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Note

Mass fragmentographic quantification of naproxen in human plasma

NIELS-ERIK LARSEN* and KARIN MARINELLI

Department of Clinical Chemistry, Division of Clinical Pharmacology, Glostrup Hospital, DK-2600 Glostrup (Denmark)

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There are publications on various methods for the determination of naproxen (Naprosyn®) (6-methoxy-α-methyl-2-naphthalene-acetic acid) (NPX) in human plasma, e.g. gas chromatography [1], liquid chromatography [2] and spectrofluorometry [3]. The plasma concentrations after ordinary therapeutic treatment do not cause any sensitivity problems in respect of any of the above-mentioned methods. However, naproxen is extensively bound to plasma albumin (>99%) at therapeutic levels [4, 5], and the degree of the protein binding has been demonstrated to be affected by the total plasma concentration [3]. This might influence the therapeutic effect, since it is generally assumed that only the unbound drug will be available to the receptors. Consequently, it is of clinical interest to examine the influence of total naproxen plasma concentration on the magnitude of the free fraction. Thus, a method sufficiently sensitive to quantify approximately 0.1% of the total concentration had to be developed, since neither gas chromatography nor liquid chromatography have the required sensitivity. The spectrofluorometric method [3] demonstrated sufficient sensitivity, but the method does not include a separation procedure. This can influence the specificity, since any chemical substance showing fluorescence will be co-determined. Therefore, a mass fragmentographic method was developed. This method can quantify naproxen far below any plasma concentration of unbound drug, and specificity tests revealed no interference with ordinary, simultaneously administered drugs.

EXPERIMENTAL

Reagents and glassware

Toluene, analytical grade from E. Merck (Darmstadt, G.F.R.) was distilled once before use. N,O-Bis(trimethylsilyl)acetamide (BSA) of a particularly

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purified grade from Pierce (Rockford, IL, U.S.A.) was used for silylation of the compounds. The glassware was cleaned with 0.1 *N* sulphuric acid in an ultrasonic bath for half an hour and rinsed with distilled water twice.

Extraction procedure

To a centrifuge tube containing 1 ml of plasma, 200 μl of 10% hydrochloric acid were added. The internal standard, trideuterium labelled NPX (NPX-D_3) (60 μg) was added, and the compounds were extracted with 6 ml of toluene by mixing for 5 min in a rotary mixer (20 rpm). After centrifugation for 5 min, the organic phase was transferred to a 10-ml glass stoppered tube containing 1 ml of distilled water and 150 μl of 6.6 *N* sodium hydroxide. The compounds were extracted into the aqueous phase by mixing for 5 min. After centrifugation, the organic phase was discarded. By adding 600 μl of 10% hydrochloric acid and 3 ml of toluene to the aqueous phase, the compounds were extracted into the organic phase, by mixing for 5 min in a rotary mixer. The organic phase was, after centrifugation, transferred to an ethanol-moistened tapered tube and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 μl of toluene containing 3% (v/v) BSA and was derivatized for 15 min at 40°C. An amount of 2 μl was injected into the combined gas chromatograph—mass spectrometer.

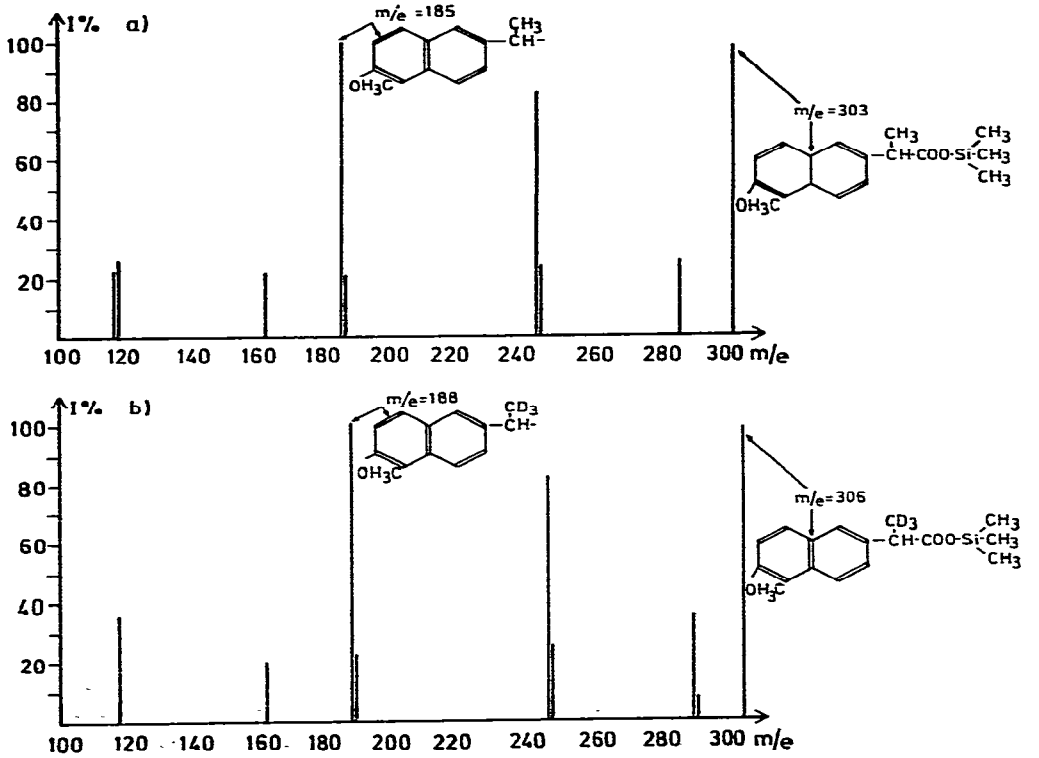

Fig. 1. Mass spectra of (a) silylated naproxen (NPX) and (b) silylated trideuterium labelled naproxen (NPX-D_3). The molecular ions for NPX and NPX-D_3 are *m/e* 303 and *m/e* 306, respectively. Fragments below *m/e* 100 and/or with intensities below 10% have been omitted.

Mass fragmentography

The instrument was a combined gas chromatograph—mass spectrometer (LKB 9000). The mass fragmentographic conditions were as follows: ionization energy, 27 eV; trap current, 60 μA; ion source temperature, 250°C; molecular separator temperature, 235°C; column temperature, 215°C. The column was glass (0.9 m × 2 mm I.D.) packed with 5% FFAP on Diatomite CLQ (100—120 mesh) (J.J.'s Chromatography, Norfolk, Great Britain). NPX and NPX-D_3 are quantified on mass fragments 303 and 306, respectively. Mass spectra of the silylated derivatives of NPX and NPX-D_3 are shown in Fig. 1a and b. Mass fragmentograms of plasma samples containing increasing amounts of NPX are shown in Fig. 2.

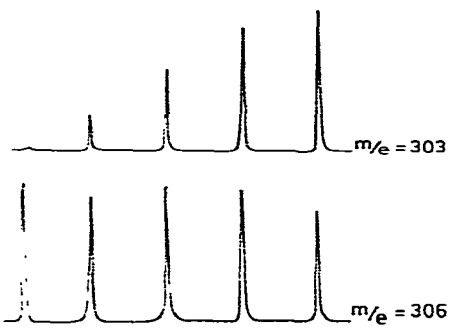

Fig. 2. Mass fragmentograms of plasma samples containing (from left to right), 0, 20, 40, 60, and 80 mg/l of naproxen.

Calculations

The plasma concentrations were read from standard curves drawn on the basis of chromatograms of plasma samples containing varying, but known amounts of naproxen giving concentrations from 20 to 80 mg/l. The peak height ratios between NPX—BSA and NPX-D_3—BSA were plotted against the concentrations. A straight line through the origin was obtained.

RESULTS AND DISCUSSION

The main reason for the development of this mass fragmentographic method was the need for an adequate method for the measurement of the unbound fraction of NPX in human plasma, i.e. concentrations of about 0.1 mg/l. The constitution formula of NPX shows the presence of a carboxylic acid group. In spite of the use of an FFAP stationary phase, which has been developed particularly for the chromatographic separation of compounds containing COOH groups, some tailing on the peaks representing NPX and the internal standard was observed. Attempts to quantify NPX in concentrations of 0.1 mg/l failed, probably owing to absorption phenomena in the glass column. However, in the case of silylation with BSA, concentrations far below 0.1 mg/l are detectable with narrow and symmetric peaks (Fig. 2). The figure illustrates mass fragmentograms of plasma samples containing NPX in varying, but known amounts. The left peak shows a plasma blank with added internal standard. The detector measures NPX on the fragment of *m/e* 303 and the internal standard on the

TABLE I

ACCURACY TEST FOR NAPROXEN AT DIFFERENT CONCENTRATION LEVELS

Concentration added	No. of samples	Observed concentration* (%)
20 μg/l	10	21.6 ± 8.4
40 μg/l	10	39.0 ± 3.8
60 μg/l	10	60.5 ± 2.1
80 μg/l	10	78.5 ± 2.1
20 mg/l	20	21.7 ± 3.6
40 mg/l	20	40.2 ± 1.8
60 mg/l	20	59.5 ± 1.9
80 mg/l	20	77.7 ± 2.1

*Mean ± C.V.

fragment of *m/e* 306. As will be seen from Fig. 2, blank plasma samples produce only a negligible signal on the fragment of *m/e* 303 because the internal standard contains small amounts of unlabelled compound. From a clinical point of view, this small deviation from zero of the peak heights of NPX and NPX-D_3 is of no significance.

Accuracy tests were performed on 120 plasma samples containing NPX in varying concentrations (Table I). The lower limit for quantification (sensitivity) was found to be below 5 μg/l in the case of a plasma volume of 1 ml. The precision of the method gave a coefficient of variation below 10% even in the lowest concentration range (20 μg/l).

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