Talanta 120 (2014) 191–197

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of cyclic and linear siloxanes in wastewater samples by ultrasound-assisted dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 23 July 2013
Received in revised form
8 November 2013
Accepted 14 November 2013
Available online 2 December 2013

Keywords:
Volatile siloxanes
Ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME)
Experimental design
Wastewater

ABSTRACT

A fast, simple and environmentally friendly ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME) procedure has been developed to preconcentrate eight cyclic and linear siloxanes from wastewater samples prior to quantification by gas chromatography–mass spectrometry (GC–MS). A two-stage multivariate optimization approach has been developed employing a Plackett–Burman design for screening and selecting the significant factors involved in the USA-DLLME procedure, which was later optimized by means of a circumscribed central composite design. The optimum conditions were: extractant solvent volume, 13 μL; solvent type, chlorobenzene; sample volume, 13 mL; centrifugation speed, 2300 rpm; centrifugation time, 5 min; and sonication time, 2 min. Under the optimized experimental conditions the method gave levels of repeatability with coefficients of variation between 10 and 24% ($n=7$). Limits of detection were between 0.002 and 1.4 μg L^{-1}. Calculated calibration curves gave high levels of linearity with correlation coefficient values between 0.991 and 0.9997. Finally, the proposed method was applied for the analysis of wastewater samples. Relative recovery values ranged between 71 and 116% showing that the matrix had a negligible effect upon extraction. To our knowledge, this is the first time that combines LLME and GC–MS for the analysis of methylsiloxanes in wastewater samples.

1. Introduction

Low molecular weight cyclic and linear methylsiloxanes are synthetic compounds which belong to the class of organosilicons. The analysis of these compounds has increased in recent years, especially due the interest in environmental and fuel industry.

Siloxanes are used in the manufacture of a great variety of products such as electronics, cosmetics, paints, food additives, medical devices, cosmetic surgery, needles coating, coating pacemaker, etc. [1]. This growing use has led to a considerable increase of these compounds in wastewaters [2]. Although the toxicological behaviour of these compounds is still poorly studied, some works have indicated that they can cause adverse toxicological effects on wildlife [3].

On the other hand, biogas is an important renewable energy source produced from the anaerobic digestion of sludge in wastewater treatment plant. The presence of siloxanes in the biogas can adversely affect the life-time of combustion engines due to abrasive effects of microcristalline silicon dioxide generated at high temperature [4]. Physical damage and poor performance of biogas are the reasons to seek new alternatives to the removal of these compounds before they reach the landfill gas [5]. Therefore, it is important that siloxanes may be detected and quantified in the wastewater and sewage sludge, as well as in the biogas, to prevent combustion engines damages and to select and design the appropriate siloxane abatement technique.

Literature describes some methods for the determination of the volatile siloxanes in biogas [6–9], in sewage sludge [10,11], but the studies on wastewaters are scarce [4,12–14]. One of the main limitations in the analysis of methylsiloxanes is the high volatility of these compounds and the potential sources of background contamination that affect their final determination. The detection technique of choice for most of the studies carried out till now has been gas chromatography–mass spectrometry (GC–MS) after a sample preparation step.

Determination of cyclic siloxanes from wastewater samples and activated or digested sludge were carried out successfully after stripping the siloxanes from samples by helium and adsorption on XAD resine [4]. Nevertheless, this method is time-consuming and labor. Sparham et al. [12] proposed a sensitive headspace (HS)-GC–MS

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http://dx.doi.org/10.1016/j.talanta.2013.11.042

method to analyze D5 in river water and treated wastewater samples. However, the static headspace method can be only used for clean water samples with low organic matters since cyclic methylsiloxanes have high organic carbon-water partition coefficients [14]. Sanchís et al. [13] analyzed methylsiloxanes on wastewater samples using liquid–liquid extraction followed by GC–MS/MS. Although the method is very sensitive it needs an evaporation step where analytes could be lost. Recently, D.G. Wang et al. [14] has developed a simple, rapid, and environmentally friendly analytical methodology for determination of three cyclic volatile methylsiloxanes (i.e., D4, D5 and D6) in industrial wastewater, sediment, soil, biota and biosolid samples. For water samples, a membrane-assisted solvent extraction technique was used followed by large-volume injection (LVI)-GC–MS. Therefore, the data about the presence of siloxanes in wastewater samples are limited and these studies were mainly focused on environmental risk assessment. Hence, more studies are needed to assess the content of siloxanes in wastewater samples used for biogas production.

Over the last two decades, liquid–liquid microextraction (LLME) techniques have been widely used in sample preparation due to their numerous advantages such as rapidity, price, easiness and environmental friendliness, among others [15]. Different modes of LLME have been developed, being ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME) [16] one of the modes that presents most of the advantages of the LLME techniques. In this technique, the disperser solvent is avoided and as a consequence the organic solvent volume is reduced and the enrichment factors increased. For this reason, USA-DLLME was considered as an excellent LLME candidate technique for the determination of siloxanes in wastewater samples.

The aim of this paper was to develop a fast, inexpensive and environmentally friendly sample preparation method based on ultrasound energy to assist the dispersion of a few microliters of extractant solvent for the preconcentration of eight volatile siloxanes from complex samples, as wastewaters, before the quantification by GC–MS. The optimization of the extraction conditions was done using experimental design. Good figures of merit were obtained and the analytical method was applied to wastewater samples.

To our knowledge, this is the first time that LLME has been combined with GC–MS for preconcentration and quantification of methylsiloxanes in complex wastewater samples.

2. Experimental

2.1. Chemicals and "real-world" water samples

Trimethylsilanol (TMS), hexamethyldisiloxane (L2), hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4), decamethyltetrasiloxane (L4), decamethylcyclopentasiloxane (D5), dodecamethylpentasiloxane (L5), and dodecamethylcyclohexasiloxane (D6) were all obtained from Sigma-Aldrich (St. Louis, MO, USA). Pesticide grade methanol, carbon tetrachlorhyde, tetrachloroethylene, and chlorobenzene were also from Sigma-Aldrich. Deionized water was prepared on a water purification system (Gradient A10) supplied by Millipore (Billerica, MA, USA). Stock standard solution of 1 g L^{-1} of target compounds was prepared in methanol. Working solutions were daily prepared by dilution of stock standard solution. All solutions were stored in the dark at 4 °C.

Wastewater samples from a municipal and industrial wastewater treatment plant located in Alicante (Spain) and from two treatment plants located in Murcia (Spain) were analyzed. Samples were stored in the dark at 4 °C before use. Analysis of samples collected at different points of the treatment plants (influent, effluent, UV reactor inlet and UV reactor outlet) in Murcia confirmed the presence of siloxanes. On the other hand, analysis of samples from Alicante confirmed that all target analytes were below the LOD of method, and these samples were used to carry out the recovery study.

2.2. Ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME)

For USA-DLLME, 13 mL of the sample solution was placed in a 20 mL glass test tube with a conical bottom and 13 μL of chlorobenzene as extractant solvent was dropped into the sample solution. The mixture was sonicated in an ultrasonic bath (Ultrasons-H, Selecta, Barcelona, Spain) for 2 min and subsequently centrifugated for 5 min at 2300 rpm in a centrifuge table (GS-6R of Belman, Fullerton, CA, USA). Finally, 2 μL of the extractant phase deposited at the bottom of the test tube was manually injected into GC–MS system for analysis.

2.3. GC–MS determination

All analyses were carried out on a Varian 3900-Saturn 2100 Gas Chromatograph/Mass Spectrometer system (Walnut Creek, CA, USA) equipped with a low bleed DB-624 Agilent J&W column (60 m × 0.25 mm, 1.40 μm) (Palo Alto, CA, USA). The inlet septa used was absent of siloxanes (CrossLab non-stick BTO inlet septa, Agilent Technologies, Palo Alto, CA, USA). The mass spectrometer employed was an ion trap (20 μA) with 0.82 s of scan time. The injector was maintained at 250 °C and operated in the splitless mode with the split closed for 0.75 min. Helium (> 99.999 % pure) was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The column oven was initially set at 40 °C for 2 min, programmed to 120 °C at 6 °C min^{-1} rate, where it was held for 5 min, followed by a 8 °C min^{-1} ramp up to 150 °C, and finally to 220 °C at 20 °C min^{-1} rate, where it was held for 5 min. The interface temperature was set at 200 °C and the detector voltage at 4 V. A solvent cut time was allowed between 21 and 22.8 min for all analysis. The base peak ion was chosen as the quantification ion and two other significant ions of each analyte were chosen for confirmation. Table 1 shows the ions selected for quantification and confirmation of methylsiloxanes using the GC–MS method. Prior to quantification, the identification of all target compounds was based on their mass spectra and GC retention times. Fig. 1 shows a typical chromatogram of deionized water spiked at 10 μg L^{-1} level of all target analytes and a chromatogram of a blank. As can be seen, contamination from the septum or chromatographic column could be considered negligible.

2.4. Data handling and processing

According to previous works, the response of the instrument used in the screening study was based on each area of the individual peaks eluted during GC–MS analysis [17]. By contrast, the optimization of the significant factors was based on the sum of

Table 1
Quantification and confirmation ions selected for the GC–MS analysis.

Analyte	Retention time (min)	Quantification ion (*m*/*z*)	Confirmation ions (*m*/*z*)
TMS	11.1	75	45;47
L2	12.6	147	148;149
D3	17.9	207	96;208
D4	24.9	281	282;283
L4	26.9	207	73;295
D5	28.6	355	73;267
L5	30.2	281	73;147
D6	32.0	341	73;429

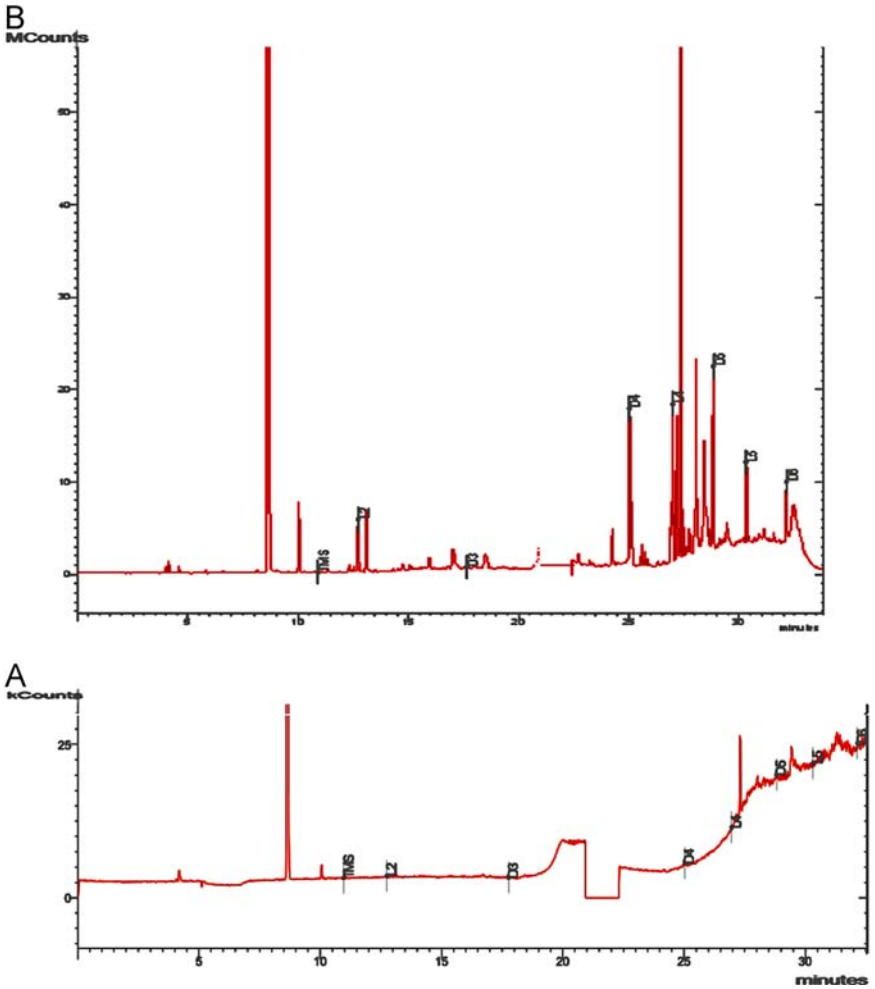

Fig. 1. (A) Chromatogram of a blank solution subjected to the optimized USA-DLLME-GC–MS method; (B) chromatogram of a standard solution (10 μg L^{-1}) subjected to the developed method. TMS, trimethylsilanol; L2, hexamethyldisiloxane; D3, hexamethylcyclotrisiloxane; D4, octamethylcyclotetrasiloxane; L4, decamethyltetrasiloxane; D5, decamethylcyclopentasiloxane; L5, dodecamethylpentasiloxane; D6, dodecamethylcyclohexasiloxane.

all the areas of the individual peaks [18–20], in order to obtain one unique set of optimum conditions for the simultaneous extraction of all siloxanes. Concentrations of 5 and 1 mg L^{-1} were used for the screening and optimization of the significant factors, respectively, in order to assure a detectable signal (peak area) in every experimental run for all the analytes.

Experimental design matrices were constructed and results were evaluated using the Statgraphics Statistical Computer Package "Statgraphics Plus 5.1" (Statpoint Technologies, Inc. Warrenton, VA, USA).

3. Results and discussion

Preliminary experiments proved that the conventional liquid–liquid extraction of these compounds in wastewater samples produced emulsion problems. Therefore, its determination using this methodology was unfeasible. However, the emulsion problem was not produced when LLME is carried out. Therefore, USA-DLLME, as an advantageous mode of LLME, was chosen in this work.

3.1. Study of experimental factors involved in USA-DLLME

3.1.1. Study of solvent extraction

The selection of an appropriate extraction solvent is very critical for developing an efficient dispersive liquid–liquid microextraction. Generally, extraction solvent used in USA-DLLME procedures must fulfil the following requirements: It is preferably to have a higher density than water, low solubility in water, high extraction capability of the target analytes, and in addition, it should be easily dispersed in water during sonication. Additionally, the extraction solvent should also have good chromatographic behaviour during the course of chromatographic separation. Based on these facts, three solvents including carbon tetrachlorhyde, tetrachloroethylene, and chlorobenzene were tested as potential acceptor phases.

Solvent selectivity was evaluated with 10 mL of sample containing 10 mg L^{-1} of target analytes and 40 μL of extractant solvent was added. The mixture was sonicated in an ultrasonic bath for 3 min and then was centrifugated for 3 min at 2300 rpm. As shown in Fig. 2, all the solvents supplied similar extraction behaviour with all the siloxanes. However, L2 co-eluted with carbon tetrachlorhyde and D3 co-eluted with tetrachloroethylene. Therefore, chlorobenzene was chosen as extractant phase.

3.1.2. Study of other experimental factors by experimental design

Different factors can affect the extraction yield in the USA-DLLME procedure and in most cases they could be correlated. Therefore, a multivariate approach is recommended for their optimization. In this study, based on the literature and previous experience of our group [17,20,21], the influence of five factors, namely extractant solvent volume, sample volume, sonication

time, centrifugation speed and centrifugation time, were studied in order to maximize the extraction yield of the USA-DLLME procedure. However, some of them might not have a significant effect and they can, thus, be obviated. In this respect, a screening study, prior to optimization of significant factors, is helpful in order to assess the significant factors involved in the analytical system under investigation.

If a large number of factors are involved, reduced factorial designs are employed for screening. A particular type of those designs is the Plackett–Burman design [22], which assumes that the interactions can be completely ignored and so the main effects are only calculated with a reduced number of experiments. A saturated Plackett–Burman matrix was employed because of the large number of factors to be tested. A matrix with 11 factors (five real factors and six dummy factors) was used. The effects of dummy factors were used for the estimation of the experimental error used in the statistical interpretation [23,24].

Two levels were considered for each factor (Table 2). The matrix of the Plackett–Burman design was composed of 12 experiments. The experiments were randomly carried out in order to nullify the effect of extraneous or nuisance factors. On these experiments standard solutions of 5 mg L^{-1} were used and GC peak area of individual analytes were used as goal function.

An ANOVA test was used to evaluate the data and statistically significant effects were determined using a *t*-test with a 95% probability [23,24] and visualized by using main effects Pareto charts (Fig. 3). The Pareto charts, shown in Fig. 3, belong to D3 and L4. The charts for the rest of the analytes are not shown as they are similar.

According to the results, centrifugation speed was the most significant factor for all target analytes showing a positive effect.

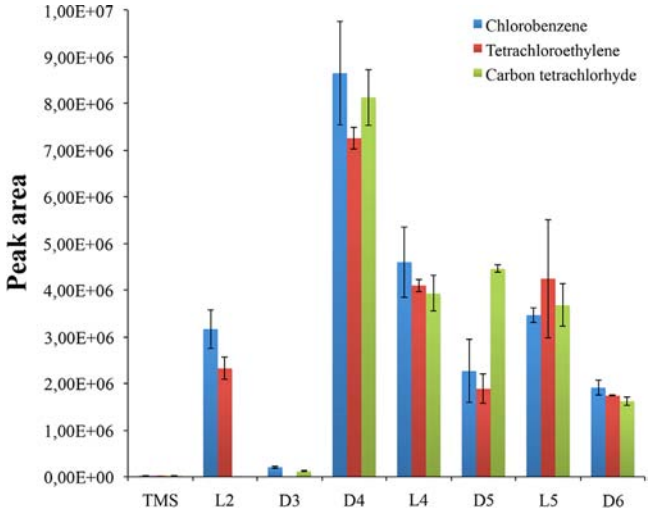

Fig. 2. Response of the organic solvents tested. Deionized water samples spiked at 10 mg L^{-1} concentration level. Error bars correspond to standard deviation.

Table 2
Experimental factors and levels studied on the Plackett–Burman design.

Factors	Level	
	Low (−1)	High (+1)
Sample volume (mL)	5	10
Extractant volume (μL)	20	50
Sonication time (min)	1	3
Centrifugation speed (rpm)	1500	2300
Centrifugation time (min)	4	6

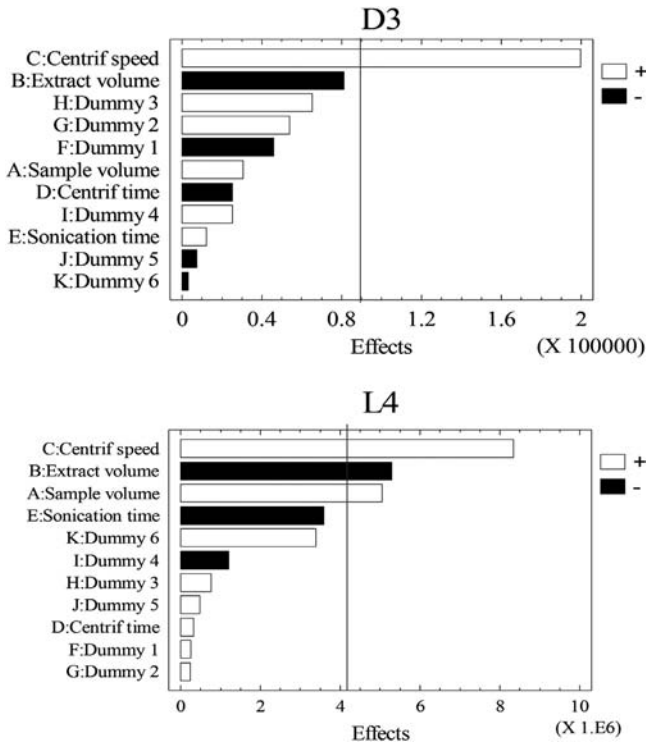

Fig. 3. Pareto charts of the main effects obtained from the Plackett–Burman design.

Higher centrifugation speed provides easier deposition of extractant solvent at the bottom of the tube. Pareto charts also reveal that sample volume appeared as a positive non-significant effect except for L4, D5 and D6 that appeared as positive significant factor. This positive effect agrees with the fact that increasing the aqueous sample volume also led to an increase in the total amount of analytes present in the solution, given that all samples were spiked at the same concentration level. Consequently, a greater amount of target pollutants was transferred to the extractant solvent.

Extractant solvent volume appeared as a non-significant effect except for L4, D5 and D6 that appeared as significant effect showing a negative sign. This is because increasing the extractant volume, the enrichment factor is reduced for dilution effect, so that the signal is larger by decreasing the ratio between extractant solvent and sample volumes. Sonication time appeared as non-significant effect for all the analytes with different sign for each target compound. Therefore, 2 min was chosen as a compromise value for all analytes.

Centrifugation time, also appeared as non-significant effect in all cases with different sign for each target compound. As previously, 5 min were chosen as a compromise value for all analytes.

The second study was concerned with optimizing the significant factors in order to obtain the best response. Different experimental designs can be found in the literature, many of them are based on the so-called response surface designs. Box-Wilson or central composite design (CCD) is one of the most used response surface designs, which is constructed by several superimposed designs. It consists of a factorial design (2^k) augmented with ($2k$) star points, where k is the number of factors to be optimized, and with a central point, which can be run n times [22]. A circumscribed central composite design (CCCD) was employed, where the star points were located at $\pm\alpha$ from the centre of the experimental domain, which was situated at 0. In order to establish the rotatability of the experimental design, n was set at 2 and $\alpha=\sqrt[4]{2^k}$ [22]. The overall matrix of CCCD design involved 16 experiments.

In this study, the three factors considered were: sample volume, extractant solvent volume and centrifugation speed. The

Table 3
Experimental factors and levels studied on the circumscribed central composite design (CCCD).

Factors	Level			Star points (α=1.682)	
	Low (−1)	Central (0)	High (+1)	$-\alpha$	$+\alpha$
Centrifugation speed (rpm)	1500	1800	2100	1295	2305
Extractant volume (μL)	20	30	40	13	47
Sample volume (mL)	8.0	10.0	12.0	6.6	13.4

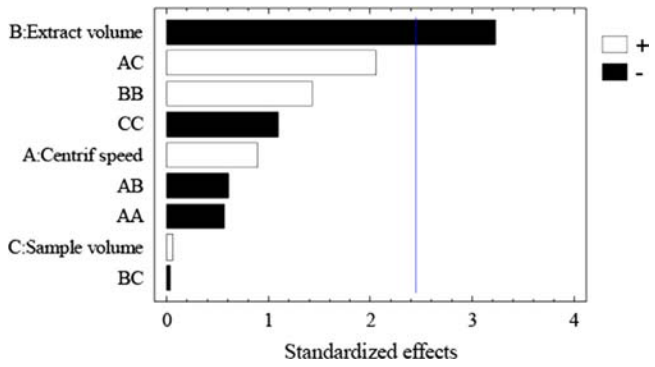

Fig. 4. Pareto chart of the main effects obtained from the circumscribed central composite design.

low (−1), central (0), and high (+1) levels of these factors, as well as the location of their star points ($\alpha=\sqrt[4]{2^k}=1.682$), are given in Table 3. Standard solutions of 1 mg L^{-1} were used and the response function was GC sum peak area of all siloxanes since the extraction conditions should be the optimum simultaneously for all model analytes.

The data obtained were evaluated by ANOVA test, and the effects were visualized by using Pareto chart (Fig. 4). As can be seen, extractant solvent volume is significant showing a negative effect, whilst sample volume shows a non-significant positive effect upon extraction. Indeed, increasing the sample volume results in an increase in the total amount of analyte extracted, reaching a maximum at 13.3 mL (+1.652). Extractant solvent volume shows a negative effect, reaching a maximum at 13 μL (−1.682). This negative effect could be attributed to a dilution effect. Centrifugation speed shows a positive non-significant effect, reaching a maximum at 2300 rpm (1.682).

Overall, the results obtained from the optimization process lead to the following experimental conditions: extraction solvent volume, 13 μL; sample volume, 13 mL; centrifugation speed, 2300 rpm; centrifugation time, 5 min; and sonication time, 2 min.

3.2. Study of performance parameters

A calibration study was performed by spiking deionized aqueous samples with analytes over the concentration range of 5–25 μg L^{-1} for TMS and D3, 2–25 μg L^{-1} for L2, D4, L4 and D5, and 2–15 μg L^{-1} for L5 and D6. In addition, L5 and D6 showed non-linear behaviour above concentrations of 15 μg L^{-1}. The calculated calibration curves gave a high level of linearity for all target analytes with correlation coefficients (r) ranged between 0.991 and 0.9997 as shown in Table 4. The repeatability of the proposed method, expressed as coefficient of variation (CV), was evaluated by extracting seven consecutive aqueous samples spiked at 10 μg L^{-1} with each target analyte and was found between 10 and 24%. The limits of detection (LODs) for all target analytes

Table 4
Performance parameters of the developed USA-DLLME-GC–MS method for the determination of siloxanes in water samples.

Analyte	Correlation coefficient (r)[a]	CV (%)[b]	LOD (μg L^{-1})[c]	LOQ (μg L^{-1})[d]
TMS	0.994	10	1.4	4.7
L2	0.993	20	0.006	0.02
D3	0.993	24	0.4	1.3
D4	0.996	22	0.002	0.007
L4	0.991	22	0.003	0.01
D5	0.993	21	0.003	0.01
L5	0.9997	22	0.02	0.07
D6	0.992	22	0.03	0.1

[a] Studied linear range: TMS and D3: 5–25 μg L^{-1} (number of standards=5, number of replicates=3 for level); L2, D4, L4 and D5: 2–25 μg L^{-1} (number of standards=6; number of replicates=3 for level); L5 and D6: 2–15 μg L^{-1} (number of standards=4, number of replicates=3 for level).
[b] Coefficient of variation (CV); mean value for seven replicate analyses; spiked level: 10 μg L^{-1}.
[c] Limits of detection (LODs) were calculated for a signal-to-noise ratio of three (S/N=3).
[d] Limits of quantification (LOQs) were calculated for a signal-to-noise ratio of ten (S/N=10).

Table 5
Enrichment factors of the optimized USA-DLLME-GC–MS method for the determination of siloxanes in water samples.

Analyte	Enrichment factor		Mean
	25 μg L^{-1}	50 μg L^{-1}	
TMS	160	146	153
L2	350	344	347
D3	619	642	631
D4	855	856	855
L4	268	303	286
D5	245	320	282
L5	260	273	266
D6	362	309	335

were determined according to a signal-to-noise-ratio (S/N) of three and the limits of quantification (LOQs) as ten times the above mentioned ratio. LODs values were found between 0.002 and 1.4 μg L^{-1} and LOQs values between 0.007 and 4.7 μg L^{-1} (Table 4). Table 5 shows USA-DLLME enrichment factors obtained with deionized water samples at 25 and 50 μg L^{-1} spiked level. The enrichment factors were obtained as the ratio of concentrations of the extractant solvent and deionized water samples (25 and 50 μg L^{-1}). As can be seen, the mean enrichment factor values range between 153 for TMS to 855 for D4.

Comparison of different analytical methods developed for the determination of siloxanes in wastewater samples is shown in Table 6. Similar LODs values are obtained, however, the sample preparation step in the present work is shorter, easy to handle and more environmentally friendly than those previously published. In addition, a higher number of siloxanes have been analyzed and some of them no previously reported (i.e., TMS). Furthermore, emulsion problem has been obviated, what is considered an important problem in other sample preparation methodologies (i.e., LLE) of wastewater samples.

3.3. "Real-world" water analysis

As described above, three replicates of each wastewater samples from different treatment plants were extracted using the USA-DLLME developed method and analyzed by GC–MS. The preliminary results showed that only samples from Murcia treatment plants contained siloxanes above the LODs of the method

Table 6
Comparison of different methods developed for the determination of siloxanes in wastewater samples.

Analyte	Separation/ detection	Extraction	Extraction time (min)	Linear range	CV (%)	LOD	Enrichment factor	Comments	Ref.
D3, D4, D6	GC-FID	XAD-resin	–	–	–	–	–	–	[4]
D5	GC–MS	Heating (headspace)	10	0–1 10^3 (ng L^{-1})	4–20	6.2 (ng L^{-1})	–	–	[12]
L3, L4, L5, D3, D4, D5	GC-(QqQ)-MS–MS	LLE	> 20	0.25–5000 (μg L^{-1})	4.5–13.2	0.1–0.4 (ng L^{-1}) 3.2–13 (ng L^{-1})	–	750 mL of hexane Two steps concentration	[13]
D4, D5, D6	GC–MS	Membrane-assisted solvent (pentane, 0.5 mL)	60	0.1–1.5 (μg L^{-1})	21–25	0.002–0.005 (μg L^{-1})	–	Two isotopic internal standards. Large volume injection	[14]
TMS, L2, L4, L5 D3, D4, D5, D6	GC–MS	USA-DLLME	7	2–25 (μg L^{-1})	10–24	1.4 (μg L^{-1}) 0.003–0.02 (μg L^{-1}) 0.002–0.4 (μg L^{-1})	153–855	–	This work

Table 7
Analysis of wastewater samples collected at different points in two treatment plants using the proposed USA-DLLME-GC–MS method.

Analyte	Concentration (μg L^{-1}) ± SD						
	Industrial & urban effluent (TPI)[a]	UV reactor inlet (TPI)[a]	UV reactor outlet (TPI)[a]	Industrial & urban influent (TPII)[b]	Industrial & urban effluent (TPII)[b]	UV reactor inlet (TPII)[b]	UV reactor outlet (TPII)[b]
TMS	≤ LOD	≤ LOD	≤ LOD	≤ LOD	≤ LOD	≤ LOD	≤ LOD
L2	≤ LOD	≤ LOD	1.7 ± 0.3	≤ LOD	≤ LOD	1.7 ± 0.3	≤ LOD
D3	≤ LOD	≤ LOD	≤ LOD	≤ LOD	≤ LOD	0.9 ± 0.2	≤ LOD
D4	1.8 ± 0.4	≤ LOD	2.5 ± 0.5	3.6 ± 0.8	2.2 ± 0.5	≤ LOD	≤ LOD
L4	≤ LOD	≤ LOD	≤ LOD	≤ LOD	≤ LOD	≤ LOD	≤ LOD
D5	22.7 ± 4.8	4.6 ± 1.0	3.7 ± 0.8	4.8 ± 1.0	≤ LOD	≤ LOD	≤ LOD
L5	≤ LOD	≤ LOD	≤ LOD	≤ LOD	≤ LOD	≤ LOD	≤ LOD
D6	≤ LOD	0.6 ± 0.1	≤ LOD	≤ LOD	1.2 ± 0.3	4.6 ± 1.0	9.5 ± 2.1

[a] Samples taken from the treatment plant (I) in Murcia.
[b] Samples taken from the treatment plant (II) in Murcia.

Table 8
Relative recoveries and CV values of the siloxanes studied in wastewater samples.

Analyte	Relative recoveries and CV values (%) in parentheses[a]		
	Industrial & urban effluent (S1)[b]	Industrial & urban effluent (S2)[b]	Industrial & urban effluent (S3)[b]
TMS	93 (18)	99 (15)	116 (13)
L2	71 (23)	73 (20)	73 (19)
D3	71 (15)	85 (6)	86 (7)
D4	77 (18)	82 (11)	82 (11)
L4	73 (16)	79 (20)	74 (21)
D5	72 (26)	77 (29)	81 (19)
L5	71 (14)	72 (24)	74 (19)
D6	73 (20)	76 (22)	81 (21)

[a] Three replicate analyses at 5 μg L^{-1} spiked level.
[b] Three samples from the treatment plant in Alicante.

Table 9
Relative recoveries and CV values of the siloxanes studied in wastewater samples.

Analyte	Relative recoveries and CV values (%) in parentheses[a]		
	Industrial & urban effluent (S1)[b]	Industrial & urban effluent (S2)[b]	Industrial & urban effluent (S3)[b]
TMS	82 (7)	94 (8)	89 (7)
L2	76 (10)	84 (10)	83 (1)
D3	82 (12)	74 (18)	82 (20)
D4	71 (10)	72 (15)	80 (12)
L4	93 (10)	81 (20)	85 (16)
D5	99 (7)	74 (11)	80 (20)
L5	80 (3)	86 (10)	81 (9)
D6	89 (4)	92 (11)	86 (21)

[a] Three replicate analyses at 10 μg L^{-1} spiked level.
[b] Three samples from the treatment plant in Alicante.

(Table 7). This table shows the analysis of wastewater samples taken at different points of two wastewater treatment plants. As can be seen, L2, D3, D4, D5 and D6 shows positive results in some of the points studied.

In order to investigate the effects of sample matrix upon the USA-DLLME procedure three replicate analyses were carried out with the effluent wastewater samples from Alicante treatment plant. Samples were spiked at 10 μg L^{-1} and 5 μg L^{-1} with each target contaminant, filtrated with common lab filter paper and analyzed under optimized experimental conditions. Relative recovery values were determined as the ratio of the concentrations found in real-world and deionized water samples, spiked at the same contamination level. The results for each set of experiments are summarized in Tables 8 and 9. Relative recovery values range between 71 and 116% for all analytes. As shown in the table, matrix effects were not significant, considering 70% and 120% as the acceptable lower and upper recovery limits, respectively.

4. Conclusions

A new and an environmentally friendly method has been developed for the analysis of eight siloxanes in wastewater samples based on ultrasound-assisted dispersive liquid–liquid microextration (USA-DLLME) coupled to GC–MS. Optimization of the microextraction method has been done by experimental

design. USA-DLLME methodology is faster, cheaper and easier to handle than those previously studied for the same purpose. The LOD values obtained satisfy the requirements for these analytes in wastewater samples for biogas production studies. Therefore, the suggested method can be an excellent alternative for laboratories that perform analysis of these compounds in this type of complex samples.

Acknowledgements

The authors would like to thank the Spanish Ministry of Science and Innovation (project n. CTQ2011-23968), Generalitat Valenciana (Spain) (project n. ACOMP/2013/072) and LABAQUA S.A (Alicante, Spain) for the financial support.

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