

HPLC determination of naproxen in plasma

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Received March 27, 2003, accepted April 2, 2003

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Pharmazie 58: 614–615 (2003)

An assay method using isocratic HPLC with fluorometric detection for the determination of naproxen sodium in plasma is presented. A reverse phase Microbondapack 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 and reproducible as was demonstrated by replicate analysis of pooled plasma sample containing 0.5–80 µg/ml naproxen sodium.

1. Introduction

Several methods of assay have been developed for naproxen in biological fluids. These include HPLC [1–4], GLC [5–7], spectrophotometry [8], colorimetry [9] and combinations of these techniques. Sample preparation for HPLC methods is, usually involving extraction, separation and then detection of naproxen in plasma. The GLC method requires a minimum plasma sample of 0.5 ml. Spectrophotometric methods lack specificity due to the interference of the metabolites. HPLC methods have been reported using UV detectors [10] and fluorometric detectors [1]. The methods require extraction and separation in order to detect naproxen sodium in plasma sample [11].

The present report describes a rapid and sensitive method for the determination of naproxen sodium in rabbit plasma using a simple isocratic HPLC with fluorometric detection. Sample preparation for this method was simple and easy and no extraction of the drug is required. The applicability of this method was demonstrated by the analysis of rabbit plasma containing 0.5 µg/ml of naproxen sodium.

2. Investigations, results and discussion

A mobile phase composed of acetonitrile 42% and water 58% adjusted to pH 3 gave a well resolved, sharp peak for naproxen sodium with a retention time of 4.2 min. Under the conditions described, 0.5 µg of naproxen sodium could be quantitated.

The quantification of the chromatogram was performed using the area of the naproxen sodium peak. 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 3.6 to 5.9%. Ampicillin and commonly used drugs did not show any interference 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 95.4 to 99.9%, which indicates excellent recovery.

The described method is simple and rapid for the analysis of naproxen sodium from biological samples since no extraction is needed and direct injection to the HPLC is achieved after simply precipitation of plasma protein. On other hand the method was accurate and precise to determine a single isocratic assay with no interference from

Table 1: Statistical analysis of linear regression

Run No.	1	2	3	4	5
Concentration (µg/ml)					
0.5	38197	56551	71010	108724	471381
1	191037	159928	172190	199758	1775515
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^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 (min)
Ampicillin	No	—
Caffeine	No	—
D,3-4dihydroxy 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 from plasma

Concentration (µg/ml)	Peak area		Recovery %
	Aqueous	Plasma	
0.5	72190	69712	96.5
1	180182	180093	99.9
5	698292	670994	96.1
10	1465876	1448003	98.7
20	2835016	2705799	95.4
40	589250	5684639	96.4
80	10974509	10913713	99.4

many commonly used drugs. This method can be recommended for routine patient monitoring and for pharmacokinetic studies.

3. Experimental

3.1. Instrumentation

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

3.2. Chemicals and reagents

Naproxen sodium was obtained from Merck Pharmaceuticals. Phosphoric acid was purchased from Fischer. All other chemicals were of analytical

grade. All solvents used were of HPLC grade. Double distilled de-ionized water was used. 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 a 0.45 µm membrane filter (Sartorius, Germany). A solution containing 1 mg/ml was prepared in water and diluted ten-fold to give a working solution of 100 µg/ml.

3.3. Sample preparation

100 µl of rabbit plasma were transferred to a 1.5 ml Eppendorf tube and 100 µl of acetonitrile were added. The mixture was mixed using a vortex mixer for 30 s, then centrifuged for 2 min. 50 µl of the supernatant were injected directly into the HPLC system.

3.4. 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.

3.5. Interference

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

3.6. 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 a plasma standard.

Acknowledgement: The authors would like to express their sincere thanks to Mr. Farouq Zughool, Faculty of Pharmacy, Jordan University of Science and Technology for his assistance during the sample analysis.

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