$$\overline{C}_{pnT} = \frac{AUC_{0\to nT}}{T} \quad \text{(Eq. 6)}$$

Therefore, the mean fraction of the steady-state plasma level, f, achieved during the nth dosing interval should be equal to:

$$f = \frac{AUC_{0\to nT}}{AUC_{0\to\infty}} \quad \text{(Eq. 7)}$$

DISCUSSION

Equation 7 indicates that if a time of nT is needed to obtain an f fraction of $AUC_{0\to\infty}$ after a single dose, it also would take that long to obtain the same mean fraction of $\overline{C}_{pss}$ during the nth interval after multiple dosing. Equation 7 also indicates that the time to reach a certain mean fraction of $\overline{C}_{pss}$ is independent of the dosing interval. The terminal biological half-life of a drug *per se* does not affect the time required to reach a certain f value.

The major advantages of this new approach are simplicity, generality, and the elimination of many pharmacokinetic parameters commonly calculated after oral and intravenous administrations. The only information needed is the compartment- and model-independent plasma area data obtained after a single-dose study.

A hypothetical example illustrates the application of this new method. The hypothetical plasma level data after administration of a single oral dose to a subject and the data analyses based on Eq. 7 are summarized in Table I. The plasma level profile is very difficult, if not impossible, to describe by an equation. When the same dose is given every 12 hr, the average plasma levels between 60 and 72 hr and between 108 and 120 hr will be 70.88 and 92.72% of the steady-state plasma level, respectively (Table I). The steady-state plasma level should be equal to 69.37 mg/liter (*i.e.*, 832.4/12). On the other hand, the average plasma levels between 48 and 72 hr and between 96 and 120 hr will still be 70.88 and 92.72% of the steady-state plasma level, respectively, if the dose is given every 24 hr. The steady-state plasma level, however, will be reduced to only 50% of 69.37 mg/liter. This new method can also be applied when there is a lag time in absorption, as shown in the example.

For drugs obeying nonlinear pharmacokinetics, such as phenytoin, a new factor, the apparent volume of distribution, can also affect the times required to reach various fractions of the steady-state plasma level after multiple dosing (11).

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Determination of Ethylene Oxide, Ethylene Chlorohydrin, and Ethylene Glycol Residues in Ophthalmic Solutions at Proposed Concentration Limits

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Abstract □ A GLC method was developed for the determination of ethylene oxide and its two reaction products, ethylene chlorohydrin and ethylene glycol, in ophthalmic solutions at the levels recently proposed by the Food and Drug Administration. The method requires no extractions, sample preparations, or elaborate trapping and concentrating techniques. All three components can be chromatographed on the same spiral glass column packed with a porous polymer adsorbent.

Keyphrases □ Ethylene oxide—analysis, GLC, ophthalmic solutions □ Ethylene chlorohydrin—analysis, GLC, ophthalmic solutions □ Ethylene glycol—analysis, GLC, ophthalmic solutions □ GLC—analysis, ethylene oxide, ethylene chlorohydrin, ethylene glycol, ophthalmic solutions

The Food and Drug Administration (FDA) recently published (1) proposed rules governing maximum residue limits for ethylene oxide (I) and its two reaction products, ethylene chlorohydrin (II) and ethylene glycol (III), in drugs and medical devices. These rules apply to ophthalmic solutions, for which maximum residue levels of 10, 20, and 60 ppm have been established for I, II, and III, respectively. These levels, proposed in conformance with current good manufacturing practices for finished pharmaceuticals, have been necessitated by the known toxicity and/or the mutagen potential of these compounds. Thus, while I is a highly effective sterilant, significant residues of it can be harmful. Its two reaction products, II, produced from ethylene oxide and free chloride ion, and III, an ethylene oxide hydrolysis product, also are harmful in significant amounts.

Ophthalmic solutions that are not treated with I but that contact treated package cap liners must be assayed for residue content according to the proposed specifications. This paper describes a successful attempt to satisfy this objective.

BACKGROUND

Extensive GLC work has been done on ethylene oxide (I) singly and in combination with its reaction products in various items including foods, fabrics, pharmaceuticals, medical and surgical devices, and plastics

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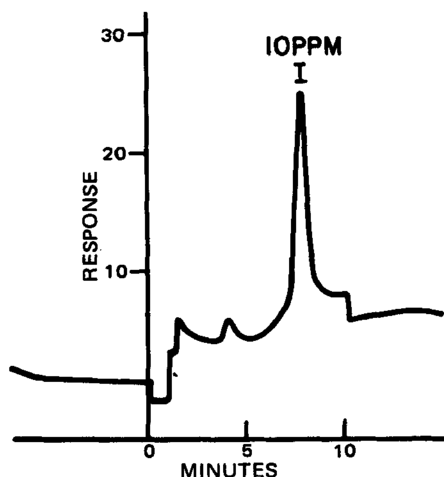

Figure 1—*Chromatogram of I reference standard at the 10-ppm level.*

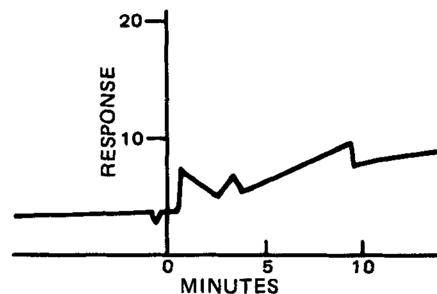

Figure 3—*Typical sample chromatogram in the analysis for I, showing none detected.*

(2–15). None of these studies applied to ophthalmic solutions specifically. Nearly all involved some type of extraction, recovery, and concentration such as solvent extractions (2, 6, 8), headspace sampling (4, 8, 12), co-sweep extractions (5, 6, 9), vacuum extractions and distillations, steam distillations (3, 7–11, 14), and chemical reactions (13).

Many of these techniques required a complex arrangement involving: (*a*) a heating system; (*b*) a combination of delivery systems such as solvent, gas, and vacuum; (*c*) a trapping device such as a coil or flask immersed in liquid nitrogen; and (*d*) a sample concentrator to improve the relative amounts of collected sample. A recent review article (16) discussed these procedures in detail. Needless to say, direct sample introduction is a distinct advantage, and ophthalmic solutions can be treated in this manner as if they were completely aqueous solutions, as in the investigation of Hartman and Bowman (15).

Additionally, a single chromatographic column was sought that could withstand the enormous deposits of sample components due to numerous injections without significantly changing and that could be used to analyze all three residues. Such a column was found and is suggested as a substitute for the two-column approach.

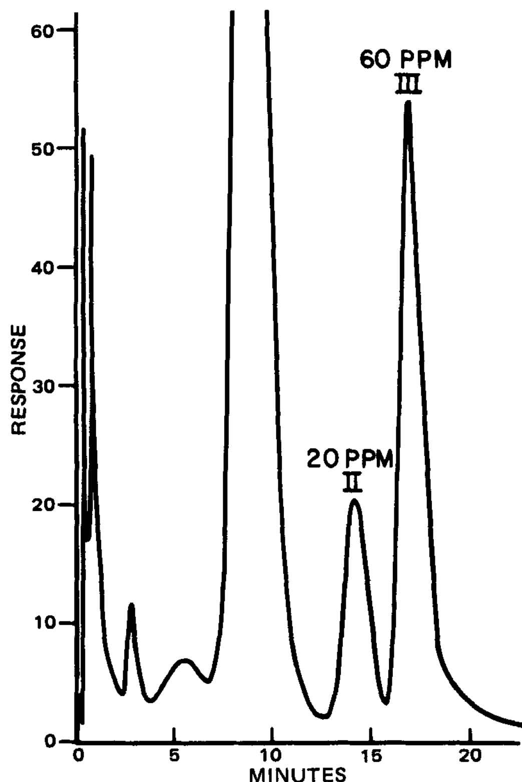

Figure 2—*Chromatogram of II and III reference standard at the 20- and 60-ppm levels, respectively.*

EXPERIMENTAL

Instrument—The gas chromatograph[1] was equipped with a flame-ionization detector and a coiled glass column, 180 cm × 2 mm i.d., which was fitted for on-column injections. The column was packed with a porous polymer[2] (60–80 mesh). The column was conditioned at 200° under a nitrogen flow of 30 ml/min for 24 hr. For the analysis, this flow rate was maintained.

The column oven temperature was held isothermally at 100° for ethylene chlorohydrin (II) and ethylene glycol (III) determinations and at 50° for ethylene oxide (I) determinations. Injector temperatures were 150° for II and III and 100° for I. The detector was set at 200° and had gas flow rates of 30 ml/min for hydrogen and 300 ml/min for air. The instrument sensitivity range was 10^{-11}, and attenuation was 4× for II and III and 2× for I determinations.

Reagents—Compounds I–III, obtained from the same source[3], were used as received.

Standard Preparations—The I standard was prepared by pipetting 1 ml of cold I liquid (0–5°) into a 100-ml volumetric flask and diluting to volume with distilled water. This solution was diluted 100-fold to give

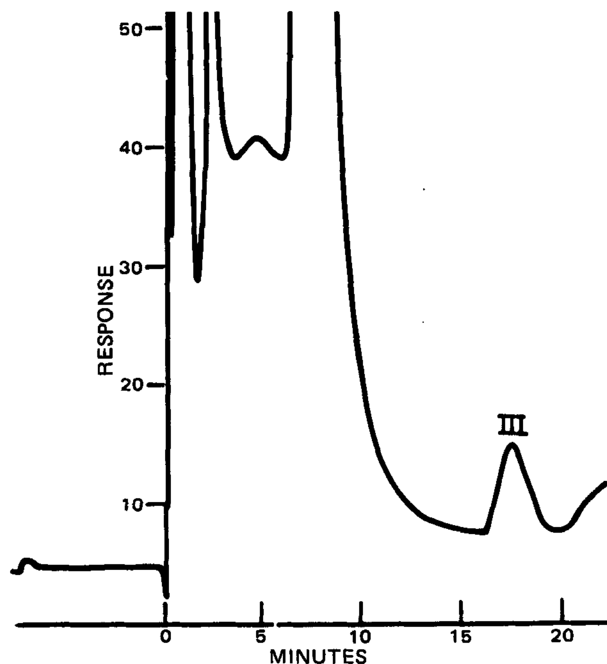

Figure 4—*Typical sample chromatogram in the analyses of II and III, showing some III.*

[1] Model 3700, Varian Instruments, Palo Alto, CA 94303.
[2] Tenax-GC, Applied Science Laboratories, State College, PA 16801.
[3] Eastman Organic Chemicals, Rochester, NY 14650.

Table I—Residue Levels in Ophthalmic Solutions

B	61	Cork and vinyl disk	0	0	26
C	59	Cork and vinyl disk	0	0	28
D	56	Cork and vinyl disk	0	2	0
E	54	Cork and vinyl disk	0	2	0
F	51	Cork and vinyl disk	0	4	23
G	18	Cork and vinyl disk	0	2	10
H	16	Cork and vinyl disk	0	2	0
I	15	Cork and vinyl disk	0	0	0
J	14	Cork and vinyl disk	0	0	0
K	11	Cork and vinyl disk	0	0	0
L	10	Polyethylene cone	0	0	0
M	8	Cork and vinyl disk	0	0	0
N	7	Cork and vinyl disk	0	0	0
O	6	Polyethylene cone	0	0	0
P	4	Polyethylene cone	0	0	0

a 100-ppm standard, which was finally diluted 10-fold to give the 10-ppm standard.

The combined II (20 ppm) and III (60 ppm) standard was prepared by transferring 20 μl of II and 60 μl of III to a 100-ml volumetric flask, diluting to volume with distilled water, and finally diluting 10-fold.

Sample Preparations—Ophthalmic solution in 15-ml bottles was hand shaken vigorously and allowed to stand in an inverted position for 10 days[4]. Prior to sampling, the bottle was reshaken. Ten-microliter aliquots were injected directly into the chromatograph.

Calculations—Quantitation was achieved by peak height measurements. Sample chromatograms were compared directly to the two standard chromatograms, showing peaks corresponding to 10, 20, and 60 ppm for I, II, and III, respectively[5].

RESULTS AND DISCUSSION

Chromatograms of the reference standards are shown in Figs. 1 and 2. Sample chromatograms are presented in Figs. 3 and 4. Elution of II was at 14 min, III eluted at 17 min, and I eluted at 7.5 min at its column temperature setting. Chromatographing all three components within the same chromatogram was impractical due to the large volatility differences between I and its two reaction products. Temperature programming resulted in extensive baseline climbing and increased ghosting distortions.

The ghosting phenomenon was the most troublesome problem encountered. Each successive injection of sample resulted in a continual buildup of peaks in both the III and I elution regions, although II remained unaffected. The first sample injection of the day barely showed these interferences, but they grew in intensity from injection to injection. The interferences became too great for meaningful interpretation of the later sample runs. By the following morning, the column (and septum) had corrected itself and sample runs could be made until the problem reoccurred.

Increasing oven temperatures for a considerable length of time to sweep away the interferences did not succeed. Fortunately, the problem was solved absolutely and completely merely by reducing injection port temperatures from an initial setting of 200 to 100° for I and to 150° for II and III. Although later eluters appeared (an additional 10-min wait is, therefore, recommended after the I region before injection of the next sample), interferences were eliminated. Other early eluting peaks (Fig. 2, excluding the desorption peak at 9 min) can be attributed to the water anomaly; although the hydrogen flame theoretically is insensitive to water, the disturbances caused by water do produce peaks (15). These extraneous peaks caused no problem in the assay.

In addition to the sample preparation described, another procedure was carried out. The cap liner, whether of vinyl with cork backing or of polyethylene, was removed, cut up, and immersed in the ophthalmic solution. The capped bottles were allowed to stand for up to 1 week, whereupon they were again sampled for residue content. No differences were observed between the chromatograms of these runs and those obtained without liner immersion (Table I).

None of the samples showed I, an indication that it readily converts to its two products. The I reference standard at the 10-ppm level did not change appreciably over a month. The most likely conversion was to III, as shown by the test data. The presence of II is mostly speculative. Some samples gave faint indications of it (miniscule blips in the II elution region), but none showed anything approaching the peak in the 20-ppm standard.

Linearity studies showed the response of the three components to be proportional to concentration within the range of 0.1–10 times the levels established as concentration limits (10, 20, and 60 ppm). Each compound could be detected at one-tenth of those levels (1, 2, and 6 ppm) at the same sensitivity settings used for the limits.

Recovery studies showed that when the three components were added at the 10-, 20-, and 60-ppm levels to a control sample of ophthalmic solution, they produced chromatograms with peak responses corresponding to those levels (Table II).

Table II—Recovery Data for Compound Additions to Control Ophthalmic Solution at Their Concentration Limits

I	8.9	8.5	96.0
II	23.9	23.6	98.7
III	66.8	76.4	114.4

A most satisfying feature of this method has been the extraordinary longevity of the column. With between 75 and 100 sample injections already made, the column continues to function well and shows changes neither in retention time nor in sensitivity[6]. Since <2 g of packing is used per column and since no liquid phase must be coated on, the separation system described here is attractive. When one adds to that system the one-column operation and the fact that sample preparation is not necessary, it appears that this method should meet the new FDA requirements.

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[4] This time duration and bottle inversion were for investigational purposes only. Test results show that sampling and injection can be performed directly after shaking.

[5] Volumes were converted to weights by using the density of I at 0° and of II and III at 20°.

[6] It would be beneficial, however, for the analyst to remove the inch or two of blackened residue from the inlet end of the column and to repack with fresh Tenax packing. This step might be done after a large number of samples (~50) has been run. Revitalizing the column in this manner should take a relatively short time.