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SCIENCE PAPERS AND DISCUSSIONS

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THE SPECTROPHOTOMETRIC ASSAY OF INJECTION SOLUTIONS CONTAINING CHLOROCRESOL

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SPECTROPHOTOMETRIC methods of assay are usually simple and rapid and for these reasons are used extensively. These methods are most useful for simple preparations which contain only one ingredient which absorbs light in the ultra-violet or visible regions. When two ingredients absorb then the problem of determining the concentration of one or both is less simple and often impossible without some preliminary separation.

Many of the injection solutions listed in the British Pharmacopœia contain two main ingredients—the active principle and an antibacterial agent. The latter is usually phenylmercuric nitrate, chlorbutol, phenol or chlorocresol, and of these the last two absorb in the ultra-violet. One of the more difficult injection solutions to assay by any method is Apomorphine Hydrochloride Injection B.P. with chlorocresol as the antibacterial agent, and whilst devising a spectrophotometric method for this preparation it seemed probable that other injections containing chlorocresol might also be assayed by similar techniques.

A single method by which any injection containing any bacteriostatic could be determined would have been an ideal solution but this is still impractical and eight preparations have been assayed by three fundamentally different methods.

THE DIRECT METHOD (I)

A direct method is applicable when the absorption of the active ingredient is so much higher than that of the chlorocresol that the latter can be ignored. Here, the determination of the active ingredient requires only a simple dilution and measurement at a suitable wavelength. Injection of Procaine and Adrenaline B.P. provides an example.

Injection of Procaine and Adrenaline B.P. Procaine hydrochloride shows a maximum absorption at 290 mμ and at this wavelength in water the absorptions of all the ingredients in the concentration present are as follows. Procaine hydrochloride $E^{2\ \text{per cent.}}_{1\ \text{cm.}}$ 290 mμ = 1360; adrenaline

hydrochloride $E^{0\cdot002\ \text{per cent.}}_{1\ \text{cm.}}$ 290 mμ = 0·09; chlorocresol $E^{0\cdot1\ \text{per cent.}}_{1\ \text{cm.}}$ 290 mμ = 4·0.

As the total absorption of all other ingredients is so small compared with that of the procaine hydrochloride, the latter is determined directly by making a 2000 times dilution in water and reading the maximum absorption at about 290 mμ.

$$\text{Per cent. procaine hydrochloride} = \frac{\text{absorption at 290 m}\mu}{680} \times 2000$$

The Simultaneous Determination Method (II)

This method can be used when the absorption characteristics of chlorocresol and the active ingredient are so different as to allow both to be determined by making measurements at two wavelengths. To achieve a sufficient degree of accuracy the absorption of the active principle should be at least as great as that of the chlorocresol. A suitable example is provided by Injection of Pethidine Hydrochloride B.P.

Injection of Pethidine Hydrochloride B.P. In 0·1N hydrochloric acid at 279 mμ chlorocresol exhibits maximum absorption while pethidine hydrochloride has none. At 257 mμ pethidine hydrochloride has maximum absorption and knowing the concentration of the chlorocresol a suitable correction can be made and the former determined. In order that both spectrophotometric measurements are made at suitable absorption values it may be necessary to use different dilutions for the two readings, depending on the relative concentrations of the pethidine and chlorocresol in the original solution. The calculations are as follows. In 0·1N hydrochloric acid for chlorocresol, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 257 mμ = 20, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 279 mμ = 105; for pethidine hydrochloride, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 257 mμ = 7·3, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 279 mμ = zero.

If the observed absorptions are, at 257 mμ = A at dilution factor = x, 279 mμ = B at dilution factor = y, then per cent. chlorocresol = $\frac{By}{105}$. At 257 mμ contribution of chlorocresol to total absorption = $\frac{20B}{105} \times \frac{y}{x}$ = C, per cent. pethidine hydrochloride = $\frac{(A - C)x}{7\cdot3}$.

Injection of methylamphetamine hydrochloride. A second example is provided by injection of methylamphetamine hydrochloride. When dissolved in 0·1N hydrochloric acid methylamphetamine hydrochloride has the following absorption characteristic. Maximum $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 257 mμ = 10·1, $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 279 mμ = zero. In mixtures containing chlorocresol both may be determined in the same way as injection of pethidine hydrochloride.

Separation Methods (III)

Preliminary separation is necessary when the absorption curves of the two ingredients are so similar that simultaneous determination is inaccurate, or when the absorption of the active ingredient is much less than that of the chlorocresol. An example is Injection of Atropine Sulphate B.P.

Injection of Atropine Sulphate B.P. The official preparation contains 0·06 per cent. atropine sulphate and when chlorocresol is used as a preservative its usual concentration is 0·2 per cent. In 0·1N acid the absorption characteristics are as follows. Atropine sulphate $E^{1\text{ per cent.}}_{1\text{ cm.}}$ 257 mμ = 6·25 (max), $E^{1\text{ per cent.}}_{1\text{ cm.}}$ 279 mμ = zero; chlorocresol $E^{1\text{ per cent.}}_{1\text{ cm.}}$ 257 mμ = 20, $E^{1\text{ per cent.}}_{1\text{ cm.}}$ 279 mμ = 105.

It might appear at first sight that the simultaneous method could be used for this preparation but it would give rise to large errors in the determination of atropine sulphate. In the concentrations at which the two are present in the original injection the absorption at 257 mμ is 0·38 for atropine sulphate and 4·0 for chlorocresol. The chlorocresol can be determined directly by diluting and reading the absorption at 279 mμ, but if this determination is used as the basis for a correction at 257 mμ as in the simultaneous method, the absolute error in determining the atropine sulphate will not be less than the absolute error in determining the chlorocresol. If the latter is 1 per cent. then it will contribute a 10 per cent. error to the atropine sulphate result. It is desirable therefore to separate the atropine sulphate from the chlorocresol and determine it directly.

Whether separation was possible was tried with 5 ml. of a solution containing 0·12 per cent. atropine sulphate and 0·2 per cent. chlorocresol. This was made alkaline with sodium hydroxide and extracted with four 15 ml. portions of chloroform. The chloroform extracts were filtered through a paper previously moistened with chloroform, and evaporated to dryness. The residue was dissolved in 10 ml. 0·1N sulphuric acid and the absorption curve measured, using in the comparison cuvette a solution obtained by evaporating to dryness 30 ml. of chloroform and dissolving the residue in 5 ml. 0·1N sulphuric acid. A comparison of this curve with that of pure atropine sulphate showed that the alkaloid was extracted quantitatively and in a reasonably pure form. The purity of the extracted alkaloid was important in order that the absorption could be used for a direct determination. Table I shows the ratios obtained on the absorptions of the four maxima of atropine sulphate both in direct solution and after extraction.

TABLE I

THE RATIOS OF ABSORPTIONS AT PEAK WAVELENGTHS FOR ATROPINE SULPHATE IN 0·1N H_2SO_4. (*a*) DIRECTLY, (*b*) AFTER CHLOROFORM EXTRACTION

	(*a*) Direct solution	(*b*) After extraction
257/246 mμ	1·48	1·41
257/251 mμ	1·21	1·18
257/263 mμ	1·29	1·29

Solutions containing 0·06 per cent. and 0·12 per cent. atropine sulphate and 0·2 per cent. chlorocresol were prepared. Five ml. was extracted as described and the atropine sulphate content determined by dissolving the residue in 0·1N sulphuric acid, the absorption being measured at 257 mμ. A further 2 ml. of the injection was diluted to 100 ml. with 0·1N hydrochloric acid and its absorption at 279 mμ used to determine chlorocresol.

Injection of Strychnine Hydrochloride B.P. A similar method was applied to strychnine hydrochloride solutions for injection. A solution was made containing 0·2 per cent. strychnine hydrochloride and 0·1 per

cent. chlorocresol, and 5 ml. of this solution diluted to 50 ml. with water. 10 ml. of this diluted solution was made alkaline with 1 ml. N sodium hydroxide and extracted as described for Injection of Atropine Sulphate. The residue was dissolved in 50 ml. 0·1N sulphuric acid and 15 ml. of this solution diluted to 50 ml. also with 0·1N sulphuric acid. The absorption of the final solution was measured at 255 mμ using 0·1N sulphuric acid in the comparison cuvette. In 0·1N sulphuric acid strychnine hydrochloride has maximum absorption at 255 mμ, $E^{1\%}_{1\,cm.}$ 255 mμ = 315. Using this information the strychnine hydrochloride of the original solution was calculated.

The alkaline solution remaining after the original chloroform extraction was diluted to 100 ml. with 0·1N sodium hydroxide and the absorption of this solution measured at 296 mμ. In 0·1N sodium hydroxide chlorocresol shows maximum absorption at 296 mμ, $E^{1\%}_{1\,cm.}$ 296 mμ = 183. The chlorocresol content of the original solution was determined. The simultaneous determination method may be used for chlorocresol and strychnine, but the extraction method gives a more accurate determination of both components.

Apomorphine Hydrochloride Injection B.P. This is an example in which the absorption curves of the ingredients are so similar as to make a separation necessary despite the fact that the apomorphine hydrochloride absorbs much more strongly than the chlorocresol. The absorption curves of the two ingredients are shown in Figure 1 and their similarity is obvious.

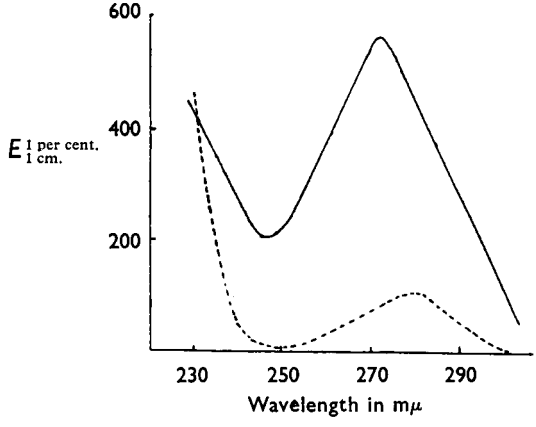

FIG. 1. Absorption curves in 0·1N hydrochloric acid of:—
——— Apomorphine hydrochloride.
- - - - Chlorocresol.

A partition chromatographic method of separating *para*- and *meta*-cresols on a buffered kieselguhr column has been described[1] and was found successful in removing chlorocresol from the injection solution. The sample after dilution is put onto a column buffered at *p*H 11·4, and eluted with *cyclo*hexane. Chlorocresol is eluted first and can be estimated in the *cyclo*hexane solution while the apomorphine is retained on the column. The total absorption of the original injection solution is measured and a correction for the chlorocresol applied. As the apomorphine hydrochloride absorbs the more strongly the method is accurate. The results summarised in Table II shows chlorocresol to be removed quantitatively and in a pure form.

The method for a solution containing 0·3 per cent. apomorphine hydrochloride and 0·2 per cent. chlorocresol is described. Dilute 2 ml. to 10 ml. with water and add 2 ml. of this solution to a beaker containing 5 g. of kieselguhr previously mixed with 5 ml. of acetate buffer solution of *p*H 11·4, and mix well. Transfer the mixture in two portions to a chromatographic tube 12 inches long, and 1 inch in diameter pressing down each portion lightly with a flat-ended glass rod. Scrub out the beaker first with 1 g. kieselguhr and then with a small piece of cotton wool, adding each to the top of the column. Elute the column with *cyclo*hexane collecting the first 25 ml. and measure the maximum absorption of the solution at about 280 mμ, using in the compensation cuvette *cyclo*hexane which has passed through the column after the first 25 ml. Determine

TABLE II

THE ABSORPTIONS OF SOLUTIONS OF APOMORPHINE HYDROCHLORIDE IN *cyclo*HEXANE (*a*) AFTER ELUTION FROM A BUFFERED KIESELGUHR COLUMN, (*b*) DIRECTLY

	λ (mμ)	Absorption of eluted solution	Absorption of direct solution
Maxima	280	0·256	0·258
	288	0·240	0·240
Minima	247	0·020	0·028
	285	0·206	0·208

TABLE III

SUMMARY OF ALL RESULTS INVOLVING THE DIFFERENT TECHNIQUES DESCRIBED. ALL SOLUTIONS USED WERE MADE UP IN THE LABORATORY FROM STANDARD MATERIALS

Active ingredient	Method	Per cent. active ingredient		Per cent. chlorocresol	
		Theory	Found	Theory	Found
Procaine hydrochloride ..	I	2·0	2·01		
Pethidine hydrochloride ..	II	2·5	2·46	0·20	0·210
		2·5	2·58	0·10	0·107
		7·5	7·54	0·20	0·206
Methylamphetamine HCl ..	II	1·0	1·05	0·10	0·108
		1·0	1·07	0·20	0·205
Atropine sulphate	III	0·06	0·062	0·20	0·200
		0·12	0·124	0·20	0·200
Strychnine hydrochloride ..	III	0·2	0·203	0·10	0·092
		0·2	0·206	0·20	0·197
Apomorphine hydrochloride	III	0·10	0·107	0·10	0·104
		0·30	0·307	0·20	0·190
Morphine sulphate	III	1·0	0·989	0·20	0·213
		3·0	2·89	0·20	0·220
Morphine sulphate (with atropine)	III	1·0	0·988	0·10	0·100
		1·0	0·990	0·20	0·224

the chlorocresol in the injection knowing that in *cyclo*hexane $E^{1\ per\ cent.}_{1\ cm.}$ 280 mμ = 129. Dilute 5 ml. of the injection to 100 ml. and a further 5 ml. to 100 ml. using 0·1N hydrochloric acid for both dilutions. Measure the absorption at 272 mμ and subtract the chlorocresol contribution. The net absorption is then directly proportional to the apomorphine hydrochloride content and the latter is calculated. The method of calculation is similar to that described for Injection of Pethidine Hydrochloride, using the following values. Chlorocresol in *cyclo*hexane $E^{1\ per\ cent.}_{1\ cm.}$ 280 mμ = 129, in 0·1N hydrochloric acid $E^{1\ per\ cent.}_{1\ cm.}$ 272 mμ = 80, apomorphine hydrochloride in 0·1N hydrochloric acid $E^{1\ per\ cent.}_{1\ cm.}$ 272 mμ = 552.

Injection of Morphine Sulphate B.P. and Injection Morphine and Atropine B.P. Morphine sulphate is retained on a kieselguhr column buffered at *p*H 11·4 when *cyclo*hexane is used for elution, and these injections can be assayed for morphine sulphate and chlorocresol by the same method as is used for Injection of Apomorphine Hydrochloride.

SUMMARY

1. Spectrophotometric methods have been used to assay eight injection solutions containing chlorocresol as an antibacterial agent.
2. The methods are accurate and are more rapid than chemical estimations.

REFERENCES

1. White and Grant, *Nature, Lond.*, 1955, **175**, 513.

DISCUSSION

The paper was presented by MR. K. A. PROCTOR.

DR. F. HARTLEY (London) said that in many official assays of injections interference might be caused by some of the permissible bacteriostatics. He hoped that the authors would go on to study the effect of other bacteriostatics in addition to chlorocresol in their recommended procedures.

DR. R. E. STUCKEY (London) said it would be of interest to know whether the figures quoted for chlorocresol were for a chemically pure sample, or one of pharmacopœial quality, what difference was found, and whether this difference could be allowed for in assaying mixtures where the absorption due to chlorocresol was as great as that due to the active principle. He also asked whether the authors had any further information as to the *p*H at which the absorption of chlorocresol started to change, as they had quoted results showing that absorption was markedly different in alkaline and acid solutions.

DR. A. H. BECKETT (London) asked for further information on the separation method III. The authors had extracted atropine with chloroform. In extracting phenols, he had experienced trouble when taking the chloroform to dryness. Had the authors done a series of blank extractions and had they obtained reproducible results?

MR. K. A. PROCTOR, in reply, said work was proceeding on other bacteriostatics. The chlorocresol used was of B.P. standard. No great variation was found with different samples, and the figure used was a mean. He had no further information of the effect of *p*H changes on the absorption. No difficulty had been encountered with chloroform in the determination of atropine sulphate by the method described.