

A Rapid and Sensitive HPLC Method for the Determination of Naproxen in Plasma

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Abstract

A sensitive, rapid method using an isocratic high-pressure liquid chromatography and fluorometric detection for the determination of naproxen sodium in plasma is presented. Reverse phase micobondapack column was used with a mobile phase consisting of 42% acetonitrile and 58% water adjusted to pH 3 using phosphoric acid. The fluorometric detector with an excitation wavelength of 270 nm and emission wavelength of 340 nm provided high sensitivity and no interferences from plasma constituents. Plasma samples were injected to HPLC without any extraction. The method was precise, reproducible as it was demonstrated by replicate analysis of pooled plasma sample containing 0.5 – 80 µg/ml naproxen sodium.

Key words: Naproxen; HPLC; fluorescence detection; plasma.

Introduction

Naproxen is a non-steroidal anti-inflammatory with analgesic and antipyretic properties. It inhibits prostaglandin synthesis (Hardman *et al.*, 1996). Naproxen is rapidly and completely absorbed from the gastrointestinal tract. It is associated with several GIT side effects ranged from relatively mild dyspepsia, gastric discomfort, and heart pain to nausea, vomiting and gastric bleeding (Golden, 1980). Therefore patient monitoring is of great interest to minimize these adverse reactions (Kimberly *et al.*, 1978).

Several methods of assay were developed in biological fluid for naproxen. It includes HPLC (Shimek *et al.*, 1982), (Dusci *et al.*, 1979), (Broquaire *et al.*, 1981) and (Slattery *et al.*, 1979), GLC (Runkel *et al.*, 1972, 1974) and (Desager *et al.*, 1976), Spectrophotometry (Anttila, 1977), Colorimetry (Populaire *et al.*, 1973) and combinations of these techniques. Sample preparations of HPLC methods were tedious. Usually it involves extraction, separation and then detection of naproxen in plasma. GLC method required a minimum plasma sample of 0.5 ml. Spectrophotometric methods lack specificity due to the interference of the metabolites. HPLC methods were reported using UV detectors (Baker *et al.*, 1980) and fluorometric detectors (Shimek *et al.*, 1982). The methods require extraction and separation in order to detect naproxen sodium in plasma sample (Hirai *et al.*, 1997).

The present method describes a rapid and sensitive method for the determination of naproxen sodium in biological rabbit plasma using a simple isocratic HPLC with fluorometric detection. Sample preparation in this method was simple, easy and no extraction of the drug is required.

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The applicability of this method was demonstrated by the analysis of rabbit plasma containing 0.5 µg/ml of naproxen sodium.

Experimental

Instrumentation: An HPLC system consisting of L-7150 pump Lachrom, connected to a fluorescent detector (Merck Hitachi, Japan), Spectra-Physics integrator S4290 (from Variant California, USA). The chromatographic separation was performed using Microbondapack 10µm C-18, 25cm x 4.6mm column (Waters, USA). The fluorescent detector was set at an excitation wavelength of 270 nm and emission wavelength of 340 nm.

Chemicals and reagents: Naproxen sodium (Merck pharmaceuticals). Phosphoric acid (Fischer). All other chemicals were of analytical grades. All solvent used were of HPLC Grades. Double distilled de-ionized water was used.

Mobile phase: The mobile phase used was composed of acetonitrile and water (42:58% V/V) adjusted to pH 3 using phosphoric acid. The mobile phase was filtered through 0.45 µm membrane filter (Sartorius, Germany).

Standard Stock Solution: A solution containing 1 mg/ml was prepared in water and diluted ten folds to give a working solution of 100 µg/ml.

Sample preparation: Rabbit plasma sample of 100µl was transferred to 1.5 ml eppendorf tube and 100 µl of acetonitrile was added. The mixture was mixed using vortex mixer for 30 second, then centrifuged for two minutes. Fifty micro liter of the supernatant was injected directly into the HPLC system.

Quantitation: A standard curve was constructed by injecting plasma samples containing naproxen sodium at concentrations ranging from 0.5-80 µg/ml. The peak area was determined and plotted versus the concentration in µg/ml plasma.

Interference: The possible interference of normal plasma constituents was tested by injecting blank plasma. The interference of other drugs was studied by direct injection of pure solution prepared in mobile phase into the HPLC system.

Recovery: For the recovery study, an exact volume of pure naproxen standard was prepared in water and then analyzed using the same HPLC system. The absolute recovery was calculated by comparing the peak area with plasma standard.

Results and Discussion

A mobile phase composed of acetonitrile 42% and water 58% adjusted to pH 3 gave well resolved, sharp peak for naproxen sodium with retention time of 4.2 minute as shown in figure 1. Under the described condition 0.5 µg of naproxen sodium could be quantitated.

The quantification of the chromatogram was performed using peak area of the naproxen sodium. The peak area versus concentration was plotted. Statistical analysis indicated excellent linearity as shown in table 1 and the precision was evaluated by replicate analysis of pooled plasma containing naproxen sodium at three different concentrations as shown in table 2. The coefficient of variance of the analyzed samples ranged from 3.66 to 5.90%. Ampicillin and commonly used drugs did not show any interference with the analysis as shown in table 3. The absolute recovery was calculated by comparing the peak area of naproxen sodium in aqueous solution and plasma. The results are shown in table 4. The absolute recoveries of the samples tested were in the range of 95.44 to 99.95%, which indicate excellent recovery.

The described method is simple and rapid for the analysis of naproxen sodium from biological samples since no extraction is needed and directed injection to the HPLC is attained after just precipitation of plasma protein. On other hand the method was accurate, precise to determine a

single isocratic assay with no interference from many commonly used drugs. This method can be recommended for routine patient monitoring and for pharmacokinetic studies.

Table 1. Statistical Analysis of Linear Regression

Run No.	1	2	3	4	5
Concentration (µg/ml)	Peak area				
0.5	38197	56551	71010	108724	47138
1	191037	159928	172190	199758	177551
5	674963	636782	625281	704665	663281
10	1336401	1250506	1342189	1327963	1385456
20	2670174	2590684	2687987	2853548	2726603
40	5672691	5256468	5348954	5559568	5585516
80	11229984	10429582	10693140	10986128	11229731
Intercept	-32215	-8759	4200	40164	-23076
Slope	140824	130619	133619	137120	140427
R- value	0.99985	0.99996	0.99998	0.99991	0.99995

Table 2. Assay Precision of Naproxen^{a)}

Theoretical Concentration (µg/ml)	Found Concentration (µg/ml)	Standard Deviation (SD)	Percent Coefficient of variance (C. V. %)
3	3.05	0.180	5.90
30	30.07	1.772	5.89
60	58.11	2.126	3.66

a N = 5

Table 3. Possible Interferences under Assay Conditions

Drug	Fluorescence at Assay Condition	Retention time (minute)
Ampicillin	No	-
Caffeine	No	-
D, 3-4 dihydroxy phenyl alanine	Yes	2.01
Diclofenac sodium	No	-
Famotidine	No	-
Ibuprofen	No	-
Naphthalene acetic acid	Yes	3.51
Naproxen	Yes	4.34
Nifedipine	No	-
Paracetamol	No	-
Phenyl propanol amine	No	-

Table 4: Absolute Recovery of Naproxen from Plasma

Concentration ($\mu\text{g/ml}$)	Peak Area		Recovery %
	Aqueous	Plasma	
0.5	72190	69712	96.57
1	180182	180093	99.95
5	698292	670994	96.10
10	1465876	1448003	98.78
20	2835016	2705799	95.44
40	5892250	5684639	96.48
80	10974509	10913713	99.45

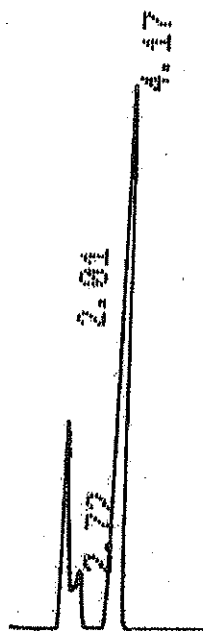


Figure 1: Typical Chromatogram of Naproxen Sodium from the Plasma Showing the Retention Time (4.17 minute).

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