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Determination of UV-filter residues in bathing waters by liquid chromatography UV-diode array and gas chromatography–mass spectrometry after micelle mediated extraction-solvent back extraction

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Abstract

A preconcentration methodology utilizing the cloud point phenomenon is described in this study for the determination of sunscreen agent residues in bathing waters by reversed phase liquid chromatography with UV detection and gas chromatography (GC) with mass spectrometric (MS) detection. The method employs the entrapment of the analytes in the micelles of the non-ionic surfactant TX-114, upon increase of the solution temperature to 60 °C. The analytes are either re-extracted or back extracted from the final micellar extract into appropriate organic solvents, a procedure that facilitates the direct application of the method not only with liquid chromatography but mostly importantly with gas chromatographic analysis. Ultrasonication was employed to assist the procedure and accelerate the extraction of the analytes into the solvent phase. Under the optimum experimental conditions, the method affords satisfactory recoveries in the range of 95–102% and relative standard deviation lower than 6% without interference from the presence of the surfactant. The method was successfully applied to the determination of UV filters in natural waters.

Keywords: Sunscreen agents; Liquid chromatography; Gas chromatography; Cloud point extraction; Bathing waters

1. Introduction

Sunscreens (or UV filters) are substances able to absorb UV radiation and thus isolate the human skin from direct exposure to the deleterious wavelengths of sunlight. These compounds usually have a single or multiple aromatic structures, often with attached hydrophobic groups, to improve their properties. However, as single UV filters have a relatively small absorption band, several combinations are made with the intention of obtaining the desired degree of protection. At present, a wide variety of commercial formulations are being marketed delivering protection against both parts of sunlight radiation (UV-A 320–400 nm and UV-B 280–320 nm). For this reason, lists of approved UV absorbers with their maximum allowed concentrations have been set by various regulatory authorities in Europe, USA and Japan.

Sunscreens are designed for external application in the uppermost layers of the skin with minimum penetration. Thus, despite their hydrophobicity, they are prone to washing off from the skin surface especially during water immersion. Studies in controlled conditions (ex vivo measurements using excited human epidermis) have shown that water immersion of the skin, to which a sunscreen formulation has been applied, results in escalated sunscreen loss from the skin surface [1]. The magnitude of this loss depends on the product as well as on water application. More specifically, waterproof or water-resistant formulations are generally maintained to a higher degree compared to products that do not claim similar properties [1]. However, field studies have shown that water and bathing activities may advocate to further washing-off compared to simple water immersion on the skin surface [2].

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In addition, any remaining quantity of sunscreens on the skin will inevitably be transferred to towels and clothes, which will be washed [2,3]. It is therefore rational to assume that sunscreens may be released into bathing waters as well as wastewater and depending on their properties accumulate into the aquatic environment [4].

Reports on the occurrence of sunscreen agents in natural waters are still scant. Only recently, studies have indicated the presence of some of these compounds in waters used for recreational purposes [5,6] but accurate data are still needed to provide a realistic construction of sunscreens fate in the aquatic environment [3]. In our previous studies, we have shown that UV absorbing chemicals hold a high accumulation factor in close systems like swimming pools [7,8]. Small pools with intermittent water recycling posses a higher potential for sunscreens accumulation [7] while lower concentrations are present in larger and better maintained pools [8]. In a different view, degradation mechanisms exert a significant effect on the diurnal variability of sunscreen concentrations in both natural and swimming pool waters [8,9].

From an analytical standpoint, the determination of UV-absorbing chemicals in bathing waters is relatively straightforward, interference free and requires an increased sensitivity allowing for their determination at the low $\mu g\,l^{-1}$ or $ng\,l^{-1}$ levels [6–9]. However, with regards to wastewater samples, traditional methods based on liquid-to-solid extraction procedures (solid phase microextraction or solid phase extraction), may be limited by the presence of high concentration of dissolved organic matter, which may compete with the target species for the reactive sites of the hydrophobic coatings. One simple solution to this problem is offered by separation procedures employing the solubilizing properties of polymer molecules in aqueous solution [10]. These procedures, usually referred to micelle mediated extractions, deploy pseudophases of non-ionic or zwitterionic surfactant micelles as means for accomplishing the separation and extraction of hydrophobic analytes from aqueous media. By simple altering the properties of the solutions (like temperature, salt content or pressure) surfactant micelles separate into two isotropic phases; a surfactant rich phase composed almost totally of the surfactant and an aqueous phase in which the surfactant concentration is close to the critical micellar concentration. That property of micellar aqueous solutions has been extensively used for the entrapment of several hydrophobic analytes, their subsequent separation and preconcentration and finally their determination with any of the available analytical techniques [11–14]. A major pitfall encountered in the conjunction of chromatographic techniques with cloud point extraction (CPE) procedures is the high concentration of the surfactant in the final condensed micellar phase. Most surfactants exhibit a significant absorbance band at 254 nm owing to the presence of an aromatic moiety which render them especially problematic even when UV-DAD detectors are employed. On the other hand, methods deploying gas chromatography (GC) analysis are scant since it is absolutely necessary to eliminate the surfactant through extensive clean-up steps, before its introduction into the GC column [15,16], a procedure that increases experimental effort as well as the time of analysis.

In view of the above, the present study reports on the development of an alternative methodology for the rapid and effective isolation and preconcentration of five major UV filters from natural waters. The method is based on the surfactant-mediated extraction of these compounds from their initial matrix followed by liquid chromatographic UV-diode array or gas chromatographic–mass spectrometric (MS) detection. Re-extraction and back extraction into appropriate organic solvents was tested as means for reducing surfactant interference in the chromatographic analysis thus enabling the direct injection of the micellar phase into the chromatographic systems. It is proved that the proposed method offers good reproducibility and low detection limits that render it suitable for the routine screening of these compounds in water samples.

2. Experimental

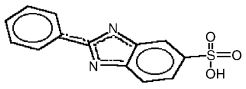
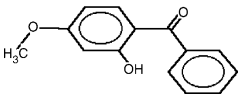
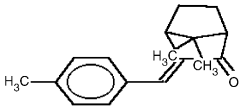
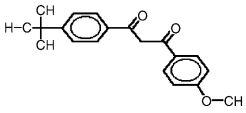
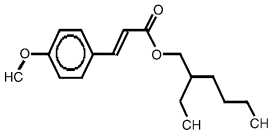
2.1. Reagents

Eusolex 232 (2-phenylbenzimidazol-5-sulfonic acid), 2-hydroxy-4-methoxybenzophenone, benzophenone-3 (Bz), Eusolex 6300 (3-(4-methylbenzyldene)-camphor), Eusolex 2292 (octyl methoxy cinnnamate) and Eusolex 9020 (1-(4-*tert*-butylphenyl)-3-(4-methoxyphenyl)1,3 propanedione)) were purchased from Merck (Darmstadt, Germany). The structure as well as some chemical properties of these substances are gathered in Table 1. Stock standard solutions of 10 mg/l were prepared weekly in methanol and stored in dark at −15 °C. Working standard solutions were prepared daily with appropriate dilution in doubly distilled water. All working solutions were stored in dark and at 4 °C. The non-ionic surfactant Triton X-114 (TX-114) was obtained from Fluka (Buchs, Switzerland) and was used without further purification. The cloud point temperature of this surface active agent is 24 °C [11]. Methanol (MeOH), acetonitrile (ACN), dichloromethane ($MeCl_2$), *n*-hexane and water were of the highest available purity (LC or GC grade) and were obtained by Labscan (Dublin, Ireland). Sodium chloride (NaCl) was supplied from Merck (Darmstadt, Germany) while HCl (32%), which was used for the pH adjustment of the solutions, was purchased from Riedel-de Haen (Sleeze, Germany). Humic acid used for the interference study was purchased from Fluka, Sodium dodecyl sulfate (SDS), obtained from Sigma (Athens, Greece), was used without further purification.

2.2. Apparatus

The chromatographic system comprised a Shimadzu online degassing system DGU-14A coupled to a FCV-10AL controller unit and a LC-10AD high-pressure solvent delivery

Table 1
Physicochemical properties of the examined UV-filters

Compound	Chemical structure	Molecular weight (g mol^{-1})	Water solubility at 25 °C (mg l^{-1})	log K_{ow}[a]	CAS reg. no
Eusolex 232		274.29	128.8	−0.16	27503-81-7
Benzophenone-3		228.25	68.56	3.52	131-57-7
Eusolex 6300		240.35	0.57	5.47	38102-62-4
Eusolex 9020		310.39	1.52	2.41	70356-09-1
Eusolex 2292		290.41	0.15	5.80	5466-77-3

[a] From EPIsuite v.3.1 [17].

pump, with a 20 μl sample loop injector. At the beginning of the investigation an SPD-M6A UV/diode-array detector was employed working under the Class M10A Software (Version 1.20). Following an update of the chromatographic system, a Shimadzu SPD-M10A UV/diode-array detector operating under the chromatography software Class-VP version 5.0 was used for recording chromatographic peaks. The column material was a Discovery C_{18} (Supelco), with particle size of 5 μm, (25 cm × 4.6 mm i.d.) and preceded by a guard column of the same material (8 mm × 3 mm). Isocratic elution was used for the elution of the analytes from the column with a mobile phase composed of water or aquatic mixture of 100 mM SDS/acetonitrile (20/80%, v/v). Column temperature was set at 30 °C and the data collection was performed by obtaining one spectrum per second with resolution of 4.0. The peaks representing the target species were recognized both by the retention time and their spectrum pattern.

GC–MS analysis was performed on a QP 5000 Shimadzu system. The GC was fitted with a DB-5-MS capillary column (J&W Scientific), 30 m × 0.25 mm × 0.25 μm, contained (5% phenyl)-methylpolysiloxane. However, only three out of the five target species were determined, the exception being Eusolex E232 and Eusolex 9020 were no signal was observed whatsoever. To achieve better detection limits and enhanced selectivity, analyses were performed in the selected ion monitoring (SIM) mode using positive electron ionization (+EI). Three ions were selected from the spectrum of each compound in order to quantify the response under SIM mode: 151, 227 and 228 for benzophenone-3, 128, 211, 254 for Eusolex 6300 and 161,178 and 290 for Eusolex 2292. For the other compounds no detector response was observed as previously mentioned.

The resolution of the analytes was accomplished with the following temperature program: 50 °C, held for 1 min, ramped at 20 °C min^{-1} to 150 °C, held for 2 min, to 250 °C at 20 °C min^{-1}, held for 12 min and 20 °C min^{-1} to 270 °C, held for 3 min. The injector temperature was 240 °C and 3 μl injections were made. Helium was used as the carrier gas at a flow of 1 ml min^{-1}. The interface was kept at 290 °C and spectra were obtained at 70 eV.

A thermostated bath maintained at the desired temperatures was used for cloud point temperature experiments and phase separation was assisted using a Jouan LP3 centrifuge. The pH was measured using a Radiometer (Copenhagen, Denmark) digital pH-meter type PHM83 with 0.01 pH resolution over the pH range of 2–10.

2.3. Sampling and sample preparation

Water samples were collected from 1 m depth in a distance of 1–1.5 m from the coast a closed gulf located in a popular touristic area in North Western Greece during July 2004. The samples were collected in methanol-washed glass mineral water bottles, immediately protected from light and refrigerated upon arrival at the laboratory. The samples were filtered through a Whatman No 40 (pore size 0.45 μm), and spiked with 0.05% (w/v) of TX-114 to prevent possible hydrolysis of the compounds [18] and stored at 4 °C until analysis.

2.4. Micelle mediated extraction procedure

The samples were treated with dilute HCl to adjust the pH to the value of 3 and appropriate amounts of NaCl were added to yield an ionic strength of 0.20 M and swirled to

ensure complete mixing. Afterwards, Triton X-114 was added to yield a final concentration of 0.1% (w/v) and the mixture was churned for 1 min and kept for 15 min in a thermostated bath at 60 °C. Separation of the phases was achieved by centrifugation for 10 min at 4000 rpm. The phases were cooled down in order to increase the viscosity of the surfactant-rich phase and the aqueous supernatant was decanted by inverting the tubes. The analytes entrapped in the remaining condensed micellar phase (about 100 μl) were either re-extracted with 100 μl of methanol for LC analysis or back-extracted with 200 μl of *n*-hexane for GC analysis. The final extraction steps were accelerated by sonication for 3 min and the vials were cooled to assist the separation of the two phases. The upper phase (hexane) was carefully transferred to an Ependorf vial with a Pasteur pipette and is volume was brought down to 50 μl under a gentle stream of nitrogen.

3. Results and discussion

3.1. Optimization of the chromatographic conditions

The optimization of both liquid and gas chromatographic conditions for the resolution of the five analytes have already been described in our previous works [8]. There it was made clear that the use of an anionic surfactant (sodium dodecyl sulfate, SDS) as an additive to the hydro-organic mobile phase is imperative in order to achieve efficient resolution of the overlapping peaks. Moreover, the presence of SDS improved the resolution of the analytes eluting adjacent to Triton X-114 thus alleviating its interference during the chromatographic analysis. Based on the results of Table 2 an SDS concentration of 100 mM was applied throughout. Fig. 1 depicts a typical liquid chromatograph of the five analytes extracted with TX-114 (and re-extracted into methanol as will be discussed further below).

3.2. Optimization of the experimental conditions

Any parameter affecting the proposed extraction scheme and micelle formation was optimised with 10 ml water solutions containing 10 μg l^{-1} of the analytes and 0.02 g of Triton X-114 unless otherwise stated. Next the procedural parameters included in the optimisation are described using LC-UV DAD analysis.

Table 2
Retention times of the sunscreen agents and Triton X-114 on the C_{18} column as a function of SDS concentration[a]

Compound	80% Acetonitrile + 20% water containing				
	0 mM SDS	25 mM SDS	50 mM SDS	100 mM SDS	200 mM SDS
Eusolex 232	1.62	1.63	1.89	2.47	2.67
Benzophenone-3	5.00	5.07	5.10	4.90	4.75
Eusolex 6300	8.58	8.52	8.66	8.05	7.85
Eusolex 9020	13.39	13.28	13.24	12.65	11.57
Eusolex 2292	13.95	13.89	13.82	13.22	12.30
Triton X-114	6.98	6.72	6.64	6.60	6.56

[a] Chromatographic conditions as described in the text.

The effect of pH on the extraction of the five UV filters was the first experimental parameter examined. As shown in Fig. 2, acidic pH values favor the extraction possibly due to protonation of the analytes towards their respective molecular forms which are sparingly soluble in water thus, exhibiting a high affinity for the micellar entities. At higher pH values the increase in the degree of ionization of the analytes reduces their transportation into the hydrophobic micellar core while hydrolysis is also probable. Based on these results a pH value of 3 was maintained throughout.

The addition of salts to aqueous solutions of non-ionic surfactants is known to alter the extraction efficiencies through changes in the solvation environment. Addition of NaCl enhanced the extraction efficiency of all species at concentration up to 0.20 M. For high concentrations there was a slight variation in the extraction efficiency possibly due to "salting-out" processes and changes in the physical properties of the surfactant [19]. Nevertheless, non-polar analytes may become less soluble in the matrix at higher salt concentrations and thus contribute to higher recoveries. It is noteworthy that the addition of salt caused less fluctuation in the analytical signals compared to those observed in our previous works with the aid of solid sorbents (SPME and SPE) [8,9]. Based on these results an ionic strength of 0.20 M was maintained throughout the remaining work.

The amount of surfactant required to achieve quantitative extraction of the analytes was then studied. The results of Fig. 3 reveal a narrow plateau (around 0.1–0.2%, w/v) within which maximum extraction efficiency and analytical signal is accomplished. Increasingly surpassing this optimum range, a deterioration in the analytical signal is observed due to the increase of the final surfactant volume causing decrease of the preconcentration factor (phase volume ratio). On the other hand, if surfactant concentration is decreased from the recommended, accuracy and reproducibility would probably suffer because the resulting surfactant-rich phase would not be sufficient for extraction, separation and reproducible measurements.

The recoveries from the cloud point extraction of the UV filters were found to increase with an increase in temperature up to 60 °C. A small reduction (about 5%) at higher temperatures may be due to thermal stability problems of the surfactant aggregates or acceleration of the hydrolysis rates of the compounds. This is further supported by the fact that prolonged heating (1 h and above) caused a gradual reduction in the recovery of almost all compounds. A compensating advantage for the use of increased temperatures is the disruption of the surfactant hydrogen bonds, causing dehydration of the micellar aggregates' inner core, which leads to increased preconcentration efficiency [12]. All things considered, a temperature of 60 °C was maintained for 15 min during the analysis of real samples.

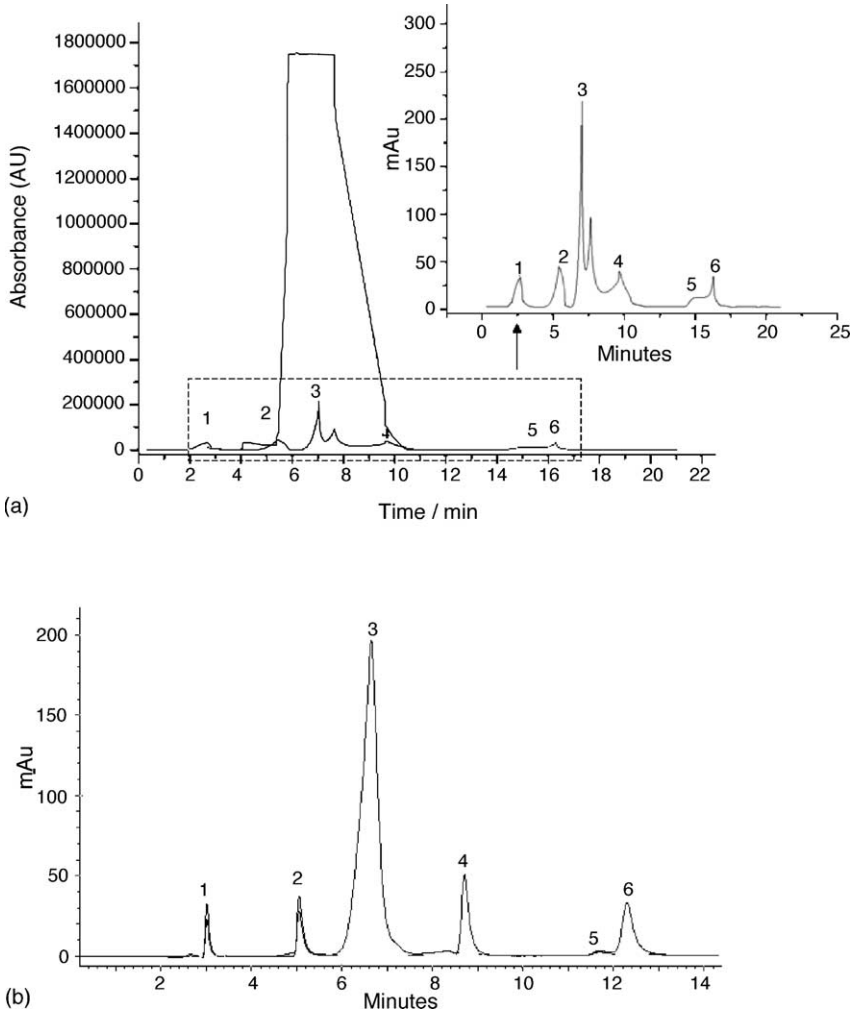


Fig. 1. A LC/UV-DAD chromatogram of a preconcentrated (1 mg l^{-1}) standard solution of the four compounds with Triton X-114 (a) direct injection of the micellar phase in ACN:H_2O (80:20). Inset: injection in ACN:H_2O (80:20) after re-extraction in MeOH. (b) Injection in ACN:H_2O (80:20) containing 100 mM SDS after re-extraction in MeOH. Peak assignment: (1) E232, (2) benzophenone-3, (3) Triton X-114, (4) Eusolex 6300, (5) Eusolex 9020, (6) Eusolex 2292. Conditions as mentioned in the text.

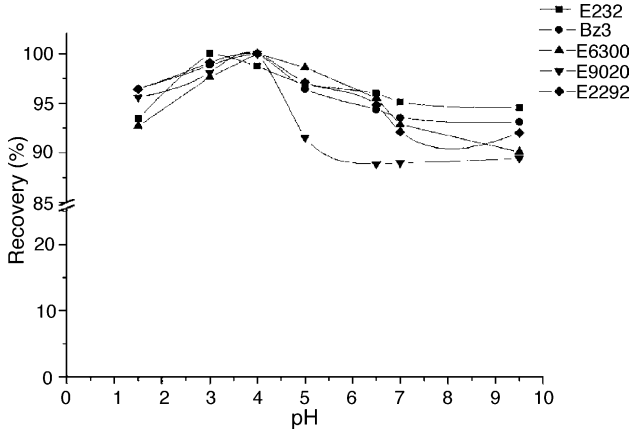


Fig. 2. Effect of pH on the extraction performance. Triton X-114 = 0.1% (w/v), temperature = 50 °C, equilibrium time = 15 min, NaCl = 0.30 M.

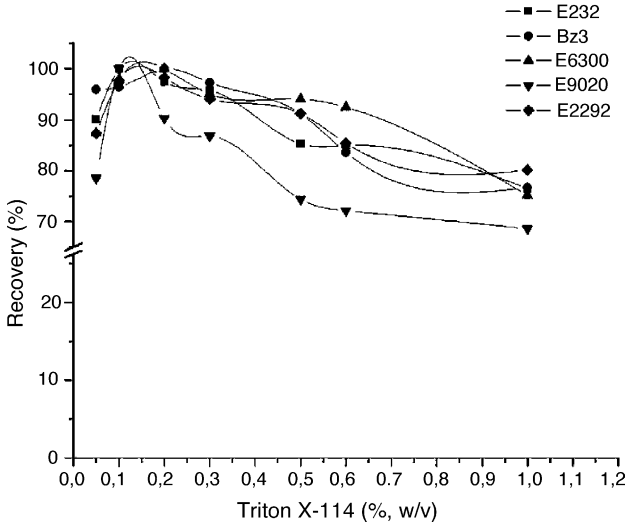


Fig. 3. Effect of Triton X-114 on the performance of the method. pH = 3, temperature = 50 °C, equilibrium time = 15 min, NaCl = 0.2 M.

3.3. Re-extraction and back extraction from the micellar phase

An important advantage of CPE is that the viscous surfactant rich phase can be redissolved in a medium suitable for the requirements of the selected or available detection method. In concurrence with previous studies, direct injection of the condensed micellar phase into the liquid chromatographic column produced a huge absorbance peak owing to the considerable amount of surfactant present in the final extract (Fig. 1a). A simple solution to this problem would be to enrich the mobile phase in organic solvent so that TX-114 could elute earlier alleviating its interference in the separation of other compounds [12]. However, this would force the analytes to elute in shorter time and perplex their resolution and differentiation. To overcome this problem, re-extraction of the analytes from the surfactant rich phase was decided.

Although Moreno-Cordero et al. (1993) reported total elimination of the surfactant in the presence of acetonitrile and with a Vydac 210 TP5415 column [11], re-extraction of the analytes from the micellar phase into a water miscible organic solvent (exposed to ultrasounds for 3 min to facilitate complete dissolution in a short time) could not totally alleviate surfactant peak with the column employed herein. However, surfactant absorbance was significantly reduced and became sharp while eluted in a double peak tail and in shorter time (Fig. 1a-inset). This can be ascribed to the fact that water miscible solvents re-dissolve the condensed micellar phase by reducing its viscosity a situation which favours surfactant mixing with the LC elution mixture. Interestingly, the placement of SDS in the mobile phase not only changed its elution time (from 6.98 min without SDS to 6.60 min with 100 mM SDS) (Table 2) but also alleviated peak tailing.

Although this procedure alleviates surfactant interference during LC analysis it does not resolve the problems encountered during GC analysis were the high concentration of surfactant still endangers blocking of the capillary column. To cope with this problem, the final condensed phase can be treated with a small volume of a water immiscible organic solvent were surfactant has limited solubility [20]. In this context, 200 μl of *n*-hexane were added to the micellar phase. Lower volumes were avoided since they lead to the production of slurries perhaps due to the formation of partially miscible ternary mixtures of water, surfactant and organic solvent [20]. To facilitate quantitative back extraction without either vigorous mixing that could force the partitioning of TX-114 into the hexane phase or prolonged standing of the tubes that would increase the time of analysis, the procedure was accelerated by ultrasonication for 3 min. Longer sonication times gave hazy solutions indicating solubilization of the surfactant into the solvent. The overlaying organic phase was carefully removed with a Pasteur pipette and brought down to 50 μl under a gentle stream of nitrogen. The chromatogram of the GC instrument (Fig. 4) reveals that although back extraction reduces the surfactant interference some scattered peaks are still detected possibly because a portion of the surfactant is transferred into *n*-hexane owing to its high concentration in the final micellar phase. However, they do not interfere with the analysis. Thus, resolution of the target species is accomplished, a task which enables the direct conjunction of CPE with GC without any laborious clean-up and pre-treatment procedures. Reasonably, surfactant peaks can be further eliminated by direct sampling from the *n*-hexane phase (avoiding the evaporation step) but the analyte response will drop accordingly.

Evaporation of the hexane residue and re-dissolution to methanol was also examined and the extract was delivered to the liquid chromatograph. Evidently, the surfactant peak was reduced but no considerable improvement was observed compared to mere methanol application to justify the adaptation of an additional extraction- back extraction step in the overall procedure.

3.4. Interference from natural organic matter

In our previous study, we have observed that the involvement of UV filters in manifold interactions with high amounts of dissolved organic matter (DOC) reduces their retention efficiency of the C_{18} solid sorbent thus deteriorating the recovery [8]. In order to investigate whether the proposed procedure suffers from the same interferences two solutions of humic acids (5 and 10 mg l^{-1} organic carbon content) were spiked with 10 μg l^{-1} of the analytes and were subjected to the CPE procedure. The results obtained (Table 3) show that quantitative extraction was achieved even at the highest concentrations examined possibly due to the charged nature of the humic material which show low affinity for the hydrophobic micellar core [21].

3.5. Analytical characteristics of the method

Calibration graphs for LC and GC analysis were constructed for 50 and 100 ml samples, respectively with 0.1% (w/v) Triton X-114. This concentration of surfactant ensures a sufficient surfactant rich phase volume (about 100 μl after water removal) that enables a high preconcentration factor (about 500) which is necessary in order to bring the concentration of the analytes within the dynamic measuring range of the detector. Furthermore, re-extraction or back extraction of the surfactant phase allows for at multitude injections per sample. Linear relationships between the produced signals

Table 3
Recoveries of sunscreens from humic acid solutions with 0.1% (w/v) Triton X-114

Compound	5 mg l^{-1} humic acid	10 mg l^{-1} humic acid
Eusolex 232	99.4 ± 1.7	98.0 ± 2.1
Benzophenone-3	98.8 ± 1.3	99.0 ± 1.8
Eusolex 6300	100.0 ± 2.0	98.3 ± 1.7
Eusolex 9020	98.0 ± 2.3	96.5 ± 2.0
Eusolex 2292	98.7 ± 2.0	97.4 ± 1.9

Average results from triplicate measurements ± standard deviation.

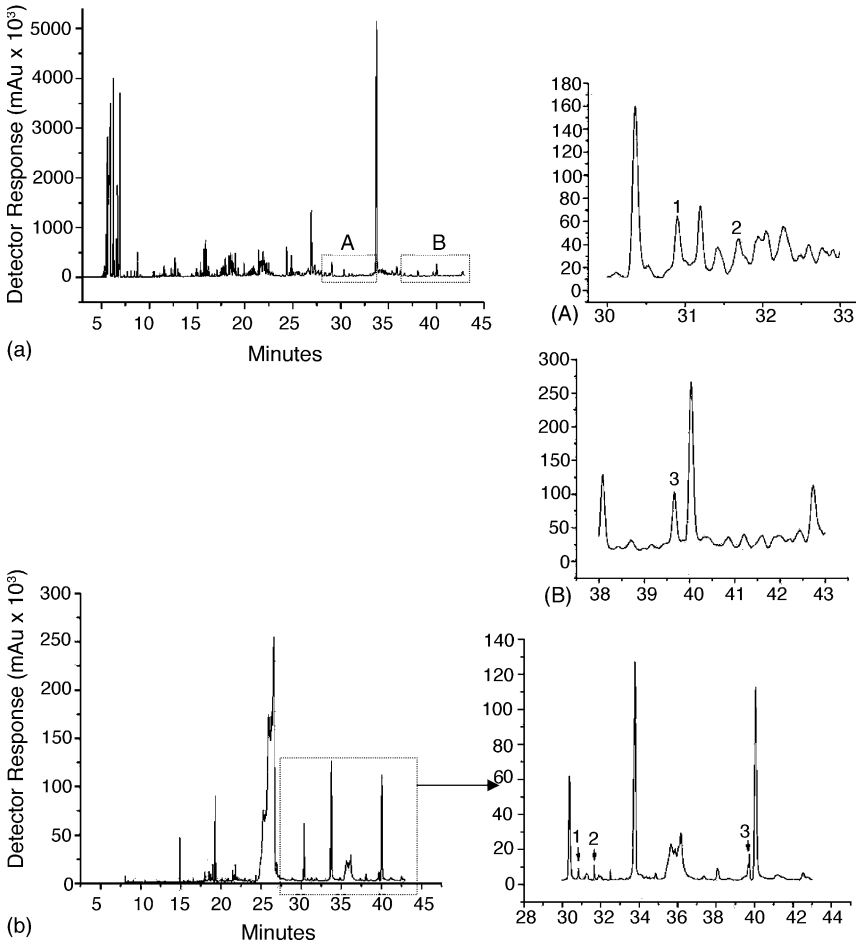


Fig. 4. A GC/MS chromatogram of preconcentrated samples back-extracted into *n*-hexane. (a) Standard mixture containing 5 μg l^{-1} in SCAN mode and (b) Standard mixture containing 2 μg l^{-1} in SIM mode. Peak assignement: (1) benzophenone-3, (2) Eusolex 6300, (3) Eusolex 2292. Conditions as mentioned in the text.

and the concentrations were found for all compounds investigated. The parameters of the individual calibration curves together with the calculated detection limits (three times the signal to noise ratio) and the relative standard deviation for five samples are gathered in Table 4. It is evident that the proposed procedure offers the required sensitivity for the determination of the studied compounds at the low and ultra-low concentration levels. Enhancement factors were above 50 for all compounds compared to non-extractive analysis. However, back extraction is the regulating parameter of the

Table 4
Analytical characteristics of the method

Compound	Linear range	Calibration curve	LOD[a]	r^2	RSD[b] (%)	Enchantment factor (%)[c]
LC-UV DAD						
E232	0.5–20 μg l^{-1}	$A = 6545 \pm 4518 + 45122 \pm 480 \times C$	0.30 μg l^{-1}	0.9995	4.4 (5)	80.0
Benzophenone−3	0.5–20 μg l^{-1}	$A = -2111 \pm 4583 + 30628 \pm 487 \times C$	0.45 μg l^{-1}	0.9989	4.3 (5)	67.7
E6300	0.5–20 μg l^{-1}	$A = -2241 \pm 2178 + 48003 \pm 232 \times C$	0.14 μg l^{-1}	0.9997	3.9 (5)	70.2
E9020	2–50 μg l^{-1}	$A = 17781 \pm 6441 + 15160 \pm 22 \times C$	1.27 μg l^{-1}	0.9988	5.2 (20)	50.0
E2292	1–30 μg l^{-1}	$A = 8048 \pm 6530 + 34862 \pm 457 \times C$	0.56 μg l^{-1}	0.9991	4.6 (5)	72.6
GC–MS (SIM)						
Benzophenone-3	30–250 ng l^{-1}	$A = 5990 \pm 4037 + 1936 \pm 28 \times C$	6.2 ng l^{-1}	0.9996	6.4 (50)	61.7
E6300	75–500 ng l^{-1}	$A = 7945 \pm 3439 + 342 \pm 13 \times C$	30.0 ng l^{-1}	0.9958	5.6 (150)	58.1
E2292	0.5–50 ng l^{-1}	$A = 78154 \pm 2015 + 2704 \pm 88 \times C$	2.2 ng l^{-1}	0.9968	5.9 (10)	81.0

[a] LOD, limit of detection.
[b] Values in parenthesis are the compound concentrations for which RSD was obtained ($n = 5$).
[c] Ratio of slopes of the calibration curves with and without the preconcentration step.

Table 5
Mean recoveries (%) from the extraction of the selected compounds from spiked water samples and results from the analysis of real samples

	E232		Bz-3		E6300		E9020		E2292	
	Measured ± SD	Recovery (%)	Measured ± SD	Recovery (%)	Measured ± SD	Recovery (%)	Measured ± SD	Recovery (%)	Measured ± SD	Recovery (%)
Distilled water[a]	n.d.	98.7	n.d.	102.0	n.d	99.2	n.d	98.5	n.d	99.5
Bathing waters (12:00 p.m.)[b]	n.d.	96.5	6.5 ± 1.4	96.7	13.1 ± 1.2	97.0	n.d.	95.2	7.4 ± 0.9	97.0
Bathing waters (15:00 p.m.)[b]	n.d.	97.6	8.2 ± 1.6	98.0	19.7 ± 1.3	97.6	n.d.	96.7	10.7 ± 1.1	97.7

All concentrations are expressed in ng l^{-1}. SD = standard deviation, n.d. = not detected. Analysis and recovery experiments of E232 and E9020 were conducted with LC UV-DAD.
[a] Recovery analysis was performed with LC-UV DAD at the spiking level of 10 μg l^{-1}; $n = 3$.
[b] Recovery analysis was performed with GC–MS (SIM) at the spiking level of 100 ng l^{-1}; $n = 3$.

enhancement factors provided of course that quantitative extraction from the CPE procedure has been accomplished.

In order to test the reliability of the proposed methodology for the extraction and preconcentration of UV filters several samples were spiked with 10 μg l^{-1} of the analytes and were subjected to the aforementioned procedure. The recoveries are summarized in Table 5.

3.6. Application

The method described above was applied to the analysis of water samples obtained from a touristic area during the summer period (July 2004), which is the period of maximum use and environmental exposure. Two samplings were conducted in one day. Whenever the concentration of the analytes was below the detection limits, larger sample volumes were extracted including the standard solutions used of the calibration curve. The results of Table 5 show that UV filters are accumulated in natural waters as a function of the bathing activity. Increasing activity and sun exposure during late noon hours results in higher sunscreen application and therefore in higher concentrations in seawater.

4. Conclusions

In this study, a new method exploiting the cloud point phenomenon has been presented for the determination of UV filter traces in bathing waters. The method is based on the preconcentration of the analytes in the micellar aggregates of a non-ionic surfactant medium upon increase of the solution temperature. One of the salient assets of the proposed method is the adaptation of a simple extraction procedure (re-extraction or back extraction of the surfactant extract) which alleviates surfactant interferences in the separation of the target analytes when either LC or GC detection is deployed. Moreover, the efficiency of the method is not negatively affected even in the presence of high amounts of natural organic matter, which enable its application in the analysis of a wide variety of natural waters. The method was successfully applied in the investigation of these compounds in bathing waters and proved to consist of an alternative tool in the monitoring of this new class of potential environmental pollutants.

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