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Note

Determination of benoxaprofen in plasma and urine by liquid chromatography

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Benoxaprofen [2-(4-chlorophenyl)-α-methyl-5-benoxazoleacetic acid] (I) is a substance reported to possess anti-inflammatory, analgetic and antipyretic properties in animal tests [1, 2]. Three methods for its determination in biological fluids have been published [3]. The most simple one is based on extraction and measurements in a UV spectrophotometer. The other two methods utilize gas chromatography of the benoxaprofen methyl ester derivative which is detected by flame ionization detector or electron-capture detector. The present liquid chromatographic method is almost as simple to perform as the spectrophotometric method and offers a specificity and sensitivity in the same order as that of the more complicated gas chromatographic—electron-capture detection method [3].

MATERIALS AND METHODS

Standards

Benoxaprofen (I) and 2-(3,5-dichlorophenyl)-α-methyl-S-benzoxazoleacetic acid (II) used as internal standard were kindly donated by Lilly (Hants, Great Britain). Their structures are shown in Fig. 1.

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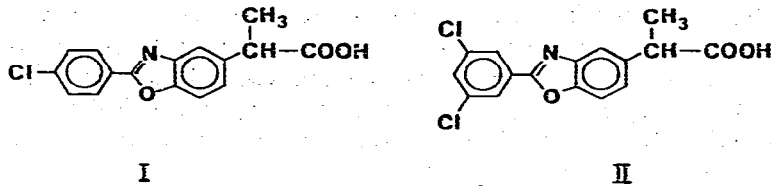


I II

Fig. 1. Structures of I, benoxaprofen [2-(4-chlorophenyl)-α-methyl-5-benoxazoleacetic acid] and II, internal standard [2-(3,5-dichlorophenyl)-α-methyl-5-benoxazoleacetic acid].

Liquid chromatography

The liquid chromatographic system used consisted of a M6000 pump, a U6K injector and a M440 filter UV detector (Waters Assoc., Milford, MA, U.S.A.). The column (0.15 m × 4.6 mm I.D., stainless steel) was slurry-packed with Spherisorb S5 ODS (particle size 5 μm). The mobile phase consisted of 0.01 *M* phosphate buffer (pH 6.5)—acetonitrile (65:35) operated at a flow-rate of 1.5 ml/min at room temperature (ca. 20—22°C). Since I had a UV maximum at 310 nm in the eluent the 313-nm filter was used in the detector.

Plasma assay

In a 10-ml screw-capped tube 100 μl plasma, 1 ml 0.1 *M* hydrochloric acid, 50 μl internal standard solution (20 μg/ml) and 4 ml diethyl ether were shaken for 5 min on a shake board. The tube was then centrifuged for 10 min at 500 *g* (Wifug X1). The diethyl ether phase was removed and transferred to a new tube (conical bottom) and the solvent was then evaporated by a stream of nitrogen. The residue was dissolved in 150 μl of the eluent mixture and after filtration through a pasteur pipette closed with fine glass-wool, 20 μl were injected in the chromatograph. When concentrations below ca. 300 ng/ml in plasma were measured 1 ml of plasma was used instead of 100 μl.

Urine assay (free I)

Urine (100 μl) was treated in the same way as the plasma sample described above.

Urine assay (free and conjugated I)

Urine (100 μl), 100 μl 0.2 *M* acetate buffer (pH 5) and about 1 mg of β-glucuronidase were incubated at 37°C overnight. One ml 0.1 *M* hydrochloric acid was added to the mixture and worked up as described above. The amount of internal standard used was adjusted so that it corresponded to the concentration of I in the urine samples.

RESULTS AND DISCUSSION

Extraction of I from an aqueous phase with diethyl ether or chloroform has been shown to yield about 98% in the organic phase [3]. Dichloromethane also gave a similar result. Diethyl ether was chosen for the extraction because it emulsified the plasma less.

The method has been described for 100-μl samples of plasma since this

volume may be easily collected from the finger tip in capillary tubes. The detection limit using this amount of plasma was 250 ng/ml. When analysing plasma containing benoxaprofen below this concentration the volume of the plasma sample was increased to 1 ml. This increase in sample volume did not, however, introduce any disturbing background peaks on the chromatogram. The detection limit of I was then about 25 ng/ml plasma.

Fig. 2 shows typical chromatograms obtained when analysing a plasma sample (100 μl) containing 5 μg/ml benoxaprofen and a blank plasma sample according to the method described above.

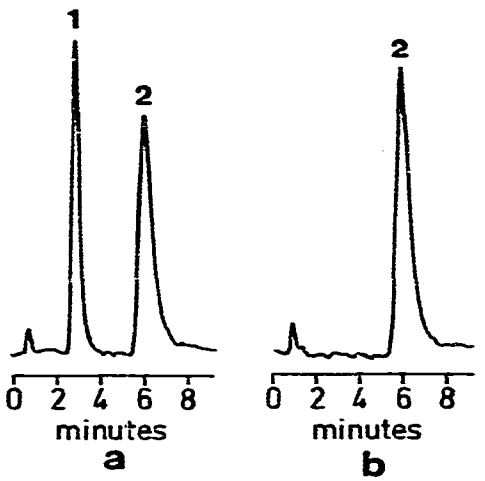
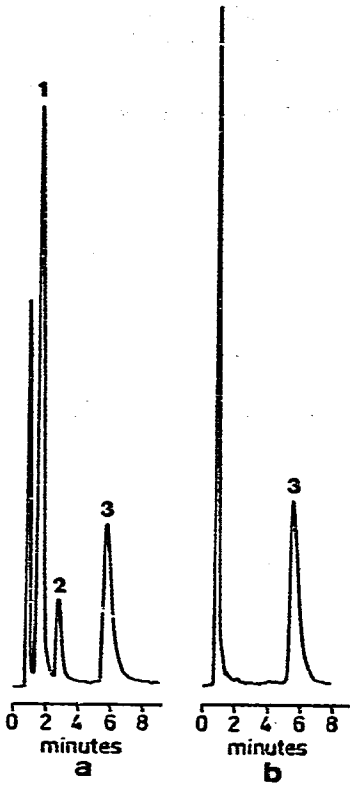


Fig. 2. Chromatograms of (a) plasma sample containing 5 μg/ml of benoxaprofen and (b) blank plasma sample treated according to the method described. Peaks: 1 = benoxaprofen; 2 = internal standard.

The reversed-phase column used in the method was eluted with acetonitrile—phosphate buffer (pH 6.5). At this pH benoxaprofen chromatographed in an ionized form. When the amount of acetonitrile in the eluent was 30% the retention time was 3.6 min. To obtain a similar retention time (3.3 min) with pH 3 phosphate buffer 80% of acetonitrile had to be present in the eluent mixture. Such a high acetonitrile concentration may cause problems with precipitation of salts from the buffer.

When urine samples were extracted in order to determine free I, a conjugate of I was also extracted to some extent under the conditions used. The peak corresponding to the conjugate can be seen in the chromatogram of Fig. 3a. This peak disappeared if the method for determination of free and conjugated I was followed, which involved incubation with β-glucuronidase prior to extraction. It is important to keep the pH of the eluent buffer at 6.5 since an increase in the pH reduces resolution between I and its conjugate and at pH 7 an overlap occurs.

Calibration graphs were constructed in the ranges 0.5—10 and 5—70 μg/ml plasma. The peak height ratios (I/internal standard) were plotted against the concentration of I. The curves were linear and passed through the origin. To



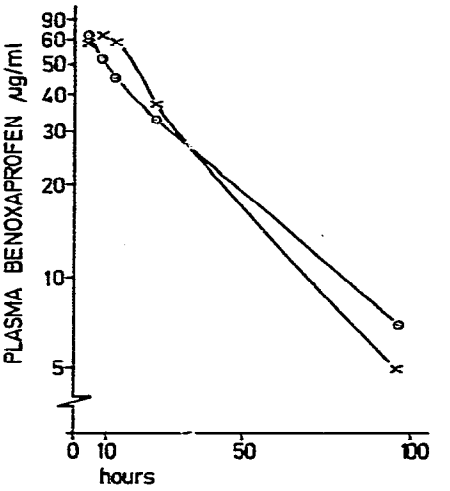


Fig. 3. Chromatograms of (a) urine sample containing 1.9 µg/ml of benoxaprofen and (b) blank urine sample treated according to the method described. Peaks: 1 = conjugate of benoxaprofen; 2 = benoxaprofen; 3 = internal standard.

Fig. 4. Plot of plasma concentration versus time from two subjects given 600 mg of benoxaprofen orally. Plasma concentrations of benoxaprofen in subject 1 (×) and in subject 2 (○).

determine the precision, I (4 µg/ml) was added to ten plasma samples and analysed by the above method. It was found to be 2.5%. The absolute recoveries of I from plasma and urine by the method were 90 and 92% respectively.

Two healthy subjects were each given 600 mg of benoxaprofen. The plasma concentration—time curves shown in Fig. 4 were constructed by analysing plasma samples from these subjects by the method described here. The result was in good agreement with earlier findings [3, 4].

REFERENCES

1 D.W. Dunwell, D. Evans, T.A. Hicks, C.H. Cashin and E.A. Kitchen, J. Med. Chem., 18 (1975) 53.
2 C.H. Cashin, W. Dawson and E.A. Kitchen, J. Pharm. Pharmacol., 29 (1977) 330.
3 D.H. Chatfield and T.J. Woodage, J. Chromatogr., 153 (1978) 101.
4 D.H. Chatfield and J.N. Green, Xenobiotica, 8 (1978) 133.