ELSEVIER

Journal of Chromatography A, 724 (1996) 398–402

JOURNAL OF
CHROMATOGRAPHY A

Short communication

Determination of glutaraldehyde and phenol in germicide products by capillary gas chromatography

James W. Danielson*, Richard D. Thompson

Food and Drug Administration, 240 Hennepin Avenue, Minneapolis, MN 55401, USA

Received 14 August 1995; accepted 18 September 1995

Abstract

A capillary gas chromatographic procedure is described for the analysis of glutaraldehyde and phenol present in germicidal formulations. Glutaraldehyde breaks down over time following activation with alkaline-buffered media and products must be used within certain time limits. Some germicidal products contain either glutaraldehyde or phenol and these are also used in combination. It is necessary to have suitable methodology for determining the concentration of glutaraldehyde and phenol as a means of evaluating the effective levels of these active ingredients. Various analytical methods are described in the literature for the determination of either glutaraldehyde or phenol. Using capillary gas chromatography with a column for compounds of intermediate polarity, both ingredients can be assayed from a single determination.

Keywords: Glutaraldehyde; Phenol; Germicides

1. Introduction

Certain germicide products contain glutaraldehyde, alone or in combination with phenol, as the active ingredient. Glutaraldehyde (1,5-pentanedial) is an amber-colored liquid that is usually stored under acidic conditions in aqueous media. The two aldehyde groups of the molecule readily react with proteins under suitable conditions [1]. The rate of reaction increases considerably under alkaline conditions [2]. Commercial germicides containing glutaraldehyde are generally activated by the addition of a slightly alkaline buffer solution (e.g., pH 7.5–8.5). Glutaraldehyde is effective against all forms of microorganisms including bacterial spores and is widely used for cold sterilization of clinical, surgical and dental instruments. However, the compound degrades over time following the activation process.

Phenol is a more stable germicide under aqueous alkaline conditions. The presence of a free hydroxyl function is responsible in part for the denaturation of protein in cell membranes of bacteria [3,4]. The efficiency of germicides is currently evaluated according to the ratio of dilutions tested in comparison to phenol in the destruction of *Salmonella typhi* [5].

The labeling of glutaraldehyde-containing products usually indicates that the germicide can be used for a given number of days following activation. It is necessary to have suitable methodology for determining the concentration of glutaraldehyde and phenol in products as a means of evaluating the

*Corresponding author.

0021-9673/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
SSDI 0021-9673(95)01035-1

effective levels of these active ingredients. Lyman et al. [6] compared iodine titration, hydroxylamine titration and gas chromatography (GC) using a packed column (7% Carbowax 20 M on Chromosorb HP) for the assay of glutaraldehyde. These authors found that the levels of glutaraldehyde were lower when determined by GC compared with those found by the titrimetric methods. This was attributed to the nonspecific nature of the titration methods that likely include the decomposition products having aldehyde groups. Millership [7] compared a similar GC procedure with ultraviolet spectrometry for the assay of undegraded and degraded glutaraldehyde and observed a similar trend. Colorimetric methods have been developed by Boratyński and Żal [8] for glutaraldehyde using a sulfuric acid–phenol (SAP) or perchloric acid–phenol (PAP) reagent mixture. The SAP assay was found to be more sensitive although the PAP assay can be performed in the presence of sugars and proteins. The U.S. Pharmacopeia [9] includes a method for the assay of glutaraldehyde by titration using hydroxylamine hydrochloride and for the assay of phenol by iodometry and GC employing a packed column. An official colorimetric method for the analysis of phenol in hazardous substances is also described [10].

Currently, there are no reported methods available for the assay of glutaraldehyde and phenol in combination. The purpose of this study was to develop a specific and sensitive procedure for the simultaneous determination of these biocidal agents in commercial products using capillary gas chromatography.

2. Experimental

2.1. Apparatus

The assays were performed with a Hewlett-Packard Model 5790A gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector and a Chromatopak C-R3A integrator (Shimadzu Scientific Instruments, Columbia, MD, USA). The column was an SPB^{TM} 20, 30 m×0.32 mm I.D. with 0.25 μm film thickness (Supelco, Bellefonte, PA, USA). The carrier gas was helium at a flow-rate of 1.1 ml/min and 1.0-μl on-column injections were made. The operating temperatures were: detector, 325°C; oven, initial 32°C (no hold time) with a ramp of 6°C/min up to a final temperature of 175°C.

2.2. Commercial products

Chemical germicides were obtained from manufacturer A with glutaraldehyde 0.3% and phenol 1.0% as the active ingredients stated on the label. The product could be used up to 45 days after activation. Products were also obtained from manufacturer B with glutaraldehyde 2.0%, phenol 7.05%, and sodium phenate 1.2% as the active ingredients. The shelf life of this product was stated as 30 days after activation.

2.3. Chemicals and reagents

Hydrochloric acid and sodium sulfate (anhydrous) were purchased from Mallinckrodt (Paris, KY, USA) and sodium chloride from J.T. Baker (Phillipsburg, NJ, USA). Methylene chloride (Optima-grade) was obtained from Fisher Scientific (Fairlawn, NJ, USA). Glutaraldehyde (50% in water) was obtained from Eastman Kodak Co. (Rochester, NY, USA) and assayed by a titration method (50.3%) provided by the manufacturer using hydroxylamine hydrochloride. Phenol USP crystals was purchased from Mallinckrodt and assayed by iodometric titration (100.2%) [9]. Water was purified with a Nanopure II system having an organic-free cartridge and 0.2-μm filter (Sybron Barnstead, Boston, MA, USA).

2.4. Standard solutions

Individual stock solutions of glutaraldehyde and phenol were prepared in methylene chloride each at a concentration of 0.25 mg/ml. The glutaraldehyde stock solution was passed through 4g anhydrous sodium sulfate into a glass-stoppered Erlenmeyer flask. These stock solutions were stored under refrigeration. Working standard solutions containing both compounds were prepared in methylene chloride at concentrations similar to that present in the diluted product extracts (e.g., 5.0 and 7.5 μg/ml for glutaraldehyde and 20.0 and 25.0 μg/ml for phenol).

2.5. Extraction procedure – determination

The germicide product was activated by addition of the buffer-containing mixture to the aqueous preparation and mixed thoroughly. A 5.0-ml volume of the activated germicide from manufacturer A was transferred to a 50-ml volumetric flask containing 1 ml 1.0 *M* HCl and diluted to volume with water. A 1.0-ml volume of the activated product from manufacturer B was transferred to a 100-ml volumetric flask containing 2 ml 1.0 *M* HCl and diluted to volume with water. A 5.0-ml volume of the acidified solution was transferred to a 125-ml separatory funnel containing 20 ml water and 1 g sodium chloride. The mixture was extracted with eight 25-ml portions of methylene chloride and the organic fractions passed through a glass funnel containing 4 g anhydrous sodium sulfate supported by a pledget of solvent-rinsed glass wool into a 200-ml volumetric flask. The combined extracts were diluted to volume with methylene chloride and mixed thoroughly. A 5.0-ml volume of a solution containing 1 ml 1.0 *M* HCl in 50 ml water was carried through the procedure to serve as an extraction blank.

Duplicate 1.0-μl volumes of the product extract, the appropriate working standard mixture and the extraction blank were injected onto the chromatographic system. Quantitation was achieved by direct comparison of the average peak area responses obtained.

2.6. Recovery experiment

A synthetic formulation containing the active ingredients present in the commercial product obtained from manufacturer B was carried through the proposed procedure.

2.7. Linearity of analyte response

Standard solutions were prepared in methylene chloride from the stock solutions of glutaraldehyde and phenol. Five concentration levels of these solutions were then prepared in the range of 2.5–75.0 μg/ml for glutaraldehyde and 3.5–85.0 μg/ml for phenol. Calibration curves were constructed based on duplicate 1.0-μl injections at each concentration level. Least squares regression was used to determine the linear characteristics [11].

3. Results and discussion

The concentrations of glutaraldehyde and phenol found in the products from both manufacturers are shown in Table 1. The shelf life of the germicide from manufacturer A was stated as 45 days and this product was assayed at 46 days following activation. The concentration of both active components in the two samples examined were within the labeled declaration. The average pH of the two activated

Table 1
Concentrations of active ingredients (% w/v) determined in products at different times after activation

Sample	pH	Glutaraldehyde (%)	Phenol (%)		
Manufacturer A[a]					
1	7.83	0.30	1.3		
2	7.85	0.37	1.0		
		Glutaraldehyde (%)		Total phenol (%)	
		0 days	30 days	0 days	30 days
Manufacturer B[b]					
1	7.22	2.25	1.46	7.56	6.98
2	7.25	2.58	1.84	8.45	8.29
3	7.16	1.90	1.48	8.49	8.49

[a] Product analyzed 46 days after activation. Concentration of active ingredients stated on the label: glutaraldehyde 0.3% and phenol 1.0%. Single determinations.

[b] Product analyzed at 0 and 30 days after activation. Concentration of active ingredients stated on the label: glutaraldehyde 2.0%, phenol 7.05%, and sodium phenate 1.2%. Single determinations.

products was 7.84. The shelf life of the germicide from manufacturer B was 30 days and three different samples were assayed for glutaraldehyde and phenol at 0 and 30 days following activation. The average pH of these products following activation was 7.21. The total phenol concentration for two of the three samples was found to exceed that stated on the label. The total amount for phenol based on the label declaration and including sodium phenate was 8.0%. The deficient sample (sample 1) remained below 8.0% for the 30-day assay even when corrected for the recovery (90.0%). For glutaraldehyde, two of the three samples were found to be below the 2.0% declared concentration at 30 days, one of which was deficient at 0 days. Samples 1 and 3 also remained below the 2.0% level when corrected for recovery (85.0%). The observed loss in potency for glutaraldehyde over time is likely due to degradation via polymerization [12], a problem of concern with medical devices in recent years [13]. The losses due to degradation for the three products ranged from 22.1 to 35.1% over the 30-day time period. Fig. 1 shows typical chromatograms for (a) a working standard mixture, (b) a product extract (manufacturer A) and (c) an extraction blank. The extraction blank and product extracts were observed to be free of any interfering responses.

The recovery values obtained using a synthetic preparation based on the product available from manufacturer B were 85.0% for glutaraldehyde and 90.0% for phenol. Glutaraldehyde was observed to have a linear detector response for over at least a 30-fold range in concentration with a correlation coefficient (r) of 0.99995. The phenol response was linear over a 24-fold range with a correlation coefficient of 1.000. The relative standard deviation values

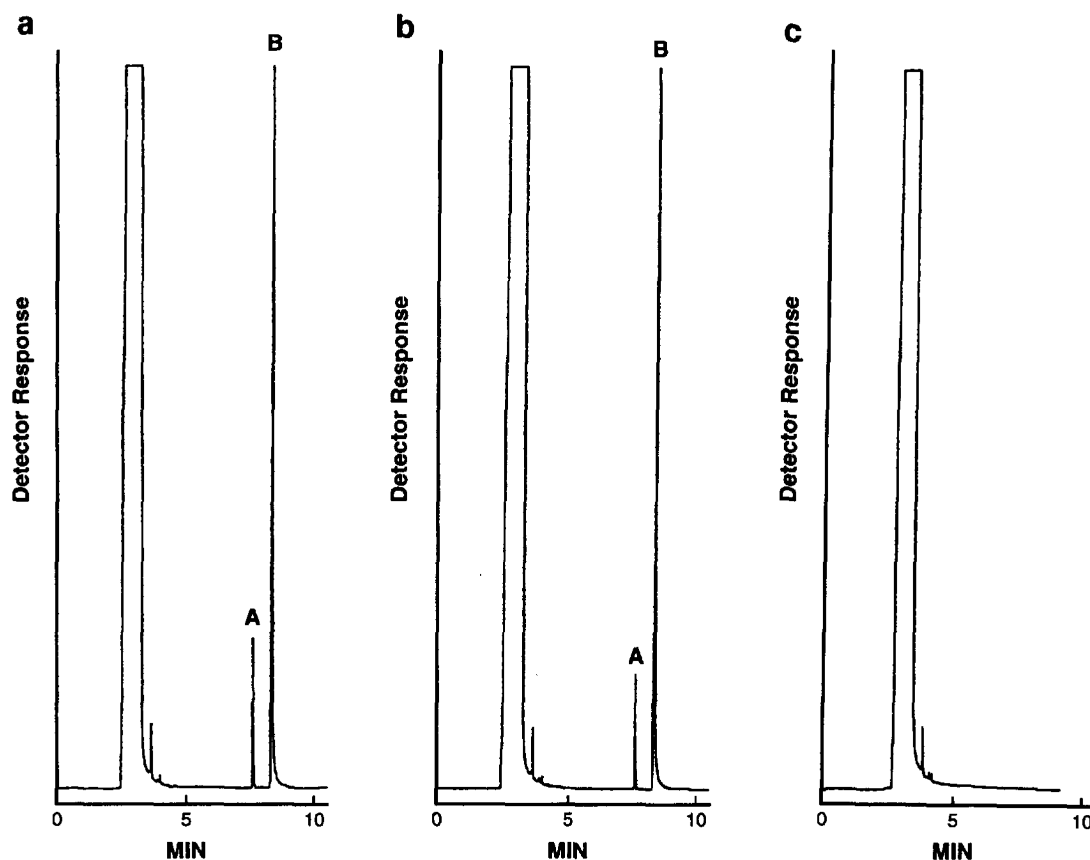

Fig. 1. (a) Chromatogram of working standard mixture containing (A) glutaraldehyde, 7.5 μg/ml and (B) phenol, 25.0 μg/ml. (b) Chromatogram of product extract containing (A) glutaraldehyde and (B) phenol. (c) Chromatogram of extraction blank.

obtained from five replicate 1.0-μl injections of a working standard mixture were 1.2% for glutaraldehyde and 2.2% for phenol.

In conclusion, the proposed capillary GC method provides a specific and efficient means for the assay of glutaraldehyde and phenol when formulated together in germicide products. The method could find utility in monitoring the shelf life of products containing glutaraldehyde, a compound known to undergo degradation in aqueous solution under slightly alkaline conditions. The data show there can be significant losses of monomeric glutaraldehyde, therefore, it is necessary for the manufacturer to provide a suitable excess of this active ingredient.

References

[1] D. Hopwood, C.R. Allen and C. McCabe, Histochem. J., 2 (1970) 137.

[2] E.M. Scott and S.P. Gorman, in S. Block (Editor), Disinfection, Sterilization, and Preservation, Lea & Febiger, Philadelphia, PA, 4th ed., 1991, pp. 596–614.

[3] D.O. O'Connor and J.R. Rubino, in S. Block (Editor), Disinfection, Sterilization, and Preservation, Lea & Febiger, Philadelphia, PA, 4th ed., 1991, pp. 204–224.

[4] S.P. Gorman, E.M. Scott and A.D. Russell, J. Appl. Bacteriol., 48 (1980) 161.

[5] A. Beloian, in P. Cunniff (Editor), Official Methods of Analysis of AOAC International, AOAC International, Arlington, VA, 16th ed., 1995, Ch. 6, pp. 1–3.

[6] G.W. Lyman, R.N. Johnson and B.T. Kho, J. Chromatogr., 156 (1978) 285.

[7] J.S. Millership, J. Clin. Pharm. Therap., 12 (1987) 33.

[8] J. Boratyński and T. Żal, Anal. Biochem., 184 (1990) 259.

[9] U.S. Pharmacopeia 23, United States Pharmacopeial Convention, Inc., Rockville, MD, 1995, p. 710–711, 1734.

[10] M.G. Torchia, in P. Cunniff (Editor), Official Methods of Analysis of AOAC International, AOAC International, Arlington, VA, 16th ed., 1995, Ch. 8, pp. 5–6.

[11] J.C. Miller and J.N. Miller, Statistics for Analytical Chemistry, Ellis Horwood, New York, NY, 1984, pp. 82–118.

[12] P.M. Hardy, A.C. Nichols and H.N. Ryden, J. Chem. Soc. (D), (1969) 565.

[13] T. Tashima, U. Kawakami, M. Harada, M. Imai, N. Satoh, T. Nakagawa and H. Tanaka, Chem. Pharm. Bull., 37 (1989) 377.