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[x] To whom inquiries should be directed.

GLC Determination of Meprobamate in Water, Plasma, and Urine

LEO MARTIS and RENÉ H. LEVY[x]

Abstract ☐ A GLC method for the determination of meprobamate in water, plasma, and urine is described. The procedure is based on alkaline hydrolysis of meprobamate followed by silylation of the hydrolysis product to give a trimethylsilyl derivative. Quantitation of the drug was effected using a homolog of the hydrolysis product as an internal standard, which is also silylated during the procedure. The method is specific, sensitive, and reproducible and has been used for the routine analysis of biological samples obtained from meprobamate-treated dogs and humans.

Keyphrases ☐ Meprobamate—GLC analysis in water, plasma, and urine ☐ GLC—analysis, meprobamate in water, plasma, and urine

The quantitative determination of meprobamate (2-methyl-2-propyl-1,3-propanediol dicarbamate) is of interest from a toxicological standpoint because of its continued widespread use as a tranquilizing agent and as a muscle relaxant. The availability of meprobamate in a large number of generic forms as well as recent awareness of questionable bioavailability (1) has increased the amount of effort devoted to determining the quantity of product in solution following dissolution studies (2) as well as the quantity found in plasma and urine. Several spectrophotometric methods (3–9) and GLC procedures (10–21) were reported for the qualitative and quantitative analysis of this drug. Many reported colorimetric methods are adequate for the determination of the drug in dissolution studies, but they lack either specificity or sensitivity for the measurement of meprobamate levels following therapeutic drug doses. Most GLC methods presently available were developed for toxicological purposes and involve direct GLC, with the inherent problem of thermal decomposition at the injection port (20). The need for an analytical procedure useful in pharmacokinetic studies of meprobamate in humans and animals treated with therapeutic or subtherapeutic doses led to the development of the method presented in this report.

EXPERIMENTAL

Reagents—The following reagents were used: stock solutions of meprobamate[1] containing 5, 10, 15, 20, and 25 μg/100 μl in water; stock solution of internal standard (2-methyl-2-ethyl-1,3-propanediol[2], I) containing 75 μg/ml in water; sodium acetate buffer (pH 5.0) (22); 0.25 *M* K_2HPO_4 (pH 7.2); β-glucuronidase[3]; *N,O*-bis-(trimethylsilyl)acetamide[4]; and reagent grade anhydrous ether.

Instrumentation—A gas chromatograph[5] equipped with a hydrogen flame-ionization detector, a recorder[6], and a glass column [1.5 m × 0.63 cm (5 ft × 0.25 in.)] packed with a 3% SE-30 on 80–100-mesh Chromosorb W[7] was employed. A gas chromatograph peak identifier[8] coupled to a recorder[9] was employed for peak identification by mass spectroscopy.

The GLC operating conditions were: column temperature, 115°; injector temperature, 175°; and detector temperature, 175°. The gas flow rates were: carrier gas (helium), 50 ml/min; hydrogen, 30 ml/min; and air, 300 ml/min.

Procedure—*Water Standards*—To 1 ml of water contained in a 15-ml Pyrex test tube, fitted with a Teflon-lined screw cap, were added 100 μl of aqueous meprobamate stock solution, 100 μl of stock solution of I, and 1 ml of 50% KOH. The hydrolysis of meprobamate was carried out at 100° for 10 min. The tubes were cooled under running water, and 3 ml of ether was added. The

[1] Wyeth Laboratories, Philadelphia, Pa.
[2] K & K Laboratories, Hollywood, Calif.
[3] Calbiochem, Los Angeles, Calif.
[4] Tri-sil/BSA, Pierce Chemical Co., Rockford, Ill.
[5] Varian model 1400, Varian Aerograph, Walnut Creek, Calif.
[6] Varian A-25, Varian Aerograph, Walnut Creek, Calif.
[7] Varian Aerograph, Walnut Creek, Calif.
[8] Finnigan Corp., Sunnyvale, Calif.
[9] Visicorder, Honeywell, Denver, Colo.

contents were shaken for 1 min on a mixer[10] and centrifuged at 2000 rpm for 5 min. About 1.9 ml of the ether layer was transferred to a 12-ml glass-stoppered centrifuge tube. The ether was evaporated at 40°, and the residue was reacted with 25 μl of silylating reagent for 10 min at 60°. About 1-2 μl was injected into the gas chromatograph.

Plasma Standards—To 1 ml of plasma were added 100 μl of aqueous meprobamate solution and 5 ml of ether. The contents were shaken for 1 min on the mixer and centrifuged at 2000 rpm for 5 min. Exactly 4 ml of the organic layer was pipeted into a clean tube, and the ether was evaporated at 40°. To the dry tubes, 100 μl of stock solution of internal standard and 1 ml of 50% KOH were added. The hydrolysis of meprobamate and the remaining steps were the same as described for the water standards.

Urine Standards—To 1 ml of urine were added 100 μl of aqueous meprobamate solution, 1 ml of 0.25 *M* K_2HPO_4 buffer, and 5 ml of ether. The drug was extracted with ether, and the remaining steps were the same as described for the plasma standards.

Biological Samples from Drug-Treated Subjects—Appropriate aliquots of samples were pipeted into 15-ml tubes. The quantitative analysis of the samples was conducted as described under their respective standards except for the addition of meprobamate stock solution.

RESULTS AND DISCUSSION

The method described in this report involves extraction of unchanged meprobamate from the biological fluid and alkaline hydrolysis of the drug to 2-methyl-2-propyl-1,3-propanediol (II), followed by silylation of II with *N,O*-bis(trimethylsilyl)acetamide. Compound I is added directly to the sample prior to the hydrolysis. After hydrolysis, ether extraction, and evaporation, the residue is reacted with *N,O*-bis(trimethylsilyl)acetamide whereby I and II are converted to the corresponding trimethylsilyl ethers, III and IV. The latter yield symmetrical peaks as shown in Fig. 1, where peak A corresponds to III and peak B to IV. Peaks C, D, and E are foreign peaks, probably due to traces of moisture present in the sample. The height of the foreign peaks is time dependent. When the samples were allowed to stand at room temperature for about 3–5 hr after the addition of silylating reagent, peaks C and D decreased and finally disappeared without any measurable change in peak height ratio of IV to III.

Determination of Meprobamate: GLC *versus* Colorimetric Methods—Various methods have been reported for the determination of meprobamate, and most of them are either spectrophotometric (3–9) or GLC (10–12) procedures. Many reported colorimetric methods seem adequate for the determination of the drug in dissolution studies, but they are either not sensitive or not specific when applied to biological fluids. Agranoff *et al.* (4) determined the amount of meprobamate by measuring the color resulting from the treatment of an extract of urine with sulfuric acid. Bedson (6) adopted this method to measure the concentration of the drug in blood. However, quantitation at therapeutic concentrations would be difficult using this method since the lowest measurable concentration shown in the calibration curves is 30 μg/ml. Furthermore, the method is not specific because compounds related to meprobamate, such as II and 2-methyl-2-*n*-propyl-3-hydroxypropyl carbamate, also react with sulfuric acid and would thus interfere (4). Hoffman and Ludwig (3) described a colorimetric procedure based on the coloration effected by treatment with *p*-dimethylaminobenzaldehyde and antimony trichloride in acetic anhydride. Several investigators used this method with success for the determination of meprobamate in plasma samples (23, 24). However, when applied to urine samples, the method is probably not specific since a major metabolite of meprobamate, 2-methyl-2-(2-hydroxypropyl)-1,3-propanediol dicarbamate, would interfere with the color reaction. Recently, a colorimetric procedure was reported (9) as an analytical method for the determination of meprobamate in dissolution studies. As pointed out in that report, the method is nonspecific since any N—H-containing substances would interfere.

Although colorimetric methods can be simple, rapid, and sensitive, they are not always specific due to interference of compounds with cognate absorption characteristics. In such instances, interfering compounds may be separated by using procedures such as TLC or solvent partition. These separation procedures can sometimes be tedious and lengthy, in which case the method becomes inadequate for routine analysis of biological samples. Hence, GLC procedures are becoming more popular for quantitative analysis of drugs in biological fluids. Several reports can be found (10–14, 16) where direct GLC was used for the measurement of meprobamate levels in biological fluids. As pointed out previously (20), thermal decomposition of meproba-

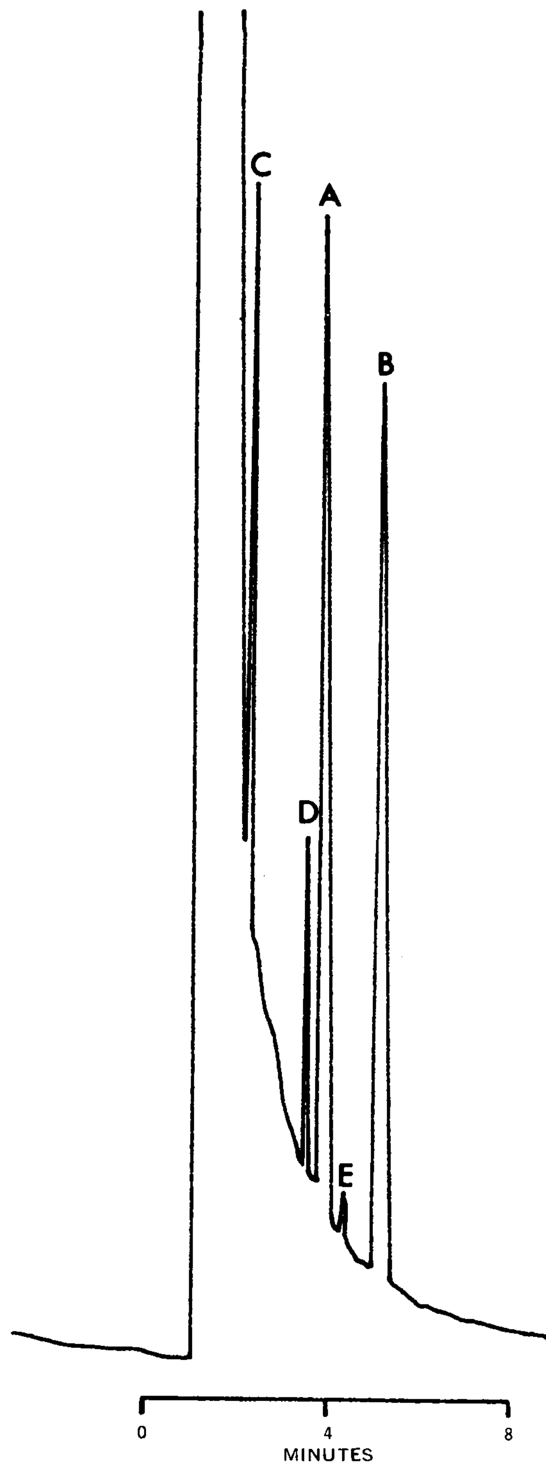

Figure 1—*Chromatogram of a plasma sample (containing 10.5 μg of meprobamate) obtained after administration of 30 mg/kg iv of meprobamate to a dog. Key: peak A, internal standard; peak B, meprobamate; and C, D, and E, foreign peaks.*

[10] Vortex.

Table I—Formation of Trimethylsilyl Derivatives of I and II at 25 and 60°

Minutes after Addition of Silylating Reagent	IV:III Peak Height Ratio Amount of Meprobamate		
	10 μg (25°)	10 μg (60°)	20 μg (60°)
5	0.791	1.00	1.810
10	0.894	1.038	1.997
20	0.993	1.040	2.031
45	1.001	1.040	2.030
120	1.003	1.037	2.020

mate at the injection port presents several problems, the lack of precision being the major drawback. Numerous reports (11, 12, 16–18, 25) document variable decomposition of the drug during direct GLC. Holch and Gjaldbaek (16) found that the main breakdown products of meprobamate are II and 2-methyl-2-propyl-1,3-propanediol monocarbamate. Hence, the decomposition of meprobamate can be overcome if it is converted to II before injecting into the gas chromatograph. However, II is a very polar compound and, when chromatographed directly, variable losses occur due to adsorption. Such losses were found in several glass columns packed with 3.8% UC-98 on Chrom W, 5% UCW-98 on Chrom W, 3% Carbowax on Chrom W, and 3% SE-30 on Chrom W. In every case, the coefficient of variation on repeated injections (five) of the same sample varied from 5 to 8%. The method proposed by Skinner (17) involves direct hydrolysis of meprobamate without extraction of unchanged drug from the biological sample. Therefore, any metabolite of meprobamate that, upon hydrolysis (as discussed later), gives II will interfere with the assay. The hydrolysis approach was also used (18), but those studies were limited to determination in water at relatively high concentrations.

Specificity of Method—Since the proposed method involves the formation of derivatives of meprobamate and the internal standard (to overcome problems associated with direct GLC), it is necessary to test for the specificity of the method. In the present method, any metabolite of meprobamate that undergoes a change in the carbamate moiety without any change in the rest of the molecule could interfere with the assay. Of the known metabolites of meprobamate in dog, rabbit (26, 27), and man (28, 29), the *N*-glucuronide of meprobamate is the only species that could thus interfere with the determination of unchanged drug in biological samples since it yields II upon alkaline hydrolysis. It was, therefore, necessary to introduce an ether extraction step before alkaline hydrolysis to remove meprobamate, leaving the *N*-glucuronide of meprobamate in the aqueous phase.

To test the specificity of the ether extraction step, the following experiment was performed. An oral dose of 800 mg of meprobamate in solution was administered to a male volunteer, and eight venous blood samples were collected at predetermined intervals. The blood samples were centrifuged for 5 min at 2000 rpm, and 3 ml of plasma was harvested from each sample. Each of the eight plasma samples was divided into three 1-ml portions, thus yielding three identical lots of Samples A, B, and C. To test whether II was present as a metabolite in drug-treated man, the plasma samples of Lot A were extracted with ether and the residue obtained after evaporation of ether was reacted with silylating reagent. Neither II nor I was present *in vivo.* The plasma samples of Lot B were assayed as described under *Plasma Standards,* except for the addition of meprobamate stock solution and the initial ether extraction step. The plasma samples of Lot C were analyzed as described under *Biological Samples from Drug-Treated Subjects* where the initial ether extraction step is included. Plasma samples collected later than 60 min after drug administration yielded higher values in the samples of Lot B than in those of Lot C. These results suggest that the *N*-glucuronide of meprobamate at least in part is not extracted by ether and that it can interfere with the determination of unchanged drug if selective separation is not achieved.

Table II—Determination of Meprobamate: Peak Height Ratios of IV to III at Five Meprobamate Levels

Sample	Amount of Meprobamate, μg	Number of Determinations	IV:III Peak Height Ratio, Mean ± *SD*
Water	5	6	1.042 ± 0.028 (2.7)[a]
Water	10	2	2.100
Water	15	6	3.133 ± 0.051 (1.6)[a]
Water	20	2	4.104
Water	25	6	5.047 ± 0.0781 (1.5)[a]
Urine	5	6	0.872 ± 0.0133 (1.5)[a]
Urine	10	2	1.836
Urine	15	6	2.535 ± 0.0237 (0.9)[a]
Urine	20	2	3.337
Urine	25	6	4.243 ± 0.0986 (2.3)[a]

[a] Corresponding coefficient of variation in percent.

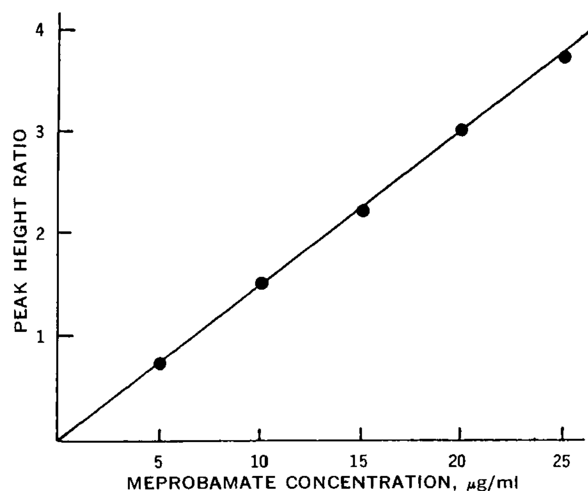

Figure 2—*Calibration curve for meprobamate extracted from rabbit plasma.*

To test whether any *N*-glucuronide at all is extracted into ether, the following experiment was performed. An oral dose of 800 mg of meprobamate was administered to a male subject and urine was collected for 48 hr. The hydrolysis of the *N*-glucuronide of meprobamate in urine samples was carried out with β-glucuronidase according to the procedure reported by Beer and Gallagher (22). The time required for maximum hydrolysis was obtained from preliminary experiments where it was found that enzymatic hydrolysis was complete in 5 days. β-Glucuronidase was then allowed to react for 5 days on four samples of evaporated ether extracts of urine which were then carried through the assay. Controls not containing β-glucuronidase were also run through this procedure. Peak height ratios obtained for samples and controls were compared using a paired *t*-test and found to be not significantly different at $p \pm 0.05$. The same experiment was repeated using a chloroform–carbon tetrachloride mixture as the extraction solvent. This solvent mixture was used previously (3) for extracting unchanged meprobamate from biological samples in that colorimetric method. This solvent mixture did not extract any *N*-glucuronide of meprobamate either. It was found, however, that when used as the extracting medium, ether removes approximately 30% more meprobamate than does a chloroform–carbon tetrachloride mixture.

Mass Spectra of Trimethylsilyl Derivative of II—Although the described experiments demonstrated that the *N*-glucuronide of meprobamate does not interfere with the assay, it is still possible that an unknown metabolite of meprobamate could yield a peak having identical retention characteristics as those of IV. To test for this possibility, the trimethylsilyl derivative of II prepared from an aliquot of a 48-hr urine sample obtained from a human subject (after oral administration of 800 mg of meprobamate) and the one prepared from pure meprobamate were studied by a combination of GLC–mass spectroscopy. The mass spectra of

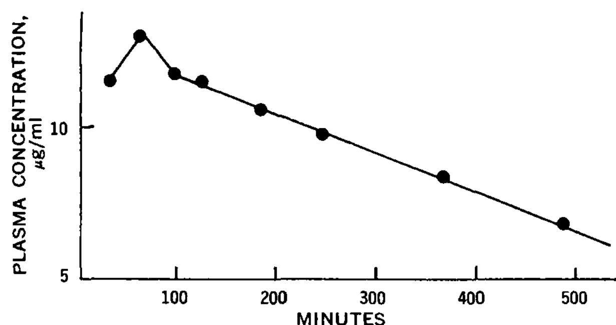

Figure 3—*Plasma levels of meprobamate following oral administration of 800 mg (in solution) to a human subject.*

IV obtained from the urine sample and the one resulting from reacting pure meprobamate were identical. Thus, it can be concluded that peak B corresponds only to meprobamate and that there is no interference from any other metabolite.

Formation of Trimethylsilyl Derivatives of I and II—To eliminate errors due to possible losses in preparation of the sample or to inaccurate injection, a known amount of an internal standard is added to the sample. Compounds to be used for this purpose should closely resemble the drug to be measured in physicochemical properties, and they should not occur spontaneously in the sample. The internal standard used in the present method is a compound very similar to II. The internal standard is added to the sample before hydrolysis of the drug, and it is carried through the steps of ether extraction and silylation. The peak due to the trimethylsilyl derivative of this internal standard (III) is symmetrical, is well resolved, and has a retention time close to that of IV. Formation of trimethylsilyl derivatives of I and II was required to overcome adsorption of these compounds on the column. Table I gives peak height ratios of IV to III as a function of time following the addition of silylating reagent at two temperatures and at two meprobamate concentrations. The peak height ratio reached the maximum value within 10 min at 60° and remained constant for at least 2 hr; subsequent experiments indicated that the peak height ratio did not change for at least 15 hr when the samples were kept in the refrigerator. At the operating conditions of the present method, III and IV were found to be stable.

Accuracy and Precision—Peak height ratios were calculated by dividing the height of the peak due to meprobamate (IV) by the height of the peak due to the internal standard (III). Calibration curves were constructed for "spiked" water, plasma, and urine samples by plotting the amount of meprobamate against the peak height ratio. A typical calibration curve obtained from spiked plasma samples is shown in Fig. 2. Table II gives peak height ratios of IV to III for various amounts of meprobamate in water and urine. In all three instances, a plot of the data (peak height ratio *versus* amount of meprobamate) yields a straight line passing through the origin, with correlation coefficients of 0.999, 0.999, and 0.998 for plasma, water, and urine, respectively. The small coefficients of variation indicate that the reproducibility of the method is adequate. The amount of drug present in biological samples obtained from a drug-treated subject is read from the calibration curve constructed simultaneously with the sample to be assayed.

Application—The proposed method was used in the determination of the biological half-life of meprobamate in a human volunteer following the oral administration of 800 mg in an aqueous solution. A semilogarithmic plot of the plasma concentration-time data is shown in Fig. 3. The half-life (7 hr) calculated from this plot is in agreement with reported half-lives for meprobamate in humans (23).

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[x] To whom inquiries should be directed.