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Journal of Chromatography A, 1026 (2004) 289–293
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Determination of residues of UV filters in natural waters by solid-phase extraction coupled to liquid chromatography–photodiode array detection and gas chromatography–mass spectrometry

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Received 13 May 2003; received in revised form 23 October 2003; accepted 28 October 2003

Abstract

This study describes a procedure for the enrichment, separation and quantification of four major UV filters in natural waters. Solid-phase extraction combined with liquid chromatography and photodiode array detection (LC–UV-DAD), and gas chromatography with mass spectroscopy (GC–MS) were employed for the analyses. LC of the four compounds with surfactant-modified hydro-organic eluents gave satisfactory resolution of overlapping peaks. In GC, a significant improvement of the detection limits was attained, but only three compounds could be detected. The method was validated for, and applied to, various water samples prone to UV filter accumulation due to recreational activities. Recoveries from real samples were 86–99% with LOQs as low as 0.5 ng/l depending on the sample volume and the analytical procedure.

Keywords: Water analysis; Solid-phase extraction; Sunscreen agents

1. Introduction

The composition of commercial sun protection products (called sunscreens) consists of various combinations of these compounds to achieve the required degree of protection. Their main characteristics are the presence of single or multiple aromatic structures, often with attached hydrophobic groups, to improve their properties. Reversed-phase liquid chromatography with UV-Vis detection is frequently employed to quantify these compounds in commercial formulations, in order to ensure compliance with the proposed maximum permissible concentrations [1–4].

Sunscreens are applied superficially to the skin, and are designed to remain on the uppermost layers with minimal penetration. Consequently, they can be washed off by the application of water [5,6]. Real-life investigations showed that water and bathing activities may cause more washing-off of these products than simple water immersion on the skin surface [7]. It is therefore reasonable to speculate that sunscreens may be emitted to the aquatic environment either directly (bathing water) or indirectly (household wastewater and sewage works).

To date, reports on the presence of UV filters in bathing waters are still scant and only a few studies deal with the determination of these compounds in natural waters [8,9]. The lack of such analytical studies probably explains why research on their fate and behavior in the water environment is limited. To improve this situation, this study discusses the determination of four major UV filters by means of solid-phase extraction and reversed-phase liquid chromatography, or gas chromatography. The method was successfully applied to the monitoring of these compounds in bathing waters and shower wastewater.

2. Experimental

2.1. Reagents

2-Hydroxy-4-methoxybenzophenone, benzophenone-3, Eusolex 6300 (3-(4-methylbenzyldene)-camphor), Eusolex 2292 (octyl methoxy cinnnamate) and Eusolex 9020 (1-(4-*tert*-butylphenyl)-3-(4-methoxyphenyl)1,3-propanedione)

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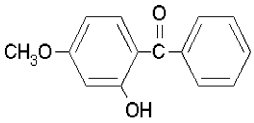
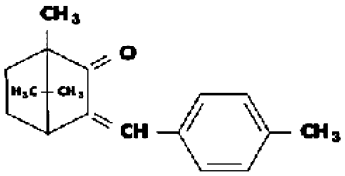
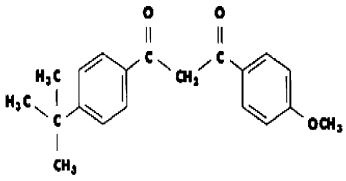
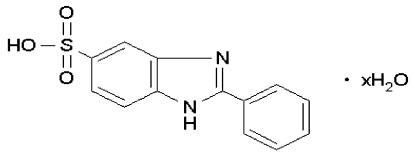
doi:10.1016/j.chroma.2003.10.114

Table 1
Physicochemical properties of the examined UV filters

UV filter	Chemical structure	Molecular weight (g/mol)	log K_{ow}[a]	Solubility (mg/l)[a]	λ_{max} (nm)	CAS registration no.
Benzophenone-3		228.25	3.52	68.56	290	131-57-7
Eusolex 6300		240.35	5.47	0.57	300	38102-62-4
Eusolex 9020		310.39	4.51	1.52	355	70356-09-1
Eusolex 2292		290.41	5.80	0.15	305	5466-77-3

[a] From EPIsuite v.3.1 [10].

were purchased from Merck (Darmstadt, Germany). Their structures and relevant properties are given in Table 1. Stock standard solutions were prepared weekly in methanol and stored in the dark at −15 °C. Working standard solutions of 5–10 μg/l were prepared daily with appropriate dilution in doubly distilled water. All working solutions were stored in the dark and at 4 °C. The solvents used, ethyl acetate (EA), dichloromethane ($MeCl_2$), methanol (MeOH), acetonitrile (ACN) and water were of the highest available purity (LC or GC grade), and were obtained from Pestiscan (Labscan, Dublin, Ireland) and Merck. Potassium chloride (KCl) was supplied by Merck and HCl (32%), which was used for pH adjustment, was from Riedel-de Haen (Seelze, Germany). Sodium dodecyl sulfate (SDS), obtained from Sigma (Greece), was used without further purification.

2.2. Separation and detection

The LC system comprised a Shimadzu on-line DGU-14A degassing system coupled to a FCV-10AL controller unit and a LC-10AD high-pressure solvent delivery pump, with a 20-μl sample loop injector and a Shimadzu SPD-M10A UV-diode array detector. The column material was a Discovery C_{18} (Supelco), with 5 μm particles (25 cm × 4.6 mm i.d.) with a guard column of the same material (8 mm × 3 mm). The column temperature was maintained at 30 °C. Water (3.5 mM SDS)/acetonitrile (20/80%, v/v) was used for the isocratic elution of the analytes. Spectrum identification of each individual compound was performed at the absorbance maximum attained at the wavelengths reported in Table 1.

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 mm × 0.25 mm × 0.25 μm, containing (5% phenyl)-methylpolysiloxane. 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: *m/z* 151, 227 and 228 for Benzophenone-3, *m/z* 128, 211 and 254 for Eusolex 6300 and *m/z* 161, 178 and 290 for Eusolex 2292.

The temperature program run was: 50 °C, held for 1 min, ramped at 20 °C/min to 150 °C, held for 2 min, ramped at 20 °C/min to 250 °C, held for 12 min and ramped at 20 °C/min 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. The interface was kept at 290 °C and spectra were obtained at 70 eV. Fig. 1a gives a typical GC–MS-SIM chromatogram of the three compounds.

2.3. Sampling and sample preparation

Samples from recreational and bathing waters were collected in dark glass bottles from the coastal line of North Western Greece (Epirus). Shower wastewater were collected from the sewage network of the hotels using the

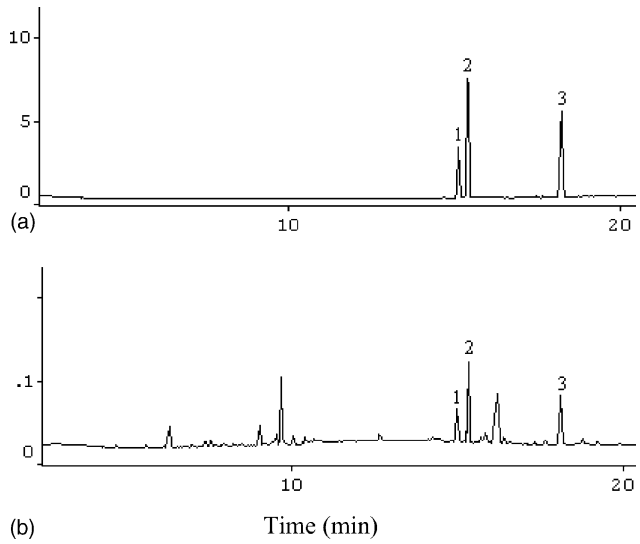


Fig. 1. A GC–MS-SIM chromatogram of: (a) a standard mixture containing 50 μg/l and (b) a real swimming pool water sample extracted with SPE (data shown in Table 3). Peak assignment: (1) benzophenone-3, (2) Eusolex 6300, and (3) Eusolex 2292. Conditions as mentioned in the text.

swimming pools under investigation. The samples, once transferred to the laboratory, were immediately extracted to avoid decomposition. For recovery studies, a portion of the samples was fortified with appropriate amounts of the standard mixtures, they were shaken vigorously to ensure homogenization and subjected to the aforementioned procedure.

2.4. Sample preconcentration and separation by SPE

The extraction of the analytes was performed with the aid of a Supelco (Bellefonte, PA, USA) vacuum apparatus, using the 500 mg C_{18} disks (47 mm) obtained from Empore (St. Paul, MN, USA).

The water samples were spiked with an amount of KCl, to obtain a final concentration of 10% (w/v) and filtered to remove any suspended particulate matter through a Whatman no. 40 filter (pore size, 0.45 μm). The filtrate was acidified with dilute HCl to pH of 3 under continuous stirring for approximately 5 min.

Before extraction, the disks were conditioned with 5 ml ethyl acetate/dichloromethane (1:1, v/v), followed by 10 ml of methanol and 5 ml of deionized water. Next, an aliquot of the sample (up to 500 ml) was percolated through the disk. A small volume of methanol was added (1%, v/v) to maintain a constant and fast sample passing rate. The disk was dried for about 5 min under vacuum. The analytes were extracted with two 5 ml aliquots of ethyl acetate/dichloromethane (1:1, v/v). The extracts were evaporated to dryness under a gentle stream of nitrogen and redissolved in scaled micro vials to 0.050 ml with methanol for LC analysis or 0.010 ml of *n*-hexane for GC–MS analysis.

3. Results and discussion

3.1. Optimization of LC conditions

The separation of the analytes on the C_{18} column was optimized with regard to the mobile phase. Isocratic elution

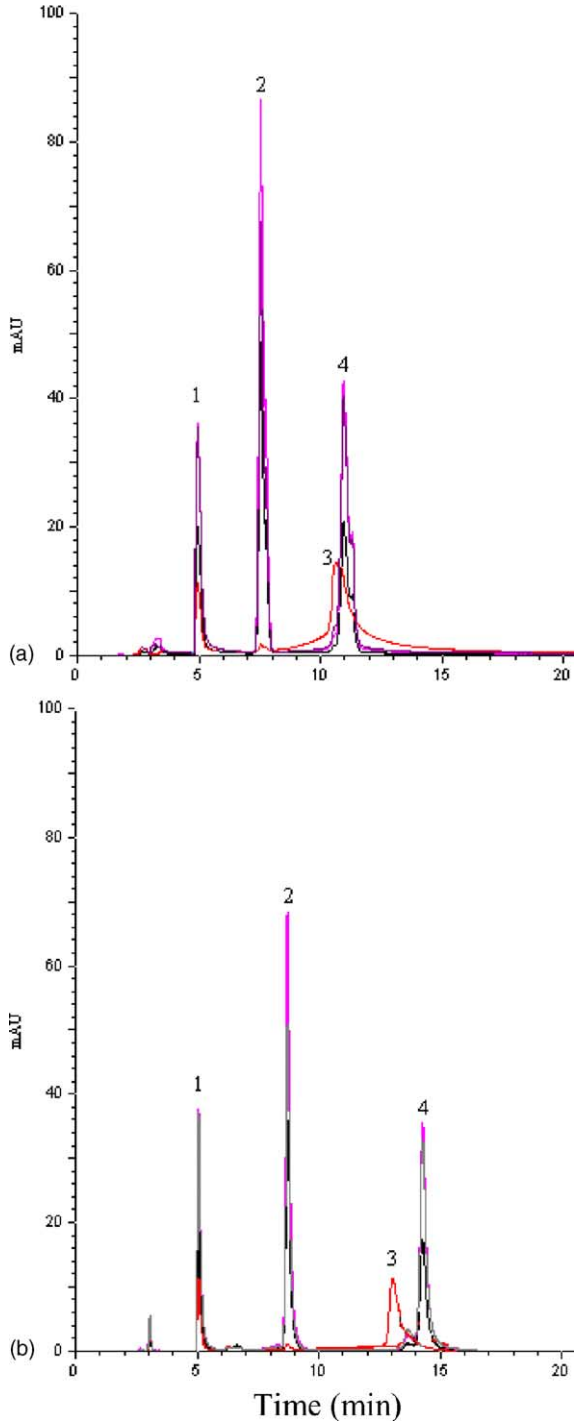


Fig. 2. A LC–UV-DAD chromatogram of a standard mixture containing 1 mg/l of the four compounds. (a) Conventional hydro-organic elution and (b) surfactant-modified hydro-organic elution of the test analytes. Peak assignment: (1) benzophenone-3, (2) Eusolex 6300, (3) Eusolex 9020, and (4) Eusolex 2292. Conditions as mentioned in the text.

Table 2
Linearities and quantitation limits of the proposed method

	GC–MS			LC–UV-DAD		
	(R^2)	Linear range (ng/l)	LOQ (ng/l)	(R^2)	Linear range (ng/l)	LOQ (ng/l)
Benzophenone-3	0.9963	5–120	1.4	0.9957	20–200	14
Eusolex 6300	0.9990	1–150	0.7	0.9988	10–150	8
Eusolex 9020	–	–	–	0.9981	50–200	24
Eusolex 2292	0.9987	2–100	0.9	0.9987	20–180	13

with acetonitrile/water (80:20, v/v) was found to produce good resolution and a short time of analysis (Fig. 2a). Unfortunately, however, separation of Eusolex 9020 and Eusolex 2292 is not accomplished. This is not a problem when photodiode array detectors are used [1] but precludes the possibility of using a simple UV-Vis detector. To overcome this problem the addition of a phase modifier, viz.: the anionic surfactant SDS, was studied to modify the polarity and solvation properties of the eluents [11]. Concentrations in the range of 1.7–7.0 mM of SDS were tested. At concentrations above 2.8 mM Eusolex 9020 and Eusolex 2292 started to be separated, with complete separation above 3.5 mM (Fig. 2b).

3.2. Optimization of SPE conditions

The effect of pH on the extraction efficiency of the SPE procedure was the first parameter studied. Acidic pH values favor the separation of sunscreen agents [12,13], probably due to a reduction of their ionic charge in agreement with earlier data [8]. At higher pH values hydrolysis probably takes place, and the increasing ionization of the analytes further reduces their retention on the hydrophobic C_{18} phase. For pH < 2, a slight decrease was also observed, possibly due to alteration of the SPE material. A pH of 3 was maintained throughout all further work.

The addition of salt to aqueous solutions is known to alter extraction efficiencies through changes in solvation environment. In addition, seawater has a relatively high salt content (approximately 35 g/l). The addition of KCl was found to enhance the extraction efficiency of benzophenone-3 and E9020, but to reduce those of E2292 and E6300. At higher concentrations, up to 12.6% (w/v), a slight improvement was attained for all compounds except E9020. A concentration of 10% (w/v) was used in all further work. Salt was added before sample filtering to avoid analyte loss on the filter, caused by their rather high K_{ow} values, especially for the wastewater samples, which had a high particle content [14].

The determination of the breakthrough volumes for all four analytes was performed by spiking the same amount of UV filters in different volumes of swimming pool water. The recoveries were above 88% when using 500 ml of sample. For larger volumes, breakthrough started to occur.

As regards the elution volume, spiked distilled water samples were extracted with 5.0–20.0 ml of ethyl acetate/dichloromethane (1:1, v/v). Maximum recoveries were observed when using 2 × 5 ml aliquots.

3.3. Analytical characteristics

The analytical features of the method were determined by spiking experiments in 500 ml doubly distilled water containing 10% (w/v) of KCl. The produced results are summarized in Table 2. The precision expressed as RSD (%) was determined by analysing three replicate spiked distilled water samples at the concentration level of 100 ng/l. In all cases the RSD (%) values were better than ±5% with both detectors and for all analytes.

Recovery was determined for all types of waters examined. Absolute recoveries were determined using external calibration. Satisfactory recoveries were obtained in all cases with lower values observed in the complex wastewater samples (Table 3). Possibly, the high amount of dissolved

Table 3
Results of GC–MS and LC–UV (only for Eusolex 9020) analysis of real samples and analytical evaluation (estimated according to the IUPAC recommendations)[a]

Sample	UV filter							
	Benzophenone-3		Eusolex 6300		Eusolex 9020		Eusolex 2292	
	Concentration ± S.D. (ng/l)	Recovery (%)	Concentration ± S.D. (ng/l)	Recovery (%)	Concentration ± S.D. (ng/l)	Recovery (%)	Concentration ± S.D. (ng/l)	Recovery (%)
Ionian sea	1.8 ± 0.4	95	Traces	96	nd	87	nd	93
Swimming pool	4.2 ± 0.9	97	6.9 ± 1.0	99	nd	88	4.5 ± 0.9	96
Game pool water	5.7 ± 0.9	–	5.4 ± 1.1	–	nd	–	3.0 ± 0.4	–
Shower wastes (game pool and toilets)	10.0 ± 1.7	97	3.8 ± 0.5	95	nd	86	4.1 ± 0.3	92

[a] Spiked = 100 ng/l.; $n = 3$; S.D. = standard deviation; nd = not detectable.

Table 4
Statistical comparison of both measurement techniques

UV filter	LC mean ± s_1 (μg/l)	GC–MS mean ± s_2 (μg/l)	F ratio[a]	t-values[b]
Benzophenone-3	101 ± 2.8	100 ± 1.7	2.71	0.75
Eusolex 6300	95 ± 1.9	97 ± 2.0	1.11	1.77
Eusolex 9020	101 ± 3.2	–	–	–
Eusolex 2292	96 ± 2.2	99 ± 1.8	1.49	1.53

[a] F ratio calculated from s_1 and s_2: the larger variance is always the numerator. Confidence probability level at 95% ($P < 0.05$). Degrees of freedom, 7 for both numerator and denominator; the tabulated F-value for $F_{P(n1,n2)} = F_{0.05(7,7)} = 3.79$.

[b] Individual experimental t-values calculated from $t = |\text{mean}_{LC} - \text{mean}_{GC-MS}|/\sqrt{\{(s_1^2 + s_1^2)/n - 1\}}$. Confidence probability level at 95% ($P < 0.05$). Degrees of freedom, 7; the tabulated t-value for $t_{P(n)} = t_{0.05(7)} = 2.36$.

organic matter (DOC) in these samples causes clogging of the C_{18} packing material and interferes with the retention of the UV sunscreens. In addition, humic substances may as well have interacted with the organic analyte molecules [15], especially at the low pH value applied, causing reduced retention on the SPE material.

To verify the confidence in the comparability of the results between LC and GC analysis, a statistical comparison was performed (Table 4). Equivalence was evaluated by applying the F-test for equality of the variance and the T-test for equality of the mean measured concentration among the seven measurements of each sample. For simplicity, the variances were considered to be constant among the various samples. Samples were fortified at 100 ng/l to ensure the robustness of the measurements. Neither the individual F-test nor the T-test revealed significant differences, indicating the equivalency of the methods, provided that the analyte concentrations lie within the dynamic measuring range of each detector.

The accuracy of the proposed procedure was tested with a commercial sunscreen product analyzed according to a previously described method [13]. Once the concentrations have been measured, properly weighted amounts were dissolved in methanol and then diluted with doubly distilled water ensuring that the final sunscreen concentrations do not exceed their water solubilities (10 μg/l). This artificial aquatic sample was then extracted according to the proposed protocol. In both cases, quantitation was performed using external calibration curves and the results are summarized in Table 5.

Table 5
Results of LC–UV-DAD procedure compared to a reference protocol [13][a]

UV filter	Reference method (%)[b]	Proposed method (%)
Benzophenone-3	4.65	4.59
Eusolex 6300	1.95	1.88
Eusolex 9020	1.65	1.60
Eusolex 2292	7.60	7.54

[a] RSD ($n = 3$) below 7%.

[b] g/100 g.

It is explicit that the proposed procedure offers the required accuracy for the determination of these compounds in the aquatic matrix.

3.4. Application

The described method was applied to the determination of sunscreen residues in bathing water from the coast of North-Western Greece (Epirus) and shower wastewater. The sampling campaign took place in the summer, which is the period of maximum use as well as environmental and light exposure. The results of this study show that sunscreen residues are present at up to 10 ng/l in water used for bathing activities.

4. Conclusions

The proposed procedure uses solid-phase extraction of UV filter residues from water with the aid of LC and GC techniques. Although LC–UV does not achieve the sensitivity of GC–MS, it offers a shorter time of analysis and can be applied to a broader range of analytes. The detection limits are at the low ng/l level with recoveries higher than 86% and RSD values below 5%.

Acknowledgements

The authors would like to extend their gratitude to Dr. D. Melissos for supplying us with information regarding the properties of the sunscreens agents as well as Mr. J. Pappas for his contribution during the sampling campaign.

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