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Determination of hydroxylated polychlorinated biphenyls by ion trap gas chromatography–tandem mass spectrometry

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Abstract

Hydroxylated polychlorinated biphenyls also known as biphenylols, are suspected estrogen mimics found in the environment. Various derivatization schemes were evaluated and a gas chromatography–ion trap mass spectrometry method was developed for the trifluoroacetyl derivative using MS–MS techniques for the analysis of eleven biphenylols. A time-segmented chromatographic method was developed using the respective MS–MS parameters to analyze all the eleven biphenylols in a single chromatographic run. Isomers were differentiated based on the MS–MS data of the trofluoroacetyl-biphenylol derivatives. The method was applied to detect 40 pg on-column of these compounds in a spiked egg sample which simulates a real world sample. © 1997 Elsevier Science B.V.

Keywords: Derivatization, GC/MS; Polychlorinated biphenyls, hydroxylated

1. Introduction

Polychlorinated biphenyls (PCBs) (trade name Aroclor) are stable, lipophilic industrial organochlorine chemicals found in the environment in almost every ecosystem. Due to their lipophilic nature, PCBs are found in high concentrations in fish, wildlife and in human adipose tissue, serum and breast milk [1–4]. Oxidative metabolism of PCBs produces hydroxylated products (hydroxylated PCBs). In mammals, PCBs are primarily metabolized to their hydroxylated forms by hepatic microsomal mixed-function oxidases. Safe and coworkers reported [5,6] that cows eliminate 4-chlorobiphenyl in the form of, 4-chloro-4′-hydroxybiphenyl. Gardner et al. [7] reported ten hydroxylated PCB metabolites containing two to four chlorine atoms per molecule were found in the milk of cows fed Aroclor 1242, and hydroxylated metabolites containing four to five chlorine atoms per molecule were found in the milk of cows fed with Aroclor 1254. The number of chlorine atoms present in the parent PCBs was reflected in the hydroxylated metabolites [8,9]. Studies have shown that the less chlorinated PCBs metabolize more readily than the higher chlorinated congeners such as pentachlorobiphenyls [10]. Therefore, the degree of chlorine substitution in a congener determines the rate of metabolism. Extensive studies have been reported on the environmental and human health impacts of PCBs. Since residues of hydroxylated PCBs are not routinely analyzed, only a few studies have reported on the biochemical and environmental effects of these compounds and their relative contributions to the overall toxicity and

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biochemical response of PCBs. The hydroxylated metabolites, being more hydrophilic than the parent PCBs, can potentially migrate in the environment with facility. This enhanced migration could be of major environmental concern if the hydroxylated PCBs are found to be more active than the parent compounds. In fact, recent studies have indicated that hydroxylated PCBs are effective estrogen mimics. Environmentally incurred residues interfere with endocrine transmission during gestation and have significant ramifications in human fertility and reproduction [11,12].

The presence of the phenolic hydroxyl groups in chlorinated biphenylols and the interaction of these groups with the basic sites in the injector and column of a gas chromatograph causes difficulties in trace analyses of hydroxylated PCBs. The acidic character of this phenolic moiety can lead to incomplete transfer from the injector to the gas chromatograph column, resulting in tailing chromatographic peaks and irreproducible peak areas. Derivatization of the hydroxlated PCBs can eliminate the above problems by eliminating the acidic site thus increasing volatility and stability which makes them more amenable to gas chromatographic analysis. Hydroxylated PCBs have been analyzed as trimethylsilyl ethers [13–15] and methyl ethers [16,17] by gas chromatography (GC). Mass spectra of certain methyl ether derivatives of di-, tri- and tetrachlorobiphenylols prepared by the Shu Huang–Cadogan coupling reaction have been reported [18]. Highly chlorinated biphenylols like the penta- and heptachloro have been synthesized and characterized using nuclear magnetic resonance (NMR) and gas chromatography–mass spectrometry (GC–MS) [19].

GC–ion-trap MS has proven to be an effective analytical technique for both qualitative and quantitative trace analyses. Advances in ion-trap technology allow the use of sophisticated tandem mass spectrometry (MS–MS) techniques in conjunction with GC analyses to enhance selectivity. The MS–MS technique is playing an increasingly important role in environmental analysis. Benchtop quadrupole ion trap mass spectrometers with both electron impact (EI) and chemical ionization (CI) and MS–MS capabilities have made it possible to achieve detection limits at parts-per-trillion (ppt) levels.

In this paper, we report the development of a method for the GC–MS–MS analysis of a mixture of eleven chlorinated biphenylols. Various derivatization schemes for the hydroxylated PCBs were evaluated based on improved sensitivity, chromatographic peak shape and MS–MS characteristics. Trifluoroacetyl derivatives of the biphenylols provided the best overall performance and were selected for incorporation in this MS–MS method. The MS–MS parameters were optimized for characteristic fragment (product) ions from the eleven compounds. The method was applied to the analysis of hydroxylated PCBs in a simulated environmental sample.

2. Experimental

2.1. Materials

Analytical standards of eleven hydroxylated PCBs were obtained from Ultra Scientific (North Kingstown, RI, USA). The bis(trimethylsilyl)trifluoroacetamide (BSTFA)+1% trimethylchlorosilane (TMCS) was obtained from Pierce (Rockford, IL, USA). Acetic anhydride, pentafluoropropionic anhydride and trifluoroacetic anhydride were obtained from Aldrich (Milwaukee, WI, USA). The hexane used was pesticide residue quality (Burdick and Jackson). Diazoethane was prepared in the laboratory according to US Environmental Protection Agency (EPA) procedure 600/8-80-038 Section 5, A, using N-ethyl-N-nitroso-N′-nitrosoguanidine from Aldrich as the precursor. A solution mixture containing the eleven hydroxylated PCBs with individual concentrations ranging from 4 ng/μl to 8 ng/μl were prepared in hexane.

2.2. Derivatization

Ethyl derivatives of the hydroxylated PCBs were prepared by mixing excess diazoethane solution with 1 ml of the hydroxylated PCBs standards. The resulting solution was reduced to 1 ml under a stream of nitrogen and analyzed.

The trimethylsilyl (TMS) ether derivative was prepared by adding 0.5 ml of BSTFA+1% TMCS to 1 ml of the biphenylol mixture. The mixture was allowed to react at room temperature for 1 h,

evaporated to 1 ml and was analyzed without further treatment.

The acetyl, trifluoroacetyl and pentafluoropropionyl derivatives of the hydroxylated PCBs were prepared by adding 0.5 ml of the respective anhydrides and 5 drops of pyridine to 1 ml of the hydroxylated PCBs mixture. The mixtures were heated for 1 h at 50°C in a water bath. Upon completion, the mixtures were allowed to cool down to room temperature. 6 ml of sodium carbonate solution (50 mg/ml) were added to the cooled mixture to quench the reaction. The derivatized hydroxylated PCBs were extracted five times with 1 ml of hexane and the resulting hexane solution was reduced to 1 ml under a stream of nitrogen.

2.3. Sample preparation

The simulated environmental sample extract was prepared using the following method. 2 g of chicken egg sample were spiked with 2–4 μg of each biphenylol. Sodium sulfate (25 g) which had been dried overnight at 400°C was mixed with egg. This mixture was transferred to a Soxhlet-extractor and extracted with 200 ml methylene chloride for 7 h. The methylene chloride extract was then partitioned three times with 50 ml of 0.25 *M* NaOH. The collected NaOH was made acidic with sulfuric acid to pH<2 and extracted three times with 100 ml methylene chloride. The combined methylene chloride extracts were reduced in a Kuderna–Danish concentrator to 1 ml and then diluted to 10 ml with hexane. A 4-ml aliquot of this solution was spiked with 31.6 μg Aroclor 1248, 15.8 μg Aroclor 1242, 10 μg Aroclor 1254 and 10 μg Aroclor 1260 to evaluate potential interferences with the hydroxylated PCBs analysis. The final step involved derivatization of a 1-ml aliquot of spiked solution with trifluoroacetic anhydride using the method described above and finally diluted to 5 ml using hexane.

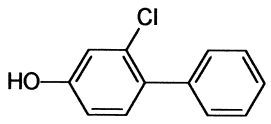
2.4. Instrumentation

A Saturn 3 ion-trap GC–MS system (Varian, Walnut Creek, CA, USA) with Wave Board technology was used for all the experiments. The GC system was equipped with a DB-5 MS fused-silica capillary column, 60 m×0.25 mm I.D. with a 0.25 μm bonded film (J&W Scientific). Helium was used as the carrier gas at a head pressure of 23 p.s.i. (1 p.s.i.=6894.76 Pa). All injections of 1 μl volume were performed in the splitless mode on a 1078 injector using a Varian 8200 Autosampler. The GC temperature program was 50°C for 1 min, temperature increased to 200°C at 30°C/min with no hold time, then increased to 280°C at 4°C/min and finally increased to 300°C at 5°C/min, and held at 300°C for 3 min. The injector temperature was maintained at 270°C and the transfer line temperature was at 300°C.

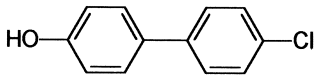
EI ionization spectra were obtained using the full scan mode (typically 50 to 550 m/z). For MS–MS experiments, collisionally induced dissociation (CID) was performed in the non-resonant excitation mode [20]. Precursor ions were isolated using a 3 u isolation window. A CID excitation time of 20 ms was used in all the non-resonant excitation experiments. Automated method development (AMD) toolkit software was used to optimize the CID parameters (low-mass cutoff and CID voltage) to obtain maximum sensitivity. Multiple reaction monitoring (MRM) was used for the analysis of eleven hydroxylated PCBs in a single chromatographic run. Various chromatographic time segments and corresponding compound- dependent non-resonant MS–MS parameters were set up for a single chromatographic run.

3. Results and discussion

The structures of the eleven hydroxylated PCBs and their molecular masses are shown in Fig. 1. Direct GC–MS analysis of the hydroxylated PCBs produced chromatographic peaks which tailed extensively. Attempts to improve this condition by injector maintenance (including silanization of the liner) were not successful. Therefore, various derivatization methods were investigated. Conversion of the hydroxylated PCBs to their corresponding ethyl ethers with diazoethane produced marginal results. The TMS derivatives produced sharp, well-resolved chromatographic peaks which addressed the GC problems in the analysis of hydroxylated PCBs. However, the TMS-4,4′-dichloro-3,3′-biphenyldiol and the TMS-2′,3′,4′,5,5′-pentachloro-2-biphenylol elute very closely in the chromatogram making it difficult



(1)
2-chloro-4-biphenylol (M_r 204)



(2)
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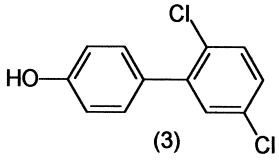
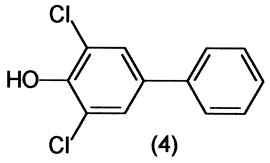
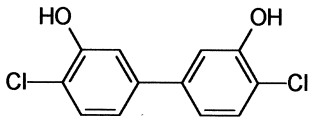


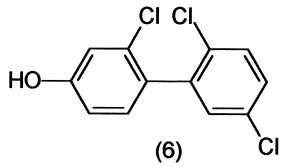
(3)
2', 5'-dichloro-4-biphenylol (M_r 238)



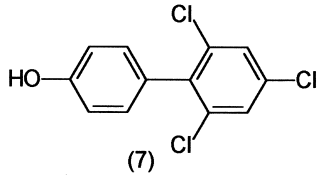
(4)
3, 5-dichloro-4-biphenylol (M_r 238)



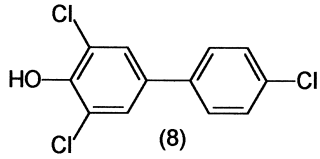
(5)
4, 4'-dichloro-3, 3'-biphenyldiol (M_r 254)



(6)
2, 2', 5'-trichloro-4-biphenylol (M_r 272)



(7)
2', 4', 6'-trichloro-4-biphenylol (M_r 272)



(8)
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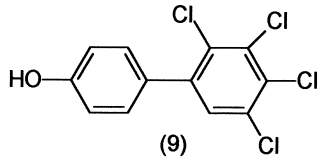
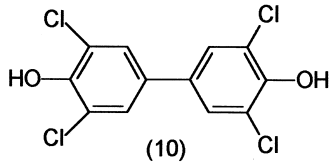
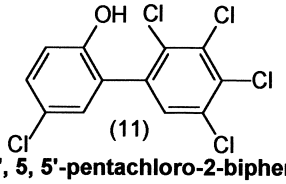


(9)
2', 3' ,4', 5'-tetrachloro-4-biphenylol (M_r 306)



(10)
3, 3', 5, 5'-tetrachloro-4, 4'-biphenyldiol (M_r 322)



(11)
2', 3', 4', 5, 5'-pentachloro-2-biphenylol (M_r 342)

Fig. 1. Structures of hydroxylated PCBs.

to carry out MS–MS using different chromatographic segments. Also, the product ion formed from the precursor ion of pentachloro-TMS biphenylol is the molecular ion of TMS-dichloro-biphenyldiol and its precursor ion which further complicates the MS–MS analysis.

Several acyl derivatives were evaluated. Acetic anhydride was used to form acetyl derivatives of the hydroxylated PCBs. The chromatographic peaks were sharp and well resolved for all eleven derivatized compounds. However, the molecular ions of the derivatives were not observed due to the apparent loss of a neutral ketene group ($O=C=CH_2$) from the derivatized biphenylols. Indeed, the mass spectra of the derivatized and the underivatized compounds were virtually identical. However, the chromatographic retention times peaks were different indicating that derivatization had occurred. To confirm the formation of the acetyl derivative and subsequent loss of the ketene group, pentafluoropropionic anhydride was used for derivatization. The mass spectra of pentafluoropropionic derivatives showed the molecular ions of the derivatized compounds indicating no fragmentation via the ketene loss. Although the pentafluoropropionyl derivatives gave sharp chromatographic peaks and relatively unique MS–MS spectra, the retention times were long and the molecular masses of the derivatives were very high and nearly reached the maximum mass range of the ion trap. Therefore, trifluoroacetic anhydride as derivatization reagent was evaluated. The idea was to retain the interesting properties of the pentafluoro derivatives and result in lower-molecular-mass products. Indeed, the trifluoroacetyl (TFA) derivatives produced very sharp chromatographic peaks with full chromatographic resolution of the hydroxylated PCBs (Fig. 2). Intense molecular ions were observed in the full scan spectra of these derivatives. Therefore, we concluded that the most satisfactory derivatization scheme for the analysis of the eleven biphenylols was to convert them to their TFA derivatives.

The eleven TFA-biphenylols were well resolved on the 60 m DB-5 column. However, the EI full scan spectrum is very similar among the congeners. The EI spectra from both TFA-monochlorobiphenylols exhibited $[M]^{+\cdot}$ and $[M-97]^{+}$ ions. However, the ratio of $[M-97]^{+}$ to the molecular ion was higher for the TFA-2-chloro-4-biphenylol than for the TFA-4′-chloro-4-biphenylol indicating possible congener differentiation. Further fragmentation occurred via subsequent loss of the CO and chlorine. The EI spectra of the two TFA-dichlorobiphenyls contained similar fragment ions, however the ion intensities were very different. The spectrum of TFA-3,5-dichloro-4-biphenylol, which contains two chlorine

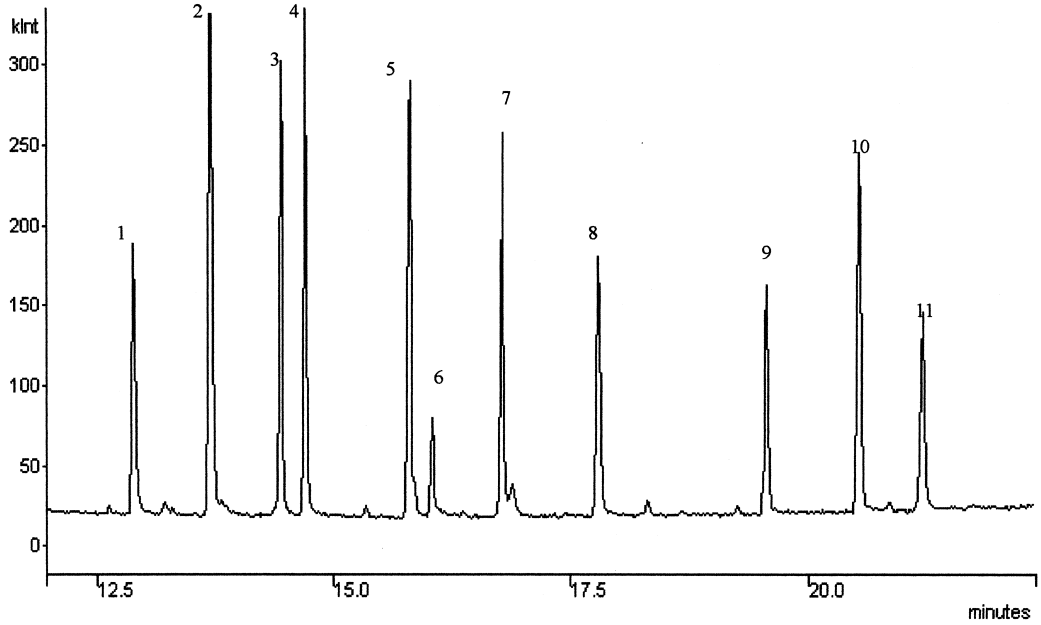


Fig. 2. Full scan EI chromatogram of trifluoroacetyl derivatives of the eleven hydroxylated PCBs. The numbers correspond to respective underivatized hydroxylated PCBs in Fig. 1.

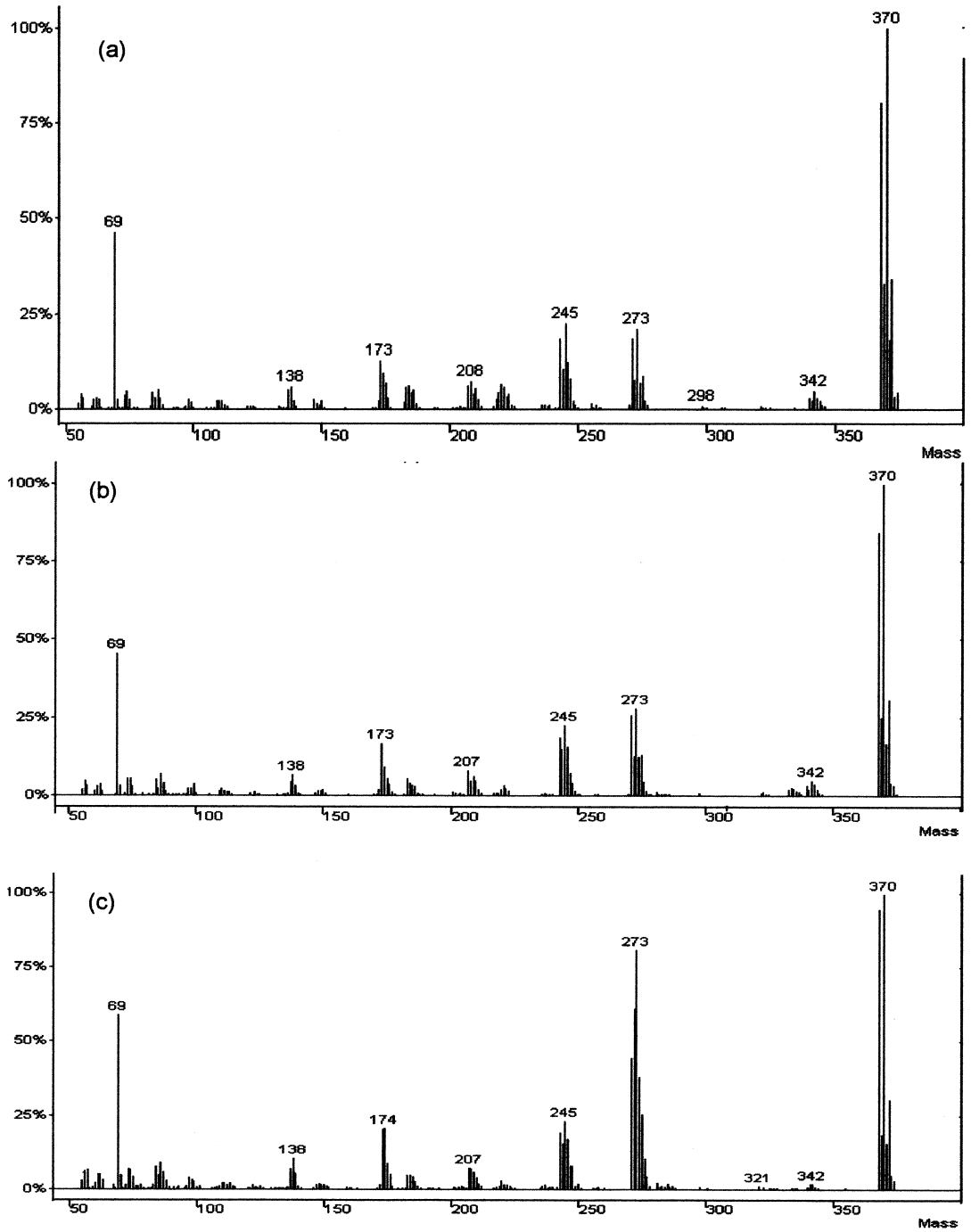


Fig. 3. Full scan EI spectrum of trifluoroacetyl derivative of (a) 2′,4′,6′-trichloro-4-biphenylol, (b) 2,2′,5′-trichloro-4-biphenylol and (c) 3,4′,5-trichloro-4-biphenylol.

substituents ortho to the trifluoroacetyl group, had $[M-97]^+$ as the base peak and a molecular ion of 98% relative intensity. The other congeners, TFA-2′,5′-dichlorobiphenylol, produced a spectrum with the molecular ion as the base peak and $[M-97]^+$ at 26% relative intensity, again indicating a possible mechanism for congener identification. The three trichloro-congeners included in this study were: TFA-2′,4′,6′-trichloro-4-biphenylol, TFA-2,2′,5′-trichloro-4-biphenylol and TFA-3,4′,5-trichloro-4-biphenylol and their mass spectra as derivatives are shown in Fig. 3. The TFA-3,4′,5- trichloro-4-biphenylol congener which has chlorine substituents *ortho* to the trifluoroacetyl group, produced a spectrum which had a higher intensity of the fragment ion $[M-97]^+$ relative to the intensity of the molecular ion (base peak) than the other two isomers. The above discussion indicates a profound *ortho*-effect for these congeners only observed when the trifluoroacetyl group is present. This effect will be exploited in the development of a MS–MS technique for residue analysis as well as congener identification.

The eleven TFA-biphenylols exhibited similar trend in fragmentation which included observation of the molecular ion, loss of the trifluoroacetyl group, loss of the CO and subsequent losses of chlorine. A slight deviation was observed for the two TFA-biphenyldiols which subsequently lost the additional trifluoroacetate group. The fragment ions formed reflect the phenol fragmentation pattern, i.e., loss of a CO and HCO group from the molecular ion or other fragment ions in the spectrum [21]. Fragmentation with the loss of chlorine or HCl is typical of that normally observed with chloroaromatic compounds.

Based on the observations made from the EI full scan experiments, parameters were developed for the non-resonant CID of the molecular ions obtained from the TFA-biphenylols. The non-resonant MS–MS parameters of the compounds were selected for production of a single or in some cases two product ions (Table 1). The extracted ion (product ions) chromatograms for all the eleven TFA-biphenylols are shown in Figs. 4 and 5. In most cases, the precursor ions (molecular ions) dissociated to a single, intense product ion (scan range 50–460 m/z). High CID energies were required due to the stable nature of the selected precursor ions. The formation of a single intense product ion during CID fragmentation is ideal for trace analysis since all the ion current belonging to the precursor ion will be transferred to the ion current of the single product ion thus improving sensitivity.

The conversion efficiencies of precursor to product ions were not calculated in this study due to the presence of chlorine isotopic clusters in ion isolation of the precursor ions using a 3 u window. Non-resonant CID mode causes universal ion excitation which results in the excitation of the isolated chlorine isotopic cloud. The CID energy is distributed over the whole ion cloud resulting in fragmentation

Table 1
Non-resonant MS–MS parameters of the trifluoroacetyl derivatives

Compound	Precursor ion	Product ion	CID parameters	
			R_t (m/z)	Amplitude (V)
2-Chloro-4-biphenylol	300	203	101	75
4′-Chloro-4-biphenylol	300	203+175	101	79
3,5-Dichloro-4-biphenylol	334	237	118	87
2′,5′-Dichloro-4-biphenylol	334	209	104	87
4,4′-Dichloro-3,3′-biphenyldiol	446	411	129	99
2′,4′,6′-Trichloro-4-biphenylol	370	342+245	117	99
2,2′,5′-Trichloro-4-biphenylol	370	245	111	96
3,4′,5-Trichloro-4-biphenylol	370	273	124	91
2′,3′,4′,5′-Tetrachloro-4-biphenylol	404	376+279	112	92
3,3′,5,5′-Tetrachloro-4,4′-biphenyldiol	516	419	155	100
2′,3′,4′,5,5′-Pentachloro-2-biphenylol	438	369	142	100

CID time was 20 ms for all MS–MS experiments. The scanning mass range varied for each time segment of the chromatographic run depending on the m/z of the product and precursor ion. Typically 10 m/z less than the m/z of the product ion to 10 m/z greater than the m/z of the precursor ion.

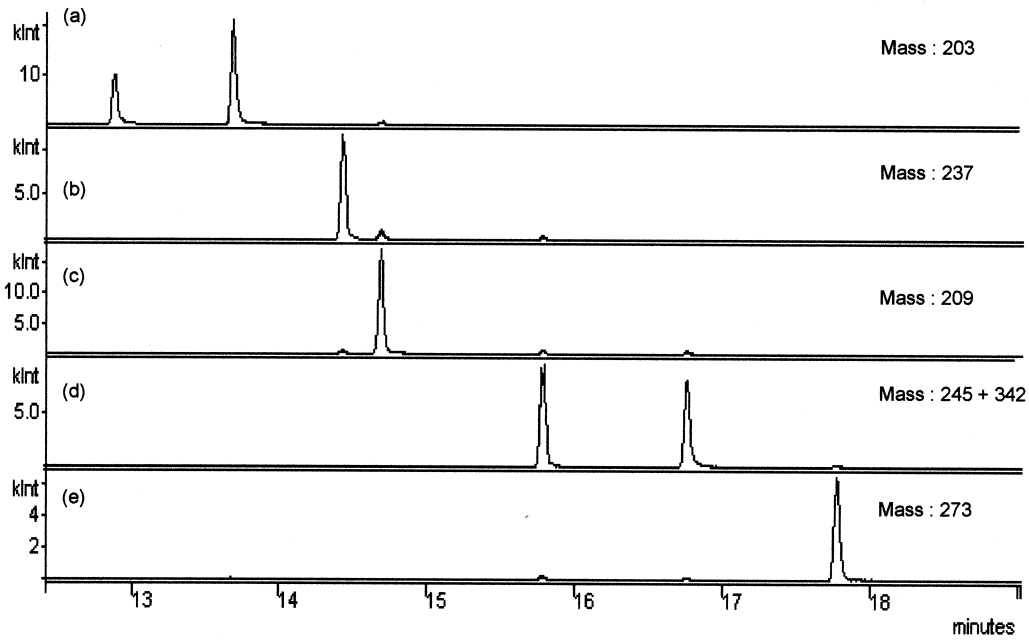


Fig. 4. Extracted ion MS–MS chromatograms of the trifluoroacetyl derivatives of the (a) monochloro-, (b, c) dichloro- and (d, e) trichloro-biphenylols. The extracted ions are the respective product ions of the compounds (see Table 1).

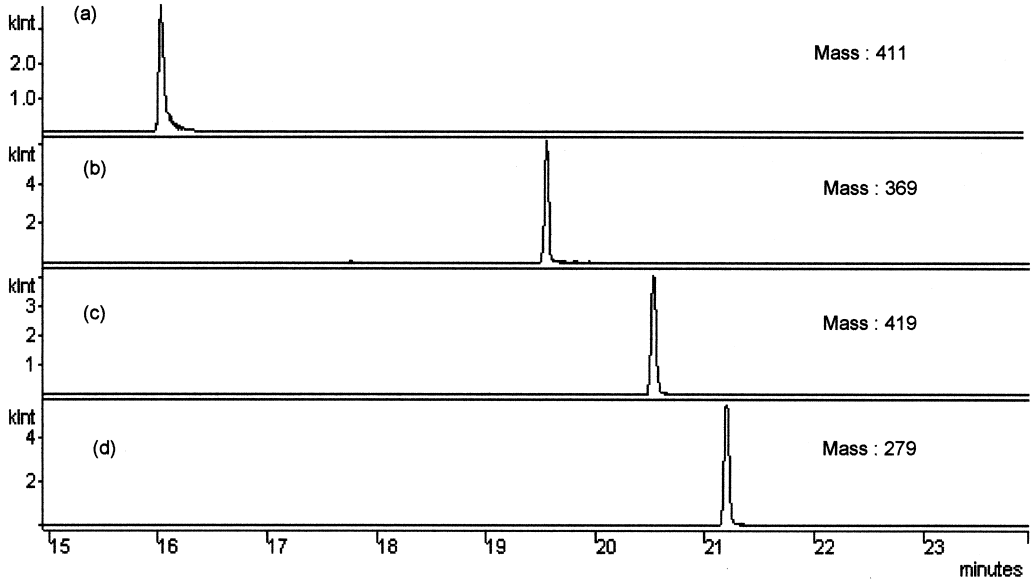


Fig. 5. Extracted ion MS–MS chromatograms of the trifluoroacetyl derivatives of the (a) dichloro-biphenyldiol, (b) pentachloro-biphenylol, (c) tetrachloro-biphenyldiol and (d) tetrachloro-biphenylol. The extracted ions are the respective product ions of the compounds (see Table 1).

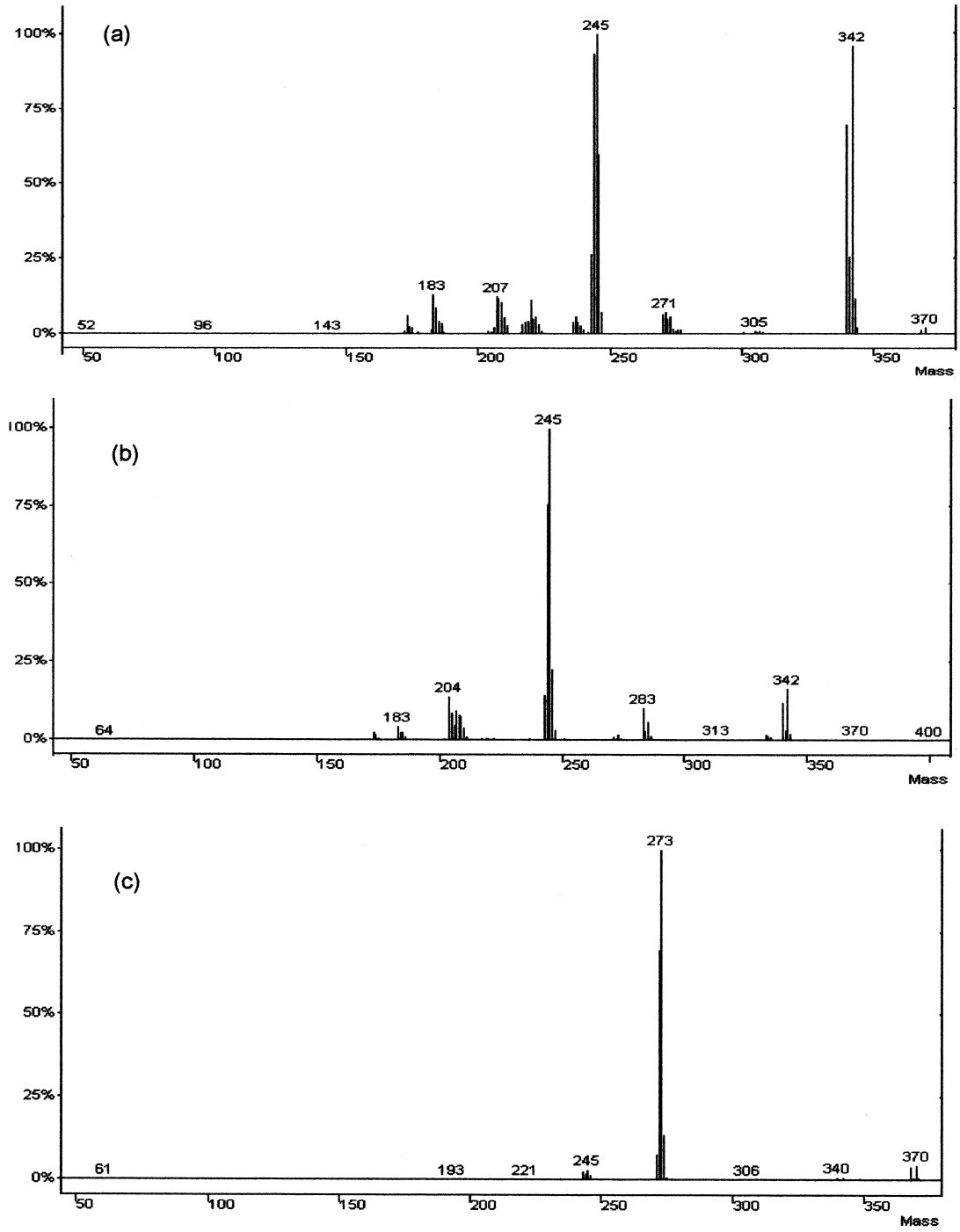


Fig. 6. Non-resonant EI-MS–MS spectrum of the trifluoroacetyl derivative of (a) 2′,4′,6′-trichloro-4-biphenylol (precursor ion m/z 370), (b) 2,2′,5′-trichloro-4-biphenylol (precursor ion m/z 370) and (c) 3,4′,5-trichloro-4-biphenylol (precursor ion m/z 370). The non-resonant MS–MS parameters given in Table 1.

which makes it difficult to calculate the conversion efficiency of a single precursor ion to its respective product ions. If a mass window of one was selected to isolate a single precursor ion, there would be a potential loss in sensitivity. In the case of highly chlorinated compounds, it is therefore difficult to get conditions for under which 100% conversion of a single parent ion to its product ions is achieved, although this will be the ideal for increasing selectivity and sensitivity.

The full scan EI spectra of the TFA-biphenylol congeners indicated that the isomers were difficult to differentiate based on their similar spectra. However, these isomers could be differentiated based on the product ions formed in their MS–MS spectra. In the case of the two monochloro isomers, the ratio of the intensity of product ion m/z 203 to that of m/z 175 is different at similar CID energies. The two dichloro isomers can be differentiated more easily based on the product ions formed and their intensities (m/z 237 and 209) formed using two different CID energies. Similarly, the three trichloro position isomers can be identified based on the difference in the intensity of their product ions (m/z 245 and 342) for 2,2′,5′-trichloro-4-biphenylol and 2′,4′,6′-trichloro-4-biphenylol and by the different product ion (m/z 273) formed for 3,4′,5-trichloro-4-biphenylol (Fig. 6).

Matrix elimination and sensitivity of the GC–MS–MS method was evaluated using a simulated environmental sample. A chicken egg was spiked with all eleven biphenylols and four Aroclors to simulate a heavily contaminated environmental sample. Fig. 7a is the EI full scan chromatogram of the egg sample. Fig. 7b–d shows the extracted CID ion chromatograms of a monochloro-, dichloro- and a trichloro-TFA-biphenylol. The spiked PCBs were eliminated during the isolation stage and did not interfere with the analysis of the hydroxylated PCBs. However, only the lower molecular mass biphenylols were detected. The isomers of these lower-molecular-mass compounds could be identified from their MS–MS spectra and the retention times. The signal-to-noise ratios of these compounds in complex

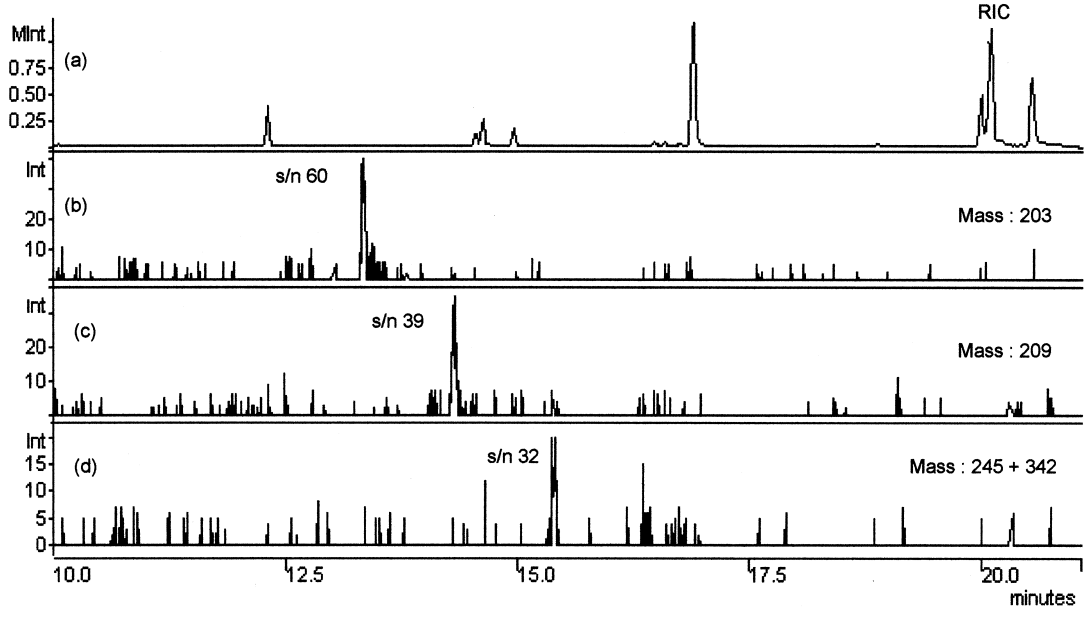


Fig. 7. (a) Full scan EI chromatogram of the simulated egg sample spiked with Aroclors and the mixture of eleven biphenylols. (b) Extracted ion MS–MS chromatogram of the trifluoroacetyl derivative of 4′-chloro-4-biphenylol, (c) extracted ion MS–MS chromatogram of the trifluoroacetyl derivative of 2′,5′-dichloro-4-biphenylol and (d) extracted ion MS–MS chromatogram of the trifluoroacetyl derivative of 2′,4′,6′-trichloro-4-biphenylol.

matrices like the chicken egg matrix indicate possible method detection limits of less than 40 pg on-column using MS–MS analysis.

4. Conclusions

Among the various derivatives prepared for the analysis of the eleven hydroxylated polychlorinated biphenyls, trifluoroacetyl derivatives gave the best chromatographic resolution and mass spectral performance. The derivatization method which was developed to form the trifluoroacetyl derivatives was successful in derivatizing all eleven biphenylols. The MS–MS spectra obtained using the MS–MS parameters helped to identify the three sets of isomers. The method developed was used to analyze picogram levels of target compounds in a biological matrix. Future applications involve extending this method for the analysis of common tern eggs which have been shown to be negatively impacted by high levels of PCBs and potentially these endocrine disrupting hydroxylated PCBs.

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