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Note

Determination of ciprofibrate in human plasma by high-performance liquid chromatography

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Ciprofibrate, 2-[4-(2,2-dichlorocyclopropyl)-phenoxy]-2-methylpropanoic acid, is an orally active hypolipidemic agent [1—3] which is currently the subject of clinical trials to evaluate its safety and efficacy in humans. The absorption and disposition of ciprofibrate in laboratory animals and human volunteers have been reported [4] and the relationship between the blood levels of ciprofibrate in rats and the duration of its hypolipidemic activity was studied [5].

A gas chromatographic (GC) technique for the determination of ciprofibrate in plasma, after conversion to the butyl ester, has been published [4] and was linear over the range of 1—60 μg/ml. This report describes a high-performance liquid chromatographic (HPLC) method for the quantitative analysis of ciprofibrate in human plasma, which is faster and less complicated than the published procedure.

EXPERIMENTAL

Materials

Ciprofibrate and the internal standard, 2-[4-(2,2-dichloro-3-phenylcyclopropyl)-phenoxy]-2-methylpropanoic acid, were synthesized at Sterling-Winthrop Research Institute. Chromatographic grade acetonitrile (Omni-Solv, MCB Manufacturing Chemists, Cincinnati, OH, U.S.A.) and *n*-hexane (Nanograde, Mallinckrodt, St. Louis, MO, U.S.A.) were purchased. All other chemicals were reagent grade and used without further purification.

The high-performance liquid chromatographic apparatus was a modular system consisting of an automatic sample injector, Model 710 WISP from

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Waters Assoc. (Milford, MA, U.S.A.), a reciprocating pump (Minipump, Laboratory Data Control, Riviera Beach, FL, U.S.A.), a C2-phenyl (10 μm) 30 cm × 3.9 mm I.D., fatty acid analysis column, a pre-column 25 mm × 3.9 mm, guard column packed with phenyl/corasil (37—50 μm, Bondapak), all from Waters Assoc. and a variable-wavelength UV detector (Schoeffel Model 770, Kratos, Westwood, NJ, U.S.A.). The signal from the detector went to an automated data system (LAS 3354, Hewlett-Packard, Palo Alto, CA, U.S.A.) which estimated both peak heights and peak height ratios (ciprofibrate:internal standard).

Preparation of standards and samples

Plasma standards were prepared by supplementing 1.0 ml of control human plasma (containing potassium oxalate as the anticoagulant) with aliquots of a solution of ciprofibrate in ethyl acetate. Duplicate standards at ciprofibrate concentrations of 0, 0.5, 1, 2, 5, 10, 20, 30 and 40 μg/ml were prepared.

Two sets of randomized and coded plasma samples, to be analyzed under single-blind conditions, were prepared as described above. Each set contained triplicate samples at final concentrations of 0, 3, 8, 15, 25 and 35 μg of ciprofibrate per ml of plasma. One set of samples was analyzed upon preparation. The second set was stored in the laboratory freezer for seven days before analysis.

Analytical procedure

To a tube containing 1.0 ml of human plasma, were added 50 μl of internal standard solution (0.1 mg/ml in ethyl acetate), 2 ml of 1 *N* hydrochloric acid and 0.2 ml of 60% perchloric acid. The mixture was extracted twice with 10 ml of hexane. The organic phases were combined and evaporated to dryness under a stream of nitrogen in a warm water bath. The residue was dissolved in 2 ml of acetonitrile. Hexane, 2 ml, was added and the tube was shaken vigorously. The hexane phase was discarded and the remaining acetonitrile was evaporated to dryness, as described above. The residue was dissolved in 0.5 ml of acetonitrile—tetrahydrofuran (10 : 1, v/v), and 0.5 ml of 0.1 *M* K_2HPO_4 buffer, pH 4, was added. The mixture was vigorously shaken and a 100-μl aliquot was injected into the liquid chromatograph for analysis.

The chromatographic analysis was performed isocratically at ambient temperature with a mobile phase of tetrahydrofuran—0.1 *M* K_2HPO_4 buffer, pH 4.0—acetonitrile (10 : 104 : 96, v/v/v), and a flow-rate of 2 ml/min. Ciprofibrate and the internal standard were eluted in approximately 4 and 6 min, respectively. The column effluent was monitored at 232 nm.

Extraction efficiency

The percent recovery of the extraction procedure for ciprofibrate was determined at four concentrations, ranging from 2 to 40 μg/ml, by comparing the peak heights of ciprofibrate obtained from extracted plasma samples with those obtained by injection of unextracted solutions. A similar procedure was used for the internal standard.

Statistical analysis

Several statistical tests were applied to the analytical data. A regression analysis of the peak height ratios (ciprofibrate:internal standard) obtained for the standards was performed to determine the linearity of the response with respect to concentration. The resulting linear regression was used to estimate the concentrations of ciprofibrate in the prepared samples. The minimum quantifiable level (MQL) of the assay was estimated as the concentration whose lower 80% confidence limit just encompassed zero [6]. The assayed levels for the prepared samples were expressed as percent differences from the nominal values and analyzed by a two-way analysis of variance with replication to test for a concentration effect, a time (fresh vs. frozen) effect, and a concentration X time effect. The percent recovery data were subjected to a one-way analysis of variance to determine if the percent recovery was a function of concentration.

RESULTS AND DISCUSSION

Representative chromatograms of an extracted plasma blank and an extracted 2 μg/ml plasma standard are shown in Figs. 1A and B, respectively (for comparison with GC, see Fig. 1C). Regression analysis on the standards indicated a linear relationship between peak height ratio and concentration over the range 0—40 μg/ml. Typically, the slope was 0.228, and the *y*-intercept was 0.0164 μg/ml.

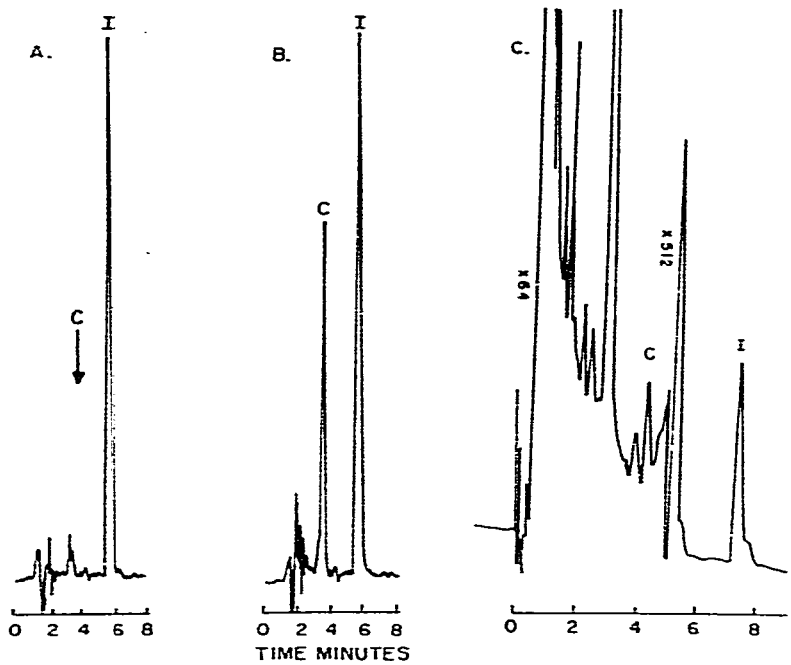

Fig. 1. Representative liquid chromatograms (see text for experimental conditions) of extracted plasma standards containing (A) 0 μg/ml, (B) 2 μg/ml. (C) Gas chromatogram of 2 μg/ml extracted plasma standard (see ref. 4 for experimental conditions). Peaks: C, ciprofibrate; I, internal standard.

TABLE I

RESULTS OF THE ANALYSIS OF PREPARED HUMAN PLASMA SAMPLES

Concentration added (μg/ml)	Concentration found, fresh* (μg/ml)	Concentration found, frozen** (μg/ml)
0	<MQL***	<MQL§
	<MQL	<MQL
	<MQL	<MQL
3	3.0	3.0
	2.8	3.0
	3.1	3.0
Mean	3.0	3.0
S.E.M. (%)	3.0	0.0
Mean percent difference	—1.1	0.0
8	7.9	8.4
	7.9	8.6
	7.8	9.0
Mean	7.9	8.7
S.E.M. (%)	0.4	2.0
Mean percent difference	—1.7	8.3
15	15.0	13.8
	14.4	13.4
	14.3	14.0
Mean	14.6	13.7
S.E.M. (%)	1.5	1.3
Mean percent difference	—2.9	—8.4
25	23.4	24.6
	27.3	24.9
	24.5	25.1
Mean	25.1	24.9
S.E.M. (%)	4.6	0.6
Mean percent difference	0.3	—0.5
35	36.4	35.4
	33.4	36.7
	34.5	35.3
Mean	34.8	35.8
S.E.M. (%)	2.5	1.2
Mean percent difference	—0.7	2.3

*Analyzed upon preparation.
**Analyzed after 7 days of storage in a laboratory freezer.
***MQL = 0.65 μg/ml.
§MQL = 0.73 μg/ml.

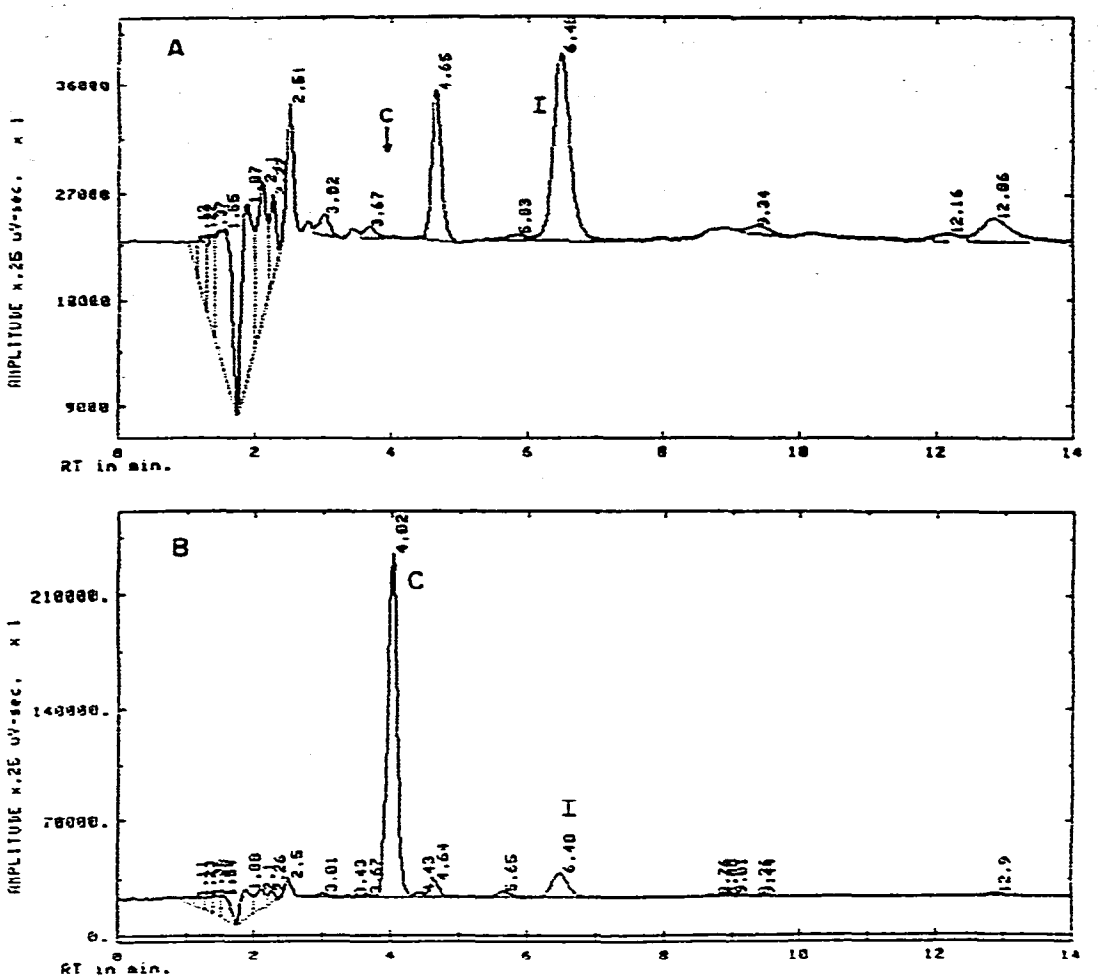

Fig. 2. Computer-reconstructed chromatograms for plasma obtained from rats after 1 year of medication. (A) Control, (B) ciprofibrate dosage = 10 mg/kg/day. See text for experimental conditions.

The concentrations of ciprofibrate in the prepared plasma samples, estimated by inverse prediction from the regression equation, are summarized in Table I. The two-way analysis of variance of the nominal values for the plasma samples indicated no significant sources (concentration, time or concentration × time) of variation at $p < 0.01$. An overall estimate of assay precision, based on the variance of the repeat determinations within each concentration level was 7.0%. The accuracy of the assay, defined by the ranges of the mean percent differences from the nominal concentration values, varied from −8.4% to +8.3%. The mean MQL was 0.69 μg/ml ($n = 2$).

The extraction efficiency for ciprofibrate was independent of the ciprofibrate concentration. The mean recoveries of ciprofibrate (± S.E.) and the internal standard were 94.8±3.2 and 86.1±7.2%, respectively.

The assay described in this report has been useful for the determination of ciprofibrate in human plasma, in the concentration range 0.69—40 μg/ml.

Furthermore, this method has been applicable in the determination of the concentration of ciprofibrate in the plasma of laboratory animals (Fig. 2). The precision and accuracy of the HPLC method are comparable to those reported in the GC method [4]; the major advantages of the former method are its speed and simplicity.

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