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Rapid and sensitive analysis of polychlorinated biphenyls and acrylamide in food samples using ionic liquid-based *in situ* dispersive liquid-liquid microextraction coupled to headspace gas chromatography

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Abstract

A simple and rapid ionic liquid (IL)-based *in situ* dispersive liquid-liquid microextraction (DLLME) method was developed and coupled to headspace gas chromatography (HS-GC) employing electron capture (ECD) and mass spectrometry (MS) detection for the analysis of polychlorinated biphenyls (PCBs) and acrylamide at trace levels from milk and coffee samples. The chemical structures of the halide-based ILs were tailored by introducing various functional groups to the cations to evaluate the effect of different structural features on the extraction efficiency of the target analytes. Extraction parameters including the molar ratio of IL to metathesis reagent and IL mass were optimized. The effects of HS oven temperature and the HS sample vial volume on the analyte response were also evaluated. The optimized *in situ* DLLME method exhibited good analytical precision, good linearity, and provided detection limits down to the low ppt level for PCBs and the low ppb level for acrylamide in aqueous samples. The matrix-compatibility of the developed method was also established by quantifying acrylamide in brewed coffee samples. This method is much simpler and faster compared to previously reported GC-MS methods using solid-phase microextraction (SPME) for the extraction/preconcentration of PCBs and acrylamide from complex food samples.

Keywords: ionic liquids; liquid-liquid extraction; headspace gas chromatography; polychlorinated biphenyls; acrylamide; food analysis

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1. Introduction

The monitoring of contaminants in foods is very important for human health risk assessment [1, 2]. The accumulation of toxic compounds such as PCBs from the environment [3] and the unintentional formation of toxic substances during the manufacturing process (e.g., generation of acrylamide during roasting of coffee beans [4]) are two major sources of food contamination. It is well known that continuous exposure to these toxic compounds can cause several chronic diseases, including cancer and serious endocrine disorders [5]. However, the identification and quantification of contaminants from food samples is a significant analytical challenge. Although GC and high-performance liquid chromatography (HPLC) coupled to MS have been widely adopted for food analysis, the direct analysis of food samples is often very challenging due to the high complexity of the sample matrices. To address this issue, highly selective, sensitive, and cost-effective sample preparation techniques must be selected and employed prior to downstream chromatographic, electrophoretic, or mass spectroscopic analysis [1, 6, 7].

SPME is a solvent free, simple, and convenient technique which combines sampling and preconcentration into one step [8]. By applying various commercially available SPME coatings in the headspace or direct-immersion mode, a wide variety of compounds have been successfully extracted from food samples [1, 2]. Structurally-tuned polymeric ionic liquids (PILs) were recently employed by our group as sorbent coatings for the extraction of PCBs and acrylamide from milk and coffee samples, respectively [9-11]. The PIL-based sorbent coatings exhibited superior selectivity and sensitivity in the extraction of these compounds compared to commercially available SPME coatings. However, it was also observed that long extraction times (from 30 min to a couple hours) were required to extract detectable amounts of analytes

from the sample matrices. Furthermore, the development of matrix-compatible SPME sorbent coatings remains a significant challenge. When analyzing very complex sample matrices, irreversible fouling of the sorbent coating can dramatically decrease the lifetime of the SPME fiber [12]. In our previous work, matrix-compatible PIL-based sorbent coatings were applied for the in-solution extraction of acrylamide from brewed coffee samples. However, the developed method required a washing and reconditioning step after each extraction, which can significantly decrease the sample throughput [10, 11]. Therefore, alternative extraction techniques that are rapid, robust, selective, and sensitive need to be explored.

Liquid phase microextraction (LPME) are techniques that employ small volumes (microliters) of solvent as extraction phase. The three main approaches of LPME are single drop microextraction (SDME), hollow fiber liquid-phase microextraction (HF-LPME) and dispersive liquid-liquid microextraction (DLLME). SDME and HF-LPME has been widely applied for the extraction of analytes from complex samples [13-15]. However, the major disadvantages for these techniques are long extraction times and low enrichment factors. To solve this issue, dispersive liquid-liquid microextraction (DLLME) was first introduced by Rezaee and co-workers in 2006 [16]. Analyte preconcentration in this technique is achieved by dispersing a water-immiscible extraction solvent into fine droplets with the assistance of a water-miscible disperser solvent. Subsequently, the hydrophobic extraction solvent can be recovered by centrifugation [17-20] or by decreasing the temperature of the solution [21-23] followed by chromatographic analysis. Due to the significantly increased surface area of the extraction solvent, very short extraction times (often less than a minute) are required resulting in high extraction efficiencies for target analytes.

Ionic liquids (ILs) were first applied as extraction solvents for DLLME in 2008 [21, 24]. Compared to conventional extraction solvents employed for DLLME (i.e., chlorobenzene, chloroform, and carbon tetrachloride), ILs exhibit many unique physical properties including negligible vapor pressures and tunable viscosities [25, 26]. Even though the environmental toxicity of some ILs has been recently reported [27, 28], ILs are also less likely to be diffused to the atmosphere and have been suggested as a green alternative to traditional organic solvents. Moreover, the chemical structures of ILs can be custom designed to enhance extraction efficiencies toward different classes of analytes. In 2009, another modified DLLME approach termed *in situ* DLLME or *in situ* solvent formation microextraction based on ILs was introduced [29, 30]. In this approach, a hydrophilic IL-based extraction solvent is dissolved in an aqueous sample solution. An anion exchange reagent such as lithium bis[(trifluoromethyl)sulfonyl)]imide ($LiNTf_2$) is then added to the solution to form fine droplets of the hydrophobic IL solvent that can be easily separated from the aqueous solution. This technique has been applied towards the analysis of many analytes from a variety of samples and has been recently reviewed [31]. Most analyses have been carried out using HPLC due to the fact that direct GC analysis can cause accumulation of the nonvolatile IL in the GC inlet. This has limited the use of IL-based *in situ* DLLME in the analysis of volatile and semi-volatile compounds. Very recently, *in situ* ionic liquid DLLME has been combined to GC analysis by direct microvial insert thermal desorption of the extract. This approach requires the use of a thermal desorption unit equipped with a programmed temperature vaporization (PTV)-cooled injector system [32].

Headspace sampling is an ideal technique for analyzing volatile and semi-volatile analytes

from a non-volatile sample matrix [33-35]. This approach minimizes the amount of non-volatile matrix components introduced in the GC and results in lower background interference and better sensitivity. ILs have been employed as a new class of diluents in headspace gas chromatography (HS-GC) analysis [36-39]. Due to their high thermal stability and low volatility, the HS oven can be operated at high temperatures, thereby broadening the application of HS-GC.

To address the aforementioned limitations of the conventional DLLME method, ILs were studied as extraction solvents in *in situ* DLLME to provide rapid preconcentration of PCBs and acrylamide from complex food samples followed by analysis of the IL-based extraction solvent by HS-GC. Five halide-based ILs containing varied cation moieties (i.e., long alkyl side chains, aromatic and hydroxyl groups) were prepared to evaluate the effect of different structural features on the extraction efficiency of the target analytes. Extraction parameters including the molar ratio of IL to metathesis reagent and mass of IL employed in the extraction were optimized. The effects of HS oven temperature and the HS sample vial volume on the analyte response were also evaluated. The matrix-compatibility of the developed method was also studied by quantifying acrylamide in brewed coffee samples. This method is much simpler and faster compared to the previously reported SPME GC-MS method [11] and has tremendous potential to be applied for the routine analysis of contaminants present in complex food samples.

2. Materials and methods

2.1 Materials

The reagents 1-methylimidazole (99%), 1-benzylimidazole (99%), 1-bromobutane (99%),

2-bromoethanol (95%), 1-bromooctane (99%), 6-chlorohexanol (96%), acrylamide (99.9%), ninhydrin, ethanol (99.9%), and centrifuge tubes (natural polypropylene conical, 5 mL) were purchased from Sigma Aldrich (St. Louis, MO, USA). Acetone (99.5%), isopropanol (99.5%), ethyl acetate (99.9%), and glass beads (Walter Stern economical solid glass beads, 3 mm diameter) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Lithium bis[(trifluoromethyl)sulfonyl]imide ($LiNTf_2$) was purchased from SynQuest Labs, Inc. (Alachua, FL, USA).The PCB mixture containing 100 μg mL^{-1} of 21 different congeners in acetone was purchased from Accustandard (New Haven, CT, USA). The names and structures for each of the PCBs are listed in Table S1 (Supplementary information). Ultrapure water (18.2 MΩ cm) was obtained from a Milli-Q water purification system (Bedford, MA, USA). Headspace vials (10 mL) were purchased from Agilent (Santa Clara, CA, USA). Fat free, low fat, and reduced fat milk samples and a commercial blend of dark roasted coffee beans were purchased from a local market (Ames, IA, USA). The coffee beans were ground with a commercial coffee grinder before being subjected to brewing.

2.2 Synthesis of ILs

Chemical structures of the ILs employed in this study are shown in Fig. 1. All ILs were synthesized according to previously published methods [18, 30, 40] and were fully characterized by ^{1}H NMR (see Fig. S1-S5 supplementary information). ^{1}H NMR spectra were collected in deuterated dimethyl sulfoxide or chloroform using a Bruker DRX 500 MHz nuclear magnetic resonance (NMR) spectrometer (Billerica, MA, USA).

2.3 Instrumentation

An Agilent 7890B gas chromatograph (Santa Clara, CA, USA) equipped with an Agilent 7697A headspace sampler, electron capture detector (ECD) as well as a 5977A mass spectrometer (MS) was employed in this study. For the HS-GC-ECD analysis of 21 PCB congeners, the HS oven was operated at an optimal sampling temperature of 250 ˚C, which was determined through optimization. The sample loop and transfer line was operated at 10 ˚C and 20 ˚C higher, respectively, than the HS oven temperature. The equilibration time was 10 min. The GC injector was maintained at 280 ˚C with a 5:1 split ratio. The separation of 21 PCB congeners was achieved using a HP-5MS UI capillary column (30 m × 250 µm I.D., d_f = 0.25 µm) obtained from Agilent Technologies (Santa Clara, CA, USA). Helium was used as the carrier gas at constant flow of 1 mL min^{-1}. The temperature program used for GC-ECD was as follows: initial temperature was set at 130 ˚C and held for 2 min, followed by a ramp from 130 ˚C to 240 ˚C at 5 ˚C min^{-1} and held for 5 min. The temperature was then increased to 320 ˚C at 20 ˚C min^{-1} and held for 5 min. The temperature for the ECD was set at 300 ˚C and the argon/methane make-up flow was maintained at 30 mL min^{-1}.

For the HS-GC-MS analysis of aqueous acrylamide solution and brewed coffee spiked with acrylamide, the HS oven was operated at 205 ˚C. The sample loop and transfer line was operated at 215 ˚C and 225 ˚C, respectively. The equilibration time was 10 min. The GC injector was maintained at 250 ˚C with a 5:1 split ratio. The separation and quantification of acrylamide by GC-MS was achieved using a Mega-FFAP-EXT column (50 m × 200 µm I.D., d_f = 0.20 µm) (Legnano, MI, Italy). Helium was used as the carrier gas at constant flow of 1 mL min^{-1}. The temperature program used was as follows: initial temperature was set at 50 ˚C

and held for 1 min, followed by a ramp of 2 ˚C min^{-1} to 165 ˚C and then increased to 250 ˚C at 7.5 ˚C min^{-1} and held for 2 min. The MS was operated in electron ionization mode (EI) at 70 eV. Data were initially acquired in SCAN mode to determine the retention time of acrylamide. Subsequently, single ion monitoring (SIM) acquisition mode was used for the detection/quantitation of acrylamide (target ion: *71 m/z*, qualifier ion: *55 m/z*).

2.4 DLLME procedure for aqueous samples and food samples

To compare different incubation temperatures on the response of PCBs, 4.5 mL of ultrapure water containing 10 μg L^{-1} of each PCB congener was added to a 5 mL conical centrifuge tube. After gentle shaking, an aqueous solution containing 120 mg of [BMIM][Br] was added into the solution. The IL was completely dissolved into the sample solution by vortexing for 30 s. An aqueous solution of $LiNTf_2$ (0.2 g mL^{-1}) was then added to achieve an IL to $LiNTf_2$ molar ratio of 1:1. The sample solution immediately became cloudy due to the metathesis reaction and the formation of hydrophobic [BMIM][NTf_2] IL. The tube was then vortexed for 30 s and centrifuged for 5 min at 4500 rpm. Approximately 100 μL of the hydrophobic IL containing preconcentrated PCBs was formed at the bottom of the centrifuge tube. A 20 μL aliquot of [BMIM][NTf_2] IL was then withdrawn via micropipette and evenly transferred to four 10 mL headspace vials for HS-GC analysis at different incubation temperatures (i.e., 220 ˚C, 240 ˚C, 250 ˚C, and 260 ˚C).

For the comparison of extraction efficiencies using different ILs, a 5 mL conical centrifuge tube was filled with 4.5 mL of the PCB working solution at a concentration of 10 μg L^{-1}. After gentle shaking, an aqueous solution containing a specific amount of halide-based IL was added

into the solution (see Table S2, supplementary information). To ensure a fair comparison, the amount of each IL was calculated to yield 80 mg of the NTf_2^--based IL after the metathesis reaction. The IL was completely dissolved into the sample solution by vortexing for 30 s. An aqueous solution of $LiNTf_2$ (0.2 g mL^{-1}) was then added to achieve an IL to $LiNTf_2$ molar ratio of 1:1 or 1:1.5. The centrifuge tube was then vortexed for 30 s and centrifuged for 5 min at 4500 rpm. A 20 μL (12 μL for [BeEOHIM][NTf_2] IL) aliquot of the hydrophobic IL solvent was then withdrawn via micropipette and transferred to a 10 mL headspace vial for HS-GC analysis.

To prepare milk samples for analysis, the bovine milk sample was diluted with ultrapure water at a 1:1 v/v ratio. The *in situ* DLLME procedure shown in Figure 2 was applied to extract PCBs from the milk samples. After centrifugation, a very viscous sedimented IL solvent containing a white precipitate from the milk sample was formed on the bottom of the centrifuge tube. A 20 μL aliquot of the hydrophobic IL solvent was then withdrawn via micropipette and transferred to a 10 mL headspace vial for HS-GC analysis.

An improved *in situ* DLLME approach employing a washing step was also designed for the extraction of PCBs from milk samples. As shown in Fig. 2, after centrifugation and removal of the upper aqueous layer, 0.4 mL of ultrapure water was added to the sedimented IL layer. The mixture was then vortexed for 30 s and centrifuged for 5 min at 4500 rpm. A phase separation between the white precipitate and hydrophobic IL was immediately observed. All of the IL solvent was then withdrawn via micropipette and transferred to a 10 mL headspace vial for HS-GC analysis.

The *in situ* DLLME approach was also applied to extract acrylamide from ultrapure water

and brewed coffee. A 2 mL solution containing 1 mg L^{-1} of acrylamide was sampled using the previously employed conventional *in situ* DLLME method. The amount of halide-based IL and $LiNTf_2$ used is listed in Table S3 (supplementary information). Brewed coffee samples were prepared using a household American coffee maker from 35 g of ground coffee extracted with 600 mL of tap water. As mentioned previously, interfering acrylamide can be produced at high temperature (220 ˚C) from free asparagine and glucose extracted from the brewed coffee [10, 11]. A quenching reaction using ninhydrin was therefore applied to inhibit this reaction. Before analysis, a 19 mL aliquot of the brewed coffee was mixed with 1 mL of 2% (w/v) ethanolic ninhydrin solution and heated on a hot plate at 80 ˚C (with constant agitation at 1500 rpm) for 10 min [10, 11]. *In situ* DLLME sampling was performed immediately after the reaction (see Figure 2). A sample volume of 2 mL was selected for brewed coffee and the amount of IL-based extraction solvent and ion-exchange reagent used in this approach is listed in Table S3 (supplementary information).

3. Results and discussion

3.1 Structural design of IL solvents for *in situ* DLLME coupled to HS-GC analysis

Five imidazolium-based ILs with varied functional groups were prepared in this study to examine their selectivity towards PCBs and acrylamide. The [BMIM][Br] IL has been previously reported as an extraction solvent in *in situ* DLLME and was employed as a reference IL in this study [30, 40]. In an effort to further increase the hydrophobicity of the sedimented IL, an IL containing an octyl side chain was prepared ([OMIM][Br]). It has been previously reported that incorporation of aromatic moieties to the imidazolium-based polymeric ionic

liquids (PILs) can enhance the extraction efficiency of PCBs [9]. In order to further study this effect, the [BeBIM][Br] and [BeEOHIM][Br] ILs were synthesized. The [BeEOHIM][Br] and [HeOHMIM][Cl] ILs were also synthesized to examine the effect of aromatic and hydroxyl groups on the extraction efficiency of acrylamide.

3.2 ILs as solvents for HS-GC analysis after *in situ* DLLME sampling

The main aim of this work is to utilize ILs as the extraction solvent in *in situ* DLLME to provide preconcentration of the analytes and exploit their non-volatile nature in direct HS-GC analysis. In order to promote the partitioning of the analytes to the headspace, the ILs must be exposed to high HS oven temperatures without significantly increasing the chromatographic background. After the *in situ* metathesis reaction, the thermal stability of the four ILs (i.e., [BMIM][NTf_2], [OMIM][NTf_2], [BeBIM][NTf_2], and [BeEOHIM][NTf_2]) employed for PCB analysis was screened. Fig. S6 (supplementary information) shows the HS-GC-ECD chromatograms generated for all four ILs at an incubation temperature of 250 °C. The [BMIM][NTf_2] IL exhibited significantly lower background compared to the other ILs containing varied cationic moieties. Even though the other three ILs exhibited some impurity peaks in their background, no significant overlay of these peaks with the PCB peaks was observed (see Fig S6E, supplementary information).

The thermal stability of the [HeOHMIM][NTf_2] IL employed for acrylamide analysis was also examined. Due to the physico-chemical properties of acrylamide, an incubation temperature of 205 °C was applied. Enlarged HS-GC-MS chromatograms of [HeOHMIM][NTf_2] are shown in Fig. S7 (supplementary information). By comparing the

chromatograms of the [HeOHMIM][NTf_2] IL with and without sampling of acrylamide, no background interference with the acrylamide peak was observed. The aforementioned results confirm that the high thermal stability and low vapor pressure of the NTf_2^--based ILs make them appropriate solvents for the HS-GC analysis of PCBs and acrylamide after *in situ* DLLME sampling.

3.3 Effect of incubation temperature on the response of analytes

In HS-GC analysis, the incubation temperature of the HS oven plays a vital role in the response of the analytes. Theoretically, decreasing the incubation temperature can decrease the chromatographic background and potentially increase the response for highly volatile analytes. The effect of incubation temperature with respect to the response of 21 PCBs was evaluated by incubating 20 μL of [BMIM][NTf_2] IL after the extraction of PCBs at varied HS oven temperatures for 10 min. As shown in Fig. S8 (supplementary information), all PCBs show relatively low response at 220 ˚C. This is especially noticeable for the late eluting PCBs (i.e., PCB 170, 195, 206, and 209), which possess higher boiling points. When the HS oven temperature was increased to 240 ˚C, the response of all PCBs was dramatically increased. Interestingly, an analyte dependent variation in response was observed when the HS oven was increased from 240 ˚C to 260 ˚C. For early eluting PCBs (i.e., PCB 8, 18, 28, and 52), a slight decrease in response was observed when the HS oven temperature was increased from 240 ˚C to 260 ˚C. However, for PCBs that possess higher boiling points, the highest response was observed at 250 ˚C. This observation is in good agreement with previously reported results and can be attributed to the shifting in the equilibrium concentrations of the analytes between the

headspace and IL solvent at elevated temperatures [36]. Based on this result, 250 ˚C was selected as the optimized incubation temperature for all subsequent PCB analyses.

The effect of incubation temperature on the response of acrylamide was also evaluated at 185 ˚C, 205 ˚C, and 225 ˚C. As shown in Fig. S9 (supplementary information), a HS oven temperature of 205 ˚C exhibited slightly higher response compared to 185 ˚C and 225 ˚C. Based on this result, an optimized incubation temperature of 205 ˚C was selected for subsequent acrylamide analyses.

3.4 Comparison of extraction efficiencies using different ILs

The extraction efficiency of PCBs in ultrapure water using the [BMIM][Br], [OMIM][Br], [BeEOHIM][Br], and [BeBIM][Br] ILs was compared. To ensure a fair comparison, the amounts of each IL applied was calculated to yield 80 mg of the NTf_2^--based IL after the metathesis reaction. After centrifugation, a 20 μL aliquot of the hydrophobic IL was withdrawn for HS-GC analysis. However, due to the high solubility of the [BeEOHIM][NTf_2] IL in water, only 12 μL of the sedimented IL could be collected. As shown in Fig. 3, even though a lower amount of the sedimented IL could be recovered when applying the [BeEOHIM][Br] IL as extraction solvent, it still exhibited higher extraction efficiencies for all PCBs compared to the other ILs. The [BMIM][Br] IL also exhibited good extraction efficiencies for most PCBs, especially for the less volatile ones. The [OMIM][Br] and [BeBIM][Br] ILs were observed to produce lower extraction efficiencies for all PCBs. Based on these results, [BeEOHIM][Br] was selected as the optimal IL for all subsequent studies.

Three ILs containing aromatic and/or hydroxyl moieties (i.e., [BeBIM][Br],

[BeEOHIM][Br], and [HeOHMIM][Cl]) were employed for the extraction of acrylamide from ultrapure water. As shown in Fig. S10 (supplementary information), the [HeOHMIM][Cl] IL exhibited significantly higher extraction efficiency compared to the [BeBIM][Br] and [BeEOHIM][Br] ILs. Based on this result, [HeOHMIM][Cl] was selected as the optimal IL for all subsequent acrylamide analyses.

3.5 Optimization of extraction parameters

The ILs that exhibited superior extraction efficiency for PCBs and acrylamide were applied for the optimization of extraction parameters. Several important extraction parameters for *in situ* DLLME including the amounts of ion-exchange reagent and IL mass were optimized. The effect of each extraction parameter on the extraction efficiency was evaluated based on the peak areas of the analytes obtained using the applied method. The conditions that generated the highest peak areas were adopted for subsequent experiments.

3.5.1 Effect of the molar ratio of IL to metathesis reagent on extraction efficiency of analytes

Two different molar ratios of IL to $LiNTf_2$ metathesis reagent (i.e., 1:1 and 1:1.5) were examined to explore the effect of the amount of ion-exchange reagent on the extraction efficiency of the target analytes. As shown in Fig. 4 for the extraction of PCBs from ultrapure water, a 1:1 molar ratio of [BeEOHIM][Br]:$LiNTf_2$ exhibited significantly higher extraction efficiency compared to the extraction employing a molar ratio of 1:1.5. The same trend was also observed for the extraction of acrylamide using the [HeOHMIM][Cl] IL as extraction solvent (see Fig. S11, supplementary information). This is in good agreement with previously reported results [30, 40]. A possible reason for this result could be that the additional ion-

exchange reagent can increase the ionic strength of the sample matrix and affect the partitioning of the analytes into the IL solvent [30, 40]. Due to the better extraction performance that was obtained using a lower molar ratio of metathesis reagent, a IL:$LiNTf_2$ molar ratio of 1:1 was employed for all subsequent studies.

3.5.2 Effect of IL mass on extraction efficiency of analytes

The quantity of [BeEOHIM][Br] used as extraction solvent was varied to examine its effect on the extraction efficiency of PCBs. Three different quantities of [BeEOHIM][Br] IL, namely, 46 mg, 53 mg, and 60 mg, were tested to yield 12 μL, 20 μL, and 24 μL of sedimented ILs, respectively. In order to maximize the sensitivity of the method, all sedimented ILs were collected after *in situ* DLLME sampling. As shown in Fig. 5, higher peak areas for all PCBs were observed when the IL mass was increased from 46 mg to 53 mg. It was previously reported that higher enrichment factors for analytes could be obtained using a smaller volume of sedimented IL in *in situ* DLLME [40-42]. However, due to the significantly increased volume of sedimented IL (20 μL versus 12 μL), higher amounts of PCBs could be extracted using 53 mg of [BeEOHIM][Br] IL. As shown in Figure 5, an interesting trend was observed when the amount of [BeEOHIM][Br] IL was increased from 53 mg to 60 mg. Peak areas were slightly decreased for all PCBs, indicating that the increased volume of sedimented IL (24 μL versus 20 μL) could not compensate for the loss of enrichment. Based on this result, an IL quantity of 53 mg was employed for subsequent PCB analyses.

The effect of [HeOHMIM][Cl] IL mass on the extraction efficiency of acrylamide was also examined. As shown in Fig. S12 (supplementary information), the extraction efficiency of

acrylamide increased when the mass of IL was increased from 38 mg to 57 mg. However, when the mass of IL was increased from 57 mg to 76 mg, a slight decrease in extraction efficiency of acrylamide was observed. Based on this result, an IL mass of 57 mg was employed as the optimum condition for subsequent acrylamide analyses.

3.6 Effect of the headspace volume on the response of analytes

After *in situ* DLLME sampling, the IL containing the target analytes must be incubated at high HS oven temperature to permit the desorption of analytes to the headspace for subsequent GC separation and quantification. As discussed previously, the incubation temperature was optimized to increase the response of the analytes. Another important parameter that can determine the final concentration of the analytes in the HS is the phase ratio (β) within the HS system (see Eq. 1). According to Eq. 1, V_g is the volume of gas phase (headspace) and V_s is the volume of the sample phase (IL solvent). It has been reported previously that lower values of β will increase the concentration of volatile analytes in headspace and yield higher response [43]. In order to decrease β, two approaches can be applied, namely, an increase in sample volume and/or a decrease in headspace volume.

$$\beta = V_g/V_s \qquad (1)$$

An increase in the volume of the IL solvent (V_s) did not yield a significant increase in the response of the PCBs (see Section 3.4.2). Moreover, this also increases the amount of IL consumed and increases the cost of the analysis. Another approach is to decrease the headspace volume in the sample vial (V_g). As shown in Fig. S13 (supplementary information), in order to make the HS vial compatible with the HS autosampler, 12.5 g of glass beads (3 mm diameter) and a glass vial with a flat bottom were transferred into a 10 mL HS vial (the smallest

commercially available vials that are compatible with the Agilent HS sampler), resulting in a HS vial containing a headspace volume of 4.2 mL. As shown in Fig. 6, a comparison of the PCB response from the HS vials with and without the addition of glass beads showed that for the early eluting PCBs (i.e., PCB 8, 18, 28, 52, 44, 66, and 101), approximately 20-40% higher responses were observed when using the vials with smaller HS volume. It should be noted that most of the background interference in the GC chromatogram appears before 20 min (see Fig. S6, supplementary information). The increased response for early eluting PCBs could potentially increase the sensitivity of the HS-GC method. Only a few late eluting PCBs exhibited less than 10% loss in peak area, which could be due to adsorption of the analytes on the surface of the glass beads.

The same approach was also applied for the HS-GC analysis of acrylamide using the [HeOHMIM][NTf_2] IL. As shown in Fig. S14 (supplementary information), more than a 50 % increase in the peak area was observed when using the modified HS vials. Based on this result, a HS vial containing a headspace volume of 4.2 mL was employed for subsequent analyses.

3.7 Analytical performance of selected ILs for the extraction of PCBs and acrylamide from water and food samples

The analytical performance of the [BeEOHIM][Br] IL was evaluated by sampling aqueous solutions spiked with PCBs at different concentration levels. Table 1 shows the figures of merit based on a six-point calibration curve where the PCBs exhibited slightly different linear ranges. Good linearity with correlation coefficients (R^2) varying from 0.995 to 0.999 was obtained. The LODs were determined by decreasing the analyte concentration until a 3:1 signal:noise

(S/N) ratio was achieved. The LODs for the PCBs varied from 2.5 to 10 ng L^{-1}. The precision of the developed method was studied at 100 ng L^{-1} and 1000 ng L^{-1}. The relative standard deviation (%RSD) values ranged from 3.9% to 13.8% at 100 ng L^{-1} and from 4.3% to 15.0% at 1000 ng L^{-1}.

To demonstrate the applicability of the proposed method, the IL-based *in situ* DLLME method was employed for the extraction of PCBs from milk samples. Milk is a very complex sample matrix containing proteins, carbohydrates, and lipids [44]. All of these components can severely interfere with the IL solvent and affect the extraction of PCBs. Following *in situ* DLLME sampling of fat free milk spiked with 10 µg L^{-1} of PCBs, a very viscous mixture containing sedimented IL and precipitate from the milk sample was observed on the bottom of the centrifuge tube (see Fig. 2). It has been reported previously that the [BMIM][Cl] IL exhibited good extraction efficiency for proteins from complex samples [45]. In the *in situ* DLLME sampling process, the proteins may preconcentrate in the halide-based IL solvent and subsequently precipitate out after the *in situ* metathesis reaction. Due to its high viscosity, it was difficult to recover all of the sedimented IL solvent from the centrifuge tube. The collected sedimented IL solvent was subjected to HS-GC analysis and, as shown in Fig. 7A, very low response of the PCBs was observed. Moreover, the background in the chromatogram was significantly higher compared to the pure [BeEOHIM][NTf_2] IL (see Fig. S6D, supplementary information), which makes peak identification and integration very challenging. The *in situ* DLLME analysis using other ILs was also tested and as shown in Fig. 7B the [BMIM][Br] IL exhibited higher extraction efficiency for all PCBs compared to the [BeEOHIM][NTf_2] IL. In an effort to decrease the matrix interference and increase the response of the PCBs, an improved

approach was designed. As shown in Fig. 2, a washing step was applied after the conventional DLLME approach to purify the sedimented IL solvent. After centrifugation, a clear layer of the sedimented IL could be collected. As depicted in Fig. 7C, the improved approach resulted in a lower background and significantly enhanced the response for all PCBs compared to the DLLME approach without any purification.

Due to the varied water solubility of different ILs, the additional washing step can affect recovery of the sedimented IL. It was observed that when employing [BeEOHIM][Br] IL as the extraction solvent, only 5 μL of the sedimented IL could be collected. This result indicates that the [BeEOHIM][Br] may not be the optimal IL for milk samples when employing the improved DLLME approach. The extraction of PCBs from milk samples using different ILs at varied IL:$LiNTf_2$ molar ratios is shown in Fig. S15 (supplementary information). Among all tested ILs, the [BMIM][Br] IL exhibited the highest extraction efficiency for all PCBs. A very strong matrix effect was also observed when comparing the extraction efficiency of PCBs for aqueous samples and milk samples. For the extraction of PCBs from ultrapure water, late eluting PCBs (i.e., PCB 180, 170, and 195) exhibited higher response compared to early eluting PCBs (see Fig. 3). However, for the sampling of milk samples, decreased analyte response was observed, especially for the late eluting PCBs.

It is well known that due to their hydrophobic nature, PCBs will primarily partition into oil or fat rather than into water [46]. In order to study the effect of fat content on the extraction efficiency of the PCBs, the [BMIM][Br] IL was employed for the extraction of PCBs from milk samples containing varied amounts of fat. As shown in Fig. S16 (supplementary information), good extraction efficiency for all PCBs could be obtained for fat free milk samples. However,

when performing extractions on low fat and reduced fat milk, a significant decrease in extraction efficiency was observed. This may be due to the competitive partitioning of the PCBs between the IL solvent and the fat from the milk samples. As a result, fat free milk was selected as the real-world sample to evaluate the analytical performance of [BMIM][Br] IL in the extraction of PCBs. Table 2 shows figures of merit based on a six-point calibration curve where the PCBs exhibited slightly different linear ranges. Good linearity with correlation coefficients (R^2) varying from 0.996 to 0.999 was obtained. The LODs for the PCBs varied from 5 to 25 ng L^{-1}. The precision of the developed method was also studied at 100 ng L^{-1} and 1000 ng L^{-1} with relative standard deviation (%RSD) values ranging from 3.2% to 13.3% at 100 ng L^{-1} and from 5.3% to 12.6% at 1000 ng L^{-1}.

The analytical performance of [HeOHMIM][Cl] IL for the extraction of acrylamide was evaluated by sampling aqueous solutions containing different concentration levels of acrylamide. The results are summarized in Table S4 (supplementary information). The precision of the developed method was studied at 100 μg L^{-1} wherein a %RSD value of 3.6% was obtained. The linearity of the calibration curve was also studied based on a four-point calibration curve and excellent linearity with a correlation coefficient (R^2) of 0.999 was obtained.

The [HeOHMIM][Cl] IL was also applied for the quantification of acrylamide in brewed coffee. Quantitative analysis was carried out by the method of standard addition. A calibration curve was generated by sampling individual brewed coffee samples spiked with varying amounts of acrylamide. Table S4 (supplementary information) reports the figures of merits of the calibration curves with the extrapolated concentration of acrylamide. The extrapolated

concentration of acrylamide in the brewed coffee was determined to be 91.2 μg L^{-1}, which is in good agreement with our previously reported result using SPME coupled to GC-MS (i.e., 77 μg L^{-1}, 82 μg L^{-1}, and 73 μg L^{-1}, respectively, using different PIL-based SPME sorbent coatings) [14]. The precision of the developed method was studied at 100 μg L^{-1} and a %RSD value of 3.6% was obtained.

After performing the aforementioned experiments, the results demonstrated that the IL-based *in situ* DLLME approach developed in this study can be used for the detection and quantification of PCBs and acrylamide at trace levels in complex sample matrices. This technique also represents an alternative to the conventional SPME technique that often requires long sampling time and relative larger sample volumes. A comparison of the important features of the two techniques is shown in Fig. 8 and Table S5 (supplementary information). Compared to SPME, the IL-based *in situ* DLLME is more robust, which requires minimal sample pretreatment and can be applied for in-solution analysis of complex samples. The time required for sampling 20 samples further demonstrates that DLLME is a high-throughput and labor-saving technique compared to SPME. Moreover, even though the DLLME method consumes a small amount of IL in each extraction, it also eliminates the carryover effect and the need to consider the lifetime of the extraction device when performing large numbers of extractions in situations that demand high-throughput analysis.

4. Conclusions

Structurally-tuned ILs were synthesized and utilized as extraction solvents in *in situ* DLLME coupled to HS-GC-ECD/or MS for the trace level analysis of PCBs and acrylamide

in water, milk, and brewed coffee samples. The *in situ* DLLME approach showed good analytical precision, good linearity, and provided detection limits down to the low ppt level for PCBs and low ppb level for acrylamide in aqueous samples. The method also exhibited good matrix-compatibility with complex real-world samples. Good extraction efficiency was obtained using the [BMIM][Br] IL for the extraction of PCBs from milk samples. The quantification of acrylamide in brewed coffee was performed by the method of standard addition using the [HeOHMIM][Cl] IL as extraction solvent. Overall, *in situ* IL-based DLLME coupled to HS-GC exhibited fast sampling times, is capable of achieving high sample throughput, and represents a significant advantage over the conventional SPME method. Further studies will focus on applying structurally-tuned ILs for the analysis of trace level analytes in matrices with higher complexity such as volatile and semi-volatile metabolites from biological samples. Moreover, a fully automated IL-based *in situ* DLLME procedure will be explored to further increase the speed of sample analysis.

Acknowledgements

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Figure Captions:

Fig. 1. Chemical structures and abbreviations of the ILs employed in this study.

Fig. 2. Schematic diagram demonstrating the IL-based *in situ* DLLME method applied in this study for the extraction of PCBs from milk and acrylamide from brewed coffee.

Fig. 3. Extraction comparison for 21 PCBs extracted from ultrapure water using different ILs: (▒) [BMIM][Br], (■) [OMIM][Br], (▓) [BeEOHIM][Br], (▩) [BeBIM][Br]. See Table S1 for list of all PCB structures and corresponding numbers of PCBs. IL:$LiNTf_2$ = 1:1. Concentration of analytes: 10 μg L^{-1}. HS oven was operated at 250 ˚C and the equilibration time was 10 min. Error bars represent the standard deviation from experiments performed in triplicate.

Fig. 4. Effect of molar ratio of the [BeEOHIM][Br] IL and $LiNTf_2$ on the extraction efficiency (based on peak area) of PCBs. (■) [BeEOHIM][Br]:$LiNTf_2$=1:1, (▒) [BeEOHIM][Br]:$LiNTf_2$=1:1.5. See Table S1 for list of all PCB structures and corresponding numbers of PCBs. Concentration of analytes: 10 μg L^{-1}. HS oven was operated at 250 ˚C and the equilibration time was 10 min. Error bars represent the standard deviation from experiments performed in triplicate.

Fig. 5. Effect of IL mass on the extraction efficiency (based on peak area) of PCBs. (▒) 46 mg, (■) 53 mg, (▓) 60 mg. See Table S1 for list of all PCB structures and corresponding

numbers of PCBs. Concentration of analytes: 10 μg L^{-1}. All sedimented ILs were collected for HS-GC analysis. HS oven was operated at 250 ˚C and the equilibration time was 10 min. Error bars represent the standard deviation from experiments performed in triplicate.

Fig. 6. Effect of headspace volume on the response (based on peak area) of PCBs. (■) headspace vial containing 10 mL of headspace volume. (▨) modified headspace vial possessing a headspace volume of 4.2 mL. See Table S1 for list of all PCB structures and corresponding numbers of PCBs. Concentration of analytes: 10 μg L^{-1}. HS oven was operated at 250 ˚C and the equilibration time was 10 min. Error bars represent the standard deviation from experiments performed in triplicate.

Fig. 7. Chromatograms for HS-GC analysis of PCBs from milk. The conventional *in situ* DLLME approach was employed for the extraction of PCBs from milk. (A) The [BeEOHIM][Br] IL was employed as extraction solvent. (B) The [BMIM][Br] IL was employed as extraction solvent. (C) An improved approach using a washing step was applied to decrease the matrix effect with the [BMIM][Br] IL as extraction solvent. See Table S1 for list of all PCB structures and corresponding numbers of PCBs. Concentration of analytes: 10 μg L^{-1}. HS oven was operated at 250 ˚C and the equilibration time was 10 min.

Fig. 8. Features of IL-based *in situ* DLLME coupled to HS-GC and SPME couples to GC methods.

Fig. 1. Chemical structures and abbreviations of the ILs employed in this study.

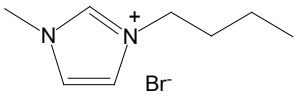
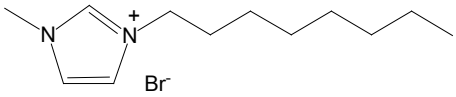
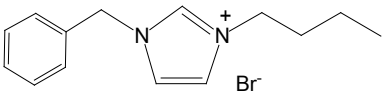
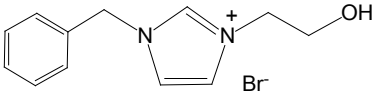
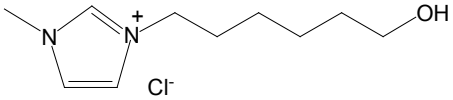
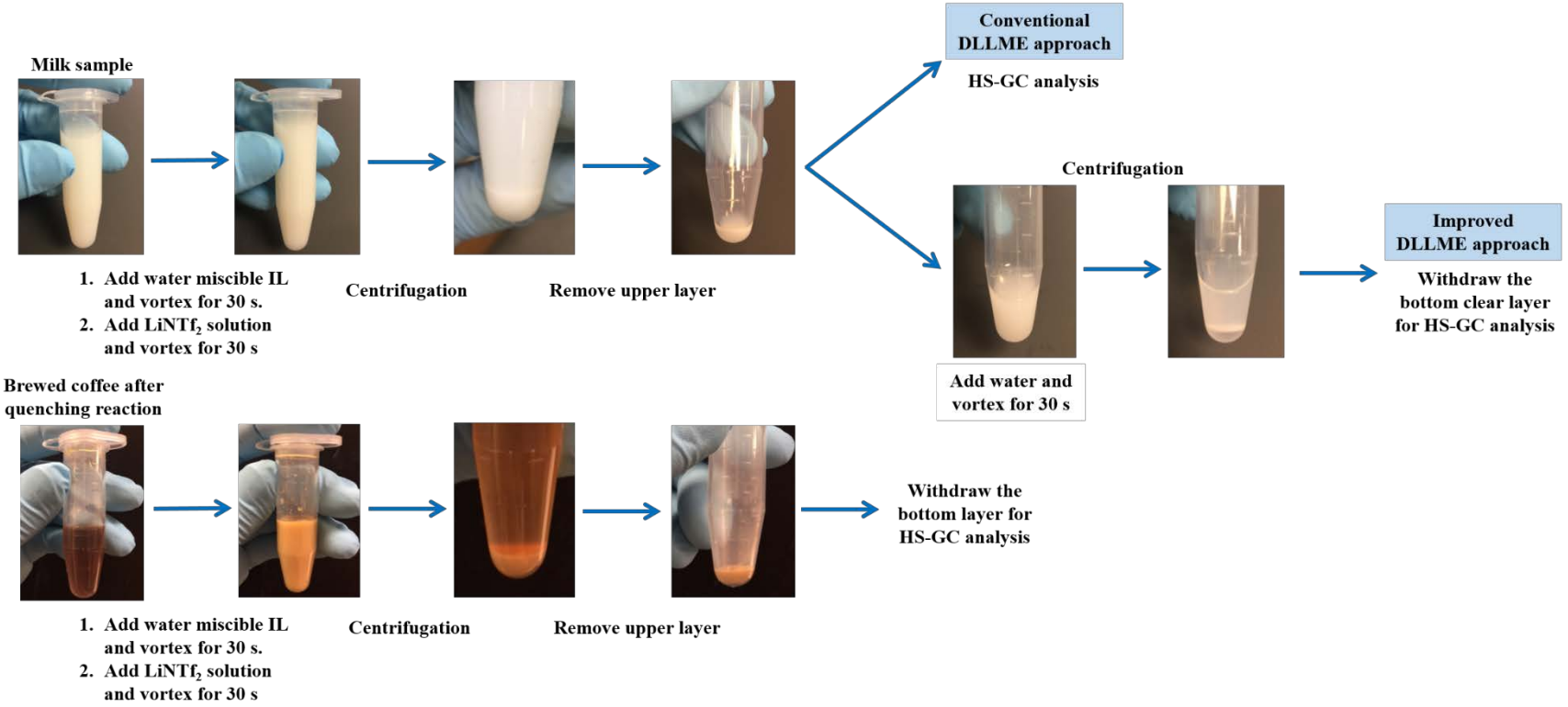
[BMIM][Br]	
[OMIM][Br]	
[BeBIM][Br]	
[BeEOHIM][Br]	
[HeOHMIM][Cl]	

Fig. 2. Schematic diagram demonstrating the IL-based *in situ* DLLME method applied in this study for the extraction of PCBs from milk.



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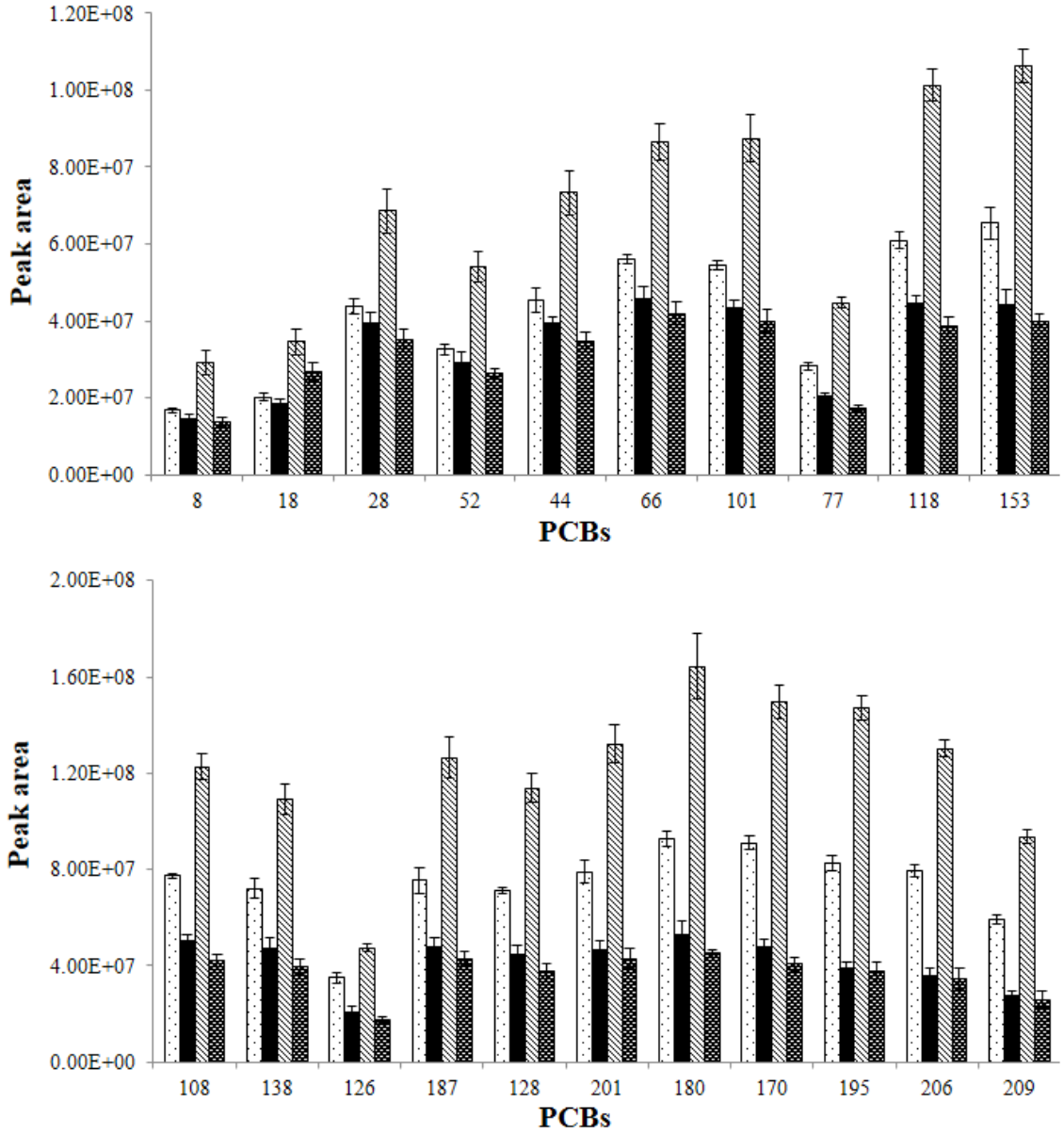

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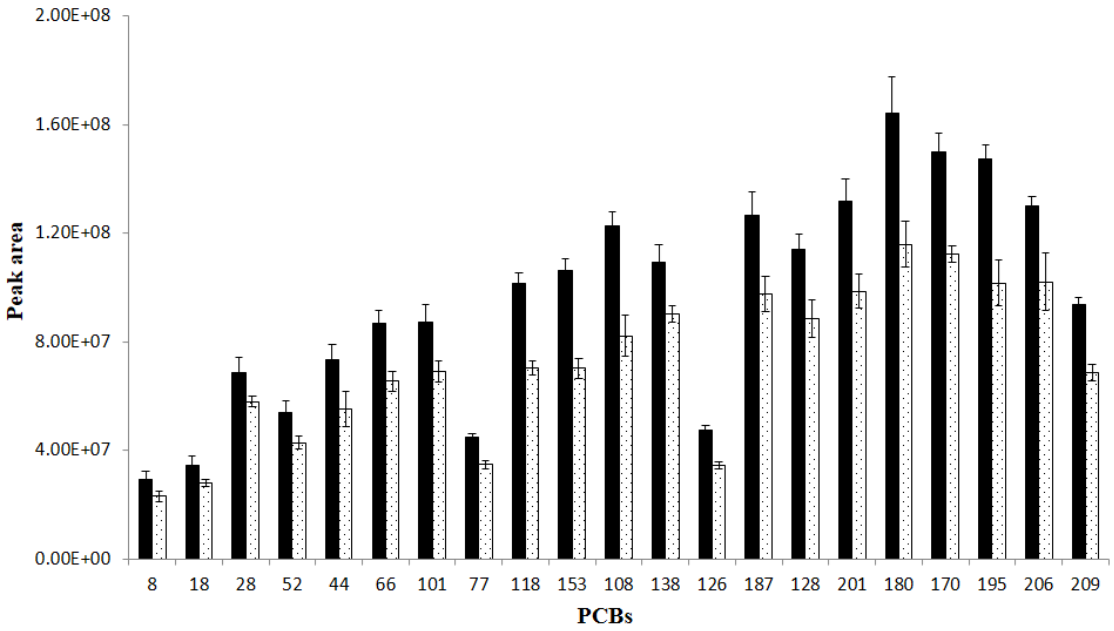

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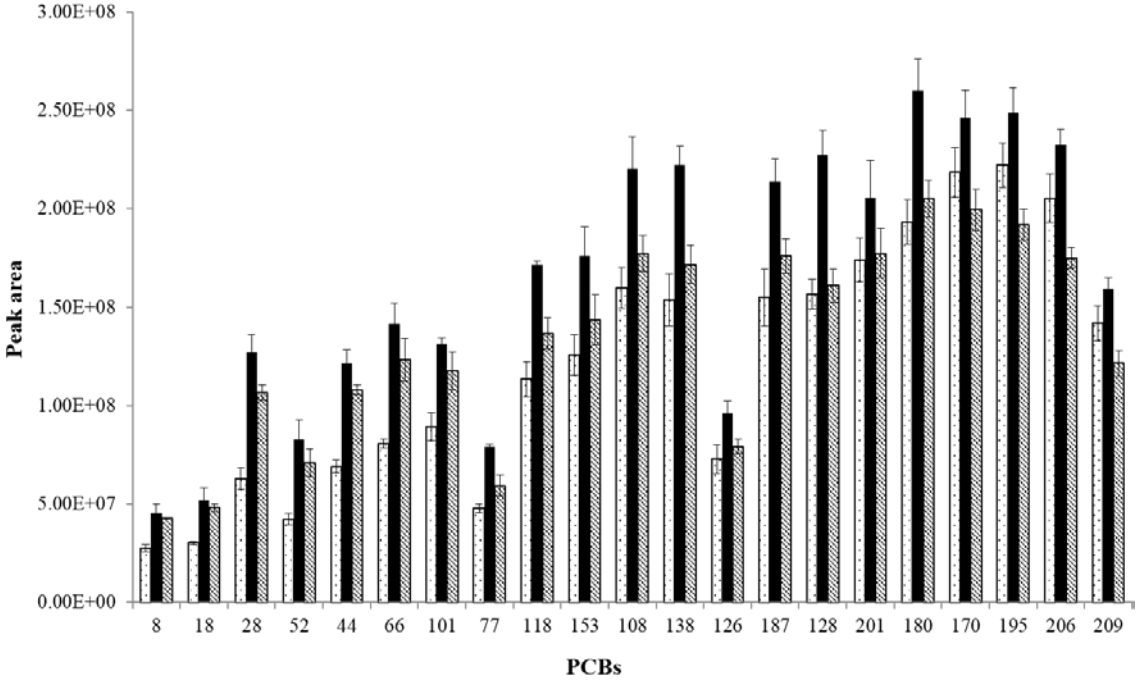

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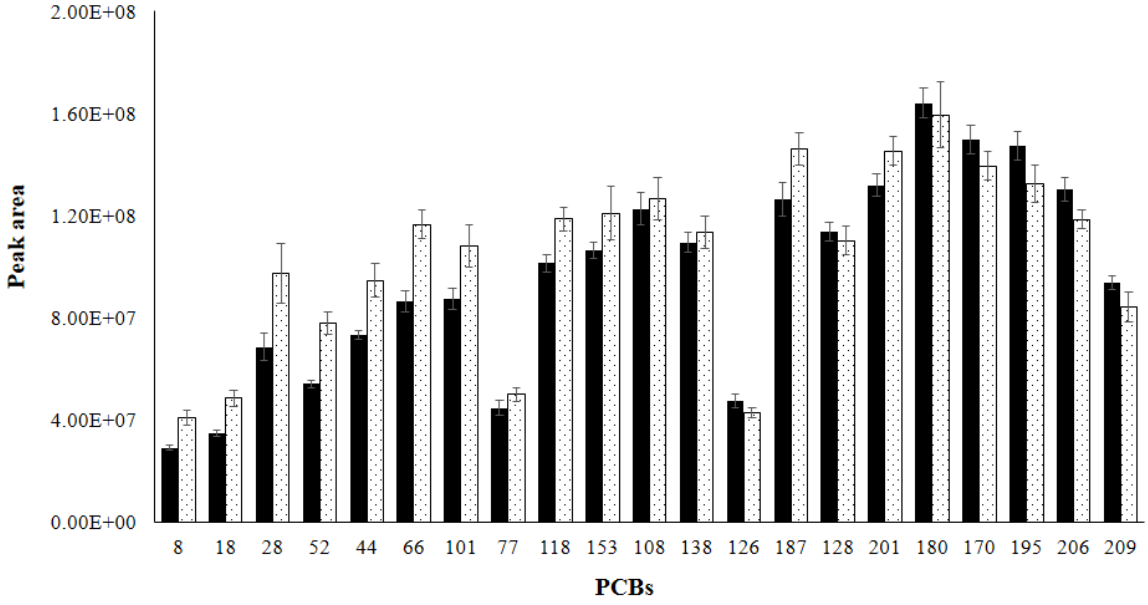

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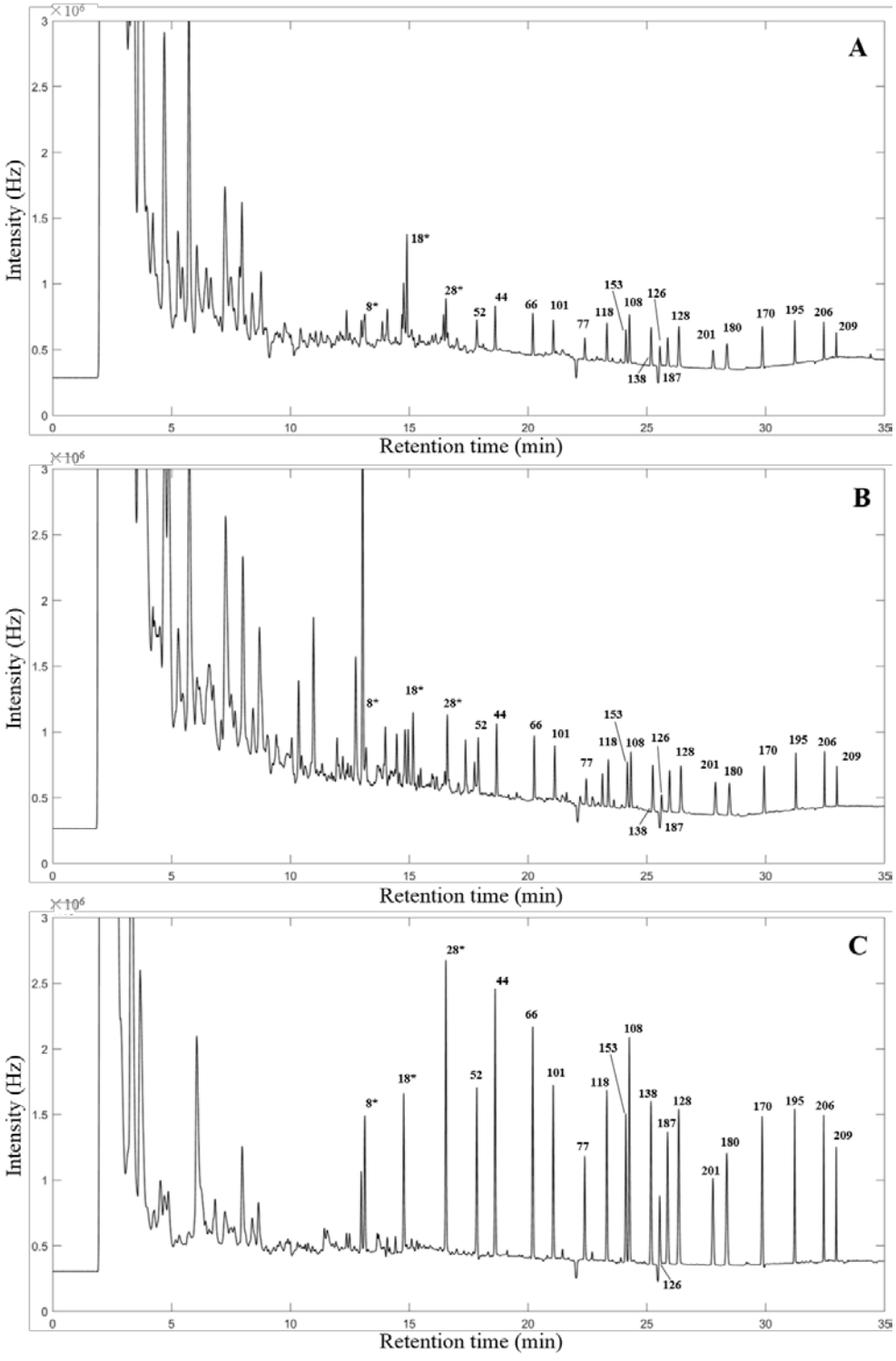
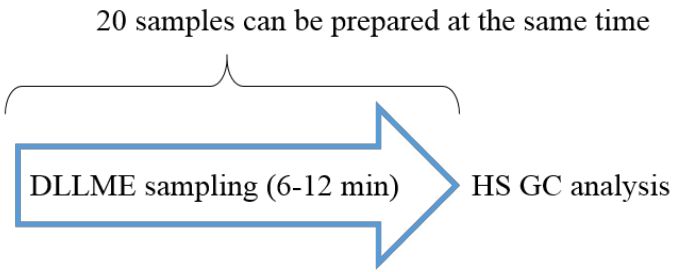
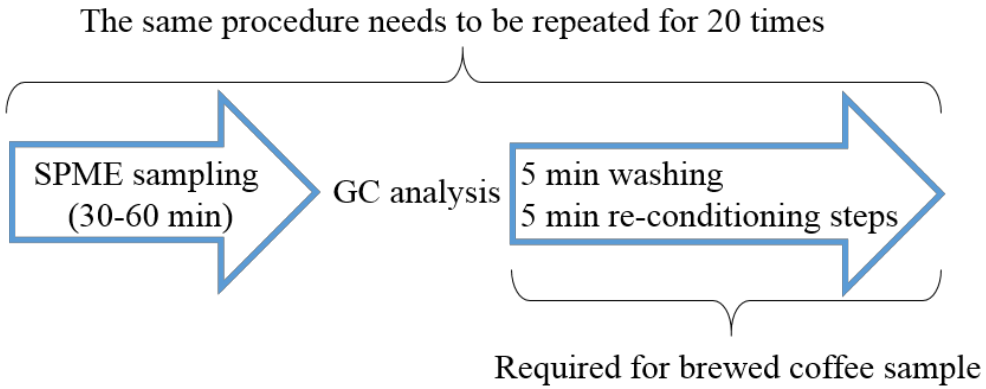


Fig. 8. Features of IL-based *in situ* DLLME coupled to HS-GC and SPME couples to GC methods

Sampling technique	Analytes/Sample matrix	Time required for sampling 20 samples	Reusability	Carryover effect
IL-based *in situ* DLLME coupled to HS-GC	PCBs/Milk Acrylamide/Brewed coffee		Consume ~50 mg of IL per extraction	No
SPME coupled to GC	PCBs/Milk [12] Acrylamide/Brewed coffee [13, 14]		~100 extractions (depending on coatings)	Needs to be considered for low volatility analytes

Supplementary Information

Rapid and sensitive analysis of polychlorinated biphenyls and acrylamide in food samples using ionic liquid-based *in situ* dispersive liquid-liquid microextraction coupled to headspace gas chromatography

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Synthesis of ILs

Synthesis of the [BMIM][Br] and [OMIM][Br] were performed by mixing 0.05 mol of 1-methylimidazole and 0.06 mol of alkyl halides (i.e., 1-bromobutane or 1-bromooctane) in 10 mL isopropanol and heating at 70 ˚C for 12 hrs. After removal of the solvent under reduced pressure, the product was then dissolved in 10 mL of water and washed with three 10 mL aliquots of ethyl acetate. The water layer containing the IL was recovered and dried under vacuum at 80 ˚C for 24 h.

Synthesis of the [BeBIM][Br] IL was performed by mixing 0.05 mol of 1-benzylimidazole and 0.06 mol of 1-bromobutane in 10 mL isopropanol and heating at 70 ˚C for 12 hrs. After removal of the solvent under reduced pressure, the product was then dissolved in 10 mL of water and washed with three 10 mL aliquots of ethyl acetate. The water layer containing the IL was recovered and dried under vacuum at 80 ˚C for 24 h.

Synthesis of the [BeEOHIM][Br] IL was carried out by mixing 0.05 mol of 1-benzylimidazole and 0.06 mol of 2-bromoethanol in 10 mL isopropanol and heating at 70 ˚C for 3 days. The [BeEOHIM][Br] was purified by following the same procedure as the [BMIM][Br]. After being dried under vacuum at 80 ˚C for 24 h, a 3 g aliquot of [BeEOHIM][Br] was dissolved in 1 mL of isopropanol and stored in a vial at 4 ˚C for 2 days. Following this storage process, clear crystals were formed on the bottom of the vial. The crystal layer was washed with 2 mL of cold isopropanol and dried under

vacuum at 80 ˚C for 12 h. The final product appeared as a viscous liquid with a faint yellow color.

Synthesis of the [HeOHMIM][Cl] IL was performed by mixing 0.05 mol of 1-methylimidazole and 0.06 mol of 6-chloro-1-hexanol in 10 mL isopropanol and heating at 70 ˚C for 3 days. After the removal of solvent under reduced pressure, the product was then dissolved in 10 mL of water and washed with three 10 mL aliquots of ethyl acetate. The water layer containing the IL was recovered and dried under vacuum at 80 ˚C for 24 h.

All final products were subsequently characterized by proton nuclear magnetic resonance spectroscopy (^{1}H NMR).

Figure S1. ^{1}H-NMR (500 MHz, Chloroform-*d*) spectrum of [BMIM][Br]: 10.46 (s, 1H), 7.47 (t, *J* = 1.8 Hz, 1H), 7.37 (t, *J* = 1.8 Hz, 1H), 4.32 (t, *J* = 7.4 Hz, 2H), 4.12 (s, 3H), 1.95-1.84 (m, 2H), 1.42-1.32 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H).

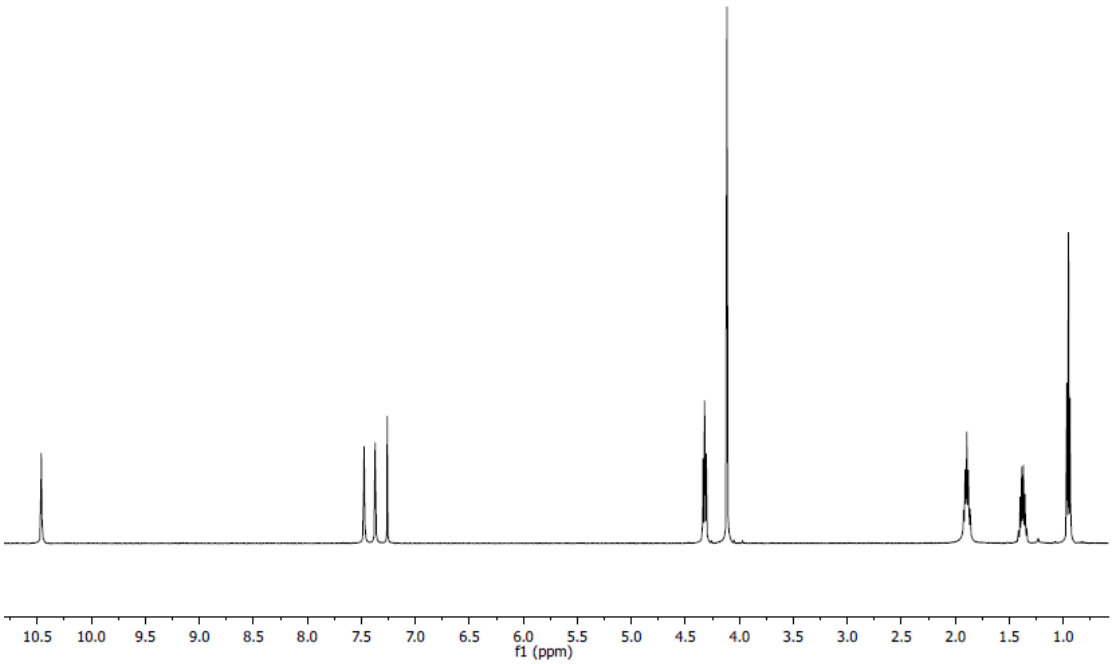

Figure S2. ^{1}H-NMR (500 MHz, Chloroform-*d*) spectrum of [OMIM][Br]: 10.45 (s, 1H), 7.46 (t, *J* = 1.8 Hz, 1H), 7.33 (t, *J* = 1.8 Hz, 1H), 4.30 (t, *J* = 7.5 Hz, 2H), 4.12 (s, 3H), 1.95-1.84 (m, 2H), 1.37 - 1.18 (m, 10H), 1.85 (t, *J* = 6.8 Hz, 3H).

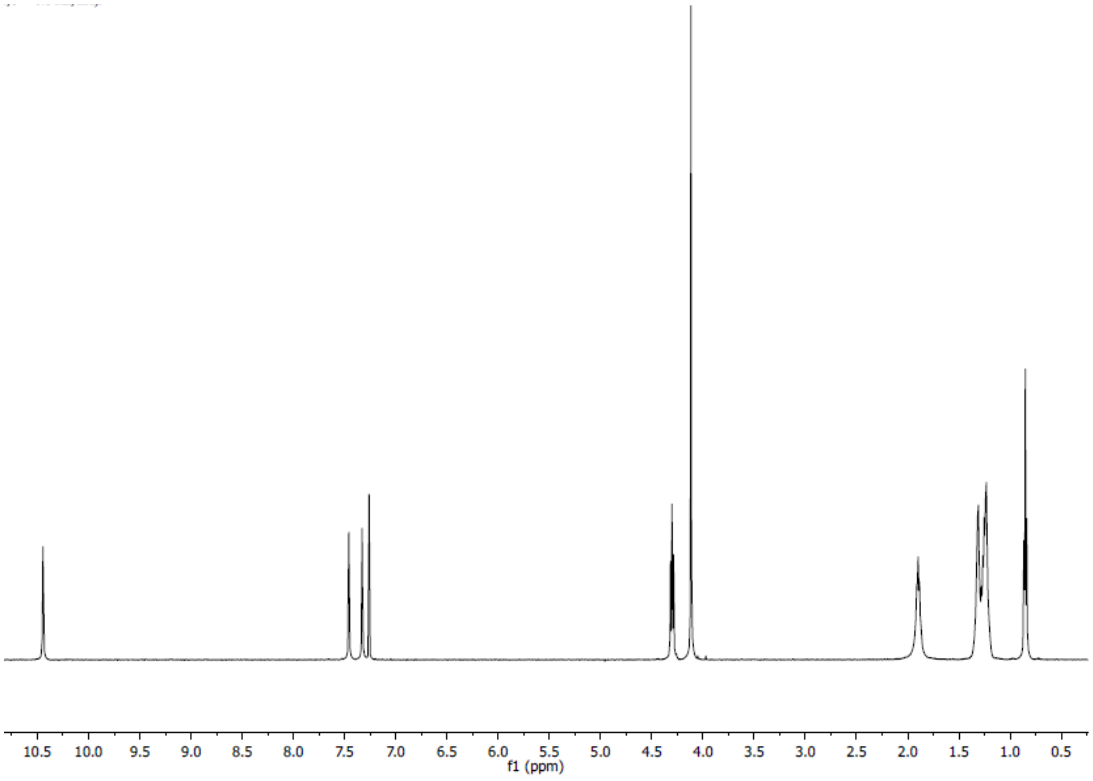

Figure S3. ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of [BeBIM][Br]: 9.35 (s, 1H), 7.83 (d, J = 1.7 Hz, 2H), 7.47-7.36 (m, 5H), 5.43 (s, 2H), 4.18 (t, J = 7.2 Hz, 2H), 1.83-1.72 (m, 2H), 1.31-1.20 (m, 2H), 0.9 (t, J = 7.4 Hz, 3H).

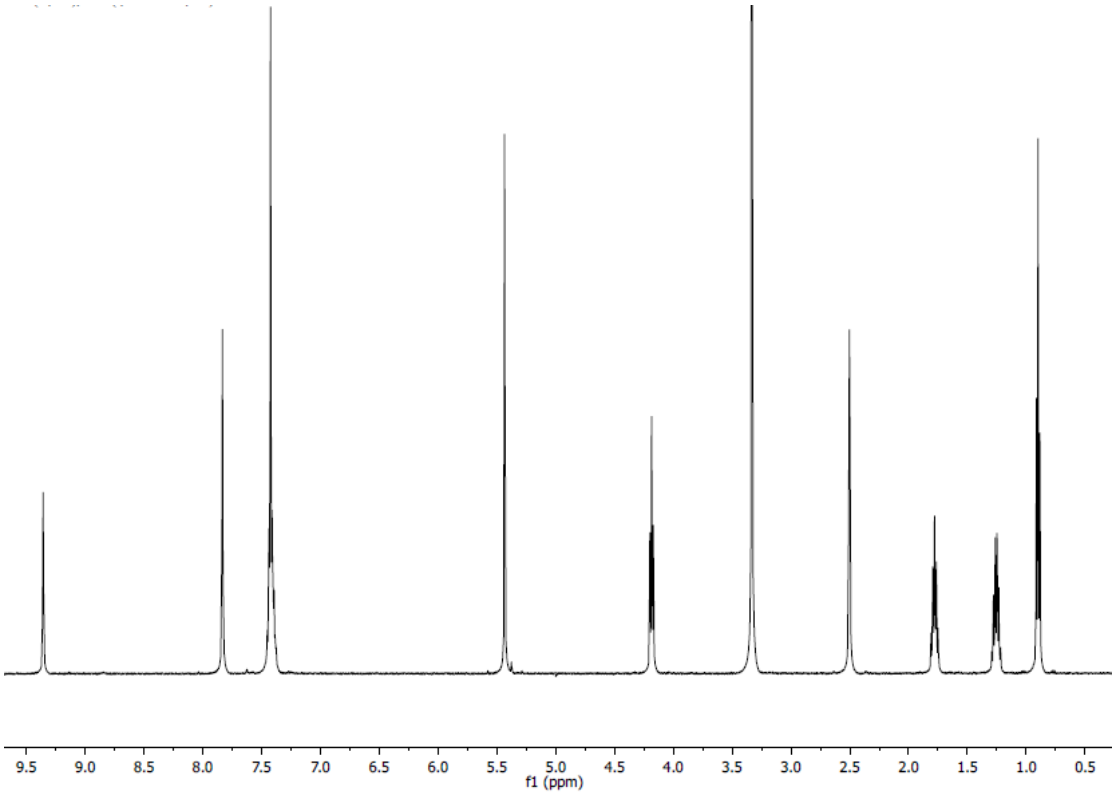

Figure S4. ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of [BeEOHIM][Br]: 9.31 (s, 1H), 7.82 (t, J = 1.8 Hz, 1H), 7.78 (t, J = 1.8 Hz, 1H), 7.46-7.37 (m, 5H), 5.45 (s, 2H), 5.20 (br. s., 1H), 4.24 (t, J = 5.0 Hz, 2H), 3.73 (t, J = 4.0 Hz, 2H).

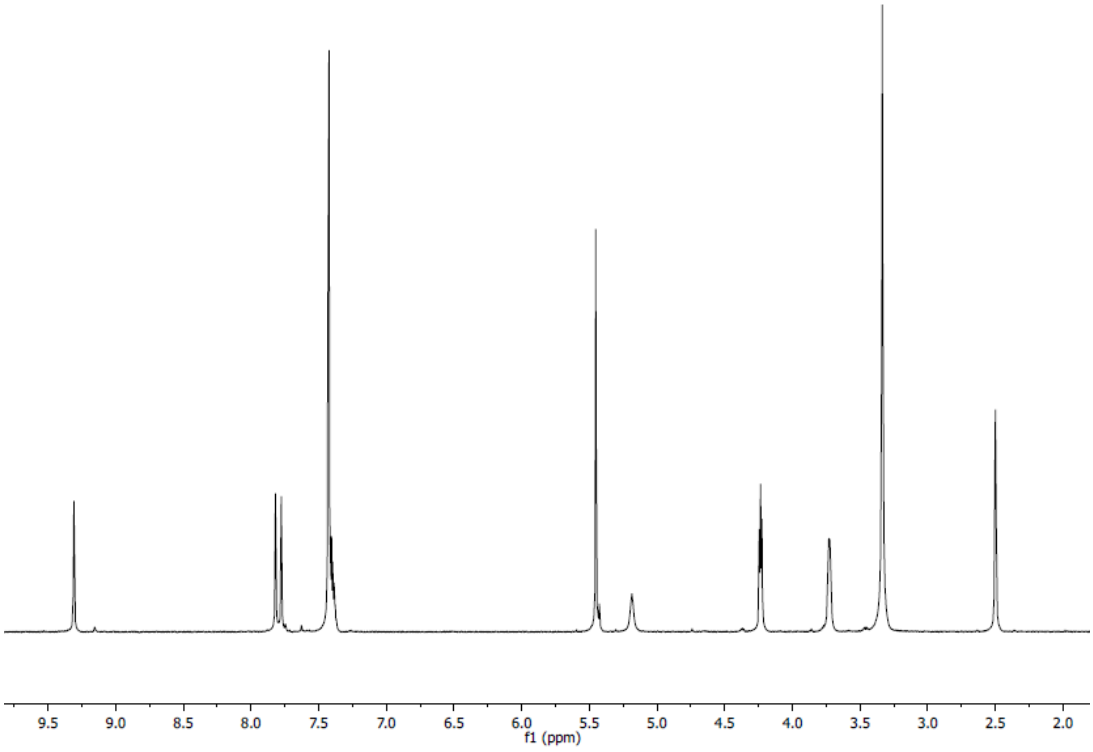

Figure S5. ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of [HeOHMIM][Cl]: 9.28 (s, 1H), 7.81 (t, J = 1.8 Hz, 1H), 7.73 (t, J = 1.8 Hz, 1H), 4.45 (br. s., 1H), 4.16 (t, J = 7.2 Hz, 2H), 3.86 (s, 3H), 3.36 (t, J = 5.7 Hz, 2H), 1.77 (td, J = 7.5, 14.8 Hz, 2H), 1.44 - 1.35 (m, 2H), 1.33 - 1.25 (m, 2H), 1.25 - 1.17 (m, 2H)

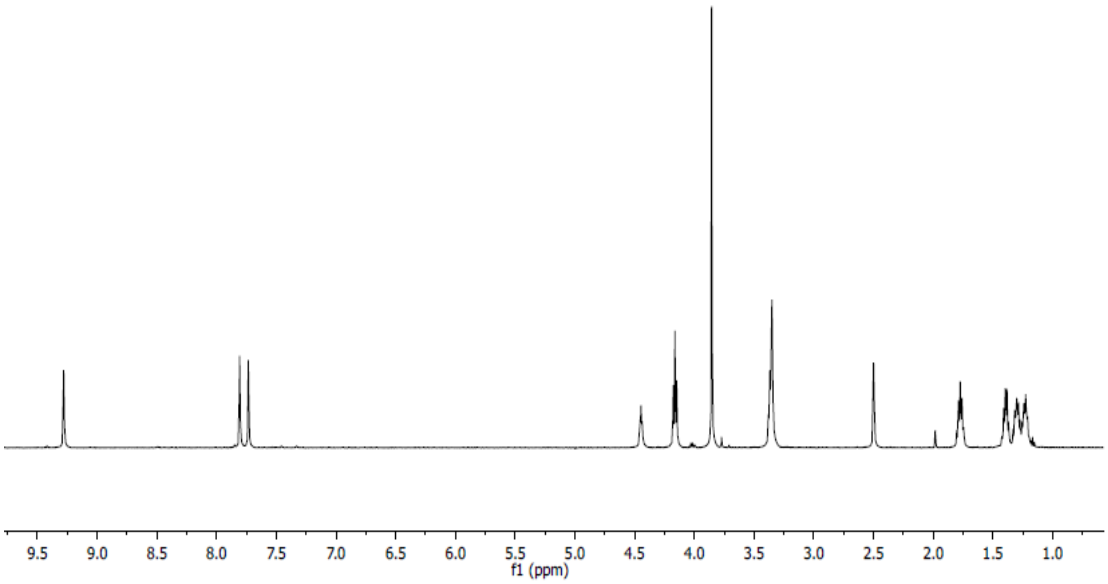

Figure S6. HS-GC-ECD chromatograms for ILs incubated at 250 ˚C for 10 min. (A) [BMIM][NTf_2], (B) [OMIM][NTf_2], (C) [BeBIM][NTf_2], (D) [BeEOHIM][NTf_2], and (E) direct injection of 21 PCBs

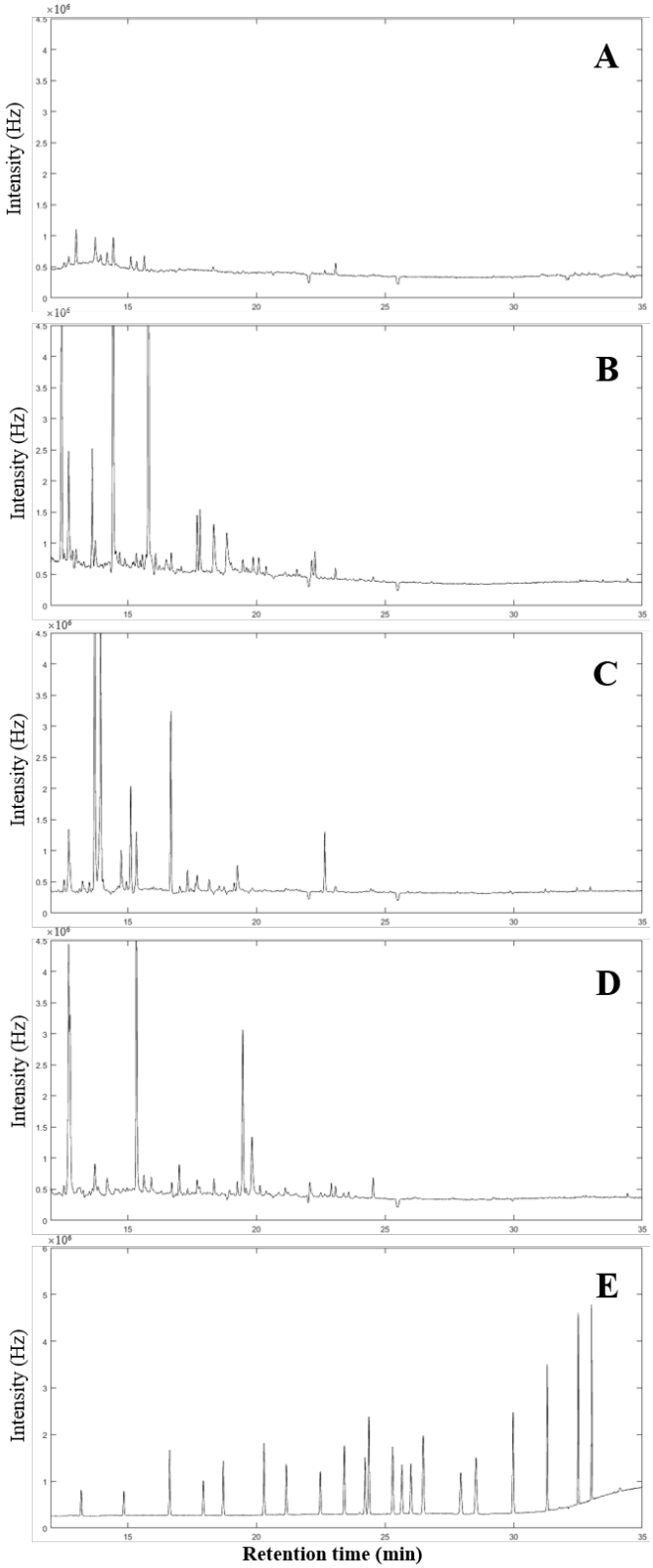

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Figure S7. (A) HS-GC-MS chromatogram for the [HeOHMIM][NTf_2] IL incubated at 205 ˚C for 10 min. (B) HS-GC chromatogram for the [HeOHMIM][NTf_2] IL incubated at 205 ˚C for 10 min after in situ DLLME sampling of ultrapure water containing 1 mg L^{-1} of acrylamide.

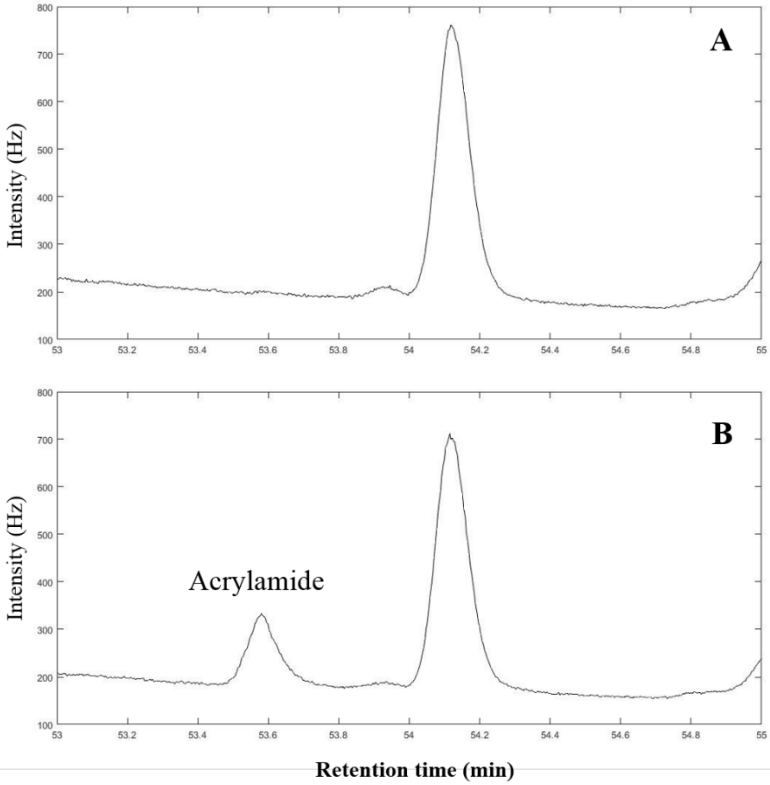


Figure S8. Incubation temperature effects on the response (peak area) of PCBs in the [BMIM][NTf_2] IL. (▒) 220 ˚C, (▒) 240 ˚C, (■) 250 ˚C, (▒) 260 ˚C. (See Table S1 for list of all PCB structures and corresponding numbers of PCBs). The equilibration time was 10 min.

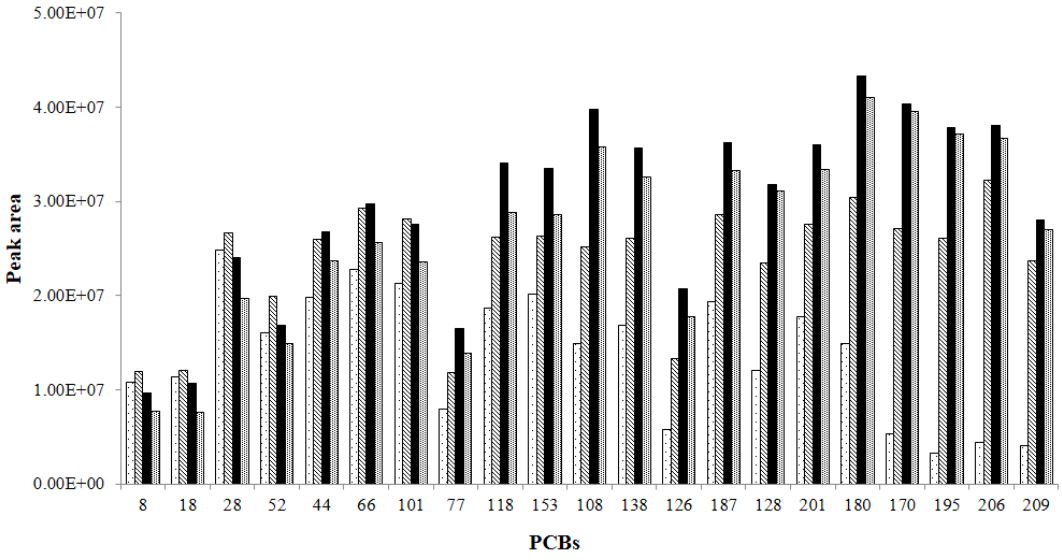


Figure S9. Effect of HS oven incubation temperature on the response (peak area) of acrylamide. The [HeOHMIM][Cl] IL was employed as extraction solvent. (⁘) 185 ˚C, (■) 205 ˚C, and (▨) 225 ˚C. The equilibration time was 10 min.

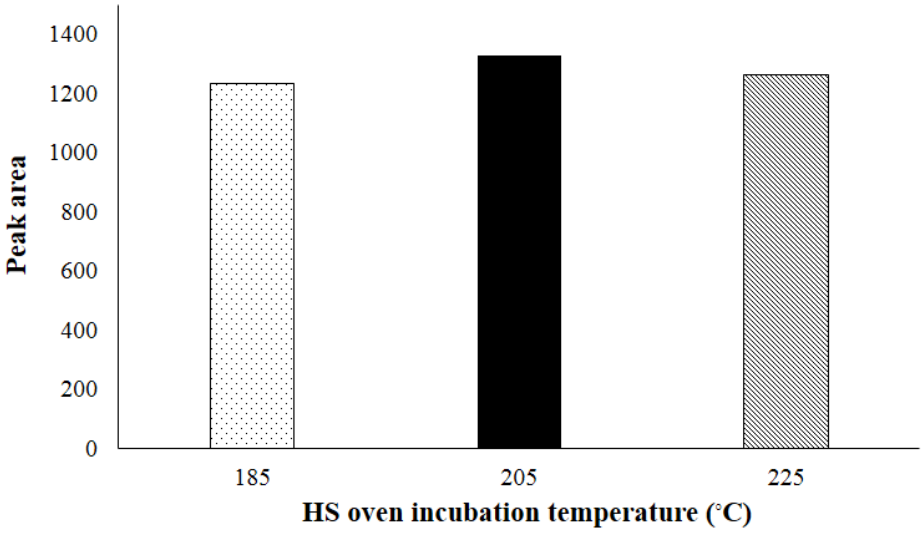


Figure S10. Comparison of extraction efficiency (peak area) of acrylamide from ultrapure water using different ILs: (▒) [BeBIM][Br], (■) [BeEOHIM][Br], and (▨) [HeOHMIM][Cl]. IL:$LiNTf_2$ = 1:1. Concentration of analyte: 1 mg L^{-1}. HS oven was operated at 205 ˚C and the equilibration time was 10 min. Error bars represent the standard deviation from experiments performed in triplicate.

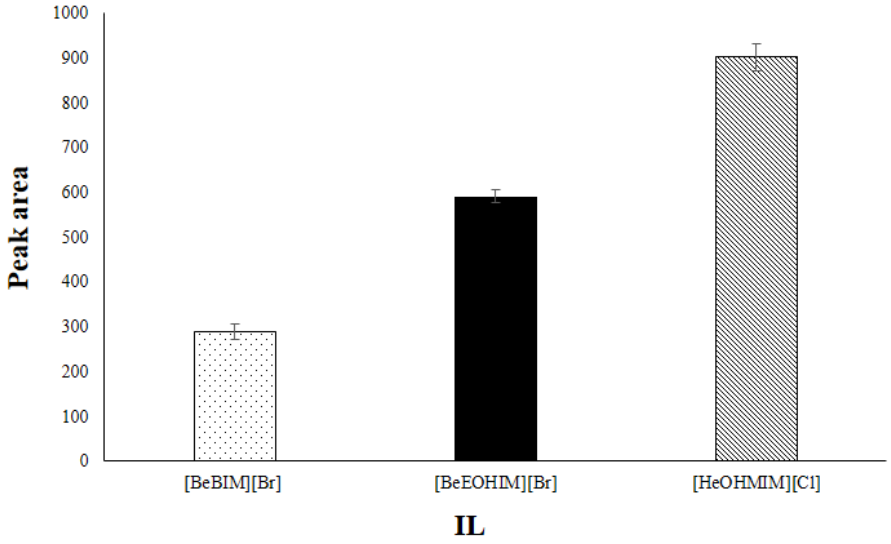


Figure S11. Effect of molar ratio of the [HeOHMIM][Cl] IL and $LiNTf_2$ on the extraction efficiency (peak area) of acrylamide. (▨) [HeOHMIM][Cl]:$LiNTf_2$=1:1, (■) [HeOHMIM][Cl]:$LiNTf_2$=1:1.5. Concentration of analyte: 1 mg L^{-1}. HS oven was operated at 205 ˚C and the equilibration time was 10 min.

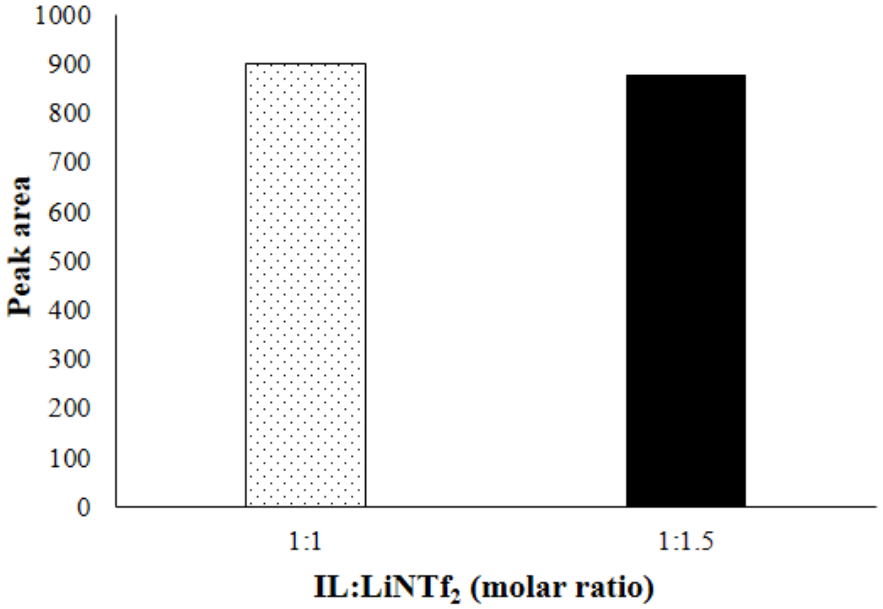


Fig. S12. Effect of the mass of the [HeOHMIM][Cl] IL on extraction efficiency (peak area) of acrylamide. (⬚) 38 mg, (■) 57 mg, (▨) 76 mg. Concentration of analyte: 1 mg L^{-1}. HS oven was operated at 205 ˚C and the equilibration time was 10 min.

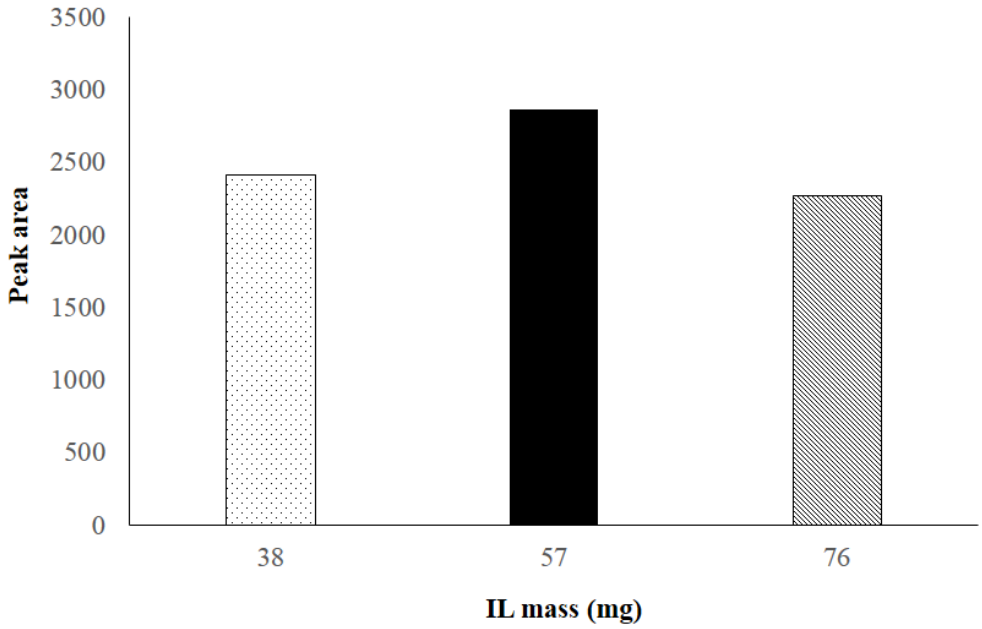


Figure S13. HS vials applied in this study with varied headspace volumes. (A) HS vial containing 10 mL of headspace volume. (B) After the addition of glass beads and glass vial, the headspace volume of the HS vial was decreased to 4.2 mL. Note: the 10 mL headspace vials are the smallest commercially available vials that are compatible with the Agilent 7697A headspace sampler.

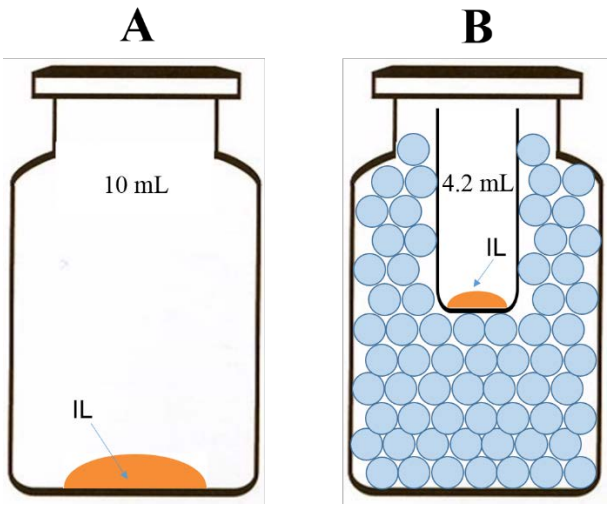


Figure S14. Effect of the headspace volume on the response (peak area) of acrylamide. (▒) HS vial containing 10 mL headspace volume. (■) headspace vial containing 4.2 mL headspace volume. Concentration of analyte: 1 mg L^{-1}. HS oven was operated at 205 ˚C and the equilibration time was 10 min.

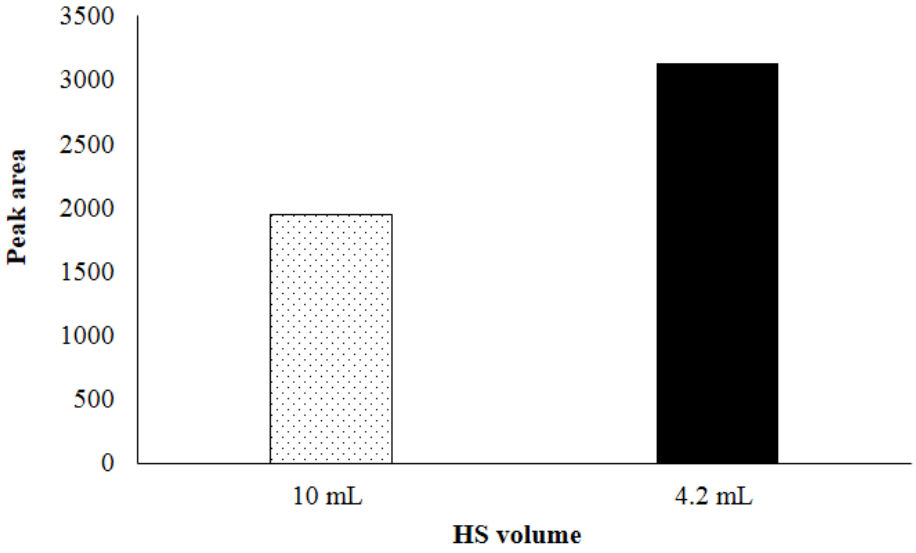


Figure S15. Comparison of extraction efficiency (peak area) of 21 PCBs from milk sample using different ILs: (▒) [BMIM][Br], IL:$LiNTf_2$ = 1:1, (■) [BMIM][Br], IL:$LiNTf_2$ = 1:1.5, (▒) [OMIM][Br], IL:$LiNTf_2$ = 1:1, (▒) [BeBIM][Br] IL:$LiNTf_2$ = 1:1, (▒) [BeEOHIM][Br]:$LiNTf_2$ = 1:1. See Table S1 for list of all PCB structures and corresponding numbers of PCBs. IL:$LiNTf_2$ = 1:1. Result obtained by *in situ* DLLME sampling of fat free milk containing 10 µg L^{-1} of PCBs. All sedimented ILs were collected for HS-GC analysis. HS oven was operated at 250 ˚C and the equilibration time was 10 min. Error bars represent the standard deviation from experiments performed in triplicate.

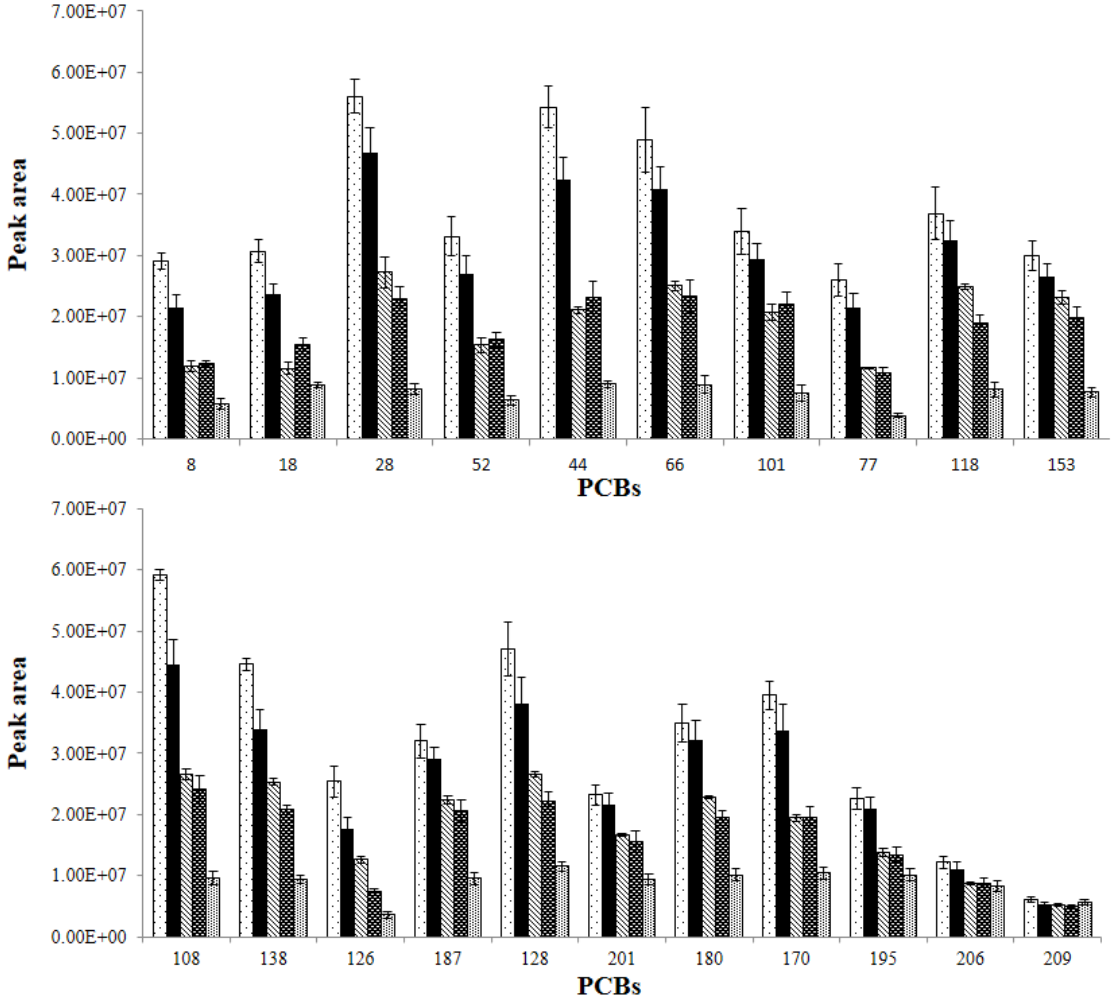


Figure S16. Comparison of extraction efficiency (peak area) of 21 PCBs from different milk samples using [BMIM][Br] IL: (⁘) fat free milk (containing 0% of fat), (■) low fat milk (containing 1% of fat), (▨) reduced fat milk (containing 2% of fat). See Table S1 for list of all PCB structures and corresponding numbers of PCBs. Result obtained by *in situ* DLLME sampling of milk samples containing 10 μg L^{-1} of PCBs. IL:$LiNTf_2$ = 1:1. All sedimented ILs were collected for HS-GC analysis. HS oven was operated at 250 ˚C and the equilibration time was 10 min. Error bars represent the standard deviation from experiments performed in triplicate.

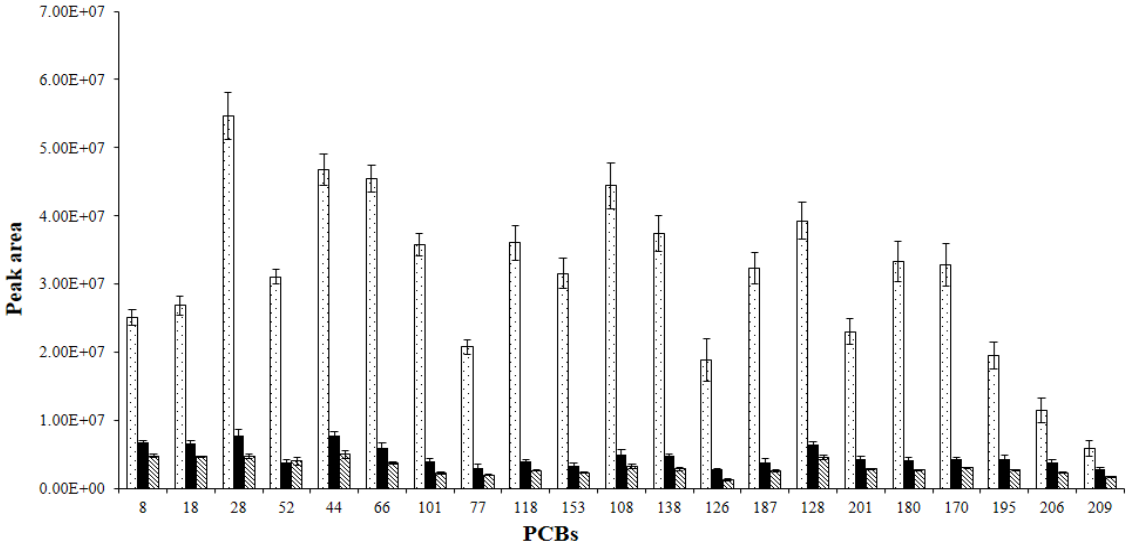
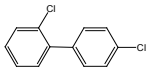
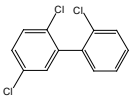
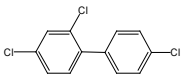
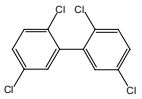
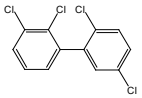
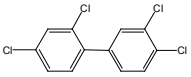
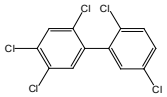
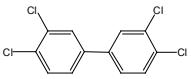
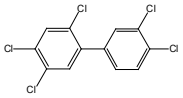
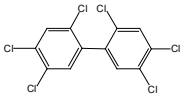
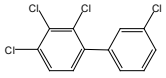
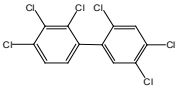
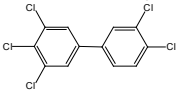
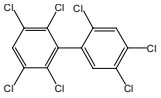


Table S1. Names and structures for all studied PCBs.

PCB	Name	Structure
8	2,4′-dichlorobiphenyl	
18	2,2′,5-trichlorobiphenyl	
28	2,4,4'-trichlorobiphenyl	
52	2,2′,5,5′-tetrachlorobiphenyl	
44	2,2′,3,5′-tetrachlorobiphenyl	
66	2,3′,4,4′-tetrachlorobiphenyl	
101	2,2′,4,5,5′-pentachlorobiphenyl	
77	3,3′,4,4′-tetrachlorobiphenyl	
118	2,3′,4,4′,5-pentachlorobiphenyl	
153	2,2′,4,4′,5,5′-hexachlorobiphenyl	
108	2,3,3′,4,4′-pentachlorobiphenyl	
138	2,2′,3,4,4′,5′-hexachlorobiphenyl	
126	3,3′,4,4′,5-pentachlorobiphenyl	
187	2,2′,3,4′,5,5′,6-heptachlorobiphenyl	

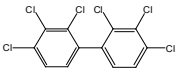
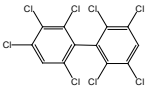
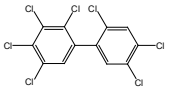
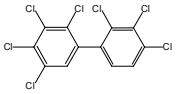
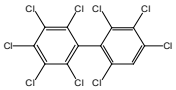
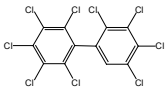
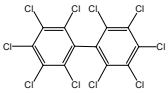
128	2,2′,3,3′,4,4′,-hexachlorobiphenyl	
201	2,2′,3,3′,4,5′,6,6′-octachlorobiphenyl	
180	2,2′,3,4,4′,5,5′-heptachlorobiphenyl	
170	2,2′,3,3′,4,4′,5-heptachlorobiphenyl	
195	2,2′,3,3′,4,4′,5,6-octachlorobiphenyl	
206	2,2′,3,3′,4,4′,5,5′,6-nonachlorobiphenyl	
209	2,2′,3,3′,4,4′,5,5′,6,6′-decachlorobiphenyl	

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Table S2. The volumes of IL and $LiNTf_2$ solution applied for *in situ* DLLME analysis of PCBs from ultrapure water.

IL applied for *in situ* DLLME	Volumes of the IL solution added (μL) [a]	Volumes of the $LiNTf_2$ solution added (μL) [b]	Theoretical yield of the NTf_2^--based ILs
[BMIM][Br]	127	273 [c] 409 [d]	80 mg
[OMIM][Br]	139	240 [c] 360 [d]	
[BeBIM][Br]	140	230 [c] 345 [d]	
[BeEOHIM][Br]	139	238 [c] 357 [d]	

[a]: The IL solution was prepared by dissolving 1.66 g of IL in 3.34 mL of ultrapure water.

[b]: The $LiNTf_2$ solution was prepared by dissolving 2 g of $LiNTf_2$ in 10 mL of ultrapure water.

[c]: Molar ratio of IL:$LiNTf^2$=1:1.

[d]: Molar ratio of IL:$LiNTf^2$=1:1.5.

Table S3. The volumes of IL and $LiNTf_2$ solution applied for *in situ* DLLME analysis of acrylamide from ultrapure water and brewed coffee.

Sample matrix	IL applied for DLLME	Volumes of the IL solution added (μL) [a]	Volumes of the $LiNTf_2$ solution added (μL) [b]	Theoretical yield of the NTf_2^--based ILs
Ultrapure water	[BeBIM][Br]	140	230	80 mg
	[BeEOHIM][Br]	139	238	
	[HeOHMIM][Cl]	112	245	
Brewed coffee	[HeOHMIM][Cl]	168	368	120 mg

[a]: The IL solution was prepared by dissolving 1.66 g of IL in 3.34 mL of ultrapure water.

[b]: The $LiNTf_2$ solution was prepared by dissolving 2 g of $LiNTf_2$ in 10 mL of ultrapure water.

Table S4. Figures of merit for [HeOHMIM][Cl] IL examined in this study for the *in situ* DLLME analysis of acrylamide in ultrapure water and brewed coffee.

Sample matrix	Linear range (μg L^{-1})	Slope ± error	LOD (μg L^{-1})	Linearity (R^2)	%RSD (n=3) 100 μg L^{-1}	Acrylamide amount
Ultrapure water	50-1000	2.93 ± 0.08	25	0.998	3.6	-
Brewed coffee	100-1000	3.09 ± 0.15	-	0.999	2.9	91.2 μg L^{-1}

Table S5. Features of IL-based *in situ* DLLME coupled to HS-GC and SPME couples to GC methods for the extraction of PCBs from milk samples.

Sampling techniques	Sample pretreatment	Extraction model	Sampling time
IL-based *in situ* DLLME coupled to HS-GC	Dilute the milk sample with ultrapure water at a 1:1 v/v ratio	In solution extraction	12 min
Saponification-SPME coupled to GC [1]	Saponification was performed at 100 ˚C for 6 min before extraction	Headspace extraction	60 min
SPME coupled to GC using polymeric ionic liquid-based sorbent coatings [2]	The milk sample needs to be diluted and incubated for 24 h, then NaCl was added to the sample and the sample was equilibrated at 65 ˚C	Headspace extraction	45 min
SPME coupled to GC [3]	The milk sample needs to be incubated for 24 h. 5% v/v of methanol and 36% m/v of NaCl were then added to the milk sample and the sample was equilibrated at 95 ˚C	Headspace extraction	60 min

References

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