

**METHOD DEVELOPMENT AND VALIDATION FOR QUANTIFICATION OF
NAPROXEN IN BULK AND TABLET DOSAGE FORM BY RP-HPLC****B. Naveen Kumar, Dr. Srikanth Lingala* and A. Venkatesham**

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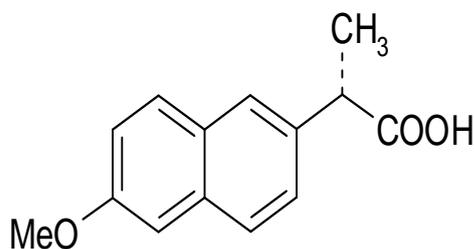
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ABSTRACT

While developing the HPLC method, basic chromatographic conditions such as the column, solvents and UV detection employed in this method were taken into account. In selecting the UPLC column, its efficiency and resolution was taken into consideration. Most commercial C₁₈ columns are not stable at high pH on the longer run, thus shortening their life span. Waters Acquity HSS T-3 C₁₈ column (100 × 2.1 mm, 1.8µm) column was found to be more suitable and stable at this pH. The peak was sharp and distinct. Developing a UPLC method was to reduce the run time of the method and solvent consumption for routine analysis such as assay, dissolution and content uniformity during quality assurance. Detection of Naproxen must adequate at 287 nm. The initial trial was conducted using HPLC and chromatographic separation obtained on a Waters HSS C₁₈ column (100 x 2.1mm, particle size 5µm). The developed method must be validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness. The method was linear for Naproxen from 20 - 60µg/ml and the linear regression obtained Precision, evaluated by intra- and inter-day assays had relative standard deviation (R.S.D) values within 1.5 %. Recovery data were in the range 99.3-103.2% with R.S.D. values < 1.5 %.

KEYWORDS: Naproxen, RP-HPLC, Sodium dihydrogen phosphate, cyclooxygenase.**INTRODUCTION**

High-performance liquid chromatography (HPLC)^[1] is an advanced form of liquid chromatography used in separating the complex mixture of molecules encountered in chemical and biological systems, in order to understand better the role of individual molecules. Among different chromatographic methods, high performance liquid chromatography (HPLC) offers a greater variety of stationary phases, which there by allows selective interactions and more possibilities for separation. In HPLC^[2] the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 3-10 µm. In HPLC mobile phase composition is changed in a programmed fashion to increase the efficiency of separation.

**Figure 1: Structure of Naproxen.**

Name	: Naproxen
Category	: Nonsteroidal antiinflammatory drug
Molecular formula	: C ₁₄ H ₁₄ O ₃
Molecular weight	: 230.25
Solubility	: It is soluble in water and in aqueous solutions, and insoluble organic solvents.
PKA	: 4.15
Chemical name	: (αS)-6-methoxy-α-methyl-2-naphthalene acetic acid.

Mechanism of Action: The mechanism of action of Naproxen, like that of other NSAIDs, is believed to be associated with the inhibition of cyclooxygenase activity. The constitutive cyclooxygenase, COX-1, synthesizes prostaglandins necessary for normal gastrointestinal and renal function. The inducible cyclooxygenase, COX-2, generates prostaglandins involved in inflammation. Inhibition of COX-1 is thought to be associated with gastrointestinal and renal toxicity while inhibition of COX-2 provides anti-inflammatory activity.

MATERIALS AND METHODS

Materials and Reagents

Naproxen Working Standard was procured from Natco laboratories, Hyderabad, India. Commercially available Naproxen purchased from local pharmacy. Methanol HPLC Grade and Ortho phosphoric acid AR grade were obtained from Merck chemicals, Mumbai. Water was prepared by using Millipore Milli Q Plus water purification system.

Preparation of Sodium Phosphate buffer: Weigh 2.5mg of sodium dihydrogen phosphate into a 1000ml beaker, dissolve and diluted to 1000ml with Millipore 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 (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µm filter under vacuum filtration.

Naproxen Standard Solution Preparation: Accurately transferred 10mg of Naproxen working standard into a 10 mL volumetric flask and about 7 mL of diluent added then sonicated to dissolve it completely and the volume was made up to the mark with the same solvent (Stock solution). Further pipetted 0.4 mL of the above stock solution into a 10mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µm filter.

Naproxen Sample Solution Preparation: Accurately transferred the sample equivalent to 10 mg of Naproxen into a 10 mL volumetric flask. About 7 mL of diluent added and sonicated to dissolve it completely and the volume is made up to the mark with diluent. Mixed well and filtered through 0.45µm filter. Further pipetted 0.4 mL of the above stock solution into a 10mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µm filter.

Instrumentation

Table 1: List of Equipment used in the method.

S.No	Name	Make/ Model
1	Analytical balance	Aicoset
2	HPLC instrument Series Software	AquityHPLC system (WATERS) Alliance e2695 EMPOWER- 2
3	Columns	Acquity HSS T-3 C ₁₈ (100 × 2.1 mm, 5 µm). Acquity BEH C ₁₈ , (50mm × 2.1 mm, 5 µm) Phenyl (100mm × 2.1 mm, 5 µm)
4	Detector	UV detector
5	Sonicator	SONICA 2200MH
6	PH meter	Metler Toledo
7	Vacuum filter	Model XI 5522050 of Millipore

Method Development

Various chromatographic conditions^[3] were tried to achieve better efficiency of the chromatographic system. Conditions such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer, and solvents (water, methanol and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Selection of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. Phenyl (100mm × 2.1mm, 5µm) column used for the elution, but the peak eluted before 4.5 minutes with a tailing factor of 2. Experiment with Acquity BEH C₁₈, (50mm × 2.1mm, 5µm) column ended with inconsistent retention time and peak fronting. Waters Acquity HSS T-3C₁₈ (100X2.1mm, 5µm) have produced a peak with adequate resolution and efficiency.

Buffers^[4] like sodium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate, and disodium hydrogen orthophosphate did not yield desired results. Use of ion pair reagents also did not yield the expected peak. At 30:70 (Sodium Phosphate buffer: methanol) ratio of the mobile phase, a perfect peak was eluted. Thus the mobile phase ratio was fixed at 30:70 (Sodium Phosphate buffer: methanol) in an isocratic mobile phase flow rate. The optimized conditions were illustrated in Table 2.

Table 2: Optimized Chromatographic conditions for Naproxen.

Parameters	Method
Stationary phase (column)	Waters Acquity HSS T-3 C ₁₈ (100 × 2.1mm, 5µm)
Mobile Phase	Sodium Phosphate buffer: methanol(30:70 v/v)
pH	7.0 ± 0.02
Flow rate (ml/min)	0.8
Run time (minutes)	6.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	287
Drug RT (min)	2.852

The typical chromatogram obtained for Naproxen from final HPLC conditions are depicted in Typical chromatogram of Naproxen by proposed method.

When the developed method^[5] was used to analyze a commercial brand of Naproxen tablet formulation, the mean recovery of five replicates recovery value indicates non-interference from the excipients present in the dosage form.

The chromatograms were depicted

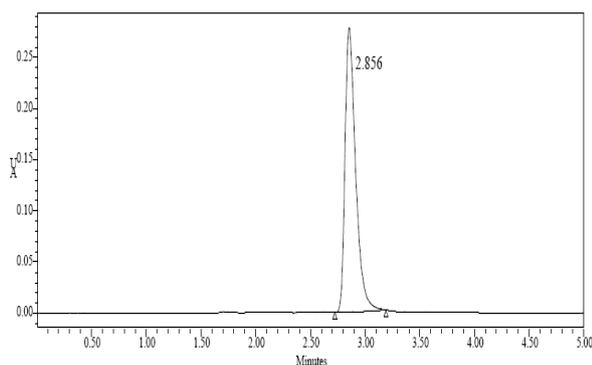


Figure 2: Chromatogram for Sample injection-1.

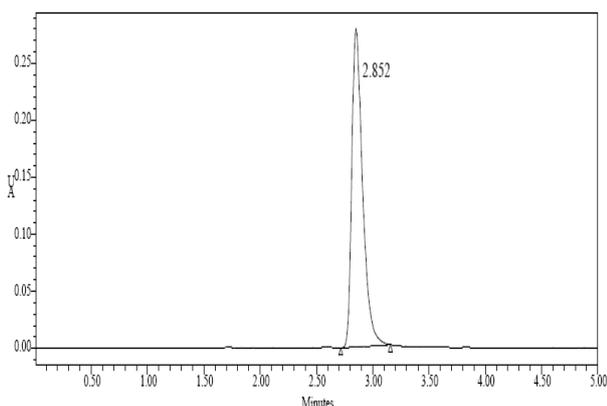


Figure 3: Chromatogram for Sample injection-2.

RESULTS AND DISCUSSION

Method validation

Based on International Conference on Harmonization (ICH) guidelines, the proposed method is validated with regard to system suitability,^[6] linearity, accuracy, precision, LOD, LOQ, robustness and sensitivity as follows.

System suitability

Standard solution of Naproxen was prepared as per procedure and was injected six times into the UPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

1. The % RSD for the retention times of principal peak from 6 replicate injections of each Standard solution should be not more than 2.0 %
2. The number of theoretical plates (N) for the Naproxen peak should be NLT 2000.
3. The Tailing factor (T) for the Naproxen peak should be NMT 2.0.

Precision

4. The precision^[7] of the method was evaluated by carrying out five independent assays of test sample against a qualified reference standard and the %RSD

of assay was calculated (% RSD should not be more than 2%).

5. **Intra-day precision:** The precision of the assay method was evaluated by carrying out six independent assays of Naproxen (50,100, 150% i.e. 5.0, 10.0, 15.0µg/ml.) test samples against qualified reference standard. The %RSD of six assay values was calculated.
6. **Intermediate precision (inter-day):** Different analyst from the same laboratory and by using different column of same brand evaluated the intermediate precision of the method. This was performed by assaying the six samples of Naproxen against qualified reference standard. The percentage of RSD for the area of six replicate injections was found to be within the specified limits (% RSD should not be more than 2%).
7. Recovery of the assay method^[8] for Naproxen was established by three determinations of test sample using tablets at 50%, 100% and 150% of analyte concentration. Each solution was injected thrice (n=3) into UPLC system and the average peak area was calculated from which Percentage recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).
8. **Linearity:**^[9] Test solutions were prepared from stock solution at 5 concentration levels (10, 20, 30, 40 and 50 µm/ml). The peak area vs. concentration data treated by least square linear regression analysis. (Correlation coefficient should be not less than 0.999.).
9. Limit of Detection (LOD) Limit of Quantification (LOQ).^[10]
10. LOD and LOQ for the were determined at signal to noise ratios of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations.

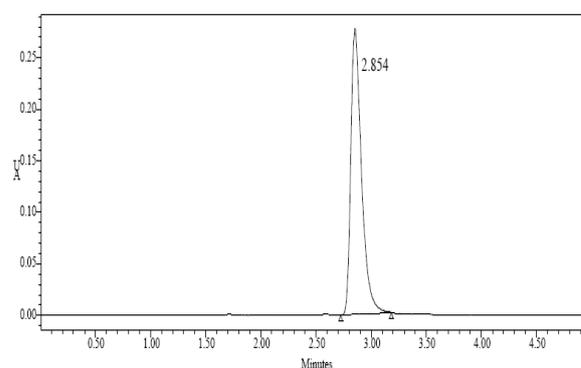


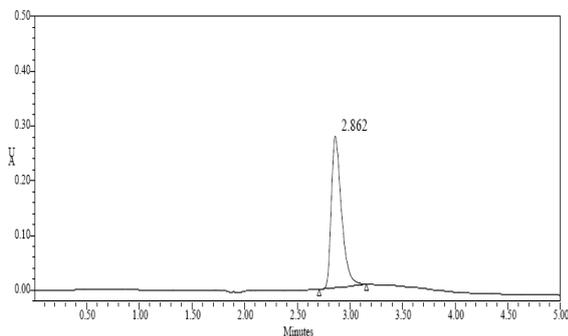
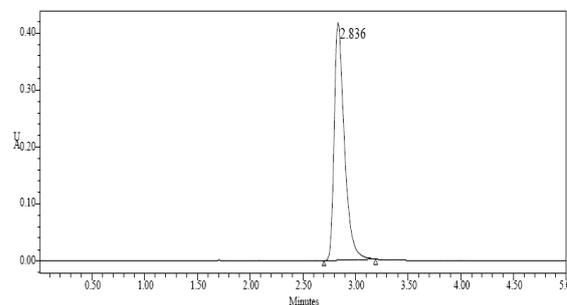
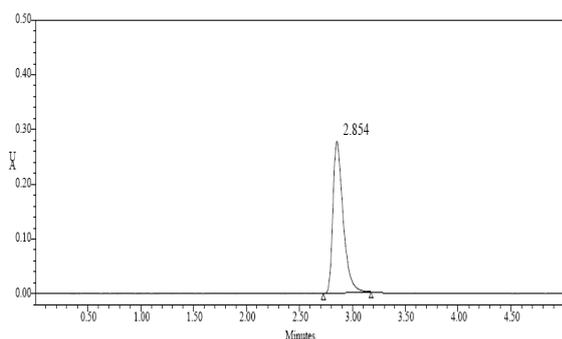
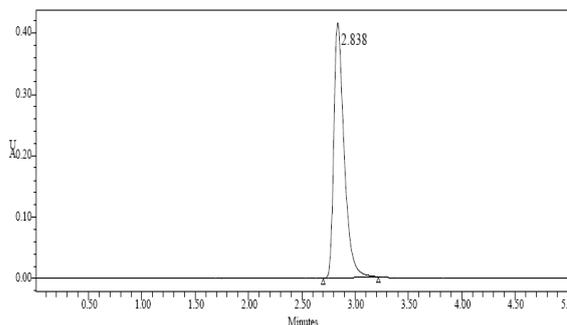
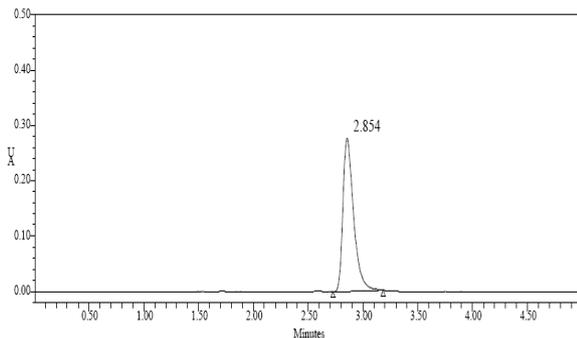
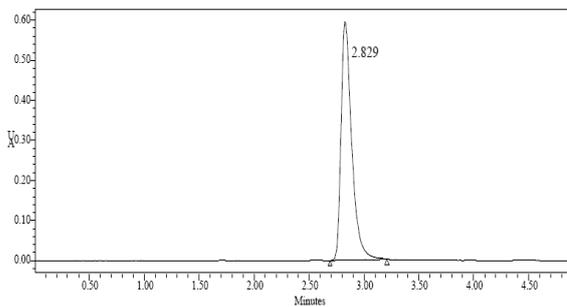
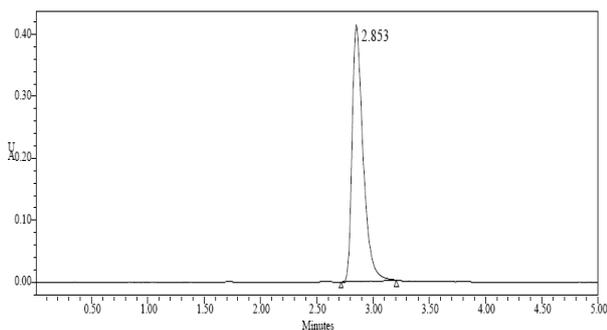
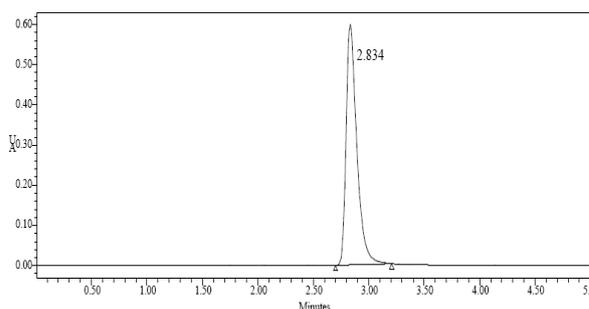
Figure 4: LOQ and LOD.

Accuracy

Percentage recovery of Naproxen samples ranged from 100.0% to 101.2% and the mean recovery is 100.5%, showing the good accuracy of the method. The result is shown in Table 5. The chromatograms were shown in figures 5 to 13.

Table 3: Results of Accuracy.

% Concentration (at specification Level)	Mean PeakArea(n=3)	Amount Added(mg)	Amount Found(mg)	Average % 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	101.2%	

**Figure 5: Chromatogram for Accuracy 50% injection-1.****Figure 9: Chromatogram for Accuracy 100% injection-2.****Figure 6: Chromatogram for Accuracy 50% injection-2.****Figure 10: Chromatogram for Accuracy 100% injection-3.****Figure 7: Chromatogram for Accuracy 50% injection-3.****Figure 11: Chromatogram for Accuracy 150% injection-1.****Figure 8: Chromatogram for Accuracy 100% injection-1.****Figure 12: Chromatogram for Accuracy 150% injection-2.**

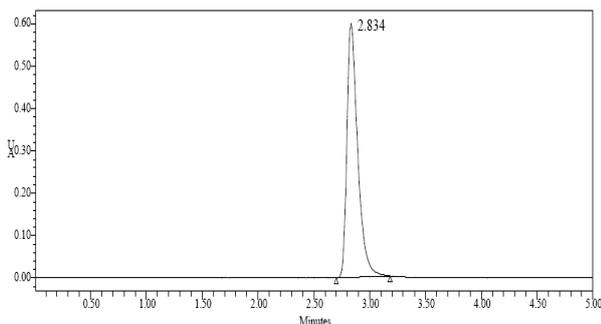


Figure 13: Chromatogram for Accuracy 150% injection-3.

Limits of detection (LOD) and quantification (LOQ)

LOD and LOQ for Naproxen^[11] were 0.01 and 0.05 $\mu\text{g/ml}$, respectively. Since the LOQ and LOD values of Naproxen are achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry. The corresponding chromatograms were given in Figure 14 and 15 for LOD and LOQ respectively.

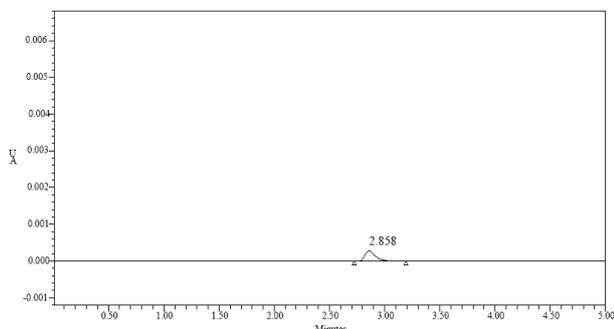


Figure 14: Chromatogram for LOD.

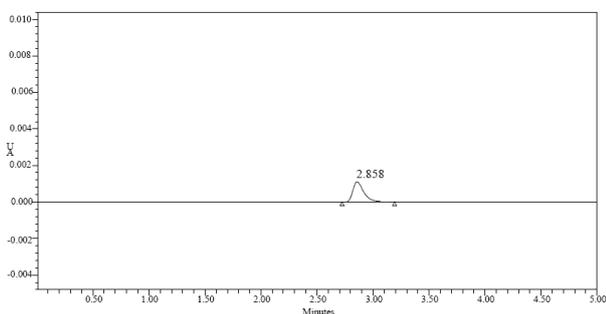


Figure 15: Chromatogram for LOQ.

SUMMARY AND CONCLUSION

The prime objective of the proposed method was to achieve separation and quantification of Naproxen using an isocratic mobile phase with UPLC system. Developing a UPLC method was to reduce the run time of the method and solvent consumption for routine analysis such as assay, dissolution and content uniformity during quality assurance. Detection of Naproxen was adequate at 287 nm. The initial trial was conducted using HPLC and chromatographic separation was obtained on a Waters HSS C₁₈ column (100 x

2.1mm, particle size 5 μm). Naproxen was resolved by using a mobile phase of Potassium di-hydrogen phosphate: methanol in the ratio 30:70 v/v. While developing the present method, basic chromatographic conditions such as the column, solvents and UV detection employed in the HPLC method were taken into account. In selecting the UPLC column, its efficiency and resolution was taken into consideration. Most commercial C₁₈ columns are not stable at high pH on the longer run, thus shortening their life span. Waters Acquity HSS T-3 C₁₈ column (100 x 2.1 mm, 1.8 μm) column was found to be more suitable and stable at this pH. The peak was sharp and distinct.

The flow rate also is scaled down from 2.0 to 0.8 mL/min. When these operating conditions were applied to the developed method, a satisfactory peak was achieved for Naproxen, which eluted at around 2.852 min giving a total run time of 6 min. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness. The present method was linear for Naproxen from 20 - 60 $\mu\text{g/ml}$ and the linear regression obtained was 0.999. Precision, evaluated by intra- and inter-day assays had relative standard deviation (R.S.D) values within 1.5 %. Recovery data were in the range 99.3-103.2% with R.S.D. values < 1.5 %.

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