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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR NAPROXEN IN PHARMACETICAL DOSAGE FORMS

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ABSTRACT

A simple, sensitive and precise reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed for the estimation of naproxen in pharmaceutical dosage forms. The method was developed using the mobile phase comprising of dibasic sodium phosphate buffer (Na 2 HPO 4) at pH 7.80 (adjusted by sodium hydroxide) and acetonitrile in the ratio of 70:30 (v/v) over C-18 column at ambient temperature. The flow rate was at 0.7 ml/min and the column washing was monitored by UV detector at 225 nm. The retention time of naproxen was 4.8 ± 0.1 min. The recovery was found to be >97% which is demonstrative of accuracy of the protocol. Inter-day and intra-day precision of the newly developed method were less than the maximum allowable limit according to ICH, USP and FDA guidelines. The method showed linear response with correlation coefficient (r 2) value of 0.9991. Therefore, the method was found to be accurate, reproducible, sensitive and less time consuming and can be successfully applied for routine analysis of naproxen in pharmaceutical formulations. The compound were well separated on a on hypersil BDS C-18,250×4mm,5µg reversed phase column by use of a mobile phase consisting of mixed phosphate buffers (K2HPO4,KH2PO4) (PH:6.5) Acetonitrile (55:45v/v) atafewrateof1.0mlmin-1 with detection wavelength at 290nm.

Keywords: Naproxen, RP-HPLC, Chromatography and retention time.

INTRODUCTION

1. Importance of drug analysis

'Health is wealth'. It is vital fact that a healthy body is desire of every human being. Good health is first condition to enjoy the life and all other things which mankind is having. Now a day's peoples are more concentrating towards health. Even governmental bodies of different countries and World health organization (WHO) are also focusing for health of human being. Health care is prevention, treatment and management of illness and preservation of mental and physical well being. Health care embraces all the goods and services designed to promote health including preventive, curative and palliative in interventions. The Health care industry is considered an industry or profession which includes people's exercise of skill or judgment or providing of a service related to the prevention or improvement of the health of the individuals or the treatment or care of individuals who are injured, sick, disabled or infirm. The delivery of modern healthcare depends on an Interdisciplinary Team.

The medical model of health focuses on the eradication of illness through diagnosis and effective treatment. A traditional view is that improvement in health results from advancements in medical science. Advancements in medical science bring varieties of medicines. Medicines are key part ofthe health care system. The numerous medicines are introducing into the world-market and also, that is increasing every year. These medicines are being either new entities or partial structural modification of the existing one. So, to evaluate quality and efficacy of these medicines is also important factor. Right from the beginning of discovery of any medicine quality and efficacy of the same are checked by quantification means. Quality and efficacy are checked by either observing effect of drug on various animal models or analytical means. The option of animal models is not practically suitable for every

batch of medicine as it's require long time, high cost and more man-power. Later option of analytical way is more suitable, highly precise, safe and selective.
The analytical way deals with quality standards which are assigned for products to have desirable efficacy of the medicines. Sample representing any batch are analyzed for these standards and it is assumed that drug/medicine which is having such standards are having desire effect on use.
Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stage of production. The decision to release or reject a product is based on one or more type of control action.

2. History of chromatography and HPLC

In 1903 a Russian botanist Mikhail Tswett produced a colorful separation of plant pigments through calcium carbonate column. Chromatography word came from Greek language chroma = color and graphein = to write i.e. color writing or chromatography[1,2].
Prior to the 1970's, few reliable chromatographic methods were commercially available to the laboratory scientist. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease flow through time, thus reducing purification times of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure was debated[3].
High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of online detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds.
By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro-column, affinity columns, and Fast HPLC began to immerge.

HPLC Gradient mixtures

HPLC gradient mixers must provide a very precise control of solvent composition to maintain are producible gradient profile. This can be complicated in HPLC by the small elution volumes required by many systems. It is much more difficult to produce a constant gradient when mixing small volumes then when mixing large volumes. For low pressure systems this requires great precision in the operation of the miniature mixing General Introduction valves used and low dispersion flows throughout the mixer. For multi-pump high pressure systems it requires a very precise control of the flow rate while making very small changes of the flow rate.

HPLC Pumps

Because of the small particles used in modern HPLC, modern LC pumps need to operate reliably and precisely at pressures of 10,000 psi or at least 6,000 psi. To operate at these pressures and remain sensibly inert to the wide variety of solvents used HPLC pumps usually have sapphire pistons, stainless steel cylinders and return valves fitted with sapphire balls and stainless steel seats. For analytical purposes HPLC pumps should have flow rates that range from 0 to 10ml/min., but for preparative HPLC, flow rates in excess of 100 ml/min may be required. It is extremely difficult to provide a very constant flow rate at very low flow rates. If 1% is considered acceptable then for 1ml/min a flow variation of less than 10µl/min is required. This level of constancy is required because most HPLC detectors are flow sensitive and errors in quantization will result from change in flow rate.

HPLC Sample Valves

Since sample valves come between the pump and the column it follows that HPLC sample valves must also tolerate pressures up to10,000 psi. For analytical HPLC, the sample.

DRUGPROFILE

Naproxen

Description

Naproxen is classified as a nonsteroidal anti-inflammatory drug (NSAID) and was initially approved for prescription use in 1976 and then for over-the-counter (OTC) use in 1994.[3] It can effectively manage

acute pain as well as pain related to rheumatic diseases, and has a well-studied adverse effectprofile.[5] Given its overall tolerability and effectiveness, naproxen can be considered a first line treatment for a variety of clinical situations requiring analgesia.[5] Naproxen is available in both immediate and delayed release formulations, in combination with sumatriptan to treat migraines and in combination with esomeprazole to lower the risk of developing gastric ulcers.

Structure

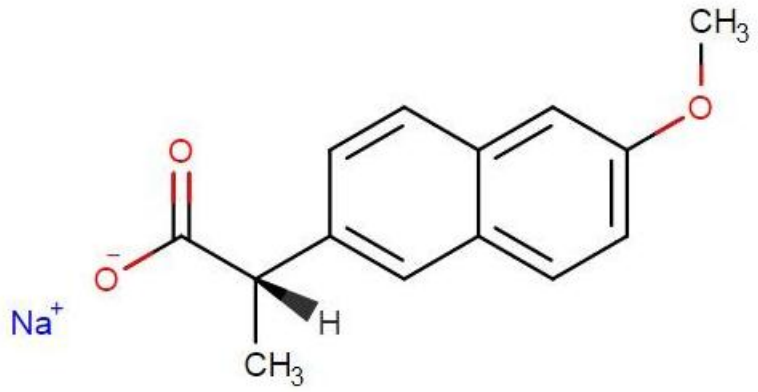

Fig. 1: Structure of Naproxen

Chemical Name
Sodium(2S)-2-(6-methoxynaphthalen-2-yl) propanoate.

Molecular formula
C14H13NaO3.

Molecular Weight
230.26g/mol.

Mechanism of action
Naproxen works by reversibly inhibiting both the COX-1 and COX-2 enzymes as a non-selective coxib. This results in the inhibition of prostaglandin synthesis. Prostaglandins act as signaling molecules in the body, inducing inflammation.

Pharmacokinetic characters
Absorption
Rapidly and well absorbed from the gastrointestinal tract after oral administration. The absolute bioavailability is approximately 70% with no substantial loss by first pass metabolism.

Metabolism
Hepatic. Four metabolites have been identified in human urine which together account for approximately 15% of an oral dose. The metabolites have antimicrobial activity, but are less active than unchanged ciprofloxacin.

Half-life
4hours.

Affected organisms
Humans and other mammals.

EXPERIMENTAL METHOD

Table 1: Instruments used

SL.No	Instruments	Model
1	HPLC	Waters, sciftware: Empower, 2695, separationmodule. 2487UV detector
2	UV/VIS spectrophotometer	LABINDIA UV 3000+
3	pH meter	Adwa—AD1020
4	Weighing machine	AfiosotER-2OOA
5	Pipettes and Burette s	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

SL. No	Chemical	Company Name
1	Naproxen	PHARMATRAIN
2	Water for HPLC	FINER chemical LTD
3	Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	MOLYCHEM
5	KH2PO4	MERCK
6	NaOH	FINER chemical LTD

HPLC METHOD DEVELOPMENT

Wavelength selection

UV spectrum of 10 µg/mL Naproxen in diluents (mobile phase composition) was recorded byscanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 290 nm. At this wavelength both the drugs show good absorbance.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water, Methanol: 0.1% OPA, Acetonitrile : Phosphate buffer and Methanol: Phosphate buffer with various combinations of Development & Validation of HPLC methods For Naproxen In Pharmaceutical Dosage forms VJ'S College of Pharmacy, Rajahmundry 25 pH as well as varying proportions. Finally, the mobile phase was optimized to Methanol : Phosphate buffer(pH4.0) in proportion 35:65v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column Phenomenex column, Inertsil ODS column and YMC column. Xterra (4.6 x 150mm, 5 m) was found to be ideal as it gave good peak shape and resolution at1.0ml/min flow.

Optimized Chromatographic Conditions

Instrument used: Waters HPLC with auto sampler and UV detector.
Temperature: Ambient
Column: Xterra (4.6x150mm,5m)Buffer: Phosphate buffer
pH : 4.0
Mobile phase: Methanol: Phosphate buffer (35:65)Flowrate:1 mlpermin
Wavelength: 290nm
Injection volume: 20
Runtime: 10 min.

Preparation of Buffer and Mobile Phase

Preparation of Phosphate buffer

Take 3.4 gms of potassium di hydrogen ortho phosphate in 1000 ml of HPLC water, pH was adjusted with NaOH up to 4.0. Final solution was filtered through 0.45 Membrane filter and sonicate it for 10mins.

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Preparation of mobile phase
Accurately measured 350 mL of Methanol (35%) and 650 mL of above buffer (65%) were mixedand degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation
The Mobile phase was used as the diluent.

ASSAY
Standard Solution Preparation
Accurately weigh and transfer 250 mg of Naproxen working standard into a 100 ml clean dry volumetric flask add about 50 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.
Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (75 ppm Naproxen)

Sample Solution Preparation
Accurately weigh 10 tablets crush in motor and pestle and transfer equivalent to 250 mg (350 mg of tablet power) of Naproxen sample into a 100 ml clean dry volumetric flask add about 50 mL of diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45micron injection filter. (Stock solution)
Further pipette 2.5 ml of the above stock solutions into a 25 ml volumetric flask and dilute up to the mark with diluent.
Further pipette 3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (75 ppm Naproxen)
Development & Validation of HPLC methods For Napraxen in Pharmaceutical Dosage forms.

Procedure
Inject 20 uL of the standard, sample into the chromatographic system and measure the areas for Naproxen peaks and calculate the %Assay by using the formulae.

VALIDATIONPARAMETERS
1. LINEARITY
Preparation of stock solution
Accurately weigh and transfer 250 mg of Naproxen working standard into a 100 ml clean dry volumetric flask add about 50 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 2.5 ml of the above stock solutions into a 25 mL volumetric flask and dilute up to the mark with diluent.

Preparation of Level – I (25 ppm of Naproxen**)**
1 mL of above stock solutions has taken in 10 mL of volumetric flask, dilute up to the mark with diluent.

Preparation of Level –II (50 ppm of Naproxen**)**
2 mL of above stock solutions has taken in 10 mL of volumetric flask, dilute upto the mark with diluent.

Preparation of Level –III (75 ppm of Naproxen**)**
3 mL of above stock solutions has taken in 10 mL of volumetric flask, dilute up to the mark with diluent.

Preparation of Level –IV (100 ppm of Naproxen**)**
4 mL of above stock solutions has taken in 10 mL of volumetric flask, dilute up to the mark with diluent.

RESULTS AND DISCUSSION

SYSTEM SUITABILITY

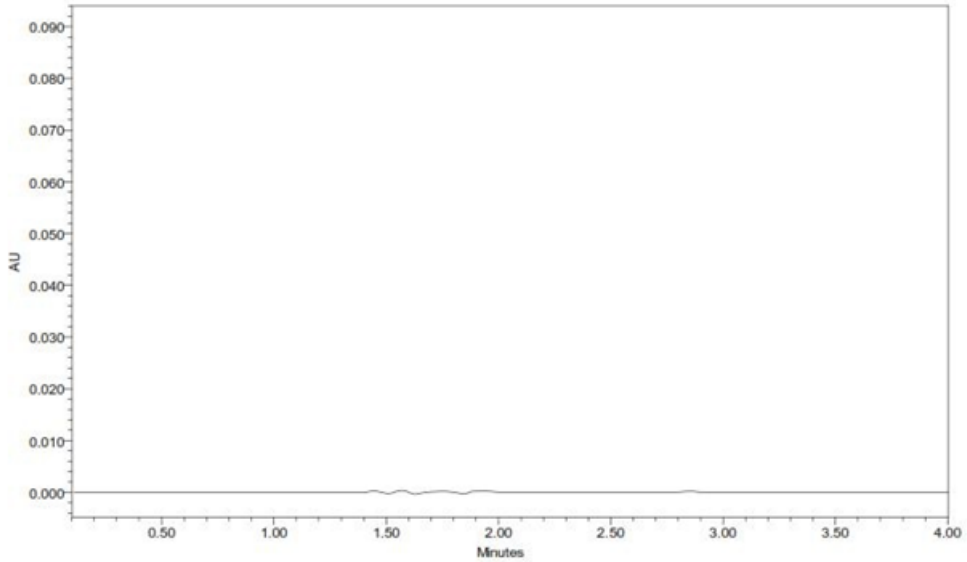

Fig. 2: Chromatogram for Blank

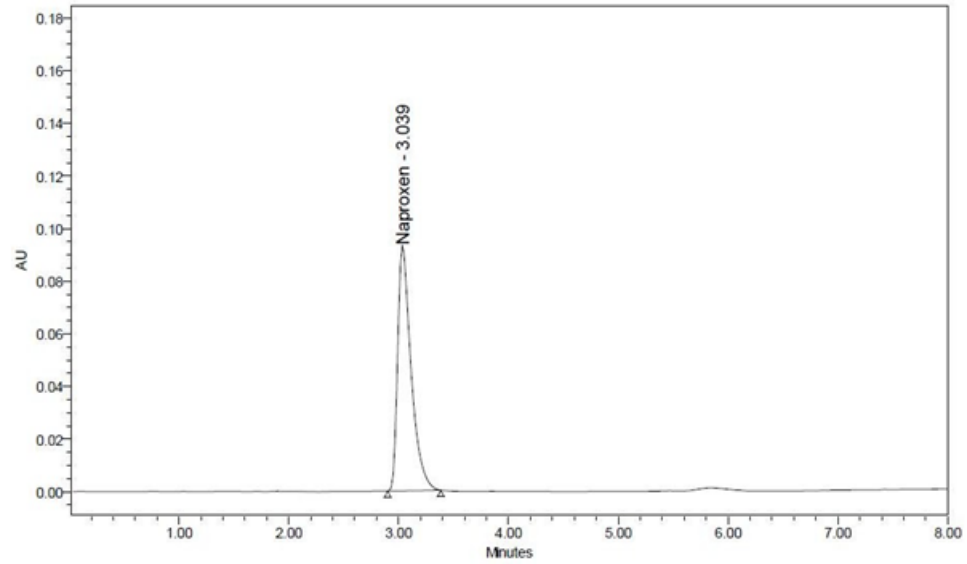

Fig. 3: Chromatogram for system suitability

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Table 3: Results of system suitability parameters

S.No.	Name	RT(min)	Area (µVsec)	Height(µV)	USP tailing	USP plate count
1	Naproxen	3.039	1863144	93497	1.41	4287.35

Acceptance criteria

- Theoretical plates must be not less than 2000.
- Tailing factor must be not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

ASSAY

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below.

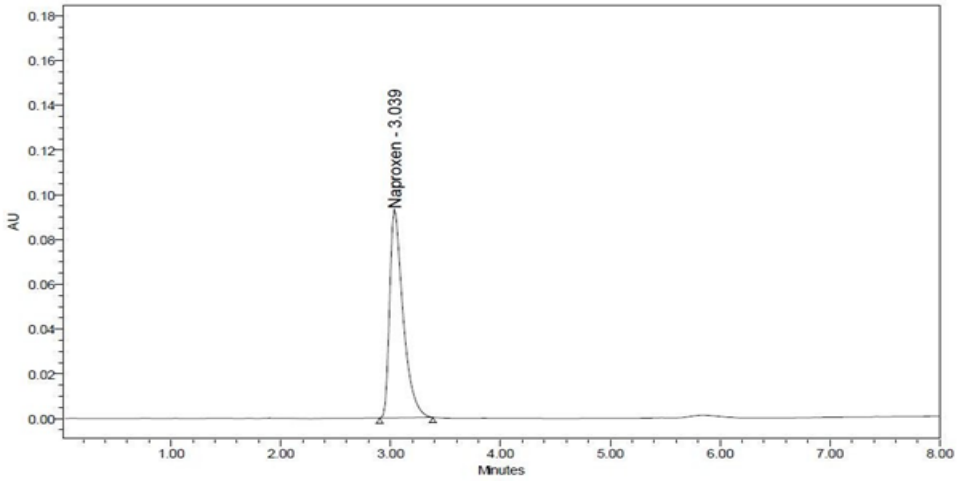

Fig. 4: Chromatogram for Standard

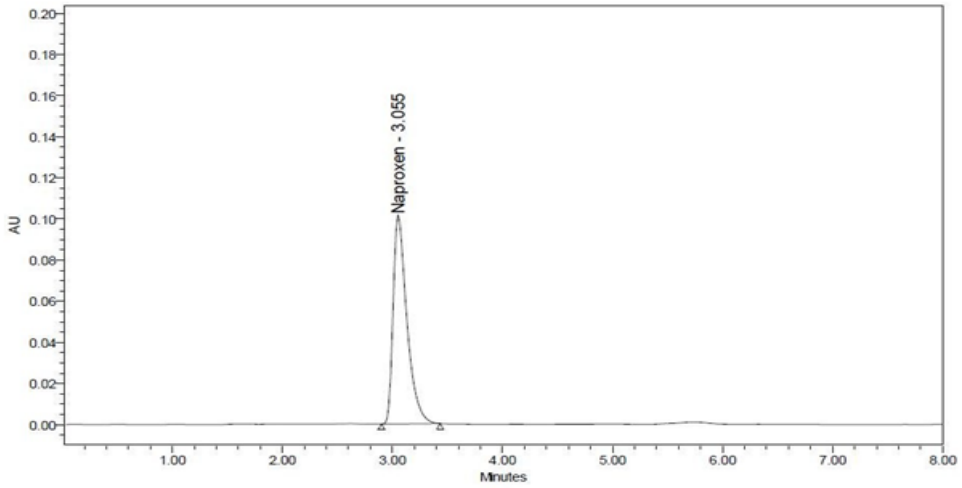

Fig. 5: Chromatogram for Sample

Formula for % Assay

$$\frac{Test\ Area}{Standard\ Area} * \frac{Standard\ Concentration}{Sample\ Concentration} * \frac{Percentage\ Purity\ of\ Drug}{100} * 100$$

$$\%\ Assay = \frac{1879959}{1873945} * \frac{75}{75} * \frac{99.8}{100} * 100 = 100.12$$

Table 4: Results of Assay for Naproxen

	Label Claim (mg)	%Assay
Naproxen	250	100.12

VALIDATION PARAMETERS

1. LINEARITY

The linearity range was found to lie from 25 µg/mL to 125 µg/mL of Naproxen and chromatograms are shown below.

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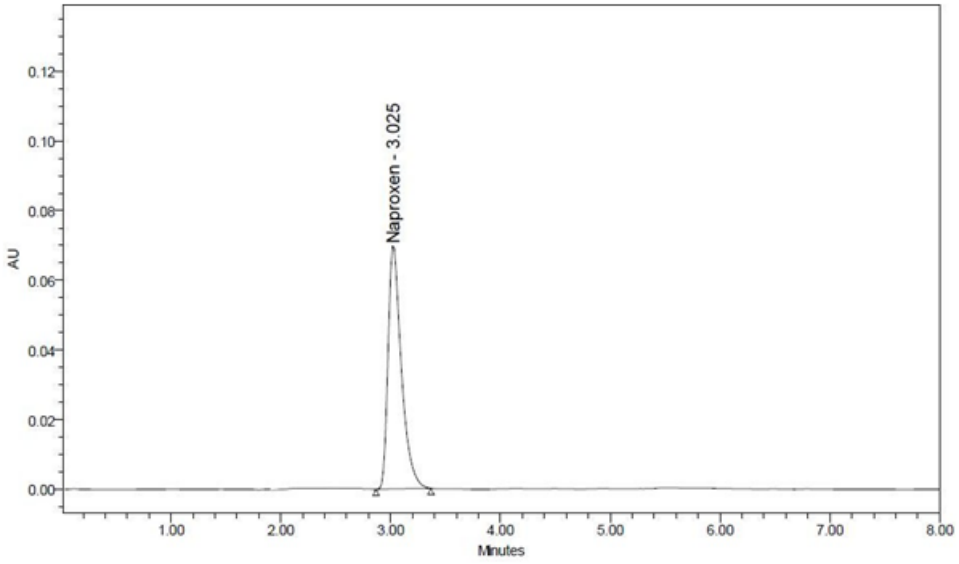

Fig. 6: Chromatogram for linearity-1

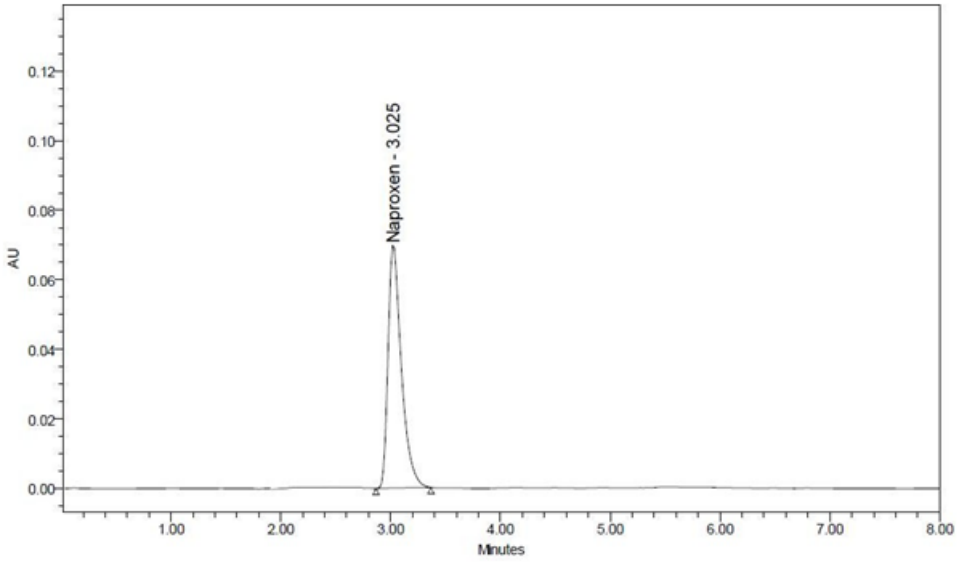

Fig. 7: Chromatogram for linearity-2

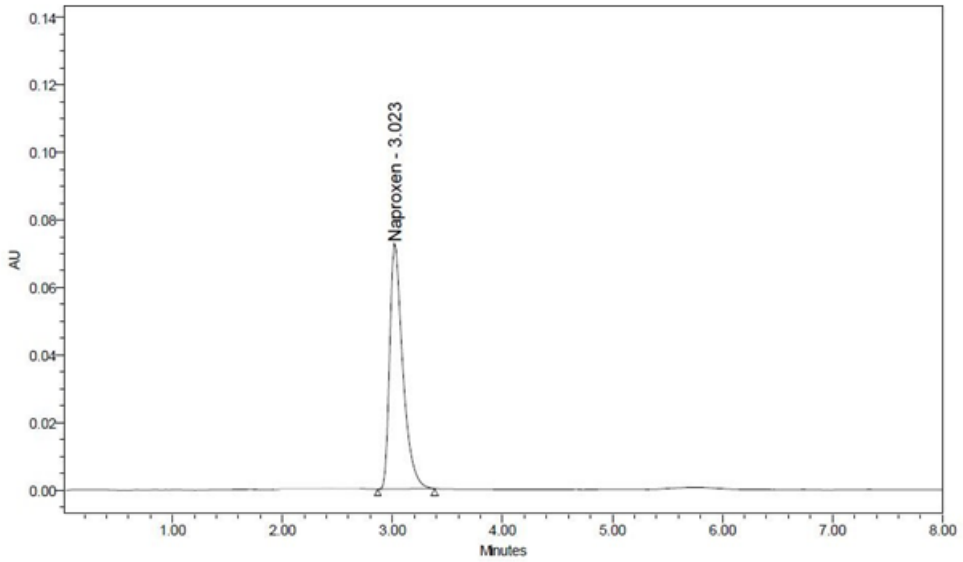

Fig. 8: Chromatogram for linearity-3

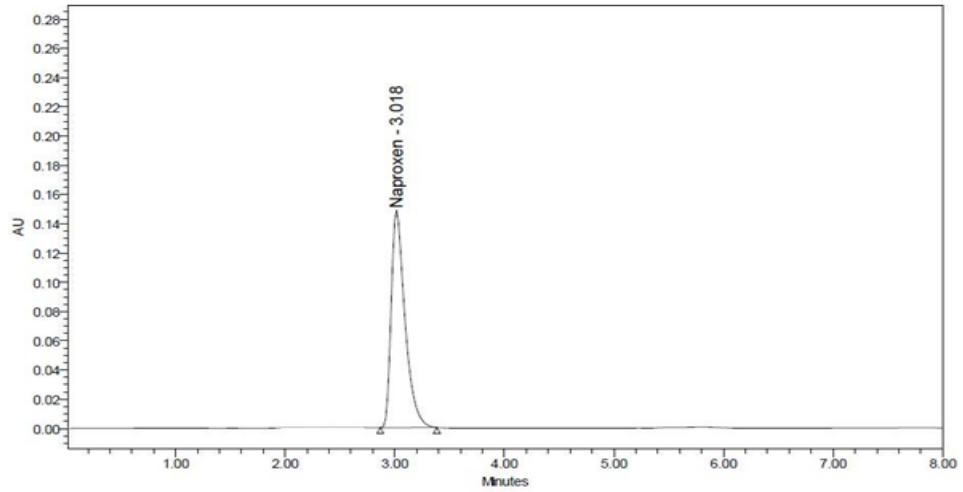

Fig. 9: Chromatogram for linearity-4

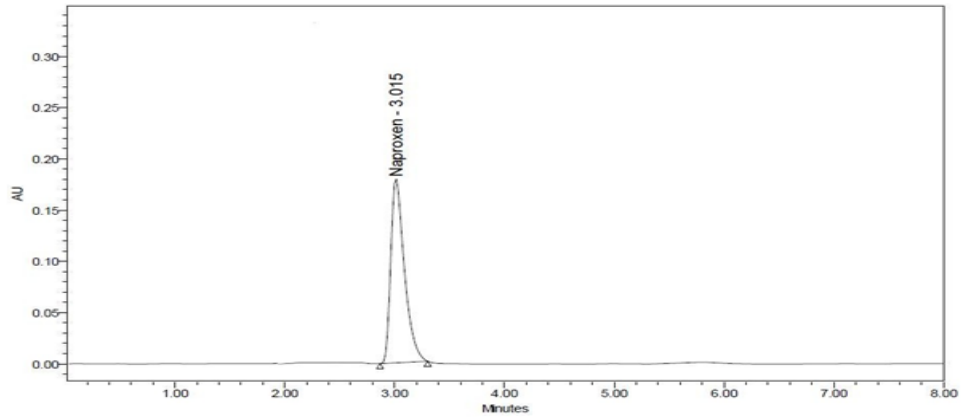

Fig. 10: Chromatogram for linearity-5

Table 5: Area of different concentration of Naproxen

Naproxen		
Concentration (µg/ml)		Area
1	**0**	**0**
2	25	587354
3	50	1204384
4	75	1835225
5	100	2463352
6	125	3025734

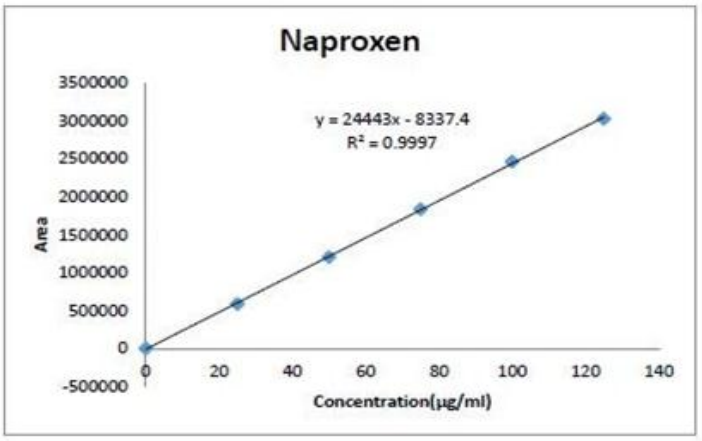

Fig. 11: Calibration graph of Naproxen

Table 6: Analytical Performance Parameters of Naproxen

Parameters	Naproxen
Slope (m)	61107
Intercept (c)	8337.4
Correlation coefficient (R^2)	0.999

Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999

- The correlation coefficient obtained was 0.999 which is in the acceptance limit.

2. PRECISION:

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.

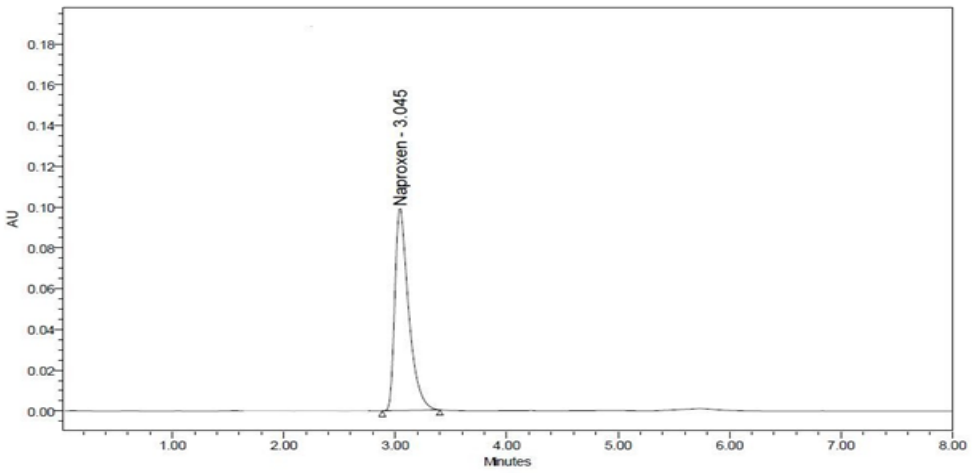

Fig. 12: Chromatogram for Precision-1

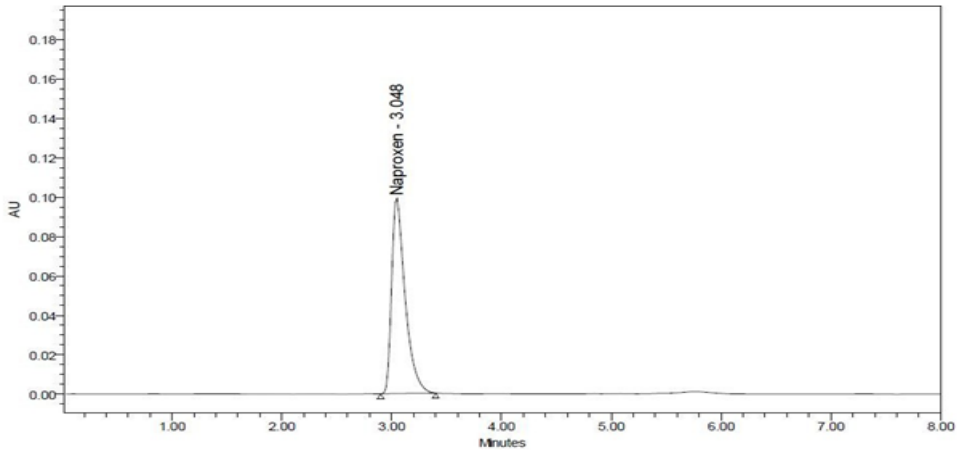

Fig. 13: Chromatogram for Precision-2

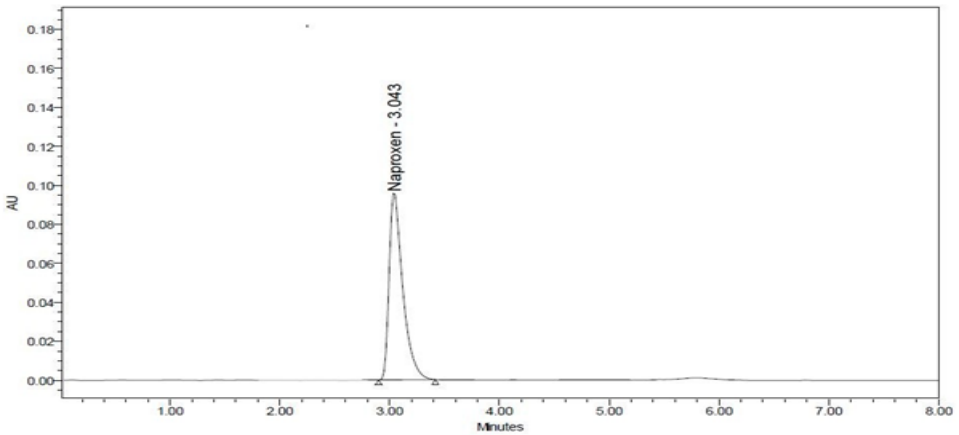

Fig. 14: Chromatogram for Precision-3

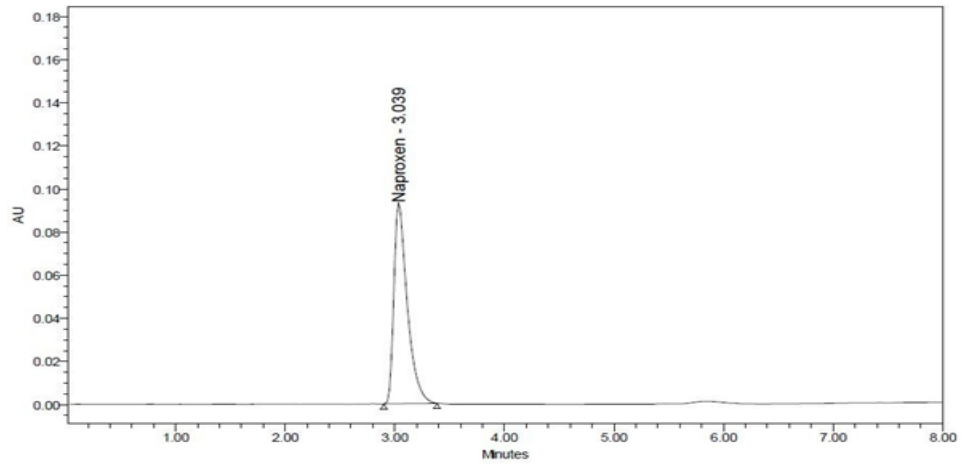

Fig. 15: Chromatogram for Precision-4

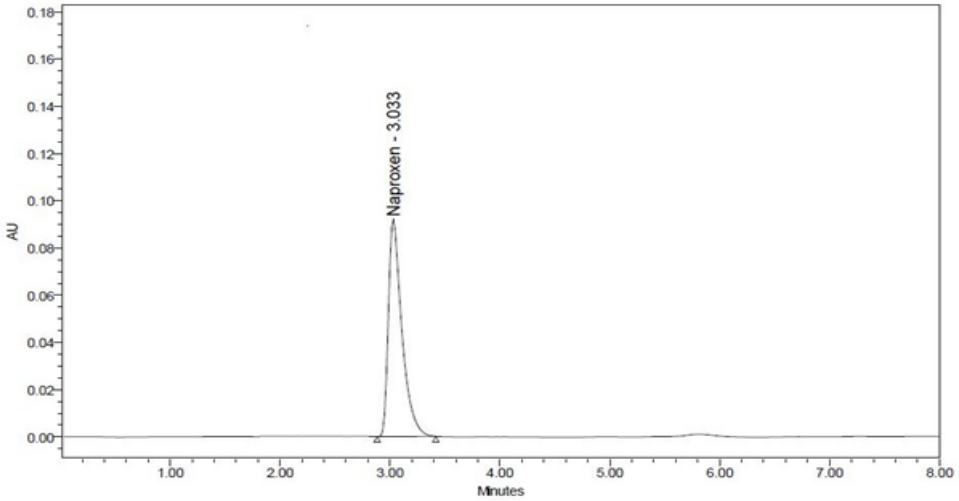

Fig. 16: Chromatogram for Precision-5

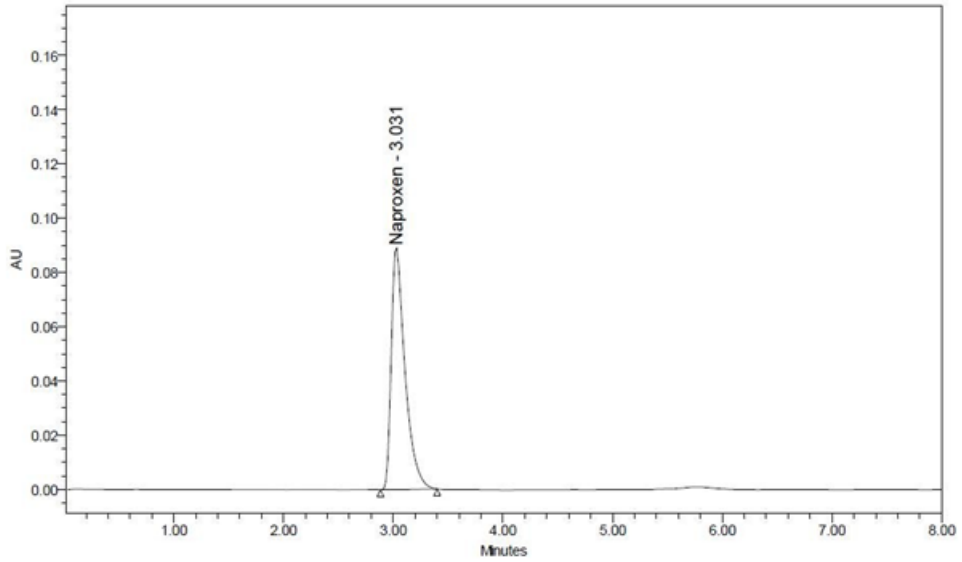

Fig. 17: Chromatogram for Precision-6

Table 7: Results of Precision for Naproxen

Injection	Area
Injection-1	1895632
Injection-2	1877893
Injection-3	1853626
Injection-4	1866732
Injection-5	1864894
Injection-6	1855663
Average	1869073.3
Standard Deviation	15649.6
%RSD	0.8

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

3. INTERMEDIATE PRECISION (ruggedness)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

SUMMARY AND CONCLUSION

The estimation of Naproxen was done by RP-HPLC.
The assay of Naproxen was performed with tablets and the % assay was found to be 100.12 which show that the method is useful for routine analysis.
The linearity of Naproxen was found to be linear with a correlation coefficient of 0.999, which shows that the method is capable of producing good sensitivity.
The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 for Naproxen which shows that the method is precise.
The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.9 for Naproxen which shows that the method is repeatable when performed in different days also.
The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 100.65% for Naproxen. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.
The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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