Chromatographic Separation and Assay of Morphine in Tablets

I. J. HOLCOMB, R. B. LUERS, and S. A. FUSARI ˣ

Received January 6, 1975, from *Analytical Development, Parke, Davis and Company, Detroit, MI 48232.* Accepted for publication November 29, 1977.

Abstract □ A method specific for the determination of morphine sulfate in tablets was developed. Morphine is separated from the excipient and 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 greater than 99%. This method is more specific for the separation and determination of morphine in tablets than is the USP XVIII procedure.

Keyphrases □ Morphine sulfate—chromatographic analysis in tablets □ Chromatography—analysis, morphine sulfate in tablets □ Narcotic analgesics—morphine sulfate, chromatographic analysis in tablets

Previously (1), morphine was separated from degradation products in injectables by a simple, rapid, chromatographic method. The same type of procedure is applicable to the separation of morphine in tablets. The USP XVIII extraction–titrimetric method (2) is time consuming, and small amounts of decomposition products as well as morphine are extracted from tablets.

The purpose of this investigation was to provide a simple, rapid, chromatographic method specific for the separation of morphine from its degradation products in tablets.

EXPERIMENTAL

Reagents—Morphine sulfate and lactose were USP grade. Acid-washed diatomaceous earth[1], sodium chloride, hydrochloric acid, isobutyl alcohol, chloroform, and anhydrous ether were reagent grade. Pseudomorphine base and morphine *N*-oxide were prepared in these laboratories (1).

Apparatus—A 25-mm i.d. × 25-cm long chromatographic column with a polytef stopcock, 1-cm quartz cells, and a spectrophotometer[2] with a recorder[3] were used.

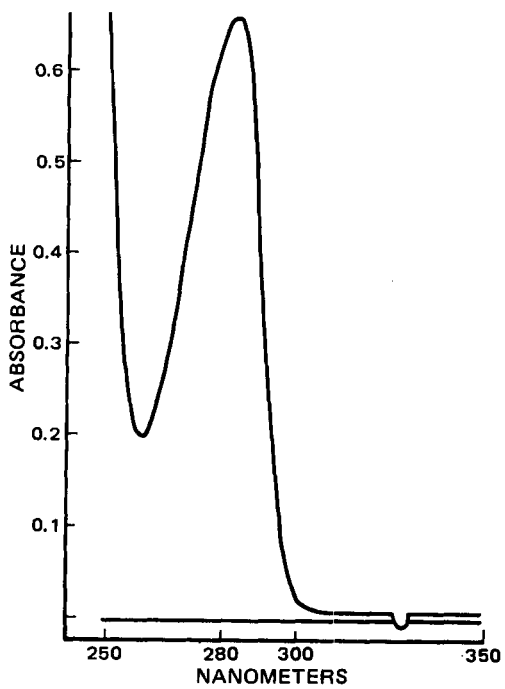

Figure 1—*UV spectrum of morphine sulfate standard, 0.1624 mg/ml, in 0.05 N HCl.*

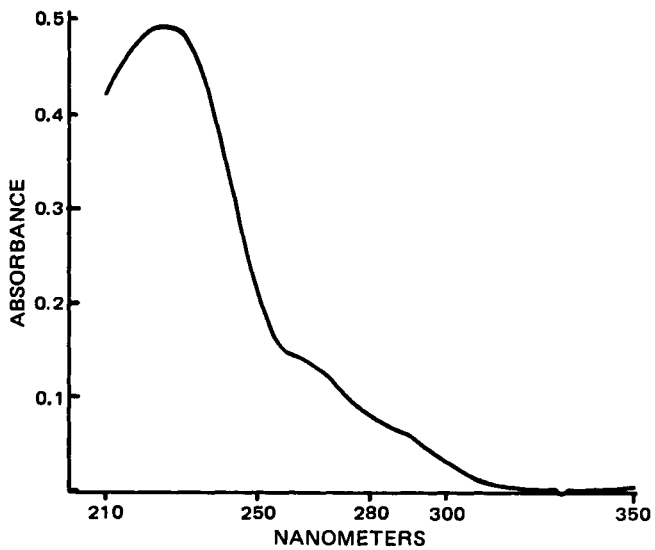

Figure 2—*UV spectrum of pseudomorphine, 0.00746 mg/ml, in 0.05 N HCl.*

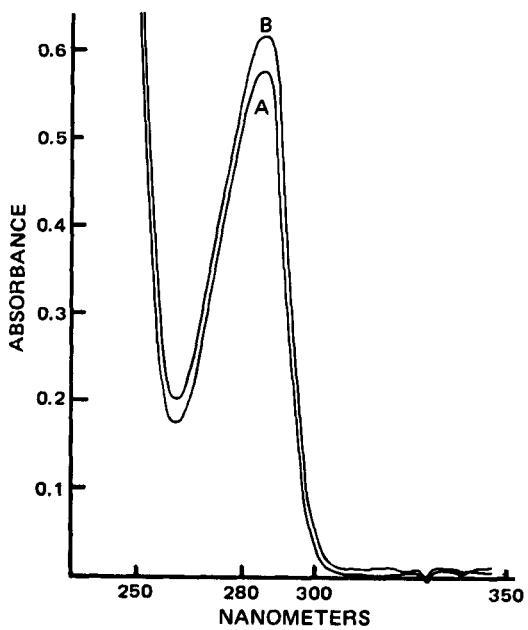

Figure 3—*UV spectra. Key: A, morphine sulfate, 0.1486 mg/ml, in 0.05 N HCl; and B, morphine sulfate, 0.1486 mg/ml, and pseudomorphine, 0.0075 mg/ml, in 0.05 N HCl.*

[1] Celite 545, Johns-Manville Co.

[2] Perkin-Elmer model 124.

[3] Perkin-Elmer model 165.

0022-3549/78/0800-1099$01.00/0

Table I—Assay Results on Morphine Tablets

Lot	Date of Manufacture	Milligrams of Morphine Sulfate Found per Tablet (Percent Label Claim)		
		Column Separation Spectrophotometry	Direct Dilution Spectrophotometry	USP XVIII Procedure
10-mg Tablets				
A	1964	10.03 (100.3)	10.50 (105.0)	10.26 (102.6)
B	1966	9.74 (97.4)	10.46 (104.6)	10.28 (102.8)
C	1973	9.55 (95.5)	9.76 (97.6)	9.81 (98.1)
15-mg Tablets				
D	1966	14.40 (96.0)	15.01 (100.1)	15.00 (100.0)
E	1966	15.30 (102.0)	15.68 (104.5)	15.58 (103.9)
30-mg Tablets				
F	1966	29.54 (98.5)	30.72 (102.4)	30.85 (102.8)
G	1968	30.57 (101.9)	31.24 (104.1)	—

Sample Preparation—The weight of 20 tablets was determined, and the tablets were reduced to a fine powder with a mortar and pestle.

Separation—In a 100-ml beaker, 2.0 ml of 1 *M* potassium phosphate buffer (pH 6.5) was mixed with 3 g of diatomaceous earth. The mixture was firmly packed in a chromatographic column with a pledget of glass wool at the base. A quantity of the powdered tablets, accurately weighed and equivalent to about 20 mg of morphine sulfate, was transferred to a 150-ml beaker. Then 1 ml of water was added to dissolve the powder, and 0.5 g of sodium bicarbonate was added and mixed well. Then 3 g of reagent grade acid-washed diatomaceous earth was added and thoroughly mixed. 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) isobutyl alcohol in chloroform, and the eluate was collected in a 250-ml separator. A 100-ml volume of ether, water washed prior to use, was added to the eluate in the separator, and the contents were mixed carefully.

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

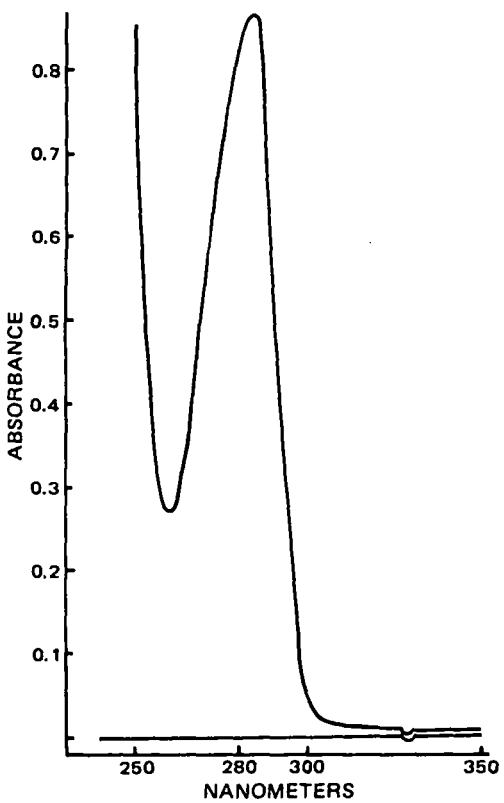

Figure 4—*UV spectrum of morphine eluate from the column chromatographic procedure for morphine tablets (in 0.05 N HCl), Lot C.*

With this chromatographic separation procedure, morphine sulfate standard formulation and tablets were assayed.

Morphine Sulfate Solutions—The excellent recovery of morphine sulfate from standard solutions using this method was established previously (1).

Morphine Sulfate Standard Formulations—Morphine sulfate standard formulations were prepared by mixing weighed quantities of morphine sulfate USP with lactose USP in 100-ml beakers. The following standard formulations were prepared and assayed by the described chromatographic procedure: A, morphine sulfate USP, 19.80 mg, plus lactose USP, 12.20 mg; B, morphine sulfate USP, 19.66 mg plus lactose USP, 15.05 mg; and C, morphine sulfate USP, 18.57 mg, plus lactose USP, 10.80 mg.

UV Spectra of Morphine and Pseudomorphine—The curves in Figs. 1–4 show the spectral differences of morphine and pseudomorphine and demonstrate the effect of pseudomorphine at the morphine maximum at 285 nm. Application of the column chromatographic method separates morphine from pseudomorphine and other degradation products.

Direct Spectrophotometric Examination of Morphine Tablets—Samples from seven lots of morphine sulfate tablets (A–G) from two manufacturers were diluted directly in 0.05 *N* HCl, and spectrophotometric curves were obtained. The purpose of this experiment was to show that interfering substances, which may or may not be decomposition products in morphine tablets, would give assay values (Table I) higher than those obtained using the column chromatographic procedure.

TLC—Separation of morphine in the standard formulation and tablets

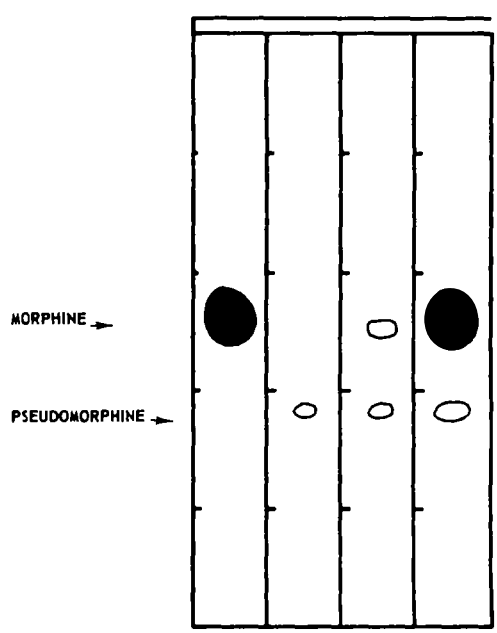

Figure 5—*TLC examination of the fractions obtained using the USP assay for morphine tablets. Key: 1, morphine sulfate; 2, pseudomorphine; 3, alkaline buffer phase after extraction; and 4, chloroform–isobutyl alcohol extract.*

Table II—Recovery of Morphine from Standard Formulations

Standard Formulation	Morphine Sulfate in Standard Formulation, mg	Amount Recovered, mg	Recovery, %
A	19.80	19.70	99.5
B	19.66	19.60	99.7
C	18.57	18.49	99.6

Table III—Content Uniformity Data on Morphine Tablets

Tablet	Milligrams of Morphine Sulfate Found per Tablet (Percent Label Claim) Lot C	Lot E
1	10.05 (100.5)	15.23 (101.5)
2	8.63 (86.3)	15.59 (103.9)
3	10.11 (101.1)	15.12 (100.8)
4	9.90 (99.0)	16.44 (109.6)
5	8.87 (88.7)	16.01 (106.7)
6	9.38 (93.8)	15.06 (100.4)
7	9.18 (91.8)	15.93 (106.2)
8	9.50 (95.0)	15.15 (101.0)
9	9.01 (90.1)	15.62 (104.1)
10	9.29 (92.9)	14.42 (96.1)
Average	9.39 (93.9)	15.45 (103.0)
Range, %	8.63–10.11 (86.3–101.1)	14.42–16.44 (96.1–109.6)

was monitored by TLC. Following the chloroform and chloroform–isobutyl alcohol elutions, the chromatographic columns were eluted with 0.05 *N* HCl in methanol to remove any remaining decomposition products and lactose.

Precoated silica gel GF plates[4] (10 × 20 cm) were used; the mobile phase was chloroform–methanol–diethylamine (80:15:5).

Eluates from the chromatographic columns were concentrated to about 1 ml prior to application to the plates. Aliquots of 20 μl of each concentrate were applied, and the plates were developed for 15 cm from the start. Then the plates were air dried and examined under long and short wavelength UV light.

RESULTS AND DISCUSSION

Extracts from the USP procedure were examined by TLC, and both morphine and pseudomorphine were found (Fig. 5), showing that the USP procedure lacks selectivity. Since most of the tablets were discolored to varying degrees, TLC was performed on the tablets to characterize them. All tablets showed that pseudomorphine was present, and other zones were also present (Fig. 6).

The procedure presented involves the release of morphine as the base with the use of sodium bicarbonate. Chloroform passed through an acidic

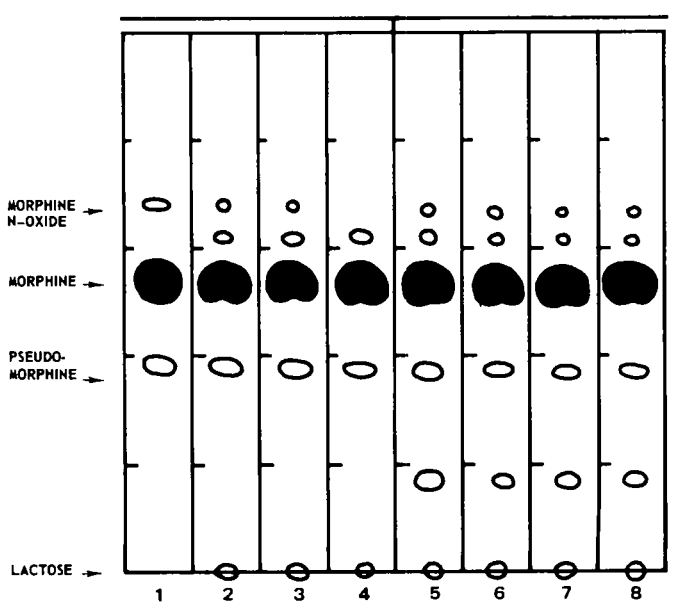

Figure 6—*TLC of morphine tablets. Solutions of tablets in water (10 μl of 60 mg/ml) were chromatographed. Key: 1, standard mixture of morphine sulfate, morphine* N*-oxide, and pseudomorphine; and 2–8, solutions of morphine tablets, Lots A–G.*

[4] Analtech, E. Merck.

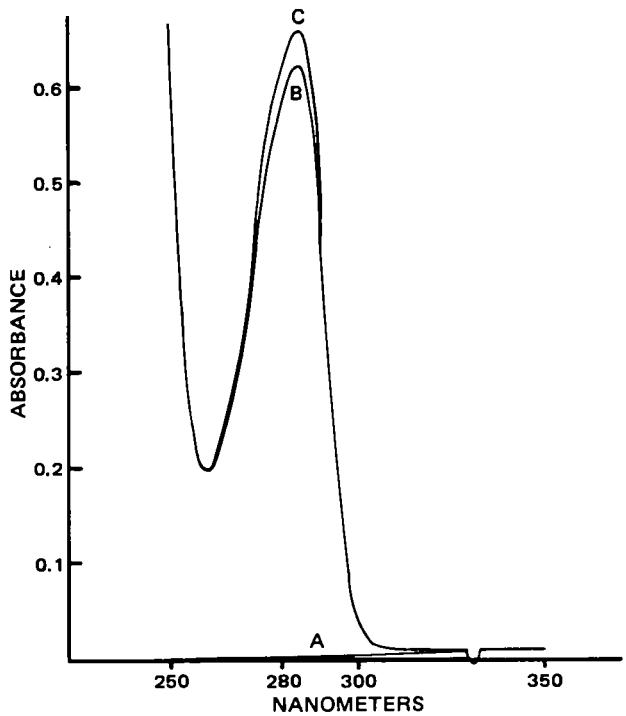

Figure 7—*UV spectra of 0.15 mg of lactose/ml in 0.05* N *HCl (A), Lot E of morphine sulfate tablets via the column chromatographic procedure (B), and Lot E of morphine sulfate tablets via direct dilution and spectrophotometry (C).*

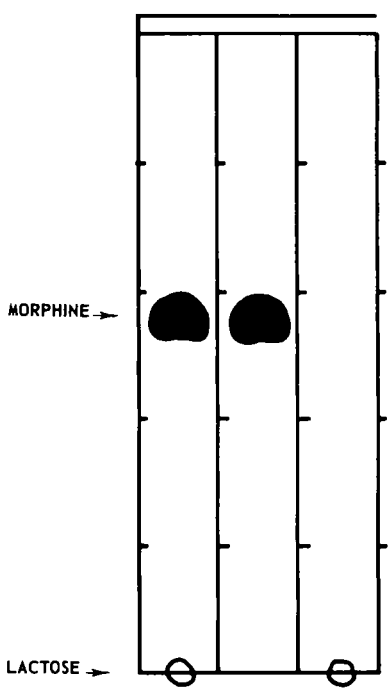

Figure 8—*Chromatogram showing the column separation of morphine in the standard formulation. Key: 1, direct application of standard formulation of morphine sulfate USP and lactose USP; 2, 20 μl of chloroform–isobutyl alcohol eluate; and 3, 20 μl of the 0.05* N *HCl in methanol eluate.*

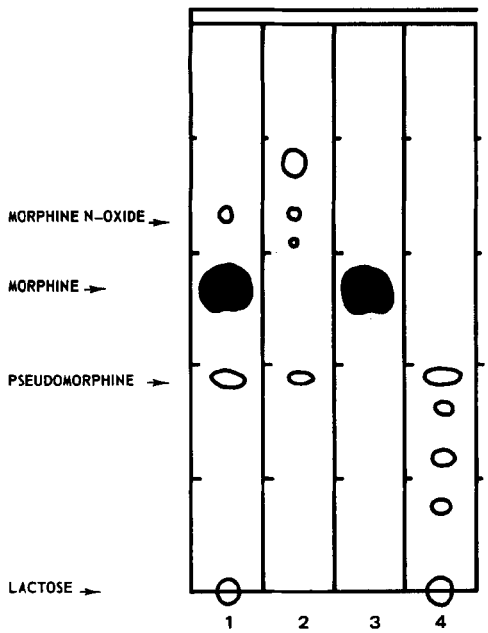

Figure 9—*Chromatogram showing the separation of morphine from decomposition products in morphine tablets. Key: 1, mixture of morphine sulfate, lactose, morphine* N*-oxide, and pseudomorphine; 2, 20 μl of chloroform eluate; 3, 20 μl of chloroform–isobutyl alcohol eluate; and 4, 20 μl of 0.05* N *HCl–methanol eluate.*

trap layer elutes morphine *N*-oxide and other impurities. Morphine, which is partially eluted by the chloroform, is retained by an acidic trap layer (1 *M* phosphate buffer, pH 6.5) and is finally eluted from the column using 15% (v/v) isobutyl alcohol in chloroform. Other tablet ingredients and remaining decomposition products are retained on the column.

On freshly manufactured lots, a direct dilution spectrophotometric assay should be possible. On aged lots, a separation step is necessary (Fig. 7).

The procedure presented was monitored by TLC to check for completeness of separation. In every instance, complete and clean separation of intact morphine was obtained from other substances either present or added (Figs. 8 and 9).

Recovery of morphine from the standard formulations was quantitative (Table II). Morphine was completely and cleanly separated from its decomposition products and lactose (Fig. 9). Seven samples of tablets from two manufacturers were assayed by the column chromatographic procedure (Table I). The results obtained by direct dilution and spectrophotometry (Table I) support the enhancing effect of interfering substances on the morphine assay results as obtained at the maximum at 285 nm. The USP XVIII procedure gave consistently higher assay values than those obtained by the proposed method. The column chromatographic procedure was applied successfully to determine content uniformity of two lots of morphine tablets. Both lots met the USP requirements for content uniformity (Table III).

REFERENCES

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Estimation of Adsorption of Drugs and Proteins on Glass Surfaces with Controlled Pore Glass as a Reference

TAKAHARU MIZUTANI ˣ **and AKIRA MIZUTANI**

Received September 2, 1977, from the *Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467, Japan.* Accepted for publication November 15, 1977.

Abstract □ The amount of drugs adsorbed to glass surfaces was studied, with controlled pore glass as a reference standard, by elution of a solution containing each drug on a pore glass column. The amount of basic drugs, such as epinephrine, physostigmine, and atropine, adsorbed was 1.5–2.0 mg/g of controlled pore glass (97 m^2) in a distilled water medium, but this amount in physiological saline or 0.1 *M* tris(hydroxymethyl)aminomethane hydrochloride (pH 8.6) was negligible. Ascorbic acid, barbital, aspirin, sulfamethoxazole, and acetylcholine were minimally adsorbed to the glass in a water medium or a physiological saline medium. Insulin was adsorbed in a water medium and a glycerin isotonic solution medium at pH 2.6; the amount was 5.5–5.9 mg/g of glass. For clarification of the adsorption mechanism of protein drugs, adsorption of proteins such as bovine serum albumin, chymotrypsin, and lysozyme to glass surfaces was investigated under the various conditions. The maximum amounts of proteins adsorbed on 1 g of controlled pore glass in a distilled water medium were 136, 233, and 84 mg, respectively. The two major forces for adsorption of proteins were ionic amine–silanol bonding and a cooperative cohesive force between proteins and glass. The amount of drugs and proteins to be adsorbed on the inner surface of a glass container and conditions for preventing this adsorption are discussed.

Keyphrases □ Adsorption—various drugs and proteins to glass surfaces studied using controlled pore glass, effect of pH □ Glass surfaces—adsorption of various drugs and proteins studied using controlled pore glass

Adsorption of biological materials on glass surfaces is well known, *e.g.*, the blood clotting reaction (1), macrophage adhesion (2), an antigen-coated column (3), and low adhesion of transformed cells (4). Controlled pore glass was developed for exclusion chromatography (5), and adsorption chromatography of proteins on controlled pore glass was recently reported (6, 7). Applications of porous glass as a carrier for immobilized enzyme were investigated, and the forces involved in the reactions between proteins and glass surfaces were suggested to be ionic amine–silanol bonding and hydrogen bonding (8).

Adsorption of biochemical materials on glass was studied (9) to clarify the adsorption mechanisms. It was reported that about 5 μmoles of cationic biological materials, such as basic amino acids and amino sugars, were adsorbed on 1 g of controlled pore glass, which had a large

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