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Note

High-performance liquid chromatographic determination of indoprofen in plasma and urine

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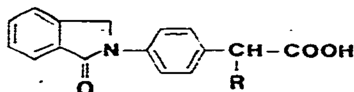
Indoprofen (I) is an isoindoline derivative which has been reported to have analgesic and anti-inflammatory activity [1, 2]. Methods for its determination in plasma [3—5] and urine [3] have been described. These methods are based on gas chromatography with flame ionisation detection and involve extraction with rather large solvent volumes and derivatisation steps. The aim with the present method was to cover the same concentration range in the biological samples as the gas chromatographic methods but with a simpler sample handling procedure and avoidance of derivatisation. In the described liquid



chromatographic method, 100 µl of plasma (or urine) samples were used to quantify indoprofen down to about 0.5 µg/ml. A homolog of indoprofen, indobufen (II), was used as internal standard.

MATERIALS AND METHODS

Standards

Indoprofen (I) and indobufen (II) were kindly donated by Pharmitalia, Carlo Erba, Milan, Italy.

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Instrumental

The liquid chromatograph consisted of an M6000 pump, a U6K injector and an M440 filter UV detector (Waters Assoc., Milford, Mass., U.S.A.). The 280 nm filter was used in the detector. A radial compression separation system (RCSS, Waters Assoc.) was used, consisting of a Radial-PAK A column, 10 cm × 1.3 cm, with octadecylsilane bonded to silica, (10 μm) and mounted in an RCM-100 radial compression module. The eluent was a mixture of 55% acetonitrile and 45% 0.01 *M* phosphate buffer (pH 3) operated at a flow-rate of 1.5 ml/min at room temperature (20—22°).

Plasma assay

To a plasma sample (100 μl) in a 10-ml screw-capped tube were added 100 μl of internal standard solution (10 μg/ml of II in methanol), 1 ml of 0.1 *M* HCl and 4 ml of diethyl ether. The extraction was carried out on a shake board for 10 min and the tube was then centrifuged for 5 min at 500 *g* (Wifug XI). The ether phase was transferred to a new tube, extracted with 2 ml of 0.5 *M* NaOH (shake board for 10 min) and centrifuged (500 *g*). The aqueous phase (ca. 1.5 ml) was then transferred to another tube to which 0.5 ml of 2 *M* HCl and 4 ml diethyl ether were added and extracted as before. The organic phase was transferred to a conical tube and the solvent evaporated under a stream of nitrogen. The residue was dissolved in 200 μl of the eluent and an aliquot (20 μl) was injected into the liquid chromatograph.

Urine assay (free indoprofen)

A 100-μl sample of urine was added to 1 ml of 0.2 *M* acetate buffer (pH 5), and from here on the same procedure was followed as in the plasma assay described above.

Urine assay (free and conjugated indoprofen)

A 100-μl volume of urine was added to 100 μl of 0.2 *M* acetate buffer (pH 5) and 1 mg of β-glucuronidase. The mixture was incubated at 37° overnight. After acidification with 1 ml of 0.1 *M* HCl the same scheme was followed as above. Since the concentrations of I in urine reach higher levels than in plasma, two calibration graphs were used. In the higher range the concentration of internal standard solution was 100 μg/ml.

RESULTS AND DISCUSSION

The extraction of indoprofen (I) from an aqueous phase (pH = 1) to diethyl ether was close to quantitative using the proportions described in the method. The same was true for the back extraction from ether into sodium hydroxide solution. On injection of a series of samples that had been extracted once with ether only, the base-line became rather unstable due to substances with long retention times. In order to reduce these interfering substances in the samples to an acceptable level, it was necessary to do an extraction into an alkaline aqueous phase from the first ether extract; after acidification this was followed by another ether extraction. This extra purification step allowed determinations of I down to about 0.5 μg/ml in both plasma and urine. Further purification steps were considered unnecessary.

When the fraction of free I in urine was determined the pH was adjusted to 5. This was done because at pH 1, but not at pH 5, the conjugate of I will be co-extracted to some extent into the ether phase. The conjugate will then be hydrolysed to I during the extraction with the sodium hydroxide solution, thus introducing an error into the determination of I. The extraction yield of I was found to be slightly lower at pH 5. It was also possible to determine I after a single extraction with ether since the conjugate does not interfere with I in the chromatographic system. However, the peak resulting from compounds slightly or not retained will be rather large, making it difficult to resolve the peak of I. Figs. 1 and 2 show typical chromatograms obtained when using the method described.

The eluent mixture used in the liquid chromatograph consisted of phosphate buffer (pH 3) and acetonitrile. At pH 7, I eluted much faster and a lower acetonitrile concentration could be used, but at this pH there was a considerable increase in background interference. In order to construct a calibration graph a series of plasma samples to which had been added 0.5—10

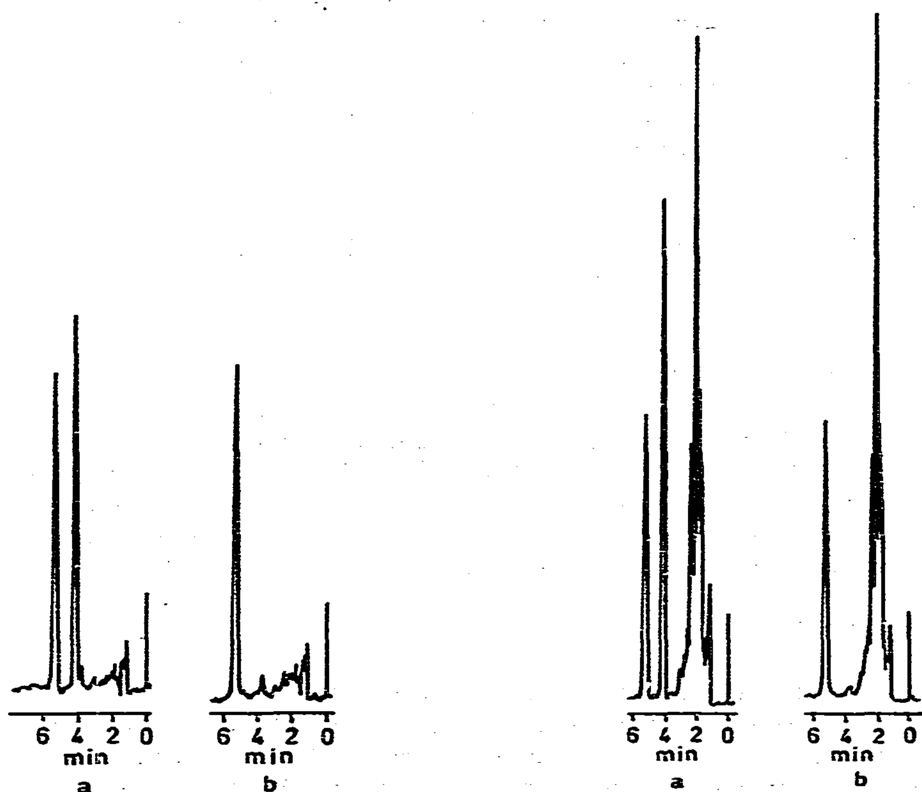

Fig. 1. (a) Chromatogram obtained on analysis of a plasma sample containing 10 μg/ml of indoprofen. The peaks at 4 min and at 5 min 15 sec correspond to indoprofen and the internal standard, respectively. (b) Chromatogram obtained on analysis of a blank plasma sample.

Fig. 2 (a) Chromatogram obtained on analysis of a urine sample containing 15 μg/ml of indoprofen. Indoprofen retention time was 4 min. Internal standard retention time was 5 min 15 sec. (b) Chromatogram obtained on analysis of a blank urine sample.

μg/ml of I were analysed according to the method described. The peak height ratios (I/II) were plotted against the concentration of I. The graph was linear and passed through the origin. The precision was determined to be 1.5% (n = 10) and 3.6% (n = 10) at the concentrations of 5 and 1 μg/ml of plasma, respectively. The reproducibility in the determination of total indoprofen in urine after enzyme hydrolysis of the conjugate was determined by repeated analysis of a sample from a patient (n = 10). This sample contained 35 μg/ml of I after hydrolysis (4 μg/ml before) and the variation was 1.9%. The absolute recovery of I from plasma and urine was 90% and 93%, respectively. The absolute recovery of I from urine, with pH 5 in the first extraction step, was 87%.

Since I is excreted to a large extent as conjugate in urine, the stability of the conjugate was investigated when exposed to the conditions of the method. To urine containing both I and the conjugate of I was added hydrochloric acid in an amount corresponding to the concentration used in the method. Samples of this urine were then analysed immediately and after 5, 10, 20 and 60 min. No significant change in the concentration of I could be detected.

Fig. 3 shows a mean plasma concentration curve of I obtained from three subjects that had been given 200 mg of indoprofen orally. The plasma samples drawn from the subjects were analysed using the described method. The plasma concentrations and $t_{1/2}$ values were in good agreement with earlier findings [3, 5].

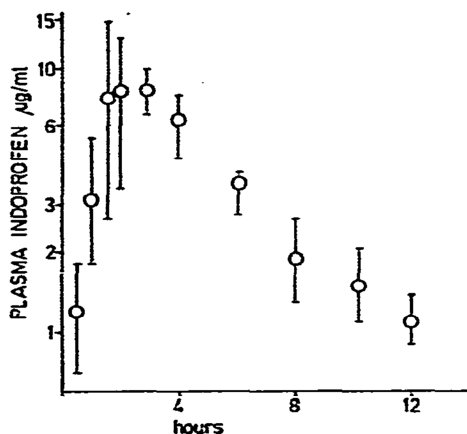

Fig. 3. Mean plasma time—concentration curve obtained from three subjects who had been given 200 mg of indoprofen orally.

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