Journal of Chromatography, 137 (1977) 218–221

CHROM. 10,036

Note

Use of high-performance liquid chromatography to measure plasma concentrations of *p*-chlorophenoxyisobutyric acid after administration of clofibrate to humans

R. N. WOODHOUSE, D. G. CRESSWELL, L. F. CHASSEAUD and R. R. BRODIE

Department of Metabolism and Pharmacokinetics, Huntingdon Research Centre, Huntingdon PE18 6ES (Great Britain)

(Received February 21st, 1977)

Clofibrate (ethyl *p*-chlorophenoxyisobutyrate) is an important hypolipidaemic agent[1–4]. It is hydrolysed during (or after) absorption from the gastrointestinal tract so that only the corresponding acid is detected in the peripheral plasma[5–7]. Plasma concentrations of this acid, *p*-chlorophenoxyisobutyric acid (CPIB), have usually been measured by the spectrophotometric method of Barrett and Thorp[8] or more recently by gas chromatography[6,9,10]. The specificity of the spectrophotometric method is adequate only for controlled studies in human volunteers; otherwise, gas chromatography has been preferred[6]. Furthermore, the variable background encountered using the spectrophotometric method limits the accuracy of measurement of low plasma concentrations of CPIB.

The use of high-performance liquid chromatography (HPLC) would improve the specificity of the method and its accuracy for measurement of lower concentrations of CPIB.

EXPERIMENTAL

Materials

Analytical grade diethyl ether, which was redistilled before use, acetonitrile and methanol (Spectrograde) were obtained from Fisons, Loughborough, Great Britain. 4-Chloro-2-methylphenoxyacetic acid (CMPA) was available from Aldrich, Wembley, Great Britain and *p*-chlorophenoxyisobutyric acid (CPIB) was a gift from Bristol-Myers, New York, U.S.A.

Extraction

The internal standard CMPA (50 μg) in methanol and 3 *M* HCl (0.5 ml) were added to plasma (1 ml) in a 12-ml glass-stoppered centrifuge tube, which was shaken for 15 sec and allowed to stand for 5 min. Diethyl ether (6 ml) was added to the contents of the tube, the mixture shaken for 30 sec and centrifuged for 5 min at 2000 *g*.

The ether layer (now containing CPIB extracted from plasma and the internal standard CMPA) was removed, evaporated to dryness under nitrogen at *ca.* 20°, and the resulting residue dissolved in methanol (100 μl). Portions (10 μl) of this solution were injected into the chromatograph using a stop-flow injection technique.

Apparatus and chromatography

A Pye Unicam LC20 liquid chromatograph (Pye Unicam, Cambridge, Great Britain) fitted with a septum injector and a SP6-400 variable wavelength ultraviolet detector linked to a Philips PM 8220 pen recorder was used. The stainless steel column (25 × 0.46 cm I.D.) was packed with C_{18} Partisil (10 μm, Reeve Angel, London, Great Britain). The mobile phase was 27% (v/v) acetonitrile containing 0.4% (w/v) orthophosphate buffer (to maintain the pH at 4.2) at a flow-rate of 2 ml/min and a typical back pressure of 50 bar.

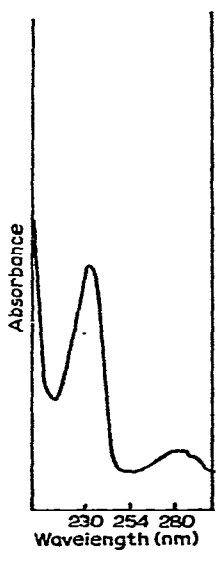
Under these conditions, the retention times of CMPA and CPIB were 6 and 7 min respectively, thus allowing repeat injections every 10 min.

Standard curves were constructed from chromatograms obtained from plasma to which known amounts of CPIB (0–100 μg/ml) had been added (Fig. 3).

The method was used to measure concentrations of CPIB in the plasma of human subjects who had been dosed orally with clofibrate (1 g) during bioavailability studies[11]. These plasma samples were also analysed by the spectrophotometric method[8].

RESULTS AND DISCUSSION

The ultraviolet absorption spectrum of CPIB shows maxima at *ca.* 230 nm and 280 nm and a minima at *ca.* 254 nm in the solvent used (Fig. 1). The spectrum of CMPA is similar. Thus measurement at 254 nm which is common on fixed wavelength detectors, provided poor sensitivity. At this wavelength there was also an interfering peak in blank plasma extracts equivalent to *ca.* 25 μg/ml of CPIB. Monitor-



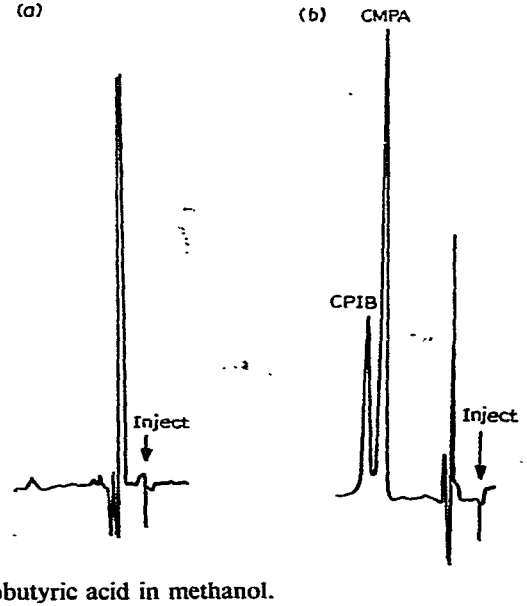


Fig. 1. Ultraviolet spectrum of *p*-chlorophenoxyisobutyric acid in methanol.

Fig. 2. High-performance liquid chromatogram obtained from (a) control plasma, (b) plasma containing a CPIB concentration of 20 μg/ml (and a CMPA concentration of 50 μg/ml).

ing the column eluate at 230 nm increased the sensitivity to CPIB and decreased the sensitivity to the interfering peak producing an overall limit of detection of about 2 μg/ml (Fig. 2).

Measurement of peak height ratios of CPIB and CMPA with respect to concentrations of CPIB provided a standard curve that was linear ($y = a + bx$, where $a = 0.0385(\pm 0.0384)$, $b = 0.0157(\pm 0.0006)$; the value of the intercept was not significantly different from zero, Fig. 3).

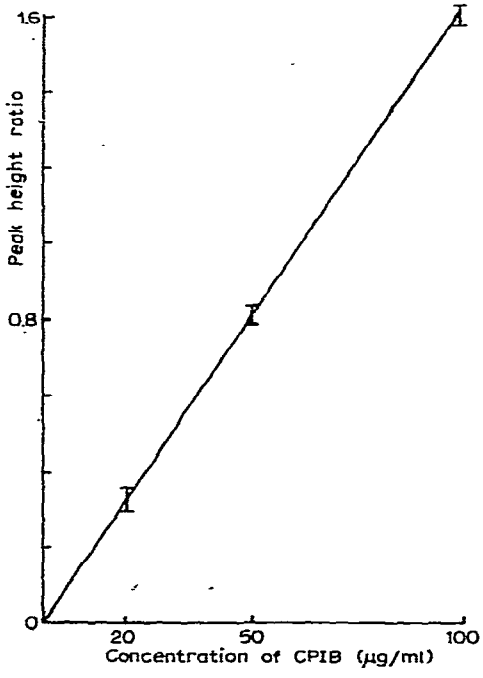


Fig. 3. Standard (calibration) curve for measurement of CPIB in plasma by HPLC. Each point represents the mean ± S.D. of three replicates.

The range of three replicate measurements of CPIB added to plasma at concentrations of 20, 50 and 100 μg/ml were 18–22, 49–51 and 99–102 μg/ml, respectively.

The recovery of CPIB added to plasma at concentrations of 20, 50 and 100 μg/ml exceeded 90% and that of the internal standard of CMPA added to plasma at a concentration of 50 μg/ml exceeded 95%.

Concentrations of CPIB in plasma samples withdrawn during bioavailability studies of clofibrate[11] were measured by both HPLC and spectrophotometric methods[8]. There was good agreement of results obtained using either method (Table I), but mean plasma concentrations of CPIB, measured by HPLC, declined with a half-life of 23 h whereas the half-life measured using the spectrophotometric method was 19 h. However, the background (blank) values of CPIB measured were lower and varied less when HPLC was used (<2 μg/ml in 8 subjects) than when the spectrophotometric method was used [16.7 ± 5.5 S.D. μg/ml ($n = 8$), range 8.6–24.9 μg/ml].

TABLE I

CONCENTRATIONS OF CPIB IN HUMAN PLASMA

CPIB in the plasma of 8 human subjects was measured using HPLC or spectrophotometry. Results are expressed as μg/ml $\pm$ S.D.

Time (h)	*HPLC*	*Spectrophotometric method**
1	28.6 $\pm$ 22.3	24.7 $\pm$ 24.6
2	48.1 $\pm$ 18.6	48.7 $\pm$ 26.5
3	55.9 $\pm$ 18.5	57.6 $\pm$ 23.8
4	60.1 $\pm$ 16.4	66.6 $\pm$ 21.0
6	64.4 $\pm$ 11.7	72.5 $\pm$ 17.1
8	64.1 $\pm$ 10.3	71.1 $\pm$ 15.1
12	60.1 $\pm$ 6.1	61.8 $\pm$ 14.5
24	43.9 $\pm$ 10.6	40.7 $\pm$ 15.7
32	33.4 $\pm$ 7.8	27.7 $\pm$ 12.4
48	19.1 $\pm$ 6.7	16.5 $\pm$ 9.5

* Values corrected for pre-dose (blank) concentration of 16.7 $\pm$ 5.5 μg/ml.

The HPLC method described, appears as accurate and is more convenient than reported gas chromatographic methods[6,9,10,12–14]. Nevertheless, the assay needs to be applied to the plasma of patients receiving multi-drug therapy in order to provide a more rigorous test of its specificity. However, many basic drugs would not be extracted from acidified plasma and the versatility of HPLC, would be expected to allow CPIB to be separated from interfering neutral and acidic drugs.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. P. Gooding of Bristol-Myers for a gift of *p*-chlorophenoxyisobutyric acid, to Dr. W. J. Price of Pye Unicam for his help and interest and to Mrs. M. J. Browne for excellent technical assistance.

REFERENCES

1 R. Howe, *Advan. Drug Res.*, 9 (1974) 7.
2 M. F. Oliver, *Brit. Med. J.*, 4 (1971) 767.
3 D. B. Hunninghake, D. R. Tucker and D. L. Azarnoff, *Circulation*, 39 (1969) 675.
4 S. M. Grundy, E. H. Ahrens, Jr., G. Salen, P. H. Schreibman and P. J. Nestel, *J. Lipid. Res.*, 13 (1972) 531.
5 J. M. Thorp, *Lancet*, I (1962) 1323.
6 H. Bruderlein, W. T. Robinson, M. Kraml and D. Dvornik, *Clin. Biochem.*, 8 (1975) 261.
7 L. F. Chasseaud, A. J. Cooper and V. H. Saggers, *J. Clin. Pharmacol.*, 14 (1974) 382.
8 A. M. Barrett and J. M. Thorp, *Brit. J. Pharmacol.*, 32 (1968) 381.
9 A. Sedaghat, H. Nakamura and E. H. Ahrens, Jr., *J. Lipid Res.*, 15 (1974) 352.
10 A. Berlin, *J. Pharm. Pharmacol.*, 27 (1975) 54.
11 L. F. Chasseaud, T. Taylor, M. J. Browne and D. D. Cracknell, Huntingdon Research Centre, Huntingdon, unpublished results, 1976.
12 C. Harvengt and J. P. Desager, *Curr. Ther. Res. Clin. Exp.*, 19 (1976) 145.
13 F. Knuchel and H. Ochs, *Arzneim. Forsch.*, 24 (1974) 576.
14 T. C. Cuong and A. Tuong, *J. Chromatogr.*, 106 (1975) 97.