TRABALHO DE CONCLUSÃO DE CURSO

ENGENHARIA DE ALIMENTOS

DETERMINATION OF THREE CRESOL ISOMERS IN SEWAGE SLUDGE BY SOLID-LIQUID EXTRACTION WITH LOW TEMPERATURE PURIFICATION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Montes Claros, 18 de junho de 2021

Dedico à minha mainha, Dasdores.

Ao meu pai, Francisco.

À minha esposa, Naiara.

Aos meus irmãos e amigos.

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"Não importa o quão estreito seja o portão e quão repleta de castigos seja a sentença, eu sou o mestre do meu destino: eu sou o capitão da minha alma". (William Ernest Henley, Invictus,1875)
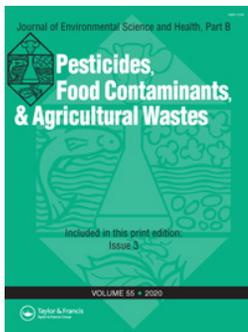
Determination of three cresol isomers in sewage sludge by solid-liquid extraction with low temperature purification and gas chromatography-mass spectrometry

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Determination of three cresol isomers in sewage sludge by solid-liquid extraction with low temperature purification and gas chromatography-mass spectrometry

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ABSTRACT

Cresols are chemical contaminants derivative from phenol which can be found in sewage sludge. However, little attention has been given to monitoring these compounds in environmental matrices in the literature. Thus, the objective of this study was to develop a simple method based on solid-liquid extraction with low temperature purification for determining three cresol isomers in sludge. The quantification of these compounds was performed by gas chromatography coupled to mass spectrometry with a previous derivatization step. After a detailed study, the cresol recovery was higher than 91%, with relative standard deviation lower than 12% and a limit of quantification of $20\,\mu g\ kg^{-1}$. Linearity was achieved between 10 and $90\,\mu g\ L^{-1}$ ($R^2 > 0.98$) with the standard solutions prepared in matrix extracts due to the trouble caused by the matrix effect. The proposed method was applied with success for monitoring cresols in sewage sludge samples coming from six different wastewater treatment plants. All samples showed contamination by cresols, mainly p-cresol with values between 32.3 and $516.9\,\mu g\ kg^{-1}$. The majority of the analyzed samples showed a total sum of the isomers higher than the maximum residue limit established by Brazilian legislation ($160\,\mu g\ kg^{-1}$).

Introduction

Cresol is a nonsystematic name used to refer to the 2-methylphenol (o-cresol), 3-methylphenol (m-cresol) and 4-methylphenol (p-cresol) isomers. These compounds are produced as by-products in steel industries during the pyrolysis process of mineral coal,[1] and are used as an important intermediate in the synthesis of various substances for chemical, food and pharmaceutical industries such as antioxidants and phenolic resins. The mixture of isomers is also marketed as disinfectants for domestic and veterinary use known by the generic names of creolin® and lysol®. In addition, cresols are naturally formed during the wood combustion process, they are present as undesirable compounds in petroleum derivates,[2,3] and p-cresol is detected in human urine due to anaerobic biodegradation of tyrosine by intestinal bacteria.[4,5]

Thus, one of the main ways for introducing cresols into the environment is through sewage sludge produced from treating domestic and industrial sewage in the wastewater treatment plants. Although cresols can be removed by some sewage treatment systems,[2,6] Fisher et al.[7] showed that p-cresol can be produced in sludge during the stocking process of the residual material. This phenomenon is attributed to the action of endogenous bacteria of the sludge in biodegrading tyrosine.[8]

In 1995, the International Program on Chemical Safety, in which the World Health Organization and the United Nations Environment Program participated, published a complete monograph about cresols due to growing concerns of the impacts of these compounds on the environment and human health.[9] In 2006, the Brazilian resolution no. 375 of the National Council for the Environment (CONAMA)[10] established the maximum residue limit (MRL) of $160\,\mu g\ kg^{-1}$ as the sum of all cresols in sewage sludge intended for application in agricultural soil in order to avoid indirect human exposure. In 2017, the United States Agency for Toxic Substances and Disease Registry inserted cresols onto its substance priority list, taking into account their frequency, toxicity and potential for human exposure.[11]

Although cresols belong to an important class of chemical contaminants denominated total phenols,[12] cresol monitoring in environmental matrices such as sewage sludge has received little attention in the literature.[13–15] The monitoring of similar compounds, for example alkylphenols and bisphenol A, has preferably been performed using gas chromatography (GC) and liquid chromatography both coupled to mass spectrometry (MS). However, for GC analysis, molecules containing hydroxyl functional group are very troublesome due to their ability to form hydrogen bonds on the injection system, resulting in low sensitivity. In these cases, derivatization of the target analytes must be considered prior

to GC-MS analysis and it is usually done by acylation, silylation or alkylation reactions.[16,17]

The most common sample preparation techniques have been ultrasound-assisted extraction and microwave-assisted extraction, generally followed by clean up with solid phase extraction.[13] Solid-liquid extraction with low temperature purification (SLE-LTP) has been an interesting alternative for determining chemical contaminants in food,[18,19] biological[20,21] and environmental[22,23] matrices. The principle of SLE-LTP is based on the addition of a homogenous mixture composed of water and organic solvent to the sample. The system is homogenized and cooled to –20 °C to freeze the matrix components and the aqueous phase. The organic phase, typically acetonitrile, remains liquid and extracts the compounds of interest. This technique is simple, easy, cheap and compatible with any chromatographic analysis. Therefore, the objectives of this study were to optimize, validate and apply the SLE-LTP for determining cresols in sewage sludge samples.

Materials and methods

Reagents and solutions

Standards of o-cresol, m-cresol and p-cresol with purity higher than 99% (w/w) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Table 1 lists the compounds studied and some of their physical-chemical properties. Derivatization reagents N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane, pyridine (99% purity) and acetic anhydride were also obtained from Sigma-Aldrich. Ethyl acetate and acetonitrile high performance liquid chromatography grade, anhydrous sodium sulfate, sodium chlorite and sodium hydrogen phosphate were all obtained from Vetec (Rio de Janeiro, Brazil). The sorbent silica gel (30–70 mesh) was obtained from Carvalhaes (Germany).

Stock standard solutions of 500 mg L^{-1} were prepared from pure standard o-cresol, m-cresol and p-cresol in acetonitrile. These solutions were used for preparing the work

Table 1. Chemical structures and physical-chemical properties for target cresols.[9]

	o-cresol	m-cresol	p-cresol
Structures			
Boiling point (1 atm, °C)	191	202.32	201.94
Vapor pressure (25 °C, mm Hg)	0.31	0.143	0.13
Solubility in water (25 °C, g L^{-1})	25.95	22.70	21.52
Sorption Coefficient (K_{OC})	22–3420	22–3420	22–3420
Log $K_{O/W}$	1.95	1.96	1.94
pK$_a$ (25 °C)	10.287	10.09	10.26

$K_{O/W}$ = partition coefficient octanol/water.

solution, simultaneously containing the cresols at 4 mg L^{-1} in acetonitrile. All solutions were put into dark bottles and maintained at –20 °C.

Aqueous solutions at pH 2 and 4 were prepared from phosphoric acid (Vetec, Brazil).

Equipments

Identification and quantification of compounds were performed on a gas chromatograph (GC 7890A) coupled to a mass spectrometer (MS 5975C) from Agilent Technology. The injector temperature was set to 270 °C and 1 μL of solution was introduced by a CombPal auto injector into the system with a 1:5 split ratio. Helium (99.9999% purity) was used as the carrier gas with flow at 1.2 mL min^{-1}. A funded silica chromatographic column with a stationary phase of 5% phenyl and 95% methyl polysiloxane (30 m × 0.25 mm i.d. × 0.25 μm) was used to separate the compounds. The oven was heated from 70 °C up to 250 °C with a rate of 15 °C min^{-1}. The interface was maintained at 280 °C and ion source at 230 °C. Mass detector was operated on an electron impact mode (70 eV) with a quadrupole mass analyzer. First, analyzes were performed with full ions at a range between 40 and 650 m/z. After, analyzes were performed in selective ion monitoring (SIM) with m/z 91, 165 and 180 selected for identification and quantification of the three cresols. Equipment control and data acquisition were done by ChemStation software (E.02.02.1431 copyright© 1989–2011, Agilent Technology).

A vortex (Phoenix), a centrifuge (Kindly), an ultrasonic bath (Unique, Brazil) and an analytical balance (Shimadzu) were used in the sample preparation step.
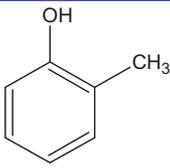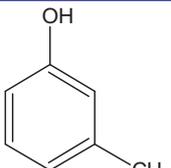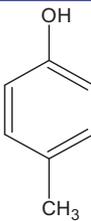
Samples

Two sewage sludge samples were used in this study in developing the method, with the first sample obtained from WWTS of Juramento City (80,000 habitants), which mainly receives domestic sewage and its treatment is based in aeration pounds followed by a drying bed. The second sample was obtained from WWTS of Montes Claros City (400,000 habitants), which receives industrial and domestic sewage, and its treatment is based on an upflow anaerobic sludge blanket (UASB) reactor and thermal drying (350 °C). Both samples were put into glass bottles and maintained at 4 °C during work development.

Fortification of the samples

During optimization of the proposed method, the sludge samples (4.00 g) were spiked with 100 μL work solution (4 mg L^{-1}) and left at rest for 3 h in order to evaporate the solvent and allow the cresols to interact with the matrix.

Optimization of the identification and quantification method of cresols

Identification and quantification of the cresols by GC-MS were evaluated by three experiments: (i) analysis of a standard solution prepared in acetonitrile; (ii) analysis of a standard solution derivatized with BSTFA/pyridine (25 °C) and (iii) analysis of a standard solution derivatized with acetic anhydride/pyridine (25 °C). Both derivatization experiments were performed by adding 100 μL of standard solution (1 mg L^{-1}), 30 μL of pyridine and 50 μL of derivatizing reagent in insert of 240 μL. The reactions were carried out at room temperature (25 °C) during 30 min. The experiments were repeated after choosing the best derivatizing reagent by heating derivatization vials into glycerin bath at 50 °C for 30 min. The stability time of the trimethylsilanized cresols formed after a derivation in acetonitrile was additionally studied at 0, 0.5, 1, 2 and 3 h.

Derivatization experiments were performed again in order to evaluate the influence of the matrix components in the chromatographic response of the cresols, replacing cresol standard solutions prepared in pure solvent by cresol standard solutions prepared in matrix extracts. Although these extracts had been obtained from domestic sewage sludge a strong matrix effect was observed. Thus, the proportion of the derivatization reagent and extract volumes was studied in three experiments: (i) 100 μL of derivatizing reagent and 50 μL of extract; (ii) 50 μL of derivatizing reagent and 100 μL of extract and (iii) 125 μL of derivatizing reagent and 25 μL of extract.

After defining the best derivatization condition, o-cresol, m-cresol and p-cresol extraction from sewage sludge was optimized by evaluating four parameters of the SLE-LTP. Details of this optimization are shown in Table 2.

As can be observed in Table 2, the extraction phase volume (i) was always maintained at 8 mL while only changing the composition of the mixture. Bearing in mind that cresols are weak acids (Table 1), the pH of the aqueous phase (ii) was adjusted to 2, 4 and 7 to increase the recovery of cresols into organic phase. In addition, a small amount of salts were diluted into aqueous phase (iii) in order to increase ionic strength, but without separation of the organic and aqueous phases. Finally, two efficient homogenization strategies (iv) of the sludge-water-organic solvent system were evaluated before the freezing step and the organic phase separation. All experiments were performed in triplicate.

Solid-liquid extraction with low temperature purification

In the optimized method, 4.00 g of sewage sludge was transferred to a 22 mL transparent vial and 2 mL of ultrapurified water, 6.5 mL of acetonitrile and 1.5 mL of ethyl acetate were added. The vial was homogenized in a vortex for 1 min and frozen at –20 °C for 1 h. Then, 2 mL organic phase (still liquid) was transferred to a 15 mL falcon tube containing 375 mg of anhydrous sodium sulfate. The tube was homogenized in a vortex for 30 s and centrifuged at 4,000 rpm (2,950 × g) for 15 min. Finally, the supernatant was stocked in a 2 mL vial until analysis.

Derivatization

In the optimized conditions, derivatization of the extracts was carried out in a 240 μL insert, which was put into a 2 mL injection vial. Next, 50 μL of extract or standard solution, 100 μL of BSTFA and 30 μL of pyridine were added to the insert. The system was left at rest for 10 min at ambient temperature (25 °C), and the derivatized product was then immediately analyzed by GC-MS.

Method validation

After achieving the best cresol extraction condition using SLE-LTP and analyzing by GC-MS, the method was validated evaluating selectivity, limit of quantification (LOQ), linearity, accuracy and precision.[24] The sludge samples used in these experiments were obtained from industrial and domestic sewage treatment.

Results and discussion

Chromatographic analysis

Individual standard solutions of cresols at 1 mg L^{-1} were analyzed by GC-MS in order to identify the retention times of the three isomers. The o-cresol signal was identified at 7.97 min, while the m-cresol and p-cresol compounds were coeluted at 8.7 min. Another observed problem was low sensitivity for two signals, compromising the method quality. This latter result can be explained by the presence of a hydroxyl group in the cresols which adsorbs on the silanol groups of the chromatographic system, for example, the injector liner.

Thus, the best option achieved to solve these problems was to derivatize the cresols before chromatographic analysis. For this, BSTFA and acetic anhydrides (50 μL) reagents, which are widely used for derivatization of phenolic compounds,[22] were assessed in the presence of pyridine (30 μL) for derivatization of a standard solution of cresols at 1 mg L^{-1} (100 μL) at room temperature (25 °C). The first point observed in this assay was separation of the three isomers using both derivatizing reagents, especially with BSTFA. It is possible that the silylation of hydroxyl groups reduced the adsorption of the cresols on polar groups of the injector and increased their interaction on the chromatographic column (non-polar) by the partition phenomenon. The second point observed in this assay was the better intensity of cresol signals when derivatized with BSTFA, which can be confirmed from the chromatographic
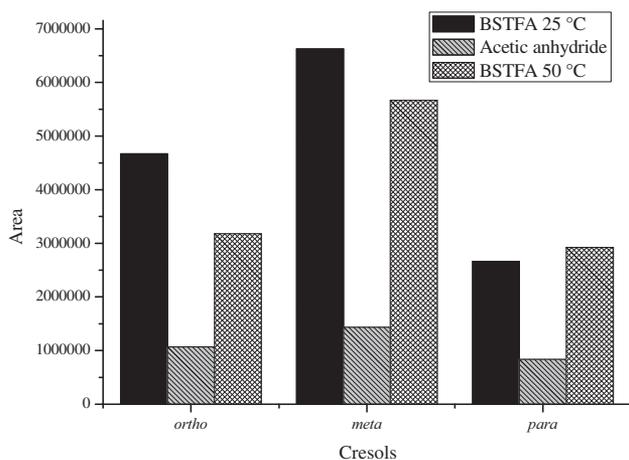
Table 2. Optimized parameters in the SLE-LTP.

	Parameters	Levels
(i)	Extraction mixture	Acetonitrile/Ethyl acetate (6.5 mL/1.5 mL)
		Acetonitrile (8.0 mL)
		Isopropanol/Ethyl acetate (6.5 mL/1.5 mL)
		Tetrahydrofuran (8.0 mL)
(ii)	pH	2, 4 and 7
(iii)	Ionic strength	0.2 mg L^{-1} NaCl
		0.2 mg L^{-1} Na$_2$HPO$_4$
(iv)	Homogenization	Vortex for 1 and 5 min
		Ultrasonic bath for 15 min

Figure 1. Areas obtained in the chromatographic analyses by GC-MS of cresols at 1 mg L^{-1} prepared in acetonitrile after derivatization with BSTFA and acetic anhydride.
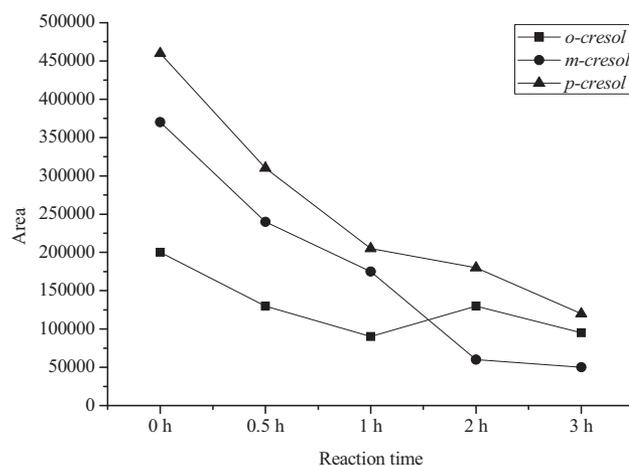

Figure 2. Chromatographic areas obtained after analyses by GC-MS of cresol standard solutions prepared in matrix extracts (100 μL), derivatized with BSTFA (50 μL) and 30 μL of pyridine. Derivatization times studied: 0, 0.5, 1, 2 and 3 h.

areas of the cresols shown in Figure 1. The areas of the *o*-cresol, *m*-cresol and *p*-cresol derivatized with BSTFA (25 °C) were about 4, 6 and 2 times, respectively, higher than the same compounds derivatized with acetic anhydride (25 °C).

BSTFA was then chosen as the derivatizing reagent based on this result, and the retention times of the target analytes were 9.12, 9.45 and 9.74 min for *o*-cresol, *m*-cresol and *p*-cresol, respectively. The derivatization step with BSTFA was also performed at 50 °C in a glycerin bath for 30 min, but the chromatographic area decreased about 33 and 17% for *o*-cresol and *m*-cresol, respectively.

New assays were then performed in order to evaluate the stability of the trimethylsilanized cresols at 0, 0.5, 1, 2 and 3 h after the derivatization procedure of the cresols in pure solvent (acetonitrile). However, no significant differences were observed in the chromatographic areas of the cresols at the studied time interval, thus the derivatized products were stable for 3 h. The same assays were then performed again, but replacing cresols prepared in pure solvent by cresols prepared in matrix extracts. Two important results were observed with these assays: (i) the chromatographic area of the cresols in matrix extract significantly reduced when compared with the area in pure solvent; and (ii) the chromatographic areas of the cresols decreased exponentially between 0 and 1 h (Fig. 2). This indicates that matrix compounds competed with the cresols for the derivatizing reagent, and beyond that they hydrolyzed the trimethylsilanized cresols over time. Thus, the derivatization time was defined as 10 min, although equilibrium was not reached in this reaction. The criteria applied for this choice were: (i) high chromatographic area; (ii) practicality of the derivatization step; (iii) repeatability of the results and (iv) low derivatization time.

Another important parameter evaluated was the quantity of BSTFA required for derivatization of the cresols in the presence of matrix components. The insert volume for derivatization was limited to 240 μL, thus the pyridine volume was maintained at 30 μL and the BSTFA + extract volume was always maintained at 150 μL. When the proportion between BSTFA:extract was 2:1 (100 μL:50 μL), the

chromatographic areas of cresols were approximately 5 times higher than the proportion of 1:2 (50 μL:100 μL). These results indicate that a greater amount of BSTFA was needed for derivatization because there was a competition between matrix components and cresols by the derivatizing reagent. Another assay was additionally performed by increasing the proportion of BSTFA:extract to 5:1 (125 μL:25 μL), but the results were similar to the proportion of 2:1, which was chosen in order to save BSTFA.

Optimization of the extraction method

This is the first time that SLE-LTP has been optimized to extract compounds with weak acid behavior in sludge samples, thus four parameters were carefully assessed in order to achieve higher recovery of cresols, extract cleaner and an easier method.

The first evaluated parameter was composition of the extraction phase (Fig. 3A), and the results showed that acetonitrile:ethyl acetate (6.5 mL:1.5 mL) provided higher extraction than 60% for all cresols. The same mixture was also used for extracting polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran in a sewage sludge sample by SLE-LTP.[25] Although tetrahydrofuran also provided similar recovery rate of cresols, it increased the matrix extraction interferences, requiring more maintenance of the GC-MS. On the other hand, the isopropanol:ethyl acetate mixture provided extractions lower than 10% for all target analytes, although it was the best extraction mixture for polychlorinated biphenyls in a sewage sludge sample by SLE-LTP.[26]

The second assessed parameter was the pH of the water used during the extraction process of the cresols by SLE-LTP because they have p$K_a \cong 10$. As expected, the extractions of the cresols were similar at values of pH 2, 4 and 7 (Fig. 3B), and no significant change was observed in the chromatograms in these three experimental conditions. Thus, the pH of ultrapurified water, which is near to 6, was not adjusted during the extraction procedure.
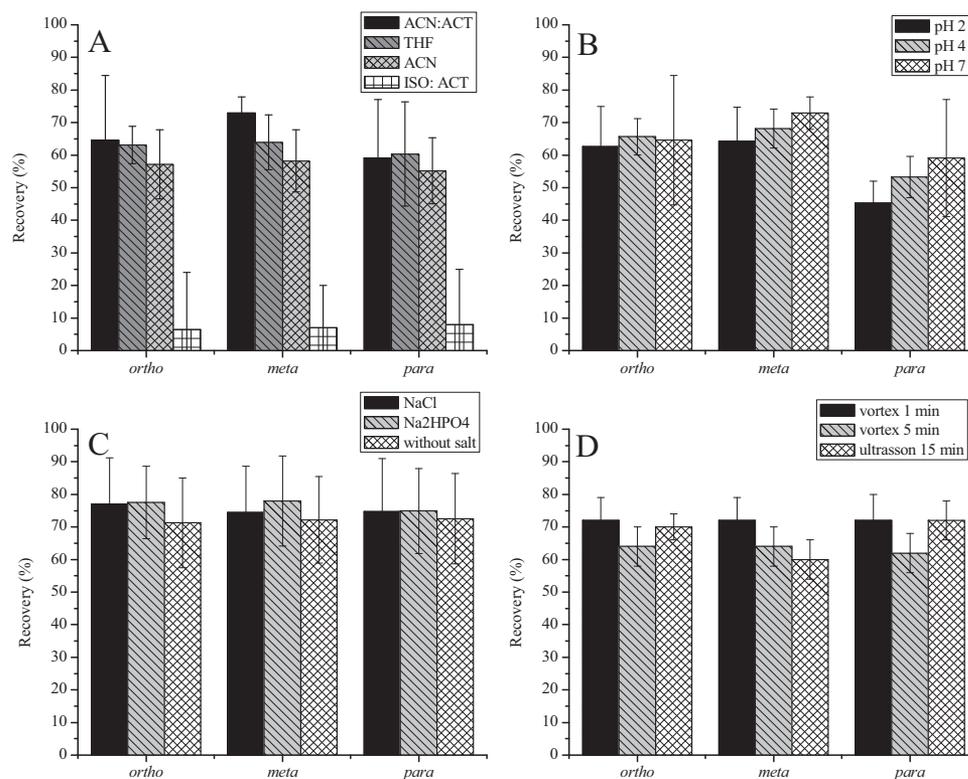
Figure 3. Percentage of recoveries for three cresols (50 μg L^{-1}) in sewage sludge obtained during optimization step of the SLE-LTP and analysis by GC-MS: (A) extraction solvent, (B) pH of the aqueous phase, (C) ionic strength of the aqueous phase and (D) homogenization mode.
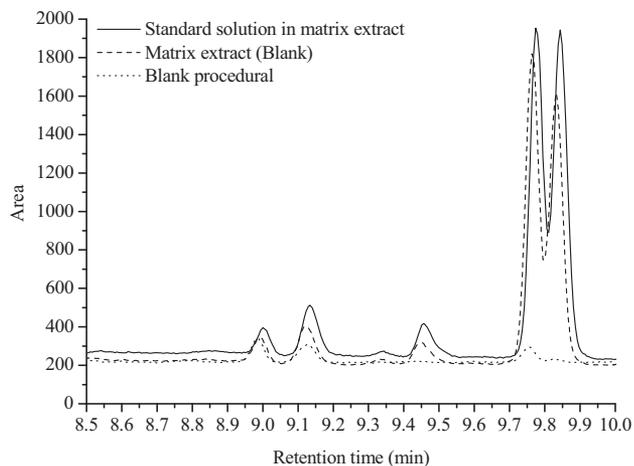

Figure 4. Chromatograms obtained by GC-MS-SIM after SLE-LTP in order to evaluate the method selectivity: cresol standard solution prepared in matrix extract at 10 μg L^{-1}, non-fortified matrix extract, and extract obtained from the proposed method without using a sludge sample, denominated "blank procedural."

The third evaluated parameter was ionic strength which was changed by adding sodium chloride or sodium hydrogen phosphate in aqueous phase during the SLE-LTP procedure. In light of the results observed in Figure 3C, the added salts did not influence the recovery of the cresols. Sewage sludge generally has a high concentration of available metals in its composition, which could contribute to increase the ionic strength of the aqueous phase during SLE-LTP.

The fourth assessed parameter was homogenization of the sludge-water-organic solvent system. The results in

Figure 3D demonstrate that both the vortex and ultrasound provided similar extractions for all cresols. A vortex was chosen to be used for 1 min considering that it has been used for extracting chemical contaminants in sewage sludge by SLE-LTP in previous studies.[25,27]

The cresols extractions were approximately 70% after optimizing the main SLE-LTP parameters. In order to investigate this low recovery of the target analytes, the sludge sample was replaced by another sample with lower mineral content and consequently higher organic matter content obtained from a complex system of sewage treatment (UASB). In this analysis, the recovery of the cresols reached values above 90%. This result was attributed to the adsorption of the cresols in the silicate material present in the first sludge sample. In order to prove this supposition, a new assay was performed by adding 2 mL of sludge organic extract obtained from SLE-LTP into a falcon tube, as well as 375 mg of anhydrous sodium sulfate and 50 mg of 30–70 mesh silica gel. The tube was homogenized in a vortex for 30 s, centrifuged at 4,000 rpm for 15 min and the supernatant was analyzed by GC-MS. The results revealed that the chromatographic area of each cresol decreased by 20% in the presence of silicate material. This kind of problem is expected because sewage sludge is a very complex matrix and has an unspecified chemical composition, which varies according to origin and treatment. These results are also supported by a wide range of sorption coefficients (22–3,420) for cresols in different soils, as shown in Table 1. Considering everything, validation of the proposed method was performed with sewage sludge obtained from domestic

Table 3. LOQ, linearity, accuracy and precision for SLE-LTP of three cresols in sewage sludge.

| Cresols | LOQ | Calibration dates | | Recovery (RSD intra-day, %) | | RSD inter-day (%) |
		Range (μg L^{-1})	R^2	50 μg L^{-1} ($n = 5$)	90 μg L^{-1} ($n = 5$)	50 μg L^{-1} ($n = 10$)
ortho	10 μg L^{-1}	10–90	0.9908	98 (6.1)	103 (6.6)	6.8
meta	10 μg L^{-1}	10–90	0.9809	103 (11.6)	95 (15.0)	10.4
para	10 μg L^{-1}	10–90	0.9953	91 (9.6)	102 (15.9)	10.7
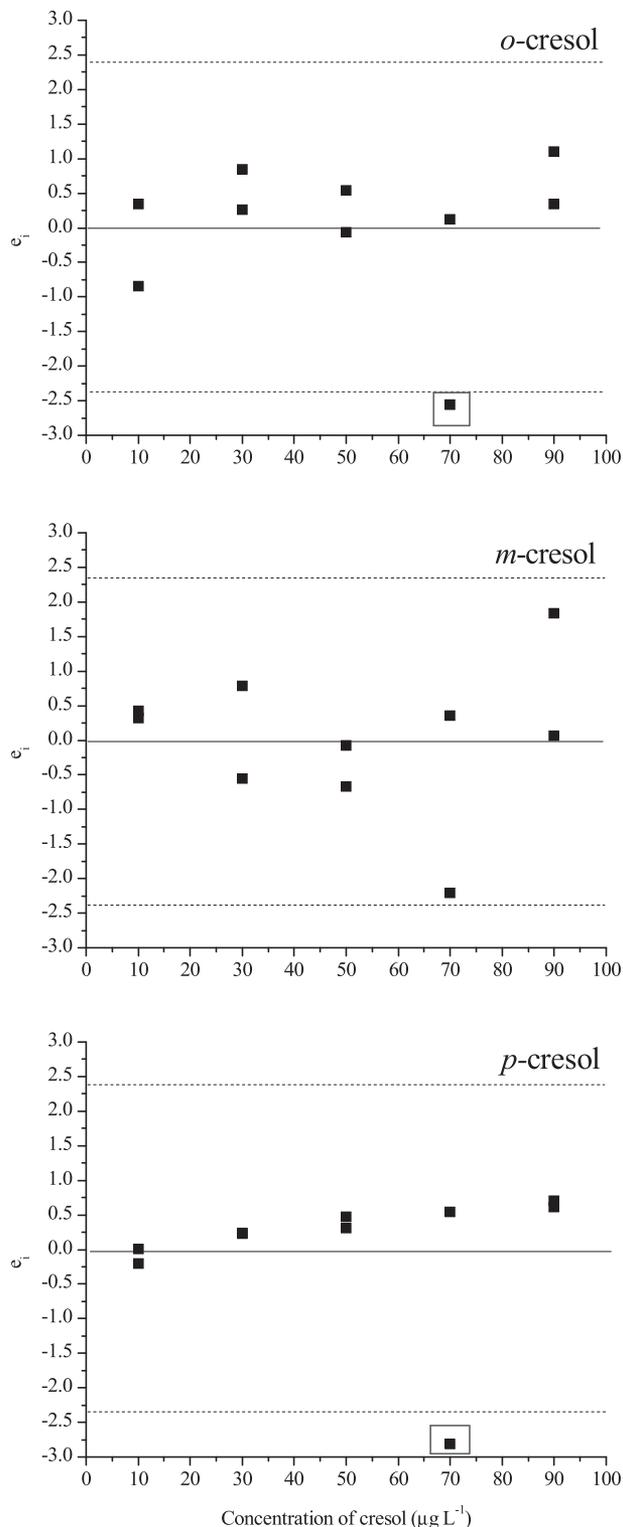
Figure 5. Residual plots for outliers diagnosed by the Jacknife standardized residual test: e_i is the residual, circled points are outliers and dashed lines are $\pm t_{crit}$ (0.975, n-p-1).

and industrial sewage treatment since there is a greater expectation of finding cresols in this type of sample.

Validation of the extraction method

The method selectivity was evaluated by comparison between chromatograms obtained from a cresol standard solution (10 μg L^{-1}) prepared in matrix extracts and from blank matrix extracts (no spiked sludge). As can be observed in Figure 4, signals were detected in the retention times of the cresols in blank extract.

In order to investigate the origin of the signals, all the extraction and derivatization procedures were performed without a sludge sample, denominated "blank procedural." The results presented in Figure 4 demonstrate that there was interference at retention times of o-cresol (9.12 min) and p-cresol (9.74 min). This interference at 9.12 min had approximately 40% area of the same signal in blank matrix extracts. A similar observation occurred for interference at 9.74 min, which had an area less than 4% when compared with the same signal in blank matrix extracts. These results indicate that the sewage sludge sample had the three cresols in its composition, which is not surprising since the sludge sample came from treatment of industrial and domestic sewage. After a thorough investigation, the interference in the "blank procedural" were identified from derivatization reagents (BSTFA/pyridine), and their presence was inevitable.

As expected, the presence of signals in the same retention times of cresols has a negative effect on determining the LOQ of these compounds in matrix extracts. In this configuration, the LOQ was determined by the analysis of standard solutions prepared in extracts of blank matrix at decreasing concentrations. It was observed that at concentration above than 10 μg L^{-1} the analytical signal, subtracting the contribution of the blank matrix, was about 10 times the signal detected in blank procedural. In addition, sludge samples spiked with cresols at concentrations equal to the LOQ (triplicates) and submitted to the SLE-LTP and analyzed by GC-MS showed recoveries between 90 and 100% and relative standard deviations (RSD) $\leq$ 15%.[24] For this reason, 10 μg L^{-1} was estimated as the LOQ for each cresol, corresponding to 20 μg kg^{-1}. This value is satisfactory because the Brazilian legislation establishes a MRL of 160 μg kg^{-1} for the sum of the concentrations of the three isomers.

Linearity was achieved in analyzing standard solutions of cresols prepared in matrix extracts at 10, 30, 50, 70 and 90 μg L^{-1} in duplicate (Table 3). This procedure is known as matrix-matched calibration. This linear range included LOQ, the method optimization concentration (50 μg L^{-1}) and MRL (80 μg L^{-1}). It is noteworthy that blank extracts were analyzed in duplicate due to problem with the method

Table 4. Comparison between proposed method and the traditional methods used for determining alkylphenols in sewage sludge samples by GC.[13]

Target analytes	Technique	Clean up	Derivatization	Limits	Recovery (%)
Cresols	SLE-LTP	–	BSTFA/pyridine	LOQ: 20 μg kg^{-1}	91–103
4-Tert-octylphenol	Microwave-assisted extraction	Gel permeation chromatography	BSTFA/pyridine	LOQ: 0.2–9.5 μg kg^{-1}	74.8–101.6
Nonylphenol, octylphenol	Liquid-solid extraction	Solid phase extraction	–	MQL: 0.5–20 μg kg^{-1}	40.2–89.4
Alkylphenols	Liquid-liquid extraction	–	–	LOD: 0.5–50 μg kg^{-1}	88–112
Nonylphenol, octylphenol	Ultrasound-assisted extraction	Solid phase extraction	MTBSTFA	LOD: 0.1–25 μg kg^{-1}	83–107
Nonylphenol	Ultrasound-assisted extraction	Silica column	HFBA/trietylamine	LOD: 0.12–5 μg kg^{-1}	39–79

BSTFA: N,O-bis(trimethylsilyl)trifluoroacetamide; HFBA: heptafluorobutyric anhydride; LOD: Limit of detection; LOQ: Limit of quantification; MQL: Method quantification limit; MTBSTFA: N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide.

Table 5. Cresol concentration in six real samples of sewage sludge.

Cresols	Concentration (μg kg^{-1}) ± relative standard deviation (%)					
	City 1	City 2	City 3	City 4	City 5	City 6
ortho	55.6 ± 6.1	55.5 ± 8.7	57.2 ± 4.9	64.2 ± 1.4	76.2 ± 0.7	21.9 ± 1.2
meta	105.2 ± 9.8	36.8 ± 3.8	41.9 ± 5.0	–	24.2 ± 16.7	51.3 ± 2.9
para	516.9 ± 4.8	88.2 ± 11.6	85.8 ± 10.8	63.8 ± 6.2	69.8 ± 2.0	32.3 ± 1.5
Total sum	677.7	180.5	184.9	128.0	170.2	105.5
	>MRL	>MRL	>MRL	<MRL	>MRL	<MRL

selectivity, and the average area was subtracted in each concentration of the curve, disregarding all the possible method interference. Calibration data were obtained by linear regression and the determination coefficients (R^2) were greater than 0.98 for all cresols (Table 3).

Linearity was assessed by the ordinary least squares method and the outliers were confirmed by the Jackknife residue test, with maximum of 22.2% removed outliers of the 10 replicates (Fig. 5).

Accuracy was assessed using fortification/recovery experiments in two concentrations 50 and 90 μg L^{-1}, corresponding to 100 and 180 μg kg^{-1}, respectively. Recovery means in each tested concentration were satisfactory, with values within the range recommended by the International Union of Pure and Applied Chemistry, which establish the range from 70 to 120% for a complex matrix.[24] Table 3 shows the mean recovery percentages for all cresols.

The method precision was evaluated under intraday and interday repeatability conditions. Thus, five sludge samples fortified at 50 μg L^{-1} (100 μg kg^{-1}) were submitted to SLE-LTP and analyzed by GC-MS within the same day (intraday). In this condition, the RSD were less than 12%, confirming the intraday precision of the method (RSD $\leq$ 20%).[24] After one month, the same procedure was performed with the same analyst, equipment, and so forth, and a new RSD was calculated for all recovery results obtained from two days ($n = 10$). The RSD achieved for each cresol was less than 11%, confirming the interday precision of the method.[24]

Taking into account that dates about optimization and validation of methods for cresols in sewage sludge are not available in the literature,[13–15] the results obtained in the proposed method were compared with dates available for similar chemical contaminants, such as alkylphenols (Table 4). Although developing a method for determining contaminants in sewage sludge is extremely challenging, it is observed that the results achieved for the proposed method were satisfactory. In addition, the SLE-LTP showed advantages because it did not require an additional clean up step since the matrix components were retained in the frozen

aqueous phase. Thus, the SLE-LTP was simpler, cheaper, faster and easier than traditional methods used for determining similar contaminants in sludge, as can be observed in Table 4.

Application of the method

The optimized method was applied in samples obtained from six WWTS, which have different sewage treatment systems. The cresol concentration in each sample is shown in Table 5.

The compound *p*-cresol was the isomer which had the higher detected concentration (516.9 μg kg^{-1}) in the sample identified by City 1. This result could be explained by expressive presence of *p*-cresol in human urine[4,5] or was produced in the sludge during the storage process.[7,8] Most of the samples had a total sum of cresols higher than the MRL (160 μg kg^{-1}) established by CONAMA Resolution no. 375.

Conclusion

A simple, easy and cheap method based on the SLE-LTP technique was developed for determining *ortho, meta* and *para*-cresol in sewage sludge samples. The quantification of the target analytes by GC-MS requires a previous derivatization step with BSTFA/pyridine to achieve complete chromatographic separation of the three signals and better sensitivity. The quantification of cresols should be done using an analytical curve prepared in matrix extracts because the presence of matrix components requires a higher quantity of BSTFA than quantification based in pure solvent. The characteristics of the sludge samples influence the recovery percentages of the cresols by the proposed method, since silicate samples presented recoveries near 70% for all compounds, while sludge samples with higher content of organic matter showed recoveries near 100%. This latter sample provided a LOQ of 20 μg kg^{-1}, linearity between 10 and 90 μg L^{-1}, and RSD $\leq$ 15%, attesting to the method's quality

for application in real samples. Analysis of real samples demonstrated the presence of cresols in the six samples, with four samples showing a concentration above the MRL established for total sum of cresols in soil conditioned with sludge by Brazilian Law. Relevant quantification was obtained for *p*-cresol in one sample because its value was greater than three times the MRL, demonstrating the importance of monitoring these compounds in sludge before disposal in agricultural soils.

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