

Research article

An expeditious method for determination of polychlorinated biphenyls in liquid humus using optimized microwave-assisted extraction methodology followed by gas chromatograph-mass spectrometry

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Abstract

An alternate method for extracting polychlorinated biphenyls (PCBs) in liquid humus using microwave-assisted extraction (MAE) was optimized through the response surface methodology (RSM). The economy in the use of solvents coupled with the decrease in extraction time and lower power consumption make MAE a technique that meets the principles of green chemistry. The extraction process was defined by a 23 factorial design. This study was realized with the aim of evaluating the efficiency of extraction and the influence of the parameters that affect the process, extrapolating the extraction conditions to other congeners as also to other matrices. In the factorial design, temperature, extraction time and hexane volume were evaluated. Samples were extracted with mixed solvent (ethyl acetate: hexane) and the data for the study were based on injection of a mix of PCBs. In optimum conditions, the extraction procedure showed recoveries in the range of 90.92 to 98.90%. The relative standard deviations were less than 3.08%. According to the defined ranges for each parameter assessed, the best extraction conditions were achieved at high temperature and a shorter extraction time

Keywords: Microwave-assisted extraction, optimization, polychlorinated biphenyls, liquid humus

Introduction

The liquid humus contains nutrients and organic matter that can bring benefits to the soil and consequently serve as an additive in agriculture, increasing the fertility of the soil or contaminate the environment if it is not controlled.[1]

Polychlorinated biphenyls (PCBs) are a class of persistent organic pollutants (POPs), which differ in the number of chlorine atoms (1-10) attached to their biphenyl rings.[2] PCBs are found in soils, water, sediments and air, since they are transported across international boundaries far from their sources, even for regions where they have never been produced.[3] Due to their toxicity and persistence in the environment, its production has gradually declined since the 70s, however, from anthropogenic sources of pollution (industry, agriculture, mining, etc.) these compounds can be introduced in the environment as byproducts of many chemical processes.[4]

Due of their high molecular stability, low water solubility and high tendency to adsorb on particulate phase, PCBs are extremely difficult to remove from the soil or sediment matrices. Because their hydrophobicity and lipophilicity, they are poorly absorbed by plants, but are susceptible to bioaccumulate in animals, mainly in adipose tissue and breast milk.[2,5] For accumulation in adipose tissues, such compounds reach the human body mainly by eating animal foods contaminated with PCBs.[6]

Depending on the number of chlorine atoms and their position in the molecule, congeners of PCBs differ in their chemical, physical and toxicological properties, which are significant to predict the fate and environmental impact of them.2 PCBs molecules may have up to 209 different structures called congeners, depending on the different positions that the chlorine atom can occupy in the molecule.[7]

Microwave-assisted extraction (MAE) of organic pollutants was originally introduced in 1986 by Ganzler et al (1986).[8] Since that time, several applications have been reported the use of microwaves for assisting the extraction of organic and organometallic compounds from liquid and solid matrices.[9] The classical method for extraction of PCBs from environmental samples is Soxhlet, which requires large amounts of solvent and about 20 hours for complete extraction. Techniques such as ultrasonic extraction, Soxhlet extraction, matrix solid-phase dispersion, pressurized fluid extraction, supercritical fluid extraction and microwave-assisted extraction have gained wide acceptance due to low temperature requirement, high extraction rate (recovery), automation and the possibility of simultaneous extraction of different types of samples.[3,10,11]

The main advantage of MAE is the ability of rapidly heat the sample-solvent mixture. By using closed vessels, the extraction can be performed at elevated temperatures accelerating the mass transfer of target compounds from the sample matrix, in most cases, reproducibility and extraction yields are improved, using less energy and less solvent volume, compared to traditional methods.[11]

There are many factors that affect the MAE efficiency, some of these are the power, temperature, extraction time, solvent ratio, and solvent.[12] Therefore, a optimization step is required.

The aim of this study was to optimize a methodology for the extraction of polychlorinated biphenyls in biofertilizers obtained from liquid humus using microwave-assisted extraction followed by gas chromatography-mass spectrometry determination.

Materials and Method

Samples

To the optimization tests, liquid humus samples obtained from the fermentation of agricultural wastes were used. The mixture consisted of 20% wastes (4 L of cattle manure + 4 L of poultry) with 80% water (32 L), in volume.

Reagents

For the optimization tests, a mix of the reference materials 2,6 dichlorobiphenyl; 2,4,4-trichlorobiphenyl; 2,2,5,5-tetrachlorobiphenyl; 2,2,4,4,5,5-hexachlorobiphenyl and 2,2,3,4,4,5-heptachlorobiphenyl, in a concentration of 100 mg L⁻¹ was used. From the reference material, a stock solution of 10 mg L⁻¹ in ethyl acetate was prepared. Ethyl acetate and hexane HPLC grade were purchased from Tedia. Helium (purity: 99.9990%) was purchased from White Martins.

Chromatographic system

The chromatographic system used was a GC/MS, Shimadzu, GC2010 Plus, equipped with split/splitless and a mass spectrometric detector. The capillary column used was a BP-5 capillary column, 30 m x 0.25 mm i.d. and 1.0 µm film thickness (Agilent). Helium at a constant flow rate of 1 mL min⁻¹ was used as carrier gas. The injector and detector temperature were 280 °C. Monitored ions for PCBs analysis were: 284, 286, 263, 261, 272, 274, 183, 353, 37, 249 (m/z). The mass spectrometer operated in full scan mode for the determination of the retention times of the compounds and the SIM mode to analyze the samples. The analytes were quantified by analytical curve constructed with 6 concentration levels in the range of 10 – 1500 µg L⁻¹ for each reference material. The mass spectrometer operating parameters were ion source temperature at 220 °C; interface temperature maintained at 250 °C. The chromatographic conditions are described in Table 1.

Table 1. Gas chromatograph conditions established for determination of PCBs in liquid humus.

Injection	Splitless (1 min)
Injector temperature	250°C
Column temperature	80°C
Flow	He, 1.0 mL min ⁻¹
Temperature program	50 °C min ⁻¹ up to 100 °C + 15 °C min ⁻¹ up to 235 °C (3:00 min) + 5 °C min ⁻¹ up to 270 °C

Microwave-assisted extraction

The extraction procedure was performed in a microwave digestion system (SPEEDWAVE FOUR®, with Built-in Non-Contact, Temperature and Pressure Measurement, BERGHOF, Germany) with a capacity of 12 vessels of 60 mL and 1450 W maximum power. To the optimization tests, a 10 mL sample plus 10 mL of a mixture of the solvents ethyl acetate and hexane were used. The extraction temperature ranged from 70 to 150 °C, hexane volume in the mixture ranged from 20 to 80%, the irradiation times evaluates were 10 and 30 minutes. The magnetron power output of the microwave unit was set at 80% (1160 W). The ramp time (8 min) was previously defined, evaluating the time necessary for the equipment to reach the programmed maximum temperature (120 °C), using the lowest possible power.

After the extraction step, the vials were cooled until room temperature before opening. Then, the material was filtered in 45 µm filter paper, concentrated under vacuum in rotary evaporator (FIZATOM) at 80 °C until dryness, reconstituted with 1 mL of ethyl acetate and placed in a vial for the chromatographic determination. For tests with

blanks, the above procedures were followed using the sample without addition of the reference material. Table 2 shows the MAE operational conditions.

Table 2. MAE operational conditions

Magnetron power	80% (1160 W)
Ramp*	8 min
Extraction temperature	70-150°C
Extraction time	10-30 min
Solvent volume	10 mL
Sample volume	10 mL
Maximum vessel pressure	35 bar

Experimental design and optimization of microwave-assisted extraction

The extraction conditions of PCBs in liquid humus samples were optimized based on the analysis of response surface methodology (RSM) plots of the investigated factors for each compound. In this study, the effects of three factors on the response were evaluated using a 23 factorial design, 16 experiments in duplicate were randomly performed according to the experimental design. The response-variable (area) was chosen to the optimization process.

Table 3 shows the settings of the three factors. All experimental parameters were defined according to previous experiments in our laboratory.

Table 3. Factors and their settings for 23 experimental design for MAE

Factor	Minimum	Maximum
Temperature (°C)	70	150
Extraction time (min)	10	30
Hexane in ethyl acetate mixture (vol.%)	20	80

A two-way interaction linear model was adjusted to the experimental data based on the equation 1.

$$\hat{y} = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 \quad (1)$$

where \hat{y} is the predicted value (area for each compound), β 's are the model coefficients, and x_1, x_2, x_3 the encoded factors: temperature (x_1 , 70-150 ° C), extraction time (x_2 , 10-30 min) and hexane volume in the mixture of solvents (x_3 , 20-80%).

Results and Discussion

Extraction

The extractions were performed using a mixture of ethyl acetate-hexane. The use of polar solvents, such as ethyl acetate, facilitates the process of absorption of microwave energy and, consequently, a rapid heating of the non-polar solvent, the hexane in this study, allowing reaching temperatures above its boiling point.[3] The use of microwaves in the extraction process generates energy, causing heating solvent and consequently in the sample which can suffer degradation or conversion of some compounds in different ways such as by exposure to high temperatures and pressure inside the extraction vessels.[13] To achieve the best extraction conditions a 2³ factorial design was used. The actual and encoded levels of the factors (temperature, time and hexane volume) for each experiment as well as the response-variable (area) for each compound are shown in Table 4.

Table 4. Experimental design 23: actual and encoded levels of the factors and average of observed responses.

Factors			Average of responses (duplicate)				
Temperature (°C)	Time (min)	Hexane (vol. %)	DCB	TCB	TTCB	HXCB	HPCB
70 (-)	10 (-)	20 (-)	2412.53	5994.66	500.24	54.79	121.19
150 (+)	10 (-)	20 (-)	1308.33	8637.70	653.12	1010.23	264.31
70 (-)	30 (+)	20 (-)	1852.21	6322.42	435.82	37.99	47.38
150 (+)	30 (+)	20 (-)	1501.14	10168.14	1866.22	103.76	110.33
70 (-)	10 (-)	80 (+)	2305.99	9684.05	474.23	1.42	57.58
150 (+)	10 (-)	80 (+)	2412.64	966.55	649.24	106.32	163.05
70 (-)	30 (+)	80 (+)	1461.69	7728.96	514.68	863.50	334.00
150 (+)	30 (+)	80 (+)	584.97	1033.61	705.90	42.84	249.23

* DCB (2,6-dichlorobiphenyl); TCB (2,4,4-trichlorobiphenyl); TTCB (2,2,5,5-tetrachlorobiphenyl); HXCB (2,2,4,4,5,5-hexachlorobiphenyl) and HPCB (2,2,3,4,4,5-heptachlorobiphenyl).

Table 5 shows the significance of the extraction process. Bold numbers indicate significant factors as identified by the analysis of variance at the 95 % confidence level.

Table 5. Significance levels of parameters (effects) identified by analysis of variance (ANOVA).

Congeners*	β_0	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}
DCB	1729.94	-556.33	-759.87	-77.23	-57.56	171.3	-576.11
TCB	6317.01	-2231.02	-7.46	-2927.43	806.33	-5473.02	-936.43

TTCB	724.93	48738	311.45	-277.84	323.43	-304.26	-262.89
HXCB	277.61	76.36	-31.16	-48.17	-453.80	-434.24	430.47
HPCB	168.38	56.70	33.70	65.16	-67.60	-46.34	147.59

β_0 (average of responses), β_1 (temperature), β_2 (time) e β_3 (hexane volume). Bold numbers indicate significant factors as identified by the analysis of variance at the 95 % confidence level.

*DCB (2,6-dichlorobiphenyl); TCB (2,4,4-trichlorobiphenyl); TTCB (2,2,5,5-tetrachlorobiphenyl); HXCB (2,2,4,4,5,5-hexachlorobiphenyl) and HPCB (2,2,3,4,4,5-heptachlorobiphenyl).

From the effects of calculated parameters, the factor 1 (temperature) was the most important for all investigated compounds, except for the congeners DCB and TCB. The extraction time was significant only for two compounds and generally negative, showing a decrease in extraction time (factor 2) leads to an enhanced response. However, in the congener TTCB the extraction time was significant. The factor 3 (hexane volume) was not significant (strongly negative) for all compounds, i.e., from this step using only of ethyl acetate as solvent may be considered. The factor interactions β_{12} (temperature x time), has the greatest significance in the congeners TCB and TTCB. The factor interaction β_{13} (temperature x hexane volume) showed significance only for the congeners DCB, i.e., this factor interactions was not significant in the process.

Analysis of variance

Table 6 shows the statistically analyses of variance for the regression model equations and significant coefficients for each congener.

Table 6. Analysis of variance (ANOVA) for the experimental results.

Source	SS	DF	MS	F	P	Source	SS	DF	MS	F	P
Model	3.6611E6	3	1.2204E6	6.8633	0.0060	Model	850971.5	3	283657.1	2.2616	0.1336
DCB*						HXCB*					
X ₁	1.2380E6	1	1.2380E6	3.8036	0.0714	X ₁	23325.69	1	23325.69	0.1399	0.7139
X ₂	2.3096E6	1	2.3096E6	9.2775	0.0087	X ₂	3885.34	1	3885.34	0.0231	0.8813
X ₃	23857.12	1	23857.12	0.0578	0.8134	X ₃	9280.91	1	9280.91	0.0553	0.8174
X ₁ X ₂	13253.20	1	13253.20	0.0712	0.7941	X ₁ X ₂	823760.4	1	823760.4	6.5678	0.0248
X ₁ X ₃	117379.8	1	117379.8	0.319	0.5826	X ₁ X ₃	754270.5	1	754270.5	5.7681	0.0334
X ₂ X ₃	1.3276E6	1	1.3276E6	7.4665	0.0181	X ₂ X ₃	741204.7	1	741204.7	5.5531	0.0362
Error	2.1337E6	12	177811.3			Error	1.5050E6	12	125424.3		
Cor. total	5.7948E6	15				Cor. total	2.3560E6	15			
R ²	0.9957					R ²	0.9909				

Model	1.7411E8	3	5.8036E7	109.4377	5.551E-9	Model	108666.3	3	36222.11	10.15862	0.0013
TCB*						HPCB*					
X ₁	1.9910E7	1	1.9910E7	1.7360	0.2088	X ₁	12857.86	1	12857.86	1.2988	0.2735
X ₂	222.6064	1	222.6064	1.727E-5	0.9967	X ₂	4543.77	1	4543.77	0.433	0.5212
X ₃	3.4280E7	1	3.4280E7	3.2827	0.0915	X ₃	16983.95	1	16983.95	1.7682	0.2048
X ₁ X ₂	2.5998E6	1	2.5998E6	0.1975	0.6646	X ₁ X ₂	18281.07	1	18281.07	1.8948	0.1938
X ₁ X ₃	1.199E8	1	1.199E8	226.130	3.77E-9	X ₁ X ₃	8590.04	1	8590.04	0.9120	0.3584
X ₂ X ₃	3.7788E7	1	3.7788E7	1.05935	0.4024	X ₂ X ₃	87138.61	1	87138.61	24.4383	3.40E-4
Error	6.3638E6	12	530314.9			Error	42787.85	12	3565.65		
Cor.total	1.8047E8	15				Cor. total	151454.2	15			
R ²	0.9981					R ²	0.9929				

Model	1.7565E6	3	585528.7	5.1885	0.0158
TTCB*					
X ₁	950147.3	1	950147.3	6.15651	0.0264
X ₂	387998.2	1	387998.2	1.995	0.1796
X ₃	308774.7	1	308774.7	1.5427	0.2346
X ₁ X ₂	418840.8	1	418840.8	3.7080	0.0782
X ₁ X ₃	370296.6	1	370296.6	2.9992	0.1089
X ₂ X ₃	276444.6	1	276444.6	1.5520	0.2366
Error	1.3542E6	12	112850.8		
Cor. total	3.1108E6	15			
R ²	0.9907				

SS (sum of square); DF (degree of freedom); MS (mean of square). X₁ = temperature, X₂ = time e X₃ = hexane volume. *DCB (2,6- dichlorobiphenyl); TCB (2,4,4- trichlorobiphenyl); TTCB (2,2,5,5- tetrachlorobiphenyl); HXCB (2,2,4,4,5,5- hexachlorobiphenyl) e HPCB (2,2,3,4,4,5- heptachlorobiphenyl). Bold numbers indicate significant factors as identified by the analysis of variance at the 95 % confidence level.

The P-value is used as a tool to check the significance of each parameter between the variables. The smaller the value of P, the more significant is the corresponding coefficient.[14] According to Table 6, the greatest significant is in the interaction between temperature and hexane volume (X₁X₃) in congener TCB (P<0.05), followed by the interaction between extraction time and hexane volume (X₂X₃) in congener HPCB. All calculated regression

coefficients (R^2) are in the range 0.99, indicating a good correlation to quantify and interpret the relationship between the experimental data and the factor effects studied. Based on the calculated effects (Table 5) on the extraction parameters, i.e., temperature (positive) and time (negative), an example of the proposed model for congener HXCB is described (Equation 2).

$$\hat{y} = 724,93 + 38,18x_1 - 15,58x_2 - 24,08x_3 - 226,9x_1x_2 - 217,12x_1x_3 + 215,23x_2x_3 \quad (2)$$

Response surface plot analysis

Response surface plots are useful to estimate the effects of two factors on the response, as well as identify the optimal values for obtaining maximum response.[15] Graphs were plotted using the z-axis (response) against the two independent variables maintaining a third one at a fixed value. Figures 1, 2 and 3 show an example of main interactions in the process for the compound HXCB. Similar plots but not identical were obtained for all the other investigated congeners.

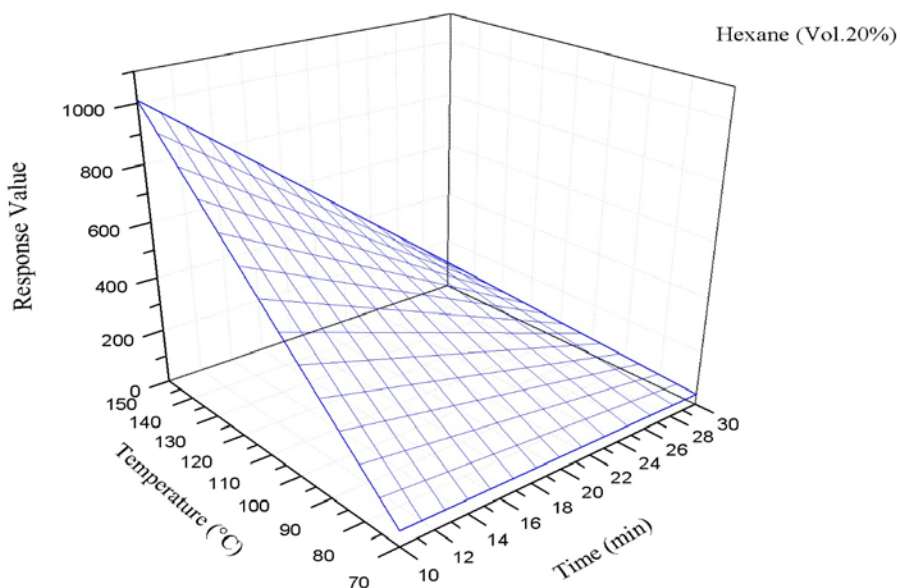


Figure 1. Response surface. Temperature x time.

Figure 1 shows the interaction between the temperature and the extraction time in response. By increasing the temperature from 70 to 150°C and decreasing the extraction time from 30 to 10 minutes, the responses is improved. Therefore, the increase of temperature (150 ° C) combined with a smaller volume of hexane (20%) improved the extraction of analytes adsorbed in the particulate matrix, avoiding the risk of loss by degradation using a shorter time (10 minutes).

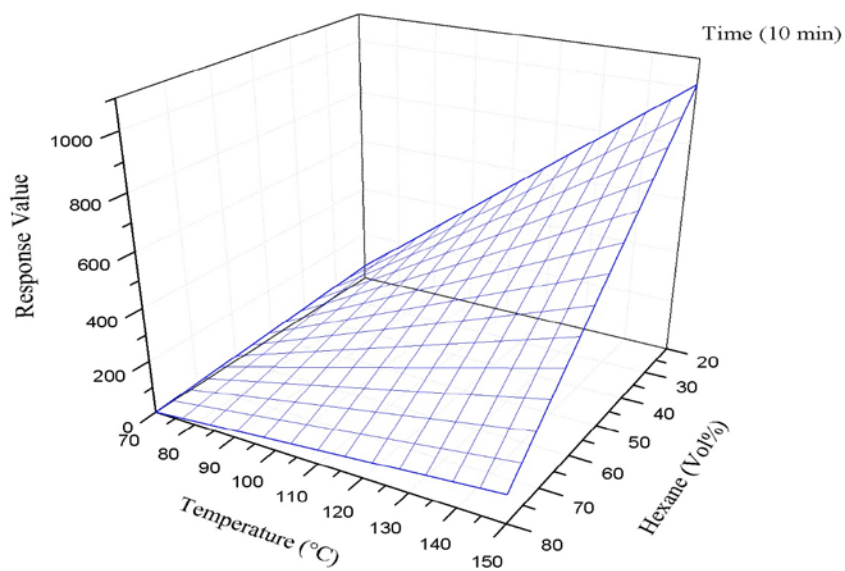


Figure 2. Response surface. Temperature x percentage of hexane (vol%).

Figure 2 shows the interaction between temperature and percentage of hexane in the solvent mixture. By increasing the temperature from 70 to 150°C and decreasing the volume of hexane from 80 to 20%, best responses are achieved. Thus, adjusting the temperature at 150 °C with 20% of hexane, the extraction is improved. Although both solvents had low dielectric loss factor ($\tan \delta < 0.1$), the improvement in the response with the decrease of the percentage of hexane in the mixture indicates the effectiveness of the ethyl acetate solvent due to its polarity and low volatility.

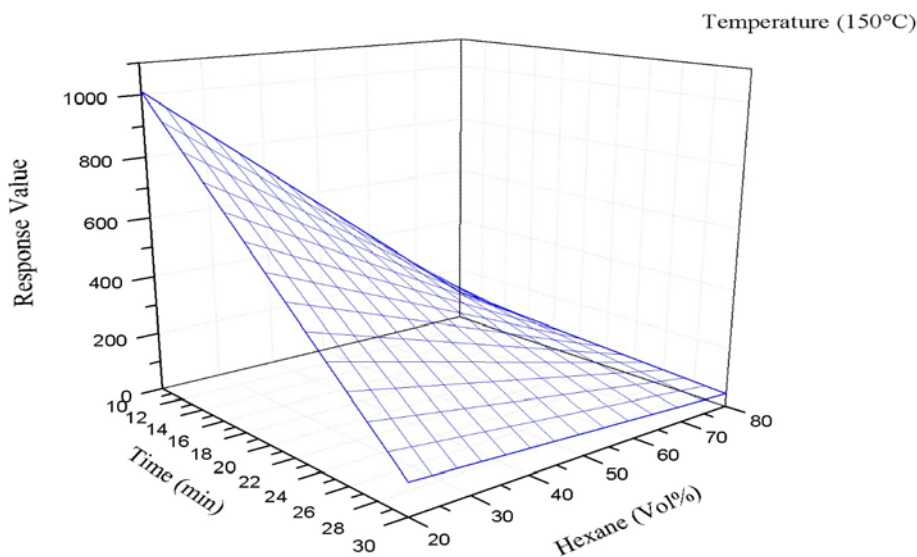


Figure 3. Response surface. Time x percentage of hexane (vol%).

Figure 3 shows that best responses are achieved with a smaller extraction time (10 min), using a hexane volume of 20% in the solvent mixture, i.e., decreasing the time of extraction and the hexane volume in the solvent mixture.

Recovery assay: yield of extraction

After optimization of the methodology, the yield of extraction was evaluated using the parameters defined as the best response in the optimization of microwave assisted extraction, ie, temperature of 150°C; mixture ethyl acetate: hexane (80:20%), and extraction time of 10 minutes. The recovery assays experiments were performed by adding 2.00 mL of a stock solution of 10 mg L⁻¹ to the extraction vessel to a vessel whose final volume was 20 mL. Thus, inside of vessels, the concentration of PCBs was 1.0 mg L⁻¹. After the extraction, the sample was determined according to the procedure previously described. A chromatogram of the reference material together with the sample is shown in Figure 4.

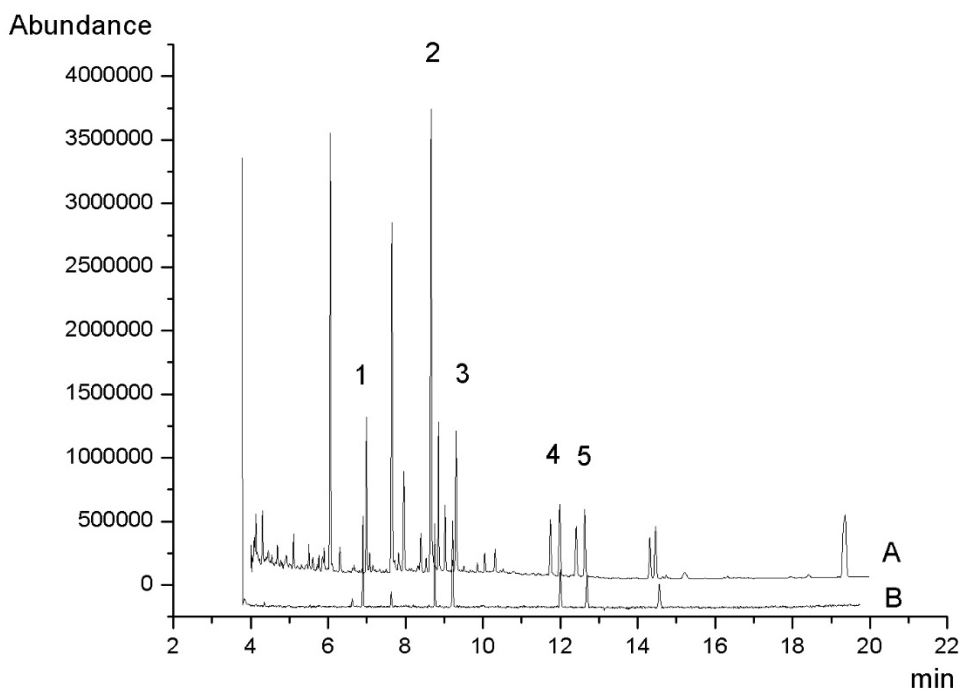


Figure.4. Chromatograms. (A) sample; (B) mixed standard. Peaks: (1) 2,6-dichlorobiphenyl; (2) 2,4,4-trichlorobiphenyl; (3) 2,2,5,5-tetrachlorobiphenyl; (4) 2,2,4,4,5,5-hexachlorobiphenyl; (5) 2,2,3,4,4,5-heptachlorobiphenyl.

Table 7 shows the linear regression, coefficient of determination, recoveries and relative standard deviation of the congeners studied.

Table 7. Linear regression, coefficient of determination, recoveries and relative standard deviation of the congeners studied.

Congeners	Calibration	R ²	Average recovery* (%)	R.S.D* (%)
2,6-dichlorobiphenyl	y= 90.6987x + 985.073	0.9981	95.88 ± 2.77	1.82
2,4,4-trichlorobiphenyl	y= 82.8818x + 986.683	0.9781	90.92 ± 1.07	1.17
2,2,5,5-tetrachlorobiphenyl	y= 61.3565x + 504.024	0.9995	95.68 ± 1.05	3.08
2,2,4,4,5,5-hexachlorobiphenyl	y= 59.5722x – 989.561	0.9805	98.90 ± 1.82	2.43
2,2,3,4,4,5-heptachlorobiphenyl	y= 95.2792x + 3743.5	0.9998	94.98 ± 1.42	1.45

*Means ± standard deviation (triplicate) and relative standard deviation (R.S.D).

Another studies using optimized methodology of microwave-assisted extraction (temperature: 80 to 120°C; extraction time of 5 to 15 min and acetonitrile as solvent) reach recoveries between 70% and 120% with RSD less than 17.2% by GC-MS in the determination of organophosphorus, organochlorine, pyrethroid and carbamate pesticides in herbal medicine (*Radix astragali*).[16]

Other example is in the determination of PCBs and chlorobenzenes by GC-MS in fly ash using microwave-assisted extraction in optimal conditions (30 mL volume of toluene/acetone or a 15 mL volume of toluene, samples with less than 60% water content, and an irradiation time of 15 min), obtaining recoveries about 90-110%. [17]

Recoveries generally depend upon the matrix, the analytes and the extraction method considered. Therefore, a direct comparison with literature is not so straightforward.

Conclusion

This paper describes an alternative method for the extraction of PCBs in liquid humus from the perspective of green analytical chemistry, as well as a means to monitoring of contamination for these pesticides in other matrices. Considering the significance of factors in the process, associated with the recovery levels achieved (90.92 - 98.90%), the maximum level of temperature combined with a smaller extraction time resulted in an increase of observed response, and hence improve yield extraction. However, temperature levels above 150 °C increase the internal pressure of the extraction vessels, considering the volatility of hexane (68 °C) in the mixture ethyl acetate-hexane, which consequently result in analyte losses and possible explosion risks. The optimization of microwave-assisted extraction was efficient and rapid (10 min.) to extract polychlorinated biphenyls from liquid humus. As shown, the choice of solvent based in polarity improve the extraction process. The extraction time and temperature were the most significant factors. In general, the experiment shows that increasing the ethyl acetate volume, the effect on the extraction is positive. Analyzing all the data and combination of them, the optimal conditions were maximum temperature of 150 °C, solvent mixture ethyl acetate-hexane (80:20%) and an extraction time of 10 minutes.

The production of liquid humus from organic waste increases the aggregated value of this waste, being a good alternative for soil organic matter replacement. However, the presence of potentially toxic compounds must be evaluated. Only after confirmation of the absence of pollutants is that their use becomes environmentally safe.

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