To check on the reliability of this correction factor, a number of solutions containing varying amounts of acetaminophen and *p*-aminophenol were run. The results of this experiment are summarized in Table I. It is seen that in those samples where acetaminophen is the predominant species, accurate values can be obtained. From a practical point of view, this is not a very limiting shortcoming of the method since one is almost always analyzing samples with less than 10% or no more than 20% degradation.

To determine the general applicability of the technique to dosage form analysis, studies were run on acetaminophen in combination with other drugs, common tablet excipients, and common components of liquid preparations. In these studies, 50 mg. of acetaminophen was combined separately with each component under study. The amount of each component was chosen to represent that level normally found in a formulation. Polarograms were obtained on these mixtures as well as on the component under investigation without any acetaminophen present. The results of this work are listed in Tables II–IV. Ascorbic acid was the only interfering component found.

The relative standard deviation of this technique for the analysis of solid dosage forms was found to be 0.5%; for liquid dosage forms it was 0.7%. This peak polarographic technique gives results that are in good agreement with those obtained by the NF XIII (13) method on commercial samples (Table V).

It should be noted that the amount of water present in the supporting electrolyte influences the magnitude of the peak current for acetaminophen. If the water content is increased over a certain level, it decreases the measured peak current for a given concentration of acetaminophen. This is demonstrated in Table VI. A maximum water concentration of 5% in the supporting electrolyte is recommended.

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Stability-Indicating Method for Analysis of Homatropine Methylbromide in Pharmaceutical Formulations

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Abstract □ A stability-indicating method of analysis for homatropine methylbromide in pharmaceutical formulations containing antacids was developed. The method is based upon the extraction of homatropine methylbromide with methanolic hydrochloric acid, followed by reaction with a modified Dragendorff reagent. The iodobismuthate complex is measured spectrophotometrically in a stabilized methanol–acetone solution at 382 nm. A homatropine methylbromide degradation product was isolated from these formulations and identified as tropine methylbromide. The Dragendorff method of analysis was found to be selective for homatropine methylbromide in that there was no interference from its major decomposition product.

Keyphrases □ Homatropine methylbromide formulations with antacids—extraction, iodobismuthate (modified Dragendorff reagent) complexation, UV analysis, compared to silver nitrate titration □ Dragendorff reagent, modified—determination of homatropine methylbromide in formulations with antacids □ UV spectrophotometry—analysis, homatropine methylbromide, Dragendorff reagent complex

A specific method was desired for analyzing homatropine methylbromide in two capsule formulations containing antacids along with other excipients. Because some problems had been encountered with a relatively nonspecific silver nitrate titration for samples stored under accelerated conditions, experimental work was initiated to develop an alternative method of analysis for homatropine methylbromide in these formulations.

The literature revealed that many researchers have investigated methods for analyzing mandelic acid esters of tropine compounds. Durick *et al.* (1) estimated some tropine alkaloids, including homatropine methylbromide, using an acid–dye procedure with bromcresol purple. Our investigations showed that the method yielded erratic results because it was very sensitive to even slight variations in the acid strength of the analytical solutions. The colorimetric determination with ammonium reineckate (2) and the UV estimations of the oxidation reaction products from cerimetric measurements (3, 4) had to be rejected due to interferences from the formulations. A colorimetric procedure was used successfully by Patel and Lemberger (5) to study the kinetics of the hydrolysis of homatropine methylbromide; however, their procedure could not be used to evaluate the stability of the alkaloid because of difficulties encountered in separating degradation products prior to color development. The phosphomolybdic acid reaction (6) has been used to analyze homatropine

methylbromide; however, low recoveries were obtained when the procedure was applied to synthetic samples.

A more promising method of analysis utilized the Dragendorff reagent (7). TLC experiments revealed that the Dragendorff reagent could be used selectively to analyze homatropine methylbromide; therefore, this colorimetric reaction was used as a basis for a spectrophotometric determination. Quantitative analysis of homatropine methylbromide was obtained by determining the absorbance of the alkaloid–iodobismuthate complex at 382 nm. in a stabilized acetone solvent (8). Recently, a colorimetric determination based on a ferric hydroxamate procedure was published. This procedure (9) was not tried on our formulations because it was published after these investigations were completed. However, it would be difficult to apply this method since it relies on an aqueous extraction of homatropine methylbromide from the formulation. The data presented in this paper utilize the Dragendorff colorimetric reaction for the quantitative analysis of homatropine methylbromide in formulations containing antacids such as aluminum hydroxide and magnesium trisilicate.

EXPERIMENTAL

Reagents—The following were used: acetone and methanol[1], spectroquality; homatropine methylbromide NF; 5% sulfuric acid; ethyl ether[1], reagent; hydrogen chloride gas[2]; methanolic hydrochloric acid solution (bubble hydrogen chloride gas into 1 l. of methanol for approximately 1 hr., allow to cool to room temperature, titrate the solution with standardized sodium hydroxide, and adjust the hydrochloric acid concentration to 0.1 *N*); and Dragendorff reagent as modified by Munier and Macheboeuf (7). Ten milliliters of glacial acetic acid and 40 ml. of distilled water are added to 850 mg. of bismuth subnitrate. To 40 ml. of this slurry, 40 ml. of 40% aqueous potassium bromide solution, 125 ml. of glacial acetic acid, and 250 ml. of distilled water are added.

Method—*Sample Procedure*—Weigh one average capsule fill (equivalent to 1.25 mg. homatropine methylbromide/capsule) into a 250-ml. iodine flask and add 25 ml. of methanolic 0.1 *N* HCl (gas). Shake the mixture on the wrist-action shaker overnight. Filter the mixture through a medium sintered-glass funnel into a 250-ml. round-bottom flask with the aid of vacuum. Wash the residue in the funnel with methanol. Evaporate the solvent using a flash evaporator. Add 10 ml. of 5% sulfuric acid and swirl the contents for 1 min. Add 40 ml. of ethyl ether and swirl the contents for an additional minute. Quantitatively transfer the contents into a 250-ml. separator, using small quantities of distilled water and ethyl ether. Extract the aqueous layer two times with 50 ml. of ethyl ether and drain the aqueous layer into a 250-ml. beaker. Evaporate the residual ether under a gentle stream of nitrogen. Add the aqueous Dragendorff reagent (1 ml./10 ml. solution), and allow to remain under the nitrogen stream until the reaction product shows evidence of coagulation (about 2 hr.). Filter the reaction product onto a medium sintered-glass funnel with the aid of vacuum and effect a quantitative transfer. Wash the complete precipitate with the mother liquor and air dry the precipitate. Water rinse any mother liquor from the underside of the funnel and stem. Place the funnel containing the reaction product onto a 50-ml. suction flask, and dissolve the product with small quantities of acetone. Transfer the acetone solution to a 50-ml. volumetric flask containing 25 ml. of methanol, and dilute to volume with acetone. Measure the absorbance of the complex at 382 nm. in 1-cm. absorption cells on a suitable spectrophotometer. Prepare a blank solution of 50% acetone–50% methanol by volume.

Standard Solution—An accurately weighed amount of homatropine methylbromide is dissolved in distilled water to yield a 0.25-mg./ml. solution. Five milliliters of this solution (equivalent to 1.25 mg. of homatropine methylbromide) is transferred to a 100-ml. beaker, and 2 drops of 5% sulfuric acid and 1 ml. of a freshly prepared Dragendorff reagent is added. (The experiments showed that there was no difference in the results obtained by adding 1 ml. of reagent to 1.25 mg. of homatropine methylbromide contained in either 5 or 10 ml. of solution.) The resulting precipitate is allowed to stand 1 hr. to facilitate filtration. The precipitate is filtered through a fine sintered-glass funnel with the aid of vacuum. The precipitate is dried by suction and dissolved in acetone directly in the sintered-glass funnel. The solution is quantitatively transferred to a 50-ml. volumetric flask containing 25 ml. of methanol. Dilute to volume with acetone. Measure the absorbance of the standard solution in 1-cm. absorption cells on a suitable spectrophotometer against a blank solution of 50% acetone–50% methanol.

TLC—Two capsule fills (equivalent to 2.5 mg. of homatropine methylbromide) are extracted overnight with 40 ml. of methanolic 0.1 *N* HCl (gas). The mixture is filtered and evaporated, and the residue is taken up in 2 ml. of 95% methanol. Twenty microliters (equivalent to 50 mcg.) are spotted on a 250-μ thick silica gel G plate. The sample is developed to a distance of 10 cm. in a saturated chamber in acetic acid–ethyl acetate–water–hydrochloric acid (35:55:10:20). The Dragendorff reagent is used as the detector after the plate has been air dried. Homatropine methylbromide is detected as an orange spot at an R_f of approximately 0.35.

[1] Baker.
[2] Matheson.

Table I—Recoveries of Homatropine Methylbromide from Synthetic Preparations

Number	Milligrams Added	Milligrams Found	Percent Recovery
1	1.250	1.270	101.6
2	1.290	1.302	100.9
3	1.230	1.230	100.0
4	1.345	1.347	100.1
5	1.283	1.295	100.9
6	1.245	1.260	101.2
7	1.280	1.285	100.4
8	1.265	1.250	98.8
9	1.300	1.317	101.3
10	1.265	1.265	100.0

Average = 100.5%
Relative *SD* = ±0.8%

RESULTS AND DISCUSSION

The Dragendorff colorimetric reaction with homatropine methylbromide was applied to synthetic capsule formulations containing antacids. Initially, low recoveries inhibited the successful application of the colorimetric reaction. Investigations showed that gelatin was extracted with aqueous dilute hydrochloric acid and interfered with the Dragendorff precipitation. To overcome these problems, methanolic hydrochloric acid was utilized as the ex-

Table II—Homatropine Methylbromide Assay Results from Formulation A

Batch Number	Storage Conditions, Temperature/Time	Assay, mg./Capsule—Dragendorff	Assay, mg./Capsule—Silver Nitrate
1	Initial	1.25	1.25
2	Initial	1.22	1.21
3	Initial	1.24	1.22
4	25°, 15 months	0.92	1.29
5	25°, 15 months	0.82	1.31
6	6°, 15 months	1.10	1.24
	25°, 15 months	1.08	1.26
	40°, 15 months	0.65	1.22
7	25°, 27 months	0.91	1.18
8	25°, 27 months	0.87	1.27
9	6°, 28 months	0.68	1.19
	25°, 28 months	0.61	1.24
	40°, 28 months	0.54	0.91
10	40°, 28 months	0.51	1.04
11	40°, 28 months	0.58	1.12
12	40°, 28 months	0.57	1.09

Table III—Homatropine Methylbromide Assay Results for Formulation B

Batch Number	Storage Conditions, Temperature/Time	Assay, mg./Capsule—Dragendorff	Assay, mg./Capsule—Silver Nitrate
1	Initial	1.29	1.31
2	Initial	1.25	1.22
3	Initial	1.22	1.22
4	6°, 3 months	1.30	1.30
	25°, 3 months	1.24	1.30
	40°, 3 months	1.20	1.30
5	6°, 6 months	1.31	1.26
	25°, 6 months	1.26	1.21
	40°, 6 months	1.20	1.17
6	6°, 12 months	0.83	1.31
	25°, 12 months	0.74	1.34
	40°, 12 months	0.67	1.26
7	25°, 15 months	0.89	1.25
8	25°, 15 months	0.95	1.33
9	25°, 27 months	0.63	1.28
10	25°, 27 months	0.70	1.37
11	25°, 36 months	0.52	1.29
	40°, 36 months	0.34	1.15
12	25°, 36 months	0.53	1.26
	40°, 36 months	0.48	0.71

tracting solvent. Recoveries from synthetic preparations were somewhat improved but were still unacceptable. An additional modification involved using completely anhydrous reagents, namely, anhydrous methyl alcohol and hydrogen chloride gas. Factors, such as acid strength in methanol and extraction time, were investigated to find the proper conditions for the quantitative extraction of homatropine methylbromide. The studies showed that the best conditions for extracting homatropine methylbromide from the synthetic preparations were the use of 0.1 *N* hydrochloric acid (gas) in anhydrous methanol and an overnight extraction.

The results of some synthetic preparations where recovery data were obtained are shown in Table I.

Low results were obtained with formulations stored under accelerated conditions. TLC investigations of these formulations revealed the presence of a component at R_f 0.25 which gave a purple color with the Dragendorff reagent. This component was isolated by preparative TLC and identified to be tropine methylbromide based on microelemental analysis, melting point, and IR (10). No other Dragendorff-sensitive decomposition products of homatropine methylbromide were detected. Tropine methylbromide was also prepared in the laboratory by refluxing homatropine methylbromide in methanol, followed by recrystallization with ethyl ether.

Efforts were made to determine the selectivity of the Dragendorff procedure. These investigations showed that although tropine methylbromide was reactive with the Dragendorff reagent, it did not interfere in the assay procedure because it did not yield any precipitate. This statement is based upon recovery experiments where the following combinations of homatropine methylbromide–tropine methylbromide were mixed together with excipient material from the formulation: (*a*) 0.25 mg./0.75 mg., (*b*) 0.50 mg./0.50 mg., and (*c*) 0.75 mg./0.25 mg. Recoveries in the order of 98% were obtained from these experiments. These studies showed that the Dragendorff colorimetric assay is specific for homatropine methylbromide in the formulations studied.

The analytical procedure was applied to recently produced batches of the formulations as well as retainer and stability samples. The results were compared to values obtained by a silver nitrate potentiometric titration and are shown in Tables II and III. A comparison of the data reveals the following:

1. On recently produced batches, there is good agreement between the Dragendorff colorimetric procedure and the silver nitrate titration method.

2. Lower results were obtained by the Dragendorff method for samples stored under accelerated conditions than by the silver nitrate method. This can be attributed to the fact that the silver nitrate method is not specific because it measures any titratable bromide, whereas the Dragendorff method is specific and does not measure the main decomposition product of homatropine methylbromide, namely, tropine methylbromide.

Additional studies were conducted to prove that the lower results obtained by the Dragendorff procedure were valid and were not due to extraction problems. The formulation residues were examined for homatropine methylbromide content by TLC. The analysis showed that homatropine methylbromide was not detected in the residues, thereby showing that all of the active ingredient was extracted and subsequently measured.

CONCLUSIONS

An assay method for the specific determination of homatropine methylbromide in two formulations containing antacids was developed. Homatropine methylbromide was found to be unstable in both formulations after 6–12 months of storage at room temperature. The main decomposition product of homatropine methylbromide in these formulations was tropine methylbromide. This decomposition product does not interfere with the Dragendorff colorimetric assay procedure. TLC was used to detect the presence or absence of the tropine methylbromide decomposition product in these formulations. Although this assay and TLC methods were applied to only two capsule formulations, the authors feel that both procedures can be used to study the stability of homatropine methylbromide in other formulations.

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