Quantitative Determination of Morphine in Poppy Capsules by Differential Spectrophotometry

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Abstract □ A procedure for a rapid quantitative analysis of morphine in poppy capsules is described. It is based on differential spectrophotometry between alkaline and acidic solutions. The method was applied to poppy capsules cultivated in Argentina. The results were statistically compared with those obtained using a TLC method. Both methods showed no significant differences.

Keyphrases □ Morphine, in poppy capsules—determination by differential spectrophotometry □ Poppy capsules, Argentine—analysis of morphine content, differential spectrophotometry □ Opium alkaloids—determination of morphine in Argentine poppy capsules, differential spectrophotometry □ Spectrophotometry, differential—determination of morphine in poppy capsules

Morphine is still important in pharmacy for its direct use and as a source of semisynthetic derivatives. It is obtained from poppy capsules and from opium. Both foreign opium and local poppy capsules are used in Argentina (1), but the latter is obtained from domestic harvests of *Papaver somniferum* var. *nigrum*.

Between 1943 and 1970, poppy was cultivated in different provinces of Argentina in search of favorable growing conditions. The province of Mendoza fulfilled the minimum requirements. It was essential to produce an homogeneous race of alkaloid-rich plants to improve the average morphine content of poppy capsules. Thus, it was necessary to devise a method for determining morphine in a single capsule.

A number of gravimetric (2–4), titrimetric (3, 5), spectrometric (6–17), polarimetric (18), chromatographic (19–28), and electrophoretic (21) methods have been reported for the quantitative determination of morphine. The differential spectrophotometric method seemed the most suitable for a crude extract in which many interfering substances were expected to be present.

Differential spectrophotometry in the analysis of pharmaceutical products was studied by Milos (6) and later by Casinelli and Sinsheimer (8) who used 1 *N* sodium hydroxide solutions, finding it inapplicable to morphine unless it had been previously separated by chromatography. Gupta (12) found 0.1 and 0.01 *N* sodium hydroxide solutions to be more suitable and developed a practical way for determining the alkaloid even in mixtures. Recently, a similar method (29) has been developed and applied to the determination of morphine in tablets, suppositories, and eardrops. Up to now, differential spectrometry has not been applied to the determination of morphine in poppy capsules.

Table I—Absorbance–Concentration Relationships

Morphine Concentration, mg./100 ml.	Absorbance
0	0
0.97	0.059
1.94	0.121
2.91	0.190
3.88	0.254
4.84	0.321
5.81	0.387
6.78	0.450
7.75	0.516
8.72	0.595
9.69	0.655

EXPERIMENTAL

Apparatus—A spectrophotometer[1] with 10-mm. path rectangular cells was used.

Reagents—Analytical grade reagents were used:

0.1 N *Sodium Hydroxide*—Dissolve 4.00 g. of sodium hydroxide in 1.00 l. of water.

30% Ammonium Phosphate—Dissolve 30 g. of ammonium phosphate, monobasic, in water to make 100 ml. total volume.

0.2 N *Hydrochloric Acid*—Add 16.6 ml. of concentrated hydrochloric acid to enough water to make 1.00 l.

2% Sodium Nitrite—Dissolve 2.0 g. of sodium nitrite in 100 ml. of water.

10% Ammonium Hydroxide—Add 350 ml. of concentrated ammonia to enough water to make 1.00 l.

Plant Material—Dried ripe capsules of *Papaver somniferum* L. var. *nigrum* DC., cultivated in the province of Mendoza (Argentina) in 1970, were used. They were chosen at random and obtained from well-grown, healthy plants.

The capsules were opened and the seeds were discarded. The material was powdered, thoroughly mixed, and dried at 60° to constant weight. The same batch was used throughout the study.

Preparation of Alkaloidal Extract—Transfer an accurately weighed aliquot (about 2.50 g.) into a conical flask with a stopper and add a mixture of 2 ml. of 10% ammonium hydroxide and 6 ml. of 96% ethanol. Add 30 ml. of isopropanol–chloroform (1:3) and stir the mixture for 30 min. Filter the solution through paper into a 50-ml. volumetric flask and bring to volume.

Procedure A: Differential Spectrophotometry—*Standard Preparation*—Accurately weigh about 10.0 mg. of anhydrous morphine or its equivalent of a suitable salt. Dissolve it in 0.1 *N* sodium hydroxide to make 100 ml. total volume. Use this solution immediately.

Calibration—Use 0.1 *N* sodium hydroxide to dilute the standard preparation to obtain 11 solutions containing between 0 and 0.100 mg. of anhydrous morphine per milliliter. Transfer two 2.7-ml. portions of each solution to separate cells (*r* and *s*). Add 0.45 ml. of 30% ammonium phosphate to the reference cell (*r*) and 0.45 ml. of 0.1 *N* sodium hydroxide to the sample cell (*s*); mix carefully. Set the wavelength control knob to 320 nm. and adjust to 100% transmittance. Turn the wavelength control knob to 299 nm. and determine the absorbance of the *s versus* the *r* cell. Use the data thus obtained to calculate the absorptivity (*a*) from Eq. 1:

$$a = \frac{\Sigma AC - (\Sigma C \Sigma A/n)}{\Sigma C^2 - [(\Sigma C)^2/n]} \qquad \text{(Eq. 1)}$$

and the A_0 value from Eq. 2:

$$A_0 = \frac{\Sigma A}{n} - a \cdot \frac{\Sigma C}{n} \qquad \text{(Eq. 2)}$$

[1] Beckman DBG.

Table II—Comparison of Assay Methods for Morphine Applied to Poppy Capsules

Sample Number	Morphine Found, g./100 g. Poppy Capsules Procedure A	Procedure B
1	0.516	0.563
2	0.538	0.542
3	0.541	0.528
4	0.513	0.515
5	0.566	0.529
6	0.537	0.501
7	0.510	0.490
8	0.507	0.470
Average	0.5285	0.5173
SD	±3.9%	±5.8%

where A is the absorbance corresponding to the concentration C, n is the number of observations, and A_0 is the theoretical absorbance when $C = 0$.

Procedure—Transfer 10 ml. of the alkaloidal extract to a separator and extract with 0.1 *N* sodium hydroxide (3 × 15 ml.). Collect the alkaline solution in a 50-ml. volumetric flask, and dilute to volume with 0.1 *N* sodium hydroxide. If necessary, centrifuge to clarify the solution. Transfer two 2.7-ml. portions to separate cells (*r* and *s*). Measure the absorbance of the *s versus* the *r* cell as stated under *Calibration*. The amount of morphine in the sample is calculated using Eq. 3:

$$\text{g. morphine/100-g. capsule} = \frac{(A - A_0)}{a} \cdot \frac{25}{q} \qquad \text{(Eq. 3)}$$

where A is the measured absorbance, a is the absorptivity calculated using Eq. 1, A_0 is the theoretical absorbance when $C = 0$ (previously calculated with Eq. 2), and q is the weight in grams of the sample.

Procedure B: TLC—*Standard Preparation*—Accurately weigh about 40.0 mg. of anhydrous morphine or its equivalent of a suitable salt. Dissolve in isopropanol–chloroform (1:3) to make 100 ml. total volume, and keep the solution in a refrigerator. Renew monthly.

Procedure—A modified version of the method of Heusser and Jackwerth (11) was used. The absorbent was silica gel PF_{254}[2]. Chromatography was carried out on 20 × 20-cm. glass plates coated with a 250-μ layer. They were heated at 130° for 30 min. before spotting. The solvent system was chloroform–acetone–methanol–25% ammonia solution (30:40:10:5). Solutions were spotted with a 1-ml. pipet in 2.5-cm. bands. A total of five bands was used per plate as follows: 1, 0.30 ml. standard preparation; 2, 1.20 ml. alkaloidal extract; 3, 0.60 ml. standard preparation; 4, 1.20 ml. alkaloidal extract; and 5, 1.20 ml. standard preparation.

The plates were developed (ascendingly) to 15 cm. After development, they were dried at room temperature and viewed under UV 254-nm. light. The morphine spots were marked and then scraped off with a spatula. Each spot was transferred to a centrifuge tube and extracted by shaking with 3 ml. of 0.2 *N* hydrochloric acid. An extra tube containing 3 ml. of 0.2 *N* hydrochloric acid was added at this point as a reagent blank. To each tube, 2 ml. of 2% sodium nitrite was added. The tubes were allowed to stand at room temperature for 15 min. and then made basic by the addition of 3 ml. of 10% ammonium hydroxide; the silica layer was separated by centrifugation.The absorbance *versus* the reagent blank was immediately determined at 470 nm. The values of a and A_0 were calculated for each plate using Eqs. 1 and 2 (where C refers to the quantity of morphine applied to each standard band). The assay values of morphine were determined using the relationship:

$$\text{g. morphine/100-g. capsule} = \frac{A_2 + A_4 - 2A_0}{12a} \cdot \frac{25}{q} \qquad \text{(Eq. 4)}$$

where A_2 and A_4 are the absorbances corresponding to bands 2 and 4 of the corresponding plate, respectively, and q is the weight in grams of the sample.

[2] Merck.

Table III—Application of the Student *t* test to the Differences between the Data from Table II

Sample Number	Difference[a]
1	−0.047
2	−0.004
3	0.013
4	−0.002
5	0.037
6	0.036
7	0.020
8	0.037
$\bar{d}$	0.01125
s^2	0.000831
$s_{\bar{d}}$	0.01019
t_0'	1.1036
$t_{0.3}$ (from tables)	1.119

[a] Procedure A − Procedure B = d_i.

RESULTS AND DISCUSSION

By using differential spectrophotometry as described, the concentration of a pure morphine solution was shown to be linear with the absorption (Table I). The average of eight determinations of morphine in the batch gave 0.5285 g. of anhydrous morphine/100-g. capsule. The relative standard deviation was 3.9%. The compatibility of these results with the 0.10–0.60% values found in the literature (21, 32, 33) and with the average industrial yield (0.2%) (32, 34) is of interest.

The method of Heusser and Jackwerth (11), in which morphine is previously isolated by TLC, was selected for comparative purposes. The alkaloidal extract was chromatographed, and then morphine was eluted and submitted to the Radulescu reaction (30), a simple case of phenol nitrosation (31). The nitrosomorphine was measured at 470 nm. An average of eight determinations gave 0.5173 g. of anhydrous morphine/100-g. capsule, and the relative standard deviation was 5.8%. Results of both methods are shown in Table II.

A comparison between averages shows a difference of 0.0112 g. of morphine/100-g. capsule. Statistical analysis of the data showed that this difference was not significant.

As the same alkaloidal extract was used for each pair of trials, the Student *t* test could also be applied to the respective differences (Procedure A − Procedure B = d_i) (Table III).

From the table of *t*, it was found that the chance of obtaining a value of *t* greater than or equal to $\pm t_0'$ (1.104) is slightly greater than 30 in 100 ($t_{0.3} = 1.119$). Hence, the null hypothesis ($\bar{d} = 0$) cannot be rejected at the 5% level of significance. The conclusion is also that the two methods of analysis did not produce significantly different results.

CONCLUSION

The present method allows a relatively rapid determination of morphine in poppy capsules without prior chromatography of the crude extract. It is accurate enough to be used in the selection of the alkaloid-rich capsules.

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Inhibition of Pyruvic Acid Oxidation by 2,5-Substituted 1,3,4-Oxadiazoles

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Abstract □ Several 2-substituted amino-5-(dichlorophenoxymethyl)-1,3,4-oxadiazoles were synthesized by cyclizing various corresponding 1-(dichloro substituted phenoxyacetyl)-4-substituted thiosemicarbazines. These compounds exhibited the ability to inhibit oxidation of pyruvic acid by rat brain homogenate and showed gross behavioral depression in mice on intraperitoneal administration.

Keyphrases □ Pyruvic acid oxidation—2,5-substituted 1,3,4-oxadiazoles, synthesized and tested as inhibitors, rats, mice □ 1,3,4-Oxadiazoles, 2,5-substituted—synthesis, studied as inhibitors of pyruvic acid oxidation, rats, mice □ Thiosemicarbazines, substituted—synthesis, studied as inhibitors of pyruvic acid oxidation, rats, mice

Several 1-acyl-4-substituted thiosemicarbazines and their cyclized 1,3,4-oxadiazoles have been reported to possess diverse biological properties. The ability of 2,5-disubstituted 1,3,4-oxadiazoles to exhibit analgesic (1, 2), anti-inflammatory (2–4), antipyretic (2), muscle relaxant (5–7), tranquilizing (5, 6), and CNS depressant (8) activities led the present authors to synthesize several 2-substituted amino-5-aryl-1,3,4-oxadiazoles. Furthermore, selective inhibition of nicotinamide adenine dinucleotide-dependent oxidation by CNS depressants such as substituted 4-quinazolones (9–11) prompted the evaluation of the intermediate thiosemicarbazines and cyclized oxadiazoles for their ability to inhibit oxidation of pyruvic acid by rat brain homogenate. Attempts also were made to determine the structure–activity relation of these compounds to their enzyme inhibitory activity. The various oxadiazoles were synthesized according to Scheme I.

EXPERIMENTAL

Ethyl 2,6-Dichlorophenoxyacetate (I)—An equimolar quantity of 2,6-dichlorophenol (8.15 g.), ethyl chloroacetate (6.15 g.), and potassium carbonate (10.35 g.) in dry acetone (50 ml.) was refluxed under anhydrous conditions for 10 hr. The reaction mixture was filtered and the filtrate was poured into 50 ml. of chilled water. The ester was extracted with ether and dried over magnesium sulfate. On removing the excess ether, the remaining liquid was fractionally distilled. The fraction boiling at 180–185° was collected, yielding 9.90 g. (80%).