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Field Amplified Sample Injection-Micellar Electrokinetic Capillary Chromatography (FASI-MECC) for the analysis of Bisphenol A, Bisphenol F, and their diglycidyl ethers and derivatives in canned soft-drinks.

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Abstract

Conditions were established for the separation and analysis of Bisphenol A (BPA), Bisphenol F (BPF), and their diglycidyl ethers, by micellar electrokinetic capillary chromatography (MECC). Good resolution was obtained for all compounds, although in order to achieve the separation of *ortho-ortho, ortho-para,* and *para-para* isomers of Bisphenol F diglycidyl ether (BFDGE), BFDGE·2H_2O and BFDGE·2HCl, it was necessary to use a 25 µm I.D. fused-silica capillary. To increase sensitivity, a field amplified sample injection (FASI)-MECC method was developed using 10 mM sodium dodecyl sulfate (SDS) solution as injection matrix and a 75 µm I.D. fused-silica capillary. Instrumental quality parameters such as limits of detection (<55 µg/L with standards), linearity (r^2>0.999), and run-to-run and day-to-day precisions (relative standard deviations (RSD) values lower than 12.5%) were determined. Finally, the suitability of the FASI-MECC method for the analysis of BPA, BPF and their diglycidyl ethers in canned soft-drinks was evaluated. Quantitation was performed by matrix-matched calibration using a plastic-bottled isotonic drink as matrix. The results showed that FASI-MECC is an economic method for the screening and quantitation of these kinds of compounds in soft-drink beverages, with no loss of reproducibility, and effective at concentrations lower than the specific migration level (SML) values established by the European Union.

1 Introduction

Bisphenol A (BPA) (2,2-bis[4-hydroxyphenyl] propane) is an industrially important chemical that is widely used as a raw material in the production of polycarbonate plastics and epoxy resins, which have a variety of applications such as plastic food containers and epoxy food-can coatings. Additional applications of BPA include printed circuit boards, composites, adhesives and tooling. Bisphenol F (BPF), which is a mixture of 3 isomers (2,2'-, 2,4'- and 4,4'-dihydroxydiphenylmethane), is also used in the manufacture of epoxy resins. Epoxy phenolic resins are the predominant protective coatings used for lining the interior of metal food cans. These resins are often polymerization products of bisphenol A-diglycidyl ether (BADGE) or novolac diglycidyl ether (NOGE, also known as epoxy novolac) and the lowest molecular weight component of NOGE is bisphenol F-diglycidyl ether (BFDGE). Furthermore, NOGE and BADGE have also been used as additives to polyvinyl-based dispersions (organosols) to remove the hydrochloric acid formed during heat treatment in the coating procedure. It is well documented that these monomers can migrate into the product during autoclavation, if the lacquer curing process is unsuccessful. Moreover, several reactions can take place in foodstuffs. For instance, the epoxy groups may be hydrolyzed when they come into contact with aqueous and acidic food during storage, generating the corresponding hydrolyzed derivatives. In addition, PVC aerosols contain chlorinated derivatives of BADGE and BFDGE, formed by the reaction with surplus hydrochloric acid generated during the production process. These chlorinated compounds can also migrate into canned food products during autoclaving. Consequently, the European Union (EU) has set specific migration limits (SML) for the sum of BADGE and its derivatives in food [1-3]. This regulation establishes a SML of 9 mg kg^{-1} or 9 mg/6 dm^2 for the sum of BADGE and its hydrolyzed derivatives and 1 mg kg^{-1} or 1 mg/6 dm^2 for the sum of BADGE·HCl, BADGE·2HCl and BADGE·HCl·H_2O. Although the use of BFDGE and the NOGE has been prohibited since 2005 because of the lack of toxicological studies, the presence of BADGE, BFDGE and NOGE is still permitted in very large containers. On the other hand, since BPA is a potential endocrine disruptor, the SML for this compound in food or

food simulants was set at 0.6 mg/kg by the EC Directive in an amending document related to plastic materials and articles intended to come into contact with food-stuffs [4]. The maximum acceptable dose and tolerable daily intake (TDI) for BPA were established at 50 µg/kg of body weight/day by both the US. Environmental Protection Agency (EPA) [5] and the European Food Safety Authority (EFSA) [6].

Both gas chromatography and liquid chromatography coupled to mass spectrometry (GC-MS, LC-MS) [7-15] and liquid chromatography with fluorescence detection [16,17] have been used to analyze BPA, BPF and their diglycidyl ether derivatives. Few studies have been published on the determination of BADGE and BFDGE by GC-MS without a derivatization step, mainly using 5% phenyl-methyl polysiloxane columns [8] which achieved sufficient resolution to separate the three isomers of BFDGE (*o,o'*-BFDGE, *o,p*-BFDGE and *p,p'*-BFDGE). Although good chromatographic separation and peak shape can be obtained for BPA [18,19], the derivatization of the hydroxyl groups was recommended to improve separation, peak shape and sensitivity when GC-MS was used. For the analysis of BPA and BPF, as well as bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE), and their chlorhydroxy- and hydrolyzed derivatives, reversed phase-liquid chromatography (RP-LC) is currently used. The mobile-phase organic modifier has an important effect on the elution of diglycidylethers. For instance, Lintschinger and Rauter [20] reported a change in the elution order of BADGE·H_2O and BADGE·HCl·H_2O when methanol was used instead of acetonitrile; this effect is probably due to the relative hydrophobicity of both solvents. In methanol, BADGE·H_2O appears before BADGE·HCl·H_2O and BFDGE elutes between these compounds, which provides good resolution between the three compounds. In general, for the analysis of this family of compounds, methanol provides good chromatographic profiles for the different isomers of BFDGE and its derivatives [17,20]. Since, the purchase of a mass spectrometer is not always an affordable expense for some laboratories, the development of alternative cheap, fast and sensitive methods is needed. In this context, capillary electrophoresis can represent a real alternative to current methods since it can provide high separation efficiencies

similar to GC and lower-cost separations compared to LC because it only requires a small amount of solvents. As these compounds are neutral and with pKa values higher than 9.7 (BPF), MECC emerges as a good electrophoretic technique for their analysis. To our knowledge, only one article has been published to date on the separation of these compounds by MECC, where the five BADGEs were used as test analytes in a comparative study of the resolution obtained using microemulsion EKC and MECC [21]. It should be noted that this work did not focus on the analysis of this family of compounds. Moreover, a cyclodextrin-modified MECC method using SDS and γ-cyclodextrin has been proposed for the analysis of BPA and some alkylphenols in serum [22], and Ha et al., [23] used BPA to evaluate a capillary zone electrophoresis with an electrochemical detector (CE/ECD) microchip. In spite of the high efficiency of CE techniques, an important drawback when using UV-detection is the relatively low sensitivity, due to the small amount of sample injected and the short optical path length used (I.D. of the capillary). To overcome this problem, several electrophoretic-based techniques such as field amplified sample injection (FASI), stacking, and sweeping can be used [24-28]. Of these, FASI is very popular since it is quite simple, only requiring the electrokinetical injection of the sample following the introduction of a short plug of a high-resistivity solvent (mainly water). Recently, Hu et al., [29] used a large-volume stacking procedure and sweeping-micellar electrokinetic chromatography to analyze BPA in beverages.

This paper describes a method for analyzing BPA, BPF, and their diglycidyl ethers and derivatives (Figure 1) using MECC. For the separation of the three isomers of BFDGEs the effect of the capillary diameter was evaluated. Moreover, in order to increase sensitivity, FASI was used as an in-line preconcentration procedure prior to MECC separation for the analysis of this family of compounds. Several parameters which affect the electrophoretic separation and the in-line preconcentration, such as the composition of the background electrolyte (BGE) (buffer concentration and pH, SDS concentration, and organic content), and sample injection solvent and time, among others, were optimized. Instrumental quality parameters such as limit of detection (LOD), linearity, and run-to-run and day-to-day precisions were established. Finally, the

applicability of the proposed FASI-MECC methodology was evaluated by analyzing this family of compounds in canned soft-drinks.

2 Materials and methods

2.1 Chemicals and consumables

Bisphenol A (BPA), Bisphenol A diglycidyl ether (BADGE), Bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O), Bisphenol A bis(2,3-dihydroxypropyl) ether (BADGE·2H_2O), Bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl), Bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl), and Bisphenol A (3-chloro-2-hydroxypropyl)(2,3-dihydroxypropyl ether (BADGE·HCl·H_2O) standards of analytical grade were obtained from Sigma-Aldrich (Steinheim, Germany). Bisphenol F (BPF) was purchased from Fluka (Steinheim, Germany). Bisphenol F diglycidyl ether (BFDGE), Bisphenol F bis(2,3-dihydroxypropyl) ether (BFDGE·2H_2O), Bisphenol F bis(3-chloro-2-hydroxypropyl) ether (BFDGE·2HCl) (all three standards are a mixture of *ortho-ortho, ortho-para* and *para-para* isomers) were also obtained from Sigma. The structures of the studied compounds are given in Figure 1.

HPLC-gradient-grade methanol, acetonitrile, ethanol, hydrochloric acid (25%), and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulphate (SDS), and sodium monohydrogen phosphate were obtained from Fluka, and phosphoric acid was supplied by Carlo Erba (Rodano, Italy).

Stock standard solutions of BPA, BPF, and their diglycidyl ether and derivatives (~ 1000 mg l^{-1}) were prepared in ethanol. Intermediate working solutions were prepared weekly from the primary standard solutions by appropriate dilution in water. Working standard solutions were prepared in the appropriate SDS solution (see section 2.2). All stock solutions were stored at 4 ^{o}C for no more than one month. Buffers were prepared daily by mixing 0.5 M phosphoric acid solution

and 0.5 M sodium monohydrogen phosphate solution at the appropriate pH. A stock solution of SDS 0.5 M was prepared weekly in purified water. BGE was obtained daily by dilution of the SDS stock solution in the appropriate phosphate buffer, and adding the appropriate amount of 2-propanol. BGE solutions were filtered using 0.45 µm nylon filters (Whatman, Clifton, NJ, USA).

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

2.2 Instrumentation

MECC experiments were performed on a Beckman P/ACE 5500 capillary electrophoresis system (Fullerton, CA, USA) equipped with a diode array detection system. The electrophoretic separation was carried out using uncoated fused-silica capillaries (Beckman) with a total length of 57 cm (effective length 50 cm) x 75 µm I.D. (or 50 µm and 25 µm I.D. in some cases), and a 25 mM phosphoric acid-monohydrogen phosphate buffer solution (pH 2.5) containing 200 mm SDS and 35% of 2-propanol as background electrolyte (BGE). Capillary temperature was held at 25 ^{o}C. The BGE was filtered through a 0.45 µm (0.22 µm with 25 µm I.D. capillaries) membrane filter, and degassed by sonication before use. It should be noted that it was necessary to change the BGE twice during the working day because of organic solvent evaporation. Samples were loaded by pressure assisted hydrodynamic injection (3.5 KPa, 15 s) or by using field amplified sample injection (FASI). This in-line preconcentration method was performed as follows: the capillary was first filled with BGE and then a small water plug (2 s, 3.5 kPa) was introduced. Samples were then loaded into the capillary by electrokinetic injection at -10 kV during 45 s. Samples were prepared by dilution with a 150 mM SDS solution (hydrodynamic injection mode) or with a 10 mM SDS solution (FASI mode). The MECC separation was performed by applying -30 kV through the capillary. Direct UV-detection was carried out at 214 nm. The CE instrument was controlled using a Beckman P/ACE station software version 1.2.

2.3 Capillary conditioning

New capillaries were pre-treated with 0.1 M hydrochloric acid for 30 min, water for 30 min, 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 sodium hydroxide for 30 min, water for 30 min, and with the BGE during 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 Sample preparation and clean-up procedure

Sample preparation and clean-up procedure was performed as follows. First, soft-drink canned samples were degassed by sonication. Then, 25 mL of samples were loaded into reversed-phase C18 solid phase extraction (SPE) cartridges (Bond Elute, 500 mg; Varian). Cartridges were previously conditioned with 5 mL of methanol and 5 mL of water. After samples had passed through the cartridges, these were washed with 10 mL methanol:water (1:9) solution to remove interferences. Finally, cartridges were dried and the analytes were eluted with 5 mL of methanol. The collected fraction was evaporated to dryness under a stream of nitrogen, the extract was reconstituted in 1 mL of 10 mM SDS solution (containing 5% ethanol) and was then directly injected using the FASI-MECC method.

3 Results and discussion

3.1 Optimization of MECC separation

In a previous paper, Poouthree *et al.* [21] obtained the separation of 5 BADGEs, which were used as test analytes, by using 25% of isopropanol as organic additive in a BGE containing a relatively high concentration of SDS and under suppressed electroosmotic conditions. The objective of the present study was to accomplish the separation of BPA, BPF, their diglycidyl ethers and derivatives (a total of 11 target compounds) by MECC. For this purpose, a mixture of the 11

compounds at a concentration level of 20 mg/L and prepared in a 180 mM SDS was used as test solution. As a first experiment, we used the conditions proposed by Poouthree *et al.* [21], and to optimize the separation, some BGE parameters such as buffer concentration, pH and SDS concentration, and organic solvent amount (methanol, acetonitrile, and isopropanol) were assayed. All the experiments were performed using a 75 µm I.D. fused-silica capillary and hydrodynamic injection (10 s, 3.5kPa).

Buffer concentration (from 10 mM to 125 mM) and SDS concentration (from 150 to 220 mM) were studied by keeping buffer pH at 2.5. It was observed that an increase in SDS concentration and a decrease in buffer concentration produced an improvement in the electrophoretic separation. For instance, the separation of the first eluting analytes was strongly affected by these two parameters, and a baseline separation could only be achieved by working at buffer concentrations between 10-50 mM and at SDS concentrations of 200-220 mM. For this reason, we chose a buffer concentration of 25 mM and an SDS concentration of 200 mM as optimal conditions for the working BGE. Buffer pH was also evaluated and as previously reported by Poouthree *et al.* [21], the best results were achieved when working under EOF suppression; consequently, we kept buffer pH at 2.5 as the optimum value.

The most notable effect on electrophoretic separation was observed with the addition of an organic solvent in the BGE. Methanol and acetonitrile did not improve the separation very much. In contrast, and as reported by Poouthree *et al.* [21], isopropanol produced better results. By adding 35 % of isopropanol to the background electrolyte, BPA, BPF and all their diglycidyl ethers and derivatives separated well, as can be seen in the electropherogram given in Figure 2a. This figure also shows that the migration order is related to the size and the hydrophobicity of the compounds. Larger and more hydrophobic compounds interact more strongly with the micelles and as a result eluted first when reverse polarity is used. For instance, BPA eluted before BPF, and the more hydrophobic BFDGE derivatives (BFDGE·2HCl, peaks 8+10+12) presented a migration time lower than the less hydrophobic one (BFDGE·2H_2O, peaks 7+9+11), as described Poouthree *et al.*

[21] for BADGEs. Moreover, as has been commented earlier, each BFDGE derivative is a mixture of three isomers (ortho-ortho, ortho-para and para-para, Figure 1) and in these conditions a slight separation of the isomers of BFDGE·2H_2O and BFDGE·2HCl occurred. An increase in isopropanol content did not improve electrophoretic separation of these isomers. In order to improve the separation, fused-silica capillaries of lower internal diameter (50 µm and 25 µm) were tested, as capillary efficiency, and consequently resolution, increase as the capillary I.D. decreases [30]. Figure 2b and 2c show the electropherograms obtained when the separation of the target compounds was performed using 50 µm I.D. and 25 µm I.D. capillaries, respectively. As can be observed, the decrease in capillary I.D. produced an improvement in isomer separation, as expected. By using 25 µm I.D. capillaries, all three isomers of BFDGE·2H_2O (peaks 7, 9 and 11) and BFDGE·2HCl (peaks 8, 10 and 12) were baseline separated, and the separation of the BFDGE isomers was also improved. Of course, an increase in analysis time was also produced. Moreover, an important decrease in detection sensitivity was observed, as was to be expected from the lower amount of sample introduced into the capillary and the shorter optical pathway when small I.D. capillaries are used. A migration order of BFDGEs isomers could be proposed taking into account the separation obtained by Gallart-Ayala *et al.* [31] using reversed-phase liquid chromatography, where the isomers were characterized using mass spectrometry. By comparing the elution profile of the commercial mixture, it was observed that the elution order in MECC (*ortho-ortho*, *ortho-para*, and *para-para*) was the opposite to that obtained by reversed-phase liquid chromatography, since the compound most highly retained in LC would interact more strongly with SDS micelles, reducing its migration time.

The effect of the amount of SDS in the sample injection matrix from 50 to 250 mM was also evaluated. It was observed that the best electrophoretic separation was achieved when an SDS concentration between 100 and 200 mM was added to the sample injection matrix. Higher SDS concentration values worsened the separation of some compounds, mainly BPF and BADGE·2H_2O

(peaks 2 and 13, respectively, in Figure 2). Thus, we used a 150 mM SDS solution as sample injection matrix for the MECC method.

Although an improvement in the electrophoretic separation of the 11 target compounds and the isomers of BFDGE, BFDGE·2H_2O, and BFDGE·2HCl was achieved by using a 25 µm I.D. capillary, for quantitation we chose the 75 µm I.D. capillary, as this allows to obtain higher sensitivity. Thus, the quantitation of BFDGE, BFDGE·2H_2O, and BFDGE·2HCl can be carried out as the sum of their three isomers. BFDGEs should rarely be found in real samples since their use has been prohibited by EU legislation [1,2]. In the case that these compounds are found in a sample, a smaller I.D. capillary can be used for the analysis if it is necessary to identify the amount of each isomer. If not, a 75 µm I.D. capillary can be used to quantify the sum of all three isomers.

Hydrodynamic injection time was also studied in order to increase sensitivity. As a compromise between peak signal and resolution, 15 s (3.5 kPa) was selected as optimal injection time for MECC.

3.2 Field amplified sample injection optimization

In order to enhance sensitivity for the analysis of BPA, BPF, and their diglycidyl ethers and derivatives, FASI was studied as an in-line preconcentration method. For this purpose a small water plug was introduced into the capillary by hydrodynamic injection prior to a long electrokinetic injection of the sample. The application of FASI with MECC for neutral compounds such as those studied in this research requires the addition of SDS in the standard or sample matrixes, which are introduced into the capillary electrokinetically.

The first assays were performed using the same injection matrix as that used in MECC (150 mM SDS). Nevertheless, the application of FASI was not satisfactory, probably due to the high conductivity of the standard matrix. Then, SDS solutions at low concentrations were evaluated as injection matrixes. Figure 3 shows the electropherograms obtained when solutions with SDS concentrations between 5 and 150 mM were used as injection matrix. FASI methodology improved

considerably with the decrease in SDS concentration in the injection matrix, showing significant enhancement at a concentration of 10 mM SDS. However, if lower SDS concentrations were used, a loss in sensitivity was again observed, which was probably due to a significant decrease in SDS micelles to interact with the analytes. Thus we selected a 10 mM SDS solution as the optimal concentration for the injection matrix. This solution was also used to reconstitute the extract of the samples after sample treatment and clean-up.

Sample electrokinetic injection (at -10 kV) and water plug hydrodynamic injection (at 3.5 kPa) were also optimized in order to achieve the best sensitivity. As expected, an increase in sensitivity was attained by increasing the sample injection time up to 45 s. Higher injection times produced a loss in separation resolution of some compounds. Thus, we used 45 s as optimum sample electrokinetic injection time. An increase in the water plug injection time up to 2-3 s also produced an enhancement in sensitivity. When higher water plugs were used (i.e. 5 s) a loss in separation resolution and efficiency of some compounds was also observed. For this reason, a 2 s water plug injection time was used as optimum. A sensitivity enhancement of up to 50 fold was observed with the application of FASI-MECC, so this was the method used for the analysis of this family of compounds.

3.3 Method performance

To verify method performance, instrumental quality parameters using the proposed FASI-MECC method under optimal conditions were obtained (Table 1). Limits of detection (LODs), based on a signal-to-noise ratio of 3:1, were calculated using standard solutions at low concentration levels. LODs with the MECC method were in the range of 2.9 to 5.4 mg/L. By using FASI-MECC, a sensitivity enhancement of between 27 and 50 times was achieved, obtaining LODs below 55 µg/L.

Calibration curves based on peak area at concentrations between 150 µg/L (75 µg/L for BADGE·$2H_2O$) and 5 mg/L for FASI-MECC were established and good linearity was observed with correlation coefficients higher than 0.999 in all cases.

Run-to-run and day-to-day precisions for compound quantification using external calibration were calculated at two concentration levels, a low concentration level (LCL = 3 x LOD) and a medium concentration level (MCL ~1 mg/L). In order to obtain the run-to-run precision, six replicate determinations for each concentration level were carried out. Similarly, day-to-day precision was calculated by performing 18 replicate determinations of each concentration level on 3 non-consecutive days (six replicates each day). The relative standard deviations (RSDs) obtained for run-to-run and day-to-day precisions were below 6% and 9%, respectively, at medium concentration level. RSDs slightly increased at low concentration levels, with values lower than 8.4% (run-to-run) and 12.5% (day-to-day). These results showed that in terms of precision, FASI-MECC is a satisfactory methodology for the quantitative analysis of BADGEs and BFDGEs at relatively low concentration levels.

Finally, in terms of migration times, good run-to-run and day-to-day precisions were obtained, with RSD values always lower than 3%.

3.4 Application

In order to evaluate the applicability of the optimized FASI-MECC method for the determination of BPA, BPF, and their diglycidyl ethers and derivatives in real samples, some canned soft-drinks were analyzed. For this purpose a simple sample treatment and clean-up procedure, where 25 mL of a soft-drink sample were passed through a C18 SPE cartridge, were used.

First, LODs and LOQs using a drink sample free of the target compounds were estimated. As the analyzed compounds are only expected to be found in canned foods and soft-drinks, a plastic bottle isotonic drink was used as sample matrix. Figure 4 shows the electropherograms of a non-

spiked plastic bottle isotonic drink (a) and a spiked one (b) at a level of 100 µg/L. As can be seen, the same separation can be achieved as that with standards, and although some matrix peaks were observed, these did not interfere with the target compound signals. LODs were determined by spiking the plastic bottle isotonic drink at low concentration levels. The LODs, based on a signal-to-noise ratio of 3:1, are given in Table 2. For all compounds, the LOD was lower than 5.4 µg/L. The decrease in LODs (9 to 18 fold) when compared to those obtained using standards (Table 1) is due to the preconcentration factors achieved with the sample treatment. According to these results, the SPE-FASI-MECC methodology described in this paper produced almost a 900-fold sensitive enhancement compared to MECC, and it can be used to analyse these compounds at concentrations higher than 9-15 µg/L (limit of quantitation, LOQ), a value which is much lower than the SML values established by the EU (0.6 mg/L for BPA, 9 mg/L for the sum of BADGE and its hydrolyzed derivatives and 1 mg/kg for the sum of BADGE·HCl, BADGE·2HCl and BADGE·HCl·H_2O) [1,2,4].

Method repeatability (sample treatment + FASI-MECC analysis) and recoveries were also evaluated, and the results are given in Table 2. For this purpose, six replicate determinations of a plastic bottle isotonic sample spiked with 1 mg/L for each compound were performed using the proposed method. The RSD values obtained ranged from 2.5 % to 5.8 %. These values were similar to those obtained for the instrumental FASI-MECC run-to-run precision at the MCL (Table 1), showing that the simple sample treatment and clean-up procedure used in this study did not significantly affect method performance. The recoveries obtained for all compounds were always higher than 90%.

Three canned soft-drinks (citrus soda, apple soda, and lemon beer) were analyzed using the proposed method and the concentrations found are given in Table 2. Quantitation was performed by matrix-matched external calibration using the plastic bottled isotonic drink as matrix in order to prevent sample matrix effects. All the matrix-matched standards ranging from 9 µg/L to 1 mg/L were treated with the same sample treatment and clean-up procedure as the real samples. As an

example, Figure 4c shows the electropherogram of a canned citrus soda sample. Neither BPF nor BFDGEs were observed in the three canned soft-drinks analyzed, as was to be expected, given that the use of BFDGE and NOGE has been prohibited since 2002 in the EU. However, their analysis is necessary because they can be found in canned foods from other countries, such as products from the Japanese market [13,32]. In contrast, some levels of BPA (23-33 µg/L), and some diglycidyl ethers such as BADGE (25-90 µg/L), BADGE·H_2O (~9 µg/L), and BADGE·$2H_2O$ (40-51 µg/L) were found. All this levels were, nevertheless, below the SML levels established by EU [1,2,4].

For method validation, samples were also analyzed following a LC-MS/MS method established by Gallart-Ayala *et al.* [31], and the results are shown in Table 2. A statistical paired-sample comparison analysis was performed with the results obtained using both methods. For a 95% confidence level, the results were not significantly different (*P* value of 0.74).

These results show that FASI-MECC is an economic method for the screening of BPA, BPF, and their diglycidyl ethers and derivatives in canned soft-drinks. By using matrix-matched external calibration, good quantitation results can also be obtained.

Concluding remarks

BPA, BPF, their diglycidyl ethers, and also the three isomers of BFDGE, BFDGE·$2H_2O$, and BFDGE·2HCl, were separated by MECC using small I.D. (25 µm) fused-silica capillaries. Moreover, a FASI-MECC method for the analysis of this family of compounds in canned soft-drinks was developed. For quantitation, the use of 75 µm I.D. fused-silica capillaries is proposed, performing the quantitation of BFDGE, BFDGE·$2H_2O$, and BFDGE·2HCl as the sum of their three isomers. A 50-fold sensitivity enhancement was achieved with FASI for most of the compounds, obtaining LODs in the range of 27-55 µg/L (for standards), and with good run-to-run and day-to-day precisions (RSD values lower than 12.5). A simple sample treatment and clean-up procedure was applied for the analysis of these compounds in canned soft-drinks by FASI-MECC without affecting method performance, and achieving a 900-fold sensitive enhancement for real samples

compared to MECC. In general, the presence of BPF and BFDGEs was not observed in the canned soft-drinks analyzed, whilst BPA and some BADGEs were found at levels lower than the legislated SML values. The results obtained in this study allow us to conclude that FASI-MECC is a reliable and economic method for the analysis of this family of compounds in canned soft-drinks at concentrations higher than 9-15 µg/L (LOQ in real samples) and below the SML values established by the EU.

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Figure captions

Figure 1. Chemical structures of BPA, BPF, and their diglycidyl ethers.

Figure 2. MECC Electropherograms of a standard mixture of 25 mg/L obtained using fused-silica capillaries of (a) 75 µm I.D., (b) 50 µm I.D., and (c) 25 µm I.D. BGE: 25 mM phosphate buffer (pH 2.5), 200 mM SDS, 35% isopropanol. Sample injection mode: hydrodynamic injection. Sample injection time: 15 s (3.5 kPa). Separation performed at -30 kV, with acquisition wavelength of 214 nm. Peak identification as in Figure 1.

Figure 3. Effect of SDS concentration in the sample matrix injection on electrophoretic separation when applying FASI-MECC. (a) 150 mM SDS, (b) 20 mM SDS, (c) 10 mM SDS, and (d) 5 mM SDS. All sample matrixes contain a 5% of ethanol. FASI injection: 2 s water plug (3.5 kPa) and 45 s electrokinetic injection (-10 kV). Other conditions as in Figure 2. Peak identification as in Figure 1.

Figure 4. Electropherograms of a non-spiked plastic bottle isotonic soft-drink (a), a plastic bottle isotonic soft-drink spiked at 100 µg/L (b), and a canned citrus soda soft-drink sample (c), obtained by FASI-MECC under optimal conditions. Peak identification as in Figure 1.

Table 1. FASI-MEKC Instrumental quality parameters.

Peak number	Compound	MEKC	FASI-MEKC						
		LOD (mg/L)	LOD (µg/L)	run-to-run precision (%RSD, n=6)			day-to-day precision (%RSD, n=3x6)		
				t_m	LCL[b]	MCL[c]	t_m	LCL[b]	MCL[c]
1	BPA	3.3	55	1.2	8.1	3.1	2.9	12.5	8.2
3	BADGE	3.0	52	1.1	2.0	4.4	1.9	3.6	9.2
16	BADGE·H_2O	3.0	50	1.1	8.4	5.2	1.2	11.5	6.2
13	BADGE·$2H_2O$	2.9	27	0.9	6.7	4.3	1.3	11.5	7.8
17	BADGE·HCl	5.3	53	1.0	3.0	4.5	2.2	3.6	9.1
14	BADGE·2HCl	3.3	55	1.0	3.8	5.7	1.9	11.1	9.3
15	BADGE·HCl·H_2O	3.2	53	0.9	4.4	4.0	1.1	10.5	5.8
2	BPF	3.2	54	1.0	8.2	4.6	1.4	12.1	8.7
4+5+6	BFDGE [a]	3.1	50	0.9	4.1	4.8	1.3	12.0	9.1
7+9+11	BFDGE·$2H_2O$ [a]	5.4	54	1.1	7.5	3.9	2.3	8.8	4.3
8+10+12	BFDGE·2HCl [a]	5.2	52	0.9	5.1	5.0	1.9	8.0	7.5

LCL: Low Concentration Level; MCL: Medium Concentration Level

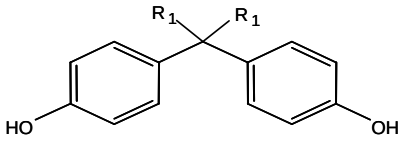
[a] Quantitation carried out for the sum of the three isomers; [b] LCL = LOQ; [c] MCL = ~1 mg/L

Table 2. LODs, method precision, recoveries and quantitation results in canned soft-drink samples

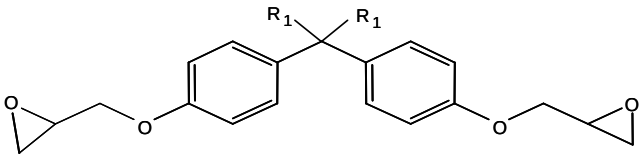
Peak number	Compound	LOD (µg/L) [a]	Method precision (RSD, n=6)	Recoveries (%) [a]	Analysis of canned soft-drink samples (µg/L)					
					Citrus soda		Apple soda		Lemon Beer	
					FASI-MEKC	LC-MS/MS	FASI-MEKC	LC-MS/MS	FASI-MEKC	LC-MS/MS
1	BPA	3.0	5.1	95	23	n.a.	33	n.a.	< LOQ	n.a.
3	BADGE	3.1	3.7	98	90	81	25	35	38	33
16	BADGE·H_2O	3.2	4.1	93	< LOQ	4	< LOQ	4	9	10
13	BADGE·2H_2O	3.0	5.0	96	51	56	< LOQ	6	40	44
17	BADGE·HCl	5.2	5.6	95	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14	BADGE·2HCl	3.0	4.8	99	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
15	BADGE·HCl·H_2O	3.0	2.5	90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	BPF	3.1	5.8	95	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4+5+6	BFDGE [b]	3.1	3.6	90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7+9+11	BFDGE·2H_2O [b]	5.4	4.2	98	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8+10+12	BFDGE·2HCl [b]	5.0	3.8	96	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] In a plastic bottle isotonic drink.
[b] Quantified as the sum of the three isomers.
n.d.: not detected
n.a.: not analyzed

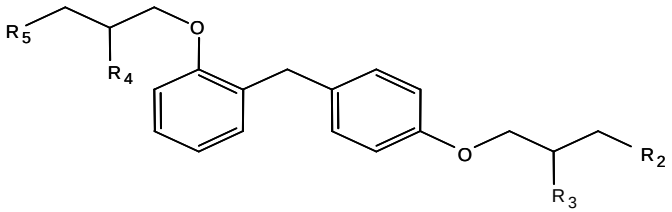
Figure 1



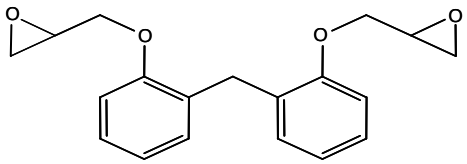
(1) Bisphenol A (BPA) $R_1 = CH_3$
(2) Bisphenol F (BPF) $R_1 = H$



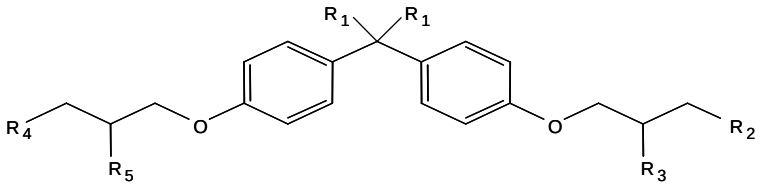
(3) BADGE $R_1 = CH_3$
(4) *p,p*-BFDGE $R_1 = H$



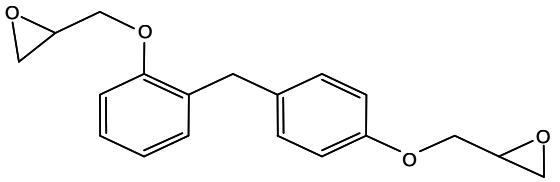
(9) *o,p*-BFDGE·2H_2O $R_2 = R_3 = R_4 = R_5 = OH$
(10) *o,p*-BFDGE·2HCl $R_2 = R_4 = OH$; $R_3 = R_5 = Cl$



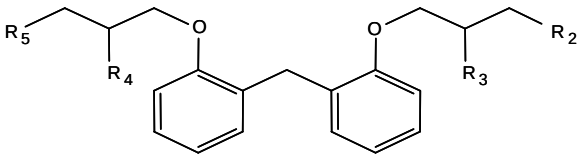
(5) *o,o*-BFDGE



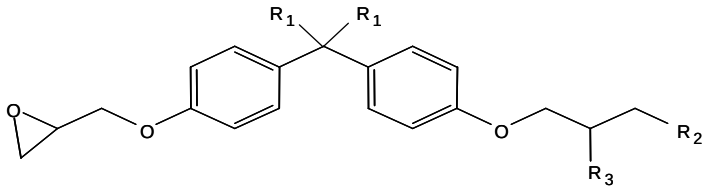
(11) *p,p*-BFDGE·2H_2O $R_1 = H$; $R_2 = R_3 = R_4 = R_5 = OH$
(12) *p,p*-BFDGE·2HCl $R_1 = H$; $R_2 = R_4 = OH$; $R_3 = R_5 = Cl$
(13) BADGE·2H_2O $R_1 = CH_3$; $R_2 = R_3 = R_4 = R_5 = OH$
(14) BADGE·2HCl $R_1 = CH_3$; $R_2 = R_4 = OH$; $R_3 = R_5 = Cl$
(15) BADGE·HCl·H_2O $R_1 = CH_3$; $R_3 = Cl$; $R_2 = R_4 = R_5 = OH$



(6) *o,p*-BFDGE



(7) *o,o*-BFDGE·2H_2O $R_2 = R_3 = R_4 = R_5 = OH$
(8) *o,o*-BFDGE·2HCl $R_2 = R_4 = OH$; $R_3 = R_5 = Cl$



(16) BADGE·H_2O $R_1 = CH_3$; $R_2 = OH$; $R_3 = OH$
(17) BADGE·HCl $R_1 = CH_3$; $R_2 = OH$; $R_3 = Cl$

Figure 2

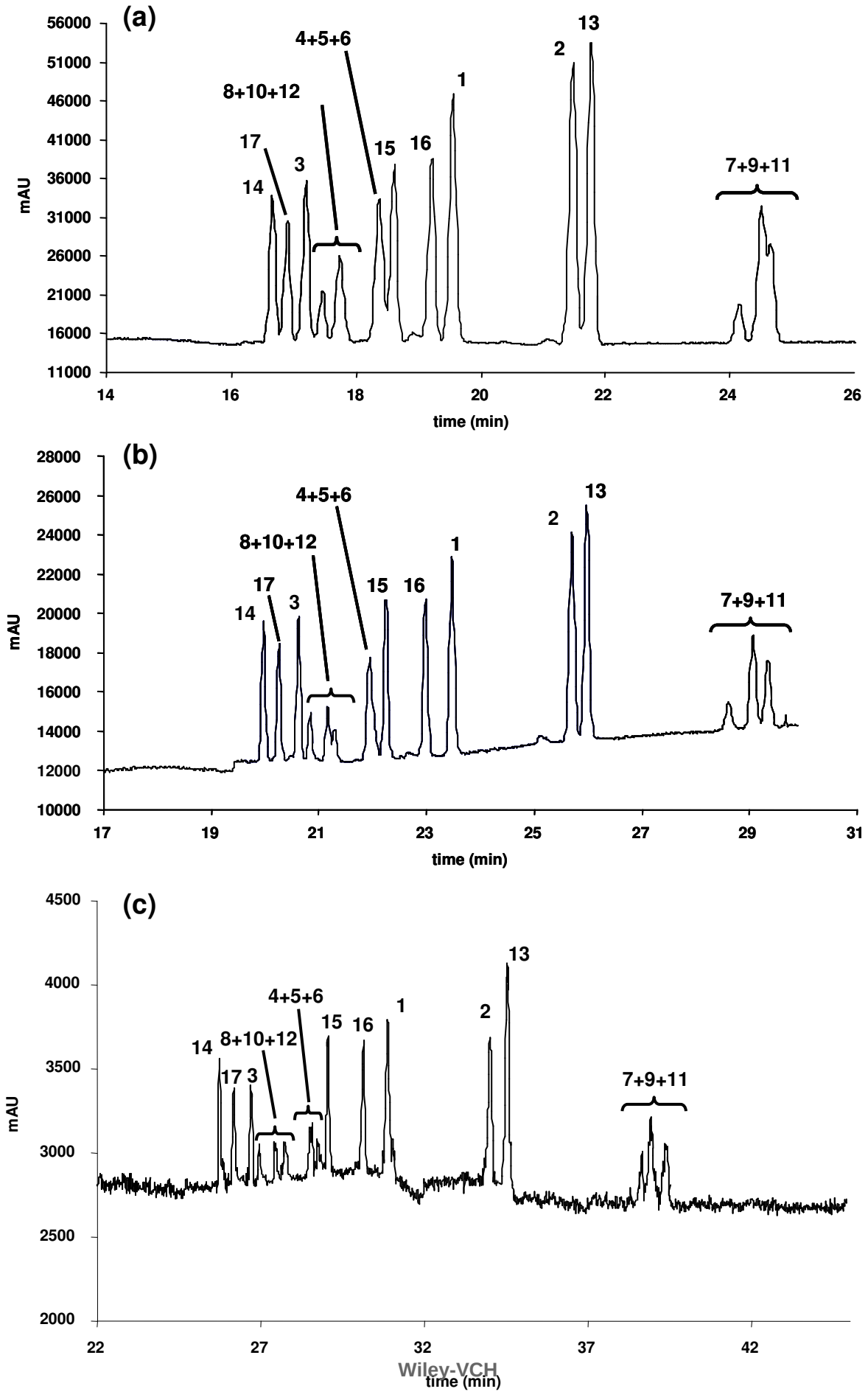


Figure 3

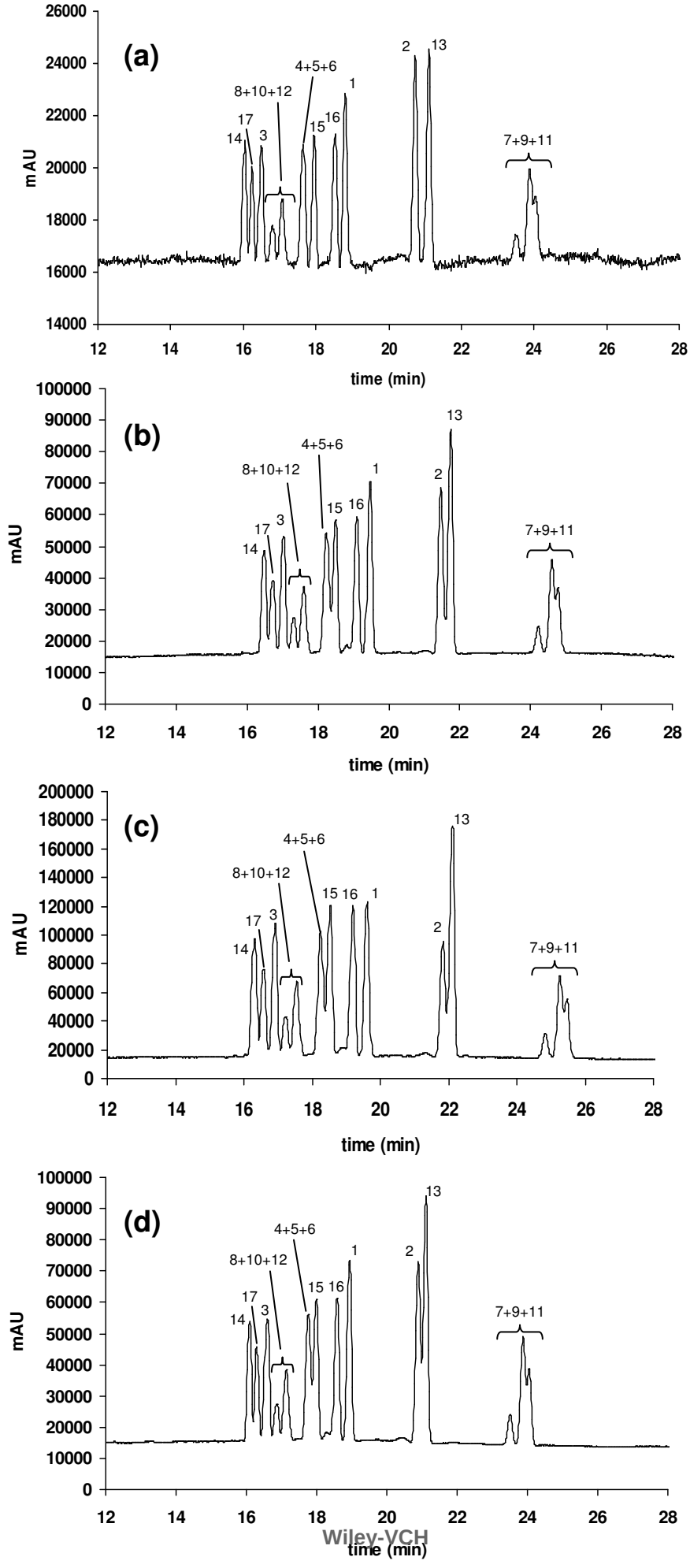
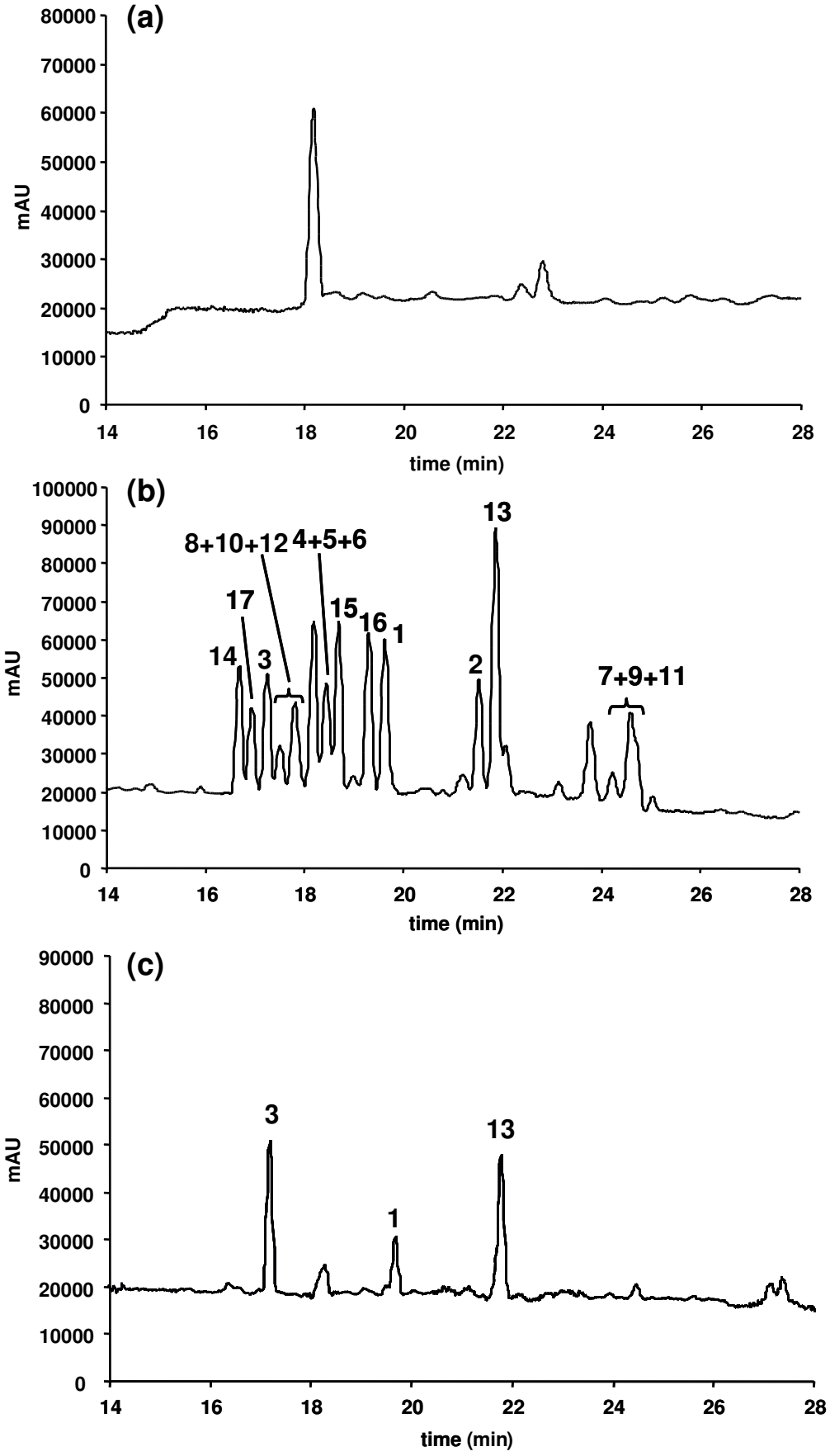


Figure 4


(a)
mAU
time (min)
(b)
mAU
time (min)
14
3
8+10+12
4+5+6
17
15
16
1
2
13
7+9+11
(c)
mAU
time (min)
3
1
13