Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1024 (2004) 155–163
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Optimization of a microwave-assisted derivatization–extraction procedure for the determination of chlorophenols in ash samples☆

M. Ramil Criado, S. Pombo da Torre, I. Rodríguez Pereiro, R. Cela Torrijos*

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Química, Universidad de Santiago de Compostela, Santiago de Compostela 15782, Spain

Received 7 March 2003; received in revised form 9 October 2003; accepted 17 October 2003

Abstract

A procedure for the determination of 17 chlorophenolic compounds in ash samples obtained from the incineration of waste materials is described. Analytes were simultaneously derivatized with acetic anhydride in presence of triethylamine (TEA), and extracted from the sample in a mixture of *n*-hexane acetone using a microwave system equipped with closed extraction vessels. Influence of five experimental parameters (volume of TEA and acetic anhydride, extraction time and temperature, as well as the volume of *n*-hexane acetone) on the yield of the derivatization–extraction procedure was systematically studied using a uniform experimental design at four levels, followed by a conventional factorial design at two levels. Under optimal extraction conditions, recoveries from 72 to 94% were obtained for a spiked ash sample with a carbon content of 8.7%. Quantification limits of the proposed procedure ranged from 2 to 5 ng/g using GC–MS as detection technique. The proposed method was applied to the determination of chlorophenols in three ash samples obtained from different incineration plants. Total chlorophenol contents of 423 and 135 ng/g were found in two of these samples.

Keywords: Microwave-assisted extraction; Extraction methods; Chlorophenols

1. Introduction

Chlorophenolic compounds are widely distributed in the environment due to their industrial use as wood preservatives, fungicides, intermediates in the production of chlorinated pesticides and application in the preparation of adhesives [1]. Other sources of chlorinated phenols in the environment are the hydrolysis of phenoxyacidic herbicides [2]and the chlorination of phenol resulting from the degradation of lignin in pulp and paper mills when chlorine is used during the bleaching process [1,3]. Furthermore, chlorophenols have been detected in vapors and fly ash from waste incineration plants [4,5]. The presence of chlorophenols in those samples has different origins such as the incineration of wastes contaminated with phenol and chlorinated phenols, the in situ chlorination of phenol, which depending on the operating conditions will produce several chlorophenols [6], and the condensation of smaller chlorinated compounds as trichloroethylene [7].

On the other hand, waste incineration plants are considered as one of the most important sources of polychlorinated dibenzo-*p*-dioxins (PCDDs). These compounds are mainly formed due to two mechanisms during incineration processes: chlorination of unburned carbon residues (de novo synthesis), and condensation reactions of precursor compounds containing in their structures an aromatic ring and atoms of chlorine. Thus, chlorophenols are known as one of the main PCDD precursors, and according to results obtained in pilot waste incineration plants, the yield of PCDDs production from certain phenols such as 2,4,6-trichloro-, 2,3,4,6-tetrachloro- and pentachlorophenol is much higher than the yield of the de novo synthesis reactions [8,9]. Therefore, as chlorophenols are present in fly ash at higher concentrations than PCDDs, they could be used as chemical indicators of the PCDDs content in these samples [10].

Chlorophenols can be measured, at the low ng/ml, using a combination of HPLC or gas chromatography (normally after a derivatization step) with mass spectrometry. The critical step in their analytical determination is doubtless their extraction from the matrix, either as native species, or

☆ Presented at the 2nd Meeting of the Spanish Society of Chromatography and Related Techniques, Barcelona, 26–29 November 2002.

* Corresponding author. Tel.: +34-981-563100x14271; fax: +34-981-595012.
E-mail address: qnrctd@usc.es (R. Cela Torrijos).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.10.068

as less polar derivatives. In the last years supercritical carbon dioxide containing polar modifiers [11,12], solvents at high pressure and temperature [13]and microwave-assisted extraction (MAE) processes [14,15] have been widely evaluated as alternatives to the Soxhlet method for the extraction of phenolic species from soils and sediments [16,17]. Conversely to those studies, the extraction of chlorophenols from ashes has been scarcely investigated. The properties of fly ash samples depend on the nature of the burned materials and on the incineration conditions; however, in general, they can be considered as highly adsorptive matrices with an important content of carbon which makes difficult the extraction of organic compounds [18], specially those ones with a planar structure such as PCDDs (the matrix needs to be digested with acid previously to the extraction step) and those with capacity to produce polar interactions with the ash sample. For phenolic species, it has been demonstrated that the yield of the extraction was only 30–40% in ash samples [18] versus more than 80% for soil samples [19], when carbon dioxide containing a 5% of methanol was used as the extraction fluid. To our knowledge, pressurized liquid extraction (PLE) and MAE have not been tested yet for the extraction of chlorophenols from fly ash samples.

The aim of this paper was to evaluate the possibilities of a microwave-assisted procedure for the extraction of 17 chlorophenols from ash samples. In the final method, compounds were derivatized and simultaneously extracted using a microwave system equipped with pressurized extraction vessels. Influence of different parameters in the yield of the extraction–derivatization step was systematically optimized using factorial designs and spiked ash samples. Chlorophenols, as acetyl derivatives, were determined using a GC–MS system. Finally, the concentration of chlorophenols in two different ash samples was compared with the dioxin profile of those samples.

2. Experimental

2.1. Reagents and standards

Methanol, acetone, *n*-hexane and dichloromethane (HPLC grade) were purchased from Merck (Darmstadt, Germany). Acetic anhydride, potassium carbonate, triethylamine (TEA), tetramethylammonium hydroxide (TMAH) and anhydrous sodium sulphate were obtained from Aldrich (Milwaukee, WI, USA).

Standards of 2-chlorophenol (2-CP), 3-chlorophenol (3-CP), 4-chlorophenol (4-CP), 2,6-dichlorophenol (26-DCP), 2,4-dichlorophenol (24-DCP), 3,5-dichlorophenol (35-DCP), 2,3-dichlorophenol (23-DCP), 3,4-dichlorophenol (34-DCP), 2,4,6-trichlorophenol (246-TCP), 2,3,6-trichlorophenol (236-TCP), 2,3,5-trichlorophenol (235-TCP), 2,4,5-trichlorophenol (245-TCP), 2,3,4-trichlorophenol (234-TCP), 2,3,5,6-tetrachlorophenol (2356-TCP), 2,3,4,6-tetrachlorophenol (2346-TCP), 2,3,4,5-tetrachlorophenol (2345-TCP) and pentachlorophenol (PCP) were obtained from Aldrich. Individual stock solutions containing around 2500 μg/ml of each compound were prepared in methanol. Diluted standards and mixtures of the chlorophenols were also prepared in methanol.

A standard of 2,4,6-trichlorobiphenyl (PCB-30) was obtained from Dr. Ehrensdorfer. The stock was diluted in *n*-hexane and used as internal standard to compensate variations in the volume of the final organic extracts containing the acetylated species.

2.2. Apparatus

Analysis of the chlorophenolic compounds, as derivatized species, were performed using two gas chromatographic systems equipped with microwave induced plasma atomic emission spectrometry (MIP-AES) and MS detection. The GC–MIP-AES system was an Agilent 6890 Series Plus gas chromatograph equipped with a split/splitless injector port and connected to an Agilent G2350A atomic emission detector (Wilmington, DE, USA). The GC–MS system consisted of a Varian CP 3800 gas chromatograph (Walnut Creek, CA, USA) also equipped with a split/splitless injector and connected to an ion-trap mass spectrometer (Varian Saturn 2000). In both cases, separations were carried out using a BP-5 type capillary column (30 m × 0.25 mm i.d., $d = 0.25$ μm). Helium was used as carrier gas at a constant flow of 1.2 ml/min. Derivatized phenolic compounds were injected in the splitless mode (1 μl volume injections, purge time 1 min), using the following GC oven program: 1 min at 50 °C, first rate at 15 °C/min to 115 °C (held for 3 min), second rate at 3 °C/min to 175 °C and third rate at 30 °C/min to 250 °C (held for 5 min).

In GC–MIP-AES, helium was used as make-up gas at 50 ml/min and oxygen was added as auxiliary gas in the plasma at a pressure of 30 psi (1 psi = 6894.76 Pa) Mass spectra were obtained in the electron impact mode (70 eV) in the range of 80–350 *m*/*z* and the trap temperature was fixed at 200 °C. The GC–MIP-AES system was used during the optimization of the extraction procedure for the selective monitoring of the chlorine emission signal at 479 nm. The determination of chlorophenols in ash samples spiked at low level, in non spiked samples, and the performance of the developed procedure were evaluated using MS detection due to the limited sensitivity of the MIP-AES detector to chlorinated species.

The gas chromatographic profile of dibenzo-*p*-dioxins, in two of the ash samples was obtained with the ion trap GC–MS system operating in the MS–MS detection mode [20].

Microwave extractions of chlorophenols from ash samples were performed in a MES-1000 microwave extraction system (CEM, Matthews, NC, USA) equipped with teflon-lined 100 ml extraction vessels. Numerical analysis of data resulting from the experimental designs were carried out by means of the statistical package Statgraphics Plus for Windows, version 3.3 (Manugistics, USA).

2.3. Samples

Three samples of ash (fraction under 60 μm) were obtained from the incineration of wood, bark and lignin wastes in a pulp production plant (sample A), from biomass wastes (sample B) and from the combustion of carbon in a power production plant (sample C). Optimization of the extraction procedure was performed using sample A which contains 8.7% of carbon. In preliminary experiments 1 g of this sample was spiked with a solution of the analytes in methanol, just 1 h before the extraction. In further experiments, to achieve a better simulation of the interaction between the chlorophenols and the matrix, a mixture was prepared by spiking a known amount of this sample with a solution of the 17 studied chlorophenols in methanol. The slurry was left in the dark, and allowed to air-dry for 2 weeks until constant weight was achieved. It was then stored at 4 °C for 1 month before analysis. Long term spiked samples were prepared at two concentration levels containing nominally 5 μg/g and 150 ng/g for each compound.

2.4. Extraction and cleanup procedure

In the optimal conditions, chlorophenolic compounds were extracted from the ash samples and simultaneously converted into their acetyl derivatives, in the closed vessels of the microwave extractor. In summary, 1 g of ash was placed in an extraction vessel with 30 ml of *n*-hexane acetone (1:1), 80 μl of TEA and 500 μl of acetic anhydride. The extraction time and temperature were 20 min and 100 °C, respectively. Once the microwave extraction had finished, vessels were cooled down to room temperature and the organic extract concentrated in a Turbo Vap II workstation (Zymark) by means of a 55 kPa nitrogen stream at 25 °C until a final volume of ca. 0.5 ml. The extract was recovered from the concentration tube, made up to approximately 1 ml with *n*-hexane, spiked with 30 μl of polychlorinated biphenyl (PCB) 30 and then washed with 5 ml of a potassium carbonate solution (5% in water) to remove the acetic acid formed in the derivatization reaction and to destroy the excess of anhydride. The *n*-hexane phase was dried with anhydrous sodium sulphate and injected in the chromatographic system.

The PCCDs were extracted from the ash samples using the Soxhlet method. Firstly, the digestion of 5 g of ash was carried out with 100 ml of 1 M HCl. The mixture was shaken for 3 h at room temperature. After filtering, the ash cake was washed with 500 ml of Milli-Q water and left to dry overnight at room temperature in a desiccator.

After that, the ash was mixed with 5 g of anhydrous powdered sodium sulphate, and extracted with 90 ml of toluene for 24 h (4–6 cycles/h). The extract was concentrated under a N_2 stream to 1 ml, and then, cleaned through a small glass column filled with basic alumina, florisil and anhydrous sodium sulphate. The final extract was concentrated to 100 μl for analysis.

2.5. Quantification

Quantification was performed using chlorophenolic compounds derivatized according to a previous published procedure [21]. In brief, 1 ml of methanol containing increasing amounts of the considered chlorophenols was added over 5 ml of a potassium carbonate solution (5% in water), then 1 ml of *n*-hexane, 30 μl of the PCB-30 standard and 200 μl of acetic anhydride were added. The mixture was manually shaken for 5 min and the organic phase dried over sodium sulphate before injection in the gas chromatograph. Calibration curves were built plotting the ratio: analyte peak area/PCB-30 peak area versus the analyte concentration. When MS was used as detection technique, the following *m*/*z* ratios were used for the quantification of each group of isomers: monochlorophenols (126 + 128), dichlorophenols (162+164), trichlorophenols (196+198), tetrachlorophenols (230+232) and pentachlorophenol (266+268). The internal standard PCB-30 was quantified at the *m*/*z* ratio 256 + 258.

3. Results and discussion

3.1. Choice of the extraction strategy

Chlorophenols can be extracted from solid matrices as native species and then derivatized prior to their gas chromatographic separation or derivatized in the matrix and then extracted and analyzed. The last option decreases the polarity of the analytes and therefore, an increase in the yield of the extraction could be expected. However, a higher number of variables should be considered in the optimization of the process., so that the first strategy was initially considered.

Methanol and mixtures of dichloromethane–methanol, which have been previously tested for the Soxhlet extraction of phenols from soils [16] were considered as possible solvents for the microwave-assisted extraction of chlorophenols from the fresh spiked ash samples. After finishing the extraction, the solvent volume was reduced to 1 ml and the phenols present in the methanolic extract derivatized according to conditions given in the experimental section for the acetylation of the calibration standards. In the most favorable extraction conditions (using 20 ml of dichloromethane–methanol, 80:20, at 100 °C for 20 min), extraction efficiencies of 30% for mono and dichlorophenols and around 15% for trichlorophenols were obtained. Tetrachlorophenols and pentachlorophenol (the compounds with the lowest pK_a values) could not be recovered from the fresh spiked sample in an appreciable extension. These compounds were only found in the obtained extract when TMAH (0.1%) was added to the extraction mixture. Probably in presence of TMAH, chlorophenols formed the corresponding ionic pairs which are more easily extracted from the ash matrix. However, the presence of TMAH in the final methanolic extract produced a significant decrease in the yield of the further acetylation reaction. In order to avoid

this inconvenient, it was decided to carry out simultaneously the derivatization and extraction steps.

Following published results for soil samples using supercritical fluid extraction, TEA and acetic anhydride were used as derivatization reagents [12]. A mixture of *n*-hexane acetone (1:1), widely explored for the microwave-assisted extraction of polar compounds from soils [14], was considered as the leaching solvent. Under these conditions, initial experiments showed that all the compounds were extracted from the fresh spiked sample with an approximate yield of 60%. Therefore, the derivatization–extraction approach was chosen and submitted to detailed optimization.

3.2. Optimization of the derivatization–extraction procedure

Five experimental variables were considered in the optimization of the derivatization–extraction of chlorophenols from ash matrices. Two of these variables (TEA and acetic anhydride volumes) potentially affect the derivatization reaction, whereas the microwave exposition time and the extraction temperature as well as the extracting solvent volume may affect the extraction efficiency. The use of a two level full factorial design to evaluate the influences of all these variables would need at least 32 experiments. Resorting to fractional factorial when the interactions between variables can be assumed negligible reduce the number of experiments. However, in any case, by using two level designs, we will model the extraction process by means of an hyperplane. Thus, it should be interesting to consider more than two levels for variables in the screening study without increasing the experimental effort. With this aim, it was decided to carry out the screening study by resorting to experimental uniform designs. Uniform designs (UDs) were developed by Fang [22]and have already been applied to some studies in chemistry [23]and chemical engineering [24]. In analytical chemistry, the only reported application was the optimization of the separation conditions for dithiocarbamates by means of capillary electrophoresis [25]. The main advantages in uniform designs are: (a) they are capable of producing samples with high representativeness in the studied experimental domain; (b) they do not impose strong assumptions in the model, and present robustness against changes of the model and (c) they accommodate the largest possible number of levels for each factor among all published experimental designs. A large number of tables with uniform designs are accessible in the Net [26], which helps practitioners to exploit the advantages of UDs without much worry about theoretical background and design construction. In our case, a UD table to study five continuous experimental factors at four levels in 12 experiments was downloaded and used. This matrix is shown in Table 1. As it can be seen, the considered experimental variables were studied in a wide range in all cases, which allows to obtain a clear insight of the experimental response surface. Results of the experiments were expressed as percentage recoveries of each chlorophenol and regression analysis was applied to these data in two stages.

Firstly, stepwise regression in forward mode (using an F to enter of 4) allowed the selection of the most statistically significant variables to build the model. Then, using these selected variables, a non linear regression model was constructed for each compound, Table 2. Empty cells mean that the corresponding variables (or interactions) did not show a statistically significant effect in the yield of the process, and thus they were not considered in the construction of the model. Signed cells denote significant effects, positive (+) or negative (−), in the yield of the derivatization–extraction for each analyte. The last column of Table 2 presents the correlation coefficients (R^2) for the regression model developed for each compound. Values near to 100, indicate a good agreement between experimental recoveries and those predicted by the proposed model.

Interaction terms corresponding to variables 1–4 (volume of acetic anhydride-extraction temperature), 3–4 (solvent volume-extraction temperature) showed a statistically significant influence in the yield of the process for most chlorophenols. Since interactions were positive in all cases, it appeared as advisable to set the three variables involved in those interactions at the highest levels. However, conversely to this finding, the main effects associated to variables

Table 1
Summary of the experiments considered in the uniform factorial design

Experiment	Volume of acetic anhydride (μl)	Volume of TEA (μl)	Volume of *n*-hexane–acetone (ml)	*T* (°C)	Time (min)
1	220	20	10	80	30
2	500	200	24	80	30
3	360	140	17	95	20
4	500	20	30	95	20
5	80	80	24	80	10
6	500	80	10	115	40
7	360	20	17	130	10
8	220	80	30	130	40
9	360	140	24	115	30
10	220	200	30	115	10
11	80	200	17	95	40
12	80	140	10	130	20

Table 2
Significant variables and first order interactions in non linear regression models produced by the uniform design screening of derivatization–extraction of chlorophenols in ash matrix

Chlorophenol	Effects of each variable[a]				First order interactions between variables.					Adjusted R^2
	1	2	3	4	1–3	1–4	2–4	3–4	4–5	
2-Chlorophenol		+	−			+	−	+		96.76
3-Chlorophenol			−	−		+		+		94.30
4-Chlorophenol	−					+				75.65
2,6-Dichlorophenol			−			+		+		90.76
2,4-Dichlorophenol			−			+		+		89.02
3,5-Dichlorophenol			−			+		+		87.50
2,3-Dichlorophenol				+		+				76.36
3,4-Dichlorophenol			−	−		+		+		88.33
2,4,6-Trichlorophenol			+		−	+				74.84
2,3,6-Trichlorophenol			−			+		+		78.88
2,3,5-Trichlorophenol						+		+		79.47
2,4,5-Trichlorophenol						+		+		82.51
2,3,4-Trichlorophenol						+		+		75.81
2,3,5,6-Tetrachlorophenol						+		+	+	82.51
2,3,4,6-Tetrachlorophenol						+				65.41
2,3,4,5-Tetrachlorophenol						+		+		81.26
Pentachlorophenol						+		+	+	85.20

[a] Variables: (1) volume of acetic anhydride, (2) volume of TEA, (3) extractant volume, (4) extraction temperature, and (5) extraction time.

3 (solvent volume) and 4 (extraction temperature) showed a negative influence in the yield of the derivatization–extraction step for several compounds. It can be noticed that the extraction time (variable 5) was not significant as main factor and only in the case of 2356-TCP and PCP it interacted positively with the extraction temperature (variable 4).

Therefore, the uniform design was considered as a screening study which allowed to identify the most significant variables. For further experiments, it was conservatively decided to fix variables without significant main effects and first order interactions (the volume of TEA and the extraction time) at medium levels (80 μl and 20 min, respectively) and the volume of acetic anhydride at the maximum explored level (500 μl) because of its positive interaction with the extraction temperature in the yield of the process for all compounds.

In the next experiment, the influence of the extraction temperature and the volume of extractant were evaluated in more detail using a well known central composite experiment (2^2 plus star) using a total of 10 randomized experiments, divided into two blocks, Table 3. From these experiments, very similar results were obtained for all considered chlorophenols: the volume of *n*-hexane–acetone showed a positive effect in the extraction efficiency while the effect of temperature and the first order interaction between both factors exhibited a negligible influence. In Fig. 1, the graph of main factors show these conclusions and clearly indicate that the effect of the significant one reached its maximum for the highest level considered in the experiments. Thus, a further increase in the extractant volume, which in any case would be limited by the size of the extraction vessels, was not considered. Temperature was fixed at a value of 100 °C.

3.3. Recoveries

Recoveries of the developed extraction procedure, for long term spiked samples (sample A) at two different concentration levels: 5 μg/g and 150 ng/g, are shown in Table 4. Analytical determinations were carried out using GC–MIP-AES and GC–MS for the analysis of samples spiked at high and low level, respectively. Blank samples were also analyzed to detect the presence of native contamination with chlorophenols. Concentrations of each phenol in the obtained extracts were determined against calibration curves built for standards derivatized in an aqueous potassium carbonate solution and further extracted to *n*-hexane. Theoretically, standards should have been acetylated in the same conditions than samples: direct derivatization in an organic medium using TEA and acetic anhydride; however, as the slopes of calibration curves for acetylated compounds were equivalent in both procedures (data not shown), the former approach,

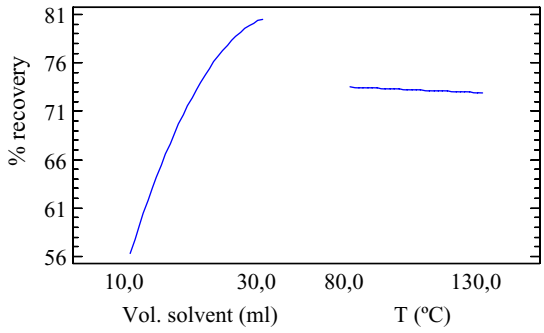

Fig. 1. Main effects of solvent (*n*-hexane–acetone) volume and extraction temperature in the yield of the extraction for 235-TCP.

Table 3
Design matrix and obtained extraction yields (% of recovery) for chlorophenolic compounds in the second factorial design

Experiment	Block	Volume of hexane–acetone (ml)	T (°C)	2-CP	3-CP	4-CP	26-DCP	24-DCP	35-DCP	23-DCP	34-DCP	246-TCP	236-TCP	235-TCP	245-TCP	234-TCP	2356-TCP	2346-TCP	2345-TCP	PCP
1	1	20	105	78	81	80	76	85	84	86	85	79	75	84	84	86	78	75	84	89
2	1	30	130	70	71	72	68	74	74	74	74	72	67	74	72	75	71	66	75	80
3	1	30	80	69	72	72	66	75	75	76	76	68	64	74	74	75	66	63	73	73
4	1	10	130	57	59	59	55	59	56	60	58	54	54	57	55	57	65	50	58	55
5	1	10	80	64	66	66	62	69	68	70	69	63	61	68	68	70	65	60	67	72
6	2	34	105	83	87	88	77	91	90	90	92	80	75	90	90	92	79	75	90	90
7	2	20	70	60	64	63	58	66	66	67	66	59	56	66	65	67	58	56	65	65
8	2	6	105	36	37	37	36	39	38	38	39	38	37	38	38	39	41	38	38	41
9	2	20	140	67	71	72	68	72	71	72	73	70	68	72	70	73	72	66	72	77
10	2	20	105	68	71	71	65	72	72	73	72	66	62	71	71	73	68	59	71	74

Table 4
Recoveries of chlorophenols from a spiked ash sample (sample A) using AES detection (spiked level 5 μg/g) and MS detection (spiked level 150 ng/g), $n = 4$ replicates

Compound	Recovery ± RSD (%)	
	Added concentration	
	5 μg/g	150 ng/g
2-CP	77 ± 5	89 ± 4
3-CP	84 ± 3	84 ± 11
4-CP	78 ± 5	84 ± 9
26-DCP	76 ± 4	72 ± 8
24-DCP	84 ± 3	89 ± 10
35-DCP	82 ± 3	86 ± 10
23-DCP	80 ± 4	90 ± 10
34-DCP	87 ± 3	92 ± 10
246-TCP	94 ± 3	84 ± 6
236-TCP	79 ± 3	72 ± 9
235-TCP	86 ± 3	84 ± 11
245-TCP	77 ± 3	96 ± 8
234-TCP	87 ± 3	89 ± 11
2356-TCP	82 ± 3	81 ± 6
2346-TCP	74 ± 4	85 ± 9
2345-TCP	92 ± 3	84 ± 5
PCP	89 ± 3	87 ± 9

which allowed a reduction in the use of organic solvents and avoided the final extract concentration step, was chosen.

Obtained recoveries ranged from 72 to 94% and relative standard deviations from 3 to 11%, depending on each compound. Obviously, the highest variability corresponded to results for the sample spiked at the low level.

3.4. Performance of the analytical procedure

The linearity of the response in GC–MS was investigated using acetylated standard mixtures of chlorophenols in the concentration range of 10–500 ng/ml. Correlation coefficients from 0.995 to 0.999 were obtained for all compounds. Repeatability for consecutive injections of a standard, containing ca. 100 ng/ml of each chlorophenol, ranged from 2 to 5% after internal standard correction. Quantification limits between 2 and 5 ng/g were obtained for a signal to noise ratio of 10, considering a sample intake of 1 g, Table 5.

3.5. Analysis of real samples

The developed method was applied to the analysis of three ash samples from different sources. Levels of chlorophenols in sample C were under the detection limits of the proposed procedure. However, in samples A and B several chlorinated species were found at quantifiable concentrations, with total chlorophenols contents of 423 and 135 ng/g, respectively, Table 6. With the only exception of 23-DCP, only *ortho*- and *para*-substituted mono-, di- and trichlorophenols were found in both samples. These results are in good agreement with predictions obtained in pilot studies for the chlorination of phenol during incineration processes [6]. As the formation of

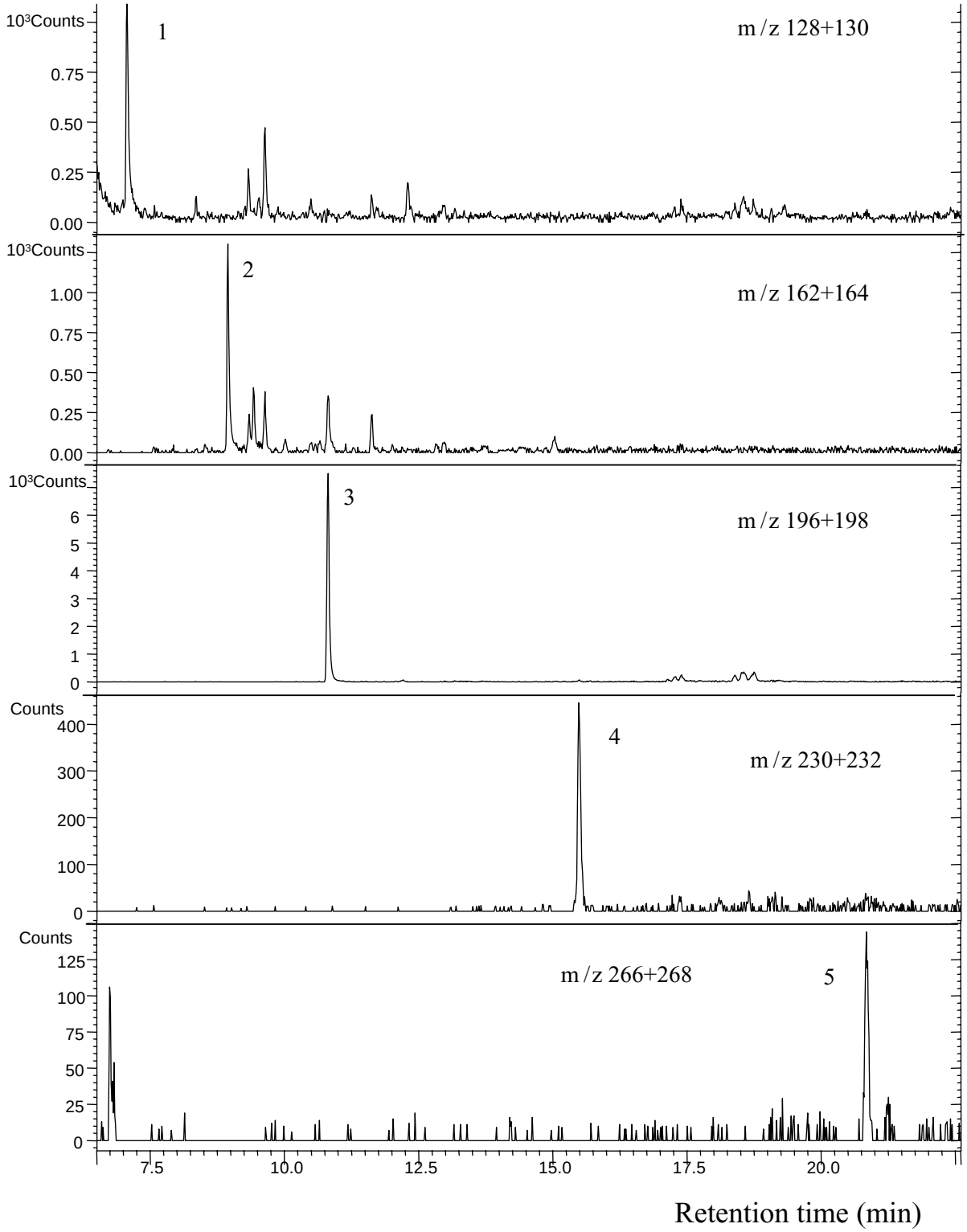

Fig. 2. GC–MS chromatogram for a non spiked ash sample (A). Compounds: (1) 4-CP, (2) 2,4-DCP, (3) 246-TCP, (4) 2346-TCP, and (5) PCP.

23-DCP, in the mentioned processes, has not been reported in the literature, it could be assumed that this compound was already present in the burned wastes and remained in the ash sample after incineration. It should be also noticed that BP-5 type capillary columns cannot separate 24-DCP and 25-DCP isomers as acetyl derivatives, and therefore, the peak assigned to 24-DCP could correspond to 25-DCP or to the sum of both species. The 2346-TCP and PCP were also found in ash samples A and B, Fig. 2. Again, the possible formation of these compounds from phenol in combustion

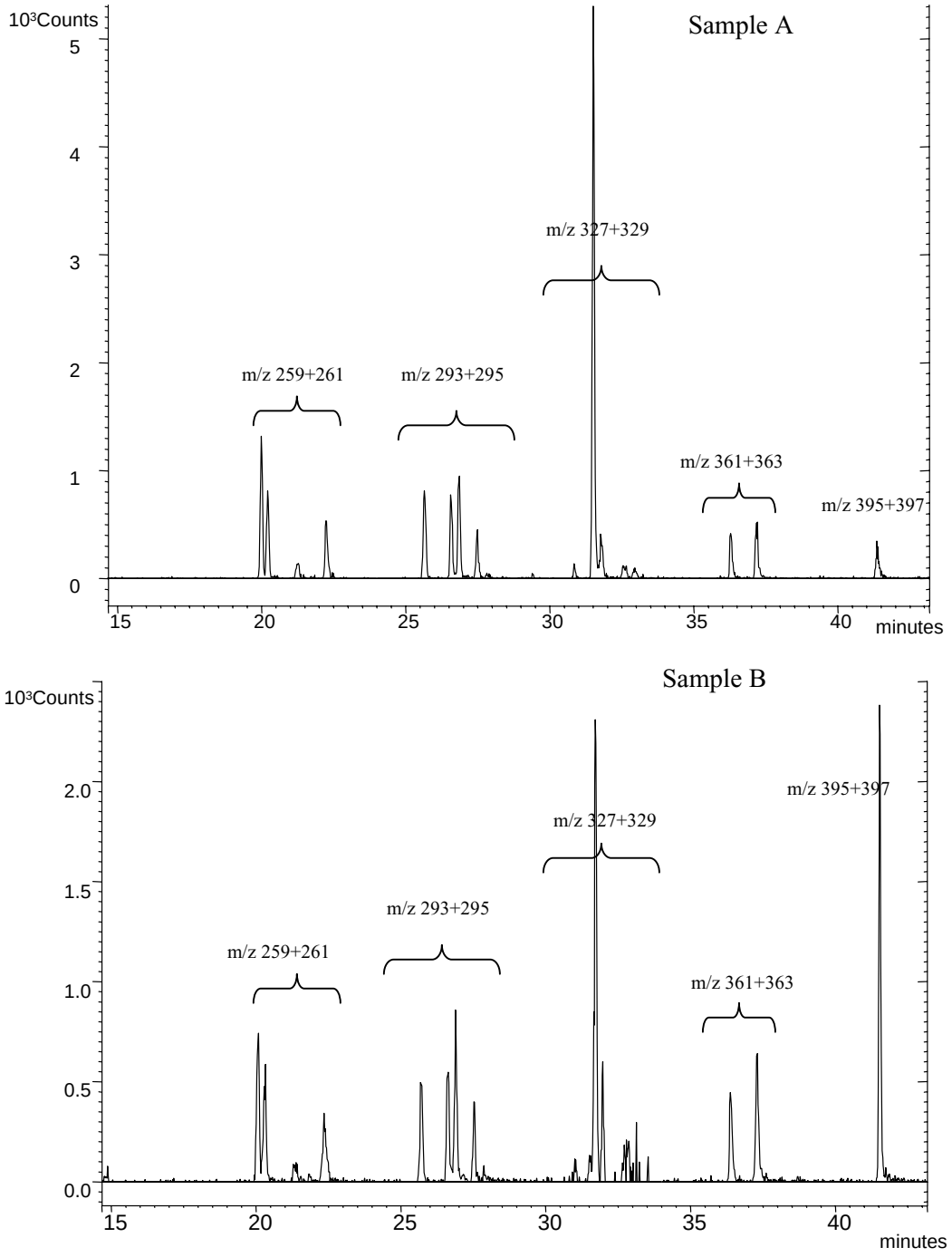

Fig. 3. GC–MS–MS profiles for tetra- (*m*/*z* 259 + 261), penta- (*m*/*z* 293 + 295), hexa- (*m*/*z* 327 + 329), hepta- (*m*/*z* 361 + 363) and octa- (*m*/*z* 395 + 397) chlorodibenzo-*p*-dioxins in ash samples A and B.

plants has been described in the literature [5,6]. Sample A contained not only a higher concentration of chlorophenols than sample B but also a different distribution pattern, with 246-TCP as the most abundant species. The 245-TCP, which has been detected in biomass materials as a result of the environmental degradation of the 2,4,5-trichlorophenoxy acetic acid herbicide [2], and is considered a direct precursor of the 2,3,7,8-TCDD, was not found in any of the analyzed ash samples. The absence of chlorophenols in sample C is probably the consequence of the lack of chlorine sources in the burned carbon.

Fig. 3 shows the dioxin profile (from tetra- to octachlorodibenzo-*p*-dioxins) for samples A and B. In sample A, the most intense peak corresponds to an hexachlorodibenzodioxin, which can be formed in condensation reactions of two 2346-TCP molecules and of 246-TCP with PCP [6,8]. In the case of sample B, the intensity of the chromatographic peaks for the hexachloro- and the octachlorodioxins were similar.

Table 5
Linearity, repeatability and quantification limits (S/N 10) of the proposed procedure using MS detection

Compound	Correlation coefficient (R^2)	Repeatability (RSD) (%) ($n = 5$)	Quantification limit ($S/N = 10$) (ng/g)
2-CP	0.997	3	4
3-CP	0.999	4	4
4-CP	0.996	5	4
26-DCP	0.996	1	5
24-DCP	0.996	5	5
35-DCP	0.997	3	5
23-DCP	0.995	3	5
34-DCP	0.995	5	5
246-TCP	0.997	2	3
236-TCP	0.998	3	4
235-TCP	0.997	4	4
245-TCP	0.997	4	4
234-TCP	0.996	4	4
2356-TCP	0.995	5	4
2346-TCP	0.995	6	4
2345-TCP	0.996	6	3
PCP	0.998	4	2

Table 6
Concentrations of chlorophenols in ash samples ($n = 3$ replicates per sample)

Compound	Mean value (ng/g) ± S.D.	
	Sample A	Sample B
4-CP	69 ± 3	40 ± 3
24-DCP	43 ± 4	20 ± 2
23-DCP	n.q.	26 ± 2
246-TCP	281 ± 31	16 ± 1
2346-TCP	26 ± 3	5 ± 0.4
PCP	4 ± 0.3	28 ± 1
Total chlorophenols	423	135

n.q.: under quantification limits.

Thus, although the establishment of correlations between chlorophenols and PCDDs concentrations in ash samples is outside of the scope of this paper, it is apparent that different chlorophenols patterns correspond to different PCDDs profiles in the ash samples.

4. Conclusions

A microwave-assisted method for the extraction of chlorophenolic compounds from ash samples has been optimized. The strong interaction of the native species with the matrix made necessary to combine the derivatization and extraction procedures in the same step in order to obtain acceptable recoveries for all compounds. Using this approach, the volume of acetic anhydride and *n*-hexane–acetone showed a higher influence in the yield of the derivatization–extraction method than extraction time, temperature and volume of TEA. Application of the proposed method to non spiked ash samples revealed that found chlorophenols isomers were mostly those ones which formation had been predicted from the chlorination of phenol in model studies using pilot incineration plants.

Acknowledgements

This work has been supported financially by the Spanish DGICT (project REN 2000-0984HIP). M.R.C. acknowledges a FPU grant from the Spanish Ministry of Education.

References

[1] B.K. Afghan, A.S.Y. Chau, Analysis of Trace Organic in the Aquatic Environment, CRC Press, Boca Raton, FL, 1989.
[2] L.H. Keith, Environmental Endocrine Disruptors, Wiley, New York, 1997.
[3] M.H. Tavendale, A.L. Wilkins, A.G. Langdon, K.L. Mackie, T.R. Stuthridge, P.N. Mcfarlane, Environ. Sci. Technol. 29 (1995) 1407.
[4] J. Kanters, R. Loouw, Chemosphere 32 (1996) 89.
[5] H. Hunsinger, S. Kreisz, H. Vogg, Chemosphere 32 (1996) 109.
[6] J.G.P. Born, P. Mulder, R. Louw, Environ. Sci. Technol. 27 (1993) 1849.
[7] K.L. Froese, O. Hutzinger, Chemosphere 28 (1994) 1977.
[8] M.S. Milligan, E.R. Altwicker, Chemosphere 30 (1996) 225.
[9] K. Hell, E.R. Altwicker, L. Stieglitz, R. Addink, Chemosphere 40 (2000) 995.
[10] T. Öberg, J. Bergström, Chemosphere 19 (1989) 337.
[11] F.J. Santos, O. Jáuregui, F.J. Pinto, M.T. Galcerán, J. Chromatogr. A 823 (1998) 249.
[12] H.B. Lee, T.E. Peart, R.L. Hong-You, J. Chromatogr. 605 (1992) 109.
[13] L. Wennrich, P. Popp, M. Moder, Anal. Chem. 72 (2000) 546.
[14] V. Lopez-Avila, R. Young, J. Benedicto, P. Ho, R. Kim, W.F. Beckert, Anal. Chem. 67 (1995) 2096.
[15] M. Llompart, R.A. Lorenzo, R. Cela, J.R. Jocelyn Paré, J.M.R. Bélanger, K. Li, J. Chromatogr. A 757 (1997) 153.
[16] M.C. Alonso, D. Puig, I. Silgoner, M. Grasserbauer, D. Barceló, J. Chromatogr. A 823 (1998) 231.
[17] P. Voogt, E.A. Maier, A. Chollot, Fresenius J. Anal. Chem. 361 (1998) 158.
[18] J.W. Hills, H.H. Hill, D.R. Hansen, S.G. Metcalf, J. Chromatogr. A 679 (1994) 319.
[19] M.H. Liu, S. Kapila, K.S. Nam, A.A. Elseewi, J. Chromatogr. 639 (1993) 151.
[20] A. Mitchell, C. Feigel, E. Almasi, Proceedings of the 17th ANZSMS (The Australian and New Zealand Society for Mass Spectrometry) Conference. Thredbo Alpine Village, February 1999, MoP-19.
[21] I. Rodríguez, M.I. Turnes, M.C. Mejuto, R. Cela, J. Chromatogr. A 721 (1996) 297.
[22] K.T. Fang, Acta Math Appl. Sin. 3 (1980) 363.
[23] Y.Z. Liang, K.T. Fang, Q.S. Xu, Chemom. Intell. Lab. Syst. 58 (2001) 43.
[24] S.Y.L. Leung, W.H. Chan, C.H. Luk, Chemon. Intell. Lab. Syst. 53 (2000) 21.
[25] L. Zhang, I.Z. Liang, J.H. Jiang, R.Q. Yu, K.T. Fang, Anal Chim. Acta 370 (1998) 65.
[26] http://www.math.hkbu.edu.hk/UniformDesign.