Determination of Ethylene Oxide, Ethylene Chlorohydrin, and Ethylene Glycol in Aqueous Solutions and Ethylene Oxide Residues in Associated Plastics

N. A. BALL

Received May 31, 1983 from *Fisons plc, London Road, Holmes Chapel, Crewe, Cheshire, England.* Accepted for publication September 8, 1983.

Abstract □ A gas chromatographic (GC) method was developed for the determination of ethylene oxide and its two reaction products, ethylene chlorohydrin and ethylene glycol, in aqueous ophthalmic solutions. Propylene oxide was used as an internal standard. All three components were determined in one isothermal chromatographic analysis in <15 min. An extraction method for the determination of ethylene oxide residues in plastic components was also developed, and certain plastics with different ethylene oxide retention characteristics were identified.

Keyphrases □ Gas chromatography—determination of three components in one analysis, ethylene oxide residues □ Ethylene oxide residues—GC, determination of three components in one analysis

The Food and Drug Administration published guidelines in 1978 for the levels of ethylene oxide and its hydrolysis products in ophthalmic solutions; ethylene oxide was used to sterilize plastic components. Manufacturers are now required to control the levels of ethylene oxide (I), ethylene chlorohydrin (II), and ethylene glycol (III) in their ophthalmic products for sale in the United States.

Many papers have been published on the analysis of ethylene oxide (1–7), ethylene chlorohydrin, and/or ethylene glycol (8–13). Very few papers deal with the analysis of all three compounds (14–16), and, of these, the analyses either involve two different gas chromatography (GC) columns (14, 15) or two analyses on one column (16).

EXPERIMENTAL SECTION

Apparatus—The gas chromatograph[1] was equipped with a flame-ionization detector and a glass column, 3% Carbowax 20M on Chromosorb 101 (80–100 mesh), 200 cm × 3 mm i.d. The gas chromatograph was linked to a data station[2] which controlled the temperatures and flow rates and processed the peak area data. The column was conditioned at 170°C under a nitrogen flow of 30 mL · min^{-1} for 14–16 h, at which time the temperature was reduced to 150°C, and several 1-μL injections of water were made before analysis commenced. This temperature and flow rate was maintained throughout the analysis. The injector temperature was 300°C and the detector temperature was 250°C.

Compounds I[3], II[3], III[4], and propylene oxide (IV)[3] were used as received.

Extraction Procedure—Approximately 15 g of cut-up plastic was weighed into a 500-mL conical flask; 100 mL of 1 M HCl was added and the flask was stoppered and sealed[5]. The flask was then placed in a water bath for 14–16 h at 80°C. The resultant solution was transferred to a 200-mL flask, neutralized to pH 7.0 ± 1.0 (using 1 M NaOH) and made to volume with water.

Approximately 15 g of plastic was exposed to an atmosphere of ethylene oxide under a pressure of 15 psi for 6 h and then sampled immediately for extraction.

Solution Preparation—Standards I and IV were prepared by accurately weighing ~50 μL of cold liquid (0–5°C) into a 10-mL flask, containing 3 mL of water, using a previously cooled (0–5°C) 100-μL syringe. The solution was then transferred quantitatively to a 100-mL flask and made to volume with water. Standards II and III were prepared in a similar way; however, the cooling stage was not necessary. A working standard was prepared by adding 1 mL of each solution to a 10-mL flask and diluting to volume with water. Standard IV was the internal standard.

The sample was prepared with 1 mL of IV which was added to a 10-mL flask and made to volume with sample solution.

RESULTS AND DISCUSSION

Column conditioning was found to be very important in achieving reproducible retention times. A high injection temperature of 300°C was used to achieve a sharper ethylene glycol peak. The peak shape was improved further by using a "hot needle" injection technique. Using a 5-μL syringe, a small volume of air was drawn into the barrel both before and after the uptake of 1 μL of sample solution. The needle was then placed in the injection port (300°C) for 5 s, the sample injected, left for a further 5 s in the port, and, finally, withdrawn. The ghosting phenomenon encountered by some authors (16) was not manifested in this analysis.

From the standard chromatogram, it can be seen that the I and IV peaks are very close to the solvent, *i.e.*, water peak. By using a peak skimming technique it was possible to achieve accurate and precise values for I content to 1 ppm.

Water flow peaks in GC are more intense with ophthalmic products. None of the ophthalmic products tested using this method gave rise to a sufficiently intense water flow peak as to overwhelm the ethylene oxide peak. The ophthalmic products[6] were tested with and without 2-phenylethanol.

The ethylene glycol peak was broad and, by altering the integration period during the analysis, accurate and precise figures for III content of samples down to 6 ppm were achieved. Below this level, III values become nonlinear. Without the advanced peak processing techniques inherent in the micro-

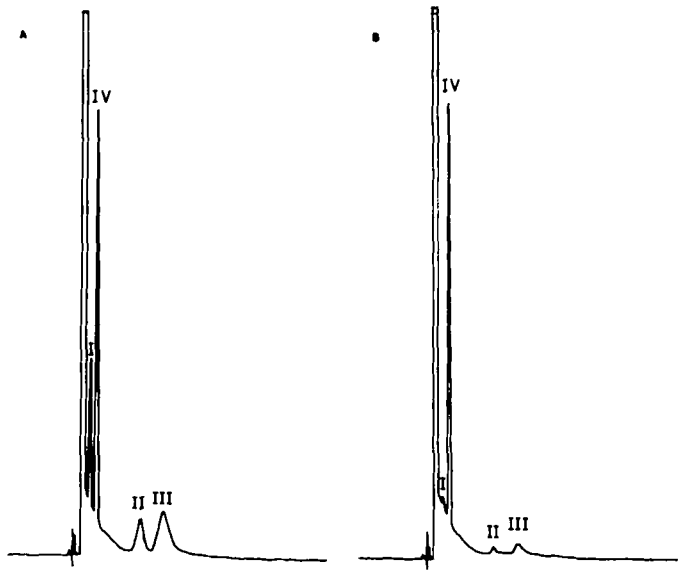

Figure 1—*Typical chromatograms of I–IV. Key: (A) 10 ppm of I, 20 ppm of II, 60 ppm of III, and 15 ppm of IV; (B) 1 ppm of I, 2 ppm of II, 6 ppm of III, and 15 ppm of IV.*

[1] Sigma 1; Perkin-Elmer.
[2] Sigma 10 Data Station; Perkin-Elmer.
[3] BDH Chemicals Ltd., Poole, Dorset.
[4] FSA Ltd., Loughborough, Leicestershire.
[5] Parafilm.
[6] Lomusol and Opticrom; registered trademarks of Fisons plc.

0022-3549/84/0900-1305$01.00/0

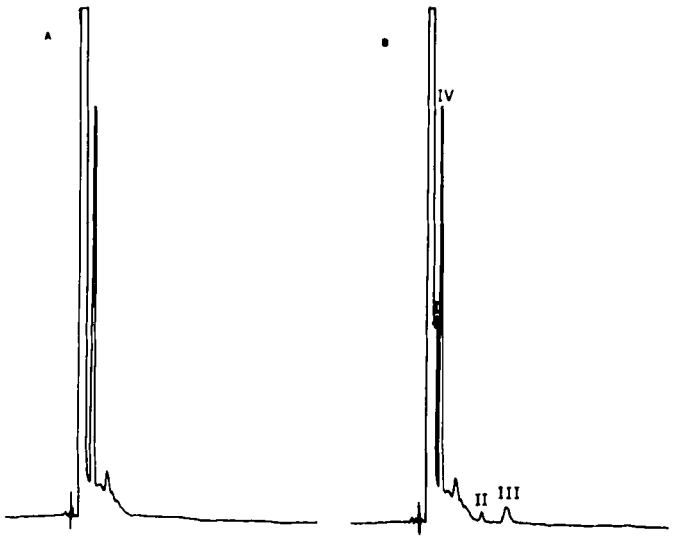

Figure 2—*Chromatograms of an ophthalmic solution (A) and an ophthalmic solution spiked with 2 ppm of I, 4 ppm of II, and 10 ppm of III (B).*

computer software, it would have been impossible to achieve the analysis in one isothermal run.

Coefficients of variation of 3.7, 2.1, and 5.7% for I, II, and III, respectively, were determined at concentrations of 18, 21, and 70 ppm, and coefficients of variation of 1.2, 1.3, and 6.2% were determined at concentrations of 90, 105, and 127 ppm, respectively. The limits of detection were 1, 2, and 6 ppm for I, II, and III, respectively (Fig. 1). Figure 2 shows a chromatogram of an ophthalmic solution and a chromatogram of an ophthalmic solution "spiked" with 2 ppm of I, 4 ppm of II, and 10 ppm of III.

Table I shows recovery data for I–III. For I and III, the recovery was worse at lower levels due to a shouldering of peaks and nonlinearity of response. Recovery values for II are very satisfactory at all levels. The system was calibrated at 10 ppm for I, 20 ppm for II, and 60 ppm for III.

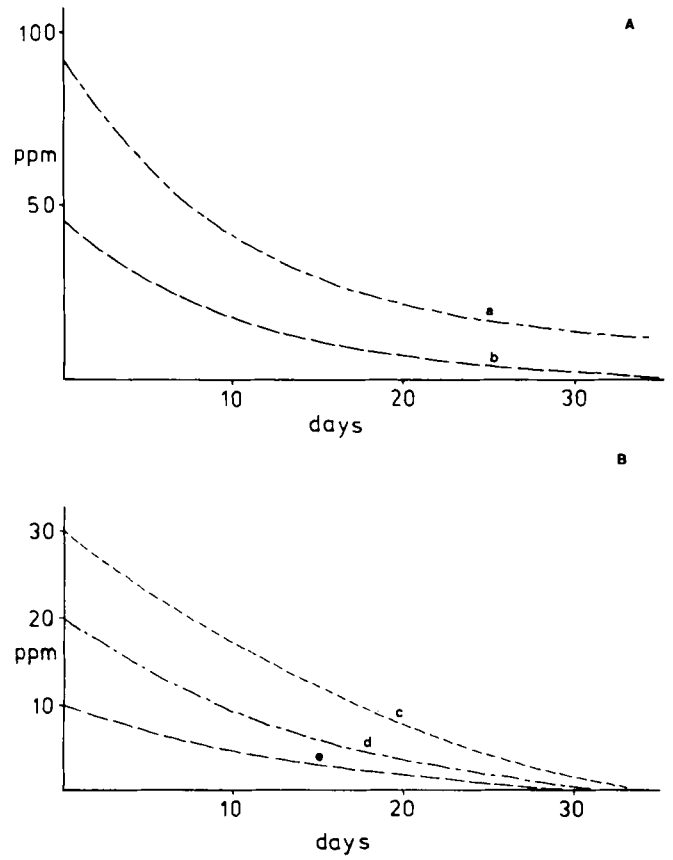

Figure 3—*Ethylene oxide hydrolysis at 25°C. Key: (A) (a) 100 ppm, (b) 50 ppm; (B) (c) 30 ppm, (d) 20 ppm, (e) 10 ppm.*

Table I—Recovery Data for Compound Additions to Control Ophthalmic Solution

Compound	Amount Added, μg/g	Amount Found, μg/g	Amount Recovered, %
I	8	6	75.0
	15	13	86.6
	30	28	93.3
	60	58	96.7
	150	150	100.0
II	13	13	100.0
	26	26	100.0
	52	51	98.1
	104	105	101.0
III	30	28	93.3
	60	59	98.3
	90	91	101.1
	120	122	101.1

This method was also employed to produce ethylene oxide hydrolysis curves in aqueous solution at 25°C (Fig. 3). It can be seen that ethylene oxide hydrolyzes steadily to ethylene glycol in solution, the reaction going to completion in <40 d at 30 ppm. At 80°C ethylene oxide fully hydrolyzes in <16 h.

An acidic medium was chosen for the extraction of ethylene oxide from plastics for two reasons. First, acid extracts I faster from plastic than does an entirely aqueous medium. Using an acid medium at 80°C, consistent ethylene oxide contents for plastics were achieved after 16 h, whereas an aqueous medium at 80°C required 7 d. At 25°C, recoveries of 18% after 16 h and 37% after 7 d were achieved for both media. Second, by choosing hydrochloric acid, the ethylene oxide thus extracted will be converted to II rather than III. Compound II is both easier to quantify and has a lower detection limit. Thus, by combining 1 M HCl with an elevated temperature (80°C), an efficient extraction procedure was developed. The retention characteristics of different plastics were studied in this way.

Solutions equivalent to 10, 20, and 30 ppm of ethylene oxide in 1 M HCl were prepared. After only 12 h at 80°C, ethylene oxide was fully converted to ethylene chlorohydrin. The recoveries ranged from 94 to 98% (Fig. 4).

The retention characteristics of polystyrene, high-density polyethylene, and low-density polyethylene were tested. The plastics had ethylene oxide contents of 430, 280, and 53 μg/g, respectively, after fumigation with ethylene oxide for 6 h at 15 psi. It was found that polystyrene retains a greater amount of I than does high-density polyethylene, which retains more I than does

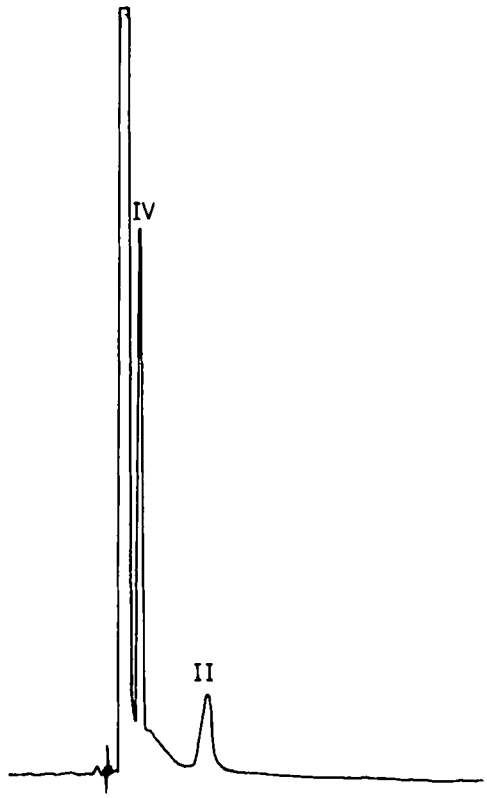

Figure 4—*Chromatogram showing complete conversion of I to II in 1 M HCl.*

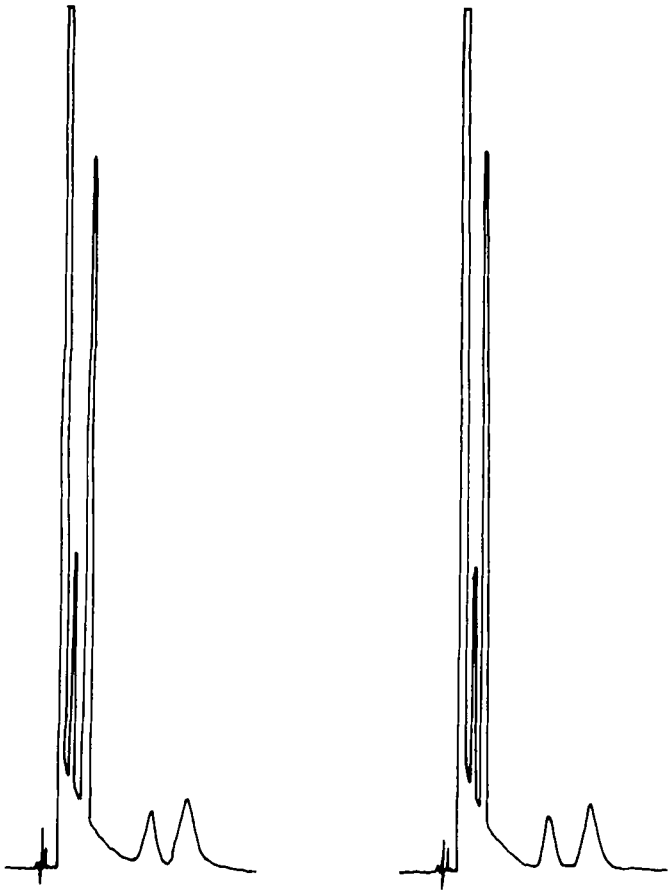

Figure 5 —*Duplication of the chromatographic column.*

low-density polyethylene. The average thickness for the materials were 1.26 mm for polystyrene and high-density polyethylene and 1.00 mm for low-density polyethylene.

When using polystyrene for manufacturing plastic components which are to be sterilized with ethylene oxide, there is the possibility of a "mesh" effect due to the composite nature of the plastic; the presence of bulky phenyl groups gives the plastic many interstitial spaces. Ethylene oxide may enter these spaces within the plastic and remain there, dissipating slowly over a long time period. Obviously, elevated temperature and reduced pressure will increase the rate of dissipation. Likewise, high-density polyethylene plastic components retain more ethylene oxide than low-density polyethylene plastics due to their higher density, where it is less likely that the fumigant will penetrate the plastic.

A most satisfying feature of this method has been the extraordinary longevity of the column. This method of analysis has been used routinely for 18 months with only two replacement columns, giving ~1000 injections per column life. The end of the useful life of a column manifests itself in the broadening of the ethylene glycol peak and a drift in retention times. Duplication of the chromatographic column has been achieved several times with no adverse effects on the separation of the components (Fig. 5). Since this method uses only one isothermal column run for the analysis of all three components and no elaborate sample preparation is necessary, it would appear that this method is ideal for use in quality control laboratories of companies which need to comply with the Food and Drug Administration proposals.

REFERENCES

(1) S. G. Heuser and K. A. Scudamore, *Analyst (London)*, **93**, 252 (1968).

(2) B. Warren, *J. Pharm. Pharmacol.*, **23**, 170S (1971).

(3) J. A. Mogenhan, J. E. Whitbourne, and R. R. Ernst, *J. Pharm. Sci.*, **60**, 222 (1971).

(4) L. A. Zagar, *J. Pharm. Sci.*, **61**, 1801 (1972).

(5) S. J. Romano, J. A. Renner, and P. M. Leitner, *Anal. Chem.*, **45**, 2327 (1973).

(6) J. D. White and T. J. Bradley, *J. Pharm. Sci.*, **62**, 1634 (1973).

(7) S. J. Romano and J. A. Renner, *J. Pharm. Sci.*, **64**, 1412 (1975).

(8) N. Adler, *J. Pharm. Sci.*, **54**, 735 (1965).

(9) E. P. Ragelis, B. S. Fisher, B. A. Klimeck, and C. Johnson, *J. Assoc. Off. Anal. Chem.*, **51**, 709 (1968).

(10) J. E. Whitbourne, J. A. Mogenhan, and R. R. Ernst, *J. Pharm. Sci.*, **58**, 1024 (1969).

(11) D. J. Brown, *J. Assoc. Off. Anal. Chem.*, **53**, 263, (1970).

(12) K. M. Brobst and H. Tai, *J. Assoc. Off. Anal. Chem.*, **54**, 1093 (1971).

(13) J. Weinberger, *J. Pharm. Sci.*, **60**, 545 (1971).

(14) H. D. Spitz and J. Weinberger, *J. Pharm. Sci.*, **60**, 271 (1971).

(15) P. A. Hartman and P. B. Bowman, *J. Pharm. Sci.*, **66**, 789 (1977).

(16) G. J. Manius, *J. Pharm. Sci.*, **68**, 1547 (1979).

Anti-Influenza A Activity of Some *N*-Substituted Bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine Hydrochlorides: Synthesis and Structure

ELENA DE LA CUESTA, PALOMA BALLESTEROS, and GREGORIO G. TRIGO[x]

Received March 9, 1983, from the *Departamento de Quimica Organica y Farmaceutica, Facultad de Farmacia, Universidad Complutense, Madrid-3, Spain.* Accepted for publication July 19, 1983.

Abstract □ Some *N*-substituted bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine hydrochlorides (IX–XII) prepared from bicyclo[3.2.1]octan-3-one (I), were assayed *in vitro* against influenza A viruses. All materials showed activity similar to 1-adamantanamine hydrochloride. A ^{1}H-NMR study revealed only one isomer at the spiro carbon atom.

Keyphrases □ *N*-Substituted bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine hydrochlorides — synthesis, structure, antiviral activity against influenza A □ Antiviral agents—potential, *N*-substituted bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine hydrochlorides, influenza A

Since Davis *et al.* (1) described the antiviral activity of 1-adamantanamine (amantadine) against influenza A viruses, a wide variety of derivatives have been prepared (for review; 2). Among them, some spiropyrrolidine derivatives of adamantane and other cyclic systems (3, 4) have been reported to show amantadine-like antiviral activity.

In this report we describe the synthesis of the *N*-substituted bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine hydrochlorides

0022-3549/84/0900-1307$01.00/0