On-line renewable solid-phase extraction hyphenated to liquid chromatography for the determination of UV filters using bead injection and multisyringe-lab-on-valve approach

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ABSTRACT

For the first time, an automatic sample pre-treatment/detection method is proposed for the multiclass determination of UV filters (namely, benzophenone-3, ethylhexylmetoxycinnamate, butylmethoxydibenzoylmethane and homosalate) in environmental samples. The new methodology comprises in-line solid-phase extraction (SPE) of the target analytes by exploiting the bead injection (BI) concept in a mesofluidic lab-on-valve (LOV) format, with subsequent determination by liquid chromatography (LC). The proposed microanalytical system, using a multisyringe burette as propulsion unit, automatically performed the overall SPE steps, including the renewal of the sorbent in each analytical cycle to prevent sample cross-contamination and the post-extraction adjustment of the eluate composition to prevent chromatographic band broadening effects. In order to expedite the LC separation, a C18 monolithic column was applied and an accelerated isocratic elution was carried out by using a cationic surfactant as mobile phase additive. The LOV-BI-LC method was proven reliable for handling and analysis of complex matrices, e.g., spiked swimming pool water and seawater, with limits of detection ranging between 0.45 and 3.2 μgL−1 for 9 mL sample volume. Linear calibration was attained up to 160 μgL−1 for homosalate and up to 35 μgL−1 for the other target analytes, with good reproducibility (RSD < 13%, for 5 different SPE columns). The hyphenated scheme is able to process a given sample simultaneously and within the same time frame than the chromatographic separation/determination of the formerly pre-treated sample, providing concentration values every 9 min. Hence, the sample throughput was enhanced up to 33 times when compared with previously reported off-line SPE methods. A drastic reduction in reagent consumption and effluent production was also attained, contributing to the development of an environment-friendly analyzer.

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1. Introduction

Organic UV filters comprise a wide range of chemical compounds that have absorptive properties of the solar radiation. The growing concern about the pathological effects of sun exposure, namely skin cancer, originated a widespread use of these compounds not only in sunscreen products but also in a wide range of personal care products used daily by millions of people. Despite being present in cosmetics at low concentrations, usually comprised between 0.1 and 10%, their physical and chemical properties, associated with the high potential for input into surface waters, introduced an additional concern about their eventual accumulation and toxicological effects [1]. Therefore, UV filters are presently considered contaminants of emerging concern [2,3] and reviews focused on this novel problem have been recently published [1,4,5].

Although the quantification of UV filters in cosmetics is well established [6], the determination in environmental and biological matrices is complex, because analyte enrichment and matrix clean-up are necessary to detect the analytes at trace levels [7]. Efforts have been made in order to achieve sensitive and selective methods to cover a wide range of target analytes, combining manual sample preparation with chromatographic separations. For sample preparation, liquid–liquid extraction [8], cloud-point extraction [9], solid-phase extraction (SPE) [10–14], solid-phase micro extraction (SPME) [15,16] and stir bar sorptive extraction (SBSE) [17,18] were proposed, followed either by gas chromatography (GC) coupled to mass-spectrometry (MS) [9,10,12–18] or by liquid chromatography coupled to diode array (DAD) [9,10] or MS [11,12] detection. Actually, these methods allowed the detection of UV-screen chemicals at μgL−1 or ngL−1 levels but at the same time they are time-consuming and labour intensive. Moreover, the information...
about the presence of UV filters in surface waters or biological fluids is scarce and the analytical methods reported so far are not amenable to use in monitoring protocols. Hence, automation through on-line coupling of extraction procedures to chromatography would provide a suitable tool for environmental research [19].

In this context, straightforward and cost-effective methodologies can be implemented by using flow injection analysis and related techniques prior to column separation systems [20]. The progress in the miniaturization resulted in the introduction of the third generation of flow injection analysis, the so-called lab-on-valve (LOV) [21]. Besides the downsizing in the amount of reagents used, the microchannels engraved on the LOV module allowed the accurate handling of bead material in a fully automatic fashion, defined as bead injection (BI) [22]. This meso-fluidic technique was applied to sorptive beads, allowing the implementation of new microSPE protocols for a wide range of analytes [22,23]. The hyphenation of BI to LC has been merely reported once for multiresidue determination of pharmaceutical compounds using a particulate LC column [24]. In this case, the BI-LOV approach was associated to the multisyringe flow injection (MSI) analysis [25,26], providing a multi-channel propulsion unit, which can handle different solutions and solvents simultaneously at the low µL level [26,27].

The objective of the present work was the development and validation of an MSI-BI-LOV system for on-line coupling of microSPE with LC for multiclass determination of UV filters in environmental samples. The target analytes were chosen in order to represent four different families of the most used UV-sunscreen agents, that is benzophenones (benzophenone-3, BP3), dibenzoylmethanes (butylmethoxydibenzoylmethane, BMDBM), cinnamates (ethylhexylocinnaminate, EHC) and salicylates (homosalate, HMS). Due to the high range of polarity of these families of compounds [Electronic Supplementary material, Table S1], it would not be possible to attain a good resolution and a short analysis time by resorting to previously described reversed-phase chromatographic methods [9,10]. Hence, we propose for the first time an ultra fast meso-fluidic LOV-BI method coupled to fast monolithic C18 column separation in surfactant media for automatic determination of sunscreen agents in different matrices.

2. Material and methods

2.1. Reagents and solutions

Ultra pure water (resistivity > 18 MΩ cm−1) obtained from a MilliQ (Millipore, Bedford, MA, USA) system was used for the preparation of all aqueous solutions. Methanolic solutions were prepared with HPLC grade methanol (Merck, Darmstadt, Germany). A 95% (v/v) MeOH/H2O was used as eluent and solution for sorbent conditioning. All glassware was periodically washed with methanol at least once a week.

Two reversed-phase co-polymeric bead sorbents with hydrophobic/hydrophilic moiety balance and of spherical shape were used: Oasis HLB (30 μm, Waters, Milford, MA, USA), and Focus (44 μm, Varian, Palo, CA, USA). Bead suspensions were prepared by mixing approximately 200 mg of sorbent with 2 mL of ethanol.

The stock standard solutions of UV-sunscreen chemicals, benzophenone-3 (BP3), butylmethoxydibenzoylmethane (BMDBM), ethylhexylocinnaminate (EHC) and homosalate (HMS) (Merck), were prepared by accurately weighing the appropriate mass of solid or liquid and making it to a final concentration of 500 mg L−1 with methanol. These solutions were stored at 4 °C in the darkness. Working standard solutions were prepared by diluting the stock solution with 10 mmol L−1 HCl (for preconcentration) or pure methanol (for experiments with direct injection). The same procedure was adopted for benzylcinnamate (BZC) and dibenzoylmethane (DBM) (Sigma–Aldrich, St. Louis, MO, USA), both used as internal standard for chromatographic analysis.

For the chromatographic assays, sodium dodecyl sulfate (SDS), cetyltrimethylammonium chloride (CTAC) and diocetyl sulfosuccinate (DOSS) (Sigma–Aldrich) were used as mobile phase additives. Solutions with concentrations in the range of 25–100 mmol L−1 were prepared by dissolving the appropriate amount of SDS or DOSS in water or by dilution of a commercial solution of 25% (w/v) CTAC. Acetonitrile (ACN) (Merck) was employed as organic modifier of the mobile phase. All mobile phase components were filtered through a 0.45 μm filter and degassed before use by ultrasound radiation during 15 min.

2.2. Chromatographic analysis

The chromatographic assays were performed on a Merck/Hitachi liquid chromatography equipment (LaChrom 7000 series, Hitachi Ltd., Tokyo, Japan), composed of an interface (D-7000), a high-pressure pump (L-7455) and a diode array detector (L-7100). Reversed-phase separation was performed in the isocratic mode with a mobile phase containing 65% of ACN and 35% of aqueous solution of 100 mmol L−1 CTAC. The analytical column consisted of a Chromolith RP-18e (100 mm × 4.6 mm i.d.) connected to a guard column of the same material (5 mm × 4.6 mm i.d.) both from Merck. A Rheodyne (Rohnert Park, CA, USA) 7725i six-port rotary valve equipped with a 400 μL loop (i.d. 0.75 mm) was employed as interface between the flow system and the LC equipment. All the chromatographic data were automatically recorded by resorting to D-7000 software. For comparison purposes, two conventional 5 μm C18-particle columns with dimensions of 150 mm × 3.9 mm i.d. (Xterra, Waters) and 250 mm × 4.6 mm i.d. (AlloSphere, Alltech, Deerfield, IL, USA) were evaluated.

For each sample, retention time, spectra, and peak area at maximum absorbance wavelength (see Table S1, Electronic Supplementary material) were compared with those obtained from standards, in order to identify and quantify the target analytes. BP3, BMDBM, EHC, and HMS were quantified at 290, 360, 305, and 235 nm, respectively. DBM (55 μg L−1) was used as internal standard for sample analysis, except for swimming pool water where degradation by free chlorine was detected. For this type of sample, BZC was added as internal standard at the same concentration level. These compounds were detected at 345 and 280 nm, respectively. Limits of detection (LOD) and quantification (LOQ) were estimated from the analysis of untreated water as the minimum concentration of a given species for which the signal-to-noise ratios are ≥3 and 10, respectively [24].

2.3. Flow manifold

The proposed flow system for the preconcentration, separation and determination of UV filters is depicted in Fig. 1. It comprises a multisyringe burette (Crisson Instruments, Allela, Spain) as propulsion unit equipped with two syringes (Hamilton, Bonaduz, Switzerland) of 5000 μL (syringe S1 and S2) and one of 2500 μL (syringe S3). A three-way commutation valve (NRsearch, Caldwell, NJ, USA) was placed at the head of each syringe, allowing the access to the solution reservoir (position off) or the flow network (position on). The multisyringe module was connected to a lab-on-valve unit made of poly(methylmethacrylate) (PMMA) containing 8 peripheral ports (with channels of 1.5 mm i.d.), which was mounted on the head of an eight-port multi-position selection valve (MPV, Crison Instruments). The central port was connected
to syringe S2 and also to any of the eight ports of the MPV, one at a time, allowing the aspiration of the sample (port 1), eluent (port 4), bead suspension (port 5) or air (port 6) into the holding coil (HC). In order to hold the beads into the cavity of channel 8, a 1-mm thick polypropylene frit with pore diameter of 10 µm (MoBiTec, Goettingen, Germany, ref. #2210) was placed between the exit of the channel and the fitting. The MPV channel 3 was used as waste. The special configuration of the dual channel 1 (Fig. 1), one of them being connected to syringe S1, enabled the exchange of samples or standards without aspiration of the solution into the HC, minimizing the risk of carryover between consecutive solutions loaded into the system. The manifold was built with 0.8 mm i.d. polytetrafluorethylene tubing (Omnifit, Cambridge, UK). The exception was the holding coil, with 5 mL of capacity, and the connections between syringes and reservoir flasks, which were built with 1.5 mm i.d. tubing. A three-way confluence, built in PMMA, was incorporated into the manifold, connecting both MPV channel 8 and syringe S3 to the injection valve (IV) of the LC equipment. The tubing length between MPV and the confluence was 8 cm while the connection between the confluence and the injection valve was 44 cm long.

The analytical procedure was executed and controlled by personal computer running software written in-house using Quick Basic 4.5 (Microsoft, Redmond, WA, USA). The software was designed to control the position of commutation valves, the speed and direction of piston movement on the multisyringe apparatus, and also the selection of ports on the MPV.

2.4. Protocol sequence

Automatic SPE and chromatographic analysis were fully synchronized, that is, one sample was processed by the chromatographic system, while the multisyringe procedure was applied in parallel to the next sample. The automatic SPE procedure comprised four main steps designated as (1) packing and conditioning of the extraction microcolumn, (2) sample loading, (3) elution and filling of the injection valve loop and (4) bead removal (see Electronic Supplementary material, Table S2). An empty SPE cartridge of 1 mL of capacity was mounted onto the port 5 of LOV by using a polyetheretherketone (PEEK) connector. Before beginning the analytical procedure, the cartridge was filled with an ethanolic suspension of beads. When the beads settled at the bottom of channel connecting to port 5, the system was ready for operation.

A brief description of the automatic MSFI-BI-LOV method is given below:

(1) **Packing and conditioning of the extraction column.** After filling S2 with 1320 µL of carrier (10 mmol L⁻¹ HCl) and the HC with 650 µL of 95% (v/v) MeOH/H₂O, 30 µL of bead suspension was collected through port 5 at a flow rate of 0.3 mL min⁻¹. The sorbent was washed by aspirating 1000 µL of water to perform washing of the beads plus methanolic solution. The conditioning procedure was completed by perfusing 1000 µL of extra acidic carrier over the sorbent bed at a higher flow rate.

(2) **Sample loading.** When new sample is to be analyzed, the connection tubing between LOV port 1 and the sample container was washed by aspirating 500 µL of liquid by S1 through the outlet of port 1. Thereafter, S2 performed the sequential aspiration of an air segment (250 µL) and sample (4000 µL). Next, 3000 µL of HC content were dispensed through port 8, the sample perfused through the sorbent and the target analytes were retained. Discarding the remaining sample and the air plug, and the washing of HC as well were ensured by pumping 4750 µL of carrier to waste (port 3). These operational steps were repeated up to 3 times, resulting in 9 mL as total volume of loaded sample. The sample loading step was finished by removing the non-retained species with 750 µL of carrier. Simultaneously, 375 µL of water were fed into the LC injection loop to avoid contamination by sample dispersion into the tubing connecting the confluence point to S3.

(3) **Elution of analytes.** First, an air segment (200 µL) was aspirated through port 6 to prevent the dispersion of the eluent into the carrier solution, followed by the aspiration of 700 µL of 95% (v/v) MeOH/H₂O. After refilling the syringes and reversing the direction of piston movement, the elution was performed by perfusing a nominal volume of 600 µL of 95% (v/v) MeOH/H₂O at 0.75 mL min⁻¹ through the sorbent bed, which were subsequently merged with 300 µL of water (nominal) at the T junction (Fig. 1). The mixture that filled the loop of the injection valve (267 µL of eluate + 133 µL of water) was immediately injected into the chromatographic system, whereupon the analytical run was started. Thereafter, the air and remaining 95% (v/v) MeOH/H₂O were removed from the HC towards waste by 1650 µL of carrier. Finally, the rotary valve was switched to the “load” position.
(4) Discarding of beads. The packed SPE microcolumn was wetted with 20 μL of 95% (v/v) MeOH/H2O whereupon the used beads were aspirated back into HC. The former column content was backflushed and disposed to waste (port 3) with carrier solution (1350 μL) followed by rinsing of the tubing connecting LOV (port 8) with the injection valve (1250 μL carrier). After this step, the system was ready for processing the next sample.

2.5. Samples

Bathing water samples, namely swimming pool water (SPW) and seawater (SW), were collected from a public swimming pool and Matosinhos beach in Porto (Portugal), respectively. To simulate water contamination from personal care products during recreational bathing activities (especially of children in small swimming pools), a volunteer applied a commercial sunscreen preparation containing 10 L of seawater for a period of 30 min, after which a sample was collected. After collection, all samples were immediately acidified to pH 2 and filtered through 0.22 μm membrane filter (Millipore). The free chlorine content of SPW samples was determined by resorting to the standard method based on N,N-diethyl-p-phenylene-diamine [28].

3. Results and discussion

3.1. Chromatographic method

Considering that screening methods should be fast, chromatographic assays of UV filters should be tailored to take the lowest time possible. To this end, we evaluated the usage of a monolithic column under isocratic elution conditions for separation. It should be borne in mind that isocratic separations do not require equilibration of the column after the analytical run and that the implementation of monolithic columns fosters a significant decrease in running time when compared with conventional particulate columns [29].

Isocratic LC methods reported in the literature [9,10] included the addition of surfactant molecules to the mobile phase for acceleration of the chromatographic runs as a result of the so-called “gradient effect”, where the most hydrophobic molecules are shifted to decreased retention times [30]. This strategy has been successfully applied using reversed-phase (RP) packed columns. However, to the best of our knowledge, this effect has not been exploited in RP separations using monolithic columns. Hence, the influence of a cationic (CTAC) and two anionic (SDS and DOSS) surfactants in the retention of the target compounds was evaluated. The flow rate was fixed at 1.5 mL min⁻¹ and 20 μL of a standard solution containing 5 mg L⁻¹ of each compound was injected into the LC system. Preliminary experiments were performed using mobile phase containing 80% acetonitrile:20% aqueous surfactant solution. However, the resolution attained was poor for BMDBM and EHMC. Hence, the organic modifier content was decreased to 60–70% (v/v) using an aqueous solution containing 100 mmol L⁻¹ of surfactant. The retention was evaluated by calculating the capacity factor (k') [30] for each analyte (Electronic Supplementary Material, Fig. S1).

The experimental results evidenced a decrease in retention times for the suite of analytes tested when compared to the values obtained for mobile phase without surfactant. Considering the concentration of surfactant (100 mmol L⁻¹) and the content of organic modifier (60–70%, v/v), the chromatographic separation takes place in conditions that can be defined as “high submicellar” [31]. In this case, the stationary phase is partially coated with surfactant molecules, where the hydrophobic tail is associated to the alkyl chains of the stationary phase and the polar head groups are oriented away from the surface, that is, the C18 column becomes eventually more hydrophilic. Furthermore, as micelle existence is unlikely at acetonitrile contents above 30%, surfactant monomers which compete with the stationary phase for the uptake of the target compounds will prevail in the bulk mobile phase [32], decreasing the retention time of the most hydrophobic compounds.

The reduction of k' was dependent on the additive and followed the order CTAC > DOSS > SDS. In fact, when comparing k' in the absence of surfactant a decrease between 41 and 60% was verified for k' upon the usage of CTAC regardless of the ACN/surfactant proportion. For DOSS and SDS the decrease was smaller (27–53% and 20–44%, respectively). Considering the absence of amine moieties and the presence of oxygen containing groups (carbonyl and hydroxyl) in the target analytes, the electrostatic interactions between the analytes and the cationic surfactant in solution would most likely explain the experimental results.

The effect of the concentration of CTAC on the retention times was also assessed. Because of the fact that the lowest k' was obtained with mobile phases of less water content (Electronic Supplementary material, Fig. S1) and that the higher the water content the better was the peak resolution, mobile phases containing either 30 or 35% of aqueous CTAC were tested (Table 1). The k' for all UV filters decreased by 25–30% when using 70% ACN compared to 65% ACN. The same trend was observed upon increasing CTAC concentration, showing a decrease of k' around 50% at 100 mmol L⁻¹ CTAC in relation to that of the mobile phase without surfactant. Hence, a mobile phase containing 65:35 (v/v) ACN/100 mmol L⁻¹ CTAC was selected as a compromise between run time and resolution. Using these conditions, BP3, BMDBM, EHMC, and HMS were quantified at 290, 360, 305, and 235 nm, respectively (see Electronic Supplementary Information Fig. S2). Though BMDBM and EHMC peaks eluted at close retention times, the detection at different wavelengths allowed their correct quantification.

The performance of the monolithic column was compared to that of conventional 5 μm C18-particulate columns (column A, 250 mm × 4.6 mm i.d., and column B, 150 mm × 3.9 mm i.d.). Calibration curves were established by injecting standard solutions
containing 2–10 mg L\(^{-1}\). The mobile phase composition was 65:35 (v/v) ACN/CTAC, except for the larger column, where the organic content was increased up to 80% following the recommendations by Giokas et al. [10]. The mobile phase was pumped at a flow rate of 1.5 mL min\(^{-1}\) for the monolithic column and column B or at 1 mL min\(^{-1}\) for column A. Monolithic column and column B showed a similar performance in terms of parameters of the calibration curves, with a maximum slope variation of 10%. On the other hand, the sensitivity of the assays was better with column A, with differences between 20 and 30% for BMDBM, EHMC and HMS, and 40% for BP3. The improvement in the separation efficiency was a consequence of the larger dimensions of this column [29]. Nevertheless, concerning run time and system backpressure, the monolithic column fostered the separation of target compounds in 9 min with a backpressure of 23 bar, in contrast with 12 min/160 bar of column B and 34 min/105 bar of column A. By the same token, the flow rate of the mobile phase in monolithic column separation can be in principle increased without loss of performance [33]. Actually, flow rates up to 3 mL min\(^{-1}\) were tested in this work, which gave rise to the decrease of run times up to 4.5 min with no significant deterioration of the separation efficiency and the operation of the chromatograph at a backpressure <50 bar. Hence, the usage of the monolithic column was proven particularly suited to the development of a screening method for monitoring purposes.

### 3.2. Design of the MSFI-BI-LOV-LC system

The implementation of microscale SPE based on the bead injection concept using the LOV format requires a precise control of volume delivering and flow rates applied, in order to attain excellent performance in all the steps of sample preparation, especially for bead trapping, sample handling and elution of analytes. These features can be provided by the MSFI module which might be furnished with syringes of varied volumes, adapted to the operational steps of the analytical procedure. In the present work, we exploited this option by using one main syringe (S2) for handling of fluids from and to the LC (bead suspension, sample, eluent and carrier) and two auxiliary syringes that performed the clean-up of the sample channel (S1) and the adjustment of the eluent composition (S3) before injection into the LC.

An important modification of previously described BI setups [24,34] was the mode of trapping the beads into the LOV microchannels. Usually, one or two pieces of PEEK tubing are placed inside the engraved LOV channels [24,34]. This strategy reduces the channel diameter and traps the beads for SPE. However, it also promotes a significant increase in backpressure, limiting the perfusion flow rates through the packed microcolumn [24]. In the present work, we replaced the PEEK stopper by a polypropylene frit (Fig. 1, port 8). Hence, an effective barrier for holding the beads without clogging the channel was available, fostering the implementation of ultra fast percolating schemes, as performed in this work, where flow rates up to 8 mL min\(^{-1}\) were used during the sample loading step. Additionally, by removing the piece of PEEK tubing, an extra portion of sorbent can be loaded, increasing the sorbent capacity available for analyte enrichment compared to earlier reported LOV-BI analyzers [34–36].

As to the choice of sorbent material for microSPE of UV filters, two factors were considered. First, it should possess reversed-phase sorptive capacity in a broad spectrum of polarity (log \(K_{\text{ow}}\) between 3.79 and 6.16 for the target analytes). Second, the physical characteristics of beads are also relevant for adequate handling within the LOV platform, requiring primarily spherical format, uniformity in size distribution and water-wettable properties. Two commercial available sorbents fulfilling these requirements were tested: Oasis HLB, a divinylbenzene-co-N-vinylpyrrolidone polymer and Focus, a patented product described as a polar enhanced styrene-divinylbenzene resin. For evaluation of their performance, the most polar analyte (BP3) was used. In both sorbents, readily and reproducible in-valve column formation and withdrawal was accomplished and a maximum breakthrough volume of 24 mL was achieved when a standard solution containing 3.0 mg L\(^{-1}\) of BP3 was percolated through the SPE microcolumn with constant monitoring of the effluent with a UV detector. The loading flow rate was varied within the range 3.0–8.0 mL min\(^{-1}\) for the preconcentration of 10 mL of 0.25 mg L\(^{-1}\) BP3 at 8.0 mL min\(^{-1}\) (using the same SPE column) was <3% for Oasis HLB and <8% for Focus. Hence, Oasis HLB was chosen for further experiments.

Considering the weakly acidic character of BP3, BMDBM and HMS and the reversed-phase mechanism of interaction with the SPE sorbent, 10 mmol L\(^{-1}\) HCl was used as carrier and sample medium for the preparation of the overall standard solutions. The same held true for the real samples. The elution of the compounds from Oasis HLB bed was carried out with a mixture of 95:5 (v/v) MeOH/H\(_2\)O. This eluent was chosen attending its capability to promote the desorption of the analytes, the negligible bubble evolvement within the flow network as a result of the addition of a low percentage of water and the chemical compatibility with the material (PMMA) from which the LOV piece was built. However, band broadening of the most polar UV filters was detected whenever 200 µL of eluate were injected in the chromatograph. This situation is commonly observed in on-line SPE when the eluent composition is significantly different from that of the mobile phase of the chromatographic separation [37]. To circumvent this problem, the multi-propulsion capability of the MSFI system was exploited for in-line dilution of the eluate with water (2:1), thereby providing a chemical composition similar to that of the mobile phase (namely, 65:35, v/v, ACN/aqueous phase). Dilution took place immediately before the injection valve loop by simultaneous activation of syringe S2 and S3. A “heart-cut” mode for injection of the eluate into the LC was utilized. Hence, the volume of the injection valve loop was increased to 400 µL, leading to enhanced method sensitivity. The nominal volume of 95% (v/v) MeOH/H\(_2\)O solution feeding the SPE microcolumn was adjusted to maximize peak areas taking into account the dead volume between the LOV apparatus and the injection valve loop. It has been proven that ≥414 µL of 95% (v/v) MeOH/H\(_2\)O (actual volume, propelled by S2) should be delivered to fill the injection valve loop with 400 µL of solution (267 µL of eluate + 133 µL of water added from S3). Nominal volumes of 95% (v/v) MeOH/H\(_2\)O solution ranging between 500 and 750 µL were tested, with maximum responses for the overall compounds at 600 µL. For all compounds, except HMS, peak areas were 75, 88, 95, 85, and 70% of that attained for 600 µL, considering nominal volumes of 500, 550, 650, 700 and 750 µL, respectively. The elution profile for HMS was however sharper as evidenced by peak areas that were 84, 84, 83, 70, and 52% of the maximum value (600 µL), considering the nominal volumes mentioned above.

The total time required for performing the automatic SPE protocol was 9 min, using a sample volume of 9 mL. Hence, the mobile phase flow rate for the chromatographic separation was fixed to 1.5 mL min\(^{-1}\) to synchronize sample preparation with chromatographic analysis.

### 3.3. Analytical performance of the MSFI-BI-LOV hyphenated to LC

The analytical performance of the proposed methodology was assessed taking into account the dynamic linear range, breakthrough volume, enhancement factor (EF), and precision (Table 2).
The use of a time-based strategy for introducing the sample into the manifold [38] allowed the implementation of mass-based calibration curves [39,40], by plotting the peak area or relative peak area against the mass of compound perfused through the SPE microcolumn. Thus, the sample volume to be processed can be adjusted according to the concentration of the analytes expected in the different real-life matrices. Calibration curves, with correlation coefficients above 0.995, were obtained by extracting amounts between 60 and 480 ng of each compound contained in a volume up to 12 mL, except for HMS. For this compound, the linear range comprised 240–1920 ng because the sensitivity of the chromatographic assay was lower than that of the other UV filters. In fact, using mobile phase as solvent, the molar absorptivity of HMS at $\lambda = 235$ nm was 0.0333 cm$^{-1}$ mg$^{-1}$ L$^{-1}$, which is, for example, ca. 40% the value attained for EHMC at $\lambda = 305$ nm (namely, 0.8080 cm$^{-1}$ mg$^{-1}$ L$^{-1}$).

Mass-based calibration was also used for the determination of analyte breakthrough volumes via the preconcentration of variable volumes over the range of 3–24 mL of a standard mixture containing 20 $\mu$g L$^{-1}$ of BP3 and BMDBM, 9 $\mu$g L$^{-1}$ of EHMC and 80 $\mu$g L$^{-1}$ of HMS. A multi-standard was applied in order to simulate the conditions that would be found in samples, where several UV filters may be present simultaneously. The combination of volumes and analyte concentrations provided mass values that were within the linear range previously established by a fixed-volume calibration using 9 mL of standards with different concentrations. Hence, by plotting the peak area against the volume percolated through the microcolumn (which is directly proportional to the mass of analyte), it was possible to identify the volume at which loss of linearity of the analytical signal occurred. From this volume and onwards the loaded analytes were pre-eluted from the LOV microcolumn by the standard/sample solvent itself. For all compounds, excepting BP3, the breakthrough volume was 12 mL. In the case of BP3, no breakthrough was observed up to the maximum volume loaded (24 mL), which is in agreement with the former results obtained by frontal analysis during the sorbent selection experiment. These results may also seem puzzling at a first glance because the more polar analyte (BP3) presented the highest breakthrough volume. However, it should be pointed out that the concentration applied for each target compound was not the same. In fact, for the same volume percolated through the sorbent, the mass of HMS (less polar analyte) was 4 times higher than that of BP3 (more polar analyte). Hence, at the breakthrough volumes established, the mass of HMS (present in 12 mL) was twice the mass of BP3 (present in 24 mL). Concerning the other two analytes (BMDBM and EHMC), the concentrations applied were closer to that of BP3. The better retention of BP3 could be explained by electrostatic interactions between the more polar analyte and the hydrophilic monomer of N-vinyl-pyrrolidone that appears at high percentages in Oasis HLB, conferring a mix-mode character to this sorbent.

The enhancement factor [41] was calculated as the ratio between the slopes of the calibration curves constructed by sub- jecting the sample to the BI protocol and direct injection (20 $\mu$L) of the compounds into the chromatographic system. This factor represents the improvement in sensitivity that was attained by combining the on-line SPE procedure with the injection of a larger volume, which is only possible due to the on-line adjustment of eluate composition. Furthermore, as a heart-cut approach was adopted, it was not possible to calculate the enrichment factor, as only part of the eluate was introduced into the loop of the injection valve. The enhancement factors obtained using a sample volume of 12 mL were between 51 and 140 (see Table 2). The 3-fold lower enhancement factors obtained for BP3 and HMS when compared to those of the BMDBM and EHMC might be a consequence of the larger molecular size of the latter two compounds. Differences in sorption distribution over the SPE column are expected to yield different elution profiles, which would affect the amount of analyte introduced into the LC via the “heart-cut” injection mode.

The repeatability and reproducibility of the LOV-BI method were calculated as the % RSD of four replicate injections of 9 mL mixture containing 20 $\mu$g L$^{-1}$ of BP3, BMDBM and EHMC, and 90 $\mu$g L$^{-1}$ of HMS, using either a permanent SPE sorbent or five renewable microcolumns, respectively. Using a reusable packed microcolumn, the RSD values ranged from 17 to 29%, while RSDs for renewable sorbent microcolumns comprised the range of 2–13%. In the first case, the high RSDs were attributed to sample carryover effects. The strong adsorption of the most hydrophobic UV filters ($\log K_{ow}$ > 4) onto polymeric materials has been reported earlier by Rodil et al. [11]. The disposable SPE mode implemented here overcomes this problem by offering automatically a new sorbent aliquot for each individual sample and replicates to be processed. The better RSDs for BP3 and HMS (see Table 2) are attributed to a more reproducible and efficient “heart-cut” injection for both compounds, which concords with the data of enhancement factors given above.

### 3.4. Application to environmental samples

Bathing waters (seawater (SW) and swimming pool water (SPW)), which represent the most important pathways of direct introduction of UV filters in the aquatic ecosystems, were processed by the proposed methodology for reliability and accuracy assessment.

As to swimming pool water samples, UV filters are usually not detected or detected at very low concentrations due to the reaction with free chlorine in the sample, which has an important role on the degradation of these compounds [42]. Hence, in order to evaluate the influence of Cl$_2$ in the assays, SPW samples were fortified at different concentration levels of the target analytes and free chlorine as well, and immediately processed by the MSFI-BI-LOV mesosfluidic setup (Table 3; Electronic Supplementary material, Fig. S3). Recoveries between 86 and 112% were obtained for all compounds in the raw sample, where the Cl$_2$ content was <0.05 mg L$^{-1}$, excepting BMDBM, for which recovery was 55% at the lowest spiked concentration. When Cl$_2$ concentration was increased to 0.1 mg L$^{-1}$, recoveries for both BP3 and BMDBM were <51% but ≥96% for the highest concentration level of EHMC and both con-

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linear range (ng)</th>
<th>Sensitivity (L µg$^{-1}$)</th>
<th>Breakthrough volume (mL)</th>
<th>Enhancement factor</th>
<th>Reproducibility (% RSD, 5 columns, n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP3</td>
<td>60–480</td>
<td>2895 ± 68</td>
<td>24</td>
<td>140 ± 7</td>
<td>2.5</td>
</tr>
<tr>
<td>BMDBM</td>
<td>60–420</td>
<td>1455 ± 39</td>
<td>12</td>
<td>51 ± 3</td>
<td>13</td>
</tr>
<tr>
<td>EHMC</td>
<td>60–420</td>
<td>2044 ± 55</td>
<td>12</td>
<td>63 ± 2</td>
<td>12</td>
</tr>
<tr>
<td>HMS</td>
<td>240–1920</td>
<td>1825 ± 38</td>
<td>12</td>
<td>140 ± 5</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Note: a $R^2 > 0.995$.

b Calculated for a sample volume of 12 mL.

c Using 4 standards, 3 replicates (n = 12).
centrations of HMS. At a concentration level of 0.5 mg L\(^{-1}\) Cl\(_2\), a complete degradation of the compounds was observed, with the exception of HMS that was still detected in the sample with recoveries as high as 104–107%. This experiment was also performed with concentrations of 1.0 and 2.0 mg L\(^{-1}\) Cl\(_2\) and, at both levels, the overall target compounds were quantitatively degraded. These results are in agreement with previously reported data [42] that indicate the fast formation of halogenated by-products in the presence of free chlorine for some UV filters, e.g., BP3. Although it was spiked at a rather high concentration level, the elevated tolerance of HMS to oxidation must be emphasized. This fact, associated to its low polarity (log \(K\text{ow} = 6.15\)) reveals the potentially high persistence of this UV-sunscreen agent in the aquatic environment.

Limits of detection (LOD) between 0.45 and 3.2 \(\mu\)g L\(^{-1}\), and limits of quantification (LOQ) within the range 1.5–10.6 \(\mu\)g L\(^{-1}\) were attained for the SPW sample whenever a sample volume of 9 mL was processed (Table 3). The reproducibility, expressed as RSD, was <15% (\(n = 5\)) for the suite of SPW samples utilizing the bead injection mode for microscale SPE.

Concerning seawater samples, the average recovery percentage at the same spike levels than those used in SPW samples was 104% with deviations <16% (Electronic Supplementary material, Table S3). Thus, the high concentration levels of salts in seawater did not pose any problem for accurate measurements.

Aimed at evaluating the putative migration of UV filters from sunscreen lotions during recreational bathing, a sample aliquot was collected 30 min after immersion of a skin area where a commercially available sunscreen preparation had been applied. The experiment was performed in a child swimming pool containing 10 L of seawater. BMDMB and EHMC, which were labelled as ingredients of the sunscreen lotion, were found in the bathing water sample (290 ± 16 and 198 ± 12 \(\mu\)g L\(^{-1}\), respectively). For quality control purposes, the sample was also spiked with 19.4 ng mL\(^{-1}\) of each of these two analytes (Fig. 2). An average recovery of 101 ± 2% was found for both UV filters with RSDs <5% (\(n = 5\)). LODs and LOQs for BMDMB and EHMC were 1.0 and 3.3 \(\mu\)g L\(^{-1}\), and 0.7 and 2.4 \(\mu\)g L\(^{-1}\), respectively. These results indicate the suitability of the proposed methodology for monitoring the eventual migration of UV filters to bathing waters.

### 3.5. Comparison with previously described methods

Previously reported methods for the determination of UV filters in environmental matrices involved LC–UV [9,10], LC–MS [11,12], or GC–MS [9,10,12–18] following extraction/preconcentration of the target analytes. The figures of merit (linear range, precision, LOD and LOQ) in the proposed MSFI-BI-LOV mesofluoridic system are in general terms comparable with those of the reported LC–UV methods [9,10] and also with a GC–MS method [15]. As expected, concentration values for linear working range, LOD and LOQ were higher than those reported in LC–MS methods [11,12]. These methods however require prior manual sample preparation based on exhaustive (SPE) or non-exhaustive analyte enrichment via SPME or SBSE. In many instances, multiple-step separation procedures comprising the conditioning of the sorbent material, sample loading, matrix removal followed by elution, evaporation and reconstitution of the extract are called for. These are time-consuming tasks that may take altogether more than 100 min per sample. Moreover, a long drying step is often mandatory and, for MS detection, a derivatization protocol has proven necessary to improve the performance of the method [11]. In contrast, the automatic microSPE procedure presented here did not take more than 9 min for the preconcentration of 9 mL of sample, providing enrichment factors similar, and in some cases better, than those encountered when extracting up to 200 mL of sample [11]. As a result of downsizing the SPE procedure, waste disposal and both solvent and sorbent consumption were minimized. Regardless the sample volume, 11.1 mL of effluent were generated and merely 1.55 mL of methanol were used. In contrast to earlier SPE methods, a decrease of 60% in waste generation and more than 90% in solvent consumption was accomplished in the LOV-BI system. A 10-fold saving in the amount of sorbent was also obtained with respect to a batchwise SPE procedure prior to LC–MS, where 60 mg Oasis HLB cartridges were employed [11].

Non-exhaustive enrichment techniques, such as SPME and SBSE [15–18], feature the minimization or even avoidance of solvents. However, large sample equilibration times within the

### Table 3

Concentrations of UV filters (\(\mu\)g L\(^{-1}\)) found in swimming pool water* containing different levels of free chlorine. Limits of detection (LOD) and quantification (LOQ) for this type of sample are also listed.

<table>
<thead>
<tr>
<th>Spike level ((\mu)g L(^{-1}))</th>
<th>LOD ((\mu)g L(^{-1}))</th>
<th>LOQ ((\mu)g L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP3(^b)</td>
<td>17.6</td>
<td>0.81</td>
</tr>
<tr>
<td>SPW (&lt;0.05 mg L(^{-1}) Cl(_2))</td>
<td>15.1 ± 1.6</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>SPW* (0.1 mg L(^{-1}) Cl(_2))</td>
<td>7.1 ± 0.5</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>SPW* (0.5 mg L(^{-1}) Cl(_2))</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>LOD ((\mu)g L(^{-1}))</td>
<td>0.08</td>
<td>0.15</td>
</tr>
<tr>
<td>BMDMB(^b)</td>
<td>35.2</td>
<td>1.5</td>
</tr>
<tr>
<td>LOD ((\mu)g L(^{-1}))</td>
<td>14.5</td>
<td>3.0</td>
</tr>
<tr>
<td>EHMC(^b)</td>
<td>29.1</td>
<td>3.0</td>
</tr>
<tr>
<td>LOD ((\mu)g L(^{-1}))</td>
<td>15.5</td>
<td>3.0</td>
</tr>
<tr>
<td>HMS(^b)</td>
<td>31.1</td>
<td>3.0</td>
</tr>
<tr>
<td>LOD ((\mu)g L(^{-1}))</td>
<td>68.2</td>
<td>3.0</td>
</tr>
<tr>
<td>LOD ((\mu)g L(^{-1}))</td>
<td>136.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Samples were immediately processed after addition of free chlorine.

b Values calculated by external calibration.

c Samples were immediately processed after addition of free chlorine.

n.a., not available.

**LOD** = Limit of Detection, **LOQ** = Limit of Quantification.
range of 45–300 min are frequently called for to warrant appropriate method sensitivity. This leads to a 5–33-fold lower sample throughput than that of the proposed LOV-BI method, making them inappropriate for fast screening of UV filters in environmental samples.

4. Conclusions

For the first time, an automatic flow-based analytical procedure hyphenating sample pre-treatment and chromatographic determination is proposed for fast quantification of UV filters in environmental samples. The novel methodology capitalizes on the combination between flexibility, miniaturization and simplification in sample preparation integrated within the LOV mesofluidic platform and the high throughput of the chromatographic assays supported on the use of a monolithic column. This leads to cost-effective analytical procedures in terms of labour and reagent expenses, contributing to the development of miniaturized robust methods with “Green Chemistry” credentials.

The proposed methodology was successfully applied to screening of four analytes in spiked samples of recreational waters (swimming pool and seawater), chosen as model compounds representative of different UV filter families (benzophenone, dibenzoylmethane, cinammate and salicylate). In this context, it should be emphasized that the UV–vis detection system employed here does not allow discrimination between structurally similar compounds, such as the following pairs: HMS/ethylhexyl salicylate, EHMC/isoamyl methoxycinnamate and BP3/benzophenone-1. The individual quantification of these compounds could be attained by using MS detectors or by adequate tailoring of the chromatographic separation.

Finally, considering the results obtained for the fast interaction between the UV filters tested and Cl2, future work should be focused on studying the kinetics and products involved in such reactions, which would provide better understanding about UV filters’ fate in the environment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.03.035.

References