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Simultaneous determination of naproxen and related compounds by HPLC using porous graphitic carbon column

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Abstract

A simple, selective and sensitive high performance liquid chromatographic (HPLC) method has been developed for the simultaneous determination of naproxen and its main degradation products such as 1-(6-methoxy-2-naphthyl) ethanol (MNE), 2-methoxy-6-ethyl naphthalene (MEN) and 2-acetyl-6-methoxy naphthalene (AMN). The separation of these compounds was achieved on porous graphitic carbon (PGC) column using tetrahydrofuran–methanol as the mobile phase, and the effluent from the column was monitored at 272 nm. At a flow rate of 1 ml min^{-1}, the retention time of the last eluting compound was less than 10 min. Correlation coefficient for calibration curves in the ranges 2–25 μg ml^{-1} for all compounds studied were greater than 0.999. The sensitivity of detection is 0.05 μg l^{-1} for naproxen, MNE and MEN and 0.20 μg ml^{-1} for AMN. The reproducibility of the peak area of these compounds using isocratic elution were quite high, and the standard deviations (S.D.) were below 2% ($n = 5$). The reproducibility of retention times of these compounds was within 1% ($n = 5$). The proposed liquid chromatographic method was successfully applied to the analysis of commercially available naproxen sodium (NS) dosage forms with recoveries of 98.8–102%. A comparative study shows that the selectivity of these compounds on PGC column was different to that obtained with octadecyl silica (ODS) columns.
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Keywords: Naproxen; Degradation products; Isocratic elution; Porous graphitic carbon

1. Introduction

Naproxen [(S)-6-methoxy-α-methyl-2-naphthalene acetic acid] is a non-steroidal anti-inflammatory drug widely used as mild to moderate pain relief and in the treatment of osteo- and rheumatoid arthritis [1]. Naproxen action is due to the inhibition of the cyclooxygenase enzyme, which in turn, presents the biosynthesis of certain prostaglandines [2]. The stability of naproxen in the raw materiel or in the final product could be altered under abnormal conditions such as: temperature, light, humidity and pH, which could yield different kinds of degradation products such as 1-(6-meth-

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oxy-2-naphthyl) ethanol (MNE) and 2-methoxy-6-ethyl naphthalene (MEN). The strict international regulations obliged most of the pharmaceutical companies to control these compounds precisely in raw materials and in final products.

Several liquid chromatography methods were reported for individual and simultaneous determination of naproxen and other anti-inflammatory drugs in human serum and urine [3–6] and in pharmaceutical preparations [7–9]. Both recommended methods, United State Pharmacopeia [10]and the British Pharmacopeia [11], describe the analysis of naproxen in formulations and raw materials for related compounds using thin layer chromatography. However, on one hand, few methods describe the analysis of naproxen and related compounds in pharmaceuticals and raw materials [8,12]. On the other, these methods are time consuming in addition to the complexity of their separation systems. One of the main reasons in the increasing interests in finding robust systems for evaluation of pharmaceutical products. Porous graphitic carbon (PGC) is a preferred packing material for this purpose that has successfully been used in the separation of pharmaceuticals and related compounds [13–17].

Thus, this study describes an isocratic HPLC method for the simultaneous determination of naproxen sodium (NS) and its main degradation products using PGC column, in order to simplify and to reduce the analysis time.

2. Experimental

2.1. Chemicals and reagents

NS, MNE, MEN and AMN were provided by the pharmaceutical society Ibn Albytar (Tunis, Tunisia). Their chemical structures are represented in Fig. 1. Drug samples were collected from local pharmacies. HPLC grade methanol, acetonitrile and tetrahydrofuran were purchased from prolabo (Paris, France).

2.2. Instrumentation and chromatographic conditions

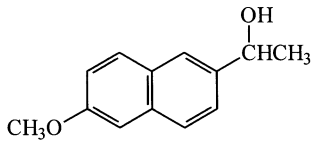
The HPLC system (Beckman Instruments Inc., USA) was equipped with a solvent module 125, a spectrophotometer detector166 and a rheodyne 7725 injector with a 20 μl loop. The carbon column (100 × 4.6 mm i.d., 7 μm particle size) was packed with Hypercarb PGC (Shandon, Runcorn, UK). The mobile phase was consisted of an isocratic mixture of 80/20 (v/v) tetrahydrofuran–methanol. The flow rate was 1 ml min^{-1}. The detector wavelength was set at 272 nm. Responses were recorded and integrated using GOLD NOUVEAU software.

2.3. Preparation of solutions

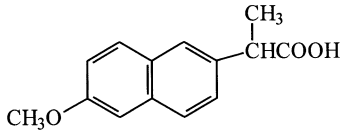
Stock solutions of naproxen, MNE, MEN and AMN (0.4 mg ml^{-1} each) were prepared in the mobile phase and stored at 4 °C. The working standards (2–25 μg ml^{-1}) were freshly prepared from the stock solutions by dilution with the appropriate volume of the mobile phase. Naproxen containing tablets were prepared by crushing 20 tablets and an accurately weighed portion of the mixed powder equivalent to naproxen content of one tablet was transferred to 100 ml volumetric flask and dissolved by sonication. The sample was filtered and diluted to make a final concentration of naproxen in the range of 10–20 μg ml^{-1}.

3. Results and discussion

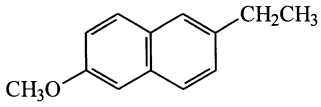
The separation of NS, MNE, MEN and AMN on PGC was initially investigated using similar mobile phase composition to that obtained by octadecyl silica (ODS) column [8] with a higher ratio of acetonitrile (60/40ACN/buffer pH 3.0). Results of this essay showed excessive retention especially for MNE ($t_R > 50$ min) and AMN, which was not eluted during 1 h of analysis. This indicates the higher affinity of these compounds towards PGC. Not much improvement was obtained by increasing acetonitrile ratio in the mobile phase or by adding methanol. Therefore, in order



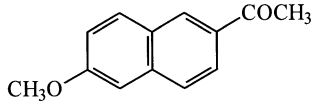
1-(6-methoxy-2-naphthyl) ethanol



Naproxen



2-methoxy-6-ethyl naphthalene



2-acetyl-6-methoxy naphthalene

Fig. 1. Chemical structure and names of studied compounds.

to enhance the separation of naproxen and related compounds on PGC column, tetrahydrofuran was added to the mobile phase. Thus the use of THF with a higher dipolar properties than acetonitrile to separate these compounds on PGC was necessary in this method to achieve a reasonable analysis time.

The separation of naproxen and related compounds on PGC was investigated by varying THF–methanol, THF–water and THF–acetonitrile ratios. The baseline separation of the four compounds was achieved (Fig. 2) in less than 10 min using 80/20 THF–methanol. Increasing the concentration of methanol could enhance the resolution between NS and MNE, but at the same time the analysis time will be increased. Therefore, the relative chromatographic resolution parameters using a mobile phase containing 20% methanol are reported in Table 1. The good chromatographic separation indicated that any one of these solutes could be used as internal standard for the assay of others. These results are within the validated standard values for the analysis of drugs and related compounds.

Results obtained with ODS columns [8,12] showed that the elution order of these compounds on ODS column was; MNE, NS, AMN and MEN while the elution order on PGC was; NS, MNE, MEN and AMN. The difference in the elution order between PGC and ODS columns, suggest that the molecular interactions determining solute retention are different for the two materials, and compounds with acetyl groups (AMN) were highly retained than that with ethyl groups (MEN).

3.1. Method validation

Method validation establishes that the method performance characteristics are suitable for the intended use. Various parameters of the method such as selectivity, accuracy, precision, linearity, sensitivity, detection limit, quantification limit and recovery should be evaluated.

3.1.1. Selectivity

The selectivity of the chromatographic method was tested in the presence of the common impurities of naproxen. The chromatogram in Fig. 2 shows an adequate separation of naproxen peak from other impurities with a resolution ≥ 1.5.

3.1.2. Linearity

The linearity of the analytical response, here the peak area using absorbance detection at 272 nm,

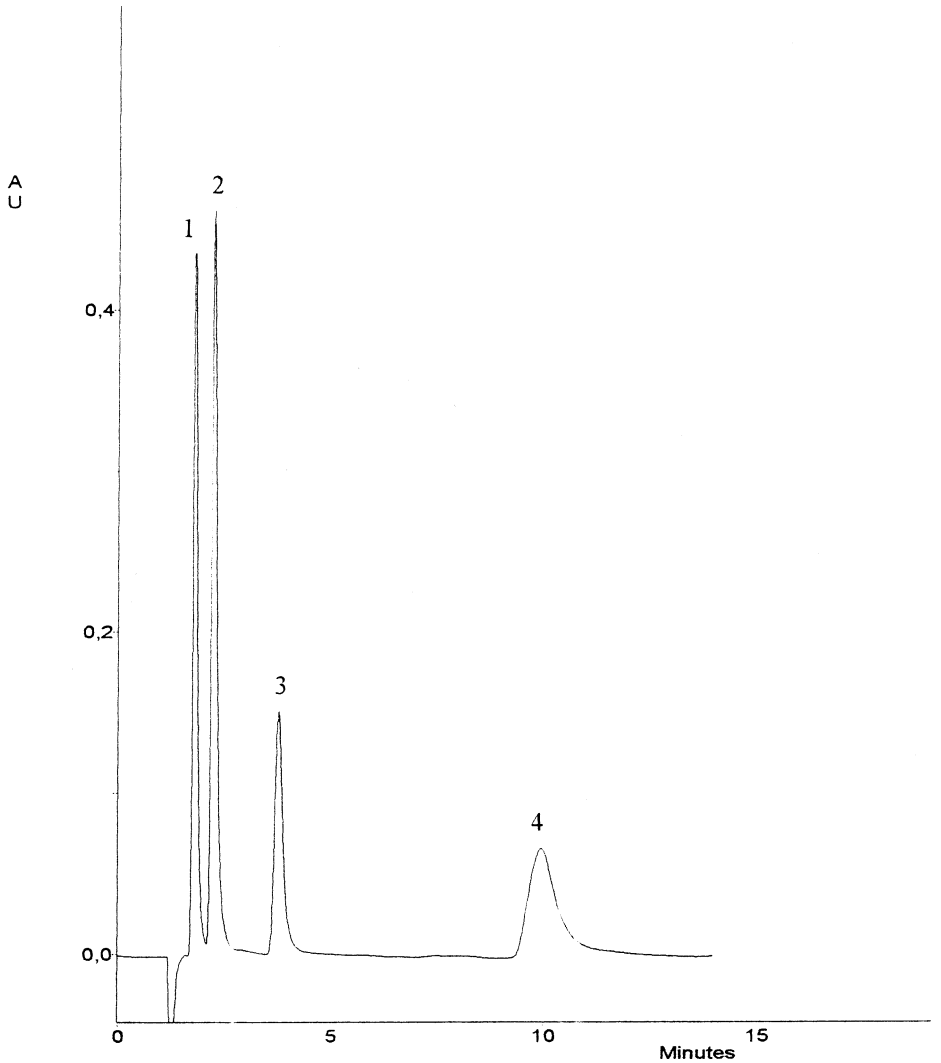


Fig. 2. Separation of naproxen and its potential impurities on PGC column with a mobile phase containing 80/20 THF–methanol, and a flow rate of 1 ml min^{-1}. Peaks: 1 = naproxen; 2 = MNE; 3 = MEN;

Table 1
Chromatographic parameters for the peaks recorded in analysis of naproxen and related compounds

Compounds	k′	A_s	N	R_s
Naproxen	0.63	1.20	1205	
				1.54
1-(6-methoxy-2-naphthyl) Ethanol	1.23	1.3	1025	
				3.5
2-methoxy-6-ethyl Naphthalene	2.42	1.28	2316	
				6.9
2-acetyl-6-methoxy Naphthalene	8.06	1.33	1337	

was studied for each compound in the concentration range from 2 to 25 μg ml^{-1}. The data was analysed by the linear lease squares fit method and the results are presented in Table 2. Excellent linearity was obtained in all cases with correlation coefficients higher than 0.999. The equation of the line ($y = ax + b$, where y is the area and x is the concentration in μg ml^{-1}) was used to calculate the concentration of naproxen in the real pharmaceutical samples.

Table 2
Linear regression (least squares fit of peak area vs. concentration in μg ml^{-1}) calibration data for the analysis of naproxen and related compounds ($n = 5$)

Compounds	Concentration range (μg ml^{-1})	Slope	R.S.D. of the slope (%)	Intercept	R.S.D. of the intercept (%)	R^2
Naproxen	2–25	16331	0.51	200745	1.2	0.9997
1-(6-methoxy-2-naphthyl) Ethanol	2–25	47931	0.40	184074	1.1	0.9999
2-methoxy-6-ethyl Naphthalene	2–25	19247	0.63	29251	1.0	0.9999
2-acetyl-6-methoxy Naphthalene	2–25	22891	1.0	29551	1.3	0.9997

3.1.3. Limit of detection and quantification

Detection limits (LOD) of all analytes were determined at a signal-to-noise ratio of three by serial dilution of standard solutions (Table 3). They were found to be 0.05 μg ml^{-1} for NS, MNE, MEN and 0.20 μg ml^{-1} for AMN. However, the quantification limits (QL) measured as ten times the noise, were 0.20 μg ml^{-1} for NS, MNE, MEN and 0.80 μg ml^{-1} for AMN. Furthermore, the QL was assessed as percent relative to naproxen concentration in the formulations. It was found that as little as 0.04% of MNE, MEN and 0.16% of AMN could be quantified in naproxen real samples.

3.1.4. Accuracy and recovery

In this study, the average recovery was calculated as the mean value obtained by spiking drug tablets with a mixture of analytes studied at three levels within the working range. Data corresponding to recovery assays for the studied analytes are presented in Table 3. The average recoveries of all solutes studied were between 99 and 100.8%.

3.1.5. Precision

The stability of the chromatographic system of the proposed method was evaluated by studying the variations (intra-day and inter-day) of the peak area and retention for all analytes. As summarised in Table 3, a relative standard deviation (R.S.D.) values less than 2% were obtained by injecting the standard mixture of NS, MNE, MEN and AMN at different concentrations for at least five injections in the same day. The inter-day R.S.D. values obtained for retentions were less than 1% and for peak areas were less than 2%. The HPLC-PGC method provides satisfactory precision and accuracy for the analysis of naproxen and related compounds.

Table 3
Method reproducibility, recovery and limit of detection for the studied analytes

Compounds	Retention time[a] Mean ±S.D. (min)	R.S.D. (%)	Peak area[b] R.S.D. (%)	Recovery[c] (%)	Limit of detection (μg ml^{-1})
Naproxen	1.77±0.02	1.12	1.90	99.6	0.05
1-(6-methoxy-2-naphthyl) Ethanol	2.25±0.02	0.88	1.30	99.8	0.05
2-methoxy-6-ethyl Naphthalene	3.77±0.04	1.01	1.80	100.8	0.05
2-acetyl-6-methoxy Naphthalene	9.87±0.08	0.80	1.25	99.0	0.2

[a] Chromatographic conditions as in Fig. 2.
[b] 20 μl injection of 5 and 10 μg ml^{-1} solutions ($n = 5$).
[c] 20 μl injection of Naproxen samples spiked with 5, 10 and 15 μg ml^{-1} standard solutions ($n = 5$).

Table 4
Content of NS expressed as% with respect to label amount claim

Drug	Naproxen (mg)		%
	Claimed	Found	
Nopain D.S.	500	494	98.8
Axer Alfa	550	546	99.3
Naprosyne	500	509	102

3.1.6. Determination of naproxen in pharmaceutical formulations

The method was applied to different pharmaceutical formulations (tablets) for determining their content in naproxen. The values of the drug percentage with respect to the label claim, were ranged within 98.8 and 102% (Table 4). According to the united state pharmacopeia, these values were acceptable.

4. Conclusion

The present method has demonstrated the simultaneous determination of NS and related compounds on PGC. The method is straightforward, simple and feasible enabling the determination of naproxen in pharmaceutical formulation in the presence of its related compounds in less than 10 min. It can be indicated to be useful for routine analysis instead of pharmacopeia standard methods.

It is interesting to note the ruggedness of PGC column after 9 years of usage. During this period, it was able to function as modified (support for chiral anchors) and non-modified analysing a variety of samples including biological and pharmaceuticals.

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