Spectrophotometric Determination of Methenamine and Its Salts by 2-Hydrazinobenzothiazole

JAMES K. LIM[▲] and CONNIE C. C. CHEN

Abstract ☐ A rapid and sensitive method is described for the assay of the pure powder form and the tablet dosage form of methenamine and its salts. The procedure is shown to be capable of measuring as little as 0.005 μmole of material and is highly reproducible. The proposed method is based on the color development of formaldehyde released from the acid hydrolysis of methenamine with a dye, 2-hydrazinobenzothiazole. Beer's law is obeyed in the concentration range of 1-8 μM of methenamine at 510-nm. wavelength. This method offers greater versatility over the official assay methods, which have been designed primarily for determining specifically either methenamine or its mandelate salt. Data comparing the assays obtained by the official methods with this new technique are presented.

Keyphrases ☐ Methenamine and salts in powders and tablets—colorimetric analysis using 2-hydrazinobenzothiazole, compared to compendial methods ☐ 2-Hydrazinobenzothiazole—used as color-forming agent in spectrophotometric analysis of methenamine and its salts ☐ Colorimetry—analysis, methenamine and salts in powders and tablets

The NF XIII (1) assay for methenamine involves its acid hydrolysis by reflux until the "odor of formaldehyde is no longer perceptible," followed by back-titration with an alkali. The procedure proved to be not only tedious but also subject to inaccuracies arising from human error in obtaining the "perceptible end-point," *i.e.*, absence of formaldehyde odor. The USP XVIII (2) method for determining methenamine mandelate, on the other hand, represents an indirect method of analysis. It essentially involves nesslerization followed by an iodometric titration of the mandelic acid moiety; therefore, this method cannot be employed for determining other salts of methenamine.

Other methods also have been developed for the quantitation of this compound, including those based on chromotropic acid reactions (3-5), the Hantzsch reaction between acetylacetone, ammonia, and formaldehyde (6), and fluorometric techniques (7). This report presents a method for determining methenamine and its salts through the formation of xanthylinum dicationic dyes with formaldehyde and formaldehyde-releasing compounds. Measurements based on such a reaction are reported (8, 9) to be more sensitive than those by chromotropic acid.

EXPERIMENTAL

Apparatus—Absorbance measurements were made on a spectrophotometer[1].

Reagents and Chemicals—2-Hydrazinobenzothiazole[2] was the dye-forming reagent used. Powdered samples of methenamine[3-5],

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[1] Bausch and Lomb Spectronic 70.
[2] Eastman Organic Chemicals.
[3] Baker Chemical Co.
[4] Amend Drug Chemical Co.
[5] Fisher Scientific Co.

Table I—Absorbance by Different Methenamine Concentrations

Determination Number	Concentration, μmole/5 ml.			
	0.005	0.01	0.02	0.04
1	0.075	0.141	0.297	0.598
2	0.084	0.161	0.290	0.598
3	0.084	0.161	0.276	0.584
Average	0.081	0.154	0.288	0.593
± SD	0.004	0.009	0.008	0.007

Table II—Assay of Commercial Methenamine Powder by the 2-Hydrazinobenzothiazole and NF Methods

Powder	Percent Found	
	2-Hydrazinobenzothiazole Method	NF Method
A	98.5[a] ± 1.08[b]	98.8
B	96.3 ± 2.07	93.5

[a] Average of three determinations. [b] Standard deviation.

methenamine mandelate[6], and methenamine hippurate[7] were used as received. All other chemicals and reagents used were USP or the highest grade of commercial materials. Several commercial brands of either the plain or enteric-coated tablets of methenamine, methenamine mandelate, and methenamine hippurate were employed for the tests comparing the official assays with the proposed method. The dye reagent consists of a 0.5% solution of 2-hydrazinobenzothiazole in 10% hydrochloric acid. It is stable for 2 weeks.

Assay Procedure—Aqueous solutions of methenamine or the methenamine salt in the concentration range of 0.01-0.08 μmole/ml. were made up in 100-ml. volumetric flasks. A 0.5-ml. volume of each solution was placed in a test tube and mixed with 0.5 ml. of 10 N sulfuric acid. The samples were refluxed in a boiling water bath for 15 min. to obtain complete hydrolysis and were allowed to cool to room temperature before the addition of 0.5 ml. of the 2-hydrazinobenzothiazole reagent. After 5 min., 0.5 ml. of a 1% aqueous solution of potassium ferricyanide was added and this reaction was allowed to proceed another 20 min. before the final admixture of 3 ml. of dimethylformamide solvent. The color thus formed was allowed to stabilize for 20 min. before absorbance readings were made at a 510-nm. wavelength against a reagent blank.

RESULTS AND DISCUSSION

Absorbance of the color obtained of known standards in the concentration range of 0.005-0.04 μmole/5 ml. of methenamine was seen to obey Beer's law. The calculated linear correlation coefficients were: methenamine, 0.9996; and methenamine mandelate and methenamine hippurate, 0.9969 and 0.9975, respectively. Initial studies showed that the slopes might vary from time to time, probably due to aging of the reagents employed with the 2-hydrazinobenzothiazole reaction. However, the experimental values were reproducible within the normal analysis period (Table I). It is, therefore, recommended that a fresh standard curve be prepared with each analysis.

Table II compares the percentages of methenamine ($C_6H_{12}N_4$) found in two different commercial brands of methenamine powder as assayed by the 2-hydrazinobenzothiazole and the NF methods.

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[6] K & K Laboratories, Inc.
[7] Riker Laboratories, Inc.

Table III—Assay of Commercial Methenamine Products by the 2-Hydrazinobenzothiazole Method

					Methe-namine
			Percent Found		
				Tablets	
	Powders				namine
	Methe-				Mandel-
Determina-	namine	Methe-		Methe-	ate,
tion	Mandel-	namine	Methe-	namine	Enteric
Number	ate	Hippurate	namine	Hippurate	Coated
1	96.6	96.6	103.8	96.6	95.2
2	96.6	101.4	99.0	101.4	91.0
3	96.6	99.0	99.0	99.0	91.0
Average	96.6	99.0	100.6	99.0	92.4
± SD	0.0	2.0	2.3	2.0	2.0

Only 0.02 μmole of material was needed per determination in this proposed procedure compared to approximately 1 g. or 7.1 mmoles in the NF method. Table III presents the results of assays on methenamine mandelate and methenamine hippurate powders and those on their tablet dosage forms. Tablets were crushed, dissolved, and filtered, if necessary, before an aliquot representing approximately 0.02 μmole was withdrawn for the determination. There appeared to be no significant interference in absorbance where color was formulated in the tablets because of the high dilutions involved in obtaining the extremely small amount of material.

It is clear from these data that the proposed 2-hydrazinobenzothiazole procedure possesses several distinct advantages over the present official methods for the analysis of methenamine or methenamine mandelate. It offers the convenience of a single method for the analysis of either the free base or salts. It has been found to be simple, sensitive, and less time consuming. It does not require prior standardization of a number of titrants, as for nessleriza-

tion with the official assay of methenamine mandelate. Furthermore, the proposed method can be used for the assay of other salts of methenamine, unlike the USP procedure. With respect to the NF method, the lengthy period needed for the acid hydrolysis of methenamine using 1 N sulfuric acid as well as the uncertain end-point of the hydrolysis can be eliminated with the 2-hydrazinobenzothiazole procedure.

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Chromatographic Separation and Assay of Morphine in Injectables

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Abstract ☐ A method specific for the determination of morphine sulfate in injectables was developed. Morphine is separated from preservatives, pseudomorphine, and other degradation products by a column chromatographic method. The eluate containing morphine is extracted into an acid solution and determined spectrophotometrically. Recovery from standard solutions is better than 99%. The method presented is more specific for the separation and determination of morphine in injectables than present official methods.

Keyphrases ☐ Morphine sulfate injection—separation from preservatives and degradation products, UV analysis ☐ Column chromatography—separation, morphine from preservatives and degradation products, morphine sulfate injection ☐ UV spectrophotometry—analysis, morphine sulfate injection

Morphine has been well established as an effective analgesic and sedative since its isolation in 1805 (1, 2). Due to the limited solubility of morphine base in aqueous media, the more soluble hydrochloride and sulfate salts have been used in pharmaceutical prepara-

tions. The dosage form commonly employed since 1941 has been morphine sulfate as an injectable aqueous solution, since it is the most stable of the morphine salts in solution (3). When stored for a long time or exposed to heat, morphine sulfate injections develop a light-brown color.

The discoloration is not desirable, since decomposition of the dosage form is suggested and is, in fact, taking place; however, the extent of the discoloration is not an indication of the extent of decomposition. The cause has been attributed to the decomposition of morphine sulfate to pseudomorphine and further oxidation products (4-8). Foster *et al.* (5) and later Lach and Yeh (9-11) demonstrated that morphine injections decomposed first to pseudomorphine and then to oxidation products of pseudomorphine. Foster *et al.* (5) also showed that rubber closures contributed to the discoloration of the morphine injection and possibly to the decomposition of morphine sulfate.
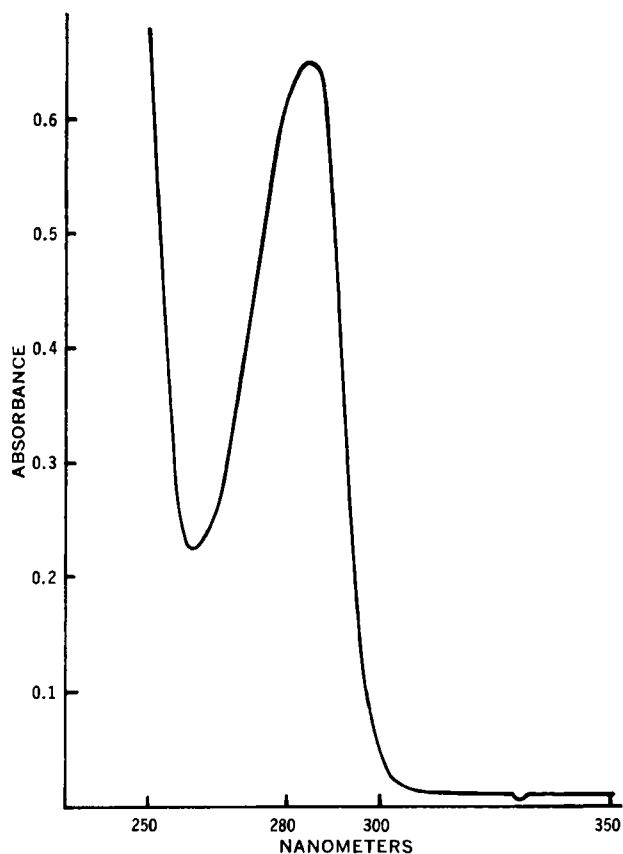
Numerous assay methods have been developed for

Figure 1—*UV spectrum of morphine sulfate, 0.1622 mg./ml., in 0.05 N hydrochloric acid.*

Table I—Recovery of Morphine Sulfate from Chromatographic Column

Theoretical, mg./ml.	Recovered, mg./ml.	Recovery, %
13.76	13.45	99.2
15.90	15.83	99.5
15.95	15.95	100.0
17.00	16.95	99.7
17.95	17.88	99.6

Table II—Recovery of Morphine Sulfate from a Standard Formulation

Morphine Sulfate in Standard Formulation, mg./ml.	Recovered, mg./ml.	Recovery, %
15.10	15.03	99.6
15.10	15.06	99.7
15.10	15.09	99.8

the determination of morphine, using gravimetric (12, 13), colorimetric (14–19), fluorometric (20, 21), titrimetric (22, 23), and spectrophotometric (24) procedures. These methods are satisfactory for freshly prepared solutions of morphine sulfate but are not always specific for the determination of morphine in the presence of its degradation products. Ion-exchange (25–27) and partition chromatographic (9, 28–30) methods have been used to separate morphine in pharmaceutical preparations. Most of these procedures are time consuming and are not applicable to routine analysis. A partition chromatographic method was developed (9,
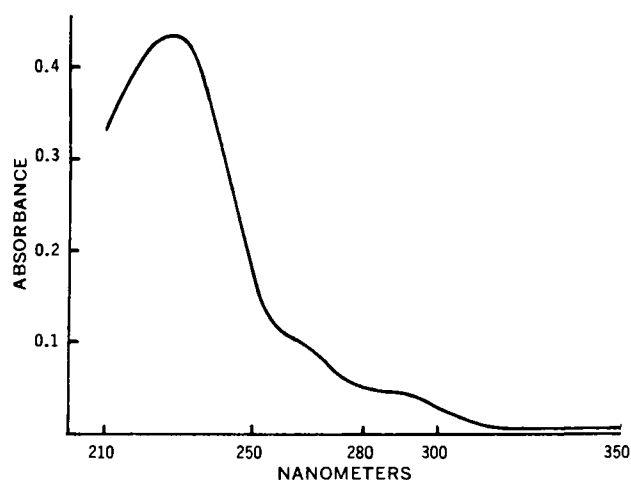
28) for the separation of intact morphine from degradation products; this method was employed in studies on the stability of morphine in aqueous solution (9–11). This procedure was also time consuming. The present USP extraction–titrimetric method (23) extracts small amounts of decomposition products as well as morphine from discolored solutions of morphine injection, giving false, high assay values. When the extracts from the USP procedure were examined by TLC, both morphine and pseudomorphine were found.

The purpose of the present investigation was to provide a simple, rapid chromatographic method specific for the separation of morphine from its degradation products. The same procedure may be employed to separate morphine from such alkaloids as atropine and codeine. The separation of morphine and
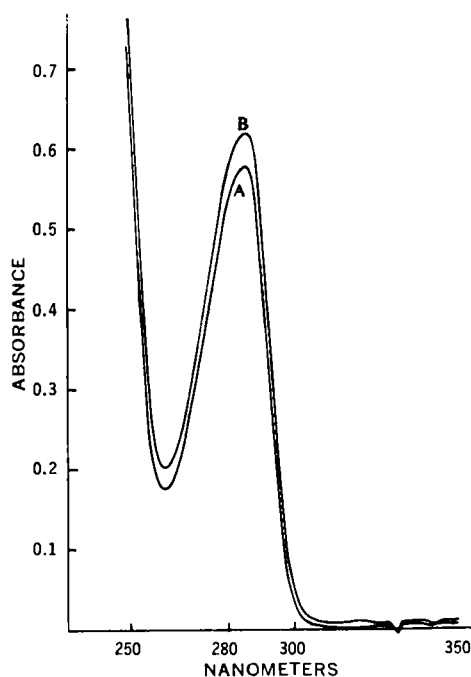

Figure 2—*UV spectrum of pseudomorphine, 0.00746 mg./ml., in 0.05 N hydrochloric acid.*

Figure 3—*UV spectra of: A, morphine sulfate, 0.1486 mg./ml., in 0.05 N hydrochloric acid; and B, morphine sulfate, 0.1486 mg./ml., and pseudomorphine, 0.0075 mg./ml., in 0.05 N hydrochloric acid.*

Table III—Recovery of Morphine Sulfate from a Standard Formulation and Other Added Substances

Morphine Sulfate in Standard Formulation, mg./ml.	Substance Added, mg./ml.	Recovered, mg./ml.	Recovery, %
15.10	Pseudomorphine (1.65)	15.06	99.7
15.10	Codeine phosphate (16.60)	15.21	100.7
15.10	Atropine sulfate (2.14)	15.19	100.6

Table IV—Assay Results on Morphine Injection USP [Sulfate], 15 mg./ml.

Manufacturer	Morphine Sulfate Found, mg./ml.	Percent Label Claim
A	14.27	95.4
	14.32	95.5
B	15.35	102.3
	15.47	103.1

atropine is significant because of the use of these alkaloids in combination for preanesthetic injection.

The procedure involves a release of morphine as the base with the use of sodium bicarbonate. Chloroform elution through an acidic trap layer removes neutral substances such as the parabens and bases such as codeine and atropine. Morphine, which is partially eluted by chloroform, is retained by the acidic trap layer and is finally eluted from the column using isobutanol in chloroform. The decomposition products are retained on the column.

EXPERIMENTAL

Reagents and Apparatus—*USP Grade Chemicals*—The following were used: morphine sulfate, codeine phosphate, atropine sulfate, methylparaben, and propylparaben.

Reagent Grade Chemicals—Reagent grade acid-washed diatomaceous earth[1], sodium bisulfite, isobutanol, chloroform, and anhydrous ether were used. Pseudomorphine base was prepared by oxidation of morphine in a basic solution with potassium ferricyanide (31). The product was washed with methanol and dried. TLC showed only one zone, confirming the purity of the base.

Apparatus—The following were used: 25 mm. i.d. × 15 cm. long chromatographic column[2] with a coarse fritted disk in the base of the column, 1-cm. quartz cells, and a spectrophotometer[3] with a recorder[4].

Separation—In a 100-ml. beaker, 2.0 ml. of 1 *M* pH 6.5 potassium phosphate buffer solution was mixed with 3 g. of reagent grade, acid-washed diatomaceous earth. The mixture was transferred to the chromatographic column and packed firmly. To 1.0 ml. of the test solution in a 150-ml. beaker, 0.5 g. sodium bicarbonate was added and mixed well. Then 3 g. of reagent grade, acid-washed diatomaceous earth was added and mixed well. The mixture was transferred to the chromatographic column and packed firmly. The beaker was wiped with a pledget of glass wool, and the glass wool was placed on the top of the packed column. The column was eluted with 25 ml. of reagent grade chloroform, and the eluate was discarded. The column was then eluted with 55 ml. of 15% (v/v) isobutanol in chloroform, and the eluate was collected in a 250-ml. separator. Water-washed ether, 100 ml., was added to the eluate in

[1] Celite 545, Johns-Manville Co.
[2] Chromaflex, Kontes Glass Co.
[3] Perkin-Elmer model 124.
[4] Perkin-Elmer model 165.

Table V—Separation of Morphine Sulfate from Degradation Products

Theoretical, mg./ml.	Conditions of Treatment	Recovery, mg./ml.	Recovery, %
17.00	Initial assay	16.95	99.7
	After 17 days at 105°	15.94	93.8

the separator, and the contents were carefully mixed. The solvent mixture was extracted with three 25-ml. portions of 0.05 *N* hydrochloric acid. The acid phases were collected in a 100-ml. volumetric flask, and 0.05 *N* hydrochloric acid was added to volume and mixed. The spectrum of the acid solution was obtained from 350 to 250 nm. (Fig. 1). The absorbance maximum obtained at 285 nm. was used to calculate the quantity of morphine sulfate.

By employing this chromatographic procedure, aqueous solutions of morphine sulfate and of morphine sulfate in combination with other substances present were assayed.

Morphine Sulfate Solutions—Aqueous solutions of morphine sulfate USP, ranging in concentration from 13.76 to 17.95 mg./ml., were prepared and assayed (Table I).

Morphine Sulfate Standard Formulation—A morphine sulfate aqueous solution was prepared using methylparaben and propylparaben as preservatives and sodium bisulfite as an antioxidant. The standard formulation consisted of the following (mg./ml.): morphine sulfate USP, 15.10; methylparaben USP, 1.69; propylparaben USP, 0.21; and sodium bisulfite, reagent grade, 1.03.

Assay results for the standard formulation are given in Table II.

The standard formulation was modified by the addition of other substances (pseudomorphine and atropine sulfate) commonly associated with morphine sulfate in injectables and with the chemi-

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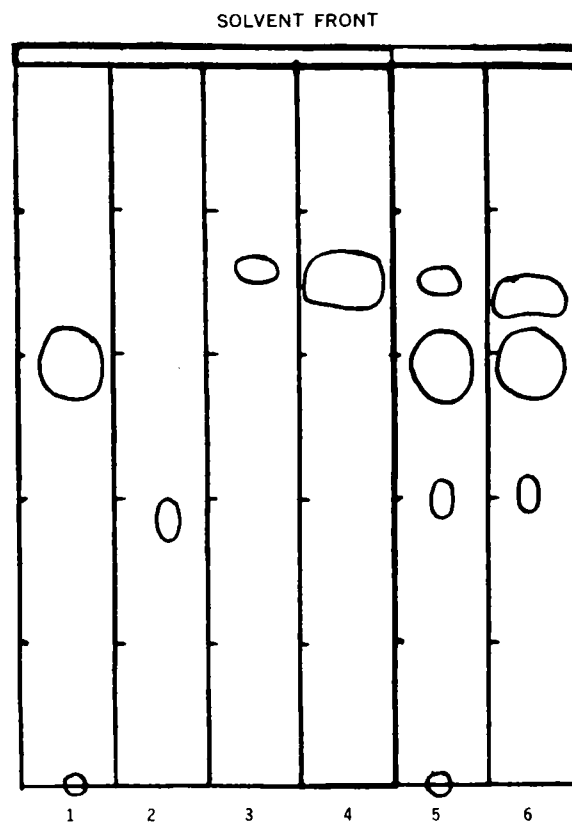
Figure 4—*Chromatogram of reference compounds used in the thin-layer examination study of morphine sulfate, standard formulation, and decomposition products. Key: 1, 150 mcg. of morphine sulfate; 2, 3 mcg. of pseudomorphine; 3, 3 mcg. of morphine N-oxide; 4, 18 mcg. of methylparaben and propylparaben mixture; 5, mixture of morphine sulfate, pseudomorphine, and morphine N-oxide; and 6, mixture of morphine sulfate, pseudomorphine, and parabens.*

cally related substance codeine. Morphine was successfully separated and quantitatively assayed in the presence of these substances (Table III). These separations were confirmed by TLC. To demonstrate the applicability of this assay method to formulations, samples of morphine injection USP [sulfate], 15 mg./ml., were obtained and assayed (Table IV).

UV Spectra: Morphine and Pseudomorphine—The UV curves of morphine sulfate (Fig. 1) and pseudomorphine (Fig. 2) in 0.05 N hydrochloric acid clearly show the spectral differences of the two compounds. The curves in Fig. 3 show the effect of pseudomorphine sulfate at the maximum at 285 nm. Application of the column chromatographic method separates morphine from pseudomorphine and other degradation products.

Separation from Degradation Products—To show the separation of morphine sulfate from degradation products, a solution of morphine sulfate was submitted to conditions to accelerate the decomposition. A solution of morphine sulfate was sealed in a glass ampul and placed in an oven at 105° for 17 days. The solution developed a distinct brown color. The ampul was opened and the contents were assayed. The morphine content definitely decreased, as indicated by the data in Table V. The brown color originally present was retained on the chromatographic column. The morphine eluate and subsequent acid extract were clear and colorless. Thus, the chromatographic system is capable of separating intact morphine from its colored degradation products.

Additional work on the aged product involved assay of room temperature samples which were 4–10 years old. These samples were assayed by both the column chromatographic procedure and GLC (Table VI).

TLC—Complete separation of morphine in the preceding standard mixtures was monitored by TLC. Following the chloroform

Table VI—Assay Values on Sterile Morphine Injection in Rubber-Stoppered Amber Glass Vials

Sample	Date of Manu-facture	Milligrams Morphine Sulfate Found per Milliliter (Percent Label Claim) Column Separation	GLC[a]
1	1962	14.26(88.0)	14.00(86.4)
2	1963	14.42(89.1)	13.91(85.9)
3	1964	14.62(90.3)	13.89(85.9)
4	1966	14.82(91.5)	14.72(90.8)
5	1968	14.84(91.6)	14.92(92.1)

[a] The GLC assays were performed by Mr. V. G. Davies.

and chloroform–isobutanol elutions, the chromatographic columns were eluted with 0.01 or 0.05 N hydrochloric acid in methanol to remove the remaining decomposition products.

Precoated silica gel GF plates[b] (10 × 20 cm.) were used as the stationary phase. The mobile phase consisted of chloroform–methanol–diethylamine (80:15:5).

Eluates from the chromatographic columns were concentrated to 1 ml. prior to application to the stationary phase. Twenty microliters of each concentrate was applied, and the plates were developed to a height of 15 cm. from the origin. Plates were air dried and examined under long and short wavelength UV light.

In every instance, complete and clean separation of intact morphine was obtained from the other substances present or added (Figs. 4–8).

RESULTS AND DISCUSSION

The recovery data in Table I indicate that quantitative recovery of morphine sulfate was obtained from the morphine sulfate solutions assayed. No morphine was detected in the initial chloroform eluate from the chromatographic column. Recovery of morphine sulfate from the morphine sulfate standard formulation was quantitative (Table II). The other ingredients in the formulation did not

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PARABENS →

MORPHINE →

PSEUDOMORPHINE →

1   2   3
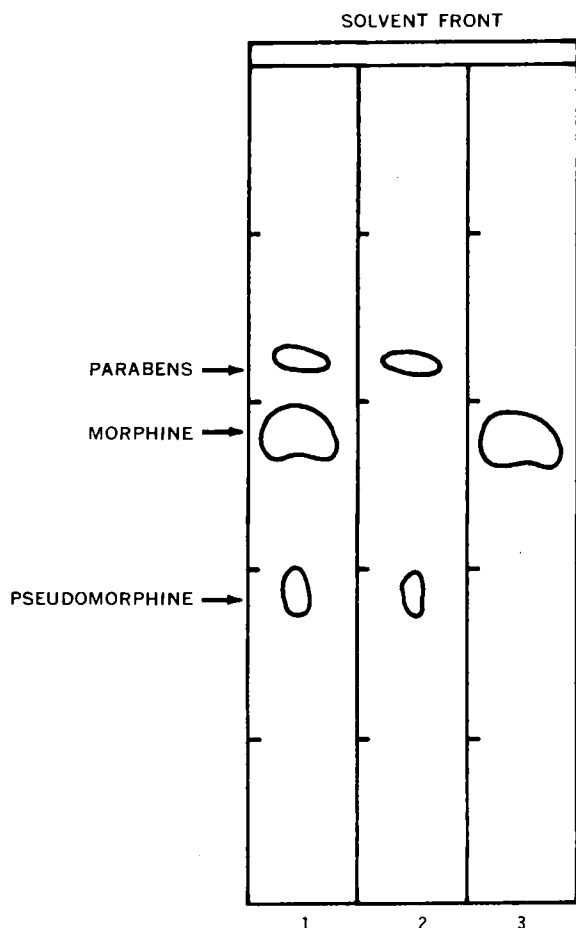
Figure 5—*Chromatogram demonstrating the column separation of morphine from pseudomorphine and other components in the standard formulation. Key: 1, 20 μl. of the standard formulation; 2, 20 μl. of the chloroform eluate; and 3, 20 μl. of the chloroform–isobutanol (85:15) eluate.*

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MORPHINE →

PSEUDO-MORPHINE →

MORPHINE N-OXIDE

1   2   3   4
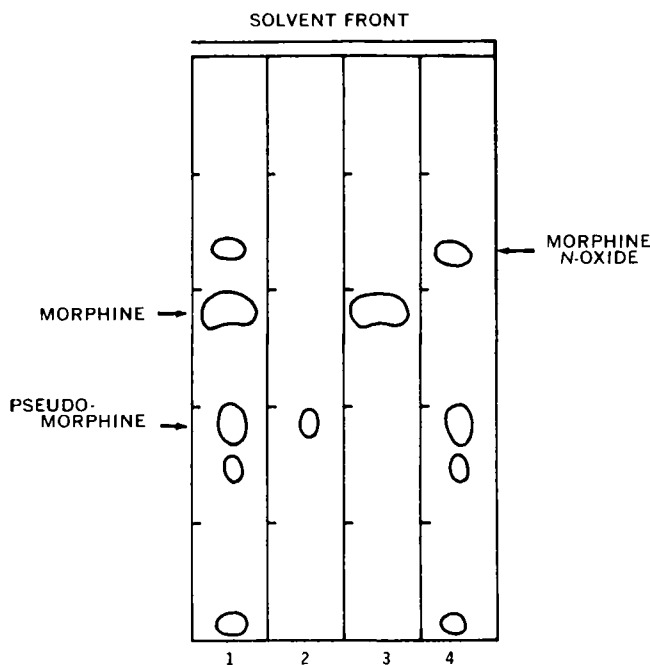
Figure 6—*Chromatogram showing the column separation of morphine from decomposition products. Key: 1, 20 μl. of degraded morphine sulfate solution; 2, 20 μl. of the chloroform eluate; 3, 20 μl. of the chloroform–isobutanol (85:15) eluate; and 4, 20 μl. of the 0.01 N hydrochloric acid eluate in methanol.*

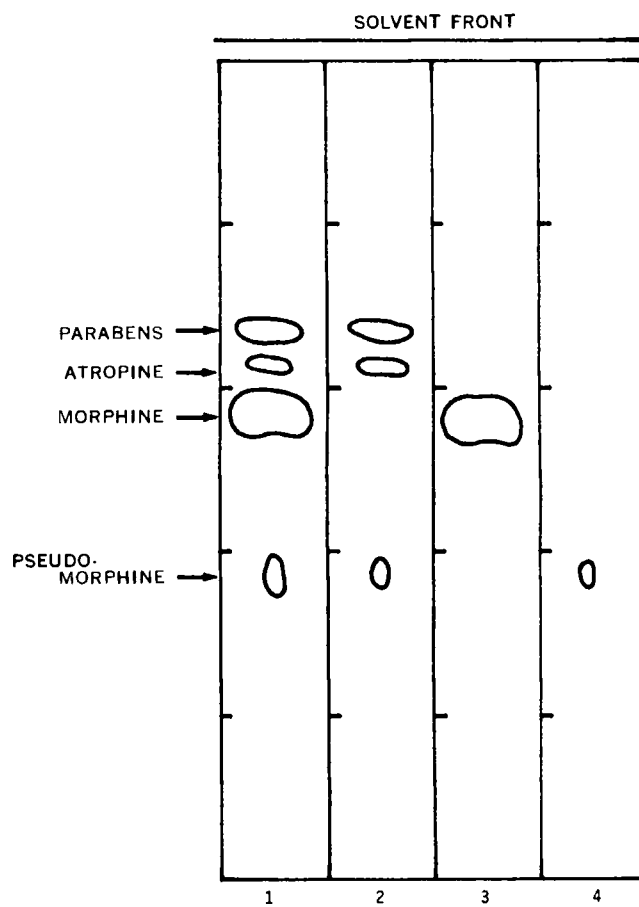[b] Analtech, E. Merck.
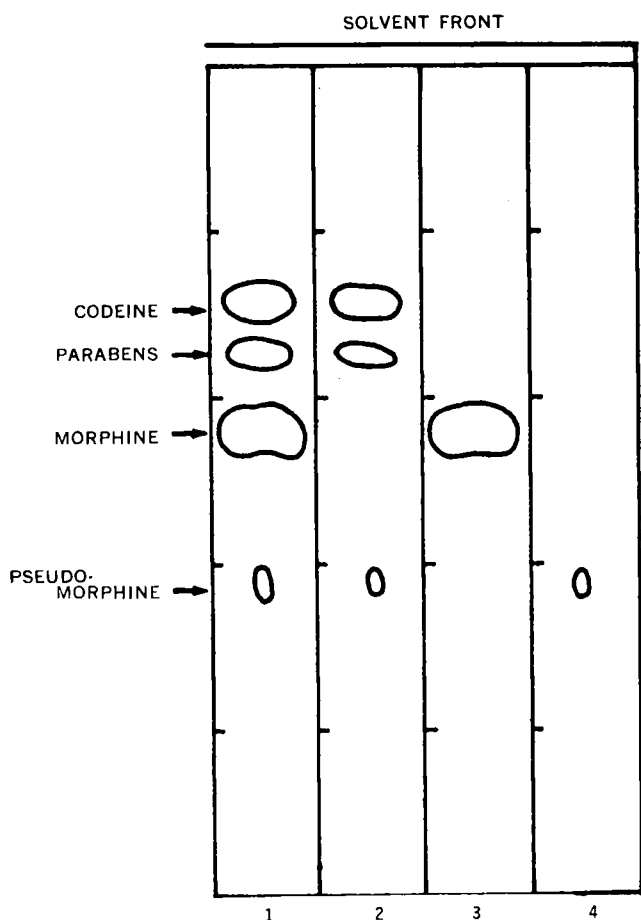
Figure 7—Chromatogram showing the column separation of morphine in the standard formulation with codeine phosphate. Key: 1, 20 μl. of the standard formulation with codeine phosphate; 2, 20 μl. of the chloroform eluate; 3, 20 μl. of the chloroform–isobutanol (85:15) eluate; and 4, 20 μl. of the 0.01 N hydrochloric acid eluate in methanol.

Figure 8—Chromatogram showing the column separation of morphine in the standard formulation with atropine sulfate. Key: 1, 20 μl. of the standard formulation with atropine sulfate; 2, 20 μl. of the chloroform eluate; 3, 20 μl. of the chloroform–isobutanol (85:15) eluate; and 4, 20 μl. of the 0.05 N hydrochloric acid eluate in methanol.

interfere with the assay of morphine. The separation of morphine sulfate from the standard formulation with pseudomorphine added was complete and quantitative (Table III). The column chromatographic separation of morphine and pseudomorphine was confirmed by TLC (Fig. 5). Morphine sulfate was quantitatively recovered from the standard formulation with codeine phosphate added (Table III). The codeine was quantitatively recovered in the initial chloroform eluate from the chromatographic column; therefore, it did not interfere with the determination of morphine sulfate. Finally, morphine sulfate was completely and quantitatively separated from the standard formulation with atropine sulfate added (Table III). The atropine was found in the initial chloroform eluate from the chromatographic column. All of the preceding tabulated data indicated quantitative recovery of morphine sulfate from the solutions and mixtures.

CONCLUSIONS

By using this chromatographic procedure, morphine sulfate was separated quantitatively from its degradation products and from other substances present in an aqueous formulation of morphine sulfate for injection and from other alkaloidal salts which might be employed with morphine sulfate in injectables. The method is simple, rapid, and stability indicating for measuring actual morphine content in aged or discolored morphine injections.

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Purity Profiles for Heroin, Morphine, and Morphine Hydrochloride

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Abstract ☐ Purity profiles were established for samples of heroin (diacetylmorphine), morphine, and morphine hydrochloride in order to define the scope of utility of each as qualitative and/or quantitative analytical standards. Systems for solubility analysis and high-pressure liquid chromatography were investigated. A heroin synthesis affording a product of high purity is described.

Keyphrases ☐ Purity profiles—heroin, morphine, and morphine hydrochloride, phase solubility analysis, high-pressure liquid chromatography ☐ Heroin—synthesis, purity profile, phase solubility analysis, high-pressure liquid chromatography ☐ Morphine and hydrochloride salt—purity profiles, phase solubility analysis, high-pressure liquid chromatography ☐ Phase solubility analysis—purity profiles for heroin, morphine, and morphine hydrochloride ☐ High-pressure liquid chromatography—analysis, purity profiles, heroin, morphine, and morphine hydrochloride

Increased concern in recent years has given rise to demands for greater specificity and accuracy of analytical procedures for various abuse drugs, notably morphine and heroin. Along with this arises a need for standards for these drugs, having known scopes of utility for known qualitative and quantitative applications. The purposes of this report are to identify analytical procedures of value in determining the purity profiles (1) of these drugs and to define the analytical utility of given samples of the drugs. A synthesis of heroin is described which has yielded material suitable for use as an analytical standard.

METHODS AND MATERIALS

Morphine and its hydrochloride salt were obtained commercially. All reagents used were USP, NF, or ACS grade, except as noted.

Phase Solubility Analysis (2)—The method described in NF XIII (3) was followed. Nine 10-ml. tared glass ampuls were flushed with nitrogen, charged with an appropriate progression of sample weights and a fixed volume (3 ml. by pipet) of solvent, and flame sealed. All solvents were freshly distilled and degassed, starting with spectroquality or commercial distilled-in-glass grades. The bath temperature was 25°, the time allowed for equilibration varied with the drug, and rotation was 28 r.p.m. The residues obtained were dried to constant weight at 130°, except for heroin which was dried at 105°. All samples were kept in the dark when possible or in subdued light. The appropriate Student's *t* value, two-tailed, was used in calculating (2) the 95% confidence limits. In computing and

graphing the system composition, the sample weights used were converted to the anhydrous basis.

Titrations—Commercial equipment[1] was used for all potentiometric titrations (*n* = 3, averages given in Table 1) and the dead-stop end-point titration (duplicates).

Nonaqueous Titration (4a, 5)—The sample was dissolved in 5 ml. of glacial acetic acid and titrated to the potentiometric end-point with 0.1 *N* acetous perchloric acid, using a glass indicating electrode and a calomel reference electrode filled with 0.02 *N* lithium chloride in glacial acetic acid. The titrant was standardized against dried potassium biphthalate. Five milliliters of glacial acetic acid served as a blank.

Chloride—The sample was dissolved in 5 ml. of 1.5 *N* sulfuric acid and was titrated to the potentiometric end-point with 0.1 *N* silver nitrate, using a silver indicating electrode and a mercurous sulfate reference electrode. The titrant was standardized with dried sodium chloride, and 5 ml. of 1.5 *N* sulfuric acid served as a blank.

Moisture (4b, 6, 7)—An aliquot of sample was dissolved in anhydrous methanol, injected into a vessel containing methanol titrated to the end-point, and titrated with Karl Fischer reagent[2], using the dead-stop end-point technique and a 20-sec. delay. An aliquot of methanol was titrated as a blank. The titrant was standardized by titrating known volumes of water, delivered by gravimetrically calibrated microsyringe. Loss on drying to constant weight was determined on 300-mg. samples at 130°, except for heroin which was dried at 105°.

Spectroscopy—Recording UV[3] and IR[4] spectrophotometers were used to obtain spectra of the samples, and rotation data were obtained using an automatic polarimeter[5]. Absorptivity data in Table I are the averages of triplicates and rotational data are the averages of duplicates.

GLC—The GLC system[6] was as follows: on-column injection; 1.2-m. × 4-mm. i.d. glass columns; methylsilicone gum on silanized, acid-washed, flux-calcined diatomite; cured, conditioned, and tested as described previously (8); helium carrier gas at 50 ml./min.; flame-ionization detector; and automatic digital integrator[7] for area normalization. Temperature programs were 150–250°, and isothermal purity determinations were at 210°. A nonlinear adsorption graph was prepared for 0.5–100-mcg. sample injections of morphine, codeine, heroin, acetylcodeine, and both acetylmor-