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Note

Separation of prostaglandins on an OV-210 whisker-wall-coated open tubular column

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Prostaglandins present in biological fluids are routinely analyzed by capillary gas chromatography where the columns usually employed are coated with apolar liquid phases[1–3]. Columns coated with the more polar OV-225 and Carbowax 20M phases have been investigated but are not widely used[1]. Adequate resolution of $PGF_{1\alpha}$ and PGE_2 may not be achieved using apolar liquid stationary phases. Although this does not present a problem in the analysis of prostaglandin mixtures when the E and F fractions are previously separated by liquid chromatography on silicic acid[1], a capillary gas chromatographic system capable of this separation would be helpful.

In our experience, packed columns containing OV-210 coated Gas-Chrom Q have found application for the separation of hydroxylated drug metabolites which were not separated on apolar or moderately polar siloxane liquid phase[4]. We felt that this liquid phase would provide a different retention pattern for the prostaglandins than the apolar variety as a consequence of the interaction with the hydroxyl and keto moieties on the prostaglandin molecules. It is very difficult to obtain a stable coating of OV-210 on a glass open tubular column. However, Sandra and Verzele[5] described the successful coating of this liquid phase on whisker-wall-coated open tubular (WWCOT) columns. This paper demonstrates the utility of a WWCOT column containing OV-210 for complete separation of $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGA_1, PGA_2, PGE_1 and PGE_2 as the methyl ester trimethylsilyl ether derivatives.

EXPERIMENTAL

Materials

All solvents were distilled in glass and purchased from Burdick & Jackson

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Labs. (Muskegon, MI, U.S.A.). The prostaglandins were generously provided by Dr. Edward Ham, Merck Sharp & Dohme Research Labs., Rahway, NJ, U.S.A. N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Regis Chemical Co. (Morton Grove, IL, U.S.A.). N-Nitrosomethylurea for preparation of ethereal diazomethane was purchased from K & K Labs.–ICN Pharmaceuticals (Plainview, NY, U.S.A.). A splitter injection system and glass-lined tubing (GLT) were purchased from Scientific Glass Engineering (SGE) (Austin, TX, U.S.A.).

Gas chromatography

A Hewlett-Packard Model 5750 gas chromatograph was modified for use with capillary columns. The major modifications were the use of an injection port from an F & M Scientific Model 810 gas chromatograph and a pressure-controlled SGE splitter injection system with needle valve regulation of the split ratio. The capillary column was connected to the injector and flame ionization detector with 1/16 in. O.D. × 0.3 mm I.D. GLT transfer lines. WWCOT columns (25 m × 0.25 mm I.D.) coated (d_f = 0.2 μm) with either SE-30 or OV-210 liquid stationary phases were prepared as described previously[6]. Helium, which was purified over a desiccant of $CaCl_2$–5 Å molecular sieves and an Oxy-Trap (Alltech, Arlington Heights, IL, U.S.A.) was employed as the carrier and make-up gas.

All operating conditions are listed in the appropriate figures.

Derivatization

The methyl esters of the prostaglandins were prepared by reaction of 200 μl of a freshly prepared 0.5 *M* ethereal diazomethane solution with 100-μl aliquots of each prostaglandin (1 mg/ml in methanol). After 10 min, the reaction mixtures were concentrated to dryness under nitrogen at 25°C. The trimethylsilyl ethers were prepared by adding 100 μl of BSTFA to the residue at room temperature and immediately concentrating the sample to dryness under dry nitrogen after brief vortex mixing. The resulting methyl ester trimethylsilyl ether derivatives were reconstituted in hexane prior to capillary gas chromatographic analysis.

RESULTS AND DISCUSSION

A typical chromatographic separation of the six prostaglandins as the methyl ester trimethylsilyl ether derivatives is illustrated in Fig. 1. Adequate resolution was obtained within 14 min for all of the components except $PGF_{1\alpha}$ and PGE_2 at a column temperature of 250°C with the SE-30 WWCOT column. This separation is similar to that reported in the literature when the methyl ester methoxime trimethylsilyl ether derivative of the PGEs was prepared[1]. Although the methyl ester trimethylsilyl ether derivatives of the PGE analogs are less stable than those prepared following oximation of the 9-keto function, the conditions of derivatization were adjusted to minimize degradation of these derivatives.

A chromatogram of the same mixture of the six prostaglandins as the methyl ester trimethylsilyl ether derivatives chromatographed on an OV-210 WWCOT column is presented in Fig. 2. At a column temperature of 230°C, all of the components are well separated within 20 min. Upon comparison of these results with those observed on an SE-30 column (Fig. 1), the retention order has changed such

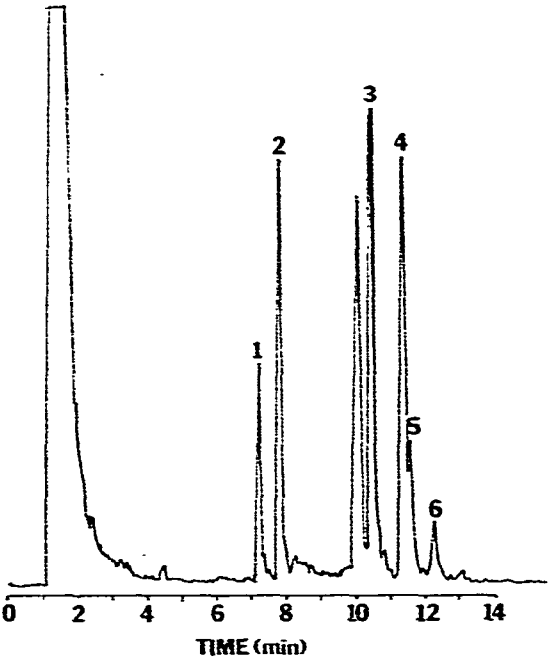

Fig. 1. Chromatogram of a mixture of prostaglandins as their methyl ester trimethylsilyl ether derivatives in hexane. Column: SE-30 whisker glass capillary (d_f = 0.2 μm) (25 m × 0.25 mm I.D.); N = 3378 plates per metre, k = 11.5. Linear velocity: 38.6 cm/sec. Carrier gas: helium, 30 p.s.i. Temperatures: detector, 245°C; injector, 250°C; column, 250°C. Splitting ratio: 10/1. Attenuation: 8 × 1. Peaks: 1 = PGA_2; 2 = PGA_1; 3 = $PGF_{2\alpha}$; 4 = $PGF_{1\alpha}$; 5 = PGE_2; 6 = PGE_1.

that the PGF analogs elute much earlier than the PGA and PGE analogues. Although the resolution of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ has diminished slightly from that observed in Fig. 1, the resolution of PGA_1 and PGA_2, as well as that of PGE_1 and PGE_2, has markedly increased.

It is well known that with the present technology only the non-polar silicone liquid stationary phases may be coated with good stability on fused-silica open tubular columns. Columns containing the moderately polar and polar silicone liquid phases may be prepared with sufficient stability on a roughened glass surface. WWCOT columns have been considered too difficult to prepare, to possess approximately ten times the activity compared to smooth wall glass columns, as well as less efficient due to the severe roughening. This view is not totally correct. It is now possible to deactivate sufficiently the whisker-walled columns for analysis of biological samples as demonstrated in this paper. It is true that these columns do not provide the theoretical plate efficiency of smooth glass columns, but the whisker-walled columns offer the flexibility of supporting almost any liquid phase. This consideration alone may be worth more than the efficiency gained with other types of open tubular columns which may not provide the necessary selectivity. The benefits of selectivity in many cases outweigh the minor loss in efficiency for many applications of WWCOT columns. The effective theoretical plate value listed in the legend of Fig. 1 is comparable to similar columns prepared on a non-whiskered surface, suggesting that WWCOT columns provide adequate efficiency.

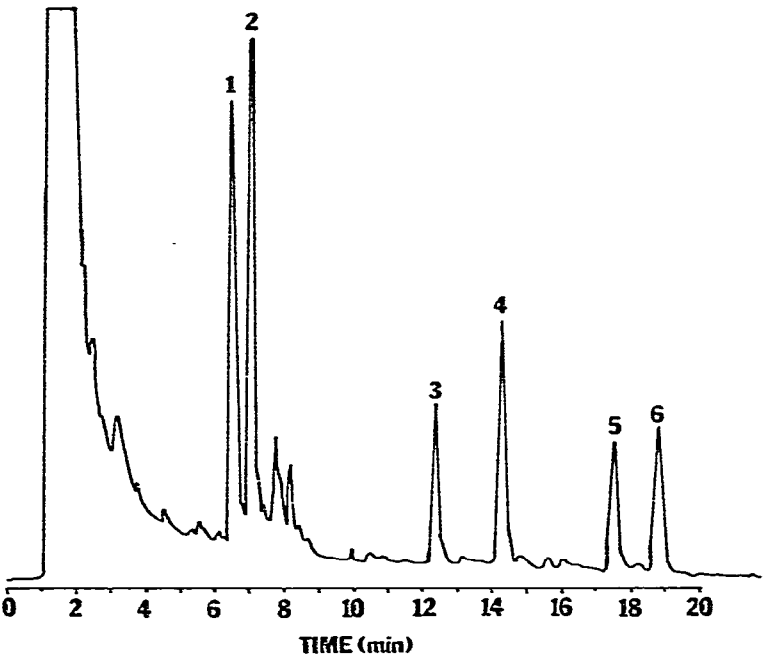

Fig. 2. Chromatogram of prostaglandins as their methyl ester trimethylsilyl ether derivatives in hexane. Column: OV-210 whisker glass capillary (d_f = 0.2 μm) (25 m × 0.25 mm I.D.); N = 1986 plates per metre, k = 21.5. Linear velocity: 37.9 cm/sec. Carrier gas: helium, 30 p.s.i. Temperatures: detector, 245°C; injector, 250°C; column, 230°C. Splitting ratio: 10/1. Attenuation: 8 × 1. Peaks: 1 = PGF_{2_2}; 2 = PGF_{1_2}; 3 = PGA_2; 4 = PGA_1; 5 = PGE_2; 6 = PGE_1.

REFERENCES

1 J. Maclauf, M. Rigaud, J. Durand and P. Chebroux, *Prostaglandin*, 11 (1976) 999.
2 F. A. Fitzpatrick, *Anal. Chem.*, 50 (1978) 47.
3 F. A. Fitzpatrick, D. A. Stringfellow, J. Maclouf and M. Rigaud, *J. Chromatogr.*, 177 (1979) 51.
4 A. G. Zacchei, T. I. Wishousky, B. H. Arison and G. Hitzenberger, *Drug Metab. Dispos.*, 6, (1978) 303.
5 P. Sandra and M. Verzele, *Chromatographia*, 10 (1977) 419.
6 T. I. Wishousky, *Ph.D. Thesis*, Villanova University, Villanova, PA, 1980.