Journal of Chromatography, 182 (1980) 379—386
Biomedical Applications

CHROMBIO. 543

DETERMINATION OF THE ANTISPASMODIC AGENT ETHAVERINE IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

R.R. BRODIE, L.F. CHASSEAUD and L.M. WALMSLEY

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

H.H. SOEGTROP

Dr. Kade Pharmazeutische Fabrik GmbH, Konstanz (G.F.R.)

and

A. DARRAGH and D.A. O'KELLY

Endocrine Pharmacology Unit, University College Dublin, St. James Hospital, Dublin (Ireland)

(Received November 30th, 1979)

SUMMARY

Ethaverine can be measured in the plasma of human subjects by reversed-phase high-performance liquid chromatography employing UV detection. The limit of detection was 2 ng/ml, and the precision was ± 14, ± 6 and ± 2% at concentrations of 5, 25 and 50 ng/ml respectively. A peak mean plasma drug concentration of 20 ng/ml occurred at 1.5 h after single oral doses of a capsule formulation to human subjects, and declined with a half-life of 2.9 h.

INTRODUCTION

Ethaverine (Fig. 1) is an antispasmodic agent structurally and pharmacologically related to the benzylisoquinoline alkaloid papaverine; it is used as a smooth muscle relaxant and vasodilator and is claimed to be more potent and less toxic than papaverine [1]. Due to the rapid rate of metabolism of this type of compound, they are frequently administered as sustained-release formulations [2—5], which provide relatively lower blood concentrations of unchanged drug that are maintained, however, over longer periods of time [6]. In order to generate reliable pharmacokinetic data, accurate, precise, sensitive (<0.01 μg/ml) and specific methods of measurement of drug concentrations in

plasma are required. Very few methods are available for the measurement of this type of drug in biological fluids; early methods [7—10] were either non-specific or lacked sensitivity. Papaverine has been determined using ion-pair extraction followed by gas—liquid chromatography (GLC) with a sensitivity of 0.01 μg/ml when analysing a 5-ml plasma sample [11]. It has also been measured by gas chromatography utilising mass spectrometric detection (GC—MS), with a sensitivity of 0.005 μg/ml when analysing a 3-ml blood sample [12]. More recently, a column extraction procedure using Amberlite XAD-7 followed by GLC achieved this same level of sensitivity from 1 ml of whole blood [13]. Ethaverine has been measured by high-performance liquid chromatography (HPLC) in an adsorption mode with UV absorption detection at 254 nm [14]; a method sensitivity of 0.025 μg/ml could be achieved from 3-ml plasma samples.

This paper describes an HPLC method in the reversed-phase mode for the measurement of ethaverine from plasma. This mode of chromatography was preferred because of its high stability and reproducibility when analysing biological extracts. The method is simple, incorporates papaverine as internal standard and is more sensitive than any of the previously reported methods for this type of compound, achieving 0.002 μg/ml from a 2-ml plasma sample. The method can also be used for the analysis of papaverine from biological fluids in which case ethaverine could serve as an internal standard.

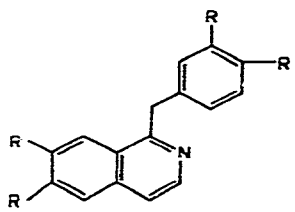

Fig. 1. Chemical structure of ethaverine (R = OC_2H_5) and papaverine (R = OCH_3).

EXPERIMENTAL

Materials

All reagents were of analytical grade and all inorganic reagents were prepared in freshly glass-distilled water. Diethyl ether was freshly redistilled prior to use. Ethaverine hydrochloride [1-(3,4-diethoxybenzyl)-6,7-diethoxyisoquinoline hydrochloride] and papaverine hydrochloride [1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride], used as internal standard (Fig. 1), were supplied by Dr. Kade Pharmazeutische Fabrik GmbH, Konstanz, G.F.R. Standard solutions of ethaverine HCl and internal standard were prepared in methanol at concentrations of 1 μg/ml and 2 μg/ml respectively and stored at 4°C.

Extraction procedure

Plasma samples (2 ml) were transferred into 10-ml pointed centrifuge tubes and spiked with internal standard (15 μl, containing 30 ng papaverine HCl). Sodium hydroxide solution (200 μl, 4 *M*) was added and the mixture was extracted by shaking it with diethyl ether (5 ml) using a vortex mixer. After

centrifugation of the extract at 2000 *g* for 10 min, the organic layer was transferred to another pointed centrifuge tube and the aqueous layer extracted with more ether (2 ml). After centrifugation, the organic layers were combined and extracted for 1 min by vortex mixing with hydrochloric acid (0.5 ml, 1 *M*). After centrigugation at 2000 *g* for 10 min, the ether layer was discarded and the aqueous acid layer made alkaline with sodium hydroxide solution (0.5 ml, 4 *M*). The alkaline solution was extracted with diethyl ether (5 ml) as previously described. The ether layer was transferred to a pointed centrifuge tube and evaporated to dryness under a stream of nitrogen at 37°C. The sides of the tube were rinsed with more ether to ensure that the residue was at the bottom of the tube, and the ether again evaporated. The residue was dissolved in methanol (25 µl) and aliquots injected into the chromatograph.

Apparatus

The liquid chromatograph consisted of a Waters M6000A pump (Waters Assoc., Northwich, Great Britain) coupled to a Cecil 212 variable-wavelength UV detector (Cecil Instruments, Cambridge, Great Britain) operated at 238 nm. Injection was by syringe (25 µl, Precision Sampling Corp., Baton Rouge, LA, U.S.A.) via a U6K universal injector (Waters Assoc.). The column was con-

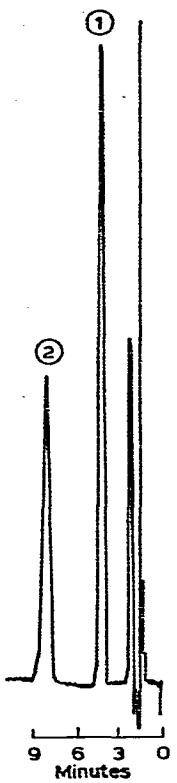

Fig. 2. Chromatogram of a standard mixture containing papaverine (1) and ethaverine (2). Column: 250 × 4.6 mm I.D., pre-packed with Partisil 10 ODS; flow-rate, 2 ml/min; solvent system: 65% (v/v) methanol—aqueous potassium dihydrogen orthophosphate (0.1% w/v), detector: UV at 238 nm, attenuation 0.01 a.u.f.s.

structed of stainless steel (25 cm × 4.6 mm I.D.), prepacked with Partisil 10 ODS (mean particle size 10 μm) (Whatman, Maidstone, Great Britain).

Chromatography was performed in a reversed-phase mode using a mobile phase of 65% (v/v) methanol in aqueous potassium dihydrogen orthophosphate (0.1%) with a flow-rate of 2 ml/min. The retention times of ethaverine and papaverine (internal standard) under these conditions were 8.5 min and 3.5 min respectively (Fig. 2).

Calibration procedure

Calibration lines of peak height ratio measurements of ethaverine HCl to internal standard against concentration of ethaverine HCl were constructed over the concentration range up to 50 ng/ml. Samples of blank (drug-free) plasma (2 ml) were spiked with ethaverine HCl at concentrations of 5, 15, 25, 35 and 50 ng/ml and with internal standard at a fixed concentration of 15 ng/ml. The samples were taken through the extraction procedure described previously.

Studies in humans

Plasma samples were obtained from six human volunteer subjects dosed orally with a sustained-release formulation of 30 mg ethaverine HCl and analysed by the foregoing procedures. The studies in volunteers were carried out under conditions similar to those described by Brodie et al. [15].

RESULTS AND DISCUSSION

Precision

Extraction and measurement at each concentration was repeated on five occasions. The precision of the method for the measurement of ethaverine HCl in plasma as indicated by the coefficient of variation of peak height ratio measurements of drug to internal standard (Table I) were ± 14%, ± 6% and ± 2% at 5 ng/ml, 25 ng/ml and 50 ng/ml respectively.

The coefficient of variation of peak height ratio measurements of a non-extracted mixture of ethaverine and internal standard analysed routinely was

TABLE I

RECOVERY AND PRECISION OF MEASUREMENT OF ETHAVERINE FROM PLASMA

Concentration of ethaverine HCl added to plasma (ng/ml)	Recovery* (%)	Coefficient of variation (%)
5	80	14
15	86	2
25	81	6
35	82	3
50	79	2
Mean	82 ± 3 S.D.	

*Means of 5 determinations at each concentration.

± 2% throughout the analysis of all the plasma samples analysed during a six-week period.

Accuracy

The calibration line for the measurement of ethaverine HCl in plasma was constructed from five replicate measurements at five concentrations over the range, and plots of peak height ratio against concentration were linear (y = 0.0271 x + 0.0210, correlation coefficient r = 0.9970) and the value of the intercept was shown to be not significantly different from zero ($P > 0.05$). The equation for the line forced through the origin was y = 0.0277 (± 0.0002 S.D.) x, where y is the peak height ratio and x is the concentration of ethaverine HCl (ng/ml). The accuracy of the method as defined by the 95% confidence limits of the least-squares regression line forced through the origin, i.e., taking the calibration line as an estimate of the concentration of ethaverine HCl in plasma, was ± 48%, ± 9% and ± 5% at 5 ng/ml, 26 ng/ml and 50 ng/ml respectively.

Recovery

The recovery of internal standard from plasma (15 ng/ml) was 86% ± 4 S.D. (n = 5). The mean recovery of ethaverine HCl from plasma over the concentration range 5—50 ng/ml was determined by comparison of peak height ratio measurements of non-extracted standards to those of extracted standards corrected for 100% recovery of internal standard, and was 82% ± 3 S.D. (Table I).

Stability of ethaverine in plasma

The stability of ethaverine HCl in plasma under the storage conditions used was tested by storing spiked plasma samples (35 ng/ml) at —20°C for one

TABLE II

CONCENTRATIONS OF ETHAVERINE HCl (ng/ml) IN THE PLASMA OF SIX HUMAN SUBJECTS AFTER SINGLE ORAL DOSES OF 30 mg

Figures in parentheses are repeat analyses up to one month after the first analysis.

Time (h)	Subject No.						Mean ± S.D.
	1	2	3	4	5	6	
0.5	<2	<2	<2	<2	<2	3	<2
0.75	6	12 (13)	<2	2	2	10	5 ± 5
1	19	35	<2	9	3	17	14 ± 13
1.5	25	36 (33)	<2	24	8	24	20 ± 13
2	24 (22)	29 (27)	<2	27	11	19	18 ± 11
3	18 (16)	21	2 (<2)	16 (18)	8 (9)	19 (20)	14 ± 7
4	18	12 (11)	32 (29)	13 (13)	5 (6)	15 (14)	16 ± 9
6	10	6	15	5	4	10	8 ± 4
8	5	3	6	3	3	6	4 ± 2
12	3	<2 (<2)	3	<2	<2	4	<2
16	<2	<2	<2	<2	<2	3	<2
24	<2	<2	<2	<2	<2	<2	<2
32	<2	<2	<2	<2	<2	<2	<2

month prior to analysis. Concentrations found (32 ng/ml ± 0.6 S.D.) indicated that a slight decomposition had occurred during this time period. The reproducibility of the method was tested by the re-analysis of a minimum of 10% of the samples up to one month after the first analysis. Concentrations of ethaverine HCl found were in very good agreement with results previously obtained (Table II).

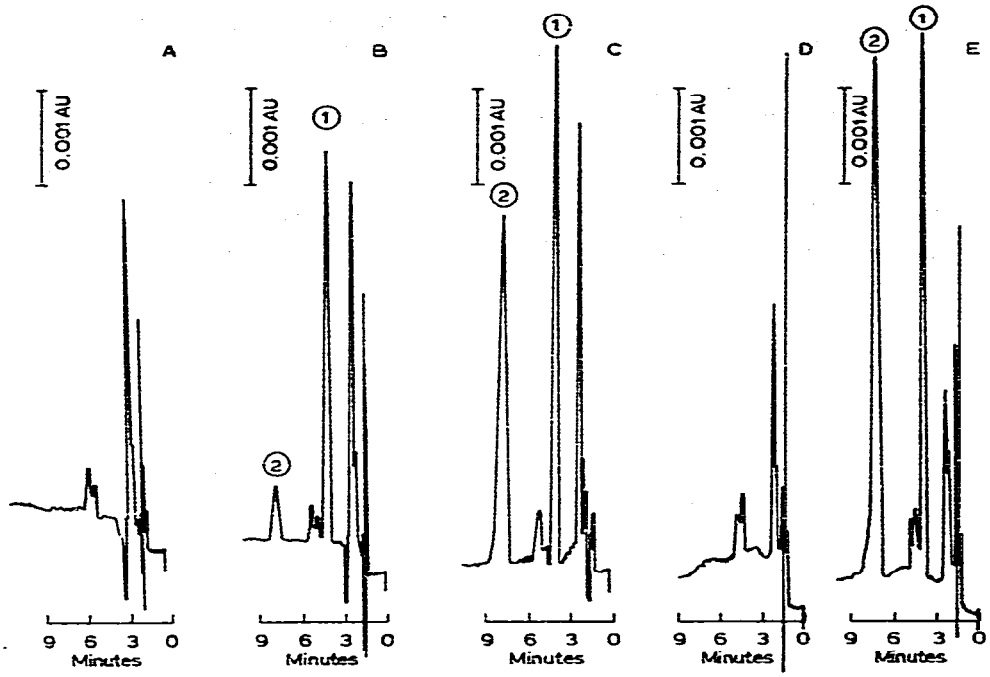

Fig. 3. Chromatograms of (A) control plasma extract used in calibration procedure; (B) control plasma spike containing internal standard (1) 15 ng/ml and ethaverine (2) 5 ng/ml; (C) control plasma spike containing internal standard (1) 15 ng/ml and ethaverine (2) 25 ng/ml; (D) predose plasma extract; (E) 1-h post-dose plasma sample containing ethaverine at a concentration of 35 ng/ml. Chromatographic conditions same as for Fig. 2.

TABLE III

HALF-LIVES OF THE TERMINAL LINEAR SECTIONS OF THE PLASMA ETHAVERINE CONCENTRATION—TIME RELATIONSHIPS

Subject	Half-life (h)	r^2
1	3.18	0.969
2	2.00	1.000
3	1.66	0.997
4	1.88	0.986
5	5.43	0.995
6	3.05	0.998
Mean ± S.D.	2.87 ± 1.41	

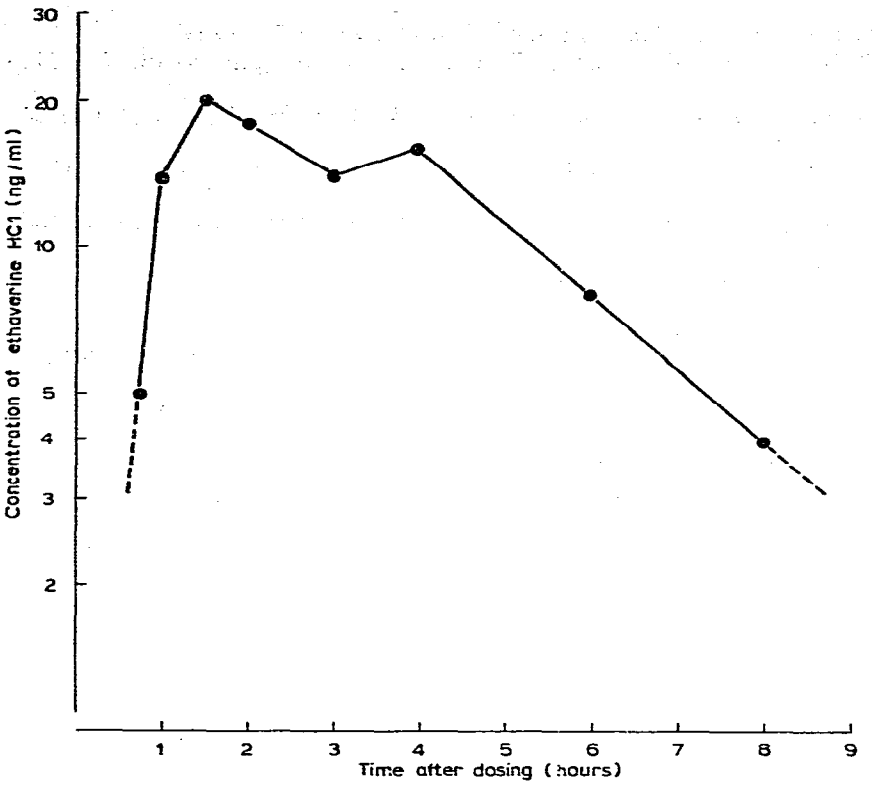

Fig. 4. Mean plasma concentrations of ethaverine HCl during 8 h after an oral dose of 30 mg of drug. Semi-logarithmic scale.

Limit of detection

Predose (blank) plasma samples taken from each subject showed no interfering peaks with similar retention times to either ethaverine or the internal standard (Fig. 3). The limit of detection of ethaverine under the experimental conditions used (signal-to-noise ratio = 5:1) with a 2-ml plasma sample was 2 ng/ml.

Concentrations of ethaverine (measured as the hydrochloride salt) in plasma

After single oral doses of capsules containing 30 mg of ethaverine (as the hydrochloride), a peak of mean concentrations of 20 ng/ml was reached at 1.5 h after dosing (Fig. 4, Table II). Mean concentrations remained between 14—20 ng/ml during 1—4 h after dosing, and thereafter declined to 4 ng/ml at 8 h. The mean half-life of ethaverine in plasma was 2.87 h ± 1.41 S.D. (n = 6) (Table III). After 8 h, mean concentrations of ethaverine were below the limit of detection.

REFERENCES

1 J.J. Lewis, An Introduction to Pharmacology, Livingstone, Edinburgh, 1965, p. 477.
2 E.R. Garrett, H. Roseboom, J.R. Green, Jr., and W. Schuerman, Int. J. Clin. Pharmacol., 16 (1978) 193.

3 G.C. Maggi, D. Cerchiari and G. Coppi, Arzneim.-Forsch., 27 (1977) 1214.
4 R.B. Miller, S.T. Leslie, F.M. Black and C. Boroda, Brit. J. Clin. Pharmacol., 5 (1978) 51.
5 E.Y. Lee, H. Sakamoto, F. Trainer, G. Brody and Y.W. Cho, Int. J. Clin. Pharmacol., 16 (1978) 32.
6 J.D. Arnold, J. Baldridge, B. Riley and G. Brody, Int. J. Clin. Pharmacol., 15 (1977) 230.
7 J. Axelrod, R. Shofer, J.K. Inscoe, W.M. King and A. Sjoerdsma, J. Pharmacol. Exp. Ther., 124 (1958) 9.
8 E. Mussini and A. Marzo, Biochem. Exp. Biol., 10 (1973) 331.
9 H.V. Street, J. Chromatogr., 29 (1967) 68.
10 N.D. Greenwood and I.W. Guppy, Analyst (London), 99 (1974) 1179.
11 D.E. Guttman, H.B. Kostenbauder, G.R. Wilkinson and P.H. Dubé, J. Pharm. Sci., 63 (1974) 1623.
12 J. De Graeve, J. Van Cantfort and J. Gielen, J. Chromatogr., 133 (1977) 153.
13 E. Zuccato, F. Marcucci and E. Mussini, J. Pharmacol. Meth., 1 (1978) 9.
14 E. Renier, K. Kjeldsen and M. Pays, Feuill. Biol., 20 (1979) 111.
15 R.R. Brodie, L.F. Chasseaud, T. Taylor, D.A. O'Kelly and A. Darragh, J. Chromatogr., 146 (1978) 152.