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Determination of polychlorinated biphenyls in biotic matrices using gas chromatography–microwave-induced plasma atomic emission spectrometry

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Abstract

Basic parameters associated with practical application of gas chromatography coupled with microwave-induced plasma atomic emission spectrometric detection GC–MIP-AED in the determination of seven "indicator" polychlorinated biphenyls (PCBs) in biotic matrices were evaluated. The detection limit for chlorine (Cl-479) was found to be 0.54 pg/s. Under the conditions used for sample analysis (1 μl of purified extract injected into the GC–MIP-AED system represented 2.5 mg of original fat), this value corresponded approximately to 0.15 mg/kg of the respective congeners in fat. The detector response was linear within the tested range of 0.5–10 ng of each injected PCB. The relative standard deviation of repeated injections for the lowest concentration level of 0.5 ng of PCB per injection ranged between 10.5 and 34.4% depending on the chlorine content of the individual analytes. The results demonstrate a high selectivity of chlorine detection. Carbon (C-496) chromatograms recorded simultaneously demonstrated the efficiency of the clean-up step used. Quantitative results (analytes at levels of 0.1–1 mg/kg) obtained with the atomic emission detector did not differ significantly from those recorded with a conventional electron-capture detector.

1. Introduction

Great efforts have been focused on the optimisation of a quantification strategy that would provide an unbiased expression of the polychlorinated biphenyl (PCB) content in biotic matrices. In spite of this fact, no general-purpose approach has been defined to date, although the applicability of various detection techniques has been widely discussed [1]. Electron-capture detection (ECD) is routinely used in analyses for these contaminants. Although ECD provides the most sensitive detection of polyhalogenated compounds, its selectivity is relatively poor. As we have demonstrated previously [2], interferences caused by the presence of various electron-capturing substances (e.g., phthalates) occur and cannot be avoided, regardless of thorough sample extract clean-up. Nevertheless, the main problem in using detection methods such as

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ECD and/or mass selective detection (MSD) is the unequal response factors of individual PCB congeners (even of those with an identical chlorine content). As a result, calibrating congener-specific analysis remains a very complicated task [3,4].

A detection technique for GC employing multi-channel atomic emission spectrometric detection (AED) with a helium microwave-induced plasma (MIP) has recently been introduced. Fragmentation of eluted substances into atoms occurs in the MIP and the light emitted by excited atoms is then simultaneously detected by a photodiode array (PDA) over a portion of the spectrum. The element selectivity of MIP-AED makes its use for the analysis of wide range of xenobiotics and other compounds very attractive [5,6].

Comparison of AED with other selective detection methods used for the analysis of pesticide residues in plant matrices was discussed in detail by Lee and Wylie [7]. Several studies [8–10] described possible ways for the determination of the partial empirical formulae of volatile organic and/or organometallic compounds. Calculations were based on elemental molar ratios measured in multiple-element chromatograms. However, some specific effects of various molecular structures on elemental responses per mole were reported by some workers [11,12]. Variation of response factors was reported, especially for oxygen. "Declining" behaviour was observed also for hydrogen; unlike most other elements, its MIP-AED response is non-linear. Nevertheless, no structure dependence was observed in this case.

Studies of chlorine-containing substances show good accordance between the experimental data and actual content of chlorine in molecules of many pesticides, as demonstrated by Wylie and Oguchi [13]. Similarly, no change in dependence on the type of molecule was demonstrated for chlorine responses of polychlorinated hydrocarbons [12]. The response of GC–MIP-AED as a function of the percentage of chlorine atoms in several pesticides was also studied by Ting and Kho [14]. The tested relationship was found to be linear within the (low) concentration range corresponding to the common residue levels in real samples.

It should be noted that the sensitivity of chlorine detection is relatively low [14,15]. Considering sulphur, phosphorus and nitrogen, which are common heteroatoms present in most environmental contaminants, the sensitivity of chlorine detection could be ranked as the third lowest. Only nitrogen (N-174) was found to give a lower signal at an equal concentration level.

Although a detailed knowledge of the advantages and limitations of ECD in the field of PCB analysis is available, there is limited experience in utilising MIP-AED. The purpose of this study was to characterize parameters for application of this novel detection technique in the trace analysis of PCBs in biotic matrices and to compare the resulting data with those obtained by "classical" approaches. Prior to the analysis of real samples, the most essential features of AED were established in model experiments employing a standard mixture of seven "indicator" congeners widely used for regulation.

2. Experimental

Chemicals

Seven analytical standards of indicator PCBs, congeners 28, 52, 101, 118, 138, 153 and 180, were purchased as solids from Promochem. Technical mixtures of PCBs, Delor 103 and Delor 106, were products of the PCB-producing plant Chemko Strázské. Both stock and working standard solutions containing either the seven indicator PCBs or individual Delors were prepared in isooctane.

All solvents (acetone, *n*-hexane, isooctane, chloroform) were of pesticide grade, supplied by J.T. Baker.

2.2. Analysed materials

A 5-kg amount of a representative sample of carp (the commonest freshwater fish on the Czech market) were collected in two localities. The first carp sample was obtained from one of

the regular river monitoring sites and the second represented a heavily polluted lake area (leakage of transformer oil containing Delor 103 into the surface water).

Bioptic fat was obtained from a cow confined in a cow-shed where paint containing Delor 106 was identified as the source of pollution.

2.3. Sample preparation

An aliquot (ca. 50 g) of homogenized tissue was mixed with anhydrous sodium sulphate and extracted with three 50-ml portions of acetone–*n*-hexane (1:1, v/v). Sonication was applied to enhance the efficiency of fat extraction. The combined extracts were filtered through anhydrous sodium sulphate and the solvent was subsequently evaporated.

To carry out the clean-up step of isolated fat, ca. 500 mg of sample were loaded on to a Celite column (300 × 25 mm I.D.) impregnated with fuming sulphuric acid. *n*-Hexane (200 ml) was used for the elution of analytes. After solvent evaporation, the residue was dissolved in 200 μl of isooctane.

2.4. GC–MIP-AED

A system consisting of a Hewlett-Packard HP 5921 A atomic emission detector coupled to an HP 5890 gas chromatograph equipped with split–splitless injector and an HP 7673 A autosampler was used. The experiments were carried out under the following conditions: injection, 1 μl, splitless, 2 min; injector temperature, 280°C; column, SPB-5 (60 m × 0.25 mm I.D., film thickness 0.25 μm); oven temperature programme, 90°C for 2 min, increased at 20°C/min to 210°C, then at 2°C/min to 280°C; carrier gas, helium; flow-rate 23.3 cm/s; detection, chlorine at 479.454 nm, carbon at 495.724 nm; cavity temperature, 320°C; and reagent gas, oxygen.

2.5. GC–ECD

An HP 5890 gas chromatograph equipped with an electronically programmed pressure (EPP) split–splitless injector, electron-capture detector and HP 7673 A autosampler were used. The conditions were as follows: injection, 1 μl, splitless, 2 min; injector temperature, 260°C; column, HP Ultra-2 (50 m × 0.2 mm I.D., film thickness 0.11 μm); oven temperature programme, 60°C for 2 min, increased at 30°C/min to 210°C, then at 2°C/min to 280°C; carrier gas, nitrogen; flow-rate, 20.5 cm/s; and detector temperature, 300°C.

3. Results and discussion

3.1. Sensitivity of detection

As was noted earlier, the attainable sensitivity of MIP-AED varies significantly for individual elements. Chlorine (Cl-479), unfortunately, does not exhibit a very high response [14]. This is well documented in Fig. 1, showing the chlorine chromatogram corresponding to the injection of 0.5 ng of each of the PCBs on to the GC column. Obviously, the concentrations of the analytes were close to the detection limit (defined as a signal-to-noise ratio of 3). Only a slightly worse MIP-AED detection sensitivity, ca. twice the higher detection limit, for organochlorine pesticides with comparable molecular contents of chlorine was reported by Miyahara et al. [16] (they used the same type of GC–MIP-AED system as used in our laboratory). Wylie and

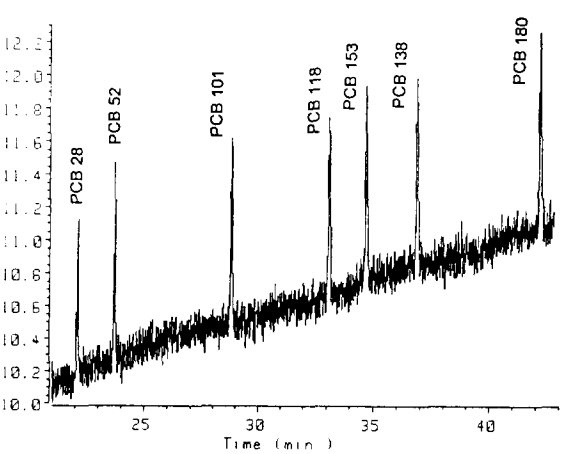

Fig. 1. Chromatogram of seven indicator congeners obtained by GC–MIP-AED (Cl-479); 0.5 ng of each analyte injected.

Oguchi [13], also using this Hewlett-Packard system, reported the Cl-479 sensitivity limit to be 39 pg/s. A similar mean value, 54 pg/s, was observed in our study.

Under the conditions used for sample preparation (see Experimental), the detection limit for PCB No. 28 with the lowest content of chlorine (consequently giving the lowest MIP-AED response) corresponded to ca. 0.15 ppm in fat. As expected, such a value was significantly higher (almost by three orders of magnitude) than that obtained by ECD. As the amount of sample processed (500 mg) is relatively high, its further increase to enhance the sensitivity of the whole analytical procedure does not represent an optimum solution. Provided that an injector with electronic pressure control (EPC) is available, a better approach lies in increasing the sample volume (in our experience, up to 6 μl of isooctane solution) loaded into the GC system. Larger injections are facilitated by the pulse pressure occurring during the splitless period. On the other hand, one should always consider the risk of column overload by other abundant sample components, especially when columns coated with thin-film stationary phases are used.

3.2. Linearity of response

In order to evaluate the applicability of MIP-AED to the determination of PCBs, the character of the relationship between its response and the content of chlorine in the analytes was tested. Table 1 summarizes the results obtained in the analysis of standard solutions. The MIP-AED response (R) as a function of the mass of chlorine (c) in injected PCBs (see Fig. 2) was found to be linear within the tested range of 0.5–10 ng, and could be described by the equation $R = -13.691 + 207.607\ c$. The calculated value of the correlation coefficient, r, was 0.99938. The results presented here demonstrate the identity of the response factors of PCBs with the same number of chlorine atoms (i.e., pentachlorobiphenyls. Nos. 101 and 118 and hexachlorobiphenyls Nos. 138 and 153); in other words, no structure dependence of the measured values was recorded.

Compared with many other elements, a relatively narrow linear dynamic range for chlorine was reported by some workers [14]. Our experience is different: in a preliminary study solutions containing a wide concentration range of hexachlorobenzene (HCB) (up to 1000 μg/ml) were repeatedly injected into the GC–MIP-AED system. Such high concentrations are not likely to be present in environmental samples, but nevertheless no deviation from the linear relationship between injected amount of analyte and detector response was observed. The calculated values of areas per nanogram of chlorine injected were virtually identical for different substances; e.g., for PCB No. 52 (10 ng of analyte injected, i.e., 4.86 ng of chlorine) this value was $2.11 \cdot 10^3$ (R.S.D. 2.6%); for HCB (44.87 ng of analyte injected, i.e., 33.52 ng of chlorine) a similar result, $2.20 \cdot 10^3$ (R.S.D. 2.9%), was obtained.

3.3. Precision of repeated injections

The plot of R.S.D. versus the amount of individual congeners (characterized by chlorine content, see Table 2) is shown in Fig. 2. The data were calculated for six repetitive 1-μl injections with the autosampler. ca. three times higher R.S.D. value was observed at the lowest concentration level A (0.5 ng of analyte per injection) for trichlorobiphenyl No. 28, compared with heptachlorobiphenyl No. 180 (the weight ratio of relative chlorine content in these two compounds is 1:1.52). In general, the repeatability of the detector response achieved in our experiments was significantly better than the results presented by Ting and Kho [14], who analysed a mixture of chlorinated pesticides using the same equipment. Fig. 3 illustrates the change in R.S.D. for individual PCBs at the lowest concentration level. For comparison, we also plotted the R.S.D. estimate calculated for the respective chlorine concentrations from the Horwitz equation. With the exception of the value for congener No. 28, more favourable data were obtained in our measurements. However, it should be noted that an additional increase in R.S.D. may occur when the whole analytical

Table 1
Results obtained in linearity and precision tests[a]

PCB No. (number of Cl atoms)	A			B			C		
	ClC in 0.5 ng	R 10^{-3}	R.S.D. (%)	ClC in 2.0 ng	R $\times 10^{3}$	R.S.D. (%)	ClC in 10.0 ng	R $\times 10^{3}$	R.S.D. (%)
28 (3)	0.2065	0.3565	34.4	0.826	1.3491	12.2	4.130	7.9893	3.3
52 (4)	0.2430	0.4806	22.6	0.972	1.7641	6.7	4.860	10.2520	2.6
101 (5)	0.2715	0.5199	14.8	1.086	1.9989	6.3	5.430	11.3742	3.0
118 (5)	0.2715	0.5225	13.9	1.086	1.9471	4.1	5.430	11.3250	3.2
138 (6)	0.2945	0.5712	11.7	1.178	2.1623	6.2	5.890	12.0993	1.6
153 (6)	0.2945	0.5815	13.5	1.178	2.1003	5.1	5.890	11.9920	2.0
180 (7)	0.3140	0.6632	10.5	1.256	2.3017	4.0	6.280	12.7902	2.0

[a] Results given are chlorine content (ClC) in injected amount of PCB congener, corresponding mean AED response (R) and relative standard deviation (R.S.D) for three concentration levels (experiments A, B and C) for the seven examined PCBs.

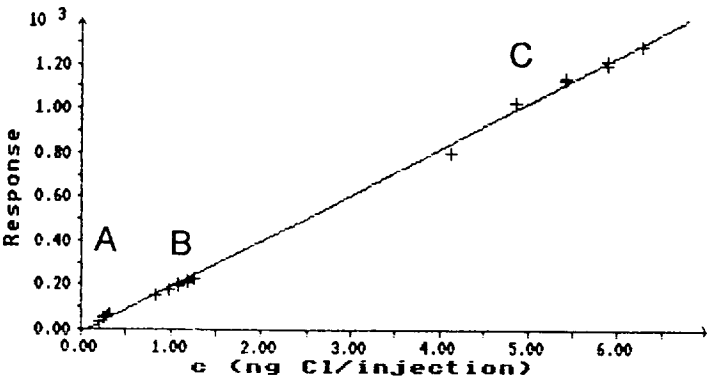

Fig. 2. Plot of MIP-AED response (Cl-479) versus mass of chlorine injected. Cluster A, 0.5 ng of the seven PCBs injected; cluster B, 2.0 ng of the seven PCBs injected; cluster C, 10 ng of the seven PCBs injected. For details, see Table 1.

procedure is applied for the processing of real samples.

3.4. Selectivity

One of the potential problems encountered when ECD is used for the routine determination of PCBs is the concurrent detection of many other even non-chlorinated substances. As will be demonstrated, the element selectivity of MIP-AED makes it possible to avoid the bias resulting from the non-specific detection of interferents. Reduction of undesirable signals is facilitated by the use of a real-time multi-point background correction method included in the MIP-AED software. A properly corrected run is well illustrated in Fig. 4, where chlorine- and carbon-selective chromatograms of contaminated fish oil are shown. No interfering signals were recorded in the chlorine trace at retention times corresponding to the elution of co-extracts detected in the carbon channel. A higher carbon detection sensitivity compared with that of chlorine is displayed. Apparently, the efficiency of the clean-up step can be conveniently controlled in this manner and, further, optical emission spectra continuously collected during the chromatographic run may provide additional proof when-

Table 2
Determination of PCBs in various matrices by GC–MIP-AED and comparison with GC–ECD (concentrations expressed in mg/kg of fat)

Analyte	Method	Origin of fat sample		
		Fish from accidentally polluted area	Fish from "unpolluted" area (monitoring site)	Beef from contaminated cow-shed
Sum of 7 indicator PCBs	GC–ECD	22.41	1.86	3.16
	GC–MIP-AED[a]	20.17	2.04	2.91
Total PCBs	GC–MIP-AED[a]	137.25	5.67	3.48
Chlorine contained in PCBs	GC–MIP-AED[a]	60.39	2.67	2.10
Contribution of PCB chlorine (% of weight) to total PCBs	GC–MIP-AED[a]	44.0	47.1	60.3

[a] Calculations are based on data obtained on carbon and chlorine channels.

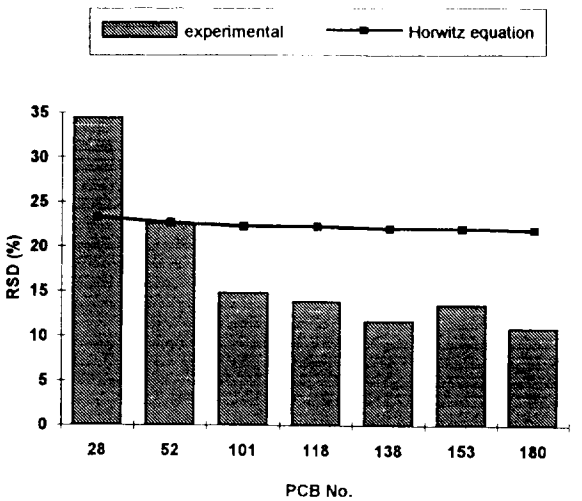

Fig. 3. Changes in experimental and calculated R.S.D.s for individual PCBs. Concentration level 0.5 ng of analyte per injection.

ever the presence of the element is doubted. A "snapshot" portion of the spectrum produced by PDA at the maximum of the peak marked with an asterisk is displayed in the inset in Fig. 4b. The triplet of atomic emission lines (479.5, 481.0 and 481.9 nm) clearly confirms the presence of chlorine in this compound.

3.5. Determination of PCBs by GC–MIP-AED and comparison with GC–ECD

Prior to the analyses of real samples (beef tallow and fish oil), optimization of the GC conditions leading to an efficient separation of a standard mixture was carried out. The model solution used in our experiments consisted of the following contaminants potentially occurring at detectable levels in fat of biota: (i) common persistent chlorinated pesticides together with some of their metabolites (isomers of DDT, DDE, DDD, HCH, methoxychlor, aldrin, dieldrin, HCB) and (ii) technical mixtures of PCBs (Delor 103 and Delor 106). Correct recognition of the peaks corresponding to PCBs in the presence of other chlorine-containing "interferences" was thus possible.

"Compound-independent calibration" was employed for the determination of PCBs based on MIP-AED data. As was mentioned above, this approach is not applicable in general owing to the possible structure dependence of the emission gain for some atoms. Nevertheless, for the present analytes, on both the chlorine (see Fig. 2) and carbon channels virtually identical area counts were obtained for isomeric PCBs (i.e., congeners with different substitution patterns) at all calibration levels. A similar quantification strategy cannot in principal be applied [1] in processing of ECD data. In our study, only the contents of the seven indicator PCBs for which a standard solution was available could be calculated.

As shown in Table 2, good accordance of the results for the sum of the seven indicator PCBs was obtained by both ECD and MIP-AED. Thanks to the unique principle of MIP–AED, it was possible to express the contribution of chlorine contained in all PCBs present (i.e., including congeners whose structure was not exactly identified) to the total weight of these contaminants (in weight %). This value reflects well the character of sample contamination. It was lower for fish from water contaminated by Delor 103 (trichlorobiphenyls predominate in this mixture) in comparison with fish representing "background" pollution. In the latter case, PCBs with a higher content of chlorine (characterized by a longer half-life in the environment) were determined in the isolated fat. The lowest value was measured in beef tallow. As opposed to fish, biodegradation of lower chlorinated PCBs occurs in mammals after exposure and, consequently, highly chlorinated PCBs prevail in such types of samples.

4. Conclusions

The main advantage of the application of GC–MIP-AED for the determination of PCBs in biotic matrices lies in the high selectivity of chlorine detection and also in the simultaneous possibility of obtaining a multi-element profile of a sample. An assessment of other element-selective chromatograms thus provides additional

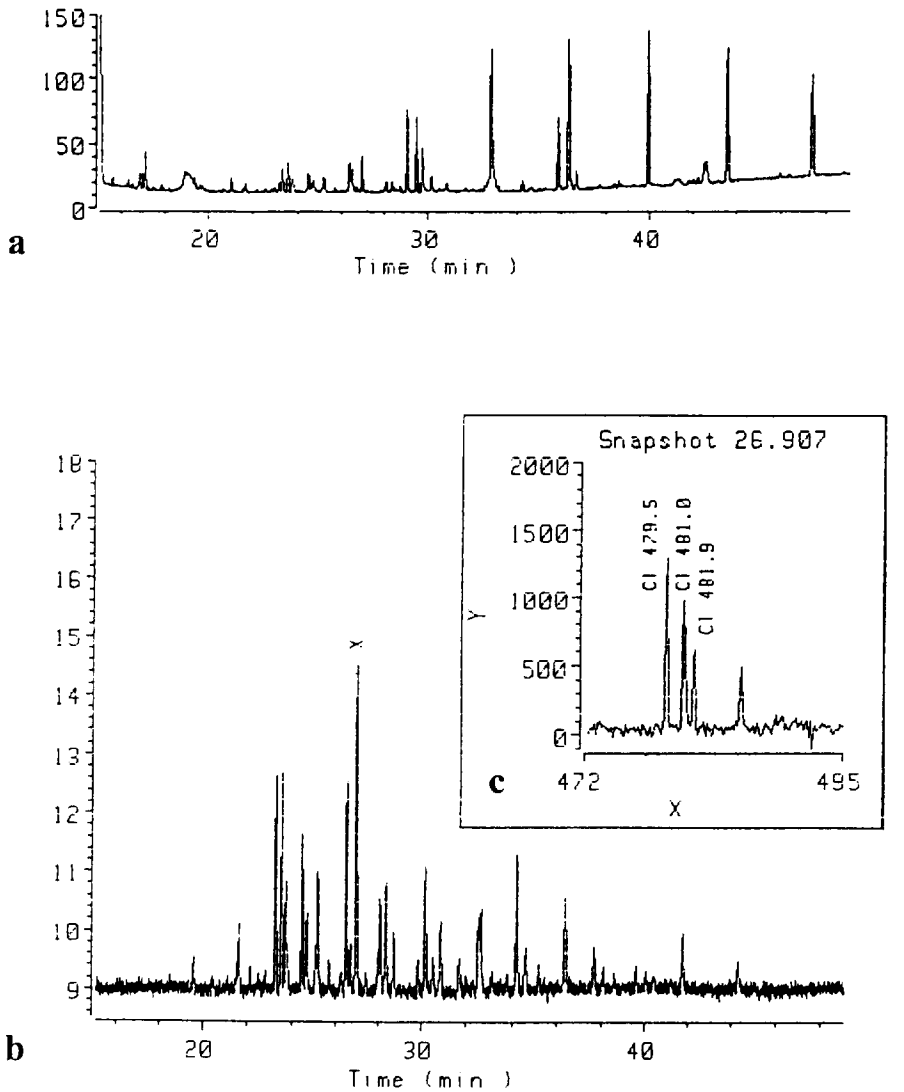

Fig. 4. GC–MIP-AED of fish oil extract. Injection corresponds to 2.5 mg of original sample. (a) Recorded on carbon (C-496) channel; (b) recorded on chlorine (Cl-479) channel.

information about the components present in examined extracts. The efficiency of clean-up procedures (seen on the carbon channel) and, in relation to that, the protection of analytical column can be easily controlled.

The distinctly lower sensitivity of MIP-AED compared with conventional GC detection methods (ECD, MSD-SIM) used for the determination of PCBs requires more efficient preconcentration of analytes, especially when matrices with a low degree of contamination are to be analysed. Further, higher R.S.D.s (lower precision) than those obtained by GC-ECD can be expected for comparable amounts of injected PCB congeners. On the other hand, an improved accuracy of the results obtained by MIP-AED can be expected because of the good selectivity of detection.

Thanks to the detection principle utilized in MIP-AED, the type of sample (extract) contami-

nation can be characterized by the content of volatile organic chlorine. The degree of PCB chlorination may be interpreted as the contribution of this element to the total content of parent substances. Nevertheless, good GC resolution of analytes from other chlorinated compounds remains crucial.

Acknowledgement

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