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Comparative Study of Two Chromatographic Columns Used in the GLC Determination of Methylmercury

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A large effort has gone into finding an adequate analytical method for the determinations of methylmercury. Various stationary phases in GC determination have been tested, such as diethyleneglycol succinate (Sumino 1968a,b; Watts et al. 1976; O'Reilly 1982), polyethyleneglycol (Westöö 1966; Uthe et al. 1972) and OV-17+QF-1 (Hartung 1972; Cappon and Smith 1977). It was obvious with every method that the stationary phase had to be saturated (or better "loaded") to give a stable response without tailing the peakes or "walking" the retention time. To accomplish this several authors have reported treatments which included the injection of the solutions containing inorganic or organic mercuric chloride metoxyethylmercuric iodide, or large amount of potassium iodide, etc. (FAO 1976; Rodriguez-Vazquez 1978; O'Reilly 1982). We report here a simple and efficient way to obtain satisfactory stable response from the chromatographic column based on the use od 10 % diethyleneglycol adipate (DEGA) and 3 % polyethyleneglycol (Carbowax 20M).

MATERIALS AND METHODS

A Hewlett-Packard Model 7530A gas chromatograph, with a 63 Ni electron capture detector and HP recorder-integrator Model 3380A was used. All results were obtained on chromatographic columns of the same lenght and inner diameter (1.8 m x 4 mm). Injector and detector temperatures were kept on 200 and 300 °C respectively. The carrier gas was nitrogen. Gas flow rates were 50 ml/min for the column coated with 10 % DEGA, and 60 ml/min for Carbowax 20M, home made coatings, operated on 170 and 150 °C respectively. Chart speed was 0.5 cm/min. The initial conditioning was performed by heating overnight at 200 °C with disconnected detector, concurrently flushing the columns with carrier gas. All chemicals used were p.a. grade for standard chromatographic use. Benzene was regular grade, but distilled twice, to eliminate a peak at the retention time of methylmercury. After initial overnight conditioning, column temperature and gas flow rate 200 °C and 30 ml/min respectively, 10 % DEGA was treated with successive injections of solutions of 1 mg mercuric chloride/ml benzene (final volume 160 µl) (Schafer et al. 1975; FAO 1976; O'Reilly 1982). The column was saturated until it gave a stable peak area and retention time changing less than 2 %. The 3 % Carbowax 20M columns conditioning consisted of an initial

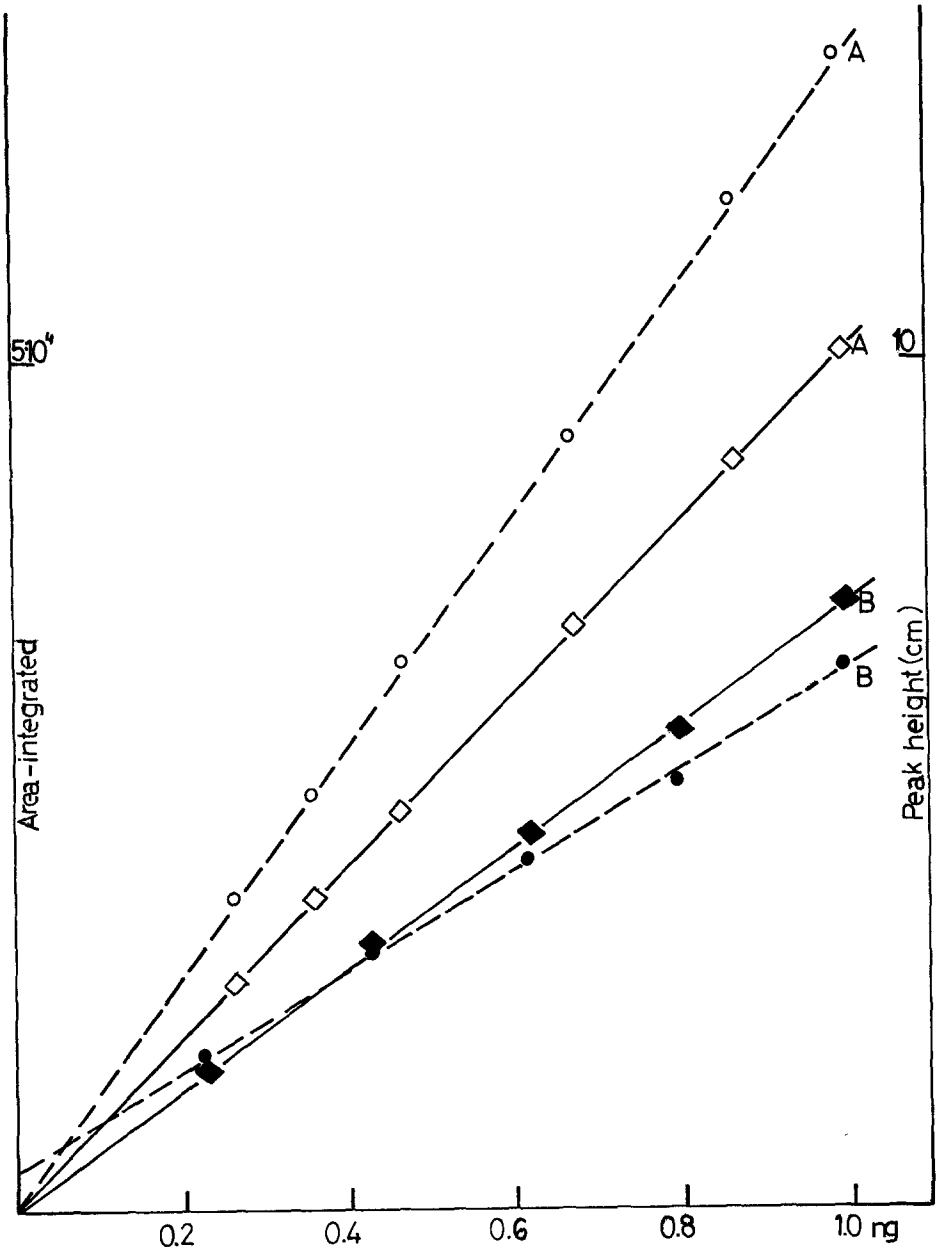

Figure 1 Calibration curves of methylmercury chloride (MMC)
A: 10% DEGA, 170°C, N_2=50 ml/min
B: 3% Carbowax 20M, 150°C, N_2=60ml/min
Area versus quantity is shown with the solid line. Peak height versus quantity is shown with the broken line.

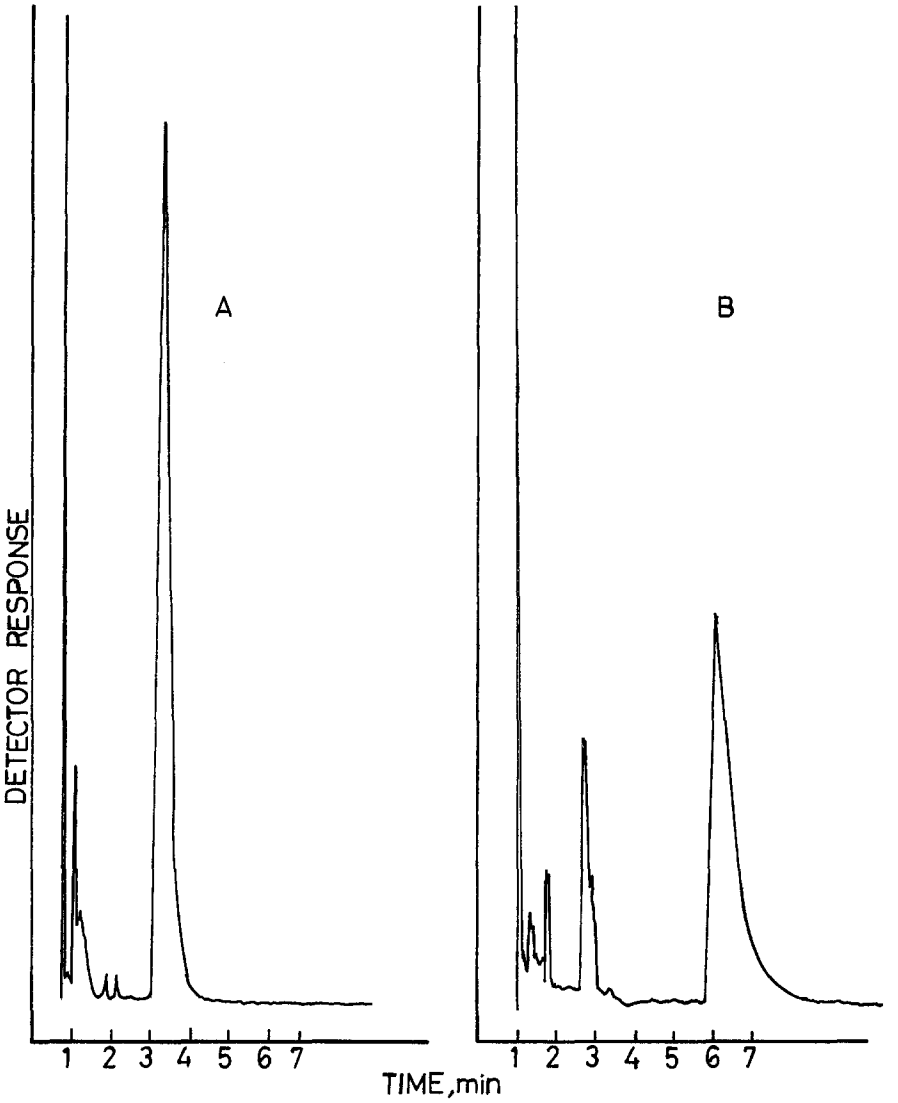

Figure 2 Chromatograms of benzene extracts of tuna liver samples containing a level of 1.7 µg/g of methylmercury: 3 µl of sample were injected on column.
A: 10% DEGA, 170°C, R_{tMMC}=3.5 min
B: 3% Carbowax 20M, 150°C, R_{tMMC}=6.5 min
A glutathione solution (50 µg/g) was used as a stripping reagent.

Table 1 Dissipation of the integrated peak areas in dependece of quantity of methylmercuric chloride (MMC) standard solutions injected.

Column	MMC (ng)	No. of injections	Average peak area ± standard deviation (integrator counts x 0.3)
10 % DEGA	0.2	6	10520 ± 560
	0.3	6	14731 ± 1034
	0.5	10	25588 ± 481
	0.8	6	40307 ± 420
	1.0	10	50060 ± 1045
3 % Carbowax	0.2	6	8186 ± 1234
	0.4	7	16075 ± 1307
	0.6	6	22776 ± 741
	0.8	8	27899 ± 933

overnight heating, followed by saturation with a 10 µl of 0.5 µg methylmercury/µl benzene (FAO 1976) and with 3 µl of 0.5 ng methylmercury/µl benzene. To check the efficiency of the columns both were tested with extracts of tuna liver tissue. During the extraction procedure the pH was maintained in the optimal range (Sumino 1968a). After a dozen samples the standard solution was injected, and peak area and retention time checked.

RESULTS AND DISCUSSION

Calibration curves for both columns and chromatograms of tuna liver tissue are presented in figures 1 and 2, respectively. In table 1 are the mean values of integrated peak areas and standard deviations for both columns. It was concluded that 3 % Carbowax 20M column needed longer conditioning, the retention time was not satisfactorily stable, and the peak area for same concentration of methylmercury was smaller than on the 10 % DEGA column. After overnight heating of the 3 % Carbowax 20M columns by keeping them on working conditions one day, 10 and 3 µl of methylmercury standard with different concentrations were injected. Only after a dozen injections peak area and retention time were stabilized. Injecting 0.5 µg methylmercury/µl in benzene (10 µl) on a recently prepared Carbowax 20M column does not give a satisfactory response. This system needs time for equilibration and the detector is soon contaminated. On the contrary, injecting only 0.5 ng methylmercury/µl in benzene (3 µl) on this column needs no regeneration and detector is not easily contaminated. In contrast, the 10 % DEGA column, not used for the determination of methylmercury to our knowledge, gave stable peak areas without change in the retention time after only one day of injecting mercuric chloride with excellent reproducibility of the peak areas. Daily use keeps the 10 % DEGA column in satisfactory condition, and further inactivation is not needed. The

column has been used for up to two months for the analyses of various samples, and stable retention time is still observed.

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