



Occurrence of multiclass UV filters in treated sewage sludge from wastewater treatment plants

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ABSTRACT

Many substances related to human activities end up in wastewater and accumulate in sewage sludge. So far, there is only one extensive survey on the occurrence of UV filter residues in sewage sludge. However, more data are required to draw a reliable picture of the fate and effects of these compounds in the environment. This study attempts to fill this gap through the determination of selected UV filters and derivatives namely 4-methylbenzylidenecamphor, benzophenone-3, octocrylene, ethylhexylmethoxycinnamate, ethylhexyldimethyl PABA, 4-hydroxybenzophenone, 2,4-dihydroxybenzophenone, and 4,4'-dihydroxybenzophenone in treated sewage sludge.

The target compounds were extracted using pressurized liquid extraction and after this, determined by ultra high resolution liquid chromatography–tandem mass spectrometry. The determination was fast and sensitive, affording limits of detection lower than 19 ng g⁻¹ dry weight (dw) except for 2,4-dihydroxybenzophenone (60 ng g⁻¹ dw). Good recovery rates, especially given the high complexity of sludge matrix (between 70% and 102% except for 2,4-dihydroxybenzophenone (30%)) were achieved.

The application of developed method allowed reporting for the first time the occurrence of two major degradation products of benzophenone-3 that have estrogenic activity in sewage sludge: 4,4'-dihydroxybenzophenone (in 5/15 WWTPs) and 4-hydroxybenzophenone (in 1/15 WWTPs). Results revealed the presence of UV filters in 15 wastewater treatment plants in Catalonia (Spain) at concentrations ranging from 0.04 to 9.17 µg g⁻¹ dw.

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1. Introduction

Organic UV filters are used as additives in formulations to absorb solar radiation, and therefore, to protect against their harmful effects. These compounds are extensively used in personal care products and are also present in a wide variety of industrial goods such as paints, plastics or textiles to prevent degradation of polymers and pigments (Lowe et al., 1997). According to an exhaustive report on personal care products, UV filters experienced the highest sales growth, 13%, in Europe from 2002 to 2003 reaching around 1000 millions US \$ mill (ACNielsen Global Services, 2004).

In this work analytes were selected according two criteria, the extent of their use and the toxicological effects they may cause. The five selected UV filters have presented estrogenic activity, some of them also anti-androgenic activity and interactions with the thyroid axis (Matsumoto et al., 2005; Schlumpf et al., 2008a,b). 4-Methylbenzylidene camphor (4MBC) was found to

interfere with development of reproductive organs, brain and behaviour in rats (Schlumpf et al., 2008a,b; Faass et al., 2009). Moreover, 4MBC is known to cause adverse effects on reproduction for some invertebrates at concentrations of 1.7 mg kg⁻¹ sediment (Schmitt et al., 2008). For ethylhexyl-methoxy cinnamate (EHMC), long-term effects on reproduction in adult ovariectomized rats (Seidlová-Wuttke et al., 2006) and on expression of genes involved in hormonal pathways in fathead minnows (*Pimephales promelas*) (Christen et al., 2011) have been described. Besides, these compounds tend to bioaccumulate even being detected in human milk (Schlumpf et al., 2008b, 2010). Concerning the benzophenone-3 (BP3) derivatives, 2,4-dihydroxybenzophenone (BP1) and 4-hydroxybenzophenone (4HB), both showed the highest estrogenicity (below 1 mg L⁻¹ exposure) in a recombinant yeast assay with rainbow trout ER α (Kunz et al., 2006). Additional data on environmental concentrations and toxicity have been reviewed by Fent et al. (2008) and Brausch and Rand (2011).

UV filters enter the aquatic environment directly as a result of water recreational activities when they are washed off from the skin and/or clothes, or indirectly through wastewater resulting

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Table 1
Characteristics of the selected wastewater treatment plant (WWTP).

WWTP	Type of treatment	HRT (d)	SRT (d)	Equivalents inhabitants	Influent flow rate (m ³ d ⁻¹)	Sludge treatment	Disposal	Dry matter (t y ⁻¹)	% Organic matter
Girona	Biological with P and N removal + Tertiary	12.5	–	147 000	37 000	Anaerobic digestion/centrifuge	Soil application (97%) Composting (2%) Land reclamation (1%)	3162	63
St. Feliu de Llobretat	Biological + Tertiary	0.36	12	198 000	46 000	Anaerobic digestion/centrifuge	Thermal drying (51%) Soil application (43%) Landfill (6%)	3789	48
Rubí	Biological with N removal	0.3	4	142 000	22 000	Anaerobic digestion	Thermal drying (84%) Landfill (16%)	1845	59
Granollers	Biological with P and N removal	4.45	–	182 000	22 000	Anaerobic digestion/centrifuge	Thermal drying (89%) Soil application (11%)	2325	64
Reus	Biological	18.96	–	124 000	17 000	Anaerobic digestion/filter press	Soil application (79%) Composting (21%)	1954	55
Montcada Llagosta	Biological Biological with P removal	0.3 0.5	– –	229 000 205 000	52 000 30 000	– Anaerobic digestion/centrifuge	Dehydration (100%) Thermal drying (93%) Landfill (7%)	6155 2142	– 53
Lleida Vilafranca	Biological Biological with P and N removal	0.16 7.5	4–6 9	177 000 87 000	58 000 12 000	Anaerobic digestion/centrifuge Anaerobic digestion/centrifuge	Soil application (100%) Soil application (99%) Composting (1%)	2045 992	54 61
Mataró	Biological	0.23	–	154 000	29 000	Anaerobic digestion/centrifuge	Thermal drying (63%) Soil application (37%)	2797	63
Prat de Llobregat	Biological with P and N removal + Tertiary	0.5	–	1 142 000	267 000	Anaerobic digestion/centrifuge	Thermal drying (76%) Soil application (24%)	14 955	51
Terrassa	Biological	29.9	17	165 000	42 000	Anaerobic digestion/centrifuge	Soil application (98%) Composting (2%)	3787	52
Gavà	Biological	0.44	10	272 000	41 000	Anaerobic digestion/centrifuge	Soil application (100%)	2750	62
Teià	Biological	0.6	–	84 000	15 000	Centrifuge	Composting (99%)	1512	77
Vic	Biological with P and N removal	1.27	16	399 000	21 000	Anaerobic digestion/filter press	Thermal drying (1%) Thermal drying (99%) Landfill (1%)	2865	53

HRT: hydraulic retention time; SRT: solid retention time; Influent flow rate: average inflow over the year. (–): data not available.

Table 2

Recovery rates (RR) and relative standard deviation (RSD) of UV filters as a function of the extraction solvent and temperature (T).

Solvent	RR \pm RSD (%)									
	T (°C)	4MBC	OCT	EHMC	OD-PABA	BP3	BP1	4HB	4DHB	
3 cycles MeOH	50	45 \pm 12	60 \pm 10	35 \pm 21	40 \pm 6	30 \pm 21	6 \pm 41	47 \pm 21	44 \pm 10	
	75	75 \pm 7	71 \pm 21	75 \pm 8	61 \pm 14	41 \pm 18	3 \pm 66	49 \pm 22	50 \pm 12	
	100	80 \pm 8	66 \pm 5	78 \pm 6	69 \pm 12	55 \pm 9	20 \pm 22	77 \pm 32	79 \pm 21	
3 cycles MeOH/H ₂ O (1:1)	50	12 \pm 11	14 \pm 5	3 \pm 4	11 \pm 5	39 \pm 22	15 \pm 7	38 \pm 12	53 \pm 4	
	75	41 \pm 5	13 \pm 7	15 \pm 43	22 \pm 6	40 \pm 6	32 \pm 3	40 \pm 14	60 \pm 5	
	100	33 \pm 25	35 \pm 7	31 \pm 5	35 \pm 10	41 \pm 4	22 \pm 14	46 \pm 12	66 \pm 8	
2 cycles MeOH + 2 cycles MeOH/H ₂ O (1:1)	50	55 \pm 5	65 \pm 12	52 \pm 9	65 \pm 12	49 \pm 10	20 \pm 8	66 \pm 5	75 \pm 12	
	75	82 \pm 8	60 \pm 10	81 \pm 12	80 \pm 5	72 \pm 8	24 \pm 23	96 \pm 17	110 \pm 4	
	100	102 \pm 6	70 \pm 7	90 \pm 6	85 \pm 4	70 \pm 6	30 \pm 16	95 \pm 8	96 \pm 9	
3 cycles DCM/hexane (1:1)	50	50 \pm 7	43 \pm 12	23 \pm 5	33 \pm 6	33 \pm 5	–	15 \pm 15	5 \pm 8	
	75	59 \pm 11	55 \pm 7	65 \pm 5	55 \pm 11	41 \pm 4	–	12 \pm 4	19 \pm 13	
	100	51 \pm 4	55 \pm 6	61 \pm 12	49 \pm 5	32 \pm 5	–	18 \pm 6	12 \pm 4	

4-Methylbenzylidene camphor (4MBC); Octocrylene (OCT); Ethylhexyl methoxycinnamate (EHMC); Ethylhexyl dimethyl PABA (OD-PABA); Benzophenone-3 (BP3); 2,4-Dihydroxybenzophenone (BP1); 4-Hydroxybenzophenone (4HB); 4,4'-Dihydroxybenzophenone (4DHB); methanol (MeOH); dichloromethane (DCM), (–): not detected.

from the use of personal care products, washing clothes and industrial discharges, among others. Residues of more polar organic UV filters have been found in all kind of water matrices (Balmer et al., 2005; Rodil et al., 2008; Fent et al., 2010; Tarazona et al., 2010). Due to the significant efforts carried out in recent years in terms of improving water quality, the new treatment plants (built to meet the requirements of European Council Directives 91/271/EEC and 98/15/EEC by 2005), have led to a substantial increase in sewage sludge loads. Data collected in the 1999 Report on the Implementation of Legislation on Waste, estimated a value of 7.2 Mt of dry matter production (dry weight (dw)) in European Union countries. The use of sludge in agriculture is the most important outlet in Europe, accounting for about 50% of sludge production (Schowanek et al., 2004).

The European Union is currently working on new legislation on the quality of biosolids (Working Document on Sludge, 3rd Draft, 2000). In most cases, direct risk would currently be considered to have adverse effects to consumers of crops (humans and animals) by virtue of uptake by crops or contamination of crops. However, also an important risk at heavily amended sites is that of groundwater pollution. The 3rd Draft proposes limit values for concentrations of heavy metals and also for organic compounds, such as PAHs and PCBs. This fact should restrict the use of sewage sludge in agriculture if the limits are exceeded. In Spain, the quality criteria of the sludge generated in wastewater treatment plants (WWTPs) for possible use in agriculture focused on its metal content (Spanish Directive 1310/1990, 10–29th-1990 on the use of sewage sludge in agriculture).

Due to the high lipophilicity and poor biodegradability of most UV filters they are expected to end up in sewage sludge during wastewater treatment as reported for other personal care products (Shek et al., 2008; Chen et al., 2009). Despite that, current knowledge on concentrations of UV filters in sewage sludge is scarce. To the author's knowledge there are only three studies on the analysis of UV filters in sewage sludge (Plagellat et al., 2006; Nieto et al., 2009; Rodil et al., 2009).

Thus, the aims of the present study were: (i) to develop and validate a selective and sensitive multi-residual analytical methodology based on pressurized liquid extraction (PLE) and ultra high resolution liquid chromatography–electrospray-tandem mass spectrometry (UPLC–ESI–MS/MS) for the determination of UV filters and derivatives having quite different polarities (log Kow from 2.55 to 7.53) in sewage sludge, and (ii) to perform a survey of multiclass UV filters and some of the major degradation products of BP3 in the 15 main WWTPs of Catalonia (Spain).

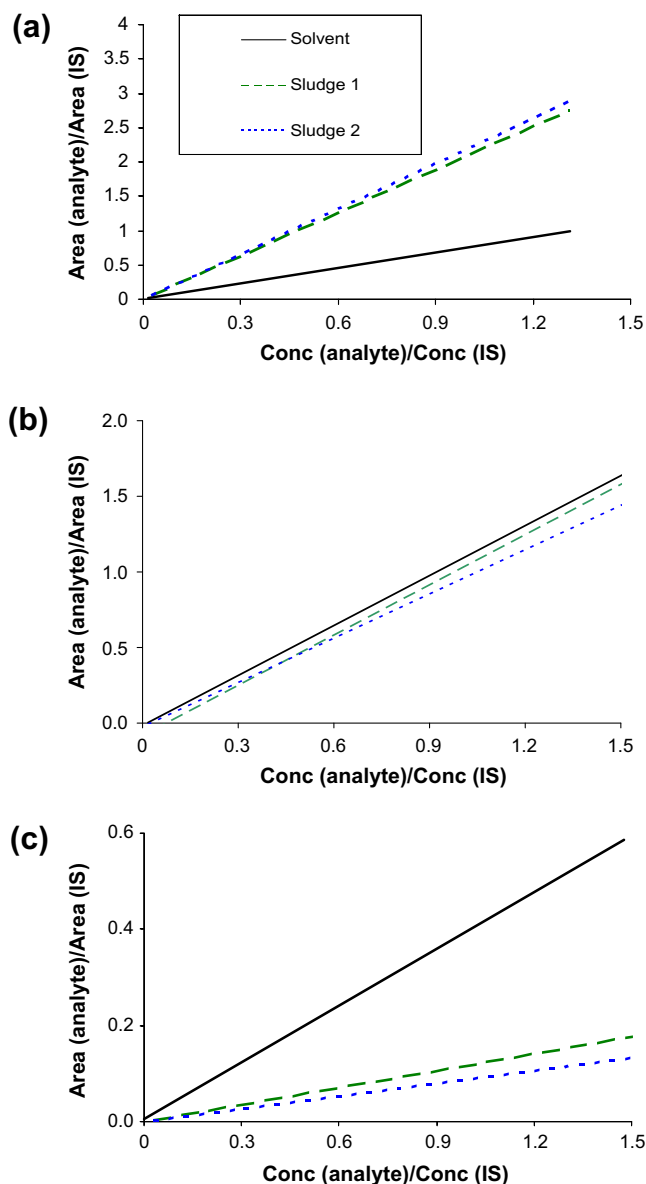


Fig. 1. Comparison of calibration curves obtained in acetonitrile and in PLE sewage sludge extracts for: (a) Benzophenone-3 (BP3), (b) 4,4'-Dihydroxybenzophenone (4DHB), and (c) 4-Methylbenzylidene camphor (4MBC). Conc: Concentration; IS: Internal standard.

2. Materials and methods

2.1. Chemicals and materials

BP3, octocrylene (OCT), OD-PABA, BP1, 4HB, 4,4'-dihydroxybenzophenone (4DHB) and the isotopically labelled compound benzo-phenone- d_{10} were used as internal standard (IS). They were of high purity and obtained from Sigma–Aldrich (Steinheim, Germany); 4MBC was supplied by Dr. Ehrenstorfer (Augsburg, Germany); and EHMC by Merck (Darmstadt, Germany). Methanol, acetone, dichloromethane (DCM), acetonitrile and HPLC grade water (Lichrosolv), as well as formic acid (98% purity), aluminium oxide and hydromatrix were provided by Merck. N_2 and Ar (purchased from Air Liquide (Barcelona, Spain)) were of 99.995% purity. The syringe and the PLE cellulose filters used were obtained from Whatman (London, UK) and from Dionex Corporation (Sunnyvale, CA, USA), respectively. Isolute C18 Cartridges used for solid phase extraction (SPE) were obtained from Biotage (Uppsala, Sweden).

Individual UV stock standard solutions and an isotopically labelled internal stock standard solution were prepared on a weight basis in methanol at 200 mg L^{-1} . The solutions were stored in the dark at -20°C . A mixture standard solution at 20 mg L^{-1} in methanol of each compound was prepared weekly. Working solutions were prepared daily by appropriate dilution of the mixture stock standard solution in methanol.

2.2. Sample collection and pre-treatment

Treated sewage sludge samples were collected in June 2009 from 15 full-scale WWTPs in Catalonia (Spain). Table 1 summarizes the main characteristics of the selected facilities. These plants are the biggest ones in Catalonia in terms of equivalent inhabitants (Eq-Inh), and in terms of dry matter tonnes generated (amount of stabilized sewage sludge produced).

Dehydrated sludge samples, about 500 g dw, were collected in clean brown glass containers and shipped in refrigerated coolers to the laboratory. Then, they were freeze-dried in a LyoAlfa 6–50 (Telstar S.A., Barcelona, Spain) and stored in the dark at -20°C until analysis.

2.3. Contamination and photo-degradation considerations

Background contamination is a common problem in the determination of UV filters at environmental levels. Therefore, several measures were taken in order to prevent this problem. All glassware used was previously washed and heated overnight at 380°C , and after this sequentially rinsed with different organic solvents and HPLC grade water. Then it was immediately used. Furthermore, gloves were worn during the sample preparation; separate solvents and only previously unopened packages of solvents, chemicals and other supplies, and glassware were used. In

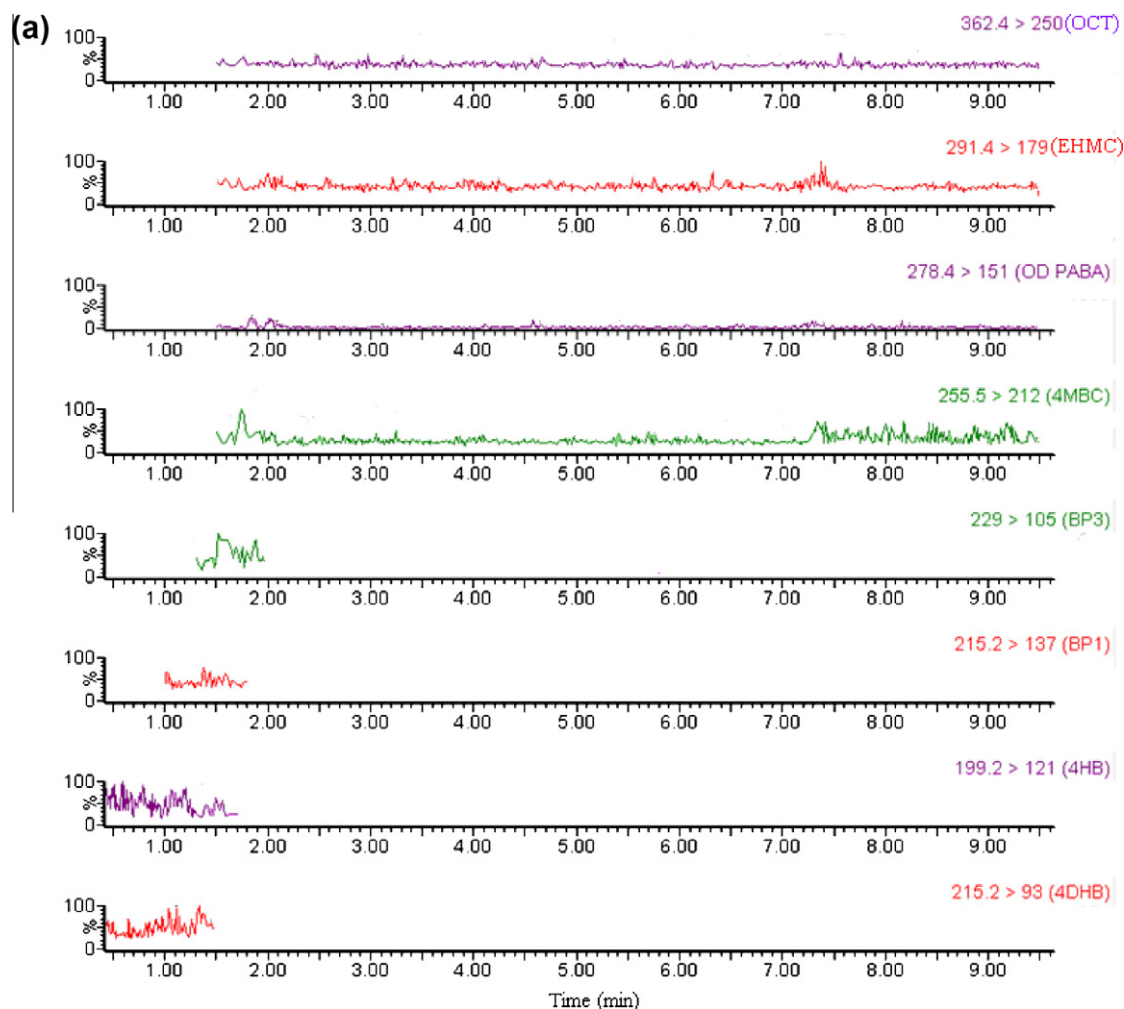


Fig. 2. Selected reaction monitoring (SRM) chromatograms obtained by UPLC–ESI(+)-MS/MS of (a) an operational blank sample and (b) the treated sewage sludge sample corresponding to the wastewater treatment plant of Granollers. 2,4-Dihydroxybenzophenone (BP1); 4,4'-Dihydroxybenzophenone (4DHB); 4-Hydroxybenzophenone (4HB); Benzophenone-3 (BP3); 4-Methylbenzylidene camphor (4MBC); Octocrylene (OCT); Ethylhexyl dimethyl PABA (OD-PABA); Ethylhexyl methoxycinnamate (EHMC).

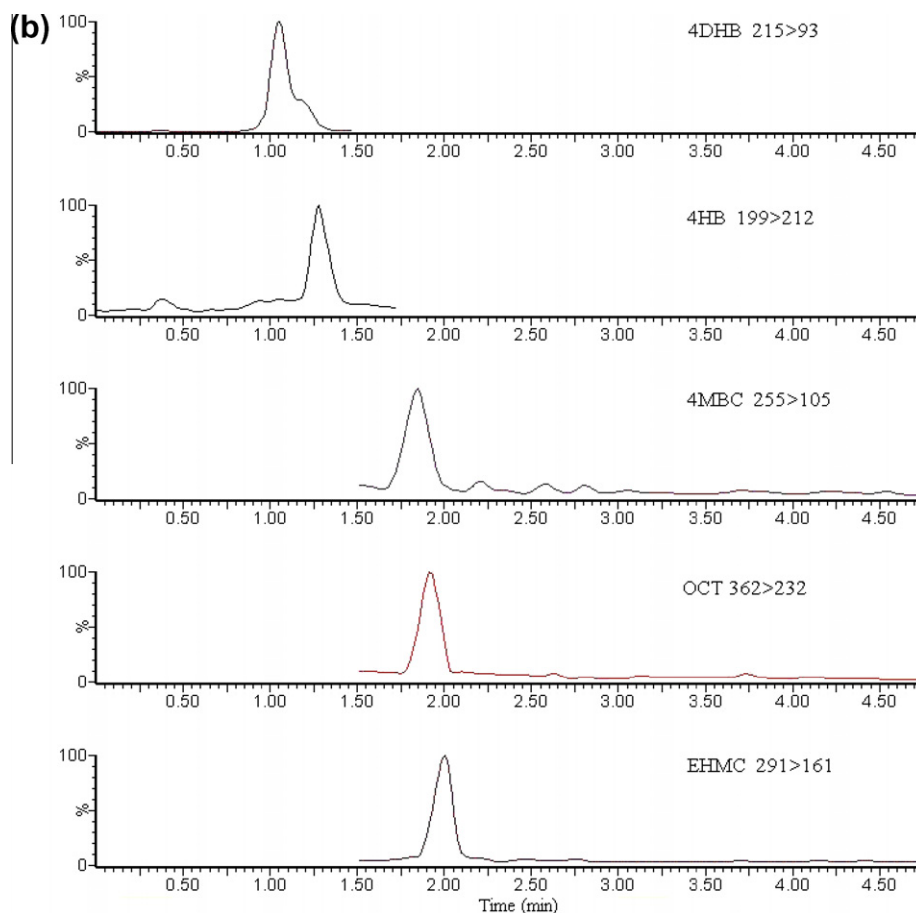


Fig. 2 (continued)

addition, a set of at least two operational blanks were processed together with each batch of samples. Since many of the compounds analyzed undergo photodegradation, stock standard solutions and samples were always covered with aluminium foil and stored in the dark.

2.4. Sample extraction and purification

Samples were extracted by PLE using an automatic extractor ASE 200 (Dionex Corporation, Sunnyvale, CA, USA). One gram of freeze-dried and grinded sludge samples were mixed in the extraction cells with aluminium oxide (alumina, previously activated by heating at 130 °C for 24 h) in order to perform the purification step within the cell (in-cell purification). The PLE optimised parameters were as follows: preheating of 5 min, two static cycles of 5 min using methanol as extraction solvent followed by other two static cycles of 5 min using methanol/water (1/1, v/v), temperature of 100 °C, pressure of 10 000 kPa, flush volume of 40% of cell and 60 s of nitrogen purge. The PLE extract obtained was brought to 25 mL with methanol. A 2 mL aliquot of this solution was passed through a syringe filter of 0.45 µm to a LC-vial. The filter was washed three times with 1.5 mL of methanol. All extracts were joint and evaporated to dryness. The residue was further reconstituted with 1 mL of acetonitrile and the internal standard was added before LC–MS analysis.

2.5. UPLC–ESI–MS/MS analysis

Analyses were performed on an Acquity UPLC chromatograph attached to a TQD mass spectrometer (Waters). A Hibar Puro-

spher® STAR® HR R-18 ec. (50 mm × 2.0 mm, 2 µm) column (Merck) was used. The solvent flow rate was set to 0.4 mL min⁻¹ and the column temperature was kept at 50 °C. The sample volume injected was 10 µL.

In order to optimize chromatographic separation several mobile phases and additives were tested. The use of formic acid at different concentration levels was evaluated, whilst methanol, acetonitrile and mixtures of both solvents were tested for the organic phase using methanol and acetonitrile as organic component and different concentrations of formic acid as modifier. The best results were obtained using as mobile phase HPLC grade water (A) and acetonitrile (B), both with 0.3% formic acid (which significantly improved the peak shape of BP3 and its derivatives). The adopted elution gradient started with 5% of eluent B, increasing to 95% in 1.20 min, kept constant for 2.30 min, and rising to 100% in the following 0.5 min. During the next 2.5 min the elution gradient was kept constant, and then back to initial conditions in 3 min.

MS/MS was operated in selected reaction monitoring (SRM) mode. ESI ionization was tested in both positive and negative modes. For all analytes a significant improvement of sensitivity was observed working in positive mode. Fragmentation voltage and collision energy were evaluated for each transition. The optimized parameters were obtained by flow injection analysis of single analyte standard solutions at 500 ng L⁻¹, and they were as follows: desolvation gas (nitrogen), 750 L h⁻¹; collision gas (Ar), 0.19 mL min⁻¹; nebulisation gas (nitrogen), 90 L h⁻¹; ion spray voltage 3.35 kV; source temperature, 130 °C and desolvation temperature, 450 °C. For each compound, two characteristic fragments of the protonated molecular ion [M+H]⁺ were recorded. The most

abundant transition was used for quantification, whereas the second most abundant was used for confirmation.

3. Results and discussion

3.1. Analytical aspects

In the optimization of the extraction and purification process, aliquots of 1 g of sample were placed in scintillation vials, covered completely with acetone and spiked with the appropriate volume of the UV filter mixture stock standard solution. The acetone was allowed to evaporate at room temperature until sludge was dry. In order to homogenize the suspension the mixtures were frequently shaken during the process. For recovery experiments samples were spiked at 2500 ng g⁻¹. A high level of spike was necessary, as sewage sludge pollutants are usually found in high concentrations. As shown in Table 2, recovery rates obtained were in the range 70–102% for all compounds except for BP1 (30%) (see Materials and Methods) under the optimized experimental conditions. The low recovery obtained for BP1 is consistent with the value reported by Nieto et al. (2009).

For extraction and purification another approach was evaluated. The method was based on PLE extraction, where the samples were mixed in the extraction cells with hidromatrix. Next, the extracts were purified using Isolute C18 SPE cartridges. In the evaluation,

a number of extraction pure and binary mixture solvents and several temperatures were tested. The best results were obtained at 75 °C using a mixture of DCM/hexane (1/2 v/v) as extraction solvent. However, recoveries achieved were below 50%.

The performance of LC–ESI–MS/MS methods is strongly affected by the ionisable impurities coming from the matrix that can interfere with the ionization processes. In the present study matrix effects were evaluated. Results evidenced three different behaviours as represented in Fig. 1; for 4MBC, OCT, OD-PABA and EHMC, signal suppression was observed, for BP3 and BP1, the opposite effect occurred, and for the rest of compounds no significant differences were found. Therefore, internal standard quantification with matrix-matched standards was finally used. With each set of samples two operational blanks were processed. Fig. 2a shows the chromatogram of a blank where it can be observed that no contamination occurs. The quality parameters of the whole method are compiled in Table 3.

3.2. Occurrence of UV filters in treated sewage sludge

As reported in Table 4, all sewage sludge samples analysed contained at least two out of the eight UV filters investigated. As an example, Fig. 2b shows the chromatogram of a sludge sample where five UV filters were found.

Table 3

Performance of the UPLC–ESI(+)-MS/MS developed method for the analysis of UV filters in sewage sludge.

	Instrumental		Method						
	Linearity range (ng L ⁻¹)	r ²	ILOD (pg)	ILOQ (pg)	Precision (RSD%) n = 5		RR ^a (RSD%, n = 5)	MLOD (ng g ⁻¹)	MLOQ (ng g ⁻¹)
					Intraday	Interday			
4MBC	20–900	0.998	13.11	43.70	6	10	102 (6)	12	40
OCT	20–900	0.997	2.53	8.44	4	9	70 (7)	18	60
EHMC	10–500	0.993	7.53	25.08	5	10	90 (6)	19	63
OD-PABA	1–300	0.994	0.07	0.22	7	7	85 (4)	0.2	0.7
BP3	1–300	0.994	1.66	5.53	5	9	70 (10)	1.0	3.3
BP1	5–300	0.994	2.01	6.70	9	14	30 (16)	60	200
4HB	5–300	0.992	3.09	10.29	4	11	95 (8)	5.0	17
4DHB	1–300	0.996	3.35	11.17	3	6	96 (9)	5.0	17

^a Samples spiked at 2500 ng g⁻¹ (dw). ILOD: instrumental limit of detection; ILOQ: instrumental limit of quantification; RSD: relative standard deviation; RR: recovery rates; MLOD: method limit of detection; MLOQ: method limit of quantification; 4-Methylbenzylidene camphor (4MBC); Octocrylene (OCT); Ethylhexyl methoxycinnamate (EHMC); Ethylhexyl dimethyl PABA (OD-PABA); Benzophenone-3 (BP3); 2,4-Dihydroxybenzophenone (BP1); 4-Hydroxybenzophenone (4HB); 4,4'-Dihydroxybenzophenone (4DHB).

Table 4

Concentration of the target UV filters in sewage sludge (μg g⁻¹ dw). In parentheses: daily mass loads (mg (day × 1000 Eq-Inh)⁻¹).

WWTP	BP1	4DHB	4HB	BP3	4MBC	OCT	OD-PABA	EHMC
Girona	n.d	<LOQ	n.d	n.d	1.63 (96.4)	2.60 (153.8)	n.d	n.d
St Feliu de Llobregat	n.d	n.d	n.d	n.d	1.61 (84.2)	2.87 (150.6)	n.d	0.75 (39.1)
Rubí	n.d	0.07 (2.3)	n.d	n.d	3.83 (136.4)	9.17 (326.9)	n.d	1.22 (43.6)
Granollers	n.d	0.04 (1.2)	0.15 (5.1)	n.d	1.52 (53.4)	2.61 (91.6)	n.d	0.78 (27.3)
Reus	n.d	n.d	n.d	n.d	2.98 (128.1)	5.39 (231.8)	n.d	1.91 (82.5)
Montcada	n.d	n.d	n.d	n.d	3.17 (232.9)	4.15 (304.8)	n.d	1.09 (80.1)
Llagosta	n.d	0.62 (17.8)	n.d	n.d	1.79 (51.4)	4.49 (128.78)	n.d	n.d
Lleida	n.d	n.d	n.d	n.d	0.73 (23.2)	2.86 (90.6)	n.d	n.d
Vilafranca	n.d	n.d	n.d	n.d	1.53 (47.6)	2.16 (67.3)	n.d	0.61 (18.9)
Mataró	n.d	n.d	n.d	n.d	1.76 (87.5)	3.63 (180.5)	n.d	1.08 (53.6)
Prat de Llobregat	n.d	n.d	n.d	n.d	1.84 (66.1)	6.60 (236.9)	n.d	3.35 (120.3)
Terrassa	n.d	n.d	n.d	n.d	1.34 (84.2)	2.25 (141.2)	n.d	n.d
Gavà	n.d	n.d	n.d	n.d	2.84 (78.6)	3.86 (106.8)	n.d	2.09 (57.7)
Teià	n.d	n.d	n.d	0.79 (9.18)	0.81 (40.0)	3.00 (148.9)	n.d	2.01 (99.8)
Vic	n.d	<LOQ	n.d	n.d	0.89 (17.5)	1.06 (20.8)	n.d	n.d
Mean over positive samples	–	0.24 (7.1)	0.15 (5.1)	0.79 (9.18)	1.88 (81.8)	3.78 (158.8)	–	1.49 (62.3)

WWTP: wastewater treatment plant; 2,4-Dihydroxybenzophenone (BP1); 4,4'-Dihydroxybenzophenone (4DHB); 4-Hydroxybenzophenone (4HB); Benzophenone-3 (BP3); 4-Methylbenzylidene camphor (4MBC); Octocrylene (OCT); Ethylhexyl dimethyl PABA (OD-PABA); Ethylhexyl methoxycinnamate (EHMC); n.d: not detected; <LOQ: below the limit of quantification.

As expected, more lipophilic compounds were present in higher quantities, with the exception of OD-PABA, which was not detected in any of the samples. This fact may be attributed to decreased use of this compound as previously reported for PABA. In addition, degradation processes of OD-PABA in the sewer and during wastewater and/or sludge treatment cannot be ruled out. Moreover, the more polar compounds, when detected, were found at low concentrations, as was the case with BP3.

Determined range and mean concentrations (corrected for recoveries) were as follows: 4MBC (0.73–3.83, $1.88 \mu\text{g g}^{-1}$ dw), OCT (1.06–9.17, $3.78 \mu\text{g g}^{-1}$ dw) and EHMC (nd–3.35, $1.49 \mu\text{g g}^{-1}$ dw). These values are comparable to those found by Plagellat et al. (2006) (4MBC (0.15–4.98, $1.78 \mu\text{g g}^{-1}$ g dw), OCT (0.32–18.74, $4.84 \mu\text{g g}^{-1}$ dw), EHMC (0.01–0.39, $0.11 \mu\text{g g}^{-1}$ dw)). In the second study on sludge samples, Nieto et al. (2009) analyzed three sludge samples from the municipal WWTP of Tarragona city (Spain) reporting lower mean concentrations of OCT, BP3, and OD-PABA (1.45, 0.017, and $0.15 \mu\text{g g}^{-1}$ dw, respectively). In the third study Rodil et al. (2009) analyzed eleven UV filters in two spot sludge samples, one from Geel (Belgium) where, among others, OCT, BP3, 4MBC and OD-PABA were determined (2.48, 0.007, 3.89, and $0.001 \mu\text{g g}^{-1}$ dw, respectively); and the other from a WWTP from Leipzig (Germany) reporting also lower levels for these compounds (OCT ($0.59 \mu\text{g g}^{-1}$ dw), BP3 ($0.029 \mu\text{g g}^{-1}$ dw), 4MBC ($0.073 \mu\text{g g}^{-1}$ dw) and OD-PABA ($0.002 \mu\text{g g}^{-1}$ dw)).

Concentration levels found for 4MBC, OCT and EHMC are notoriously high, reflecting that there is no effective elimination of such compounds through the treatments performed to the sewage sludge. In addition to the selected UV filters, we determined two major BP3 degradation products 4HB and 4DHB, both exhibiting endocrine disrupting activity (Kunz and Fent, 2006) in four samples at concentrations in the range 0.04–0.62 $\mu\text{g g}^{-1}$ dw, 4DHB being the most frequently found. It is noteworthy that these results constitute the first data on the occurrence of these derivatives in sewage sludge.

Considering the lipophilicity of UV filters, an attempt to correlate concentrations detected with the organic matter of the sludge was made, however no correlation was found. Further, normalized concentrations of the target compounds to the content of organic matter were calculated (data not shown). Again, no correlation could be established.

In order to compare the WWTPs investigated, the specific loads ($\text{mg} (\text{day} \times 1000 \text{ Eq-Inhab})^{-1}$) were estimated for each compound (Table 4). The WWTP that generally showed higher concentrations were those with higher specific loads. The WWTPs of Rubí, Montcada and Prat de Llobregat, (with a big industrial contribution) were those with the highest specific loads. In contrast, the lower specific load corresponded to Vic, an area where the main economic activity is agriculture/livestock.

According to the 2008 Annual Report of the Catalan Water Agency (ACA), the sludge generated in most WWTPs with high concentrations of UV filters were usually applied as fertilizer to agricultural soils. This common practice can lead to certain environmental issues such as: an excess and/or imbalance of nutrients, the spread of human, animal and plant pathogens, and the introduction of inorganic contaminants such as heavy metals and organic pollutants (including UV filters). UV filters have scarcely been studied in sludge. However, if more experience and monitoring results (on sludge and soil) are gathered, a decision about submitting these compounds under legislation might be reached.

4. Conclusions

A multi residue analytical methodology based on ultra high resolution liquid chromatography separation and detection by elec-

troscopy-tandem mass spectrometry (UPLC–ESI-MS/MS) was developed and validated for the determination of UV filters in sewage sludge. The method was fast (chromatographic run time of 9.5 min), sensitive (LODs below 19 ng g^{-1}) and selective with good recovery rates (70–102%).

The widespread occurrence of UV filter residues in the 15 major WWTPs in Catalonia (Spain) was evidenced. Both, the frequency of detection and the mean concentrations found for lipophilic compounds (4MBC (100%, $1.88 \mu\text{g g}^{-1}$ dw), OCT (100%, $3.78 \mu\text{g g}^{-1}$ dw) and EHMC (66.6%, $1.49 \mu\text{g g}^{-1}$ dw)) were notoriously high. In contrast, BP3 were rarely detected (1/15 WWTPs) and BP1 and OD-PABA were not detected in any sample. Moreover, two major BP3 degradation products 4DHB and 4HB, both well-known endocrine disrupting compounds, were determined in sewage sludge for the first time. More information on UV filters consumption, degradability and WWTPs technology is needed to provide a reliable explanation to the estimated occurrence data. It should be pointed out that the treated sludge generated in most WWTPs is used as fertilizer in agriculture. Therefore, in light of these results, a greater knowledge over the presence and potential effects of UV filters in the environment is needed to ensure a safer management of the generated biosolids.

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