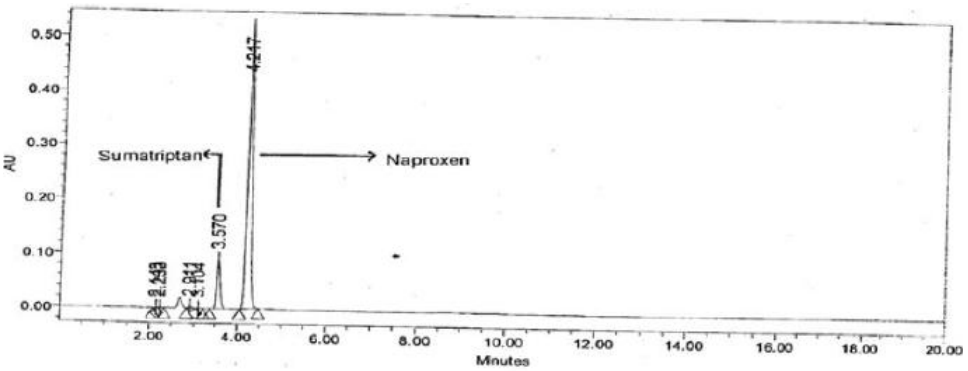

Figure 6 : Chromatogram of oxidation stress sample

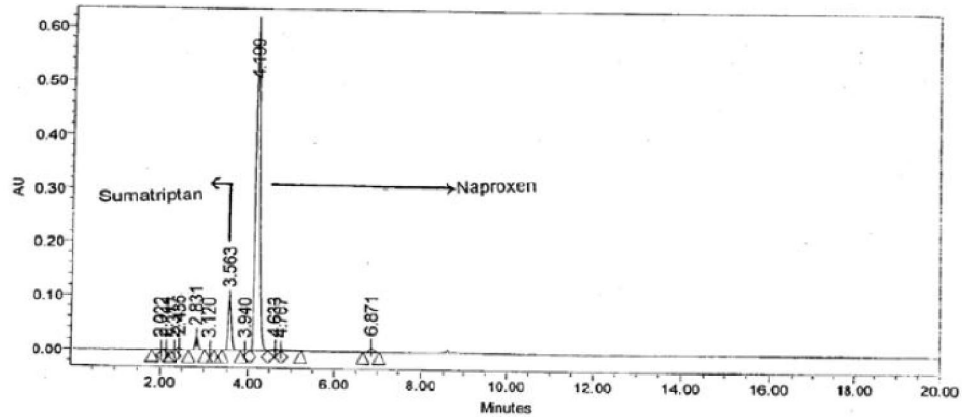

Figure 7 : Chromatogram of water stress sample

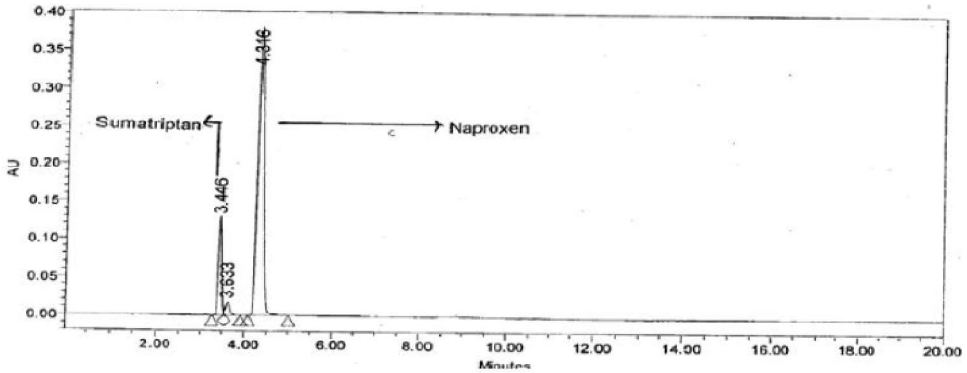

Figure 8 : Chromatogram of UV light stress sample

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TABLE 2 : System suitability results for Sumatriptan Succinate and Naproxen Sodium

S.No:	System suitability Parameter	Result	Acceptance Criteria
01	Relative standard deviation for peak areas of Sumatriptan Succinate for five injections of standard	0.4%	NMT 2.0
02	Relative standard deviation for peak areas of Naproxen Sodium for five injections of standard	0.2%	NMT 2.0
03	Tailing factor for Sumatriptan succinate peak	1.2	NMT 2.0
04	Tailing factor for Naproxen Sodium peak	1.2	NMT 2.0

TABLE 3A : Linearity of Sumatriptan

Linearity of Sumatriptan		
S.No.	Concentration (ppm)	Peak Area
1	2.1	18257
2	25.4	229024
3	84.5	774018
4	101.4	932837
5	135.3	1236026
Slope	9171.347779	
Y-Intercept	-1577.394084	
Correlation Coefficient	0.999981908	

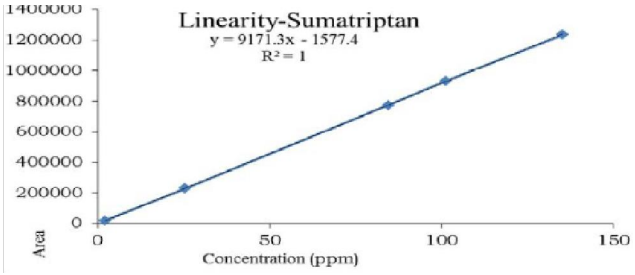

Figure 9 : Linearity plot of Sumatriptan

TABLE 3B : Linearity of Naproxen

Linearity of Naproxen		
S.No.	Concentration (ppm)	Peak Area
1	100	670399
2	200	1341260
3	500	3327948
4	600	3968727
5	900	5983091
Slope	6629.195388	
Y-Intercept	8855.121359	
Correlation Coefficient	0.999987851	

ies are summarized in the TABLE 3A, 3B.

Linearity

Linear calibration plot of the method was obtained over the calibration ranges tested, i.e. from 2.5µg/ml to 135µg/ml for Sumatriptan Succinate and from 100µg/ml to 900µg/ml for Naproxen Sodium. Correlation co-efficient, slope, y-intercept for both the components are presented in TABLE 3A, 3B. The correlation coefficient obtained was greater than 0.999 indicating linear response of the both Sumatriptan succinate and Naproxen Sodium (Supporting Information- Figure-3A, 3B).

Accuracy

The percentage recovery of Sumatriptan succinate ranged from 98.5% to 101.4% and Naproxen Sodium ranged from 98% to 101.9%. The percentage recovery values of the Sumatriptan succinate and Naproxen Sodium are listed in TABLE 4.

Range

Range of the method was established from precision, accuracy and linearity parameters. Range of the method is from concentrations of 8.5µgm/ml to 127.5µgm/ml and 50µgm/ml to 750µgm/ml for Sumatriptan Succinate and Naproxen Sodium respectively.

Robustness

All the results for robustness changes were well with in set limits of system suitability.

Solution stability

Standard solution and test solutions were stable for a period of 5 days on bench top. Similarity factor was calculated for standard solution and difference in assay value was calculated for test solution to establish stability of the solutions.

CONCLUSION

A simple specific liquid chromatographic method

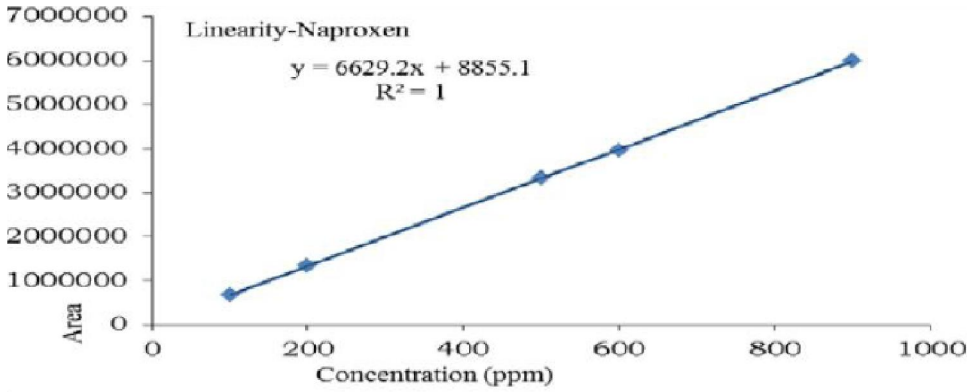

Figure 10 : Linearity plot of Naproxen

TABLE 4: Percentage recovery of Sumatriptan Succinate and Naproxen Sodium

Recovery Level	mg added		mg found		Recovery (%) [a]	
	Sumatriptan succinate	Naproxen Na	Sumatriptan succinate	Naproxen Na	Sumatriptan succinate	Naproxen Na
10%	85	500	83.86	509.6	98.7	101.9
50%	425	2500	418.48	2468.3	98.5	98.7
100%	850	5000	854.25	4901.6	100.5	98.0
120%	1020	6000	1025.78	5950	100.6	99.2
150%	1275	7500	1292.85	7452.5	101.4	99.4

[a] **Average of three determinations**

with isocratic elution is developed for quantification of Sumatriptan succinate and Naproxen Sodium. This method is validated and it is found to be specific, precise, accurate, linear and rugged for the detection and quantification of Sumatriptan succinate and Naproxen Sodium.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The Authors are thankful to the authorities of Jawaharlal Nehru Technological University, Hyderabad, India, for providing the laboratory facilities.

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