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Note

Determination of meprobamate in serum by alkaline hydrolysis, trimethylsilyl derivatization and detection by gas chromatography–mass spectrometry

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Cases of toxic overdose of meprobamate, a mild tranquilizer, are still prevalent We encountered several cases of accidental or suicidal overdoses from the emergency room due to this drug, making a reliable and accurate serum assay necessary in clinical and forensic labs Meprobamate is readily absorbed after oral administration and has a serum half life of 6–17 h with a mean of 11 h [1] The half-life may be much longer after chronic administration and may be dose-dependent [1] Therapeutic serum concentrations usually range from 5 to 20 μg/ml, with 90% of the dose excreted in urine in 24 h and concentrations greater than 50 μg/ml are usually considered toxic [1]

Methods for quantitation are limited to gas chromatographic (GC) methods since these aliphatic compounds lack any appreciable UV absorbance or fluorescence and are thus not suitable for high-performance liquid chromato-

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graphy (HPLC) unless they are derivatized [2]. Problems encountered with current GC methodology [3,4] are due to the heat instability of meprobamate at the injection port leading to thermal decomposition [3] or the lack of derivatization which results in poor chromatography Other previously described derivatization methods use *n*-butylboronate to form cyclic esters prior to analysis [5] We describe here a simple alkaline hydrolysis to produce free hydroxyl groups followed by rapid trimethylsilyl derivatization for the analysis of meprobamate in serum by gas chromatography–mass spectrometry (GC–MS)

EXPERIMENTAL

Reagents and standards

Prepare meprobamate (Wallace Labs , Cranbury, NJ, U S A) and the mebutamate (internal standard, I S) (Aldrich, Milwaukee, WI, U.S A) standard solutions in methanol Prepare working meprobamate standards in 0 5 ml of pooled serum to establish a standard curve with meprobamate concentrations of 50, 10 and 1 μg/ml The I S. concentration is 520 μg/ml Additional materials are 5 *M* potassium hydroxide, methylene chloride and the derivatizing solution bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylsilane (TMS), which is available commercially (Pierce, Rockford, IL, U S A)

Sample extraction, hydrolysis and derivatization

Add 25 μl of the I S to 0 5 ml of each standard and unknown sample To alkalinize each sample prior to extraction, add 0.1 ml of 5 *M* potassium hydroxide to each tube and then extract with 2 ml of methylene chloride by rotating gently for 5 min Transfer the organic layer (bottom layer) into a 12-ml screw-top test tube and evaporate to dryness under nitrogen at 37°C For alkaline hydrolysis, add 1 0 ml of 5 *M* potassium hydroxide to the dried extract and then heat in a boiling water bath for 10 min Extract the hydrolysis product with 3 ml of methylene chloride and transfer the methylene chloride layer to a small test tube (75 mm × 10 mm) and evaporate to dryness under nitrogen at 37°C Derivatize the dried extract by addition of 50 μl of BSTFA with 1% TMS and heat at 70°C for 20 min to form the trimethylsilyl derivative (see Fig 1) To assure that the derivatized hydrolysis product is homogeneous in solution, add an additional 50 μl of methylene chloride and inject 2 μl into the GC–MS system

GC–MS assay

The instrumentation is from Hewlett-Packard (Palo Alto, CA, U S.A.) consisting of an HP 5890 gas chromatograph with a 5988A mass spectrometer set for electron-impact ionization with a 5970B work station with a 7946 55-megabyte disk drive for data collection For GC separation, we use a 12 m × 0 2

mm I D. fused-silica capillary column coated with 0.33 μm film thickness of cross-linked methylsilicone (HP column No 19091A-102) The flow-rate of helium through the column is 0 9 ml/min with a splitless injection liner, with a total flow-rate of 60 ml/min measured with a bubble flowmeter at the exit port The head pressure is approximately 35 kPa with the linear velocity of carrier gas through the column at 43 cm/s Operating parameters for the mass spectrometer were adjusted with the HP 'autotune' provided with perfluorotributylamine (PFTBA) as the calibration standard

The GC–MS system has the following settings: injection port temperature, 250°C, oven temperature, 100°C with a 1 min initial hold and oven temperature programming at 20°C/min to a final temperature of 270°C. The equilibration time is 0 5 min and the splitless on time is 0 5 min. The ion source temperature is 200°C and the transfer line temperature 280°C. We used an initial solvent delay of 2 min. The detector employed selected-ion monitoring (SIM) of masses 143, 103 and 147 The electron multiplier voltage was 2103 V and the ion source pressure was approximately $1 \cdot 10^{-6}$ Torr

RESULTS AND DISCUSSION

Due to the heat instability of meprobamate, we used the hydrolysis and derivatization steps outlined in Fig. 1 to convert meprobamate into the hydrolysis product, 2-methyl-2-propyl-1,3-propanediol, in order to assay meprobamate by GC. We chose as the I.S., mebutamate (2-methyl-2-*sec* -butyl-1,3-propanediol dicarbamate) which differs only in one side-group (Fig 2) from meprobamate (2-methyl-2-propyl-1,3-propanediol dicarbamate) and this compound has been successfully used as an internal standard for both GC and HPLC assays [2]. On hydrolysis, mebutamate is converted into 2-methyl-2-*sec* -butyl-1,3-propanediol which then can be derivatized as shown in Fig. 1 Retention times are 2.7 and 3.3 min for the derivatized hydrolysis products of meprobamate and mebutamate (I.S.), respectively (Fig. 3). Other structurally similar carbamates such as carisoprodol and methocarbamol (Fig. 2) did not interfere with the assay and are separated by chromatography (Fig. 3). Carisoprodol and methocarbamol had retention times of 5.0 and 6 3 min, respectively Meprobamate is metabolized by hydroxylation of the propyl side-chain, with alkaline hydrolysis, this metabolite will be converted to 2-methyl-2-hy-

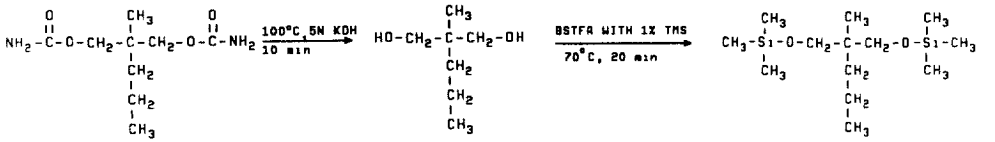

Fig 1 Alkaline hydrolysis of meprobamate into 2-methyl-2-propyl-1,3-propanediol and formation of the trimethylsilyl derivative

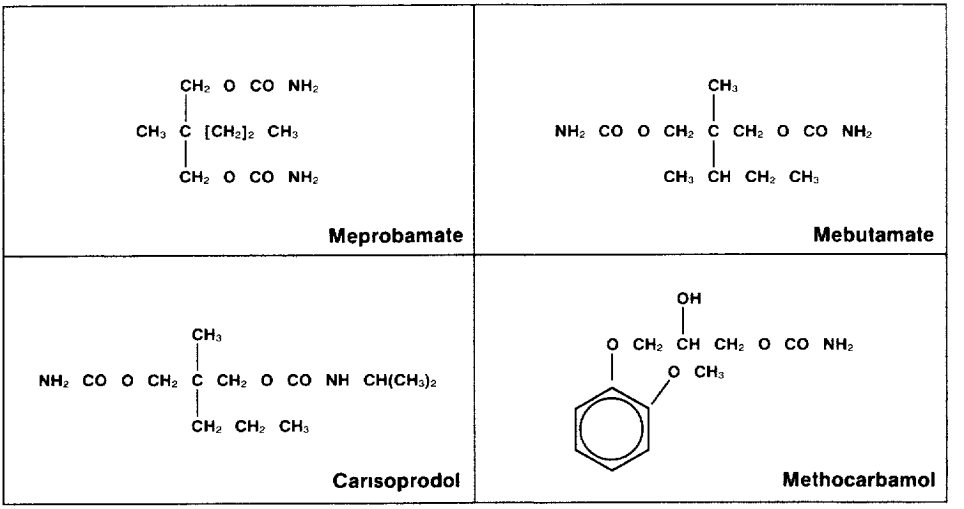

Fig 2 Structures of meprobamate, mebutamate (I S), carisoprodol and methocarbamol

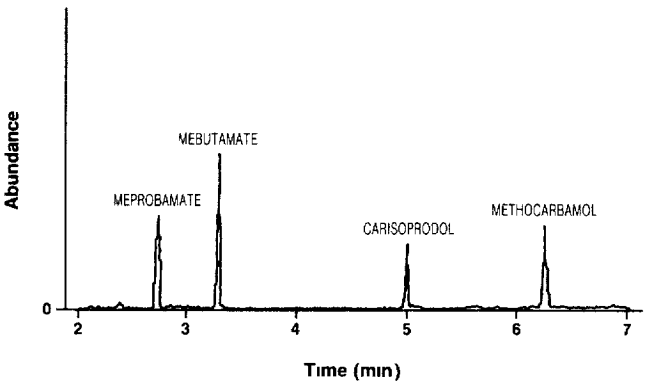

Fig 3 Selected-ion monitoring of masses 147, 143 and 103, showing GC separation of the trimethylsilyl derivatives of the hydrolysis products for meprobamate, mebutamate (I S), carisoprodol and methocarbamol from an unextracted standard of 20 μg of each compound

droxypropyl-1,3-propanediol, which is structurally different from the hydrolysis product of meprobamate, 2-methyl-2-propyl-1,3-propanediol. Ten serum blanks from different patients were taken through these steps and showed to interferences

The abundance of masses for meprobamate and the internal standard are shown in Table I. The three most abundant masses are 143 (base peak), 103 and 147, and these ions were used for quantitation The fragmentation pattern of ions as shown in Table I is very characteristic of trimethylsilyl derivatives of aliphatic glycols [6] Ion 147 represents the $(CH_3)_2Si{=}O^+Si(CH_3)_3$ fragment which occurs following expulsion of the central portion of the glycol (see Fig 1) Mass 103 represents the loss of $CH_2{=}O^+Si(CH_3)_3$ which occurs due to

TABLE I

ABUNDANCE OF MASSES OF THE ALKALINE HYDROLYSIS PRODUCT OF MEPROBAMATE AND MEBUTAMATE (TRIMETHYLSILYL DERIVATIVES)

Mass	Abundance (% of base peak)	
	Meprobamate	Mebutamate (L S)
55	11 8	12 9
69	—	15 5
73	72 3	67 5
74	7 0	5 90
75	30 8	21 3
97	—	11 6
103	21 3	28 1
133	11 3	—
143	100 0	100 0
144	13 8	14 1
147	43 7	20 6
171	7 2	—

this mass loss on both sides of the trimethylsilyl glycol (Fig 1) It is surprising that the 103 fragment represents only 21 3 and 28 1% of the base peak for meprobamate and mebutamate, respectively, since this fragment is present on both sides of the molecule This lower abundance than expected for mass 103 may be due to cleavage through the central carbon of the molecule, resulting in a mass 143, which seems plausible since both meprobamate and mebutamate share the same central structure Masses 75 and 73 represent the characteristic trimethylsilyl fragments, $HO^+=Si(CH_3)_2$ and $(CH_3)_3Si^+$, respectively

We tested the precision and recovery of the method at concentrations of 10 and 50 μg/ml We found mean values of 9 7 μg/ml ($n=12$) and 48 6 μg/ml ($n=12$) corresponding to a coefficient of variation of 6 2 and 6 1%, respectively. We evaluated the recovery by comparing the peak-area ratios (meprobamate/I S) of the hydrolysis products, without and with the addition of 0 5 ml of serum in the overall extraction process The mean recovery of meprobamate after addition of 20 μg/ml pure drug to human serum was 94 6% With this method, meprobamate was detectable down to a level of 1 μg/ml A plot of peak-area ratio versus concentration showed that the assay is linear from 1 to 50 μg/ml with a correlation coefficient of 0 9999

The method presented here overcomes problems associated with previous assays for meprobamate such as heat instability at the injection port [3] or no hydrolysis pretreatment step to allow for derivatization [4] Alkaline hydrolysis, extraction and derivatization are essential steps With the addition of a structurally similar internal standard, mebutamate, this method is very reli-

able, reproducible and sensitive for the determination of serum levels of meprobamate.

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