Morphine Determination in Kaolin Pectin Formulations

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A method has been developed for the quantification of morphine in kaolin pectin formulations containing opium. The method consists of lyophilizing the suspension, washing of the solids with ammonia methanol, complexing with bromothymol blue, extraction, silanization, and gas chromatography.

THE ALKALOIDS of opium are frequently formulated with kaolin and pectin for the symptomatic relief of acute nonspecific diarrhea. The suspensions combine the adsorbent and detoxifying demulcent action of kaolin and pectin with the sedative and analgesic effects of opium alkaloids.

A number of assay methods are available for the quantification of opium alkaloids (1–3). Brochmann-Hanssen and Svendsen (4) describe the gas chromatography of opium alkaloids and Brochmann-Hanssen and Furuya (5) identified the alkaloids using a combination of gas, thin-layer, and paper chromatography. No procedure, however, has been reported involving the quantification of opium alkaloids in kaolin pectin suspensions. Bracey and Selzer (6) described a method combining liquid–liquid extraction and column chromatography for the determination of microgram quantities of belladonna alkaloids in kaolin pectin suspensions. Similar methods, when applied to the extraction of morphine from these suspensions, met with little success in this laboratory.

This paper presents a method for simple elution of morphine from the lyophilized kaolin pectin formulation. The morphine is then complexed with bromothymol blue (7, 8) and the complex extracted with chloroform to remove interfering excipients. The morphine is measured by gas chromatographing the trimethylsilyl derivative. Since the concentration of morphine in opium extracts is known (10%) the assay of the extracts in the product is based on the quantification of morphine.

EXPERIMENTAL

Instrument—An F & M model 402 gas chromatograph equipped with a flame-ionization detector and glass columns, 1.2 m. long × 3 mm. i.d. was used for this work. The columns were packed with 3.8% SE-30 on 80–100 mesh Diatoport S. The carrier gas was helium at a flow rate of approximately 50 ml./min. Hydrogen and air flows were adjusted for maximum response. The column was operated isothermally at 200°, the flash heater at 220°, and the detector at 260°. The recorder was equipped with a model 227 disk chart integrator for peak area measurements.

Solvents and Reagents—The following reagent grade materials were used: chloroform, dimethylformamide, sodium hydroxide, citric acid monohydrate, sodium phosphate dibasic anhydrous, anhydrous methanol, and ammonium hydroxide. Other reagents used were tetraphenyl tin,[1] bromothymol blue,[2] hexamethyldisilazane,[3] and tetramethylammonium iodide.[4]

Solutions—*Dye-Buffer Solution*—Prepare 500 ml. of a water solution containing 200 mg. bromothymol blue, 3.2 ml. of 0.1 *N* sodium hydroxide, 577.5 mg. of citric acid monohydrate, and 6.32 g. of sodium phosphate dibasic anhydrous. The pH of the resulting solution should be between 7.2 and 7.4.

Internal Standard Solution—Prepare a dimethylformamide solution containing 1 mg./ml. of tetraphenyl tin.

Reference Solution—Transfer an accurately weighed portion of morphine sulfate equivalent to about 100 mg. of morphine and dissolve it in exactly 100 ml. of water. Transfer 2.0 ml. of this solution to a separator, add 20 ml. of dye-buffer solution, and extract with four 30-ml. portions of chloroform. Take the combined chloroform extracts to dryness. Dissolve the residue in exactly 2.0 ml. of internal standard solution, add 2 ml. of hexamethyldisilazane, 10 mg. of tetramethylammonium iodide, and allow it to stand for 2 hr.

Sample Preparation—Accurately measure and lyophilize a volume of well mixed sample equivalent to about 2 mg. of morphine. To lyophilize or freeze-dry the sample, shell freeze it in a dry ice–acetone bath and place the sample in sufficient vacuum to maintain the frozen condition until the water is removed.[5] Quantitatively transfer the residues to a 2.5 × 40 cm. glass column with a

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[1] Aldrich Chemical Co., Milwaukee, Wisc.
[2] Eastman Organic Chemicals, Rochester, N. Y.
[3] Applied Science Laboratories, Inc., State College, Pa.
[4] Matheson Coleman and Bell, Cincinnati, Ohio.
[5] Freeze dryer, model FDC-6-V15 MRS, available from Thermovac Industries, Inc., was used in this laboratory.

TABLE I—DETERMINATION OF OPIUM IN KAOLIN PECTIN FORMULATIONS

Formulation	Opium Content (Label)	Found
U	20.0 mg./30 ml.	19.4 mg./30 ml.
P	15.0 mg./30 ml.	15.2 mg./30 ml.
D	24.0 mg./30 ml.	23.5 mg./30 ml.

fritted disk. Wash the solids with 250 ml. of 5% ammonia methanol and evaporate the eluate to dryness. Dissolve the residue in 10 ml. of dye-buffer solution and 20 ml. of chloroform. Transfer this mixture to a separator and rinse with similar portions of dye-buffer solution and chloroform. Extract the dye with the chloroform and repeat with three additional 30-ml. portions of chloroform and take the combined extracts to dryness. Dissolve the residue in 2.0 ml. of the internal standard solution, add 2 ml. of hexamethyldisilazane, 10 mg. of tetramethylammonium iodide, and allow it to stand for at least 2 hr.

Chromatography—Chromatograph 5 μl. each of the sample and reference preparations. Using the conditions described under *Instrument*, retention times of 10 and 18 min. were obtained for morphine and the internal standard, respectively.

Calculations—Calculate the mg. of morphine in each 30.0 ml. of the formulation from the formula:

$$\frac{R_1 \times A \times 30}{R_2 \times B}$$

where

R_1 = sample morphine peak area/internal standard peak area
R_2 = reference morphine peak area/internal standard peak area
A = weight in milligrams of morphine in the reference preparation
B = volume in milliliters of the formulation used in the sample preparation

RESULTS AND DISCUSSION

The desirable absorptive properties of kaolin pectin formulations makes difficult the quantitative removal of morphine for analysis. Several approaches to wet extraction including salting out and complexing met with little success. Removal of the water by lyophilization from the clays apparently reduces their adsorptive properties permitting elution of morphine with an ammonia–methanol mixture. The complexing with bromothymol blue and subsequent extraction are necessary to separate the morphine from water-soluble excipients. The tetramethylammonium iodide is added to break the morphine bromothymol blue complex prior to silanization. There is no apparent chromatographic response from the bromothymol blue.

A sample was prepared in this laboratory containing 1.95 mg. of morphine/30 ml. of formulation. Ten replicate assays of this sample showed 99.5% recovery with a coefficient of variation of 1.96%.

Three commmercial preparations were assayed by this procedure and the results are shown in Table I.

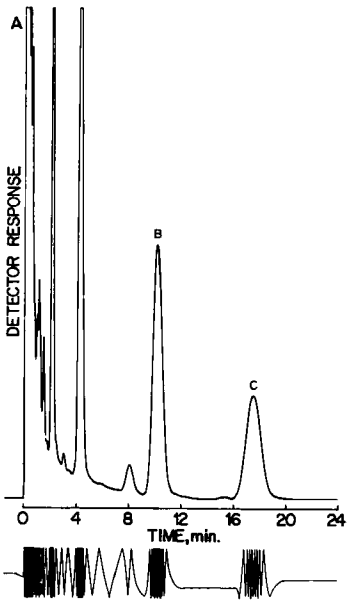

Fig. 1—Chromatogram of an assay preparation from a kaolin pectin formulation. Key: A, solvent; B, morphine; C, internal standard.

Figure 1 shows a chromatogram resulting from the assay of Formulation D. Combination gas chromatography-mass spectrometry (LKB-900GC-MS) was used to verify the morphine trimethylsilyl derivative peak. No effort was made to determine the nature of the excipient peaks.

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Keyphrases

Morphine analysis—kaolin pectin formulations
Bromothymol blue-morphine complex-extraction
GLC—analysis
Dimethylformamide-tetraphenyl tin solution—internal standard