Ghosting of Ethylene Glycol in GC

Keyphrases □ Ethylene glycol—GC analysis, reversible adsorption on column, ghosting □ Ghosting of ethylene glycol—GC analysis, reversible adsorption on column □ GC—analysis, ethylene glycol, reversible adsorption on column, ghosting

Sir:

The phenomenon of ghosting (1–5), also known as the repeater or memory effect, is now becoming recognized as a potential source of error in the qualitative and quantitative GC analyses of various types of polar solutes.

Ghosting may be considered as a desorption process whereby a small amount of the solute, which was previously injected onto the GC column, is removed from the column by subsequent injections of a solvent that does not contain the solute in question.

Spitz and Weinberger (6) observed ghosting of ethylene glycol on a column containing 3% polyethylene glycol coated on a styrene–divinylbenzene copolymer resin. The purpose of this paper is to introduce new experimental data employing their procedure and column to provide some insight into the mechanism of the reversible adsorption process and at the same time alert the reader to possible sources of error which could occur during the GC analysis of ethylene glycol.

The experimental data were obtained from 1-μl. injections of several different concentrations of an aqueous glycol solution, the order of injection being the most dilute to the most concentrated. After each sample injection, there was at least one injection of 1 μl. of water to see if any ghosting would occur at that particular level of ethylene glycol previously injected. This 1-μl. water injection procedure was carried out until there was no evidence of ethylene glycol appearing as a ghost peak.

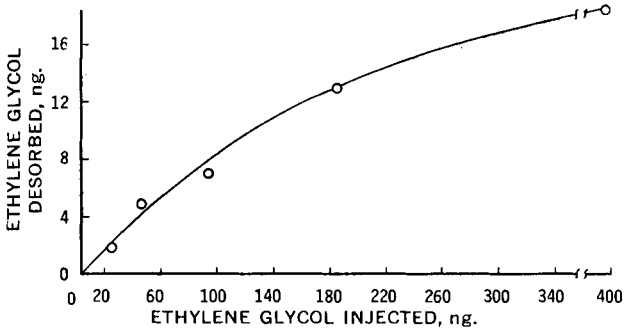

Figure 1—*Desorption curve of ethylene glycol based on the data from Table I.*

The data, as listed in Table I, reveal that the higher concentrations of ethylene glycol show this ghosting effect even after two 1-μl. injections of water. The approximate concentrations of ethylene glycol that were desorbed were calculated, using the two lowest concentrations of ethylene glycol as reference standards. This mathematical approach seems reasonable because these two concentration levels exhibit a linear response within the concentration range studied and they do not indicate any ghosting.

The sum of the desorbed ethylene glycol at each concentration level originally injected was plotted *versus* the original amount of solute injected at that level. These data are graphically presented in Fig. 1.

The ghost peak has the same characteristics as an ethylene glycol standard, namely, the same peak shape

Table I—Ghosting of Ethylene Glycol

		Number of Water Injections (1 μl.)							
		No. 1		No. 2		No. 3		No. 4	
Ethylene Glycol Injected, ng.	Peak Height[a], att.[b]	Peak Height[a], att.[b]	Ethylene Glycol Found, ng.	Peak Height[a], att.[b]	Ethylene Glycol Found, ng.	Peak Height[a], att.[b]	Ethylene Glycol Found, ng.	Peak Height[a], att.[b]	Sum of Desorbed Ethylene Glycol, ng.
2.5	1.9 (1 × 2)	0 (1 × 2)	0	—	—	—	—	—	—
12.4	10.0 (1 × 2)	0 (1 × 2)	0	0 (1 × 2)	0	—	—	—	—
24.7	17.9 (1 × 2)	1.4 (1 × 2)	1.9	0 (1 × 2)	0	—	—	—	1.9
49.5	18.8 (1 × 4)	4.0 (1 × 2)	5.5	0 (1 × 2)	0	—	—	—	5.5
99.0	23.1 (1 × 8)	6.1 (1 × 2)	7.1	0 (1 × 2)	0	—	—	—	7.1
198.0	12.2 (1 × 32)	8.1 (1 × 2)	9.5	2.1 (1 × 2)	2.9	0.4 (1 × 2)	0.5	0	12.9
396.0	14.3 (1 × 64)	10.8 (1 × 2)	12.4	3.4 (1 × 2)	4.8	1.0 (1 × 2)	1.3	0	18.5

[a] Peak height measured in centimeters. [b] Refers to attenuation range used on the gas chromatograph.

and retention time. Evidently, the adsorption takes place at the beginning of the column in order to meet the criteria of obtaining the same peak shape and retention time as the standard glycol solution.

We found that as long as an ethylene glycol standard is employed in the same concentration range as is found in the sample, the results are quantitative and no significant error is obtained even upon duplicate injections. Again, it is important to keep in mind that this phenomenon does exist, and it should be considered in the quantitative as well as the qualitative analysis of ethylene glycol.

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Use of Confidence Intervals in Analysis of Comparative Bioavailability Trials

Keyphrases ☐ Bioavailability trials, comparative—analysis, use of confidence intervals ☐ Confidence intervals—analysis of comparative bioavailability trials ☐ Drug formulations, comparison—use of confidence intervals in analysis of comparative bioavailability trials ☐ Clinical equivalence of drug formulations—use of confidence intervals in analysis of comparative bioavailability trials

Sir:

The current emphasis on the comparative bioavailability trial, in which a new formulation of a drug is compared against a standard formulation in human subjects, is attracting considerable attention. Today such a trial is a key element in the submission of an abbreviated New Drug Application, in which the object is to show that the *in vivo* characteristics of the new formulation are essentially identical to those of an already approved, standard formulation. Typically, a crossover trial is employed, and blood levels of the drug at various times after administration and/or amounts of drug excreted in the urine are determined. Results are often analyzed using the classical statistical theory of hypothesis testing, in which the null hypothesis of no difference between the formulations is tested with respect to some characteristics of the blood or urine levels of the drug. Presumably, if the difference between the formulations turns out to be significant at the 0.05 or 0.01 level, one rejects the null hypothesis and concludes that the new formulation does not match the standard.

The purposes of this communication are to suggest that classical hypothesis testing techniques may not be particularly relevant to this problem and to propose, as an alternative, an approach based on confidence intervals. To bring the problem into focus, it is assumed that the new and standard formulations are to be compared with respect to total amount of drug excreted in the urine for some specified period following administration. If the trial is then run under tightly controlled conditions (resulting in small error variance in the analysis) with a large number of subjects, it could happen that the formulation would give a urinary recovery only 1% different from the standard but that this difference would still be significant statistically. It should be kept in mind that however small the difference between the formulations, it will be detected as significant if the trial is sufficiently well controlled and the number of subjects employed is large enough. In such a situation, the reviewing clinical pharmacologist or physician might well feel that a 1% difference in absorption is of no clinical significance, but he might also be concerned that this difference is termed significant in the statistical sense. The dilemma is occasioned by use of an inappropriate tool—hypothesis testing. What he may need is not a test of whether the two formulations are identical but rather some degree of assurance that the mean amount of drug excreted using the new formulation is sufficiently close to the amount excreted using the standard. The usual hypothesis testing approach does not give this assurance; nevertheless, the analysis can easily be modified to provide it in a manner that will be meaningful and that can provide the basis for a rational decision by the clinical pharmacologist. The proposed approach is based on the use of confidence intervals and is described here.

Suppose, to fix ideas, that the total urinary excretion of the drug (or the mean drug blood level over a number of sampling times) is to be analyzed in a crossover trial in 12 subjects, with both formulations—new and standard—administered to each subject. With the usual analysis of variance, which is based on the presence of additive effects due to subject (11 degrees of freedom), day of administration (1 degree of freedom), and formulation (1 degree of freedom), one can easily verify that the error mean square, s^2, is based on the remaining 10 degrees of freedom. If μ_s and μ_n are the true population means of the mean total urinary excretion of drug for the standard and new formulations, respectively, and $\bar{x}_s$ and $\bar{x}_n$ are the corresponding sample means obtained from the trial, then with the usual normality assumptions, $\{(\bar{x}_s - \bar{x}_n) - (\mu_s - \mu_n)\}/(s/\sqrt{6})$ has the t-distribution with 10 degrees of freedom. Two constants, k_1 and k_2, can be chosen so that the integral of the t-distribution from k_2 to k_1 is 0.95. Then with 95% probability, the inequality:

$$k_2 s/\sqrt{6} \leq \{(\bar{x}_s - \bar{x}_n) - (\mu_s - \mu_n)\} \leq k_1 s/\sqrt{6} \quad \text{(Eq. 1)}$$

holds. This inequality can be rearranged to give:

$$\{\mu_s + k_2 s/\sqrt{6} - (\bar{x}_s - \bar{x}_n)\} \leq \mu_n \leq \{\mu_s + k_1 s/\sqrt{6} - (\bar{x}_s - \bar{x}_n)\} \quad \text{(Eq. 2)}$$