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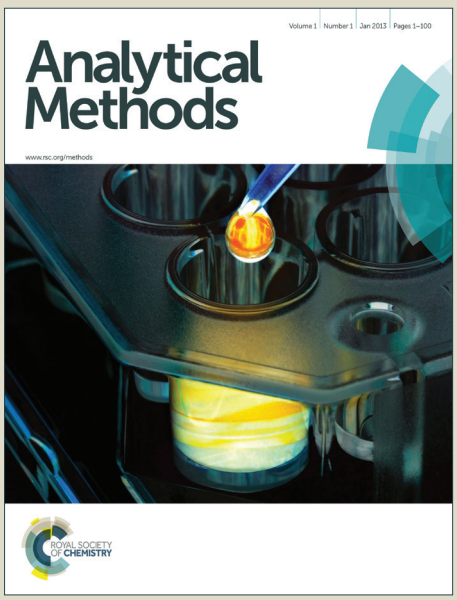

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Development of a gas chromatography-mass spectrometry method for the determination of ultraviolet filters in beach sand samples

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Abstract

An analytical method for the determination of eight fat-soluble ultraviolet (UV) filters in beach sand samples is presented for the first time. The method is based on a leaching process of the target compounds from sand samples using vortex mixer agitation and further centrifugation, followed by dispersive liquid-liquid microextraction (DLLME) of the supernatant and gas chromatography-mass spectrometry (GC-MS) analysis of the DLLME extract. The variables involved in the leaching and in the DLLME processes were studied to provide the best enrichment factors. In the first case, the leaching solvent type and volume, and the vortex mixer agitation time were studied. In the case of the DLLME, the type and volume of both disperser and extraction solvent and the influence of the pH and the ionic strength of the supporting aqueous solution were studied. Under the selected conditions, the method was successfully validated showing good linearity (R^2 > 0.995), method limits of detection in the pg g^{-1} level, enrichment factors in the range of 8 to 50 (depending on the analyte) and good intra- and inter-day precision. No significant matrix effects were found, thus external calibration can be used. However, internal calibration was recommended to improve repeatability in both the DLLME and the GC-injection. Moreover, in order to correct losses during the leaching process, the surrogate was added to the samples before the leaching step. The validated method was successfully applied to the analysis of several beach sand samples from different origin.

Keywords: Beach sand; Dispersive liquid-liquid microextraction; Gas chromatography-mass spectrometry; ultraviolet filters; Vortex-assisted leaching

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1. Introduction

It is well-known that sun exposure provides many health benefits on the human health, such as an improvement in the endogenous production of vitamin D or prevention of some diseases as rickets or osteoporosis. However, sun overexposure causes adverse effects, such as skin cancer, cutaneous photoaging and damage to the skin's immunological system [1].

The concern about the health risks commented above has led to an increase in the use of cosmetics containing the so-called UV filters as active ingredients to prevent or minimize the harmful effects of the UV radiation. These active compounds have an organic or inorganic nature, and they act absorbing and/or reflecting, respectively, the UV radiation. The compounds that can be used as UV filters in cosmetic products, and their maximum allowed concentrations are regulated by the legislations in force in each country [1-3].

The excessive use of cosmetics containing these compounds (not only those cosmetics intended specifically for sun protection but also all type of daily products such as moisturizes, after shave products, shampoos, etc) had led to an appearance of the UV filters in the aquatic environment, through direct and indirect sources, where they are being accumulated [4,5]. The high lipophilic characteristics of some of them makes them susceptible to be accumulated in the suspended particles contained in water, sediments, sludge or even biota [4]. Furthermore, different *in vitro* and/or *in vivo* studies show that some UV filters, even at trace levels, present endocrine disrupting activity that might affect the reproduction of fish [5-8]. For this reason, UV filters are currently considered as emerging contaminants and it is interesting to develop analytical methods that allow their determination in the environment at trace levels.

Most publications about the development of analytical methods to determine UV filters in environmental samples are focused on the analysis of environmental water samples [4,5,9,10]. However, different analytical methods can be found in the literature dealing with the determination of UV filters in environmental soil samples, such as river and/or lake sediments [11-17], coastal sediments [16], bight sediments [18], ground soil [12], sewage sludge [10,15,19-23] or even indoor dust [24].

In order to improve the method sensitivity and/or to eliminate some potentially interfering compounds, preconcentration and/or clean-up techniques have been employed. Thus, the determination of UV filters in this type of samples was carried out by extracting (usually in consecutive steps) the analytes from the solid sample into various organic solvents, such as methanol or acetone, by solid-liquid partitioning

[11,12,15,18,19]. This traditional extraction technique is time-consuming, poorly selective (i.e., many interferents may be co-extracted) and it often requires large amounts of organic solvents, which causes dilution of the target analytes in the extract. So, an additional clean-up and/or preconcentration step is needed in some cases [12,15]. Newer extraction techniques, such as microwave-assisted extraction (MAE) [16], pressurized liquid extraction (PLE) [13,14,17,20-23] or matrix solid-phase dispersion (MSPD) [24] were employed in subsequent works. In these techniques, both the organic solvent consumption and the time required to carry out the extraction are considerably decreased, but PLE often requires additional clean-up and/or preconcentration steps [13,14,20,22,23].

A good alternative to the above mentioned extraction techniques is the so-called dispersive liquid-liquid microextraction (DLLME) [25]. Due to the several advantages that this extraction technique presents (i.e., fast, inexpensive, easy to operate and low consumption of organic solvent) it has become a very popular extraction technique that has been used for the determination of organic and inorganic compounds in different type of samples [26]. Specifically, this microextraction technique has already been used before for the determination of UV filters in water samples [27-35], but it has not ever been employed for the determination of UV filters in sediment samples, especially in case of beach sand samples, most probably due to the fact that these are solid samples. Nevertheless, in this type of samples, a leaching process of the target compounds from the matrix sample prior to the DLLME procedure could overcome this drawback.

In this sense, the aim of this paper is to draw on the high potential of the DLLME to develop a rapid, selective and sensitive method for the determination in beach sand samples of eight typical organic UV filters (Table 1). The developed method, which is expected to be used in environmental surveillance studies, is based on the leaching of the analytes from the sand sample prior to DLLME and followed by GC-MS analysis.

<Table 1>

2. Experimental

2.1. Reagents and samples

2-Ethylhexyl salicylate (ES) 99%, 2-hydroxy-4-methoxybenzophenone (benzophenone-3 (BZ3)) 98%, 2-ethylhexyl 4-methoxycinnamate (EMC) 99.8% and 2-

ethylhexyl 4-(dimethylamino)benzoate (ethylhexyl dimethyl PABA (EDP)) 98% from Sigma-Aldrich, 3,3,5-trimethylciclohexyl salicylate (homosalate (HS)) >98% from Merck (Darmstadt, Germany), isoamyl 4-methoxycinnamate (IMC) 99.3% from Haarmann and Reimer (Parets del Vallés, Spain), 3-(4'-methylbenzylidene)camphor (MBC) 99.7% from Guinama S.L. (Valencia, Spain) and 2-ethylhexyl 2-cyano-3,3-diphenylacrylate (octocrylene (OCR)) >98% from F.Hoffman-La Roche Ltd. (Basel, Switzerland) were used as standards. Deuterated benzophenone (benzophenone-d_{10} (BZ-d_{10})) 99% from Isotec (Miamisburg, Ohio, USA) was used as surrogate to minimize possible deviations occurred during the DLLME and GC injection processes.

LC-grade absolute ethanol from Scharlau Chemie (Barcelona, Spain) was used as solvent to prepare the multicomponent and surrogate standard stock solutions. Analytical reagent-grade acetone also from Scharlau Chemie was used as solvent to prepare the working standard solutions and as leaching/disperser solvent. Analytical reagent-grade chloroform from Scharlau Chemie was used as extraction solvent. De-ionized water, obtained by means of a NANOpure II water purification system from Barnstead (Boston, USA), was used as supporting solvent in the DLLME process.

Analytical reagent-grade sodium chloride (NaCl) 99.5% from Scharlau Chemie was used to adjust the ionic strength of the DLLME aqueous supporting solutions. Sodium dihydrogen phosphate (NaH_2PO_4) and phosphoric acid (H_3PO_4), both also from Scharlau Chemie, were used to adjust the pH of these solutions.

High-purity helium (99.9999%) from Carburos Metálicos S.A. (Paterna, Spain) was used as carrier gas in the GC-MS system.

Sand samples were all collected from the beach shore of different Spanish beaches located in Valencia (Sample 1: *Malvarrosa beach* (June 2013); Sample 2: *Pinedo beach* (June 2013); Sample 3: *Patacona beach* (July 2013)) and Gran Canaria Island (Sample 4: *Los ingleses beach,* (August 2011)). An additional sand sample from *Malvarrosa beach* collected away from the shore and out of beach season (February 2013) was used as blank. All they were stored in the dark and dried at 60 ºC in porcelain capsules overnight before sample analysis.

2.2. Apparatus

A Focus GC gas chromatograph, equipped with an AS 3000 autosampler and coupled to a DSQ II mass spectrometric detector (operated in positive electron ionization mode at ionization energy of 70 eV, with a multiplier voltage set at 1300 V), from Thermo Fisher Scientific (Austin, TX, USA) was employed.

A Hettich (Tuttlingem, Germany) EBA 21 centrifuge and a Crison (Alella, Spain) Basic 20 pH meter were used in sample treatment. An ultrasound bath (50 Hz, 360 W) from J.P. Selecta S.A. (Barcelona, Spain) was also used in the leaching optimization.

2.3. Proposed method

Multicomponent and surrogate standard stock solutions were prepared separately in ethanol at 500 and 1000 μg mL^{-1}, respectively. From these solutions, multicomponent and surrogate solutions were prepared daily in acetone at 2 and 10 μg mL^{-1}, respectively. Calibration standard solutions (10-50 ng mL^{-1}) in acetone, containing 40 ng mL^{-1} of surrogate, were also prepared daily. An additional 40 ng mL^{-1} surrogate solution in acetone was prepared as blank. These solutions were subjected to the DLLME procedure.

Besides, by triplicate, 10 g of dry sand sample were weighted and placed into 50 mL screw cap glass centrifuge tubes with conic bottom. Then, 20 μL of the surrogate solution were added in all cases (i.e., at 20 ng g^{-1}). The mixture was homogenized and left to solvent evaporation. A volume of 5 mL of acetone was added and the tube was vigorously shaken with vortex mixer during 20 s and centrifuged at 5000 rpm for 10 min. The supernatant (ca. 2 mL) was separated, and this operation was repeated twice with 1 mL of acetone. Then, the supernatants of each sample were merged in a 5 mL volumetric flask and acetone was added up to the mark. After that, an aliquot was filtered through 0.45 μm nylon membrane filters and subjected to the DLLME procedure.

2.3.1. DLLME procedure

Different aliquots of 5 mL of deionized water, used as supporting solvent, were adjusted to pH 4 and placed into 7.5 mL screw cap glass centrifuge tubes. Then, 2 mL of the acetone standard solutions (or sample extracts) containing 60 μL of chloroform, were rapidly injected into the water. The formed cloudy solutions were vigorously shaken with vortex mixer during 5 s. Finally, they were centrifuged at 3000 rpm for 3 min for phase separation. The sedimented phases were collected and transferred into 1.5 mL GC injection vials.

2.3.2. GC-MS analysis

Two µL of each one of the aforementioned sedimented phases were injected into the GC injection port set at 280 ºC in splitless mode, and run at 1 mL min^{-1} helium constant flow rate by using a HP-5MS Ultra Inert (95% dimethyl-5% diphenylpolysiloxane, 30 m length, 0.25 mm i.d., 0.25 µm film thickness) column from Agilent Technologies (Palo Alto, CA, USA). The oven temperature program was: from 70 ºC (1 min) to 170 ºC at 10 ºC min^{-1}, then to 200 ºC at 2 ºC min^{-1} and finally to 280 ºC (6 min) at 10 ºC min^{-1}. The transfer line and ion source temperatures were set at 280 ºC and 250 ºC, respectively. The chromatograms were recorded in selected ion monitoring (SIM) mode at the mass/charge (m/z) ratios shown in Table 2.

<Table 2>

Figure 1 shows, as an example, the obtained chromatogram for a sand blank spiked with the target compounds at 20 ng g^{-1} and subjected to the described DLLME-GC-MS procedure.

<Fig 1>

3. Results and discussion

3.1. Study of the experimental variables involved in the DLLME procedure

Different variables may affect the DLLME process, such as the type and volume of both extraction and disperser solvents, and the pH and ionic strength of the aqueous phase [25]. The influence of all these variables was evaluated in terms of the analytical signal (i.e. chromatographic peak area of each target analyte).

As the DLLME is carried out after the analytes leaching, the leaching solvent was also employed as disperser solvent in DLLME in order to make compatible both techniques. Thus, unlike conventional DLLME, in this case the disperser solvent, instead of the aqueous phase, contains the target compounds. The aqueous phase is not used as donor phase but as supporting solvent to make the DLLME possible (i.e., to form the cloudy solution and to transfer the analytes to the extraction solvent). Hence, a multicomponent solution of 100 ng mL^{-1} of the target analytes was employed as disperser solvent in the different DLLME studies. Later, the mixture of disperser and extraction solvent were injected into 5 mL of deionized water.

The extraction time was not studied in this work because it is well known that in this extraction technique the surface area between the extraction solvent and the aqueous phase is infinitely large so the transfer of the analytes is fast. The equilibrium state is achieved quickly and the extraction time is very short. This is the most important advantage of DLLME technique [25].

The surrogate (BZ-d_{10}) was not used to perform the DLLME optimization since it could be affected in the same or different way as analytes and could provide wrong conclusions.

3.1.1. Study of the extraction solvent and disperser solvent

The combination of the extraction solvent and the disperser solvent is an important issue in the DLLME process that requires an exhaustive study prior to the final selection. The extraction solvent should extract the target analytes efficiently and have low solubility in the aqueous phase. Moreover, as only a few microliters of extraction solvent are employed, a solvent with a higher density than water is recommended in order to remain in the bottom of the extraction tube and ease its collection. On the other hand, the disperser solvent should be miscible in both the supporting aqueous solution and the organic extraction solvent, and has also to form the so-called cloudy solution. Moreover, after centrifugation, a phase separation has to be achieved. In this sense, dichloromethane and chloroform were studied as extraction solvents, and acetone, acetonitrile and ethanol were studied as disperser solvents.

Therefore, a bivariant study considering all the possible combinations was performed. For this purpose, mixtures of 1 mL of each disperser solvent (940 µL) with each extraction solvent (60 µL) were injected into 5 mL of deionized water. When dichloromethane was used as extraction solvent, no cloudy solution was formed. Furthermore, when the combination ethanol-chloroform was tested, no phase separation occurred after centrifugation. The best results were accomplished when the mixture acetone-chloroform was used. Thus, acetone and chloroform were selected as disperser and extraction solvents, respectively, for further studies.

3.1.2. Effect of the disperser solvent volume

As the disperser solvent contains the target analytes, the higher the volume of acetone injected the higher will be the amount of analyte extracted. Thus, mixtures of different volumes of the acetone standard solution and 60 µL of chloroform, with a total

mixture volume ranging from 0.5 to 5 mL, were tested. Volumes above 2 mL redounded in no phase separation. Therefore, a total volume mixture of 2 mL (1940 µL of disperser solvent in this case) was finally chosen.

3.1.3. Effect of the extraction solvent volume

When the extraction solvent volume is increased, the amount of extracted analyte is expected to increase too, but it should be taken into account that the dilution effect is also increased. Thus, a careful study is needed in order to achieve the best results.

In this sense, mixtures of chloroform (ranging from 40 to 120 µL) and the disperser solvent, with a total volume of 2 mL were tested. 40 µL of chloroform was disregarded because there was no phase separation. The rest of the results are plotted in Figure 2, which shows that the higher analytical signals were obtained when the smaller extraction solvent volume was employed, probably due to the dilution effect. Thus, 60 µL of extraction solvent was employed in the subsequent experiments.

<Figure 2>

3.1.4. Effect of the pH of the supporting aqueous solution

The influence of the pH of the supporting aqueous solution on the extraction efficiency was studied. Different aqueous solutions were adjusted to pH values ranging from 2 to 8. For non-ionisable compounds (i.e., IMC, 4-MBC, EMC and OCR) no significant changes were observed. In case of phenolic compounds such as ES, HS and BZ3 are better extracted at acidic pH rather than alkaline pH, since at acidic pH their phenolic moieties are not ionized and the extraction is favoured. However, the extraction of EDP is not favoured at very low pH since its amine moiety is protonated (and thus charged). In summary, the best responses were obtained at mild acidic pHs rather than high pHs. Hence, the aqueous solutions employed as supporting solvent were adjusted to pH 4 before the injection of the disperser-extraction solvent mixture.

3.1.5. Effect of the ionic strength of the supporting aqueous solution

In general terms, the addition of salt reduces the solubility of the organic compounds in water and forces them to pass to the extraction solvent improving the extraction efficiency (*salting-out* effect). Thus, in order to study this effect, NaCl was

added to the supporting aqueous solution at concentration values up to 15 % (m/v). For saline contents of 10 to 15 % the extraction solvent floated on the aqueous phase as an extremely thin layer, with the subsequent difficulty to collect it. For this reason NaCl contents higher that 7.5% were discarded. Figure 3 shows that the higher was the ionic strength the lower were the responses. This could be explained by the fact that increasing the saline content of the aqueous phase significantly increases the volume of the sedimented phase obtained (from 20 to 70 µL). The obtained results indicate that the dilution becomes more important than the *salting-out* effect for this case. Therefore, the ionic strength of the supporting aqueous solution was not adjusted in further experiments.

<Figure 3>

3.2. Study of the experimental variables involved in the leaching procedure

For the determination of the UV filters in sand samples by DLLME, firstly, is necessary to leach them from the solid matrix. The leaching solvent volume and vortex mixer time were studied to achieve the higher analytical responses. A sand blank sample spiked with 100 ng g^{-1} of the target analytes was employed to carry out this study.

As in the case of DLLME study, the surrogate was not employed in the leaching study since it could be affected in the same or different way as analytes and could lead to wrong conclusions.

3.2.1. Effect of the leaching solvent type

The selection of the leaching solvent is a critical variable since the leaching and the DLLME processes must be compatible. On one hand, it should effectively leach the target analytes to the samples and, on the other hand, behave as a good disperser solvent in the DLLME. For the last reason, acetone was selected as leaching solvent because of the results obtained in *3.1.1.*

3.2.2. Effect of the leaching solvent volume

The influence of the leaching solvent volume in the analytical signal was studied. For this purpose, different volumes ranged from 5 to 20 mL of acetone were

added to the spiked sand blanks placed into the 50 mL screw cap glass centrifuge tubes. After that, the tubes were vigorously shaken with vortex mixer during 5 s and centrifuged at 5000 rpm for 10 min. The supernatant acetone was collected with a syringe and filtered through 0.45 μm nylon membrane filters. Then, the acetone-chloroform mixture was prepared and subjected to the DLLME process (see section *2.3.1*). As can be seen in Figure 4 the analytical signal decreases when the volume of acetone is increased. This is due to the dilution of the target analytes in the leached phase. Volumes below 5 mL did not provide satisfactory results, since a high amount of acetone remained soaking the sand sample. Then, the volume of acetone employed in the leaching process in further experiments was 5 mL. Nevertheless, two additional consecutive extractions with 1 mL of acetone each were carried out in order to increase the extraction efficiency (see Section 3.4).

<Figure 4>

3.2.3. Effect of the vortex mixer agitation time

The vortex mixer agitation time was studied up to 60 s. The results are shown in Figure 5. As can be seen, shaking times longer that 20 s did not provide better responses. Therefore, 20 s were selected for further experiments.

<Figure 5>

Besides, it is worth remarking that direct evaporation of the leaching solvent after the leaching of the analytes from the sand sample instead of carrying out the DLLME process was tested. The residue obtained after the evaporation was redissolved in a low volume of chloroform (50 µL) and injected into the GC-MS system. Although this methodology is simpler, worse results were achieved since the analytical signals observed for the analytes were considerably lower than those obtained when the DLLME process was carried out. It could be attributed to losses during the evaporation or to the adsorption into the walls of the evaporation tube. Moreover, it should be emphasized that an additional clean-up is achieved by DLLME.

3.3. Use of surrogate

In order to reduce the variability of the measurements, especially caused by the GC injection and the handling of low volumes in the DLLME process, the use of deuterated benzophenone, i.e., benzophenone-d_{10} (BZd_{10}), as surrogate was considered. Thus, A_i/A_{sur} (where A_i is the peak area of the target analyte and A_{sur} that of the surrogate) was used as response function for quantification purposes. BZ-d_{10} was selected for various reasons: (1) it is extractable in chloroform by the DLLME proposed method; (2) its volatility is suitable to be measured by GC; (3) as it is a deuterated compound, its possible presence in the environmental samples is nil, on the contrary of its non-deuterated homologous; and (4) it does not present ionisable functional groups in its structure, and thus, its extraction is not influenced by pH. Thus internal calibration was used instead of external calibration.

3.4. Study of matrix effects and leaching efficiency

In order to evaluate matrix effects, the following experiments were performed by triplicate: on one hand, a dried sand blank was subjected to the leaching process. After centrifugation, 2 mL of the supernatant were spiked with the target analytes at 200 ng mL^{-1} and the surrogate at 100 ng mL^{-1}; on the other hand, 2 mL of an acetone standard solution containing the analytes and surrogate at the same concentration than the above-mentioned solution was also prepared. Both solutions were subjected to the DLLME and measured in the GC-MS system. The obtained recoveries were 80±12, 94±7, 86±11, 92±6, 106±15, 82±9, 84±12 and 95±12 % for ES, HS, IMC, MBC, BZ3, EMC, EDP and OCR, respectively. These results show that matrix effects caused by the sand sample are negligible.

In order to evaluate the leaching efficiency, the following experiments were performed in triplicate: on one hand, a dried sand blank was spiked with the target analytes at 100 ng g^{-1}, and subjected to the leaching process. After centrifugation, 2 mL of the supernatant were collected and spiked with the surrogate at 100 ng mL^{-1}. On the other hand, the same dried sand blank was subjected to the leaching process, and after centrifugation, 2 mL of the supernatant were spiked with the target analytes at 200 ng mL^{-1}, in order to simulate 100% leaching efficiency, and then with the surrogate at 100 ng mL^{-1}. All these solutions were subjected to the DLLME and measured in the GC-MS system. The leaching efficiencies obtained were below 70%. The experiments were repeated by performing two additional consecutive extractions with 1 mL of acetone each. The results showed that the extraction efficiency increased, but not quantitatively, since the target analytes partially remained in the acetone soaking the

sand sample. Then, an additional experiment was carried out in the same way to a dried sand blank spiked with the target analytes at 100 ng g^{-1} but also with the surrogate at 50 ng g^{-1}, and then subjected to the leaching process. After centrifugation, 2 mL of the supernatant were collected and subjected to the DLLME process. The results revealed quantitative apparent extraction efficiencies for all the target compounds (i.e., 105±14, 96±9, 104±10, 84±9, 87±4, 96±14, 100±14 and 104±14 % for ES, HS, IMC, MBC, BZ3, EMC, EDP and OCR, respectively) if the surrogate was added before the leaching process. These results show that the losses during the leaching process, presumably due to the volume of acetone cannot be totally recovered, are corrected with the use of the surrogate.

Based on these both experiments, it can be concluded that internal calibration, using standard solutions of the target compounds and surrogate in acetone can be used. In the case of samples, they need to be spiked with the surrogate before the leaching and DLLME processes.

3.5. Study of the drying temperature

In order to remove the water in the beach sand samples, which could affect the leaching and/or the DLLME processes, they must be dried. However, this could redound in analyte losses due to their volatilization and/or degradation. In this sense, the drying temperature was studied. Preliminary studies showed that several hours at around 100 ºC were needed to dry the samples. Therefore, in duplicate, a dried sand blank sample was spiked with 100 ng g^{-1} of the target analytes using an acetone standard solution. It was homogenized and left to evaporate at room temperature. Then, it was divided into three portions; one of them was left overnight at room temperature whereas the other two were left at 60 ºC and 100 ºC, respectively. Later, they were subjected to the proposed method. Results (Figure 6) show that losses were significant at 100 ºC, whereas they were negligible at 60 ºC. Thus, samples were dried at this temperature.

3.6. Analytical figures of merit of the proposed DLLME-GC-MS method

The quality parameters of the proposed method, such as enrichment factor, linearity, method limits of detection (MLOD) and quantification (MLOQ), and intra- and inter-day precision, were evaluated under the final optimized conditions. The results are summarized in Table 3.

The enrichment factors (EF) obtained for the DLLME process (defined as EF = C_{sed}/C_0, where C_{sed} is the concentration of the target compound in the organic sedimented phase and C_0 is the initial concentration of this compound, in this case, in the disperser solvent) ranged from 8.2±0.7 (OCR) to 50±4 (BZ3) (Table 3). The maximum EF value that could be obtained, corresponding to a total transfer of the target analytes from the disperser solvent to the extraction solvent, is calculated as V_0/V_{sed}, where V_0 is the disperser solvent volume and V_{sed} the sedimented phase volume. In the present work, V_0 corresponds to 2 mL and the V_{sed} obtained was around 25 µL. Thus, the maximum EF value that could be obtained in the present method corresponds to values around 80. Although the values obtained for some of the target compounds are relatively low, especially for OCR, it should not be forgotten that also an additional clean-up is achieved when the DLLME is performed.

The linearity was studied by measuring standard solutions in acetone containing the surrogate at 40 ng mL^{-1}, which were subjected to the DLLME process. A solution of the surrogate in acetone at 40 ng mL^{-1} was also analyzed as blank. Calibration curves were plotted using the ratio of the peak area of each target analyte to the surrogate (A_i/A_S) *versus* the analyte concentration. Results indicated that linearity reached at least 1000 ng mL^{-1} for all the target compounds. However, due to the low concentration levels expected for the target analytes, the calibration range was set from 10 to 50 ng mL^{-1}. The calibrations parameters are shown in Table 3 and reveal a high level of linearity in all cases.

The method limit of detection (MLOD) and quantification (MLOQ) of the target analytes are also shown in Table 3. As can be seen, the MLODs and MLOQs values were found to be in the pg g^{-1} level ranging from 18±1 to 53±6 pg g^{-1} and from 61±5 to 180±20 pg g^{-1}, respectively, which shows that the proposed method is suitable to determine these compounds at trace levels.

The precision of the method was evaluated applying the proposed method to a sand blank spiked at three concentration levels of the target analytes (5, 20 and 50 ng g^{-1}) and the surrogate at 20 ng g^{-1}, during the same working session (intra-day precision) or in different working sessions (inter-day precision). Results, expressed as relative standard deviation (RSD) of five measurements, are shown in Table 3 and reveal that good precision was achieved for all the target analytes.

3.7. Application of the proposed method to the analysis of real samples

Four beach sand samples collected in the summer season were analyzed using the proposed DLLME-GC-MS method. Results are shown in Table 4. As can be seen, all the samples analyzed contained appreciable amounts of several of the UV filters under study. Specifically, the samples with higher content of UV filters (concentration and type) were Samples 1 (*Malvarrosa beach*) and Sample 4 (*Los ingleses beach*) in accordance with the fact that these beaches are more crowded than the other two. Moreover, ES and OCR are the most abundant UV filters in beach sediments samples since these compounds are widely employed in sunscreen creams formulations nowadays.

However, it should be noted that the concentration of UV filters found in this environmental samples could be highly variable as it depends on the people concourse, the number of users of sunscreens products, the water tide and the sampling date, among other factors.

Nevertheless, the data obtained by this method, jointly to those obtained by those methods focused in the analysis of water samples, could aid to evaluate the impact of the UV filters in the marine ecosystem [36], thus obtaining important conclusions from an environmental standpoint.

4. Conclusions

A sensitive analytical method based on vortex-assisted leaching followed by DLLME and GC-MS determination is proposed to determine eight fat-soluble UV filters at trace levels in beach sand samples.

The study of the matrix effects and the leaching efficiency reveal that internal calibration using standard solutions of the target compounds and surrogate in acetone can be used. The beach sediment samples were spiked with the surrogate and subjected to both the leaching and DLLME processes.

Good analytical features, including limits of detection, sensitivity and intra- and inter-day precision are obtained.

The proposed method can be considered both user and environmentally-friendly since although organic solvents are necessary to carry out the extraction process, their amounts have been minimized by the use of the DLLME procedure.

The proposed method was successfully applied to the analysis of four samples from different origin. In all cases, ES and OCR are the UV filters found at higher

concentration. This is a reasonable fact taking into account that these are two of the most commonly used UV filters in cosmetic formulations today.

Finally, it should be said that the proposed method can be used from an environmental surveillance standpoint to evaluate the fate of these emerging pollutants

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References

[1] A. Chisvert, A. Salvador, in *Analysis of cosmetic products,* ed. A. Salvador, A. Chisvert, Elsevier, Amsterdam, 2007, ch.3, pp. 83-140.

[2] A. Salvador, A. Chisvert, Sunscreen analysis: A critical survey on UV filters determination, *Anal. Chim. Acta*, 2005, 537, 1-14.

[3] Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.

[4] D.L. Giokas, A. Salvador, A. Chisvert, *Trends Anal. Chem.*, 2007, 26, 360-374.

[5] M.S. Díaz-Cruz, D. Barceló, *Trends Anal. Chem.,* 2009, 28, 708-717.

[6] P.Y. Kunz, H.F. Galicia, K. Fent, *Toxicol. Sci.,* 2006, 90, 349-361.

[7] M. Coronado, H. de Haro, X. Deng, M.A. Rempel, R. Lavado, D. Schlenk, *Aquatic Toxicol.*, 2008, 90, 182-187.

[8] M. Schlumpf, P. Schmid, S. Durrer, M. Conscience, K. Maerkel, M. Henseler, M. Gruetter, I. Herzog, S. Reolon, R. Ceccatelli, O. Faass, E. Stutz, H. Jarry, W. Wuttke, W. Lichtensteiger, *Toxicology* 2004, 205, 113-122.

[9] S.D. Richardson, *Anal. Chem.*, 2011, 83, 4614-4648.

[10] P. Gago-Ferrero, M.S. Díaz-Cruz, D. Barceló, *Anal. Methods* 2013, 5, 355-366.

[11] M. Ricking, J. Schwarzbauer, S. Franke, *Water Res.,* 2003, 37, 2607-2617.

[12] H.Y. Jeon, Y. Chung, J.C. Ryu, *J. Chromatogr. A,* 2006, 1131, 192-202.

[13] R. Rodil, M. Moeker, *Anal. Chim. Acta*, 2008, 612, 152-159.

[14] P. Gago-Ferrero, M.S. Díaz-Cruz, D. Barceló, *Anal. Bioanal. Chem.*, 2011, 400, 2195-2204.

[15] Y. Kameda, K. Kimura, M. Miyazaki, *Environ. Pollut.*, 2011, 159, 1570-1576.

[16] H. Amine, E. Gomez, J. Halwani, C. Casellas, H. Fenet, *Marine Poll. Bull.*, 2012, 64, 2435-2442.

[17] E. Barón, P. Gago-Ferrero, M. Gorga, I. Rudolph, G. Mendoza, A. Mauricio Zapata, S. Díaz-Cruz, R. Barra, W. Ocampo-Duque, M. Páez, R.M. Darbra, E. Eljarrat, D. Barceló, *Chemosphere*, 2013, 92, 309-316.

[18] J. Schwarzbauer, V. Weigelt, R. Littke, *Org. Geochem.*, 2000, 31, 1713-1731.

[19] C. Plagellat, T. Kupper, R. Furrer, L.F. de Alencastro, D. Grandjean, J. Tarradellas, *Chemosphere*, 2006, 62, 915-925.

[20] R. Rodil, S. Schrader, M. Moeder, *J. Chromatogr. A,* 2009, 1216, 8851-8858.

[21] A. Nieto, F. Borrull, E. Pocurull, R.M. Marce, *Trends Anal. Chem.*, 2010, 29, 752-764.

[22] A. Wick, F. Thomas, A. Ternes, *J. Chromatogr. A*, 2010, 1217, 2088-2103.

[23] N. Negreira, I. Rodríguez, E. Rubí, R. Cela, *J. Chromatogr. A*, 2011, 1218, 211-217.

[24] N. Negreira, I. Rodríguez, E. Rubí, R. Cela, *J. Chromatogr. A*, 2009, 1216, 5895-5902.

[25] M. Rezaee, Y. Assadi, MR. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, *J. Chromatogr. A*, 2006, 1116, 1-9.

[26] M. Rezaee, Y. Yamini, M. Faraji, *J. Chromatogr. A*, 2010, 1217, 2342-2357.

[27] I. Tarazona, A. Chisvert, Z. León, A. Salvador, *J. Chromatogr. A*, 2010, 1217, 4771-4778.

[28] N. Negreira, I. Rodriguez, E. Rubi, R. Cela, *Anal. Bioanal. Chem.*, 2010, 398, 995-1004.

[29] P. Zhang, Z. Shi, Q. Yu, Y. Feng, *Talanta*, 2011, 83, 1711-1715.

[30] Y. Zhang, H.K. Lee, *J. Chromatogr. A*, 2012, 1249, 25-31.

[31] JW. Wu, HC. Chen, WH. Ding, *J. Chromatogr. A*, 2013, 1302, 20-27.

[32] YC. Ku, MI. Leong, WT. Wang, SD. Huang, *J. Sep. Sci.*, 2013, 36, 1470-1477.

[33] Y. Zhang, H.K. Lee, *Anal. Chim. Acta* 2012, 750, 120-126.

[34] Y. Zhang, H.K. Lee, *J. Chromatogr. A*, 2013, 1271, 56-61.

[35] D. Ge, H.K. Lee, *J. Chromatogr. A,* 2012, 1251, 27-32.

[36] A. Tovar-Sánchez, D. Sánchez-Quiles, G. Basterretxea, J.L. Benedé, A. Chisvert, A. Salvador, I. Moreno-Garrido, J. Blasco, PLOS One 8 (2013) e65451.

Figure captions.

Fig. 1 A chromatogram obtained applying the proposed DLLME-GC/MS method to a sand blank spiked with 20 ng g^{-1} of the target analytes and the surrogate (BZ-d_{10}) (see text for experimental details)

Fig. 2 Effect of the extraction solvent volume on the DLLME process (extraction conditions: 5 mL of deionized water solution, mixtures of 2 mL of acetone containing 100 µg L^{-1} of the target analytes as disperser solvent and chloroform as extraction solvent, with different volumes of chloroform)

Fig. 3 Effect of the ionic strength of the aqueous phase on the DLLME process (extraction conditions: 5 mL of deionized water adjusted at pH 2-4 and at different ionic strength values, 1940 µL of acetone containing 100 µg L^{-1} of the target analytes as disperser solvent mixed with 60 µL of chloroform as extraction solvent)

Fig. 4 Effect of the leaching solvent volume on the analytical signal (leaching conditions: 10 g of sand blank spiked with the target analytes at 100 µg L^{-1}, different acetone volumes and 5 s of vortex mixer agitation)

Fig. 5 Effect of the vortex mixer agitation time on the analytical signal (leaching conditions: 10 g of sand blank spiked with the target analytes at 100 µg L^{-1}, 5 mL of acetone and different times of vortex mixer agitation)

Fig. 6 Effect of the drying temperature on the analytical signal (see text for experimental conditions. A_i/A_S corresponds to the ratio of the peak area of each target analyte to the surrogate (BZ-d_{10}))

Fig. 1

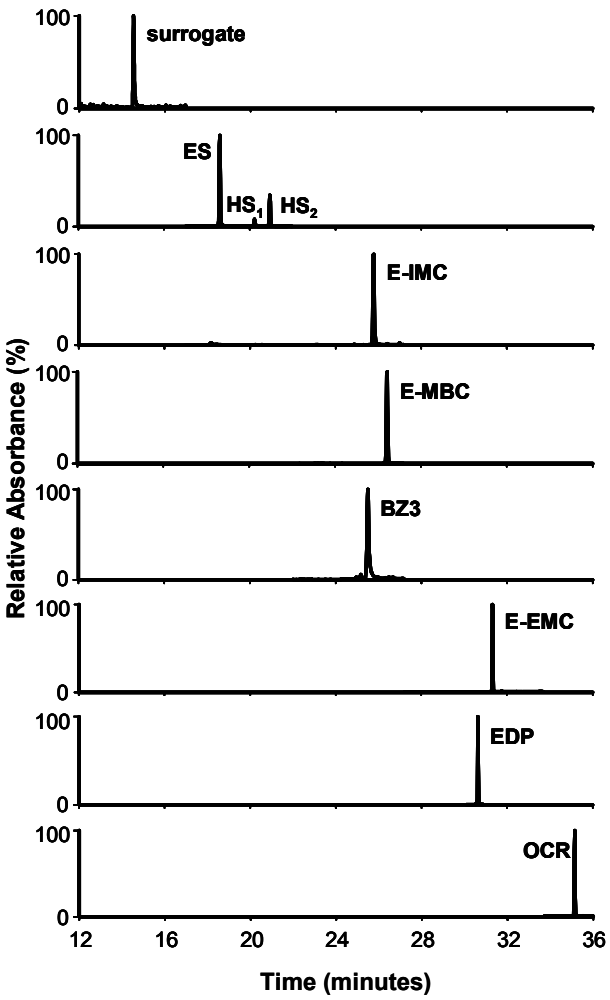


Fig. 2

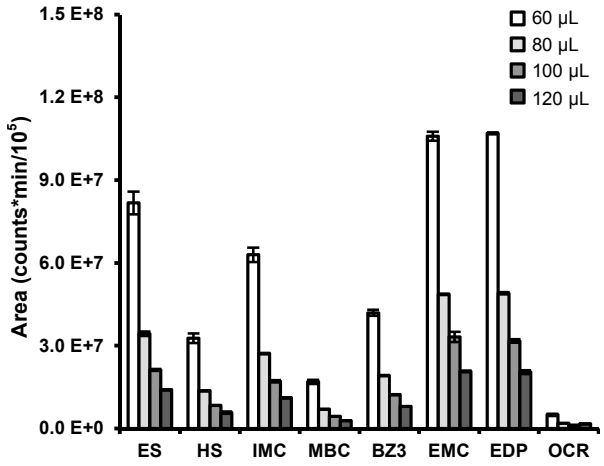


Fig. 3

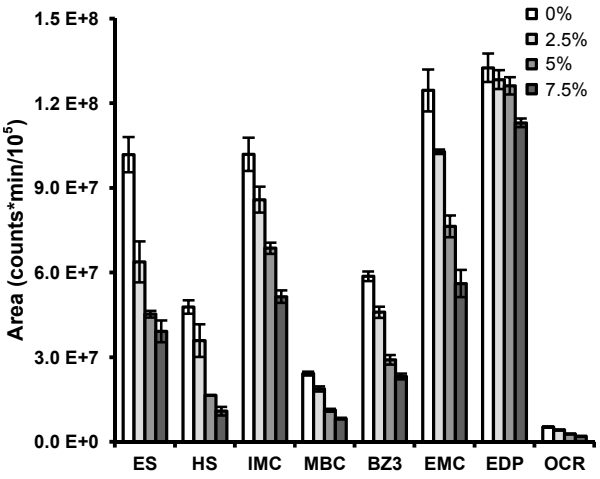


Fig. 4

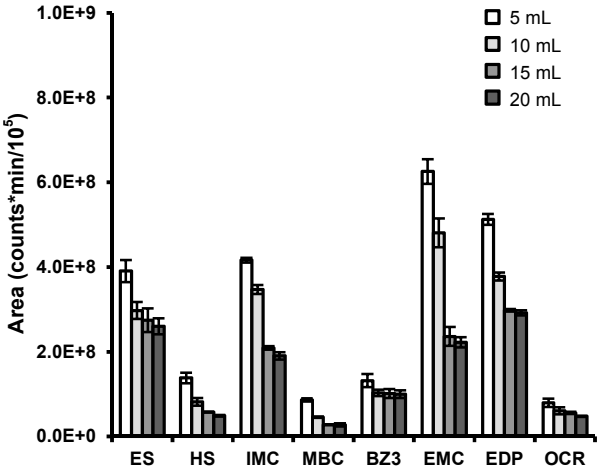


Fig. 5

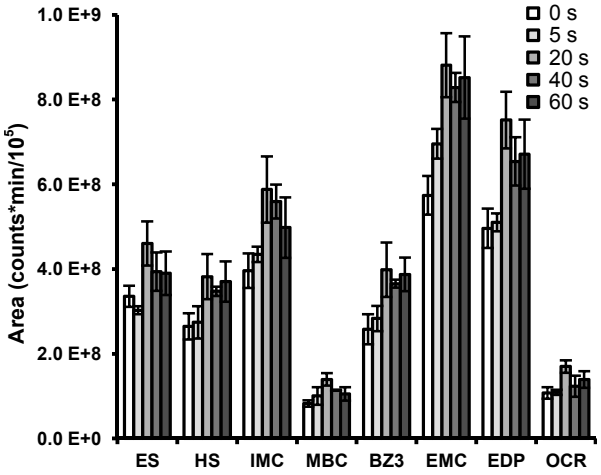


Fig. 6

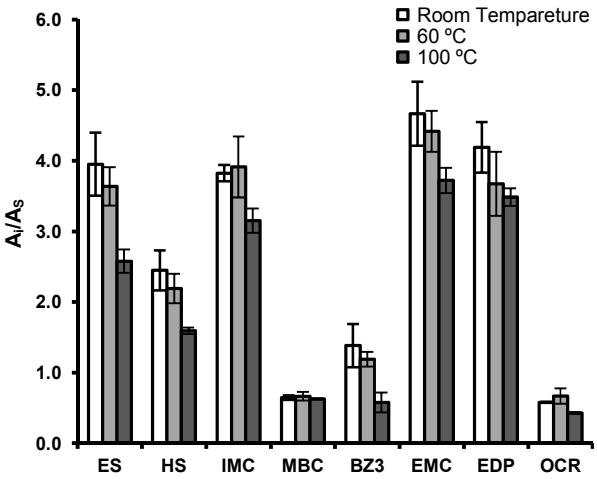
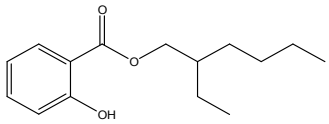
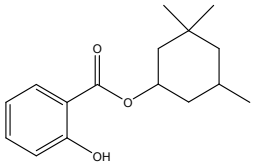
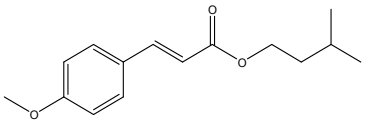
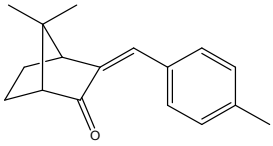
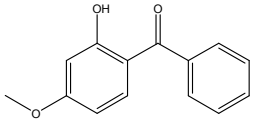
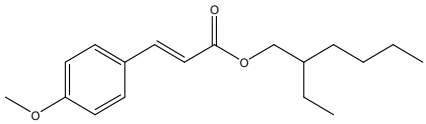
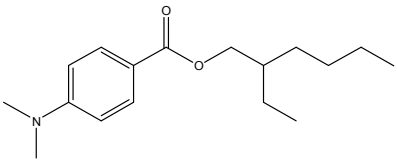
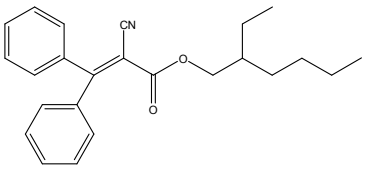


Table 1. Chemical structure and some data of the target compounds.

UV filter	Chemical structure	Molecular formula	CAS number
2-ethylhexyl salicylate (ES)		$C_{15}H_{22}O_3$	118-60-5
Homosalate (HS)[a]		$C_{16}H_{22}O_3$	118-56-9
Isoamyl 4-methoxycinnamate (IMC)[b]		$C_{15}H_{20}O_3$	71617-10-2
3-(4-methylbencylidene) camphor (4-MBC)[b]		$C_{18}H_{22}O$	36861-47-9
Benzophenone-3 (BZ3)		$C_{14}H_{12}O_3$	131-57-7
2-ethylhexyl 4-methoxycinnamate (EMC)[b]		$C_{18}H_{26}O_3$	5466-77-3
Ethylhexyl dimethyl PABA (EDP)		$C_{17}H_{27}NO_2$	21245-02-3
Octocrylene (OCR)		$C_{24}H_{27}NO_2$	6197-30-4

[a] There are two isomers (HS_1 and HS_2).

[b] There are two geometrical isomers (Z and E) when exposed to light.

Table 2. GC-MS features of the target compounds.

UV filter	Retention time (min)	m/z [a]	Acquisition time window (min)
BZ-d_{10} (surrogate)	14.44	82, **110**, 192	10.0-17.0
ES	18.45	**120**, 138, 250	17.0-22.0
HS	20.04 (*HS*$_1$), 20.75 (*HS*$_2$)	120, **138**, 262	17.0-22.0
IMC	21.20 (*Z*), 25.54 (*E*)	161, **178**, 248	20.0-27.2
MBC	24.50 (*Z*), 26.23 (*E*)	128, 211, **254**	22.0-27.2
BZ3	25.33	151, **227**, 228	22.0-27.2
EMC	29.78 (*Z*), 31.22 (*E*)	161, **178**, 290	27.2-30.0 31.0-33.7
EDP	30.62	148, **165**, 277	30.0-31.0
OCR	35.04	**204**, 232, 360	33.7-40.0

[a] The m/z values used as quantifiers are shown in bold.

Table 3. Main analytical parameters of the proposed DLLME-GC-MS method.

UV filter	EF[a]	Slope[b] ± deviation (ng^{-1} mL)/ 10^5	Regression coefficient (r^2)[b]	MLOD[c] (pg g^{-1})	MLOQ[d] (pg g^{-1})	Precision, RSD[e] (%)					
						Intra-day			Inter-day		
						5 ng g^{-1}	20 ng g^{-1}	50 ng g^{-1}	5 ng g^{-1}	20 ng g^{-1}	50 ng g^{-1}
ES	25 ± 2	4900 ± 200	0.995	38 ± 5	130 ± 20	14	8	6	16	8	7
HS	19 ± 1	4000 ± 100	0.997	53 ± 6	180 ± 20	13	9	5	15	9	8
IMC	38 ±3	6100 ± 200	0.998	41 ± 5	140 ± 20	11	6	4	14	7	6
MBC	42 ± 2	1140 ± 70	0.997	29 ± 2	96 ± 8	10	8	5	13	8	7
BZ3	50 ± 4	2000 ± 100	0.997	41 ± 5	140 ± 20	11	5	4	11	10	7
EMC	21 ± 2	9200 ± 500	0.9991	18 ± 1	61 ± 5	9	7	8	12	9	9
EDP	31 ± 3	8100 ± 500	0.998	46 ± 9	150 ± 30	7	7	5	15	12	8
OCR	8.2 ± 0.7	1700 ± 100	0.997	35 ± 3	117 ± 9	7	7	7	13	11	10

[a] EF: Enrichment factor, as the mean of three replicates.

[b] Working range: 10-50 ng mL^{-1}. Number of calibration points: 6.

[c] MLOD: method limit of detection, calculated as 3 times the signal-to-noise ratio.

[d] MLOQ: method limit of quantification, calculated as 10 times the signal-to-noise ratio.

[e] Relative standard deviation (RSD); five replicate analysis of spiked sand blank at different concentrations of the target analytes during the same working session (intra-day precision) or in different working sessions (inter-day precision)

Table 4. UV filters content found in beach sand samples after applying the proposed DLLME-GC-MS method.

UV filter	Concentration (ng g^{-1})			
	Sample 1[a]	Sample 2[b]	Sample 3[c]	Sample 4[d]
ES	5.3 ± 0.2	2.6 ± 0.2	1.8 ± 0.5	12 ± 1
HS	1.8 ± 0.2	1.06 ± 0.04	< LOQ	4.9 ± 0.7
IMC	1.3 ± 0.3	< LOQ	< LOQ	1.2 ± 0.3
MBC	0.9 ± 0.1	< LOQ	< LOQ	2.0 ± 0.4
BZ3	1.0 ± 0.1	< LOQ	< LOQ	< LOQ
EMC	2.1 ± 0.3	0.9 ± 0.2	< LOQ	10 ± 1
EDP	< LOQ	< LOQ	< LOQ	< LOQ
OCR	8 ± 1	1.7 ± 0.4	5.2 ± 0.9	25 ± 3

[a] Sample 1: *Malvarrosa beach* (Valencia, Spain).

[b] Sample 2: *Pinedo beach* (Valencia, Spain).

[c] Sample 3: *Patacona beach* (Valencia, Spain).

[d] Sample 4: *Los ingleses beach* (Gran Canaria Island, Spain).