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QUANTITATIVE ANALYSIS OF VERALIPRIDE IN PLASMA AND URINE BY GAS CHROMATOGRAPHY—MASS SPECTROMETRY AND GAS CHROMATOGRAPHY WITH FLAME-IONIZATION DETECTION

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SUMMARY

A highly sensitive and selective quantitative assay for unchanged veralipride has been developed. The compound is extracted from alkalized samples (plasma or urine) with dichloromethane and converted to its trimethylated derivative by reaction with trimethylanilinium hydroxide. The reaction mixture is then chromatographed on a 3% OV-1 column. Trimethylated derivatives of plasma samples were assayed by selected-ion monitoring in the chemical-ionization mode and quantified by comparing the intensity of the quasi-molecular ion *m/z* 426 (M + H) with the intensity of the corresponding ion from trideuterated internal standard, *m/z* 429 (M + H). Flame-ionization detection was used for the assay of urine samples. The peak height ratio of trimethylated veralipride over trimethylated sulpiride, the internal standard, was used for quantitation of urine samples. A relative standard deviation of less than 10% was found when quantifying 10 ng/ml veralipride in plasma or 1 μg/ml in urine.

INTRODUCTION

Veralipride is a new non-hormonal non-steroidal agent displaying antidopaminergic and antigonadotropic acitivity [1—3]. It is used as regulator of sudden elevations in body temperature and other physiological disturbances associated with menopause. Veralipride is classified in the *o*-methoxybenzamide family because of its structural analogy.

The present paper deals with the quantitative analysis of veralipride in human biological fluids, plasma and urine, after oral or intravenous administration. Gas chromatography (GC)—mass spectrometry (MS) and GC with flame-ionization detection (FID) were used for the quantitative analysis of veralipride

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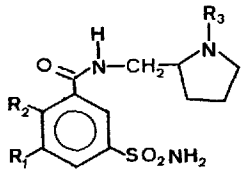
in plasma and urine, respectively. Internal standards were veralipride-d_3 for plasma samples and sulpiride for urine samples.

EXPERIMENTAL

Chemicals

Veralipride, [N-(1-allyl-2-pyrrolidinyl-methyl)] 2,3-dimethoxy-5-sulfamoyl benzamide, and sulpiride (Fig. 1) were supplied by Delagrange (Paris, France). Veralipride-d_3 [4, 5] was synthesized in our laboratory by a slight modification of the manufacturer's method [4]. The C^2H_3 was introduced on the methoxy group in the 2 position (Fig. 1).



	R_1	R_2	R_3
Veralipride	OCH_3	OCH_3	CH_2—CH=CH_2
Sulpiride	H	OCH_3	CH_2—CH_3
Veralipride-d_3	OCH_3	OC^2H_3	CH_2—CH=CH_2

Fig. 1. Chemical structures of veralipride, sulpiride and veralipride-OC^2H_3.

Trimethylanilinium hydroxide [6, 7] (Methelute) was obtained from Pierce (Spiral, Dijon, France). All solvents and chemicals were of analytical grade and supplied by Merck (Darmstadt, F.R.G.).

Instrumentation

The gas chromatograph—mass spectrometer was a Ribermag R10-10 (Delsi, Rueil-Malmaison, France) equipped with a 2.1-m glass column (2 mm I.D.) packed with 3% OV-1 on Chromosorb W AW DMCS 80—100 mesh (Spiral). Helium was used as carrier gas at a flow-rate of 20 ml/min. Chromatograph oven, injector block and interface temperatures were set at 290°C. The ion source temperature was about 100°C. Mass spectra were recorded at an electron energy of 70 eV in both electron-impact (EI) and chemical-ionization (CI) modes. Data acquisition and reduction were done with the Nermag Sidar computing system. Quantitative analyses of plasma samples were performed on the system described above by selected-ion monitoring at *m/z* 426 and *m/z* 429 in ammonia-CI-MS, including peak area calculations.

A Girdel Series 30 gas chromatograph (Suresnes, France) equipped with a flame-ionization detector was used for urine samples. For the chromatographic separations, a 2.7-m glass column (2 mm I.D.) packed with 3% OV-1 on Chromosorb W AW DMCS, 80—100 mesh, was used. Nitrogen was used as a carrier gas at a flow-rate of 30 ml/min. The column oven temperature was 270°C; injector block and detector temperatures were 300°C and 290°C, respectively. Flow-rates of hydrogen and oxygen used for the detector were 25 and 370 ml/min, respectively.

Glassware

Extraction and derivatization tubes were washed with RBS R25 (Fluka, Basle, Switzerland), rinsed sufficiently with deionized water and dried at 100°C. Pipettes, micropipettes and other labware were of disposable glassware.

Internal standards and standard solutions

Veralipride-d_3, 10 μg/ml in methanol, was used as internal standard for the plasma sample; 50 μl were mixed with plasma to obtain a final concentration of 500 ng/ml of plasma. For urine analyses, sulpiride was used as the internal standard at a concentration of 200 μg/ml in dichloromethane and 50 μl of this solution were mixed with each urine sample to give a final concentration of 10 μg/ml. Standard calibration curves were obtained by enrichment of blank plasma with 10, 20, 50, 100, 250, 500, 1000 and 1500 ng/ml veralipride and 500 ng/ml veralipride-d_3. For urine analyses blank urine was enriched with 1, 2.5, 5, 7.5, 10, 25, 50, 75 and 100 μg/ml veralipride and 10 μg/ml sulpiride as internal standard.

Analytical procedure

A 50-μl volume of internal standard solution was introduced into a 10-ml tube fitted with a PTFE-lined screw cap; after evaporation of the organic solvent, 1 ml of plasma or urine was first added and then 0.5 ml of sodium carbonate buffer (0.5 *M*, pH 9.5) for alkalization. The mixture was extracted with 4 ml of dichloromethane. After shaking for 3 min and centrifugation, the organic phase was transferred to a 4-ml tube (fitted with a PTFE-lined open screw cap). The extraction of the alkaline aqueous phase was repeated with 4 ml of dichloromethane. Organic phases were combined and evaporated to dryness under a flow of nitrogen. After addition of 50 μl of trimethylanilinium hydroxide the reaction mixture was allowed to stand for 5—10 min at room temperature; 1—3 μl of the mixture were injected into the gas chromatograph.

RESULTS AND DISCUSSION

Plasma analysis and quantitation

The pK_a of veralipride, determined by potentiometric titration, was 7.9 ± 0.0076. The solubilities of veralipride in some common solvents are listed in Table I. The percentage of veralipride base extracted by chloroform as a function of pH is given in Table II. The extracted veralipride was measured by spectrophotometry at 291.7 nm for 10^{-3} *M* solutions. It can be seen that veralipride is quantitatively extracted at pH > 9.

TABLE I

SOLUBILITY OF VERALIPRIDE BASE IN VARIOUS SOLVENTS

Solvent	Solubility (g per 100 ml at 20°C)
Acetone	18.6
Chloroform	18.9
Cyclohexane	0.009
Dichloromethane	36.3
Ethanol	7.3
Methanol	18.7
Toluene	0.05

TABLE II

PERCENTAGE OF VERALIPRIDE BASE EXTRACTED BY CHLOROFORM AT DIFFERENT pH VALUES

pH	Veralipride base extracted (%)	pH	Veralipride base extracted (%)
7.0	28.5	9.6	100.9
8.0	48.7	10.0	99.3
8.4	79.3	10.4	98.2
8.8	92.4	10.8	100.4
9.0	98.7	11.0	97.3
9.2	98.6		

A study of the stability and reproducibility of the overall GC—MS method was made. Three samples of different concentration were extracted and analysed in a series of multiple consecutive injections. The results obtained, 2146.8 (± 25.7) ng/ml, coefficient of variation (C.V.) 1.2% (n = 5), 433.8 (± 4.5) ng/ml, C.V. 1.0% (n = 10) and 41.4 (± 0.5) ng/ml, C.V. 1% (n = 5), showed good reproducibility for the GC—MS method and good stability of the trimethylated derivatives of veralipride.

The integrated analytical procedure with extraction and derivatization was controlled using two blank plasmas spiked with veralipride and the internal standard; ten aliquots were extracted. After derivatization the samples were analysed by GC—MS. Results obtained gave 7% variation for 50 ng/ml samples and 3.9% for 1000 ng/ml samples.

The isotopic purity of veralipride-d_3 was verified to be 95.5%. Thus 4.5% of unlabelled compound remained (Table III). This quantity corresponded to 22.5 ng of veralipride for 500 ng of internal standard used per ml of plasma sample.

This result permitted us to calculate the extraction yield of veralipride from plasma using dichloromethane. This was determined by spiking 1-ml plasma samples with four different concentrations of veralipride (Table IV). After the extraction 500 ng/ml internal standard was added and the samples were

TABLE III

DETERMINATION OF THE QUANTITY OF VERALIPRIDE CONTAINED IN THE VERALIPRIDE-d_3

Injection	Quantity of veralipride in the veralipride-d_3, 500 ng/ml	
	Peak area ratio	%
1	0.044	4.4
2	0.045	4.5
3	0.046	4.6
4	0.046	4.6
5	0.045	4.5
Mean	0.045	4.5
S.D.	0.0008	0.08
C.V. (%)	1.85	1.85

TABLE IV

RECOVERY OF VERALIPRIDE EXTRACTED FROM PLASMA BY DICHLOROMETHANE

Quantity (ng/ml)	Measured quantity (mean ± S.D.)	C.V. (%) (n = 5)	Theoretically expected quantity	Extraction yield (%)
10	17.05 ± 2.45	70.5	32.5	50.4
25	28.75 ± 4.17	12.0	47.5	60.4
50	66.6 ± 6.5	13.2	72.5	91.9
1000	1032.9 ± 35.1	3.5	1022.5	101.0

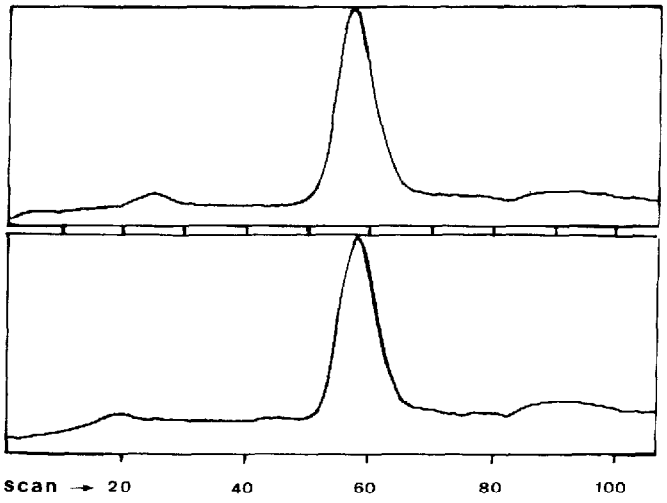


Fig. 2. Reconstructed mass chromatogram of ions m/z 426.3 (bottom) and 429.3 (top) of trimethylated veralipride (retention time 2 min 9 sec) and veralipride-d_3 (internal standard, retention time 2 min 8 sec), respectively, using a 3% OV-1 packed column.

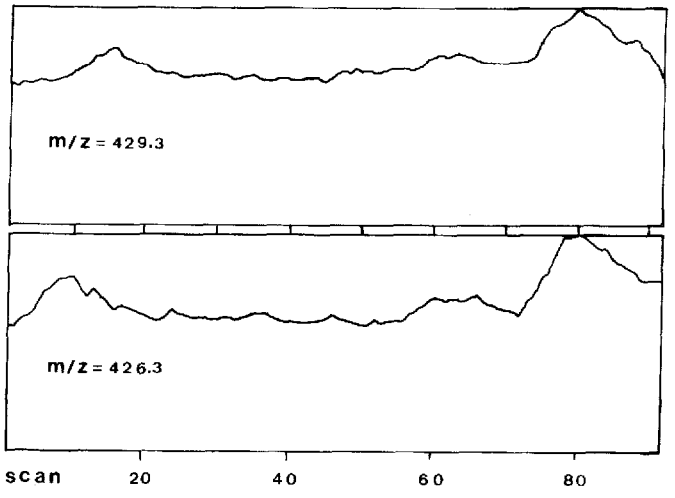


Fig. 3. Reconstructed mass chromatogram of ions m/z 426.3 and 429.3 of a blank plasma without internal standard because of the residual veralipride contained in the veralipride-d_3. No contamination from plasma or reagents interfered in the analysis.

analysed. The difference between the measured quantities and those theoretically expected permitted calculation of the extraction efficiency (Table IV). The apparent over-estimation of the low-concentration samples was compensated by the calibration curves established daily in the same conditions as the unknown samples. In other words, a standard small quantity of veralipride was systematically introduced in all samples and standards. A good linearity was observed in the range 10—1500 ng/ml of plasma. The equation was $y = ax + b$ where $a = 1$ (± 0.03) and $b = 2.97$ (± 1.7) for $n = 30$ with $r > 0.999$.

Fig. 2 shows a reconstructed ion chromatograph of veralipride and veralipride-d_3. This method allowed the quantification of about 10 ng/ml veralipride in plasma with a precision better than 10%. No contamination interfered with the analysis, as is shown by a blank obtained without veralipride and internal standard (Fig. 3).

Urine analysis and quantitation

Urine samples were analysed and quantified by GC—FID using sulpiride as the internal standard. The reproducibility tested by repeated injections of samples of two different concentrations (2.7 ± 0.18 μg/ml, C.V. 6.9%, $n = 10$, and 23.75 ± 0.15 μg/ml, C.V. 0.6%, $n = 10$) was satisfactory. Repeated extractions and analyses demonstrated good stability of the overall process (Table V). Extraction yield was about 50% for low-concentration samples and rapidly increased to ± 80% (Table VI) at around 20 μg/ml. Therefore no attempt was made to improve the extraction yields because large quantities of veralipride were found in urine and good signals were obtained. For quantitation peak height ratios of trimethylated veralipride over trimethylated sulpiride have been used instead of peak area measurement since the results were found to be

TABLE V

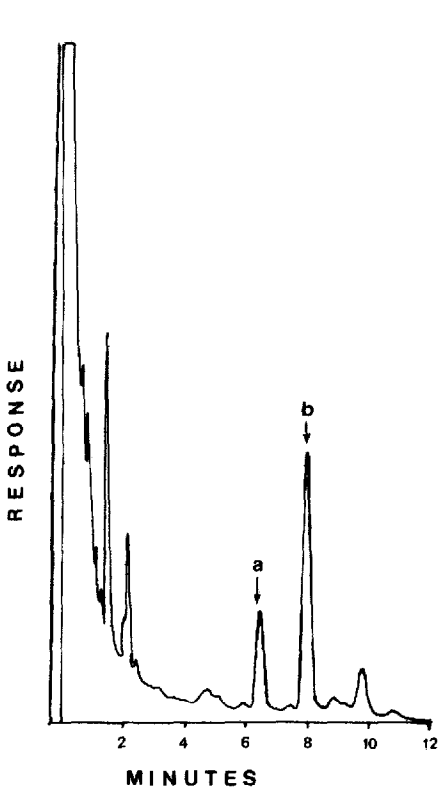
REPRODUCIBILITY OF THE EXTRACTION AND DERIVATIZATION PROCEDURE AND THE GC—FID QUANTITATION OF VERALIPRIDE IN URINE

Quantity (μg/ml)	Recovered (mean ± S.D.)	C.V. (%) ($n = 10$)
2.6	2.35 ± 0.08	3.6
7.78	7.43 ± 0.33	3.0
25.95	23.95 ± 0.55	2.3

TABLE VI

RECOVERY OF VERALIPRIDE EXTRACTED FROM URINE BY DICHLOROMETHANE USING SULPIRIDE AS EXTERNAL STANDARD

Quantity (μg/ml)	Measured (mean ± S.D.)	C.V. (%) ($n = 5$)	Extraction yield (%)
2.59	1.29 ± 0.069	5.3	50
7.78	5.17 ± 0.34	6.5	66.5
25.9	21.70 ± 2.91	13.3	83.8



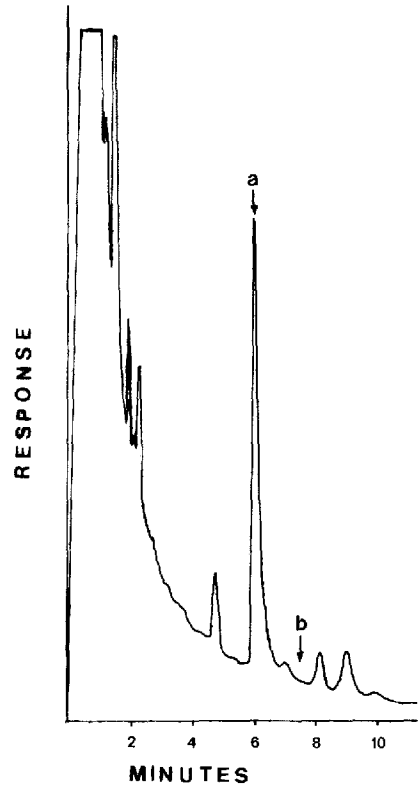


Fig. 4. Chromatogram obtained after GC—FID analysis, on a 3% OV-1 column, of urinary veralipride (b) using sulpiride (a) as internal standard.

Fig. 5. Chromatogram of a blank urine sample. Same experimental conditions as described in Fig. 4. a = Sulpiride; b = veralipride.

identical. Good chromatographic separation has been obtained in these conditions (Fig. 4) without any interferences (Fig. 5).

Calibration curves were established periodically ($y = 1.08x - 0.95$, $r = 0.998$) and gave good linearity in the range 1—100 μg/ml of urine. Standards with known quantities were injected daily to check the accuracy of the method.

Blood and urinary levels

The methods discussed above were used to determine blood and urine concentrations of unchanged veralipride in man after a single intravenous infusion or single oral administration. Two different oral dosage forms were administered: hard gelatine capsules and an oral solution. This paper reports typical biological data which illustrate the analytical method. Plasma concentrations obtained after a 30-min, 100-mg intravenous infusion of veralipride are shown in Fig. 6. The theoretical curves given by computerized calculation were close to the experimental points. Similar results have been obtained for all twelve volunteers participating in this study. Fig. 7 shows a typical curve obtained after analysis of unchanged veralipride excreted in urine after intravenous or oral administration. Different plasma concentration curves were

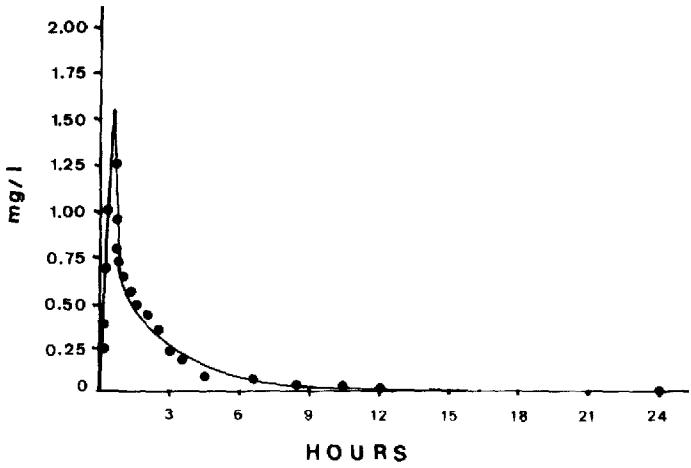


Fig. 6. Veralipride plasma levels after a 100-mg, 30-min intravenous infusion.

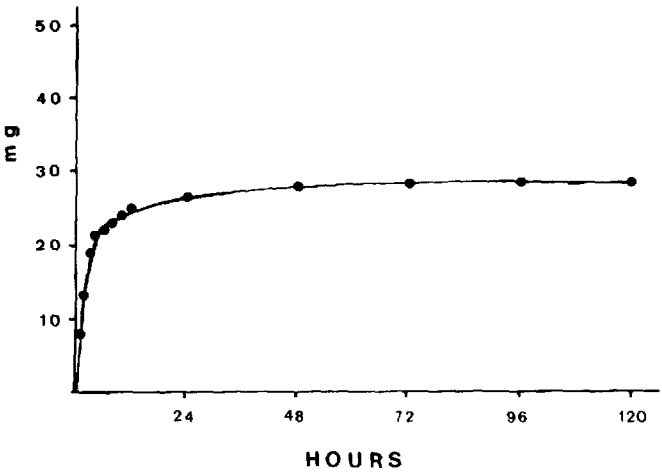


Fig. 7. Cumulated amounts of unchanged veralipride after a 100-mg, 30-min intravenous infusion. The general aspect of the curves after oral administration was similar except that amounts of eliminated veralipride were different.

obtained after oral administration. Fig. 8 shows curves obtained from one volunteer after administration of four single doses of veralipride as an oral solution. Two peaks of maximum concentration appear for the three doses. This phenomenon was observed regularly when this dosage was administered. It was proved to be due to distribution of veralipride in plasma and not to analytical error since it was constantly observed when repeating the same analysis at different dates and with different operators. Samples were injected into the GC—MS system in various working conditions. The same results were always obtained with less than 10% standard error. On the other hand, no double maximum concentration peaks were observed when veralipride was administered in 100-mg hard gelatine capsules (Fig. 9). Only two volunteers among twelve presented a slight rebound effect.

The analytical assays described above permitted successful analysis of

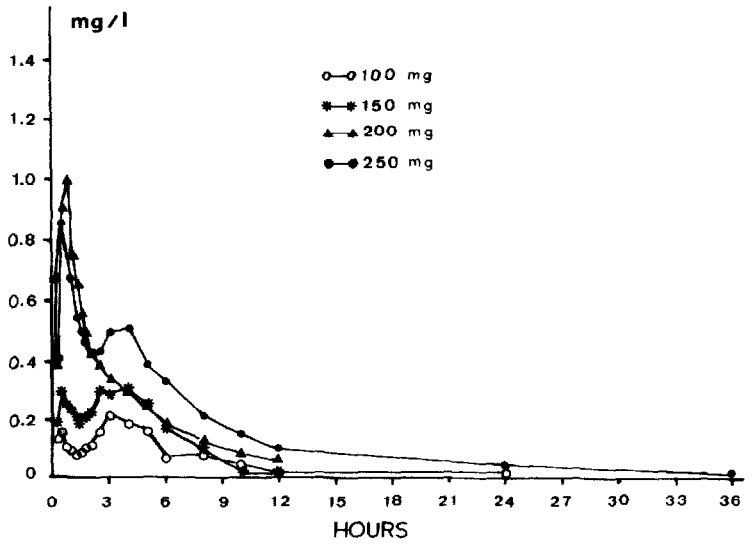


Fig. 8. Plasma levels after single oral administration of various doses of veralipride to one volunteer.

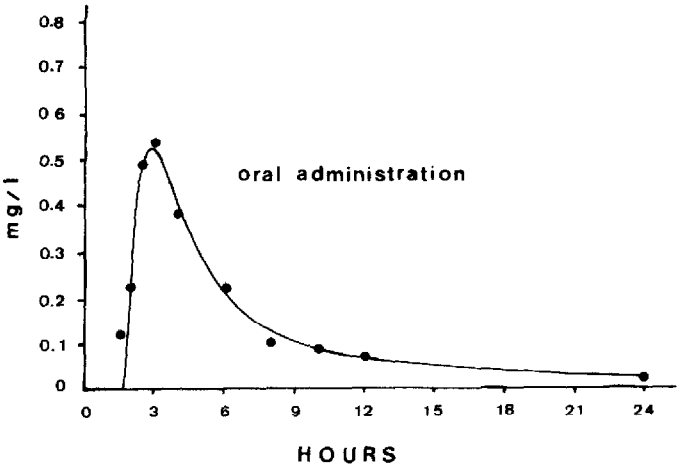


Fig. 9. Plasma levels after oral administration of hard gelatine capsules containing 100 mg of veralipride.

unchanged veralipride in human plasma and urine. This method has been used to establish the pharmacokinetic parameters of veralipride in man and its bioavailability from two oral dosage forms [8, 9].

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