THE DETERMINATION OF BENZENE HEXACHLORIDE (HEXACHLOROCYCLOHEXANE) IN PHARMACEUTICAL PREPARATIONS

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In addition to its use as an industrial insecticide benzene hexachloride (Gammexane) has proved of value as an external parasiticide in human and veterinary medicine. For this purpose, the biologically active γ-isomer (Lorexane) has been isolated and formulated to yield a variety of pharmaceutical products. The object of the work described here was to provide a simple method for the determination of benzene hexachloride in the presence of the excipients likely to be found in such formulations. Earlier analytical studies of benzene hexachloride have been concerned chiefly with mixed isomers and have described the determination of either (a) γ-isomer in presence of others, or (b) total benzene hexachlorides. Of these, (a) usually involve biological or physicochemical methods, while (b) nearly all depend on the alkaline dehydrochlorination to trichlorobenzene originally reported by Van der Linden[1].

$$C_6H_6Cl_6 + 3OH' = C_6H_3Cl_3 + 3Cl' + 3H_2O$$

Several workers have shown that this reaction is quantitative for all isomers when benzene hexachloride is refluxed with alcoholic potassium hydroxide, and that the determination may be completed by either acid or silver nitrate titration of the resultant solution. Goldenson and Sass[2] have studied the effect of replacing potassium hydroxide by other alkalis, and Howard[3] has drawn attention to the possible use of monoethanolamine, which has the advantages of being easily freed from chloride impurity and of not reacting with vegetable oils.

Since the quality of the active agent is capable of independent analytical control, it was considered sufficient that the method should determine total benzene hexachloride, i.e., there was no need for it to be specific for γ-isomer. The main criterion was that it should be effective in the presence of relatively large amounts of excipients and diluents, since the biological potency of the γ-isomer is such that it is rarely used at concentrations above 1 per cent. In view of the simplicity of the reaction, it was decided to investigate more fully alkaline dehydrochlorination as a basis for a general method.

Methods of Dehydrochlorination:

(1) *By Alcoholic Potassium Hydroxide:* The limits of time, temperature and concentration to ensure a quantitative reaction were investigated as follows. A known weight of pure γ-isomer (about 0·4 g.) was reacted with an excess of alcoholic potassium hydroxide at controlled time and temperature, and the ionisable chlorine produced was titrated with silver nitrate. The results obtained are recorded in Table I, and

show that : (a) Using 1 per cent, potassium hydroxide the reaction is quantitative in 15 minutes at room temperature, provided that sufficient alcohol is present to dissolve the benzene hexachloride. (b) No further reaction involving the trichlorobenzenes takes place after at least 1 hour's refluxing with 4 per cent. potassium hydroxide in alcohol (95 per cent.).

TABLE I

	Reaction time	Alcohol strength	Potassium hydroxide solution	Recovery (percentage of theory)
	minutes	per cent. v/v	per cent. w/v	
Room temperature 20° C. approx.	1	95	4	66
	2			89
	5			100·0
	15			100·2
	15		1	99·4
Refluxing	15	Nil (aqueous)	1	16
	15	20	1	51
	15	70	1	100·1
	15	95	1	100·1
	60	95	4	100·0

(2) *By Monoethanolamine:* Howard[3] has already shown that monoethanolamine produces quantitative dehydrochlorination when heated either with solid benzene hexachloride or its oily solutions. To compare this method with (1) the action of an excess of monoethanolamine when used both alone and in alcoholic solution was investigated under similar conditions of time, temperature, and concentration. The results are recorded in Table II and show that:—

(a) *For the alcoholic solution:* (i) Even a large excess of monoethanolamine produced practically no reaction after 1 hour at room temperature. (ii) To obtain a quantitative reaction it was necessary to boil for 2 hours with 8 per cent. of monoethanolamine in alcohol (95 per cent.).

(b) *For monoethanolamine alone:* (i) The reaction was not complete in 18 hours at room temperature. (ii) The reaction was quantitative after 5 minutes heating on the water-bath, with frequent shaking.

The action of monoethanolamine in alcoholic solution is thus much

TABLE II

	Reaction time	Monoethanolamine alone (ml.)	Monoethanolamine (ml. in 25 ml. of alcohol)	Recovery (percentage of theory)
Room temperature	1 hour	—	1	Trace
	18 hours	—	1	16
	18 hours	2	—	91
Refluxing	15 minutes	—	1	24
	60 ,,	—	1	75
	120 ,,	—	1	94
	60 ,,	—	2	97·5
	120 ,,	—	2	100·0
	5 minutes	2	—	100·0
	15 ,,	2	—	99·7

slower than that of potassium hydroxide. This confirms the findings of Howard, who recommended heating with undiluted monoethanolamine for 1 hour at 100°C. as a general procedure for dehydrochlorination.

Application to Pharmaceutical Preparations:

The relative merits of the potash and monoethanolomine methods were assessed for the following types of pharmaceutical preparations, selected as being the most commonly encountered. (a) Solutions in mineral oil (including ointments with paraffin base); (b) solutions in vegetable oil (including sulphonated oils); (c) alcoholic preparations; (d) dusting powders; (e) emulsions and creams. In testing the suitability of each method the following processes, described here as General Methods, were first applied and subsequently modified, where necessary, to suit special requirements.

General Method A (Potassium Hydroxide): To an appropriate weight of sample is added 25 ml. of alcohol, with sufficient potassium hydroxide to react with the benzene hexachloride and with any saponifiable matter present. The mixture is refluxed for 1 hour, cooled, and acidified with dilute nitric acid. Fatty acids, trichlorobenzene, and unsaponifiable matter are extracted with ether and the ionisable chlorine in the aqueous layer determined by any convenient method. A control titration is made to compensate for the presence of ionisable chlorine in sample and reagents. For this purpose, dilute nitric acid is first added in sufficient quantity to ensure that the solution remains acid during and after the addition of potassium hydroxide. It has already been established that benzene hexachloride itself is unaffected by nitric acid[4].

General Method B (Monoethanolamine): To an appropriate weight of sample is added an excess of monoethanolamine (1 ml. per g. of benzene hexachloride) with 0·1 ml. extra per 1 ml. of oil, and the mixture heated for 1 hour on the water-bath, with frequent shaking. After cooling, the mixture is acidified with dilute nitric acid, diluted with water, and extracted with ether. The ionisable chlorine in the aqueous layer is then determined as before. A blank titration is also carried out as described in Method A. Experimental results are summarised in Table III.

Discussion

The potassium hydroxide method is, in general, the more attractive since the reagent is readily available and the reaction proceeds rapidly in alcohol even at room temperature. It is particularly useful when the analysis involves a break-down of emulsions where monoethanolamine is markedly inefficient. The presence of a trace of chloride impurity in potassium hydroxide of A.R. quality is not a serious disadvantage, firstly because it is small in relation to the total ionisable chlorine produced in the assay and, secondly, because it is compensated by a control titration. This method is thus the more suitable for preparations of types (a), (c), (d) and (e). The only circumstances in which the monoethanolamine method is to be preferred are when it is desired to avoid

TABLE III

Preparation	Prepared Strength per cent.w/w	Modifications to Method A	Strength Found per cent.w/w	Modifications to Method B	Strength Found per cent.w/w
1. Solution in liquid paraffin	1·970	Nil	1·97	Nil	1·96
2. Ointment containing liquid, hard and soft paraffins and wool fat.	1·964	Nil	1·96	Not suitable, due to formation of emulsions on extraction.	—
3. Solution in arachis oil	1·027	Nil	1·01	Nil	1·02
4. Solution in sulphonated castor oil ...	5·058	Reflux with nitric acid for control titration	5·09	As for Method A	5·06
5. Solution in aqueous alcohol	0·1013	Add 50 per cent. aqueous solution of potassium hydroxide to give a concentration of 1 per cent. and allow to stand for 15 minutes. No heating necessary.	0·101	Add momoethanolamine to give a concentration of 8 per cent. Reflux 2 hours. Cool, acidify, and complete by General Method.	0·101
6. Self emulsifying concentrate containing castor oil and polyglyceryl ricinoleate in alcohol.	0·2014	Reflux with dilute nitric acid for control titration.	0·202	As for No. 5	0·200
7. Dusting powder containing talc and starch.	0·102	Extract by shaking with a known volume of cold alcohol (95 per cent.). Filter or centrifuge. To an aliquot add sufficient 50 per cent. aqueous potassium hydroxide to give a concentration of 1 per cent. Allow to stand 15 minutes, acidify, and complete.	0·105	Less suitable than potassium hydroxide due to long procedure.	—
8. Ointment with oil in water emulsion base containing sulphonated castor oil, diethylene glycol distearate, and emulsifying wax.	0·111	Reflux with dilute nitric acid for control titration.	0·107	10 per cent. of added momoethanolamine at 100° C. for 2 hours failed to give quantitative recovery.	—
9. Ointment with oil in water emulsion base containing castor oil, cetyl alcohol, diethylene glycol distearate, polyglyceryl ricinoleate and non-ionic emulsifying agent.	0·100	After saponification extract oils, etc. with petroleum ether in presence of a high concentration of alcohol.	0·103	Comments as for No. 8	—
10. Fluid oil in water emulsion containing kerosene and sulphonated castor oil	0·211	Reflux with dilute nitric acid for control titration.	0·214	Comments as for No. 8	—

saponification of oils and fats, where the comparatively large quantities of potash required for saponification may introduce an undesirably large amount of chloride. Otherwise, monoethanolamine has the disadvantage of always requiring heat and agitation unless used in high concentration in the presence of alcohol, which eliminates the necessity for shaking. It also becomes less efficient as a dehydrochlorinating agent in the presence of solvents. It is thus recommended, as far as pharmaceutical preparations are concerned, only for the determination of benzene hexachloride in simple solutions in vegetable oils, particularly when the concentration is very small.

Summary

Methods are suggested for the determination of benzene hexachloride in certain pharmaceutical preparations. These are based on alkaline dehydrochlorination using monoethanolamine or alcoholic potassium hydroxide.

References

1. Van der Linden, *Ber. dtsch. chem. Ges.*, 1912, **45**, 231.
2. Goldenson and Sass, *Anal. Chem.*, 1947, **19**, 320.
3. Howard, *Analyst*, 1947, **72**, 427.
4. Slade, *Chem. and Ind.*, 1945, **64**, 314.

DISCUSSION

DR. C. GARRATT (Nottingham) suggested that the author had obtained a surprising degree of accuracy in the analysis of the difficult mixtures mentioned in Table III. He asked whether the author could give some idea of the degree of variation obtained in successive determinations, and whether the results recorded were typical, or picked from a series which showed the method to advantage.

MR. SEYMOUR (Welwyn) asked whether the author had considered the use of bases other than ethanolamine.

MR. W. H. C. SHAW, replying, said that a certain amount of variation in the analytical results was to be expected. This really depended on the strength of the preparation which was being assayed. With the stronger preparations, the results came in general within $\pm$ 0·5 per cent. of the actual amount of benzene hexachloride used, but for the less concentrated preparations such as a 0·1 per cent. cream or emulsion the accuracy probably fell to about $\pm$ 2 per cent. As to bases other than monoethanolamine, pyridine had been tried by Howard in the work which led him to recommend monoethanolamine.