THE COLORIMETRIC DETERMINATION OF MORPHINE IN GALENICAL PREPARATIONS

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Methods by which morphine may be determined colorimetrically are discussed and the present official method criticised. The reaction described by Pride and Stern, which depends upon the oxidation of morphine with iodic acid followed by treatment with ammonium bicarbonate and nickel chloride to give a green colour has been found satisfactory. Improved extraction procedures for the separation of morphine from extraneous material in a number of galenical preparations are suggested and application of the colorimetric reaction to morphine residues so obtained, is described.

In an investigation to find better assay procedures for galenical preparations of opium than have been used hitherto the reaction of morphine with fluorodinitrobenzene was studied in detail[1]. The procedure is applicable to preparations containing 0·1 per cent or more of morphine but not less since the size of sample required is then too large. Many colorimetric reactions have been proposed; these have been reinvestigated in a search for one which would be specific, reliable and applicable to pharmaceutical preparations containing small quantities of opium.

The nitrosomorphine reaction[2] is the official procedure in this country. Besides being a general phenolic reaction[3] the colour produced depends on many variable factors, while the presence of coloured extractive from other ingredients often means that the final colours are of different tints so that a compensating technique may be required[4]. Many of the variables were described by Stephens[5] but two pharmacopoeias have been issued since without modification of the standard method. Our investigation of the nitrosomorphine method confirmed and extended that of Stephens. We concluded that improvements could not be expected by modifying the official method.

In 1906 Georges and Gascard[6] described a determination of morphine based on the action of iodic acid followed by ammonia to give a brown colour and subsequently other workers modified the method and extended its use[7–9]. Although many reducing substances undergo this reaction morphine is one of the few alkaloids to do so. In 1946 Guarino[10] described a series of reactions claimed to provide a specific test for morphine. This depended on the formation of a violet-red colour when the oxidation product resulting from the action of iodic acid followed by ammonium carbonate was treated with ferric chloride solution. The procedure was adapted to the quantitative determination of morphine[11–13] and its specificity was evaluated by Javicoli[14]. Cramer and Voerman[15] found difficulties with Guarino's method and proposed the substitution of ferric chloride by nickel sulphate. In 1954 Pride and Stern[16] investigated the procedure and described its application to opium and to poppy capsules.

Little attention[17] seems to have been paid to Pride and Stern's work, yet it seemed to the present authors that the procedure would be satisfactory for general application to galenical preparations, provided that suitable extracts could be prepared. Much of the present work has been directed to this problem of the effective separation of morphine from interfering substances.

Examination of Experimental Conditions and Reproducibility of the Pride and Stern Reaction

As well as confirming the results of Pride and Stern we have extended their work to cover the following points.

The reproducibility of the method was checked over the range of 0·4 to 4·0 mg. anhydrous morphine on different days under similar conditions of light intensity and temperature. Beer's Law is obeyed over this range and consistently reproducible results were obtained. Complexes were developed in the dark, in daylight and in artificial light. Similar extinction values were obtained showing the reaction to be independent of light intensity.

The optical density was recorded at intervals of 15 minutes after the addition of the ammonium bicarbonate–nickel chloride reagent. Maximum absorption was developed at 90 minutes after the addition of the complexing solution and the complex was stable for at least a further 2½ hours.

Pride and Stern made all measurements within the limits 20° ± 1·5°. Since the working temperatures might be outside this range, colours were formed at temperatures ranging from 15 to 25° but without significant differences in optical density.

Pride and Stern's investigations into the effect of varying the concentration of ammonium bicarbonate in the complexing reagent were extended. Variations of ± 7·5 per cent were tolerable.

Specificity of the Method

Pride and Stern investigated the specificity of the reaction by examining firstly some of the naturally occurring alkaloids of opium and secondly a series of natural and synthetic bases, with structures closely related to morphine.

Non-phenolic alkaloids. The reported non-interference by the principal non-phenolic alkaloids occurring in opium was confirmed.

In opium, 0·1 to 0·5 per cent of an "unknown base" has been quantitatively determined by the United Nations Secretariat for Opium Analysis[18]. Isolation of this base, believed to be hydrocotarnine, a hydrolysis product of narcotine, was made from a sample of Turkish opium and a suitable portion taken for colour development. Although visually there was no apparent reaction with either iodic acid or ammonium bicarbonate–nickel chloride reagent an absorption curve of extinction against wavelength between 400 and 800 mμ gave a calculated E(1 per cent, 1 cm.) at 670 mμ of 4·4. (The E(1 per cent, 1 cm.) for morphine at 670 mμ was 54·2.) On the assumption that the unknown base occurs in opium to a maximum

extent of 0·5 per cent the amount of interference would appear to be negligible. Recoveries of morphine determined in the presence of amounts of the unknown base in excess of what would occur naturally were quantitative.

Phenolic alkaloids. Of these only pseudomorphine is mentioned by Pride and Stern. Although this alkaloid was obtained by Pelletier it is still not known whether it actually occurs in opium or is formed during extraction[19]; the amounts obtained are small, about 0·02 per cent. The "minor phenolic alkaloids" are known to occur, possibly up to 2 per cent.

Pseudomorphine. A sample of pseudomorphine was prepared by the method of Polstorff[20].

The liberation of iodine from iodic acid by pseudomorphine and the formation of a brown colour which merely deepens on the addition of the ammonium bicarbonate–nickel chloride reagent was confirmed. The absorption curve of the complex shows minimum absorption at wavelengths >650 and the calculated E(1 per cent, 1 cm.) at 670 mμ was 1·9. The amount of interference when the method is applied to natural products is therefore negligible and a correction procedure detailed by Pride and Stern is unnecessary. Recoveries of morphine in the presence of amounts of pseudomorphine in excess of that which would be obtained from natural sources were quantitative.

Minor phenolic alkaloids. These are porphyroxine-meconidine, the substance responsible for the formation of the red-coloured material on treatment of opium extracts with acid, representing about 0·5 per cent of the opium, and three alkaloids, laudanine and the related codamine and narcotoline, together representing about 1 per cent of the opium. No supply of these alkaloids was available and they were therefore extracted from a sample of opium by the method of the United Nations Unified Analysis of Opium.

Porphyroxine-meconidine. The fact that iodine was liberated from iodic acid could not readily be seen owing to the dark red colour of the solution but was verified by shaking with carbon tetrachloride which became purple after about 2 minutes. Addition of the ammonium bicarbonate–nickel chloride reagent produced a yellowish-brown solution which on standing gave a dark brown turbidity. After removal of the precipitate by centrifuging the extinction was negligible.

Recoveries of morphine in the presence of such proportions of porphyroxine–meconidine as would be extracted with it from an opium preparation, however, were quantitative; the reaction mixture failed to show the formation of a precipitate during the time that the green morphine complex was stable.

Laudanine, codamine and narcotoline. These three alkaloids are collectively extracted by the United Nations method, and were not separated before applying the colour reaction.

Iodine was liberated from the iodic acid; addition of ammonium bicarbonate–nickel chloride gave a yellow-brown solution which on standing produced a greyish-green turbidity. At greater dilutions the reactivity of these alkaloids appears to be so much reduced that no

precipitate is formed while the morphine complex is stable. Recoveries of morphine in the presence of these phenolic alkaloids were quantitative.

Application to the Determination of Morphine in Galenical Preparations

The United Nations Unified assay of opium for alkaloids recommends a 3 : 1 chloroform-*iso*butyl alcohol mixture for the extraction of morphine. We have found this to be satisfactory for the preparations discussed below.

Preparation of Standard Samples of the Galenicals under Test

Since many official preparations containing morphine use Camphorated Tincture of Opium which is made from Tincture of Opium, the latter was taken as a standard, samples being prepared from both Turkish and

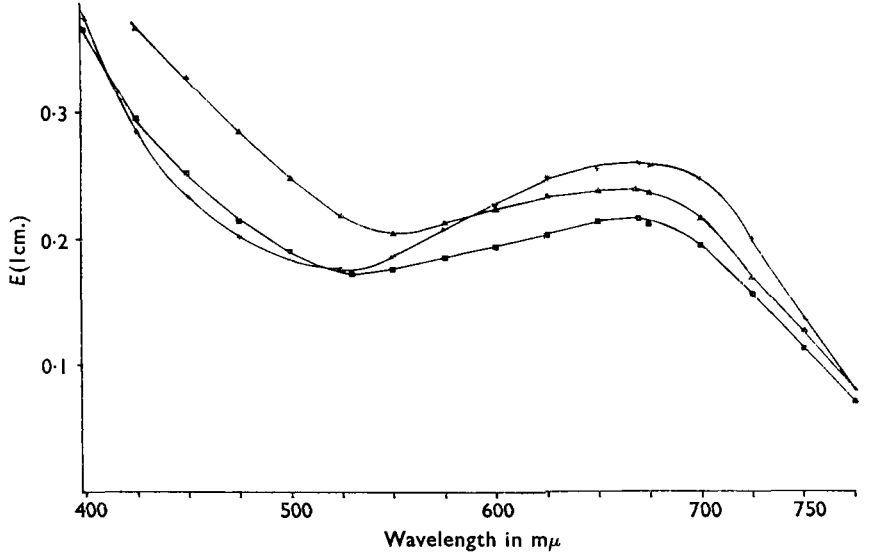

FIG. 1. Absorption curves of colours developed from morphine standard and that obtained from Camphorated Tinctures of Opium B.P.
—*—*— Morphine standard.
—▲—▲— Camphorated Tincture of Opium B.P. prepared from Indian opium.
—■—■— Camphorated Tincture of Opium B.P. prepared from Turkish opium.

Indian opiums. The anhydrous morphine content of the prepared tinctures was determined by the 2 : 4-dinitrofluorobenzene precipitation method[1].

Camphorated Tincture of Opium B.P. A direct extraction of the morphine from ammoniacal solution with chloroform-*iso*butyl alcohol mixtures led to emulsification. Removal of the alcohol on the steam bath and addition of 0·05N HCl to the residue gave a solution which was not only strongly coloured but had a pH above the critical value of 1·6 established by Pride and Stern[16].

A procedure similar to that recommended for the preliminary separation of morphine from extraneous material in galenicals was adopted[1].

The preparation is evaporated to a small volume and after liberation of the free base mixed with aluminium oxide to form a column. The alkaloid is then eluted from the column with a solvent. This method has the advantage of retaining much extraneous material, avoiding the formation of emulsions and requiring a minimum of solvent for extraction. Satisfactory results were obtained with this method. pH measurements on the solution before colour development ranged from 1·48 to 1·52, and of the complex they varied between 7·98 and 8·03. Pride and Stern have shown that the pH of the solution after colour development should be 8·0 $\pm$ 0·05. Blank mixtures containing oil of anise, benzoic acid and camphor gave no colour by the same procedure. Absorption curves for the morphine complexes derived from Turkish and Indian opiums and pure morphine are shown in Figure 1.

The recommended method of assay is as follows.

Apparatus. Unless otherwise stated all optical densities were recorded on a Unicam S.P. 600 spectrophotometer using 1 cm. cells.

Reagents. Dry ammonium bicarbonate. Ammonium bicarbonate B.P. 1953 dried over silica gel, at room temperature, and stored in the same manner. Chloroform—*iso*butyl alcohol mixture. Chloroform B.P. 3 parts, *iso*butyl alcohol 1 part. Iodic acid solution. A 4·5 per cent w/v aqueous solution of reagent grade iodic acid. Ammonium bicarbonate–nickel chloride reagent. Transfer 8 g. of dry ammonium bicarbonate to a 100 ml. graduated flask, add 25 ml. of 4M ammonium chloride solution, 20 ml. of N ammonia solution and 10 ml. of a 1 per cent w/v aqueous solution of nickel chloride hexahydrate and dilute to about 90 ml. with water. Stopper the flask, shake vigorously until the ammonium bicarbonate has dissolved and dilute to volume with water. This reagent should be freshly prepared but the various solutions from which it is made, with the exception of the N ammonia, may be kept as stock solutions.

Method

Take 10 ml. of sample in a small dish and evaporate to dryness on a steam bath. Triturate the residue with 1 ml. of dilute solution of ammonia. Add aluminium oxide gradually and continue triturating until a dry free-flowing powder is obtained. Transfer the powder to a dry chromatographic tube about 40 cm. in length and 1·5 cm. in diameter, previously plugged lightly above the tap with cotton-wool. Remove any adhering powder from dish and pestle with cotton-wool moistened with *iso*butyl alcohol and add to the tube. Insert the lower end of the tube through a bung fitting into the neck of a 150 ml. separator and elute with 50 ml. of chloroform-*iso*butyl alcohol mixture, adjusting the rate of elution to about 1·5 ml. per minute. Wash the solution in the separator with 10 ml. of water. Allow to separate. Filter the organic phase through a cotton-wool plug moistened with solvent into a 150 ml. beaker. Shake the aqueous phase with 10 ml. chloroform-*iso*butyl alcohol and after allowing to separate, filter the organic phase into the same beaker. Wash the filter with a little more of the solvent mixture and evaporate the solvent on a steam bath under a gentle current of air. Heat the residue on the

steam bath for a further 5 minutes to ensure removal of ammonia. Take up the residue in 5 ml. 0·5N hydrochloric acid accurately measured, gently warming for a few seconds on a steam bath if necessary. Filter the solution through a Whatman No. 41 filter paper into a 50 ml. graduated flask washing the beaker well with small quantities of water and transferring to the filter. Cool to laboratory temperature if necessary, then make up to volume with water. Take 10 ml. of the solution prepared above in each of two 25 ml. graduated flasks. To one (the experiment) add 2 ml. of the iodic acid solution with thorough mixing and after exactly

TABLE I

COMPARISON OF THE DETERMINATION OF MORPHINE BY THE B.P. METHOD AND THAT OF PRIDE AND STERN IN FIVE SAMPLES OF CAMPHORATED TINCTURE OF OPIUM B.P.

	Per cent w/v anhydrous morphine		
	Calculated	B.P. method	Proposed method
Sample A (Indian)	0·057	0·060 0·060	0·057 (Mean of 16 results; range 0·056 to 0·059)
Sample B (Indian)	0·050	0·053 0·054	0·051 (Mean of 12 results; range 0·049 to 0·052)
Sample C (Indian)	0·048	0·051 0·050	0·048 (Mean of 8 results; range 0·047 to 0·049)
Sample D (Turkish)	0·053	0·058 0·057	0·054 (Mean of 8 results; range 0·053 to 0·055)
Sample E (Turkish)	0·048	0·051 0·053	0·049 (Mean of 7 results; range 0·048 to 0·050)

2 minutes add 10 ml. of the ammonium bicarbonate–nickel chloride solution. Dilute to volume with water and mix. To the other flask (the blank) add 5 ml. of 0·1N hydrochloric acid and 10 ml. of the ammonium bicarbonate–nickel chloride solution and mix.

Read the optical density after 90 minutes in a 1 cm. cell at 670 mμ on a suitable spectrophotometer or with an Ilford Filter No. 608 if using a "Spekker" or similar instrument.

Determine the weight of anhydrous morphine present by reference to a calibration curve. Alternatively compare the optical density with that of a complex developed at the same time on a standard solution of morphine of suitable dilution. The results obtained on a number of prepared samples are given in Table I.

Opiate Linctus of Squill B.P.C. Standard samples were prepared by accurately diluting the Camphorated Tinctures of Opium referred to above.

A direct column purification cannot be applied to this preparation because of the large quantity of the sample required. An extraction method which separates the morphine from interfering ingredients without the formation of emulsions is detailed below. Oxymel of Squill and Syrup of Tolu gave no colour when similarly treated. Recoveries of morphine

made in the presence of a "blank" sample were quantitative. The method of assay is as follows.

Transfer 30 ml. of sample to a separator. Add 1 ml. dilute solution of ammonia. Mix. Shake vigorously for 2 to 3 minutes with 30 ml. of the chloroform-*iso*butyl alcohol mixture. Allow to separate and run the solvent phase into another separator containing 10 ml. of water. Shake well and after separation filter the solvent through a plug of cotton-wool into a small beaker. Repeat the extraction in the first separator with two further quantities of chloroform-*iso*butyl alcohol, each of 30 ml., washing

TABLE II

COMPARISON OF THE DETERMINATION OF MORPHINE BY THE B.P.C. METHOD AND THAT OF PRIDE AND STERN IN THREE SAMPLES OF OPIATE LINCTUS OF SQUILL. B.P.C.

	Per cent w/v anhydrous morphine		
	Calculated	B.P.C. method	Proposed method
Sample A (Indian)	0·0156	0·0180	0·0158 (Mean of 13 results; range 0·0153 to 0·0161)
Sample B (Indian)	0·0176	0·0200	0·0177 (Mean of 13 results; range 0·0175 to 0·0180)
Sample C (Turkish)	0·0145	0·0180	0·0146 (Mean of 5 results; range 0·0145 to 0·0147)

successively with the same 10 ml. as before, and filtering into the beaker. Rinse the filter and plug with a little of the solvent. Evaporate the solvent on a steam bath under a current of air and leave the residue on the bath for a further 5 minutes. Cool. Take up the residue in 5 ml. 0·5N hydrochloric acid, accurately measured, warming slightly for a few seconds if necessary. Filter the solution through a Whatman No. 41 filter paper into a 50 ml. graduated flask washing the beaker well with small quantities of water and transferring to the filter. Cool to laboratory temperature if necessary then make up to volume with water.

Continue by the method for Camphorated Tincture of Opium from the words "Take 10 ml. of the solution prepared above. . . ."

Results obtained on a number of samples are given in Table II.

Compound Camphorated Linctus of Opium B.P.C. 1949

Standard samples were again prepared by accurate dilution of the Camphorated Tinctures of Opium.

By the official method some extracted material gives a bright yellow colour on adding ammonia, even in the absence of nitrite. The proposed method separates morphine from other ingredients; a sample made up with all ingredients except Tincture of Opium gave negligible absorption at wavelengths between 400 and 800 mμ.

Transfer 50 ml. of sample to a separator. Add 3 ml. dilute solution of ammonia. Mix well. Shake vigorously for 2 to 3 minutes with 50 ml. of the chloroform-*iso*butyl alcohol mixture. Allow to separate and run the solvent into a 250 ml. separator. Extract the solution in the first

separator with two further portions, each of 50 ml. of chloroform-*iso*butyl alcohol mixture and bulk with the first extract. Reject the aqueous phase. Extract the morphine from the solvent by shaking with two portions each of 10 ml. followed by one portion of 5 ml. of 2 per cent sulphuric acid solution, passing each extract through the same plug of

TABLE III

COMPARISON OF THE DETERMINATION OF MORPHINE BY THE B.P.C. METHOD AND THAT OF PRIDE AND STERN IN TWO SAMPLES OF COMPOUND CAMPHORATED LINCTUS OF OPIUM B.P.C. 1949

	Per cent w/v anhydrous morphine		
	Calculated	B.P.C. 1949 method	Proposed method
Sample A (Indian)	0·0143	0·0190	0·0145 (Mean of 4 results; range 0·0144 to 0·0146)
Sample B (Turkish)	0·0125	0·0180	(Mean of 4 results; range 0·0126 to 0·0128)

cotton-wool into another separator. Wash the filter with a few drops of water and make the bulked acid extracts alkaline to litmus paper with dilute solution of ammonia. Continue by the method for Opiate Linctus of Squill beginning at the words "Shake vigorously for 2 to 3 minutes with 30 ml. of the chloroform-*iso*butyl alcohol mixture. . . ." Results obtained on several samples are given in Table III.

TABLE IV

COMPARISON OF THE DETERMINATION OF MORPHINE BY THE B.P.C. METHOD AND THAT OF PRIDE AND STERN IN TWO SAMPLES OF AMMONIATED TINCTURE OF OPIUM B.P.C. 1949

	Per cent w/v anhydrous morphine		
	Calculated	B.P.C. 1949 method	Proposed method
Sample A (Indian)	0·100	0·115	0·099 (Mean of 6 results; range 0·097 to 0·103)
Sample B (Indian)	0·096	0·112	0·097 (Mean of 5 results; range 0·096 to 0·098)

Ammoniated Tincture of Opium B.P.C. 1949

Samples were prepared from standard Tinctures of Opium. The procedure adopted for the assay of Camphorated Tincture of Opium may be applied since benzoic acid and oil of anise do not interfere. Because of its high pH the sample is made just acid before evaporation, to avoid possible oxidation of the morphine. The recommended method is as follows.

Take 5 ml. of sample in a small evaporating dish and add 3N sulphuric acid dropwise with stirring until the mixture is just acid to litmus paper. Evaporate the solution to dryness on a steam bath and continue by the method for Camphorated Tincture of Opium beginning at the words "Triturate the residue with 1 ml. dilute solution of ammonia". Results obtained are given in Table IV.

Opiate Pastilles of Squill B.P.C.

Since it was not possible to prepare pastilles containing an accurately known amount of morphine tests were made on production batches. Different methods of extracting the morphine and of preparing solutions for colour formation were used and where the solution had a pH below the critical value of 1·6 colours were developed and extinction values

TABLE V

COMPARISON OF THE DETERMINATION OF MORPHINE BY THE B.P.C. METHOD AND THAT OF PRIDE AND STERN IN TWO SAMPLES OF PASTILLES OF OPIATE LINCTUS OF SQUILL B.P.C.

	Anhydrous morphine per pastille in g.	
	B.P.C. method	Proposed method
Batch 2374	0·00036	0·00029 (Mean of 5 results; range 0·00029 to 0·00030)
Batch 2493	0·00037	0·00028 (Mean of 3 results; range 0·00027 to 0·00028)

measured. The method yielding the optimum recovery of morphine and a minimum of interference from large excesses of other ingredients are as follows.

Take a sample of 20 pastilles and determine the average weight. Cut up some and accurately weigh into a separator an amount equivalent to about 5. Add 30 ml. of water, stopper the separator and shake vigorously to dissolve. Make the solution alkaline with dilute solution of ammonia

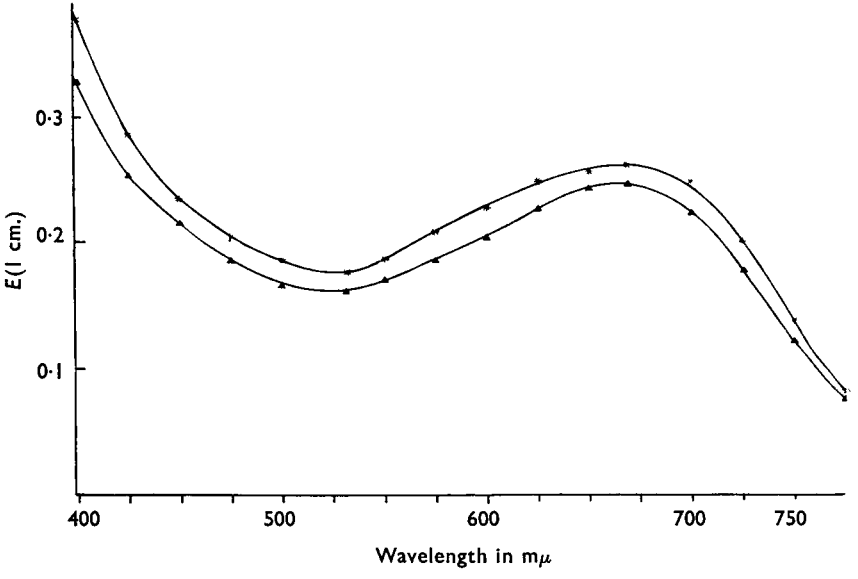

FIG. 2. Absorption curves of colours developed from morphine standard and that obtained from Tincture of Chloroform and Morphine B.P.C.
—*—*— Morphine standard.
—▲—▲— Tincture of Chloroform and Morphine B.P.C.

and extract with three successive portions, each of 30 ml. of chloroform-*iso*butyl alcohol mixture shaking well at each extraction for 2 to 3 minutes. Collect the extracts in a second separator and reject the aqueous phase. Extract the solvent mixture with two successive portions, each of 10 ml. followed by one of 5 ml. of 2 per cent sulphuric acid solution, bulking all three acid extracts in the same separator. Make ammoniacal and continue by the method for Opiate Linctus of Squill beginning at the words "Shake vigorously for 2 to 3 minutes with 30 ml. of the chloroform-*iso*butyl alcohol mixture". Read the optical density of the green complex in a 4 cm. cell.

Results obtained on production batches sampled at random are given in Table V.

Tincture of Chloroform and Morphine B.P.C.

That the nitroso-phenol reaction when applied to extractives of chlorodyne is subject to interference from other substances present in the residue has been known for some time. Garratt[21] in 1946 drew attention

TABLE VI

COMPARISON OF THE DETERMINATION OF MORPHINE BY THE B.P.C. METHOD AND THAT OF PRIDE AND STERN IN THREE SAMPLES OF TINCTURE OF CHLOROFORM AND MORPHINE B.P.C.

	Per cent w/v anhydrous morphine			
			Proposed method	
	Calculated	B.P.C. method	Sample alone	With 5-fold excess of Liquorice
Sample 1 (Liquid Extract of Liquorice P9216)	0·174	0·190	0·175 (Mean of 11 results; range 0·174 to 0·179)	0·174
Sample 2 (Liquid Extract of Liquorice 1872M)	0·177	0·210	0·176 Mean of 5 results; range 0·173 to 0·176)	0·176
Sample 3 (Liquid Extract of Liquorice P9240)	0·174	0·192	0·172 (Mean of 4 results; range 0·168 to 0·175)	0·174

to apparent "morphine" contents of treacle. In 1951 McLachlan[22] reported the interference from liquorice as being due to the colour which develops with ammonia and glycyrrhizin and some of its hydrolysis products. In 1956 the "apparent morphine contents" of both treacle and liquid extract of liquorice were determined in the laboratory of the Pharmaceutical Society[17], and high recoveries of morphine were obtained by application of the Pride and Stern reaction.

By the extraction procedure detailed below morphine can be effectively separated from the substances present in liquorice which give a colour with ammonia: no yellow colour was developed on addition of the strongly ammoniacal complexing reagent to the blank solution.

Determinations on blank mixtures containing all ingredients except morphine hydrochloride gave negligible extinction values. No colour was obtained on treating Liquid Extract of Liquorice B.P. by the same

procedure. Figure 2 shows absorption curves for the colours obtained from a morphine standard and from chlorodyne. Quantitative recoveries of morphine were obtained using a number of different batches of Liquid Extract of Liquorice, each present in a five-fold excess. For recorded figures obtained on samples prepared from morphine hydrochloride standardised by the fluorodinitrobenzene method, see Table VI. The recommended method of assay is as follows.

Determine the weight/ml. of the sample.

Accurately weigh about 4 g. of sample into a small dish and evaporate the volatile solvents on a steam bath. Cool. Triturate the residue to a smooth cream with 1 ml. dilute solution of ammonia and continue by the method for Camphorated Tincture of Opium beginning at the words "add aluminium oxide gradually . . ." and ending at ". . . adjusting the rate of elution to about 1·5 ml. per minute". Extract the morphine from the eluate with two successive portions each of 10 ml. followed by one of 5 ml. of 2 per cent sulphuric acid solution. Bulk the acid extracts in another separator. Make alkaline to litmus paper with dilute solution of ammonia and proceed by the method for Opiate Linctus of Squill beginning at the words "shake vigorously for 2 to 3 minutes with 30 ml. of the chloroform-*iso*butyl alcohol mixture".

References

1. Garratt, Johnson and Lloyd, *J. Pharm. Pharmacol.*, 1957, **9**, 914.
2. British Pharmacopoeia, 1958, p. 444.
3. Lykken, Treseder and Zahn, *Industr. Engng Chem. (Anal.)*, 1946, **18**, 103.
4. Garratt, *Quart. J. Pharm. Pharmacol.*, 1937, **10**, 466.
5. Stephens, *J. Pharm. Pharmacol.*, 1951, **3**, 815.
6. Georges and Gascard, *J. Pharm. Chim.*, 1906, **25**, 513.
7. Heiduschka and Faul, *Arch. Pharm.*, 1917, **255**, 172.
8. van Itallie and Harmsma, *Chem. Zent.*, 1926, i.2612.
9. van Itallie and Steenhauer, *Arch. Pharm.*, 1927, **265**, 698.
10. Guarino, *Arch. Sci. biol.*, 1946, **31**, 115.
11. Nicolini, *Ann. pharm. franç.*, 1947, **5**, 528.
12. Moorhoff, *Pharm. Weekbl.*, 1952, **87**, 593.
13. Mariani, Guarino and Marelli, *Ann. Chim. appl., Roma*, 1951, **51**, 661.
14. Javicoli, *Soc. Ital. Biol. Sper.*, 1946, **22**, 815.
15. Cramer and Voerman, *Pharm. Weekbl.*, 1949, **84**, 129.
16. Pride and Stern, *J. Pharm. Pharmacol.*, 1954, **6**, 590.
17. Pharmaceutical Society Laboratory Report, *Pharm. J.*, 1956, **176**, 384.
18. United Nations Secretariat, ST/SOA/Ser.K/34. Unified Analysis of Opium for alkaloids.
19. Bentley, *The Chemistry of the Morphine Alkaloids*, Oxford University Press, London, 1954.
20. Polstorff, *Ber.*, 1880, **13**, 86.
21. Garratt, *Quart. J. Pharm. Pharmacol.*, 1946, **19**, 419.
22. McLachlan, *Pharm. J.*, 1951, **167**, 24.

DISCUSSION

The paper was presented by MR. C. A. JOHNSON.

The CHAIRMAN. The nitrosomorphine method had not always been satisfactory, often because of impurities in the final reaction mixture. Better methods of separation of the morphine might improve the results. Had the authors applied the nitrosomorphine method to their final reaction mixtures?

MR. S. G. E. STEVENS (London). How had the Authors arrived at the 'calculated' figures in the Tables?

DR. A. H. BECKETT (London). Was it possible to determine morphine in biological fluids by this method and would it then distinguish between morphine and normorphine?

DR. G. F. SOMERS (Liverpool). How many assays were represented by the figures quoted for the B.P. and B.P.C. assays?

DR. R. E. STUCKEY (London). Recoveries from pastilles were often poor but the authors' method had given a slightly lower figure than the B.P.C. method and no theoretical figure was stated.

MR. JOHNSON replied. 'Calculated' referred to samples prepared on a laboratory scale. The nitrosomorphine reaction had been applied to solutions prepared as described in the Paper and showed a great improvement, but there were other objections to the method. Normorphine had been shown by Pride and Stern to give less colour than morphine. If the two occurred together there would be some interference. The results quoted in the second columns of the Tables were supported by experience over many years. The figures for the pastilles were included to indicate that the proposed method gave lower results than the official method.