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Analysis of salicylate and benzophenone-type UV filters in soils and sediments by simultaneous extraction cleanup and gas chromatography–mass spectrometry

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ABSTRACT

An analytical method for the determination of UV filters in soil and sediment has been developed and validated considering benzophenones (BP) and salicylates as target analytes. Soil and sediment samples were extracted with ethyl acetate–methanol (90:10, v/v) assisted with sonication, performing a simultaneous clean-up step. Quantification of these compounds was carried out by gas chromatography-mass spectrometry (GC–MS) after derivatization of the extracts with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Recoveries from spiked soil samples ranged from 89.8% to 104.4% and they were between 88.4% and 105.3% for spiked sediment samples. The effect of the residence time and soil moisture content on the recovery of these compounds was also studied. The precision, expressed as relative standard deviation, was in all cases below 6.1% and the limits of detection (S/N = 3) varied from 0.07 to 0.10 ng g^{-1} and from 0.11 to 0.28 ng g^{-1} for soils and sediments, respectively. The validated method was applied to the analysis of five benzophenone and two salicylate UV filters in soil and sediment samples collected in different areas of Spain.

1. Introduction

UV filters are a broad class of substances used in a wide range of products, such as plastics, adhesives, rubber, cosmetics and sunscreen lotions. UV filters are designed to protect those products or skin, in the case of cosmetic sunscreens, from damage caused by the UV component of sunlight. Different studies have indicated that these substances are environmentally persistent, bioactive and have a bioaccumulation potential [1]. The use of these products is increasing and, in 2008, approximately 10,000 tonnes/year was the total amount used in European Union (EU) [2]. They are considered of environmental concern due to their endocrine disrupting effects [3] and, therefore, the approved UV filters and their maximum allowed concentrations in commercial products have been legislated by various regulatory authorities in Europe [4] and USA [5]. The maximum authorized concentration of 2-hydroxy-4-methoxybenzophenone (HMB) in sunscreens is 10% in EU and 6% in USA. UV filters may enter the environment in two ways, either indirectly via wastewater treatment plants (WWTPs) or directly from swimming and bathing in lakes and rivers. It has been reported that when HMB was applied on the skin, it was absorbed by the human body and at least two more polar metabolites, 2,4-dihydroxybenzophenone (DHB) and 2,2-dihydroxy-4-methoxybenzophenone (DHMB) were excreted. These metabolites are also used in cosmetic products as sunscreen agents to protect the skin against UV radiation [6]. Due to their high lipophilicity (log K_{ow} 4–8), water insolubility and relative stability against biotic degradation, they are expected to be principally transferred to sewage sludge during wastewater treatment and, with sewage sludge application in agriculture, they may end up in soil. The available monitoring data show that these compounds have been found in wastewater [7–10], surface water [11–14], sewage sludge [15–17] and fish [18,19]. These data confirm the presence of UV filters in the environment from consumer use of these substances in sunscreen products.

Different instrumental techniques have been used to determine UV filters in various matrices. UV filters have been determined by high-performance liquid chromatography (HPLC) with UV or mass spectrometric detection (MS) [20–25], HPLC tandem mass spectrometry (HPLC–MS/MS) [10,12,19,26], gas chromatography–mass spectrometry (GC–MS) [7,8,13,27–29] and gas chromatography–tandem mass spectrometry (GC–MS/MS) [9,10]. Since these UV filters contain phenolic hydroxyl groups, poor reproducibility and low sensitivity are achieved with GC analysis due to their insufficient volatility and thermal stability, hence derivatization is required to obtain sharper peaks, better separation and higher sensitivity.

Although the occurrence of UV filters in water samples is well documented, the information regarding the presence of UV filters in soil and sediment is scarce [7,28]. Only a method using pressurized liquid extraction (PLE) for the determination of some UV filters in sediments [28] and another one for the determination of

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benzophenones in soils [7] by shaking with methanol have been found in the available literature.

The objective of this paper was the development of a rapid and sensitive method for the determination of benzophenone and salicylate UV filters in soil and sediment samples. The developed method was applied to the analysis of agricultural and industrial soils and sediments (fluvial and marine) located in Spain to investigate the presence of these compounds. The target analytes were chosen in order to represent two different families of the UV filters, five "hydroxylated benzophenones", 4-hydroxybenzophenone (HBP), 2,4-dihydroxybenzophenone (DHB), 2-hydroxy-4-methoxybenzophenone (HMB), 2,2-dihydroxy-4-methoxybenzophenone (DHMB) and 2,2-dihydroxy-4,4-dimethoxybenzophenone (DHDMB) and two "salicylates", ethylhexyl salicylate (EHS) and 3,3,5-trimethylcyclohexyl salicylate (HMS).

2. Materials and methods

2.1. Reagents and standards

Ethyl acetate, methanol, acetonitrile and *n*-hexane, residue analysis grade, were purchased from Scharlab (Barcelona, Spain). Anhydrous sodium sulphate was obtained from Aldrich (Steinheim, Germany), heated for 24 h at 180 °C and then allowed to cool down in a desiccator before use. Florisil, a magnesium silicate adsorbent, 150–250 μm (60–100 mesh) for chromatography and silica Bondesil-C_{18}, particle diameter of 40 μm, were purchased from Scharlab (Barcelona, Spain). A Milli-Q water purification system from Millipore (Bedford, MA, USA) was used to provide ultrapure water. N-(ter-butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA) and a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (99:1, v/v) were purchased from Aldrich (Steinheim, Germany) as silylation reagents.

The substances analysed in this study, HBP, DHB, HMB, DHMB, DHDMB, EHS and HMS purity >97%, were obtained from Sigma–Aldrich (St Louis, MO, USA). $^{13}C_{12}$-bisphenol-A ($^{13}C_{12}$–BPA, purity >99%, 50 μg ml^{-1} in methanol) was provided by Cambridge Isotope Laboratories (Andover, MA, USA). The physical–chemical properties, molecular weight and abbreviations of the UV filters are given in Table 1. Separate stock solutions of each compound were made up at 5 μg ml^{-1} level in ethyl acetate. A working standard solution containing each compound at 150 ng ml^{-1} was prepared by dilution of the stock solution in ethyl acetate, acetonitrile and *n*-hexane. A set of standard solutions of 200, 100 and 50 ng ml^{-1} was prepared weekly by dilution in acetonitrile and was used to fortify samples. The final concentration of the internal standard $^{13}C_{12}$–BPA in the calibration solutions was 1 μg ml^{-1}. All standard and working solutions were protected from light and stored in the fridge at 4 °C.

2.2. Apparatus

2.2.1. GC–MS analysis

GC–MS analysis was performed with an Agilent 6890 (Waldbronn, Germany) gas chromatograph equipped with an automatic injector, Model HP 7683, and a mass spectrometric detector (MSD), Model HP 5973N, equipped with an inert ion source. A fused silica capillary column ZB-5MS, 5% phenyl polysiloxane as nonpolar stationary phase (30 m × 0.25 mm i.d. and 0.25 μm film thickness), from Phenomenex (Torrance, CA), was used.

Operating conditions were as follows: injector port temperature 250 °C; helium (purity 99.995%) as carrier gas at a flow-rate of 1.0 ml min^{-1} and pulsed splitless mode (pulsed pressure 45 psi = 310 kPa for 1.5 min) with the splitless injector purge valve activated 1.5 min after sample injection, in a double-taper glass liner with a nominal volume of 800 μl. The column temperature was maintained at 70 °C for 2 min, then programmed at 10 °C min^{-1} to 220 °C, followed by a final ramp to 280 °C at a rate of 12 °C min^{-1}, and held for 5 min. The total analysis time was 27.00 min and the equilibration time 2 min. A 2 μl volume was injected splitless, with the split valve closed for 1 min.

The mass spectrometric detector (MSD) was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from *m*/*z* 100 to 600, an ion source temperature of 300 °C and a quadrupole temperature of 150 °C. The electron multiplier voltage (EM voltage) was maintained 100 V above autotune with a solvent delay of 10 min.

Table 2 lists the compounds and the labelled internal standard along with their retention times, selected ions and limits of detection and quantification. The SIM program used to determine and confirm these compounds in soil and sediment samples has seven acquisition windows, with an ion dwell time of 100 ms. The target and qualifier abundances were determined by injection of standards under the same chromatographic conditions using full-scan with the mass/charge ratio ranging from 100 to 600 *m*/*z*. The analytes were confirmed by their retention times, the identification of target and qualifier ions and the determination of qualifier to target ratios. Retention times must be within ±0.3 min of the expected time and qualifier-to-target ratios within a 20% range for positive confirmation. The quantification of the analytes was accomplished by calibration with a labelled internal standard.

2.2.2. Extraction equipment

Glass columns (20 ml) of 10 cm × 20 mm i.d., Afora, Spain, and Whatman No. 1 filter paper circles of 2 cm diameter (Whatman, Maidstone, UK) were used.

An ultrasonic water bath (Raypa, Barcelona, Spain) was used in the extraction step. The generator of this ultrasonic water bath has an output of 150 W and a frequency of 35 kHz. A vacuum manifold (Supelco, Visiprep, Madrid) was employed for removing the extraction solvent.

2.3. Samples

2.3.1. Sample collection

Soil samples were taken from two agricultural fields located in the region of Madrid (Spain), fertilized with sewage sludge at 12 ton/ha (0.36% dry weight of sewage sludge) and from one industrial soil in the area of Bilbao. Soil was sampled from the upper layer (0–30 cm) with a stainless steel corer and transported to the laboratory where it was air dried, sieved through a 2 mm mesh, thoroughly mixed and kept frozen (−18 °C) in glass containers until analysis.

Sediments were collected during June-July 2010 in different rivers of Madrid, Manzanares (Sed 1), Jarama (Sed 2), Henares (Sed 3), Guadarrama (Sed 4) and Lozoya (Sed 5) and in the Mediterranean coast (Sed 6), at Piles, Valencia, Spain. These sediment sampling sites were selected because of their location in areas of bathing or recreational activities. Sediments were collected into a glass container rinsed several times with acetone, a stored in a refrigerated bag during transport to the laboratory. Before analysis, sediments were dried at room temperature, homogenized and kept frozen at −18 °C.

2.3.2. Extraction procedure

C_{18} (1.5 g) was mixed thoroughly with anhydrous sodium sulphate (1 g) at room temperature and, in order to carry out the simultaneous extraction-cleanup procedure, this mixture was transferred to a glass column (20 ml) containing two filter paper

Table 1
Physico-chemical properties of the target compounds.

IUPAC name	INCI[a] name	Abbreviation	MW	Log K_{ow}[b]	pK_a
4-Hydroxybenzophenone	–	HBP	198.22	3.07	–
2,4-Dihydroxybenzophenone	Benzophenone-1	DHB	214.22	2.96	7.53
2-Hydroxy-4-methoxybenzophenone	Benzophenone-3	HMB	228.24	3.52	7.56
2,2-Dihydroxy-4-methoxybenzophenone	Benzophenone-8	DHMB	244.24	3.82	6.99
2,2-Dihydroxy-4,4-dimethoxybenzophenone	Benzophenone-6	DHDMB	274.27	3.1	–
2-Ethylhexyl salicylate	Ethylhexyl salicylate	EHS	250.35	5.97	8.13
3,3,5-Trimethylcyclohexyl salicylate	Homosalate	HMS	262.33	6.16	8.09

[a] INCI (International Nomenclature for Cosmetic Ingredient) elaborated by COLIPA, (Committee de Liaison des Associations Europeans de L'industrie de la Perfumerie, de Products Cosmetics et de Toilette).
[b] K_{ow}, octanol–water partition coefficient.

Table 2
Retention times, limits of detection and quantification (LOD, LOQ, ng g^{-1}), and selected ions[a] of the studied compounds.

Compound	MW-TMS	t_R	Soil		Sediment		m/z[a]
			LOD	LOQ	LOD	LOQ	
1 EHS	323	15. 890	0.08	0.26	0.11	0.36	195, 196
2 HMS	334	16.294	0.07	0.23	0.12	0.40	195, 196, 210
3 HBP	270	16.794	0.07	0.23	0.23	0.76	193, 255, 270
4 HMB	300	17.262	0.10	0.33	0.28	0.90	285, 242, 299
5 DHB	358	17.814	0.10	0.33	0.21	0.70	343, 344, 357
6 $^{13}C_{12}$–BPA, IS	384	18.288	–	–	–	–	369, 371, 384
7 DHMB	388	18.622	0.07	0.23	0.14	0.46	299, 373, 388
8 DHDMB	418	20.183	0.09	0.30	0.15	0.50	403, 404, 329

MW-TMS: molecular weight of the trimethylsilyl derivative. IS: internal standard.
[a] Target ions are underlined.

circles of 2 cm diameter at the end. Then, sieved soil or sediment (2 g ± 0.001) was weighed into a 10 ml weighing funnel and placed in the column. For the recovery studies, samples were previously fortified with a 100 μl mixture of the different analytes to reach final concentrations of 50 ng g^{-1}, 30 ng g^{-1} or 15 ng g^{-1} and the $^{13}C_{12}$–BPA internal standard at 30 μg g^{-1} and they were kept at room temperature during 60 min to allow solvent evaporation. Samples were extracted with 8 ml of ethyl acetate–methanol (90:10, v/v) for 15 min in an ultrasonic water bath at room temperature. The water level in the bath was adjusted to equal the extraction solvent level inside the columns, which were supported upright in a tube rack and closed with 1-way stopcocks. After extraction, the columns were placed on the multiport vacuum manifold where the solvent was filtered and collected in graduated tubes. Samples were extracted again with another 8 ml of ethyl acetate–methanol (90:10, v/v) (15 min). The extracting solvent was filtered and samples washed with 1 ml of additional solvent. The combined extracts were concentrated using a gentle stream of nitrogen to 0.5 ml, diluted with acetonitrile to 1 ml and an aliquot (0.1 ml) was transferred to the reaction minivial for the derivatization step.

To investigate the influence of moisture content of samples and aging of residues on the recoveries, 2 g of sample were placed in glass columns, where they were fortified to give a final concentration of 30 ng g^{-1} and water was added to adjust the moisture content. The capped columns were then stored at 4 °C during 72 h before the analysis.

2.3.3. Derivatization

An aliquot (0.1 ml) of the standard or extract solution was transferred into a 2 ml reaction vial, followed by the addition of 50 μl of BSTFA containing TMCS (99:1, v/v). The vials were closed and the mixture left react for 10 min at 60 °C. After the derivatization process, an aliquot (2 μl) of these solutions was injected in GC–MS.

2.4. Assessment of matrix effect

In order to state if there was a matrix enhancement response effect and in that case to obtain a correction function for each compound, calibration solutions prepared in solvent and the corresponding calibration solutions prepared using blank matrix extracts, instead of pure solvent, were prepared. The analyte concentrations were the same in both cases: 10, 20, 25, 40, 50, 80, 100, 160 and 200 μg l^{-1}. These calibration solutions were prepared by dilution with acetonitrile in the case of pure solvent and matrix matched in the case of blank matrix extracts. The internal standard ($^{13}C_{12}$–BPA) was added to both calibration solutions at 100 μg l^{-1}.

Table 3
Recoveries[a] of UV filters in soils.

UV filters	Fortification levels (ng g^{-1})			Moisture content[b]	Aged residues[b]
	50	30	15	10%	72 h
EHS	97.9 ± 1.4	104.4 ± 1.6	102.3 ± 4.0	104.2 ± 3.8	103.5 ± 4.1
HMS	97.4 ± 2.4	95.9 ± 1.9	91.3 ± 4.1	99.1 ± 2.8	96.9 ± 3.8
HBP	100.2 ± 1.8	101.1 ± 2.1	97.5 ± 3.2	99.4 ± 3.5	100.7 ± 5.1
HMB	92.8 ± 3.8	96.9 ± 3.3	95.3 ± 2.4	102.0 ± 4.6	95.7 ± 4.0
DHB	93.6 ± 4.2	95.9 ± 2.7	103.8 ± 3.1	99.8 ± 5.0	93.9 ± 2.4
DHMB	93.7 ± 4.8	89.8 ± 3.8	89.9 ± 2.7	95.9 ± 2.8	92.6 ± 3.6
DHDMB	98.9 ± 3.0	92.4 ± 4.6	91.9 ± 3.3	99.5 ± 4.2	93.9 ± 2.7

[a] Results are the mean of four replicates ± standard deviation.
[b] Results are the mean of four replicates ± standard deviation at the 30 ng g^{-1} level.

2.5. Quality assurance/quality control

The quality assurance and quality control criteria used for this method included analyses of laboratory blanks (reagent blank), laboratory control samples (LCS) and surrogate standard recoveries.

One laboratory blank was run with each set of samples to check for contamination from the preparative steps and to demonstrate laboratory background levels. No benzophenone and salicylate compounds were detected in reagent blanks, which consisted of solvents and reagents passed through the entire analytical procedure. LCS were used in the recovery assay and the concentration of the studied compounds determined in blank samples were subtracted. With each set of samples to be analysed, a standard mixture, a LCS, and a laboratory blank were run in sequence to check for contamination and instrumental performance.

For surrogate standard recoveries, a 100 μl of a standard mixture containing 30 ng of the I.S. was added to each LCS prior to analysis. The average recovery of the surrogate standard in these samples, as measured by the external standard method, was 98.7 ± 3.6%.

3. Results and discussion

3.1. Derivatization

Initially, MTBSTFA was used as derivatization reagent, because it has been employed for the silylation of various organic compounds [30,31]. Different experimental conditions (reaction time, temperature and volume of MTBSTFA) were tested, but the reaction was not found quantitative and dirty extracts and poor resolution of peaks were obtained. However, using BSTFA all compounds were converted in the corresponding trimethylsilyl derivatives. After silylation, all the studied UV filters achieved complete derivatization showing remarkable increase of sensitivities and also cleaner extracts were obtained. Therefore, BSTFA was selected as derivatization reagent.

In order to check the influence of solvent on the silylation reaction, the use of ethyl acetate, acetonitrile and *n*-hexane was evaluated. In terms of analytical responses, it was found that peak areas of derivatives obtained in acetonitrile were higher than those of the other solvents, ethyl acetate and *n*-hexane, which produced responses of similar intensity for the majority of analytes studied. The effect of the solvent on the derivatization of a standard mixture solution at a concentration of 10 μg l^{-1} is shown in Fig. 1.

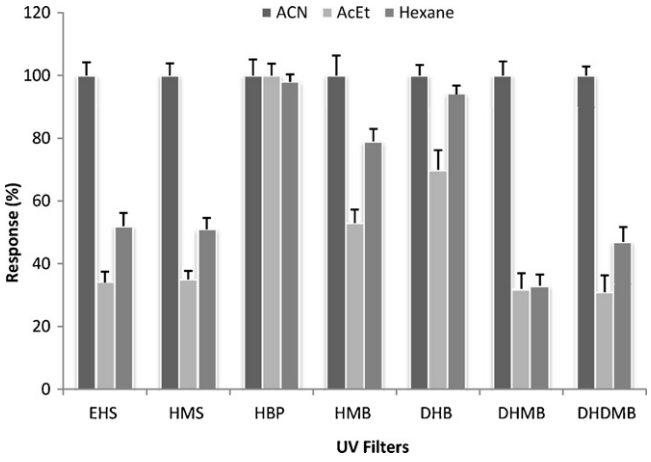

Fig. 1. Effect of the solvent on the derivatization of a 10 μg l^{-1} mixture of benzophenones and salicylates, $n = 4$. Responses are normalized to acetonitrile. See Table 1 for abbreviations of UV filters.

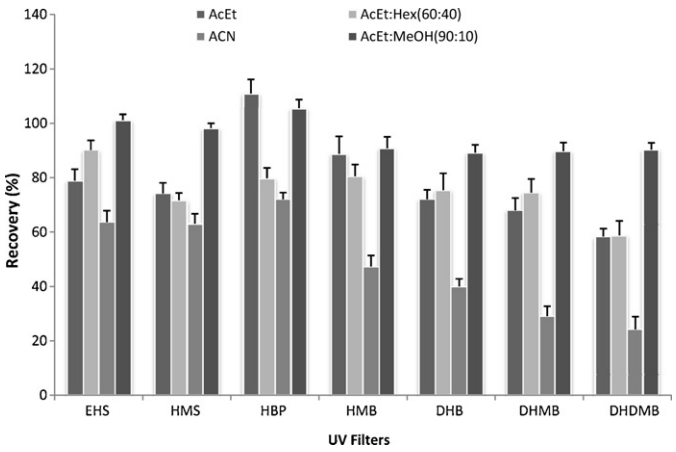

Fig. 2. Comparison of the extraction efficiency of different solvents in samples spiked at 30 ng g^{-1}, $n = 4$. See Table 1 for abbreviations of compounds.

The stability of the derivatized analytes was evaluated at different times after preparation, and they were found stable for at least 1 month when stored at 4 °C (RSD <4%) in darkness.

The effect of temperature on the derivatization was studied at 60 and 80 °C, whereas the effect of derivatization time and volume of BSTFA was studied at 10, 30 and 60 min and 50 and 100 μl, respectively. As similar result were obtained, the experimental conditions for the simultaneous derivatization of these compounds were set as follows: acetonitrile was selected as solvent for the silylation reaction and 50 μl of BSTFA + 1% TMCS were added to a 100 μl sample and the mixture was kept at 60 °C during 10 min. Relative standard deviations (RSD) in the range of 2–6% were obtained in these conditions for the compounds studied, which indicate the satisfactory reproducibility of the derivatization step.

3.2. Sample preparation

It must be emphasized that the determination of UV filters required rigorous clean-up of the material used because these compounds are ingredients in many cosmetics and personal care products in consumer goods. The use of plastic material was avoided and glassware material was used instead. Due to their lipophilic nature, these compounds can be transferred to glassware during sample preparation. To avoid background contamination, all glassware was rinsed several times with acetone before use and reagent blanks were routinely measured to check for contamination.

Selective extraction of analytes from complex matrices, such as industrial or amended soils and sediments, is recognized as a very complicated task because these matrices contain a large variety of compounds that may difficult analysis and make necessary lengthy purification processes. In this sense, we have developed a rapid method based on the extraction assisted with sonication with a simultaneous clean-up. For the method development procedure, different organic solvents were compared for extraction efficiency, such as ethyl acetate, acetonitrile, ethyl acetate–hexane (60:40, v/v) and ethyl acetate–methanol (90:10, v/v). Recoveries obtained from samples with acetonitrile at the 30 ng g^{-1} fortification level were lower than 60% for some compounds, whereas those achieved with ethyl acetate and ethyl acetate–hexane mixture were around 80% for the majority of UV filters studied (Fig. 2). Nevertheless, to increase the recoveries of compounds of highest polarity such as DHMB and DHDMB, ethyl acetate–methanol (90:10, v/v) was used. In these conditions, the recoveries of all compounds were around 90% or higher. Therefore, ethyl acetate–methanol (90:10,

Table 4
Recoveries[a] of UV filters in sediments.

UV filters	Fortification levels (ng g^{-1})			Moisture content[b]	Aged residues[b]
	50	30	15	10%	72 h
EHS	99.4 ± 5.4	100.1 ± 4.1	102.0 ± 4.0	104.2 ± 3.8	103.5 ± 4.1
HMS	101.3 ± 1.2	97.4 ± 1.0	97.6 ± 2.8	99.1 ± 2.8	96.9 ± 3.8
HBP	104.2 ± 3.3	105.3 ± 2.0	102.0 ± 1.5	99.4 ± 3.5	100.7 ± 5.1
HMB	98.9 ± 1.7	99.3 ± 3.5	101.3 ± 3.3	102.0 ± 4.6	95.7 ± 4.0
DHB	94.3 ± 2.2	101.9 ± 2.6	96.0 ± 4.1	99.8 ± 5.0	93.9 ± 2.4
DHMB	91.4 ± 1.9	88.4 ± 2.9	88.9 ± 5.7	95.9 ± 2.8	91.6 ± 3.6
DHDMB	92.4 ± 2.0	90.4 ± 4.7	89.9 ± 6.1	99.5 ± 4.2	95.9 ± 2.7

[a] Results are the mean of four replicates ± standard deviation.
[b] Results are the mean of four replicates ± standard deviation at the 30 ng g^{-1} level.

v/v) provided the highest extraction efficiency and it was the solvent selected for the extraction of these compounds.

3.3. Detection of UV filters by GC–MS

The studied compounds were determined, after their derivatization, by gas chromatography–mass spectrometry with selected ion monitoring (SIM). Fig. 3 presents a SIM chromatogram of a standard mixture solution derivatized in conditions indicated above.

The base peak in the MS spectra of the benzophenones showed the loss of a methyl group, at *m/z* 285, 343, 373 and 403 for DHB, HMB, DHMB and DHDMB, respectively, whereas for HBP, the base peak was the loss of a benzyl group, *m/z* 193 $[M-C_6H_5]^+$. The molecular ions $[M]^+$ were present at low relative intensities, except for HBP. The base peaks and other characteristic ions of these compounds are listed in Table 2. In the case of salicylates, EHS and HMS, an intense peak at *m/z* 195 was observed, due to the 2-dimethylsilyloxybenzoic acid moiety.

3.4. Matrix effect

The matrix effect occurring in the GC analysis of some organic compounds has a negative impact on the accuracy of the results. Elimination of this effect is essential for quantification of pollutants at trace levels in complex environmental matrices [32,33], such as industrial or amended soils and sediments. Blank sample extracts, prepared according to the method described above, were fortified and used to compare the response of analytes in sample extracts with that of external standards prepared in pure solvents. In this study, we evaluated the matrix effect on the UV filters analysed and this effect increased in the range from 3% for HMS to 27% for DHDMB. To overcome this effect, matrix matched standards can be used, nevertheless, this approach is more laborious than conventional calibration using standards prepared in neat solvents. On the other hand, some regulatory agencies, like the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA), in the USA, do not permit matrix-matched standardization for enforcement purposes.

Isotope labelled compounds are suitable internal standards when using mass spectrometric detection and can be used to correct the matrix induced enhancement. The concentration difference between the analyte and the internal standard should be small to represent a similar analyte matrix concentration ratio. In the present work, we studied the use of $^{13}C_{12}$–BPA to overcome the matrix effect found.

Two multipoint calibration curves [matrix-free calibration curve in pure solvent (SC) and matrix-matched calibration curve (MC)] with nine standard solutions of different concentration levels, in the range of 10–200 μg l^{-1}, appropriate to the levels expected in our samples, were obtained. The labelled internal standard at a concentration of 100 μg l^{-1} was added to the standard solutions prior to derivatization and injection. In both cases, a good linearity of the calibrations curves was obtained in the studied range. Plots of calibration curves for HMS (3% matrix effect) and DHDMB (27% matrix effect) in neat solvent (SC) and matrix-matched (MC) soil extracts are shown in Fig. 4A and B, respectively. Analysis of UV filters in neat solvent produced for most compounds calibration curves with lower slopes as compared with matrix matched standards.

Fig. 4C and D show calibration curves of HMS and DHDMB quantified with the isotope labelled internal standard. The addition of $^{13}C_{12}$–BPA as internal standard practically eliminated differences between calibrations obtained in matrix extracts versus matrix free solutions. $^{13}C_{12}$–BPA was chosen as internal standard due to the lack of isotopically labelled hydroxy benzophenones and its similar physicochemical properties.

3.5. Method validation

3.5.1. Recovery

The recovery assay was conducted by sample spiking at three concentrations (15, 30 and 50 ng g^{-1}) for each analyte with a sample that had been found not to contain any of the compounds. Four replicates per fortification level were carried out and the obtained results are shown in Tables 3 and 4. In all the fortification levels, recoveries were in the range of 89.8–104.4%, whereas RSD values ranged from 1.4% to 4.8%, thus fulfilling the requirements of the IUPAC [34]. The range of recoveries achieved is similar to that obtained by other authors for the analysis of UV filters in environmental samples [10].

With the aim of assessing the effect of soil and sediment moisture content on UV filters recovery, samples were fortified at 30 ng g^{-1}, soil and sediment moisture content was adjusted to 10% and UV filters extracted with ethyl acetate–methanol (90:10, v/v) following the procedure described above. Tables 3 and 4 shows the recoveries obtained for the compounds studied. Good recoveries were obtained with values higher than 95%, therefore, moisture content did not affect recovery of these compounds. RSD were lower than 5% for all compounds.

To study the influence of the residence time of residues on these recoveries, soil and sediment samples were fortified at 30 ng g^{-1}, kept at 4 °C and analysed 72 h after application of compounds. Tables 3 and 4 summarized the results obtained. It can be observed that the recoveries are very similar and always higher than 93% for all compounds after 72 h of storage with RSD lower than 5.1%.

3.5.2. Repeatability

The repeatability of the chromatographic method was determined by injecting 10 times a standard solution of 10 μg l^{-1} with an automatic injector. RSD obtained for the retention times ranged from 0.01% to 0.02%, whereas for peak areas those values ranged from 2.1% to 6.4% (Table 5). Within-laboratory reproducibility of the chromatographic determination was evaluated at different days during 2 consecutive weeks and was found to be lower than 11% for all of the compounds, expressed as RSD. The repeatability of

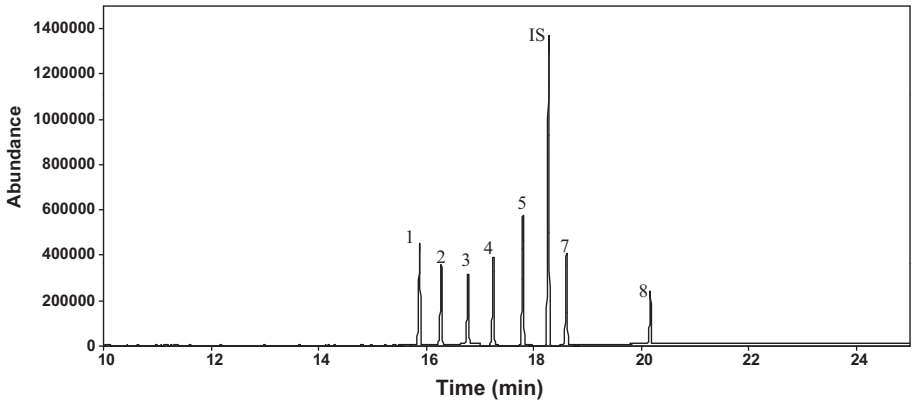

Fig. 3. A GC–MS-SIM chromatogram of a standard mixture solution of UV filters (20 μg l^{-1}) with $^{13}C_{12}$ BPA as internal standard (40 μg l^{-1}). See Table 2 for peak identification.

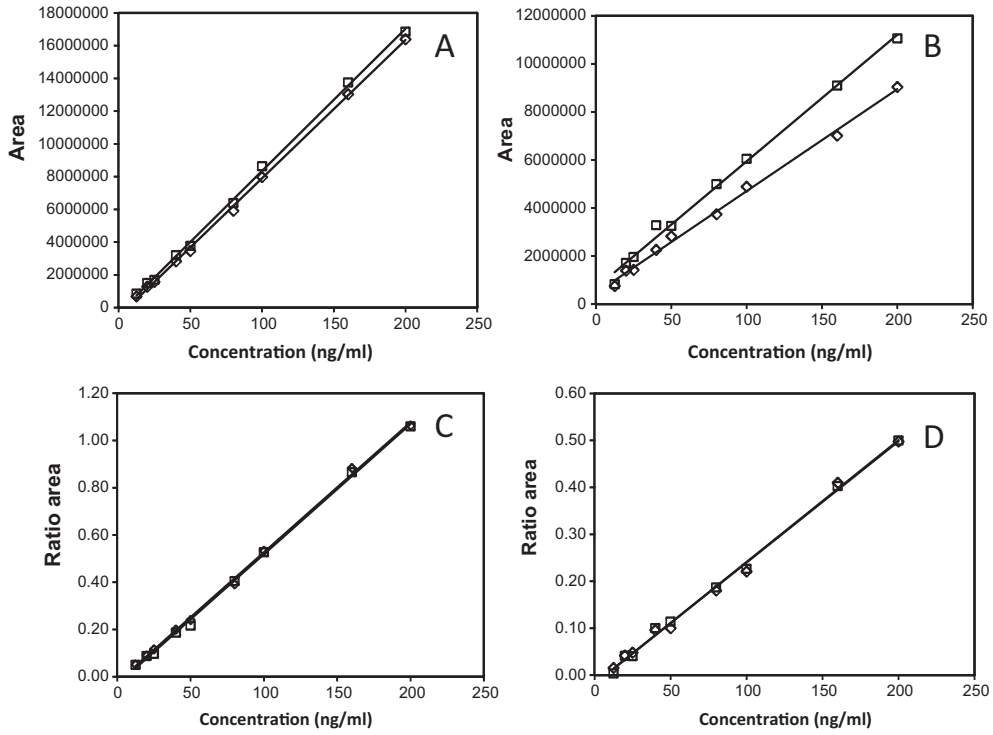

Fig. 4. Comparison of calibration curves of HMS (A) and DHDMB (B), obtained by injection of standards in neat solvent (SC, ◊) and spiked sample extracts (MC, □). Calibration curves of HMS (C) and DHDMB (D) obtained by injection of standards in neat solvent (◊) and spiked sample extracts (□) with $^{13}C_{12}$ BPA as internal standard.

the whole analytical procedure was determined by analyzing seven samples spiked at 30 ng g^{-1} within a given day and the RSD calculated for the studied compounds ranged from 3.4% to 7.5%. These results indicate that the analytical method developed for the analysis of benzophenone and salicylate compounds in soil and sediment samples provides a good precision.

Table 5
Calibration data and repeatability[a] of the studied compounds.

Compound	Calibration data		Repeatability (RSD, %)[b]	
	Equation	r	Peak area	t_R
EHS	$y = 9.54 \times 10^{-1}x - 5.65 \times 10^{-2}$	0.999	2.1	0.02
HMS	$y = 7.59 \times 10^{-1}x - 5.24 \times 10^{-2}$	0.998	2.2	0.02
HBP	$y = 3.77 \times 10^{-1}x - 3.10 \times 10^{-2}$	0.999	2.2	0.01
HMB	$y = 7.55 \times 10^{-1}x - 3.49 \times 10^{-2}$	0.997	2.7	0.02
DHB	$y = 6.00 \times 10^{-1}x - 3.05 \times 10^{-2}$	0.998	2.9	0.01
DHMB	$y = 7.39 \times 10^{-1}x - 5.13 \times 10^{-2}$	0.999	3.1	0.01
DHDMB	$y = 2.94 \times 10^{-1}x - 1.03 \times 10^{-2}$	0.998	6.4	0.01

[a] Repeatability of the chromatographic method.
[b] RSD of retention times and peak areas ($n = 10$).

3.5.3. Linearity

Linearity was studied by performing a multipoint calibration curve with nine standard solutions at different concentration levels, in the range of the content expected in samples. Each calibration level of the curve (10, 20, 25, 40, 50, 80, 100, 160, 200 μg l^{-1}) was spiked prior to analysis with 100 μg l^{-1} of $^{13}C_{12}$–BPA. The calibration data, correlation coefficients and regression equations of the calibration curves are listed in Table 5, which shows a good linearity of the results obtained with correlation coefficients equal or higher than 0.997.

3.5.4. Limits of detection (LOD) and quantification (LOQ)

LODs and LOQs considered as the minimum amount of target analyte that produces a chromatographic peak with a signal-to-noise ratio of 3 and 10 times the background noise, respectively,

Table 6
Concentration[a] of the studied compounds (ng g^{-1}) in sediment and soil samples collected in various areas of Spain.

	Samples									
	Sed 1	Sed 2	Sed 3	Sed 4	Sed 5	Sed 6	Soil 1	Soil 2	Soil 3	Soil 4
EHS	7.5 ± 0.3	20.0 ± 0.5	5.3 ± 0.2	n.d.	3.5 ± 0.2	13.3 ± 0.4	n.d.	n.d.	n.d	n.d
HMS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d
HBP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d
HMB	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d
DHB	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d	5.7 ± 0.3
DHMB	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d
DHDMB	6.1 ± 0.3	n.d.	n.d.	1.4 ± 0.07	1.2 ± 0.1	n.d.	n.d.	0.6 ± 0.4	n.d	n.d

n.d.: <method detection limit. Sed 1–5: fluvial sediments; Sed 6: marine sediment. Soil 1–3: soils amended with sludge; Soil 4: industrial soil.
[a] Results are the mean of four replicates ± standard deviation.

were determined. Low limits were obtained due to the selectivity of the analytical procedure and the high sensitivity of GC–MS-SIM, allowing the determination of analytes at the levels found in soil and sediment samples. The LODs and LOQs corresponding to the different analytes are shown in Table 2. The LODs range from 0.07 ng g^{-1} to 0.10 ng g^{-1} and from 0.11 ng g^{-1} to 0.28 ng g^{-1} for soils and sediments, respectively. These values are in the lower end of those reported by other authors [7].

3.6. Application to real samples

The developed method was applied to the analysis of UV filters in soils and sediments collected from different areas of Spain. Four soil samples were analysed comprising agricultural soils amended with sludge (soils 1–3) and one industrial soil (soil 4). Table 6 shows the concentrations of the UV filters found in these samples, expressed as ng g^{-1} dry weight.

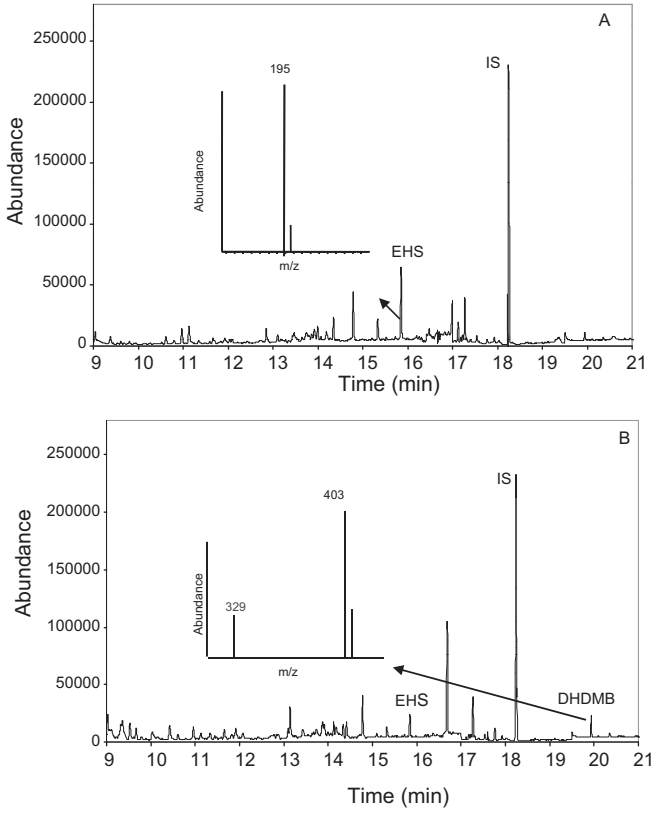

Fig. 5. Ion chromatograms of (A) a marine sediment extract containing EHS (13.3 ng g^{-1}) with the main ions of its mass spectrum and (B) a river sediment extract containing EHS (7.5 ng g^{-1}) and DHDMB (6.1 ng g^{-1}) with the main ions of its mass spectrum.

The concentrations of the studied compounds in soil were below the LOD for most of the samples. DHB was found at 5.7 ng g^{-1} in the industrial soil analysed. DHB is the major metabolite formed from HMB, but it is also used as a personal care product, therefore its occurrence may be higher than that of HMB [35]. DHDMB was found at 0.6 ng g^{-1} in one amended soil sample. DHDMB is a frequently used UV filter as well as a metabolite with high lipophilicity, therefore it may accumulate in sludge and, after its application in agriculture, be transferred to soil.

Fluvial and marine sediment samples were collected in sites located in bathing or recreational areas. The most frequently UV filters detected in sediments were EHS (3.5–20.0 ng g^{-1}) and DHDMB (1.2–6.1 ng g^{-1}). DHDMB is used in large quantities in cosmetics and food additives to protect human skin and food from UV radiation and may enter the environment indirectly though wastewater [35]. Concentrations of DHDMB in the sediment samples analysed in this study were lower than the values reported in samples from South Korea [7], which were in the range of 4.0–18.4 ng g^{-1}. EHS has been frequently detected in water [8,28] and due to its lipophillic properties it should also be present in sediments, as it has been found in our study.

A chromatogram of a marine sediment extract containing EHS (13.3 ng g^{-1}) and another one of a river sediment extract containing EHS (7.5 ng g^{-1}) and DHDMB (6.1 ng g^{-1}) showing the main ions of their mass spectrum, are depicted in Fig. 5A and B, respectively.

4. Conclusions

The analytical method developed in the present paper allows the simultaneous detection and quantification of trace amounts of benzophenone and salicylate UV filters in soils and sediments. The combination of extraction and clean-up into a single step proved to be efficient and selective for the determination of these compounds in both types of samples. Recoveries of UV filters through the method were higher than 90% and they were not affected by the moisture content or aging of these compounds in the samples. The silylated derivatives of UV filters have good sensitivities with LODs below 0.3 ng g^{-1} in the studied samples. The present method showed good linearity and the use of an isotope labelled internal standard overcame the matrix effect observed. The results presented in this work show that this method fulfills the validation criteria for trace analysis. The method was applied to the quantification of UV filters in soils and sediments collected in different areas of Spain.

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