THE DETERMINATION OF MORPHINE IN OPIUM AND SOME OF ITS GALENICAL PREPARATIONS

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A method of assay for morphine in opium depending upon precipitation of the dinitrophenylether with 1-fluoro-2:4-dinitrobenzene has been critically examined. The morphine is first separated from other alkaloids and extraneous matter by elution from an alumina column with an organic solvent and subsequent extraction with alkali. The method, which is rapid, has been applied to many galenical preparations and, in cases where the pharmacopoeial method based on the colorimetric nitroso reactions may give high results, figures in accordance with the expected values have been obtained.

In 1935 Mannich described a method of determination of mophine in which the dinitrophenylether was precipitated with 1-chloro-2:4-dinitrobenzene[1], and the application of such a method to the determination of morphine in opium was attempted. The procedure was critically assessed in 1937 by Nicholls[2], who concluded that it was unsatisfactory for opium since he obtained very discoloured residues which contained an appreciable proportion of methoxyl group indicating that other phenolic alkaloids such as laudanine had also been precipitated. In 1951 Dann and Wippern[3] investigated and recommended the use of 1-fluoro-2:4-dinitrobenzene for the precipitation of morphine since they found it more rapid than the chloro-reagent. This material was used for the determination of morphine in opium by Svendsen and Aarnes in 1955[4], but in view of the principle shortcoming of this method, namely the possible co-precipitation of other phenolic alkaloids, the validity of the procedure does not appear to have received much investigation. Moreover the results quoted for a number of samples of opium of unspecified origin were erratic, a spread of over 5 per cent being obtained with 10 determinations on a single sample. The method appeared to be relatively simple to operate and worth further investigation in an attempt to obtain a suitable assay for opium also applicable to its galenical preparations. The method described by Svendsen and Aarnes depends upon the precipitation of morphine with fluorodinitrobenzene after separation from other alkaloids and much extraneous matter by elution from an alumina column with an organic solvent and subsequent extraction with alkali. To assess the method the precipitation stage was first examined.

The Precipitation of Morphine with Fluorodinitrobenzene

In the published method an alkaline solution of the morphine is just neutralised with hydrochloric acid and adjusted to a weight of 30 g. with water; a solution containing 250 mg. of fluorodinitrobenzene in 30 ml. of acetone is added, followed by 5 ml. of 25 per cent ammonia. After

standing for four hours at room temperature the precipitate is filtered, washed twice with 2 ml. quantities of acetone followed by two 2 ml. quantities of water, dried for 1 hour at 80° and weighed.

Recovery experiments on known weights of a recrystallised sample of morphine alkaloid gave a series of erratic results, all being in excess of the expected value, some by as much as 10 per cent. It was thought that the washing and drying conditions might contribute to these results since they seemed inadequate. We therefore examined the effect of washing the residue with acetone and water. Aliquot portions of a solution of morphine were precipitated and washed with varying quantities of acetone, or with acetone followed by water, and the weights of residues after drying for 1 hour at 80° were recorded. To assess the purity of each residue, as anhydrous morphine dinitrophenylether, the methods described by Mannich[1,5] which are based on aqueous titration procedures, were applied but found to be unsatisfactory. Non-aqueous titration with perchloric acid in glacial acetic acid proved to be satisfactory, giving a readily detectable end point with crystal violet as an indicator; a typical titration curve is shown in Figure 1. Table I shows the results of the washing tests, the recovery of morphine being calculated both from the weight of residue and from the titration figure.

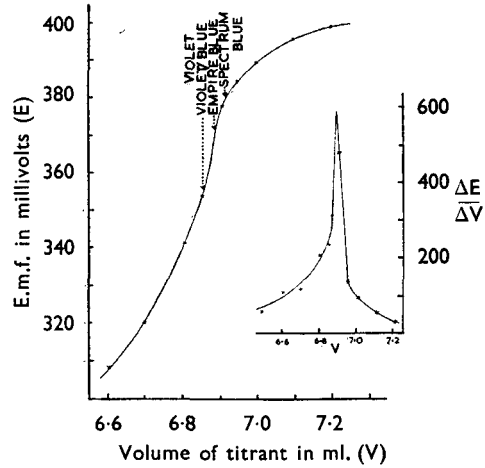

FIG. 1. Titration curves of morphine dinitrophenylether in glacial acetic acid with 0·05N perchloric acid.

TABLE I

EXAMINATION OF WASHING CONDITIONS FOR MORPHINE DINITROPHENYLETHER

Washing employed	Wt. of residue* (g.)	Apparent recovery, per cent	
		Volumetric	Gravimetric
None	0·2375	119·8	160·3
2 ml. acetone	0·2200	105·1	148·4
2 × 2 ml. acetone	0·1967	101·5	132·8
2 × 2 ml. acetone and 1 × 2 ml. water	0·1603	99·7	108·1
†2 × 2 ml. acetone and 2 × 2 ml. water	0·1630	99·7	110·0
3 × 2 ml. acetone	0·1611	100·1	108·7
4 × 2 ml. acetone	0·1481	99·9	100·0
5 × 2 ml. acetone	0·1480	99·9	99·9
6 × 2 ml. acetone	0·1479	99·7	99·8
7 × 2 ml. acetone	0·1479	99·7	99·8
8 × 2 ml. acetone	0·1478	99·6	99·7
9 × 2 ml. acetone	0·1475	99·4	99·5
10 × 2 ml. acetone	0·1473	99·4	99·4

* The quantity of morphine precipitated in each test should yield 0·1481 g. of dinitrophenylether.

† The conditions prescribed by Svendsen and Aarnes.

Some titratable impurity is present in the precipitate but is removed by washing with acetone; the high titration values for unwashed material might be accounted for by a trace presence of ammonia or of ammonium chloride but this would not account for the high gravimetric values. Washing with water increases the gravimetric values, presumably due to inadequate drying, whilst continued washing with acetone causes a gradual loss due to solubility. Little is published on the solubility of morphine dinitrophenylether, Mannich[1] stating it to be soluble with difficulty in acetone; a solubility-time test was therefore made. Finely powdered morphine dinitrophenylether was shaken vigorously with acetone at 17° for periods up to one hour. The solubility at 17° in 100 ml. of acetone was: 10 minutes 8 mg., at 20 minutes 12 mg., at 30 minutes 15 mg., at 40 minutes 19 mg., at 50 minutes 22·5 mg., at 60 minutes 26 mg., showing a slow progressive solubility with time.

TABLE II

COMPARATIVE REACTIVITY OF FLUORO- AND CHLORODINITROBENZENE

Per cent recovery of anhydrous morphine

	Precipitation time, hours					
	2	3	4	6	18	24
Fluorodinitrobenzene A ..	100·0	100·1	100·1	—	100·2	—
,, B ..	—	—	100·9	—	—	—
			100·2			
,, C ..	99·9	100·2	100·0	—	100·5	—
,, D ..	99·9	100·0	100·0	—	100·5	—
Chlorodinitrobenzene ..	—	—	92·4	96·0	97·7	100·1

(A) Commercial liquid I. (B) Crystalline material. (C) Commercial liquid II. (D) Laboratory prepared liquid.

The time of standing necessary before filtration of the precipitate was next examined, and a comparison was made between the use of the fluoro- and the chloro- reagents. The results recorded in Table II illustrate that, as reported by Dann and Wippern, the fluoro- compound is much more reactive than the chloro- compound. Several different samples of 1-fluoro-2:4-dinitrobenzene were used. A note on the reagent is included as an appendix.

In an examination of conditions for drying the precipitate aliquot portions of a solution of morphine were precipitated, the residue obtained being dried at different temperatures for 1 hour. Drying at 80° gave a pale yellow residue of weight closest to theoretical (0·1599 g. found; 0·1598 g. theoretical). Increasing the temperature by stages to 160° resulted in progressive increase in discolouration and weight of residue to a dark brown colour and 0·1612 g. at 160°. These results suggests that some oxidation occurs at higher temperatures. To confirm this a weight of morphine dinitrophenylether was heated for periods, each of 1½ hours, at temperature intervals of 10° between 80° and the melting point at about 250°. Darkening commenced at 110° becoming more marked until, after 160° the precipitate had become brown; further periods of heating caused a fall in weight, and an uncertain melting point in the region of

250°. Professor Clement Duval, of the Sorbonne, kindly made a thermogravimetric analysis of morphine dinitrophenylether prepared by the proposed method and his results support the view that a gradual oxidation occurs as the temperature is raised and that there is a sudden fall in weight at a temperature of about 250°. For these reasons the drying conditions of 80° for 1 hour were considered satisfactory.

The nature of the acetone-soluble impurity in morphine dinitrophenylether precipitated by the fluoro- reagent was next examined. Blank determinations in which 30 ml. of water replaced the morphine solution showed that a precipitate was rapidly formed when the fluoro- reagent was used, but that no precipitate resulted from use of the chloro- reagent. The precipitate was found to consist of 2:4-dinitroaniline, a further demonstration of the remarkable reactivity of the fluorine-substituted nitrobenzene.

An investigation was now made of the extraction procedure of Svendsen and Aarnes, the principles of which had been earlier suggested by Graf[6]. In this procedure the morphine-containing material (1 g. of opium) is triturated with 3 ml. of methanol and 1 ml. of 25 per cent ammonia until a homogeneous mass is formed. It is then triturated with 15 g. of aluminium oxide and the powder mixture so obtained is run into a glass tube; extraction is by eluting the column with 240 ml. of a mixture in the proportion 3 parts of chloroform to one of *iso*propyl alcohol. The morphine is extracted from the eluate by shaking with successive quantities of 0·1N sodium hydroxide and the bulked extracts, after neutralisation with hydrochloric acid, are evaporated to 30 ml. After cooling, the reagent solution is added and the determination made as previously described. Known quantities of morphine were subjected to this procedure and recoveries between 99·5 and 100 per cent were obtained in all cases. All of the morphine was found to be eluted from the column with less solvent than the 240 ml. prescribed. In every experiment 100 ml. of eluting solvent was found to be more than sufficient.

The Determination of Morphine in Opium

The determination of morphine in a sample of Turkish opium was then examined.

Reagents

Acetone B.P.C.; alcohol 95 per cent B.P.; aluminium oxide, Brockmann chromatographic grade; dilute solution of ammonia B.P.; chloroform—*iso*propyl alcohol mixture (chloroform B.P. 3 parts, *iso*propyl alcohol B.P.C., 1 part); solution of 1-fluoro-2:4-dinitrobenzene (a 0·8 per cent w/v solution in acetone); hydrochloric acid solution, 1N; sodium bicarbonate solution (a saturated solution of sodium bicarbonate B.P. in water); sodium hydroxide solution 0·1N.

Method

Powder a representative sample of the opium and accurately weigh about 1 g. into a small porcelain dish. Triturate with 4 ml. of a 3:1

mixture of 95 per cent ethanol and dilute solution of ammonia to an homogeneous cream. Add aluminium oxide gradually and continue triturating until a free-flowing powder is obtained. Transfer the powder to a dry chromatographic tube of about 1·5 cm. diameter and 40 cm. long, previously plugged lightly above the tap with cotton wool. Remove any adhering powder from dish and pestle with cotton wool moistened with alcohol, and add to the tube. Insert the lower end of the tube through a bung fitting into the neck of a 250 ml. separator and elute with 100 ml. of chloroform-*iso*propyl alcohol mixture, adjusting the rate of elution to about 1·5 ml./minute using slight positive pressure if necessary.

Extract the chloroform-alcohol solution in a separator by shaking gently with 20 ml. of 0·1N solution of sodium hydroxide. Allow to separate. Run off the organic phase into a second separator. Filter the aqueous phase through a cotton wool plug into a 150 ml. beaker.

TABLE III

ANALYSIS OF TURKISH OPIUM

Batch	Per cent anhydrous morphine, calculated to the dried opium			
	Proposed method		B.P. method	U.N. method[7]
5402	15·78	15·75	15·55	15·68
	15·51	15·84	15·60	—
6227	15·70*		15·40	15·70
			15·45	—

* Mean of 15 determinations, spread −0·13 to +0·14.

Extract the organic phase with two further quantities of 0·1N sodium hydroxide solution, each of 15 ml., filtering the extract into the same beaker. Add N HCl to the bulked aqueous solutions until just acid to litmus paper and concentrate on a steam bath to 30 ml. Cool, add 30 ml. of solution of 1-fluoro-2:4-dinitrobenzene, followed by 5 ml. of dilute solution of ammonia. Stir gently to mix, cover the beaker and set aside at 15 to 20° for 4 hours, after which decant the supernatant through a tared sintered-glass filter (porosity No. 3). Use the filtrate in portions of 2 to 3 ml. to quantitatively transfer the dinitrophenyl ether to the filter. Rinse the beaker with 2 ml. of acetone and transfer to the filter. After several seconds contact with the crystals remove the acetone by applying gentle suction. Repeat this washing procedure with three further portions of acetone, each of 2 ml. Dry the residue for 1 hour at a temperature of 80°; each g. of residue is equivalent to 0·6319 g. $C_{17}H_{19}O_3N$.

Total working time is about $1\frac{1}{2}$ to $1\frac{3}{4}$ hours and the whole determination may be made in less than a day. Results in Table III show the reproducibility of the method and a comparison of results with those obtained by two other methods.

Difficulties were encountered when the method was applied to opium of Indian origin. The eluates were much darker than those from Turkish opium and in the subsequent extraction with sodium hydroxide emulsions tended to form which were sometimes difficult to break. Moreover the precipitate was much darker than that from morphine or Turkish opium

and appeared to be contaminated with a resin-like material. Morphine figures calculated from the weight of the precipitate were erratic and, in general, were substantially higher than those by the B.P. method. When titrated by the non-aqueous procedure previously described, the residues from Indian opium gave figures for purity ranging between 88 and 95 per cent compared with 99 per cent from Turkish opium. Because the interfering material was readily extracted from the organic layer by sodium hydroxide it was assumed to be either acidic or phenolic in nature.

TABLE IV

EFFECT OF WASHING ELUATES FROM INDIAN OPIUM WITH SODIUM BICARBONATE SOLUTION

Vol. of saturated $NaHCO_3$ solution used for washing	Per cent anhydrous morphine		
	Proposed method		
	Gravimetric	Volumetric	B.P. method
2 × 25 ml.	13·32	12·88	12·75
2 × 25 ml.	13·47	12·96	12·78
3 × 20 ml.	12·86	12·85	—
4 × 20 ml.	12·98	12·98	—
4 × 20 ml.	12·92	12·91	—

Attempts were therefore made to separate it from the morphine by extracting the organic eluate with sodium bicarbonate solution before removal of the morphine with sodium hydroxide. The bicarbonate washings, while not removing morphine, extracted some material which, on subsequent treatment with fluorodinitrobenzene, gave an amorphous precipitate, soluble in glacial acetic acid, but the solution did not titrate with perchloric acid. A number of estimations were made, varying the volume of bicarbonate solution used in washing. In each, washings were bulked, shaken with two 10 ml.-portions of chloroform-*iso*propyl alcohol mixture, and the three organic phases combined before extraction of the morphine with sodium hydroxide. On shaking the washed organic phases with sodium hydroxide there was little or no emulsification; morphine dinitrophenylether was precipitated in the usual way and the purity of the residues determined by titration with perchloric acid. Results in terms of anhydrous morphine are given in Table IV.

Results obtained by different methods on two samples of Indian opium are shown in Table V.

Titration of the impure residue is not satisfactory as the end point is sometimes difficult to detect because of the colour; furthermore the volume of 0·05N perchloric acid used is only about 7 or 8 ml. The method of choice is the gravimetric procedure after washing with a standard solution of sodium bicarbonate and is as follows:

Proceed by the method described for Turkish opium to the words ". . . elute with 100 ml. of chloroform-*iso*propyl alcohol mixture." Wash the eluate in the separator by shaking gently with four 20 ml.-portions of saturated sodium bicarbonate solution. Combine the four washings and extract with two 10 ml.-volumes of chloroform-*iso*propyl alcohol mixture. Reject the aqueous phase, combine the two washings

with the original washed eluate and continue by the method for Turkish opium from the words "Extract the chloroform-*iso*propyl alcohol solution in the separator. . . ."

As with Turkish opium the results are a little higher than those obtained by the B.P. method but are in close agreement with those obtained by the United Nations method.[7]

TABLE V

ANALYSIS OF INDIAN OPIUM

	Per cent anhydrous morphine					
	Precipitation of morphine as dinitrophenylether					
	No washing with $NaHCO_3$		Washing with $NaHCO_3$			
Batch	Gravimetric	Volumetric	Gravimetric	Volumetric	B.P.	U.N.[7]
4749	13·32	12·66	12·67	12·66	12·43	12·58
	13·21	12·69	12·69	12·67	12·31	—
	—	—	12·58	12·57	—	—
	—	—	12·72	12·70	—	—
	—	—	12·58	12·54	—	—
	—	—	12·74	12·70	—	—
2458	13·64	13·00	12·98	12·98	12·75	12·99
	13·60	12·91	12·92	12·92	12·78	13·01
	13·70	—	—	—	—	—

COMPARISON OF THE RESIDUES OBTAINED FROM OPIUM WITH PURE MORPHINE DINITROPHENYLETHER

Samples of morphine dinitrophenylether prepared from pure morphine, Turkish opium and Indian opium using 1-fluoro-2:4-dinitrobenzene and from pure morphine using the chloro- reagent were compared.

Assuming the empirical formula $C_{17}H_{18}O_3N{\cdot}C_6H_3(NO_2)_2$ with a molecular weight of 451·42 theoretical values are C, 61·2 per cent, H, 4·69 per cent, N, 9·30 per cent, methoxyl content nil. Residues contaminated with other phenolic alkaloids of opium would be expected to give a significant methoxyl value. Values found are, per cent:

	C	H	N	From non-aqueous titration	Methoxyl
From morphine with F cpd	60·9	5·0	9·2	99·9	less than 0·1
From morphine with Cl cpd	60·7	4·8	9·0	99·6	—
From Turkish Opium..	61·2	4·8	9·3	99·8	less than 0·1
From Indian Opium	61·0	5·0	9·5	99·7	less than 0·1

Infra-red spectra suggested that samples precipitated from opium are identical with those precipitated from morphine. The presence of dintroaniline in insufficiently washed samples prepared with fluorodinitrobenzene influences the spectrum of morphine dinitrophenylether, causing modifications at 9·40 μ, 10·85 μ, 11·97 μ and 13·05 μ. Spectra of samples prepared by the proposed assay process confirmed that all the dinitroaniline had been removed. The spectrum of pure morphine dinitrophenylether over the range 5 μ to 15 μ is shown for reference in Figure 2.

Thus the possibility that the precipitates from the opium samples might be contaminated with other phenolic alkaloids is neither supported by the infra-red spectra, nor by the very low methoxyl contents found.

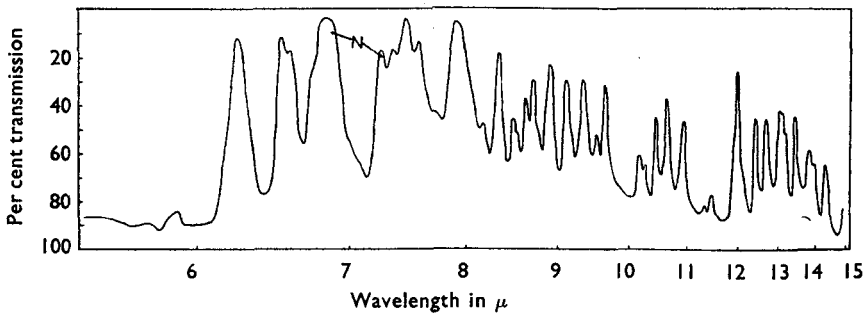

FIG. 2. Infra-red spectrum of morphine dinitrophenylether in Nujol mull. N = Nujol bands.

THE INFLUENCE OF OTHER ALKALOIDS OF OPIUM AND RELATED MATERIALS ON THE PRECIPITATION OF MORPHINE DINITROPHENYLETHER

Codeine, thebaine, papaverine and narcotine. 0·1 g. of each was subjected to the precipitation procedure already described and since no phenolic groups are present, no acetone-insoluble precipitate was obtained. Recoveries of anhydrous morphine in the presence of these alkaloids are shown in Table VI.

TABLE VI

RECOVERY EXPERIMENTS ON ANHYDROUS MORPHINE IN THE PRESENCE OF OTHER ALKALOIDS OF OPIUM AND RELATED MATERIALS

Alkaloid or group of alkaloids	Amount present (g.)	Anhydrous morphine		Per cent recovery of morphine
		Added (g.)	Recovered (g.)	
Codeine	0·1	0·1206	0·1205	99·9
Papaverine	0·1	0·1215	0·1216	100·1
Narcotine	0·1	0·1324	0·1324	100·0
Thebaine	0·1	0·0895	0·0896	100·1
*"Porphyroxine-meconidine"	ε 0·015	0·1190	0·1189	99·9
*Laudanine, etc.	ε 0·015	0·1074	0·1077	100·2
*"Unknown Base"	ε 0·005	0·1014	0·1015	100·1
*Codeine and cryptopine	ε 0·05	0·1076	0·1075	99·9
Pseudomorphine	0·1	0·1071	0·1072	100·1
Apomorphine	0·1	0·1011	0·1014	100·3
Ethylmorphine	0·1	0·1154	0·1158	100·3
Diamorphine	0·1	0·1029	0·1030	100·1

* Residues obtained in the U.N. assay.

Minor phenolic alkaloids, principally laudanine, codamine and narcotoline. No supply of these was available. In the United Nations "Unified Analysis of Opium for alkaloids"[7], however, a method is given whereby various groups of alkaloids are separated and estimated. Two groups of phenolic alkaloids are separated, one, the so-called "porphyroxine-meconidine" group, the other laudanine and related alkaloids. The complete assays were made on two samples of opium of Indian origin and one of Turkish and the residues from each separation were precipitated. No acetone-insoluble residues were obtained from any of the fractions other than the morphine one. Further supplies of these groups of alkaloids were then similarly obtained from one of the Indian opiums

and recovery experiments on anhydrous morphine were made in their presence. The results are included in Table VI. Appreciable colours were obtained when small quantities of the minor phenolic residues were tested by the nitroso-morphine reaction.

Pseudomorphine. This is 2-2′-dimorphine[8] and although it was obtained from opium by Pelletier[9] it is not known whether it is actually present in opium or formed during extraction[10]. A sample was prepared by the method described by Polstorff[11] and subjected to the proposed precipitation procedure, after identification by the micro-technique of Clarke and Williams[12]. From the crystalline nature of the material obtained it appeared that something other than 2:4-dinitroaniline had precipitated but the residue was entirely soluble in the stipulated quantity of acetone used for washing. A recovery experiment on morphine made in the presence of pseudomorphine is included in Table VI.

Apomorphine, ethylmorphine and diamorphine. The precipitation procedure described was carried out on samples of these alkaloids. Whereas ethylmorphine and diamorphine gave no precipitates other than of 2:4-dinitroaniline, apomorphine gave a resinous residue but this was completely soluble in the volume of acetone used for washing. Recoveries of anhydrous morphine in the presence of these alkaloids are shown in Table VI.

Application of the Method to Galenical Preparations

Having obtained satisfactory results on both Turkish and Indian opiums the method was extended to the determination of morphine in galenical preparations; most preparations to which the method has been applied are official and with a few exceptions were compounded from Turkish opium.

Preparations of the British Pharmacopoeia

Tincture of Opium. Take 10 ml. of sample in a porcelain dish and evaporate to dryness on a steam bath. Continue by the appropriate method for raw opium.

The results per cent w/v anhydrous morphine in tincture of opium from Turkish and Indian sources, obtained on routine batches are:

Turkish,	proposed method,	1·04, 1·04;	B.P. method,	1·03, 1·03
,,	,,	,,	1·00, 1·00;	,,	,,	1·00, 0·99
,,	,,	,,	1·06, 1·07;	,,	,,	1·07, 1·04
Indian (unadjusted)	,,	2·13,	;	,,	,,	2·12

Camphorated Tincture of Opium. With this preparation we find that a high result may be obtained by the official colorimetric method and this has also been observed by others[13]. A number of samples were prepared from Tinctures of Opium already assayed and the results obtained by the proposed method and the B.P. method are shown in Table VII. The theoretical morphine contents quoted are based upon results by the B.P.

method of assay on the original tinctures. The recommended method is as follows:—

Take 100 ml.* in a porcelain dish and evaporate to about 10 ml. on a steam bath. Add about 5 g. of aluminium oxide and continue the evaporation to dryness. Continue by the appropriate method for raw opium.

TABLE VII

PER CENT W/V ANHYDROUS MORPHINE IN CAMPHORATED TINCTURE OF OPIUM

Tincture of Opium used	Sample No.	Per cent anhydrous morphine w/v		
		Theoretical	Proposed method	B.P. method
8243	1	0·049	0·049	0·058
	2	0·053	0·053	0·055
			0·053	0·054
8303	3	0·053	0·054	0·062
8597	4	0·050	0·051	0·060
	5	0·050	0·051	0·056
				0·058

Powdered Ipecacuanha and Opium. The official assay is based on the measurement of colour by the nitroso reaction first reported by Radulescu[14]. Being a general reaction for most phenols[15,16] a colour may be given by the minor phenolic alkaloids of opium or by the phenolic alkaloids of ipecacuanha. Despite the use of a compensating technique described in the B.P., routine analyses may yield high figures (Table VIII). Another source of error in the official method arises from a difference of tint in the final matching between the standard and the sample.

TABLE VIII

PER CENT W/W ANHYDROUS MORPHINE IN POWDERED IPECACUANHA AND OPIUM

Batch No.	Proposed method	B.P. method
24338	0·97	1·17
	0·97	1·16
4564	0·99	1·11
	0·98	—
301	0·98	1·31
26647*	1·01	1·26
	1·01	—
25595*	1·01	1·25
	1·02	—
26645	0·97	1·31
2467	0·97	1·14
23559†	Operator 1 1·01	1·21

* B.P. 1914 formula.
† For batch 23559, Operators 1–7 had the following further results by the proposed method: (Op. 1) 1·01, 1·00; (Op. 2‡) 0·99, 1·01; (Op. 3‡) 1·00; (Op. 4‡) 0·97; (Op. 5‡) 0·99; (Op. 6) 1·01; (Op. 7‡) 0·98.
‡ These operators were carrying out the assay for the first time.

The possibility of interference with the proposed gravimetric method by alkaloids of ipecacuanha was considered and emetine, cephaëline and psychotrine were extracted from powdered ipecacuanha and subjected to precipitation tests. No acetone insoluble precipitate was obtained from any of the alkaloids under consideration and results of recovery experiments on anhydrous morphine in the presence of these alkaloids are:

	g.	Morphine, g.		Per cent recovery
		Added	Recovered	
Emetine	0·1	0·1143	0·1141	99·8
Cephaëline	0·1	0·1129	0·1134	100·4
Psychotrine	0·05	0·1146	0·1148	100·1

* For manufacturing control purposes this volume presents no difficulty, but with a suitable semi-micro technique it could be reduced to 25 ml.

The recommended method of assay is as follows:—

Take about 5 g. accurately weighed and continue by the appropriate method for raw opium.

Aromatic Powder of Chalk and Opium. Satisfactory results cannot be obtained on this material. The trituration and column procedure is far too cumbersome because of the large bulk of material and extraction and precipitation after lime treatment yielded results which were only about 90 per cent of theory.

Preparations of the British Pharmaceutical Codex

Dry Extract of Opium. This can be assayed directly as for raw opium using about 0·5 g. accurately weighed. A sample which gave a figure of just under 20·2 by the B.P.C. method gave results of 20·29 and 20·27 per cent w/v anhydrous morphine.

Concentrated Camphorated Tincture of Opium. The recommended method of assay is as follows:—

Take 25 ml. of sample in a porcelain dish and evaporate to about 10 ml. on a steam bath; continue by the method for Camphorated Tincture of Opium from the words "Add about 5 g. of aluminium oxide. . . ."

The results, per cent anhydrous morphine content, from tincture prepared from two batches of Tinctures of Opium are: (1) proposed method 0·414, 0·414; B.P.C. method 0·462. (2) (manufacturing batch) proposed method 0·431, 0·433; B.P.C. 0·483. A theoretical figure of 0·413 for batch (1) is derived from the B.P. assay of the Tincture of Opium used.

Liniment of Opium B.P.C. 1949. There is no interference from Liniment of Soap; the recommended method of assay is as follows:—

Take 10 ml. of sample in a porcelain dish and evaporate to dryness on a steam bath; continue by the method for raw opium. A sample which gave a result of 0·54 per cent w/v by the B.P.C. method gave a figure of 0·55 per cent by the proposed method.

Papaveretum. Officially, papaveretum may be prepared either ". . . from opium by converting the total alkaloids into the hydrochlorides . . ." or by ". . . mixing suitable proportions of the hydrochlorides of morphine, codeine, narcotine and papaverine". Since codeine, narcotine and papaverine have been shown to cause no interference in the precipitation of morphine as the dinitrophenylether, it is unnecessary to adopt the column separation where papaveretum is a mixture of pure alkaloids.

Recommended assays are as follows:—

For material prepared from the total alkaloids of opium take 0·2 g. accurately weighed in a porcelain dish and proceed by the method for raw opium.

For material prepared by mixing the hydrochlorides of morphine, codeine, narcotine and papaverine—take 0·2 g. accurately weighed in a 150 ml. beaker, add 30 ml. of water and stir to dissolve. Continue by the method for raw opium commencing with the words "To the cooled solution add 30 ml. of solution of 1-fluoro-2:4-dinitrobenzene. . . ."

The results, per cent anhydrous morphine, in 2 batches of papaveretum, are: (1) column treatment, 49·9, 49·9; B.P.C. method 49·8; (2) column treatment, 48·5; direct precipitation, 48·6; B.P.C., 48·5.

Injection of Papaveretum. Phenylmercuric nitrate does not interfere with the method. Even in the presence of 20 mg. (hundredfold excess) quantitative recoveries of anhydrous morphine were obtained. Morphine may be determined, using 10 ml. with or without column treatment. A number of samples of the injection was prepared and the recovery of anhydrous morphine in g. calculated. The theoretical amount calculated from the estimate made on the papaveretum used is given in parentheses. With column treatment, 0·1041 (0·1043); 0·1028 (0·1029); 0·1046 (0·1044); and without column treatment, 0·1128 (0·1128); 0·1068 (0·1067).

Tablets of Papaveretum. Take a quantity of powdered tablets, accurately weighed, equivalent to about 0·2 g. of papaveretum and proceed by the method for raw opium.

The content of anhydrous morphine in grains/tablet of two batches was: (1) proposed method 0·089, B.P.C. method 0·099; proposed method 0·090, B.P.C. 0·097; (2) proposed method 0·085, B.P.C. 0·103; proposed method 0·087, B.P.C. no figure. The nominal content of papaveretum in each tablet was $\frac{1}{6}$ grain.

Liquid Extract of Poppy, B.P.C. 1949. On evaporating the sample to dryness, it becomes resinous and cannot be completely homogenised with the aluminium oxide. If the extract is treated by the method of Bennett and Garratt[17] the dinitrophenylether procedure may be applied satisfactorily. The recommended method of assay is as follows:—

Take 50 ml. of sample in a 250 ml. stoppered flask, add 20 ml. of water and 150 ml. of *iso*propyl alcohol and shake vigorously for 2 to 3 minutes. Allow to stand for 5 minutes and pour off the alcohol into a 500 ml. separator. Redissolve the residue by shaking with a further 10 to 20 ml. of water, then add 50 ml. of *iso*propyl alcohol, shake again and pour off the alcohol into the separator. Repeat the process of dissolving the residue in 10 ml. of water and shaking with 20 ml. of *iso*propyl alcohol twice more, bulking the alcoholic extracts in the separator. Shake the separator and filter the alcoholic extracts through a plug of cotton-wool into an evaporating dish. Evaporate the solvent on a steam bath, triturate the residue with 3 ml. of 95 per cent alcohol and sufficient dilute solution of ammonia to ensure alkalinity to litmus paper. Continue by the general method for Turkish opium.

On a batch of Liquid Extract of Poppy B.P.C. 1949 results of 0·172 per cent and 0·169 per cent w/v of anhydrous morphine were obtained by the B.P.C. method and 0·160 per cent and 0·159 per cent w/v by the proposed method.

The method has not been applied to a number of products such as Liquid Extract of Opium B.P.C., Sedative solution of Opium B.P.C. 1949 and Ammoniated Tincture of Opium B.P.C. 1949 which would obviously present no difficulty. One preparation has not been assayed satisfactorily: Ointment of Gall with Opium B.P.C.

APPENDIX

A Note on 1-*Fluoro*-2:4-*dinitrobenzene*

In the initial experiments on the determination of morphine in opium a pale yellow crystalline solid (m.p. 26·5°) of commercial origin was used. Further supplies of reagent from this source were unobtainable, and a supply was obtained from a second commercial source. This was a yellow liquid, b.p. 167° at 11 mm. pressure, wt./ml. at 20°, 1·5640, refractive index at 20° 1·5686. Doubt arose whether this liquid was the same material as the crystalline solid and a search of the literature was made.

A synthesis of fluorodinitrobenzene is described by Finger and Finnerty[18] which, when carried out, yielded a pale yellow viscous oil. Material prepared in this way has been used for the majority of the determinations described in the present work. The method of preparation was as follows:

Prepare a mixture of 900 ml. of concentrated sulphuric acid and 300 ml. of concentrated nitric acid, heat to 50 to 60° and add, with constant stirring, 192 g. of fluorobenzene at such a rate as to maintain the temperature between 70 and 75°. When the addition is complete maintain the reaction mixture at a temperature of 85° for 30 minutes, cool and pour into 4 litres of ice-cold water. Leave overnight and distil the oil under reduced pressure on the following day. A yield of about 250 g. was obtained, b.p. 166° at 11 mm. pressure, wt./ml. at 20° 1·5429, refractive index at 20° 1·5682. A nitrogen determination by Dumas' method gave 15·30 per cent (theory 15·06 per cent). Two derivatives were prepared. (i) anilide, m.p. 157·4 to 157·5° (156°)[19]; (ii) *p*-chlorophenylmercaptan, m.p. 124·8 to 125·3° (123°)[19]. Zahn and Würz[20], however, described a synthesis (not attempted) which yielded a product with b.p. 108° and m.p. 25·8°. Heilbron and Bunbury[21] quote the *p*-fluoromononitrobenzene as existing in two forms, the stable one of which has m.p. 26·5 to 27°. As it seemed improbable that the crystalline material could have been the mono-nitro compound since morphine recoveries would then have been about 11 per cent higher than theory, the possibility of polymorphism in the dinitro compound was considered. Rheinboldt and Perrier[22,23] quote the 1-fluorodinitrobenzene as having m.p. of 26° and 28° and state[24] that polymorphism in this compound, as in many others where 2:4-dinitrobenzene is substituted in the 1- position, is very pronounced.

The manufacturers of the crystalline sample later supplied a further quantity of 1-fluoro-2:4-dinitrobenzene. This was a yellow liquid, b.p. 167° at 11 mm. pressure and refractive index at 20° = 1·5684. The various samples gave essentially identical infra-red spectra and since the spectrum does not appear to have been recorded in the literature, this is reproduced in Figure 3 over the range 5 μ to 15 μ. Furthermore, quantitative recoveries of morphine were obtained with the various samples, and the solid and liquids would thus appear to be polymorphic forms of 1-fluoro-2:4-dinitrobenzene.

During the precipitation of morphine with fluoro-dinitrobenzene the solution gradually changes colour from yellow to dark reddish orange.

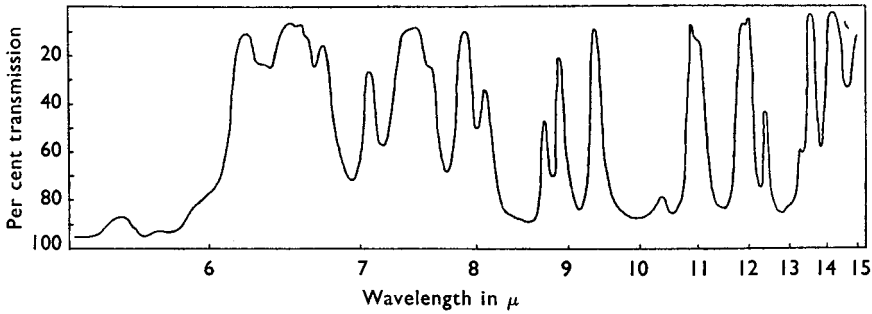

FIG. 3. Infra-red spectrum of 1-fluoro-2:4-dinitrobenzene (capillary film).

After about two hours a bright yellow material of different crystalline form and readily distinguishable from morphine dinitrophenylether begins to deposit slowly. These changes are not observed when chlorodinitrobenzene is used. In this case the solution becomes pinkish-purple after only a few minutes standing, gradually increasing in intensity to a reddish-purple which remains for about 30 hours before fading to an orange-yellow. No co-precipitate appears within about 36 hours.

Tests were made with the fluoro reagent in the absence of morphine; the colour changes and deposition of a bright yellow precipitate as described above were again noticed. This precipitate was insoluble in water, soluble with difficulty in cold alcohol but more readily soluble in hot alcohol and very readily soluble in acetone. After filtering and washing several times the material was recrystallised from aqueous alcohol and shown to be 2:4-dinitroaniline as follows:

(i) M.p. 179·6 to 180·2° (180°)[19]. (ii) Mixed m.p. with pure 2:4-dinitroaniline 179·8 to 180·2°. (iii) Nitrogen content by the method of McCutchan and Roth[25] 22·80 per cent (theory 22·94 per cent). (iv) Acetyl derivitive m.p. 121·8 to 122·3° (120°). (v) Identity of infra-red spectrum with that of pure 2:4-dinitroaniline. The ease with which this material may be prepared using fluorodinitrobenzene as opposed to chlorodinitrobenzene[26] again illustrates the remarkable reactivity of the fluorine substituted compound.

It should be noted that 1-fluoro-2:4-dinitrobenzene is a vesicant and care should be taken to avoid contact with the skin.

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DISCUSSION

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

MR. H. B. HEATH (Sudbury). Why was the opium and ammonia first mixed with the alumina? How much alumina was used?

DR. G. E. FOSTER (Dartford). A similar method was published in the Australian Chemical Institute proceedings in 1946 by Dr. Trautner. What was the minimum quantity of morphine which could be estimated by their method?

DR. L. SAUNDERS (London). About three years ago there appeared in *Analytical Chemistry* a method describing the use of ion exchange resins for extracting morphine. Had this method been tried?

MR. R. L. STEPHENS (Portsmouth). Had the authors tried to estimate morphine in Tincture of chloroform and morphine?

MR. W. SMITH (Ware). Was the vesicant nature of fluorodinitrobenzene any disadvantage to the method.

MR. C. A. JOHNSON replied. The quantity of alumina varied a little with the product being assayed, but was about 10 to 15 g. Alumina was added until a free flowing powder was obtained. The method could be operated successfully with about 25 ml. of camphorated tincture of opium. The method with ion exchange resins published in *Analytical Chemistry* had been tried, but they had not obtained quantitative results. Attempts had been made to apply the method to Tincture of chloroform and morphine, but the trouble involved in setting up the column was such as to make it not worth while. The quantity of water required cancelled out any variation in the alumina used. With reasonable care the vesicant nature of fluorodinitrobenzene was no disadvantage.