Solid-Phase Extraction and Field-Amplified Sample Injection-Capillary Zone Electrophoresis for the Analysis of Benzophenone UV-filters in Environmental Water Samples

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Abstract

A field amplified sample injection-capillary zone electrophoresis (FASI-CZE) method for the analysis of benzophenone (BP) UV-filters in environmental water samples was developed, allowing the separation of all compounds in less than 8 minutes. A 9- to 25-fold sensitive enhancement was obtained with FASI-CZE, achieving limits of detection down to 21-59 µg/L for most of the analyzed BPs, with acceptable run-to-run and day-to-day precisions (relative standard deviations lower than 17%). In order to remove water sample salinity and to enhance FASI sensitivity, an off-line solid-phase extraction (SPE) procedure using a Strata X polymeric reversed-phase sorbent was proposed, obtaining recoveries up to 72-90% for most of benzophenones. With the combination of off-line SPE and FASI-CZE, limits of detection in the range 0.06-0.6 µg/L in a river water matrix, representing a 2400- to 6500-fold enhancement, were obtained. Method performance was evaluated by quantifying a blank river water sample spiked at 1 µg/L. For a 95% confidence level, no statistical differences were observed between found concentrations and spiked concentrations (probability at the confidence level, *p* value, of 0.60), showing that the proposed off-line SPE-FASI-CZE method is suitable for the analysis of benzophenone UV-filters in environmental water samples at low µg/L levels. The method was successfully applied to the analysis of BPs in river water samples collected before and after industrialized and urban areas, and in some drinking water samples.

KEYWORDS: Solid-Phase Extraction; Field-Amplified Sample Injection; Capillary Zone Electrophoresis; Benzophenone UV-filters; Water Analysis

1. Introduction

Nowadays it has been well established that excessive UV radiation is clearly detrimental and may cause sunburn, premature aging of the skin, development of skin cancers and cataracts, immune suppression, and even the activation of latent viruses [1,2]. In order to reduce the harmful effects of UV radiation to human health, national and international health authorities have advised the public to take protective measures, and among them sunscreen agents are often the most feasible to use in order to absorb harmful UV radiation [1]. For that purpose, UV-filters which can reflect or absorb harmful UV radiation are commonly added to various sunscreen products as well as in several personal care products [3]. Among them, benzophenones (BPs) UV-filters are widely used because of their excellent absorbing abilities for the UVA (320-400 nm wavelengths) component of the solar radiation [4,5]. The European Union has established a list of allowed European cosmetic UV-filters which include several BPs [6]. These chemicals can easily reach the aquatic environment by direct sources (e.g. sunbathing or swimming) and/or indirect sources (wastewater-treatment plants, showering or domestic washing), thus being accumulated in environmental water reservoirs such as sea, lakes or rivers [3,7]. Additionally, some studies have shown that organic UV-filters, and among them several BPs, could cause hormonal disruption on the reproduction of fish [8], and possess endocrine activity [9], even at low concentration levels. UV-filters have been recently classified as emerging contaminants. For this reason, the development of sensitive and reliable methods for their analysis in environmental samples is needed..

Different analytical methods have been employed for determining benzophenone UV-filters in environmental samples. Liquid chromatography (LC), using basically C18 reversed-phase columns, together with gas chromatography (GC), both of them mainly coupled with mass spectrometry (MS), are the techniques of choice for the quantitative determination of UV filters [10-17]. Regarding GC, derivatization with silylating reagents is frequently necessary to increase the volatility of these compounds. In addition, taking into account that the UV-filters are in the low µg/L to ng/L range in environmental samples, enrichment techniques are usually employed to improve the sensitivity and limits of detection.

Lately, the use of capillary electrophoresis (CE) techniques has increased as an alternative to LC because of its high efficiency, rapid analysis, and low reagent consumption, and several applications dealing with the analysis of UV-filters in

cosmetics are described in the literature [5,18-21]. To the best of our knowledge, there is only one publication in the literature describing the use of a capillary electrophoresis-mass spectrometry (CE-MS) method for the analysis of several UV-filters, including some BPs, in river water samples [22]. Despite the high efficiency of CE methods they present relatively low sensitivity because of the small volume of sample injected (2-10 nL) and the short optical-path length (25-100 μm). This problem can be overcome by on-line preconcentration techniques such as field-amplified sample injection (FASI), stacking, and sweeping [23]. Among these techniques, FASI is very popular since it is quite simple only requiring the electrokinetical injection of the sample after the introduction of a short plug of a high-resistivity solvent such as methanol or water [24]. FASI is taking advantage of the higher amount of analytes introduced into the capillary when electrokinetic injections are used. The pre-injection of a short plug of a high-resistivity solvent such as water allow the enhancement of the sample electrokinetic injection because of the conductivity differences between the sample and the water plug. Once the analytes enter into the capillary they will stack-up in the boundary region between the high-resistivity solvent and the background electrolyte (BGE) used, and separation will take place.

This work was aimed at developing a capillary zone electrophoretic (CZE) method for the simultaneous determination of eight benzophenone UV-filters in environmental water samples. In order to improve method sensitivity, the applicability of FASI was also evaluated. The influence of several parameters such as buffer composition and electrophoretic acquisition conditions on the analysis of benzophenones was studied. Quality parameters, such as limits of detection (LODs), limits of quantification (LOQs), linearity, and run-to-run and day-to-day precisions, were established with both CZE-UV and FASI-CZE methods. Despite the expected improvement on sensitivity with FASI, environmental water sample salinity could be a problem to an efficient FASI application. For this reason, a solid-phase extraction (SPE) step previous to FASI-CZE analysis was evaluated in order to remove water sample salinity, and at the same time as an additional enrichment procedure to enhance sensitivity (taking into account the very low concentration levels of BPs in environmental waters). Several SPE sorbents were compared, and recoveries and breakthrough volumes were established. Method performance (LODs, precision, accuracy) of the proposed method (combination of off-line SPE and FASI-CZE) for the analysis of 8 BPs in a spiked blank river water sample was established. Finally, the

method was applied to the analysis of BP UV-filters in river water samples, as well as in a mineral and a tap water samples.

2. Materials and Methods

2.1. Chemicals

The benzophenone UV-filters studied, which are shown in Table 1, were 4-hydroxybenzophenone (HBP), 2,4-dihydroxybenzophenone (24DHBP or BENZ-1), 4,4'-dihydroxybenzophenone (44DHBP), 2,3,4-trihydroxybenzophenone (TrHBP), 2,2',4,4'-tetrahydroxybenzophenone (THBP or BENZ-2), 2-hydroxy-4-methoxybenzophenone (HMBP or BENZ-3), 2,2'-dihydroxy-4-methoxybenzophenone (DHMBP or BENZ-8), and 2,2'-dihydroxy-4,4'-dimethoxybenzophenone (DHDMBP or BENZ-6), all of them obtained from Sigma-Aldrich (Steinheim, Germany).

HPLC gradient-grade methanol, dichloromethane, hydrochloric acid (25%), sodium hydroxide, and sodium tetraborate were also obtained from Sigma-Aldrich.

Stock standard solutions of all benzophenones (~1000 mg/L) were prepared in methanol in amber-glass vials. Intermediate working solutions were prepared weekly from these stock standard solutions by appropriate dilution with water (CZE) or with a 2.5 mM sodium tetraborate aqueous solution (FASI). All stock solutions were stored at 4 ^{o}C for no more than 1 month. Background electrolyte (BGE) was prepared daily by diluting a 100 mM sodium tetraborate solution with water.

Water was purified using an Elix 3 coupled to a Milli-Q system (Millipore, Bedford, MA, USA) and filtered through a 0.22 µm nylon filter integrated into the Milli-Q system.

2.2. Instrumentation and methods

CZE-UV and FASI experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis instrument equipped with a diode array detector. Electrophoretic separations were carried out using uncoated fused-silica capillaries with a total length of 50 cm (40 cm effective length) x 75 µm I.D. (360 µm O.D.). BGE consisted of a 35 mM sodium tetraborate buffer solution (pH 9.2). Capillary temperature was held at 25 ^{0}C. The BGE was filtered through a 0.45 µm nylon membrane filter (Whatman, Clifton, NJ, USA) and degassed by sonication for 5 minutes before use. For CZE-UV, samples were loaded by pressure-assisted hydrodynamic injection (10 s, 3.5 kPa). The electrophoretic separation of BP UV-filters was performed by applying a

capillary voltage of +30 kV (normal polarity) (capillary current of ~180 µA). Direct UV absorption detection was carried out from 190 to 400 nm, and sample quantification was performed at three UV wavelengths depending on the compound: 240 nm (HMBP), 285 nm (DHMBP and DHDMBP) and 345 nm (other BPs). FASI experiments were performed as follows: the capillary was first filled with BGE (35 mM sodium tetraborate buffer) and then a water plug was introduced into the capillary by pressure assisted hydrodynamic injection (20 s, 3.5 kPa). Samples were then introduced into the capillary by electrokinetic injection at -10 kV (reversed polarity) during 25 s. The electrophoretic separation was then performed by applying +30 kV (normal polarity) through the capillary. For FASI, standards were prepared in a 2.5 mM sodium tetraborate buffer solution used as sample matrix to guarantee the ionization of benzophenone UV filters (pka values below 8.14). The CE instrument was controlled using a Beckman P/ACE station software version 1.2.

New CE capillaries were pre-treated with 0.1 M hydrochloric acid for 30 min, water for 30 min, 0.1 M sodium hydroxide for 30 min, and finally they were washed with water for 30 min. At the beginning of each session, the capillary was rinsed with 0.1 M sodium hydroxide for 15 min, water for 15 min, and with the BGE during 30 min. The capillary was rinsed with BGE for 5 min between runs and stored after rinsing with water at the end of each session.

2.3. Sample treatment

Four SPE cartridges were evaluated for the off-line SPE preconcentration of BPs in water samples: Oasis HLB (500 mg) (Waters, Millford, MA, USA), Supelclean ENVI-18 (500 mg) (Supelco, St. Louis, MO, USA), Strata X 33u polymeric reversed-phase (200 mg) (Phenomenex, Torrance, USA), and Bond Elut Plexa (200 mg) (Varian, Middeelburg, The Netherlands).

Sample treatment was carried out as follows: SPE cartridges were first conditioned with 5 mL of methanol and 5 mL of Milli-Q water. Water samples of 500 mL and adjusted to pH 3.0 with 1 M hydrochloric acid immediately before use were passed through the cartridges at a flow-rate of 2-3 mL/min using a Visiprep System (Supelco). Cartridges were then washed with 5 mL of Milli-Q water and dried with air. BP UV-filters elution was carried out with 3 mL of methanol followed by 3 mL of dichlormethane and the eluate collected in an amber-glass vial. Eluate was then evaporated to dryness under a nitrogen stream, and finally reconstituted in 1 mL of a 2.5

196 mM tetraborate sodium buffer (pH 9.2) aqueous solution and directly analyzed by
197 FASI-CZE.
198
199 **3. Results and discussion**
200 **3.1. Capillary zone electrophoretic conditions**
201 The present work is aimed at developing a CZE method for the analysis of
202 several BP UV-filters in environmental water samples. Several years ago Wang *et al.*
203 [21] proposed a CZE method where they improved the separation of benzophenones by
204 adding Tween 20 (a non-ionic surfactant) into a sodium tetraborate buffer. But in order
205 to improve sensitivity, a BGE compatible with on-line preconcentration methods such
206 as FASI is required, and for this reason we aimed to achieve baseline separation of the
207 eight studied BPs with a simpler BGE. For that purpose, a 2.5 mM sodium tetraborate
208 buffer solution (pH 9.2) was used as initial BGE to study the electrophoretic separation
209 of BPs. Under these BGE conditions, BPs were in an anionic form (pka values from
210 6.81 to 8.14, Table 1), but because of the high pH value used, the electrophoretic
211 separation was carried out by applying a capillary voltage in positive polarity (+25 kV)
212 in order to work at counter electroosmotic flow (EOF) conditions. Under these
213 conditions, all BPs were detected in less than 4 min although with co-migration of some
214 of them: DHMBP and DHDMBP (peaks 2 and 3 in Figure 1a), and HBP, 24DHBP and
215 TrHBP (peaks 4, 5 and 6 in Figure 1a). In order to achieve base-line separation of all
216 studied BPs, and the highest sensitivity in the shorter analysis time, the effect of sodium
217 tetraborate buffer concentration (from 2.5 mM to 50 mM) in the BGE was evaluated,
218 and the electropherograms obtained are shown in Figure 1a. Better separation can be
219 achieved with the increase of buffer concentration due to the EOF reduction caused by
220 the increase on BGE ionic strength. This study allowed us to conclude that a BGE
221 consisting of a sodium tetraborate buffer solution at a concentration between 30 and 40
222 mM will be suitable for the separation of the studied BPs without the necessity of
223 adding any other BGE modifier such as organic solvents or non-ionic surfactants as
224 previously reported in the literature [21], and it will be completely compatible with the
225 application of on-line preconcentration procedures such as FASI. For that purpose, a
226 BGE of 35 mM sodium tetraborate buffer solution was proposed as optimum for the
227 CZE separation of BPs (Figure 1b, bottom electropherogram).
228 Hydrodynamic injection time was also optimized (from 5 to 40 s) and an
229 injection time of 10 s was selected as optimal since higher values produced peak

broadening and the loss of electrophoretic separation. Finally, in order to reduce a little the analysis time, the capillary voltage was increased to +30 kV (highest value attainable with the MDQ CE instrument used). Under these conditions, baseline separation of all compounds was achieved within 8 min and keeping a similar separation than the one observed at +25 kV (Figure 1b, top electropherogram).

3.2. Field amplified sample injection optimization

The development of methods sensitive enough to determine low concentration levels of UV-filters in environmental waters is necessary due to the potential harmful effects of these compounds even at low concentrations. For this reason, and in order to increase sensitivity, the use of an on-line CZE preconcentration method was investigated. Among on-line enrichment procedures, FASI is very popular since it is quite simple only requiring the electrokinetical injection of the sample after the introduction of a short plug of a high-resistivity solvent. This technique takes advantage of the differences in mobility and conductivity between the sample matrix and the BGE to preconcentrate the analyte. In this study, the electrolyte previously optimized for the conventional CZE separation (35 mM sodium tetraborate buffer at pH 9.2) was used as BGE for the FASI-CZE procedure, and water was used as the high resistivity solvent. Other solvents such as methanol were also tested but the electrophoretic voltage frequently failed, probably due to the formation of bubbles into the capillary.

Additionally, sample matrix will also play an important role during FASI application and even more with low acidic compounds such as BPs due to the requirement of using a matrix with a pH higher than BPs pka values in order to guarantee the presence of ionic compounds and, consequently, a good introduction of the analytes into the capillary when electrokinetic injection is used. For this purpose, sodium tetraborate buffer solutions were used as sample matrix and the effect of its concentration (from 1 to 10 mM) was evaluated when FASI was applied under some preliminary conditions, i.e. hydrodynamic injection of a water plug for 10 s (3.5 kPa) and sample electrokinetic injection at -10 kV for 10 s. Milli-Q water was also evaluated as sample matrix. The electropherograms obtained in this study are shown in Figure 2a. As can be seen, when only water was used no effective FASI injection was observed due to the fact that at the pH value of Milli-Q water (~7.0) most of the BPs are mainly in the neutral form and consequently not well electrokinetically introduced into the capillary. Obviously, the use of sodium tetraborate buffer solutions (pH 9.2) allowed the

deprotonation of BPs and their introduction into the capillary by electrokinetic injection. However, the increase on buffer concentration in the sample matrix makes its mobility and conductivity more similar to those of the BGE, making less effective the FASI injection. This can be observed on the important reduction on BP signals (Figure 2a) when sample matrix buffer concentrations higher than 2.5 mM were used. Thus, a sample matrix consisting of a 2.5 mM sodium tetraborate buffer solution was selected as optimal sample matrix for FASI.

Injection times for both the plug of water (hydrodynamic mode) and the sample (electrokinetic mode) were simultaneously optimized. Hydrodynamic injection (at 3.5 kPa) of a water plug from 0 to 40 s and electrokinetic sample injection (at -10 kV) from 5 to 40 s were tested. When short plugs of water were used, BPs showing low electrophoretic mobilities (HMBP, DHM and DHDMBP, which were the first compounds detected under counter-EOF separation conditions) did not appear on the electropherograms registered with high electrokinetic injection times (see Figure 2b, top electropherogram). This is caused by the removal of these compounds from the capillary by the EOF during sample injection. In contrast, when large plugs of water were used, a double peak was observed for some BPs such as 44DHBP and THBP (see Figure 2b, bottom electropherogram) which were the last migrating compounds under counter-EOF separation conditions. This effect is probably due to the presence of an equilibrium reaction between both acid-basic forms of these benzophenones through the capillary. A plug of water previous to sample injection not only ensures a proper enhancement of the electric field at the injection point during FASI but also provides a void region to concentrate negative BP ions deeper into the capillary away from the injection point [25]. However, if this void region is too large pH could decrease and become similar to benzophenone pKa values favoring the presence of both BP acid-basic forms in equilibrium. For this reason, a compromise between both hydrodynamic injection time of a water plug and sample electrokinetic injection time must be achieved. Obviously, when increasing sample injection time an enhancement of the response was also observed, however, peak broadening also occurred. The best results were obtained with a water plug hydrodynamic injection time of 20 s and a sample electrokinetic injection time of 25 s, values that were selected for the optimum FASI conditions (see electropherogram in Figure 2c). Under these conditions, an instrumental sensitive enhancement up to 25-fold for some BPs with respect to CZE hydrodynamic

injection was achieved. It should be pointed out that these were conditions taken as a compromise in order to achieve good FASI of all analyzed BPs.

3.3. Instrumental quality parameters

Instrumental quality parameters for both CZE-UV and FASI-CZE methods under optimal conditions were calculated and the figures of merit are summarized in Table 2. The limits of detection (LODs), based on a signal-to-noise ratio of 3:1, were obtained by analyzing BP standard solutions at decreasing concentration levels. The use of CZE-UV with hydrodynamic injection provided LODs between 0.2 and 1.4 mg/L, being HBP, 24DHBP, 44DHBP and THBP the most sensitive BPs. When FASI-CZE was applied, LODs in the range 21 to 136 μg/L were achieved, which means between a 9-fold (24DHBP) and a 25-fold (HMBP) sensitive enhancement. The limits of quantification (LOQs), based on a signal-to-noise ration of 10:1, were established in the range of 0.7 to 4.6 mg/L for CZE-UV and between 70 to 450 μg/L for FASI-CZE.

Run-to-run and day-to-day precisions for BP quantification were calculated at two concentration levels, a low level (LOQ) and a medium level (~20 mg/L for CZE-UV, and ~1 mg/L for FASI-CZE). In order to obtain the run-to-run precision, five replicate determinations for each concentration level were carried out using the two proposed methods under optimal conditions. On the other hand, day-to-day precision was calculated by performing 15 replicate determinations of each concentration level on 3 non-consecutive days (five replicates each day). The relative standard deviations (% RSDs) obtained with conventional CZE-UV at medium-concentration level were between 0.8 and 5.6% and between 2.9 and 11.5% for run-to-run and day-to-day precisions, respectively. The values were slightly higher for the low-concentration level, as it can be expected, but always RSD values lower than 13.0 and 14.5% for the run-to-run and the day-to-day, respectively, were obtained. Regarding FASI precision at medium concentration level, RSD values were similar or only slightly higher than those previously obtained by CZE-UV. However, when quantification was performed at the low concentration level (LOQ), RSD values generally increased (up to 15.2% and 17.6% for run-to-run and day-to-day precision, respectively), which can be explained because of the poor reproducibility of electrokinetic injection [26] and the low concentration level quantified (70-150 μg/L for most of the studied BPs).

External calibration curves based on peak area at concentrations between LOQ and 50 mg/L (CZE-UV) and between LOQ and 2 mg/L (FASI-CZE) were obtained and

good linearity was observed ($r^2 > 0.994$). Accuracy was also evaluated by the triplicate analysis using external calibration of standard solutions at concentrations of 10 mg/L (CZE-UV) and 500 µg/L (FASI-CZE) achieving acceptable results, with relative errors ranging from 0.4 to 7.8% and from 1.1 to 8.1% for CZE-UV and FASI-CZE, respectively.

3.4. Off-line solid-phase extraction

Despite the considerable improvement on LODs achieved by the application of FASI-CZE for the analysis of BPs, the sensitivity is not yet good enough for the application of this methodology in environmental water samples where lower BP concentration levels are expected. For this reason, an off-line SPE preconcentration step prior to FASI-CZE analysis was evaluated as sample treatment. For the off-line SPE procedure four different SPE sorbents, Oasis HLB (hydrophilic lipophilic balanced) (500 mg), Supelclean ENVI-18 (500 mg), Strata X 33u polymeric reversed-phase (200 mg), and Bond Elut Plexa (200 mg), were tested. Four water matrices with differences in sample salinity were studied for comparison: Milli-Q water, Barcelona (Spain) tap water, still mineral water, and blank river water. Sample volumes of 100 mL of each water sample spiked with 30 µg of each BP (final concentration of 300 µg/L) were preconcentrated with each SPE cartridge following the procedure described in section 2.3, although final extracts were reconstituted in 1 mL of Milli-Q water. After preconcentration, samples were injected into the CZE-UV system and peak areas were measured, and the recoveries were calculated by comparing the peak areas with those of a control sample (30 mg/L) representing 100% recovery. All experiments were carried-out by triplicate. In general, recoveries where higher when Milli-Q water was used, but similar recoveries were obtained for the other three water samples, showing the effectiveness of the SPE procedure to remove sample salinity. As an example, Figure 3a compares the recoveries obtained with each SPE cartridge when the blank river water sample was used. As regards the recoveries of studied BPs, two behaviors can be observed. A group of five BPs (HBP, 24DMBP, TrHBP, 44DHBP and THBP) have recoveries, in general, higher than 85%. In contrast, the other three BPs (HMBP, DHMBP and DHDMBP) show recoveries lower than 60% and, in most of the cases, even lower than 10-30%. This different behavior can be explained by the differences in BP structures and in their interactions with the SPE sorbents. For instance, HMBP and DHDMBP have one or two epoxy groups in their structures, with lower polarity than

365 the hydroxyl groups found in other BPs, although they can interact with the SPE
366 sorbents by dipole-dipole interactions. However, these interactions are weaker than the
367 hydrogen bonding interactions that can be obtained by the hydroxyl groups. In the case
368 of DHMBP, only one hydroxyl group is present in its structure explaining is lower
369 interaction with the SPE sorbents when compared to the other poly-hydroxyl
370 benzophenones.

371 A notable difference in recoveries depending on the SPE cartridge was also
372 observed, although it seems that the Strata X sorbent showed the best recoveries for
373 almost all evaluated BPs. Thus, as a compromise, Strata X sorbent was selected as the
374 optimum one for the off-line SPE preconcentration of benzophenones in water samples.

375 Breakthrough volume of the proposed SPE cartridge was determined by using
376 the blank river water sample. For that purpose, different water sample volumes (from 50
377 to 1000 mL) spiked at a constant amount of analyte (30 µg of each BP), and
378 consequently a decreasing concentration (from 600 µg/L to 30 µg/L), were
379 preconcentrated as previously indicated and analyzed with the proposed CZE-UV
380 method. All experiments were carried-out by triplicate. Figure 3b shows the
381 breakthrough curve obtained for the Strata X cartridge. In general, practically constant
382 recoveries up to 500 mL were obtained for all BPs, and then a decrease in recoveries
383 was observed, being quite important for several BPs such as TrHBP and DHDMBP.
384 Thus, 500 mL was selected as optimal sample volume for the off-line SPE
385 preconcentration of BPs in water samples by using the Strata X cartridge.

386

387 **3.5. Off-line SPE-FASI-CZE method performance**

388 Method performance of the combination of both off-line SPE preconcentration
389 sample treatment and the on-line FASI-CZE method was evaluated and the figures of
390 merit are given in Table 3. LODs, based on a signal-to-noise ratio of 3:1, were obtained
391 by analyzing blank river water samples spiked at low concentrations (below 1 µg/L)
392 with the proposed FASI-CZE method after off-line preconcentration with the Strata X
393 SPE cartridges. Very good sensitivity was achieved, with LOD values down to 60-72
394 ng/L for HBP, 24DHBP, 44DHBP and TFBP benzophenones and in the range 400-600
395 ng/L for the other compounds. Thus, between a ~2300-fold (TrHBP) and a ~6500-fold
396 (THBP) sensitive enhancement was achieved with the combination of both off-line SPE
397 and FASI in comparison to conventional CZE-UV methodology. Regarding the off-line
398 SPE step, preconcentration factors between 132 (HMBP) and 472 (24DHBP) were

obtained. The LODs obtained in this work are only slightly higher than those previously reported by using an in-line SPE-CE-MS method (10-50 ng L^{-1}) for the analysis of similar BP UV-filters [22], although in the mentioned work LODs were calculated using standard solutions. It should be pointed out that, if necessary, sensitivity could be improved by reconstituting the extracts after the off-line SPE step with less than 1 mL of 2.5 mM sodium tetraborate solution because only a small amount of sample extract (~100 µL) is required for injection into the FASI-CZE system.

Recoveries at low concentration levels (~1 µg/L) were also evaluated as described in section 3.4. For that purpose, after SPE preconcentration, final extracts were reconstituted in 1 mL of 2.5 mM sodium tetraborate aqueous solution and injected into the FASI-CZE system. Peak areas were measured and the recoveries were calculated by comparing the peak areas with those of a control sample (0.5 mg/L) representing 100% recovery. All experiments were carried-out by triplicate. Values in the range 72-90% for most of the BPs and 24% and 36% for DHMBP and HMBP, respectively, were obtained (Table 3), which were similar to those previously obtained at higher concentrations (300 µg/L) (Figure 3b). Off-line SPE-FASI-CZE run-to-run method precision for BP quantification at ~1 µg/L was calculated by performing five replicate determinations of a spiked blank river water sample, obtaining an acceptable precision for this kind of method with RSD values lower than 22.9% for all BPs (see Table 3).

For method validation, a blank river water sample was spiked at around 1 µg/L of each benzophenone and quantified by external calibration following the proposed off-line SPE-FASI-CZE method, and the found concentrations, as well as the accuracies in terms of relative errors (%), are also summarized in Table 3. As can be seen, good accuracies, taking into account the method and concentration level, in the range 1.9-17.9% were obtained. A statistical paired-sample comparison analysis was performed between the spiked concentrations and found concentrations in the analyzed blank river water sample. For a 95% confidence level, the quantification results obtained were not significantly different to those of the target sample, with a *p* value (probability at the confidence level) of 0.60..

The results obtained in the method performance, i.e. low LODs, and good precision and accuracy when analyzing a spiked blank river water sample, show that the proposed off-line SPE-FASI-CZE method is suitable for the analysis of benzophenone UV-filters in environmental water samples at low µg/L levels.

3.6. Application to environmental water samples

The proposed off-line SPE-FASI-CZE method was applied for the first time to the analysis of several river water samples, as well as a mineral and tap water sample from Barcelona (Spain). For that purpose, after sampling, water samples were adjusted to pH 3.0 with 1 M hydrochloric acid and immediately processed by the off-line SPE method. Extracts were then analyzed by FASI-CZE as soon as possible, or kept in amber-glass vials at the refrigerator at 4 ^{o}C for no more than 1 week to prevent degradation. Sample volumes of 500 mL were processed by triplicate, and quantified by external calibration using BP standards prepared in 2.5 mM sodium tetraborate solution, and the results were corrected by the corresponding recoveries. Figure 4 shows the electropherograms obtained for a blank river water sample (Figure 4a, which was the one used to study the method performance), for Barcelona's tap water (Figure 4b), and for a water sample collected from Segre River (Catalonia, Spain) after industrialized and urban areas (Figure 4c). Peak identification was carried-out by the addition of benzophenone standards and by the comparison of retention times. As an example, Figure 4d shows the electropherogram obtained for an SPE extract obtained from the blank river water sample and spiked with BPs at a concentration of ~1 mg/L. In all samples, peak purity was checked through the electrophoretic peak by comparing the UV-spectrum of each benzophenone. The quantification results of the analyzed samples are summarized in Table 4.

As can be seen, none of the analyzed BPs was detected in the mineral water sample, as expected. However, Barcelona's tap water showed the presence of HBP, 24DHBP, 44DHBP and THBP, although all of them at the LOD of the proposed method or bellow the LOQ (THBP). It should be mention that the presence of some BPs in Barcelona's tap water was detected only occasionally, and in most of the cases negative results were obtained after analyzing this kind of sample. Environmental water samples from two rivers, Segre and Llobregat (Catalonia, Spain) were analyzed. Sampling was carried out in two locations on each river: (1) at the beginning of the river course before industrialized and urban areas and (2) at the middle of the river course after some industrialized and urban areas. No BPs were detected on those river water samples collected before industrialized and urban areas, as expected, while the presence of some BPs at quantified levels (see Table 4) was observed when the sample was collected after industrialized and urban areas. It should be noted the presence of relatively higher

concentrations (between 10-82 µg/L) for some BPs such as HMBP, DHMPB and TrHBP in the river water samples taken after industrialized and urban areas. Regarding the levels of other found BPs, they are between 0.25 and 0.45 µg/L, concentrations that are more common compared to the values described in the literature for these compounds in environmental water samples.

4. Conclusions

A sensitive field amplified sample injection-capillary zone electrophoresis method for the analysis of eight benzophenone UV-filters in environmental water samples has been developed. With the application of FASI, a 9-fold to 25-fold sensitive enhancement was observed, obtaining limits of detection down to 21-60 µg/L for most of the analyzed BPs, with good linearity, run-to-run and day-to-day precisions (RSD values lower than 17%), and accuracy (relative errors lower than 8%).

In order to remove sample salinity from environmental waters which can become an important handicap for FASI efficient application, solid-phase extraction was evaluated as off-line preconcentration and sample treatment prior to FASI-CZE analysis. Strata X polymeric reversed-phase sorbent was selected as a compromise providing good recoveries (72-90%) for most of analyzed BPs. A 2400- to 6500-fold sensitive enhancement was obtained when combining both off-line SPE and FASI-CZE for the analysis of BPs in a blank river water sample, achieving LODs down to 0.06-0.6 µg/L with good precision (RSDs in the range 6.8-22.9%). The proposed off-line SPE-FASI-CZE method was applied for the first time in environmental river water samples as well as in some drinking water samples (mineral and tap water). Benzophenones were detected in a tap water from Barcelona (Spain) although at LOD or below LOQ levels. None of the analyzed BPs was detected in river water samples collected before industrialized and urban areas, although the presence of some BPs, in some cases at relatively high concentrations (10-82 µg/L), was observed in river water samples collected after industrialized and urban areas.

The good results obtained in this study shown that the proposed off-line SPE-FASI-CZE is suitable for the analysis of benzophenone UV-filters in environmental water samples at low µg/L levels.

Acknowledgements

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Figure captions

Fig. 1. (a) Effect of sodium tetraborate buffer concentration in the BGE for the CZE separation of BPs. Standard solution of BPs at 30 mg/L in water. Capillary voltage: +25 kV; sample injection: hydrodynamic 10 s (3.5 kPa); UV detection: λ 345 nm (electropherograms at λ 285 nm are also shown for the three first BPs). (b) Electropherograms obtained under optimal BGE conditions (35 mM sodium tetraborate buffer solution) at a capillary voltage of 25 and 30 kV. Standard solution of BPs at 30 mg/L in water. Capillary voltage: +25 kV; sample injection: hydrodynamic 10 s (3.5 kPa); UV detection: λ 345 nm. Peak identification: 1, HMBP; 2, DHMBP; 3, DHDMPB; 4, HBP; 5, 24DHBP; 6, TrHBP; 7, 44DGBP; and 8, THBP.

Fig. 2. (a) Effect of sodium tetraborate buffer concentration in the sample matrix during FASI. Water plug hydrodynamic injection: 10 s (3.5 kPa); Sample electrokinetic injection: 10 s (-10 kV); UV detection: λ 345 nm (electropherograms at λ 285 nm are also shown for the three first BPs). (b) Examples of FASI-CZE electropherograms during simultaneous optimization of water plug hydrodynamic injection time and sample electrokinetic injection time. Sample matrix: 2.5 mM sodium tetraborate buffer; UV detection: λ 345 nm (c) Separation of BPs obtained under optimal FASI-CZE conditions. Sample matrix: 2.5 mM sodium tetraborate buffer; Water plug hydrodynamic injection: 20 s (3.5 kPa); Sample electrokinetic injection: 25 s (-10 kV); UV detection: λ 345 nm (electropherograms at λ 285 nm are also shown for the three first BPs). Peak identification: 1, HMBP; 2, DHMBP; 3, DHDMPB; 4, HBP; 5, 24DHBP; 6, TrHBP; 7, 44DGBP; and 8, THBP. In all cases a standard solution of all BPs at 0.5 mg/L was used.

Fig. 3. (a) Comparison of different SPE sorbents for the off-line SPE preconcentration of benzophenone UV-filters. Sample: 100 mL of a blank river water sample spiked at 300 µg/L with each BP. (b) Breakthrough curve for the preconcentration of BP UV-filters with the Strata X SPE cartridge. Sample: different blank river water sample volumes spiked with a constant amount of each BP (30 µg).

Fig. 4. Off-line SPE-FASI-CZE electropherograms of (a) blank river water sample, (b) Barcelona's tap water, (c) Segre River water, and (d) SPE extract of a blank river water

628 sample spiked with BPs at ~1 mg/L. UV detection: λ 345 nm. Peak identification: 1,
629 HMBP; 2, DHMBP; 3, DHDMPB; 4, HBP; 5, 24DHBP; 6, TrHBP; 7, 44DGBP; and 8,
630 THBP.
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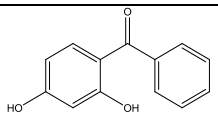
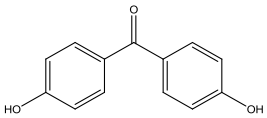
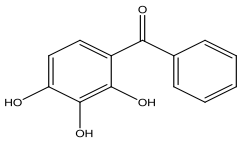
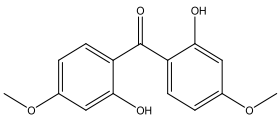
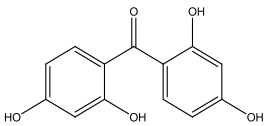
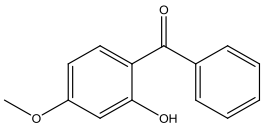
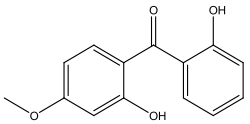
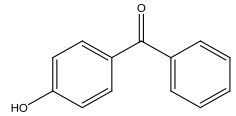
633 **Table 1. Structures, abbreviations, pKa values, and CAS numbers of studied benzophenones.**

Benzophenone	Abbreviation	pKa value[a]	Structure	CAS number
2,4-dihydroxybenzophenone	24DHBP (BENZ-1)	7.72±0.85		131-56-6
4,4'-dihydroxybenzophenone	44DHBP	7.67±0.15		611-99-4
2,3,4-trihydroxybenzophenone	TrHBP	7.51±0.40		1143-72-2
2,2'-dihydroxy-4,4'-dimethoxybenzophenone	DHDMBP (BENZ-6)	6.81±0.35		131-54-4
2,2',4,4'-tetrahydroxybenzophenone	THBP (BENZ-2)	6.98±0.35		131-55-5
2-hydroxy-4-methoxybenzophenone	HMBP (BENZ-3)	7.56±0.35		131-57-7
2,2'-dihydroxy-4-methoxybenzophenone	DHMBP (BENZ-8)	7.11±0.35		131-53-3
4-hydroxybenzophenone	HBP	8.14±0.13		1137-42-4

634 [a] Calculated using Advanced Chemistry Development (ACD/Labs) software v 11.02 (® 1994-2013 ACD/Labs)

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Table 2. CZE and FASI-CZE instrumental quality parameters.

Compound	Method	LODs (µg L^{-1})	Sensitive enhancement (SE_c)[a]	run-to-run precision, % RSD (n=5)			day-to-day precision % RSD (n=5x3)		
				Migration time	Conc. (low level)[b]	Conc. (medium level)[c]	Migration time	Conc. (low level)[b]	Conc. (medium level)[c]
HMBP	**CZE**	1300	-	0.01	1.9	1.9	1.9	10.8	9.4
	FASI	53	25	0.3	15.2	4.3	4.7	17.2	8.4
DHMBP	**CZE**	1000	-	0.1	1.8	5.6	1.6	8.1	11.5
	FASI	59	17	0.4	14.2	3.7	4.3	16.5	9.1
DHDMBP	**CZE**	1000	-	0.1	4.2	0.9	1.7	11.0	6.8
	FASI	59	17	0.5	14.2	6.5	4.3	17.6	8.1
HBP	**CZE**	200	-	0.1	5.8	1.2	2.0	12.7	4.6
	FASI	21	10	0.4	13.9	1.9	4.8	16.2	6.8
24DHBP	**CZE**	300	-	0.2	10.0	1.8	2.1	11.6	5.3
	FASI	34	9	0.5	13.5	4.6	4.9	15.4	5.4
TrHBP	**CZE**	1400	-	0.1	6.7	3.9	2.0	10.4	7.7
	FASI	136	10	0.4	9.7	3.3	5.1	15.4	5.1
44DHBP	**CZE**	300	-	0.4	11.1	1.0	3.2	11.5	5.2
	FASI	26	11	0.3	10.7	2.2	8.3	15.6	5.4
THBP	**CZE**	400	-	0.2	13.0	0.8	3.4	14.5	2.9
	FASI	27	15	0.4	8.7	5.8	8.6	11.7	7.1

[a] SE_c = LOD (CZE) / LOD (FASI-CZE)
[b] low level concentration = 3 x LOD
[c] medium level concentration: CZE: ~20 mg/L; FASI: ~1 mg/L

Table 3. Off-line SPE-FASI-CZE method performance.

Compound	LODs (ng L^{-1})	Sensitive enhancement (SE_c)[a]	off-line SPE preconcentration factor[b]	Recoveries (%)[c]	Working range (mg L^{-1})[d]	Linearity (r^2)	Run-to-run precision (%RSD)[e]	Method validation		
								Spiked value (µg/L)	Found value (µg/L)[f]	% Relative error
HMBP	400	3250	132	36	0.13-2	0.994	21.2	1.02	0.97	4.9
DHMBP	410	2440	143	24	0.1-2	0.996	22.9	0.95	1.12	17.9
DHDMBP	415	2410	142	72	0.1-2	0.994	12.2	1.03	0.90	12.6
HBP	60	3333	350	86	0.1-2	0.996	8.3	0.93	0.95	2.2
24DHBP	72	4166	472	85	0.17-2	0.997	6.8	1.10	0.96	12.7
TrHBP	600	2333	227	90	0.14-2	0.996	9.3	0.94	1.04	10.6
44DHBP	65	4615	400	82	0.13-2	0.998	11.6	1.05	1.03	1.9
THBP	62	6450	436	90	0.14-2	0.995	6.9	1.05	0.93	11.4

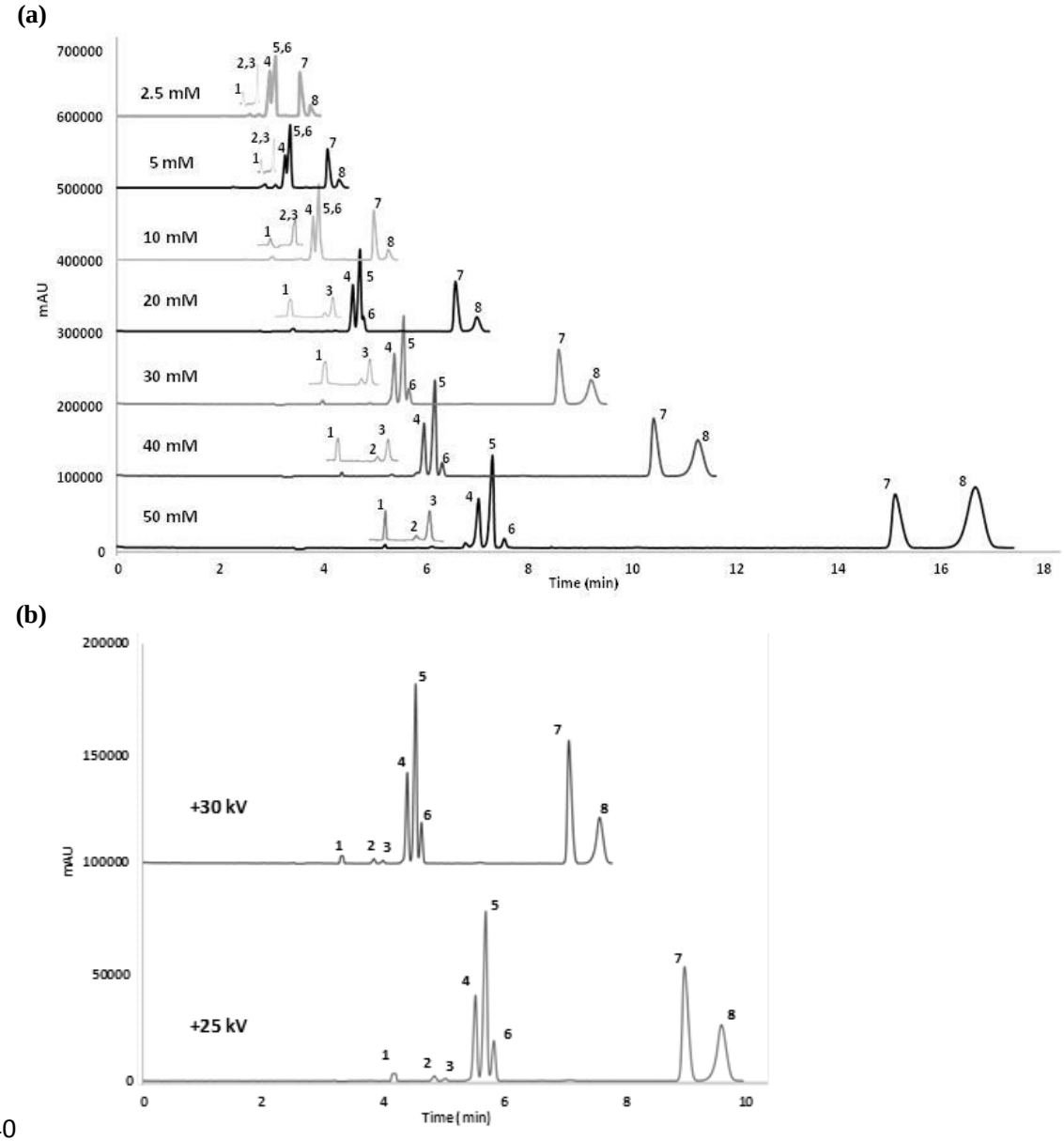
[a] SE_c = LOD (CZE) / LOD (off-line SPE-FASI-CZE)
[b] Calculated as LOD (FASI-CZE) / LOD (off-line SPE-FASI-CZE)
[c] Determined at 1 µg/L
[d] Working range of standards for the external calibration by FASI-CZE
[e] n=5, concentration level 1 µg/L
[f] n=3, quantified by external calibration

Table 2. Analysis of water samples by off-line SPE-FASI-CZE.

Sample	Concentration (µg/L)[a]							
	HMBP	**DHMBP**	**DHDMBP**	**HBP**	**24DHBP**	**TrHBP**	**44DHBP**	**THBP**
Mineral water	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Barcelona Tap water	n.d.	n.d.	n.d.	~LOD	~LOD	n.d.	~LOD	<LOQ
Segre River (1)[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Segre River (2)	82.0 ± 11.1	n.d.	12.5 ± 1.3	0.37 ± 0.03	0.45 ± 0.03	n.d.	n.d.	n.d.
Llobregat River (1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Llobregat River (2)	n.d.	n.d.	n.d.	n.d.	0.60 ± 0.05	10.0 ± 1.2	0.25 ± 0.04	0.35 ± 0.03

[a] Results given as average ± standard deviation (n=3)
[b] Sample used for the study of method validation
n.d.: not detected

Figure 3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5 mM
10 mM
20 mM
30 mM
40 mM
50 mM
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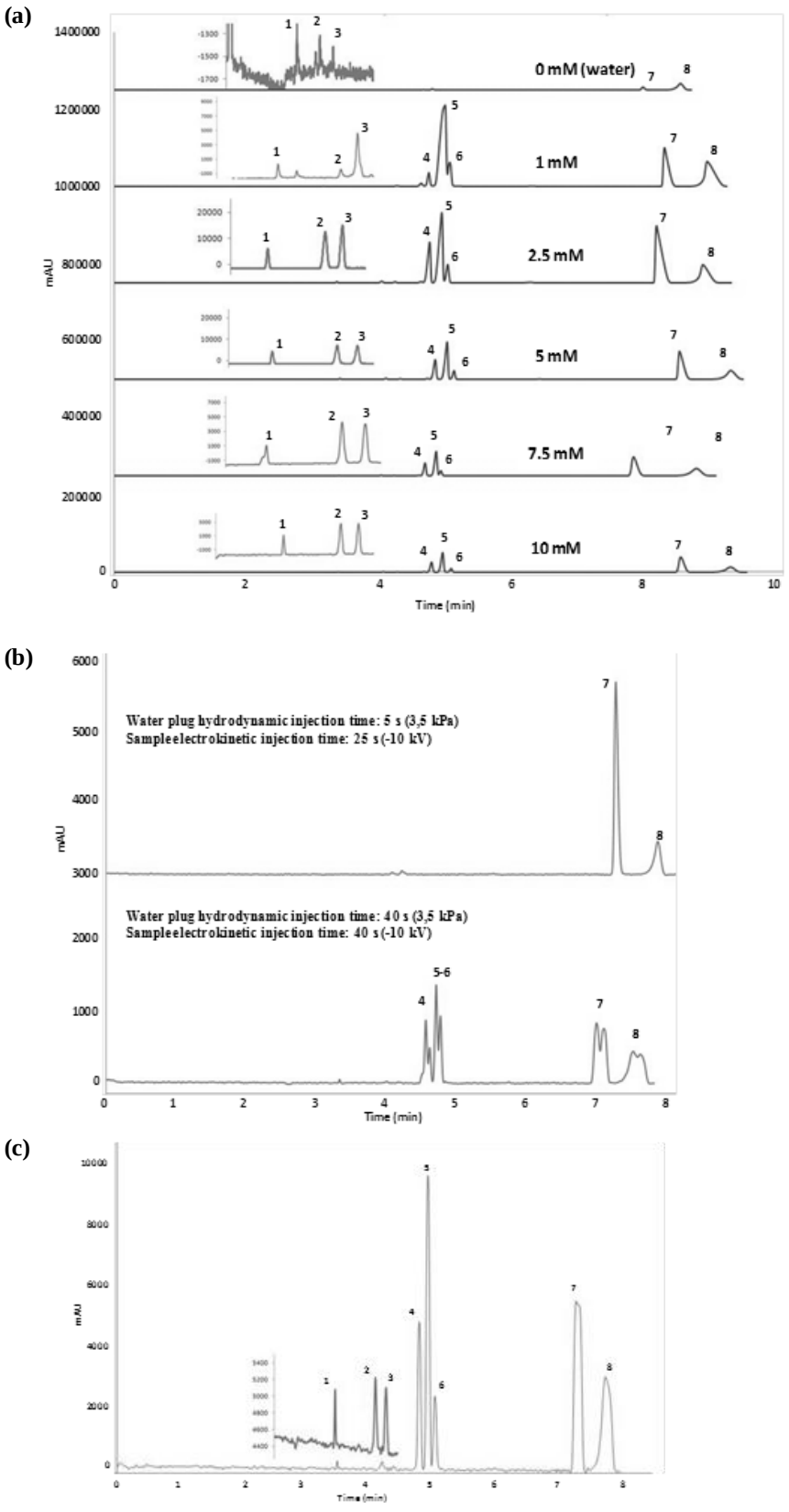
(b)
+30 kV
+25 kV
mAU
Time (min)

Figure 5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0 mM (water)
1 mM
2.5 mM
5 mM
7.5 mM
10 mM
mAU
Time (min)
(b)
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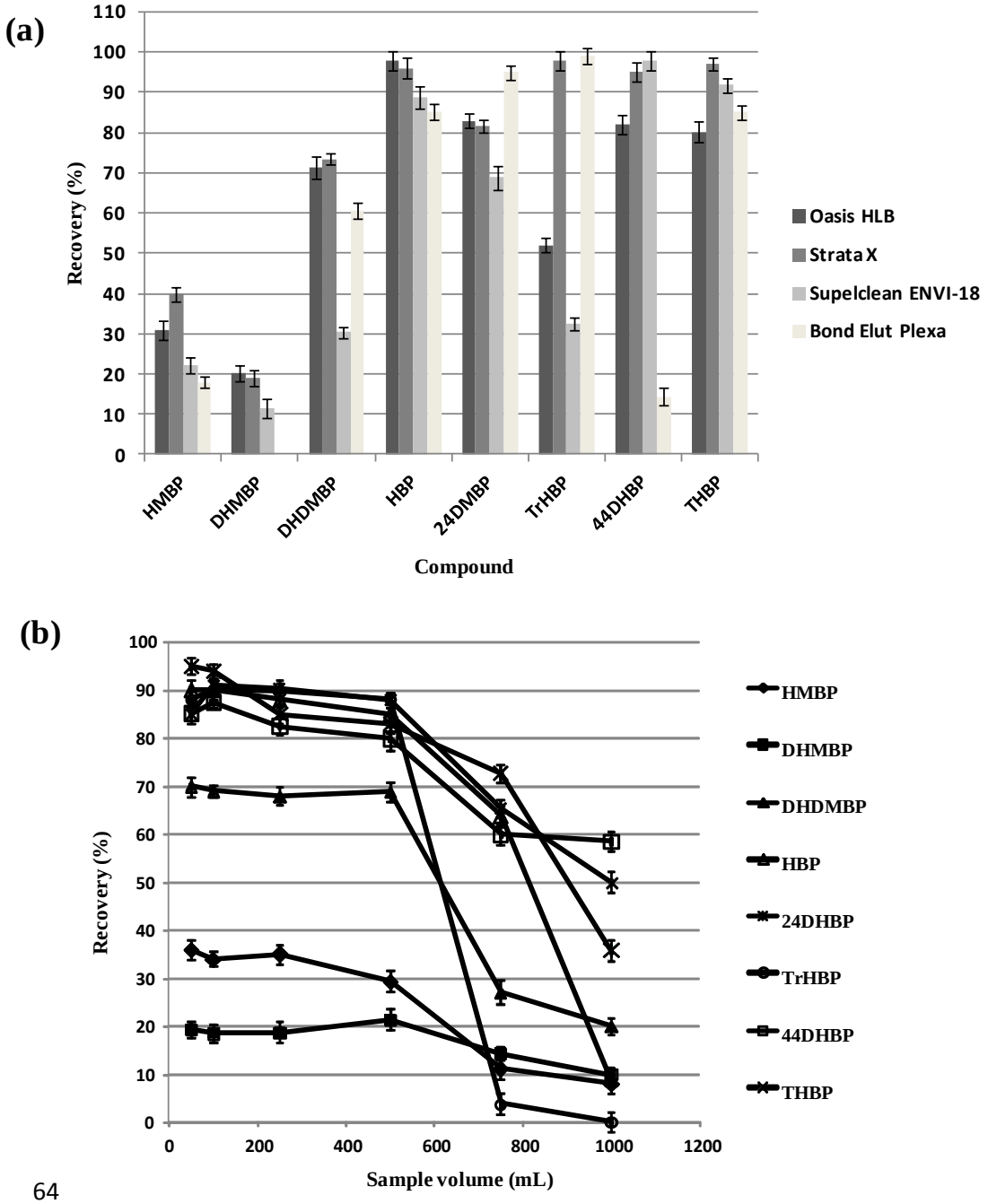
Sample electrokinetic injection time: 25 s (-10 kV)
Water plug hydrodynamic injection time: 40 s (3,5 kPa)
Sample electrokinetic injection time: 40 s (-10 kV)
mAU
Time (min)
(c)
mAU
Time (min)

Figure 2


(a)
Recovery (%)
Compound
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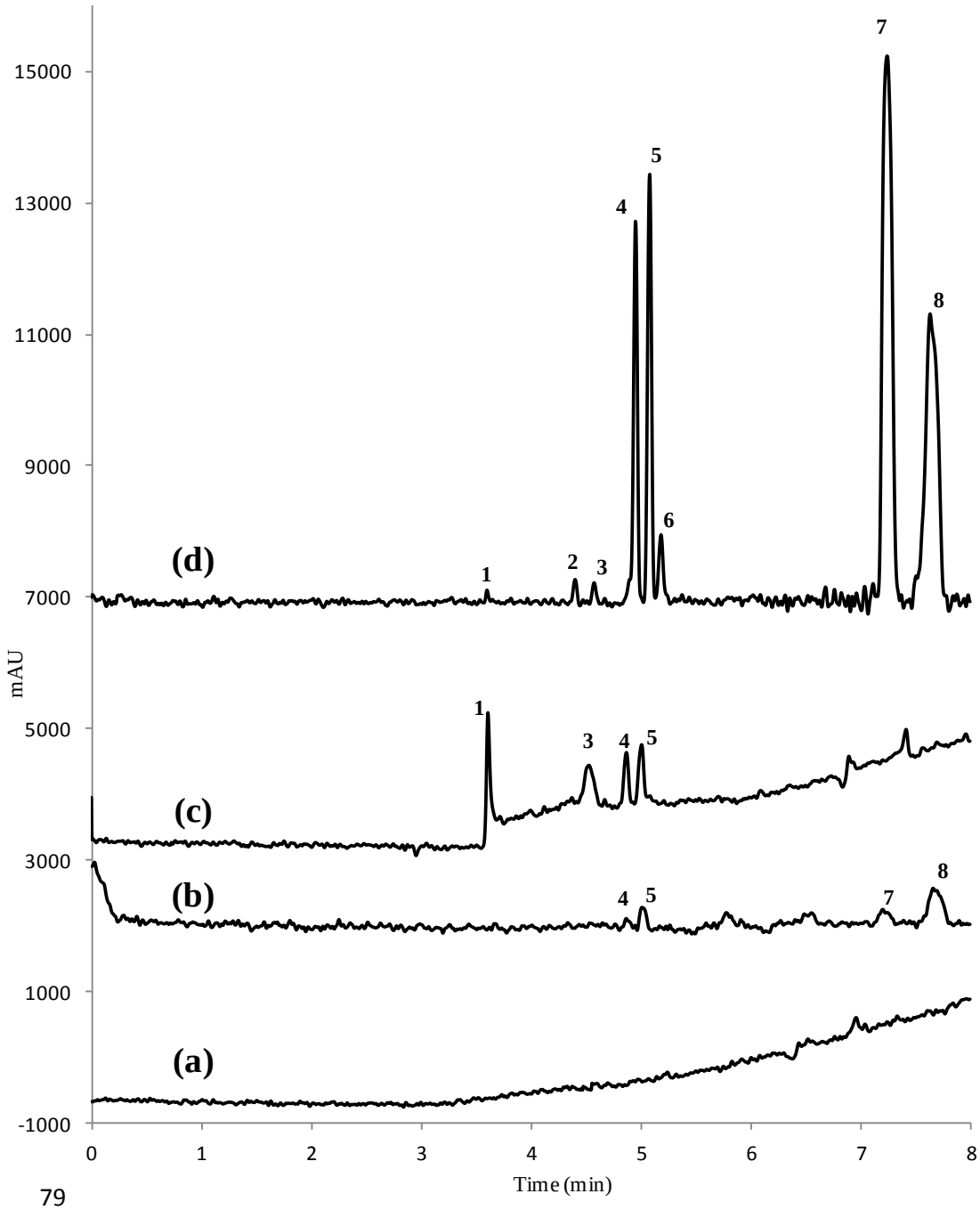
DHMBP
DHDMBP
HBP
24DMBP
TrHBP
44DHBP
THBP
Oasis HLB
Strata X
Supelclean ENVI-18
Bond Elut Plexa
(b)
Recovery (%)
Sample volume (mL)
HMBP
DHMBP
DHDMBP
HBP
24DHBP
TrHBP
44DHBP
THBP

Figure 7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8
Time (min)
(d)
(c)
(b)
(a)
1
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