Journal of Chromatography, 223 (1981) 365—370
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 825

SIMULTANEOUS DETERMINATION OF CYCLANDELATE AND ITS METABOLITE IN HUMAN PLASMA BY CAPILLARY COLUMN GAS—LIQUID CHROMATOGRAPHY

G. ANDERMANN* and M. DIETZ

Laboratoires P.O.S., 68240 Kaysersberg (France)

(Received October 28th, 1980)

SUMMARY

A method was developed for the simultaneous determination of cyclandelate and mandelic acid concentrations in plasma, involving extraction from plasma followed by trimethylsilylation and chromatography of the derivatives on a glass capillary column with hydrogen flame-ionization detection. Calibration graphs were linear down to at least 20 μg/ml for each substance. The precision was excellent with a pooled relative standard deviation of 6.3% and 6.4% for cyclandelate and mandelic acid serum samples, respectively. Concentrations below 500 ng/ml of each substance could be detected in human plasma. The method was developed for use in bioavailability and metabolism studies.

INTRODUCTION

Since cyclandelate (I) was first synthesized, it has become a much used drug for the treatment of vascular disease [1—5], but little information is available concerning its pharmacokinetic behaviour in humans [6—10]. The use of polarography [6] for the determination of cyclandelate in urine is not sensitive enough for the study of its pharmacokinetic properties in plasma. A spectrophotometric method [11] has been used to measure cyclandelate in biological samples, but it is non-specific for the determination of metabolites.

A chromatographic method was described previously for the determination of cyclandelate in human plasma [12], but the determination of its metabolite (mandelic acid) is difficult because of interfering peaks. This paper presents a modified method for the simultaneous determination of cyclandelate (I) and mandelic acid (II) using a capillary column and ethyl mandelate (III) as an internal standard.

0378-4347/81/0000—0000/$02.50

EXPERIMENTAL

Reagents

Ethyl mandelate, anhydrous sodium sulphate, hydrochloric acid and pyridine were of analytical-reagent grade and were used without further purification. Diethyl ether was purified by distillation in an all-glass apparatus. A commercially prepared mixture of N-bis(trimethylsilyl)fluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (99:1) was used for methylating the derivatives.

Standard solutions of ethyl mandelate (Roth, Karlsruhe, G.F.R.), mandelic acid (Fluka, Buchs, Switzerland) and cyclandelate (Synthedis, Bobigny, France) were prepared in ethyl acetate.

Instrumentation

The analysis was performed on a gas chromatograph (Girdel) equipped with a flame-ionization detector and a splitless mode injector (Ross) connected to a recorder fitted with an automatic programmable integrator (Sigma 1, Perkin-Elmer). The gas chromatograph input attenuation was $1 \cdot 10^{-11}$ A/mV and the integrator attenuation was $\times 4$. The 25 m $\times$ 0.3 mm I.D. glass capillary column was deactivated and wall-coated with SE-30 (Girdel, Paris, France). The carrier gas was nitrogen at a flow-rate of 2 ml/min.

The injector and detector temperatures were 250°C. The column oven was operated at 125°C, with a programme involving a 13-min initial hold, a 3°C/min increase to 180°C and a final hold at 180°C for 1 min.

Extraction and preparation of samples for chromatography

The extraction procedure reported earlier [12] was slightly modified. To 1 ml of plasma in a glass-stoppered 15-ml centrifuge tube were added 20 μl of the internal standard, ethyl mandelate (20 μg/ml in ethyl acetate). The plasma was extracted with 10 ml of diethyl ether by mixing for 1 min on a vortex mixer. This organic portion contained cyclandelate. After separation from the aqueous phase by centrifugation (5 min at 4300 *g*) the organic phase was removed into another 30-ml tube containing anhydrous sodium sulphate.

Mandelic acid was obtained by another 10-ml extraction with diethyl ether, after the plasma had been acidified with 30% hydrochloric acid and shaken as described above. The organic phases were pooled, filtered and evaporated to 1—2 ml in a rotary flash evaporator with a 20—30°C water-bath. The residual material was transferred to a silylation vial and the solvent was removed by evaporation with a stream of nitrogen at room temperature. The dried residue in each vial was dissolved in 50 μl of pyridine, 25 μl of the trimethylsilylating reagent were added and the solution was allowed to stand for 5 min at 60°C. Approximately 1 μl of this solution was injected into the gas—liquid chromatographic (GLC) column.

Method of quantitation

The peak heights of I and II were measured by computer. Peak-height ratios were obtained by dividing the peak heights of I and II by the peak height of III.

Calibration graphs for known concentrations of I and II in plasma were

prepared by plotting peak-height ratios versus I and II concentrations. Values for unknown concentrations of I and II in plasma samples, obtained in the same manner, were calculated by the Sigma 1 computer from the slope of the calibration graph. The slopes were obtained by a least-squares linear regression analysis for the cyclandelate and mandelic acid standards.

RESULTS AND DISCUSSION

Analysis of plasma

The GLC analysis of control (blank) human plasma carried through the extraction procedure, is presented in Fig. 1A. Fig. 1B shows a gas chromatogram of cyclandelate (I), mandelic acid (II) and the internal standard (III), obtained by adding cyclandelate, mandelic acid and ethyl mandelate (internal standard) to control plasma and carrying the mixture through the extraction procedure.

The spiked samples showed excellent resolution. The retention times were 31.3, 8.2 and 7.1 min for I, II and III, respectively.

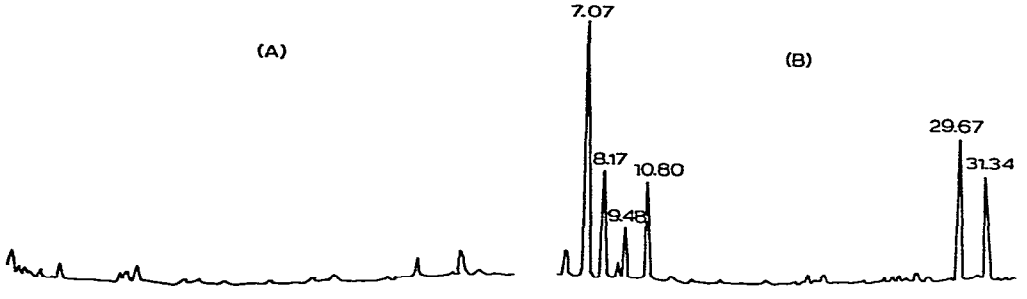

Fig. 1. Gas chromatograms of 1-ml human plasma extracts. (A) Blank plasma; (B) plasma spiked with 20 μg of ethyl mandelate (retention time 7.07 min), 10 μg of mandelic acid (8.17 min) and cyclandelate (31.34 min).

Recovery and linearity

Cyclandelate and mandelic acid recoveries were determined by adding various known amounts of product to human plasma and analysing each sample in quadruplicate according to the described procedure (Table I). Compared to a similar series of unextracted reference standards, the mean recoveries varied from 95% to 106% (mean 101%) for mandelic acid and from 95% to 102% (mean 98%) for cyclandelate. They were independent of concentration within the range used for plasma.

The calibration graphs obtained from plasma spiked with cyclandelate and mandelic acid in the range 1—20 μg/ml were linear for both products, as reflected by the values of the correlation coefficients ($r = 0.997$ for cyclandelate and $r = 0.996$ for mandelic acid) (Fig. 2).

Precision

The precision was determined by carrying out eight consecutive calibration runs on plasma samples containing both cyclandelate and mandelic acid (Table II). The coefficients of variation (C.V.) ranged from 4 to 8% and 3 to 7% for

TABLE I

RECOVERY OF MANDELIC ACID AND CYCLANDELATE ADDED TO HUMAN PLASMA

Compound	Amount added (μg/ml)	n	Amount recovered (μg/ml)	Recovery (mean ± S.D.) (%)
Mandelic acid	1	8	1.06 ± 0.08	106 ± 8
	5	8	5.02 ± 0.19	100.4 ± 3.8
	10	8	9.57 ± 0.69	95.7 ± 6.9
	20	8	20.17 ± 0.91	100.9 ± 4.6
Cyclandelate	1	7	0.95 ± 0.12	95 ± 12
	5	7	4.76 ± 0.19	95.2 ± 3.8
	10	7	10.27 ± 0.72	102.7 ± 7.2
	20	7	19.84 ± 0.66	99.2 ± 3.3

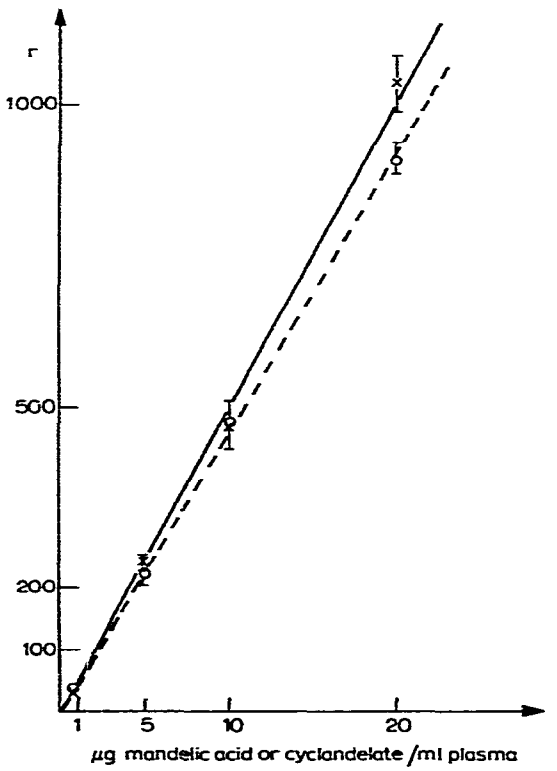

Fig. 2. Calibration graphs of amounts of cyclandelate (— — —) and mandelic acid (——) added to 1 ml of human control plasma versus measured peak-area ratio (r) of mandelic acid or cyclandelate to internal standard (ethyl mandelate). The concentration of the ethyl mandelate was 20 μl/ml.

TABLE II

PRECISION OBSERVED ON ANALYSIS OF CONSECUTIVE CALIBRATION GRAPHS FOR PLASMA CONTAINING CYCLANDELATE AND MANDELIC ACID AT THE CONCENTRATIONS INDICATED

Amount added to plasma (μg/ml)	Peak-height ratio			
	Mandelic acid:ethyl mandelate		Cyclandelate:ethyl mandelate	
	Mean ± S.D. (n = 8)	C.V.(%)	Mean ± S.D. (n = 7)	C.V.(%)
1	0.048 ± 0.004	8.3	0.052 ± 0.006	11.5
5	0.239 ± 0.010	4.2	0.224 ± 0.009	4
10	0.471 ± 0.038	8.1	0.474 ± 0.033	7
20	1.031 ± 0.047	4.6	0.907 ± 0.030	3.3

mandelic acid and cyclandelate, respectively, for 5—20 μg/ml levels. Only the 1 μg/ml level for cyclandelate had a high coefficient of variation (11.5%). These results indicate the good reproducibility of the technique.

Detection limits

The detection limit, as determined at the 95% confidence level, was 500 ng/ml for both cyclandelate and mandelic acid.

Application to pharmacokinetic studies

The suitability of the proposed method for the determination of cyclandelate and its metabolite in plasma was tested with six healthy volunteers who received 400 mg of cyclandelate as a single oral dose. Blood samples (10 ml) were drawn by venipuncture at 0, 0.5, 1, 1.5 and 2 h. Plasma was separated, and the samples were frozen until taken for the determination of the cyclandelate and mandelic acid concentrations by the described method. An interesting observation concerning metabolism of cyclandelate was made; these results will be published elsewhere [13].

CONCLUSION

The method described is linear, precise, accurate and sensitive. It has been used for over 6 months in our pharmacokinetic laboratory, where it has been applied to the generation of pharmacokinetic profiles on humans and animals who have been administered cyclandelate orally.

The reason for preparing a derivative of cyclandelate and mandelic acid is that the unchanged drugs are highly polar compounds that show marked tailing during chromatography. However, there are two important disadvantages with I and II derivatives: they are time consuming to prepare and are readily hydrolysed by moisture, even in the presence of excess of reagents.

REFERENCES

1 Ö. Algan and H. Kienle, Med. Welt, 9 (1969) 471.
2 J.A.C. Ball and A.R. Taylor, Brit. Med. J., 3 (1967) 525.

3 U.G. Bijlsma, A.B.H. Funcke, H.M. Tersteege, R.F. Rekker, M.J.E. Ernsting and W.Th. Nauta, Arch. Int. Pharmacodyn., 105 (1956) 145.
4 M.D. O'Brien and N. Veall, Lancet, i (1966) 729.
5 O. Eichhorn, Vasc. Dis., 2 (1965) 305.
6 M.J.E. Ernsting, R.F. Rekker, H.M. Tersteege and W.Th. Nauta, Arzneim.-Forsch., 6 (1956) 145.
7 M.J.E. Ernsting, R.F. Rekker, H.M. Tersteege and W.Th. Nauta, Arzneim.-Forsch., 12 (1962) 632.
8 M.J.E. Ernsting, R.F. Rekker, H.M. Tersteege and W.Th. Nauta, Arzneim.-Forsch., 12 (1962) 691.
9 M.J.E. Ernsting, R.F. Rekker, H.M. Tersteege and W.Th. Nauta, Arzneim.-Forsch., 12 (1962) 853.
10 M.J.E. Ernsting, R.F. Rekker, H.M. Tersteege and W.Th. Nauta, Arzneim.-Forsch., 7 (1962) 930.
11 G. Andermann, M. Dietz and D. Mergel, J. Pharm. Belg., 34 (1979) 233.
12 M. Dietz and G. Andermann, J. High Resolut. Chromatogr. Chromatogr. Commun., 2 (1979) 635.
13 G. Andermann and M. Dietz, Biopharm. Drug Dispos., submitted for publication.