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DETERMINATION OF CONDITIONS FOR DERIVATIZATION AND CHROMATOGRAPHIC ANALYSIS BEFORE SIMULTANEOUS ANALYSIS OF CHLOROVERATROLES AND PENTAFLUOROBENZYL DERIVATIVES OF CHLOROCATECHOLS AND CHLOROGUAIACOLS IN ENVIRONMENTAL AND FOOD SAMPLES

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SUMMARY

Gas chromatography–mass spectrometry and gas chromatography with electron-capture detection have been investigated for analysis of persistent chlorinated aromatic compounds in the aquatic environment. A range of polychlorinated veratroles, guaiacols, and catechols produced during pulp bleaching and during PCB degradation were derivatized with pentafluorobenzyl bromide to improve their chromatographic properties. The reaction was conducted in the presence of triethylamine as neutralization reagent and the derivatized standards obtained were used to estimate the linear range, detection limits, and precision of the method. The conditions used for the derivatization reaction and for chromatographic analysis were optimized. Detector response for the derivatized guaiacols was a linear function of concentration in the range 0.5–100 ng mL^{-1}; for chloroguaiacols and underivatized chloroveratroles the range was 0.5 to 80 ng mL^{-1}. Detection limits ranged from 0.18 ng mL^{-1} for tetrachlorocatechol (TeCC) to 2.72 ng mL^{-1} for 4-chloroguaiacol (4-CG). GC–MS was used for final confirmation of the identity of the compounds analyzed.

INTRODUCTION

A range of chlorinated aromatic compounds, for example chlorocatechols, chloroveratroles, and chloroguaiacols are known components of pulp bleaching effluents and are also products of biotransformation and

biodegradation of PCB. The formation of hazardous, chlorinated aromatic compounds in the effluents occurs as a result of reaction of residual lignin in the pulp with molecular chlorine or its oxygenated derivatives, hypochlorite or chlorine dioxide. Some of these compounds, for example chlorinated guaiacols have been shown to be toxic at concentrations of 1 mg L^{-1} or less, so persistence of such compounds in the aquatic environment, sediments, or soil may be a matter of serious concern [1–4]. It has also been shown that under aerobic conditions in sediments trichloro and tetrachloroguaiacols and chloroveratroles can undergo biotransformation to the corresponding trichloro and tetrachlorocatechols, which slowly undergo further biodegradation [5].

The biodegradation of PCB by microorganisms may occur in two ways, depending on the number of chlorine atoms in the molecule. Dechlorination in general removes chlorine atoms from PCB molecules without reducing the number of molecules and leaving *ortho* chlorine atoms untouched. The *ortho*-chlorocatechols contribute to DNA degradation, especially when associated with heavy metals. The higher chlorinated PCB congeners (more than five chlorine atoms per molecule) undergo anaerobic dechlorination. Anaerobic biodegradation of low-chlorinated PCB occurs in the top layers (only a few millimetres) of sediment or soil. These PCB congeners are decomposed to products such as 4-chlorocatechol and, then, CO_2, H_2O, HCl, and biomass [6–11]. Thousands of organochloroaromatic compounds, for example chlorocatechols, chlorophenols, chloroguaiacols, and chloroveratroles can be transformed into precursors of dioxins during complicated processes such as combustion. Their persistence in the aquatic environment suggests that these toxic and hazardous compounds may be dispersed over wide areas [12–14]. This occurrence illustrates the importance of developing methods for determination of chloroveratroles, chlorocatechols, and chloroguaiacols in environmental and food matrices.

Although increasing interest in hydroxylated PCB and dioxin metabolites, for example chlorophenols, chlorocatechols, and chloroguaiacols, has resulted in the development of new analytical procedures and techniques for different matrices [11,15–17], these methods give no information about chloroveratroles and most microbiological experiments yet reported have barely discussed (or tested) such problems as recovery of the analytical procedures applied. These biodegradation, biotransformation, and toxicological studies have also been performed using high concentrations (μg L^{-1}) and phenolic compounds have usually been examined as their acetylated, never pentafluorobenzyl, derivatives.

The objective of this work was determination of conditions for derivatization and chromatographic analysis for simultaneous determination of chloroveratroles and pentafluorobenzyl derivatives of chlorocatechols and chloroguaiacols by GC–ECD and GC–MS.

EXPERIMENTAL

Materials

4-Chloroveratrole (4-CV), 4,5-dichloroveratrole (4,5-DCV), 3,4,5-trichloroveratrole (3,4,5-TCV), 3,4,5,6-tetrachloroveratrole (TeCV), 4-chloroguaiacol (4-CG), 4,5-dichloroguaiacol (4,5-DCG), 4,5,6-trichloroguaiacol (4,5,6-TCG), 4-chlorocatechol (4-CC), 4,5-dichlorocatechol (4,5-DCC), and 3,4,5-trichlorocatechol (3,4,5-TCC) (all purity >95%) were obtained from Helix Biotech (Vancouver, BC, Canada). 3,4,5,6-Tetrachlorocatechol (TeCC) and 3,4,5,6-tetrachloroguaiacol (TeCG) (both purity >99%) were purchased from Cambridge Isotope Laboratories (Warszawa, Poland). The derivatization reagents pentafluorobenzyl bromide (PFBBr) and triethylamine were obtained from Sigma–Aldrich (Poznań, Poland). Pure hexane and 2-propanol were from POCh (Gliwice, Poland) and J.T. Baker (USA) respectively.

Instrumentation and Analytical Conditions

GC–ECD analysis was conducted using a Varian GC-3800 gas chromatograph equipped with a split/splitless injector and a 30 m × 0.32 mm i.d. × 0.25 μm film thickness CP-Sil 5 CB capillary column (Varian, USA). The injection port of the gas chromatograph was operated at 250°C and the detector temperature was 330°C. The oven temperature was maintained at 100°C for 1 min then programmed at 20° min^{-1} to 160°C, then at 5° min^{-1} to 260°C, and finally at 25° min^{-1} to 300°C, which was held for 3 min. The carrier gas was helium, flow rate 1 mL min^{-1}.

GC–MS analysis was performed with a Trace 2000 Series gas chromatograph (Finnigan, USA) equipped with a 30 m × 0.25 mm i.d. × 0.25 μm film thickness ZB-5 capillary column with helium as carrier gas (flow rate 1 mL min^{-1}) and an ion-trap mass spectrometer operating in the mass range 50–650 *m*/*z*. The column temperature was maintained at 60°C for 4 min then programmed at 20° min^{-1} to 160, then at 5° min^{-1} to 260°C (which was held for 5 min) and finally at 20° min^{-1} to 300°C which was

held for 3 min. An OA-SYS N-EVAP 111 heating system (nitrogen evaporator) was used for sample evaporation.

Derivatization and Preparation of Standards

The functionality of chloroguaiacols and chlorocatechols must be derivatized to modify the chromatographic properties of the compounds. Although BSTFA is a suitable silylation agent for phenolic compounds and does not require additional neutralization reagents, it does not improve ECD detection, especially of poorly chlorinated guaiacols and catechols. This was achieved by reaction of phenolic standards with pentafluorobenzyl bromide (PFBBr) in the presence of triethylamine (TEA) as neutralization reagent. PFBBr converts not only phenols but also carboxylic acids, mercaptans, and sulfonoamides to halogenated derivatives that are easily detected by ECD, but during the reaction acidic by-products are formed. To avoid column problems TEA was added to guarantee alkaline conditions. Standard solutions (600 µg mL^{-1}) of the chloroveratroles, chlorocatechols, and chloroguaiacols were prepared by dissolving 15 mg of each compound in 25 mL 20% 2-propanol in hexane.

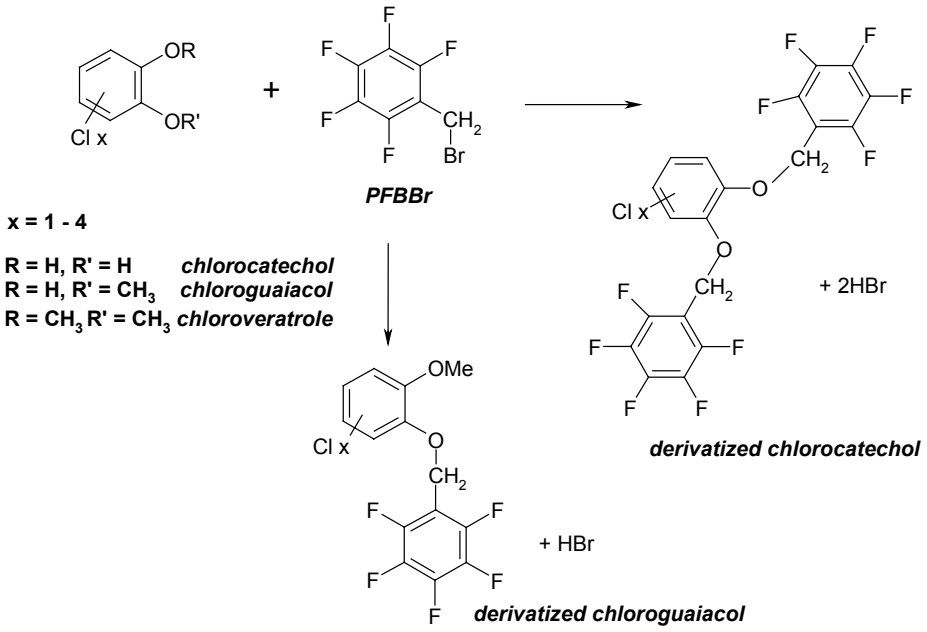

Fig. 1

Reaction of PFBBr with chloroguaiacols and chlorocatechols. Chloroveratroles do not undergo this reaction

Derivatization was achieved by first evaporating 17 μL of standard solution to dryness in a 1.5 mL reaction vial under a gentle stream of nitrogen and then adding 30 μL TEA and 20 μL PFBBr. The reaction vessel was closed and heated at 60°C for 1 h. After this time excess PFBBr and triethylamine was removed by evaporation for 10 min under a stream of nitrogen and the derivatization products were dissolved in 1 mL hexane, resulting in 10.2 μg mL^{-1} of each compound. The derivatization reaction is illustrated in Fig. 1.

The standard solution containing the derivatized chlorocatechols, chloroguaiacols, and underivatized chloroveratroles was further diluted with hexane for calibration and GC–MS identification.

RESULTS AND DISCUSSION

Derivatization Conditions

Derivatization time and temperature were established. The reaction was conducted at 50, 60, or 80°C for 0.5, 1, or 1.5 h. Results obtained after 1 and 1.5 h were not significantly different, but after 0.5 h peak sizes were smaller. Because evaporation and reaction temperature might lead to serious losses of volatile analytes (especially the lowest chlorinated, for example 4-chlorocatechol and 4-chloroguaiacol) a gentle stream of nitrogen should be used both before and after derivatization and the reaction temperature should be <70°C. The optimum temperature for the derivatization was found to be 60°C. If 80°C was used the sizes of the peaks of derivatized 4-chloroguaiacol and 4-chlorocatechol were slightly reduced. Figure 2 shows a chromatogram obtained from underivatized chloroveratroles and derivatized chloroguaiacols and chlorocatechols (concentration 75 ng mL^{-1}) after reaction at 60°C for 1 h.

Although 4-chloroveratrole is not detectable by ECD, its identification and quantification can be successfully achieved by use of GC–MS (data below).

GC–MS Analysis of Derivatized and Underivatized Compounds

Gas chromatographic–mass spectrometric analysis was used to confirm that derivatization occurred under the conditions selected, and for final identification of each compound. The order of elution, retention times, and characteristic ions (relative intensity, %) obtained in GC–MS analysis are listed in Table I.

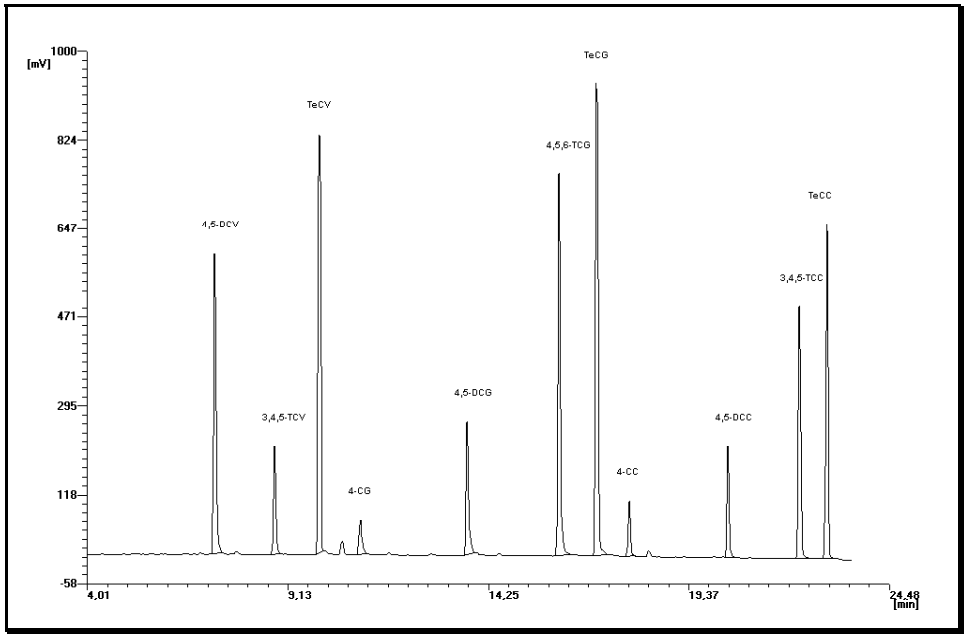

Fig. 2

GC–ECD chromatogram obtained from chlorinated underivatized veratroles and pentafluorobenzyl derivatives of chlorocatechols and chloroguaiacols

The spectra of the chloroveratroles indicate that these compounds undergo fragmentation by eliminating a methyl group and a chlorine atom. Fragmentation of derivatized chloroguaiacols and chlorocatechols furnishes PFB ions that are very intense whereas the molecular ions and other characteristic ions also obtained after elimination of chlorine atoms are relatively small.

Linearity, Precision, and Detection Limits in GC–ECD Analysis

For estimation of linearity, solutions of the compounds were prepared at concentrations in the range 0.25–102 ng mL^{-1}. For all the compounds response was linearly dependent on concentration from 0.5 ng mL^{-1} up to 100 ng mL^{-1} for derivatized chloroguaiacols and up to 80 ng mL^{-1} for chloroveratoles and PFB derivatives of chlorocatechols. Calibration plots were constructed from results obtained by triplicate injection of 1 μL of solutions of concentration 0.5, 1.0, 10.2, 24.5, 51.0, 81.6, and 102.0 ng mL^{-1}. Figures 3–5 show the plots and the corresponding r^2 values.

Table I

Chromatographic data and characteristic ions

Analyte	RT (min)	MW (g mol^{-1})	M^+ (*m*/*z*) (relative intensity, %)	Characteristic ions (*m*/*z*) (relative intensity)
4-CV	9.76	173.39	172 (100)	157 (52); 142 (13)
4,5-DCV	11.62	207.74	206 (100)	191 (66); 163 (23); 128 (33)
3,4,5-TCV	13.23	242.09	240 (95)	229 (100); 197 (26); 162 (53); 133 (23)
TeCV	14.36	276.44	276 (100)	261 (86); 233 (23); 218 (37)
4-CG	15.57	339.40	338 (49)	181 (36); 157 (100); 129 (22)
4,5-DCG	18.51	373.75	372 (63)	191 (100); 181 (61); 163 (23); 128 (35)
4,5,6-TCG	20.95	408.10	407 (41)	225 (100); 199 (17); 181 (57); 162 (34)
TeCG	21.88	442.45	441 (15)	405 (13); 261 (40); 181 (100)
4-CC	23.00	505.37	504 (23)	323 (14); 323 (12); 295 (10); 181 (100)
4,5-DCC	25.86	539.72	538 (17)	357 (10); 329 (6); 181 (100)
3,4,5-TCC	27.84	574.07	573 (8)	393 (6); 363 (5), 181 (100)
TeCC	28.44	608.42	605 (6)	364 (6); 181 (100)

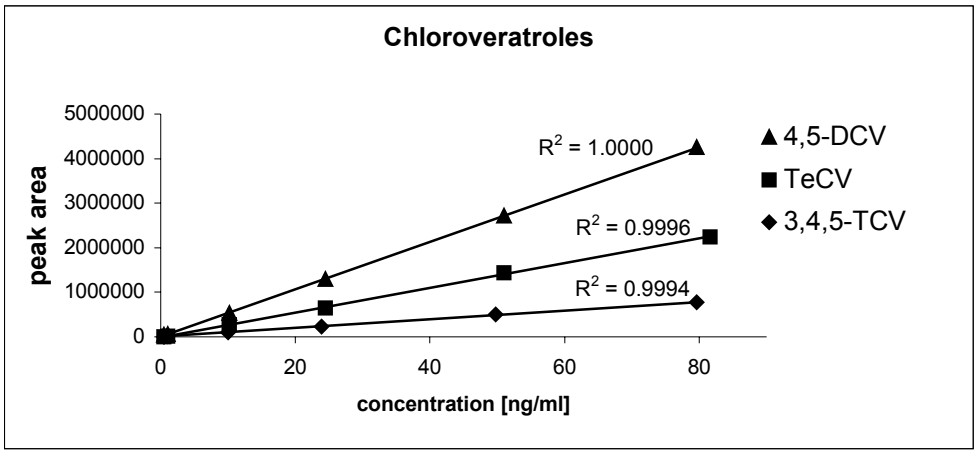

Fig. 3

Calibration plots for chloroveratroles

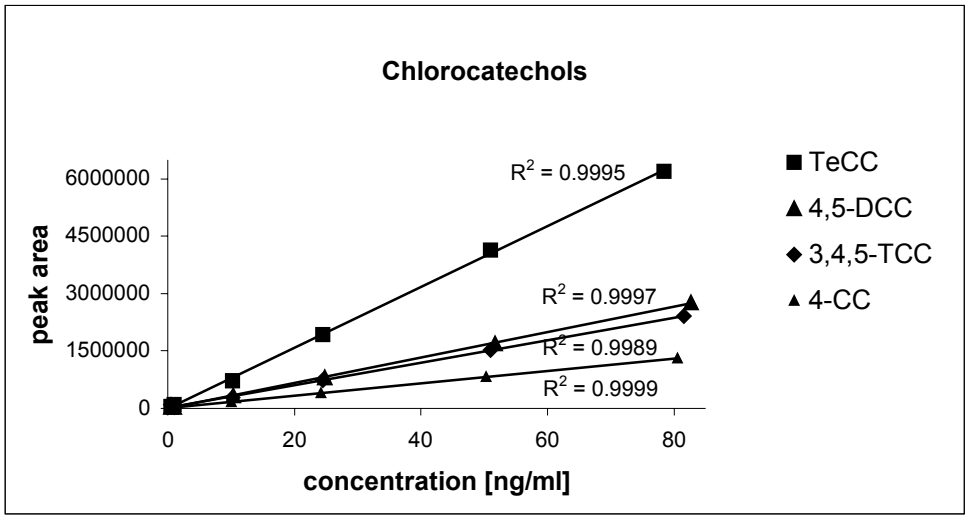

Fig. 4

Calibration plots for derivatized chlorocatechols

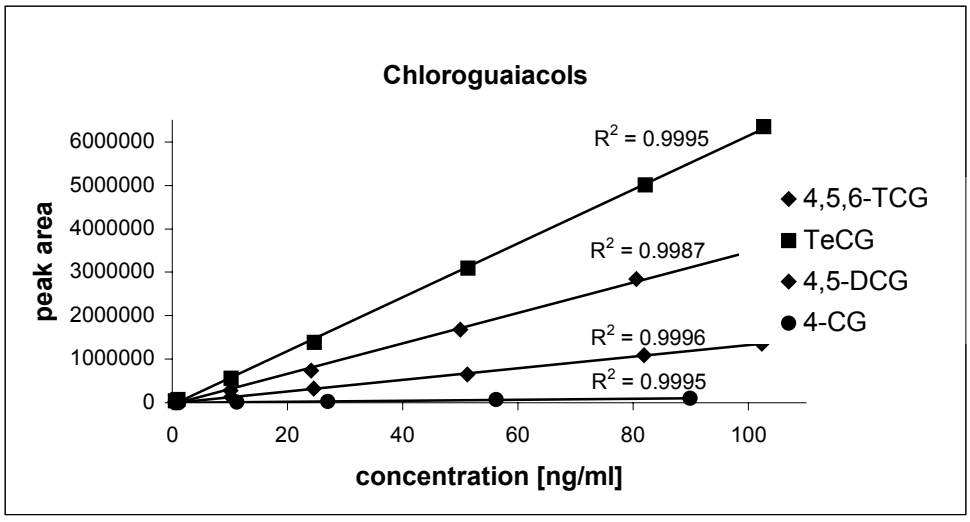

Fig. 5

Calibration plots for derivatized chloroguaiacols

Estimated detection limits ($DL = 3 \times SD$) and precision (evaluated for 10 ng mL^{-1}, $n = 10$) are listed in Table II.

Table II

Linearity, detection limit (*DL*), and precision in GC–ECD analysis

Analyte	RT (min)	Linear range (ng mL^{-1})	*RSD* (%)	*DL* (ng mL^{-1})
4-CV	–	–	–	–
4,5-DCV	7.28	0.51–81.60	0.30	0.28
3,4,5-TCV	8.81	0.50–79.59	2.37	0.58
TeCV	9.95	0.51–81.60	0.11	0.65
4-CG	10.97	0.56–90.40	2.26	2.72
4,5-DCG	13.71	0.51–102.34	2.03	0.47
4,5,6-TCG	16.07	0.50–100.64	1.61	1.74
TeCG	17.02	0.51–102.61	0.14	0.99
4-CC	17.85	0.50–80.51	1.86	1.01
4,5-DCC	20.33	0.52–82.68	1.93	0.41
3,4,5-TCC	22.20	0.51–81.60	1.53	1.14
TeCC	22.90	0.51–81.60	0.16	0.18

CONCLUSIONS

PFBBr may be successfully used as a derivatization reagent not only in GC–ECD but also in GC–MS analysis. The derivatization reagents and condition tested in this work prove that halogenated phenolic compounds are readily derivatized with PFBBr with good efficiency, detection limit, and precision. Although significant losses of volatile compounds such as chlorocatechols can occur, the products of derivatization are relatively stable for long periods of time (more than 50 days). Their lower volatility, non-planar structure, and higher molecular weight might be helpful in further extraction and clean-up experiments that might otherwise lead to low recovery. Use of internal labelled standards (especially ^{13}C 4-chlorocatechol) might also be helpful in such investigations. Although 4-chloroveratrole is not detectable by GC–ECD, the results obtained showed that this method has many significant advantages; it should be considered for analysis of these compounds in environmental and food samples.

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