Determination of Ethylene Oxide, Ethylene Chlorohydrin, and Ethylene Glycol by Gas Chromatography

HARVEY D. SPITZ and JOSEPH WEINBERGER

Abstract □ The technique of gas chromatography was employed for the quantitative determination of ethylene oxide, ethylene chlorohydrin, and ethylene glycol. The simultaneous determination of ethylene chlorohydrin and ethylene glycol in an aqueous solution using a single polyethylene glycol column was accomplished under isothermal conditions, while ethylene oxide was determined employing a styrene–divinylbenzene copolymer column. A key step in obtaining useful and reproducible columns for determining trace quantities of ethylene oxide, ethylene chlorohydrin, and ethylene glycol was found to be related to the aging procedure employed. Experimental data indicate that one can quantitatively recover low levels of ethylene chlorohydrin and ethylene glycol from a water-absorbable fabric. The lower limits of detection of ethylene oxide, ethylene chlorohydrin, and ethylene glycol were found to be in the nanogram range.

Keyphrases □ Ethylene oxide, chlorohydrin, and glycol—determination □ Quantitative recovery from fabric—ethylene oxide, chlorohydrin, and glycol □ GLC—analysis

The increasing demand for ethylene oxide as a sterilizing agent has stimulated a great deal of research on the possible toxicological effects of ethylene chlorohydrin (2-chloroethanol) and ethylene glycol, which are associated side products of this sterilant. Although the literature contains a significant number of reports on the toxicity of ethylene oxide (1–12), ethylene chlorohydrin (13–17), and ethylene glycol (13, 18–24), the large variation in the experimental systems employed and the conclusions reached have prompted further investigations.

Several analytical methods have been reported in the literature for the determination of ethylene oxide (25–33), ethylene glycol (27, 34), and ethylene chlorohydrin (35–39). However, the present analytical requirements with respect to sensitivity, specificity, and time of analysis for the quantitative determination of ethylene oxide, ethylene chlorohydrin, and ethylene glycol in water-absorbable fabrics did not readily adapt to these methods.

Some workers (40) advocated a weighing procedure based on the premise that the loss in weight of a sterilized sample with respect to time represents the amount of ethylene oxide lost. The poor sensitivity one obtains with this approach, coupled with other possible concomitant loss of material (such as a loss due to absorbed gases used in diluting the sterilant or a loss of volatile materials from the sample) or even gain in material (such as the formation of ethylene glycol, diethylene glycol, *etc.*), makes this method less attractive as compared to other techniques.

Since low levels of the three residues may be toxicologically significant, the technique of gas chromatography was chosen over other available analytical methods because of its excellent sensitivity and selectivity.

In this paper, emphasis is placed on the quantitative determination of ethylene oxide, ethylene chlorohydrin, and ethylene glycol in the nanogram range, employing two different gas chromatographic columns. One column is capable of determining trace quantities of ethylene oxide, while the second column is capable of simultaneously determining trace amounts of ethylene chlorohydrin and ethylene glycol.

EXPERIMENTAL

Apparatus—F&M model 5750, equipped with a dual-flame ionization detector, connected to a 1-mv. recorder[1] was used.

Column A—A coiled stainless steel column, 1.83 m. (6 ft.) × 0.32 cm. (0.125 in.) i.d., containing a styrene–divinylbenzene copolymer resin (80–100 mesh, 300–400 m.²/g. surface area)[2] was employed for the analysis of ethylene oxide.

Column B—A coiled glass column, 1.83 m. (6 ft.) × 2 mm. i.d., containing 3% polyethylene glycol[3] coated on a styrene–divinylbenzene copolymer resin (80–100 mesh, less than 50 m.²/g. surface area)[4] was used for the analysis of ethylene chlorohydrin and ethylene glycol.

Preparation of Column Packing—To prepare the packing for Column B, the following method was employed. A 1% solution of polyethylene glycol in chloroform was prepared by dissolving 0.6 g. of the polyethylene glycol in 60 ml. of chloroform. To this solution, 19.4 g. of the styrene–divinylbenzene resin[4] was slowly added with gentle stirring. The mixture was allowed to stand for 10 min. before being transferred to a large watchglass. The packing was spread out to a height no greater than 0.64 cm. (0.25 in.). The watchglass was then placed in a ventilation hood with occasional stirring during this drying procedure until all traces of chloroform were removed from the packing.

Column Packing—Column A was vibrated while being packed under vacuum and then coiled to the necessary configuration of the chromatograph oven. The coiled glass column (Column B) was packed in a similar manner.

Column Conditioning—Column B was initially conditioned in the gas chromatograph overnight at 200° with helium flow. The following day, the column was connected to the detector system and 1-μl. injections of distilled water were made approximately every 15 min. for several hours at a column temperature of 180°. Similarly, Column A was aged overnight at 200°, and several 1-μl. injections of acetone were made at this temperature.

With this aging technique, one is able to analyze all three residues in the nanogram range, employing the maximum sensitivity of the instrument.

Instrumental Parameters—For the analysis of ethylene chlorohydrin and ethylene glycol (Column B), the instrument was operated isothermally at a column temperature of 168°, an injector temperature of 195°, and a detector temperature of 220°. Helium was used as the carrier gas with a flow rate of 30 ml./min., while a flow rate of 300 ml./min. was used for air and 30 ml./min. for hydrogen. For the analysis of ethylene oxide (Column A), the instrument was operated at an injector temperature of 125°, a detector temperature of 220°, and a column temperature of 100° for 9 min.; then the instrument was temperature programmed at 50°/min. up to a maximum of 200° and held for 4 min. at this temperature. Flow rates for helium, hydrogen, and air were approximately the same as used for Column B. A chart speed of 0.51 cm. (0.2 in.)/min. was used for the determination of ethylene oxide and 1.3 cm. (0.5 in.)/min. for the simultaneous determination of ethylene chlorohydrin and ethylene glycol.

[1] Sargent recorder, model No. SRG.
[2] Chromosorb 102, Johns-Manville Products Corp.
[3] Carbowax 20M, Union Carbide Corp.
[4] Chromosorb 101, Johns-Manville Products Corp.

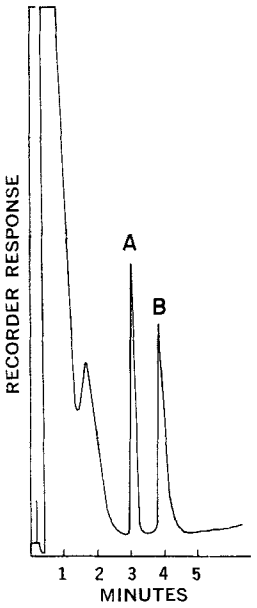

Figure 1—*Typical gas chromatogram of 5.7 ng. of ethylene chlorohydrin (A) and 6.4 ng. of ethylene glycol (B) on Column B. Attenuation range = 1 × 1. Injection volume = 1 μl.*

Recovery Study for the Simultaneous Determination of Ethylene Chlorohydrin and Ethylene Glycol—A water-absorbable fabric was simultaneously spiked with known amounts of ethylene chlorohydrin and ethylene glycol at four different levels of concentration. Approximately 1-g. samples of fabric were placed in 20-ml. serum vials to which 5.0 ml. of different standard solutions was added. These standard solutions were prepared (in water) in such a manner that 5.0 ml. of each standard solution contained ethylene chlorohydrin and ethylene glycol in varying amounts. Sample 1 was simultaneously spiked with 0.022 mg. of ethylene chlorohydrin and 0.099 mg. of ethylene glycol, while succeeding samples were spiked with higher levels of both compounds (Table I). After allowing the samples to stand for 15 min., 5.0 ml. of distilled water was added to each vial. The vials were sealed by crimping an aluminum seal over a rubber septum. The samples were then placed in a mechanical shaker[5] and were shaken for 5 min. Duplicate samples were also prepared in the same manner as previously described. Likewise, corresponding standards were prepared in the same fashion, employing the same standard solutions used to spike the samples except that the vials did not contain any fabric.

One-microliter injections of each of the foregoing solutions were injected into Column B in duplicate, the order of injection being: sample, corresponding standard, and duplicate sample. The peak height method was used to calculate all the experimental data.

A lower concentration range of ethylene chlorohydrin as compared to ethylene glycol was chosen as it is presently considered to be more toxic than ethylene glycol.

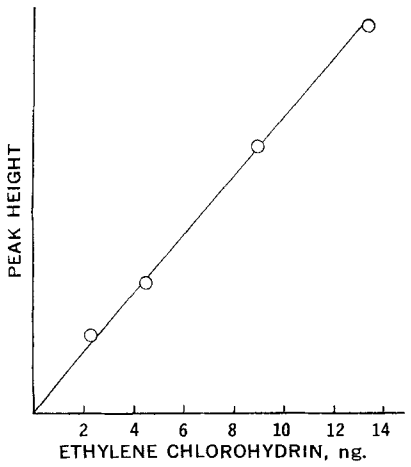

Figure 2—*Plot of the concentration of ethylene chlorohydrin* versus *peak height. Attenuation range = 1 × 1 to 1 × 2. Injection volume = 1 μl.*

[5] Model No. 3800, Pitchford Manufacturing Corp., Pittsburgh, Pa.

Table I—Recovery of Ethylene Chlorohydrin and Ethylene Glycol from Spiked Fabric

Samples	ECH[a] and EG[b] Added to Fabric, mg. ECH[a]	EG[b]	ECH[a] and EG[b] Recovered from Spiked Fabric,[c] mg. ECH[a]	EG[b]
Sample 1	0.022	0.099	0.021	0.112
Duplicate Sample 1	0.022	0.099	0.019	0.099
Sample 2	0.044	0.495	0.046	0.476
Duplicate Sample 2	0.044	0.495	0.047	0.540
Sample 3	0.088	1.48	0.085	1.44
Duplicate Sample 3	0.088	1.48	0.084	1.48
Sample 4	0.133	2.97	0.131	2.93
Duplicate Sample 4	0.133	2.97	0.131	2.98

[a] Ethylene chlorohydrin. [b] Ethylene glycol. [c] Average value of duplicate injections for each sample.

RESULTS AND DISCUSSION

The experimental data shown in Table I indicate that low levels of ethylene chlorohydrin and ethylene glycol can be quantitatively determined in the presence of each other. These results also indicate that no detectable irreversible adsorption takes place on the fabric with either compound.

One should keep in mind that 1 μl. of the final sample and standard solutions, which was injected into the gas chromatograph, contained from 2 to 13 ng. of ethylene chlorohydrin and 10 to 297 ng. of ethylene glycol. To obtain respectable peak heights for the lower concentrations of ethylene chlorohydrin and the lowest concentration of ethylene glycol, the maximum sensitivity of the instrument was required (attenuation 1 × 1). As shown in Fig. 1, the separation of the two compounds is good and baseline noise is negligible. Since the concentration of ethylene glycol was much higher in Samples 2, 3, and 4 (Table I), it was necessary to change the attenuation manually after the elution of the ethylene chlorohydrin peak to accommodate properly the ethylene glycol peak. This very minor inconvenience of manual attenuation can be alleviated by having a recorder or integrator equipped with an automatic attenuator.

This experiment was also designed to obtain linearity curves for both compounds based on the different standard solutions injected into the chromatograph (those used for the recovery study). Since each standard solution contained both compounds, one was able to obtain simultaneously from the data the individual linearity relationships for each compound. Plots of the data are shown in Figs. 2 and 3. Figure 2 covers an attenuation range for ethylene chlorohydrin from 1 × 1 to 1 × 2, while the ethylene glycol plot covers an attenuation range from 1 × 1 to 1 × 32.

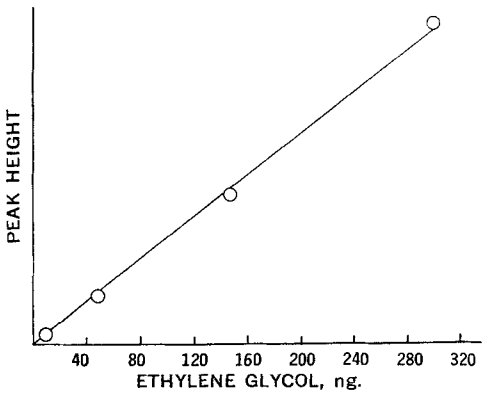

Figure 3—*Plot of the concentration of ethylene glycol* versus *peak height. Attenuation range = 1 × 1 to 1 × 32. Injection volume = 1 μl.*

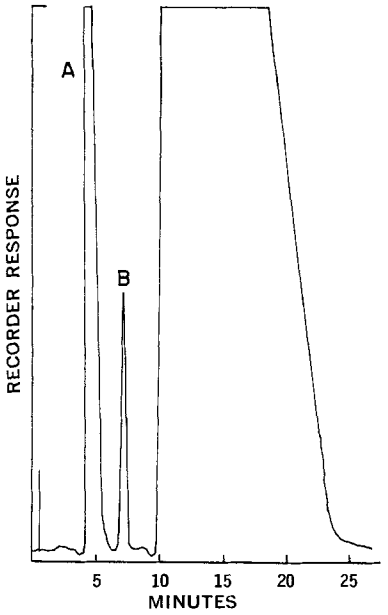

Figure 4—*Typical gas chromatogram of 5.7 ng. of ethylene oxide (B) on Column A. Attenuation range = 1 × 1. Injection volume = 1 μl. (A) is an acetone impurity peak.*

The familiar problem of ghosting (41–43) was observed with ethylene glycol on Column B. Subsequent 1-μl. injections of water after previous injections of the higher concentrations of ethylene glycol produced ethylene glycol peaks that were observable only at the most sensitive attenuations of the instrument. The possible error contributed by this ghosting phenomenon was found to be negligible. Details of this work will be discussed in a future paper.

As in the case of ethylene chlorohydrin and ethylene glycol, a gas chromatographic method was developed for determining trace levels of ethylene oxide. The excellent stability of the styrene–divinylbenzene resin,[2] coupled with its ability to resolve ethylene oxide from a solvent such as acetone (and its impurities), makes Column A highly desirable. A typical chromatogram is shown in Fig. 4. The major acetone impurity, as shown in Fig. 4, was observed in several different brands of acetone only at the most sensitive attenuations of the instrument. In most cases, distillation of the acetone will reduce the concentration of this impurity; however, it is usually separated from the ethylene oxide peak. Temperature programming is required to elute the acetone from the column so that a complete analysis can be accomplished within 25 min. As shown in Fig. 5, a linear relationship is present at low levels of ethylene oxide. The analysis for residual ethylene oxide has been carried out successfully in ethylene oxide sterilized samples, such as fabrics and plastics with acetone or tetrahydrofuran as the solvent. Recent private communications with other workers in the field have informed the authors that the method has performed satisfactorily for the analysis of residual ethylene oxide in their sterilized samples.

The approximate lower limit of detection for ethylene oxide on Column A was found to be 0.7 ng./μl. (representing 7% of the chart paper), employing a 1-μl. injection of acetone. Similarly, the approximate lower limits of detection for ethylene chlorohydrin and ethylene glycol in aqueous solution on Column B were found to be 0.6 ng./μl. and 1.6 ng./μl., respectively (both representing 8% of the chart paper).

Since more than 1 μl. of solution can be injected into the column without any deleterious effects upon the resolution, the limits of detection are further increased for any given sample. Of course, by decreasing the volume of solvent and increasing the sample size and injection volume, the limits of detection can be increased even further.

Recent experiments in this laboratory showed that all three residues can be determined simultaneously on the polyethylene glycol column in the presence of water and/or acetone. The authors hope to present in the near future the optimum conditions and limits of detection for separating and quantitating all three compounds.

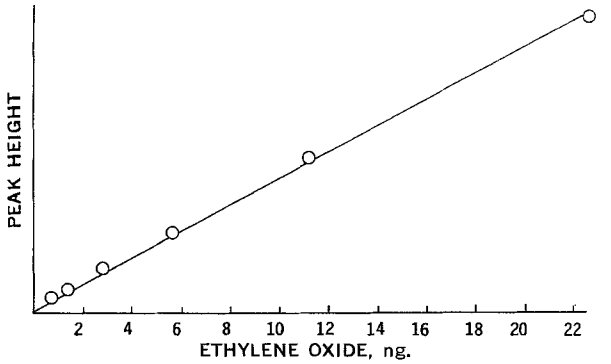

Figure 5—*Plot of the concentration of ethylene oxide* versus *peak height. Attenuation range = 1 × 1. Injection volume = 1 μl.*

Additional work is proposed on separating low molecular weight epoxides and the corresponding glycols and chlorohydrins on the same polyethylene glycol column. Preliminary experiments indicate this is feasible.

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Pharmacodynamics and Biotransformation of Pentaerythritol Tetranitrate in Man

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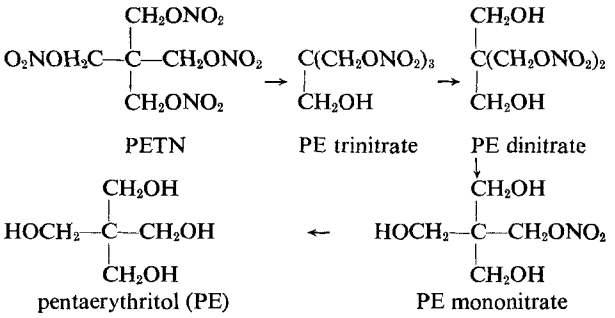
Abstract □ The absorption, biotransformation, and excretion of pentaerythritol tetranitrate was studied after oral administration of two dosages, 20 and 40 mg., to patients. The drug was given as ^{14}C-pentaerythritol tetranitrate incorporated into tablets of a type used clinically. The total ^{14}C excretion in 48 hr. was approximately 92% of both doses. However, a greater proportion of the lower dose was excreted in the urine: 60% of the 20-mg. dose and 50% of the 40-mg. dose. Drug radioactivity was detected in the blood within 15 min., and peak levels occurred from 4 to 8 hr. after administration. The only radioactive compounds found in the blood were pentaerythritol, pentaerythritol mononitrate, and pentaerythritol dinitrate. These drug metabolites were also present in the urine and feces. The kinetics of renal excretion of the principal urinary metabolites, pentaerythritol and pentaerythritol mononitrate, were first order. The renal elimination-rate constant, k_e, of pentaerythritol was independent of the dose, but k_e for pentaerythritol mononitrate was dose related and significantly smaller for the higher dose. The ratio of pentaerythritol mononitrate/pentaerythritol excreted in the urine was approximately 1:1 for the lower dose and 3:1 for the higher dose. The findings indicate a rapid deesterification of pentaerythritol tetranitrate by the human to pentaerythritol mononitrate after oral ingestion, but a limited capacity for the conversion of pentaerythritol mononitrate to pentaerythritol.

Keyphrases □ Pentaerythritol tetranitrate and ^{14}C-substituted—human pharmacodynamics, biotransformation □ Biotransformation, pharmacodynamics—pentaerythritol tetranitrate □ Urinary, fecal excretion—pentaerythritol tetranitrate □ TLC—separation □ Scintillometry—analysis

It has been generally assumed that all organic nitrates exert qualitatively similar actions and that the extended duration of action ascribed to the "long-acting" nitrates relates either to differences of absorption and metabolic stability or to specific properties of the drug molecule itself (1). Since little specific information is available on the pharmacodynamics and biotransformation of this group of drugs in man, a study was performed with pentaerythritol tetranitrate (PETN), a "long-acting" organic nitrate in wide clinical use. Biotransformation of PETN (Scheme I) was followed qualitatively and quantitatively by modifying procedures developed earlier using ^{14}C-labeled drug (2). Drug pharmacodynamics were examined at two dose levels, 20 and 40 mg., with a clinical dosage form prepared from ^{14}C-PETN.



Scheme I

EXPERIMENTAL

Subjects—The subjects were 15 male volunteers between the ages of 30 and 68 years who presented no history or evidence of malabsorption, intestinal motility disturbances, or renal disease. For the period of study (4 days), the subjects were restricted to the Clinical Research Unit at Bowman Gray School of Medicine. A complete medical history and physical examination were taken on each subject. Prestudy laboratory data included the serum levels of electrolytes (Na^+, K^+, Cl^-, and CO_2), urea nitrogen, uric acid, blood sugar, cholesterol, inorganic phosphate, lactate dehydrogenase, total protein, calcium, bilirubin, alkaline phosphatase, and glutamic-oxaloacetate transaminase. Additional laboratory tests performed were EKG, chest X-ray, sedimentation rate, hematocrit, CBC, and urinalysis.

Drug Administration—^{14}C-Labeled and nonradioactive PETN were used to prepare compressed tablets, which contained a total of 20 mg. of PETN and 44 μc. each and met the chemical assay and disintegration-time specifications for the manufacture of a commercial product.[1] After an overnight fast, one tablet was administered *per os* to each of 10 subjects and two tablets were administered similarly to each of five subjects. All subjects remained in the fasting state for an additional 2 hr.

Collection of Specimens—Urine was voided directly into plastic bottles stored in a dry-ice chest. The collection periods were 0–2, 2–4, 4–8, 8–12, 12–24, and 24–48 hr. after drug administration.

Immediately after defecation into a plastic container, each stool collection was covered with cold dioxane and stored in a dry-ice chest. For each subject the feces were pooled from 0–24, 24–48, and 48–72 hr.

Blood specimens (10 ml.) were withdrawn into 15-ml. EDTA-Vacutainers[2] at the following intervals postadministration: 15 and

[1] Peritrate.
[2] Becton, Dickinson Co.