TURBIDIMETRIC DETERMINATION OF MORPHINE IN SUBMICRO QUANTITIES

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A turbidimetric method is proposed for the determination of morphine in the range of 10—80 μ g. The procedure has been applied to the estimation of morphine after its chromatographic separation from other alkaloids in synthetic mixtures and pharmaceutical preparations. The method is rapid and accurate within ± 1.0%.

Introduction

The determination of morphine has been effected by a variety of methods. Clark and McBay [1] have determined it along with codeine spectrophotometrically. Kudakova [2] has passed its hydrochloride through a resin and titrated the resultant acid with sodium hydroxide with an error of 1%, Kakuma *et al.*[3] have determined it in opium alkaloids by means of paper chromatography with an accuracy of ±2%. Buchi and Huber [4] have separated morphine chromatographically and precipitated it as dinitrophenyl ether. Sbornik and Robot [5] have determined it gravimetrically with sodium tetraphenyl boron as the precipitant.

The gravimetric and titrimetric procedures are difficult to apply to submicro amounts of morphine after its chromatographic separation from other alkaloids or compounds in pharmaceutical preparations. Therefore, a submicro turbidimetric method, based on the application of sodium tetraphenyl boron and already described by Shah and Nargis [6] for the estimation of ajmaline has now been adapted to the determination of morphine. This communication deals with the conditions and procedure for the determination of the alkaloid.

The conditions that have been investigated for obtaining accurate results are (i) the critical volume of the solution at the time of the addition of sodium tetraphenyl boron, (ii) the pH of the solution and (iii) the time allowed for the formation of maximum turbidity. Besides, a procedure for the separation of morphine from other alkaloids through paper chromatography has been laid out.

Experimental

(1) Morphine sulphate or chloride solution 1 μl ≡ μg of morphine sulphate or chloride ≡

(2) Sodium tetraphenyl boron solution 0.3%.

(3) Buffer pH2: 21.008 g. of citric acid were dissolved in 200 ml. of 1M sodium hydroxide solution and diluted to one litre. 30.9 ml. of this solution were mixed with 69.1 ml. of 0.1M hydrochloric acid.

(4) Buffer pH 3.5: 92 ml. of 0.05 N succinic acid and 8 ml. of 0.05 M borax solution were mixed to make 100 ml.

(5) Solvent: Isobutanol + toluene 1:1 saturated with water.

(6) Dragendorff's Reagent: Solution (a) 0.85 g. of bismuth subnitrate were dissolved in 10 ml. of glacial acetic acid and 40 ml. of water.

Solution (b) 8.0 g. of potassium iodide were dissolved in 20 ml. of water. 5 ml. of solution (a) were mixed with 5 ml. of solution (b) and 20 ml. glacial acetic acid added, the solution was made to 100 ml. with water.

Preparation of the Calibration Curves

With an 'Agla' micrometer syringe 10, 20, 30, 40 and 50μl of the morphine sulphate solution were taken in graduated cones and the initial volume was brought to 0.1 ml. with the buffer solution of pH 2. 100μl of the sodium tetraphenyl boron solution were added and the solution was made to 1 ml. by adding the buffer solution. The solution in the cone was mixed by inverting the tube two or three times. For more than 50μg morphine, the initial volume was kept at 0.2 ml. and the final volume of the solution was made to 2 ml. The turbidity was measured after 5 minutes by introducing two drops of these solutions into the cell of a "Spinco" colorimeter. The calibration curves are straight lines (Fig. 1).

1. *Morphine Sulphate or Chloride in Samples (Solids).*—A sample containing 20-40μg or 50-70 μg. of morphine sulphate was weighed on a submicro balance. Alternatively, a solution of the solid containing the alkaloid, was prepared and a

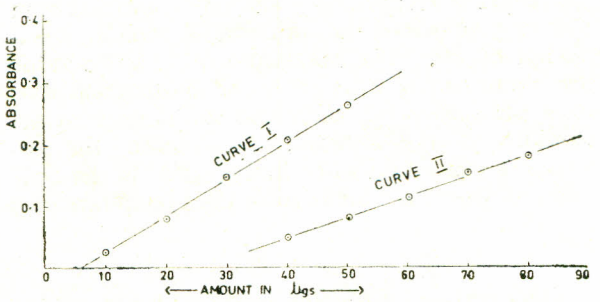

Fig. 1.—Showing calibration curves for morphine sulphate curve I (10-50 µg.) Curve II (50-80 µg·)

suitable volume containing 20-40 µg or 50-70µg of the alkaloid was measured out in graduated cones. The rest of the procedure for determining morphine was the same as has been outlined under the calibration curves.

2. *Morphine Hydrochloride Injections:* (30 *mg. of Morphine per ml.*)—The contents of an ampoule were diluted to 30 ml. with deionised water. 50µl of this solution were taken in a graduated cone and made to 0.1 ml. The method described in (1) was then followed.

3. *Morphine Sulphate Tablets:* (32 *mg. of Morphine Sulphate per Tablet*).—A tablet was dissolved in 25 ml. of deionized water and 23.4µl (3≡0 µg) of the solution were taken in a graduated cone. The procedure described in (1) was then followed.

4. *Morphine Sulphate in Combination with Papavarine Hydrochloride or a Narcotine Hydrochloride.*—The separation of morphine from papavarine and narcotine was effected by paper chromatography. A prewashed paper was impregnated with the buffer solution of pH 3.5 and (isobutanol+ toluene) 1:1 saturated with water was used as solvent.

The mixture of morphine with papavarine or narcotine in methanol was applied as three different spots on the prewashed chromatograhic paper each containing 120µg of the mixture. The chromatogram was run for 6 hours. The separated alkaloids were located by spraying the migration zone of one of the spots with the Dragendorff's reagent. The alkaloids separated from the other two spots were then circumscribed with a pencil corresponding to the position of the alkaloids made visible by the Dragendorff's reagent. The portion encircled with the pencil was then cut into small pieces and soaked overnight in 5 ml. of methanol-deionized water mixture (4:1). A piece of prewashed chromatographic paper, equal in size to the spot was similarly treated for use as a blank. 4 ml. of the clear sclution were evaporated to dryness in an atmosphere of nitrogen at 60°. The residue was dissolved in 0.1 ml. of the buffer solution of pH 2 and the method described in (1) was followed.

5. *Morphine Sulphate from Bismu'h Diarrhoea Tablets.*—The tablet was finely ground in a mortar and transferred to a 50 ml. beaker with 15 ml. of methanol and deionized water mixture and kept overnight. The solution was then filtered and volume made to 20 ml. One or two ml. of this solution were evaporated and the method described in (4) was followed.

6. *Morphine Hydrochloride in "Chlorodyne".*—10 ml. of "Chlorodyne" were taken in a separating funnel followed by 0.5 ml. of dilute ammonium hydroxide solution, 12 5 ml. of water, 10 ml. of ethyl alcohol (95%) and 15 ml. of chloroform. The mixture was shaken for sometime. The lower layer was run off into a second separating funnel. The upper layer was washed with 20 ml. of a mixture of equal volumes of alcohol and water. Further extraction in the first separating funnel was carried out with two more portions of a mixture of 15 ml. chloroform and 7 5 ml. of alcohol (95%) and each alcohol-chloroform extract was washed with alcohol-water mixture as above. The total volume of the extracted liquid was made to 25 ml. One ml. of this solution was evaporated under nitrogen at 60°C. The procedure described in (1) was then followed.

Results and Discussion

Because morphine tetraphenyl boron salt has the tendency to form a crystalline precipitate, the initial and final volumes of the reactants had, therefore, to be controlled to prevent crystallization.

Sodium tetraphenyl boron when added to a solution containing 50 µg of morphine sulphate in 1 ml. did not give any turbidity. When the concentration of the solution was increased to 50µg in 0 2 ml., a precipitate was obtained which on dilution to 1 ml. gave a turbid solution. It was, therefore, inferred that the concentration of morphine sulphate in solution at the time of the addition of sodium tetraphenyl boron was of considerable significance and must be above a certain value in order to get a turbid solution or a precipitate. The influence of the volume at the time of the addition of the precipitant is shown in Table 1, it does not show the expected uniformity

when dealing with a pure solution of the alkaloid. It would be seen that the concentration of morphine in solution is the same in (1c), 2a and 3a, but turbidity is produced in (1c) only and not in the others. Although these observations were repeated and the order of the addition of reagents was changed, no turbidity appeared. Reason for this anomaly cannot yet be explained.

TABLE 1.—SIGNIFICANCE OF THE INITIAL VOLUME OF THE SOLUTION TO OBTAIN MAXIMUM TURBIDITY.

S. No.	Morphine µg.	Initial volume ml.	Final volume ml.	Absorbance	Remarks
1.	a=5	0.1	1.0	0.005	Clear solution
	b=8			0.005	,, ,,
	c=10			0.025	Turbid.
2.	a=20	0.2	1.0	0.005	Clear Solution
	b=30			0.005	,, ,,
	c=40			0.025	Turbid.
3.	a=40	0.4	1.0	0.005	Clear solution
	b=50			0.005	,, ,,
	c=60			0.054	Turbid.
4.	a=70	0.6	1.0	—	Precipitation
	b=80			—	,,
5.	a=80	0.8	1 ml.	—	,,
	b=90			—	,,
6.	a=80	0.6	2 ml.	0.022	Turbid
	b=90			0.06	,,
7.	a=80	0.8	2 ml.	0.005	Clear
	b=90			0.005	Clear

However, from these observations it was clear that if the initial volume was kept at 0.1 ml., it would be possible to get turbidity with morphine sulphate in the range of 10-50µ g. For amounts greater than 50µg. the initial volume had to be changed to 0.2 ml. so as to prevent crystallization.

The amount of sodium tetraphenyl boron necessary to produce maximum turbidity was investigated; 0.3% sodium tetraphenyl boron solution was found optimum and, therefore, used in all experiments.

The pH of the morphine solution was varied from 2.0 to 5.0 before the addition of sodium tetraphenyl boron. The maximum absorbance value was obtained when the pH of the solution was 2.0; from pH 3.0 to 5.0 there was no increase in the turbidity of the solution. In addition, the absorbance was found to be independent of the time allowed after the formation of the precipitate in 5 minutes.

Table 2 shows the results obtained for morphine in some of the pharmaceutical products and synthetic mixtures of morphine sulphate with

TABLE 2.—RECOVERY OF MORPHINE FROM INJECTIONS MIXTURES OF MORPHINE WITH OTHER ALKALOIDS, TABLETS AND OTHER PHARMACEUTICAL PRODUCTS.

Name of the preparation	Morphine sulphate	Morphine sulphate in 4 ml. µg.	Absorbance curve II	Amount in 4 ml. µg.	Difference µg.
Morphine sulphate injections.	30 µg	—	0 15	30	0.0
		—	0.15	30	0.0
			0.15	30	0.0
			0.15	30	0.0
Morphine sulphate + 60 µg papavarine.	60 µg	48	0.250	47.5	–0.5
			0.255	48.0	0.0
			0.250	47.5	–0.5
Morphine sulphate + 60 µg narcotine	60 µg	48	0.25	47.5	–0.5
			0.255	48.0	0.0
			0.255	48.0	0.0
			0.260	48.5	+0.5
Chlorodyne syrup.					
0.1 ml.		—	0.045	14.5	—
			0.045	14.5	—
0.2 ml.		—	0.140	29.0	—
			0.145	29.5	—
Diarrhoea Tablets	(0.4 mg. morphine sulphate per Table)		0.30	11.5	0.39 mg.
			0.35	12.0	

papavarine and narcotine. These results are accurate and reproducible. The method has the advantage of being rapid and the interference due to the presence of other opium alkaloids can be easily overcome by effecting the chromatographic separations before analysis. Difficulty was experienced in the extraction of morphine from the chromatographic spots; the results obtained by a procedure involving extraction in a Soxhlet or by soaking the spot overnight and then evaporating the solution were low, although the amount of morphine sulphate estimated from the solution by direct precipitation without evaporation were quantitative. It was found that during evaporation, morphine, which is susceptible to oxidation, was being changed to pseudo morphine. This difficulty was, nevertheless, overcome by evaporating the solution in an atmosphere of nitrogen.

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