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Optimization of pressurized liquid extraction and purification conditions for gas chromatography–mass spectrometry determination of UV filters in sludge

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ABSTRACT

This work presents an effective sample preparation method for the determination of eight UV filter compounds, belonging to different chemical classes, in freeze-dried sludge samples. Pressurized liquid extraction (PLE) and gas chromatography–mass spectrometry (GC–MS) were selected as extraction and determination techniques, respectively. Normal-phase, reversed-phase and anionic exchange materials were tested as clean-up sorbents to reduce the complexity of raw PLE extracts. Under final working conditions, graphitized carbon (0.5 g) was used as in-cell purification sorbent for the retention of co-extracted pigments. Thereafter, a solid-phase extraction cartridge, containing 0.5 g of primary secondary amine (PSA) bonded silica, was employed for off-line removal of other interferences, mainly fatty acids, overlapping the chromatographic peaks of some UV filters. Extractions were performed with a n-hexane:dichloromethane (80:20, v:v) solution at 75 °C, using a single extraction cycle of 5 min at 1500 psi. Flush volume and purge time were set at 100% and 2 min, respectively. Considering 0.5 g of sample and 1 mL as the final volume of the purified extract, the developed method provided recoveries between 73% and 112%, with limits of quantification (LOQs) from 17 to 61 ng g^{-1} and a linear response range up to 10 μg g^{-1}. Total solvent consumption remained around 30 mL per sample. The analysis of non-spiked samples confirmed the sorption of significant amounts of several UV filters in sludge with average concentrations above 0.6 μg g^{-1} for 3-(4-methylbenzylidene) camphor (4-MBC), 2-ethylhexyl-p-methoxycinnamate (EHMC) and octocrylene (OC).

1. Introduction

Organic UV filters are compounds designed to absorb the ultraviolet wavelengths of solar radiation preventing photo-aging and other harmful effects in human health. The concentration of UV filters in sunscreen lotions may represent up to 10% of the product weight; moreover, they are included, at lower levels, in the formulation of many other personal care products [1,2]. The above uses contribute to the direct input of UV filters in bathing waters and their indirect release in the aquatic environment through domestic sewage water [3–7]. The activity of some UV filters as endocrine disrupters [8–10], added to their ubiquity in sewage and surface water, has awaken the concern about their potential medium-term environmental effects.

Gas and liquid chromatography–mass spectrometry techniques, combined with effective sample concentration approaches [5,11–13], have been applied to obtain an overview of UV filters occurrence in different water samples, including wastewater from sewage treatment plants (STPs). However, understanding the behaviour of UV filters in STPs requires not only measuring their concentrations in the water phase, but also determining the fraction which remains attached to sludge particles [7]. This latter information is necessary to distinguish between biodegradation and sorption processes, and to assess the risk of introducing the UV filters in the terrestrial environment through the application of sludge as fertilizer in agriculture.

From the analytical point of view, sludge is an extremely complex matrix which requires well-tuned sample preparation approaches providing a balance among efficiency, selectivity, extraction time and cost. These constraints explain the limited number of studies dealing with the analysis of UV filters in sludge versus the plethora of publications focussed on water samples. The first method for sludge was proposed by Plagellat et al. [14]. It involved three consecutive liquid–liquid extractions of fresh sludge samples (60 g), followed by dryness evaporation of the combined extract and column purification with activated silica. Solvent consumption stayed above 200 mL per sample.

Pressurized liquid extraction (PLE) is a popular sample preparation technique for solid matrices showing limited solvent consumption, excellent extraction yields and possibility to inte-

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Table 1
Abbreviations, retention times, selected ions and instrumental limits of quantification (LOQs) of the GC–MS system of target analytes.

Compound	Abbreviation	Retention time (min)	Segment	Quantification ion (*m*/*z*)	Qualification ion (*m*/*z*)	LOQs (ng mL^{-1}) (S/N 10)
2-Ethylhexyl salicylate	EHS	9.65	1	120	138	2
Homosalate	HMS	10.26, 10.40	1	120	138	4
Isoamyl-p-methoxycinnamate	IAMC	11.63[a]	2	178	161	2
2-Hydroxy-4-methoxybenzophenone	BP-3	11.63	2	227	151	6
3-(4-Methylbenzylidene) camphor	4-MBC	11.87[a]	2	254	239	3
2-Ethylhexyl-p-dimethylaminobenzoate	EHPABA	13.41	2	277	165	1
2-Ethylhexyl-p-methoxycinnamate	EHMC	13.73[a]	2	178	161	1
Octocrylene	OC	16.15	3	360	249, 232	2

[a] Retention time values for the *E* isomers.

grate extraction and purification steps. The applications of PLE to the extraction of personal care compounds from sludge have been compiled in a recent review [15]. PLE, combined with in-cell clean-up using activated silica, has been reported as a straight forward alternative for gas chromatography–mass spectrometry (GC–MS) determination of UV filters in low carbon content sediment samples [16]; however, the above strategy provided too complex extracts in the case of sludge [17]. Although, the selectivity of the extraction could be improved by enclosing the sludge sample in a non-porous polyethylene membrane bag, within the cell, the efficiency of the extraction underwent a dramatic reduction, with recoveries around or below 50% for most UV filters [17]. In addition to the above procedures, Nieto et al. [18] have developed a PLE method for the extraction of several personal care products, including three UV filters (benzophenone-3, BP-3; octocrylene, OC; and 2-ethylhexyl-p-dimethylaminobenzoate, EHPABA), from sludge samples. Analytes were recovered with methanol followed by methanol:water mixtures and on-line purified with alumina Considering a sample intake of 1 g, and 25 mL as the volume of the final extract, recoveries over 79% and low signal suppression effects (below 15%) were observed in the further LC–(ESI)-MS/MS determination.

In this study, we optimize an alternative sample preparation method for the determination of eight UV filters, belonging to different chemical classes, in freeze-dried sludge samples. PLE was selected as extraction technique due to its high automation capabilities. Purification conditions were optimized in order (1) to reduce the content of interferences (coloured matter and fatty acids) in the final extract and (2) to maintain the consumption of organic solvents and the complexity of the method at acceptable levels. GC–MS was considered as determination technique on the basis of the poor detection limits reported for salicylate type UV filters using LC–(ESI)-MS systems [19]. Finally, the applicability of the method was demonstrated with sludge samples from urban STPs.

2. Experimental

2.1. Solvents, standards and sorbents

N-hexane, isooctane, acetone, dichloromethane and ethyl ether (trace analysis grade) and HPLC-grade methanol were supplied by Merck (Darmstadt, Germany). The list of UV filters included in this study is compiled in Table 1. Standards of target analytes were acquired from Aldrich (Milwaukee, WI, USA) and Merck, except isoamyl-p-methoxycinnamate (IAMC), which was kindly provided by Dr. R. Rodil (University of Santiago de Compostela, Spain). Individual solutions of each species (ca. 1000 μg mL^{-1}) were prepared in methanol. Further dilutions and mixtures of them were dissolved in acetone (when used to prepare the spiked sludge samples employed during optimization and validation of sample preparation conditions) and in isooctane (case of calibration standards).

Alumina, Florisil and silica solid-phase extraction (SPE) cartridges (0.5 g) were acquired from Waters (Milford, MA, USA). Cartridges containing 0.5 g of silica bonded to ethylenediamine-N-propyl groups (PSA sorbent) and 0.25 g of graphitized carbon were purchased from Supelco (Bellefonte, PA, USA). Both sorbents, in the bulk format, were also obtained from Supelco. Diatomaceous earth was provided by Aldrich.

2.2. Samples

Optimization of sample preparation (extraction and purification) conditions was performed with a freeze-dried pooled matrix of primary and biological sludge, fortified with 5 μg g^{-1} of each UV filter. The total carbon (TC) content of the pooled matrix was 33%. The spiking procedure consisted of the addition of a measured volume of a standard in acetone to an accurately weighed fraction of sludge. The resulting slurry was protected from light, homogenized periodically and kept in a hood until complete elimination of the acetone. The recoveries of the method were evaluated with individual samples of primary and biological sludge fortified at different concentrations. All spiked samples were aged for a minimum of 2 weeks before extraction. The optimized method was applied to grab samples of non-digested sludge (primary, secondary and mixtures of both) from several urban STPs located in the Northwest of Spain. Some samples were received as wet sludge (ca. 3–4% of dry matter) and freeze-dried after reception. Others were already lyophilized in the STPs.

2.3. Sample preparation

Extractions were performed with a pressurized liquid extractor, ASE 200 Dionex (Sunnyvale, CA, USA), furnished with 11 mL stainless-steel cells. A cellulose filter, followed by a glass fibre one, was placed on the bottom of each cell. Under final working conditions, cells were filled (bottom to top) with 1 g of diatomaceous earth, 0.5 g of graphitized carbon, 0.5 g of diatomaceous earth and 0.5 g of sludge, previously homogenized with 2 g of diatomaceous earth. Analytes were extracted with n-hexane:dichloromethane (80:20), at 75 °C, considering a single static extraction cycle of 5 min with the cell pressurized at 1500 psi. The flush volume was 100% and the purge time 2 min.

PLE extracts were evaporated, ca. 1 mL, and additionally purified with a PSA cartridge (0.5 g) previously conditioned with n-hexane:ether (1:1) and n-hexane (5 mL each). After loading the concentrated extract, the sorbent was rinsed with n-hexane (1 mL). Analytes were further recovered with 5 mL of n-hexane:ether (1:1). Thereafter, 1 mL of isooctane was added as a keeper to the purified extract, which was evaporated and adjusted to a final volume of 1 mL with the same solvent.

2.4. GC–MS analysis

UV filters were determined with a GC–MS system consisting of an Agilent (Wilmington, DE, USA) 7890A gas chromatograph connected to a quadrupole type mass spectrometer (Agilent MS 5975C), furnished with an electron-impact (EI) ionization source. Separations were carried out in a HP-5ms type capillary column (30 m × 0.25 mm i.d., d_f: 0.25 μm) supplied by Agilent. Helium (99.999%) was used as carrier gas at a constant flow of 1.2 mL min^{-1}. The GC oven was programmed as follows: 110 °C (held for 1 min), increased at 12 °C min^{-1} to 280 °C (held for 10 min). Ionization source, mass analyzer and transfer line temperatures were set at 230, 150 and 290 °C, respectively. Standards and sample extracts were injected in the splitless mode, maintaining the injection port at 280 °C. The splitless time and the split flow were set at 1 min and 20 mL min^{-1}, respectively. The mass spectrometer was operated in the SCAN mode (m/z range from 45 to 400) to assess the efficiency of the purification process, and in the SIM mode for quantification purposes. Retention times and ions monitored for each compound are summarized in Table 1. Analytes were grouped in three chromatographic segments. The dwell time per ion was 100 ms in the first and third segment and 50 ms for the second one.

2.5. Recoveries and procedural blanks

Levels of target analytes in the extracts from spiked and non-spiked sludge samples were established by comparison with calibration standards prepared in isooctane. Recoveries were calculated as the difference between concentrations obtained for spiked and non-spiked fractions of the same sludge sample divided by the added amount and multiplied by 100. Procedural blanks represent the whole sample preparation process (extraction plus purification) performed without sludge.

3. Results and discussion

3.1. Performance of GC–MS determination

The linear response range of the GC–MS instrument was investigated with standards at seven different concentrations between 5 and 5000 ng mL^{-1}. Within this interval, the system provided linear response plots (peak area versus concentration) with determination coefficients (R^2) higher than 0.996 for all compounds. The instrumental limits of quantification (LOQs), defined as the concentration of each compound producing a response 10 times higher than the baseline noise in the SIM acquisition mode, ranged from 1 to 6 ng mL^{-1}, Table 1.

3.2. Preliminary experiments

Previous applications of PLE to the extraction of UV filters from sludge employed rather different conditions as regards the extraction solvent and the temperature of the cell [17,18]. Likely, the selected in-cell clean up strategies, based on the use of permeable non-porous membranes [17] or a normal-phase sorbent [18], conditioned the optimum extraction parameters.

In this study, in order to prevent the influence of clean-up conditions on the yield of PLE, a first series of extractions was carried out considering just diatomaceous earth as inert dispersant of sludge (0.5 g of sludge plus 2 g of diatomaceous earth) and filling material in the extraction cell. Flush volume, pressure and extraction time were set at 100%, 1500 psi and 5 min, respectively. Samples were first extracted with n-hexane (50 °C, 1 cycle) followed by dichloromethane (60 °C, 3 cycles). Extracts were collected in separated vessels, adjusted to 25 mL, filtered (0.45 μm) and injected in the GC–MS system. N-hexane, at low temperature,

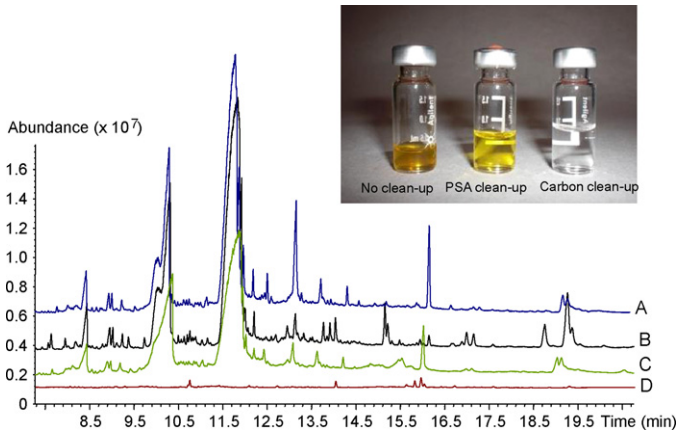

Fig. 1. GC–MS chromatograms (SCAN mode) and pictures corresponding to PLE extracts from sludge purified with different SPE cartridges: A, without clean-up; B, Florisil; C, graphitized carbon; D, PSA. Extraction conditions: n-hexane:dichloromethane (80:20), 70 °C, 2 cycles of 2 min,100% flush volume, 1500 psi.

has been proposed to remove interfering lipophilic matrix components previously to analytes extraction from complex samples [20,21]. On the other hand, Chu and Metcalfe [22] described the use of dichloromethane, under above instrumental conditions, for the PLE extraction of medium-polar personal care compounds from sludge.

Analysis of n-hexane and dichloromethane extracts revealed that BP-3 and OC were distributed between both fractions, whereas 95% of the responses measured for the rest of UV filters corresponded to the n-hexane fraction, data not shown. Despite the high dilution of sample extracts (25 mL), their GC–MS chromatograms showed a considerable complexity. This preliminary data indicate the suitability of n-hexane:dichloromethane mixtures for the extraction of UV filters from sludge at relatively low temperatures. However, there is no possibility to improve the selectivity of the process using n-hexane as pre-extraction solvent.

3.3. Clean-up conditions

Several SPE sorbents were tested in order to reduce the complexity of PLE extracts from sludge samples. In all cases, extractions were carried out at 70 °C using a n-hexane:dichlorometane (8:2) mixture with the cells pressurized at 1500 psi. Two static cycles of 2 min each and a flush volume of 100% were employed. Extracts were concentrated to 1 mL and loaded on top of the considered SPE cartridge, previously conditioned as described in Section 2. Thereafter, 1 mL of n-hexane was passed through the sorbent and discarded. Subsequently, analytes were eluted using 5 mL of a n-hexane:ether (1:1, v:v) mixture. The purified extract was mixed with 1 mL of isooctane and evaporated to a final volume of 1 mL. No differences were noticed between the turbidity and the colour of raw PLE extracts versus those purified with alumina and silica cartridges. Florisil and PSA cartridges rendered transparent, although yellowish, extracts and graphitized carbon transparent, colourless ones. The efficiency of the above clean-up sorbents was evaluated operating the GC–MS system in the SCAN mode. PSA was the only sorbent able to remove two broad chromatographic bands (tentatively identified as fatty acids with 16 and 18 carbons) overlapping the peaks of HMS, BP-3, IAMC and 4-MBC, and to reduce significantly the baseline level of the GC–MS chromatograms, Fig. 1. Likely, fatty acids and other interferences with anionic moieties remain strongly retained in the PSA cartridge, as it has been early described for the purification of extracts from vegetal samples [23]. Except graphitized carbon, the rest of sorbents failed to remove pig-

Table 2
Experimental domain of the Box–Behnken design.

Factor	Code	Level		
		Low	Medium	High
Time (min)	A	2	6	10
CH_2Cl_2 (%)	B	5	22.5	40
Temperature (°C)	C	40	65	90

ments contained in the raw extract. Although pigments exerted a little effect in the complexity of the GC–MS chromatograms (Fig. 1), they might impair the efficiency of the GC column due to irreversible contamination of the stationary phase. Thus, PSA and graphitized carbon were selected as clean-up sorbents.

In a second series of extractions, the feasibility of integrating extraction and clean-up steps, placing a layer of the above sorbents (from 0.5 to 2 g) inside the PLE cell, was investigated. Using the above described extraction parameters, graphitized carbon (0.5 g) allowed an efficient removal of pigments; however, the purification efficiency of PSA underwent a dramatic reduction. Probably, the ability of this sorbent to retain fatty acids interferences is reduced due to the temperature of the PLE cell (70 °C versus room temperature in the off-line modality), as well as the differences in the volume and the composition of the organic mixture flowing through the layer of PSA, packed inside the cell, versus those used in the SPE mode [24]. Thus, graphitized carbon (0.5 g) was introduced in the PLE cell for on-line removal of pigments; thereafter, the extract was submitted to an additional off-line clean-up with a SPE cartridge containing 0.5 g of PSA.

3.4. PLE parameters

3.4.1. Time, dichloromethane percentage and temperature

The influence of the above factors on the efficiency of the extraction step was simultaneously investigated using a Box–Behnken experimental factorial design with each variable considered at three levels, Table 2. The flush volume was 100%, the pressure 1500 psi and two extraction cycles were applied. The purified extracts were injected in the GC–MS system, operated in the SIM acquisition mode, and peak areas were used as the response variable in order to calculate the main effects associated with each experimental factor, their quadratic terms and the two-factor interactions. Table 3 summarizes the numerical values of the standardized main effects and their quadratic terms. The absolute value of a main effect is proportional to the influence of the associated factor on the efficiency of the PLE extraction. A positive sign indicates an improvement in the yield of the process when the factor varies from the low to the high level, within the domain of the design, and a negative one the opposite trend.

Table 3
Standardized main effects and quadratic terms provided by the Box–Behnken design.

Compound	Main effects			Quadratic terms		
	A	B	C	AA	BB	CC
EHS	−0.42	−2.7[a]	0.03	−2.0	−3.7[a]	−0.94
HMS	−2.7[a]	−3.2[a]	−0.52	−2.8[a]	−3.6[a]	−1.0
IAMC	−1.0	−1.5	0.82	−1.4	−3.3[a]	−0.84
BP-3	−0.37	7.7[a]	6.3[a]	−0.96	−7.6[a]	−1.1
4-MBC	−2.8[a]	−3.5[a]	−1.7	−3.6[a]	−4.3[a]	−0.98
EHPABA	−1.7	−4.1[a]	−0.60	−3.3[a]	−2.5	−0.78
EHMC	−2.2	−1.7	0.96	−3.0[a]	−3.8[a]	−1.5
OC	−4.0[a]	9.4[a]	−0.75	−2.5	−14.4[a]	0.56

[a] Statistical significant factors and quadratic terms.

Data summarized in Table 3 show that the percentage of dichloromethane (code B) played a positive and statistical significant effect (95% confidence level) in the extraction of BP-3 and OC, whereas the opposite trend was observed for the rest of analytes. The temperature of the cell (code C) affected positively and significantly to the extraction of BP-3 and the extraction time (code A) showed a negative influence on the yield of the extraction, being statistically significant for three (HMS, 4-MBC and OC) of the investigated species. Quadratic terms associated with the extraction time (AA) and the percentage of dichloromethane (BB) also presented statistically significant effects for many compounds (Table 3). These data suggest a non-linear variation in the efficiency of the extraction within the domain of the design. The main effect plots for selected compounds confirmed that maximum yields were achieved at intermediate extraction times and dichloromethane percentages, Fig. 2. Finally, two-factor interactions remained below the statistical significance threshold, data not shown.

The best compromise conditions, which maximized the efficiency of the extraction for all analytes, were calculated with a global desirability (D) function. D is defined as the geometric mean of the normalized (between 0 for the minimum and 1 for the maximum) individual responses (d_i) predicted by the Box–Behnken design for each UV-filter. The maximum value of D (0.89) was obtained at 75 °C, using a n-hexane:dichloromethane

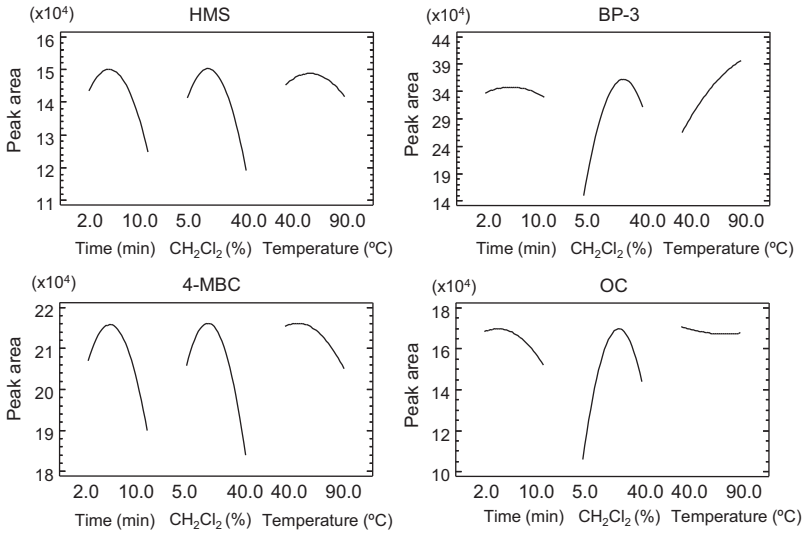

Fig. 2. Main effect graphs provided by the experimental factorial design for selected compounds.

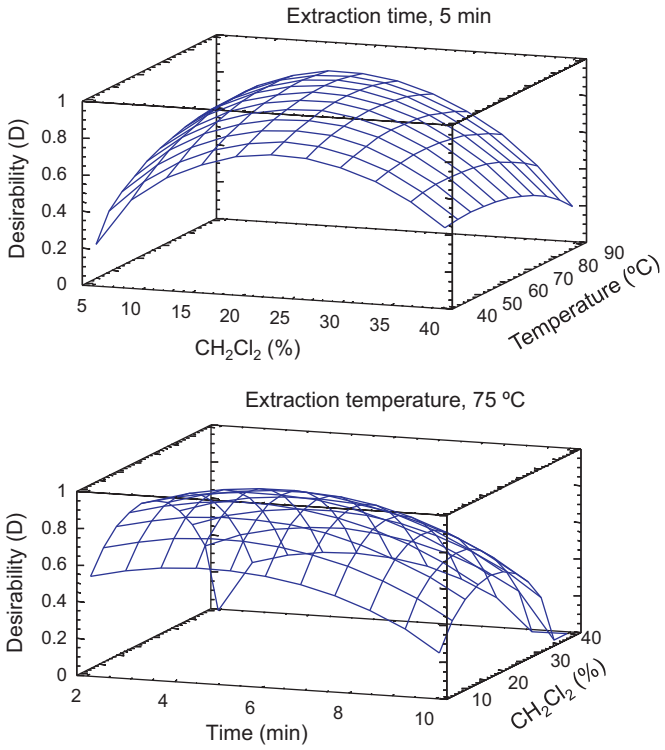

Fig. 3. Plots of the global desirability function.

(80:20, v:v) mixture and considering an extraction time of 5 min, Fig. 3.

3.4.2. Extraction cycles, flush volume and purge time

The potential influence of these parameters on the efficiency of the extraction was evaluated with an univariant approach. No differences were observed using 1, 2 or 3 extraction cycles of 5 min. Thus, a single cycle was considered to speed up the extraction step. The investigated values for the flush volume were 60%, 100% and 140%. Similar responses (peak areas) were measured for flush values of 100% and 140%, whereas a slight reduction was appreciated for several analytes using a percentage of 60%, data not shown. This factor was set at 100%. Operating under above conditions (1 cycle and 100% flush), the volume of the extract collected from the PLE cell remained around 20 mL. Purge times higher than 2 min were also studied without significant changes in the extraction efficiency; thus, 2 min was maintained as working value for this variable.

3.5. Recoveries, quantification limits and linear response range

The recoveries of the method were evaluated using two freeze-dried samples of primary and biological sludge spiked at two different concentration levels (300 and 1000 ng g^{-1}). Non-spiked fractions of each matrix and procedural blanks were also processed, Fig. 4. Found recoveries ranged from 73% to 112%, with relative standard deviation values below 12%, Table 4. The above data are similar to those reported by Plagellat et al. [14] for 4-MBC, EHMC and OC using liquid–liquid extraction of wet sludge samples and Nieto et al. [18] for BP-3, EHPABA and OC considering methanol:water mixtures for PLE of several personal care compounds from freeze-dried sludge.

The reproducibility of the method was investigated with a sample of biological sludge fortified at 500 ng mL^{-1}. The relative standard deviations (RSDs, %) for nine extractions in three consecutive days varied between 6 and 13%.

As shown in Fig. 4, analytes were not detected in the procedural blanks; therefore, the LOQs of the method (defined for a S/N of 10) were estimated from chromatographic peaks of UV filters in non-spiked samples, or in the low level spiked fraction for those species not detected in sludge (IAMC and EHPABA). The achieved LOQs varied between 17 ng g^{-1} for EHS and 61 ng g^{-1} for BP-3, Table 4. They are similar to the LOQs (from 7 to 67 ng g^{-1}) reported for same compounds in sediment samples with TC below 0.2%, using GC–MS as detection technique and a less elaborated clean-up procedure [16]. Plagellat et al. [14] achieved LOQs between 9 and 18 ng g^{-1} for 4-MBC, EHMC and OC considering a three times larger sample intake (60 g of fresh sludge at 3%) and using also GC–MS as determination technique. The linear response range of the developed method extended from LOQs reported in Table 4 up to 10,000 ng g^{-1}.

3.6. Application to real samples

The proposed method was applied to freeze-dried sludge from different urban sewage plants. EHPABA and IAMC were not detected in any of the processed samples. The concentrations measured for the rest of species are compiled in Table 5. 4-MBC and EHMC appear in sludge as mixtures of *E* and *Z* forms. The sum of peak areas for both isomers was compared with calibration curves obtained for the commercial available *E* forms. Samples code 1 and 2, in Table 5, corresponded to wet sludge from a plant receiving the wastewater from a 100,000 inhabitants city, located in the Northwest of Spain. Both samples were obtained in March of 2010 and lyophilized in our laboratory. The rest of specimens (codes 3–9) were from STPs in the same geographical area, although their exact locations are not revealed due to a confidentiality agreement. They were collected between February and May 2010, in situ lyophilized and further submitted to the laboratory for analysis. Samples code 5–9 (Table 5) are mixtures of primary and biological sludge.

4-MBC, EHMC and OC were ubiquitous pollutants in sludge, with average concentrations increasing the following order: 4-

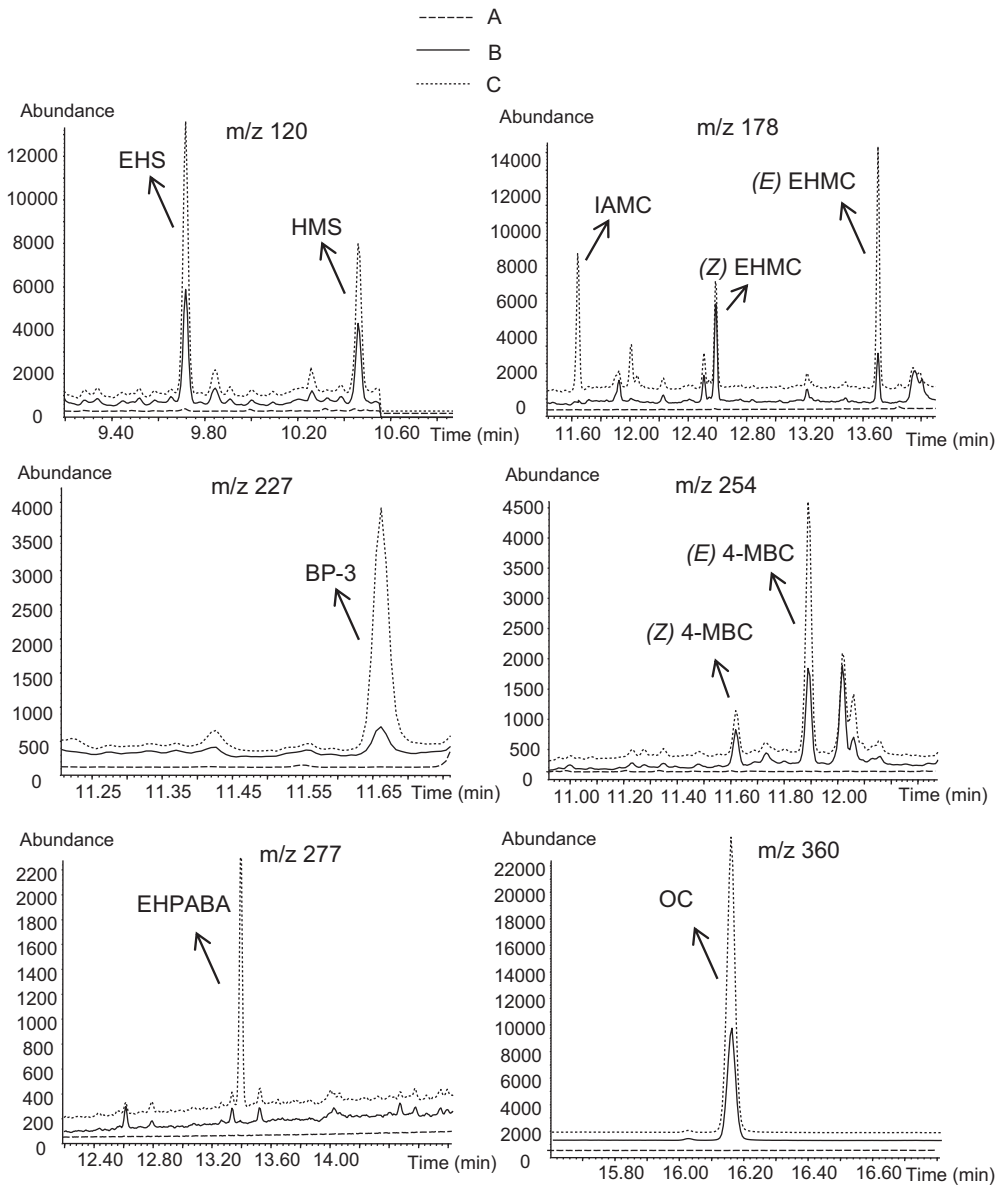

Fig. 4. Selected ion monitoring chromatograms corresponding to a procedural blank (A), a non-spiked sample of biological sludge (B), and same sample fortified with 300 ng g^{-1} of each analyte (C).

MBC < EHMC < OC, Table 5. The mean levels of 4-MBC and OC were lower than those reported for sewage sludge samples collected in Switzerland; however, a higher value was obtained for EHMC [14]. EHS and HMS were quantified in six of nine samples with maximum values below 400 ng g^{-1}, and BP-3 showed a lower detection frequency, Table 5. Globally, the occurrence frequency and the relative concentrations of UV filters in sludge followed the same pattern as in water samples taken in the same geographic area [13]. Moreover, they are in agreement with the high sorption coefficients reported for 4-MBC, EHMC and OC in sludge [6].

Table 4
Recoveries of the method for spiked samples (n = 3 replicates) and estimated limits of quantification (LOQs) of the method.

Analyte	Primary sludge (TC 30%)		Biological sludge TC (35%)		LOQs (ng g^{-1})
	[a]300 ng g^{-1}	[a]1000 ng g^{-1}	[a]300 ng g^{-1}	[a]1000 ng g^{-1}	
EHS	101 ± 7	95 ± 7	102 ± 8	103 ± 3	17
HMS	96 ± 6	78 ± 5	103 ± 1	100 ± 7	34
IAMC	107 ± 6	80 ± 4	90 ± 4	98 ± 6	34
BP-3	89 ± 11	106 ± 6	112 ± 4	100 ± 4	61
4-MBC	86 ± 5	79 ± 4	91 ± 7	107 ± 3	26
EHPABA	93 ± 7	88 ± 6	83 ± 7	104 ± 3	22
EHMC	90 ± 5	73 ± 5	90 ± 9	88 ± 7	24
OC	85 ± 5	84 ± 12	112 ± 5	98 ± 8	33

[a] Added concentration.

Table 5
Summary of concentrations ($ng\,g^{-1}$) measured in sludge samples, $n = 3$ replicates. IAMC and EHPABA were not detected in any sample.

Code	Type	Concentration ($ng\,g^{-1}$) ± SD					
		EHS	HMS	BP-3	4-MBC	EHMC	OC
1	Primary	n.d.	n.d.	n.d.	1543 ± 26	3287 ± 98	2242 ± 16
2	Biological	270 ± 14	207 ± 31	n.d.	1439 ± 49	856 ± 98	3263 ± 176
3	Primary	n.d.	n.d.	n.d.	106 ± 5	213 ± 3	1039 ± 50
4	Biological	133 ± 26	110 ± 10	93 ± 11	97 ± 8	104 ± 5	377 ± 30
5	Mixture	298 ± 5	401 ± 35	n.d.	223 ± 9	160 ± 7	1766 ± 72
6	Mixture	n.d.	n.d.	n.d.	120 ± 3	192 ± 15	1038 ± 63
7	Mixture	200 ± 36	240 ± 8	n.d.	372 ± 10	125 ± 5	1934 ± 222
8	Mixture	188 ± 9	256 ± 18	n.d.	351 ± 41	100 ± 10	523 ± 58
9	Mixture	268 ± 11	180 ± 27	295 ± 14	1579 ± 51	2776 ± 137	2240 ± 45
Mean[a]		226	232	194	648	868	1602

[a] Average value of quantified concentrations.

4. Conclusions

PLE extraction, combined with the use of graphitized carbon for in-cell retention of pigments and additional clean-up with a PSA cartridge, constitutes a suitable approach in terms of extraction efficiency and selectivity for the GC–MS determination of a broad group of UV filters in sludge samples. As far as we could trace, this study reports the first application of both materials for the clean-up of PLE extracts from sludge samples, achieving an improved selectivity in comparison with the commonly used normal-phase sorbents. The analysis of sludge samples confirmed the significant accumulation of three UV filters (4-MBC, EHMC and OC) in this matrix, with average concentrations higher than $600\,ng\,g^{-1}$. This information must be considered in order to (1) properly calculate their removal rates during wastewater treatments and (2) to evaluate the risk of re-introducing the above species in the terrestrial environment through the disposal of sludge as fertilizer in agriculture fields.

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