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Note

Relative precision in the determination of some structurally-similar O-pentafluorophenylsulfonylphenols by ultratrace gas chromatography

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While limitations in their performance have been pointed out[1–5], internal standards are commonly used in analysis by gas chromatography (GC) to enhance both the accuracy and precision. A common recommendation for best results is that the internal standard should be similar to the analyte in both molecular structure and chromatographic retention[6]. While the meaning of these aspects is fairly clear, little work has been done on the finer details that may be involved.

The structural similarities between an analyte and its internal standard may become more critical at ultratrace (*e.g.* picogram–femtogram) analyte levels. This is because of the potential for highly specific solute losses onto surfaces at such levels[7]. In order to achieve a high degree of confidence and precision in ultratrace analysis by GC, it may therefore be important to optimize fully the structure of the internal standard.

Since polar groups tend to play a more critical role than non-polar groups in GC solute behavior, it is appropriate to match especially the polar features between an analyte and its internal standard. This leads to the reasonable hypothesis that the structural variation between an analyte and internal standard in ultratrace GC should consist of a non-polar difference remote from any polar groups, where the remoteness refers to both through-bond and through-space effects. In this way, the properties of polar groups toward surface losses will be minimally perturbed by the non-polar variation.

EXPERIMENTAL

Phenol (>99%), 4-methylphenol (>99%), 4-ethylphenol (97%), 2,4,6-trimethylphenol (99%) and pentafluorophenylsulfonyl chloride (99%) were purchased from Aldrich (Milwaukee, WI, U.S.A.). 4-Methylmorpholine (sequanal grade) was obtained from Pierce (Rockford, IL, U.S.A.). Acetonitrile (HPLC grade) was pur-

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chased from Fisher Scientific (Medford, MA, U.S.A.). Toluene, distilled in glass, was acquired from Burdick & Jackson Labs. (Muskegan, MI, U.S.A.). Chloroform (reagent grade), cyclohexane (HPLC grade), hydrochloric acid (reagent grade) and sodium bicarbonate (reagent grade) were purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). Silica gel 60, 230–400 mesh (E. Merck) was obtained from VWR Scientific (Boston, MA, U.S.A.). GHLF silica gel Uniplates with fluorescence indicator for thin-layer chromatography (TLC) were purchased from Analtech (Newark, DE, U.S.A.).

Preparative scale high-performance liquid chromatographic (HPLC) separations were done on a 25 cm × 10 mm I.D. column packed with Davisil C_8 (bonded and packed in house) with a mobile phase of 70% (v/v) acetontrile in water. Analytical separations were performed on a 15 cm × 4.5 mm I.D. Supelcosil LC-8 column (Supelco, Bellefonte, PA, U.S.A.) with an acetonitrile (50–90%, v/v) gradient in water. The water was deionized, distilled, filtered (0.6-μm polyvinylchloride membrane, Millipore, Bedford, MA, U.S.A.) and degassed under vacuum; the acetonitrile was filtered (0.5-μm fluoropore, Millipore) and degassed under vacuum.

A Varian Model 3700 gas chromatograph, equipped with a Varian 1095 on-column capillary injector and an experimental, 350 μl Varian ^{63}Ni electron capture detector[8] was employed. A 10 m × 0.25 mm I.D. DB5 fused-silica-capillary column (J&W Scientific, Rancho Cordova, CA, U.S.A.) was used. Ultra high purity helium and nitrogen (Matheson, Gloucester, MA, U.S.A.) were used as carrier and make-up gases at flow-rates of 5 ml min^{-1} and 25 ml min^{-1}, respectively, measured at room temperature and uncorrected. Injections of the solutes in toluene were made with a 5-μl on-column syringe (Scientific Glass, Austin, TX, U.S.A.). Chromatograms were recorded and peak areas integrated with a Spectra-Physics SP 4270 integrator. When the chromatographic run was initiated, the injector temperature was programmed from 30 to 150°C at a setting of 180°C min^{-1}; the column oven temperature was held at 70°C for 1 min and then programmed to 150°C at a setting of 50°C min^{-1}. The detector temperature was 340°C.

Phenol, 4-methylphenol, 4-ethylphenol and 2,4,6-trimethylphenol were derivatized with pentafluorophenylsulfonyl chloride according to the following procedure. An amount of 20 mg of the phenol was dissolved in 2 ml of acetonitrile. Approximately 3 equiv. each of 4-methylmorpholine (63 μl) and pentafluorophenylsulfonyl chloride (80 μl) were added. The reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated on a rotary evaporator. In the cases of phenol, 4-methylphenol and 2,4,6-trimethylphenol, the oily residue was dissolved in 3 ml of chloroform, washed three times with 0.01 *M* hydrochloric acid, three times with 5% sodium bicarbonate and three times with distilled water. The chloroform phase was reduced to 0.5 ml and applied to an 18 cm × 1.5 cm I.D. pre-conditioned silica gel column. In the case of 4-ethylphenol the liquid–liquid extraction was not used. The volume of the reaction mixture was reduced to 0.5 ml under vacuum, 1 g of silica gel was added, and the resulting mixture was concentrated on a rotary evaporator. The silica gel was then applied to the top of the above preconditioned silica gel column. Hexane was used as the elution solvent in all cases. The fractions containing product were identified by TLC. Development media employed for TLC were hexane–ether (2:1) for 4-methylphenol and 2,4,6-trimethylphenol, hexane–ethylacetate (4:1) for phenol, and hexane for 4-ethylphenol. The solvent was evaporated from these column

fractions. For 4-methylphenol and 4-ethylphenol, the derivatives were obtained as oils. The 2,4,6-trimethylphenol derivative, a white solid, was recrystallized two times from cyclohexane (m.p. 88–90°C). The derivative of phenol required further purification by preparative LC. A Savant Speed Vac Concentrator (Savant Instruments, Hicksville, NY, U.S.A.) was used to remove the acetonitrile–water from the HPLC collected fractions containing product. White crystals were obtained (m.p. 70–71°C). The structures of these four derivatives were confirmed by mass spectrometry, giving a molecular ion in each case. All products were single peaks by analytical HPLC.

RESULTS AND DISCUSSION

We chose to investigate the importance of keeping non-polar variation remote from polar groups in the performance of an internal standard in ultratrace GC by synthesizing and testing the compounds shown in Fig. 1. Electrophoric compounds were selected because of our interest in GC with electron-capture detection (GC–ED). As seen, only non-polar structural differences involving hydrogen *vs.* methyl substitution exist between these compounds. However, compound 4 was prepared with the intention that it would be most different relative to the others based on the closeness of its polar $-OSO_2-$ group and the two adjacent CH_3 groups. Thus, according to our hypothesis, the GC behavior of this compound should least match that of the others.

A typical GC chromatogram from the determination of this solute mixture is shown in Fig. 2. As seen, the relative spacing of the bands is farily constant, allowing any contribution of retention differences to be insignificant for some of the solute pairs.

The results of a total of 22 injections of the sample mixture over a 1000 fold

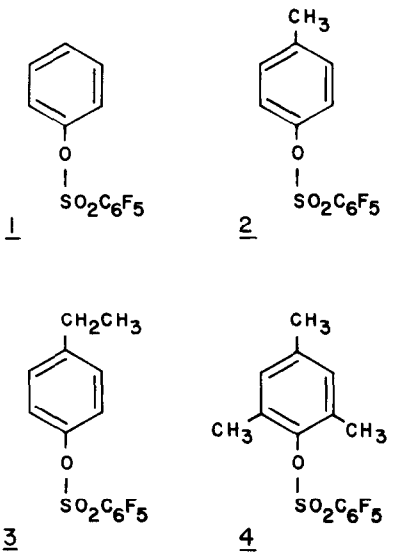

Fig. 1. Structures of O-pentafluorophenylsulfonyl derivatives of phenol (1), cresol (2), 4-ethylphenol (3), and mesitol (4).

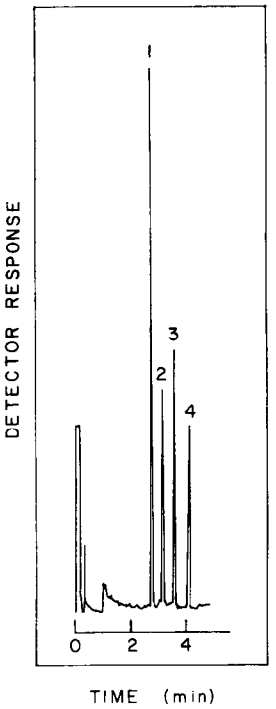

Fig. 2. Gas chromatogram of a mixture of the four phenolic derivatives. Attenuation: 0–1.3 min, 4096; 2 min, end of run, 512. Chart speed: 1 cm min^{-1}. Amount injected and retention times: (1) 5.77 pg, 2.9 min; (2) 5.75 pg, 3.24 min; (3) 5.91 pg, 3.64 min; and (4) 6.26 pg, 4.13 min. See Experimental for GC conditions.

TABLE I

PRECISION IN THE ANALYSIS OF COMPOUNDS 1–4 AS PEAK AREA RATIOS BY GC–ED

*Solute pair**	*Standard deviation of the area ratio for a solute range or level (pg)*		
	*0.1–100 (n = 22)**	*100 (n = 11)*	*5 (n = 23)*
2/3	0.064	0.017	0.010
2/1	0.076	0.016	0.007
3/1	0.094	0.014	0.009
4/3	0.106	0.019	0.035
2/4	0.098	0.023	0.025
4/1	0.104	0.025	0.025

* This average ratio was based on the data from 22 injections distributed over a 1000 fold concentration range. Triplicate injections were made at six concentration levels in the range of 0.2–100 pg, where the relative standard deviation at each level was less than 5% for each of the ratios. Four injections were made at the 0.1 pg level where the precision was poorer (see Fig. 3).

concentration range are shown in the first data column in Table I. Consistent with our hypothesis, all of the lowest precisions derive from solute pairs involving compound 4. However, the relative differences are small. Thus we continued to analyze mixtures of these four compounds, making 11 injections at the 100 pg level, and 23 injections at the 5 pg level. This data is also shown in Table I. Once again, response ratios for all the solute pairs involving compound 4 consistently have a poorer precision.

No solute pair consistently has the best precision. The data therefore does not reveal any role for chromatographic retention in the mutual monitoring of these compounds. This is not surprising since the retention of all of these compounds is similar. Also, aside from the more independent behavior of compound 4, the other compounds behave similarly for internal standard purposes. Thus, no finer details for internal standard structure beyond the aspect of remoteness of non-polar variation relative to the polar structure are suggested by this experiment.

Since the peak area ratios were in the range of 0.6–1.0, we see that the relative standard deviation ranges from about 1 to 10% for the data in Table I. The least precise values in these terms are contributed by the analyses involving the solute range of 0.2–1.0 pg, largely due to the increased baseline noise encountered at the lower concentration levels. A variation in response factors contributes to this latter variation as well; see Fig. 3, discussed below. At the 5 pg solute level, where less baseline noise was encountered, the coefficient of variation ranges from 0.7 to 1%

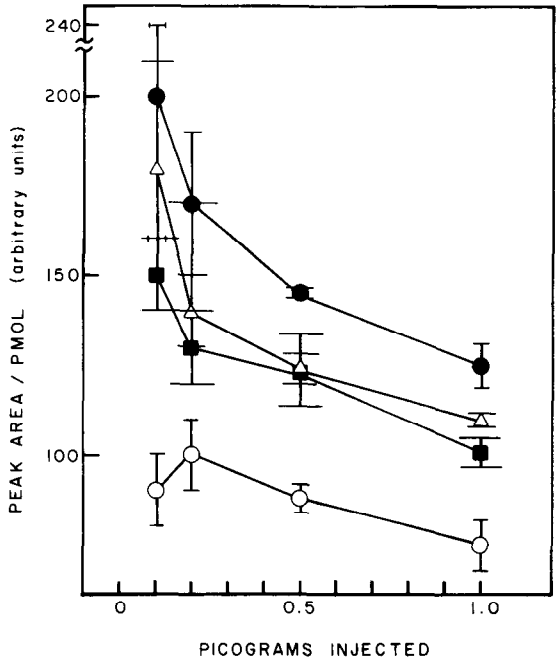

Fig. 3. Absolute response factor *vs.* amount injected for compounds 1–4 for the lower concentration data points from the $n = 22$ column in Table I. (●) = 1, (○) = 2, (■) = 3 and (△) = 4. The error bars represent ±1 S.D. about the mean.

for the three solute pairs involving compounds 1, 2 and 3, illustrating a good performance at this level.

Fig. 3 shows that the absolute response factor tends to increase at the lower sample concentrations (below 1.0 pg) in the experiment involving the solute range of 0.1–100 pg. Not shown is the corresponding data for the upper concentration range in this experiment of 5 to 100 pg, where the response factor is essentially constant for all of these compounds. Other strong electrophores behave similarly, and this phenomenon is attributed to surface effects in the ECD[7].

CONCLUSION

The hypothesis that the variation in structure between an analyte and its internal standard in ultratrace GC should keep non-polar differences remote from polar groups is supported by the relevant experimental results in this paper. Although only a single example was studied, and other factors such as differences in interferences could play a role, the hypothesis is reasonable, and should be considered when designing an internal standard for ultratrace GC analysis.

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REFERENCES

1 A. Shatkay and S. Flavian, *Anal. Chem.*, 49 (1977) 2222–2228.
2 G. C. Royston, *Anal. Chem.*, 50 (1978) 1005.
3 A. Shatkay, *Anal. Chem.*, 50 (1978) 1423–1429.
4 M. Ravey, *Anal. Chem.*, 50 (1978) 1006.
5 P. Haefelfinger, *J. Chromatogr.*, 218 (1981) 73–81.
6 B. N. Colby, P. W. Ryan and J. E. Wilkinson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 72–76.
7 A. Nazareth, K. O'Connell, A. Sentissi and R. W. Giese, *J. Chromatogr.*, 314 (1984) 219–232.
8 G. Wells, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 651–654.