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Rapid and selective determination of UV filters in seawater by liquid chromatography–tandem mass spectrometry combined with stir bar sorptive extraction

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ABSTRACT

Fast liquid chromatography coupled to triple-quadrupole tandem mass spectrometry was employed for the determination of six UV filters in seawater. The separation of the analytes was achieved in less than 5 min; polarity switching was used as four of the analytes were ionized in positive mode and the remaining two in negative mode. Two ionization sources were employed and compared: atmospheric pressure chemical ionization (APCI) gave better results than electrospray ionization (ESI) for all analytes, with higher reproducibility and lower detection limits. Therefore APCI was chosen for the determination of the analytes in seawater samples using stir bar sorptive extraction-liquid desorption (SBSE-LD).

Quantitative analysis was performed in multiple reaction monitoring (MRM) mode; fragmentation pathways of the analytes with regard to the formation of the MRM ions were also proposed.

For the analysis of seawater samples, calibration curves were drawn using SBSE in spiked seawater. All figures of merit of the method were satisfactory; limits of detection were particularly low for the four analytes ionized in positive mode, being in the range 8–31 ng/L. The method was applied to the determination of the six UV filters in seawater samples from Liguria, Italy. Only benzophenone-3 (BP-3) and ethylhexyl methoxycinnamate (EHMC) were measured in the analyzed samples; some of the remaining analytes were also detected but always below the limit of quantitation.

1. Introduction

In the last ten years stir bar sorptive extraction (SBSE) has become a widespread analytical technique for the preconcentration of organic compounds, resulting in faster analysis, higher sample throughput, lower solvent consumption, and less workload per sample [1]. This technique is based on the extraction of the analyte from the liquid matrix onto a thick film of polydimethylsiloxane (PDMS) coated on a magnetic stir bar. SBSE is characterized by highly effective sampling capability and recoveries.

Most SBSE applications involve the use of thermal desorption (TD) followed by gas chromatography (GC) to recover the analytes accumulated in the coated stir-bar [2,3]. Liquid desorption (LD) is an alternative to TD for thermally labile analytes or when the subsequent separation is carried out using liquid chromatography (LC). During LD mode, the polymer-coated stir-bar is immersed in a stripping solvent or solvent mixture for the chemical desorption of the extracted solutes. LD offers additional interesting features such as cost-effectiveness, the opportunity for method development and possible re-analysis [4].

Among trace organic contaminants in water, UV filters have been recently studied. UV filters are chemical compounds that can filter UV-A and/or UV-B radiation from sunlight in order to shield human skin from their negative effects [5]. UV filter compounds are integrated in many cosmetic formulations (e.g. sunscreen creams, lotions, shampoos, lipsticks, hair sprays, hair dyes, etc.) in amounts between 0.1% and 10% [6]. UV filters are reaching surface waters (rivers, lakes, coastal sea water) via release from the skin during swimming and bathing or through wastewater. Most UV filters are highly lipophilic and hardly degradable in sewage treatment plants and are therefore supposed to accumulate in the environment (e.g. biota and sediments) [6]. Furthermore, recent studies have shown estrogenic and other endocrine effects for several UV filters with a special emphasis to humans [7–9]. Therefore, the increasing use of UV filters along with their impact on health and environment calls for considerable environmental control by modern analytical methods.

Various methods were reported in the literature for the study of UV filters in environmental matrices with GC–MS [10–12]. In water samples, UV filters are present at very low concentrations, usually varying between nanograms and micrograms per liter; therefore

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liquid chromatography coupled to mass spectrometry (LC–MS) is probably the technique of choice due to its high sensitivity and selectivity [5,13–18].

Although during the last two decades various interfaces for coupling LC and MS were effectively employed for the analysis of pollutants in various matrices [19–22], the introduction of the atmospheric pressure ionization (API) sources significantly boosted the success of LC–MS. Both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) can generate ions at atmospheric pressure but ion formation mechanisms are different. In APCI the ionization takes place in gas phase under the form of chemical ionization while in ESI the ionization occurs in the liquid phase under the influence of an intense electric field [23,24].

In the development of a LC–MS/MS method, matrix effect is one of the main issues. In general APCI is less susceptible to matrix effect than ESI because ionization takes place in the gas phase [25,26]. Some methods were described to investigate matrix effect, such as the use of spiked matrices after extraction [27] and the postcolumn infusion system [28,29].

The aim of this work was the development of a rapid method by LC–MS/MS comparing APCI and ESI ionization sources for the determination of six common UV filters which were reported to have endocrine disrupting potential [7].

A selective sample preparation method to extract and preconcentrate the analytes from seawater was optimized by means of stir bar sorptive extraction followed by liquid desorption (SBSE-LD). The method was then applied to the determination of these compounds in seawater samples from various sites in Liguria, Italy.

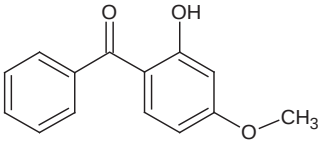
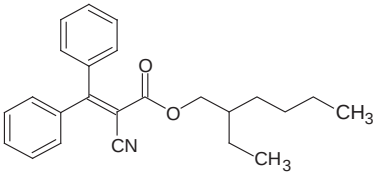
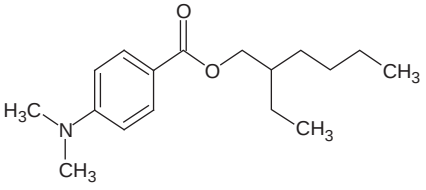
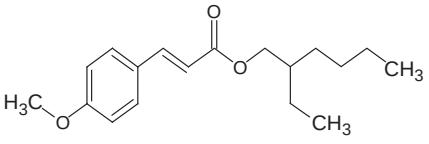
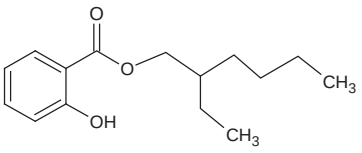
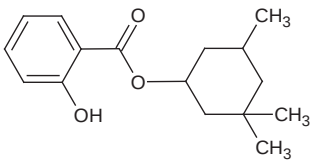
2. Experimental

2.1. Reagents and standards

Names of analytes, abbreviations and relevant data are shown in Table 1. UV filters (BP-3, OC, OD-PABA, EHMC, EHS and HMS), formic acid (HCOOH) and ammonium formate ($HCOONH_4$) were obtained from Sigma–Aldrich (Milan, Italy).

Methanol (MeOH), acetonitrile (ACN), dichloromethane (DCM), and acetic acid (AcOH) were of HPLC grade and obtained from Merck (Darmstadt, Germany). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) from VWR International (Milan, Italy) were used to adjust pH of sample.

Table 1
Analyte abbreviations, structures and analytically relevant data of UV filters.

Abbreviation	INCI name[a]	CAS no.	Empirical formula	Structure	Log $K_{O/W}$
BP-3	Benzophenone-3	131-57-7	$C_{14}H_{12}O_3$		3.79[b]
OC	Octocrylene	6197-30-4	$C_{24}H_{27}NO_2$		6.88[b]
OD-PABA	Ethylhexyl dimethyl p-aminobenzoate	21245-02-3	$C_{17}H_{27}NO_2$		6.15[c]
EHMC	Ethylhexyl methoxycinnamate	5466-77-3	$C_{18}H_{26}O_3$		5.80[b]
EHS	Ethylhexyl salicylate	118-60-5	$C_{15}H_{22}O_3$		5.97[b]
HMS	Homosalate	118-56-9	$C_{16}H_{22}O_3$		6.16[b]

[a] INCI (International Nomenclature for Cosmetic Ingredient) elaborated by CTFA and COLIPA.
[b] Experimental values, from database of physico-chemical properties. Syracuse Research Corparation: Http://www.syrres.com/esc/physdemo.htm.
[c] Software calculated value, from Scifinder Scholar Database 2006: http://www.cas.org/products/sfacad/.

Table 2
MS/MS experimental parameters.

Analyte	Precursor ion (*m*/*z*)	First transition		Second transition		FV[b] (APCI)	FV[b] (ESI)
		Product ion (*m*/*z*)	CE[a]	Product ion (*m*/*z*)	CE[a]		
BP-3	229 $[M+H]^+$	151	22	105	42	110	118
OC	362 $[M+H]^+$	250	2	232	14	60	100
OD-PABA	278 $[M+H]^+$	151	34	166	18	158	130
EHMC	291 $[M+H]^+$	161	14	179	2	72	82
HMS	261 $[M-H]^-$	137	10	93	30	154	118
EHS	249 $[M-H]^-$	137	10	93	22	156	134

[a] Collision energy (V).
[b] Fragmentor voltage (V).

Stock solutions of individual standards were prepared by dissolving each compound in MeOH at concentration of 5000 mg/L. From these, standard mixtures containing BP-3, OC, OD-PABA, EHMC at 20 mg/L, EHS and HMS at 50 mg/L were prepared in methanol and subsequently diluted with Milli-Q water in ratio of MeOH–H_2O (80:20) for working solutions. All standards and working solutions were stored in the dark at 4 °C.

Commercial stir bars (Twister) with 0.5 mm in film thickness and 10 mm in length were obtained from Gerstel (Müllheim, Germany).

2.2. Sample collection

A sea water sample was collected in March 2010 at the Sturla beach in Genoa to be used as a blank.

All surface water samples were collected during Summer 2010 along the Ligurian coast of Italy.

Nine sea water samples were collected in Santa Margherita, San Fruttuoso and Camogli, once a month in the period June–August. Other three samples were collected in a seawater swimming-pool located in a bathing establishment in Varazze, during the same period.

All samples were collected in pre-cleaned amber glass containers with caps and stored in the dark at 4 °C. They were filtered through 0.2 μm membrane filters before the analysis.

2.3. SBSE extraction procedure

Optimization of SBSE-LD was performed using 10 mL of aqueous sample spiked at the concentration of 100 ng/mL for BP-3, OC, OD-PABA, EHMC and of 400 ng/mL for HMS and EHS. The factors influencing the procedure were evaluated. Prior to use, all stir bars were preconditioned by stirring in 5 mL of methanol for 30 min at 800 rpm. After being dried with a lint-free tissue, each stir bar was placed in a clean 25 mL screw-capped amber vial containing 10 mL of water sample at pH 6 and 0.5 mL of methanol. The extraction was performed at room temperature (RT) and at a stirring speed of 800 rpm. After the extraction for 180 min, the stir bar was removed from the sample by tweezers, rinsed with Milli-Q water and dried with a lint-free tissue, then placed in a screw-capped amber vial containing 1 mL of methanol and stirred at 800 rpm for 30 min at RT. After desorption of the analytes, the stir bar was removed from the vial by a cleaned magnetic rod. An aliquot of 0.4 mL of this solution was used for LC–MS/MS analysis after the addition of 0.1 mL of Milli-Q water.

After use, reconditioning of stir bars was done by soaking in a mixture of DCM–MeOH (1:1, v/v) for 24 h. Stir bars were then removed from the solvent and stirred in DCM–MeOH (1:1, v/v) for 30 min at RT at 800 rpm. Finally, the stir bars were dried with a lint-free tissue and kept in a clean vial for the subsequent analyses.

2.4. LC and MS analysis

Separation of analytes was carried out by an Agilent 1200 SL Liquid Chromatograph with a Zorbax SB-C18 column (Agilent Technologies, 1.8 μm, 2.1 × 50 mm) thermostatted at 30 °C; flow rate was 0.4 mL/min and the isocratic elution was performed using 80% methanol and 20% Milli-Q water. The injection volume was 10 μL.

The mass spectrometer was an Agilent 6430 Triple Quadrupole equipped with both ESI and APCI interfaces. For each analyte, fragmentor voltage (FV) and collision energy (CE) were optimized (Table 2). The quantitative analysis was performed using the polarity switching mode (positive and negative ions) in multiple reaction monitoring (MRM) (Table 2). The most abundant transition was used for the quantitative analysis and the second transition for confirmation.

ESI conditions for positive and negative ionization were: dwell time 50 ms, drying gas flow (N_2) 12 L/min, capillary potential 4000 V, nebulizer pressure 50 psi and drying gas temperature 350 °C.

APCI conditions were: dwell time 50 ms, drying gas flow (N_2) 4 L/min, capillary potential 2100 V for positive ion mode and 1300 V for negative ion mode, nebulizer pressure 40 psi, drying gas temperature 350 °C, vaporizer temperature 280 °C, corona current 5 μA for positive ion mode and 23 μA for negative ion mode.

3. Results and discussion

3.1. LC separation and ionization with APCI and ESI

The separation of the six UV filters was developed with a particular attention to obtain a short time of analysis. For this reason a fast chromatographic separation was developed by testing different sub-2 μm particle size HPLC columns. For each column, different mixtures (H_2O, MeOH, and ACN) in isocratic and gradient conditions were tested. Flow rate was also tested in the range 0.2–0.7 mL/min.

The best result was obtained by means of a Zorbax SB-C18 column which allowed an efficient separation of the analytes in the shortest time (less than 5 min); the elution was performed in isocratic conditions using 80% methanol and 20% Milli-Q water at the flow rate of 0.4 mL/min. The flow rate value was a good compromise for both interfaces.

Using the optimized chromatographic conditions, the full-scan analysis of each compound was performed to choose the ionization conditions for ESI and APCI. Generally, phenolic compounds are detected with a greater sensitivity in the negative ion mode. Preliminary tests confirmed this behavior for HMS and EHS while BP-3 provided greater response in the positive mode. Remaining analytes have no acidic sites and were ionized in positive mode.

Fig. 1 shows the APCI and ESI full-scan mass spectra of the six analytes. The two analytes that were ionized in the negative mode, HMS and EHS, showed the same behavior both in APCI and ESI, generating the deprotonated molecule $[M-H]^-$ without any fragment. The other four analytes provided different spectra with the two ionization sources.

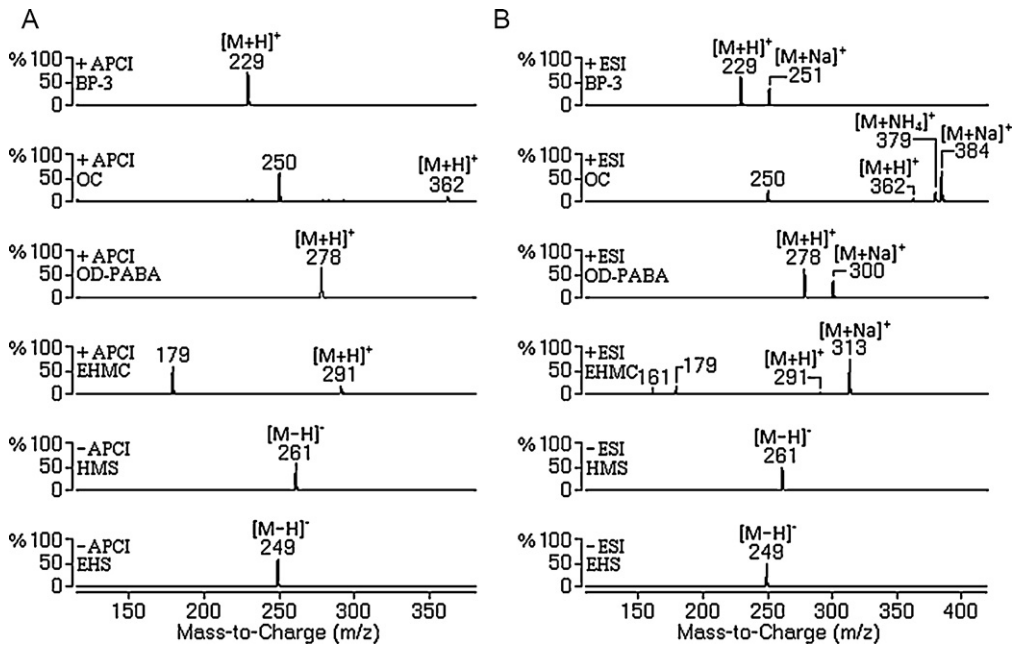


Fig. 1. Full-scan mass spectra of the six analytes: (A) APCI and (B) ESI.

APCI spectra were rather simple (Fig. 1A): BP-3 and OD-PABA produced only the $[M+H]^+$ ion, while OC and EHMC showed also the ions *m*/*z* 250 and *m*/*z* 179 respectively, due to the in-source fragmentation of $[M+H]^+$.

As regards to ESI spectra (Fig. 1B), besides the protonated molecule $[M+H]^+$ the adduct ions $[M+Na]^+$can be also observed. Sodiated ions were intense in all these four spectra, being even the base peaks for OC and EHMC. OC spectrum presented also the ion *m*/*z* 379 corresponding to the adduct $[M+NH_4]^+$.

The sodium adducts produced at high intensity in the ESI source could represent a shortcoming if chosen as parent ions in multiple reaction monitoring (MRM); in fact they are less reproducible and more resistant to fragmentation in the collision cell, thus compromising sensitivity and precision of the quantitative analysis. The formation of sodium adducts can be reduced by adding a chemical modifier to the mobile phase: this approach is discussed in the next paragraph.

For OC and EHMC, we explored the possibility to choose the in-source fragments at *m*/*z* 250 and 179 as parent ions for MRM. Although the intensity of these ions was higher than $[M+H]^+$, preliminary MRM experiments showed that they were not suitable as precursor ions as the sensitivity was poor. Therefore, the $[M+H]^+$ or $[M-H]^-$ species were selected as precursor ions for all the analytes.

3.2. Effect of modifiers added to the mobile phase

The effect of modifiers added to the mobile phase on ESI and APCI sensitivity for the analysis of the six UV filters was investigated. Weak organic acids are often employed as modifiers; in positive ion mode, the presence of an acid in the mobile phase usually facilitates the protonation of analytes with basic functional groups. The effect of weak acids on negative ionization depends on the acidic modifier, its concentration and, obviously, on the chemical properties of the analyte.

Three modifiers (AcOH, HCOOH and $HCOONH_4$) were tested individually at the concentration of 0.1% in the mobile phase; no significant effect on the chromatographic separation was observed.

It was clear at once that the modifiers were not suitable for HMS and EHS; in fact they underwent complete suppression with both ionization sources.

The effect of the three modifiers on BP-3, OC, OD-PABA and EHMC was studied by measuring, for each analyte, the peak area of the most abundant fragment obtained in MRM mode using the protonated molecule $[M+H]^+$ as precursor ion (called "first transition" in Table 2).

Results obtained in ESI are represented in the histogram of Fig. 2. When AcOH or HCOOH was used, the intensity of the analytes decreased; the only exception is OD-PABA which gave similar responses with and without HCOOH. On the contrary, a remarkable improvement was observed for all these analytes using $HCOONH_4$. The effectiveness of the modifier $HCOONH_4$ can be further appreciated comparing the ESI full-scan mass spectra reported in Fig. 3 with those obtained without modifiers (Fig. 1); sodium adducts were significantly suppressed while the $[M+H]^+$ peaks generally increased. This was particularly evident for BP-3, OD-PABA and EHMC, while the OC spectra showed also the enhancement of the adduct $[M+NH_4]^+$.

In APCI, the effect of the modifiers was less clear and none of them showed a positive influence on all these four analytes; for this reason the related histogram was not reported. OD-PABA was not affected by any of the three modifiers. Similarly, EHMC was not

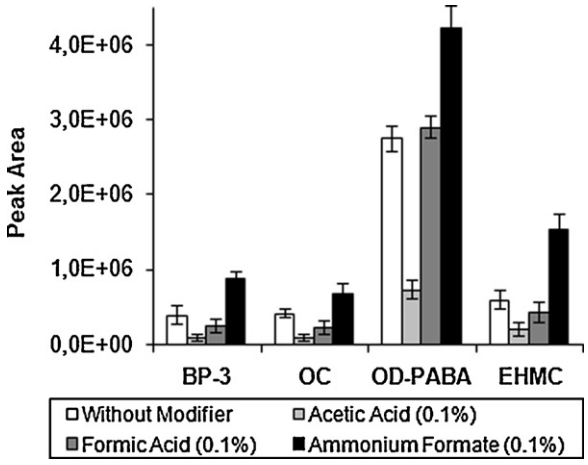


Fig. 2. Responses of BP-3, OC, OD-PABA and EHMC in ESI with different modifiers.

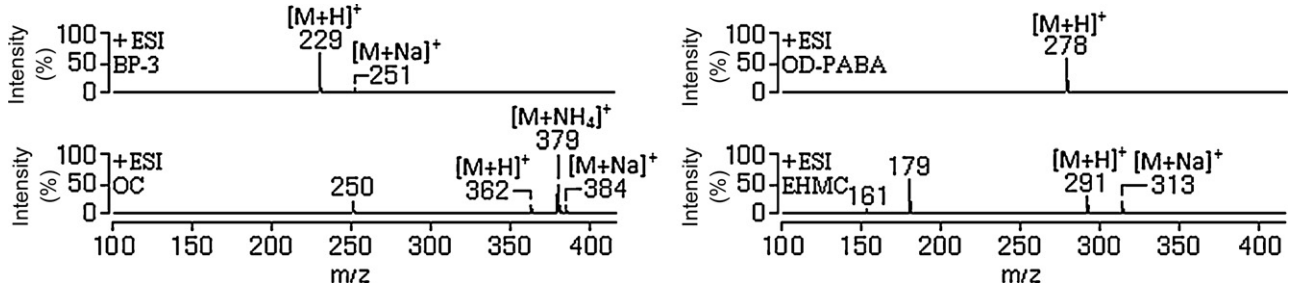


Fig. 3. Full-scan mass spectra of BP-3, OC, OD-PABA and EHMC in ESI with 0.1% $HCOONH_4$.

influenced when AcOH and HCOOH were used, while its intensity decreased using $HCOONH_4$. The latter modifier strongly suppressed the responses of both BP-3 and OC; on the contrary, their response was improved by AcOH and HCOOH.

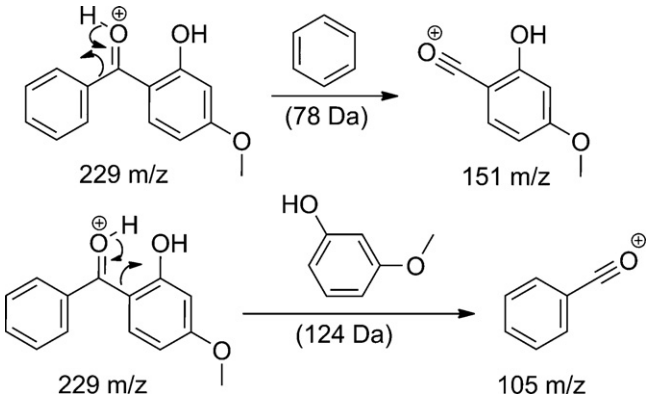
Since the goal of the present work was the determination of all six analytes at the same time, the use of modifiers was abandoned in the rest of this work, although the results obtained in ESI suggest that $HCOONH_4$ could be employed effectively if only BP-3, OC, O D-PABA and EHMC have to be detected.

3.3. MS/MS fragmentations

Two MRM transitions were used for quantitative analysis; the first was selected for quantification and the second was chosen for confirmation. The MRM transitions along with fragmentor voltage and collision energy in ESI and APCI were optimized using the "Optimizer" application of the Agilent Mass Hunter software. Selected transitions and optimal values are shown in Table 2.

The fragmentation pathways of the analytes with regard to the formation of the two MRM ions were considered; proposed mechanisms are presented in Schemes 1–5.

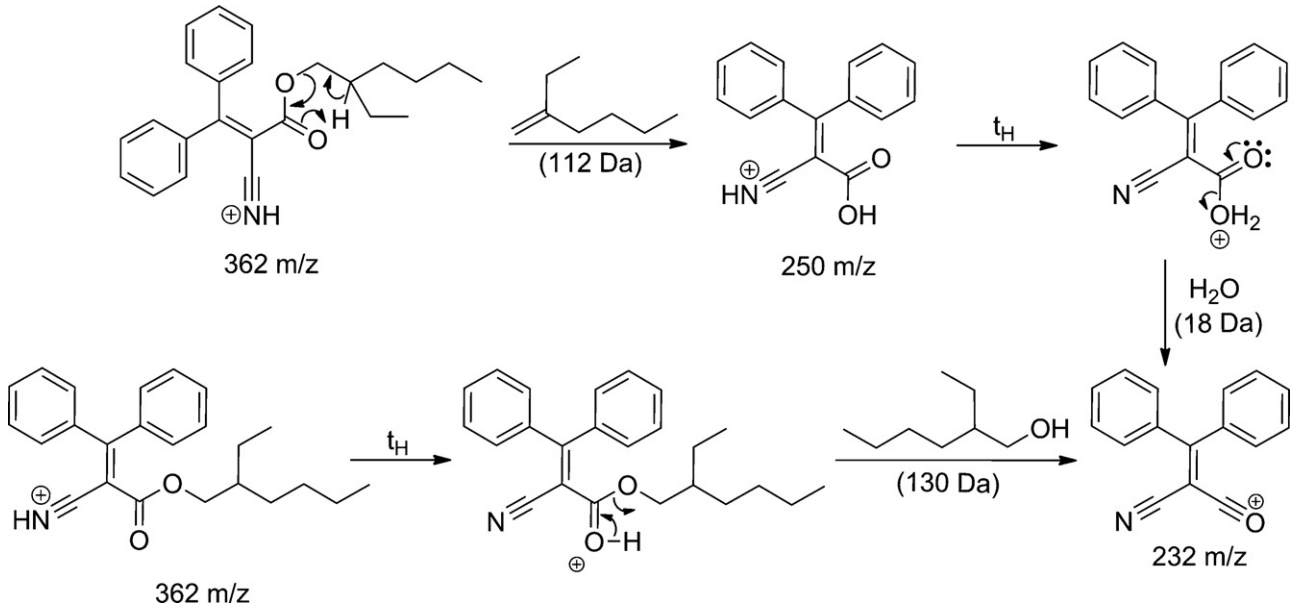
In two cases (OC and OD-PABA) before studying fragmentation it was necessary to establish the protonation site. To this aim, semi-empirical calculations were performed using the approach proposed by Madeira et al. [30]. Heats of formation of the neutral molecule and its possible protonated forms were calculated using the PM6 method [31] as implemented in MOPAC 2009 software package [32]. The protonation reaction enthalpy ($\Delta_r H$) were then calculated and compared.



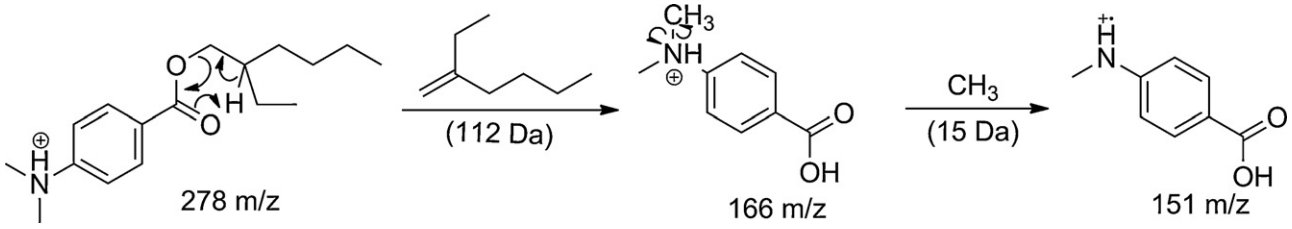
Scheme 1. Fragmentation pathway of $[M+H]^+$ ion of BP-3.

The proposed mechanism for BP-3 is shown in Scheme 1; the two fragment ions (m/z 151 and m/z 105) both originate from the $[M+H]^+$ ion (m/z 229) by α-cleavage. The ion at m/z 151 is obtained by neutral loss of benzene while the fragment at m/z 105 by neutral loss of 3-methoxyphenol.

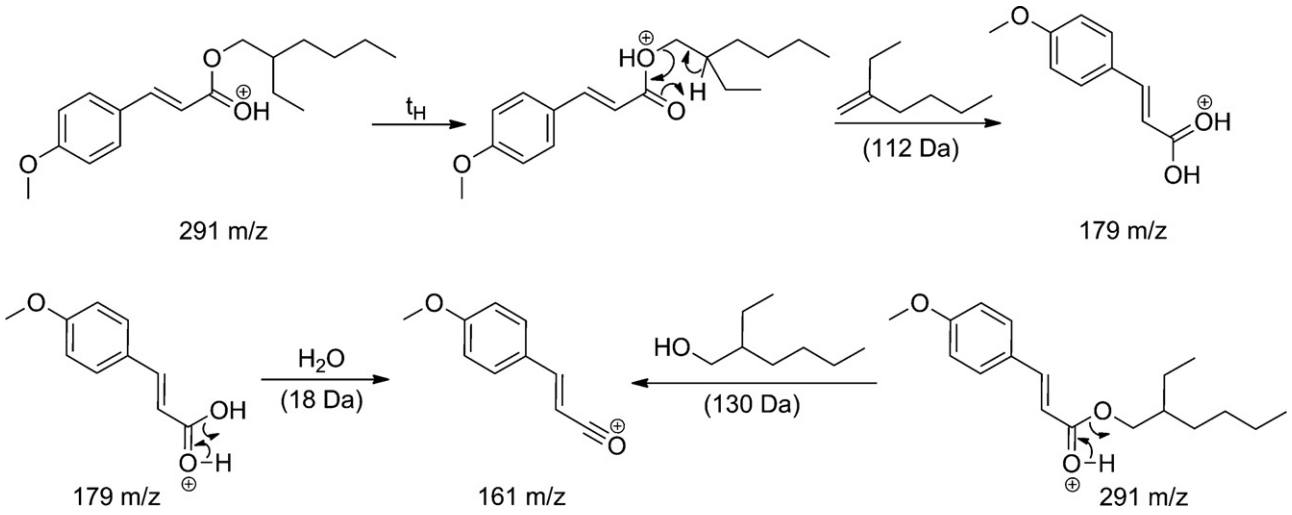
Scheme 2 describes the proposed fragmentations of OC ($[M+H]^+$ 362 m/z) driving to the two product ions at m/z 250 and m/z 232. A fragmentation pathway was already proposed by Choi and Song [33], although the authors did not assign the protonation site on the molecule. In fact this molecule may be protonated at carbonyl oxygen or nitrogen of the nitrile group. The semi-empirical calculations



Scheme 2. Fragmentation pathway of $[M+H]^+$ ion of OC.



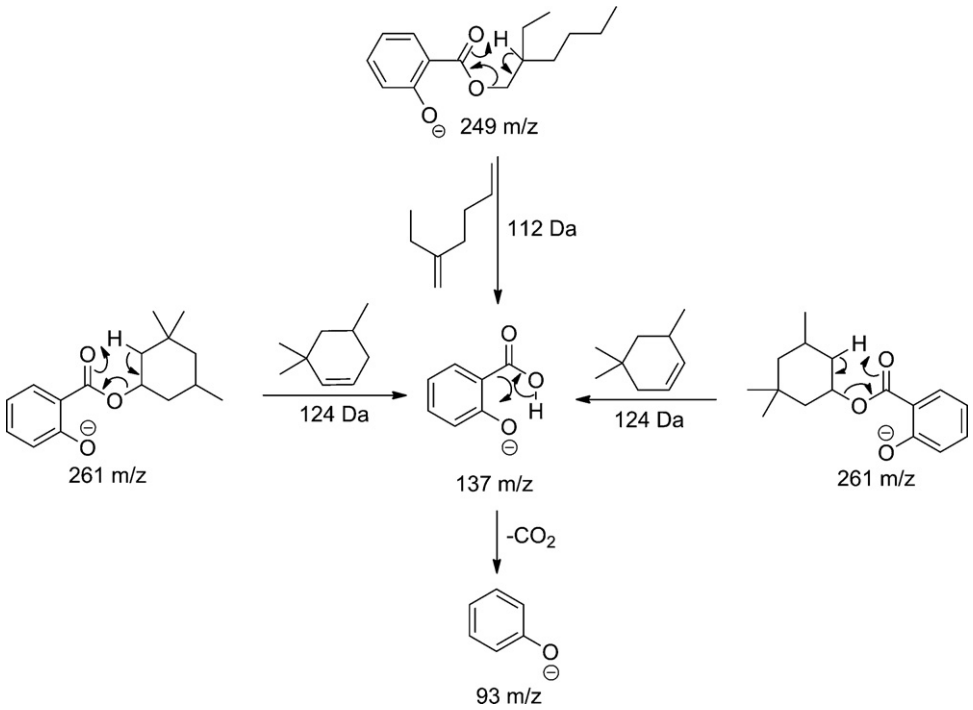
Scheme 3. Fragmentation pathway of $[M+H]^+$ ion of OD-PABA.



Scheme 4. Fragmentation pathway of $[M+H]^+$ ion of EHMC.

indicate that the nitrogen is the most favored site of protonation as its reaction enthalpy is lower ($\Delta_r H_{nitrogen}$ = 648 kJ/mol; $\Delta_r H_{oxigen}$ = 685 kJ/mol).

The fragment ion at *m*/*z* 250 is obtained by McLafferty rearrangement and neutral loss of 3-methylene-heptane. The fragment ion at *m*/*z* 232 may be obtained by the loss of a water molecule from the ion at *m*/*z* 250, as proposed by Choi et al.; in our opinion it can be also formed from the protonated molecular ion by intramolecular proton shift and 2-ethylhexanol neutral loss, as shown in Scheme 2. To establish whether one fragmentation pathway prevails over the



Scheme 5. Fragmentation pathways of $[M-H]^-$ ions of HMS and EHS.

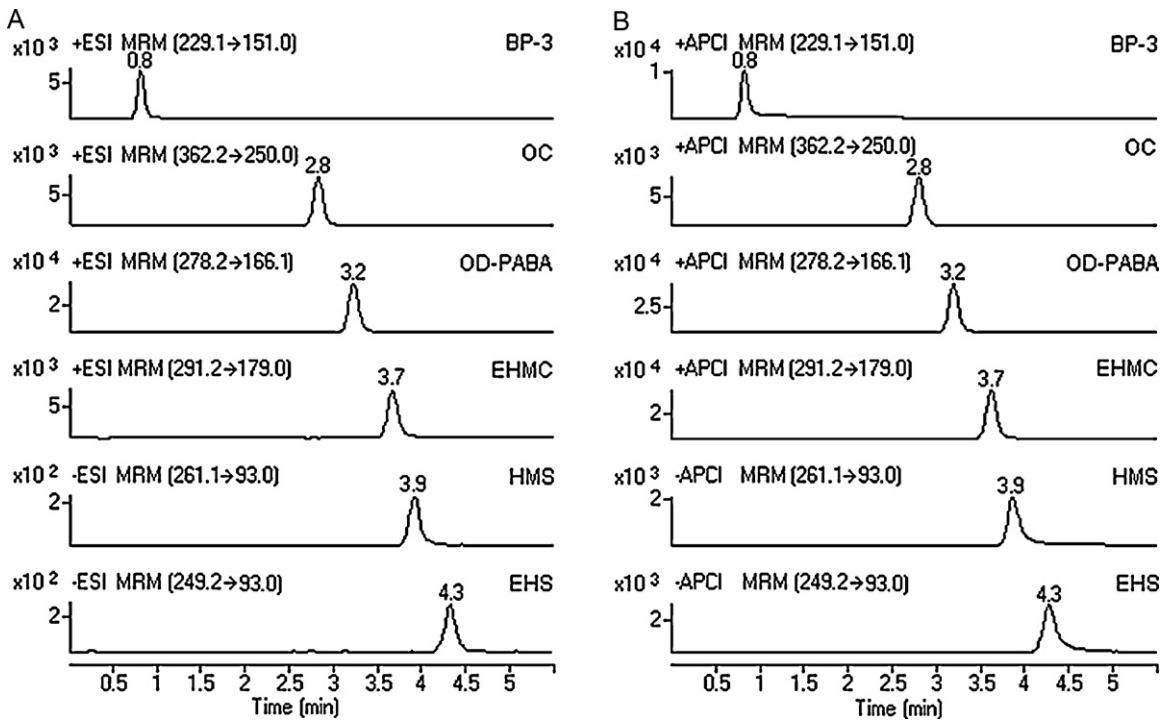


Fig. 4. MRM chromatograms of the analytes: (A) ESI-MS/MS and (B) APCI-MS/MS. Concentration was 100 ng/mL for BP-3, OC, OD-PABA, EHMC and 1000 ng/mL for HMS, EHS.

other, further calculations would be necessary, which were not in the aim of the present work.

OD-PABA gives product ions at *m*/*z* 166 and *m*/*z* 151 (Scheme 3); similarly to OC this molecule may be protonated in two different positions, at carbonyl oxygen or nitrogen of the amino group. Again, the results of semi-empirical calculations indicate that nitrogen is the most probable protonation site ($\Delta_r H_{nitrogen}$ = 619 kJ/mol; $\Delta_r H_{oxigen}$ = 627 kJ/mol). The fragment ion at *m*/*z* 166 is obtained by McLafferty rearrangement of the $[M+H]^+$ ion (278 *m*/*z*) and neutral loss of 3-methylene-heptane. The further fragmentation of ion at *m*/*z* 166 by methyl radical loss produces the fragment 151 *m*/*z*. The protonated molecular ion of EHMC (*m*/*z* 291) produces the ions at *m*/*z* 179 and 161 (Scheme 4). After intramolecular proton shift, the fragment ion at *m*/*z* 179 is obtained by McLafferty rearrangement and neutral loss of 3-methylene-heptane. The fragment ion at *m*/*z* 161 may be obtained through two different fragmentation pathways: from the ion at *m*/*z* 179 by water neutral loss or directly from the protonated molecular ion, by 2-ethylhexanol neutral loss.

The remaining compounds EHS and HMS were analyzed in negative ion mode; they both give raise to two fragment ions at *m*/*z* 137 and 93 (Scheme 5). For EHS, the 2-carboxyphenoxide anion at *m*/*z* 137 is formed from its deprotonated molecular ion (249 *m*/*z*) by McLafferty rearrangement and neutral loss of 3-methylene-heptane.

Considering HMS, the fragment at *m*/*z* 137 derives from the $[M-H]^-$ ion (261 *m*/*z*) but, in this case, two McLafferty rearrangements are possible, determining the neutral loss of one out of two isomers (3,3,5-trimethylcyclohexene or 3,5,5-trimethylcyclohexene).

The proposed mechanism for the other fragment, the phenoxide anion at *m*/*z* 93, is identical for both EHS and HMS, arising from the ion at *m*/*z* 137 by neutral loss of CO_2.

3.4. Ionization sources comparison and matrix effect

In order to compare the two ionization sources, sensitivity was primarily considered. As shown in Fig. 4, response of the six analytes in APCI was higher than in ESI; this was particularly marked for slightly polar analytes ionized in negative mode, HMS and EHS, which yielded 10-fold and 16-fold higher signals (comparing peak areas), respectively.

Some figures of merit of the method are reported in Table 3; detection limits of the analytes (LODs), calculated as a signal to noise (S/N) ratio of 3/1, confirm the higher sensitivity of APCI:

Table 3
Figures of merit of APCI and ESI.

Compound	LOD (ng/mL) (S/N = 3)		RSD (%)[a]				Correlation coefficient (R^2)[b]	
	APCI	ESI	Within-day		Interday		APCI	ESI
			APCI	ESI	APCI	ESI		
BP-3	0.08	0.12	3	4	11	12	0.9983	0.9992
OC	0.20	0.24	2	5	5	5	0.9984	0.9974
OD-PABA	0.01	0.02	1	3	6	10	0.9993	0.9992
EHMC	0.07	0.46	1	2	9	12	0.9982	0.9984
HMS	1.70	4.07	4	11	11	12	0.9984	0.9969
EHS	2.65	10.89	4	9	13	15	0.9963	0.9972

[a] BP-3, OC, OD-PABA, EHMC: spiked at 10 ng/mL; HMS & EHS: spiked at 400 ng/mL. Five replicates were carried out; interday analyses were performed in three days.
[b] Six concentration levels. BP-3, OC, OD-PABA, EHMC: 1–100 ng/mL; HMS & EHS: 100–1000 ng/mL.

in fact its values range between 0.01 and 2.65 ng/mL against 0.02–10.8 ng/mL for ESI. The same table shows the repeatability and reproducibility of the method expressed as relative standard deviation (RSD) calculated on five replicates. For all the analytes, APCI provided a slightly better precision than ESI.

Calibration curves were performed at six concentration levels in the range of 1–100 ng/mL for BP-3, OC, OD-PABA, EHMC and 100–1000 ng/mL for HMS and EHS in both ionization sources. Correlation coefficients R^2 obtained with APCI and ESI were comparable and always higher than 0.996 (Table 3).

The two ionization sources were also compared as regards to matrix effect, which can be defined as the alteration in response observed for target analyte when coelution of sample components occurs. The presence of matrix components in a real sample can cause either suppression or augmentation of response of the target analyte in a standard solution. In a recent review, Niessen et al. [34] reported different methods to evaluate matrix effect, suggesting a quantitative assessment approach that was followed in this paper.

To evaluate the matrix effect due to ionization process in both ionization sources, two sample sets were prepared and compared. The first sample set consisted of a standard solution containing each analyte dissolved in mobile phase (MeOH:H_2O, 80:20); this sample was called standard solution. The second sample set was prepared using seawater collected during winter, in which no peak of the analytes was found, and performing SBSE-LD with the optimized procedure presented in the next paragraph; then extracts were spiked with analytes at the same concentration as in the first sample set. This sample was called post-extracted spiked sample.

The analyte response in APCI and ESI was compared measuring peak areas of the two samples. The quantitative assessment of matrix effect (ME) was evaluated using the following formula:

$$\%\mathrm{ME} = \left(\frac{\text{area of post-extracted spiked sample}}{\text{area of standard solution}}\right) \times 100.$$

Three concentration levels were evaluated: 0.4, 0.8, 1.6 ng/mL for BP-3, OC, OD-PABA, and EHMC; 100, 200, 300 ng/mL for HMS and EHS. Each concentration level was prepared in triplicate.

The %ME values of the six analytes ranged from 101 to 109% in APCI and from 93 to 98% and ESI. To our knowledge, no guiding principles about the values of %ME are reported in the literature; nevertheless, Wang et al. [35] in a paper regarding human plasma suggested that matrix effect should be considered when %ME values exceed the range 85–115%.

The values of %ME obtained in our work are in the range 93–109% for both ionization sources, apparently showing a slight ion enhancement in APCI and a slight ion suppression in ESI. However, in our opinion, it is possible to conclude that both ion sources do not significantly suffer from matrix effects.

The figures of merit described above together with the considerations on matrix effect suggest that both ionization sources are indicated for the determination of the UV filter compounds considered. One shortcoming could be the relatively limited sensitivity verified for HMS and EHS, more pronounced for ESI. Considering the very low concentration levels expected in seawater, APCI was then chosen for the determination of the analytes in real samples.

3.5. SBSE-LD optimization

SBSE is an equilibrium technique based on the sorption of the investigated analytes onto a film of polydimethylsiloxane which is coated on a magnetic stir bar. For water samples, the extraction of solutes from the aqueous phase into the extraction medium is controlled by the partitioning coefficient of the solutes between the PDMS phase and the aqueous phase ($K_{PDMS/W}$). Recent studies have correlated this coefficient with the octanol–water distribution coefficient ($K_{O/W}$) [36,37] which gives a good indication of whether and how well a given analyte can be extracted with SBSE.

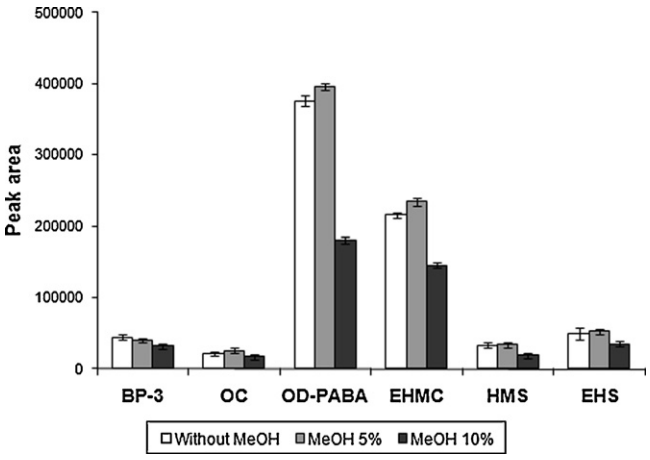


Fig. 5. Optimization of MeOH addition to water sample before SBSE.

To this aim, the theoretical recovery of each analyte (under equilibrium conditions) was calculated using $K_{O/W}$ instead of $K_{PDMS/W}$, following the equation proposed by David and Sandra [36]. The lowest value was obtained for BP-3 (93%); the theoretical recoveries calculated for all the other analytes were close to 100%.

Several parameters affect SBSE extraction efficiency such as aqueous medium characteristics, extraction time and stirring rate. After extraction, analytes are desorbed by liquid desorption; the physicochemical properties of the solvent and desorption time also influence the final recovery. All these parameters were evaluated and optimized.

Preliminary experiments allowed to fix the best operative conditions for the extraction time (3 h) and stirring rate (800 rpm). The influence of pH was tested in the range 2–8; the pH value of 6 provided best results and was then selected.

The effect of adding an organic solvent to the seawater sample before extraction to minimize adsorption of the analytes on the vial walls was also investigated. Two different levels of MeOH (5% and 10%) were added to the aqueous samples. As Fig. 5 shows, the response of BP-3 is slightly reduced while increasing MeOH percentage. The efficiency of extraction of the remaining analytes was highest with 5% MeOH. Hence, this percentage of methanol was added to seawater samples before SBSE.

Regarding the solvent choice for liquid desorption, methanol provided better results than acetonitrile, yielding to higher extraction efficiency and better reproducibility for all the analytes.

Desorption time was tested in the range 15–120 min. The amount of extracted analytes increased with time during the first 30 min and then remained constant; consequently this desorption time was chosen for the SBSE extraction procedure.

3.6. Validation of SBSE-LD and application to seawater samples

For quantitative analysis of seawater samples, new calibration curves were drawn using SBSE. To this aim, sea water collected during winter, in which the analytes were not detected, was spiked with standard solutions of the analytes at six concentration levels. Each spiked solution was extracted using the SBSE extraction procedure reported in Section 2 and analyzed in triplicate by APCI LC–MS/MS. The resulting calibration curves (in the range 0.2–20 ng/mL for BP-3, OC, OD-PABA and EHMC; in the range 20–600 ng/mL for HMS and EHS) showed very good linearity for all the analytes ($R^2 > 0.998$). Repeatability was excellent for the compounds determined in positive mode, with RSD values in the

Table 4
Concentrations and standard deviation ($n = 3$) of UV filters measured in seawater.

Sample	BP-3 (ng/L)			EHMC (ng/L)		
	June	July	August	June	July	August
Santa Margherita	<LOQ	<LOQ	33 ± 2	<LOQ	27 ± 3	<LOQ
San Fruttuoso	101 ± 5	118 ± 6	88 ± 5	<LOQ	28 ± 2	83 ± 2
Camogli	<LOQ	<LOQ	<LOQ	28 ± 3	25 ± 2	47 ± 4
Swimming-pool	25 ± 1	216 ± 4	112 ± 6	71 ± 2	53 ± 2	86 ± 7

range 3–5%, while HMS and EHS showed RSD values of 9% and 7% respectively. Interday reproducibility was lower than 10% for all the compounds except for EHS (13%).

Limits of detection and quantitation in seawater were calculated as a signal to noise (S/N) ratio of 3/1 and 10/1 respectively. LODs of the analytes determined in positive ion mode were in the range 8–31 ng/L while LODs of the analytes determined in negative mode were more than one order of magnitude higher (1.2 μg/L).

LOQs were 25 ng/L for BP-3, OD-PABA and EHMC, 101 ng/L for OC, and 3.9 μg/L for HMS and EHS.

According to Niessen et al. [34] the process efficiency was evaluated by running five replicates at two concentration levels (0.4 ng/mL and 1.6 ng/mL for the compounds in positive ion mode; 100 and 300 ng/mL for those in negative ion mode). Spiked seawater was submitted to SBSE-LD and analyzed; then the response of the analytes was compared with that of standard solutions at the same concentration directly injected in LC–MS/MS.

As discussed in previous section, APCI does not significantly suffer from matrix effects, therefore in this case the process efficiency corresponds to the recovery. The obtained values were close to 100% for all the analytes except for BP-3 (83%) and OC (71%).

The method was then applied to seawater samples collected in four seaside resorts of Liguria, during summer 2010. Only BP-3 and EHMC were measured in the analyzed samples; some of the remaining analytes were also detected but always below the limit of quantitation. Results are presented in Table 4; BP-3 was found in all the studied sites, although, in the seawater sampled in Camogli, its concentration was below the limit of quantitation. During the sampling period, the contamination level of this compound in San Fruttuoso was rather constant. Usually, EHMC values were slightly lower than BP-3. It was not surprising that the swimming-pool was the only site in which both the analytes were measured in all the considered samples, presenting the highest values in July for BP-3 (216 ng/L) and in August for EHMC (86 ng/L); anyway such values do not seem to be alarming.

In general, the measured concentrations of both UV filters are similar to those usually reported for freshwaters [26,38,39]. To our knowledge, only two reports show values for seawater. Rodil et al. in a paper of 2008 [17] studied different UV filters (BP-4 and PBSA) which were detected in the concentration range 38–138 ng/L. A very recent work by Tarazona et al. [40] concerns the determination of four hydroxylated benzophenones in seawater collected along Spanish beaches; reported values for BP-3 are unexpectedly higher, ranging from 1340 and 3300 ng/L. Monitoring studies are necessary to obtain an estimation of average values in coastal seawater.

4. Conclusions

A rapid and selective method for the determination of six UV filter compounds in seawater by LC–MS/MS was developed. The separation of the six analytes was obtained in less than 5 min using a fast chromatographic column (sub-2 μm particle size) with an isocratic elution (80% methanol-20% Milli-Q water).

Performances of APCI and ESI were compared; both ionization sources provided good figures of merit. APCI was chosen for seawater analysis due to its higher sensitivity and reproducibility. The use of $HCOONH_4$ as modifier in the mobile phase provided good results in ESI but only for BP-3, OC, OD-PABA and EHMC and was then abandoned.

Real sample preparation was performed using stir bar sorptive extraction-liquid desorption (SBSE-LD) enabling the selective extraction of the analytes from seawater without significant matrix effect. The optimized SBSE method is simple, avoids further clean-up steps and uses small solvent volumes.

Quantitative analysis was carried out by tandem mass spectrometry in MRM mode. The methods provided a very good sensitivity although HMS and EHS presented definitely higher detection limits than the other analytes. Results obtained from real samples indicate a rather low contamination level of the considered seawaters.

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