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Short Communication

Regeneration of column activity after the gas chromatographic separation of membrane lipids on thermostable SE-52 phase

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ABSTRACT

The phosphoglyceride/cholesterol ratio of membrane lipids can be approximately determined by gas chromatography by direct injection into a short capillary column of the lipid material extracted from tissues according, for instance, to the Folch procedure. However, as a result of the pyrolysis of the injected material, in the course of the separation acidic substances are produced that deactivate the stationary phase. With the aid of a suitable scavenger, such as diethylenetriamine, it is possible to regenerate the column activity.

INTRODUCTION

Changes in the lipid composition of cellular membranes, such as those observed in some pathological conditions [1], may be indicated by variations in the phosphoglyceride/cholesterol ratio. This ratio can be rapidly determined by gas chromatography (GC) using a short capillary column coated with a highly thermostable phase such as SE-52 [2,3]. However, as a result of the pyrolysis of the injected material, in the course of the separation [2,3] substances are produced during continuous use that modify the stationary phase and lead to deactivation. With the aid of a suitable scavenger, however, it is possible to regenerate the column activity.

EXPERIMENTAL

A Carlo Erba Model 4160 HRGC gas chromatograph with a Mega SE-52 fused-silica column (10 m × 0.32 mm I.D.; film thickness 0.1 μm) was used. The injection temperature was initially 40°C, programmed at 20°C/min to 340°C, the final temperature being maintained for 10 min. The carrier gas was hydrogen at a flow-rate of 8 ml/min. On-column injection of 1 μl of a 0.2% benzene solution of membrane lipid extract, *i.e.*, a mixture of cholesterol and phosphoglyceride, was applied.

RESULTS AND DISCUSSION

Extraction of membrane lipids by the method of Folch *et al.* [4] and injection into a new column programmed rapidly to 340°C gave a chromatogram similar to that shown at the top of Fig. 1. The peak at 11 min corresponded to cholesterol and those between 13 and 20 min were ascribable to the diglycerides arising from the decomposition of the corresponding phosphoglycerides [2,3] in the course of the separation.

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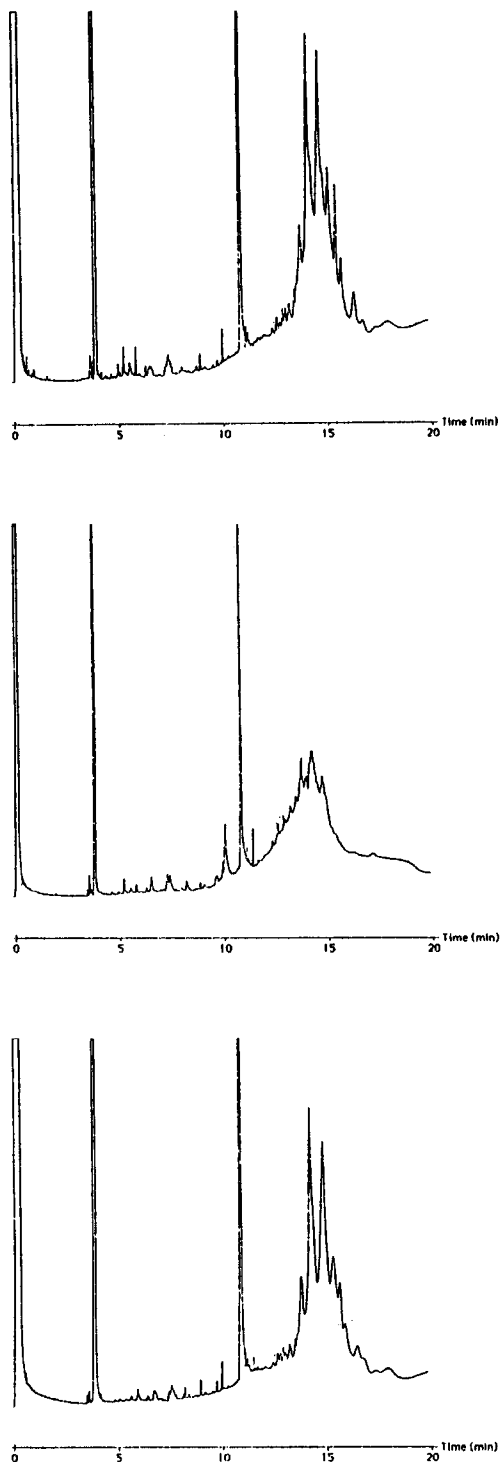

According to the relationship

area of cholesterol peak/100 = area of diglyceride peaks/x

it is possible to relate the composite area of the diglyceride peaks to the single cholesterol peak area normalized at 100 and obtain indirect information about the variation in the lipid composition of the cellular membranes of a tissue in the course of recurring experiments.

When samples are being continuously analysed, progressive deactivation of the stationary phase is observed as a result of adsorption of acidic, non-volatile products arising from decomposition of the phosphoglyceride components. Comparisons between consecutive chromatograms during continuous use may not give rise to any observable change. However, analytical comparisons between chromatograms obtained on consecutive days show considerable differences as the lack of use of the column during the overnight period allows the firm deposition of the acidic breakdown material with a consequential extensive effect on separation (see the middle chromatogram in Fig. 1).

Our investigations have shown that the interfering, non-volatile, acidic compounds that contaminate the column can be satisfactorily eliminated by the use of diethylenetriamine (m.p. = −39°C; b.p. = 207°C). A 1-μl volume of diethylenetriamine in the free state was injected into the column on completion of daily use, under conditions identical with those used for the separation of the membrane lipid fractions. An injection temperature of 40°C resulted in uniform application of the reagent throughout the stationary phase. Increasing the column temperature at 20°C/min resulted in complete elimination of the reagent in 5 min, i.e., at a column temperature of 140°C. Therefore, the

Fig. 1. Chromatograms produced by on-column injection of 1 μl of a 0.2% benzene solution of membrane lipid into a short capillary column coated with SE-52 under the described conditions. Top, chromatogram produced from a new column; middle, chromatogram produced from the same column deactivated by lack of use during the overnight period; bottom, chromatogram produced from the same column after reactivation by injecting 1 μl of the scavenger diethylenetriamine. The peak at 3.6 min corresponds to butylhydroxytoluene.

reagent in the liquid state washes the surface layers of the stationary phase. The effectiveness of such a treatment in the regeneration of the separation ability of the column can be seen in the bottom chromatogram in Fig. 1. In addition to maintaining the optimum separation, the overall working life of the column is also considerably extended.

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