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# 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

## Intoduction

Naproxen is a proprionic 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 ¬soluble, 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:-

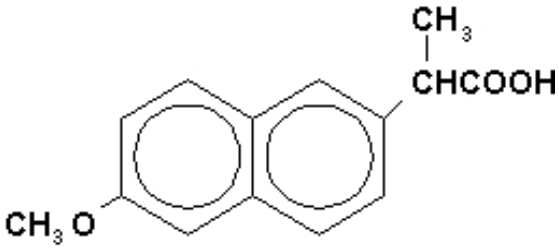



Fig.1: *Chemical structure of Naproxen*

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 mammals[8]. 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 inflammation[11]. 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

a. Water HPLC Grade.
b. Naproxen Working Standard.
c. Methanol
d. 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.

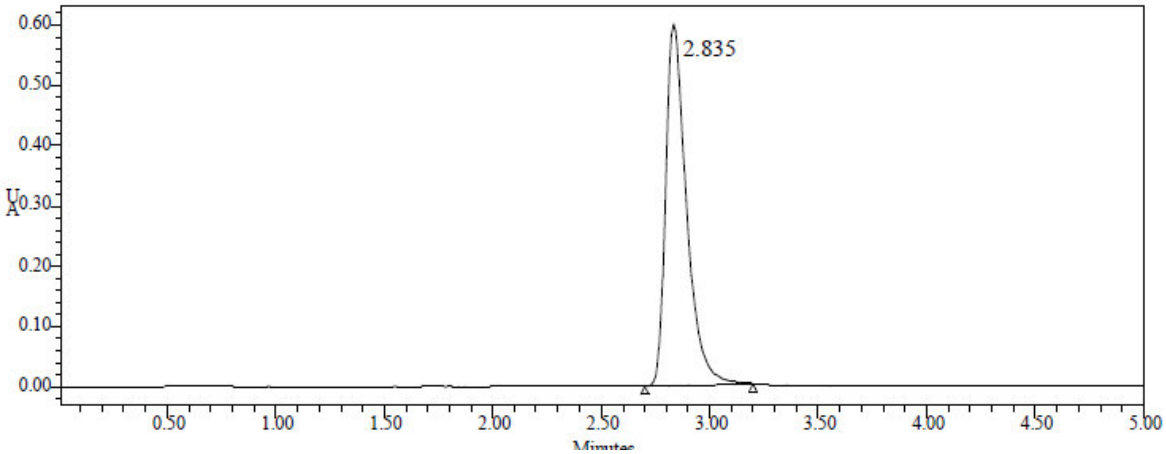
**5.2 Sample Solution Preparation:**
Weigh 5 Naproxen Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Naproxen into a 10 mL volumetric flask. Add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette0.4 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

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




**METHOD VALIDATION SUMMARY:**

**9.1 Precision:**

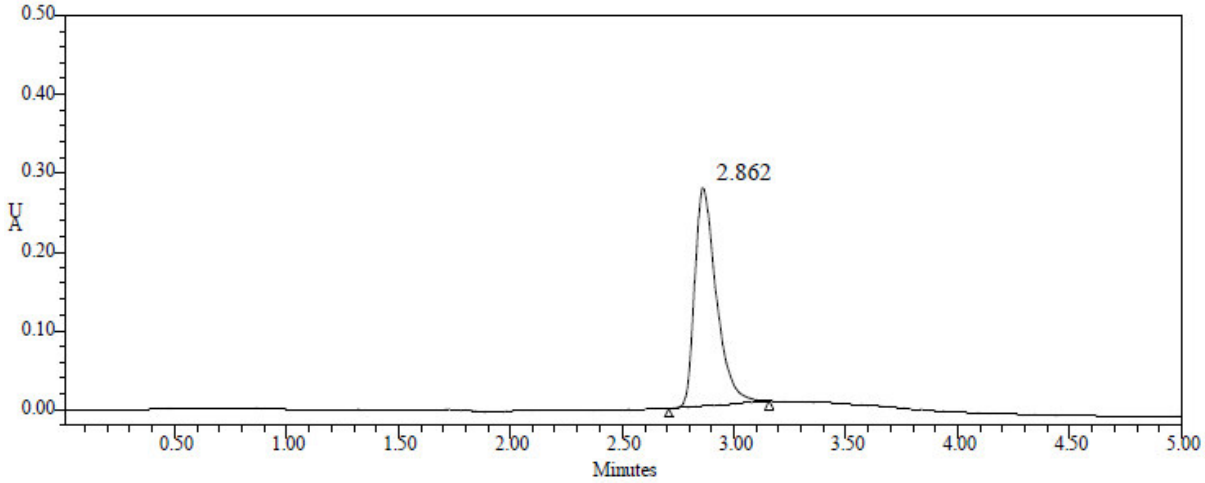
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

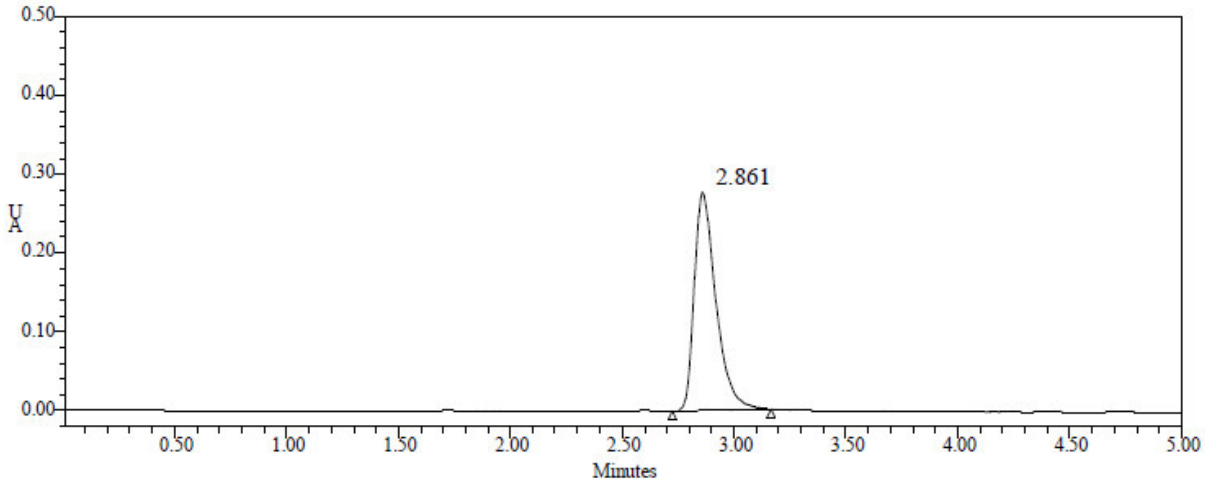
The results are summarized

| **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.

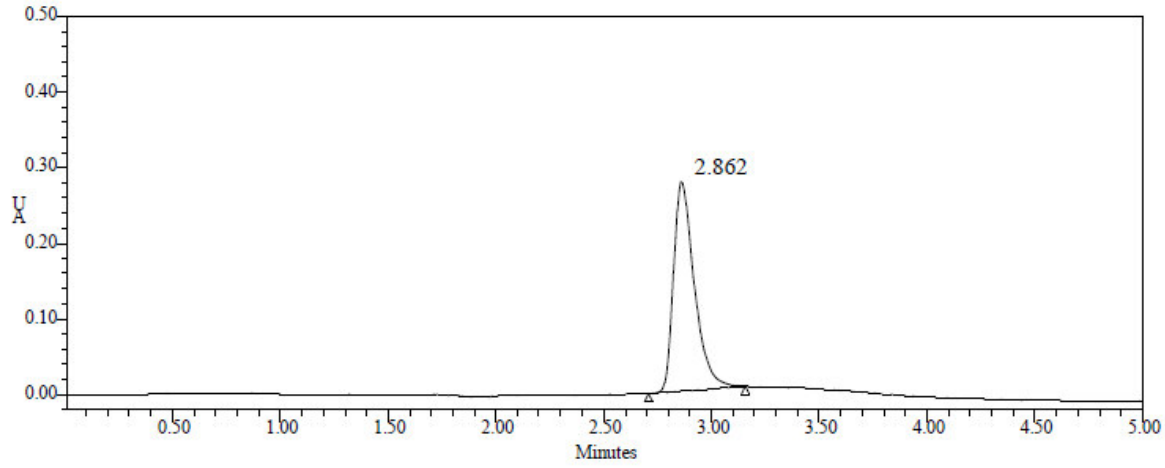
The standard solution was injected for Five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits

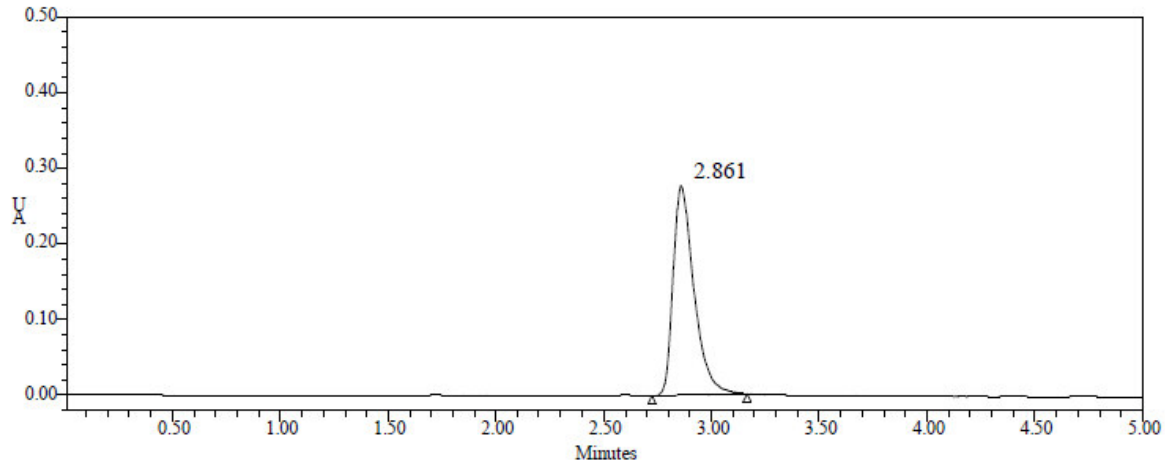
The results are summarized in

| **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.

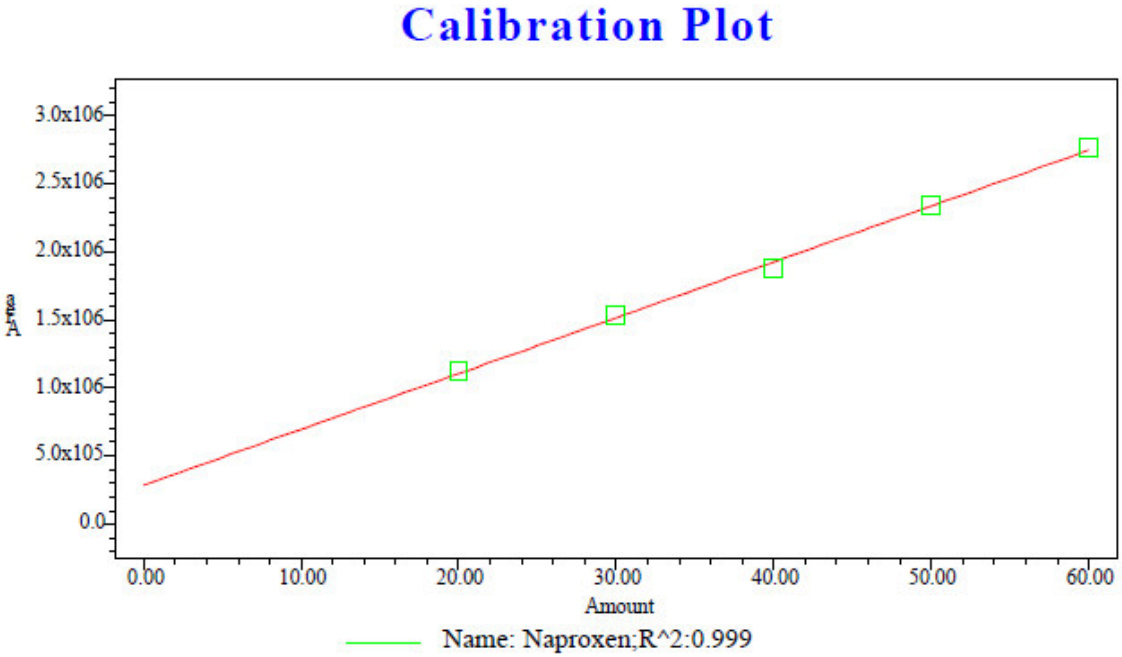
**Preparation of Level – III (50µg/ml):**

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.




**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 from70% to 50%.

| No | Change in Organic Composition in the Mobile Phase | stem Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | SP Tailing |
| | 10% less | 4758 | 1.5 |
| | ctual | 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 absorbence. 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:<br>A)System Precision<br>B).Method precision | RSD NMT 2.0%<br>RSD NMT 2.0% | 0.2<br>0.2 |
| 4 | Linearity | Correlation coefficient NLT 0.999 | 1.000 |
| 5 | Accuracy | %Recovery range98-102 % | 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 µg/ml The LOQ for Naproxen were found to be 9.98µg/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 robusted.Hence it was concluded that the RP-HPLC method developed was very much suit for routine analysis.Naproxen in tablet formulations and future planings use this method for estimation of Naproxen in clinical trials.

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**REFERENCES**
1. Ulla Grove Sidelmann, Inga Bjørnsdottir,et al Directly coupled HPLC-NMR and HPLC-MS approaches for the rapid characterisation of drug metabolites in urine: application to the human metabolism of naproxen *Journal of Pharmaceutical and Biomedical Analysis*, Volume 24, Issue 4, February 2001, Pages 569-579
2. Lotfi Monser, Frida Darghouth, et alSimultaneous determination of naproxen and related compounds by HPLC using porous graphitic carbon column *Journal of Pharmaceutical and Biomedical Analysis,* Volume 32, Issues 4–5, 8 August 2003, Pages 1087-1092
3. F.J. Otero-Espinar, S. Anguiano-Igea, N, et al Oral bioavailability of naproxen-β-cyclodextrin inclusion compound *International Journal of Pharmaceutics***,** Volume 75, Issue 1, 30 August 1991, Pages 37-44
4. Ernest Marco-Urrea, Miriam Pérez-Trujillo, et al Biodegradation of the analgesic naproxen by Trametes versicolor and identification of intermediates using HPLC-DAD-MS and NMR *Bioresource Technology*, Volume 101, Issue 7, April 2010, Pages 2159-2166
5. Wei Fan, Xian-Kun Li, et al Design synthesis and biological evaluation of brain-specific glucosyl thiamine disulfide prodrugs of naproxen *European Journal of Medicinal Chemistry,* Volume 46, Issue 9, September 2011, Pages 3651-3661
6. Lucia Steenkamp, Dean Brady,et al Screening of commercial enzymes for the enantioselective hydrolysis of R,S-naproxen ester *Enzyme and Microbial Technology,* Volume 32, Issues 3–4, 3 March 2003, Pages 472-477
7. Eduard Borràs, Guillem Llorens-Blanch, et al Soil colonization by Trametes versicolor grown on lignocellulosic materials: Substrate selection and naproxen degradation *International Biodeterioration & Biodegradation,* Volume 65, Issue 6, September 2011, Pages 846-852
8. Rodríguez-Rodríguez, Ernest Marco-Urrea, et al Naproxen degradation test to monitor Trametes versicolor activity in solid-state bioremediation processes *Journal of Hazardous Materials,* Volume 179, Issues 1–3, 15 July 2010, Pages 1152-1155
9. Li-Juan Wang, Yu-Hai Tang, et al Flow injection chemiluminescence determination of loxoprofen and naproxen with the acidic permanganate-sulfite system *Journal of Pharmaceutical Analysis,* Volume 1, Issue 1, February 2011, Pages 51-56
10. Junichi Goto, Nobuharu Goto, et al Separation and determination of naproxen enantiomers in serum by high-performance liquid chromatography *Journal of Chromatography A,* Volume 239, 30 April 1982, Pages 559-564
11. Xianglei Cheng, Lixia Zhao, et al In vitro monitoring of nanogram levels of naproxen in human urine using flow injection chemiluminescence *Analytica Chimica Acta,* Volume 558, Issues 1–2, 3 February 2006, Pages 296-301
12. N. Lane, H. Maeda, et al Cancellous bone behavior in hindlimb immobilized rats during and after naproxen treatment Bone and Mineral, Volume 26, Issue 1, 1994, Pages 43-59
13. Hiroyuki Yokoyama, Toshiharu Horie, et al Naproxen-induced oxidative stress in the isolated perfused rat liver *Chemico-Biological Interactions,* Volume 160, Issue 2, 25 March 2006, Pages 150-158
14. Nafaâ Adhoum, Lotfi Monser, et al Determination of naproxen in pharmaceuticals by differential pulse voltammetry at a platinum electrode *Analytica Chimica Acta,* Volume 495, Issues 1–2, 24 October 2003, Pages 69-75
15. Aarbakke, J., Gadeholt, G, et al Pharmacokinetics of naproxen after oral administration oftwo tablet formulations in healthy volunteers *International Journal of Pharmaceutics* Tox., 21, 281-283.
16. Abdel-Moety, E. M., Al-Obaid, et al Coupling of TLC and UV measurement for quantification of naproxen and its main metabolite in urine *European Journal of drug metabolism and pharmacokinetic* 13, 267-271.
17. Anttila, M. (1977).Fluorimetric determination of naproxen in serum *International Journal of Pharmaceutics sciences.* 66, 433-434.
18. Anttila, M., Haataja, M,et al Pharmacokinetics of naproxen in subjects with normal and impaired renal function *European Journal of clinical pharmaceutics.* 18,263-268.

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