Journal of Chromatography, 368 (1986) 370–373
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 942

Note

Preparation of thermostable cyanosilicone capillary columns

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(First received June 10th, 1986; revised manuscript received July 15th, 1986)

Highly efficient non-polar capillary columns have become commercially available in recent years because of the tremendous research effort devoted to standardising methods of preparation for this type of column[1,2], but the corresponding techniques for the preparation of polar capillary columns have still to be achieved although some progress has recently been reported[3–6]. The basic difficulty in the preparation of such columns lies in the stabilisation of a homogeneous thin film of the polar phase on a glass surface with which it is essentially incompatible. In order to achieve thermal stability the surface must be treated in some way to increase the ability of the phase to wet the surface, *i.e.* reduce the contact angle. In fact, thermal stability is achieved by the control of two factors of which contact angle is one, the other being inertness of the glass since active sites on the glass will promote thermal decomposition or polymerisation of the phase. Either will cause loss of column performance.

Stabilisation of the stationary liquid phase can be achieved either by etching the surface[7] or by deposition of particulate matter[8–11] onto the surface of the glass. Each will produce a roughness which will hold the phase in place. This technique prevents the phase being a film of constant thickness and thus results in some loss of potential column efficiency. Leaching of the glass surface[12] improves the inertness of the surface but does little to improve wettability.

Recent research has centred on the chemical modification of the surface to control surface activity and wettability and the resulting surfaces produce excellent columns with non-polar phases. The *in situ* polymerisation (immobilisation) of stationary phases may also involve a chemical reaction with the column wall. The equivalent chemistry for the preparation of polar columns has still to be defined. Instead polar capillary columns are prepared by complex procedures which require either etching[13] or surface roughening[14]. Either approach produces a surface which is active and exceedingly difficult to deactivate[15]. Conventional deactivation techniques involve the use of Carbowax (PEG) 20M as a barrier layer between the phase and the surface.

In this preliminary communication we describe a simple method for the preparation of capillary columns coated with the highly polar cyanosilicone phases. Such

0021-9673/86/$03.50

phases are particularly useful for the analysis of fatty acid methyl esters where isomeric mono-unsaturation is present.

EXPERIMENTAL

Soda glass (Ruhr AR, Schott, F.R.G.) was washed with chromic acid overnight, rinsed with water, then acetone and finally air dried prior to drawing. Capillaries (0.25 and 0.30 mm I.D.) were drawn on a Shimadzu Type GDM1 glass drawing machine (Shimadzu, Tokyo, Japan).

Capillary columns were etched with dry hydrogen chloride gas. Typically a column was filled with hydrogen chloride gas from a cylinder, the ends sealed with a flame and the column was then heated (350°C, 16 h). A well etched column is milky white in appearance and shows an even opacity along its length. Upon opening the column was rinsed with water (removing sodium chloride crystals and residual hydrogen chloride), then acetone and dried by nitrogen flow. After drying a plug of 3% (v/v) solution of silicic acid suspension (DUDOX colloidal silica; Dupont, Wilmington, U.S.A.) in acetone was pushed through the column at a steady rate (0.8 cm s^{-1}) by nitrogen pressure. This was followed by a plug of mercury to remove excess coating solution. A buffer column was attached to the end of the true column to ensure an even coating velocity across the entire column length. After coating with silicic acid the column was dried in a water bath at 90°C to remove acetone. The dried column was then dehydrated (150°C, 16 h) under a flow of nitrogen.

Columns were coated by standard static coating procedures[16,17]. The concentration of the coating solution was such that a film thickness of *ca.* 0.15 μm resulted.

Gas chromatography was performed on a Carlo Erba Series 2150 gas chromatograph fitted with a split/splitles injector and a flame ionization detector. Care was taken to ensure that the capillary column penetrated inside the jet of the detector. The carrier gas was hydrogen (1 ml min^{-1}), the split ratio 1:60 and the splitless period 40 s.

RESULTS AND DISCUSSION

Etching with hydrogen chloride gas produces a roughened surface which is therefore rather active. This roughness tends to limit the coating efficiency which may be achieved. The use of silicic acid is intended to smooth out the roughness generated by the etching process by deposition of microparticulate silica in the column cavities and crevices. In this respect the final effect is somewhat similar to the generation of a very thin porous layer at the column wall surface. Polar phases will be retained easily on such surfaces. Incidental to this process is a degree of homogenisation of the surface.

The cyanosilicone phases OV-225 (25% cyano groups) and Silar 10C (100% cyano groups) were used as these were considered the phases most likely to achieve the desired separation. Our particular application was the resolution of the methyl esters of fatty acids found in olive oil. Adulteraton of olive oil with other low grade oils is difficult to detect by conventional analytical methods. However, adulteration does result in the introduction of spurious acids into the oil and thus the characteristic fatty acid profile of olive oil is altered. Monitoring of the fatty acid profile is thus a

desirable objective. The particular chromatographic difficulty is the separation of the mono-unsaturated *cis/trans* isomers of C_{18} and for this a very polar phase and a high-efficiency column is required.

Using the procedure described above a column coated with OV-225 was prepared and evaluated. Its separation efficiency was 90% but its separation number (TZ) was only 11. A useful improvement in performance was achieved when Silar 10C was used as the phase. Typical separation efficiencies were 95% and the TZ was of the order of 25. It is usual to evaluate a capillary column by chromatographing a Grob test mixture of compounds in order to examine the column for acidic or basic active sites, for phase thickness, and for efficiency. Our particular concern was the resolution of *cis/trans* isomers and the standard test mixture gives no information on this topic. High efficiency is seldom sufficient to achieve this by itself and phase selectivity is important. All our compounds are methyl esters and are thus relatively non-polar. Hence we chose to judge the usefulness of a column solely by its ability to separate the *cis* 18:1 isomer from the *trans* 18:1 isomer. In fact columns prepared by this procedure usually turn out to be slightly acidic in character.

Using a Silar 10C column prepared by this route a set of fatty acid methyl esters was chromatographed (Fig. 1). Baseline separation of *cis* 18:1 from *trans* 18:1 was achieved as was resolution from the 18:0 and 18:2 compounds. Subsequently olive oil was fractionated and a fraction containing the fatty acids chromatographed (after derivatisation) to give Fig. 2. The various isomers of the C_{18} chain are clearly resolved. The Silar 10C column prepared has now been used to analyse more than

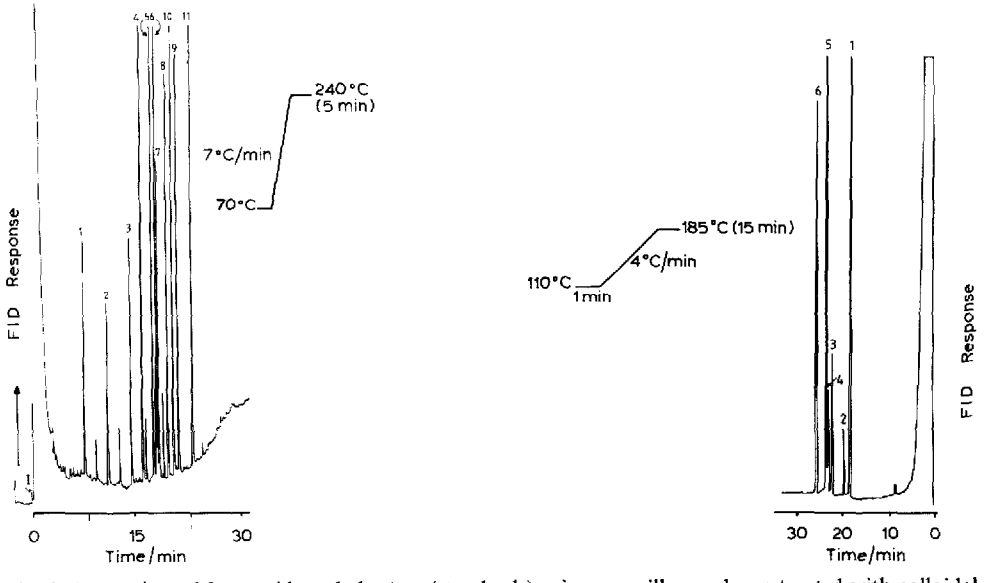

Fig. 1. Separation of fatty acid methyl esters (standards) using a capillary column treated with colloidal silica and coated with Silar 10C. 1 = 12:0; 2 = 14:0; 3 = 16:0; 4 = 16:1; 5 = 18:0; 6 = 18:1 *trans*; 7 = 18:1 *cis*; 8 = 18:2; 9 = 18:3; 10 = 20:0; 11 = 22:0. Detection, FID; injection, 1 μl splitless for 40 s.

Fig. 2. Separation of fatty acid fraction (methyl esters) from Libyan olive oil. 1 = 16:0; 2 = 16:1; 3 = 18:0; 4 = 18:1 *trans*; 5 = 18:1 *cis*; 6 = 18:2. Other conditions as for Fig. 1.

250 samples of Libyan olive oil without appreciable loss of efficiency or thermal stability.

CONCLUSIONS

A simple method for preparing thermally stable and highly efficient polar capillary columns is described. Such columns are capable of resolving complex isomeric mixtures of fatty acid methyl esters and can thus be used to detect adulteration in naturally occurring vegetable oils intended for human consumption.

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