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Article

Determination of Chlorobenzenes in Sewage Sludge by Solid-Liquid Extraction with Purification at Low Temperature and Gas Chromatography Mass Spectrometry

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Nesse artigo, a técnica de extração sólido-líquido com purificação em baixa temperatura para a determinação de nove clorobenzenos (CB) em lodo de esgoto foi otimizada, validada e aplicada. Análises foram realizadas por cromatografia gasosa acoplada ao espectrômetro de massas. Depois da otimização da fase extratora, um planejamento fatorial foi usado para avaliar a influência de três variáveis, as quais foram pH, força iônica (NaCl) e tempo de homogeneização. O método mostrou ser seletivo, com limites de detecção e quantificação menores que 4,1 e 12,5 µg kg^{-1}, respectivamente. As porcentagens de extração foram cerca de 70% e o desvio padrão relativo menor que 13% para os nove clorobenzenos analisados. Durante o monitoramento mensal os compostos 1,4-CB; 1,2,4-CB e 1,2,3-CB foram detectados em amostras de lodo de esgoto. Somente o composto 1,2,3-CB foi quantificado em concentrações superiores ao limite máximo aceitável para clorobenzenos em solos agrícolas condicionados com lodo de esgoto, mas sem considerar o efeito de diluição do lodo no solo.

In this paper, the technique of solid-liquid extraction with purification at low temperature for the determination of nine chlorobenzenes (CB) in sewage sludge was optimized, validated and applied. Analyses were carried out by using gas chromatography coupled with mass spectrometry. After univariate optimization of the extraction phase, a factorial design was used in order to evaluate the influence of pH, ionic strength (NaCl) and homogenization time. The method proved to be selective, with detection and quantification limits less than 4.1 and 12.5 µg kg^{-1}, respectively. Recoveries were about 70% and the relative standard deviation was less than 13% for the chlorobenzenes analyzed. During the monthly monitoring of sewage sludge samples 1,4-CB, 1,2,4-CB and 1,2,3-CB were detected. Only 1,2,3-CB was quantified in concentrations higher than the maximum acceptable limit for chlorobenzenes in agricultural soils amended with sewage sludge disregarding the dilution effect of the sludge on soil.

Keywords: biosolids, chlorobenzenes, extraction methods

Introduction

Chlorobenzenes (CB) are widely used in industry as organic solvents, pesticides, dielectric fluids, deodorants and intermediates in the synthesis of chemicals.[1] When exposed to the environment some chlorobenzenes are highly toxic, carcinogenic, teratogenic and mutagenic.[1,2] Furthermore, these compounds are persistent in the environment and have the ability to bioaccumulate in living organisms.[3] These contaminants can be found in several environmental matrices such as superficial and underground water,[1,4] soil,[5] air,[6] biota,[7] sediments,[8] fertilizers[9] and sewage sludge.[10,11]

Sewage sludge is a byproduct of the domestic and/or industrial sludge treatment and has very complex chemical composition.[12] Its characteristics depend mainly on its origin and on the degree of treatment at the Sewage Treatment Plants (STP).[13] One of the ways to dispose of sewage sludge is to utilize it as a soil conditioner and fertilizer in agricultural and forest soils due to its richness in nutrients and organic matter.[14,15] Sludge has been applied to crops such as sunflower,[16] eucalypt,[17] acacia,[18] corn and beans.[19] However, when sewage sludge is used arbitrarily it can cause environmental problems due to the presence of pathogens, heavy metals, and organic contaminants such as chlorobenzenes.[12] For safe and proper use of sewage sludge, the resolution No. 375/2006 of the National Council for the

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Environment (CONAMA)[20] has established concentration limits of chlorobenzenes in soil that range between 6.5 to 730 μg kg^{-1}.

The monitoring of chlorobenzenes in sewage sludge has traditionally been carried out by soxhlet extraction; however, this method is laborious and consumes high amount of organic solvents.[20] Other techniques have also been used for the extraction of chlorobenzenes, such as headspace[21] and solid phase microextraction.[22] Given the lack of modern techniques for the extraction of chlorobenzenes in sewage sludge and the complexity of this matrix, the solid-liquid extraction with purification at low temperature (SLE-PLT) is presented as a promising alternative. This technique has been applied for the analysis of contaminants in samples of tomato, soil, potato, apple and butter,[23-25] etc. The principle is based upon the partitioning of analytes between the sample and the organic phase at −20 °C resulted from a difference in the freezing point. The advantage of this method is that the sample components are frozen within the aqueous phase, whereas analytes are extracted by the organic phase. The method is practical and has a reduced number of steps in addition to low sample and organic solvent consumption.[26]

The aim of this study was to optimize and validate the SLE-PLT for the analysis of nine chlorobenzenes specified by CONAMA[20] in sewage sludge samples by gas chromatography and mass spectrometry. The characteristics and results obtained in the optimized method were compared to the reference method (soxhlet) of the Environmental Protection Agency (EPA). SLE-PLT was applied to real sludge samples for the monitoring of chlorobenzenes over a period of 11 months.

Experimental

Reagents and solutions

The standards of 1,2-Dichlorobenzene (1,2-CB), 1,3-Dichlorobenzene (1,3-CB), 1,4-Dichlorobenzene (1,4-CB), 1,2,3-Trichlorobenzene (1,2,3-CB), 1,2,4-Trichlorobenzene (1,2,4-CB), 1,3,5-Trichlorobenzene (1,3,5-CB), 1,2,3,4-Tetrachlorobenzene (1,2,3,4-CB), 1,2,3,5-Tetrachlorobenzene (1,2,3,5-CB), 1,2,4,5-Tetrachlorobenzene (1,2,4,5-CB) and the analytical-grade naphthalene were provided by Sigma-Aldrich (St. Louis, USA). Naphthalene was used as the internal standard (IS).

Stock standard solutions of the nine chlorobenzenes and naphthalene were individually prepared in acetonitrile at a concentration of 500 mg L^{-1} and were stored at 4 °C. By diluting stock solutions, a working standard solution containing nine chlorobenzenes and a solution containing naphthalene at a concentration of 25 mg L^{-1} were prepared in the same solvent.

Acetonitrile, ethyl acetate, and dichloromethane, HPLC-UV grade, were used as solvents and obtained from Vetec (Rio de Janeiro, Brazil). Anhydrous sodium sulphate, sodium chloride and sodium hydroxide, with purity over 99%, were obtained from Vetec (Rio de Janeiro, Brazil). Silica gel 230-400 mesh (Carvalhaes, Germany) was used and conditioned with a mixture of acetonitrile, dichloromethane and ethyl acetate and then oven dried at ± 45 °C.

Samples

Sewage sludge samples used to optimize the method were obtained at the STP in Juramento-MG, Brazil, from domestic sludge only. Samples were randomly collected from the drying bed. After that, they were stored in glass flasks in a refrigerator at 4 °C. The moisture content was gravimetrically measured after drying samples (2.00 g) in an oven at 105 °C until constant mass.[12]

Equipment and condition of analysis

During optimization and validation of the extraction method, a Phoenix vortex (São Paulo, Brazil) and a Quimis microprocessor pHmeter (São Paulo, Brazil) were used.

Analyses were performed in a gas chromatograph by Agilent Technologies (GC 7890A) coupled with a mass spectrometer (MS 5975C) using an HP-5 MS capillary column (Agilent Technologies) with a stationary phase consisting of 5% phenyl and 95% methylpolysiloxane (30 m × 0.32 mm inner diameter × 0.25 μm film thickness). Helium (purity 99.999%) was used as a carrier gas at a flow rate of 1.2 mL min^{-1}. Split/splitless injector was maintained at 280 °C. The chromatography column temperature was maintained at 70 °C for 4 min. Then, it was heated at a rate of 5 °C min^{-1} to 180 °C. After separation of the compounds the column temperature was heated up to 300 °C and held for 3 min (post run). One microliter (1 μL) of the sample was injected into the equipment under split injection mode at a ratio of 1:5 using a CombiPAL injector. The mass spectrometer was operated in electron ionization mode at 70 eV and quadrupole mass analyzer. The interface was kept at 240 °C and the ion source at 230 °C.

For the method optimization, data acquisition was performed in full scan mode in the range of 50-450 (*m/z*). At the validation step, analyses were performed in selective ion monitoring mode (SIM) and data acquisition was divided into three groups of ions as shown in Table 1.

Table 1. Groups of chlorobenzene ions used for chromatographic analysis (GC-MS) of the compounds at the validation of the method

Group		Compound	R_t[a]	*m/z*
1	Dichlorobenzenes	1,3-CB	6.73	84, 111, 146, 148, 150
		1,4-CB	6.91	
		1,2-CB	7.56	
2	Trichlorobenzenes and IS[b]	1,3,5-CB	10.43	74, 109, 128, 145, 180
		1,2,4-CB	11.76	
		1,2,3-CB	12.80	
		Naphthalene[b]	12.01	
3	Tetrachlorobenzenes	1,2,3,5-CB	16.01	89, 108, 143, 179, 216
		1,2,4,5-CB	16.07	
		1,2,3,4-CB	17.36	

[a]Retention time; [b]Internal Standard

Spiked sewage sludge samples

For the optimization of the method, sludge samples from the drying bed (free of chlorobenzenes) were sieved (16 mesh) and homogenized. 4.00 g of sewage sludge were measured in a 22.0 mL transparent glass vial and spiked with 100 µL of the working solution containing all chlorobenzenes at a concentration of 25.0 mg L^{-1}. The spiked samples (0.625 mg kg^{-1}) were allowed to stand for three hours in a closed vial at room temperature.

Solid-liquid extraction with purification at low temperature

Several parameters that affect extraction efficiency were evaluated as detailed in section "optimization of extraction parameters". In the optimised method, 4.00 g of sludge samples were added 2.40 mL sodium hydroxide solution (pH 14.0). The system was heated at 50 °C in a water bath for 10 min. After, 8.00 mL of the extraction phase consisting of acetonitrile, ethyl acetate and dichloromethane (6.50, 1.50 and 0.70 mL, respectively) were added and the system was homogenized using a vortex for 5 min. The vials were cooled down to −18 °C for 45 min for the freezing of the sludge and water. The organic phase still liquid was transferred into a glass column (20 cm length × 10 mm diameter) containing 1.50 g anhydrous sodium sulphate and 2.00 g silica. The flow rate was maintained at 0.50 mL min^{-1} and the column was eluted with acetonitrile until the final volume of the extract reached of 5.00 mL. 100 µL naphthalene solution (internal standard) at 25.0 mg L^{-1} were added to the eluate. Then, 1.00 mL extract was filtered through a 0.45 µm nylon membrane and transferred into an injection vial. The extraction procedure was performed in triplicate along with a blank sample in order to assess the presence of any interference.

Optimization of extraction parameters

The SLE-PLT optimization for chlorobenzenes was divided into two steps: (*i*) evaluation of the extraction phase and (*ii*) use of a 2-level full factorial design with three factors.

The composition of the extraction phase (single phase) was obtained using acetonitrile (ACN), ethyl acetate (ACT) and dichloromethane (DCM). Four extraction phases were evaluated with different proportions of solvents: (*i*) 6.50 mL acetonitrile, 1.00 mL ethyl acetate and 0.50 mL dichloromethane; (*ii*) 7.30 mL acetonitrile and 0.70 mL dichloromethane; (*iii*) 7.00 mL acetonitrile and 1.00 mL ethyl acetate; (*iv*) 8.00 mL acetonitrile. The results were examined by the analysis of variance. When significant differences were found ($p < 0.05$), the Duncan's test was applied ($\alpha = 0.05$).

A full factorial design with three factors and two levels was used to obtain extracts with higher rates of recovery of chlorobenzenes. A combination of all factors was adopted: (*i*) pH, (*ii*) NaCl, and (*iii*) homogenization time in a vortex (Table 2).

The 2^3 factorial design requires 8 tests in duplicate, which means a total of 16 tests. For the lowest levels, negative signs were employed and for the highest levels, positive signs. In order to evaluate the effects of the factors of this factorial design, recovery percentages of each chlorobenzene were obtained after extraction of samples by SLE-PLT.

Thus, the tests were designed as follows: 2.40 mL distilled water (approximately pH 7.0) and 2.40 mL sodium hydroxide solution (pH 14.0) for the factor (*i*) were added to the spiked samples, corresponding respectively to the levels (−) and (+); 0 g and 0.48 g NaCl for the factor (*ii*), corresponding sequentially to the levels (−) and (+). Then, 8 mL extraction phase were added and the system

Table 2. 2^3 Factorial design for spiked sewage sludge samples (0.625 µg g^{-1})

Test	Encoded factor			Original factor		
	F1	F2	F3	pH	NaCl / g	Homogenization time / min
1	−	−	−	7.0	0	2
2	−	−	+	7.0	0	5
3	−	+	−	7.0	0.48	2
4	−	+	+	7.0	0.48	5
5	+	−	−	14	0	2
6	+	−	+	14	0	5
7	+	+	−	14	0.48	2
8	+	+	+	14	0.48	5

was homogenized, factor (*iii*), in vortex for 2 min (−) and 5 min (+). After homogenization, the system was cooled down to −18 °C for 45 min.

Matrix effect

In order to evaluate the influence of matrix components on the detector response, two sets of standard solutions containing nine chlorobenzenes at concentration ranges of 3.3 to 650 µg L^{-1} were prepared. The first set was obtained by diluting the working solution containing the chlorobenzenes in pure acetonitrile (triplicate) and the second one was prepared by diluting the same working solution in organic extracts of the matrix (triplicate) obtained by SLE-PLT (chlorobenzene-free samples). The quantification of analytes was performed by GC-MS.

Validation

The proposed analytical method (SLE-PLT and analysis by GC-MS) was validated in relation to the main figures of merit: selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity, precision and accuracy as suggested by the National Institute of Metrology, Standardization and Quality (INMETRO).[27]

Comparison of extraction methods

The SLE-PLT technique was compared to sohxlet extraction. The procedure used by this traditional technique was based on the modified methods 3550C and 3630 set by the EPA for the analysis of organic compounds in solid samples.[28-30]

Approximately 20.0 g of sludge samples were mixed with previously activated copper pieces. The mixture was wrapped with filter paper and placed in a soxhlet extractor. The extraction was carried out with 250 mL of extraction phase consisting of acetone:dichloromethane (1:1, v/v) for 24 h. The extracts were concentrated on a rotary evaporator and recovered in 5 mL of acetone:dichloromethane (1:1, v/v).

Then, the extracts were purified using columns packed with 3 cm alumina, 10 cm silica gel and 2 cm anhydrous sodium sulphate. The extracts were eluted with 20.0 mL dichloromethane and collected in a 25.0 mL volumetric flask. The extracts were again concentrated in a rotary evaporator and recovered in 0.50 mL acetonitrile.

Application to real samples

The optimized and validated method was used for the monitoring of chlorobenzenes in sludge samples collected at the sewage treatment plant in Montes Claros - MG, Brazil. Two types of sludge samples were collected: (*i*) after centrifugation step (centrifuged sludge) and (*ii*) after thermal drying step at 300 °C (dried sludge), with 72% (w/w) and 20% (w/w) humidity, respectively.

The samples were collected monthly from December 2011 to October 2012. After collection, the samples were properly stored in a refrigerator at 4 °C until analysis.

Results and Discussion

Chromatographic analysis

The extracts obtained in the optimization step were analyzed by GC-MS in scan mode. It was observed that the chlorobenzenes are completely eluted within 18 min, whereas most of the matrix components have a longer retention time. Thus, the chromatography column was heated to 300 °C after elution of chlorobenzenes for the cleaning of the chromatographic system.

In the validation step, the extracts were analyzed in the selective ion monitoring mode (SIM). It was found that only the isomers 1,2,3,5-CB (R_t = 16.01 min) and 1,2,4,5-CB (R_t = 16.07 min) were not well resolved; however, the partial coelution did not prevent the individual quantification of these compounds. Similar results have been reported in the literature.[1,31]

Optimization of SLE-PLT

Sewage sludge is a complex matrix having interferents that vary from one sample to another. Although the solid-liquid extraction with purification at low temperature has been applied to food matrices, it has not been used for the analysis of chemical contaminants in sludge samples. In this way, some parameters of the proposed technique were evaluated in order to obtain higher extraction efficiency and less interference from the sample.

Evaluation of the extraction phase

Previous works have shown that the technique of SLE-PLT has higher extraction efficiency when the extraction phase : water ratio is equal to 8.00 mL : 4.00 mL.[23-26] In the moisture content test, the sludge samples had 42.5% (w/w) of water, i.e., they had about 1.7 mL of water. Thus, 2.30 mL of water were added into the samples in the SLE-PLT.

The principle of the technique is based on the formation of a single phase between the organic solvent and the water. Disruption of the equilibrium occurs only by lowering the temperature. The water and the sample should freeze and the organic solvent, less dense than the water, should be liquid. However, the separation of phases (organic and aqueous) was observed in ambient condition due to the complexity of the matrix (sewage sludge). Thus, the acetonitrile has shown appropriate characteristics for SLE-PLT of polar compounds. For the extraction of less polar compounds, the addition of small quantities of non-polar solvents into the organic phase is required, for example, ethyl acetate and/or dichloromethane. The results obtained with different phases for the extraction of chlorobenzenes in sewage sludge are shown in Figure 1.

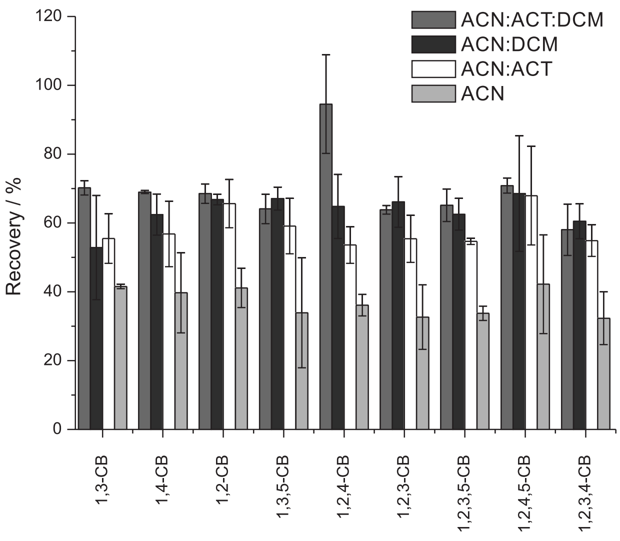

Figure 1. Influence of the extraction phase on the recovery percentage of chlorobenzenes (CB) in sewage sludge. ACN: acetonitrile; DCM: dichloromethane; ACT: ethyl acetate.

It was observed that ACN had the lowest percentage of extraction for all compounds quantified, ranging from 32 to 42% extraction ($p < 0.05$ by Duncan's test). The remaining extraction phases evaluated showed similar results although the mixture of acetonitrile/ethyl acetate/dichloromethane stood out due to its highest percentage of extraction for compounds 1,3-CB and 1,2,4-CB ($p < 0.05$ by Duncan's test). This extraction phase made up of three solvents gave 58 to 94% extraction for all chlorobenzenes analyzed. The polarity order of the organic solvents is ACN > DCM > ACT, based on the partition coefficient octanol / water (K_{OW}). Thus, the polarity order of the extraction phases is ACN > ACN:DCM > ACN:ACT:DCM > ACN:ACT. The homogeneous mixture composed of three solvents provided appropriate polarity for the extraction of chlorobenzenes. In addition, the volume of the extraction phase reduced from 8 mL to 5 mL. This can be explained given that the sludge samples retain part of the organic solvent.

Factorial design

From the results of the recovery percentage of the eight tests of the factorial design performed in duplicate, it was possible to calculate the average recovery rates for each chlorobenzene, the effect of each factor, their interactions, and estimates of experimental error. Estimates of the effects correspond to significant influences at 95% probability. The results obtained in the statistical analysis are presented in Table 3.

It was found that the homogenization time had a significant influence on the percentage of extraction of 1,2,4,5-CB and 1,2,3,4-CB at the level of 95% probability by *t*-test, increasing 9.7% and 11.1%, respectively. These results can be explained by the greater contact time between sample and extraction phase which favoured the migration of chlorobenzenes to the organic phase.[32] The variation of pH did not significantly influence the extraction of the chlorobenzenes analyzed.

Although the addition of salt is common in many extraction methods, it showed a negative effect in this study (Table 3). In addition to reducing extraction of 1,3-CB, 1,2-CB, 1,2,4-CB and 1,2,3,4-CB, the presence of salt increased baseline noise on the chromatograms. This can be justified

Table 3. Mean recovery percentages, effects of each factor and interactions between factors (± experimental error estimate) of extraction of each chlorobenzene obtained in the factorial design experiments for sewage sludge samples

CB [a]	MR / %[b]	(1) pH	(2) NaCl	(3) HT[c]	(1) & (3)	(2) & (3)	(1) & (2)	(1), (2) & (3)
1,3	54.4 ± 0.98	0.45 ± 1.9	**−8.8 ± 1.9***	3.1 ± 1.9	−2.6 ± 1.9	−3.1 ± 1.9	0.23 ± 1.9	−3.0 ± 1.9
1,4	56.3 ± 1.1	1.3 ± 2.1	−8.0 ± 2.1	−0.56 ± 2.1	−0.62 ± 2.1	−4.11 ± 2.1	−2.5 ± 2.1	0.12 ± 2.1
1,2	54.7 ± 0.84	2.4 ± 1.6	**−9.3 ± 1.7***	1.6 ± 1.7	−1.7 ± 1.7	−4.01 ± 1.7	−0.82 ± 1.7	1.3 ± 1.7
1,3,5	52.2 ± 0.53	2.2 ± 1.1	−6.1 ± 1.1	3.4 ± 1.1	−1.1 ± 1.1	−4.6 ± 1.1	0.48 ± 1.1	−0.41 ± 1.1
1,2,4	52.3 ± 0.71	2.6 ± 1.4	**−8.4 ± 1.4***	5.3 ± 1.4	−0.66 ± 1.4	−3.1 ± 1.4	−1.2 ± 1.4	−0.26 ± 1.4
1,2,3	52.9 ± 0.66	1.8 ± 1.3	−8.4 ± 1.3	6.5 ± 1.3	−3.2 ± 1.3	−4.2 ± 1.3	−0.13 ± 1.3	−0.69 ± 1.3
1,2,3,5	48.3 ± 0.67	5.5 ± 1.3	−5.5 ± 1.3	5.8 ± 1.3	−4.1 ± 1.3	−5.9 ± 1.3	−0.38 ± 1.3	−2.9 ± 1.3
1,2,4,5	62.9 ± 0.63	3.8 ± 1.3	−5.6 ± 1.3	**9.7 ± 1.3***	−3.4 ± 1.3	−4.4 ± 1.3	3.7 ± 1.3	−4.4 ± 1.3
1,2,3,4	49.3 ± 0.57	1.5 ± 1.1	**−6.2 ± 1.1***	**11.1 ± 1.1***	−0.58 ± 1.1	**−6.1 ± 1.1**[d]	0.83 ± 1.1	−1.7 ± 1.1

[a]Chlorobenzenes; [b]mean recovery; [c]homogenization time and [d]statistically significant effect at 95% probability by *t*-test.

by the complexity of the matrix, as well as by the greater extraction of interferents. It was also observed that there is an interaction effect between the factors (*ii*) and (*iii*) when the extraction is carried out with longer homogenization time and with an increase in ionic strength (NaCl). The extraction of 1,2,3,4-CB is significantly reduced by about 6.1%.

Although the interaction between the factors (*i*) and (*iii*) was not statistically significant, it was decided to adopt longer homogenization time and pH 14.0 in this methodology. This combination increased the percentages of extraction of chlorobenzenes to values ranging from 59.26% to 73.40% and improved the quality of the chromatograms. It is possible that these positive factors may be due to the ionization of fatty acids in basic medium (matrix rich in lipids) and lower extraction of interferents.

Table 4. Percentage of variation of slope and linear coefficients of the calibration curves obtained in acetonitrile and in sewage sludge extracts

Compound	Concentration / (μg L^{-1})	Slope coefficients / %[a]	Linear coefficients /%[a]
1,3-CB	6.7 - 650	15.99	53.84
1,4-CB	3.3 - 650	15.19	−684.77
1,2-CB	6.7 - 650	19.73	59.03
1,3,5-CB	3.3 - 650	24.22	13,43
1,2,4-CB	6.7 - 650	14.32	−5.86
1,2,3-CB	8.0 - 650	17.78	51.72
1,2,3,5-CB	5.2 - 650	−11.27	93.34
1,2,4,5-CB	8.0 - 650	14.93	3.48
1,2,3,4-CB	10 - 650	−8.03	45.86

[a](1 – coefficient matrix/coefficient solvent) × 100

Matrix effect

The effect of coextractives on the chromatographic response of chlorobenzenes was assessed by comparing analytical curves prepared from extracts of the matrix and acetonitrile. It is characterized as a proportional systematic error when only the slope varies; when the linear coefficient varies, a constant systematic error takes place.[33] Both calibration curves prepared in acetonitrile and in sludge extract showed different slopes and linear coefficients for the chlorobenzenes on GC-MS. The percentage of variation of the angular and linear coefficients is presented in Table 4.

It was observed that for the chlorobenzenes evaluated (except 1,3,5-CB; 1,2,4-CB and 1,2,4,5-CB) the matrix effect modified the linear coefficient more significantly, indicating that the amount of analyte adsorbed was constant regardless of concentration. Thus, a greater matrix effect was observed at low concentrations, which is in agreement with the literature.[33,34] Furthermore, the chromatographic response for seven chlorobenzenes in the matrix extract was lower compared to the standard solution prepared in acetonitrile (negative matrix effect). In this type of error new adsorption sites are formed inside the chromatographic system which retains chlorobenzenes during the analysis and thus lower amount is quantified. Thus, in order to minimize errors due to the matrix effect, the analytical curves were prepared adding the standards into the matrix extract.

Validation

Selectivity

Selectivity of the proposed method was assessed by comparing the chromatograms of the matrix blank extract and the extract spiked with the nine chlorobenzenes. After investigations, the presence of 1,4-CB was observed in the sewage sludge although no interference was observed for any other retention time (Figure 2).

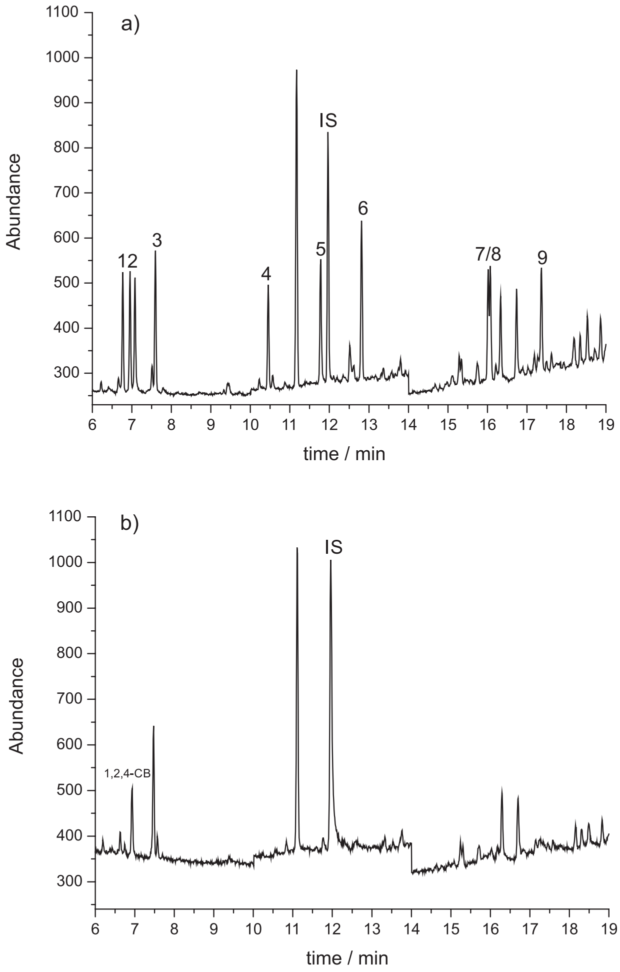

Figure 2. Chromatograms of the extract obtained after SLE-PLT: a) sludge extract spiked with chlorobenzenes from 6.6 to 20 µg L^{-1}, b) sludge extract (blank). Identification of the peaks: (1) 1,3-CB, (2) 1,4-CB, (3) 1,2-CB, (4) 1,3,5-CB, (5) 1,2,4-CB, IS, (6) 1,2,3-CB, (7) 1,2,3,5-CB, (8) 1,2,4,5-CB and (9) 1,2,3,4-CB.

Limit of detection, limit of quantification and linearity

The limit of detection (LOD) was determined by using the matrix extract spiked with the chlorobenzenes at the lowest acceptable concentration. LOD was adopted as three times the baseline noise signal obtained in the analysis of sewage sludge by GC-MS. The results found for the compounds were less than 3.75 µg kg^{-1} (Table 5).

The limit of quantification of the chlorobenzenes analyzed in the sludge was determined as ten times the baseline noise signal obtained for the analyte-free samples (blank). The values obtained for the nine chlorobenzenes were below 12.5 µg kg^{-1}. These values were less than the maximum residue limits established by CONAMA resolution[20] for chlorobenzenes in soil samples conditioned with sludge (Table 5). This parameter was adopted due to the absence of legislation that establishes maximum residue limits of chlorobenzenes in sewage sludge.

The linearity of the detector response was evaluated by using extracts from sludge samples spiked with known concentrations of the nine chlorobenzenes ranging from 4.1 to 650 µg kg^{-1}. The respective straight-line equations were obtained by linear regression and the coefficients of determination (r^2) were greater than 0.99 for the nine compounds (Table 5).

Accuracy and precision of the method

In order to evaluate the accuracy of the method, sewage sludge samples free of chlorobenzenes were spiked at concentrations of 1 × LOQ, 2 × LOQ and 5 × LOQ. Recovery percentages for the nine chlorobenzenes were greater than 63.96%. Precision was evaluated by the relative standard deviation (RSD) of the results of seven repetitions of extractions of chlorobenzenes in sludge samples spiked at a concentration of 2 × LOQ. The RSD was less than 13% for all compounds although a RSD of up to 20% is acceptable for complex matrices.[35] The data obtained is shown in Table 6.

Table 5. Limit of detection (LOD), limit of quantification (LOQ), linearity of the method for chlorobenzenes in sewage sludge samples and maximum residue limits (MRL) established by CONAMA

	Calibration data				
CB	Equation	r^2	LOD / (µg kg^{-1})	LOQ / (µg kg^{-1})	MRL / (µg kg^{-1})
1,3	$y = 0.025(\pm 0.00021)x + 0.085(\pm 0.063)$	0.9982	2.5	8.4	390
1,4	$y = 0.028(\pm 0.00030)x - 0.0010(\pm 0.091)$	0.9971	1.2	4.1	390
1,2	$y = 0.028(\pm 0.00030)x + 0.083(\pm 0.091)$	0.9988	2.5	8.4	730
1,3,5	$y = 0.021(\pm 0.00024)x + 0.13(\pm 0.074)$	0.9965	1.2	4.1	500
1,2,4	$y = 0.019(\pm 0.00025)x + 0.21(\pm 0.075)$	0.9959	2.5	8.4	11
1,2,3	$y = 0.020(\pm 0.00027)x + 0.15(\pm 0.083)$	0.9953	3.0	10	10
1,2,3,5	$y = 0.020(\pm 0.00040)x - 0.061(\pm 0.12)$	0.9900	1.9	6.5	6.5
1,2,4,5	$y = 0.017(\pm 0.00017)x + 0.14(\pm 0.051)$	0.9974	3.0	10	10
1,2,3,4	$y = 0.017(\pm 0.00016)x + 0.015(\pm 0.049)$	0.9999	3.7	12	16

Table 6. Recovery percentages of chlorobenzenes after extraction of sewage sludge samples spiked with three different concentrations of each compound

Compound	Recovery ± RSD / %		
	1 × LOQ[a]	2 × LOQ[b]	5 × LOQ[a]
1,3-CB	66.7 ± 3.1	67.9 ± 2.3	66.4 ± 9.9
1,4-CB	108 ± 13	100 ± 6.4	98.5 ± 4.2
1,2-CB	66.2 ± 4.9	64.2 ± 5.9	64.0 ± 11.2
1,3,5-CB	70.2 ± 2.0	66.3 ± 5.2	67.7 ± 12
1,2,4-CB	70.3 ± 12	72.5 ± 6.5	69.7 ± 12
1,2,3-CB	70.3 ± 12	69.2 ± 5.2	71.2 ± 11
1,2,3,5-CB	70.4 ± 9.6	71.8 ± 14	69.1 ± 4.3
1,2,4,5-CB	73.3 ± 6.3	69.7 ± 4.4	72.2 ± 12
1,2,3,4-CB	69.4 ± 1.7	67.6 ± 7.8	67.3 ± 12

[a]Three repetitions; [b]seven repetitions; LOQ: limit of quantification; RSD: relative standard deviation.

Comparison of methods

Figure 3 shows the chromatograms of the extracts free of chlorobenzenes (blank) in scan mode obtained by SLE-PLT and by sohxlet extraction.

It was observed that the amount of matrix components extracted by sohxlet is much higher than by SLE-PLT, which requires chromatographic system maintenance more frequently. Compared with studies in the literature, the soxhlet extraction showed LOD from 0.19 to 1.1 µg kg^{-1} for four chlorobenzenes,[10,28] and LOD of 5-10 µg kg^{-1} (dichlorobenzenes), 0.6-1 µg kg^{-1} (trichlorobenzenes) and 0.5 µg kg^{-1} (tetrachlorobenzenes)[36] whereas the SLE-PLT obtained values between 4.1 and 12 µg kg^{-1}. Better results were also obtained with soxhlet regarding recovery percentage, where results were greater than 76%,[28] while with SLE-PLT recovery was greater than 63%. However, comparing the cost/benefit relation between sohxlet extraction and SLE-PLT, the latter was very advantageous because the consumption of organic solvent was about 27 times lower. Time spent in the determination of chlorobenzenes was approximately four hours by SLE-PLT, whereas soxhlet extraction was over 30 hours. In addition to the amount of sample used in the proposed method to be five times lower, the limits of quantification are lower than the maximum allowable limits established by CONAMA.[20]

Application of the method to real samples

Centrifuged and dried sludge samples were monthly collected during the period of 11 months. The samples were subjected to the proposed methodology and the compounds 1,4-CB; 1,2,4-CB and 1,2,3-CB were detected (Table 7). The values were compared with the maximum residue limits of chlorobenzenes on soil (CONAMA Resolution No. 375), disregarding the dilution effect of the sludge on soil.

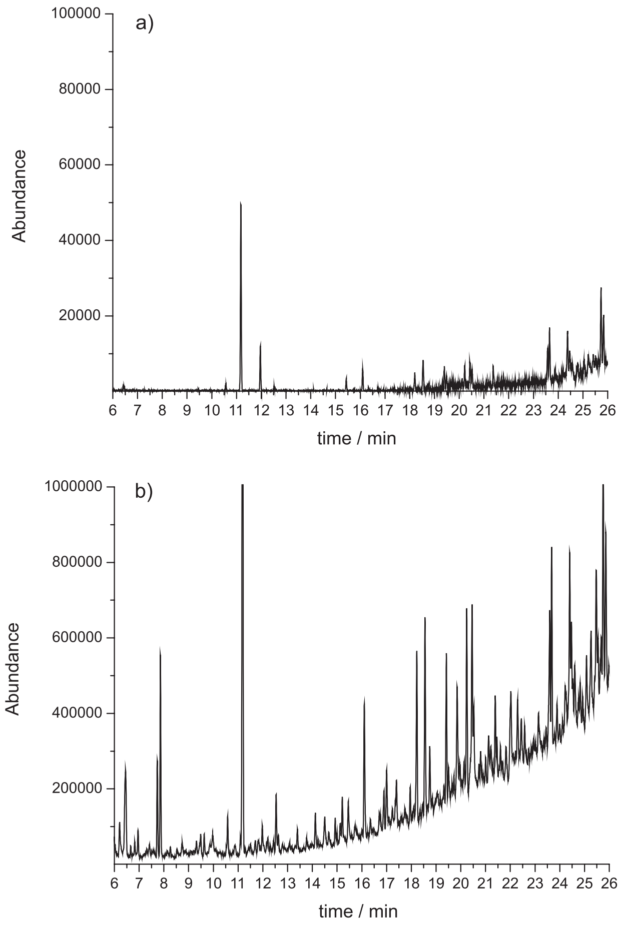

Figure 3. Chromatogram obtained by GC-MS-SCAN of the extracts free of chlorobenzenes: a) by SLE-PLT and b) by sohxlet extraction.

1,2,4-CB was detected in six centrifuged sludge samples and in five dried sludge samples, and it was quantified close to the maximum residue limit (11.0 µg kg^{-1}) once. 1,2,3-CB was detected in all centrifuged sludge samples and in nine dried sludge samples out of which four centrifuged sludge samples and two sludge samples from the thermal drying had concentrations above the maximum residue limit (10.0 µg kg^{-1}).

The compound 1,4-CB was quantified in 95% of samples; however, its concentrations were below the acceptable concentrations (390 µg kg^{-1}).This compound has various commercial applications such as disinfectant, air freshener, mosquito repellent and deodorant, thus explaining the presence of this analyte in the sludge samples.[2,37]

There was a greater amount of chlorobenzenes in centrifuged sludge samples in relation to the sludge obtained after the thermal drying. This is expected because volatilization of some chlorobenzenes may occur during the thermal drying at 300 °C at the STP since these

Table 7. Concentrations of 1,4-CB, 1,2,4-CB and 1,2,3-CB in sludge samples collected at the STP in Montes Claros

Month	Chlorobenzenes / (µg kg^{-1} ± RSD)					
	1,4-CB		1,2,4-CB		1,2,3-CB	
	CS	DS	CS	DS	CS	DS
Dec/11	17.4 ± 10.0	13.9 ± 2.74	6.90 ± 26.8	4.10 ± 8.57	14.4 ± 0.89	10.7 ± 3.91
Jan/12	16.7 ± 6.42	14.1 ± 8.80	8.60 ± 10.0	5.34 ± 6.37	13.1 ± 8.67	8.46 ± 6.21
Feb/12	15.3 ± 6.38	14.7 ± 6.51	7.09 ± 1.10	3.95 ± 0.78	14.9 ± 1.95	7.18 ± 11.2
Mar/12	19.1 ± 0.58	16.2 ± 0.89	8.11 ± 9.28	5.17 ± 5.74	16.5 ± 0.78	11.3 ± 4.51
Apr/12	42.6 ± 9.56	16.7 ± 10.8	ND[d]	ND	6.23 ± 3.53	4.48 ± 11.3
May/12	32.6 ± 7.64	28.7 ± 1.59	ND	2.95 ± 8.05	6.48 ± 10.7	5.47 ± 4.41
Jun/12	29.6 ± 6.82	9.86 ± 8.25	4.21 ± 6.43	ND	7.14 ± 2.48	2.22 ± 0.8
Jul/12	26.8 ± 0.22	23.2 ± 6.77	3.98 ± 8.37	ND	7.58 ± 6.24	6.55 ± 0.78
Aug/12	27.2 ± 2.41	ND	ND	ND	8.30 ± 5.58	ND
Sep/12	27.0 ± 4.53	28.0 ± 6.26	ND	ND	7.06 ± 5.16	5.38 ± 0.90
Oct/12	36.7 ± 6.29	8.26 ± 8.91	ND	ND	5.68 ± 4.02	ND

CS: centrifuged sludge; DS: dried sludge; RSD: relative standard deviation; ND: not detectable.

compounds have low vapour pressure.[38] In Brazil, only cities with a population over 300,000 inhabitants have the thermal drying as a step in the treatment of sewage sludge. In smaller cities, the centrifuged sewage sludge from STPs is air dried and disposed of according to CONAMA Resolution No. 375. The presence of chlorobenzenes in sludge samples has also been reported in the literature and with higher values than those found in this work.[39,40]

Although the maximum limits of chlorobenzenes have not been established for chlorobenzenes in sludge samples, the constant use of the sludge as a soil conditioner in agriculture increases the content of chlorobenzenes in the soil, which indirectly exposes humans to these chemicals. Studies have shown the carcinogenic and tumoral potential of some compounds such as 1,4-CB.[41,42]

Conclusion

In the present study, the technique of solid-liquid extraction with purification at low temperature was developed for the determination of nine chlorobenzenes in sewage sludge samples. The technique had advantages over the traditional soxhlet extraction because the proposed procedure is easy to perform and environmentally friendly because it reduces the use of toxic organic solvents. The recovery percentages were between 66 and 108% with relative standard deviation less than 13%. Limits of detection were between 1.2 and 3.7 µg kg^{-1}. In the monitoring of samples collected within the period of 11 months, three chlorobenzenes were detected in the sewage sludge (1,4-CB; 1,2,4-CB and 1,2,3-CB) at concentrations ranging between 2.22 and 42.6 µg kg^{-1}. The compound 1,2,3-CB showed concentration above the one established by CONAMA, without considering its dilution on the soil.

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