RESEARCH ARTICLE

Stability Indicating RP-HPLC Method Development and Validation for the Determination of Naproxen Sodium in Bulk Drug and Tablet Dosage Form

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ABSTRACT

A simple, rapid, accurate, precise and reproducible stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method for the estimation of Naproxen sodium in bulk and tablet dosage form was developed and validated as per ICH guidelines. The separation was done using Kromosil C_{18} 150 x 4.6mm, 5µ column. The mobile phase (Buffer and Acetonitrile 50:50%v/v) was pumped at 1.0ml/min and effluent was detected at 220 nm using a PDA detector. The retention time was 2.20 ± 0.1min and the method produced linear response in the concentration range of 2-12 µg/mL (r^2- 0.999). In recovery studies, %RSD from reproducibility was found to be below 2%. LoD and LoQ were 0.95 µg/mL and 2.88 µg/mL respectively. The drug was subjected to different stress conditions such as acidic, alkaline, oxidative, photo thermal and hydrolysis. The drug showed more degradation in acidic condition and no degradation was observed in hydrolysis and photo condition. The developed RP-HPLC method was found to be effective, sensitive and specific for the estimation of Naproxen sodium in bulk and tablet dosage form.

Keywords: Naproxen sodium, RP-HPLC, Validation

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INTRODUCTION

Naproxen sodium, a propionic acid derivative (Figure 1) is a drug used to relieve pain, fever, migraine head ache, swelling and stiffness.[1] It reversibly and competitively inhibits cyclooxygenases (COX), thereby blocking the conversion of arachidonic acid to pro-inflammatory prostaglandins. This inhibits the formation of prostaglandins that are involved in pain, inflammation and fever.[1] Migraine is about 3 times more common in women than in men.[2] Migraine is characterized by recurrent moderate to severe headache often in association with a number of autonomic symptoms. The severity, duration and frequency of attacks vary in patients with aura and without aura. Symptoms of migraine are due to local cranial vasodilatation and/or to the release of sensory neuropeptides and pro-inflammatory peptides from sensory nerve endings in an activated trigeminal system.[3-5] Naproxen Sodium is chemically, sodium;(2S)-2-(6-methoxynaphthalen-2-yl) propanoate. It is white fine powder, freely soluble in water and sparingly soluble in alcohol.[6] Literature survey revealed that several RP-HPLC based methods[7-10] have been reported for the estimation of Naproxen, but there is no method reported with 0.1%OPA: Acetonitrile (50:50%v/v) as mobile phase. The aim of the present work was to develop simple, rapid, sensitive, specific, accurate, precise, economic and reliable RP-HPLC method for the estimation of Naproxen sodium in bulk and tablet dosage form suitable for quality control analysis.

MATERIALS AND METHODS

Chemicals

Naproxen sodium working standard was received as gift sample from Dr. Reddy's Laboratories Pvt. Ltd., Hyderabad, and sample tablets (Label claim: 500mg; Naprosyn tablets) were procured from a local medical shop. HPLC grade acetonitrile, Ortho phosphoric acid and water were purchased from Merck Specialties Private Ltd., Mumbai and Sd Fine Chem. Limited, Mumbai.

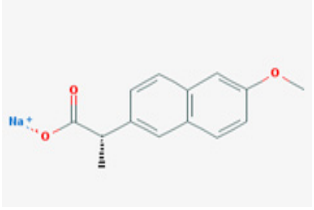

Figure 1: Structure of Naproxen Sodium

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Chromatographic Conditions

The HPLC-Shimadzu prominence binary system with PDA detector was used for the method development. The output signal was monitored and processed using LC solutions software. Chromatographic separation was performed on Kromosil C_{18} column at 30°C. The mobile phase containing 0.1% OPA and acetonitrile in the ratio of 50:50 (v/v) was pumped at 1.0 mL/min and detection was carried out at 220 nm. The injection volume for standard and sample was 10 µL (fixed loop) and the total run time was 5 min (Table 1).
Diluent: HPLC water : Acetonitrile (50:50%v/v)

Preparation of Standard Stock Solution

About 10 mg of Naproxen sodium working standard was accurately weighed and transferred into 10 mL volumetric flask, dissolved in diluent. The sample solution was filtered through 0.45 µm Ultipor N66 nylon filter and the volume was made up to the mark with the diluent to get 1000 µg/mL of Naproxen sodium.

Preparation of standard solution

Naproxen sodium (100 µg/ml) was prepared from the standard stock solution with the diluent. This solution is diluted with the diluent to get 10 µg/mL concentration of Naproxen sodium and filtered through 0.45 µm UltiPro N66 nylon filter. Accurately 10 µL was injected into the HPLC system and chromatogram was recorded.

Preparation of Sample solution

Twenty tablets were weighed, average weight determined and finely powdered. An accurately weighed quantity of powder equivalent to 10 mg of Naproxen sodium was transferred into a 10 mL volumetric flask. The tablet powder was dissolved in sufficient volume of diluent, sonicated for 20 minutes and degassed. The volume was made up to the mark with the diluent and the sample solution was filtered through 0.45 µm nylon filter. From this sample solution appropriate aliquot was prepared using the diluent. Accurately 10 µL was injected into the HPLC system and the peak area was recorded at 220 nm.

VALIDATION OF THE DEVELOPED METHOD

The method developed was validated as per ICH guidelines[11] for linearity, accuracy, precision, LoD, LoQ, ruggedness and specificity.

Table 1: Optimized chromatographic conditions

Parameter	*Optimized condition*
Chromatograph	HPLC (Shimadzu prominence with PDA detector)
Column	Kromosil C_{18} G 150mm x 4.6mm, 5µ
Mobile phase	0.1% OPA and Acetonitrile (50:50)
Flow rate	1.0 ml/min
Detection wavelength	PDA at 220 nm
Injection volume	10µL
Column temperature	30° C

Linearity

The linearity of the developed method was demonstrated over the concentration range of 2–12 µg/mL of Naproxen sodium prepared from the stock solution. A calibration curve of the drug was plotted for concentration v/s peak area. The regression equation of calibration curve was $y = 151145x + 19220$ and $R^2 = 0.9996$.

Accuracy

The accuracy of the method was determined by recovery studies in triplicate for each level. Fixed amount of sample was taken and Naproxen sodium equivalent to 80, 100 and 120% of the standard was injected into the HPLC system. The method was repeated three times for each level. The average % recovery was calculated.

Precision

The precision of the method was studied by estimation of multiple samplings from the homogeneous sample of the drug at three different concentrations on the same day and on three different days. The precision was expressed as %RSD and was calculated for intra day and inter day precision.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The calibration curve of the drug was prepared using 2-12µg/mL concentrations of Naproxen sodium. The Standard deviation of Y intercepts of regression lines were determined and substituted in the following equation for the determination of LoD and LoQ. Limit of Detection (LoD) $=3.3\sigma / S$ and Limit of quantitation (LoQ)$= 10\sigma/S$. In this equation, σ is the standard deviation of Y intercept of regression lines and S is the slope of calibration curve. The LoD and LoQ for Naproxen sodium were found to be 0.95 and 2.88µg/ml respectively.

Robustness

Robustness of the method was determined by making slight changes in the composition of mobile phase ± 2%, flow rate by ± 0.1 mL/min and temperature by ±2ºC. Retention time and chromatograms were determined for the drug.

Specificity

Commonly used excipients such as starch, lactose and magnesium stearate were spiked into weighed quantity of the drug. The chromatograms were recorded by making suitable dilutions and the amount of drug present in the sample was determined.

Stability

Stability of both the standard and sample solutions was tested during analysis up to 24hours at room temperature.

Stability Indicating Assay

The drug was subjected to acidic (0.1N HCl), alkaline (0.1N NaOH), oxidative (0.3% H_2O_2), photo (UV light), thermal (sand bath at 50ºC) and hydrolytic (water) conditions and the % degradation was calculated.

RESULTS AND DISCUSSION

In the present study, RP-HPLC method developed for the estimation of Naproxen sodium in bulk and tablet dosage form

using Kromosil C_{18} column (150 mm × 4.6 mm × 5 µ particle size) at 30°C. To develop an effective method for the estimation of Naproxen sodium, conditions such as detection wavelength, ideal mobile phase and concentration of the standard were optimized in preliminary trials. Naproxen sodium standard concentration was scanned in UV- region between 200–400 nm. λ_{max} of Naproxen sodium was found to be at 220 nm Figure 2. The Naproxen sodium peak in the sample was identified by comparing with the Naproxen sodium standard and the retention time was found to be around 2.20 ± 0.1 minutes [Figures 3 and 4].

The estimation of Naproxen sodium tablets was carried out by RP-HPLC using mobile phase, 0.1%OPA and Acetonitrile

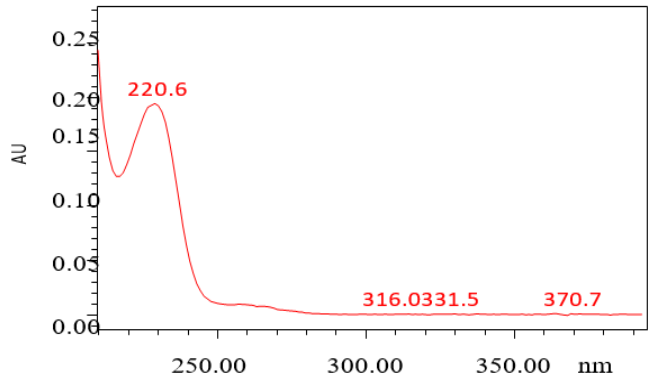

Figure 2: UV Spectrum of Naproxen sodium

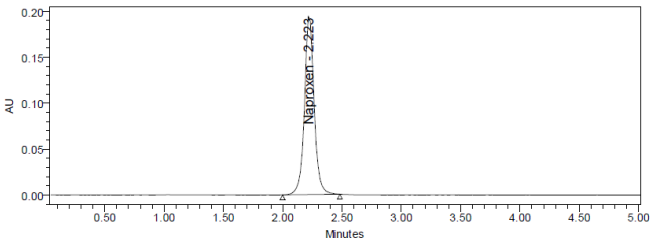

Figure 3: Chromatogram of standard solution of Naproxen sodium

Table 2: System suitability and validation parameters of the developed method

Parameter	*Naproxen sodium*
Theoretical plates	4122
Tailing factor	1.04
Retention time (min)	2.223
Linearity range (µg/mL)	2-12µg/mL
Regression equation Y = mx = c	Y = 151145x + 19220
Slope (m)	151145
Intercept (c)	19220
Correlation coefficient	0.9996
Percent RSD	< 2
Precision Intra day (n = 6)	0.719
Precision Inter day (n = 6)	0.643
LOD (µg/mL)	0.95
LOQ (µg/mL)	2.88

in the ratio of 50:50%v/v with flow rate of 1.0 mL/min. The retention time was found to be 2.203 minutes. System suitability parameters such as RSD for six replicate injections were carried out on freshly prepared standard solution and parameters were given in (Table 2). %RSD found to be less than 2%, theoretical plates 3829, and tailing factor 1.04 indicating the suitability of the system for the estimation of the drug.

The typical chromatogram of Naproxen sodium is shown in Figure 1. The calibration curve of the drug was constructed by plotting peak area of the drug (Y-axis) and concentration of the drug on (X-axis). A good linear relationship was observed between concentration of the drug and the respective ratio of peak areas in the range of 2–12 mcg/mL (target concentration for Naproxen sodium standard) with a correlation coefficient of 0.9996 reflecting that good correlation exists between peak area and the concentration (Figure 5).

The quantitative estimation of the drug in tablet was determined by taking concentration of the drug same to that of standard solution and the assay result was found to be 100.04% (Table 3). The acceptance criterion of repeatability is RSD and should not be more than 2.0%. The method repeatability was 0.2% shows that the method was precise. The developed method was validated for its intra-day and inter-day precision. The results obtained were within the acceptable limit (Table 3). Estimation of the drug by the developed RP-HPLC method for finding out intra and inter day variations show low coefficient of variation values which indicate that the developed method is highly precise.

By spiking various concentrations of the drug ranging from 80–100–120% into previously analyzed samples the amount of the drug recovered was calculated and the results were shown in Table 4. The Accuracy limit was the %recovery and

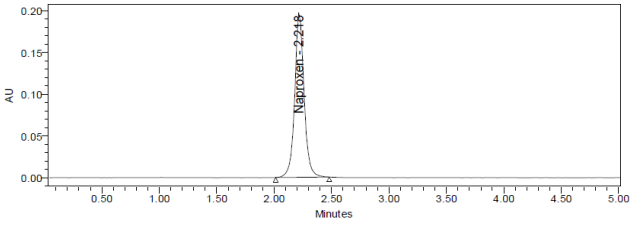

Figure 4: Chromatogram of sample solution of Naproxen sodium

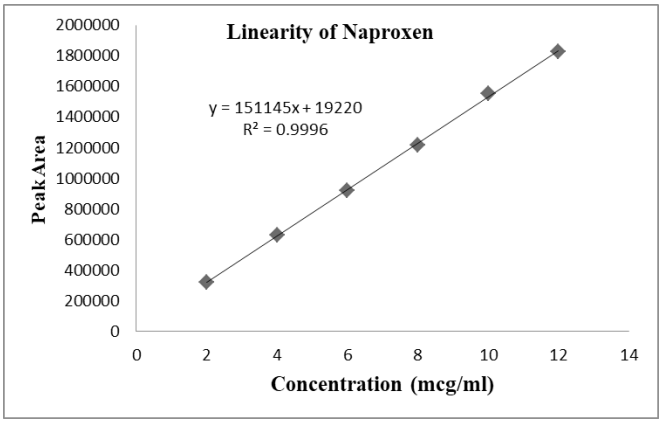

Figure 5: Calibration curve of Naproxen sodium

was in the range of 100.02 to 100.35%. From the validation of the developed method, the accuracy was within the limit, indicating that the proposed RP-HPLC method was highly accurate. LOD 0.95 µg/mL and LOQ 2.88 µg/mL (Table 2) of the drug suggest that less than a microgram of the drug can be estimated accurately.

Robustness of the method was studied by changing the chromatographic conditions slightly and results were presented in (Table 5). From the method developed it was observed that there were no significant changes in the retention time and area of the chromatograms by making slight alterations in temperature, composition and flow rate of the mobile phase. The %RSD was less than 1%, which demonstrated that the RP-HPLC method developed was robust.

The RP-HPLC method developed in the present study was used to quantify Naproxen sodium in bulk and tablet dosage form and the results were comparable with the corresponding labeled quantity (Table 3). High recovery values and no additional peaks in the chromatogram indicate that the developed method was free from interference of the commonly used excipients in the tablet dosage form. In stability studies the peak area and retention time of the drug remained almost unchanged and no significant degradation was observed up to 24 hours indicating stability of the developed method.

Stability indicating assay method was done by subjecting the drug to different stress conditions. Acidic (0.1 N HCl for 24 hours), Alkaline (0.1 N NaOH for 24 hours), oxidative (0.3% H_2O_2 for 24 hours), Photo (UV light 200watt hours/ m^2 for 6 hours), thermal (sand bath at 50ºC for 6 hours) and hydrolytic (HPLC water for 24 hours) conditions were exposed to the drug. The drug showed significant degradation in acidic (9.25%), alkaline (7.62%), less degradation in oxidative

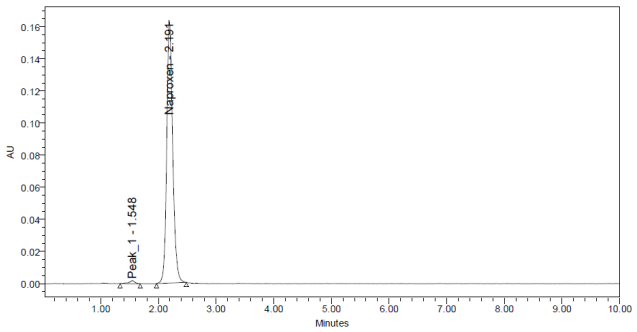

Figure 6: Acidic Degradation of Naproxen sodium

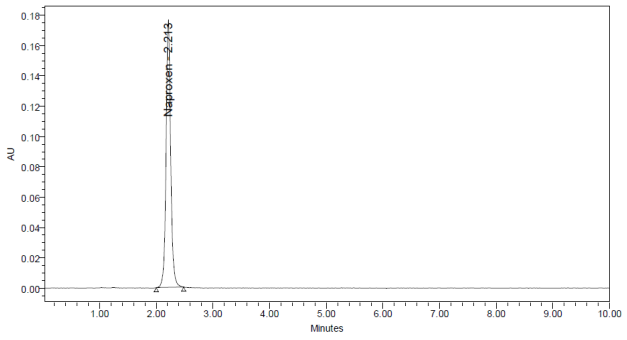

Figure 7: Alkaline Degradation of Naproxen sodium

Table 3: Results of Analysis of the tablet dosage form

Formulation	*Label claim*	*Amount Found ± SD (n = 5)*	*% recovery*	*% RSD*
Naprosyn	500mg	500.2mg ± 0.0071	100.04%	0.082
		Intra day		
		Session- 1		0.483
		Session- 2	—	0.235
		Session- 3		0.369
		Inter day		
		Day 1	—	0.101
		Day 2		0.548
		Day 3		1.126

*Average of 6 determinations

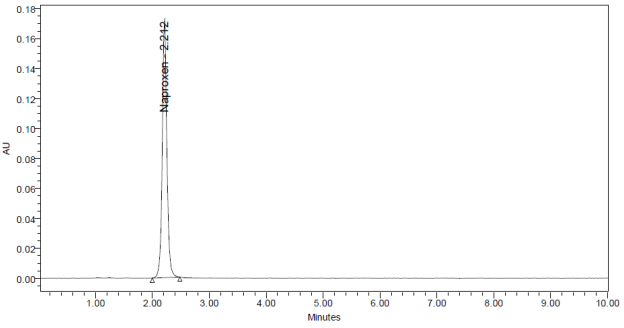

Figure 8: Oxidation Degradation of Naproxen sodium

Table 4: Recovery studies of the developed method

Reanalyzed Sample Conc(µg/mL)	*Recovery Level*	*Amount Added (µg/mL)*	*Total Amount Found (µg/mL)*	*% Recovery*
8	80%	6.4	14.45	100.35%
	100%	8	16.05	100.31%
	120%	9.6	17.604	100.02%

Table 5: Robustness data of the developed method

S.No	*Parameter*	*Proposed*	*Modification*	*%RSD*	*Retention time (min)*	*Tailing factor*
1.	Flow Rate (± 0.1ml/min)	1.0	1.1 0.9	0.4 0.5	2.206 2.318	1.237 1.102
2.	Mobile Phase (±2 %) (B:A)	50:50	48:52 52:48	0.3 0.3	2.097 2.594	1.267 1.22
3.	Temperature (± 2ºC)	30ºC	28º C 32º C	1.1 0.334	2.403 2.161	1.28 1.28

Table 6: Stability indicating method data of Naproxen sodium

S.No	*Stress condition*	*% Degradation*
1.	Acidic (0.1 N HCl for 24 hours)	9.25
2.	Alkaline (0.1N NaOH for 24hours)	7.62
3.	Oxidative (0.3% H_2O_2 for 24hours)	4.77
4.	Photo (UV light 200watt hours/ m^2 for 6 hours)	1.48
5.	Thermal (sand bath at 50^oC for 6 hours)	3.39
6.	Hydrolytic (HPLC water for 24 hours)	0.65

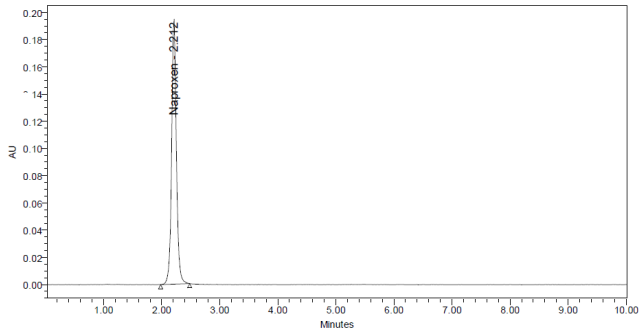

Figure 9: Photo Degradation of Naproxen sodium

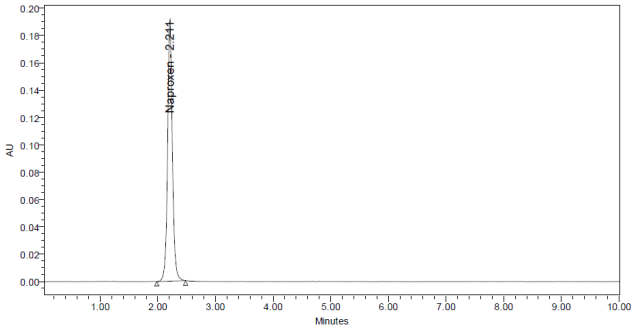

Figure 10: Thermal Degradation of Naproxen sodium

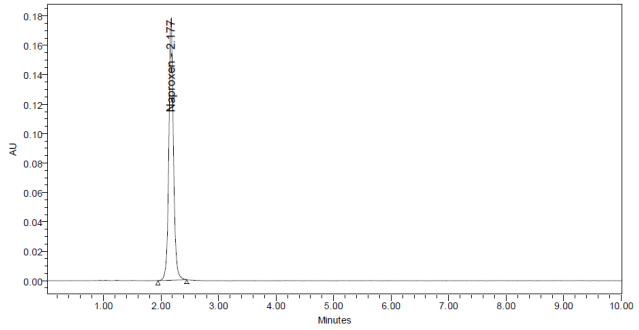

Figure 11: Hydrolytic Degradation of Naproxen sodium

(4.77%), thermal (3.39%) and no degradation in photo and hydrolytic conditions. The results were presented in Table 6 and Figures 6-11. So, the developed RP-HPLC method is accurate and specific and could be used in routine analysis of Naproxen sodium in bulk and tablet dosage form.

CONCLUSION

The developed new RP-HPLC method in the present study was found to be simple, rapid, specific, accurate, precise, linear, and robust. Thus, the method is suitable for the estimation of Naproxen sodium in raw material and tablet formulation in quality control with a high degree of Accuracy and Precision.

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