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Article

Field-Amplified Sample Injection-Micellar Electrokinetic Chromatography for the Determination of Benzophenones in Food Simulants

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Abstract: A field-amplified sample injection-micellar electrokinetic chromatography (FASI-MEKC) method for the determination of 14 benzophenones (BPs) in a food simulant used in migration studies of food packaging materials was developed, allowing almost baseline separation in less than 21 min. The use of a 10 mM sodium dodecyl sulfate (SDS) solution as sample matrix was mandatory to achieve FASI enhancement of the analyzed BPs. A 21- to 784-fold sensitivity enhancement was achieved with FASI-MEKC, obtaining limits of detection down to 5.1–68.4 µg/L, with acceptable run-to-run precisions (RSD values lower than 22.3%) and accuracy (relative errors lower than 21.0%). Method performance was evaluated by quantifying BPs in the food simulant spiked at 500 µg/L (bellow the established specific migration limit for BP (600 µg/L) by EU legislation). For a 95% confidence level, no statistical differences were observed between found and spiked concentrations (probability at the confidence level, *p* value, of 0.55), showing that the proposed FASI-MEKC method is suitable for the analysis of BPs in food packaging migration studies at the levels established by EU legislation.

Keywords: field-amplified sample injection; micellar electrokinetic chromatography; benzophenones; migration studies; food simulants

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1. Introduction

Today, food packaging is an important way to store food at different temperatures, to extend the shelf-life of the product, and enable its distribution safely. In addition, food packaging safeguards food from natural agents such as air or solar light, and can retard product deterioration, retain the beneficial effects of processing, and maintain or increase the quality, organoleptic properties and safety of food. For these reasons, numerous packaging materials (plastic, paper cardboard, *etc.*) are used. BPs are a family of UV filters added to food packaging materials in order to protect them from degradation, as well as the food contained within from harmful UV light. However, there is an increasing concern for the migration of chemicals from packaging into the foods they contain. For instance, an alert for food contamination by UV ink photoinitiators arose in Europe in November 2005, when the Italian Food Control Authority detected that 2-isopropylthioxanthone migrated into baby milk at concentrations of 120–300 μg/L, resulting in the withdrawal from the market of more than 30 million liters of milk [1]. Since then, residues of other UV filters such as benzophenone (BP) have also been found in packaged foods [2–6]. Even though most of them are not considered extremely dangerous due to their low concentration levels in food, there is a great preoccupation with the possible damaging effect caused by a daily exposition to a high variety of these toxic compounds, which have been associated with endocrine activity, cancer and contact sensitization [7]. For this reason, the European Union has established legislation to regulate the materials that are in contact with foods and to minimize the specific migration limits of some of these compounds [8]. Apart from being used in polymer-based materials, UV filters are added to printing inks. Here they act as photoinitiators, which start the reaction that eventually dries the ink rapidly and prevents set-off effects of other substances contained in ink into the food [9]. BPs are non-substituted diphenylketones used also as UV stabilizers to prevent discoloration, cracking and loss of physical properties due to sunlight [10]. They can be used as an additive or polymer production aid and as photoinitiator catalyzers for inks and lacquers that are cured with UV light.

Food-contact materials must not transfer their components into the foods in unacceptable quantities. The safety in the use of polymeric materials is a subject of concern due to the transfer of plastic material constituents to the food by a diffusion process called migration, which is a term used to describe the transfer of components from a certain material to the foodstuff in contact [7]. A compound placed in these materials may migrate either because it did not react during manufacturing or it was released as a consequence of degradation by the contact with foodstuff or environment such as food acidity or UV light. According to the Commission Regulation (UE) No 10/2011 [8], several solutions are used for specific migration studies of packaging materials depending on the nature of the food they contain: distilled water, ethanol 10% (v/v), acetic acid 3% (v/v), ethanol 20% (v/v), ethanol 50% (v/v), vegetable oil, poly(2,6-diphenyl-p-phenylene oxide), ethanol 95% (v/v), and isooctane. This regulation also established a list of permitted substances that can be used in plastic productions for food packaging containers, as well as requirements regarding global migration limits and specific migration limits for these substances. Regarding BPs, only some of them such as BP, 2,2'-dihydroxy-4-methoxybenzophenone, 2,4-dihydroxybenzophenone (24DHBP), 2-hydroxy-4-methoxybenzophenone (HMBP), 4,4'-dihydroxybenzophenone (44DHBP) and 2-hydroxy-4-(octyloxy)benzophenone (HOBP) are allowed. However, only a specific migration limit (SML) of 600 μg/kg for BP is established. Thus, the development of analytical methods for the determination of BPs, among other food packaging contaminants, to perform migration studies of food packaging materials is necessary.

Different analytical methods have been employed for determining BPs in a variety of matrices. Liquid chromatography (LC), using basically C18 reversed-phase columns, and gas chromatography (GC), both of them mainly coupled with mass spectrometry (MS) or high resolution mass spectrometry (HRMS), are the techniques of choice for the quantitative determination of this family of compounds [3–5,11–19]. Regarding GC, derivatization with silylating reagents is frequently necessary to increase the volatility of these compounds. In addition, taking into account that BPs are in the low microgram to nanogram per liter range in many environmental and food matrices, 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 BPs in environmental [20,21] and cosmetic [22–28] samples can be found in the literature. Among CE techniques, micellar electrokinetic chromatography (MEKC), a CE mode that allows the separation of hydrophobic analytes by the combination of electrophoretic and chromatographic effects, and the only CE mode able to separate both neutral and charged analytes, has also been employed for the analysis of BPs although mainly in cosmetic matrices [22,24,26,27]. 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 (25–100 μm) typically employed when on-column UV detection is performed. This problem can be overcome by on-line electrophoretic-based preconcentration techniques such as field-amplified sample injection (FASI), stacking, and sweeping [29–36]. Among these techniques, FASI is very popular because it is quite simple, only requiring a difference on the sample matrix conductivity at least 10-fold lower than the one of the BGE conductivity, and the electrokinetic injection of the sample after the introduction of a short plug of a high-resistivity solvent such as methanol or water [32,37]. FASI takes 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, allows for the enhancement of the sample electrokinetic injection because of the conductivity differences between the sample and the water plug. Once the analytes enter the capillary they stack up in the boundary region between the high-resistivity solvent and the background electrolyte (BGE) and separation will take place. An off-line SPE-FASI-CZE method was recently developed for the analysis of eight BPs in river water samples [21].

The aim of this work is to develop a micellar electrokinetic chromatography (MEKC) method for the simultaneous separation and determination of fourteen BPs to be used in migration studies of packaging materials. In order to improve method sensitivity, the applicability of FASI was also evaluated. The influence of several parameters such as BGE composition (buffer concentration, sodium dodecyl sulfate (SDS) concentration and isopropanol content) on the analysis of BPs was studied. Quality parameters such as limits of detection (LODs), limits of quantification (LOQs), linearity, run-to-run precision, and accuracy, were established with both MEKC-UV and FASI-MEKC methods. The proposed FASI-MEKC method was applied to the determination of BPs in a 3% acetic acid solution used as food simulant in food packaging migration studies.

2. Materials and Methods

2.1. Chemicals

The BPs studied, which are shown in Table 1, were 4,4'-dihydroxybenzophenone (44DHBP), 2,2',4,4'-tetrahydroxybenzophenone (THBP), 2,3,4-trihydroxybenzophenone (TrHBP), 4-hydroxybenzophenone (HBP), 2,4-dihydroxybenzophenone (24DHBP), methyl 2-benzoylbenzoate (MBB), benzophenone (BP), 2,2'-dihydroxy-4,4'-dimethoxybenzophenone (DHDMBP), 2-hydroxy-4-methoxybenzophenone (HMBP), 4-methylbenzophenone (MBP), 4-allyloxy-2-hydroxybenzophenone (AHBP), 4-benzoyl biphenyl (PBZ), 4,4'-bis(diethylamino)benzophenone (DEAB) and 2-hydroxy-4-(octyloxy)benzophenone (HOBP), all of them obtained from Sigma-Aldrich (Steinheim, Germany).

HPLC-grade methanol and 2-propanol (isopropanol), acetic acid (99.7%), and SDS were also obtained from Sigma-Aldrich. Ortho-phosphoric acid (85%), sodium dihydrogenphosphate monohydrate, hydrochloric acid (25%), and sodium hydroxide were supplied by Merck (Darmstadt, Germany).

Stock standard solutions of all BPs (*ca.* 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 (MEKC) or with a 10 mM SDS solution (FASI). All stock solutions were stored at 4 °C for no more than 1 month. Background electrolyte (BGE) was prepared daily by diluting 500 mM SDS and 500 mM phosphoric acid-dihydrogenphosphate buffer (pH 2.5) solutions with water to achieve the appropriate concentration and by adding the appropriate amount of isopropanol.

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.

Table 1. Structures and abbreviations of the studied benzophenones (BPs).

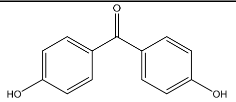
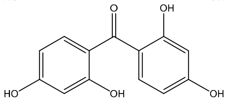
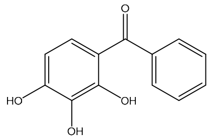
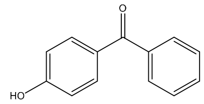
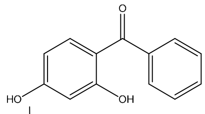
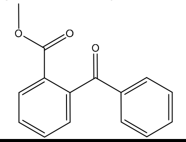
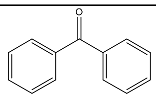
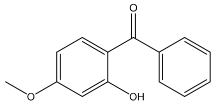
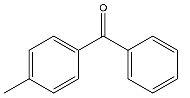
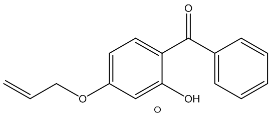
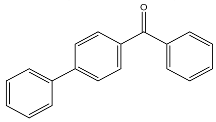
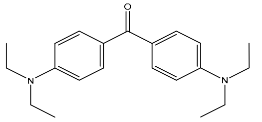
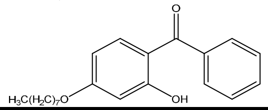
Compound	**Abbreviation**	**pka value** [a]	**Structure**	**CAS number**
4,4'-dihydroxybenzophenone	44DHBP	7.67 ± 0.15		611-99-4
2,2',4,4'-tetrahydroxybenzophenone	THBP	6.98 ± 0.35		131-55-5
2,3,4-trihydroxybenzophenone	TrHB	7.51 ± 0.40		1143-72-2
4-hydroxybenzophenone	HBP	8.14 ± 0.13		1137-42-4
2,4-dihydroxybenzophenone	24DHBP	7.72 ± 0.35		131-56-6
methyl 2-benzoylbenzoate	MBB	-		606-28-0

Table 1. *Cont.*

Compound	Abbreviation	pka value [a]	Structure	CAS number
benzophenone	BP	-		119-61-9
2-hydroxy-4-methoxybenzophenone	HMBP	7.56 ± 0.35		131-57-7
4-methylbenzophenone	MBP	-		134-84-9
4-allyloxy-2-hydroxybenzophenone	AHBP	7.63 ± 0.35		2549-87-3
4-benzoylbiphenyl	PBZ	-		2128-93-0
4,4'-bis(diethylamine)benzophenone	DEAB	4.14 ± 0.32		90-93-7
2-hydroxy-4-(octyloxy)benzophenone	HOBP	7.59 ± 0.35		1843-05-6

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

2.2. Instrumentation and Methods

MEKC-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 60 cm (50 cm effective length) ×75 µm I.D. (360 µm O.D.). BGE consisted of a 25 mM phosphoric acid-sodium dihydrogenphosphate buffer solution (pH 2.5) containing 200 mM SDS and 30% isopropanol. Capillary temperature was held at 25 °C. BGE solutions were filtered through 0.45 µm nylon filters (Whatman, Clifton, NJ, USA) and degassed by sonication for 5 min before use. For MEKC-UV, samples were loaded by pressure-assisted hydrodynamic injection (15 s, 3.5 kPa). The MEKC electrophoretic separation of BPs was performed by applying a capillary voltage of −25 kV (reverse polarity) through the capillary and working under electroosmotic flow (EOF) suppressed conditions (pH 2.5). Direct UV absorption detection was carried out from 190 nm to 400 nm, and sample quantitation was performed at three UV wavelengths depending on the compound: 240 nM (MBP, BP, MBB and ThHBP), 285 nm (HOBP, PBZ, AMBP, HMBP, 24DHBP, HBP and 44DHBP) and 345 nm (DHDMBP, DEAB and THBP).

FASI experiments were performed as follows: the capillary was first filled with BGE (25 mM phosphoric acid-sodium dihydrogenphosphate buffer solution (pH 2.5), 200 mM SDS and 30% isopropanol) and then a water plug was introduced into the capillary by pressure-assisted hydrodynamic injection (25 s, 3.5 kPa). Samples were then introduced into the capillary by electrokinetic injection at −10 kV (reversed polarity) during 20 s. The electrophoretic separation was then performed by applying −25 kV (reversed polarity) through the capillary. For FASI, standards were prepared in a 10 mM SDS solution used as sample matrix. The CE instrument was controlled using a Beckman P/ACE station software version 1.2.

2.3. Capillary Conditioning

New CE capillaries were pretreated with 0.1 M hydrochloric acid for 60 min, water for 60 min, 0.1 M sodium hydroxide for 60 min, and finally they were washed with water for 60 min. At the beginning of each session, the capillary was rinsed with 0.1 sodium hydroxide for 30 min, water for 15 min, and with the BGE for 60 min. The capillary was rinsed with BGE for 5 min between runs and stored after rinsing with water at the end of each session.

2.4. Food Simulant

In this work, a 3% acetic acid solution was used as food simulant matrix intended for migration studies. According to the European Regulation (EU) No 10/2011 [8] this solution (Simulant B in the cited regulation) is assigned to foods with hydrophilic character and with a pH below 4.5. Therefore, it is employed for migration studies of food packaging materials intended to contain clear drinks such as water, ciders, clear fruit or vegetable juices, infusions, coffee, soft drinks, energy drinks, cloudy drinks such as juices with fruit pulp and liquid chocolate, purées, *etc.*

For FASI-MEKC experiments, the food simulant employed was a 3% acetic acid solution containing 10 mM SDS, prepared daily by adequate dilution of acetic acid (99.7%) and 500 mM SDS solutions.

3. Results and Discussion

3.1. Micellar Electrokinetic Capillary Chromatography Conditions

The present work aimed to develop a MEKC method for the separation of fourteen BPs. The working BGE pH was chosen based on two premises: that it was lower than the pka values of the ionizable BPs studied (Table 1) and that it was below the pka value of the silanol groups located in the inner wall of the silica capillary in order to reduce EOF. Under these conditions, all the compounds that were subjected to this study were in their neutral form and the separation will be performed under EOF suppression. The use of an ionic surfactant in the BGE to separate the neutral BPs by MEKC was necessary. Phosphoric acid-sodium dihydrogenphosphate solution was therefore chosen as the buffer solution at pH 2.5, and sodium dodecyl sulfate (SDS) as the ionic surfactant.

The first experiments were carried out by analyzing a standard solution of the fourteen BPs (15 mg/L) in water and using a 25 mM phosphate buffer (pH 2.5) containing 100 mM SDS as preliminary BGE, and the electropherogram obtained is shown in Figure 1. Under these conditions EOF is suppressed and the separation of BPs is achieved by their hydrophobic interaction with the charged SDS micelles. However, although all BPs can be detected in less than 8.5 min, no base-line MEKC separation of the fourteen BPs was observed.

In order to optimize the electrophoretic separation of the studied BPs the effect of several parameters in the BGE such as phosphate buffer (pH 2.5) concentration, SDS concentration and the addition of organic solvents was evaluated.

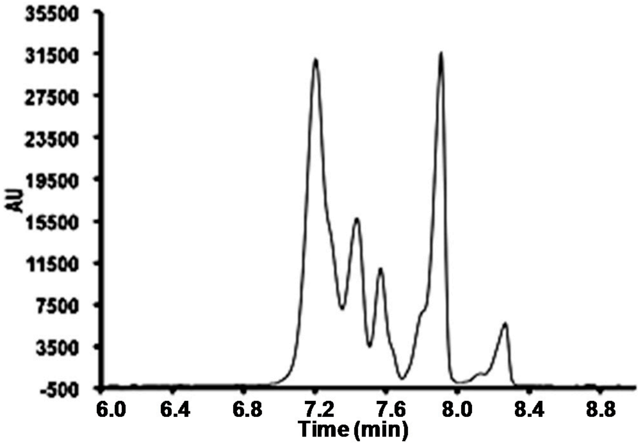


Figure 1. Preliminary micellar electrokinetic chromatography (MEKC) separation of benzophenones (BPs). Sample: standard solution of Benyophenones (BPs) at 15 mg/L in water. Background electrolyte (BGE): 25 mM phosphoric acid-sodium dihydrogenphosphate buffer (pH 2.5) with 100 mM sodium dodecyl sulfate (SDS). Capillary voltage: −25 kV (reversed polarity). Sample injection: hydrodynamic, 15 s (3.5 kPa). UV detection: λ 345 nm.

Phosphate buffer concentration was evaluated between 12.5 mM and 50 mM, keeping SDS concentration constant at 100 mM, and the electropherograms obtained for the MEKC separation of a 15 mg/L standard of BPs in water are shown in Figure 2.

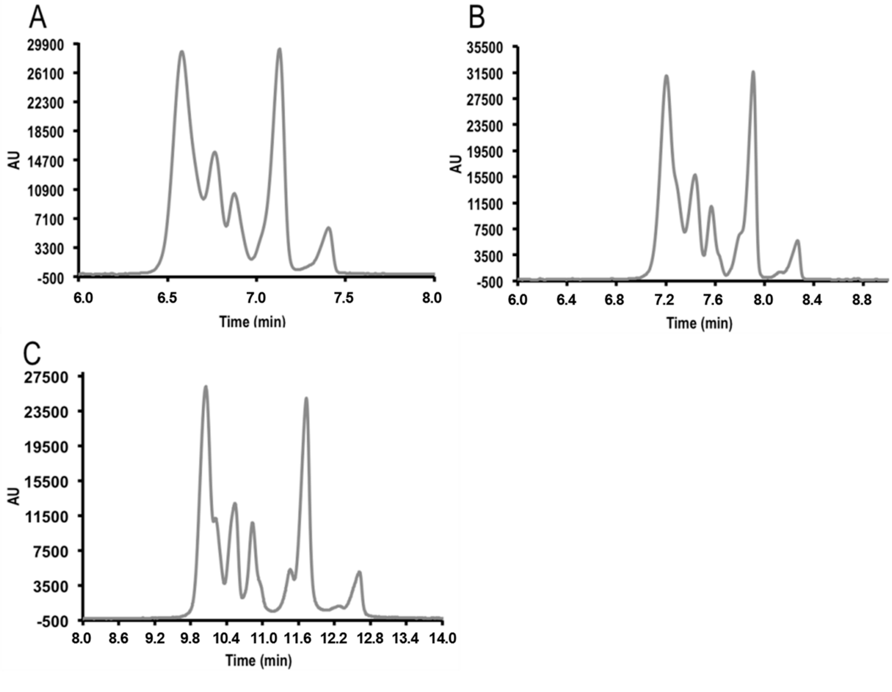


Figure 2. Effect of BGE buffer concentration in the MEKC separation of BPs. Sample: standard solution of BPs at 15 mg/L in water. BGE: 100 mM SDS and phosphoric acid-sodium dihydrogenphosphate buffer (pH 2.5) at (**A**) 12.5 mM, (**B**) 25 mM and (**C**) 50 mM. Capillary voltage: −25 kV (reversed polarity). Sample injection: hydrodynamic, 15 s (3.5 kPa). UV detection: λ 345 nm.

As expected, the increase in buffer concentration produced longer analysis times. Buffer concentration has a direct effect on ionic strength which will result in a higher resistivity in the separation medium and, consequently, a decrease in the electrophoretic mobility of the micelle-analyte structures. A small improvement in the electrophoretic separation of BPs can also be observed by increasing buffer concentration, as can be seen for the better electrophoretic peak resolution in Figure 2C. However, none of the evaluated conditions allowed the complete baseline separation of the studied BPs. Higher buffer concentrations were not employed because of the increase in capillary current. Nevertheless, based on these results and as a compromise between resolution and analysis time, 25 mM phosphoric acid-sodium dihydrogenphosphate buffer concentration was selected for further studies.

The effect of SDS concentration will be similar to that described above for the buffer concentration in that it will produce an important change in the BGE ionic strength, but it will also modify the BGE viscosity. SDS concentrations were studied between 25 mM and 200 mM, keeping the phosphate buffer concentration at 25 mM, and the results obtained are depicted in Figure 3.

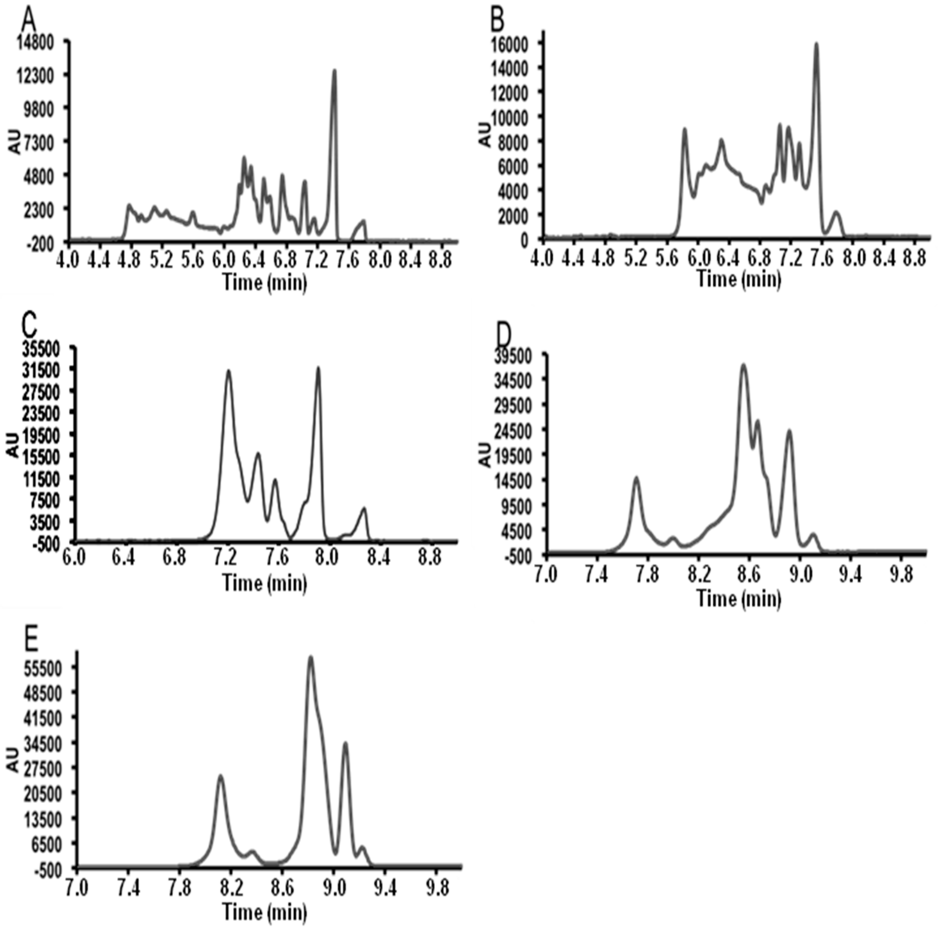


Figure 3. Effect of SDS concentration of the BGE in the MEKC separation of BPs. Sample: standard solution of BPs at 15 mg/L in water. BGE: 25 mM phosphoric acid-sodium dihydrogenphosphate buffer (pH 2.5) and (**A**) 12.5 mM SDS, (**B**) 50 mM SDS, (**C**) 100 mM SDS, (**D**) 150 mM SDS and (**E**) 200 mM SDS. Capillary voltage: −25 kV (reversed polarity). Sample injection: hydrodynamic, 15 s (3.5 kPa). UV detection: λ 345 nm.

As can be seen, increasing SDS concentration in the BGE also produced longer analysis times (as expected), because of the reduction in the electrophoretic mobility of the micelle-analyte structures caused by the increase on ionic strength and, consequently, the higher resistivity observed when applying an electric field through the capillary. Although it is difficult to see from the experimental results obtained, in Figure 3A,B a very small interaction between the analytes and the micelles is produced, probably due to the low SDS concentration. Moreover, very noisy electropherograms are observed probably due to the fact that the distribution equilibrium between micelle and analytes to form the micelle-analyte structure is not favored. However, the interaction between BPs and SDS micelles is improving when SDS concentration increases, as can be seen with the higher UV intensity signal observed (up to 50,000 AU) when 200 mM SDS was used (Figure 3E). In view of these results and given the improvement in signal intensity observed for the studied compounds, an SDS concentration of 200 mM was chosen for further experiments.

Finally, in order to achieve the separation of the studied BPs, the effect of adding an organic modifier to the BGE was also evaluated. In general, the use of organic modifiers in MEKC may alter the selectivity and modifies the viscosity of the separation media. The addition of isopropanol, a common organic modifier frequently used in MEKC, was studied from 10% to 40% and the electropherograms obtained are shown in Figure 4. The addition of isopropanol produced the most noticeable effect on the separation of BPs compared to phosphate buffer and SDS concentrations, improving electrophoretic separation and showing more and better resolved peaks. The addition of 30% isopropanol allowed almost baseline separation of all compounds in less than 21 min. This behavior is achieved because the organic solvent not only produces a change in BGE viscosity but it also changes the distribution constant of these compounds with the micellar phase. It has also an important effect on peak selectivity for some BPs, such as TrHBP and THBP (peaks 12 and 13, respectively) as can be seen when comparing the electropherograms at 30% (Figure 4C) and 40% (Figure 4E) isopropanol.

Although baseline separation can be achieved by increasing isopropanol content up to 40%, extremely large analysis times (36 min) are obtained. For this reason, and as a compromise between acceptable separation and analysis time, 30% isopropanol was selected as the optimum value for the separation of this family of compounds. Moreover, it should be mentioned that several instrumental problems arose when 40% isopropanol content was used, preventing to obtain good results due to the clogging of the capillary. This behavior was expected since high contents of organic solvents can disrupt SDS micellar structures and produce SDS precipitation.

Summarizing, under the optimal BGE conditions achieved, 25 mM phosphoric acid-sodium dihydrogenphosphate buffer (pH 2.5) with 200 mM SDS and 30% isopropanol, almost baseline separation of the fourteen studied BPs was achieved within a very reasonable analysis time (21 min).

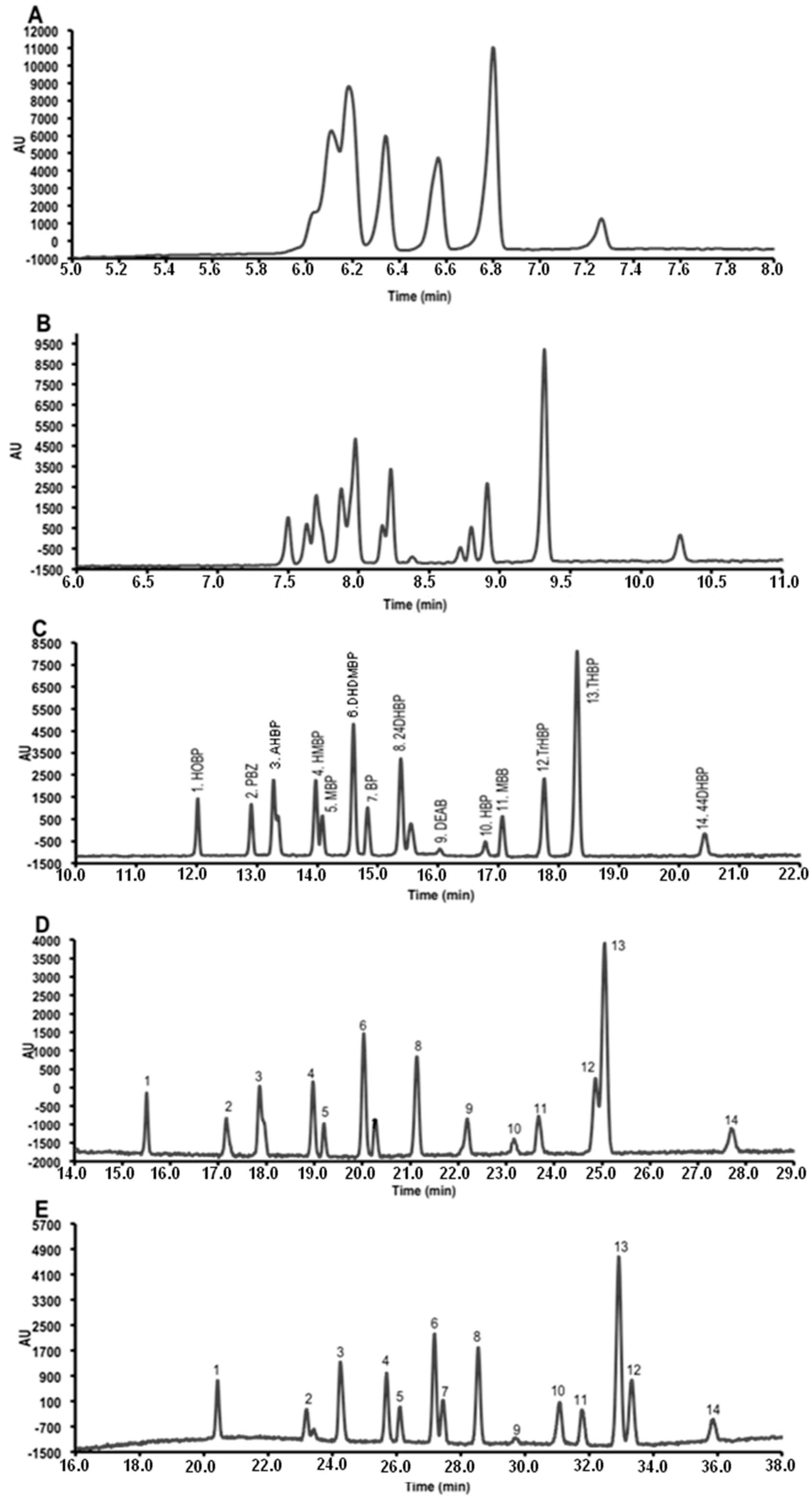


Figure 4. Effect of the isopropanol content of the BGE in the MEKC separation of BPs. Sample: standard solution of BPs at 15 mg/L in water. BGE: 25 mM phosphoric acid-sodium dihydrogenphosphate buffer (pH 2.5) with 200 mM SDS and (**A**) 10% isopropanol, (**B**) 20% isopropanol, (**C**) 30% isopropanol, (**D**) 35% isopropanol, and (**E**) 40% isopropanol. Capillary voltage: −25 kV (reversed polarity). Sample injection: hydrodynamic, 15 s (3.5 kPa). UV detection: λ 345 nm.

3.2. Field Amplified Sample Injection

The development of methods sensitive enough to determine low concentration levels of BPs in migration studies of food packaging materials is necessary because of 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 electrophoretic-based preconcentration method for MEKC was investigated. Among on-line electrophoretic-based enrichment procedures [30–32,36], FASI is very popular because it is quite simple only requiring a difference on the sample matrix conductivity at least 10-fold lower than the one of the BGE conductivity, and the electrokinetic injection of the sample after the introduction of a short plug of a high-resistivity solvent. In this study, the electrolyte previously optimized for the conventional MEKC separation (25 mM phosphoric acid-sodium dihydrogenphosphate buffer (pH 2.5) with 200 mM SDS and 30% isopropanol) was used as BGE for the FASI-MEKC procedure, and water was used as the high-resistivity solvent.

The sample matrix will also play an important role during FASI application, requiring a lower conductivity than the BGE as previously mention, but its role is even more important with compounds such as the analyzed BPs that are neutral under the previously developed MEKC conditions (pH 2.5). This behavior will also occur for most of the ionizable BPs when they are dissolved in water solutions or in the acidic food simulant that will be evaluated in this work. As enhanced electrokinetic injections are used during the application of FASI, charged analytes are required. One way to produce charged analytes while keeping the optimal conditions for the MEKC separation is to take advantage of the surfactant used for MEKC, as previously reported by other authors [32,38]. By adding the negatively charged SDS surfactant to the samples containing BPs, an interaction between BPs and SDS micelles will be obtained, and then FASI of the BP-SDS structures (with negative charges) can be performed. However, the concentration of SDS in the sample matrix needs to be optimized because the increase in the sample conductivity produced by the addition of SDS will affect FASI performance.

SDS concentration in the sample matrix was studied between 10 mM and 40 mM, and the results obtained are shown in Figure 5. The highest signal intensity for the FASI determination of BPs (above 7000 AU) was achieved when 10 mM SDS was added to the sample matrix (Figure 5A). It should be noted that BPs were not even detected when SDS was not added to the sample matrix, as expected.

However, when higher SDS concentrations were used as sample matrix (above 10 mM) lower signals were observed and the electrophoretic separation worsened. Even some of the BPs such as PBZ and DEAB (peaks 2 and 9 in Figure 5B,C) were not sufficiently introduced into the capillary. This is due to the increase on sample conductivity which is becoming closer to the one of the BGE solution, worsening the FASI enhancement. The higher the differences between sample matrix and BGE conductivities, the higher the FASI enhancements (Figure 5A). Thus, the use of 10 mM SDS solutions was selected as optimal sample matrix for the FASI-MEKC determination of BPs.

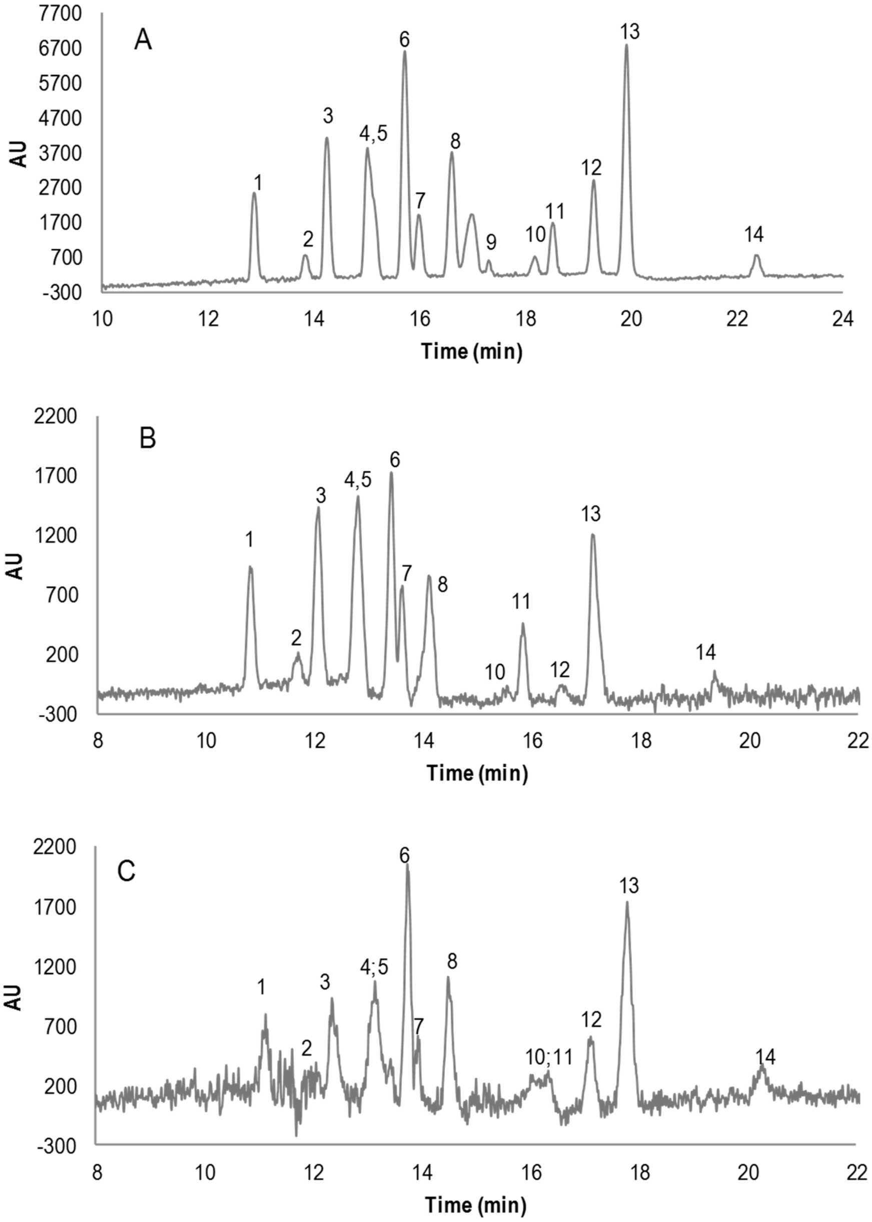


Figure 5. Effect of SDS concentration in the sample matrix in the field-amplified sampleinjection-micellar electrokinetic chromatography (FASI-MEKC) determination of BPs. Sample matrix: standard solution of BPs at 1.5 mg/L containing (**A**) 10 mM SDS, (**B**) 20 mM SDS, and (**C**) 40 mM SDS. BGE: 25 mM phosphoric acid-sodium dihydrogenphosphate buffer (pH 2.5) with 200 mM SDS and 30% isopropanol. Capillary voltage: −25 kV (reversed polarity). Water plug injection: hydrodynamic (2 s, 3.5 kPa). Sample injection: electrokinetic (20 s, −10 kV). UV detection: λ 345 nm. Peak identification as indicated in Figure 4.

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 5 s to 30 s and electrokinetic sample injection (at −10 kV) from 5 s to 60 s were tested. When short plugs of water were used, BPs showing low electrophoretic mobilities such as THBP and 44DHBP, which are the last compounds detected under the acquisition conditions (reversed polarity) did not appear in the electropherograms registered with high sample electrokinetic injection times. This is caused by the removal of these compounds from the capillary by the EOF generated in the water plug region. In contrast, when large plugs of water were used (higher than 25 s), double peaks for some BPs such as HOBP and PBZ were observed, effect caused by the long exposition of the BP-micelle structure to the water plug that can produce the breaking of the BP-SDS structure and the detection of both BP and BP-SDS complex simultaneously. For all these reasons, a compromise must be achieved between the water plug hydrodynamic injection time and sample electrokinetic injection time. Obviously, when sample injection time was increased 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 25 s and a sample electrokinetic injection time of 20 s, values that were selected for the optimum FASI-MEKC conditions (see electropherogram in Figure 6).

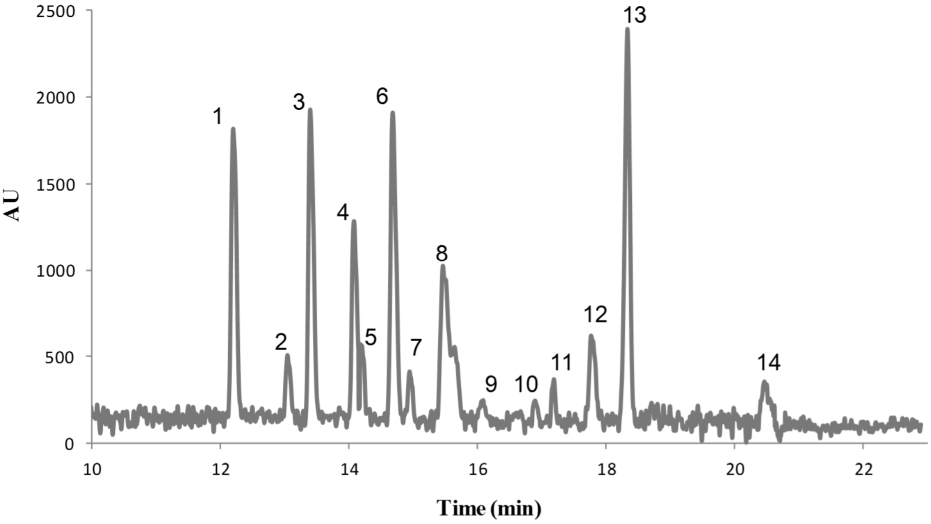


Figure 6. Separation of BPs under optimal FASI-MEKC conditions. Sample: standard solution of BPs at 500 µg/L in 10 mM SDS. BGE: 25 mM phosphoric acid-sodium dihydrogenphosphate buffer (pH 2.5) with 200 mM SDS and 30% isopropanol. Capillary voltage: −25 kV (reversed polarity). Water plug injection: hydrodynamic (25 s, 3.5 kPa). Sample injection: electrokinetic (20 s, −10 kV). UV detection: λ 345 nm. Peak identification as indicated in Figure 4.

3.3. Instrumental Quality Parameters

Instrumental quality parameters for both MEKC-UV and FASI-MEKC methods under optimal conditions were calculated and the figures of merit are summarized in Table 2. The 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 MEKC-UV with hydrodynamic injection provided LODs between 1.1 mg/L and 4.6 mg/L, the most sensitive BPs being BP, HBP, MBB, TrHBP and 44DHBP. When FASI-MEKC was applied, LODs in the range 5.1 to 68.4 µg/L were achieved, which means between a 21.6-fold (TrHBP) and a 784.3-fold (PBZ) sensitivity enhancement. The limits of quantification (LOQs), based on a signal-to-noise ratio of 10:1, were in the range of 3.6 mg/L to 15.1 mg/L for MEKC-UV and 16.8 µg/L to 225.7 µg/L for FASI-MEKC.

Table 2. Micellar electrokinetic chromatography (MEKC) and field-amplified sample injection-micellar electrokinetic chromatography (FASI-MEKC) instrumental quality parameters.

Compound	Method	LODs (μg/L)	Sensitivity enhancement (SE) [a]	LOQ (μg/L)	Run-to-run precision (% RSD, $n = 5$)		Accuracy [c] (%)
					Migration time	Concentration [b]	
HOBP	MEKC	4400	-	14,500	1.2	6.4	4.0
	FASI	14.1	314.3	46.5	1.0	22.3	5.6
PBZ	MEKC	4000	-	13,200	1.3	11.3	7.3
	FASI	5.1	784.3	16.8	1.0	17.0	15.0
AHBP	MEKC	4300	-	14,200	1.3	4.3	11.2
	FASI	10.1	425.7	33.3	1.0	15.5	8.3
HMBP	MEKC	3200	-	10,600	1.3	5.4	15.4
	FASI	12.5	256.0	41.3	1.0	17.6	16.5
MBP	MEKC	2700	-	8800	1.3	6.1	14.7
	FASI	18.1	150.0	59.7	1.0	12.4	18.8
DHDMBP	MEKC	3800	-	12,600	1.4	5.9	12.6
	FASI	58.9	64.5	194.5	1.0	10.6	18.5
BP	MEKC	1800	-	6000	1.4	4.1	11.7
	FASI	60.6	30.0	200.0	2.8	6.8	18.9
24DHBP	MEKC	2000	-	6600	1.4	5.3	0.4
	FASI	49.1	40.7	162.0	1.1	15.5	18.4
DEAB	MEKC	4600	-	15,100	1.4	9.6	12.2
	FASI	68.4	67.3	225.7	1.3	15.9	17.7
HBP	MEKC	1600	-	5200	1.5	6.6	1.5
	FASI	59.4	26.9	178.2	2.5	13.7	19.9
MBB	MEKC	1200	-	3900	1.5	6.6	7.1
	FASI	47.6	25.2	157.1	1.1	5.6	18.4
TrHBP	MEKC	1100	-	3600	1.5	6.0	8.3
	FASI	50.8	21.6	167.6	1.2	5.9	18.8
THBP	MEKC	2500	-	8300	1.5	5.2	9.6
	FASI	56.3	44.4	185.8	4.3	5.7	21.0
44DHBP	MEKC	1400	-	4700	1.6	5.6	0.4
	FASI	38.0	36.8	125.4	1.0	6.0	16.2

[a] SE = LOD (MEKC)/LOD (FASI-MEKC); [b] Concentration level: MEKC, *ca.* 15 mg/L; FASI, *ca.* 500 μg/L; [c] Accuracy: % relative error at: MEKC, *ca.* 15 mg/L; FASI, *ca.* 500 μg/L.

Run-to-run precisions for BP quantification were calculated at a concentration level of 15 mg/L and 500 μg/L for MEKC-UV and FASI-MEKC, respectively. In order to obtain the run-to-run precision, five replicate determinations were carried out. The relative standard deviations (% RSDs) obtained with conventional MEKC-UV were between 4.1% and 11.3%. Regarding FASI-MEKC precision, RSD values were similar or only slightly higher than those previously obtained by MEKC-UV, obtaining RSDs values below 22.3%. This can be explained because of the poor reproducibility of electrokinetic injection [39] and the low concentration level quantified. Run-to-run precision of migration times were also calculated, obtaining RSD values lower than 1.6% and 4.3% for MEKC-UV and FASI-MEKC, respectively.

External calibration curves based on peak area at concentrations between LOQ and 50 mg/L (MEKC-UV) and between LOQ and 1.5 mg/L (FASI-MEKC) were obtained and good linearity was observed ($r^2 > 0.991$). Accuracy was also evaluated by the triplicate analysis using external calibration of standard solutions at concentrations of 15 mg/L (MEKC-UV) and 500 µg/L (FASI-MEKC) achieving acceptable results, with relative errors ranging from 0.4% to 15.4% and from 5.6% to 21.0% for MEKC-UV and FASI-MEKC, respectively.

3.4. Application

The proposed FASI-MEKC method was applied to the analysis of BPs in a food simulant typically used in the migration studies of packaging materials. As previously commented in the introduction section, EU legislation established a specific migration limit for the BP compound of 600 µg/kg [8]. In the present work, a 3% acetic acid solution (v/v) (simulant B in the cited regulation) was used as food simulant. This food simulant is generally used for migration assays of packaging plastic materials intended to contain clear drinks such as water, ciders, clear fruit or vegetable juices, infusions, coffee, soft drinks, energy drinks, cloudy drinks such as juices with fruit pulp and liquid chocolate, fruit and vegetables in the form of purée or preserves, fermented milk as yoghurt, cream and sour cream, vinegar, sauces, mustard, and concentrated extracts of an alcoholic strength ≥6%.

FASI-MEKC method performance for the intended application was evaluated by determining LODs, LOQs, linearity within a working range (performing the quantification using matrix-matched calibration), run-to-run precision and accuracy, using as sample matrix the intended food simulant containing 10 mM SDS to make it compatible with the FASI procedure. The results obtained are shown in Table 3.

Table 3. FASI-MEKC method performance in the determination of BPs in a food simulant.

Compound	**LODs (µg/L)**	**LOQ (µg/L)**	**Run-to-run precision (% RSD, *n* = 5)**		**Accuracy [b] (%)**
			Migration time	**Concentration [a]**	
HOBP	33.0	108.9	0.7	24.9	11.0
PBZ	52.1	172.1	0.9	19.6	13.9
AHBP	68.7	226.6	1.0	15.4	16.9
HMBP	64.3	212.1	0.8	15.5	11.0
MBP	72.4	239.0	1.3	16.0	22.8
DHDMBP	102.6	338.6	1.0	15.4	23.0
BP	100.4	331.3	1.0	13.3	15.0
24DHBP	39.3	129.6	1.3	23.7	8.7
DEAB	72.4	238.9	2.1	14.5	12.5
HBP	47.1	155.4	1.0	4.7	6.1
MBB	72.4	238.8	1.2	16.0	17.5
TrHBP	65.1	214.8	0.2	10.1	11.5
THBP	56.7	187.1	1.0	7.9	18.4
44DHBP	65.3	215.5	1.0	5.8	3.2

[a] Concentration level of 500 µg/L; [b] Accuracy: % relative error at 500 µg/L.

The LODs of BPs in the food simulant, based on a signal-to-noise ratio of 3:1, were between 33.0 μg/L and 102.6 μg/L, and LOQs, based on a signal-to-noise ratio of 10:1, were in the range 108.9 μg/L to 338.6 μg/L. These values were slightly higher than those previously obtained in water (Table 2) as can be expected because of the higher resistivity of the food simulant, which make less effective the FASI enhancement. However, for all compounds the LOQ values were lower than the required specific migration limits legislated, making FASI-MEKC suitable for migration studies of packaging materials.

Run-to-run precisions for BP quantification were also calculated in the food simulant at a concentration level of 500 μg/L, by performing quantification with matrix-matched calibration using the food simulant as matrix for the preparation of calibration curve standards. In this case, RSD values in the range 4.7% to 24.9% were obtained. These values were similar or only slight higher than those previously established in water, behavior again expected due to the poor reproducibility of electrokinetic injection and the higher resistivity of the food simulant. Regarding migration times, RSD values lower than 2.1% were obtained.

External calibration curves based on peak area at concentrations between LOQ and 1.5 mg/L were obtained by matrix-matched calibration, and good linearity was observed ($r^2 > 0.989$). Accuracy was also evaluated by the triplicate analysis using matrix-matched calibration of a food simulant spiked at a concentration of 500 μg/L achieving acceptable results, with relative errors ranging from 3.2% to 23.0%. Figure 7 shows the electropherogram obtained in the determination of BPs in the evaluated food simulant. A statistical paired-sample comparison analysis was performed between the spiked concentrations and found concentrations. For a 95% confidence level, the quantification results obtained were not significantly different to those of the target samples, with a *p* value (probability at the confidence level) of 0.55.

These results prove that the proposed FASI-MEKC method is suitable for the determination of BPs in the migration studies of packaging food materials when 3% acetic acid solution is used as food simulant.

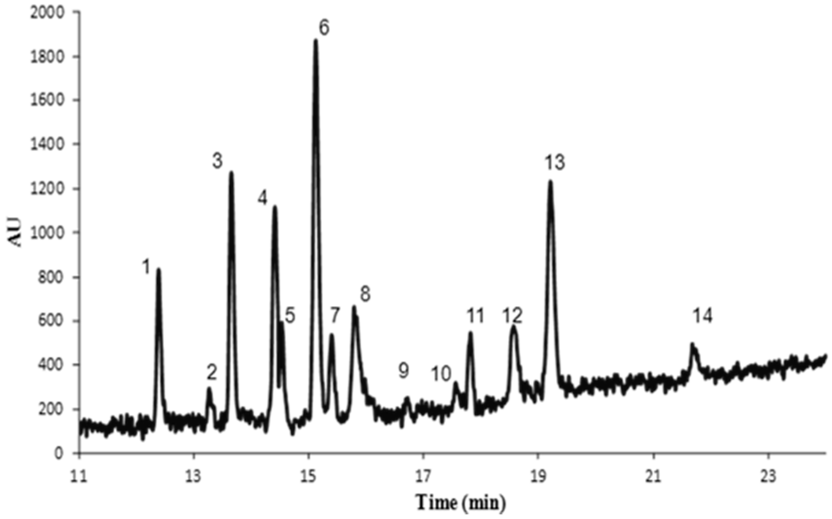


Figure 7. FASI-MEKC electropherogram in the analysis of a 3% acetic acid solution (containing 10 mM SDS) spiked with BPs at 500 μg/L. BGE: 25 mM phosphoric acid-sodium dihydrogenphosphate buffer (pH 2.5) with 200 mM SDS and 30% isopropanol. Capillary voltage: −25 kV (reversed polarity). Water plug injection: hydrodynamic (25 s, 3.5 kPa). Sample injection: electrokinetic (20 s, −10 kV). UV detection: λ 345 nm. Peak identification as indicated in Figure 4.

4. Conclusions

A sensitive field-amplified sample injection-capillary zone electrophoresis method for the determination of fourteen BPs in a food simulant has been developed. With the application of FASI, a 21-fold to 784-fold sensitivity enhancement was observed in comparison to conventional MEKC, obtaining limits of detection down to 5.1–68.4 µg/L, with good linearity, run-to-run precision (RSD values lower than 22.3%) and accuracy (relative errors lower than 22.3%).

A 25 mM phosphoric acid-sodium dihydrogenphosphate buffer (pH 2.5) with 200 mM SDS with 30% isopropanol solution was necessary to achieve almost baseline MEKC separation of the 14 BPs in less than 21 min. For FASI, the addition of 10 mM SDS to the sample matrix was mandatory in order to achieve BP FASI enhancement.

The proposed FASI-MEKC method was applied to the determination of BPs in a 3% acetic acid solution used as food simulant in the migration studies of these compounds from food packaging materials. Quantification at concentration levels below specific migration limits established for BP by EU legislation was performed and, for a 95% confidence level, no statistical differences were observed between found concentrations and spiked concentrations. The good results obtained in this study show that the proposed FASI-MEKC method is suitable and reliable for the determination of BPs in food packaging migration studies, when a 3% acetic acid solution is used as food simulant, at the levels required by EU legislation.

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Author Contributions

Cristina Félez performed the experiments and analyzed the data regarding MEKC optimization. Anna Molet performed the experiments and analyzed the data regarding FASI-MEKC optimization and application, with the close supervision of Cristina Félez. Both, Cristina Félez and Anna Molet worked under the supervision of Oscar Núñez who designed the experiments and wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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