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Evaluation of capillary columns used in the routine determination of methylmercury in biological and environmental materials

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ABSTRACT

The factors determining the effective life of the capillary columns commonly used in the determination of organomercury compounds in biological and environmental materials were examined. The effects of pretreatment of these columns with mercury(II) salts on their behaviour and the duration of the treatments in terms of the type of stationary phase considered are discussed, in addition to the influence of the thickness of the stationary phase and the external protective covering of the columns. The use of diphenyldimethylsiloxane columns with a film thickness of 5 μm seems to be the most advantageous option for routine analysis.

INTRODUCTION

Mercury is a widely distributed toxic substance in the environment. Organomercurial compounds are generally more toxic than elemental mercury or its inorganic salts. Among its organic forms, the alkylmercurials and methylmercury in particular are more toxic than the arylmercurials. The changes that have been made in the traditional methods for the analytical evaluation of the mercury content in biological and environmental materials may be attributed to the toxicity of methylmercury and the fact that methylation of inorganic mercury caused by bacteria is a natural process. Consequently, the bioaccumulation of mercury in large marine predators usually occurs as methylmercury owing to its greater lipophilic properties. The present tendency is towards the speciation of mercury and, preferably, towards the determination of methylmercury present in these materials [1–4].

With these objectives in mind, several methods have been proposed, ranging from titrimetry [5] and thin-layer chromatography [6–9] to inductively coupled plasma mass spectrometry [10–12], and including spectrometric techniques of absorption and fluorescence (cold vapour) [13–19] and emission microwave-induced plasma [20–24], associated, in most instances, with an earlier stage of chromatographic separation or clean-up, allowing for speciation. High-performance liquid chromatography (HPLC) has also been used [25–28]. The literature up to 1971 was critically reviewed by Uthe and Armstrong [29]. However, the most common technique, and the only one being used for routine analysis and quality control, is separation by gas chromatography with species detection using electron-capture detection (ECD) [6–8,30–43]. In this instance, and based on the classical studies by Westöö [6–8], in which columns packed with 10% Carbowax 20M on Chromosorb W were used, several workers have studied the problem of organomercu-

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rial compounds with completely different stationary phases. Sumino [30,31] studied sixteen stationary phases and concluded that those with the greatest polarity [diethylene glycol succinate (DEGS) and 1,4-butanediol succinate (DBS)] produce the best separations. DEGS has been proposed by several other workers [34,35,44] and is probably the most commonly used stationary phase in packed columns with these objectives, although other phases (*e.g.*, OV-17 [36,37], ethylene glycol adipate [38], Carbowax 20 M [6,7,34] and polyethylene glycol succinate [9]) have also been proposed with apparently equivalent results.

More recently, other workers have studied the use of capillary and semicapillary columns with the same objectives [45–48]. In general, the results appear to be excellent for both polar and non-polar columns, and it is evident that by using these, some of the problems associated with packed columns can be solved (*e.g.*, low response to methyl- and ethylmercury due to supposed interactions with the column and/or decomposition; peak tailing; low efficiency of the column; variable decrease in the areas and heights of methyl- and ethylmercury peaks when injected with extracts of fish and sediment.

The fact that columns having such different polarities gave equivalent results is curious. However, no studies have been carried out on the separation mechanism of these compounds in the column. There is another important fact. Most workers, whether using packed or capillary columns, have stressed the need to carry out pretreatments of the stationary phase, which generally entails the injection of large amounts of mercury(II) chloride or iodide at regular intervals [44,49,50]. No rational explanation have been found to justify this treatment, which has a number of drawbacks (rapid deterioration of the performance characteristics of the columns, progressive and irreversible contamination of electron-capture detectors, dead periods during the operation, which usually take up more time than the periods spent working on the analyses, etc.)

This paper presents the results obtained after trying, for routine purposes, various types of fused-silica semicapillary columns having different polarities (BP-1; dimethylsiloxane, BP-20; polyethylene glycol, BP-5 and AT-5; and 5% diphenyldimethylsiloxane) with external protective layers of polyimide and aluminium.

EXPERIMENTAL

Apparatus and reagents

All the experiments were carried out with a Hewlett-Packard Model 5890A gas chromatograph, equipped with a nickel-63 electron-capture detector using N52 nitrogen as carrier and make-up gas. Chromatographic data was acquired by means of a

TABLE I

COLUMNS STUDIED AND EXPERIMENTAL CONDITIONS

Characteristic	Column			
	BP-1	BP-20	BP-5	AT-5
Type	Bonded-phase	Bonded-phase	Bonded-phase	Bonded-phase
Stationary phase	Dimethylsiloxane	Polyethylene glycol	5% Diphenyl-dimethylsiloxane	5% Diphenyl-dimethylsiloxane
Polarity	Low	High	Low	Low
Length (m)	25	25	12	30
Material	Fused silica	Fused silica	Fused silica	Fused silica
Film thickness (μm)	1.0	1.0	1.0	5.0
Inside diameter (mm)	0.53	0.53	0.53	0.54
Oven temperature (°C)	90	175	90	170
Injector temperature (°C)	200	250	200	250
Detector temperature (°C)	250	250	250	250
Carrier flow-rate (ml/min)	14.2	20	14	10

Hewlett-Packard Model 3396A integrator. Table I shows the characteristics of the columns evaluated and the operating conditions used in each instance.

All reagents were of analytical-reagent grade and solvents were of spectroscopic or HPLC quality. Tests were carried out by injecting methylmercury chloride (Merck) standard solutions of appropriate concentration in toluene. These solutions were prepared by dilution from a 1 g/l stock solution and were kept refrigerated and protected from light. The treatment solution used was 1% mercury chloride (Merck) in toluene.

Column treatment

The treatment of the different columns used evaluated here coincides basically with O'Reilly's [44], except for the temperature, which depended on the column used. Thus, with columns of low polarity (BP-1 and BP-5), injections of the mercury chloride solution were made at an oven temperature of 115°C, whereas with high-polarity columns (BP-20) these injections were made at 200°C. The difference is explained by the temperature at which the separation of the compounds in question is to take place, which must always be higher than the above treatment temperature. With the BP-1 and BP-5 columns the separation temperature is 90°C and with the BP-20 type it is 175°C. According to our experiments, if treatment is carried out at a temperature 25°C higher than the separation temperature, the conditions were found to be more efficient, the system became cleaner sooner and the effect of the treatment lasted longer.

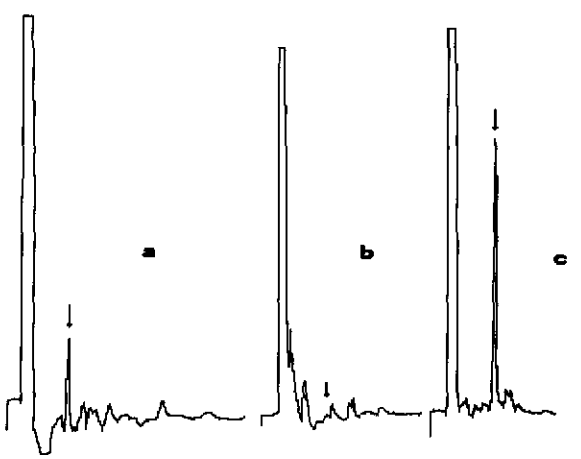


Fig. 1. Chromatograms of (a) toluene after recent treatment of the column, (b) toluene when the column is ready for analysis and (c) standard solution of methylmercury in toluene (all injections made in a BP-1 column).

Procedure for the analysis of real samples

The basic procedure used for the speciation of organomercurials in mussel samples and other materials of biological origin and in samples of marine sediment was described by Hight and Corcoran [42]. The changes made involve the extract volumes in the final stages of the process (four times lower than recommended by Hight and Corcoran), in order to improve the sensitivity, which in this work is *ca.* 50 ng/g expressed as methylmercury chloride (as opposed to 250 ng/g reported by Hight and Corcoran).

RESULTS AND DISCUSSION

Effect of previous treatment of columns

The effect of the previous treatment of the columns is decisive. If it is not applied, the injection of methylmercury chloride in toluene very often do not produce a visible peak on the chromatograms. Curiously, however, when a competely new column was mounted in the chromatograph and conditioned in the usual way as recommended by the supplier, the first injections of methylmercury standard solutions showed a relatively favourable response, especially with BP-5. However, if this or another standard continues to be injected, the height of the methylmercury peak gradually decreased and eventually disappeared. If injections of concentrated solutions of methylmercury were continued to be made, the peak reappeared in the chromatograms, which implies that part of the methylmercury had remained in the column, either retained as such or in a decomposed state, and once a certain level had been achieved, it had an effect similar to the conventional treatment.

On the other hand, after conventional treatment has been applied and once the baseline has been recovered, which takes between 10 and 15 h, during which time the chromatographic system cannot be used, it is necessary to carry out frequent injections of solvent to create a situation in which no trace of the treatment appears in the retention zone of the methylmercury peak. Fig. 1 shows one of these tol-

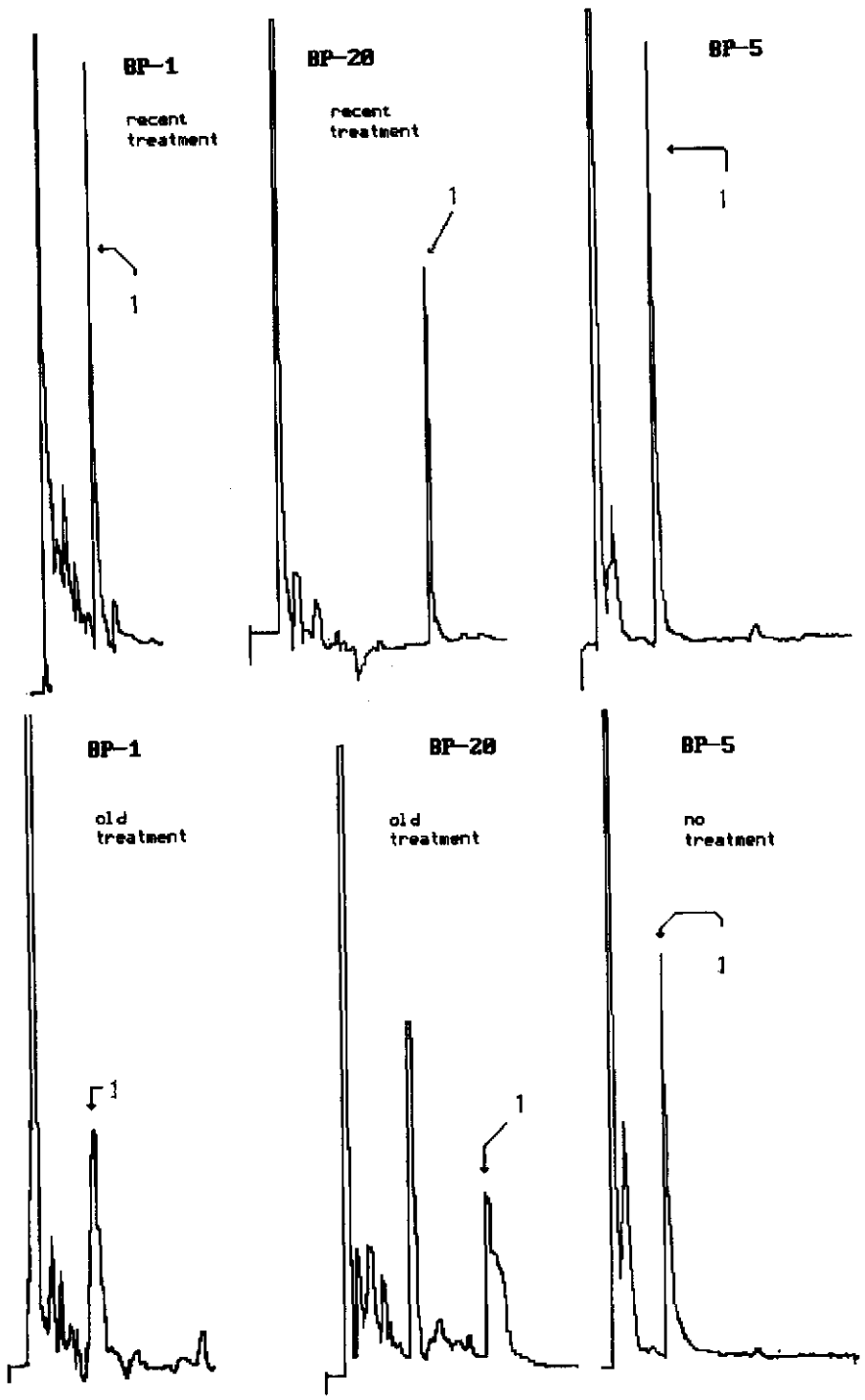


Fig. 2. Response of the different columns to injections of a standard solution of methylmercury (1) in toluene as a function of the column type and the treatment state.

uene injections after treatment, compared with the last in the series of flushing injections (usually 10–12 injections are necessary to obtain an acceptable and reproducible blank) and compared with a chromatogram for methylmercury. It can be seen that the traces of the treatment flushed out by the solvent injections appear at exactly the same retention time as the methylmercury peak, which is surprising, as these species have considerably different vapour pressures. Moreover, it is obvious that we cannot attempt to calibrate the system, which should be carried out without fail after each treatment, until the blanks are reproducible and low, meaning that the chromatograph will be unusable for practical purposes for another 2–3 h. Once the optimum situation for calibration has been attained, this should be carried out and then the series of analyses should be started.

After doing several sample injections, the height of the peaks usually tends to decrease. This effect has been described [44,49,50] and interruption of the sequence of analysis has been suggested, in order to carry out one or two "follow-up" treatment injections, so that the system will recover its analytical capabilities and be able to analyse a few more samples. Logically, this is when the baseline has been recovered and the blanks are acceptable. These "follow-up" treatments are useful for a while, until not only a decrease in peak height with the injections but also a deterioration in peak shape are observed. This means that the treatment has lost its effectiveness and that a completely new treatment is required. Thus, some workers [42] distinguish between "long" and "short" treatments, the latter being the treatments we have termed "follow-up". The chromatograms in Fig. 2 show some of the situations described above.

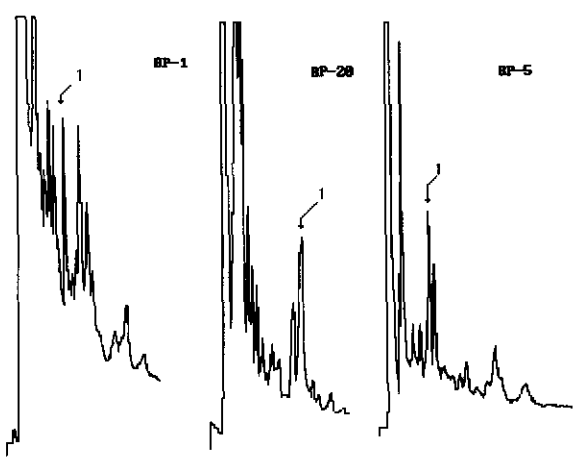


Fig. 3. Chromatograms of a spiked mussel sample as a function of the column type. For chromatographic conditions, see table I. Peak 1 = Methylmercury.

Some workers [50] even recommend treatment injections before analysing each sample in order to eliminate the variability in the time during which treatments remain effective.

In spite of the numerous objections that may be raised against this methodology, our experience has shown that the nature of the stationary phase used affects the way in which the treatments should be carried out, especially regarding duration and efficiency.

The chromatograms in Fig. 3 show the methylmercury peaks (spiked real mussel samples) obtained using BP-1, BP-5 and BP-20 columns with a polyimide covering after finishing the respective treatments and ensuring the repeatability of the blanks. Here, the three columns are seen to produce excellent separations after treatment. However, the duration of the treatment with the BP-20 columns is much shorter, and in practice, this (long) treatment must be repeated at least once every 48 h, and not more than 20–30 samples could be analysed between treatments.

After several months of work (about every 1000–4000 injections, depending of the type of column used, a number of which will have corresponded to the treatments) the shape of the peaks show that the treatments are no longer efficient and, therefore, the column has been rendered useless. As bonded-phase columns can be washed relatively easily, we attempted to "regenerate" these columns by flushing them with different solvents. The best results were obtained by flushing with acetone, although the quality of the peaks after regeneration was far from satisfactory.

Logically, if we compare the behaviours of the three types of columns considered, it is obvious that the more frequent treatments required by the BP-20 column reduce its effective lifetime. Also, for the same reason, the contamination of the electron-capture detector is much greater when this column is used. This is also a determining factor in the selection of the type of column.

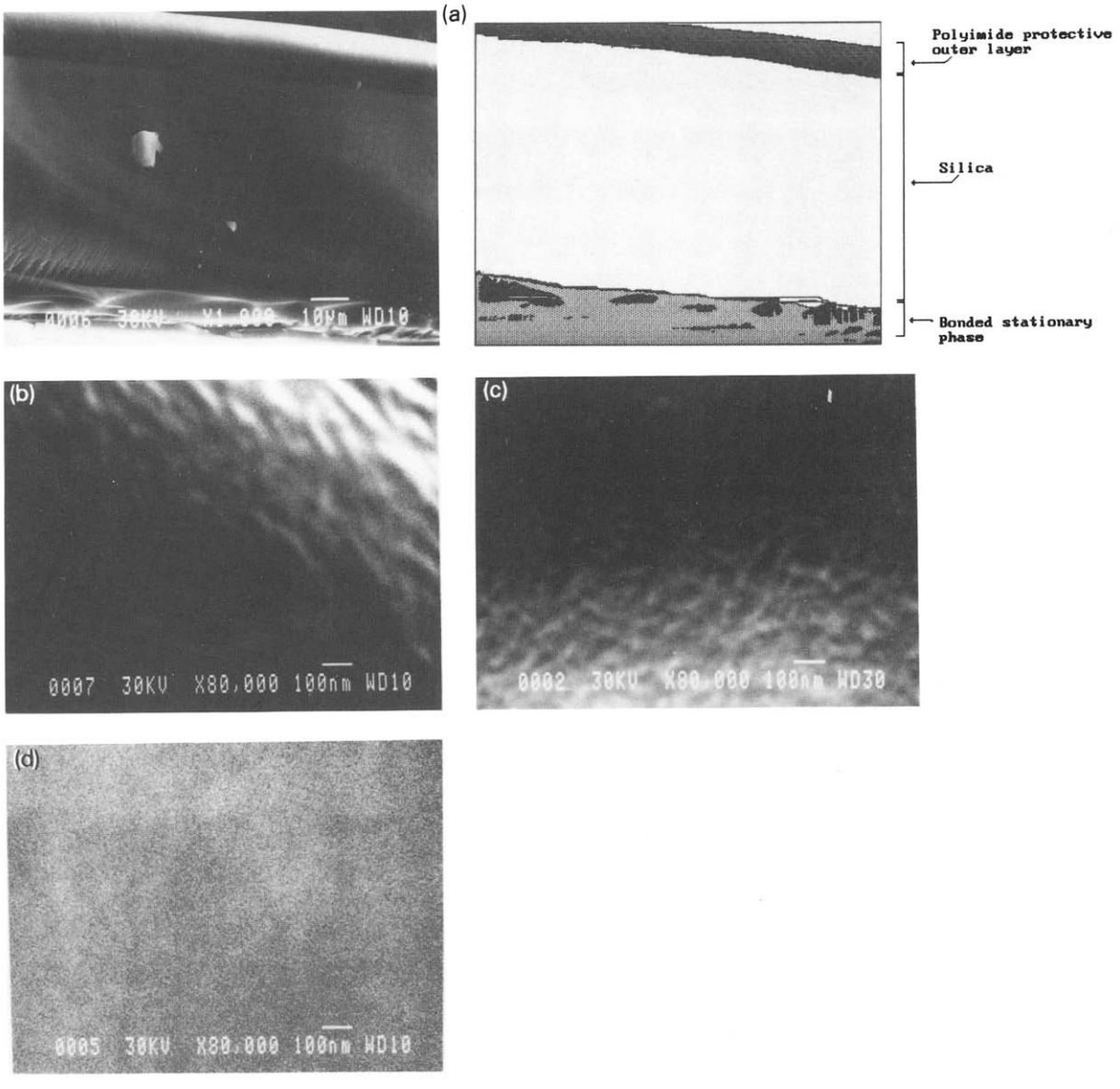


Fig. 4. Electron microscope photographs of (a) cross-section of a new BP-1 column, (b) inner surface of a new BP-1 column, (c) inner surface of a barely used BP-1 column after treatment and (d) inner surface of the same column after several treatments and analyses.

For the purpose of understanding these phenomena, the inner surface of the columns was studied by electron microscopy and the results are shown in Fig. 4. Fig. 4a shows a cross-section of a BP-1 column (bottom of the photograph) where we can observe the appearance of the bonded-phase layer. Fig. 4b, c and d are the corresponding cross-sections of the inner surface of (b) a new BP-1 column never used before, (c) the same column after being subjected to three treatments with mercury(II) chloride for 1 month of work and (d) the same column after having been rejected (useless after numerous treatments). The appearance of the surface of the bonded phase is clearly different in Fig. 4d, al-

though differences are already noticeable between Fig. 4b and c, which implies that the effect of the treatment on the condition of the surface is really noteworthy and is quickly demonstrated.

Based on these results, our hypothesis accepts the fact that there are active points on these columns at which the methylmercury may connect and attach itself irreversibly to the silanol groups on the silica wall of the column (Fig. 2). Logically, when the column is new, the number of active points is relatively small, which allows us to carry out injections and obtain acceptable results without the need for any treatment at all. When the number of active points increases, they must be blocked by means of injections of mercury(II) chloride. However, these injections also destroy the phase, which means that as the column is used more and more, it becomes necessary to carry out treatments at more frequent intervals. Finally (Fig. 4d), the stationary phase is almost non-existent and is seen to be clearly discontinuous, so the effect of the treatments barely last long enough to carry out the calibration injections.

If this hypothesis is correct, it would seem logical to assume that there are two major effects when a particular stationary phase is used: the nature of the phase and its effect on the uniformity of the bonded layer, and the thickness of the bonded layer.

In the first instance, considering the molecular structures of the different phases tested (see Fig. 5), it seems logical to accept an order of efficiency of BP-5 > BP-1 > BP-20 phases. In the latter instance, experiments were done using a BP-5 column with a thicker bonded layer (Alltech Non-Pakd AT-5, 30 m × 0.54 mm I.D., 5.0-μm film thickness). The results obtained with this column were completely satisfactory and so far it has been used in the routine analysis of mussel samples for several months without needing any treatment at all, which confirms the hypothesis that was formulated.

Effect of the external covering of the column

In recent times there has been a tendency to replace fused-silica capillary columns with a protective covering of polyimide with equivalent columns having a protective layer of aluminium. This type of covering has some advantages from a mechanical point of view, and especially when nitrogen–phosphorus detectors are used. In this instance, this type of column was initially used in the determination of organomercurial compounds because it was available in the laboratory at that time. However, the results showed that it was impossible to obtain peaks for methylmercury even after prolonged treatments. After a series of tests, we found that the

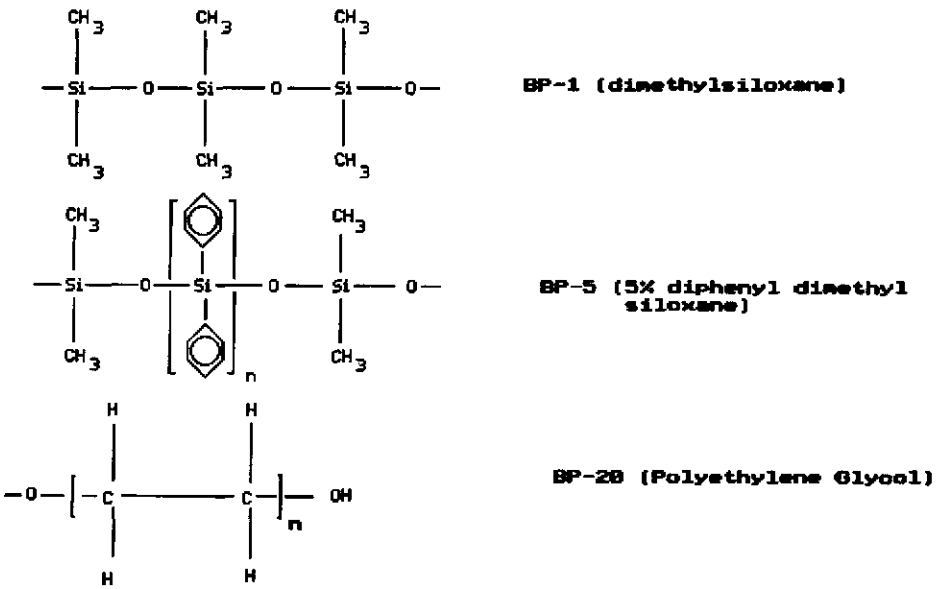
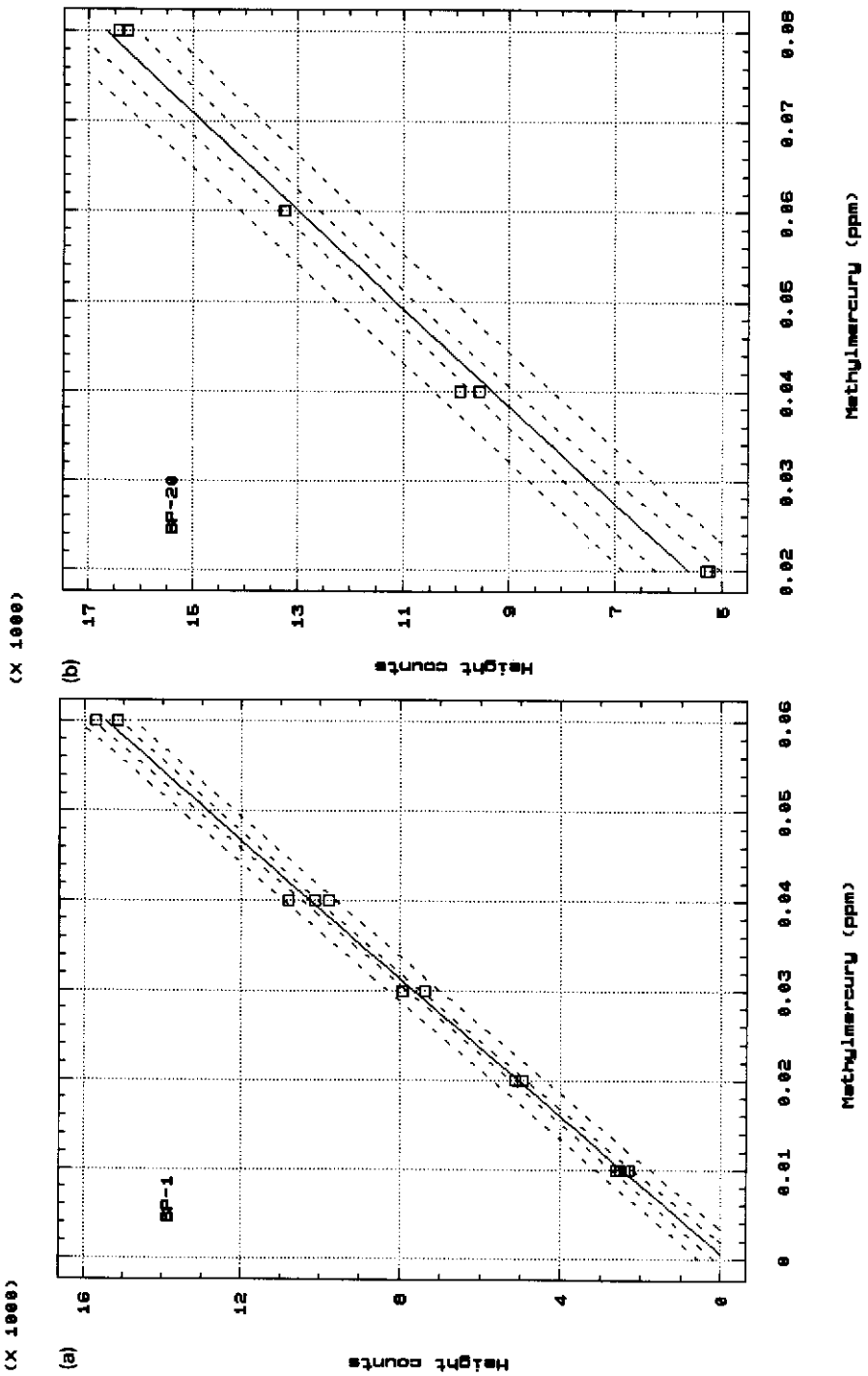
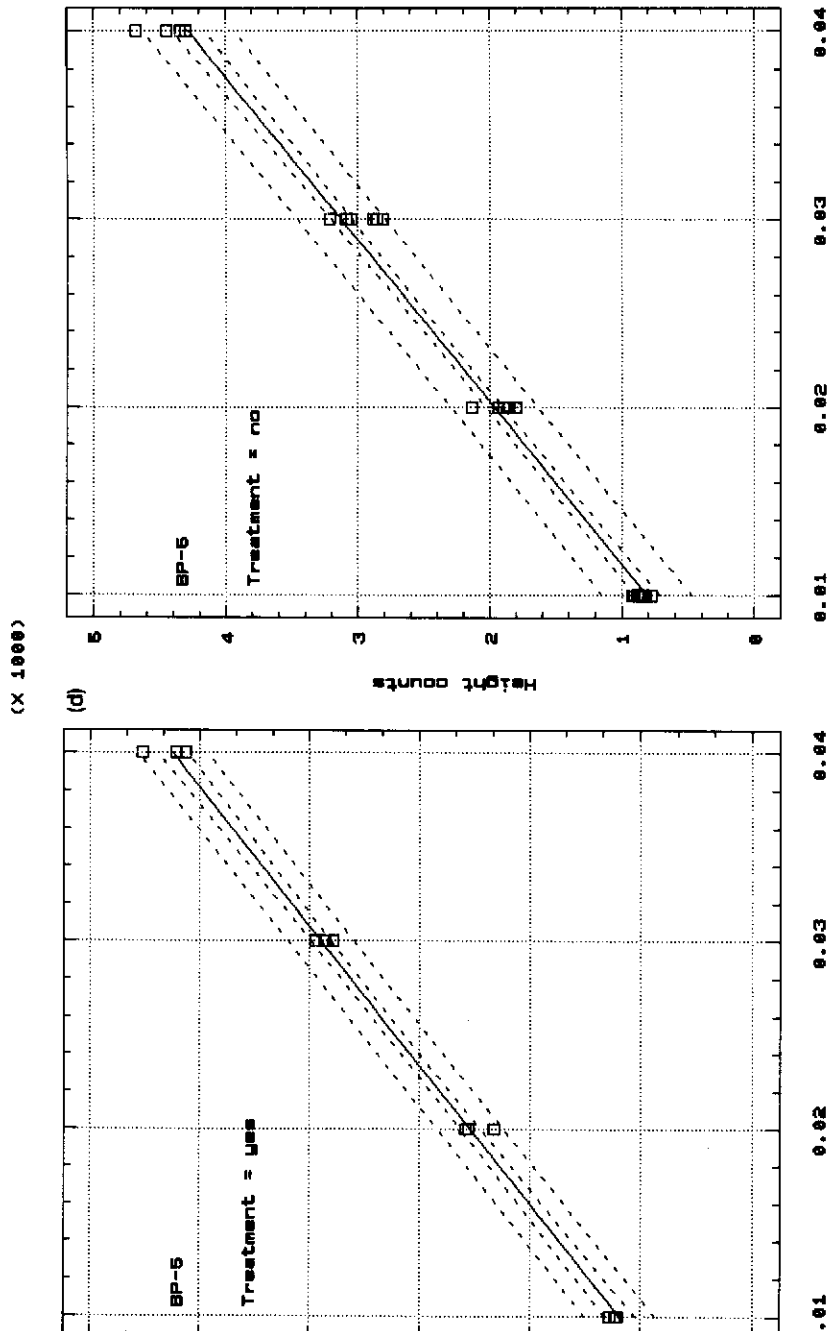


Fig. 5. Structures of the stationary phases considered.


(a)
(X 1000)
Height counts
16
12
8
4
0
BP-1
0
0.01
0.02
0.03
0.04
0.05
0.06
Methylmercury (ppm)
(b)
(X 1000)
Height counts
17
15
13
11
9
7
5
BP-20
0.02
0.03
0.04
0.05
0.06
0.07
0.08
Methylmercury (ppm)



(Continued on p. 78)

Fig. 6.

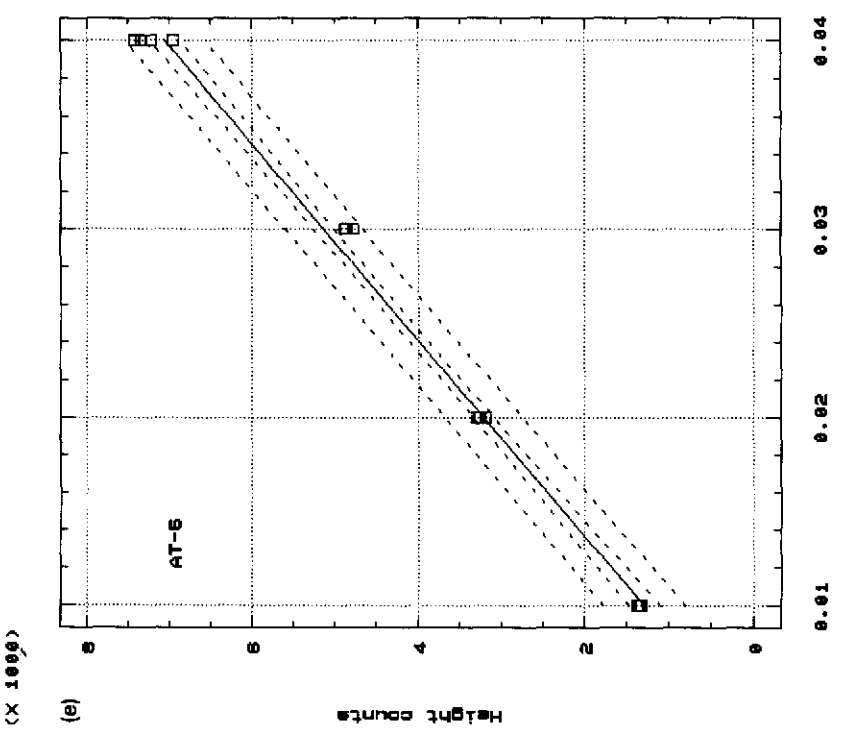


Fig. 6. Calibration graphs for methylmercury as a function of the column type and treatment.

reason for this behaviour resided in the external covering of the column in the length (barely 2 mm) that the column penetrates the injector body. The presence of metallic aluminium at high injector temperatures causes all mercury compounds in the injector to be reduced to metallic mercury, which means that no peaks are observed. Consequently, the use of this type of column was concluded to be impossible in analyses for compounds containing mercury.

Effect of the type of column on the response linearity of the chromatographic system

In addition to the practical reasons for the selection of a particular stationary phase or column type explained earlier, another study was carried out on the effect on the linearity of response of the system.

In order to do this, a multi-level point-to-point calibration (external standard method) was carried out. In Fig. 6 the respective calibration graphs are shown (together with 95% confidence and predictions bands) for the columns tested. With the BP-5 column, there is also a calibration graph corresponding to its first few days of life, when the column has not undergone any treatment. It can be seen that a linear calibration graph can be obtained also in this situation. The regression coefficients are BP-1 0.9981, BP-20 0.9960, BP-5 (no treatment) 0.9930, BP-5 (with treatment) 0.9970 and AT-5 0.9958. Hence, when treatment was administered, the different types of columns tested showed almost identical results in terms of linearity of response. On the other hand, on comparing the calibration graphs for the BP-5 column, it can be appreciated that the absolute response to methylmercury is higher when treatment had been applied. Note also that the linearity range is greater when using the BP-1 and BP-20 columns. This is because the response to methylmercury in these columns was lower than with the BP-5 column, enabling a larger dynamic range to be used. As the AT-5 column shows identical results, and for the reasons cited earlier, it must be concluded that this type of column is the most suitable for routine analysis.

CONCLUSIONS

The results presented here suggest that columns having a substantial stationary phase thicknesses must be used in the determination of organomercurial compounds by gas chromatography. When more common columns used in the laboratory (1.0-μm phase thickness) are applied, they must undergo frequent treatments with mercury(II) salts in order to obtain satisfactory separations and reproducible results. Among these, heavy phases of low polarity may behave better than polar phases in terms of the effect and duration of the treatments, for which reason they are recommended for use in occasional analyses for these compounds. However, this type of treatment has negative effects on the effective life of the columns and on the contamination of electron-capture detectors.

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