Journal of Chromatography, 307 (1984) 210—215
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2019

Note

Gas chromatographic determination of gemfibrozil and its metabolites in plasma and urine

EDWARD J. RANDINITIS*, A.W. KINKEL, C. NELSON and T.D. PARKER, III

Pharmacokinetics/Drug Metabolism Department, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105 (U.S.A.)

(First received September 22nd, 1983; revised manuscript received November 25th, 1983)

Gemfibrozil, (Lopid®, Parke-Davis), 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid, is a recently marketed lipid regulating agent [1—4]. Its metabolism has been studied using radiolabelled material as described by Okerholm et al. [5]. A gas chromatographic (GC) procedure has been described and a metabolism scheme has been proposed [6]. The major metabolite has been shown to be metabolite III, the benzoic acid metabolite. The bioavailability of the capsule has been reported by Smith [7] but no method of assay was described in this report. Prior to its marketing several pharmacokinetic and biopharmaceutic studies were conducted to determine the optimum dose and dosage form. In order to carry out such studies a GC method [8] was developed to monitor plasma concentrations of gemfibrozil and urinary excretion of unchanged gemfibrozil as well as its major metabolite, and their glucuronides. The previously reported method used a N,O-bis(trimethylsilyl)acetamide (BSA) derivative which was unstable in our hands.

This report describes the GC method for the determination of gemfibrozil and its major metabolite, 3-[(4-carboxy-4-methylpentyl)oxy]-4-methylbenzoic acid from plasma, as well as gemfibrozil, and their glucuronides from plasma and urine. The method involves in situ derivatization by methylation with trimethylanilinium hydroxide in methanol (TMAH) prior to its GC analysis. The method has been employed in several pharmacokinetic studies and some typical results will be reported to demonstrate the applicability of the method.

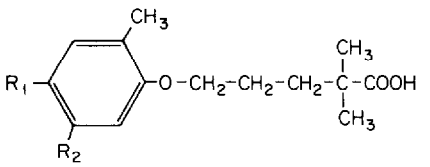
EXPERIMENTAL

Reagents

Acetic acid, hydrochloric acid, and sodium hydroxide were analytical rea-

0378-4347/84/$03.00

gent grade. Diethyl ether and chloroform were absolute reagent ACS grade. Chloroform was freshly redistilled in glass prior to its use. Glucuronidase-sulfatase (Glusulase, Endo Labs., Garden City, NY, U.S.A.) and TMAH (0.2 *M* trimethylanilinium hydroxide in methanol, Supelco, Bellefonte, PA, U.S.A.) were also used. Gemfibrozil (I), metabolite III (II), and internal standard (III) were synthesized in the Warner-Lambert/Parke-Davis Pharmaceutical Research Labs. (Ann Arbor, MI, U.S.A.). The structures of gemfibrozil, metabolite III, and internal standard are presented in Fig. 1.



	R_1	R_2
GEMFIBROZIL	$-H$	$-CH_3$
METABOLITE III	$-H$	$-COOH$
INTERNAL STANDARD	$-CH_3$	$-CH_3$

Fig. 1. Structures of gemfibrozil, metabolite III, and internal standard.

Stock solutions of gemfibrozil and internal standard, 2,2-dimethyl-5-(2,4,6-trimethylphenoxy)pentanoic acid (1 mg/ml and 100 μg/ml, respectively) and metabolite III (1 mg/ml) were prepared in 0.1 *M* sodium hydroxide. Plasma standard curves were prepared by adding 0.025, 0.05, 0.1, 0.2, and 0.3 ml of the 100 μg/ml stock solution of gemfibrozil to 1 ml of blank human plasma. Urine standard curves were prepared by adding 0.025, 0.05, 0.1, 0.2, and 0.3 ml of the 1 mg/ml stock solutions of gemfibrozil and metabolite III to 1 ml of blank human urine.

Extraction

For the assay of plasma samples or standards for free gemfibrozil, 0.2 ml of internal standard (at 100 μg/ml in 0.1 *M* sodium hydroxide), 1 ml of water, 1 ml of 1 *M* hydrochloric acid, and 10 ml of chloroform are added to 1 ml of plasma. The mixture is shaken on a reciprocating shaker for 15 min, centrifuged, and the aqueous phase discarded. The organic layer is transferred to a clean tube and evaporated to dryness in a 60°C water bath with the aid of a current of air. The residue is dissolved in 75 μl of TMAH and 100 μl of diethyl ether, and 1—2 μl are injected onto the GC column.

The urine samples are analyzed for total (free plus conjugated) gemfibrozil and metabolite III by hydrolyzing the glucuronides present in 1 ml of urine with 25 μl of glucuronidase-sulfatase adding 1 ml of water, 0.15 ml of internal standard (at 1 mg/ml in 0.1 *M* sodium hydroxide), and 1 ml of 2 *M* acetate buffer at pH 5.2. After overnight hydrolysis at 37°C, 2 ml of 1 *M* hydrochloric acid, and 10 ml of chloroform are added and the mixture is shaken for 15 min on a reciprocating shaker. After centrifugation the aqueous layer is discarded and 5 ml of 0.1 *M* sodium hydroxide are added to the organic phase. This mixture is shaken for 15 min, centrifuged, and the aqueous phase discarded. The

organic phase is removed to a clean tube and evaporated to dryness using a water bath at 60°C and a current of air. The residue is dissolved in 100 μl of TMAH and 200 μl of diethyl ether, and 1—2 μl are injected onto the gas chromatograph.

Apparatus

A Varian Model 2100 gas chromatograph equipped with a flame ionization detector was used for the assays. For the plasma samples, a 1.8 m × 2 mm I.D. glass column packed with 3% OV-22 on 80—100 mesh support was used (Applied Science, Deerfield, IL, U.S.A.). The column temperature was 180°C and the injector and detector temperatures were 230°C and 250°C, respectively. For the urine samples, a 1.8 m × 2 mm I.D. glass column packed with 10% Poly I-110 on 80—100 mesh GCQ (Supelco), maintained at 240°C was used. The injector and detector temperatures were 280°C. Nitrogen was used as a carrier gas at a flow-rate of 50 ml/min. A Shimadzu C-RIA recording integrator was used to quantitate the results using peak heights and peak height ratios.

RESULTS AND DISCUSSION

Typical chromatograms for plasma extracts are shown in Fig. 2. The retention times for the gemfibrozil and internal standard are 3.8 and 5.0 min, respectively. A peak at a retention time of about 3.1 min occurred in all of the plasma samples including the blank. This peak was well resolved from the gemfibrozil peak and did not interfere using the OV-22 column. This column was not utilized for the determination of gemfibrozil and metabolite III in urine because of the long retention time of metabolite III.

Typical chromatograms for the urine extracts are shown in Fig. 3. The retention times for the gemfibrozil, internal standard, and metabolite III are 2.8,

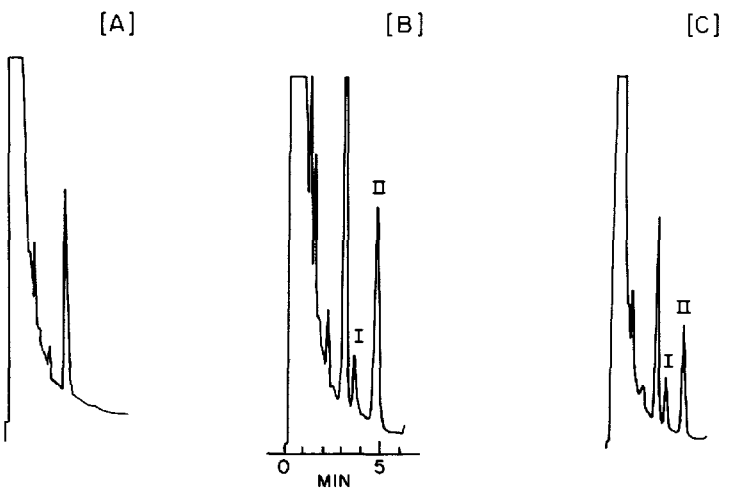


Fig. 2. Typical chromatograms of plasma extracts. A = Blank plasma; B = spiked plasma, 5 μg/ml; C = plasma sample 3 h after a 300-mg oral dose of gemfibrozil. Peaks: I = gemfibrozil; and II = internal standard.

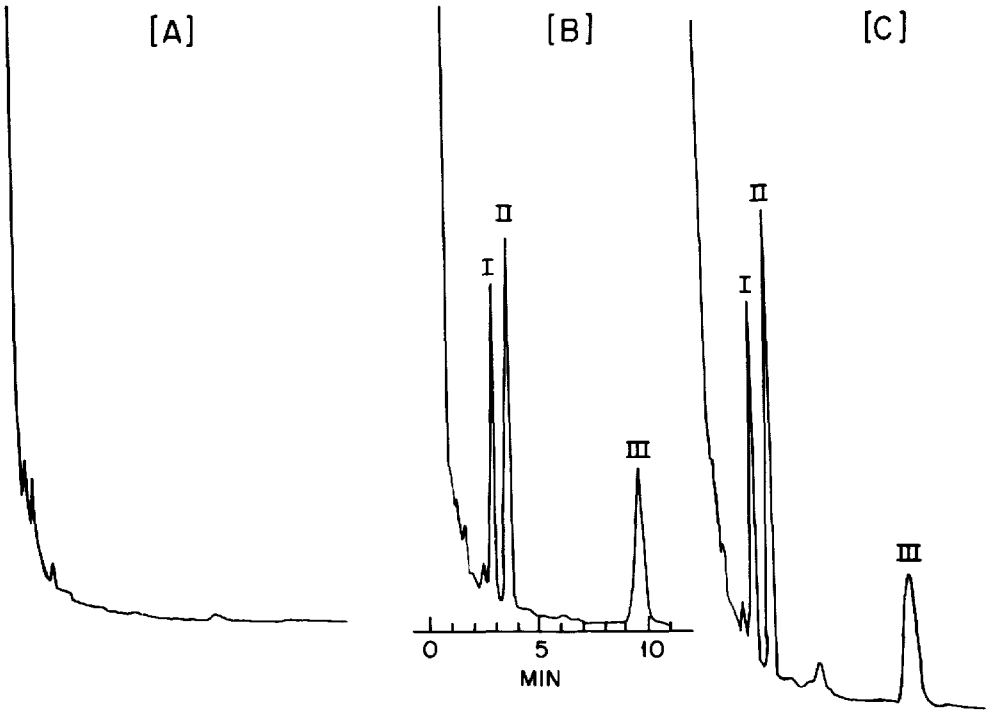


Fig. 3. Typical chromatograms of urine extracts. A = Blank urine; B = spiked urine, 100 μg/ml each of gemfibrozil and metabolite III; C = 12—24 h urine sample after a 300-mg dose of gemfibrozil. Peaks: I = gemfibrozil; II = internal standard; and III = metabolite III.

3.5, and 9.5 min, respectively. Urine extracts did not contain any interfering peaks, and the retention time for the metabolite was reasonable, therefore the urine was assayed for both gemfibrozil and metabolite III on the same injection. This column was not used for the plasma samples due to an interfering peak at the retention time of gemfibrozil.

Extraction recovery as measured against a non-extracted standard solution of gemfibrozil was about 85% and consistent over the range of concentrations occurring in plasma and urine. The minimum detectable concentration of gemfibrozil was 0.5 μg/ml from plasma and 5 μg/ml from urine as measured by three times the standard deviation of the lowest concentration used in the precision studies. Similarily the minimum detectable concentration of the metabolite was determined to be 7 μg/ml from urine.

Calibration curves were linear ($r > 0.99$) for gemfibrozil in plasma in the range 2.5—30 μg/ml and for gemfibrozil and metabolite III in urine in the range 25—300 μg/ml. The results of the precision studies are listed in Table I. Precision was assessed by the determination of percent R.S.D. on a within-day basis as well as an overall basis. The relative standard derivations were less than 10% in both measures of precision and the method was considered valid.

The method was used in several studies including the assessment of bioavailability of a 300-mg capsule formulation. Complete details of the bioavailability study will be reported elsewhere. The mean plasma concentration—

TABLE I

PRECISION STUDIES

Added	Mean backcalculated (percent R.S.D.)*							
	Day No. 1	(n=3)	Day No. 2	(n=3)	Day No. 3	(n=3)	Overall	(n=9)
Plasma concentrations (μg/ml) gemfibrozil								
0	0		0		0		0	
2.5	2.7	(9.2)	2.8	(4.1)	2.9	(7.3)	2.8	(6.6)
5	5.0	(2.0)	5.2	(1.1)	5.2	(1.1)	5.1	(2.4)
10	10.2	(3.1)	10.1	(1.7)	10.0	(1.7)	10.1	(2.2)
20	19.8	(2.6)	19.8	(1.3)	19.8	(1.3)	19.8	(1.6)
30	29.8	(1.9)	29.6	(1.1)	29.6	(0.3)	29.7	(1.1)
Urine concentrations (μg/ml) gemfibrozil								
0	0		0		0		0	
25	21.0	(10.0)	19.5	(5.5)	21.5	(8.5)	20.7	(8.4)
50	45.4	(9.4)	46.4	(6.7)	39.6	(2.5)	43.8	(9.6)
100	97.5	(2.4)	99.3	(4.6)	92.6	(4.0)	96.5	(4.5)
200	200.0	(3.3)	204.3	(1.4)	204.8	(2.3)	203.8	(5.1)
300	311.2	(1.6)	301.3	(3.7)	316.4	(1.1)	309.6	(3.0)
Urine concentrations (μg/ml) metabolite III								
0	0		0		0		0	
25	24.3	(2.2)	20.3	(9.7)	22.1	(7.6)	22.3	(9.8)
50	54.7	(7.5)	50.9	(4.6)	45.6	(1.8)	50.4	(9.2)
100	112.3	(9.6)	99.7	(7.4)	98.7	(6.4)	103.6	(9.5)
200	198.0	(6.3)	219.7	(3.0)	196.5	(8.9)	204.8	(7.8)
300	279.7	(1.7)	294.9	(1.5)	310.3	(6.8)	294.9	(5.9)

*Percent R.S.D. was assessed on both a within-day and overall basis.

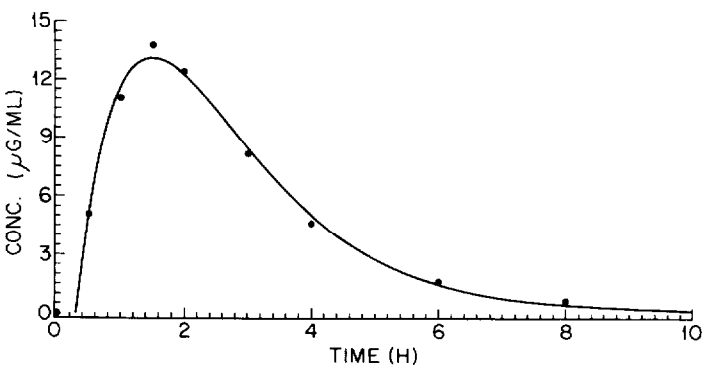


Fig. 4. Plasma concentration—time profile for gemfibrozil following a 300-mg oral capsule. The points are mean of twelve subjects. The line in the computer best-fit to a mono-exponential elimination process following first-order absorption.

time profile of a 300-mg capsule in twelve subjects is given in Fig. 4. The plasma concentrations follow mono-exponential elimination kinetics with an elimination half-life of about 1.4 h. The mean maximum plasma concentration was 16.1 μg/ml (range 10—27 μg/ml). The 48-h urinary excretion of total (free plus conjugated) gemfibrozil and metabolite III was about 27.4% (range 14—45%) and 33.2% (range 19—57%) of the dose, respectively. The relative bioavailability of the capsule as compared to the oral solution, as measured by both area

under the plasma concentration—time curve and urinary excretion data was about 0.97.

CONCLUSION

A simple rapid GC method for the analysis of gemfibrozil in plasma and total (free plus conjugated) gemfibrozil and metabolite III in urine has been developed and validated. The method is reproducible (percent R.S.D. $< 10\%$) with a minimum detectable concentration of gemfibrozil in plasma of 0.5 μg/ml and of gemfibrozil and metabolite III in urine of 5 μg/ml and 7 μg/ml, respectively. The method has been employed in several pharmacokinetic and bioavailability studies of single and multiple doses of gemfibrozil in man and an example of such a study is presented to demonstrate the applicability of the method.

REFERENCES

1 A.K. Jain, J.R. Ryan, W.S.S. Lacorte and F.G. McMahan, Clin. Pharmacol. Ther., 29 (1981) 254.
2 Anonymous, Med. World News, 23 (1982) 40.
3 Anonymous, J. Amer. Med. Ass., 247 (1982) 1540.
4 H.D. Peabooy, Jr., Cardiovasc. Rev. Rep., 3 (1982) 1194.
5 R.A. Okerholm, F.J. Keeley, F.E. Peterson and A.J. Glazko, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 35 (1976) Abstract 675.
6 R.A. Okerholm, F.J. Keeley, F.E. Peterson and A.J. Glazko, Proc. R. Soc. Med., 69 Suppl 2 (1976) 11.
7 T.C. Smith, Proc. R. Soc. Med., 69 Suppl 2 (1976) 24.
8 E.J. Randinitis, A.W. Kinkel, C. Nelson and T.D. Parker, III, Abstracts of Papers, 33rd National Meeting Academy of Pharmaceutical Sciences, San Diego, CA, November 15, 1982, Vol. 12, No. 2, 1982, p. 159.