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Short Communication

Gas chromatographic determination of meprobamate in human plasma

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ABSTRACT

A simplified and rapid gas chromatographic method has been developed for the determination of meprobamate in human plasma. The procedure includes a single-step extraction of alkalinized sample with chloroform, and chromatography on a non-polar fused-silica capillary column with flame ionization detection. The method is accurate (97.7 ± 5.7% at 20 mg/l) and precise (maximum coefficient of variation of 9.5%). It provides an alternative to existing methods and is particularly suitable for toxicological studies.

INTRODUCTION

Meprobamate, which belongs to the carbonate group of anxiolytics introduced in 1955, has been used for many years as a tranquilizer. The identification and quantification of meprobamate are routine in toxicological laboratories. In adults, concentrations in blood are usually between 5 and 20 mg/l, a overdose is over 30 mg/l, and coma hemodynamic troubles occur with concentrations greater than 150 mg/l [1]. Meprobamate is absorbed after oral administration, peak concentrations are reached to plasma in 1–3 h, and the half-life of a single dose in plasma ranges from 6 to 12 h [1].

Previously described methods for the analysis of meprobamate in biological material are: gas chromatography (GC) [2–8] column liquid chromatography [6], thin-layer chromatography (TLC) [9,10] and colorimetry [9]. However, the published methods require extended time [3,6,7],

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a large volume of plasma [2–8], derivatization [6–8] or a double extraction [2]. TLC and colorimetry techniques are time-consuming and lack specificity [9,10].

This paper describes a rapid, sensitive and reproducible GC method for the quantitation of meprobamate in serum.

EXPERIMENTAL

Materials

Meprobamate and carisoprodol (internal standard) were purchased from Clin-Midy Labs (Paris, France). The chloroform and methanol used were HPLC grade from Prolabo (Paris, France).

Instrumentation

A Delsi Instrument Series 30 gas chromatograph (Suresnes, France) equipped with a flame ionization detector and a split–splitless injector was used for the analysis in the split mode with a 1:30 split ratio. GC was carried out on a CP SIL 5 CB fused-silica capillary column (25 m × 0.25 mm I.D., 0.12 μm film thickness) (Chrompack, Les Ulis, France).

The injection, column and detection temperatures were 280, 190 and 280°C, respectively. The flow-rates of hydrogen and air for the detector were 25 and 330 ml/min, respectively. The recorder chart speed was 0.5 cm/min (Servotrace, Paris, France).

Preparation of standards

Stock solutions of meprobamate and the internal standard were prepared by dissolving the appropriate amounts in methanol to make 1 g/l free-base solutions. They were stored in glass volumetric flasks at 4°C. Calibration curves were prepared by diluting appropriate volumes of meprobamate solution in drug-free plasma to give final concentrations of 10, 20, 50, 100 and 200 mg/l.

Extraction

An aliquot (100 μl) of plasma was placed in a screw-cap glass centrifuge tube (10 ml). Internal standard (25 μl for plasma) and 20 μl of sodium hydroxide (4 *M*) were added. After shaking, the sample was extracted with 2.5 ml of chloroform for 1 min by means of a Vortex (Bioblock, Strasbourg, France).

After centrifugation (2600 *g*) for 5 min to separate phases, the upper aqueous and protein layers were discarded by suction, and 2 ml of the organic phase were transferred to a 5-ml conical glass tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved in 50 μl of methanol, and 2 μl of the solution were injected into the chromatograph.

Quantitation

The standards were extracted daily according to the above extraction procedure. Calibration curves were calculated using the peak-height ratio of meprobamate to the internal standard, and the amounts of drug added to plasma by the least-squares method. The peak-height ratios of unknown samples were compared with the standard curve in plasma.

Extraction recovery

The extraction recovery was determined by comparing the peak following injection of the dry residues of plasma spiked with known amounts of meprobamate (20, 50 and 200 mg/l) and extracted as described above, and the responses following direct injection of the same amounts without extraction.

Precision

Low-, medium- and high-concentration quality controls were prepared to contain 20, 50, 200 mg/l in plasma, respectively. Intra-assay precision was determined by analysing each quality control ten times on the same day. Inter-assay precision was determined by analysing one aliquot of each quality control per day for ten days.

Toxicological application

Plasma levels of meprobamate have been measured during 36 h after an intoxication observed in a 65-year-old female patient.

RESULTS AND DISCUSSION

Typical gas chromatograms are shown in Fig. 1. The retention times are 3 min for meprobamate and 3.70 min for carisoprodol. Barbiturates, benzodiazepines, tricyclic antidepressants, neuroleptics do not interfere because retention times are different (Table I). Salicylates and acetominophen are not detected.

The standard curve is linear in the range 0–200 mg/l. The regression equations for six calibrations curves were $y = 0.19x - 0.04$ ($r = 0.999$). Repeated assays of plasma spiked with meprobamate indicated that the reproducibility of the procedure is satisfactory over the calibration range (Table II) from 5 to 6% for within-day and from 4.4 to 9.5% for between-day studies.

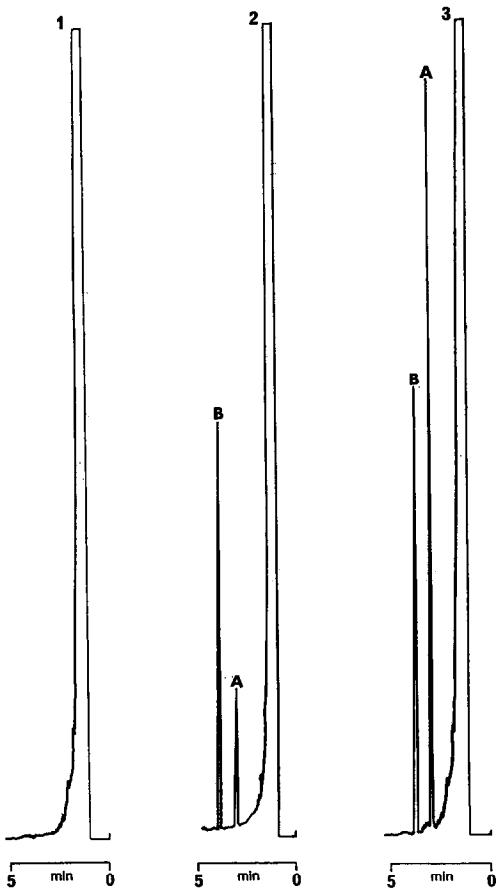

Fig. 1. Gas chromatograms of (1) a blank sample, (2) a plasma sample spiked with 20 mg/l meprobamate and (3) a patient's serum containing meprobamate. Peaks: A = meprobamate; B = internal standard (50 μg/l).

The detection limit of this assay (defined at a signal-to-noise ratio of 2) is 1 mg/l, which corresponds to 0.133 ng injected into the column, and can be further improved by extraction of larger sample volumes.

The extraction recovery of meprobamate from 100 μl of human plasma, which is greater than 94% at concentrations of 20, 50 and 200 mg/l, is comparable with that of a solid-phase extraction method recently developed [4] and better than that of another related liquid phase method [5].

Several authors using GC have described difficulties arising from thermal decomposition of meprobamate [2,7,8]. In our method, the gas chromatogram did not indicate any detectable degradation of the drug under the conditions employed. If degradation did occur, it was constant because the linearity was unchanged over the range 0–200 mg/l.

TABLE I

INTERFERENCE STUDIES

Drug	Concentration (mg/l)	Retention time (min)
Butalbital	100	2.80
Amobarbital	100	2.40
Pentobarbital	100	2.80
Secobarbital	100	3.60
Phenobarbital	100	5.30
Vinbarbital	100	2.50
Diazepam	1000	20.00
Nordiazepam	1000	15.20
Oxazepam	100	8.30
Bromazepam	1000	ND[a]
Lorazepam	1000	14.00
Clomipramine	200	15.60
Declomipramine	200	16.60
Maprotiline	1000	9.20
Amitriptyline	1000	10.20
Nortriptyline	1000	9.00
Pipotiazine	1000	ND[a]
Acepromazine	1000	ND[a]
Aceprometazine	1000	15.00
Cyamemazine	1000	10.60
Chlorpromazine	1000	15.00

[a] ND = not detected.

TABLE II

VALIDATION OF THE METHOD: EXTRACTION EFFICIENCY, WITHIN-DAY AND BETWEEN-DAY REPRODUCIBILITY

Values in parentheses are coefficients of variation (%).

Concentration added (mg/l)	Extraction efficiency (mean ± S.D.)	Measured concentration (mean ± S.D.) (mg/l)	
		Within-day (n = 10)	Between-day (n = 10)
20	97.00 ± 5.70	20.01 ± 0.95 (4.74)	19.98 ± 1.90 (9.50)
50	94.62 ± 4.94	50.25 ± 2.72 (5.41)	50.00 ± 2.23 (4.46)
200	96.83 ± 3.84	200.04 ± 9.98 (4.98)	200.03 ± 12.78 (6.40)

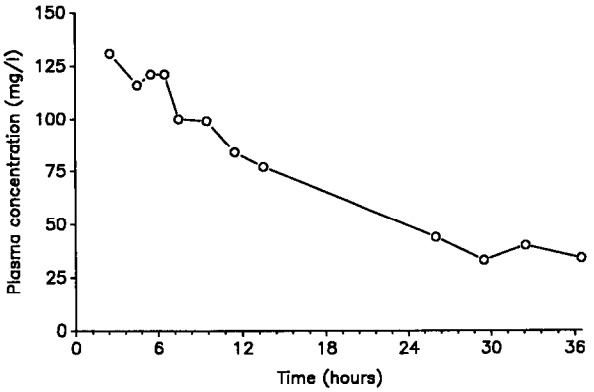

Fig. 2. Plasma concentration–time profile of meprobamate after oral intoxication in one female subject.

Fig. 2 shows a representative profile after oral intoxication; the maximum concentration is 131 mg/l.

CONCLUSION

This assay has several advantages in comparison with previous methods. It requires a smaller sample volume (0.1 ml against 0.5 ml or more [2–8]), which is important in toxicological studies. The extraction is simple to perform and not time-consuming (without derivatization or double extraction). The run-time has been reduced from 10 min [4] to 4 min. The GC procedure is sensitive, accurate, and suitable for large-scale routine analysis.

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