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Note

Liquid chromatographic determination of gemfibrozil and its metabolite in plasma

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Gemfibrozil (Lopid®, Parke-Davis), 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid, is a recently marketed lipid-regulating agent [1, 2] undergoing clinical and product line extension studies. We recently reported a gas chromatographic (GC) method to determine gemfibrozil and its metabolites in plasma and urine [3]. A liquid chromatographic (LC) method has been reported recently [4]. An LC method for determining gemfibrozil and its major metabolite was developed and validated in these laboratories [5] and is described in detail in this report.

The method involves one-step extraction before chromatography. No derivatization steps are required. Plasma concentrations of the major metabolite, 3-[(4-carboxy-4-methylpentyl)oxy]-4-methylbenzoic acid, may be determined on the same chromatogram. Modifications are required to assay conjugated gemfibrozil and metabolite. The method can be adapted for whole blood and urine analysis of conjugated as well as unchanged gemfibrozil and metabolite, and is currently used in bioavailability studies. Some typical results are presented to illustrate the applicability of the method.

EXPERIMENTAL

Materials

Acetic, hydrochloric, and phosphoric acids were analytical-reagent grade. Diethyl ether was absolute-reagent ACS grade. Glucuronidase—sulfatase (Glusulase, 90 000 U glucuronidase and 10 000 U sulfatase per ml, Dupont Pharmaceutical, Wilmington, DE, U.S.A.) was also used. Structures of

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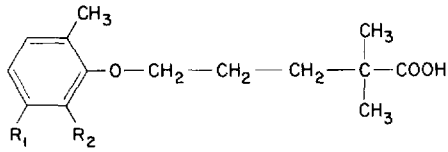

	R_1	R_2
Gemfibrozil (I)	$-CH_3$	$-H$
Metabolite (II)	$-COOH$	$-H$
Internal Standard (III)	$-H$	$-CH_3$

Fig. 1. Structures of gemfibrozil (I), metabolite (II), and internal standard (III).

gemfibrozil (I), metabolite (II), and internal standard 2,2′-dimethyl-5-(2,6-xylyloxy)valeric acid (III), which were synthesized in the Warner-Lambert/Parke-Davis Pharmaceutical Research Labs. (Ann Arbor, MI, U.S.A.), are presented in Fig. 1. Stock solutions of gemfibrozil and compound II (100 μg/ml) were prepared in 5% acetonitrile. Internal standard stock solution (200 μg/ml) was prepared in 5% acetonitrile. Standard curves were prepared by adding 0.005, 0.01, 0.015, 0.02, 0.05, 0.1, 0.2, and 0.3 ml of stock solutions of both gemfibrozil and compound II to 1 ml of blank human plasma.

Extraction

To assay plasma or standards for gemfibrozil and compound II, 0.25 ml of internal standard (200 μg/ml in 5% acetonitrile), 1 ml of 1 *M* hydrochloric acid, and 10 ml of diethyl ether are added to 1 ml of sample plasma or standard. This mixture is shaken on a reciprocating shaker for 15 min, centrifuged at 700 *g*, and the aqueous phase discarded. The ether layer is transferred to a clean tube and evaporated to dryness in a 55°C water bath with the aid of a current of air. The residue is dissolved in 0.5 ml of mobile phase, mixed, and 40 μl are injected on column.

To assay for total (unchanged plus glucuronide) gemfibrozil and compound II, the glucuronides in 1 ml of plasma (or urine) are hydrolyzed with 25 μl of glucuronidase–sulfatase, 1 ml of water, and 1 ml of 2 *M* acetate buffer at pH 5.2. After overnight incubation at 37°C, 2 ml of 1 *M* hydrochloric acid are added and the mixture is treated in the same manner as described for gemfibrozil.

Apparatus

Mobile phase, consisting of acetonitrile—0.007 *M* phosphoric acid (45:55), was pumped through the column at 2 ml/min using a Waters M-45 pump. Detection was at 276 nm using a Waters Lambda-Max Model 480 spectrophotometer. The column was a 10 cm × 4.6 mm I.D. Whatman Partisil 5-μm ODS-3 RAC II column held at room temperature. Injections of 40 μl were made with a Waters WISP automatic sample processor. A Hewlett-Packard reporting integrator, set to calculate peak-height ratios, was used to record the chromatograms.

RESULTS AND DISCUSSION

Typical chromatograms for plasma extracts are shown in Fig. 2. The reten-

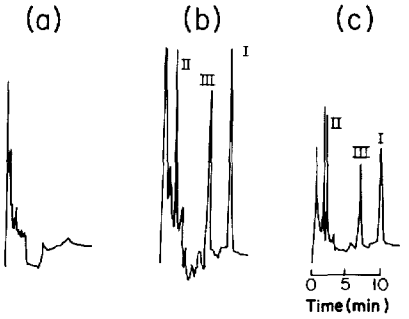

Fig. 2. Typical chromatograms of plasma extracts. (a) Blank plasma; (b) spiked plasma, 2.0 μg/ml; (c) plasma sample 6 h after a 300-mg oral dose of gemfibrozil. Peaks: I = gemfibrozil; II = metabolite; III = internal standard.

tion times for compound II, internal standard, and gemfibrozil were 2.5, 7.4, and 10.4 min, respectively. The internal standard used for LC was different than that used for the GC method [3]. The GC internal standard has a longer retention time in the LC assay resulting in poor precision of the assay of compound II. Precisions of gemfibrozil assays were equivalent using either internal standard. Chromatograms from the assay for total gemfibrozil in whole blood and plasma contained many endogenous peaks at earlier retention times and made quantitation of total II difficult in many samples.

TABLE I

PRECISION STUDIES

Values represent back-calculated concentrations of gemfibrozil and metabolite. Values in parentheses are relative standard deviations (%).

Added (μg/ml)	Found (μg/ml)			
	Day 1 (n = 3)	Day 2 (n = 3)	Day 3 (n = 3)	Overall (n = 9)
Gemfibrozil				
0	0	0	0	0
0.5	0.56 (17.2)	0.47 (0.0)	0.43 (4.8)	0.49 (15.4)
1.0	1.05 (4.4)	0.91 (3.4)	0.89 (3.6)	0.95 (8.6)
1.5	1.58 (7.7)	1.33 (5.3)	1.41 (6.6)	1.44 (9.7)
2.0	2.06 (3.7)	1.78 (0.9)	1.80 (2.2)	1.88 (7.5)
5.0	4.91 (8.2)	4.68 (5.7)	4.64 (3.5)	4.74 (6.0)
10.0	9.47 (2.0)	9.45 (1.3)	9.54 (2.2)	9.49 (1.7)
20.0	20.35 (1.5)	20.51 (5.5)	19.74 (4.6)	20.21 (4.1)
30.0	29.40 (3.9)	30.91 (2.6)	31.68 (3.2)	30.66 (4.0)
Metabolite				
0	0	0	0	0
0.5	0.57 (10.2)	0.53 (8.2)	0.65 (21.3)	0.57 (16.4)
1.0	0.97 (0.6)	0.98 (9.7)	1.08 (5.6)	1.02 (7.8)
1.5	1.44 (1.8)	1.45 (3.6)	1.56 (0.4)	1.48 (4.3)
2.0	1.90 (2.0)	1.79 (8.8)	1.83 (2.6)	1.84 (5.2)
5.0	4.99 (5.5)	4.92 (3.5)	4.83 (4.3)	4.91 (4.2)
10.0	10.40 (2.5)	9.81 (1.0)	9.72 (3.0)	9.98 (3.8)
20.0	20.16 (2.6)	22.29 (0.9)	20.76 (3.3)	21.07 (5.0)
30.0	29.76 (5.0)	28.57 (2.5)	28.61 (3.0)	28.98 (3.8)

Extraction recovery of both gemfibrozil and compound II from plasma was approximately 98% as compared to non-extracted standards, over the range of concentrations studied. The minimum quantifiable concentration was 0.5 μg/ml of plasma for both gemfibrozil and II with relative standard deviations (R.S.D.) of 12.4 and 16.4%, respectively. Standard curves were linear in the concentration range 0.5—30 μg/ml. Results of the precision study over a three-day period are presented in Table I. Precision was assessed on a within-day and an overall basis using relative standard deviations. R.S.D. was less than 10% in both measures, for both compounds in the concentration range 1—30 μg/ml, and the method was considered valid.

The method has been used in bioavailability and pharmacokinetic studies of gemfibrozil. Complete details of a bioavailability study will be reported elsewhere [6]. Mean plasma concentrations of both gemfibrozil and compound II following a single 300-mg capsule of gemfibrozil are presented in Fig. 3. Gemfibrozil follows mono-expenential elimination kinetics with a half-life of approximately 1.6 h. Plasma concentrations of II reach a plateau in about 3 h and remain relatively constant to at least 12 h.

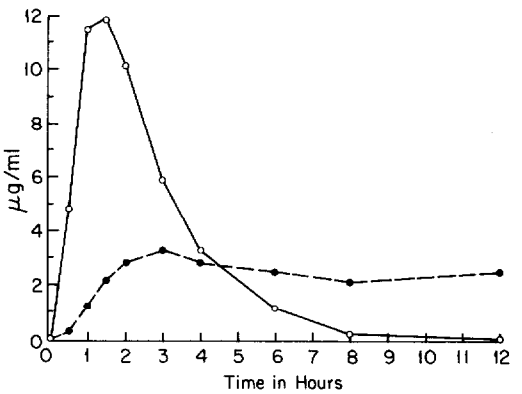

Fig. 3. Mean plasma concentration—time profile for gemfibrozil and metabolite following a 300-mg dose of gemfibrozil in twelve subjects. (○) Gemfibrozil; (●) metabolite.

CONCLUSION

A simple LC method to determine gemfibrozil and its major metabolite in plasma has been developed and validated. The method is reproducible (R.S.D. < 10%) with a minimum quantifiable concentration of 0.5 μg/ml for both gemfibrozil and metabolite. R.S.D. values at this level are 12.4 and 16.4%, respectively. The method is currently employed in these laboratories for the analysis of samples from clinical and biopharmaceutical studies, an example of which is presented to demonstrate its applicability. The method is easily adapted to the automated assay of plasma, whole blood, or urine for unchanged and/or total gemfibrozil and metabolite.

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