Modification of Glass Capillary Gas Chromatographic Columns by Alkylation of the Glass Surface with Pentafluorobenzyl Bromide

I. Kari/A. Huhtikangas/J. Gynther
Department of Pharmacy, University of Kuopio, P.O.B. 138, 70101 Kuopio 10, Finland

T. Vartiainen
Department of Chemistry, University of Kuopio, P.O.B. 138, 70101 Kuopio 10, Finland

R. Hiltunen
Department of Pharmacognosy, University of Helsinki, 00170 Helsinki 17, Finland

Key Words
GLC
Glass capillaries
Surface alkylation
Pentafluorobenzyl bromide

Summary
Glass surface alkylation with pentafluorobenzyl (PFB) bromide yields glass capillary gas chromatographic columns with modified retention characteristics. Glass-alkylated OV-225 columns have been tested in the analysis of PFB fluoroacetate, and the substantial increase in retention time of this highly volatile compound was found to improve the precision of analysis. PFB-alkylated columns should prove generally useful in gas chromatographic analysis of small relative molecular mass compounds.

Introduction
In gas chromatographic analysis difficulty is often encountered in the separation of highly volatile sample components from, for example, solvents, side products and reagents used in derivatization reactions, especially when the compounds of interest are present in low concentrations. Obviously, some form of column activation should prove useful in slowing elution in such cases. Several reports on chemical modification of glass capillary surfaces have been published mainly dealing with surface deactivation by various silylation methods [1]. The present paper describes the effect of pentafluorobenzylation of glass capillaries subsequently coated with OV-225 liquid phase.

Experimental
Reagents
Pentafluorobenzyl (PFB) bromide was obtained from EGA-chemie Steinheim/Allbuch, FRG. OV-225 was made by Analabs, No. Haven, Ct., USA. All solvents used were analytical grade.

Preparation of the Columns
The glass capillary columns were drawn from Pyrex glass using an apparatus based on the design of Desty et al. [2]. The columns, 20m × 0.3m, were flushed with a 0.1% HCl, then distilled water and acetone. After evaporation of acetone, the columns were filled with 2% PFB bromide in dichloromethane at 60°C. After 30 min, the columns were flushed with dichloromethane. Following evaporation of dichloromethane, the columns were coated using a dynamic method and an 18% solution of OV-225 liquid phase. The reference columns were prepared by the method described by Grob and Grob [3].

GLC and GLC-MS Conditions
The columns were used for the gas chromatographic analysis of monofluoroacetate in a Carlo Erba Fractovap 2300 chromatograph with flame ionization detector, using hydrogen as carrier gas (0.3 kg cm⁻¹). In mass fragmentographic analysis the gas chromatograph was operated in connection with a JEOL JMS-D 300 mass spectrometer and a JMA 2000 mass data analysis system, using helium carrier gas. The injector temperature was 175°C; the temperature program for column oven: 1 min at 60°C, then 39°C/min to 100°C and then 8°C/min to 220°C. The sample was injected using the splitless technique. The conditions in the
mass spectrometer were: ionization current 300 μA and ionization potential 23 eV, and it was calibrated using perfluorokerosene.

Preparation of Test Samples

Pentafluorobenzylation of the fluoroacetate samples, consisting of 1–2 g homogenized plant tissues, was performed by the extractive PFB alkylation method [4–8], using tetrahexylammoniumhydroxide counter ions. Authentic monofluoroacetate was used in the preparation of standard curves. As an internal standard, 1 mg 2-dichlorobutanoic acid was added to the samples prior to the extractive alkylation step.

A mixture containing octadecane, nonadecane and eicosane (100 ppm of each in hexane) was used in the determination of separation factors for the columns.

Results and Discussion

The retention times of octadecane, nonadecane and eicosane in the PFB alkylated columns were found to be more than three times longer than the corresponding values in the untreated columns. The separation factors, calculated from these retention times, did not differ significantly between the treated and untreated columns.

A mass fragmentogram of PFB fluoroacetate (molecular ion m/z 258.0) obtained with an untreated OV-225 glass capillary column is shown in Fig. 1a. The retention time is 6.3 min, corresponding to a GC oven temperature of 120 °C. The standard curve obtained with this column for quantitative analysis is shown in Fig. 2a. The mass fragmentogram obtained using a PFB alkylated column is shown in Fig. 1b. The retention time of PFB fluoroacetate is 10.1 min corresponding to an oven temperature of 185 °C. The standard curve obtained with this column is shown in Fig. 2b.

The improved standard curve obtained with the alkylated column in mass fragmentographic analysis of standard PFB fluoroacetate samples (Fig. 2) indicates that the slight peak broadening has no adverse effects on the analysis. Of course, the narrower peaks obtained with the untreated column provide a slightly lower limit of detection in these chromatographic conditions for PFB fluorooacetate when compared with the alkylated column, these limits being 10 and 25 picograms, respectively. However, the alkylated column provides generally improved conditions for quantitative GC analysis of fluoroacetate (c.f. standard curves in Fig. 2), and this type of column should prove especially useful in the analysis of gases and other low relative molecular mass compounds.

In a previous investigation [9] concerning the GC analysis of morphine as the corresponding PFB derivative, it was found that PFB morphine is not eluted from glass capillaries containing standard GC liquid phases, with the exception of the polar silicones OV-225 and Silar 10 C. The elution temperature of PFB morphine is quite high in these columns and extensive peak broadening is observed with Silar 10 C, but surprisingly, a narrow PFB morphine peak, allowing reliable quantitation in the nanogram range, is obtained when using OV-225 as the liquid phase. On the other hand, PFB morphine is not eluted from a PFB-treated OV-225 column.

Silar 10 C is considerably more polar than OV-225, the McReynolds constants for n-butanol being 757 for Silar 10 C and 369 for OV-225. A common feature of these two liquids is that both contain cyanopropyl groups, which seem to be essential for a reasonable distribution of high relative molecular mass PFB derivatives in the gas phase. The 100% cyanopropyl content of Silar 10 C is likely to be too high for PFB morphine, while the cyanopropyl group spacing in OV-225, with 25% cyanopropyl groups among...