

# THE COLORIMETRIC DETERMINATION OF MORPHINE AND DIAMORPHINE

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THE assay for diamorphine in linctus of diamorphine which is given in the British Pharmaceutical Codex, and is used for all the liquid preparations of diamorphine, was found to give results 20 per cent. too low. Pure diamorphine hydrochloride was also found to give similarly low results when used as a control material for the assay.

This assay is based on the colorimetric determination of the morphine produced by the hydrolysis of the diacetylmorphine, the morphine reaction with nitrous acid followed by ammonia being that given in the British Pharmacopœia for camphorated tincture of opium. The accuracy of this method as modified by using a Spekker absorptiometer was discussed by Adamson and Handisyde<sup>1</sup>, who gave a full bibliography of the method.

Comparison of the conditions for the reaction show that the quantity of acid specified in the British Pharmaceutical Codex for the hydrolysis of the diacetylmorphine, while it is sufficient for the hydrolysis, is not enough for the full development of the nitroso-morphine. The quantity of acid given is nearer to that employed in the morphine and nitrite reagent of the British Pharmaceutical Codex 1934, 7th. addendum. Correction of the quantity of acid to make the final solution 0.2 N with respect to hydrochloric acid produces the correct result.

The sharp change, in the colour produced, which is brought about by small differences in the concentration of hydrochloric acid suggests that the reaction depends closely on the pH of the solution and that better control might be obtained if a weaker acid were employed. A number of acids were tried and phosphoric acid and oxalic acid were both found to be suitable and to give consistent results over a wide range of concentrations.

The conditions affecting the accuracy of the results were tested using photoelectric measurements with the Spekker absorptiometer. It has been found that the substitution of phosphoric acid for hydrochloric acid in the B.P. assay for morphine in camphorated tincture of opium gives an assay that is less liable to errors due to small changes in the concentration of acid or to small changes in the volume of sodium nitrite solution employed. Sodium nitrite has less effect in raising the pH of the solution when the higher concentration of phosphoric acid is used than when the dilute solution of hydrochloric acid specified in the British Pharmacopœia is employed.

## EXPERIMENTAL

*Other acids than hydrochloric*: Table I shows the acids that were tried in place of hydrochloric acid.

The morphine and nitrite reagent of the British Pharmacopœia containing 1.0 mg. of morphine in 50 ml. was made up in 50-ml. graduated

TABLE I

Acid	Dissociation Constant $K_a$	M acid used ml.
Tartaric Acid ... ..	$1.1 \times 10^{-3}$	2 to 16
Phosphoric Acid ... ..	$1.1 \times 10^{-2}$	2 to 16
Oxalic acid ... ..	$6.5 \times 10^{-2}$	1 to 8
Sulphuric acid ... ..	$1.15 \times 10^{-2}$	1 to 16

flasks. The amount of acid and type of acid were varied, but the other conditions were all kept constant. The extinction was measured with a Spekker absorptiometer using 4 cm. cells, heat filters H 503 and Ilford 601 (spectrum violet) light filters. A reagent blank was used to set the drum at 1.00 and the drum reading recorded subtracted from 1.00.

Table II gives the light absorption using varied quantities of acids. It will be seen that oxalic and phosphoric acid each give a constant absorption over a wide range of concentrations, while the other acids give continuously variable absorptions with increasing concentration.

TABLE II  
SPEKKER EXTINCTIONS OF 4 cm.

Volume of acid used ml.	N Hydrochloric acid	N Sulphuric acid	M Tartaric acid	M Phosphoric acid	0.5 M Oxalic acid
1	.83	.74	.655	.66	.63
2	.98	.94	.72	.82	.72
3	.95	.93	.77	.89	.87
4	.92	.91	.815	.93	.925
5	.87	.88	.85	.94	.935
6	.83	.86	.87	.935	.935
7	.81	.84	.905	.94	.935
8	.78	.82	.93	.94	.925

Figure 1 shows the relation between hydrochloric acid and phosphoric acid. The sharp peak at the optimum concentration of hydrochloric acid emphasises the need for strict control when this acid is used.

*Effect of Nitrite concentration.* Various volumes of 1 per cent. sodium nitrite were used and the extinctions determined using 1 mg. of morphine and 6 ml. of dilute phosphoric acid. The results given in Figure 2 with the results using 10 ml. of 0.2 N hydrochloric acid, indicate that the volume of nitrite may be varied from 8 to 12 ml. without loss of accuracy if phosphoric acid is used, but using hydrochloric acid a difference of 2 ml. in measurement produces an error of about 5 per cent. in the result.

*Calibration Curves.* Figure 3 shows the extinction plotted against the quantity of morphine using M phosphoric acid and 0.5 M oxalic acid to liberate the nitrous acid and catalyse the formation of nitroso-morphine. For each determination various amounts of morphine as hydrochloride

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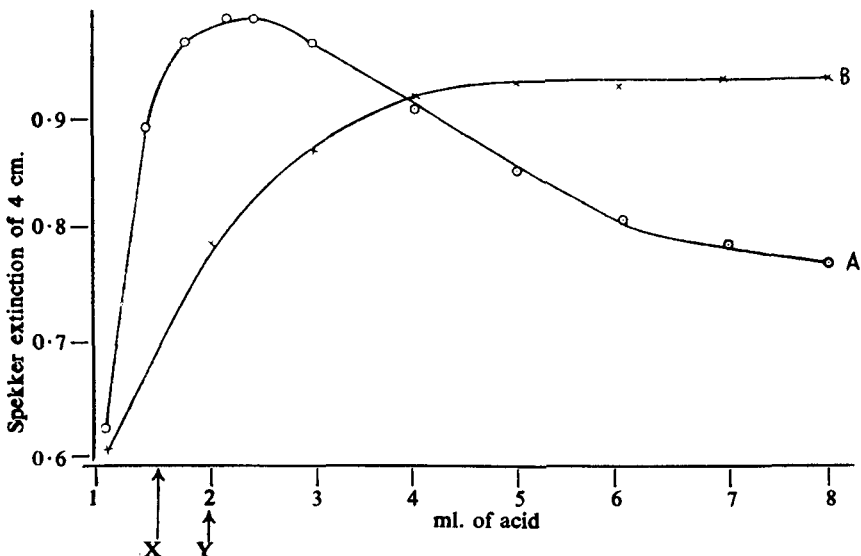


FIG. 1. Diamorphine: A, with N hydrochloric acid; B, with N phosphoric acid. X indicates the quantity of hydrochloric acid used in the B.P.C. assay (1.4 ml.) and Y the quantity used in the B.P. morphine and nitrite reagent.

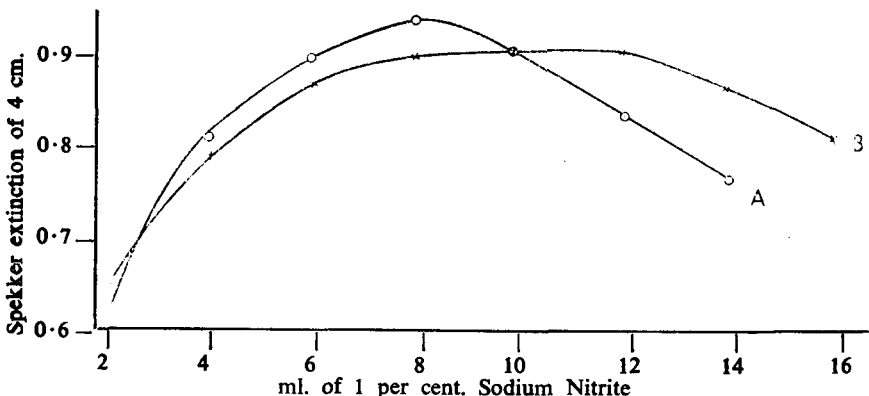


FIG. 2. Extinction curves for 1 mg. of morphine showing the variation with the volume of 1 per cent. sodium nitrite used; A, in the absence of 10 ml. of 0.2 N hydrochloric acid and B, with 6 ml. of M phosphoric acid.

were used with 6 ml. of acid and 8 ml. of 1 per cent. sodium nitrite made up to 28 ml. After 15 minutes 12 ml. of 10 per cent. ammonia was added and the solution made up to 50 ml.

It will be seen that there is very close agreement between M phosphoric acid and 0.5 M oxalic acid. Since dilute phosphoric acid of the British Pharmacopœia is approximately M the rest of the experimental work was done using this acid.

*Temperature.* The British Pharmacopœia does not specify at what temperature this reaction should be carried out. It was accordingly

decided to prepare morphine and nitrite reagent with 6 ml. of dilute phosphoric acid at various temperatures. Tables III and IV show the results obtained.

TABLE III  
EXTINCTION COEFFICIENTS OF MORPHINE AND NITRITE REAGENT CONTAINING  
10 ML. OF 0.2 N HYDROCHLORIC ACID AT VARIOUS TEMPERATURES

Temperature °C.	Spekker extinction of 4 cm.	Percentage of maximum reading
1.0 ... ..	.808	84.1
9.5 ... ..	.917	95.2
12.5 ... ..	.961	99.7
14.0 ... ..	.961	99.7
16.25 ... ..	.964	100
20.0 ... ..	.961	99.7
24 ... ..	.932	96.7

TABLE IV  
EXTINCTION COEFFICIENTS OF MORPHINE AND NITRITE REAGENT CONTAINING  
6 ML. OF DILUTE PHOSPHORIC ACID AT VARIOUS TEMPERATURES

Temperature °C.	Spekker extinction of 4 cm.	Percentage of maximum reading
2.0 ... ..	.792	80.5
6.0 ... ..	.882	94.0
10.0 ... ..	.913	97.0
12.5 ... ..	.922	98.3
15.5 ... ..	.938	100
18.5 ... ..	.934	99.3
24.5 ... ..	.885	94.3
29.5 ... ..	.835	89.0

The results show that if an accuracy of better than  $\pm 1$  per cent. is required the temperature must be controlled within the limits of 15.5° and 18.5°C.

*Effect of Time of Reaction.* The British Pharmacopœia allows 15 minutes for the reaction between the morphine and nitrous acid. Various times were tried using hydrochloric acid and phosphoric acid. Table V shows that the results are substantially consistent between 10 minutes and 20 minutes with phosphoric acid and between 12½ and 22½ minutes with hydrochloric acid.

*Relative Stability of Colour.* Two solutions each containing 1 mg. of morphine were reacted with 8 ml. of sodium nitrite solution in the presence of 2 ml. of N hydrochloric acid and 6 ml. of dilute phosphoric acid for 15 minutes. After adding 12 ml. of 10 per cent. ammonia and making up to 50 ml. the solutions were placed in 4 cm. cells and the

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TABLE V

EFFECT OF TIME ON THE EXTINCTION VALUES OBTAINED DURING THE REACTION OF MORPHINE WITH HYDROCHLORIC AND PHOSPHORIC ACIDS

Time	Hydrochloric acid		Phosphoric acid	
	Spekker extinction of 4cm	Percentage of maximum reading	Spekker extinction of 4 cm.	Percentage of maximum reading
5 minutes ... ..	0.83	85.4	0.83	89.6
7½ .. .. .	0.891	91.7	0.88	94.7
10 .. .. .	0.926	95.3	0.901	97.5
12½ .. .. .	0.962	99.0	0.924	100
15 .. .. .	0.972	100	0.920	99.6
17½ .. .. .	0.972	100	0.922	99.8
20 .. .. .	0.967	99.5	0.917	99.2
22½ .. .. .	0.962	99.0	0.907	98.2

extinction measured at intervals. Table VI shows that the solution containing phosphoric acid was more stable than the standard morphine and nitrite reagent. Both solutions remained almost in darkness during the experiment.

TABLE VI

RELATIVE STABILITIES OF COLOURS OBTAINED USING HYDROCHLORIC AND PHOSPHORIC ACIDS AS SHOWN BY EXTINCTION VALUES

Time in minutes	1	2	5	10	15	20	25	30	40
Spekker extinction of 4 cm using hydrochloric acid ... ..	.989	.984	.982	.976	.962	.958	.948	.940	.928
Spekker extinction of 4 cm using phosphoric acid ... ..	.964	.968	.972	.972	.972	.971	.970	.969	.967

It will be seen that with phosphoric acid the full colour did not develop for about 5 minutes and it then remained stable for more than 20 minutes. The B.P. morphine and nitrite reagent faded by 5 per cent. in the same time.

*Effect of Light during Acid Reaction.* This work was all carried out in normal daylight except that on sunny days the blinds were drawn to enable the galvanometer light spot to be seen clearly. Solutions were prepared in which the acid reaction was carried out in diffuse light and darkness.

TABLE VII

	Diffuse light	Darkness
Spekker extinction of 4cm. using hydrochloric acid	0.963	0.989
.. .. using phosphoric acid	0.964	0.964

It appears that when phosphoric acid is used the reaction is less sensitive to light.

*Determination of Diamorphine.* A solution of diamorphine hydrochloride containing 0.1233 g. in 100 ml. was made up. 10 ml. was boiled

under a reflux condenser for 10 minutes with 5 ml. of 10 per cent. hydrochloric acid, made up to 100 ml. and the morphine determined as given in the British Pharmaceutical Codex under "Linctus Diamorphinæ" commencing with the words "To 10 ml. of this solution add 8 ml. of a 1 per cent. w/v solution of sodium nitrite" except that Spekker comparison was substituted for visual comparison. Diamorphine hydrochloride found, 0.925 mg. = 80 per cent recovery.

A further 10 ml. was boiled under a reflux condenser with 5 ml. of 4 N hydrochloric acid (14.6 per cent.) and estimated as before. Diamorphine hydrochloride found 1.220 mg. = 99 per cent. recovery.

A further 10 ml. was boiled under a reflux condenser for 30 minutes with 20 ml. of 10 per cent. phosphoric acid and estimated as before except that 4 ml. of dilute phosphoric acid was added before the nitrite solution and the result was calculated from Figure 3. Diamorphine found, 0.935 mg. = 76 per cent. recovery. It is clear from this result that hydrolysis is not complete in half an hour when phosphoric acid is used.

A further 10 ml. was hydrolysed with 5 ml. of dilute hydrochloric

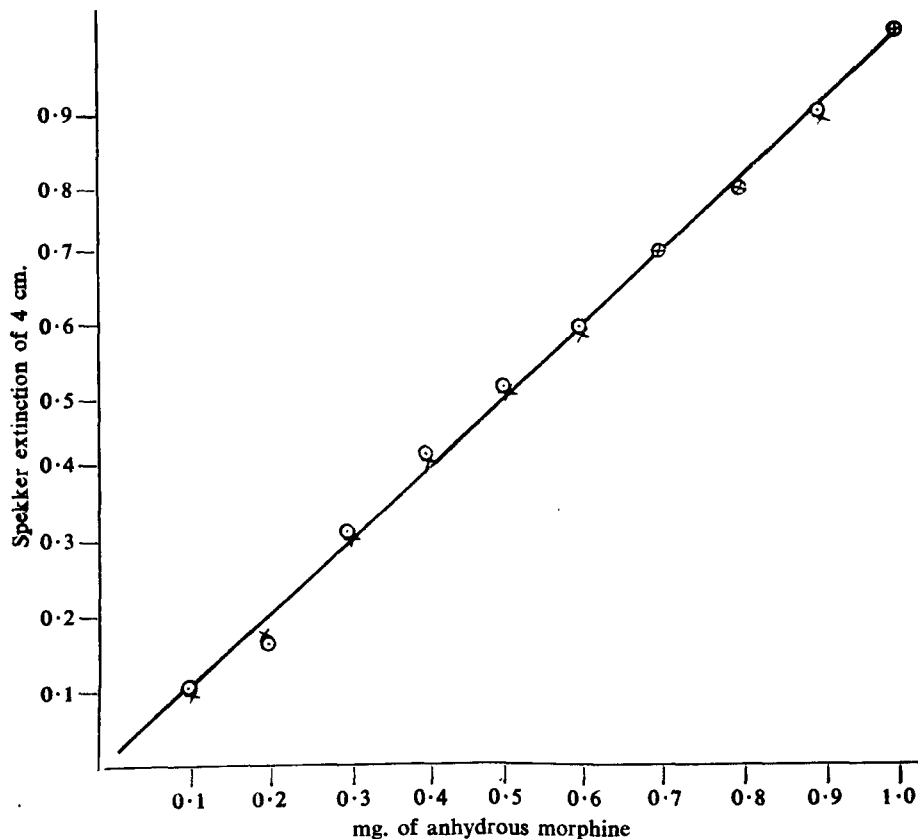


FIG. 3. Calibration curve for morphine. O, Results with oxalic acid; x, Results with phosphoric acid.

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acid and buffered with 0.3 g. of sodium dihydrogen phosphate before adding 6 ml. of diluted phosphoric acid. The result was calculated by reference to Figure 3. Diamorphine found 0.1225 mg. = 99.4 per cent. recovery.

*Linctus of Diamorphine.* The method found to give the best results was as follows: 10 ml. of linctus of diamorphine was extracted according to the method given in the British Pharmaceutical Codex and hydrolysed with 5 ml. of dilute hydrochloric acid. After extraction and making up to 100 ml., 10 ml. was buffered with 0.3 g. of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  and 6 ml. of dilute phosphoric acid; 4 ml. of water, and 8 ml. of 1 per cent. sodium nitrite were then added. After 15 minutes standing at 16° to 18°C., 12 ml. of 10 per cent. ammonia were added and the extinction measured, using as a blank a further 10 ml. made up to 50 ml., but with the omission of the nitrite. The morphine was read off from a calibration curve similar to Figure 3 and the result calculated in the usual way.

*Visual estimation.* It is interesting to note that the colour produced by substituting phosphoric acid or oxalic acid is different from the ordinary morphine and nitrite reagent and visually it is stronger and more easily matched. The differences are shown in Table VIII.

TABLE VIII  
SPEKKER EXTINCTIONS OF 4 CM. USING A RANGE OF LIGHT FILTERS WITH  
HYDROCHLORIC AND PHOSPHORIC ACIDS.

Filter	Spekker extinction of 4 cm using hydrochloric acid	Spekker extinction of 4 cm. using phosphoric acid
Spectrum violet 601	0.94	0.92
Spectrum Blue 602	0.76	0.81
Spectrum Blue-green 603	0.55	0.63
Spectrum Green 604	0.37	0.46
Spectrum Yellow-green 605	0.15	0.21
Spectrum Yellow 606	0.06	0.10
Spectrum Orange 607	0.02	0.03
Spectrum Red 608	0.00	0.08
no filter	0.151	0.184

The extinction without a filter is actually 20 per cent. greater using phosphoric acid.

### SUMMARY

1. The quantity of hydrochloric acid given for the hydrolysis of the diamorphine in the British Pharmaceutical Codex assay of linctus of diamorphine and elixir of diamorphine is insufficient and is not sufficiently accurately defined. 10.0 ml. of 2 N hydrochloric acid should be substituted for 5 ml. of dilute hydrochloric acid.

2. Accurate results can be obtained with less attention to the exact concentration of acid if phosphoric acid or oxalic acid is used in place

of hydrochloric acid in the British Pharmacopœia determination of morphine in camphorated tincture of opium.

3. The effects of most of the variables on the consistency of the results using phosphoric acid in place of hydrochloric acid have been investigated, and temperature has been found to be important. The rate of fading of the reagent is much less if phosphoric acid is used.

I wish to express my thanks to the Directors of Wright Layman and Umney, Limited, for permission to publish these results.

#### REFERENCE

1. Adamson and Handisyde, *Quart. J. Pharm. Pharmacol.*, 1946, **19**, 350.

#### DISCUSSION

The paper was presented by Mr. R. L. STEPHENS

The CHAIRMAN commented on the increased accuracy obtainable by the use of a spectrophotometer.

Dr. D. C. GARRATT (Nottingham) said that the paper appeared to indicate that the assay for linctus of diamorphine B.P.C. gave inaccurate results. However, numerous workers had found that the B.P.C. assay gave accurate results. It had previously been shown that the normality of the acid should be within a fairly close range, and it was known that the quantities of acid specified in the B.P.C. could be varied within  $\pm 5$  per cent.

Mr. W. H. STEPHENSON (Nottingham) was able to confirm Dr. Garratt's observations and gave experimental evidence in support of the B.P.C. assay. He pointed out that in the paper it was stated that the concentrations of acid used in the B.P. 7th Addendum and B.P.C. 1949 were similar. Those concentrations were calculated by Mr. Stephenson as 0.05 N and 0.075 N respectively which in his opinion was a significant difference. He also stated that various diamorphine-containing elixirs had been assayed satisfactorily by the B.P.C. method.

Mr. W. H. C. SHAW (Greenford) suggested that the *pH* of the solutions listed in Table I should have been given. He agreed that temperature control was critical. He asked whether the colour obtained when a mixture of phosphoric and hydrochloric acids was used was closer to that obtained with hydrochloric acid, whether it was closer to that obtained with phosphoric acid, or whether it was an intermediate colour.

Mr. STEPHENS, in reply, agreed that the B.P.C. assay gave accurate results provided that one started with a pure reference substance, preferably diamorphine and not morphine and nitrite reagent as had been described elsewhere. His object had been to draw attention to the many variables in the assay process of which he considered the most important to be the *pH*. The *pH* values for the solutions had not been given as they were all low values. The colour obtained with the use of mixed acids was the same as that obtained with phosphoric acid.