AN APPLICATION OF THE VITALI-MORIN REACTION TO THE DETERMINATION OF SMALL QUANTITIES OF HYOSCINE HYDROBROMIDE IN SOME PHARMACEUTICAL PREPARATIONS

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SEVERAL methods for the determination of hyoscine hydrobromide in pharmaceutical preparations have been described in the literature.[1,2,3] In general, however, these methods are not suitable for the determination of very low proportions of the alkaloid, particularly in the presence of interfering substances, and the object of the work described here was to devise a method capable of determining amounts of hyoscine hydrobromide of the order of 0·005 per cent. w/v of solution.

Kirkpatrick[4] has studied the catalytic reduction of a number of alkaloids, including hyoscine at the dropping mercury electrode. A method based on this principle has been found to give quantitative results when applied to solutions of hyoscine hydrobromide but the presence of electrolytes (other than those specified for the base solution) and of traces of other alkaloids renders the method unsuitable for routine application. Allport and Wilson[5] have studied Morin's[6] modification of Vitali's test for solanaceous alkaloids and have applied it successfully to belladonna, stramonium and their galenical preparations, whilst Allport and Jones[7] have extended the application of this reaction to the determination of atropine, hyoscyamine and hyoscine in hypodermic tablets and injection solutions. Other workers[8] have reported that this method gives accurate and reproducible results only when the reagents, the reaction times and the water content of the acetone are rigidly controlled. Preliminary experiments in these laboratories on hyoscine hydrobromide solutions have confirmed these findings.

Attention was, therefore, directed to investigating the factors affecting Allport and Wilson's method and to applying the method to the determination of hyoscine hydrobromide (*a*) in official preparations and (*b*) in solutions of pethidine hydrochloride containing hyoscine: such solutions are commercially available as injection preparations containing usually 0·0108 per cent. w/v or 0·0216 per cent. w/v hyoscine hydrobromide in a 5·0 per cent. w/v solution of pethidine hydrochloride containing a preservative.

The sample of hyoscine hydrobromide used throughout the investigation conformed to the requirements of the British Pharmacopœia 1948 and except where otherwise stated other reagents were of "Analar" quality.

Investigation of the Original* Method using Hyoscine Hydrobromide

(*a*) *Effect of solvent.* James and Roberts[9] have reported that pyridine, malonic ester, acetone and methyl ethyl ketone produce strong colours when used as solvents in the Vitali reaction with atropine. Tests carried out using Allport's method on an aqueous solution of hyoscine hydrobromide indicated that of these solvents only pyridine and acetone gave

TABLE I

Effect after 6 minutes of different amounts of potassium hydroxide added to pyridine and acetone (10 ml. quantities) of various water contents

Water content per cent. w/v	Pyridine		Acetone	
	0·1 ml. 3·0 per cent. potassium hydroxide solution	0·1 ml. 0·5 per cent. potassium hydroxide solution	0·1 ml. 3·0 per cent. potassium hydroxide solution	0·1 ml. 0·5 per cent. potassium hydroxide solution
0·075	flocculent crystalline precipitate	clear	clear	clear
0·095	flocculent crystalline precipitate	clear	clear	clear
0·15	flocculent crystalline precipitate	clear	clear	clear
0·225	strongly opalescent	clear	clear	clear
0·30	strongly opalescent	very slightly opalescent almost clear	very slightly opalescent	clear
0·45	strongly opalescent	very slightly opalescent	slightly opalescent	very slightly opalescent
0·60	strongly opalescent	slightly opalescent	opalescent	very slightly opalescent
0·75	turbid	slightly opalescent	opalescent	slightly opalescent
1·00	turbid	opalescent	clear	clear

strong purple colours. A range of other solvents were tested but none produced a purple colour as intense as that produced with pyridine or acetone. In view of these findings it was decided to investigate quantitatively the possibilities of pyridine as solvent and to determine whether it offered any advantage over acetone as used hitherto.

(*b*) *Effect of potassium hydroxide concentration and water content of solvent.* Preliminary tests with pyridine as solvent showed that the water content of the pyridine and the concentration of caustic alkali used had an important bearing on the results. Addition of 0·1 ml. of a 3 per cent. solution of potassium hydroxide in methanol to 10 ml. quantities of

* The essential details of Allport and Wilson's method are as follows. Hyoscine alkaloid is extracted by means of chloroform from ammoniacal solution, converted into its water-soluble acetate by acetic acid and an aliquot, evaporated to dryness, is nitrated by treatment with fuming nitric acid. The dry nitrated base is dissolved in acetone and the intensity of the purple colour produced on the addition of potassium hydroxide solution is measured in a Lovibond tintometer.

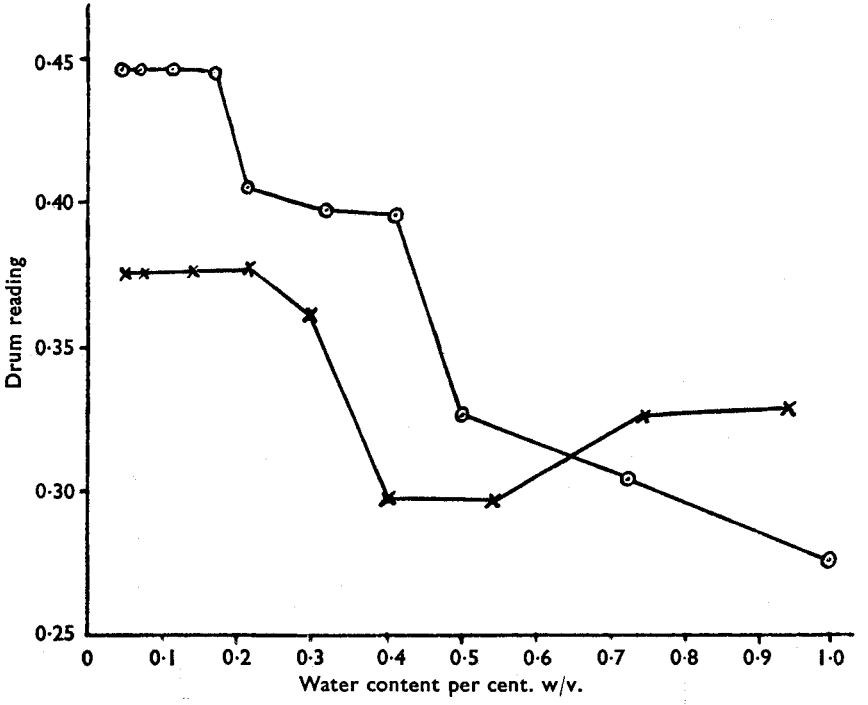

FIG. 1. Effect of water content of the solvent on the intensity of the purple colour developed in ⊙ pyridine and × acetone.

pyridine containing known amounts of water produced an opalescence or a crystalline precipitate according to the water content of the pyridine, after about 5 to 6 minutes. A similar effect, though to a less marked extent, was observed with acetone. Tests were, therefore, carried out to ascertain the maximum amount of potassium hydroxide which could be added to 10 ml. quantities of either solvent without the formation of a precipitate or turbidity which would interfere with the subsequent colorimetric comparison. It was found that the addition of 0·1 ml. of a 0·5 per cent. solution of potassium hydroxide in methanol to 10 ml. of solvent produced no precipitate or turbidity after 6 minutes standing in either acetone or pyridine containing less than 0·3 per cent. w/v of water. Comparison of the results using 3·0 per cent. and 0·5 per cent. solution of potassium hydroxide and solvent of varying water contents is shown in Table I.

The effect of the water content* of the solvent on the intensity and stability of the colour produced in the presence of hyoscine was next investigated, the lower concentration of potash being used in view of the foregoing results.

To a series of 10 ml. quantities of a 0·02 per cent. w/v aqueous solution of hyoscine hydrobromide, 2 ml. of dilute solution of ammonia (10 per

* The maximum permitted water content of "Analar" acetone is 1·0 per cent. w/v whilst that of "Analar" pyridine is 0·25 per cent. w/v.

cent. w/w) was added and the hyoscine base extracted from each solution with 3 quantities, each of 3 ml., of chloroform, the subsequent procedure being as in the original method. Each portion of nitrated base was dissolved in 10 ml. of acetone of various contents up to 1·0 per cent. w/v and 0·1 ml. of 0·5 per cent. potassium hydroxide solution added to each solution. The solutions were well mixed and the intensity of the purple colours produced measured in a 1 cm. cell using a Spekker photoelectric absorptiometer, exactly 5 minutes after the addition of the potassium hydroxide reagent. Ilford 605 gelatin filters were used.

TABLE II

HYOSCINE HYDROBROMIDE IN AQUEOUS SOLUTION. REPRODUCIBILITY OF RESULTS BY THE MODIFIED METHOD USING PYRIDINE (WATER CONTENT < 0·17 PER CENT. W/V)

	Hyoscine hydrobromide	
	Amount added mg.	Amount found* mg.
A	0·50	(0·52 0·51)
B	0·937	(0·945 0·940)
C	1·50	(1·52 1·51)
D	2·125	(2·160 2·115)
E	2·811	(2·74 2·76)
		(2·74 2·78)

* Results in brackets indicate duplicate results on the same acetate solution.

This series of tests was repeated using 10 ml. quantities of pyridine as solvent for colour development, the water content of each portion having previously been adjusted to give a range up to 1·0 per cent. w/v. The results are shown in Figure 1.

It is evident from the results that: (i) the intensity of the colour produced using pyridine as solvent is approximately 20 per cent. greater than that produced using acetone as solvent, provided the water content of the solvent does not exceed 0·17 per cent. w/v. Furthermore, with this limiting water content reproducible results are obtained; (ii) water contents of each solvent in excess of 0·17 per cent. w/v adversely affect the intensity of the colours produced, giving erratic results.

In view of these findings it is apparent that under the prescribed conditions pyridine is superior to acetone as solvent and in view of the low water content of "Analar" pyridine it was decided to use this solvent in all subsequent work. The water content of "Analar" pyridine can be reduced, if necessary, to well below the suggested upper limit of 0·17 per cent. w/v by allowing to stand over barium oxide (which has been previously heated to 200° C. for 4 hours and allowed to cool) with occasional shaking over a period of 2 to 3 days. The clear layer can be decanted off as required. It has been found, however, that of the

numerous batches of "Analar" pyridine used during routine testing, the water content is frequently below 0·17 per cent. and further dehydration is unnecessary.

The upper limit (1·0 per cent. w/v) of "Analar" acetone on the other hand allows for a considerably wider variation in water content between batches of solvent. Furthermore, it has been found that the production of acetone containing not more than 0·17 per cent. of water is not an operation applicable to routine use—the usual drying reagents do not reduce the water content sufficiently even after prolonged standing.

(*c*) *Effect of light.* It was found that the purple colour produced both in pyridine and acetone faded rapidly when placed in strong sunlight. Similar solutions kept in the dark faded less rapidly. Such solutions (both in acetone or pyridine) fade fairly rapidly for the first 2 to 3 minutes following the addition of the potassium hydroxide solution, but after this period the rate of fading of the colour is appreciably diminished.

It is essential, therefore, that the solution be kept in the dark prior to measurement of the colour and that the actual measurement be carried out as rapidly as possible.

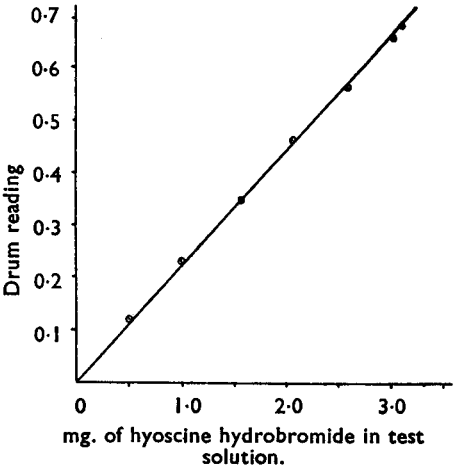

FIG. 2. Calibration curve of hyoscine hydrobromide.

(*d*) *Results.* A typical calibration graph, using aliquots of a solution containing 2·0 mg. of hyoscine hydrobromide per 10 ml. by the general method given below is shown in Figure 2 while the reproducibility on a series of test solutions is given in Table II.

APPLICATION TO PHARMACEUTICAL PREPARATIONS

The general method quoted below is essentially that as described by Allport *et al.* but has been modified in addition to slight differences in technique by: (*a*) replacing "Analar" acetone with "Analar" pyridine of a water content less than 0·17 per cent. w/v, (*b*) reducing the concentration of the potassium hydroxide solution from 3·0 to 0·5 per cent. w/v, (*c*) using the Spekker photoelectric absorptiometer in preference to the Lovibond tintometer as a means of recording the colour intensity.

(A) *General Method*

Special reagents. (*a*) Acetic acid: 6 per cent. w/v acetic acid containing approximately 5 per cent. of absolute ethanol.

(*b*) "Analar" pyridine containing less than 0·17 per cent. w/v of water.

(*c*) Potassium hydroxide: 0·5 per cent. w/v of "Analar" potassium hydroxide in "Analar" methanol. This reagent must be freshly prepared.

Procedure. To 10 ml. of the solution under test or a suitable volume containing up to 3·0 mg. of hyoscine hydrobromide add 3 ml. of dilute solution of ammonia (10 per cent. w/w) and 3 ml. of chloroform. Shake the mixture for 2 minutes, allow to separate and run off the chloroform layer. Repeat the extraction with 2 further 3 ml. quantities of chloroform shaking each for 2 minutes, allow to separate and mix each with the first chloroform extract. Wash the mixed chloroform solution once with 3 ml. of water, shaking for 2 minutes. Allow to separate, run off the chloroform layer into a 50 ml. stoppered cylinder and wash the aqueous portion with a further 1 ml. of chloroform which is added to the chloroform solution in the cylinder. Add exactly 20 ml. of 6 per cent. acetic acid reagent and shake the mixture for 1 minute. After allowing to stand until the immiscible liquids have separated, transfer 1·0 ml. of the acid layer (accurately measured by means of a micro-burette) to a Pyrex evaporating dish and evaporate just to dryness on a water-bath. Add 0·2 ml. of "Analar" fuming nitric acid (ensure that the acid comes into contact with the whole of the residue) and evaporate until the nitrated base is just dry. Dissolve the residue in 3 ml. of pyridine and transfer to a 10 ml. dry stoppered cylinder. Wash the dish separately with further quantities of pyridine adding each washing to the contents of the cylinder and adjust the total volume to 10 ml. with pyridine. Add 0·1 ml. of potassium hydroxide solution and thoroughly mix the contents by inverting several times. Transfer the solution to a 1 cm. cell and keep in the dark. Exactly 5 minutes after the addition of the potassium hydroxide solution measure the intensity of the purple colour as rapidly as possible in a Spekker photoelectric absorptiometer using Ilford 605 gelatin filters and a solution blank prepared by the addition of 0·1 ml. of potassium hydroxide solution to 10 ml. of pyridine.

Prepare a calibration graph in the same manner using suitable dilutions of a standard hyoscine hydrobromide solution covering the required range. Read off from the graph thus prepared the amount of hyoscine hydrobromide contained in the test solution.

(B) *Applications*

The method has been successfully applied to aqueous solutions of hyoscyamine sulphate B.P.C. and of atropine sulphate B.P. The intensity of the colours produced using equal weights of hyoscyamine and atropine sulphates are inversely proportional to the molecular weights of these compounds.

(*a*) *Official preparations.* The following preparations have been studied in detail and the results obtained are shown in Table III.

(i) *Injectio Hyoscinæ Hydrobromidi B.P.* Take 5 ml. for assay and proceed as by the general method. The presence of chlorocresol does not interfere with the determination.

(ii) *Guttæ Hyoscinæ B.P.C.* Dilute 10 ml. to 100 ml. with water. Take a 10 ml. aliquot and proceed as detailed in the general method.

The presence of methyl and propyl hydroxybenzoates does not interfere with the determination.

(iii) *Tabellæ Hyoscinæ Hydrobromidi B.P.C.* Take an appropriate number of tablets dependent on the active agent content, disintegrate by the addition of a few ml. of water and wash into a separator. Dilute to approximately 10 ml. with water and proceed as before.

(iv) *Oculentum Hyoscinæ B.P.* Consistently low results were obtained with this preparation. This was found to be due to incomplete extraction of the active agent from the ointment base. Several different methods of extraction were attempted but all yielded results approximately 10 per

TABLE III

OFFICIAL PREPARATIONS. RESULTS OBTAINED USING THE MODIFIED METHOD

Official preparation	Amount taken for test	Hyoscine hydrobromide	
		Added mg.	Found mg.
Injectio Hyoscinæ Hydrobromidi B.P. 1948	5 ml.	2·06	2·09 2·05
			2·11 2·11
			2·04 2·00
Guttæ Hyoscinæ B.P.C. 1949	1 ml. (by aliquot)	2·29	2·29 2·21
			2·25 2·29
			2·29 3·00
Tabellæ Hyoscinæ Hydrobromidi 1/100 gr. (≡ 0·648 mg.)	0·2579 g.	2·592	2·63 2·60
			2·60 2·52
	0·1935 g.	1·944	1·94 1·91

cent. low. No suitable method giving accurate and reproducible results has been found up to the present.

(*b*) *Solutions containing pethidine hydrochloride.* Preliminary experiments indicated that pethidine interferes with the measurement of the final colour by producing a turbidity in the pyridine solution and inducing rapid fading of the purple colour. Application of the general method without modification was, therefore, useless and an investigation was undertaken to evolve a suitable method whereby the pethidine could be removed from the solution without reducing the hyoscine content. The solubility of hyoscine alkaloid in light petroleum (b.pt. 60° to 80° C.) has been reported[10] as 1 in 510 and the solubility in water[11] as 1 in 9·5. Pethidine base, on the other hand, is miscible with light petroleum (b.pt. 60° to 80° C.) but relatively insoluble in water. This offered a convenient method of separating the alkaloids.

Preliminary experiments indicated that no detectable amount of hyoscine was lost when 4 ml. of dilute solution of ammonia was added

to 10 ml. of a solution containing 0·03 per cent. w/v of hyoscine hydrobromide and 5·0 per cent. w/v of pethidine hydrochloride and the precipitated pethidine base extracted separately 6 times with 3 ml. quantities of light petroleum (b.pt. 60° to 80° C.), the mixed petroleum extracts being washed with 3 ml. of water which was returned to the ammoniacal hyoscine solution.

Subsequent tests indicated that 4 extractions (each of 3 ml.) with light petroleum was sufficient to remove most of the pethidine base from the aqueous phase using the above conditions. The small amount of pethidine remaining in the ammoniacal solution did not interfere with the subsequent determination of hyoscine. The proposed method quoted below was applied to a series of pethidine hydrochloride solutions 5·0 per cent. w/v containing varying amounts of hyoscine hydrobromide. The results obtained are given in Table IV.

TABLE IV

RESULTS OBTAINED USING THE LIGHT PETROLEUM EXTRACTION PROCEDURE ON SOLUTIONS OF HYOSCINE HYDROBROMIDE CONTAINING 5 PER CENT. W/V OF PETHIDINE HYDROCHLORIDE

Solution (volume = 10 ml.)	Hyoscine hydrobromide added mg.	Hyoscine hydrobromide found mg.
A	1·00	1·02 0·99
B	2·00	1·99 1·98
C	3·00	3·05 3·05
D	0·50	0·49 0·48
E	1·08	1·05 1·08
F	2·16	2·12 2·15

Proposed method. To 10 ml. of the test solution containing 5 per cent. w/v of pethidine hydrochloride contained in a separator add 4 ml. of dilute solution of ammonia (10 per cent. w/w) and 3 ml. of light petroleum (b.pt. 60° to 80° C.). Shake the mixture for 2 minutes, allow to separate and run off the ammoniacal layer into a second separator and shake for 2 minutes separately with 3 further 3 ml. quantities of light petroleum (b.pt. 60° to 80° C.) allowing each to separate. Add the light petroleum extracts in turn to the first light petroleum solution contained in the first separator and wash with 3 ml. of distilled water. Run off the lower aqueous layer into the ammoniacal solution containing the hyoscine base and reject the light petroleum solution. Add 3 ml. of chloroform and continue as described in the general method with the exception that prior to taking the 1 ml. aliquot of acetic acid solution for evaporation the solution is filtered through a small pleated filter-paper rejecting the first few ml. of filtrate. The latter precaution was adopted due to the fact that the acetic acid solution was invariably turbid (in contrast to

the clear solution obtained in the absence of pethidine hydrochloride). Filtration yielded a clear filtrate. The presence of chlorocresol in the test solution does not affect the results.

During the extraction process both with light petroleum and chloroform low results were obtained if the hyoscine was allowed to remain in contact with the alkaline solution for a longer time than necessary to complete the extraction procedure. This is apparently due to the unstable nature of hyoscine alkaloid under such conditions of alkalinity. It was also found that the hyoscine present in the acetic acid solution is not very stable. All tests carried out on the same acetic acid solution should be done within 1 to 2 hours of preparation.

Attempts were made to extract the pethidine base from alkaline solutions with the aid of a continuous extraction apparatus using light petroleum (b.pt. 30° to 40° C.) as the refluxing solvent, but the results were low and in some instances this procedure resulted in total destruction of the hyoscine alkaloid present.

Summary

1. The colorimetric method described by Allport *et al.* for the estimation of hyoscine hydrobromide has been shown to give variable results if the water content of the solvent in which the colour develops is not controlled. "Analar" pyridine of low water content has been shown to be a more suitable solvent than "Analar" acetone in the original method.

2. The modified method has been shown to give accurate and reproducible results using official preparations containing hyoscine hydrobromide, except in the case of Oculentum Hyoscinæ B.P., for which a satisfactory extraction procedure has still to be devised.

3. The method has been successfully adapted to the determination of hyoscine hydrobromide in the presence of pethidine hydrochloride.

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