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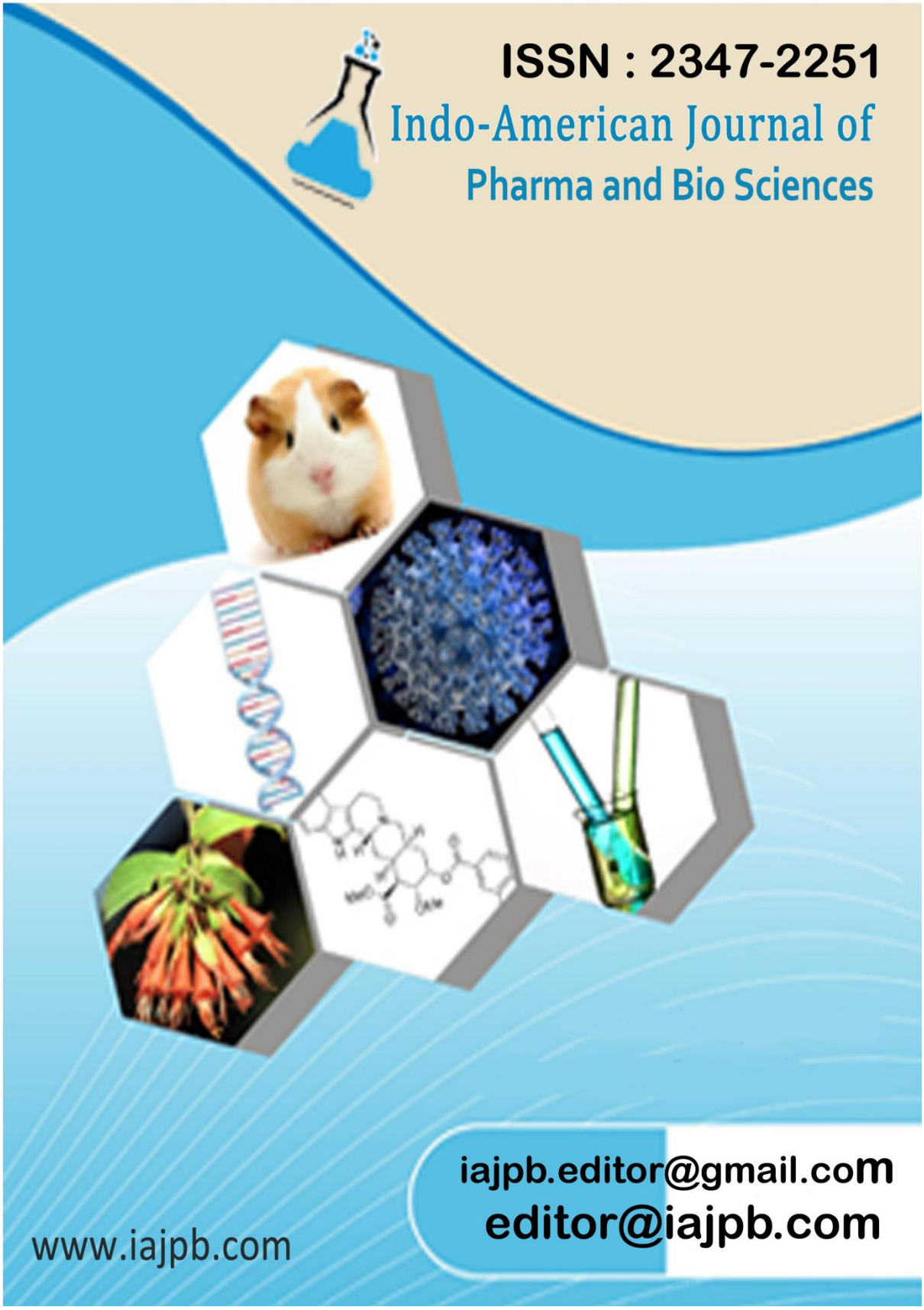

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Featured Compilation on Progress in Drug Testing and Formulation Sciences

K.Srikanth Kumar[1], S.Deepthi[2], P.Swathi[3], J.Dharuman[4]

1.Associate Professor, Department of Pharm.chemistry, QIS College of pharmacy,Ongole,AP

2.Assistant Professor, Department of pharmanalysis, QIS College of pharmacy,Ongole,AP

3.Associate Professor, Department of pharamchemistry, QIS College of pharmacy,Ongole,AP

4.Associate Professor, Department of Pharm.chemistry, QIS College of pharmacy,Ongole,AP

ABSTRACT

Goal: In accordance with ICH guidelines, a straightforward, accurate, fast, and cost-effective reverse phase high-pressure liquid chromatographic technique has been created for the measurement of naproxen in pharmaceutical formulations. Techniques: A Kromosil-C18 ODS column (150 mm x 4.6 mm; 5 µ) was used for the procedure. The mobile phase was made up of methanol (40:60 v/v) and ammonium acetate buffer (pH 4.0 adjusted with 1% triethyl amine), which was then filtered with 0.45 µ cellulose nitrate filters. In isocratic mode, the flow rate was kept constant at 1.0 mL/min. At 210 nm, the detection was performed. It ran for 7.0 minutes.

Results: Naproxen had a retention time of 3.063 minutes. Accuracy, precision, linearity, limit of detection, limit of quantification, and solution stability were all evaluated for the proposed technique.

Conclusion: For the measurement of naproxen in both bulk and tablet dose forms, the suggested approach was sufficient in terms of sensitivity, reproducibility, and specificity.

*Corressponding Author

K.Srikanth Kumar

srikanthk@gmail.com

INTRODUCTION

A popular non-steroidal anti-inflammatory medicine (NSAID) for moderate to severe pain, fever, inflammation, and stiffness is naproxen [(S)-6-methoxy-α-methyl-2-naphthalene acetic acid] (Fig. 1). It works by blocking the COX-1 and COX-2 enzymes. similar to other NSAIDs [1–3]. The literature reports stability showing simultaneous estimation of the RP-HPLC test technique for naproxen and esomeprazole in pharmaceutical formulations [4]. Numerous chromatographic techniques have been documented for the determination of naproxen in solid dosage forms, namely tablets and blood-plasma, as well as raw materials using RP-HPLC [5–8]. However, a review of the literature shows that several approaches were documented for naproxen, both alone and in combination with other medications. However, the mobile phase preparation was costly and time-consuming; the detection was carried out at a higher wavelength; and the retention period was longer. A successful effort to quantify naproxen using RP-HPLC with a photo diode array detector has been conducted in light of all these factors

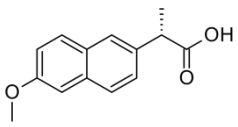

Fig. 1: Chemical Structure of Naproxen

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MATERIALS AND METHOD

substances and Reagents utilized: Triethylamine, ammonium acetate, methanol [HPLC Grade], water [HPLC Grade], and naproxen [working standards] were the substances utilized in the procedure. The source of all the chemicals was Standard Solutions in Hyderabad, Andhra Pradesh.The several solvents and solutions that were going to be injected into the column were filtered using 0.45 µ membrane filters (Advanced Micro Devices Pvt. Ltd., Chandigarh, India).

Chromatographic Conditions and Apparatus: High Performance Liquid Chromatography with Auto Sampler and UV or DAD Detector was the apparatus used. A Kromosil-C18 ODS column of 150 mm by 4.6 mm and 5 µ was used. A flow rate of 1.0 mL/min was observed. At 210 nm, the detection was performed. 20 µL was chosen as the injection volume, the column oven was kept at 25 °C, a photo diode array was employed as the detector, and the run duration was 7.0 minutes.

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.

A Lab India UV 3000 spectrophotometer was used to get the ultra violet spectra of the medications employed in the study in order to determine their λmax values. Sonication on an ultra sonicator (Power Sonic 510, Hwashin Technology) improved the compounds' solubility. An Afcoset electronic balance was used for all of the tests' weighings. The centrifugation procedure was carried out using a Remi equipment (model no. CM101DX) Cyclomixer and a HermLe microliter centrifuge Z100 (model no. 292 P01).

Glassware: All of the volumetric glassware utilized in the research was Borosil Grade A. Making the buffer solution [11]: 3.85 grams of ammonium acetate were precisely weighed and then put to a 1000 milliliter beaker. Initially, 900 milliliters of HPLC-grade water were added to dissolve the buffer. Ultimately, the diluent was used to bring the volume up to par. Triethyl amine was used to bring the pH down to 4.0.

Mobile phase preparation: 400 mL of the aforementioned buffer (40%) and 600 mL of methanol HPLC (60%) were combined to create the mobile phase, which was then degassed for five minutes in an ultrasonic water bath. After that, the solution was vacuum-filtered using a 0.45 µ filter.

Making the Naproxen standard solution: After precisely weighing 100 mg of naproxen, it was put into a 100 mL volumetric flask that had been cleaned and dried. The medication was first combined with seven milliliters of diluent. To ensure the medication was completely dissolved, the solution was sonicated for fifteen minutes. The same solvent was used to make up the remaining volume. About 5 mL of the previously produced solution was pipetted out and put into a 100 mL volumetric flask that had been cleaned and dried. First, 70 milliliters of diluent were added to the solution. To ensure the medication was completely dissolved, the solution was sonicated for fifteen minutes. To achieve a concentration of 50 µg/mL of Naproxen, the final volume was adjusted using the same solvent.

Naproxen sample solution preparation included precisely weighing twenty tablets, then using ultrasonication for 20 minutes to dissolve an amount of tablet powder equal to 100 mg of Naproxen in 70 mL of mobile phase. To prepare the stock solution, the substance was diluted with 100 milliliters of mobile phase. To achieve the required concentration of 50.0 µg/mL of naproxen, the stock solution was filtered using a 0.45 µm nylon syringe filter, and 5.0 mL of the filtrate was diluted into a 100.0 mL volumetric flask.

System suitability: Naproxen-induced peaks in standard solution should have a tailing factor of no more than 2.0. There should be at least 2000 theoretical plates for naproxen peaks in standard solution. Five distinct Naproxen formulations were injected to test the method's system appropriateness. The system suitability parameters were examined.

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VALIDATION DEVELOPMENT

System Suitability: A Standard solution was prepared by using Naproxen 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 Naproxen sodium, retention times and peak areas. The data are represented in table no. 1.

Table no. 1: System Suitability data for Naproxen

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.063	4437.5151	10168	1.106
2	3.064	4439.6279	10214	1.109
3	3.061	4437.5151	10200	1.110
4	3.059	4440.1612	10198	1.107
5	3.054	4446.1712	10210	1.108
Mean	3.0602	4440.198	10198	1.108
SD	0.003962	3.1749	-------	-------
% RSD	0.129479	0.0715	-------	-------

Specificity:

The chromatographic system is filled with standard and sample solutions that have been prepared in accordance with the test procedure. The standard and sample chromatograms need to be almost comparable in terms of retention time. A representation of the specificity may be seen in Figures 2-3.

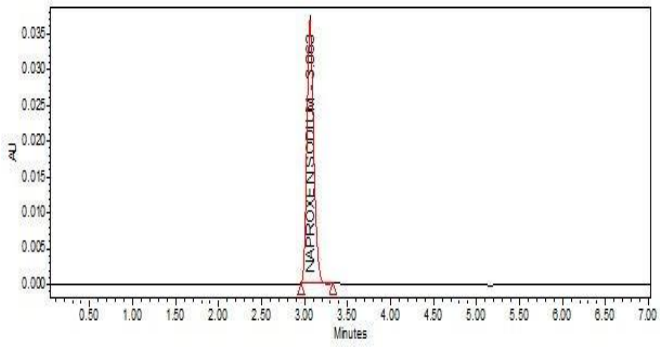

Fig. No. 3 A typical chromatogram for standard drug

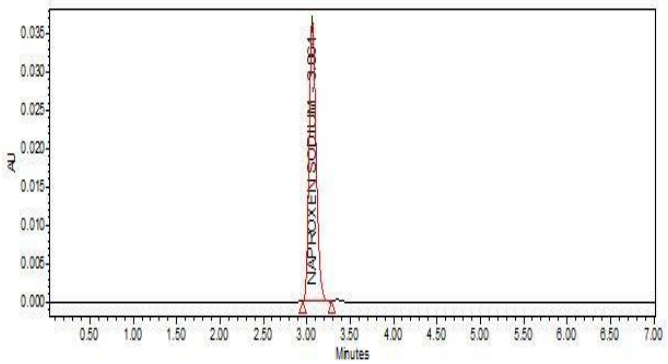

Fig. No. 3 A typical chromatogram for sample drug

Precision, which is often represented as a percentage of relative standard deviation (% RSD), is a measure of how repeatable an analytical process is under typical operating conditions. Five injections of the standard solution were made, and the area of each injection was measured in an HPLC. It was discovered that the region of five duplicate injections' percentage RSD fell inside the designated bounds. Tables two and three give the data.

Table no. 2: Precision results for Naproxen (System Precision)

Injection	Peak Areas	% Assay
1	4435.56	100.56
2	4437.58	100.88
3	4435.56	100.78
4	4440.15	100.06
5	4445.13	101.02
Mean	4438.796	100.06
SD	62.64	52.3
% RSD	1.23	0.09

Table no. 3: Precision results for Naproxen (Method Precision)

Injection	Peak Areas	% Assay
1	4437.5151	100.86
2	4439.6279	100.91
3	4437.5151	100.86
4	4440.1612	100.92
5	4446.1712	100.06
6	4445.1312	101.03
Mean	4448.67	100.77
SD	58.90	44.5
% RSD	1.56	0.08

Intermediate Precision/Ruggedness: In order to assess the method's intermediate precision, also referred to as its ruggedness, precision was carried out on several days using different-made columns of the same

measurements. Five injections of the standard solution were made, and the area of each injection was measured in an HPLC. The region of five duplicate injections' percentage RSD was confirmed to be within the designated bounds. Table No. 4 presents the information.

Table no. 4: Ruggedness results for Naproxen

Injection	Peak Areas	% Assay
1	4434.01	100.54
2	4436.79	100.86
3	4439.451	100.12
4	4442.512	100.56
5	4448.112	100.04
6	4461.012	101.26
Mean	4448.98	100.88
SD	78.90	67.0
% RSD	1.5	0.8

Accuracy: The degree of agreement between the value discovered and the value that is recognized as either a conventional true value or an approved reference value is a measure of an analytical procedure's accuracy. In order to determine the quantity of Naproxen detected and added, as well as the individual recovery and mean recovery values, the standard solution with accuracy levels of -50, 100, and 150 percent was injected into the chromatographic system. Table No. 5 presents the information.

Table No. 5: Naproxen accuracy findings

Concentration % of spiked level	Amount added (mg)	Amount found (mg)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	24.98	25.02	99.82	MEAN	99.82
50% Sample 2	23.89	24.15	98.89		
50% Sample 3	24.89	25.14	98.98	%RSD	0.82
100 % Sample 1	50.47	49.54	100.92	MEAN	100.3
100 % Sample 2	50.45	50.03	100.83		
100% Sample 3	51.46	51.2	100.56	%RSD	1.62
150% Sample 1	76.03	74.99	101.38	MEAN	101.5
150% Sample 2	75.78	74.66	101.50		
150% Sample 3	75.86	74.79	101.42	%RSD	0.62

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. 4. The data are represented in table no. 6.

Table no. 6: Linearity results for Naproxen

Concentration (µg/mL)	Average Area	Statistical Analysis	
20	1621.89	Slope	190.9x
40	5141.73	y-Intercept	-2361
50	7066.67	Correlation Coefficient	0.999
60	9054.19		
70	10980.2		
80	13060.1		

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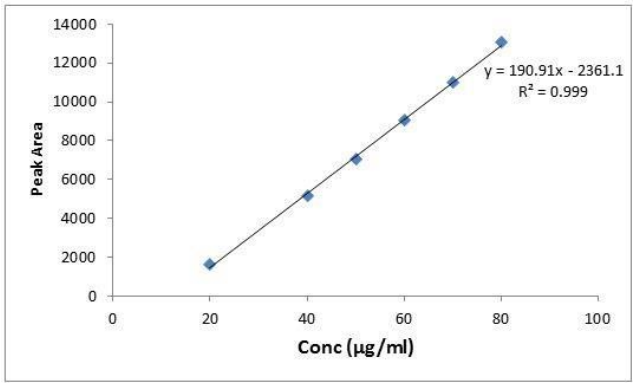


Fig. No. 4 Calibration curve for Naproxen

Limit of Detection The lowest quantity of analyte in a sample that can be identified, but not always in precise proportions, is known as the detection limit of a particular analytical process. Limit of Naproxen Detection: The signal to noise ratio was assessed after the sample with the lowest concentration was created in relation to the baseline noise. The lowest concentration of the material that may be detected—which the technology may not be able to quantify—is known as the limit of detection. (Statistical analysis of regression) The linearity curve is used to calculate the lowest concentration at which the analyte can be detected using the formula below.

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

Where S – slope of the calibration curve

σ – Residual standard deviation

$$\frac{3.43}{87.06} \times 3.43 = 0.13$$

Limit of Quantification It is the lowest analyte concentration in a sample that a certain technique can detect under specified experimental circumstances with a satisfactory level of precision, accuracy, and reliability. A concentration at a certain signal to noise ratio is used to represent LOQ. Naproxen's Quantification Limit: The signal to noise ratio was assessed after the sample with the lowest concentration was created in relation to the baseline noise. The lowest concentration of a material that can be quantitatively measured is known as the limit of quantification. By using the following formula, it may be ascertained from the linearity curve.

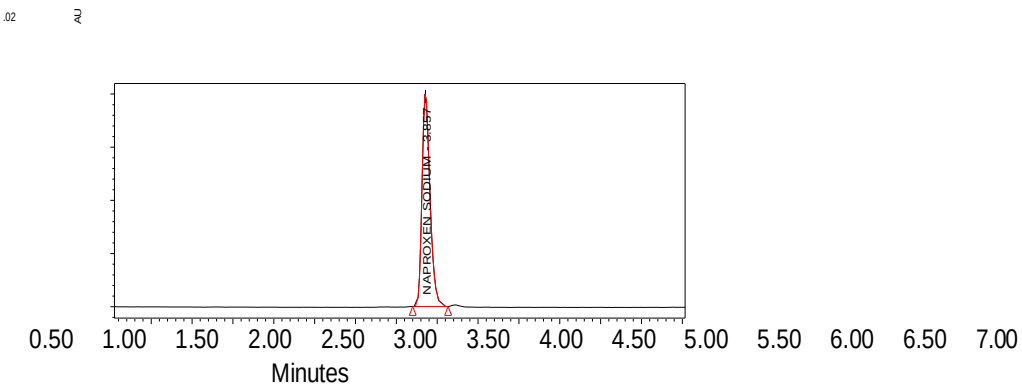
$$\text{Limit of Quantification (LOQ)} = \frac{\sigma}{S} \times 10$$

$$\frac{3.34}{87.46} \times 10 = 0.40$$

Robustness: In order to assess the effect on the approach, intentional changes were performed to the flow rate, mobile phase composition, and temperature fluctuation as part of the robustness. The chromatographic settings were changed in order to inject the Naproxen standard and samples. Parameters such as resolution, tailing factor, asymmetry factor, and plate count did not significantly alter. Table No. 7 and Figures Nos. 5, 6, and 7 display the data.

Table No. 7: System Suitability Results for Naproxen (Change in Flow Rate)

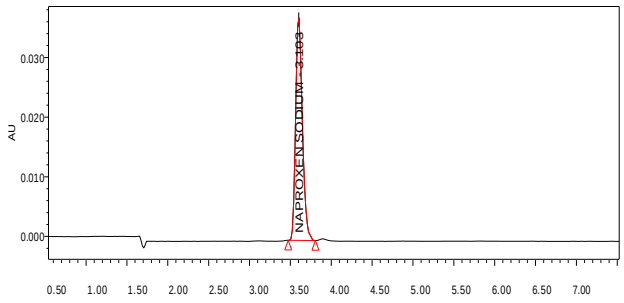
Flow 0.8 mL/min.	Std. Area	Tailing factor	Flow 1.0 mL/min.	Std. Area	Tailing factor	Flow 1.2 mL/min.	Std. Area	Tailing factor
	6079.40	1.106		4882.35	1.110		4076.02	1.123
	5895.63	1.110		4970.64	1.112		4167.62	1.125
	5935.37	1.112		4900.20	1.110		4138.32	1.124
	6056.36	1.118		4924.73	1.111		4140.31	1.124
	6059.63	1.117		4781.37	1.112		4098.21	1.123
Avg	6005.081	1.112	Avg	4891.86	1.111	Avg	4124.10	1.1238
SD	74.977	0.0044	SD	62.697	0.00089	SD	32.683	0.0007
% RSD	1.248	0.4003	% RSD	1.281	0.0804	% RSD	0.7925	0.0065



RESULTS AND DISCUSSION

Different ratios of buffer (pH 4.0) with methanol [HPLC Grade] were explored in order to improve the mobile phase. Peaks with excellent forms and resolution were produced by using methanol [HPLC Grade] and buffer (pH 4.0) in a 40:60 (v/v) ratio. It was discovered that the ideal flow rate in the 0.4–1.5 mL/min range was 1.0 mL/min, which led to a brief retention period.

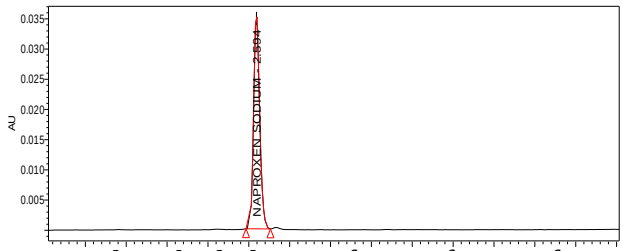
Fig. no. 5: A typical chromatogram for robustness with flow rate (for 0.8 mL/min flow)



minimal noise and baseline stability. The retention period of naproxen was found to be 3.063 minutes at 210 nm using the suggested technique. In the concentration range of 20–80 µg/mL for naproxen, quantitative linearity was observed. y = 86.83x + 57.31(R2 = 0.999) was the pertinent regression equation, where x is the Naproxen concentration (µg/mL) and y is the peak area ratio. The suggested method's intra-day and inter-day medication changes had an RSD of less than 2%, suggesting that

Minutes

Fig. no. 6: A typical chromatogram for robustness with flow rate (for 1.0 mL/min flow)



The suggested method's limits of quantification for naproxen were 0.40 µg/mL and.13 µg/mL, demonstrating the method's sensitivity. Effective performance was shown by the method's strong resilience, which allowed it to withstand little fluctuations in optimal chromatographic settings..of the column.

No interfering peaks were found in thecFig. no. 7: Excipients utilized in tablet formulations did not impede the drug's estimation using the suggested HPLC approach, according to a typical chromatogram for robustness with flow rate (for 1.2 mL/min flow).

CONCLUSION

It was discovered that the suggested HPLC technique for determining naproxen was straightforward, accurate, sensitive, and exact. Every parameter satisfied the acceptance requirements, and the procedure was verified in accordance with ICH norms. Usefulness of our technique for concurrent Naproxen tablet estimate dosage forms were verified. As a result, this approach is precise and effective for estimating naproxen in pharmaceutical dosage forms and bulk medication samples. For regular quality control examination of the aforementioned medication, this approach may thus be readily and simply implemented.

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