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GAS CHROMATOGRAPHIC DETERMINATION OF METHYL AND ETHYL MERCURY: "PASSIVATION" OF THE CHROMATOGRAPHIC COLUMN

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SUMMARY

A conditioning procedure for chromatographic columns is described in which a benzene solution of mercuric chloride is repeatedly injected onto columns of diethylene glycol succinate; hitherto unparalleled column efficiency is demonstrated for the determination of methylmercuric and ethylmercuric compounds. More than 2700 theoretical plates are attainable, with an absolute detection limit of about 0.2 pg of methylmercuric chloride per injection. After treatment, peak areas are reasonably stable but do tend to decrease about 2–6% over a 4- to 5-h period. The beneficial effects of the treatment are only temporary, however, and it must be repeated daily; the cycle of improvement and subsequent decline in column efficiency and sensitivity seems to be repeatable indefinitely. Fundamental aspects of the chromatography involved and its practical application to the analysis of fish are discussed in detail.

INTRODUCTION

Since the pioneering work of Westöö in Sweden[1–3] and of Sumino in Japan[4,5], considerable effort has been expended in the development of reliable, precise, and sensitive methods for the gas chromatographic determination of methylmercuric chloride (MMC) and ethylmercuric chloride (EMC), particularly in fish and in other biological samples. Recent reports on the chromatographic determination of organic mercury compounds include those of Cappon and Smith[6–8], Watts *et al.*[9], and Goolvard and Smith[10]. The variety and ingenuity of the analytical procedures developed, from sample treatment to the determinative step, attest to the ongoing need for alkyl mercury analyses and the continuing difficulty with analytical methodology. Variations of sample-preparation procedures include use of a radioactive methyl mercury tracer to correct for incomplete recoveries[6,11]; EMC as an internal standard[9,10]; alkaline digestion of the sample[6–8] in contrast to direct acidification and extraction; thiosulfate or cysteine re-extractions to minimize background peaks[1–3,6–8,10,12]; extraction of methyl mercury as the chloride, the bromide, or the iodide; and cupric ion[3,13] and urea[11] to increase recoveries of methyl mercury.

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Despite the diversity of sample-preparation procedures, the determinative step invariably involves gas chromatography with an electron-capture detector. A wide variety of stationary phases have been recommended for use in methyl mercury determinations: diethylene glycol succinate (DEGS)[4,5,9], OV-17 + QF-1[6,8], phenyldiethanolamine succinate[3,12,14–16], ethylene glycol adipate[10], butanediol succinate (BUDS)[4,5,17], Carbowax 20M[1,2,13], and polyethylene glycol succinate[18]. All of these columns have exhibited in some laboratories one or more of the following deficiencies: (a) poor and often variable response to MMC or EMC because of apparent interactions with the column or their decomposition on it; (b) moderate to very severe tailing; (c) poor column efficiency, which can then lead to problems with interferences. On the basis of calculations made with published chromatograms or from statements in reports, the number of theoretical plates for MMC often seems to be only about 100–200[6,8,12–14] or 300–500[1,2,5,15,16]; the highest number of theoretical plates reported is about 900 on 5% BUDS[4] and 1200 on 15% DEGS[9]; (d) very long times to initially condition the column, as much as 3–6 days in some cases[9,10,12,14]; (e) a variable decrease in the peak areas (heights) for MMC and EMC from injections of fish extracts, although MMC and EMC standard solutions furnish good chromatograms before injection of the sample extracts.

In 1979, this laboratory sent samples of fish to a dozen cooperating laboratories to analyze for methyl mercury by the method of Watts *et al.*[9]. Eight of these laboratories experienced little or only moderate difficulty with the method and reported results that agreed very well. Four, however, experienced severe difficulties with the chromatographic column specified (15% DEGS) although excellent results had been obtained previously in this laboratory over a several-year period. For that reason, we decided to investigate in more detail the chromatographic behavior of MMC and EMC on DEGS columns. Our detailed findings are presented in this report.

EXPERIMENTAL

The gas chromatograph used for most of the work was a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 5830A, equipped with Model 18803A ^{63}Ni electron-capture detector; some work was performed on a Hewlett-Packard Model 5710A gas chromatograph with Model 18713A detector. Injector and detector temperatures were maintained at 200 and 300°C, respectively. Carrier gas (argon–methane, 95:5) flow-rates were 30 and 60 ml/min for 2- and 4-mm I.D. columns, respectively. All columns were 0.25-in. O.D. silanized glass. Because the column effluent contains mercury compounds and trace radioactivity, it must be properly vented.

Twelve DEGS columns, differing in length, inner diameter, solid support and loading level were prepared and evaluated during the course of this study with both commercially prepared and "home-loaded" packing. Columns were packed no closer than about 2 cm to the threaded ends of the high temperature injection and detection ports because decomposition of nonstabilized DEGS, in particular, tends to occur at elevated temperatures, resulting in high and noisy baselines. All the columns produced more or less suitable results. Most of the results reported here were obtained with a (6 ft. × 2 mm I.D.) column packed either with 5% (stabilized) DEGS-PS on 100–120 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.) and operated at 145°C or with 10% (stabilized) DEGS on 90–100 mesh Chromosorb W AW (Analabs,

North Haven, CT, U.S.A.) and operated at 160–170°C because these columns afforded generally the highest performance of those prepared. For convenience, these two particular columns will be referred to as the "5% DEGS" and the "10% DEGS" columns throughout this report.

Stabilized DEGS was conditioned by flushing the column with carrier gas for 0.5 h, heating at 100°C for 1 h, then increasing the temperature at a rate of 4°C/min to 225°C and maintaining it overnight —all with normal carrier-gas flow[19]. Columns of nonstabilized DEGS (HI-EFF-1BP) were conditioned by modifying the recommendations of Watts *et al.*[9]: 0.5 h at room temperature, 2 h at 100°C, overnight at 200°C and 2 h at 225°C— all with normal carrier-gas flow.

Mercuric chloride, bromide, and iodide were reagent grade (Fisher Scientific, Pittsburgh, PA, U.S.A.). The acetone used to wash fish samples was distilled-in-glass (Burdick & Jackson, Muskegon, MI, U.S.A.).

The other chemicals, apparatus, and procedures used in this study have been reported elsewhere[9].

Generally, 5-μl samples were injected into the chromatograph for analysis.

RESULTS AND DISCUSSION

Although a column of nonstabilized DEGS (HI-EFF-1BP) prepared and conditioned in this laboratory in the manner recommended by Watts *et al.*[9] functioned satisfactorily and produced about 950 theoretical plates [$N = 16(t_R/w_b)^2$, where t_R = retention time and w_b = peak width at base] for MMC, a number of other laboratories experienced severe difficulties with this procedure —high and noisy baselines that would not decline even after several days. It was noted that the HI-EFF-1BP column prepared was a light tan after conditioning, and another such column, used successfully by Watts, was quite brown[20]. It seemed, therefore, that the conditioning procedure recommended by Watts, which followed suggestions by Westöö[1,2], probably involved a partial pyrolysis which was somewhat difficult to reproduce. For this reason, we chose to investigate some of the newer, stabilized varieties of DEGS coated on modern high-quality supports.

Initial results with stabilized DEGS were very disappointing. After the initial overnight conditioning, standard solutions of MMC and EMC exhibited very small, broad, tailed peaks and actual overlap of the two peaks with an efficiency of perhaps 30–40 plates.

We then decided to investigate the possible beneficial aspects of treating the column with high levels of mercuric compounds. There are several precedents for this approach: Westöö[3] noted that sample solutions containing sulfur compounds apparently poisoned the chromatographic system, which could be rejuvenated by injection of benzene solutions of methoxyethylmercury iodide or mercuric chloride. Kamps and McMahon[12] reported the necessity of conditioning their column initially by injecting solutions containing high levels of organic and inorganic mercury compounds; degradation of column performance with time could be reversed, for some unknown reason, by injection of extracts of certain blood samples. Uthe *et al.*[13] recommended rejuvenating (Carbowax) columns for MMC determinations by injecting aqueous 3 *M* potassium iodide and waiting for an hour. Schafer *et al.*[15] found it necessary to inject 5 μl of a 1 mg/ml solution of mercuric chloride in benzene twice

before the injection of each sample and several standards in order to obtain reproducible results. Finally, the analytical methods manual of the Environmental Protection Agency[14] specifies an initial 4-day conditioning of the column, including a sequence of 12 injections of a solution containing high levels of organic and inorganic mercury compounds.

Injection of mercuric chloride solutions in benzene at the milligram per milliliter level onto DEGS columns produced a peak with the same retention time as MMC; the peak area was about 0.05% that of an equal quantity of MMC. (Goolvard and Smith[10] have already noted that mercuric chloride produces a small interferent effect in MMC determinations.) With repeated injections of mercuric chloride this peak shifted to a shorter retention time, sharpened, increased in height considerably, and exhibited much less tailing. Fig. 1 illustrates the effects of mercuric chloride conditioning on a 10% DEGS column. In this particular case, injections of an MMC and EMC standard solution (5 μl of 0.20 μg/ml of each) onto a new column produced hardly any detector response (Fig. 1A). The very small, broadened peak at 6–9 min corresponds to MMC; EMC elutes at a much longer retention time. After repeated injections of a 1 mg/ml solution of mercuric chloride, the peak corresponding to MMC essentially stabilized; and about an hour later, after equilibration was reached,

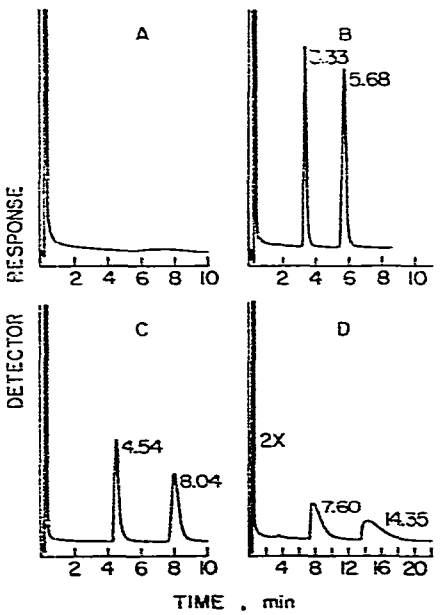

Fig. 1. Typical chromatograms for 5 μl of a standard solution of 0.20 μg/ml methylmercuric chloride (MMC) and ethylmercuric chloride (EMC) in benzene before and after treatment of the column with mercuric chloride (five 20-μl injections of a 1 mg/ml solution of mercuric chloride in benzene). Retention times are listed to the right of the peaks for MMC and EMC. MMC elutes first. Column: 10% DEGS on 90–100 mesh Chromosorb W AW operated at 170°C and 30 ml/min carrier-gas flow-rate. A, Chromatogram on a freshly (overnight) conditioned column before mercuric chloride treatment; very little response is apparent. B, Chromatogram obtained about 1.5 h after mercuric chloride treatment had begun and column had stabilized; note the dramatic improvement in sensitivity and that only a slight amount of tailing is evident, and only on the EMC peak. C, Chromatogram obtained on the day after the mercuric chloride treatment. D, Chromatogram obtained the second day after mercuric chloride treatment; detector-amplifier sensitivity increased to twice that for the other chromatograms.

injection of standard solutions produced chromatograms such as the one shown in Fig. 1B. Note that there is almost no tailing evident in chromatogram 1B, compared to what is often seen for MMC[1,2,13–15]. A freshly treated 10% DEGS column, such as that used in Fig. 1, often produced upwards of 2700 plates and somewhat more than this for EMC. Resolution between MMC and EMC was typically about 5–6. The net effect of mercuric chloride treatment was a dramatic reduction in the specific and strong interaction of organomercurials with the column, as evidenced by the disappearance of tailing. For this reason, we prefer to call the treatment a *passivation* rather than an "activation", a term sometimes used to refer to a special treatment of a column, or a "conditioning", which usually refers to the initial elevated-temperature purging of a new column.

Generally, about five 20-μl injections of a 1 mg/ml solution of mercuric chloride at 5-min intervals were required to produce the desired degree of passivation for a freshly conditioned DEGS column. Over the next 1.5 h or so, the peak areas for MMC and EMC increased, after which they began to decrease and level off. Peak areas were then stable to within about 2–6% for the next 4–5 h.

In addition to the peak with the same retention time as MMC, injection of mercuric chloride produced several very broad peaks at longer retention times (about 50 min on a 10% DEGS column operated at 170°C, about 120 min for 5% DEGS at 145°C), which sharply decreased in area with successive injections. After elution of these broad peaks, the peaks for MMC and EMC sharpened markedly and increased in height by a factor of 2–3. It is the passage of these broad peaks that finally resulted in a more or less stable column. Although the natures of these peaks are unknown at present, it is suspected that they may represent a flushing of adsorbed compounds (possibly containing sulfur) from the column.

It seems likely, therefore, that at least one important cause for the severe tailing of organomercurials is an interaction with compounds contained within the column in addition to possible interaction with active sites on the solid support. For example, there was little difference in the behavior of columns if the support was silanized (Chromosorb W HP) or simply acid-washed (Chromosorb W AW); after overnight conditioning, columns still required mercuric chloride treatment, and the decreases in peak areas with time after mercuric chloride treatment were similar to those in Fig. 1C and D. Several of the DEGS columns prepared, as well as some others (5%, 1,4-BUDS, 3% ECNSS-M and 10% EGSS-X), exhibited fairly stable chromatograms for standard solutions, but then showed a progressive decrease in the MMC and EMC peak areas with injection of fish-sample extracts. Sometimes this decrease in sensitivity was reversed after overnight standing at operating temperatures or after repeated injection of standards; sometimes it was not. Some columns had a reasonable stability for MMC but exhibited a pronounced drop in sensitivity for EMC on injection of fish extracts.

Interaction with the support cannot be totally excluded, however. The 5% DEGS column tested in this study always showed a small amount of tailing, even with extended mercuric chloride treatment (generally a maximum of 1200–1800 theoretical plates), whereas the 10% DEGS column could be treated to the point where no tailing at all was visible (2200–2700 plates typically; asymmetry, 10% peak height criterion, could usually be reduced to 1.3 or less). In the latter case, the higher loading presumably more effectively insulated the diatomaceous surface.

The range for typical absolute detection limits ($S/N = 2$ criterion, based on baseline noise levels) for "clean" standard solutions has been about 0.2–0.5 pg of MMC or EMC. Fig. 2 is a chromatogram for the injection of 25 pg each of MMC and EMC. Even at this sensitivity, the baseline noise is hardly visible. The operational limit of quantitation for the overall Watts method (based on a 5-g fish sample, 25 ml final solution volume and injection of 5 μl) is, therefore, about 2–5 ppb in the original sample.

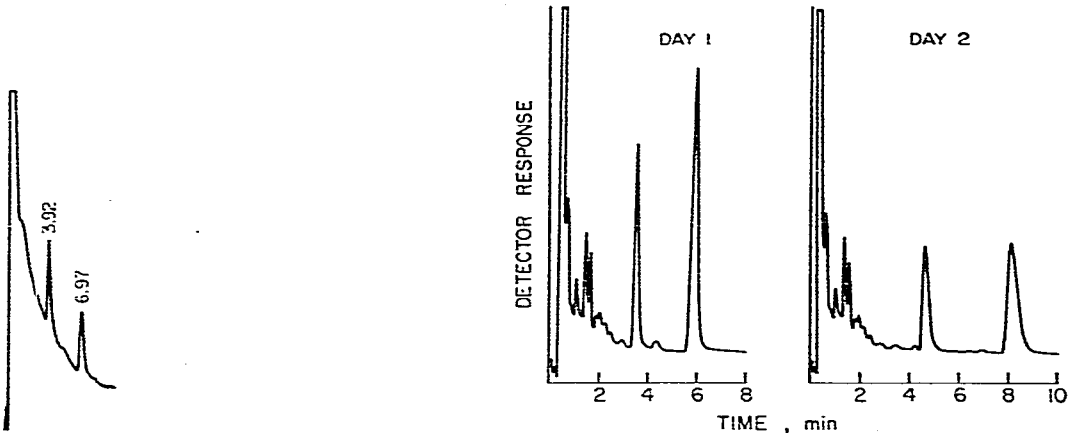

Fig. 2. Chromatogram of 25 pg each of methylmercuric chloride (MMC) and ethylmercuric chloride (EMC) (5 μl of 0.005 μg/ml) in benzene. Column: 5% DEGS-PS on 100–120 mesh Supelcoport operated at 145°C and 30 ml/min carrier-gas flow-rate. Retention times (min) for the peaks are shown. MMC elutes first.

Fig. 3. Chromatograms of a benzene extract of a tuna sample containing a natural level of 0.50 ppm of mercury (as methyl mercury) on the day of (Day 1) and the day after (Day 2) mercuric chloride passivation of the column. A small aliquot of a concentrated solution of ethylmercuric chloride (EMC) was added to the benzene extract to bring its concentration to 0.20 μg/ml. Column: 10% DEGS on 90–100 mesh Chromosorb W AW operated at 160°C and 30 ml/min carrier-gas flow-rate.

Time variation of column sensitivity and resolution

The beneficial effects of mercuric chloride passivation of DEGS columns, unfortunately, are only temporary. Typically, overnight standing of a column at the operating temperature of 170°C (10% DEGS column) caused decreases of about 10–35% in the peak heights, peak areas and the number of theoretical plates. Retention times increased by about 30%, and peak tailing was also much more evident. On the second day after the mercuric chloride treatment, column performance was much worse; peaks were quite small and drawn out. Fig. 1 illustrates these changes for chromatograms of standard solutions.

This deterioration in performance occurred whether or not fish extracts were injected onto the column and was invariably worse for EMC than for MMC. The higher the column temperature, the faster this degradation occurred. For example, a 5% DEGS column maintained at 145°C gave usable results for 3–4 days, whereas a column at 160°C was good for only about 2 days. The entire cycle of treatment with mercuric chloride, the resultant improvement, and the subsequent decline in column performance seemed to be repeatable indefinitely.

Older columns that had been subjected to a number of mercuric chloride injections over a period of time exhibited a better time stability than newer columns.

Although it is rather annoying to have the MMC and EMC peaks shift to longer retention times on the day after mercuric chloride treatment, these shifts do provide one considerable analytical benefit. Particularly for those samples that are apparently very low in MMC, there is always the concern that the "peak for MMC" may actually be due to some other component in the sample —an interferent that happens to have (nearly) the same retention time. Or a particular sample may simply have a very large interferent peak where MMC "normally" occurs. It would be most extraordinary for any interferent peak to shift by exactly the same amount as the MMC (or EMC) peak on the day after mercuric chloride treatment. This procedure provides additional evidence for the presence of MMC or EMC in a sample.

In Fig. 3, the chromatograms of one particular fish extract on the day of and the day after mercuric chloride-column passivation clearly illustrate the MMC and EMC peak shifts. The large, sharp peaks for MMC and EMC at 3.6 and 6.0 min, respectively, shifted to 4.7 and 8.2 min on the second day, whereas the background peaks did not change at all.

Mercuric chloride-treatment procedure to increase sample throughput

The mercuric chloride-passivation procedure recommended and the time for the column to equilibrate afterwards require almost an entire morning. One is therefore limited to about 50–60 injections of sample and standard (if no EMC or late eluters are present) per day. In order to increase the sample throughput, several procedures were tested to improve stability. The deliberate addition of a small, constant level of mercuric chloride to all samples and standards to provide a more or less continuous flow of the passivation agent did not seem to improve long-term column stability. Moreover, mercuric chloride gave a small positive interference for MMC. Loading of a few milligrams of solid potassium chloride into the front end of the column, to serve as a chloride source, also had no discernible effect.

An improvement was obtained, however, by lowering the column temperature to 115°C at the end of a working day, waiting a few minutes, and then injecting 20 μl of a 1 mg/ml solution of mercuric chloride in benzene. The next morning, the column was raised to its operating temperature and was stable and ready for use within about 45 min. The overnight temperature of the column is somewhat critical: Too low a temperature requires a longer time for stabilization the next morning; too high a temperature results in somewhat lower column performance.

Table I illustrates the day-to-day variation in the average peak areas for a standard solution of MMC and EMC on a column injected each night with mercuric chloride. On a day-to-day basis, the peak area for MMC is reasonably constant; the standard deviation of the composite average is only about twice that of the deviation within a day. The peak areas for EMC vary considerably more than those for MMC from day to day; the standard deviation of the composite average is about four times as great as that for MMC. Within a day, however, the precision for EMC peak areas is about the same as that for MMC.

The data in Table II were collected to illustrate that lowering the column temperature to 115°C and injecting mercuric chloride does not adversely affect analyses. In this series of analyses two subsamples (*ca.* 60 g each) of a large sample of

TABLE I

DAY-TO-DAY VARIATION IN AVERAGE PEAK AREAS FOR INJECTION OF 1 ng EACH OF METHYLMERCURIC (MMC) AND ETHYLMERCURIC (EMC) CHLORIDES

Column: 6 ft. × 2 mm I.D. silanized glass, packed with 5% DEGS-PS on 100–120 mesh Supelcoport, operated at 145°C and 30 ml/min carrier-gas flow-rate; 5-μl injections of 0.20 μg/ml standard. At the end of each working day, 20 μl of a solution of 1 mg/ml of mercuric chloride in benzene was injected into the column after the temperature was lowered to 115°C. At the start of the working day, the column temperature was raised to 145°C and allowed to stabilize for about 30 min prior to injections.

Age of column (days)	*Number of injections*	*Average peak area (standard deviation) (integrator counts × 0.01)*	
		MMC	*EMC*
1	3	9902 (71)	9023 (214)
2	6	9350 (402)	7417 (120)
3	8	9469 (261)	8986 (99)
4	5	9675 (93)	10692 (193)
8	6	9553 (115)	11983 (125)
14	7	9124 (141)	–
16	5	9240 (237)	8629 (373)
17	5	9821 (264)	10616 (652)
	5	9271 (199)	9223 (312)
21	4	8754 (51)	8873 (65)
22	6	9148 (154)	9769 (144)
23	3	9209 (68)	10783 (190)
	5	8994 (137)	10165 (162)
24	6	–	11203 (406)
Grand average		9347 (330)	9797 (1252)
Median of standard deviations		(141)	(190)

homogenized oil-packed tuna were taken; one was spiked with MMC. Two splits of each subsample were taken and extracted by the method of Watts *et al.*[9]. The benzene extracts were then analyzed on three separate days. The data illustrate good day-to-day and split-to-split precision for the overall analysis, as well as good recovery of the added MMC. Because there was sometimes a small, gradual decrease (*ca.* 2–6%) in peak areas during the working day, it is recommended that one standard solution of comparable concentration be injected for no more than two injections of sample to maintain accuracy of results.

After a 10% DEGS column is used for 3–4 h at 160–170°C, an additional 20-μl injection of a 1 mg/ml solution of mercuric chloride followed by a 1-h waiting period helps to maintain column sensitivity.

With this overnight-conditioning treatment, the number of sample and standard injections that can be made during one working day is effectively doubled.

Nature of the on-column reaction

A number of reports[1,4,12,17,21] have indicated that small quantities of all methylmercuric compounds elute at nearly the same retention time and with roughly the

TABLE II

DETERMINATION OF METHYL MERCURY IN TWO SPLITS OF NATURAL AND SPIKED OIL-PACKED TUNA

Two jars of a homogenized tuna sample (*ca.* 60 g each) were taken. The spiked sample was prepared by slowly adding a small volume of a concentrated solution of methylmercuric chloride (MMC) in ethanol to one of them with stirring (after weighing) so that the amount added would be equivalent to 0.396 ppm of mercury in the original sample. The level of mercury (in the form of methyl mercury) in the original sample was found to be 0.40 ± 0.05 ppm by eight laboratories in an interlaboratory study using the method of Watts *et al.*[9]. Column: 5% DEGS-PS on 100–120 mesh Supelcoport operated at 145°C and 31 ml/min carrier-gas flow-rate. Each table value is the average from 2–4 injections of sample and each was compared to the average value from 2–4 injections of a standard solution.

Split	*Day*	*ppm Mercury (as methyl mercury)*		*MMC recovery (%)*
		Natural	*Spiked*	
A and A′	1	0.381	0.796	105
	2	0.378	0.763	97
	3	0.399	0.800	101
Mean ± S.D.		0.386 ± 0.011	0.786 ± 0.020	101 ± 4
B and B′	1	0.402	0.775	94
	2	0.383	0.773	98
	3	0.394	0.790	100
Mean ± S.D.		0.393 ± 0.010	0.779 ± 0.009	97 ± 3

same response, regardless of the anion used in making the standard solutions, Johansson *et al.*[21] have reported that the actual molecular species responsible for the "methyl mercury peak" produced by an electron-capture detector was a mixture of MMC and methylmercuric iodide (MMI), regardless of whether the standard solution injected was prepared from the chloride or from the iodide salt. Nishi and Horimoto[22] reported that large injections ($>10^{-5}$ g) of various methyl mercury compounds eluted with different retention times (iodide < bromide < chloride) with stainless-steel columns of 5% DEGS and thermal-conductivity detection, whereas small injections ($<10^{-8}$ g) with electron-capture detection had identical retention times. With larger injections, the small amount of decomposition that occurs is negligible. With glass columns, injections of 10^{-7}- to 10^{-9}-g quantities of various methyl mercury compounds produced peaks of different retention times as well as different heights, depending on the particular compound injected. These results, as well as the severe tailing often seen in methyl mercury chromatograms, clearly indicate that a partial on-column decomposition and anion-exchange process occurs for methyl mercury. Analogous results have been noted for ethyl mercury and other organomercury compounds.

In order to further delineate what may be happening when a DEGS column undergoes mercuric chloride passivation, a column was heated overnight at 225°C and treated with several injections of a 1 mg/ml solution of mercuric bromide or mercuric iodide. Standard solutions of MMC and EMC in benzene were then injected. The data in Table III show that the retention times for the MMC or EMC peak varied, depending on the particular mercuric halide used to treat the column, and increased in the order mercuric iodide < mercuric bromide < mercuric chloride.

TABLE III

RETENTION TIMES FOR METHYLMERCURIC CHLORIDE OR ETHYLMERCURIC CHLORIDE INJECTED ONTO A COLUMN TREATED WITH MERCURIC HALIDE COMPOUNDS AND FOR VARIOUS ORGANOMERCURIC COMPOUNDS INJECTED ONTO A STAINLESS-STEEL COLUMN

MMI = Methylmercuric iodide, MMB = methylmercuric bromide, MMC = methylmercuric chloride, EMI = ethylmercuric iodide, EMB ethylmercuric bromide and EMC = ethylmercuric chloride.

*This work**				*Nishi and Horimoto*[22]**		
Column treatment	*Compound injected*	t_R *(min)*	$t_R/t_{R,Cl}$	*Compound injected*	t_R *(min)*	$t_R/t_{R,Cl}$ *(min)*
Mercuric iodide	MMC	3.30	0.77	MMI	1.60	0.65
Mercuric bromide		3.57	0.83	MMB	2.15	0.88
Mercuric chloride		4.28	1.00	MMC	2.45	1.00
Mercuric iodide	EMC	5.40	0.74	EMI	2.85	0.62
Mercuric bromide		5.92	0.82	EMB	4.00	0.87
Mercuric chloride		7.25	1.00	EMC	4.60	1.00

* Conditions: 6 ft. × 2 mm I.D. silanized glass column packed with 15% (stabilized) DEGS on 80–100 mesh Chromosorb W AW operated at 170°C and 30 ml/min carrier-gas flow-rate. Each value is the average of 4–7 injections. Quantities injected were 10^{-9} g (1 ng).

** Conditions: 0.3 × 200 cm stainless-steel column packed with 5% DEGS on 60–80 mesh Chromosorb W operated at 150°C and 45 ml/min He carrier-gas flow-rate. Quantities injected were $>10^{-5}$ g.

The relative retention times compare quite favorably to those reported by Nishi and Horimoto[22] for injection of large ($>10^{-5}$ g) quantities of authentic MMI, methylmercuric bromide (MMB) and MMC onto a stainless-steel column of 5% DEGS.

Peak heights and areas for MMC and EMC are also affected by the particular mercuric halide used to treat the column (Table IV). On a 15% DEGS column, peak areas increased in the order mercuric iodide < mercuric bromide < mercuric chloride treatment. There is some correspondence of the relative peak heights shown

TABLE IV

COMPARISON OF PEAK HEIGHTS AND AREAS FOR METHYLMERCURIC CHLORIDE (MMC) AND ETHYLMERCURIC CHLORIDE (EMC) INJECTED ONTO COLUMNS TREATED WITH VARIOUS MERCURIC HALIDE COMPOUNDS

Chromatographic conditions are the same as in Table III. Each value is the average of 4–7 injections. Peak areas are integrator counts × 10^{-2}; peak heights are in mm at an attenuator setting of 2^{10}. Quantities injected were 1 ng.

Column treatment	*Compound injected*	*Peak area*	*Relative peak area*	*Peak height*	*Relative peak height*
Mercuric iodide	MMC	7720	0.57	80.4	0.66
Mercuric bromide		8420	0.62	90.7	0.75
Mercuric chloride		13640	1.00	121.1	1.00
Mercuric iodide	EMC	13580	0.69	90.3	0.83
Mercuric bromide		15044	0.77	104.4	0.95
Mercuric chloride		19580	1.00	109.4	1.00

in Table IV to those reported by Nishi and Horimoto[22] for direct injection of MMI, MMB and MMC standard solutions; however, the correspondence of the relative retention times (Table III) is much closer.

Therefore, treatment of DEGS columns with large (10–100 μg) injections of mercuric chloride either: (a) provides a trace level of chloride ion or molecular mercuric chloride in the column that maintains MMC predominantly in its molecular form throughout the column and minimizes decomposition; or (b) temporarily ties up active sites with mercury, chlorine, or some compound containing either or both. The fact that authentic MMI injected onto a column elutes primarily as the corresponding chloride[21] clearly indicates an on-column reaction with some form of chloride bound to or adsorbed on the column packing. The fact that the performance of mercuric chloride-treated columns degrades after standing at an elevated temperature, even if no samples or standards are injected, indicates that a somewhat nonvolatile compound (or compounds) remains in the column to decrease the decomposition of the organomercury halides and the resultant tailing.

The only DEGS column prepared in this laboratory that did not require mercuric chloride treatment to exhibit reasonable efficiency is precisely the one specified by Watts *et al.*[9]: 15% HI-EFF-IBP on Gas-Chrom P. However, some other laboratories have experienced extreme difficulties with this packing, as mentioned earlier. Gas-Chrom P is unique among the solid supports tested in that it is both acid-washed (with hydrochloric acid) and then base-washed (with sodium hydroxide). It seems reasonable to speculate that Gas-Chrom P (or perhaps just some batches) contains a low level of sodium chloride that serves as a source of chloride to maintain organomercury compounds in a molecular form during passage through the column.

A second effect of mercuric chloride injections seems to be the "flushing" of impurities, quite possibly sulfur compounds[12,13], from the column. Certain DEGS (and other) columns which were prepared maintained reasonable efficiency for several days, as long as only "clean" MMC and EMC standard solutions were injected, but performance degraded as soon as fish-extract solutions were injected. Experiments were performed in which six or seven 50-μl injections of fish extracts were made over a 20-min period in an attempt to load the column with impurities. After a 1-h wait to allow the column to clear, injections of MMC and EMC standard solutions were made; decreased efficiency and increased tailing showed that column performance had degraded. Injection of a 1 mg/ml mercuric chloride solution at this point produced large, broad peaks at about 50 min (at 170°C), and column performance was restored. If a mercuric chloride-treated column was simply left to stand at an elevated temperature without injection of fish extracts, column performance degraded and was then restored by further mercuric chloride treatment, but only a small peak was seen at 50 min after injection of the mercuric chloride.

Clearly, therefore, there are things intrinsic to the column —active sites, perhaps— as well as impurities in fish extracts adsorbed or bonded to column materials that cause decomposition and poor chromatograms for organomercury compounds. The multiplicity of these reactons is no doubt one reason why the precise conditions needed for good chromatographic determinations of organomercury compounds are often still difficult to define after nearly two decades of research in methods development.

CONCLUSIONS

The mercuric chloride-passivation treatment of DEGS columns outlined in this report produces extraordinarily efficient columns. The combination of sharp, non-tailing peaks and the sensitivity of the electron-capture detector result in a routine, absolute detection limit of better than 1 pg of MMC per injection. The practical limit of quantitation is about 2–5 ppb in the original sample, and there is a relatively high freedom from interferences because of the high performance of the column.

Fish extracts have been routinely analyzed by using these treated columns, and they should be useful for other types of samples as well. Long-term use has indicated that these columns are serviceable for months, and that the detector is not adversely affected by repeated injections of a 1 mg/ml mercuric chloride solution. New columns require only an initial overnight conditioning, plus about 3 h for the first mercuric chloride treatment and stabilization; thus the several days of conditioning specified by a number of other reports are eliminated. A new column can be packed, conditioned, and ready for use in less than one day.

The only disadvantages of the treatment are a small increase in the complexity of the procedures, and the requirement that standards be frequently re-injected because of a slow decline in sensitivity over 4–5 h.

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