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Note

Silylation of microgram samples in a gas chromatography trap*

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Trimethylsilylation is a well established method of forming derivatives of polar compounds for gas chromatography[1]. The derivatives are less polar and often more stable and more volatile than the parent compounds. Adsorption on gas–liquid chromatography (GLC) columns is decreased and tailing of peaks is reduced.

The most common method of preparing the derivative is to add the silylation reagent to a solution of the polar compound in a solvent (*e.g.* pyridine). However, Esposito[2] showed that derivatives of several classes of compounds can be formed on the GLC column by injecting the reagent a few seconds after injecting the sample.

In this note, a variation of Esposito's technique will be described in which microgram amounts of compounds are silylated in a trap prior to being flushed onto the GLC column.

EXPERIMENTAL

Fig. 1 presents a schematic diagram of the GLC and trapping system. The trap is 1.2 mm I.D. × 1.6 mm O.D. stainless-steel tubing. A 23-cm length packed with 10% OV-101 on 80–100 mesh Chromosorb W-HP forms the bottom of the loop.

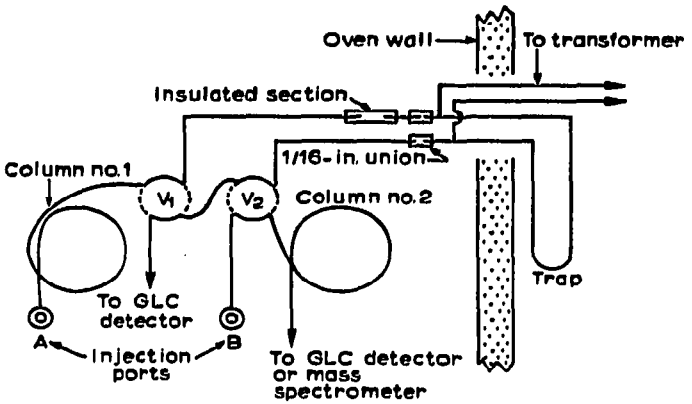

Fig. 1. Schematic diagram of gas–liquid chromatograph and trap. V_1 and V_2 are Carle microvolume valves.

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The remainder of the trap (15 cm of each arm extending to the inside of the oven wall) is packed with 80–100 mesh glass beads. The trap is heated by the current from a 3-V transformer, the input to which is controlled by a variable transformer. To avoid shorting the heating current, an insulated section is placed in the line to the inlet side of the trap. The insulated section is formed by joining the stainless-steel tubes with a short piece of PTFE tubing. The PTFE is enclosed in glass tubing, the ends of which are sealed to the stainless-steel tubes with silicone rubber (GE Silicone Seal).

The trap is cooled in a Dewar containing isopentane at −140 to −150°. By appropriate manipulation of the Carle valves a sample is introduced to the trap either by injection of a hexane solution through port B or by trapping a component from the effluent of column No. 1. Then 0.5–1.0 μl of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) is injected through port B and flushed into the trap. The valves are positioned to isolate the trap; the coolant is removed, and the trap is heated to 180° in 50 sec and held at this temperature for 5 min. (Use of a 5-V, 30-A) transformer enables the trap to be heated to 180° in 8 sec.) 30 sec after the heating current has been switched on, V_2 is turned to route the carrier gas through the trap to column No. 2. Column No. 2 is connected to the GLC detector or to a mass spectrometer.

DISCUSSION

MSTFA was chosen as the silylation reagent since its retention time is lower than those of N,O-bis(trimethylsilyl)-acetamide or N,O-bis(trimethylsilyl)-trifluoroacetamide and consequently interferes less with trimethylsilyl (TMS) derivatives with low retention times.

When small samples of free fatty acids, diols or phenols were chromatographed on column No. 2 (213 cm × 1.2 mm I.D. stainless-steel packed with 10% OV-101 on Chromosorb W-HP, 80–100 mesh), the detector response was small or absent. Similar samples silylated in the trap produced much larger and more symmetrical peaks. For example, 10 μg of butyric acid produced a broad peak with a maximum of 4×10^{-11} A (flame ionization detector), whereas 0.5 μg silylated in the trap produced a peak maximum of 3×10^{-10} A. The peak maxima for 10 μg of *o*-ethylphenol were 1.7×10^{-9} A, unsilylated, and 7.3×10^{-9}, silylated.

Comparisons were made of samples silylated in the trap with samples silylated in a vial. The peak maximum produced by 8 μg heptanoic acid silylated in the trap was 1.4×10^{-9} A. The same amount of heptanoic acid from a sample silylated in a vial produced a response of 2.3×10^{-9} A. Responses from 10 μg of *o*-ethylphenol silylated in the trap and vial were 7.3×10^{-9} and 6.3×10^{-9} A, respectively.

Acetoin chromatographed on column No. 2 emerged at 5 min with an extended tail. Under the same GLC conditions, the TMS derivative produced in the trap emerged from the column at 14 min in a sharp symmetrical peak. A plot of peak height produced by the derivative against quantity of acetoin injected into the trap was linear from 0.5 to 10 μg. The regression equation was $Y = -15 + 28.1X$ and the standard deviation from regression was 15.8 ($n = 6$).

To determine if silylation in the trap was effective for keto–enols, comparisons were made with 2,4-pentanedione silylated in a vial and in the trap. Silylation in the

vial produced a mono- and a di-TMS derivative, the ratio of di to mono increasing with time. Silylation in the trap produced only the mono-TMS derivative.

The method has been applied successfully in conjunction with combined GLC–mass spectrometric identification of cheese flavor volatiles. One fraction of neutral cheddar volatiles from column No. 1 was isolated in the trap, then rechromatographed on column No. 2. The chromatogram showed a peak at 29 min on the tail of a peak due to 2-nonanone. The mass spectrum of the 29-min component was weak and the parent ion was missing. When the fraction was silylated in the trap before rechromatography, the TMS derivative of 2-nonanol was clearly separated at 39.2 min. Similarly, a peak at 20.5 min on silylation had a retention time of 29 min, providing evidence for identification of *n*-heptanol. In another fraction, cresol isomers were unresolved at 28.5–29.5 min, with an extended tail. The silylated fraction showed the resolved isomers of the TMS derivatives at 32.7 and 33.5 min. The presence of 2-phenylethanol in cheese volatiles was confirmed by a shift in retention time from 31 to 38.3 min on silylation, as well as by mass spectral data.

The foregoing examples demonstrate that silylation in a trapping loop can provide supplementary data useful in identifying microgram samples of polar compounds.

REFERENCES

1 A. E. Pierce, *Silylation of Organic Compounds*, Pierce Chemical Co., Rockford, Ill., 1968.
2 G. E. Esposito, *Anal. Chem.*, 40 (1968) 1902.