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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF NAPROXEN SODIUM IN BULK DRUG AND TABLET DOSAGE FORM

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INTRODUCTION

Naproxen sodium, a propionic acid derivative (fig 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 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 Specialities Private Ltd., Mumbai and Sd Fine Chem. Limited, Mumbai.

Chromatographic Conditions

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 10µl (fixed loop) and the total run time was 5 min [table1].

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.

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 Ultipor 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 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 220nm.

VALIDATION OF THE DEVELOPED METHOD

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

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 intraday and inter day precision.

Limit of detection (LOD) and limit of quantitation (LOQ)

The calibration curve of the drug was prepared using 2-12 µg/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) =3.3σ /S and Limit of quantitation (LOQ)= 10σ/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 Rizatriptan 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 ±2°C. 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 24 hours at room temperature.

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 x 4.6 mm x 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 220nm [fig. 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 [fig. 3 & 4].

The estimation of Naproxen sodium tablets was carried out by RP-HPLC using mobile phase, 0.1%OPA and Acetonitrile 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 No 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 2-12mcg/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 [fig. 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 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. 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.

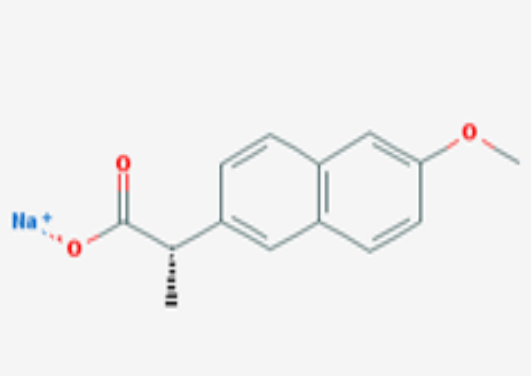

Fig. 1: Structure of Naproxen Sodium

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

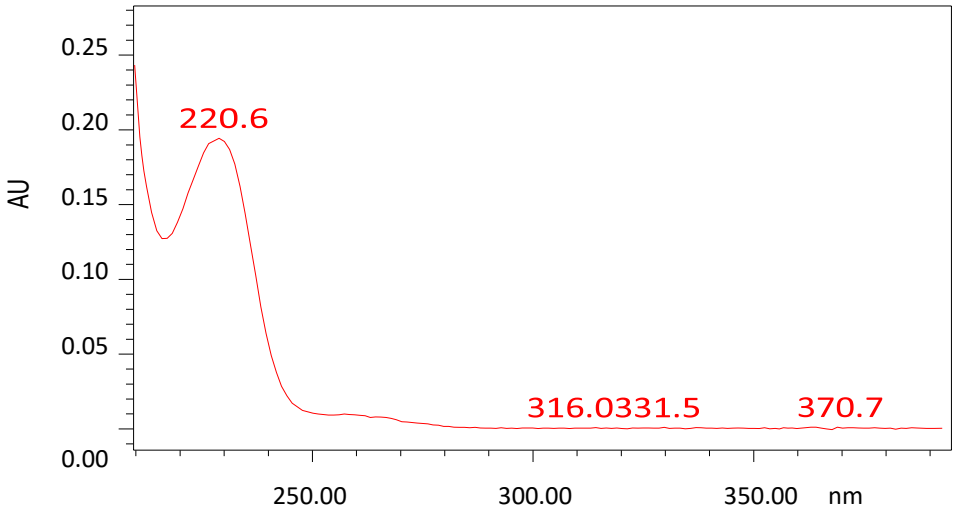

Fig.2. UV Spectrum of Naproxen

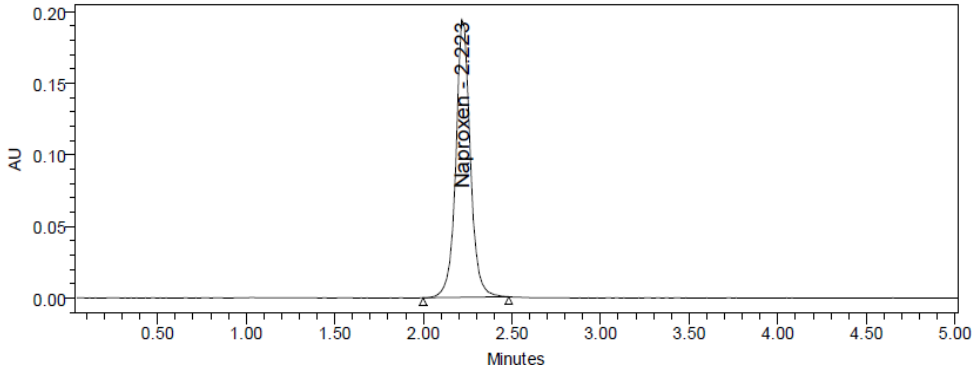

Fig.3. Chromatogram of standard solution of Naproxen

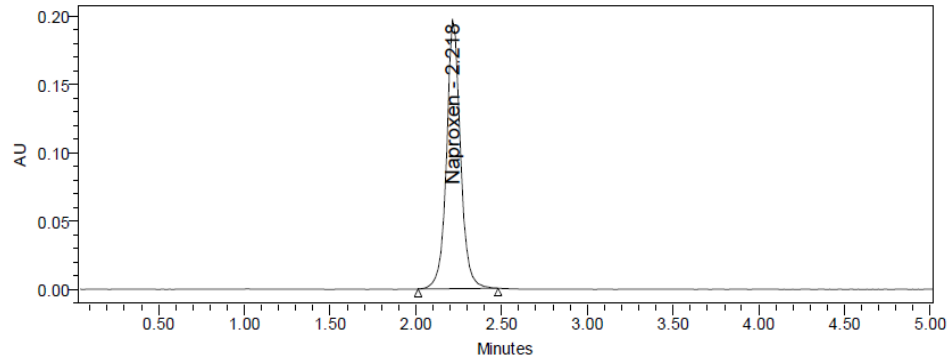

Fig.4. Chromatogram of sample solution of Rizatriptan

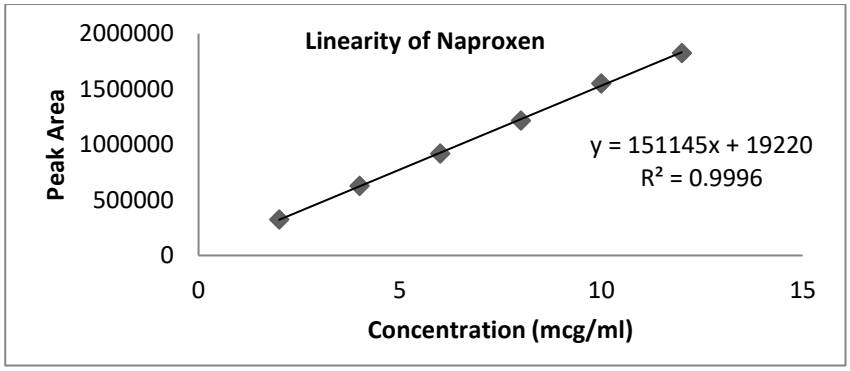

Fig.5. Calibration curve of Naproxen

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

Parameter	Naproxen
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 Intraday (n=6)	0.719
Precision Inter day (n=6)	0.643
LOD (µg/ml)	0.95
LOQ (µg/ml)	2.88

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**

Table 4: Recovery studies of the developed method

Preanalysed 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		1.1	0.4	2.206	1.237
	(± 0.1ml/min)	1.0	0.9	0.5	2.318	1.102
2.	Mobile Phase		48:52	0.3	2.097	1.267
	(±2 %) (B:A)	50:50	52:48	0.3	2.594	1.22
3.	Temperature	30ºC	28º C	1.1	2.403	1.28
	(± 2ºC)		32º C	0.334	2.161	1.28

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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REFERENCES

1. The American Society of Health-System Pharmacists. Retrieved Dec 1, 2014.
2. Stovner LJ, Zwart JA, Hagen K, Terwindt GM, Pascual J. Epidemiology of headache in Europe. Eur J Neurol.2006; 13:333–45. [PubMed]
3. Goldberg MR, Lee Y, Ermlich S., Rizatriptan, a novel 5-HT1B/1D agonist for migraine: single- and multiple-dose tolerability and pharmacokinetics in healthy subjects. J Clin Pharmacol, 40: 74-83, (2000).
4. Bou J, Domenech T, Puig J, Heredia A, Gras, J, Fernandez-Forner D, Beleta J. Palacios JM., Pharmacological characterization of almotriptan:an indolic 5-HT receptor agonist for the treatment of migraine. Eur J Pharmacol, 410: 33-41, (2000).
5. Emile H, Joseph H, Hashmonai D., J Emer Med, 3: 245-249, (2003).
6. Martindale: The Complete Drug Reference. Pharmaceutical Press. Retrieved 5 April 2014.
7. Akiful haque, Hasan Amrohi S, Mahesh Nasare, Prashanth Kumar K, Pradeep Kumar T, Nivedita G, Prakash V Diwan., Analytical method development and validation for the estimation of Naproxen using RP-HPLC. IOSR Journal of Pharmacy, 2(4):19-24, (2012).
8. Tsvetkova B., RP-HPLC method for determination of Naproxen in pharmaceutical dosage form. Pharmacia, 62(3):15-19, (2015).
9. Saleha Tanjin, Farhana Islam, Md Zakir Sultan, Asma Rahman, Sharmin Reza Chowdhury, Tasnuva Sharmin, Mohammad A Rashid., Development and Validation of a Simple RP-HPLC Method for Determination of Naproxen in Pharmaceutical Dosage Forms. Bangladesh Pharmaceutical Journal, 16(2): 137-141, (2013).
10. Yi-Hsin Hsu, Yi-Bo Liou, Jen-Ai Lee, Chau-Yang Chen, An-Bang Wu., Assay of naproxen by high-performance liquid chromatography and identification of its photoproducts by LC-ESI MS. Biomed. Chromatogr, 20: 787–793, (2006).
11. International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, Validation of Analytical Procedures: Text and Methodology. ICH Q2 (R1), 2005.

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