THE DETERMINATION OF HEXACHLOROPHANE AND OTHER PHENOLS IN PHARMACEUTICAL PREPARATIONS BY A Δ E SPECTROPHOTOMETRIC METHOD

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The changes in spectral characteristics of phenolic compounds which occur with variations in pH have been used to develop a simple, rapid method for their determination in pharmaceutical preparations. Results compare favourably with those obtained by chemical analysis. The method is more specific than the chemical method.

In recent years derivatives of phenol such as hexachlorophane have found increasing use in the cosmetic field and this has necessitated more rapid and specific methods for their determination in complex formulations.

The method described in the U.S.P. XVI for the determination of hexachlorophane in Hexachlorophane Liquid Soap is based on that of Childs and Parks (1956). This depends on the fact that the spectrum of hexachlorophane at pH 3 is different from that at pH 8 whilst the soap base shows no such change. The maximum difference in extinction occurs at 312 mμ and the hexachlorophane content of the soap is determined by comparing the "difference" extinction (ΔE) of the sample with that of a standard solution of hexachlorophane.

The present work describes the application of the "difference" or ΔE method to preparations containing hexachlorophane, phenol, resorcinol, cresol, and methyl-*p*-hydroxybenzoate.

The U.S.P. XVI method for hexachlorophane recommends the use of two buffers, one (pH 1·4) obtained by diluting acetic acid (5 ml.) and hydrochloric acid (0·3 ml.) to 100 ml. with 90 per cent methanol; the other is obtained by adjusting 90 per cent methanol to pH 8·0 with sodium hydroxide. Batch to batch variation in the pH of the latter buffer solution led us to select tris-(hydroxymethyl)aminomethane (Bates and Bower, 1956). It has a good buffering action between pH 7 and 9, is fairly soluble in 90 per cent methanol, and is transparent to ultra-violet light. Using a 0·05M solution of tris-(hydroxymethyl)aminomethane in 90 per cent methanol it was found that to adjust the pH to 8·0, 50 ml. of 0·5N hydrochloric acid was required per litre of buffer solution.

Phenol, resorcinol and cresol were not ionised at pH 8·0 and a more alkaline solution was used for these compounds. This consisted of a 0·2M solution of potassium hydroxide in 90 per cent methanol.

Methyl *p*-hydroxybenzoate did not appear to show changes in spectral characteristics in the presence of large amounts of methanol but differences were observed in aqueous solutions. In phosphate buffer at pH 7·5 methyl *p*-hydroxybenzoate showed maximum absorption at 257 mμ and in 0·1N sodium hydroxide, at 296 mμ.

EXPERIMENTAL

Reagents

A. *Buffer solution pH* 8·0. Dissolve tris-(hydroxymethyl)aminomethane (6·07 g.) in methanol (900 ml.). Add 0·5N hydrochloric acid (50 ml.) and make up to 1 litre with water.

B. *Buffer solution pH* 1·4. Add glacial acetic acid (18 ml.) and concentrated hydrochloric acid (3 ml.) to methanol (900 ml.) and make up to 1 litre with water.

C. *Potassium hydroxide* 0·2 N *in methanol.*

D. *Buffer solution pH* 7·5. Dissolve potassium dihydrogen phosphate (22·2 g.) and dipotassium hydrogen phosphate (178 g.) in 1 litre of water.

E. 0·1N Sodium hydroxide.

F. Methanol.

TABLE I

SPECTROPHOTOMETRIC CHARACTERISTICS OF VARIOUS PHENOLS

Compound	Sample buffer	Blank buffer	ΔE (1 per cent, 1 cm.)	λ max. mμ
Hexachlorophane ..	A	B	144	312
Phenol	C	A	280	289
Resorcinol	C	A	305	291
Cresols B.P.	C	A	266	293
Methyl-*p*-hydroxybenzoate	E	D	1,290	296

Spectral Determinations on Pure Phenols

The "difference absorption spectra" of the alkaline solutions of hexachlorophane, phenol, resorcinol, cresol and methyl hydroxybenzoate were measured in a 1 cm. cuvette from 220 to 350 mμ using the more acid solution of the same strength of phenol in the reference cuvette. With a series of varying concentrations it was found that Beer's Law was obeyed in all instances. The appropriate buffer, ΔE (1 per cent, 1 cm.) values and wavelength criteria are given in Table I.

Some preparations contained salicylic acid or dichlorophane in addition to hexachlorophane. The absorption spectra of these two compounds were measured in the buffer solutions used for hexachlorophane. It was found that at the wavelength where hexachlorophane showed a maximum value of ΔE (1 per cent, 1 cm.) both salicylic acid and dichlorophane also showed changes in E (1 per cent, 1 cm.) with pH. It was found, however, that at 257·5 and 263 mμ respectively the extinctions of salicylic acid and dichlorophane were independent of changes in pH. At these wavelengths the corresponding ΔE (1 per cent, 1 cm.) values for hexachlorophane were 111 and 69·6, and the results in Table III for the samples containing salicylic acid and dichlorophane were obtained using these values.

Recovery Experiments

To check the efficiency of the "difference" method we examined where possible the effects of the ingredients of the base of each preparation.

Known amounts of the phenolic compound concerned were added to the appropriate ingredients and where these were not available, known amounts of the phenols were added to the samples themselves. The range of recoveries was 97 to 104 per cent (see Table II).

Methods of Extraction

Three general methods were used.

(*a*) Direct dilution or extraction with the appropriate buffer solution.

(*b*) Extraction with chloroform.

(*c*) Extraction with light petroleum (40–60°)-methanol mixtures.

TABLE II

RECOVERY OF PHENOLS IN VARIOUS PREPARATIONS

Sample	Extraction		Recovery of added phenol per cent
	Procedure‡	Reagent‡	
HEXACHLOROPHANE			
Shampoo	(*a*)	A	98·0
Soap (1)	(*a*)	A	103·0
Soap (2)	(*a*)	A	103·0
Deodorant stick (1)	(*a*)	A	104·0
Deodorant stick (2)	(*a*)	A	104·0
Talcum powder (1)	(*b*)	A	99·7
Talcum powder (2)	(*b*)	A	99·0
Talcum Powder (3)	(*b*)	A	100·0
Pre-shave lotion	(*a*)	A	99·3
Shaving lather	(*a*)	A	98·8
Veterinary shampoo	(*a*)	A	99·3
Veterinary cream	(*a*)	A	97·0
Foot talc*	(*b*)	A	100·0
Hair lotion†	(*a*)	A	101·5
PHENOL			
Compound solution of Sodium Phenate B.P.C. 1949	(*a*)	F	101·0
Glycerin of Phenol B.P.	(*a*)	F	99·0, 97·0
Calamine Lotion B.P.	(*a*)	C	100·0
Zinc Oxide Cream	(*a*)	C	101·0
RESORCINOL			
Resorcinol Ointment B.P.C. 1949	(*c*)	F	101·0
Compound Resorcinol Ointment B.P.C.	(*c*)	F	99·4
CRESOLS B.P.			
Veterinary shampoo	(*a*)	F	98·0
Lysol B.P.	(*a*)	F	100·0
METHYL HYDROXYBENZOATE			
A complex proprietary liquid preparation	(*c*)	F	99·4

* Contains dichlorophane. The hexachlorophane content was calculated using the ΔE (1 per cent, 1 cm. value at 263 mμ.

† Contains salicylic acid. The hexachlorophane content was calculated using the ΔE (1 per cent, 1 cm. value at 257·5 mμ.

‡ Refer to text for description.

With one exception, method (*a*) was applied to all liquid preparations. Preparation of sample: Weigh or pipette a suitable amount into a 100 ml. stoppered conical flask, add about 50 ml. of buffer solution appropriate to the phenol being examined (Table I). Shake or warm until the sample is completely dispersed then filter under vacuum through a Whatman No. 42 filter paper. Wash the conical flask and filter paper with three portions, each of 10 ml., of buffer solution and transfer the filtrate quantitatively to a 100 ml. graduated flask. Make up to volume with buffer solution.

Method (*b*) was applicable to samples in powder form. Preparation of sample: Weigh a suitable amount into a sintered glass funnel (No. 3 porosity). Extract the powder with five successive portions, each of about 20 ml., of chloroform, drawing each extract through the sinter with gentle suction and collecting the extract in a Buchner flask. Remove the chloroform on a steam-bath taking care to avoid volatilisation of the phenol and dissolve the residue in a suitable volume of the appropriate buffer solution.

Method (*c*) was found suitable for ointments. Preparation of sample: Weigh a suitable amount into a small beaker, add 20 ml. of light petroleum

TABLE III

PHENOL CONTENT OF VARIOUS PREPARATIONS

Sample	Phenol expected per cent	Phenol found by proposed method per cent	Phenol found by chemical method per cent
HEXACHLOROPHANE			
Shampoo	1·0	1·02, 1·04, 1·02, 1·02	—
Soap (1)	1·0	1·16, 1·17	—
Soap (2)	1·0	1·04, 1·03, 1·03	—
Deodorant stick (1)	0·25	0·29, 0·30	—
Deodorant stick (2)	0·25	0·31, 0·32, 0·32	—
Talcum powder (1)	0·25	0·25, 0·25	—
Talcum powder (2)	0·25	0·24, 0·25	—
Talcum powder (3)	0·20	0·20, 0·20	—
Pre-shave lotion	0·20	0·20, 0·21	—
Shaving lather	0·20	0·20, 0·20	—
Veterinary shampoo	0·50	0·50, 0·50	—
Veterinary cream	0·50	0·48, 0·49	—
Foot talc.	0·50	0·51, 0·51	—
Hair lotion	0·20	0·20, 0·20	—
PHENOL			
Compound Solution of Sodium Phenate B.P.C. 1949	2·8–3·4	3·31, 3·31	3·12
Glycerin of Phenol B.P.	15·0–16·5	16·7, 16·9	15·8, 15·8
Calamine Lotion B.P.	0·39–0·47	0·46, 0·45	0·46
Zinc Oxide Cream	0·36–0·41	0·39, 0·39	0·40
RESORCINOL			
Resorcinol Ointment B.P.C. 1949	12·5	12·6, 12·6	12·2
Compound Resorcinol Ointment B.P.C.	4·0	4·07, 4·07	4·34
CRESOLS			
Veterinary shampoo	4·0	3·9, 3·9	4·10
Lysol B.P.	47–53	51·0, 51·0	51·0
METHYL HYDROXYBENZOATE			
A complex proprietary liquid preparation	0·12	0·11, 0·12	—

and disperse the sample as far as possible with a glass rod. Allow to settle and decant the supernatant liquid into a 150 ml. separator. Wash the beaker successively with the following solutions, transferring each in turn to the separator. Three portions, each of 20 ml., of light petroleum-methanol (1 : 1). Two portions, each of 10 ml., of methanol. One portion of 10 ml. of light petroleum-methanol (1 : 1). Finally add 15 ml. of water to the separator, stopper and shake gently for 1 min. Allow the layers to separate, filter the lower layer through a small plug of cotton wool into a 100 ml. graduated flask, wash the light petroleum layer in the separator with 10 ml. of methanol-water (1 : 1) and add these washings to the graduated flask through the cotton wool plug. Make up to volume with methanol.

With the complex proprietary liquid preparation examined, the methyl *p*-hydroxybenzoate was extracted directly with ether, the ether removed by evaporation and the residue dissolved in methanol. Subsequent dilutions were made in phosphate buffer pH 7·5 and 0·1N sodium hydroxide, the spectrophotometric measurements being made as rapidly as possible to avoid possible hydrolysis effects.

Spectrophotometric Determination

Dilute a suitable aliquot of the extracted phenolic compound with the "sample" buffer solution and a second aliquot in the appropriate "blank" buffer solution (listed in Table I). It is important that the concentration

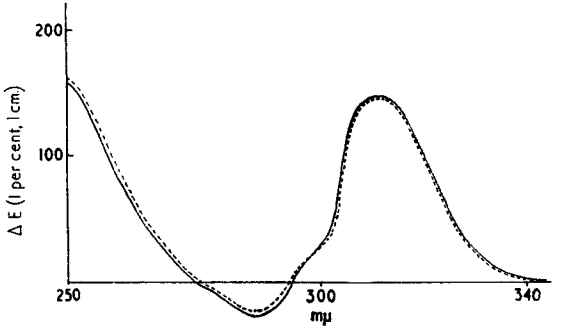

FIG. 1. △ *E* curve for pure hexachlorophane. (——) compared with △ *E* curve for hexachlorophane in a talcum powder. (– – –).

of the phenolic compound is the same in both "sample" and "blank" buffer solutions. For all the samples examined, the final dilution with the appropriate buffer solution was at least tenfold. Under these conditions the pH value of the final solution was found to be within 0·1 units of that of the diluent buffer.

Measure the extinction of the more alkaline solution in a 1 cm. cuvette at the appropriate wavelength (see Table I) using the more acid solution in the reference cuvette. Calculate the content of phenolic compound in the sample using the ΔE (1 per cent, 1 cm.) value found for the pure phenol in the same pair of buffer solutions. The figures quoted in Table I are intended only as a guide.

RESULTS

Details of extraction procedure, dilutions and recoveries obtained are shown in Table II. Results on the samples examined are given in Table III. It will be seen that quantitative determinations of a variety of phenols in a wide range of cosmetics and pharmaceutical preparations have been achieved. Comparison with chemical analysis, when available, shows good agreement. By obtaining the complete difference spectrum of the extracted phenolic compound a qualitative identification is also

achieved, as shown in the example in Fig. 1 for the hexachlorophane content of a talcum powder.

REFERENCES

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