

New RP-HPLC Technique was Development and Validation of Effective and Economical Tool for Quantitative Estimation of Naproxen in Dosage Forms

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ABSTRACT

A New RP-HPLC technique was development and validation of effective and economical tool for quantitative estimation of Naproxen in dosage forms rapid, reproducible and selective reverse phase HPLC method has been developed for the estimation of Naproxen in dosage form. It was resolved by using a mobile phase of Potassium dihydrogen phosphate: methanol in the ratio 30:70 v/v at a flow rate of 0.8 ml/min. on HPLC system using UV - Visible detector at the wavelength of 287 nm. The column used was C18 (4.6 x 150mm, 3 mm, Make: Zorbax). The linearity range was found to be 10-50 µg/ml. The proposed new method is found to be economic, sensitive, precise, rapid and reproducible.

Keywords: Naproxen, RP-HPLC, new method development, validation

Introduction

Naproxen is a propionic acid derivative related to the arylacetic acid group of nonsteroidal anti-inflammatory drugs^{1,2}. Naproxen has a molecular weight of 230.26 and a molecular formula of C₁₄H₁₄O₃. It is lipid insoluble, practically insoluble in water at low pH and freely soluble in water at high pH. The octanol/water partition coefficient of naproxen at pH 7.4 is 1.6 to 1.8. The chemical name for naproxen^{3,4}, USP is (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid. It has the following structural formula show in fig 1:-

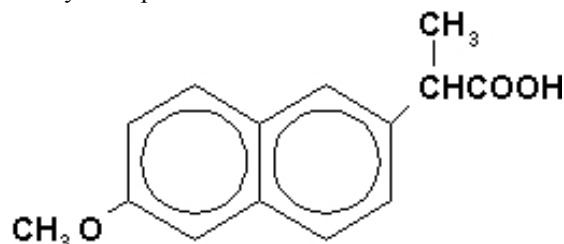


Fig.1: Chemical structure of Naproxen

These drugs are used for the management of mild to moderate pain, fever, and inflammation. They work by reducing the levels of prostaglandins, chemicals that are responsible for pain, fever, and inflammation. Naproxen blocks the enzyme that makes prostaglandins (cyclooxygenase), resulting in lower concentrations of prostaglandins. Naproxen has analgesic and antipyretic properties^{5,6,7}. As with other NSAIDs, its mode of action is not fully understood; however, its ability to inhibit prostaglandin synthesis may be involved in the anti-inflammatory effect. The mechanism of action of naproxen, like that of other NSAIDs, is believed to be associated with the inhibition of cyclooxygenase activity. Two unique cyclooxygenases have been described in mammals⁸. The constitutive cyclooxygenase, COX-1, synthesizes prostaglandins necessary for normal gastrointestinal and renal function^{9,10}. The inducible cyclooxygenase, COX-2, generates prostaglandins involved in inflammation¹¹. Inhibition of COX-1 is thought to be associated with gastrointestinal and renal toxicity while inhibition of COX-2 provides anti-inflammatory activity^{12,13,14}.

MATERIALS AND METHODS

1. Reagents and Standard – Naproxen tablets

- Water HPLC Grade.
- Naproxen Working Standard.
- Methanol
- Sodium hydroxide

2 Chromatographic Parameters

Equipment : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.

Column :Symmetry C18 (4.6 x 150mm, 3 mm, Make: Zorbax) Flow rate
 : 0.8mL per min
 Wavelength : 287 nm
 Injection volume : 20 ml
 Column oven : Ambient
 Run time : 6.0 min

3. Preparation of Sodium Phosphate buffer:

Weigh 2.5milli grams of sodium di hydrogen phosphate into a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water. Adjusted the pH to 7.0 with Sodium hydroxide

Preparation of mobile phase

Mix a mixture of above buffer 300mL (30%) and 700 mL of Methanol HPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 μ filter under vacuum filtration.

4. Diluent Preparation: Mobile phase as diluent

5. Preparation of the Naproxen Standard & Sample Solution:

5.1 Standard Solution Preparation:

Accurately weigh and transfer 10mg of Naproxen Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45μm filter.

5.2 Sample Solution Preparation:

Weigh 5 Naproxen Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Naproxen into a 10 mL volumetric flask. Add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45μm filter. Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45μm filter.

6. System Suitability:

Tailing factor for the peak due to Naproxen in Standard solution should not be more Than 2.0
 Theoretical plates for the Naproxen peak in Standard solution should not less than 2000

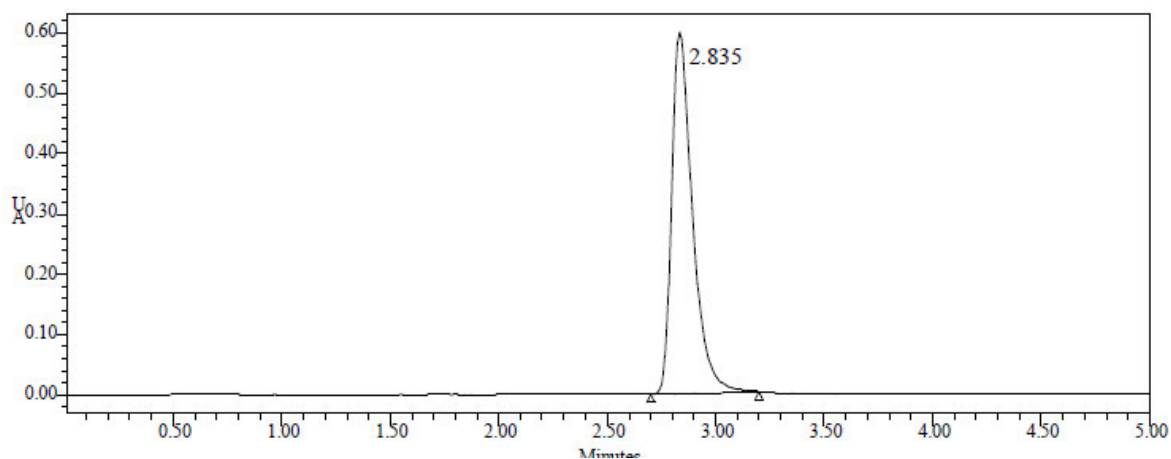
7. System Suitability Results:

- 1). Tailing factor Obtained from the standard injection is 1.5
- 2). Theoretical Plates Obtained from the standard injection is 4324.4

8. Assay Results:

Weight of 5 tablets: 0.5510 grams
 Average Weight : 0.1030grams

$$\frac{1867403 \times 10 \times 0.4 \times 10 \times 10 \times 99.8 \times 325}{1875180 \times 10 \times 10 \times 13 \times 0.4 \times 100 \times 250} \times 100 = 99.4\%$$



METHOD VALIDATION SUMMARY:

9.1 Precision:

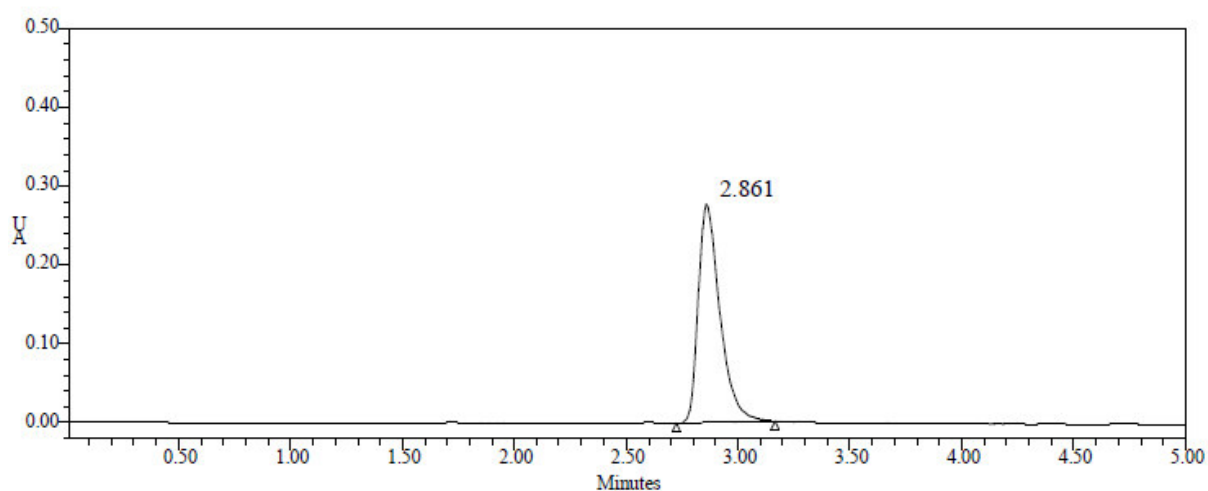
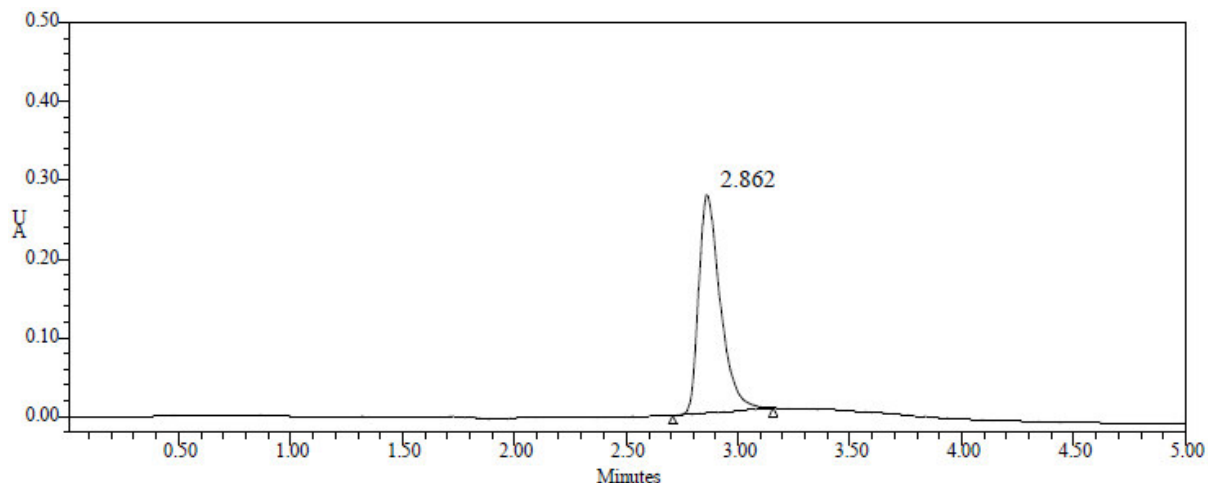
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

The results are summarized

Injection	Area
Injection-1	1871423
Injection-2	1876279
Injection-3	1874529
Injection-4	1879273
Injection-5	1873436
Average	1874987.9
Standard Deviation	2973.1
%RSD	0.2

Acceptance Criteria:

The % RSD for the area of Five standard injections results should not be more than 2%.



9.2 Intermediate Precision:

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.

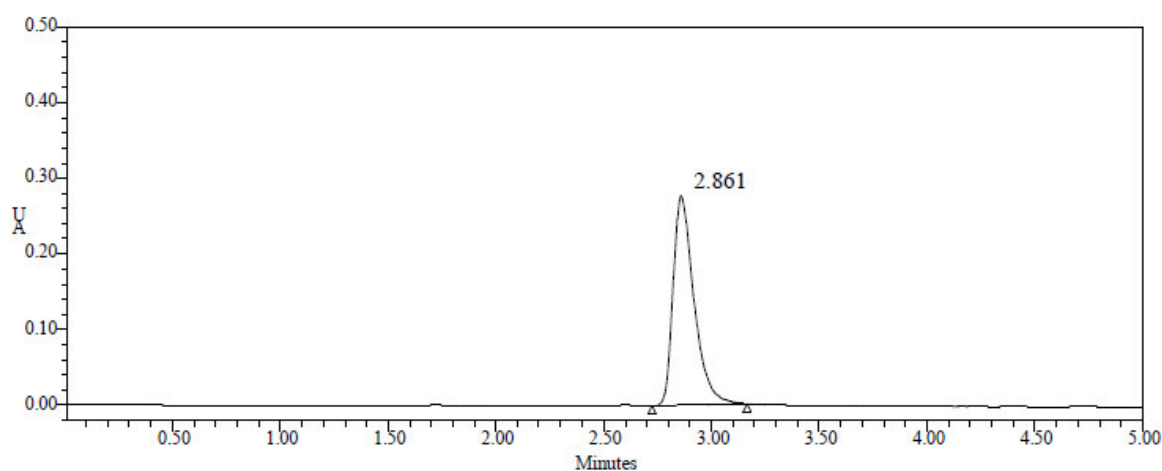
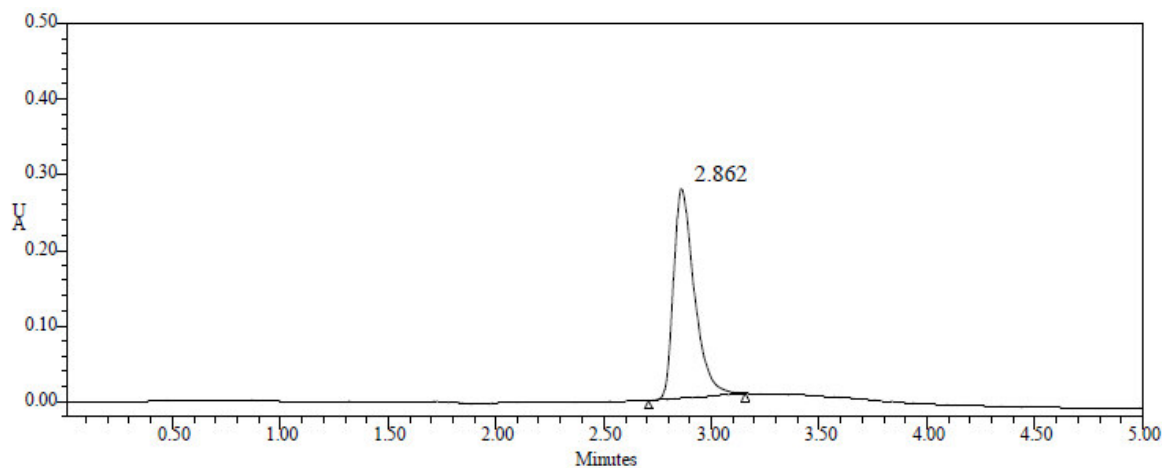
The standard solution was injected for Five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits

The results are summarized in

Injection	Area
Injection-1	1869365
Injection-2	1868938
Injection-3	1861814
Injection-4	1867522
Injection-5	1866552
Average	1866837.9
Standard Deviation	3023.9
%RSD	0.2

Acceptance Criteria:

The % RSD for the area of Five standard injections results should not be more than 2%.



10. Accuracy:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Naproxen and calculate the individual recovery and mean recovery values.

The results are summarized

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2006872	5.0	5.0	100.0%	100.5%
100%	4014113	10.0	10.0	100.0%	
150%	6104804	15.0	15.2	101.4%	

Acceptance Criteria:

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

11. LINEARITY:1

Preparation of stock solution: Accurately weigh and transfer 10mg of Naproxen API sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark

with the same solvent. (Stock solution)

Preparation of Level – I (20µg/ml):

0.2ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent

Preparation of Level – I (30µg/ml):

0.3ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (40µg/ml):

0.4ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (50µg/ml):

0.5ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (60µg/ml):

0.6ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

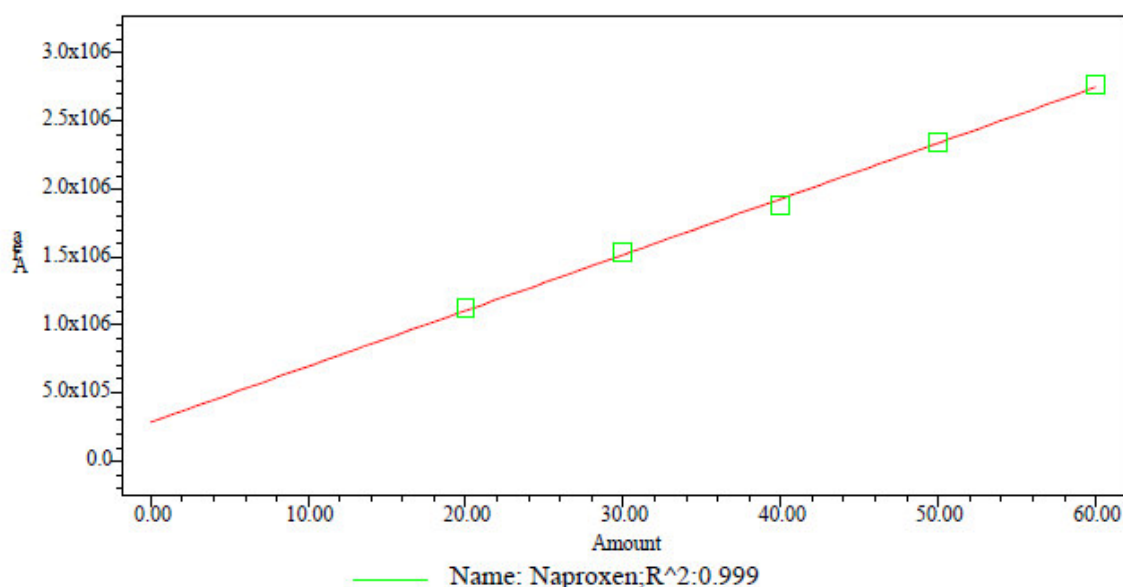
Linearity Results:

S.No	Linearity Level	Concentration	Area
1	I	20µg/ml	1121401
2	II	30µg/ml	1529276
3	III	40µg/ml	1879755
4	IV	50µg/ml	2344717
5	V	60µg/ml	2766815
Correlation Coefficient			0.999

Acceptance Criteria:

Correlation coefficient should be not less than 0.999.

Calibration Plot



12. LIMIT OF DETECTION:

Average Baseline Noise obtained from Blank : 51µV
 Signal Obtained from LOD solution (0.25% of target assay concentration) : 152 µV
 S/N = 152/51= 2.98

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

13. LIMIT OF QUANTIFICATION:

Average Baseline Noise obtained from Blank : 51µV
 Signal Obtained from LOQ solution (0.75% of target assay concentration) : 509 µV
 S/N = 509/51 = 9.98

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

14. 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.

No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	SP Tailing
	0.7	4486	1.5
8		4324.4	1.5
	0.9	4306	1.4

Results for actual flow (0.8 ml/min) have been considered from Assay standard.

b). The Organic composition in the Mobile phase was varied from 70% to 50%.

No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	SP Tailing
	10% less	4758	1.5
	Actual	4324.4	1.5
	10% more	3807	1.5

Results for actual Mobile phase composition (70:30Methanol: Buffer) have been considered From Assay standard

RESULT AND DISCUSSION

A New simple, precision and accuracy HPLC method was developed the estimation of Naproxen analysis , consisting of an Acetonitrile: buffer system (60: 40 % v/v). The chromatographic condition was set at a low rate of 0.8 ml/min with the UV detector at 230 nm. The above method was optimized with a view to develop an assay method for Naproxen. Several mobile phase compositions were tried to resolve the peaks of Naproxen. The optimum mobile phase containing KH_2PO_4 buffer : methanol (30;70 % v/v) was selected because it was found ideal to resolve the analytic peaks of the drugs^{15,16}. Quantification was achieved with UV detections at 287 nm based on peak area and absorbance. As per USP requirements system suitability studies were carried out and freshly prepared standard solutions are Naproxen^{17,18}. Various parameters obtained with 20 μl of injection volume are summarized in the table given below. Validation and system suitability parameters

Table:5

S.NO	PARAMETERS	LIMIT	OBSERVATION
1	System suitability (%RSD of tailing factor)	suitable	1.5
2.	Specificity	No interferences	Specific
3	Precision: A)System Precision B).Method precision	RSD NMT 2.0% RSD NMT 2.0%	0.2 0.2
4	Linearity	Correlation coefficient NLT 0.999	1.000
5	Accuracy	%Recovery range 98-102 %	100.5
6	Robustness	RSD NMT 2%	Robustted
7	LOD	S:N Ratio should be more than 3:1	2.98
8	LOQ	S:N ratio should be more than 10:1	9.98

The system is suitable for tailing factor, theoretical plate, resolution. The data obtained from the precision experiments. The R.S.D. value for precision was indication that the method was efficiently precise. Percentage recovery was calculated from 80% to 120% by injecting to HPLC. The excellent recovery was made at each added concentration. There is allowable variation in flow rate, wave length which indicates that method is robust enough. The LOD for Naproxen were found to be 2.98 $\mu\text{g/ml}$ The LOQ for Naproxen were found to be 9.98 $\mu\text{g/ml}$ The chromatogram of sample showed a single peak at the retention time of Naproxen indicating that there is no interference of the changing the persons for injecting the sample to the instrument.

CONCLUSION

The reliability and suitability of the method could be seen from recovery studies. Further there is no interference due to excipients . System suitability parameters were calculated which includes efficiency, resolution and tailing factor. Precision of the methods were studied by making repeated injections of the samples and system precision values were determined. The method was validated for linearity, accuracy, precision, robustness. The method is new simple, specific & easy to perform and requires short to analyse the samples. Low limit of Quantification and limit of detection makes this method suitable for Quality control. This new method enables Simultaneous determination of because of good separation and Resolution of the Chromatographic Peaks. The

method was found to be accurate, precise and robust. Hence it was concluded that the RP-HPLC method developed was very much suitable for routine analysis. Naproxen in tablet formulations and future plans use this method for estimation of Naproxen in clinical trials.

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