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Short Communication

Gas chromatographic determination of meprobamate in serum or plasma after solid-phase extraction

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ABSTRACT

This gas chromatographic technique of determining meprobamate is based on a solid-phase extraction permitting a time reduction of the analysis and improving sensitivity. Quantification is realized on 500 μl of plasma. The method uses etidocaine as internal standard and does not require derivatization. Thus it is simple, rapid, sensitive and applicable in forensic and clinical toxicological laboratories.

INTRODUCTION

Meprobamate is a mild sedative-hypnotic drug in therapeutic use since the 1950s. Overdose of this drug, even when taken alone, produces intoxication that is often serious and sometimes life-threatening. If therapeutic plasma concentrations are in the range 5–20 mg/l, toxic effects occur at greater than 50 mg/l. Hemodynamic events, such as dramatic acute circulatory failure, are the principal causes of death and ususally appear for concentrations up to 200 mg/l [1,2]. For all these reasons, the quantification of meprobamate represents a classical operation in toxicology.

Various methods for assaying meprobamate have been reported: colorimetry [3,4], thin-layer chromatography [4,5], gas chromatography (GC) [6,7], and column liquid chromatography [8]. Among them, the method of Kintz *et al.* [7] is the most interesting. However, the liquid–liquid extraction required for this analysis is time-consuming and lacks specificity. Therefore, following Arbin and Edjerfjall [6], we have developed a solid-phase extraction (SPE) well suited for emergency cases. It allows a rapid and sensitive GC assay of meprobamate in serum or plasma.

EXPERIMENTAL

Materials

For SPE, we used a Vac Elut sample processing station (Analytichem International) from Prolabo (Paris, France) and LIDA extraction columns Extra-Sep, C_8, 100 mg/1 ml, from Touzart et Matignon (Vitry sur Seine, France).

Stock solutions of meprobamate and etidocaine (internal standard) and standards were prepared with an SMI Uni Pump 200 liquid-handling unit, from Unipath (Lyon, France).

The GC workstation consisted of a 5710 A chromatograph equipped with a flame ionization detector and a 3390 A integrator from Hewlett-Packard (Les Ulis, France) and a 180 cm × 2 mm

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I.D. glass column filled with 3% OV 17 on 100–120 mesh Chromosorb W HP from Interchim (Montluçon, France). Operating conditions were as follows: column, injection port and detector temperatures, 190, 200 and 250°C, respectively; nitrogen carrier gas pressure, 0.95 bar. Analysis was achieved in 11 min. Retention times were: meprobamate, 6.6 min; etidocaine, 9.8 min.

Reagents

Meprobamate was a gift from Sanofi Pharma Industries (Montpellier, France) and etidocaine was a gift from Roger Bellon SA (Neuilly sur Seine, France).

Methanol and acetone were HPLC grade from Carlo Erba (Rueil Malmaison, France). Ammonia and potassium dihydrogenphosphate were of analytical grade from Prolabo. Carbon tetrachloride (HPLC grade) was from Merck (Nogent sur Marne, France).

Phosphate buffers were prepared from a saturated solution of KH_2PO_4 diluted to 1:4 or 1:9 with deionized water (pH 5.4).

Stock solutions (1 mg/ml) of meprobamate and etidocaine were prepared by dissolving 100 mg of free base in 100 ml of methanol and stored at −30°C. These solutions were placed in microtubes (Eppendorff type 2 ml) from ATGC (Paris, France) to produce a range of calibration for meprobamate assay (5, 10, 50, 100, 200, 300 mg/l); the internal standard was at a 100 mg/l concentration. For unknown samples, microtubes with only the internal standard were prepared. These methanolic solutions in microtubes were evaporated to dryness and stored at 4°C until assay.

Procedure

Drug-free plasma (500 μl) was added to each calibration microtube containing meprobamate and internal standard, and 500 μl of unknown sample were added to microtubes containing internal standard only. Plasma samples were vortex-mixed for 30 s. Then, 500 μl of phosphate buffer diluted 1:4 were added to all microtubes, which were vortex-mixed for 30 s and centrifuged for 2 min at 7500 *g*.

Extraction columns installed on the Vac Elut were conditioned with 2 ml of methanol followed by 1 ml of phosphate buffer diluted 1:9. Then prepared plasma samples (500 μl of plasma and 500 μl of phosphate buffer diluted 1:4) were added to the corresponding extraction columns and aspirated through under vacuum [9]. Columns were washed twice with 200 μl of deionized water and twice with 200 μl of carbon tetrachloride. The analytes were eluted with three 200-μl volumes of 2% ammoniacal acetone. The eluate was evaporated under a stream of nitrogen at 40°C. Next, just before injection on the GC column, the residue was dissolved in 150 μl of chloroform, and 3 μl were injected.

RESULTS AND DISCUSSION

This method of assaying meprobamate is linear over the range 5–300 mg/l. The linear regres-

TABLE I

REPRODUCIBILITY AND EXTRACTION EFFICIENCY IN PLASMA

Known concentration (mg/ml)	Within-day precision (n = 10)		Between-day precision (n = 16)		Extraction efficiency (%)
	Mean ± S.D. (mg/ml)	C.V. (%)	Mean ± S.D. (mg/ml)	C.V. (%)	
10	9.45 ± 0.75	7.9	9.30 ± 0.80	8.4	N.D.[a]
50	52.30 ± 3.45	6.6	51.10 ± 3.50	6.9	97.2 ± 2.3
100	101.60 ± 4.80	4.7	102.75 ± 6.15	6.0	97.0 ± 2.7
200	199.40 ± 9.60	4.8	203.00 ± 10.75	5.3	N.D.[a]
300	281.75 ± 12.70	4.5	278.15 ± 13.90	5	95.3 ± 3.7

[a] Not determined.

sion is $y = 0.968x + 1.073$, with a correlation coefficient $r = 0.996$. The within- and between-day precision results can be seen in Table I. The extraction recovery, determined by comparing peak areas of extracted plasma and methanolic standards at the same concentration, is 97.0% over the range of calibration. The limit of detection, at a signal-to-noise ratio of 2, is 1 mg/l meprobamate, which corresponds to 10 ng injected.

The SPE technique enhances the extraction efficiency of meprobamate to 97%, compared with 82.5% with a liquid–liquid method [7] tested in our laboratory. Moreover, the extraction columns can be used twice without any interference. Salicylates, acetaminophen, benzodiazepines and tricyclic antidepressants are not detected by this method.

CONCLUSION

This first SPE of meprobamate provides faster, more efficient and cheaper sample preparation than was possible with traditional liquid–liquid procedures. This rapid and sensitive GC analysis of meprobamate is applicable in all toxicological laboratories.

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