

Multivariate Optimization of Pressurized Solvent Extraction of Alkylphenols and Alkylphenol Ethoxylates from Biosolids

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Neste estudo, uma metodologia de extração em solvente pressurizado de alquilfenóis (APs) e alquilfenóis etoxilados (APEOs) a partir de biosólidos foi otimizada utilizando-se um biosólido contendo nativamente estes compostos em um nível de concentração elevada. A otimização da extração foi realizada através de um planejamento experimental de análise multivariada com um planejamento composto central (CCD) produzindo os seguintes valores otimizados: temperatura de 129 °C, tempo de extração por ciclo de 34 min em dois ciclos e acetona como solvente de extração. Em comparação com a extração Soxhlet, esta metodologia acabou por ser mais eficiente para a extração de APEOs e de eficiência semelhante para a extração de APs. Por outro lado, a metodologia é consideravelmente mais rápida do que a de Soxhlet e usa uma pequena quantidade de solvente de extração. As concentrações de 204 ± 20 e 1053 ± 23 mg kg⁻¹ de nonilfenol e nonilfenol-etoxilato, respectivamente, foram determinadas na amostras de biosólido.

In this study, a methodology for pressurized solvent extraction of alkylphenols (APs) and alkylphenol ethoxylates (APEOs) from biosolids was optimized by utilizing a biosolid natively containing these compounds at a high concentration level. Optimization of the extraction was carried out through a multivariate analysis experimental design with a central composite design (CCD) producing the following optimum values for the variables: temperature of 129 °C, extraction time *per cycle* of 34 min in two cycles and acetone as extraction solvent. Upon comparison with Soxhlet extraction, this methodology turned out to be more efficient for the extraction of APEOs and of similar efficiency for APs. On the other hand, the methodology is considerably faster than Soxhlet and uses a smaller amount of extraction solvent. Concentrations of 204 ± 20 and 1053 ± 23 mg kg⁻¹ for nonylphenol and nonylphenol-ethoxylate, respectively, were determined in the biosolid sample assayed.

Keywords: pressurized solvent extraction, biosolid, alkylphenols, alkylphenol ethoxylates

Introduction

The presence of alkylphenol polyethoxylates (APEOs) and their metabolites, alkylphenols (APs), in the environment is the result of anthropogenic activity, as no natural sources are known for these compounds.¹ These contaminants are classified as endocrine disruptors (EDCs) since they interfere with endocrine functions, causing alterations and modifications in the sexual and reproductive development of wild animals (mammals and fishes). Moreover, it has been reported that they can induce several effects in humans, such as low sperm count, precocious puberty, thyroid dysfunction, growth of mammary cancer cells, among others.^{2,3}

The determination of organic contaminants in solid matrices often implies in extensive and complex operations of sample preparation, mainly because of the difficulty of quantitative analyte extraction from the solid sample. Since on some occasion interactions between analytes and matrix are very strong, traditional methodologies based on Soxhlet extraction do not provide enough energy for quick analyte release, and thus, they require long extraction times (8-48 h).⁴ Several modern extraction methods (alternatives to Soxhlet) have been proposed such as ultrasonic extraction (USE), supercritical fluid extraction (SFE), pressurized solvent extraction (PSE) and microwave-assisted solvent extraction (MASE), among others, which have produced similar or even better recoveries than Soxhlet extraction.⁵ Many of these modern extraction methods have been applied in different matrices to extract these APs and

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APEOs.⁶ As to PSE, even though it is a methodology with great advantages compared with Soxhlet extraction, such as prompt extraction and small amount of extracting solvent and sample, there is no consensus in relation to the adequate extraction solvent and the optimum extraction conditions in order to extract APs and APEOs from a complex matrix such as biosolid. Thus, several studies have been carried out using a (1:1 v/v) mixture of acetone/hexane with good recovery results,⁷⁻⁹ however the use of (1:1 v/v) acetone/methanol¹⁰ and (1:1 v/v) acetonitrile/methanol¹¹ mixtures have also successfully applied for this purpose.

The objective of this study was to carry out a multivariate optimization of PSE considering the variables temperature, solvent and extraction time, which affect APEOs and APs extractions from the biosolid matrix, so that, the separation methodology together with quantification methodologies will be efficient and constitute a quantitative method to determine these analytes.

Experimental

Reagents

Standards in use were 4-octylphenol ethoxylate (4-OPEO), 4-nonylphenol ethoxylate (4-NPEO), 4-*n*-octylphenol (4-*n*-OP), 4-nonylphenol (4-NP) from Dr. Ehrenstorfer (Germany) and 4-*t*-octylphenol obtained from Supelco (USA). These standards were dissolved in methanol in order to make calibration curves.

Ethyl acetate, acetonitrile, acetone, formic acid, hexane, cyclohexane, dichloromethane, diethyl ether, methanol and toluene (high-performance liquid chromatography grade) were purchased at Merck (Germany). Aluminum oxide 90, neutral, activity I, was activated for 8 h at 105 °C. Sodium sulfate (Na₂SO₄) was dried for 4 h at 400 °C. Both reagents are chromatography grade and were purchased at Merck.

Biosolids

A biosolid was sampled from a wastewater treatment plant in Santiago de Chile City (Chile). The sample corresponds to sludge disposed of in January 2008 in the plant monofill.

The biosolid was air dried, ground and passed through a 2 mm-sieve. It was stored at low temperature (−18 °C) to prevent analyte degradation.

Pressurized solvent extraction (PSE)

An ASE[®]100 pressurized solvent extraction system was used. For each extraction, 0.5 g of dried and sieved

sludge was used, plus a similar quantity of diatomaceous earth. The choice of the extraction solvent included polar, nonpolar solvents and their 1:1 v/v mixtures. The solvents were acetone, dichloromethane and hexane.

The compound extraction was optimized by making use of the following variables, inherent in the working range of the utilized system: extraction temperature (40-200 °C), extraction time (5-60 min) and solvent percentage (acetone-dichloromethane, DCM (dichloromethane) or acetone-hexane mixtures).

The variables associated to the ASE extractor were selected according to the manufacturer recommendations and to standard conditions of most of the methods programmed in the system. These were: purge time of 100 s, number of static cycles of 2, cell volume of 10 mL, flush volume of 100% and working pressure of 1650 ± 50 psi.

In order to optimize the variables, a central composite design (CCD) was used: 2^N + 2N + C, where N is the number of experimental factors and C is the number of central points. It was considered 3 central points, which led to a total of 17 experiments in one block. The matrix of experiments (Table 1) contains the encoded values and their natural value is in brackets. Encoding is used to assign the same statistical weight to all of the factors.

Table 1. Optimization matrix for the PSE method (natural values are between brackets)

Experiment	Temperature / °C	Static time / min	Acetone / %
1	−1 (72)	−1 (16)	−1 (80)
2	1 (168)	−1 (16)	−1 (80)
3	−1 (72)	1 (49)	−1 (80)
4	1 (168)	1 (49)	−1 (80)
5	−1 (72)	−1 (16)	1 (20)
6	1 (168)	−1 (16)	1 (20)
7	−1 (72)	1 (49)	1 (20)
8	1 (168)	1 (49)	1 (20)
9	−1.68 (40)	0 (33)	0 (50)
10	1.68 (200)	0 (33)	0 (50)
11	0 (120)	−1.68 (5)	0 (50)
12	0 (120)	1.68 (60)	0 (50)
13	0 (120)	0 (33)	−1.68 (100)
14	0 (120)	0 (33)	1.68 (0)
15	0 (120)	0 (33)	0 (50)
16	0 (120)	0 (33)	0 (50)
17	0 (120)	0 (33)	0 (50)

Soxhlet extraction

In order to compare the efficiency of the methodology in use, a Soxhlet extraction was carried out according to methods described in the literature.^{12,13} The solvent utilized was acetone or hexane at 5 cycles *per* h, for 24 h.

Methodology of extract drying and clean-up

The extracts were dried in a glass column with 10 cm Na_2SO_4 .¹² Then, they were concentrated to 1 mL in an N_2 stream in order to subject them to the extract clean-up process.

The clean-up was carried out in 8 cm \times 1 cm i.d. glass columns (Visiprep, Supelco), filled with 5 g aluminum oxide and set up on a manifold vacuum system. The columns were conditioned with 10 mL of a mixture of (3:1, v/v) ethyl acetate/toluene. The extract was later eluted with 30 mL of the same solvent mixture, which was collected and disposed of, constituting fraction I. Next, 20 mL of a mixture of 1:1:0.05, v/v/v diethyl ether/methanol/formic acid were eluted, constituting fraction II, which was collected in 40 mL glass vials.¹³ The solvent was changed to methanol, which was finally concentrated to 1 mL by means of an N_2 stream.

Chromatographic method

A gas chromatograph with a mass detector (GC-MS) was utilized, in this case an Agilent 6890 Plus chromatograph coupled to an Agilent 5973N mass spectrometer, supplied with an HP5 column (30 m \times 0.25 mm \times 250 μm). Helium was used as a carrier gas. Injection was performed in the splitless modality, with 1 μL injection volume. The temperature schedule was the following: starting temperature of 100 $^\circ\text{C}$ for 1 min, with a slope of 10 $^\circ\text{C min}^{-1}$, to reach a final temperature of 280 $^\circ\text{C}$ for 3 min.

Results and Discussion

Table 2 shows chromatographic parameters of the methodology such as retention time, target and qualifier ions (m/z).

The performance of some preliminary tests using ultrasound permitted to determine the extraction capacity of the different solvents in the biosolid sample that naturally

Table 2. Main features of the GC-MS method for the analytes under study

Analyte	Retention time / min	Target ion / m/z	Qualifier ions / m/z
4- <i>t</i> -OP	8.5	135	107/136
TBA	8.7	346	329
4-NP	9.48-10.17	135	107/121
4- <i>n</i> -OP	10.23	107	206/108
4-OPEO	11.25	179	135/107
4-NPEO	12.04-12.98	179	135/107

contains these analytes. As can be seen in Table 3, the most efficient solvents for the set of analytes in this matrix in particular are acetone and dichloromethane. As to the 1:1, v/v solvent mixtures, the best for the quantitative extraction of the set of analytes is the DCM/acetone and hexane/acetone mixtures. Within the process of extraction, solvent or solvent mixture polarity is strongly related to that of the target compounds and to the matrix nature. Thus, some solvents are recommended in order to extract specific analytes like hexane in the case of lipophilic analytes (short-chain alkylphenols and alkylphenol ethoxylates), while more polar solvents are required for the extraction of hydrophilic compounds.¹⁴ As a function of this, the solvents that should quantitatively extract these analytes are hexane and dichloromethane, but it is observed that hexane does not extract as exhaustively as dichloromethane. On the other hand, it is well known that a fraction of a hydrophobic compound may be “trapped” in the matrix by a barrier of water molecules and consequently the penetration of a nonpolar solvent will be diminished. It has been well documented that the extraction of low-polarity compounds from highly complex matrices is benefited by the use of mixtures of nonpolar and polar solvents, a more efficient extraction being obtained if only nonpolar solvents are used.⁴ This would account for the fact that extraction with DCM/acetone and hexane/acetone mixtures have turned out more quantitative (Table 3).

Table 3. Study of the solvent extraction capability of target analytes by ultrasound extraction

Solvent	4- <i>t</i> -OP	4-NPEO	4- <i>n</i> -OP	4-OPEO
	(mg kg^{-1})			
Acetone	0.7 \pm 0.2	121 \pm 18	1.8 \pm 0.1	1.8 \pm 0.2
DCM	0.67 \pm 0.02	173 \pm 13	2.9 \pm 0.4	1.68 \pm 0.02
Hexane	0.46 \pm 0.05	65 \pm 31	1.0 \pm 0.6	1.71 \pm 0.02
MetOH	0.38 \pm 0.04	16 \pm 13	0.27 \pm 0.04	1.6 \pm 0.1
DCM/acetone	0.6 \pm 0.1	119 \pm 48	1.9 \pm 0.8	1.8 \pm 0.1
DCM/MetOH	0.44 \pm 0.01	48 \pm 2	0.6 \pm 0.3	1.66 \pm 0.04
Hexane/acetone	0.60 \pm 0.01	126 \pm 5	2.3 \pm 0.1	1.75 \pm 0.01
Hexane/MetOH	0.40 \pm 0.01	21 \pm 6	0.22 \pm 0.03	1.69 \pm 0.02

Biosolid is a very complex matrix from an analytical viewpoint, on account of the high content of organic matter. It was determined that for these analytes, the use of methanol, acetone and DCM/acetone (1:1, v/v) mixtures has not yielded satisfactory recoveries,¹² being in contrast with our results (Table 3). On the other hand, good recovery percentages were obtained for these analytes from sediment samples through the use of hexane/acetone (1:1, v/v) mixture.¹⁵ Along these lines, since it is not easy to select the best solvent or solvent mixture to extract these compounds from a matrix such as biosolid, an optimization process becomes a key aspect for correct analytical measurement of these compounds. Even though the extractions shown in Table 3 were carried out under normal temperature and pressure conditions, they show a good approach to their efficiencies under more drastic conditions. As a function of these results, acetone extraction was included as a variable to be optimized in relation to dichloromethane or hexane percentage in binary mixtures. Thus, the extraction methodology was optimized with pressurized solvent, considering acetone/dichloromethane and acetone/hexane mixtures.

Optimization of the acetone/DCM mixture

Since an optimum compromise condition is required from the responses of each of the analytes under study, it was applied the Derringer's desirability function (D), which uses the geometric mean of the normalized individual responses. For the acetone/DCM mixtures, the following equation was obtained:

$$D = 0.644 + 0.019 T + 0.049 ET - 0.078 A - 0.230 T^2 - 0.015 T ET - 0.039 T A - 0.111 ET^2 + 0.016 ET A - 0.019 A^2 \quad (1)$$

where T = temperature, ET = extraction time and A = % of acetone in the mixture, expressed in encoded values. This model exhibits a determination coefficient (R^2) of 73.39%. As observed in the Pareto chart (Figure 1a), when grouping the responses of all the compounds in the desirability function, all the variables turn out significant to 95% of confidence, being the reason why the model cannot be simplified.

According to the model, Table 4 shows the optimum values of each variable. It may be observed that acetone is

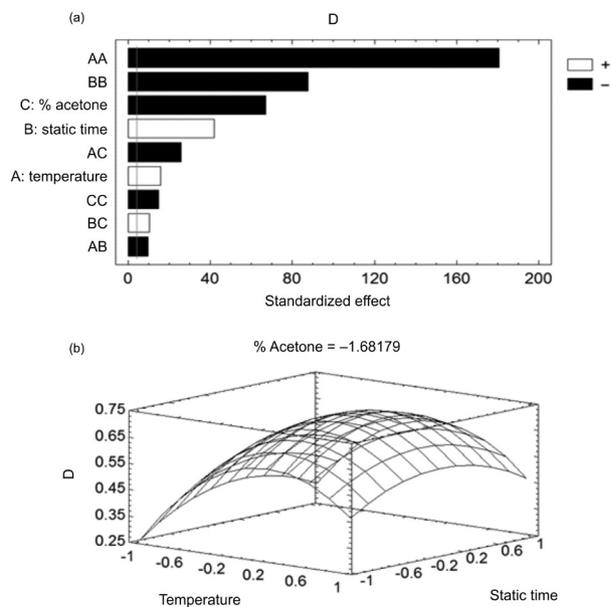


Figure 1. (a) Standardized Pareto chart for the Derringer's desirability function (D) and (b) estimated response surface for the Derringer's desirability function (D) for acetone/dichloromethane mixtures.

the solvent to select under these conditions. In turn, when observing the estimated response surface (Figure 1b), it may be seen that as the temperature increases to a value near the central value of the utilized range, an optimum value is reached, which then decreases again. Similarly, as it was gotten to an intermediate value in the range of static extraction time, an optimum value is reached.

The extraction by PSE of the analytes from a biosolid sample was carried out by applying the predicted optimum conditions for the whole set of compounds ($n = 6$). In addition, a Soxhlet extraction was carried out using acetone as a solvent, since this was the recommended by the model to extract the set of analytes (Table 5).

Optimization of the acetone/hexane mixture

In the case of acetone/hexane mixtures, the following Derringer's desirability function equation (D) was obtained:

$$D = 0.127 - 0.028 T + 0.024 ET + 0.043 A + 0.015 T^2 - 0.034 T ET - 0.032 T A + 0.022 ET^2 + 0.159 ET A + 0.043 A^2 \quad (2)$$

Table 4. Optimum values found for each variable for all grouped analytes, acetone/DCM and acetone/hexane mixtures

Solvent mixture	Temperature		Static time		Acetone	
	Encoded value	Natural value / °C	Encoded value	Natural value / min	Encoded value	Natural value / %
Acetone/DCM	0.179747	129	0.0854945	34	-1.68179	100
Acetone/hexane	-	-	-	-	1.68179	0

Table 5. Concentrations found in the biosolid for the different analytes by PSE and Soxhlet

Analyte	PSE acetone		Soxhlet acetone		PSE hexane		Soxhlet hexane	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	(mg kg ⁻¹)							
4- <i>t</i> -OP	1.2 ^a	0.1	1.22 ^a	0.04	1.4 ^a	0.6	1.2 ^a	0.4
4-NP	204 ^b	20	298 ^c	7	162 ^a	20	180 ^{ab}	2
4- <i>n</i> -OP	6.4 ^a	4	6.4 ^a	0.4	3.3 ^a	0.8	4 ^a	1
4-OPEO	1.6 ^b	0.1	1.5 ^{ab}	0.1	1.4 ^{ab}	0.3	1.0 ^a	0.3
4-NPEO	1053 ^c	23	621 ^a	18	730 ^b	30	635 ^a	13

Same letter on the same row indicates no statistical differences between the means (multiple range test for the mean, by Tukey HSD procedure (honestly significant difference)) at 95% level of confidence.

This model presents a determination coefficient (R^2) of 52.44%.

Table 4 shows optimum extraction values once the compounds are grouped in the Derringer's desirability function. Pareto's chart standardized for the desirability function (Figure 2a) shows that, when grouping the responses of all the compounds, the significant variables turn out to be the percentage of acetone in the mixture and the interaction between static time and acetone percentage, thus the model may be simplified to a shorter equation:

$$D = 0.127 + 0.043 A + 0.159 ET A \quad (3)$$

As can be seen in Table 4, in this case, hexane should be the solvent selected for extraction. In the estimated response surface for the desirability function (Figure 2b), it is observed that as the acetone percentage decreases in the

solvent mixture, an optimum value is reached in the studied interval. As to static time, an optimum value is reached as it increases. These predicted values for each variable are also found in Table 4.

The analyte PSE extraction from the biosolid sample was carried out under the optimum conditions predicted for the whole set of compounds ($n = 6$). The optimization was compared with Soxhlet extraction, under the same solvent conditions, but using a quantity of 2 g dry sludge (Table 5).

Table 5 shows that the use of acetone as an extraction solvent gave better results, both in the case of the optimized method and in Soxhlet extraction. The comparison of optimized extraction conditions of both systems by using the different solvent mixtures (Table 4) permits to observe that they differ in all the variables. The concordance should be expected between the optimum conditions predicted for each optimization due to the use of a binary mixture of acetone and another solvent (whether dichloromethane or hexane), which are similar in polarity and are miscible with one another.

When only acetone is used as an extraction solvent, the optimum recommended temperature is 129 °C, higher than its boiling temperature (56.1 °C).¹⁶ Under this condition, the solvent would be in a subcritical state since the working pressure is above the critical pressure of that solvent (681.7 psi).¹⁷ Under these conditions, acetone would have a smaller dielectric constant and, hence, its polarity would have also decreased. These phenomena was extensively studied in superheated water.¹⁶ According to Marshall's model,¹⁸ it may be possible to determine that the dielectric constant of acetone decreases considerably, from 20.56 under normal conditions to 10.68 under these extraction conditions.

Taking these considerations into account in relation to the solvent polarity, the process of extraction with acetone at 129 °C in 2 cycles of 34 min each would be similar to the extraction process with hexane, at a slightly higher temperature than its boiling point in two cycles of 60 min each. On the other hand, the choice between

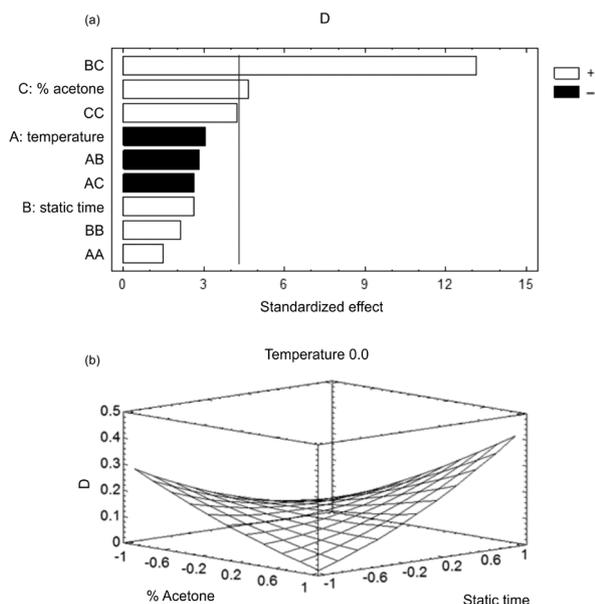


Figure 2. (a) Standardized Pareto chart for the Derringer's desirability function (D) and (b) estimated response surface for the Derringer's desirability function (D) for acetone/hexane mixtures.

dichloromethane and hexane may depend on the solvation capacity of each in front of the compounds under study. Thus, hexane would be more efficient for the solvation of the aliphatic part of alkylphenols and their ethoxylates, compared with dichloromethane, whose chlorines may exert important steric hindrance, compared with the alkyl chain of hexane, under the extraction conditions of the system.

Conclusions

Based on the present results and on the proposed objectives, it may be concluded that optimization was carried out to separate and determine alkylphenols and alkylphenol ethoxylates (4-*t*-OP, 4-NP, 4-*n*-OP, 4-OPEO and 4-NPEO) by gas chromatography with mass detector, reaching reproducible responses.

A methodology for the extraction of alkylphenols and alkylphenol ethoxylates from biosolids was optimized using a biosolid that naturally contained these compounds, with a high concentration of 4-NP and 4-NPEO. The methodology was based on batch extraction with pressurized solvents. Since the extraction of these compounds from the selected matrix depends on various factors, the optimization was carried out through a multivariate analysis experimental design with a central composite design (CCD), delivering optimum values to the methodology.

The optimum conditions for this methodology were: 129 °C, 34 min *per* cycle in two cycles and acetone as extraction solvent. In these conditions, acetone decreases its dielectric constant and, hence, its polarity is decreased.

Compared with Soxhlet extraction, this optimized methodology turned out more efficient to extract APEOs and similarly efficient to extract APs. Probably, analyte-biosolid interaction is considerably higher in ethoxylates, requiring additional energy (temperature and pressure), so that, acetone can quantitatively release the analytes from the matrix. The methodology also turned out to be considerably faster than Soxhlet extraction and requires lower amount of extraction solvent.

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