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Note

Silica capillary gas chromatographic determination of ibuprofen in serum

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Ibuprofen (Fig. 1, I), 2-(4-isobutylphenyl)propionic acid, is a drug possessing analgesic, anti-inflammatory and antipyretic effects. Several analytical methods, including gas chromatography [1—4], have been presented for the determination of ibuprofen in serum. The method described in this paper is based on the determination of ibuprofen as the free acid on a fused-silica capillary column. Derivatization is not required. Ibufenac (Fig. 1, II) is used as the internal standard.

This procedure has been devised for rapid estimation of the drug at therapeutic levels and in cases of possible overdosage.

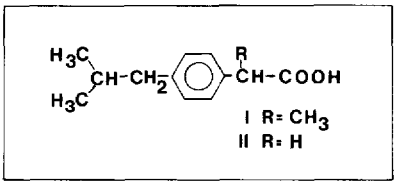

Fig. 1. Chemical structure of ibuprofen (I) and of the internal standard (II).

EXPERIMENTAL

Materials and reagents

Ibuprofen was kindly supplied by Medipolar (Oulu, Finland) and ibufenac by the Boots Company (U.K.). The identity and purity of the substances were verified by different chromatographic (thin-layer, gas) and spectrometric (ultraviolet, infrared) methods. The control serum, M + D Moni-trol IE, was obtained from Dade-Fennica Oy (Helsinki, Finland). The following reagents and stock

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solutions were used: 1 *M* hydrochloric acid (Orion, Finland), as the extraction solvent, petroleum ether (b.p. 40—60°C) (May & Baker, U.K.), and the stock solutions ibuprofen 100 μg/ml and the internal standard, ibufenac, 100 μg/ml in petroleum ether.

Gas chromatography

Gas chromatographic analyses were performed with a Fractovap 4200 gas chromatograph (Carlo Erba, Italy) equipped with a flame-ionization detector and connected to a Hewlett-Packard 3380A peak integrator. The fused-silica capillary column was OV-351 (25 m × 0.32 mm I.D.) with a film thickness of 0.20 μm (Orion Analytica, Finland). The operating temperatures were column 220°C, injection port and detector 250°C. Helium was employed as the carrier gas (156.9 kPa) and the splitting ratio was 1:20. Hydrogen (39.2 kPa) and compressed air (78.5 kPa) flow-rates were adjusted to give maximum response. Under these conditions the retention times of ibuprofen and ibufenac were 4.53 and 4.84 min, respectively. The calibration curves were calculated using a Hewlett-Packard 85 computer.

Sample preparation

First 0.30 ml (30 μg) of the internal standard solution was pipetted into each of series of tubes and evaporated to dryness with a gentle stream of nitrogen. A 1.0-ml sample of serum was added to each tube and mixed well. Samples were acidified with 0.25 ml of 1 *M* hydrochloric acid and extracted with 6.0 ml of petroleum ether by vortexing for 30 sec. The solutions were centrifuged for 5 min at 2600 *g*, after which 4.0 ml of the petroleum ether layer were transferred to a fresh tube and evaporated to dryness with a gentle stream of nitrogen. The residue was dissolved in 100 μl of petroleum ether and 0.5—1.0 μl of this solution was injected into the gas chromatograph.

A calibration curve was constructed at ibuprofen concentrations of 5.0—50.0 μg/ml control serum and the peak area ratios of I to internal standard were plotted against the amount of ibuprofen added.

RESULTS AND DISCUSSION

Gas chromatographic determination of acidic drugs in serum involves two major problems. Because of the strong polarity of these compounds they usually have to be derivatized before gas chromatographic analysis [1, 4]. Furthermore, it is often impossible to extract them from serum with sufficient recovery without extracting interfering compounds. The polar OV-351 fused-silica capillary column gives good separation of ibuprofen and ibufenac under the conditions used and permits the determination of ibuprofen as the free acid. The OV-351 column is specially designed for the determination of free fatty acids and phenols. Fig. 2 shows gas chromatograms of the serum extracts. Clearly the extraneous peaks do not interfere with the peaks of I and II.

The precision of the method is demonstrated in Table I. Results are based on at least five determinations of each ibuprofen concentration, ranging from 5.0 to 50.0 μg/ml, which were treated as described in the experimental part. The calibration curves are linear over the range 5.0—50.0 μg/ml and can be expressed

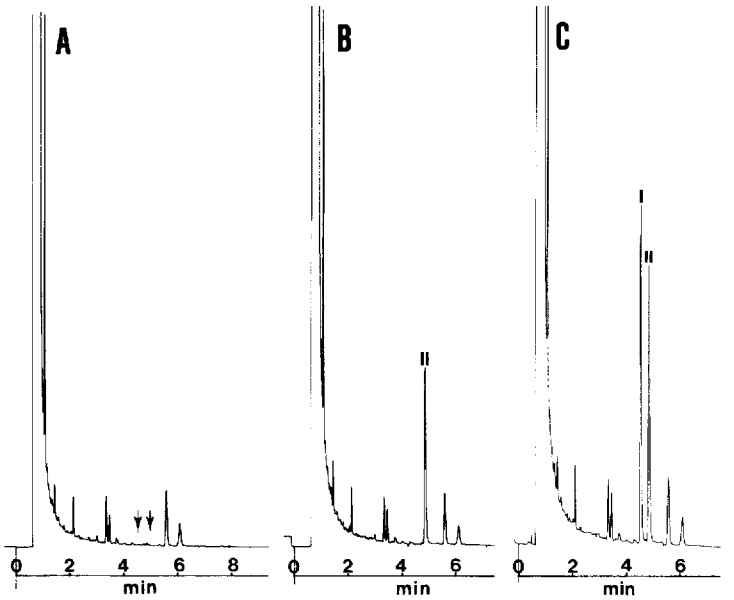

Fig. 2. Chromatograms of serum extracts. (A) Serum blank; (B) serum blank containing internal standard (II); (C) serum containing 25 μg/ml ibuprofen and 30 μg/ml ibufenac. Peaks: I = ibuprofen; II = ibufenac (internal standard).

TABLE I

PRECISION IN MEASUREMENT OF IBUPROFEN (I) ADDED TO SERUM

$Y = 0.037X + 0.019$, $r = 0.999$.

Amount of I added (μg/ml)	n	Mean peak area ratio	S.D.	R.S.D. ± %
5.0	6	0.189	0.004	2.12
10.0	5	0.416	0.008	1.92
20.0	6	0.781	0.013	1.66
30.0	6	1.063	0.016	1.50
40.0	5	1.506	0.032	2.12
50.0	6	1.880	0.025	1.33

TABLE II

RECOVERY OF IBUPROFEN (I) AND IBUFENAC (II) FROM SERUM

Recovery of I and II from serum was studied by adding 25 μg of I or II to 1.0 ml of blank serum, by extracting and adding the internal standard at the end. The reference samples were prepared by first extracting blank serum and then by adding 25 μg of I or II and the internal standard at the end. The peak area ratios of the reference samples were designated as the 100% value.

Amount added (μg/ml)	Recovery*		R.S.D. ± %
	μg	%	
I: 25.0	16.9	67.6	4.2
II: 25.0	13.5	53.9	3.1

*Each result is the mean of six determinations.

by the equations $Y = 0.036X + 0.008$, $r = 0.999$ for pure substance, and $Y = 0.037X + 0.019$, $r = 0.999$ for the spiked serum specimens.

The accuracy of the method was studied by analysing six samples, each containing an equal amount of ibuprofen (25 μg/ml) under the experimental conditions described above. The accuracy of the method was 98.1% for the spiked serum specimens, with a relative standard deviation of 1.1%.

The results of the recovery studies are presented in Table II. The recovery of ibuprofen is only about 70%, but the method gives very good precision (1.33—2.12%) and accuracy (98.1%). The extraction solvent, isopropanol—dichloromethane (1:20, v/v), gives slightly better recovery, but it extracts interfering compounds from serum. Petroleum ether is better for routine analysis because the layer of petroleum ether is the upper phase, which makes the analysis quicker to perform in practice. Speed and simplicity of the sample preparation methods are great benefits in routine clinical chemistry work.

Use of the fused-silica capillary column makes it possible to determine ibuprofen as the free acid without derivatization. The total procedure is simple and specific and allows rapid estimation of ibuprofen at therapeutic and overdose levels.

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