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Note

Simple and rapid method for determining procetofenic acid, an active metabolite of procetofen, in biological fluids by solid-phase extraction and high-performance liquid chromatography

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Procetofen is a widely used hypolipidaemic agent, more effective than clofibrate in lowering plasma levels of cholesterol and triglycerides. The active compound is procetofenic acid, the major metabolite of procetofen [1–4]. Several analytical methods for the determination of procetofenic acid have been reported, including gas chromatography and high-performance liquid chromatography (HPLC) [5–10]. However, almost all previously published methods require the extraction of the procetofenic acid from plasma or urine by an organic solvent, the evaporation to dryness of the dehydrated organic phase under a stream of nitrogen and the reconstitution of the residue prior to analysis. Sample preparation is tedious and time-consuming and requires many steps, with possible losses or contamination of the analyte.

The method described here is based on a solid-phase extraction of drug from human plasma or urine prior to HPLC analysis and it is rapid, simple and reproducible. It requires very little organic solvent and equipment, and sample preparation takes ca. 15 min per batch of ten. Furthermore, the selective elution procedure yields a final eluate with fewer background interferences than are produced by liquid–liquid extraction. This factor could improve the accuracy and precision of analysis.

EXPERIMENTAL

Chemicals and reagents

Procetofenic acid and naproxen were available in-house, (Selvi 3M, Milan, Italy). The methanol and acetonitrile were HPLC grade (Merck, Darmstadt,

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F.R.G.); the hydrochloric acid, phosphoric acid, potassium dihydrogen phosphate were analytical grade (Merck). Water was a HPLC-grade ultrapure water (conductivity > 16 mΩ) generated by a water purification system (Millipore, Milford, MA, U.S.A.), consisting of a 1-μm particulate filter, one organic filter and two ion-exchange filters connected in series.

Apparatus

The following instruments were used: Mettler Model AC 100 analytical balance (Mettler Instruments, Greifensee, Switzerland); Bransonic 32 ultrasonic bath (Branson Europe, Soest, The Netherlands); Waters liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) equipped with an M45 solvent delivery system, Model U6K injector, Model 440 absorbance detector, Model 730 data module and RCM 100 compression module; Milli-Q reagent-grade water system (Millipore); Chrompack vacuum device (Chrompack, Middelburg, The Netherlands). A reversed-phase Radial-Pak C_8 cartridge, 10 μm, 10 cm $\times$ 8 mm radially compressed in the RCM-100 compression module (Waters) was used with a guard column packed with Bondapak C_{18}/Corasil (Waters).

Extraction method

Sample preparation. To 1 ml of biological sample (plasma or urine) were added the internal standard (5 μg/ml in methanolic solution), 0.5 ml of distilled water and 1 *M* hydrochloric acid to pH 1 (0.25 ml).

Extraction column activation. Analytichem C_8 (200 mg) columns (Analytichem, Harbor City, CA, U.S.A.) were inserted into the vacuum device (the extraction columns were drained under ca. 400 mmHg), washed twice with methanol and twice with distilled water. It is important not to let columns dry out.

Extraction. With the vacuum disconnected, each sample, prepared as above, was pipetted onto an activated extraction column. Then, with the vacuum turned on, samples were drawn through and washed twice with distilled water. Procetofenic acid and naproxen were retained on the column. With the vacuum turned off and sample collection tubes (3-ml LP polypropylene tubes) (LP Italiana, Milan, Italy) placed under the tip of the columns, 0.5 ml of acetonitrile were added; after 1 min, it was drawn through the column under vacuum. For procetofenic acid levels below 0.5 μg/ml, reduction of the eluate volume was necessary. The acetonitrile eluent was reduced to ca. 150 μl under vacuum, and 100 μl were injected.

Chromatographic conditions

The mobile phase was acetonitrile–phosphate buffer (35:65) and the flow-rate was 2.5 ml/min. Detection was performed at 254 nm. Phosphate buffer was prepared by dissolving 2.72 g potassium dihydrogen phosphate in 1 l of distilled water acidified with phosphoric acid to pH 3.

RESULTS

Figs. 1 and 2 show typical chromatograms obtained from human plasma and urine samples, blank and spiked with procetofenic acid and naproxen. As can be

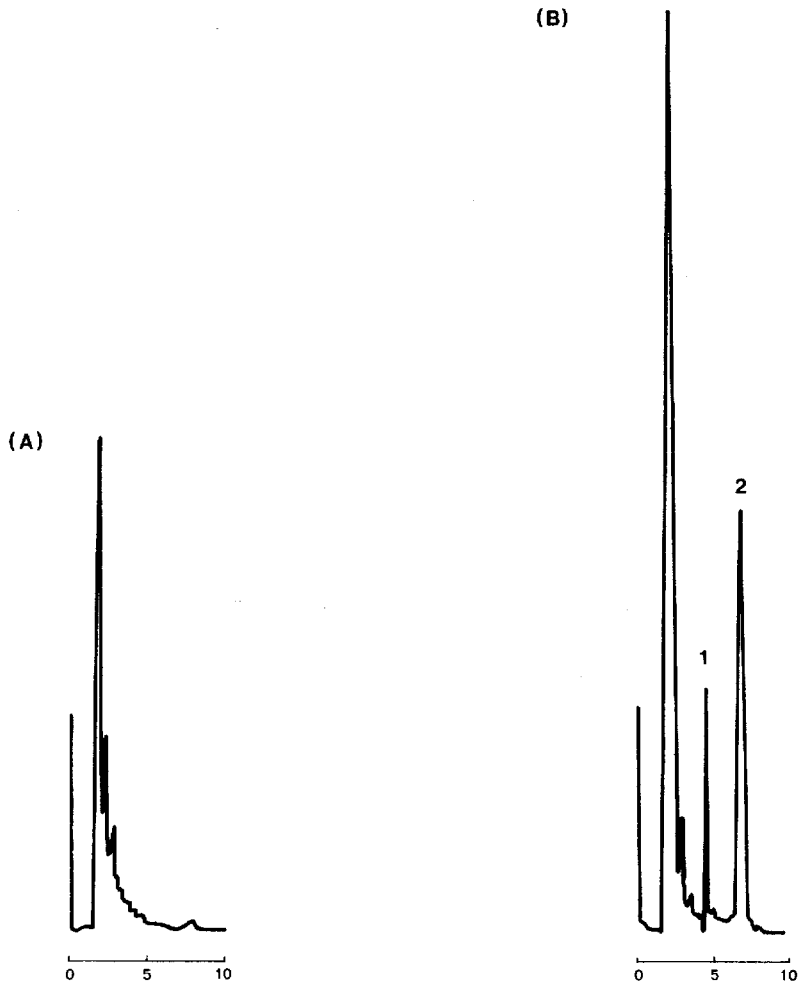

Fig. 1. Chromatograms of plasma samples. (A) Blank human plasma; (B) plasma from a patient 4 h after a single dose of 200 mg of procetofen. Peaks: 1 = naproxen; 2 = procetofenic acid.

seen, there are no chromatographic interferences with the two standards. The linearity range is 0.5–10 μg/ml for both plasma and urine samples. The plasma calibration curve was: $y = 0.2207x + 0.0065$ (y = peak-height ratio of procetofenic acid to internal standard; x = procetofenic acid concentration expressed in μg/ml). The correlation coefficient (r) was 0.9996. The urine calibration curve was: $y = 0.2024x - 0.0032$ ($r = 0.9999$). Precision and accuracy were determined by analysing replicate plasma samples containing 0.5, 1, 2.5, 5, 7.5 or 10 μg/ml of procetofenic acid. The precision was expressed as the coefficient of variation (C.V.) and the accuracy as relative error. The C.V. was $< 3\%$ and the relative error was between -0.4% to 1.5%. The results are tabulated in Table I. Similar results were obtained from human urine samples and are tabulated in Table II. The extraction efficiency of the procedure was tested by performing recovery

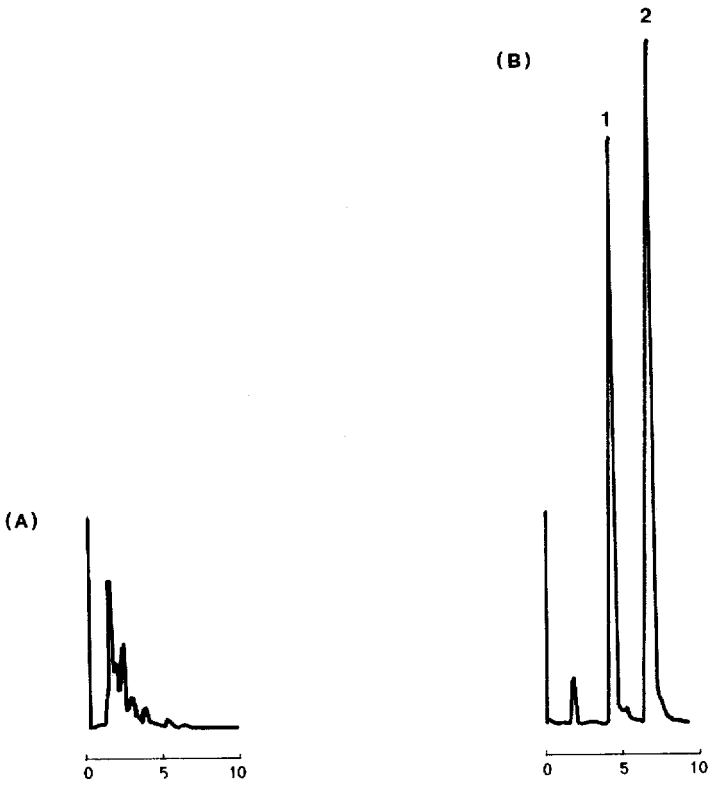

Fig. 2. Chromatograms of urine samples. (A) Blank human urine; (B) human urine spiked with procetofenic acid and internal standard naproxen. Peaks: 1 = naproxen; 2 = procetofenic acid.

experiments on drug-free plasma and urine to which procetofenic acid had been added.

The mean recoveries were 70% for plasma and 95% for urine. During the method development, many organic solvents and saline buffers for washing plasma samples were examined. No improvement was observed following a flush with one or two volumes (1 ml) of distilled water. The method has been validated by com-

TABLE I

PRECISION AND ACCURACY (INTRA-DAY) IN HUMAN PLASMA ($n=6$)

Sample concentration (μg/ml)	Coefficient of variation (%)	Relative error (%)
0.5	2.01	−0.40
1	1.97	+1.53
2.5	1.98	+1.20
5	1.29	+1.16
7.5	3.05	+0.67
10	1.57	+1.60

TABLE II

PRECISION AND ACCURACY (INTRA-DAY) IN HUMAN URINE ($n=6$)

Sample concentration (μg/ml)	Coefficient of variation (%)	Relative error (%)
1	1.84	+0.50
2.5	1.04	−0.90
5	1.94	−1.26
7.5	0.53	−0.27
10	1.20	−0.30

TABLE III

COMPARISON OF PLASMA LEVELS OF PROCETOFENIC ACID IN PATIENTS

A single oral dose of 200 mg of procetofen was administered at time zero. Values under A are obtained with the new procedure, those under B with an established procedure [9].

Time (h)	Level (μg/ml)					
	B.N. (female)		G.R. (Male)		L.F. (male)	
	A	B	A	B	A	B
0	-	-	-	-	-	-
1	3.20	3.05	0.29	0.30	1.44	1.50
2	6.40	6.29	2.50	2.40	5.28	5.15
4	5.75	5.65	3.74	3.70	5.66	5.50
6	5.10	4.95	3.93	4.10	5.12	5.00
9	3.80	3.85	2.86	2.80	3.31	3.40
12	3.80	3.80	2.40	2.35	3.00	3.00
24	1.65	1.45	0.78	0.70	1.43	1.35
48	0.50	0.40	0.31	0.25	0.64	0.60
72	0.19	0.22	0.15	0.18	0.27	0.30

paring procetofenic acid levels in the plasma of three patients obtained by this new procedure and a published one (Table III) [9].

CONCLUSIONS

A new method for the quantitation of procetofenic acid in biological fluids based on a solid-phase extraction by disposable reversed-phase columns and HPLC analysis has been developed. The method is simple, rapid and accurate. Data obtained with this new procedure and a well documented liquid–liquid extraction method [9] showed good correlation.

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