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### Determination of trimetazidine in biological fluids by gas chromatography–mass spectrometry

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Trimetazidine, 1-(2,3,4-trimethoxybenzyl)piperazine, is an anti-ischæmic drug synthesized by the Research Institute Servier (Suresnes, France). In this paper we present the results of a gas chromatographic–mass spectrometric (GC–MS) method for the determination of trimetazidine in human samples.

Trimetazidine was extracted with dichloromethane after alkalization of blood, plasma or urine. After addition of 1 M hydrochloric acid the trimetazidine remained in the aqueous acid phase and impurities in the organic layer, which was discarded. Trimetazidine was then back-extracted into dichloromethane following alkalization. Trimetazidine was derivatized into the *tert.*-butyldimethylsilyl (TBDMS) ether prior to GC–MS analysis [1–3]. The internal standard was an isomer of trimetazidine, 1-(3,4,5-trimethoxybenzyl)piperazine. Ammonia was used as the reagent gas for chemical ionization to increase sensitivity and selectivity of the method [4–6].

## EXPERIMENTAL

### *Chemicals and reagents*

Trimetazidine and the internal standard (I) (base form, MW = 266.34; dichlorhydrate form, MW = 339.27), supplied by Technologie Servier (Orléans, France), were used without further purification. Methanol, ethanol, dichloromethanol, sodium hydroxide, hydrochloric acid, sodium chloride and acetonitrile were of analytical-reagent grade from Carlo Erba (Spiral, Dijon, France). N-Methyl-N-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was purchased from Chrompack (Les Ulis, France).

### *Gas chromatography-mass spectrometry*

A Nermag R-10-10 GC-MS system (Nermag, Rueil Malmaison, France) was used. The gas chromatograph was equipped with an OV-1701 fused-silica capillary column (26 m × 0.32 mm I.D.) (Spiral) and with an all-glass solid-injector (Spiral) [7]. The operating conditions were as follows: column oven temperature, 200°C for 1 min, programmed at 10°C/min to 290°C; injection port temperature, 280°C; transfer line temperature, 280°C; carrier gas, helium, at a flow-rate of 2 ml/min; ion source temperature, 200°C; reagent gas, ammonia.

### *Standard solutions*

Stock standard solutions of trimetazidine and the internal standard were prepared at a concentration of 1 mg/ml in ethanol (base form) and stored at 4°C. Working standard solutions were prepared at concentrations of 10 and 1 ng/μl in distilled water (base form).

### *Procedure for samples of human blood and plasma*

Plasma and blood samples were stored at -20°C. After thawing at 4°C, 1 ml of plasma or blood was added to 100 ng of I in a disposable 15-ml screw-capped tube. The sample was then alkalinized with 500 μl of 20% sodium hydroxide solution. For blood 250 μl of methanol were added to complete clarification prior to alkalinization. After addition of 9 ml of dichloromethane, the mixture was shaken for 30 min and centrifuged at 1000 g for 10 min. The dichloromethane phase was transferred into a 10-ml derivatization tube. The solution was evaporated to dryness under nitrogen and the residue taken up in 50 μl of MTBSTFA-acetonitrile (2:1, v/v), followed by reaction at 65°C for 30 min.

### *Preparation of human urine samples*

A 1-ml volume of thawed urine was added to 5 μg of I and saturated with sodium chloride. After addition of 1 ml of 20% sodium hydroxide solution and 8 ml of dichloromethane, the mixture was shaken for 30 min. After centrifugation

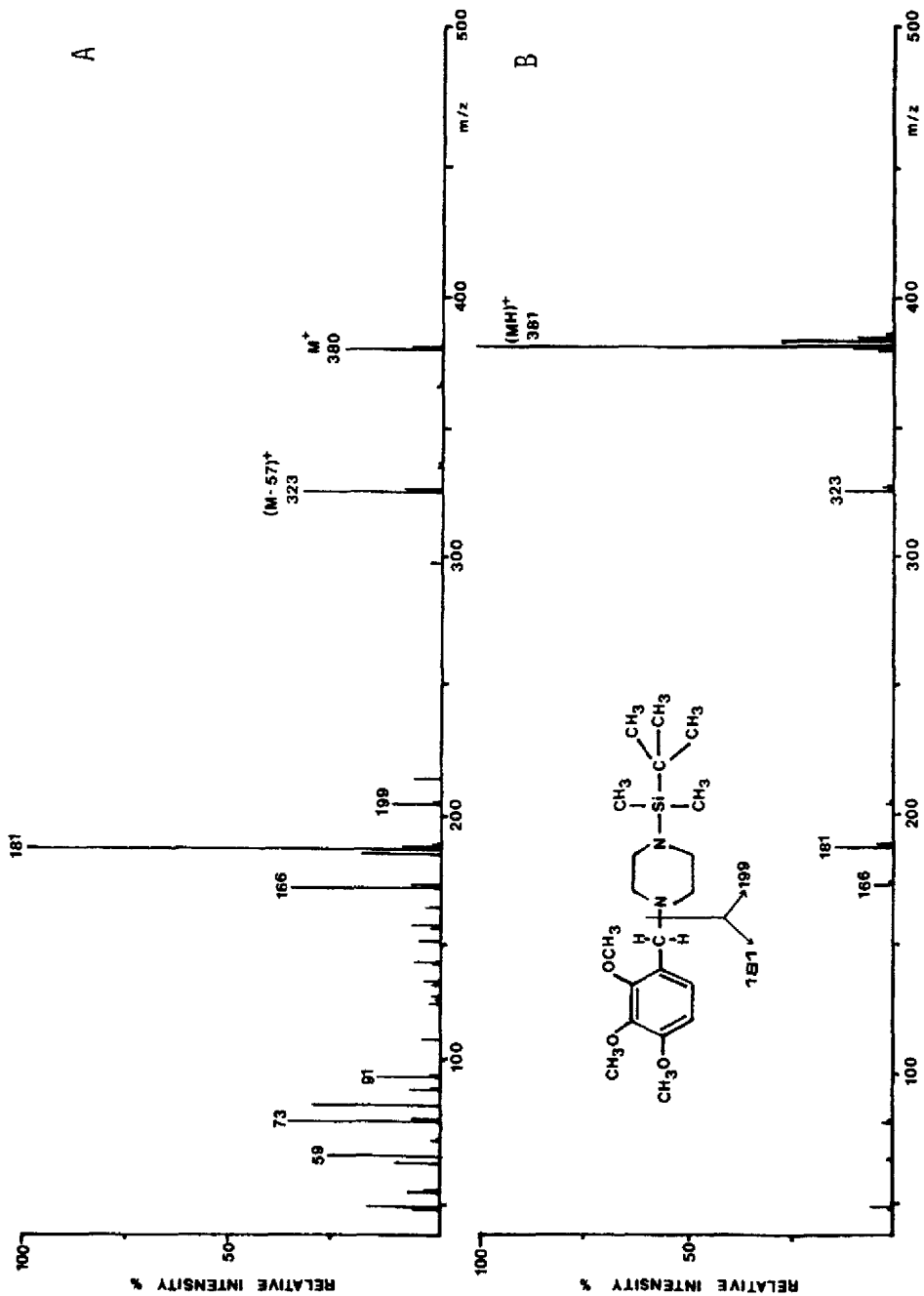


Fig. 1. Normalized 70-eV electron-impact (A) and chemical ionization (B) mass spectra of trimetazidine N-TBDMS.

gation at 1000 g, 2 ml of 1 M hydrochloric acid were added to the dichloromethane phase and the mixture was shaken for 1 min and centrifuged at 1000 g for 10 min. The dichloromethane phase was discarded, the acidic layer was alkalized with 1 ml of 20% sodium hydroxide solution, 8 ml of dichloromethane were added and the mixture was shaken for 1 min. After centrifugation at 1000 g, the organic layer was evaporated to dryness prior to derivatization as for blood or plasma.

## RESULTS AND DISCUSSION

### *GC-MS characteristics*

Fig. 1 shows the electron-impact (EI) and chemical ionization (CI) mass spectra of the TBDMS derivative of trimetazidine. The EI mass spectrum (Fig. 1A) is characterized by ions at  $m/z$  380 ( $M^+$ ),  $m/z$  323 ( $M-57$ ) specific to TBDMS derivatives [1-3] and the ion at  $m/z$  181 due to the loss of the piperazine ring.

Fig. 1B shows the CI mass spectrum of trimetazidine with the pseudo-molecular ion at  $m/z$  381 characteristic of chemical ionization. The internal standard gave similar mass spectra. CI gave a higher sensitivity than EI and led to better specificity by high-mass scanning. The N-TBDMS derivatives used for the GC-MS analysis were stable (more than one week at room temperature).

Typical mass fragmentograms obtained from a blood extract (Fig. 2) show that trimetazidine and I TBDMS have good chromatographic properties after injection on to the OV-1701 capillary.

### *Extraction properties*

The extraction and derivatization recoveries of trimetazidine from human sample using dichloromethane were tested by spiking 1 ml of blank sample with known amounts of trimetazidine and internal standard. Table I shows the extraction recoveries of trimetazidine and I. Dichloromethane appears to be a very suitable solvent and the trimetazidine isomer is a suitable internal standard [8].

### *Calibrations graphs and accuracy*

Calibration graphs were checked by spiking blank samples with different amounts of trimetazidine and 100 ng/ml (blood and plasma) or 5  $\mu\text{g}/\text{ml}$  (urine) I. The results show linear responses in the ranges 1-200 ng/ml (blood and plasma) and 0.5-10  $\mu\text{g}/\text{ml}$  (urine).

Table II gives the characteristics of four identical calibration graphs obtained with pure solutions, spiked plasma, spiked blood and spiked urine. These calibration graphs led to the same characteristics and showed the selectivity of the GC-MS detection. In these four human samples the detection limit is 1 ng/ml, which is similar to the result obtained by Courte and Bromet [9]. The



TABLE I

## EXTRACTION RECOVERY OF TRIMETAZIDINE AND THE INTERNAL STANDARD

Sample	Extraction recovery (mean $\pm$ S.D., $n=5$ ) (%)	
	Trimetazidine	Internal standard
Blood	86.3 $\pm$ 12.9	83.9 $\pm$ 11.4
Plasma	86.5 $\pm$ 9.0	84.8 $\pm$ 9.1
Urine	78.2 $\pm$ 6.1	76.9 $\pm$ 5.5

TABLE II

## CALIBRATION GRAPHS

The samples were spiked with 100 ng/ml internal standard and with trimetazidine in the range 1–100 ng/ml.

Sample	Equation of linear regression	Correlation coefficient	$n$
Pure solutions	$y = 0.0124x + 0.031$	0.99651	8
Blood	$y = 0.0109x - 0.002$	0.99845	8
Plasma	$y = 0.0108x - 0.013$	0.99843	8
Urine	$y = 0.0109x - 0.020$	0.99344	8

TABLE III

## ACCURACY OF GC-MS DETERMINATION OF TRIMETAZIDINE IN BIOLOGICAL FLUIDS

Sample	Concentration added	Mean concentration recovered	Coefficient of variation ( $n=5$ ) (%)
Blood	100 ng/ml	102.2 ng/ml	3.4
Plasma	100 ng/ml	102.0 ng/ml	4.5
Urine	5 $\mu$ g/ml	5.1 $\mu$ g/ml	0.3

centration–time profiles in a man who had received a 40-mg single dose of trimetazidine are presented in Fig. 3.

In conclusion, the proposed method offers a high sensitivity and allows the determination of trimetazidine of human blood, plasma and urine at nanogram levels. It permits the determination of all pharmacokinetic parameters after administration of single doses of this drug to human subjects.

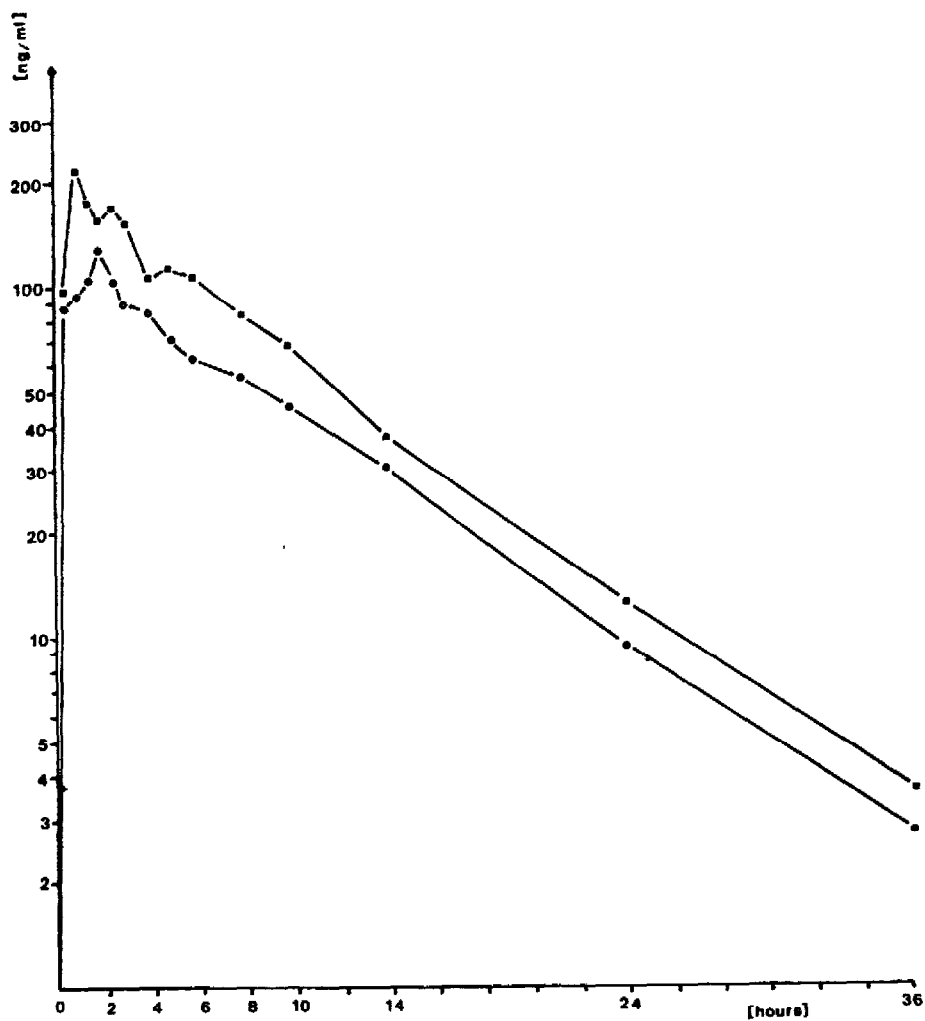


Fig. 3. Blood (■) and plasma (○) concentration-time profiles of trimetazidine in one subject after a single 40-mg oral dose.

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