Determination of polychlorinated biphenyls in biosolids using continuous ultrasound-assisted pressurized solvent extraction and gas chromatography–mass spectrometry

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ABSTRACT

An efficient continuous pressurized solvent extraction (PSE) method assisted by ultrasound energy was developed for the extraction of polychlorinated biphenyls (PCBs) from biosolids. Analytes were determined in the extracts by gas chromatography–mass spectrometry in selected ion monitoring (SIM) mode. A screening-type experimental design pointing to dynamic extraction time as the only significant variable in the extraction process was carried out to optimize PCB extraction from the biosolids. Final selected conditions for PSE were extraction temperature, 50 °C; static extraction time, 0 min; and dynamic extraction time, 30 min. Recovery of the PSE method was 73%, which was significantly improved (103%) when PSE was assisted with 30-min ultrasound (US-PSE). Precision of the overall method, expressed as relative standard deviation, was 3.6% and the detection limit was 0.037 mg/kg. The method was applied to the determination of PCBs in biosolids from different water treatment plants from central Chile.

1. Introduction

The big volumes of biosolids generated during the treatment of household wastewaters constitute an environmental problem that calls for an effective solution. Biosolid beneficial utilization as potential fertilizers and their features as soil improvers turn them into a viable alternative, as opposed to a simple buildup of solid wastes. For a safe alternative in the use of biosolids in forest, agricultural or degraded soils it is necessary to discard the presence of pathogenic organisms, heavy metals and organic pollutants [1]. Polychlorinated biphenyls (PCBs) are among the immense variety of toxic organic components which can be found in sewage sludges. The PCBs are a class of 209 congeners synthesized by chloration (1–10 molecules of chlorine) of both biphenyl rings [2]. These contaminants have been widely used in industrial processes as dielectrics and in heat transfer systems mainly because they are highly stable. Nevertheless, because of the same features, these compounds are highly persistent in the environment and very resistant to degradation, which together with their weakly polar character, make them easily accumulated, mainly due to depositional processes into organic matter present in biosolids and other environmental matrices [3].

Analytical determinations of organic compounds like PCBs in solid matrices are based on extensive procedures during the stage of sample preparation, mainly due to the difficulty in weakening the strong interactions established between analytes and matrix. This is particularly true of biosolids, considering their high content in organic matter. In this context, the traditional Soxhlet extraction technique needs a high investment in terms of time (8–36 h) and use of large amounts of solvents.

During the last years, a number of new methodologies have been developed in order to provide the suitable energy that allows more efficient extraction of the analytes from the matrix by using either less solvent or time involved in the extraction. Pressurized solvent extraction (PSE) has showed good extraction efficiency compared with that of Soxhlet extraction for PAHs, PCBs, and chlorinated pesticides in air particulates, sediment, mussel tissue, and fish materials, and greater extraction efficiencies for the higher molecular weight PAHs in diesel particulate materials [4]. Similar results were obtained by our team [5] when PSE was compared with Soxhlet in natural contaminated soil samples, in relation to PAH and diesel range organics (DRO) recoveries. On the other hand, Wang et al. [6] studied the extraction of polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) from soils using Soxhlet extraction, microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE). They observed that ASE had the best extraction efficiency compared with MAE and Soxhlet extraction, and the extraction efficiencies of MAE and Soxhlet extraction were comparable to each other depending on properties such as TOC

content of the studied soil. Similarly, PSE of steroids from coastal sediments showed average recoveries 15% higher than Soxhlet [7].

Particularly in the case of PCBs, the effectiveness of six different extraction techniques was compared for PCBs in soils (Soxhlet, Soxtec, supercritical fluid extraction, MAE, ultrasonication extraction and ASE) [8]. Although in that study none of the techniques was optimized and the authors selected the extraction parameters based on experience from previous successful investigations, most of the techniques under study are capable of providing accurate results. On the other hand, Soxhlet, shaking and batch ultrasound extraction have also been applied to PCBs from sludges coming from city sewage-treatment plants [9]. In that case, each method of extraction with the use of a particular solvent was effective and selective for particular PCB isomers.

In any case, even though improvement of effectiveness of modern extraction approaches compared with Soxhlet is not clear enough, important factors such as solvent consumption and total time of analysis, which nowadays is becoming one of the most important factors, have shown in all the studies that modern extraction techniques are preferable to Soxhlet extraction.

In this study, an efficient pressurized solvent extraction method was developed and optimized for the extraction of PCBs from biosolids. The PSE method was assisted by ultrasound (US-PSE) showing a significant effect on the recoveries. Recovery of PCBs was compared with the conventional reference Soxhlet extraction technique. As far as we know, this is the first study about the content of PCBs in biosolids from Chile.

2. Experimental

2.1. Reagents

De-ionized water (NANOpure ultrapure water system; Barnstead, Dubuque, IA, USA) was used throughout. Working standard solutions of PCBs were prepared by dilution of Aroclor 1254 and 1260, containing concentrations from $1000 \mu g/mL$ (SUPELCO Park, Bellefonte, PA, USA, 44808 and 44809). A mixed standard solution of both Aroclors prepared in methanol (GC–MS/Pesticides grade analysis, Fisher Scientific, Fair Lawn, NJ, USA), was used both to spike the biosolids and for calibration purposes. Phenanthrene-d10 (AccuStandard Inc. New Haven, CT 06513, USA) was used as internal standard. During each sequence of sample analysis, linearity was checked by including the mixed standard solutions in the sequence. Dichloromethane–acetone solvent mixture (1:1, v/v) was used in PSE, US-PSE and Soxhlet extractions.

Hexane (GC–MS/Pesticides grade analysis, Fisher Scientific, Fair Lawn, NJ, USA), anhydrous sodium sulfate (Merck), and silica gel (100–200 mesh, Sigma–Aldrich) were used in the clean-up stage.

Nitrogen 5.0 (AGA, Chile) and He 5.0 (AGA, Chile) were used for final evaporation of the extracts and as a gas chromatographic carrier gas, respectively.

2.2. Instruments and apparatus

The schematic buildup of the extraction unit has been shown previously [5]. All the tubing (1/16 in., 1/8 in. O.D.) was made of SS 306 stainless steel. Connections were made using Swagelok fittings (Solon, Ohio, USA). The valve employed was Swagelok needle valve SS-ORS2 (5000 psi allowed pressure).

The extraction chamber for PSE consisted of a homemade oven (a $28 cm \times 12 cm \times 5 cm$ aluminum block with controlled temperature). A temperature controller BTC-704-41521000; Spec.: J, $0–400 °C$ (Brainchild Electronic Co. Ltd., Taipei, Taiwan) was used to keep the temperature at the desired value. Inside the chamber a

preheated coil (2 m stainless steel tube SS-316, 1/16 in., 0.1 mm I.D., Supelco, Bellefonte, PA, USA) was fitted to keep the programmed temperature and was followed by the extraction cell (a 12 mm I.D. empty HPLC column, Supelco, Bellefonte, PA, USA). In the case of US-PSE, an ultrasonic bath (Elma, Model Transsonic Digital D-78224 Singen/Htw, Übach-Palenberg, Germany) with programmable temperature was used instead of the oven. The solvent used for the extractions was pumped using an HPLC pump (PerkinElmer series 200, Wellesley, MA, USA). For extraction, the pressure inside the system was kept in a working range of 1800 ± 50 psi.

Extract concentration was carried out in a Kuderna Danish system (Supelco, Bellefonte, PA, USA-64685-U) and for cleanup a Visiprep SPE 12-port vacuum manifold (Supelco, Bellefonte, PA, USA-57030-U) was employed.

Quantitation was performed using a gas chromatograph Hewlett-Packard model 5890 series II coupled to a mass selective detector Fisons Instruments model MD 800.

2.3. Spiking procedure

For optimization of variables and recovery studies, a real biosolid from the Metropolitan Region of Chile was considered. This biosolid contains 51.7% organic matter and 26.6% dry matter content. A spiked biosolid preparation was made by diluting a stock mixture of the Aroclor standards in 25 ml of DCM and mixing in amber bottles with 20 g of biosolid to obtain a final concentration of 50 mg/kg of total PCBs. The bottles were placed in a hood, and the solvent was slowly evaporated with constant stirring. The spiked biosolids were aged for 90 days at room temperature and then stored at $4 °C$.

2.4. General analytical procedure

The spiked biosolid and real biosolid samples were separately extracted according to the following procedures.

2.4.1. Pressurized solvent extraction (PSE)

Biosolid samples were weighed (500 mg) and loaded into an extraction cell located inside the aluminum chamber—oven extractor. The oven was coupled to a heating device fitted on the upper part of the chamber and electronically controlled through a thermocouple to reach the temperature fixed by the factorial design depending of the experiment (the temperature in the oven was checked monthly with an external probe, a Digi-Sense Thermocouple Thermometer EW-93000-00, Cole Parmer, Vernon Hills, IL, USA). Then, the DCM–acetone mixture was pumped through the system (dynamic extraction) to extract the analytes from the samples at a flow rate of 2 ml/min for the time fixed by the factorial design depending of the experiment. After PSE was done, the extract was concentrated, cleaned up and characterized by GC–MS. The solvent was evaporated to 1 ml prior to injection, and spiked with internal standard at 0.5 $\mu g/ml$. Optimization of extraction considered the following variables: extraction temperature, static and dynamic extraction time, and flow rate of solvent.

In each set of experiments one blank was always processed and one standard was injected to check linearity.

2.4.2. Ultrasound-assisted pressurized solvent extraction

The same procedure and manifold as implemented for PSE was used in this case, except for the aluminum oven, which was substituted with an ultrasonic bath at a temperature of $50 °C$. Ultrasound was applied for 0–30 min at 35 kHz. A mixture of DCM–acetone (1:1, v/v) was used for extraction.

Factors	Levels		
	−1	0	1
Temperature, X_1 (°C)	50	125	200
Dynamic extraction time, X_2 (min)	5	17.5	30
Static extraction time, X_3 (min)	0	10	20

2.4.3. Soxhlet extraction

Biosolid samples were treated using the conventional Soxhlet extraction procedure. A detailed description of the analytical procedure used for extraction of those samples has been previously described [10]. It consists basically in extraction by Soxhlet using DCM–acetone (1:1, v/v) as an extraction solvent system (24 h), then the extract is characterized by GC–MS after evaporation in a Kuderna–Danish concentrator and cleanup procedure.

2.4.4. Cleanup of the extracts

The cleanup procedure for the extracts was carried out according to US-EPA method [11] which basically consists in the use of activated silica gel and elution with hexane. The clean extracts were concentrated to 1 ml with nitrogen prior to injection into the GC–MS.

2.4.5. GC–MS determination

Final determination was carried out by GC–MS using a ZB-5 fused silica capillary column (30 m × 0.25 mm I.D., and 0.25 μm film thickness) coated with 5% phenyl–95% methylpolysiloxane. One microliter of sample was injected into the column using splitless mode. Injector temperature was 280 °C. Initial column temperature was 80 °C, raised up to 280 °C at 10 °C/min (5.0 min). A constant flow of 1.0 ml/min of helium as carrier gas was used.

The MS transfer line was held at 280 °C and quantitations were based on calibration with standard analytes using mass spectrometric parameters (SIM mode). The ions monitored were **222.0**, 224.0 (di-chlorinated PCBs); **256.0**, 258.0 (tri-); **292.0**, 290.0 (tetra-); **326.0**, 328,0 (penta-); **360.0**, 362.0 (hexa-); **394.0**, 396.0 (hepta-); **430.0**, 432.0 (octa-); **464.0**, 466.0 (nona-). In all cases, the ion in boldtype was used for quantification (target ion) and the second one as qualifier. The relative abundance ion ratio should match the comparison standard within ±25%.

2.4.6. Experimental design

A screening-type experimental design was carried out to optimize PCBs extraction from the biosolids. Table 1 shows the factors and levels considered in the experimental design (2^3 + 3 center points). Considering the variables extraction temperature, dynamic and static extraction times, Table 2 shows the experimental design matrix for the optimization experiments. For this study, a biosolid

Table 2
Experimental design matrix for optimization experiments

Run	X_1	X_2	X_3
1	1	−1	1
2	−1	1	−1
3	−1	1	1
4	−1	−1	1
5	0	0	0
6	−1	−1	−1
7	0	0	0
8	1	−1	−1
9	1	1	−1
10	0	0	0
11	1	1	1
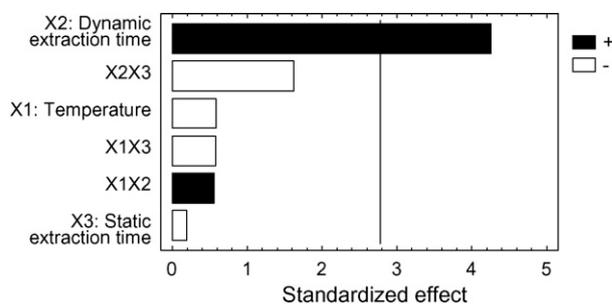
Fig. 1. Standardized Pareto chart.

sample was spiked with a known standard concentration (50 mg/kg of total PCBs) containing Aroclor 1254 and 1260.

The analytical response used for optimization was the sum of the signals attributed to the different congeners present in both Aroclor standards (mainly tetra- to hepta-chlorinated PCBs). These signals were identified by the retention time of the signals appearing in the SIM mode.

3. Results and discussion

3.1. Pressurized solvent extraction

Extraction temperature, static and dynamic extraction times, which in addition can interact with each other, were considered the three variables affecting PCB extraction from biosolids most significantly. Flow rate of solvent and pressure were kept constant at 2 ml/min and 1800 psi, respectively, considering that in previous studies [5,12] with low-polarity analytes in soils no significant effects were observed regarding these variables. Bearing these facts in mind, the three variables were optimized through a 2^3 mixed-level factorial design with three central points randomly distributed, which accounts for possible interactions.

As can be seen in Fig. 1, the Pareto chart clearly indicates that the only significant variable (p-value <0.05) corresponds to dynamic extraction time (X_2). Temperature and static extraction time, and any possible interaction among the variables, were not significant to the response. The response considers the relationship between the sum of the chromatographic areas of PCBs and the internal standard area.
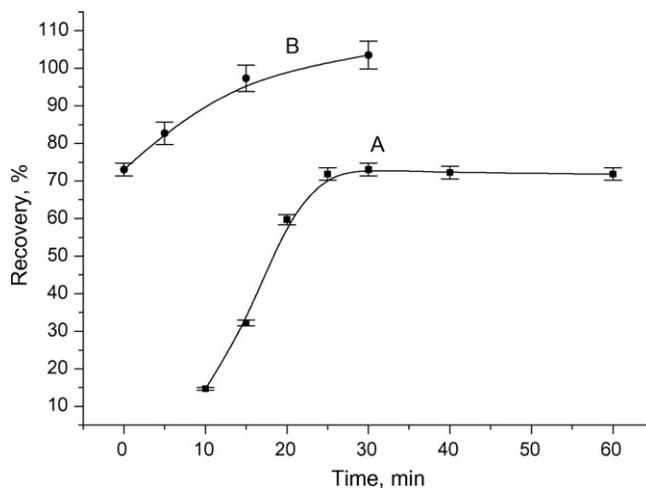
Fig. 2. Recovery of PCBs from biosolid. (A) Effect of dynamic extraction time on recovery. Other conditions: extraction temperature, 50 °C (without ultrasound assistance). (B) Effect of ultrasound time on recovery. Other conditions: extraction temperature, 50 °C; dynamic extraction time, 30 min.

After determination of significance of dynamic extraction time, this variable was optimized by the univariate method by keeping temperature and static time steady at their minimum values, 50 °C and 0 min, respectively. Fig. 2A shows that the response increases concomitantly with dynamic extraction time until 30 min are reached. After this time, the signal remains constant.

Taking these results into account, the selected variable values for further experiments were extraction temperature, 50 °C; static time, 0 min; and dynamic time, 30 min.

Calibration curves were constructed ($n = 6$) in a concentration range of 0.1–30 μg/ml of total PCBs, using a standard mixture of Aroclor 1254 and 1260.

The calibration equation by using linear regression square method was

$$\text{Response} = (0.104 \pm 0.002)\,[\text{PCBs tot}] + (0.009 \pm 0.004); \quad R = 0.9992$$

where [PCBs$_{\text{tot}}$] corresponds to the total PCB concentration in μg/ml.

The limit of detection, calculated according to the 3-σ criteria, was 0.037 mg/kg (total PCBs). Recovery and repeatability were determined by analysis of the spiked sample containing 50 mg/kg total PCBs. Average recovery was only 72.01% with a relative standard deviation of 2.3%. A previous study [13] using Soxhlet extraction reported a recovery of 89% for PCBs in biosolids. This means that in our case a considerable portion of the spiked PCBs (near 30%) is strongly held on the matrix during the extraction process.

In order to release a greater amount of PCBs from the matrix and consequently increase the recovery rate, the pressurized solvent extraction process was assisted with additional ultrasound energy.

3.2. US-PSE

In previous studies, it has been demonstrated that a correctly performed batch sonication procedure is capable of producing data similar to or better than those obtained by Soxhlet, for PCB extraction from soils [8]. By keeping this in mind, we expected that recoveries from PSE could be improved if the continuous extraction process were further assisted with ultrasound energy. However, on the other hand, the destruction of 4-chlorobiphenyl by ultrasound in homogeneous aqueous media has also been reported [14] but the presence of humic acids and alumina particles decreases degradation efficiency.

In the present case, the oven used in PSE was replaced with an ultrasonic water bath at 50 °C and 35 kHz in which the pre-heater and sample cell were immersed.

Assessment was done of ultrasound time applied on the sample, from 0 to 30 min, using in all instances a dynamic extraction time of 30 min and a flow rate of 2 ml/min. As can be seen in Fig. 2B, ultrasound improved extraction efficiency considerably, discarding the possibility of PCB degradation, and 30 min of dynamic extraction is quite satisfactory for quantitative extraction of PCBs. Consequently, under optimum conditions (same as PSE, plus 30 min of US), the mean recovery was 103.5% with a relative standard deviation of 3.6%. A similar effect of improvement of PSE extraction of aromatic and aliphatic hydrocarbons from soils has been recently reported by us [5].

On the other hand, comparative studies were made in the same sample with the conventional Soxhlet extraction technique. In this case, the recovery obtained was 87.9%, showing that the ultrasound energy incorporated into the system US-PSE promotes efficient PCB desorption from the active sorption sites of the matrix, a higher

Table 3
Determination of PCBs in biosolids coming from different wastewater treatment plants from central Chile

Wastewater treatment plant location	Total PCBs (mg/kg)	RSD (%)
Valparaíso, V region (2005)	0.88	0.09
Santiago, Metropolitan region (2004)	0.30	0.06
Santiago, Metropolitan region (2005)	0.29	0.08
Santiago, Metropolitan region (2006)	0.22	0.01
Rancagua, VI region (2005)	0.18	0.02
Talca, VII region (2005)	<LOD	–

recovery (103.2%) being reached in comparison with the technology of reference.

3.3. Application in real biosolid samples

The optimized US-PSE method was applied in order to determine total PCBs in biosolid samples (Table 3) taken from different wastewater treatment plants in central Chile. In all cases, PCB signals were confirmed according to the relative abundance ion ratio (target/qualifier) should match the comparison standard within ±25%. Analytes were quantified in all samples except in the biosolid coming from the VII Region of the country. As observed in previous studies, PCBs are manly distributed in congeners from tetra- to hepta-chlorinated [15–17]. The concentration values found in Chilean biosolids are comparable to those obtained in a wastewater treatment plant in England [15,16], but considerably lower than those reported for Brazilian biosolid samples [17]. The Chilean regulation for biosolids considers concentration limits only for trace elements. Organic compounds are not yet regulated. However, according to biosolid quality criteria in Europe, the standard for concentration of PCBs is 0.8 mg/kg [18]. Consequently, according to the results obtained in this study, all biosolids, except those coming from Valparaiso, could be used for land application.

4. Conclusions

A continuous pressurized solvent extraction method assisted by ultrasound energy was developed for the extraction of polychlorinated biphenyls from biosolids. A screening-type experimental design was carried out to optimize the analyte extraction from the biosolids indicating that the only significant variable in the extraction process was the dynamic extraction time. The recovery of the PSE method was 73%, which was significantly improved at 103% when the PSE was assisted by 30 min ultrasound (US-PSE). The precision of the overall method, expressed as relative standard deviation was 3.6% and the detection limit was 0.037 mg/kg.

The method was applied to the determination of PCBs in biosolids from different water treatment plants from central Chile. The concentrations found are comparable with those obtained in a wastewater treatment plant from England [15,16], but considerably lower than that reported from Brazilian biosolid samples [17].

References

[1] E.Z. Harrison, S.R. Oakes, M. Hysell, A. Hay, Sci. Total Environ. 367 (2006) 481.
[2] O. Hutzinger, S. Safe, V. Zitko, The Chemistry of PCBs, CRC Press Inc., USA, 1974, p. 269.
[3] C.N. Sawyer, L.P.L. McCarty, G.F. Parkin, Química para Ingeniería Ambiental, 4th ed., McGraw Hill, Inc., Colombia, 2001, p. 247.

[4] M.M. Schantz, J.J. Nichols, S.A. Wise, Anal. Chem. 69 (1997) 4210.

[5] P. Richter, M. Jiménez, R. Salazar, A. Maricán, J. Chromatogr. A 1132 (2006) 15.

[6] W.T. Wang, B.J. Meng, X.X. Lu, Y. Liu, S. Tao, Anal. Chim. Acta 602 (2007) 211.

[7] D. Li, M. Dong, W.J. Shim, N. Kannan, J. Chromatogr. A 1160 (2007) 64.

[8] S. Sporring, S. Bøwadt, B. Svensmark, E. Björklund, J. Chromatogr. A 1090 (2005) 1.

[9] W. Sulkowski, A. Rosińska, J. Chromatogr. A 845 (1999) 349.

[10] Method 3540C, U.S. Environmental Protection Agency, Office of Solid Waste, Test Methods for Evaluating Solid Waste Physical/Chemical Methods, SW-846 on CD-ROM, May 1996, National Technical Information Service (NTIS), U.S. Department of Commerce, Springfield, VA, USA.

[11] Method 3630C, U.S. Environmental Protection Agency, Office of Solid Waste, Test Methods for Evaluating Solid Waste Physical/Chemical Methods, SW-846 on CD-ROM, May 1996, National Technical Information Service (NTIS), U.S. Department of Commerce, Springfield, VA, USA.

[12] P. Richter, B. Sepúlveda, R. Oliva, K. Calderón, R. Seguel, J. Chromatogr. A 994 (2003) 169.

[13] E. Mangas, M.T. Vaquero, L. Comellas, F. Broto-Puig, Chemosphere 36 (1998) 61.

[14] Y. Lu, L.K. Weavers, Environ. Sci. Technol. 36 (2002) 232.

[15] R. Gibson, M.J. Wang, E. Padgett, A.J. Beck, Chemosphere 61 (2005) 1336.

[16] R.E. Alcock, J. Bacon, R.D. Bardget, A.J. Beck, P.M. Haygarth, R.G.M. Lee, C.A. Parker, K.C. Jones, Environ. Pollut. 93 (1996) 83.

[17] M. de Souza-Pereira, B. Kuch, Chemosphere 60 (2005) 844.

[18] E. Epstein, Land Application of Sewage Sludge and Biosolids, Lewis Publishers, USA, 2003, p. 189.