Determination of Chlorobutanol in Pharmaceuticals by Gas Chromatography

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A gas chromatographic procedure has been developed for the determination of chlorobutanol in a variety of pharmaceutical preparations. Numerous columns were investigated and found useful. Chlorobutanol was separated from its degradation products on all of these columns. Analysis of synthetic mixtures and some commercial preparations containing chlorobutanol indicated that the procedure was applicable to such systems. Peak heights relative to a standard were used to calculate unknown concentrations. If desired, cyclohexanol, camphor, or menthol can be used as the internal standard.

CHLOROBUTANOL U.S.P. is widely used as a preservative and/or an antiseptic and local anesthetic in ophthalmic, otic, nasal, and parenteral preparations. There is considerable interest, therefore, in methods for analyzing these preparations.

Several analytical procedures have been reported for the determination of chlorobutanol. Most of these methods involve the decomposition of chlorobutanol by heating with alkali and subsequent determination of the liberated chloride ion by one of the conventional chloride procedures. The official assay in U.S.P. XVII is based on this principle, utilizing the Volhard method for the determination of the chloride ion. Sinton (1) employed a gravimetric method and Taub and Luckey (2) a turbidimetric procedure. Jensen and Jannke (3) have described a method based on the decomposition of chlorobutanol by a known amount of alkali and determination of the excess alkali. They also reported an assay involving hydrolysis of chlorobutanol to acetone, steam distillation, and iodometric determination of the acetone in the distillate. Vastagh (4) and Ray and Basu (5) distilled chlorobutanol from pharmaceutical formulations and determined chloride ion in the distillate, either argentometrically or iodometrically, following treatment with alkali. All these methods have the inherent disadvantage of not differentiating between free chloride ion present in the formulation or that formed on normal decomposition or alkaline hydrolysis of chlorobutanol. In addition, these procedures are not applicable if other components of the formulation produce halide ions when hydrolyzed with alkali.

Lach *et al.* (6) have described a sensitive amperometric titration for determination of the chloride ion liberated by alkaline hydrolysis of chlorobutanol. For those materials already containing chloride ions from other components in the sample or from normal decomposition of chlorobutanol, they determined the free chloride ion initially and then the total chloride ions after treatment with alkali. The difference represents the chloride contribution by chlorobutanol. This procedure is not directly applicable to samples containing organic compounds which liberate halide ions on treatment with alkali.

Birner (7) has described a procedure for the determination of chlorobutanol by polarography. While this procedure is very sensitive and specific for chlorobutanol, its application to pharmaceutical formulations involves prior separation by steam distillation.

Rehm and Mader (8) reported a procedure based on measurement of the colored ferric hydroxamate complex formed from ferric ion and the reaction product of hydroxylamine and chlorobutanol in alkaline solution. Ketones, aldehydes, esters, imides, acid chlorides, and acid anhydrides interfere since they also form hydroxamates; ferric ion complexing anions such as tartrate, citrate, phosphate, and phthalate also interfere unless a prior distillation step is used (9). Application of the Fujiwara alkali–pyridine reaction for chloroform to the colorimetric determination of chlorobutanol in commercial parenteral dosage forms was recently reported by Chafetz and Mahoney (10). Other polyhalogen compounds interfere as would also chlorobutanol degradation products containing the polyhalo moiety.

From the above discussion it is evident that there is a need for an improved method for the analysis of chlorobutanol preparations. Chlorobutanol is volatile and should lend itself to gas chromatographic analysis, but no such method has been reported in the literature. The purpose of this study was to develop a rapid, specific, gas–liquid chromatographic procedure for the determination of chlorobutanol. Such a method would not be subject to the limitations of the techniques discussed above and would be suitable for evaluating stability samples containing

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chlorobutanol, especially those involving new packaging materials. A number of chromatographic columns were investigated in order that chlorobutanol could be effectively separated from the wide variety of drugs and solvents with which it is commonly used.

EXPERIMENTAL

An F and M model 810 dual column gas chromatograph with a dual flame ionization detector and a Minneapolis Honeywell recorder was used.

Anhydrous chlorobutanol U.S.P. (S. B. Penick and Co., New York, N. Y.) was used without further purification as a standard and in the preparation of synthetic mixtures. The material was found to contain 98.4% chlorobutanol when assayed by the official procedure.

A number of chromatographic columns were investigated. Table I lists the different stationary phases and column conditions that were found satisfactory for chlorobutanol separation. Silanized Chromosorb W, 70–80 mesh (Johns-Manville, New York, N. Y.) packed in 0.25-in. aluminum tubing was used as column support. The gas flow rates were: helium, 60 ml./min.; hydrogen, 60 ml./min.; air, 300 ml./min.

Applicability of gas chromatography to the evaluation of stability samples containing chlorobutanol was investigated. A 0.125% solution was hydrolyzed in 1.0 *M* phosphate buffer, pH 7.9 at 70°, aliquots were withdrawn at periodic intervals, and these were analyzed with the polypropylene glycol column listed in Table I.

Synthetic mixtures containing known amounts of chlorobutanol were prepared with three solvents: water, light liquid petrolatum, and propylene glycol. Thiamine mononitrate and methapyrilene hydrochloride were used as active constituents in aqueous solutions, menthol and camphor in the light liquid petrolatum solutions, and benzocaine in the propylene glycol solutions. These solvents are representative of those usually present in formulations containing chlorobutanol. Compositions of these mixtures are shown in Table II. Diisodecyl phthalate was used as the stationary phase in the analysis of these solutions. Concentrations were calculated from peak heights, using a standard chlorobutanol solution chromatographed concurrently with the sample.

The utility of the gas chromatographic procedure for the analysis of commercial chlorobutanol preparations was also investigated. Various samples of unknown age, obtained from retail pharmacies, were analyzed for chlorobutanol content using the diisodecyl phthalate column.

Table I—Columns Found Satisfactory for Chlorobutanol Separation[a]

Stationary Phase	Column Length, ft.	Column Temp.[b]	Retention Time, min.
Diisodecyl phthalate,[c] 10%	6	140	4.95
Silicone 550,[c] 10%	8	130	3.15
Diethyleneglycol succinate,[c] 20%	5.5	150	3.45
Paraffin grease,[d] 10%	8	150	2.10
1,2,3-Tris(2-cyanoethoxy)-propane,[e] 10%	8	140	4.65
Polypropylene glycol,[f] 10%	6	150	4.20
Polyethylene glycol 4000,[g] 10%	6	160	2.70
Polyethylene glycol 1000,[g] 10%	8	150	5.10

[a] Silanized Chromosorb W, 70–80 mesh packed in 0.25-in. aluminum tubing as support. [b] Injection port and detector were maintained at 225°. [c] Wilkens Instrument and Research, Inc., Walnut Creek, Calif. [d] Available as Apiezon L from Applied Science Laboratories, Inc., State College, Pa. [e] Applied Science Laboratories, Inc., State College, Pa. [f] Marketed as Ucon 50 HB 5100 by Union Carbide Corp., New York, N. Y. [g] Marketed as Carbowax by Union Carbide Corp., New York, N. Y.

Table II—Analysis of Known Solutions of Chlorobutanol

	Solvent	Chlorobutanol, % Added	Found	Recovery
Thiamine mononitrate, 1.0%	Water	0.150	0.148	98.7
		0.300	0.300	100.0
		0.400	0.404	101.0
		0.500	0.497	99.4
Methapyrilene hydrochloride, 2.0%	Water	0.200	0.199	99.5
		0.300	0.299	99.7
		0.400	0.399	99.8
Camphor, 2.5%	Light liquid petrolatum	0.80	0.79	98.8
		1.00	0.99	99.0
Menthol, 1.8%		1.20	1.19	99.2
Benzocaine, 5.0%	Propylene glycol	0.50	0.50	100.0
		1.00	1.00	100.0
		1.50	1.54	102.7

RESULTS AND DISCUSSION

The various stationary phases that were found satisfactory for chlorobutanol separation along with column conditions and respective retention times obtained from a 0.5% aqueous solution of chlorobutanol are listed in Table I. Chlorobutanol was separated from its primary degradation products, chloroform and acetone, on all of these columns. The choice of such a variety of columns increases the applicability of the gas chromatographic procedure to the separation of chlorobutanol from the ingredients commonly used with this preservative. A typical chromatogram of a mixture of chlorobutanol, menthol, camphor, and eucalyptus and cinnamon oils obtained on the diisodecyl phthalate column is shown in Fig. 1. The good resolution of such a complex mixture indicates the versatility of this column in separating volatile constituents that are commonly present with chlorobutanol.

Nair and Lach (11) have shown that the degradation of chlorobutanol in buffered aqueous solution is first order with respect to chlorobutanol concentration. Figure 2, based on gas chromatographic analysis of degraded solutions, demonstrates the pseudo first-order kinetics for chlorobutanol decomposition and suggests the usefulness of this technique in stability evaluation of chlorobutanol formulations. The polypropylene glycol column, as described in Table I, was used in this experiment.

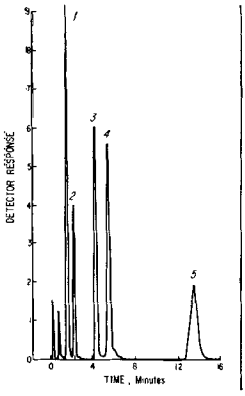

Fig. 1—Chromatogram of a mixture containing chlorobutanol on the diisodecyl phthalate column. Key: 1, eucalyptus oil; 2, chlorobutanol; 3, camphor; 4, menthol; 5, cinnamon oil.

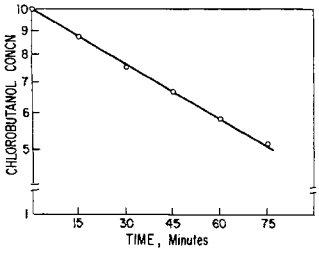

Fig. 2—Plot showing the pseudo first-order degradation of chlorobutanol in buffer pH 7.9 at 70° as determined by gas chromatographic analysis.

Results of the analysis of known mixtures containing chlorobutanol are given in Table II. The diisodecyl phthalate column was used as the stationary phase. The aqueous preparations were injected directly. Reproducibility was poor when the light liquid petrolatum preparations were injected directly but dilution with chloroform prior to injection eliminated this problem and the data presented were obtained in this manner. Tailing of the product solvent peak caused interference in the preparations containing propylene glycol. These were diluted with water and the chlorobutanol extracted with chloroform prior to analysis by comparison with a standard treated in a similar manner. The results indicate quantitative recovery of concentrations ranging from 0.15 to 1.50% in the three solvent systems, and per cent recoveries indicate no significant differences between these solvent systems.

Some commercial preparations containing chlorobutanol were analyzed, and the results are shown in Table III. Depending on the nature of the solvent, these formulations were treated as outlined above. The glycerin-containing product was too viscous to inject directly and was, therefore, analyzed in the same manner as the propylene glycol preparations.

TABLE III—ANALYSIS OF COMMERCIAL PREPARATIONS CONTAINING CHLOROBUTANOL

Prepn.	Solvent	Chlorobutanol, % Label Claim	Chlorobutanol, % Found
A_1	Water	0.15	0.05
A_2	Water	0.15	trace
B	Light liq. pet.	1.0	0.28
C	Water	0.3	0.41
D	Glycerin	3.0	2.98
E	Propylene glycol	0.4	0.39
F	Light liq. pet.	1.0	1.0
G	Water	0.5	0.51

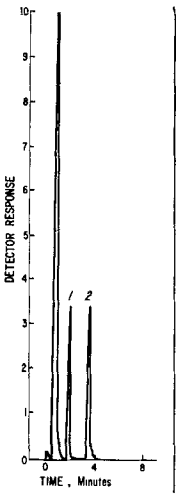

Fig. 3—Chromatogram of a chloroform solution of chlorobutanol and cyclohexanol on the diisodecyl phthalate column. Key: 1, chlorobutanol; 2, cyclohexanol.

Preparation A was an ophthalmic preparation packaged in a plastic container. Two different samples were obtained, and it is obvious that the container did not afford sufficient protection against chlorobutanol loss. Preparation B was a nasal decongestant packaged in a glass bottle with a rubber dropper assembly. The general appearance of the package indicated that this product was of considerable age, and analysis showed that the chlorobutanol content was considerably below the label claim, probably due to loss through the closure. Product C, packaged in a plastic container, was found to contain chlorobutanol in excess of the label claim, presumably the result of the excess needed to maintain the label claim through the product shelf life. Preparations D through G were found to conform to the label claim.

All chlorobutanol determinations reported in this paper were calculated from peak heights by comparison with a standard chlorobutanol solution. A standard was used for each analysis. The relationship between peak height and concentration was found to be linear in the concentration ranges investigated. However, internal standards are very useful and popular, and it appears that several compounds could be used for this purpose. The potentiality of camphor and menthol as internal standards is evident from Fig. 1, and Fig. 3 illustrates the possibility of using cyclohexanol as an internal standard.

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