An Automated Colorimetric Method for the Determination of Meprobamate and Other *N*-Unsubstituted Carbamates in Pharmaceutical Products

L. F. CULLEN, L. J. HECKMAN, and G. J. PAPARIELLO

Abstract □ An automated colorimetric procedure, based on the adaptation of the cobalt complex method for *N*-unsubstituted carbamates, has been developed for the analysis of meprobamate and other structurally related compounds in pharmaceutical products. The technique is capable of analyzing 15 samples per hour with a relative standard deviation of 0.9% at the 400-mg. meprobamate level. Data collected on commercial formulations of a number of meprobamate combination products in addition to results on several trade packages containing structurally related therapeutic components describe the wide applicability of the method. Specificity of the procedure with respect to the analysis of intact meprobamate in the presence of its photochemical, hydrolytic, and thermal degradation products was demonstrated by comparison to quantitative thin-layer chromatography values.

Keyphrases □ Meprobamate dosage forms—automated analysis □ Carbamates, *N*-unsubstituted, dosage forms—automated analysis □ Diagram, automated procedure—meprobamate, related compounds, analysis □ Colorimetric analysis—automated spectrophotometric procedure □ TLC—analysis confirmation

Meprobamate, effectively and widely employed as a skeletal muscle relaxant and tranquilizing agent, is the essential therapeutic component of numerous pharmaceutical preparations. Requests for a large number of meprobamate assays in the pharmacy development and quality control areas necessitated the development of an automated method of analysis for tablet formulations.

Several colorimetric methods (1–7) and a near-IR spectrophotometric method (8) have been reported for the quantitative determination of meprobamate. The methodology of the procedures, however, are not readily adaptable to an automated system.

Devaux *et al.* (9) described a sensitive and specific colorimetric method for the manual determination of meprobamate and structurally related, physiologically active compounds in pharmaceutical formulations. The procedure is based upon the formation of an alkaline cyanate resulting from the reaction of potassium hydroxide on the unsubstituted carbamate group, and subsequent spectrophotometric measurement of the blue cobalt-cyanate complex at 605 mμ. This method has been adapted to an automatic analyzer system[1] following modifications, *i.e.*, the use of different reagent concentrations, volume ratios of reagents, and reaction conditions to satisfy the essential flow dynamics of the automated technique.

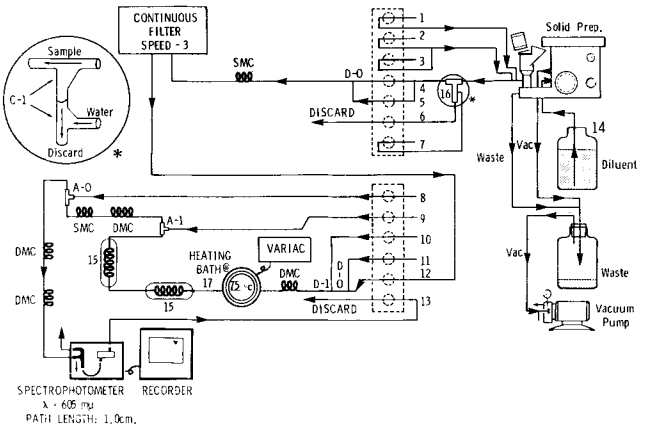

Figure 1—*Automated flow diagram. Key: 1, 0.8 ml./min. air (Tygon); 2,3, 2.76 ml./min. SDA No. 30 alcohol; 4,5, 2.03 ml./min. sample; 6, 0.60 ml./min. discard (Tygon); 7, 0.42 ml./min. SDA No. 30 alcohol (Tygon); 8, 2.03 ml./min. 0.2% cobalt chloride reagent; 9, 1.44 ml./min. acetic acid reagent; 10, 2.50 ml./min. air (Tygon); 11, 1.20 ml./min. 2.5N potassium hydroxide reagent (Tygon); 12, 2.76 ml./min. sample; 13, 2.76 ml./min. flowcell; 14, reservoir- SDA No. 30 alcohol; 15, water-cooled mixing coil (No. 114–209); 16, decantation trap; 17, heating bath with 2 × 40 ft. glass coils, 2.4 mm. i.d.*

Details of the automated procedure and analytical data on a number of meprobamate products on the market in addition to results on trade packages of several structurally related active drugs are described. Specificity of the method for the analysis of meprobamate in the presence of common active and inactive product components and validation of the technique as a stability indicating procedure will also be discussed.

EXPERIMENTAL

Apparatus—A Technicon automatic analyzer system consisting of the following modules: (*a*) Solid-prep sampler, programmed at 15 samples per hour; (*b*) proportioning pumps (two required); (*c*) continuous filter, speed 3; (*d*) spectrophotometer[2] equipped with two 1.0-cm. liquid micro-aperture flow cells[3]; (*e*) linear-log potentiometric recorder[4]; (*f*) heating bath with Variac attachment[5]; and

[1] AutoAnalyzer, Technicon Corp., Tarrytown, N. Y.

[2] Beckman model DB, Beckman Instruments, Inc., Fullerton, Calif.
[3] Beckman catalog No. 97290.
[4] Beckman model 93506.
[5] General Radio Co., West Concord, Mass.

Table I—Effect of Common Active and Inactive Components on Color Development

Component	Component: Meprobamate Ratio	Interference
Alginic acid	0.1:1	Nil
Amberlite	0.5:1	Nil
Aspirin	3.0:1	Nil
Caffeine	0.5:1	Nil
Calcium stearate	0.2:1	Nil
Codeine phosphate	0.5:1	Nil
Ethoheptazine citrate	2.0:1	Nil
Lactose	4.0:1	Nil
Magnesium stearate	0.2:1	Nil
Methocel	0.5:1	Nil
Phenacetin	2.0:1	Nil
Pentaerythritol tetranitrate	0.5:1	Nil
Starch	1.0:1	Nil
Stearic acid	0.5:1	Nil
Sterotex	0.1:1	Nil
Talc	1.0:1	Nil

(*g*) transmission lines and proportioning tubes (in the flow diagram those which are not designated as Tygon are Acidflex).

Reagents and Solutions—(*a*) SDA No. 30 alcohol (ethanol–methanol 10:1); (*b*) 2.5 *N* potassium hydroxide in 95% ethanol in water (v/v)—on the automated manifold this reagent is aspirated from a closed polyethylene bottle with drying tube attachment containing anhydrous calcium sulfate; (*c*) glacial acetic acid in 95% ethanol (2:1 v/v); (*d*) 0.2% cobalt chloride hexahydrate in 90% ethanol in water (v/v). The cobalt chloride is heated at 100° for 12 hr. before placing in solution. On the automated manifold this reagent is aspirated from a closed polyethylene bottle with drying tube attachment containing anhydrous calcium sulfate; (*e*) meprobamate analytical reference standard material.[6]

Prior to the addition of the cobalt chloride reagent line, the manifold is conditioned by pumping all other reagents for 0.5 hr. This buffers the system at a pH of about 6.0, thus preventing precipitation of cobalt hydroxide which would occur in an alkaline medium.

Automated Methodology—Figure 1 is the flow diagram indicating the automated equipment arrangement for this analytical procedure. In operation, three standards of the appropriate level of meprobamate reference material powder are placed on the sample plate, followed by samples of the intact or powdered tablets. Standardization is repeated at the end of a series of 15 samples in order to minimize the effects of reagent changes and instrumental variations. Samples are introduced into the Solid-prep sampler, programmed at 15 samples per hour, and homogenized in the denatured alcohol. During the period of homogenization the sample is diluted and dissolution of meprobamate occurs. A small portion of the mixture is aspirated into the flow system and is automatically filtered to remove insolubles. To prevent mechanical obstruction from the insoluble excipient materials at the tubing connections, a decantation trap is placed between the Solid-prep unit and the proportioning pump to remove the majority of the solid matter. The residual insolubles are removed by the continuous filter module. After passage through the filter module, the filtrate is combined with a stream of 2.5 *N* potassium hydroxide solution, segmented with air, mixed, and then heated at 75°. Following a heating period of approximately 12 min., the sample stream is immediately cooled in water-jacketed coils and combined with a stream of ethanolic acetic acid. This stream is joined with the cobalt chloride reagent and passed through two double mixing coils, which permit a thorough mixing and act as time-delay coils for complete color development. The resultant blue complex is spectrophotometrically measured at a wavelength of 605 mμ. Calculations are made using corresponding absorbances of standards and solid dosage formulation samples.

TLC System—Silica Gel GF precoated glass plates (10 × 20 cm. with a 250-μ adsorbent layer)[7] were activated by heating at 105° for 0.5 hr. before use. The chromatogram was developed in a saturated chamber containing ethyl acetate. When the solvent front ascended approximately 15 cm. from the origin, the plate was removed and air-dried. Visualization was effected by spraying the plate with 5% vanillin in concentrated sulfuric acid, and heating at approximately 115° for 15 min.

[6] NF reference standard, American Pharmaceutical Association, Washington, D. C.

[7] Analtech, Inc., Wilmington, Del.

Table II—Comparison of Colorimetric and Quantitative TLC Analysis of Intentionally Degraded Meprobamate Samples

Sample Treatment	% of Initial—Colorimetric Method	% of Initial—TLC Method
Meprobamate powder stored at 120° for 2 months	76	78
Meprobamate powder stored at 220° for 6 hr.	69	68
Meprobamate powder exposed to short-wave UV light for 4 months	83	85
Solution of the meprobamate in 0.2 *N* NaOH stored at 60° for 1 week	49	46
Solution of the meprobamate in 0.2 *N* HCl stored at 60° for 1 week	63	59
Meprobamate tablet formulation stored at 220° for 6 hr.	65	68
Meprobamate tablet formulation exposed to short-wave UV light for 4 months	87	89

RESULTS AND DISCUSSION

Specificity—Initially, it was important to ascertain the specificity of the automated procedure by establishing the effect of common active and inactive components, which may be present in a meprobamate solid dosage formulation, on the color development. This phase of the experimental evaluation was carried out by adding each of the various components to 400 mg. of meprobamate. The values obtained from these mixtures were compared to those of reference standard meprobamate. In order to emphasize any interference, the active or excipient materials were added at a level several-fold greater than the amounts usually found in meprobamate products. The remarkable selectivity of the reaction is illustrated in Table I. No interference was experienced from these materials at the indicated component:meprobamate ratios.

Suitability as Stability Method—Specificity of the method for analysis of intact meprobamate in the presence of its photochemical, hydrolytic, and thermal degradation products was demonstrated by comparing analytical values of intentionally degraded samples to those obtained by quantitative TLC. In the TLC procedure, the meprobamate was separated from its degradation products on a chromatographic plate. The intact meprobamate was removed from the plate, eluted then assayed to obtain a quantitative value. The data obtained are summarized in Table II. Since there is good agreement between the values by the two techniques, it is concluded that the colorimetric procedure is stability indicating.

Linearity—Figure 2 is a reproduction of an actual recording curve obtained by analyzing standards of meprobamate reference

Table III—Comparative Data Obtained on Meprobamate Tablets by Automated Procedure and Manual NF XII Procedure

Manufacturer	Labeled Amount, mg./Tablet	% of Claim[a]—Automated	% of Claim[a]—NF
A	200	100	100
	400	99	99
B	200	101	100
	400	99	100
C	200	98	99
	400	101	101
D	200	100	100
	400	99	100

[a] Data from a single assay on a 10-tablet composite sample.

Table IV—Recovery of Meprobamate Added to Meprobamate Combination Products

Product	Meprobamate, mg./Tablet Present	Added	Total Recovered	% Recovered
Meprobamate with conjugated estrogens	200	20	218	99
Manufacturer A	200	50	245	98
Manufacturer B	398	40	436	100
	398	100	503	101
Meprobamate with tridihexethyl chloride	203	20	224	100
Manufacture A	203	50	251	99
Manufacturer B	405	40	441	99
	405	100	507	100
Meprobamate with dextroamphetamine	396	40	437	100
	396	100	507	102
Meprobamate with hydrochlorothiazide	199	20	215	98
	199	50	252	101
Meprobamate with pentaerythritol tetranitrate	198	20	223	102
	198	50	247	100
Meprobamate with aspirin and ethoheptazine citrate	148	15	161	99
	148	40	189	100

material in the 100 to 625-mg. range. This curve also contains a recording of the steady state, during which time a 625-mg. standard was continuously sampled, which further defines the excellent flow characteristics of the automated system. When the values for the standards in Fig. 2 are plotted, a linear relationship exists between absorbance and meprobamate concentration.

Precision—Relative standard deviations of 1.3 and 0.9% were determined by performing 20 replicate assays on meprobamate reference material at the 200 and 400-mg. levels, respectively. A series of 20 replicate assays run on a composite sample of a tablet formulation at the 200-mg. meprobamate per tablet level produced a relative standard deviation of 1.3%.

Accuracy—In order to establish the accuracy of the proposed method for the analysis of meprobamate in marketed tablets, comparative results between the automated procedure and the official, manual NF XII titrimetric procedure (10) were collected (Table III). As indicated by the excellent correlation in values, it is evident that the automated technique provides an accurate means of evaluating this product.

With respect to meprobamate combination products, the accuracy of the procedure was determined by adding known quantities of meprobamate in the 15–100-mg. range to several powdered commercial tablet formulations and measuring the percentages recovered. The results, summarized in Table IV, indicate that the

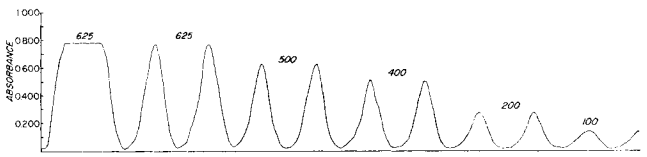

Figure 2—*Reproduction of recording curve on meprobamate standards (mg.) at the rate of 15 samples per hour.*

Table V—Results of Analysis of Pharmaceutical Products Containing an *N*-Unsubstituted Carbamate

Product	Carbamate	mg./Dosage Labeled Amount	Found[a]	% of Claim
Carisoprodol Manufacturer A	Carisoprodol	350	344	98
Manufacturer B	Carisoprodol	350	352	100
Carisoprodol with prednisolone	Carisoprodol	350	354	101
Carisoprodol with phenacetin and caffeine	Carisoprodol	200	204	102
Carisoprodol with phenacetin, caffeine, and codeine phosphate	Carisoprodol	200	198	99
Mebutamate	Mebutamate	300	293	98
Mebutamate with hydrochlorothiazide	Mebutamate	150	152	101
Tybamate capsules Manufacturer A	Tybamate	350	346	99
Manufacturer B	Tybamate	350	352	101

[a] Average value from a duplicate assay on a 10-dosage unit composite sample.

recovery for each sample was essentially complete and unaffected by the actives and the inactive excipient components in the products.

Application to Other N-Unsubstituted Carbamates—Devaux *et al.* (9) reported that the cobalt-cyanate complex reaction could be employed for other *N*-unsubstituted carbamates. There are on the market three dicarbamate compounds; namely, carisoprodol, mebutamate, and tybamate, which have at least one *N*-unsubstituted carbamate. It was of interest, therefore, to investigate the possible application of the automated technique to such related medicinals. Results obtained on commercial samples of the compounds were in good agreement with the declared values (see Table V).

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