RP-HPLC METHOD FOR DETERMINATION OF NAPROXEN IN PHARMACEUTICAL DOSAGE FORM

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Abstract. A simple, sensitive and specific liquid chromatographic method with UV detection was developed for the determination of Naproxen in tablet dosage form. Separation was achieved with a C_{18} (250 mm x 4.6 mm, 10 μm) column, ambient temperature with isocratic mode with mobile phase containing acetonitrile:0.5 M potassium dihydrogen phosphate buffer pH 2.5 adjusted with ortho-phosphoric acid:tetrahydrofuran (45:53:2 v/v/v). The flow rate was 1.0 ml/min and eluent was monitored at 254 nm. The selected chromatographic conditions were found to effectively separate naproxen with retention time of 3.25 min. The calibration curve was linear in the concentration range of 12.50-100.00 μg/ml. The proposed method was found to be accurate, precise, reproducible and specific and it can also be used for routine quality control of the analysed drug in tablets.

Key Words: liquid chromatography, validation, Naproxen, tablet dosage form

INTRODUCTION

Naproxen [(+)-2-(6-methoxy-2-naphthyl) propionic acid, is a non-steroidal anti-inflammatory drug with antiinflammatory, analgesic and antipyretic properties often preferred to acetylsalicylic acid because of its better absorption following oral administration and fewer adverse effects. Anti-inflammatory effects of Naproxen are generally thought to be related to its inhibition of cyclo-oxygenase and consequent decrease in prostaglandin levels in various fluids and tissues [1]. Formulated in tablets or suppositories, Naproxen is widely applied in the treatment of rheumatoid arthritis and other rheumatic or musculoskeletal disorders, dysmenorrhea and acute gout [2].

The official method for determination of Naproxen is by acid-base titration with phenolphthalein solution of a substance and liquid chromatography with reversed phase and isocratic elution according to tablets and suppositories [3]. Extensive literature survey revealed determination of Naproxen in dosage form by UV spectrophotometeric methods [4–10], spectrofluorimetry [11, 12], Colorimetry [13], HPLC in pharmaceutical dosage form [14–19], HPLC using CD detector [20], HPTLC [21, 22], potentiometry [23– 26], molecular imprinted polymerization for extraction from urine [27,28], and from pharmaceutical dosage forms [29].

Present work describes the development of simple, rapid, accurate and precise RP-HPLC method for the determination of Naproxen in tablets.

EXPERIMENTAL SECTION
MATERIALS AND METHODS

Reagents and chemicals

Tablets containing Naproxen (250 mg) were obtained commercially. Analytically pure powder Naproxen was procured as gift sample from Bulgarian Drug Agency. LC-grade acetonitrile and tetrahydrofuran were supplied from Merck (Germany). All other chemical reagents were of analytical grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on a modular HPLC system LC-10AShimadzu (Japan) comprising a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector with 20 μl loop, column oven CTO-10A, SPD-M10A UV detector with

Table 1. *Preparation of standard solutions for linearity*

%	Concentration (µg/mL)	Volume pipetted of solution A (mL)	Volume to made up (mL)
25	12.5	5.0	100.0
50	25	5.0	50.0
100	50	10.0	50.0
150	75	15.0	50.0
200	100	10.0	25.0

fixed wavelength and communication bus module CBM-10A. Separation was achieved isocratically with a LiChrosorb C_{18}, 250 mm x 4.6 mm, 10 µm column eluted with a mixture of acetonitrile:0.5 M potassium dihydrogen phosphate buffer pH 2.5 adjusted with ortho-phosphoric acid:tetrahydrofuran (45:53:2 v/v/v) as the mobile phase at flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45 µm membrane filter and degassed. Detection was carried out by absorbance at 254 nm. The analysis was carried out at ambient column temperature and injection volume was 20 µl.

Preparation of reference solutions

Reference solution (A): The solution was prepared by dissolving 25.0 mg of accurately weighed Naproxen RS in methanol in a 100.0 mL volumetric flask. Reference solution (B): The solution was prepared by diluting 5.0 mL of reference solution (A) with methanol into a 25.0 mL volumetric flask.

Preparation of standard solutions for evaluation of linearity

Standard solutions used in estimation of linearity were prepared as follows (Table 1).

Sample preparation

Tablets working solution was prepared as follows: an accurately weighed quantity of powdered tablet sample containing an equivalent of 25.0 mg Naproxen was transferred into 100 ml volumetric flask, then 70 ml methanol was added. The mixture was sonicated for ten minutes and the volume was made up using methanol, then the sample was filtered. 10.0 mL of the filtrate was diluted with methanol into a 50.0 mL volumetric flask to give a test solution containing 50 µg/mL Naproxen.

RESULTS AND DISCUSSION

In this work an LC method with UV detection for analysis of Naproxen in a tablet formulation was developed and validated. From the chromatogram shown in Figure 1, it is evident that, under the proposed chromatographic conditions Naproxen is eluted with short elution time – Tr =3.25 min. The specificity analysis revealed the HPLC method did not suffer from interference by the formulation excipients, since there was not another peaks on the retention time of the drug of interest.

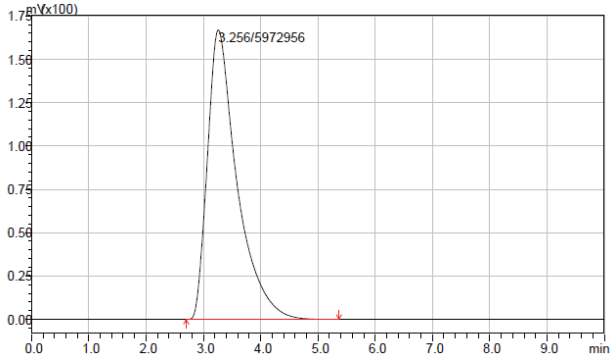

Fig. 1. Chromatogram of analysis of Naproxen RS

Method validation

The proposed method was validated with respect to linearity, accuracy, precision, limit of quantitation (LOQ) and limit of detection (LOD).

Linearity

Calibration curve was constructed in the range of 12.50-100.0 µg/ml for Naproxen to encompass the expected concentration in measured samples. An excellent correlation existed between the peak areas and the concentrations as can be seen from correlation coefficients. The limit of quantitation and limit of detection were calculated from the standard devia-

tions and slopes of the responses using a signal-to-noise ratio as per ICH guidelines. Data concerning linearity and sensitivity of the method were shown in Table 2.

Table 2. *Linearity Data*

Parameter	Naproxen
Linearity range	12.50-100.0 μg/ml
Slope	12584
Intercept	-1261
Regression coefficient	0.9998
Limit of quantitation, ng	2.5
Limit of detection, ng	0.5

Accuracy

Accuracy was determined by applying the proposed method to synthetic mixtures of the drug product components to which known quantities of Naproxen substance had been added (corresponding to 75, 100 and 125 % of the label claim of the drug). The accuracy was expressed as the percentage of analyte recovered by the assay. Mean recoveries for Naproxen from the specific formulations were shown in Table 3. The results indicated good accuracy of the method for the determination of analysed drug as revealed by mean recovery data.

Precision

The precision of the method was evaluated by performing six independent determinations of the test sample preparation and calculating RSD (%). The RSD value measured during assessment of precision was <2.0% for Naproxen, confirming the method is precise (Table 4).

Table 4. *Precision of the method*

№	Amount found, mg/tablet	Statistical data	
1.	250.02	Mean	249.5
2.	249.95		
3.	249.26	SD	0.722
4.	250.17		
5.	249.30	%RSD	0.29
6.	248.24		

CONCLUSION

The newly developed LC method for determination of Naproxen in tablet dosage forms is specific, precise, accurate and rapid. The proposed method can be conveniently adopted for routine quality control analysis.

Table 3. *Accuracy of the HPLC method*

Level (%)	Theoretical concentration (μg/ml)	Observed concentration (μg/ml)	Mean recovery (%) ± SD	RSD (%)
75	25.05	24.95	99.18±1.187	1.20
		25.08		
		24.51		
100	49.53	49.68	100.1±0.793	0.79
		49.12		
		49.87		
125	74.28	75.06	100.8±0.774	0.77
		74.18		
		75.27		

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