Comparing the Extraction Performance of Cyclodextrin-containing Supramolecular Deep Eutectic Solvents Versus Conventional Deep Eutectic Solvents by Headspace Single Drop Microextraction

Muhammad Qamar Farooq,[a,b] Victoria R. Zeger,[a] Jared L. Anderson[a,b,]*

[a]Department of Chemistry, Iowa State University, Ames, Iowa 50011, USA
[b]Ames Laboratory-USDOE, Iowa State University, Ames, Iowa 50011, USA

Abstract

A headspace single drop microextraction (HS-SDME) method coupled with high performance liquid chromatography was developed to compare the extraction of eighteen aromatic organic pollutants from aqueous solutions using cyclodextrin-based supramolecular deep eutectic solvents (SUPRADESs) and alkylammonium halide-based conventional deep eutectic solvents (DESs). Different derivatives of beta-cyclodextrin (β-CD) were employed as hydrogen bond acceptors (HBA) in SUPRADESs and the extraction performance investigated. SUPRADES comprised of the 20 wt% native β-CD HBA provided the highest enrichment factors of analytes compared to SUPRADESs comprised of other derivatives of β-CD (random methylated β-cyclodextrin, heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin, and 2-hydroxypropyl β-cyclodextrin). In addition, native β-CD and its derivatives were dissolved in the neat DESs and their effect on the extraction of analytes examined. Dissolution of 20 wt% native β-CD in the choline chloride ($[Ch^+][Cl^-]$):2Urea DES resulted in a significant increase in the extraction efficiencies of target analytes compared to the neat $[Ch^+][Cl^-]$:2Urea DES. Under optimum conditions, the extraction method required a solvent microdroplet of 6.5 μL, 1000 rpm stir rate, 30% (w/v) salt concentration, and a temperature of 40 °C. The tetrabutylammonium chloride : 2 lactic acid DES resulted in the highest enrichment factors while the $[Ch^+][Cl^-]$:2Urea DES had the lowest for most of the analytes among the evaluated solvents. The method provided limits of detection (LODs) down to 35 μg L^{-1}. Furthermore, the developed method was applied for the analysis of spiked tap and lake water, where relative recoveries ranging from 83.7%–119.7% and relative standard deviations lower than 19.2% were achieved.

Keywords: Headspace single drop microextraction; Supramolecular deep eutectic solvents; Organic pollutants; High performance liquid chromatography; Cyclodextrins

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Corresponding Author:
Jared L. Anderson
Ames Laboratory-USDOE and Department of Chemistry
Iowa State University
1605 Gilman Hall
Ames, IA 50011
Tel.: +1 515-294-8356
E-mail address: andersoj@iastate.edu
ORCID: 0000-0001-6915-8752

1. Introduction

Sample pretreatment and preconcentration are crucial steps in the chemical analysis of complex matrices. These steps often represent a bottleneck in the development of analytical methods capable of determining analytes at ultra-trace levels. Conventional sample preparation techniques such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are tedious, labor intensive, and can require large amounts of toxic organic solvents [1]. To overcome the shortcomings of traditional sample preparation approaches, various microextraction methods in which the volume of extraction phase is small compared to that of the sample have been developed. Current trends in the microextraction field are focused on miniaturization, simplification of sample preparation steps, minimization of organic solvents, and developing faster methods that provide high enrichment factors and selectivity [2], [3]. Over the last few decades, microextraction techniques such as solid-phase microextraction (SPME) [4], dispersive liquid-liquid microextraction (DLLME) [5], hollow fiber liquid-phase microextraction (HF-LPME) [6], and single drop microextraction (SDME) [7] have been introduced to overcome many limitations of traditional extraction procedures.

SDME, introduced by Liu and Dasgupta in 1996 [7], is an efficient microextraction technique that utilizes a solvent microdroplet for the preconcentration of analytes. SDME can be operated in either direct-immersion (DI) [8] or headspace (HS) modes [9]. DI-SDME enables the preconcentration of non-volatile compounds by directly immersing the microdroplet in the sample solution, while HS-SDME is used for the extraction of semi-volatile and volatile compounds by exposing the microdroplet to the sample’s headspace [10]. HS-SDME is an efficient and cost-effective technique that avoids the influence of complex matrices [11], while also providing low analyte carryover, being simple in operation, and having low extraction solvent consumption [11].

However, the technique has important shortcomings, such as limited microdroplet surface area and volume, as well as volatility of the microdroplet [12]. These issues are related to both the small surface area of the microsyringe needle tip and loss of the microdroplet due to volatilization, especially when exposing the solvent over long periods of time at elevated temperatures in the HS-SDME mode [13].

An ideal HS-SDME solvent should possess the following features: (1) high boiling point and low vapor pressure, (2) high viscosity to stabilize the microdroplet, (3) capability of solubilizing target analytes, and (4) low toxicity. Most of the commonly used organic solvents for HS-SDME, such as toluene, hexane, decane, and n-octyl alcohol, are not environmentally green and do not have a negligible vapor pressure, resulting in loss of the microdroplet by evaporation during the extraction [14]. Ionic liquids (ILs) and deep eutectic solvents (DESs) possess a number of attractive properties as HS-SDME solvents. ILs are non-molecular ionic compounds that possess melting points lower than 100 °C. Their structural tunability, negligible vapor pressure, and high thermal stability has made them attractive solvent systems for various applications [15]. DESs, introduced by Abbott et al., are prepared by mixing a hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) to yield a homogenous mixture possessing lower melting points than both of the individual components [16]. The physico-chemical properties of DESs can be easily tuned by changing either the HBA, HBD, or their relative molar ratio [17]. In addition to retaining many of the same properties as ILs, DESs provide additional advantages such as low cost, easy preparation, low toxicity, and higher biodegradability than ILs [18].

DESs have been employed as extraction solvents in a number of microextraction techniques, such as DLLME [19], SPME [20], microwave-assisted extraction [21], and ultrasound-assisted extraction [22]. The employed DESs are generally hydrophilic making them unsuitable for extractions that require the solvent to be directly immersed in aqueous samples [23]. Most DESs typically exhibit very short retention times in reverse phase liquid chromatography due to their polar nature and also do not typically interfere with the separation of target analytes that may elute at higher retention times [24]. Until now, only a few studies have employed DESs in HS-SDME for the extraction of polycylic aromatic hydrocarbons (PAHs) [25], terpenes [26], volatile aromatic hydrocarbons [27], pesticides [28], and bioactive compounds [29].

Cyclodextrins (CDs) are a class of cyclic oligosaccharides typically comprised of six (α-CD), seven (β-CD) or eight (γ-CD) glucopyranose units possessing an α-(1-4) linkage [30]. Their hydrophobic internal cavity and hydrophilic external surface makes them suitable for non-covalent guest-host interactions with hydrophobic and volatile compounds [30], [31]. CDs have attracted interest in a wide range of applications and have been used as excipients in drug delivery [32], pharmaceutical solubilizers for various drugs [33], catalysts for organic reactions [34], and chiral selectors in separations [35]. Recent studies have explored the possibility of combining the properties of CDs and DESs, either by dissolving CDs in DESs [36], [37], or by preparing supramolecular deep eutectic solvents (SUPRADESs) where the CD is used as the HBA and combined with carboxylic acid-based HBDs [38], [39]. It has been previously reported that the solubility of CDs can be significantly enhanced in DESs compared to water. For example, native β-CD can be dissolved as high as 50 wt% in a DES comprised of choline chloride : 2 urea ($[Ch^+][Cl^-]$:2Urea) compared to only 1.8 wt% in water [36]. In another study, the addition of native β-CD to a DES comprised of the ammonium acetate HBA and lactic acid HBD increased the extraction efficiency of polyphenols by approximately 21% compared to the neat DES [40]. Moreover, SUPRADESs prepared by mixing various β-CD derivative as HBAs with urea derivative as HBDs (N-methylurea and N,N’-dimethylurea) have been employed as reaction media for various organic reactions such as the hydroformylation [41], Tsuji–Trost reactions [42], and Suzuki and Heck coupling [43]. However, melting points greater than 80 °C limit their scope in other applications besides catalysis [38]. Recently, Fourmentin and co-workers have reported the formation of room temperature liquid SUPRADES with the random methylated β-cyclodextrin (RAMEB) HBA and levulinic acid HBD [38]. A 1300-fold increase in solubility of trans-anethole was observed in the RAMEB:Levulinic acid SUPRADES compared to water [38]. These CD-based SUPRADESs not only retain the physico-chemical properties of DESs but also possess the capability of forming guest-host inclusion complexes [38], [39]. Until now, no study has reported the use of cyclodextrin-containing SUPRADESs in the extraction of organic pollutants.

This study evaluates the performance of SUPRADESs comprised of native β-CD and its derivatives (RAMEB, heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD), and 2-hydroxypropyl β-cyclodextrin (HP-β-CD)) as HBAs and the L-lactic acid (LcA) HBD as extraction solvents in HS-SDME. Native β-CD and its derivatives are employed as additives to the $[Ch^+][Cl^-]$:2Urea and $[N_{4444}^+][Cl^-]$:2LcA DESs and their extraction performance compared to the analogous neat DESs. Neither SUPRADESs or the DESs containing the native β-CD and its derivatives as additives, have been employed as solvents for HS-SDME. The 1.7% (w/v) β-CD in aqueous solution (previously reported for HS-SDME of PAHs [44]) has also been examined and a comparison carried out with SUPRADES and DESs. The effect of various experimental parameters including microdroplet volume, stir rate, salt concentration, extraction temperature, and extraction time have been evaluated and optimized. The analytical performance of the solvents was evaluated through the analysis of real matrices including spiked tap water and lake water. This study provides an efficient method for the extraction of organic pollutants at ultra-trace levels using HS-SDME and also offers insight towards the use of CDs and their derivatives in greener classes of microextraction solvents.

2. Experimental

2.1 Materials

Tetrabutylammonium chloride ([N_{4444}^+][Cl^-], >97%), [Ch^+][Cl^-] (≥99%), urea (99%), TM-β-CD (>90%), HP-β-CD (>98%), and acetonitrile (≥99.9%) were obtained from MilliporeSigma (St. Louis, MO). LcA (98%) was purchased from Alfa Aesar (Ward Hill, MA). Native β-CD (98%) was purchased from Acros Organics (Morris Plains, NJ). RAMEB (95%) was obtained from Tokyo Chemical Industry (Portland, OR).

Eighteen different organic pollutants including UV-filters, PAHs, alkylphenols, plasticizers, and food ingredients were analyzed. The analytes acetophenone (AP, 99.0%), 2-chloroaniline (2-Cla, >98.0%), benzophenone (BP, 99.0%), 2-nitronaphthalene (2-NiNap, 85.0%), biphenyl (BiPh, 99.5%), 3-tert-butylphenol (3-tBP, 94%), 2-ethylhexyl salicylate (ES, ≥99.0%), and 1-chloro-4-nitrobenzene (CNB, 99.0%) were purchased from MilliporeSigma (St. Louis, MO). The analyte 2-bromo-4-fluorobenzaldehyde (BFB, 99.0%) was purchased from Oakwood Products, Inc. (West Columbia, SC). Naphthalene (Nap), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), and pyrene (Py), all possessing purities greater than 96.0%, and 20 mL glass vials capped with screw cap and PTFE septa were purchased from Supelco (Bellefonte, PA). The analytes 4-octylphenol (4-OP, 99%), 2-nitrophenol (2-NiPh, 99%), and ethyl benzoate (EB, 99%) were acquired from Alfa Aesar (Ward Hill, MA), Acros Organics (Morris Plains, NJ), and J. T. Baker (Phillipsburg, NJ), respectively. Dipentyl phthalate (DPP, >99.0%) was obtained from AccuStandard (New Haven, CT). Sodium chloride was purchased from Fisher Scientific (Fair Lawn, NJ). A 25 μL microsyringe gas tight syringe with a flat-cut needle tip used to perform extractions was purchased from Hamilton (Reno, NV). Ultrapure water (18.2 MΩ cm) was produced by a Milli-Q water filtration system (Millipore, Bedford, MA). Lake water was obtained from Ada Hayden Lake (Ames, IA). Acronyms of all chemicals are also provided in Table S1.

Pure analytes were dissolved in HPLC grade acetonitrile to prepare stock standard solutions with concentration values ranging from 1,000 to 20,000 mg L^{-1}, depending on the solubility and final concentration of the compound. These standard solutions were stored at 4 °C and used for the preparation of the stock mixture solution. The stock mixture solution was prepared using the following concentration of analytes: 250 mg L^{-1} of AP, 300 mg L^{-1} of 2-NiPh, 300 mg L^{-1} of 2-Cla, 300 mg L^{-1} of 3-tBP, 250 mg L^{-1} of BFB, 300 mg L^{-1} of CNB, 125 mg L^{-1} of EB, 300 mg L^{-1} of BP, 250 mg L^{-1} of 2-NiNap, 50 mg L^{-1} of Nap, 20 mg L^{-1} of BiPh, 50 mg L^{-1} of Fl, Phe, and Ant, 600 mg L^{-1} of 4-OP, 500 mg L^{-1} of Py, 1500 mg L^{-1} of DPP, and 600 mg L^{-1} of ES. Aqueous working standard solutions were prepared by adding 20 µL of this stock mixture solution to deionized water. The total acetonitrile content in the daily aqueous solution was kept at a constant value of 0.2 % (v/v). The final concentration of the target analytes used for optimization experiments was 500 µg L^{-1} of AP, 600 µg L^{-1} of 2-NiPh, 600 µg L^{-1} of 2-Cla, 600 µg L^{-1} of 3-tBP, 500 µg L^{-1} of BFB, 600 µg L^{-1} of CNB, 250 µg L^{-1} of EB, 600 µg L^{-1} of BP, 500 µg L^{-1} of 2-NiNap, 100 µg L^{-1} of Nap, 40 µg L^{-1} of BiPh, 100 µg L^{-1} of Fl, Phe, and Ant, 1200 µg L^{-1} of 4-OP, 1000 µg L^{-1} of Py, 3000 µg L^{-1} of DPP, and 1200 µg L^{-1} of ES.

2.2 Instrumentation

Two Shimadzu LC-20A high performance liquid chromatographs (Kyoto, Japan) were used for the separation, detection, and quantification of analytes after extraction. Each liquid chromatograph was equipped with a 20 µL sample loop, a DGU-20A$_3$ degasser, two LC-20AT pumps and a SPD-20A UV–vis detector. A Restek Ultra C$_{18}$ column (250 mm x 4.6 mm i.d., 5 µm particle size, Bellefonte, PA, USA) was used to perform all separations with mobile phases comprised of acetonitrile and water. The separation gradient employed in this study started from 53% (v/v) acetonitrile, and was linearly increased up to 60% (v/v) over 3 min, then to 70% (v/v) over the next 8 min, then to 100% acetonitrile in 8.25 min and held for 8.75 min. The flow rate was kept constant at 1.00 mL min^{-1}. The analytes 3-tBP, EB, 4-OP, DPP, and ES were monitored at a UV detection wavelength of 220 nm while all other analytes were analyzed at 254 nm. Acquisition and processing of data was performed using Shimadzu LC solution software.

2.3 Preparation of DESs

All DES were prepared using similar methods to those previously reported [16], [37], [38]. Firstly, appropriate amounts of the HBA and HBD were weighed in a 20 mL vial containing a magnetic stirrer. The vial was then heated for three hours at 80 °C, after which a uniform and homogenous DES formed. The $[N_{4444}^{+}][Cl^{-}]$:2LcA DES was prepared by mixing one mole of $[N_{4444}^{+}][Cl^{-}]$ HBA with two moles of LcA, while the $[Ch^{+}][Cl^{-}]$:2Urea DES was prepared by mixing one mole of $[Ch^{+}][Cl^{-}]$ with two moles of urea. SUPRADES comprised of β-CD and LcA was prepared by mixing 200 mg of β-CD and 800 mg of LcA to attain a homogenous mixture of 20 wt% β-CD:LcA. A similar preparation procedure was followed to obtain various wt% compositions of β-CD and its derivatives with LcA for the preparation of different SUPRADES. Figure S1 shows the generic structure of β-CD and its derivatives as well as the chemical structures of the $[N_{4444}^{+}][Cl^{-}]$:2LcA and $[Ch^{+}][Cl^{-}]$:2Urea DESs. For dissolution of β-CD and its derivatives in the $[N_{4444}^{+}][Cl^{-}]$:2LcA and $[Ch^{+}][Cl^{-}]$:2Urea DESs, the required amount of β-CD or its derivatives was weighed and added to the DES and the mixture stirred for three hours at 80 °C to obtain a solution of CD in the DES.

The water content of all solvents, provided in Table S2, was measured by a Metrohm 831 Karl Fischer coulometric titrator and the viscosity of all DESs, provided in Table S3, was measured using a Brookfield DV1 cone and plate viscometer with a CPA-51Z cone spindle. Conventional DESs examined in this study including $[N_{4444}^{+}][Cl^{-}]$:2LcA and $[Ch^{+}][Cl^{-}]$:2Urea were also characterized by ^{13}C and ^{1}H NMR, and all spectra are provided in the Supporting Information (SI).

2.4 HS-SDME procedure

A small stir bar (12 x 4.5 mm) was added to a 10 mL aqueous solution containing 30% (w/v) NaCl. Then, a 20 μL volume of the stock mixture solution was added to the vial and the vial capped. A microsyringe was used to withdraw a 6.5 μL microdroplet of solvent. A section of parafilm (2 cm x 1 cm) was carefully and tightly wrapped on the bottom edge of a pipette tip and then subsequently pulled onto the microsyringe needle to aid in stabilization of the microdroplet. The microsyringe, equipped with the pipette tip, was then inserted into the septum of the sample vial and clamped to a support to ensure consistent immersion of the syringe into the sample headspace. The distance between the surface of the sample solution and the DES microdroplet was kept constant at 1.5 cm. The plunger was then carefully depressed to expose a 6.5 μL solvent microdroplet to the sample vial. Magnetic stirring was then initiated at a specific stir rate. The schematic diagram of the experimental setup is shown in Figure 1. Following extraction, the microdroplet was retracted into the syringe and injected into the sample loop of the HPLC. To avoid any carryover effects, the syringes, stir bars, and glass vials were washed and dried thoroughly following every extraction. Throughout the entire study, all extractions were performed in triplicate (unless otherwise specified) to study the precision and accuracy of results.

Results and Discussion

3.1 Optimization of HS-SDME parameters

Factors such as microdroplet volume, stir rate, ionic strength of the aqueous sample solution, extraction time, amount of native β-CD or its derivatives in the CD-based SUPRADES, and extraction temperature were optimized to obtain the maximum extraction efficiencies of analytes. All parameters were optimized using a factor-by-factor approach. Optimization conditions were evaluated for four different solvents including 20 wt% β-CD:LcA SUPRADES, $[N_{4444}^{+}][Cl^{-}]$:2LcA DES, $[Ch^{+}][Cl^{-}]$:2Urea DES, and 1.7% (w/v) β-CD in aqueous solution. No studies have reported the use of the 20 wt% β-CD:LcA SUPRADES and $[N_{4444}^{+}][Cl^{-}]$:2LcA DES in HS-SDME. The $[Ch^{+}][Cl^{-}]$:2Urea DES and aqueous solution of native β-CD have been previously used for the extraction of PAHs using HS-SDME [25], [44]. The purpose of evaluating the $[Ch^{+}][Cl^{-}]$:2Urea DES and the 1.7% (w/v) β-CD in aqueous solution was to compare their extraction performance with the 20 wt% β-CD:LcA SUPRADES and $[N_{4444}^{+}][Cl^{-}]$:2LcA DES under the optimized conditions. Moreover, the capability of the $[Ch^{+}][Cl^{-}]$:2Urea DES and the 1.7% (w/v) β-CD in aqueous solution to extract other analytes besides PAHs, including alkylphenols and plasticizers, was also evaluated in this study.

3.1.1 Effect of microdroplet volume

The microdroplet extraction solvent volume directly affects the preconcentration of analytes. Generally, increasing the volume of extraction solvent results in higher analyte extraction efficiencies in the HS-SDME mode [45]. Microdroplet volumes of 4.0, 5.0, and 6.5 μL were selected to evaluate the extraction efficiencies of the solvents. Native β-CD, $[Ch^{+}][Cl^{-}]$, and lactic acid are all considered hygroscopic [46] and can absorb water from the sample headspace during extraction. Therefore, microdroplet volumes greater than 6.5 μL could not be employed as the droplet became too large to be sustained on the microsyringe tip over prolonged extraction times.

Figure 2 shows the influence of microdroplet volume on the analyte peak areas using the 20 wt% β-CD:LcA SUPRADES extraction solvent system at an extraction time of 10 min. Results for the other three solvents are shown in Figures S2-S4 of SI. Peak areas of all the analytes increased as the microdroplet volume became larger. Moreover, the increase in peak areas was generally higher for solvents containing native β-CD, (i.e, 20 wt% β-CD:LcA SUPRADES and 1.7% (w/v) β-CD in aqueous solution) when the microdroplet volume was increased from 4 μL to 6.5 μL. For example, the increase in microdroplet volume from 4 μL to 6.5 μL resulted in a 45.6% and 32.3% increase in peak area of 2-NiPh and 2-Cla, respectively, using the 20 wt% β-CD:LcA SUPRADES compared to only a 2.6% and 8.4% increase in peak area of 2-NiPh and 2-Cla, respectively, using the $[Ch^+][Cl^-]$:2Urea DES. These results indicate that the amount of extracted analytes can be increased by using a larger microdroplet volume. A microdroplet volume of 6.5 μL was chosen for subsequent experiments to obtain the maximum extraction efficiencies for all analytes.

3.1.2 Effect of stir rate

Sample agitation is an important factor that affects the extraction efficiencies and equilibration times of analytes by influencing their mass transfer between the aqueous solution, headspace, and extraction solvent. Upon increasing the stir rate, analyte mass transfer to the microdroplet can be accelerated and generally reduces the equilibration time [47]. However, too vigorous agitation can cause detachment of the solvent microdroplet from the syringe whereas sluggish agitation can result in the formation of a “depletion zone” around the extraction solvent and decreased extraction efficiency [48].

In this study, the stir rate was varied from 400-1000 rpm using an extraction time of 10 min for all four solvents. Figure 3 demonstrates the effect of stir rate on the extraction of analytes using the 20 wt% β-CD:LcA SUPRADES solvent. Results from all other solvents are presented in Figures S5-S7. As expected, the extraction efficiencies of AP, BFB, CNB, EB, Nap, BiPh, Fl, Phe, Ant, Py, and ES increased gradually as the stir rate was increased. However, the effect was smaller for 2-NiNap, BP, 3-tBP, 2-Cla, and 2-NiPh. Among the four extraction solvents, the $[Ch^+][Cl^-]$:2Urea DES exhibited the smallest increase in analyte peak areas when the stir rate was increased.

3.1.3 Effect of ionic strength

The addition of salt to the aqueous sample solution can be used to enhance the extraction of analytes in HS-SDME since water molecules preferably solvate kosmotropic salts prompting the transfer of analytes to the headspace due to their lower solubility. However, the addition of salts is not always beneficial in SDME for some analytes [44], [49]. In this study, sample solutions containing varying concentration of NaCl (0, 10, 20, and 30% (w/v)) were examined to investigate the salting-out effect.

Employing the 20 wt% β-CD:LcA SUPRADES as extraction solvent, extraction efficiencies of all analytes were observed to increase upon addition of salt compared to extractions performed in the absence of salt, as shown in Figure 4. Figures S8-S10 show the peak areas at varying salt concentrations using the other three solvents ($[N_{4444}^+][Cl^-]$:2LcA DES, $[Ch^+][Cl^-]$:2Urea DES, and 1.7% (w/v) β-CD in aqueous solution). Peak areas of smaller molecular weight analytes such as AP, 2-NiPh, and 2-Cla were much higher compared to larger molecular weight molecules such as the PAHs, DPP, and ES. For example, the peak areas of 3-tBP and Phe increased by 66.7-fold and 1.4-fold, respectively, when the salt concentration was increased from 0 to 30% (w/v). The increase in extraction efficiencies of small molecular weight analytes upon increasing the salt concentration in the aqueous solution can be related to their higher polarity. For high molecular weight analytes, the extraction efficiencies increased after addition of 10% (w/v) salt and then remained constant up to 30% (w/v). For example, a 3.9 and 2.5-fold increase was observed for BiPh and Fl, respectively, when the salt content was increased from 0 to 10% (w/v). Further increasing the salt concentration to 30% (w/v) resulted in similar peak areas as that of 10% (w/v) salt content. In some cases, a decrease in the extraction efficiencies was observed upon increasing the salt content from 10% (w/v) to 30% (w/v). The decreased peak areas of the PAHs and other large hydrophobic molecules such as 4-OP, DPP, and ES can be related to their highly hydrophobic character and their interaction with the glass vial when their solubility decreases in the aqueous solution [50]. Moreover, the higher ionic strength increases the viscosity of the sample solution and can decrease the diffusion of large molecular weight analytes, resulting in a decrease in their extraction efficiency [25], [51]. Overall, the decrease in extraction efficiencies of hydrophobic high molecular weight analytes was much smaller compared to the increase in extraction efficiencies of polar analytes; therefore, a salt concentration of 30% (w/v) was used for further experiments.

3.1.4 CD composition in SUPRADESs

Comparing the extraction behavior of SUPRADESs comprised of different β-CD derivatives (native β-CD, RAMEB, TM-β-CD, and HP-β-CD) as HBAs mixed with the LcA HBD are among the main purposes of this study. The effect of β-CD derivative concentration within each SUPRADES was also examined. The amount of native β-CD in the native β-CD:LcA SUPRADES was increased from 10-30 wt% to evaluate its effect on extraction performance. SUPRADESs comprised of the native β-CD HBA and LcA HBD containing less than 10 wt% of native β-CD were observed to be solids at room temperature; moreover, SUPRADES containing greater than 30 wt% of native β-CD were too viscous to be withdrawn into the microsyringe. Figure S11 shows the extraction performance of SUPRADES comprised of the native β-CD HBA and the

LcA HBD where the concentration of native β-CD in the SUPRADES was increased gradually (10, 12.5, 15, 17.5, 20, 30 wt%). The SUPRADES containing 20 wt% native β-CD provided superior extraction of BFB, EB, Nap, BiPh, and Ant compared to other concentrations of native β-CD.

Different derivatives of β-CD with varying degrees of substitution (DS) including RAMEB (DS = 12.9, R = -H or $-CH_3$) [39], HP-β-CD (DS = 5.6, R = -H or $-CH_2-CH(OH)-CH_3$) [39], and TM-β-CD (DS = 21, R = $-CH_3$) [52] employed as HBAs are shown in Figure S1. SUPRADESs were prepared by mixing 20 wt% of a β-CD derivative with LcA HBD and their extraction performance evaluated. Figure 5 shows an extraction performance comparison for SUPRADESs comprised of various CD-derivatives. The 20 wt% native β-CD:LcA SUPRADES provided the highest extraction of AP, 2-NiPh, BFB, EB, 2-NiNap, Nap, BiPh, Fl, Phe, Ant, and ES. The extraction of other analytes using the 20 wt% native β-CD:LcA SUPRADES was similar to the RAMEB and HP-β-CD-based SUPRADESs. Overall, the SUPRADES comprised of the TM-β-CD HBA provided the lowest extraction of analytes compared to native β-CD, RAMEB, and HP-β-CD. Based on these results, the 20 wt% native β-CD:LcA SUPRADES was chosen as the solvent for subsequent studies.

The dissolution of CDs in the $[N_{4444}^+][Cl^-]$:2LcA and $[Ch^+][Cl^-]$:2Urea DESs and the use of these solvents was also investigated. It has been previously reported that native β-CD is highly soluble in the $[Ch^+][Cl^-]$:2Urea DES [37], therefore, 1.7, 10, 20, and 30 wt% of native β-CD was dissolved in the $[Ch^+][Cl^-]$:2Urea DES and used as extraction solvent. Figure 6 shows a significant increase in the peak areas of AP, 2-Cla, BFB, CNB, EB, Nap, BiPh, Fl, and ES upon increasing the native β-CD concentration to 20 wt% in the $[Ch^+][Cl^-]$:2Urea DES while the extraction efficiencies of other analytes were observed to be similar to the neat $[Ch^+][Cl^-]$:2Urea DES. For example, the peak areas of BiPh and ES increased by 11.2 and 31.2-fold, respectively, while that of Ant and Py increased by 1.1 and 1.3-fold, respectively, upon increasing the native β-CD concentration from 0 to 20 wt% in the $[Ch^+][Cl^-]$:2Urea DES. The peak areas of analytes did not change upon further increasing the β-CD concentration to 30 wt% in the $[Ch^+][Cl^-]$:2Urea DES.

The dissolution of other CD-derivatives was also evaluated in the $[Ch^+][Cl^-]$:2Urea DES. RAMEB and TM-β-CD were found to be insoluble in the neat $[Ch^+][Cl^-]$:2Urea DES, but HP-β-CD could be solubilized at levels up to 20 wt%. Its extraction performance was evaluated and compared with the 20 wt% native β-CD solution in $[Ch^+][Cl^-]$:2Urea DES and the neat $[Ch^+][Cl^-]$:2Urea DES, as shown in Figure S12. The solution of 20 wt% HP-β-CD in $[Ch^+][Cl^-]$:2Urea resulted in similar peak areas as that of the solution of 20 wt% native β-CD in $[Ch^+][Cl^-]$:2Urea for most of the analytes except BFB and CNB, whose peak areas were smaller in the solution of 20 wt% HP-β-CD in $[Ch^+][Cl^-]$:2Urea DES. Due to the significant increase in extraction efficiencies of target analytes by the solution of 20 wt% native β-CD in $[Ch^+][Cl^-]$:2Urea DES, it was employed as extraction solvent for further experiments.

Neither the native β-CD or TM-β-CD were found to be soluble in the $[N_{4444}^+][Cl^-]$:2LcA DES. HP-β-CD was observed to be soluble at 20 wt% in the $[N_{4444}^+][Cl^-]$:2LcA DES and RAMEB was found to be soluble at 5 wt% in the $[N_{4444}^+][Cl^-]$:2LcA DES; both of these solvents were examined and the resulting analyte extraction efficiencies are shown in Figure S13. The extraction performance of the 5 wt% RAMEB:LcA SUPRADES was found to be similar to the neat $[N_{4444}^+][Cl^-]$:2LcA DES. However, the 20 wt% HP-β-CD:LcA SUPRADES provided better extraction of 3-tBP and 2-NiPh and peak areas were lower in the case of BFB, EB, Nap, BiPh, and Ant compared to the neat $[N_{4444}^+][Cl^-]$:2LcA DES. Peaks areas of the other analytes were observed to be similar for both the solution of 20 wt% HP-β-CD in $[N_{4444}^+][Cl^-]$:2LcA and neat $[N_{4444}^+][Cl^-$

]:2LcA DES. These results show that the effect of CD on the extraction performance of DESs is dependent upon the HBA and HBD of the DESs.

3.1.5 Effect of extraction temperature

Temperature plays a very important role in HS-SDME as it affects the kinetic and thermodynamic parameters of the extraction process. Increasing the extraction temperature typically results in enhanced Henry's constant values and diffusion coefficients, leading to an increased concentration of analytes in the headspace and improved analyte mass transfer into the solvent microdroplet [53], [54].

Extraction efficiencies of the target analytes were studied by performing the extractions at 20 ℃ and 40 ℃. As mentioned previously, the microdroplet volume increases over time due to the hygroscopic nature of the studied solvents. Although the microdroplets were stable for 2 hours at 20 ℃, they became dislodged from the microsyringe within the first hour of extraction at 40 ℃. To overcome this challenge, a 2 cm x 1 cm segment of parafilm was wrapped around the end of the pipette tip, resulting in an increased contact surface area of the syringe needle and permitting stabilization of larger microdroplet volumes. Microdroplets up to 15 μL were observed to be stable using this modification. An extraction temperature of 60 ℃ was also investigated; however, the microdroplets became unstable at approximately 75 min, even when employing the modified syringe tip.

Figure 7 shows a comparison of analyte peak areas at 20 ℃ and 40 ℃ for the 20 wt% β-CD:LcA SUPRADES using an extraction time of 90 min, salt concentration of 30 % (w/v), and stir rate of 1000 rpm. Results for other solvents are shown in Figures S14-S16. Peak areas for most of the analytes increased with an increase in temperature from 20 ℃ to 40 ℃. The peak area of 4-OP increased by 19.1-fold when the temperature was increased from 20 ℃ to 40 ℃. However, a decrease in peak areas for BFB, EB, Nap, BiPh, and Fl was observed with the same temperature increase using the 20 wt% β-CD: LcA SUPRADES. Similar peak areas for 2-NiPh and CNB were measured at 20 ℃ and 40 ℃. A significant increase in peak areas for most of the analytes was observed when the [N_{4444}^+][Cl^-]:2LcA DES was employed as the extraction solvent. The extraction efficiency of BFB did not change with increasing temperature, while EB, Nap, and BiPh were extracted less with an increase in temperature, as shown in Figure S14. In the case of the [Ch^+][Cl^-]:2Urea DES, the increase in peak areas at 40 ℃ ranged from 1.1 to 4.4-fold for most analytes compared to 20 ℃, except for EB, Nap, BiPh, Fl, and Ant where a decrease in peak areas was observed at 40 ℃. Increased extraction temperatures were least beneficial for the 1.7% (w/v) β-CD in aqueous solution, where the peak areas of 9 analytes increased and the other 9 analytes decreased. However, as demonstrated in Figure S15, the extraction efficiencies of high molecular weight analytes such as 4-OP, Py, DPP, and ES at 20 ℃ was much smaller and increased at 40 ℃. To obtain reasonable extraction of analytes, 40 ℃ was chosen as an extraction temperature for the 1.7% (w/v) β-CD in aqueous solution solvent. For all of the extraction solvents, a considerable increase in extraction efficiencies was observed at 40 ℃ for analytes that possess high molecular weight and low vapor pressure; therefore, 40 ℃ was selected for the subsequent studies.

3.1.6 Effect of extraction time

Typically, the amount of analyte extracted increases at longer extraction times when equilibrium is attained [55]. Figure 8 shows the sorption-time profile for all analytes at 40 ℃ using the 20 wt% β-CD:LcA SUPRADES. Sorption-time profiles generated for all other solvents at 20 ℃ and 40 ℃ are presented in Figures S17-S24. A time-course from 10, 30, 60, 90, 120, and 150 min was used to construct the sorption-time profiles in the case of 20 wt% β-CD:LcA and [N_{4444}^+][Cl^-]:2LcA DESs. For the 1.7% (w/v) β-CD in aqueous solution and the [Ch^+][Cl^-]:2Urea

DES, extraction times up to 120 minutes were evaluated. At 150 minutes, the microdroplet volume for the [Ch^+][Cl^-]:2Urea DES was too large and unstable whereas the microdroplet for the 1.7% (w/v) β-CD in aqueous solution became too small to recover and attain reproducible results. At 20 °C, most of the analytes did not equilibrate even after extraction times of 150 minutes; therefore, only sorption-time profiles at 40 °C were evaluated.

Sorption-time profiles at 40 °C revealed that 2-NiNap, BP, Phe, Ant, 4-OP, Py, DPP, and ES reached equilibrium at 120 min using the 20 wt% β-CD:LcA SUPRADES solvent. The increase in analyte peak area from 90 min to 120 min was much lower than the increase from 60 to 90 min. As an example, the extraction of Phe and Ant increased by 43.8% and 40.6%, respectively, when the extraction time was increased from 60 min to 90 min, but increased by only 11.9% and 5.6%, respectively, when the extraction time was increased from 90 min to 120 min. The analyte 3-tBP, which exhibited the highest peak area among all of the analytes, did not equilibrate until 150 minutes. An equilibration time of 90 min was observed for AP and 2-Cla, 30 min for BFB, CNB, and Fl, and 10 min for Nap, BiPh, and EB, as shown in Figure 8. A decrease in peak areas was observed for analytes that possess high vapor pressure and attain equilibrium within 30 min. Similar trends were observed for the [N_{4444}^+][Cl^-]:2LcA DES, as shown in Figure S21. To avoid excessively long extraction times and loss of extraction efficiencies for analytes that rapidly attain equilibrium, 90 min was selected as the optimum extraction time for the 20 wt% β-CD:LcA SUPRADES and [N_{4444}^+][Cl^-]:2LcA DES.

In the case of the 1.7% (w/v) β-CD in aqueous solution and [Ch^+][Cl^-]:2Urea DES, maximum extraction efficiencies for most analytes were obtained at 60 min (see Figures S22 and S23); therefore, this time was selected for these solvents. For the solution of 20 wt% β-CD in [Ch^+][Cl^-]:2Urea, most of the analytes equilibrated at 90 minutes, except for ES which did not reach a plateau until 120 minutes, as shown in Figure S24. Additionally, no decrease in extraction efficiencies was observed for EB, Nap, and BiPh; therefore, 90 minutes was also selected as the optimal extraction time for this solvent.

3.2 Analytical figures of merit

The developed extraction method was used to determine the analytical figures of merit under optimum conditions. Calibration curves for each analyte were constructed in ultrapure water using the two best performing solvents, namely, 20 wt% β-CD:LcA SUPRADES and $[N_{4444}^+][Cl^-]$:2LcA DES at an extraction time of 90 min, 40 °C temperature, 30% (w/v) salt concentration, 6.5 μL microdroplet volume, and stir rate of 1000 rpm. Figures of merit including the slope and error of calibration of curve, standard deviation of regression ($S_{y/x}$), correlation coefficient (R), limit of detection (LOD), and limit of quantification (LOQ) are listed in Tables 1 and 2 for 20 wt% β-CD:LcA SUPRADES and $[N_{4444}^+][Cl^-]$:2LcA DES, respectively.

Calibration curves for all analytes demonstrated good linearity with correlation coefficients ranging from 0.983-0.999. Higher calibration slopes were obtained for the $[N_{4444}^+][Cl^-]$:2LcA DES compared to the 20 wt% β-CD:LcA SUPRADES for most of the analytes except 2-Cla, 3-tBP, and 4-OP, where an opposite trend was observed. The enrichment factor (E_F) and relative standard deviation (RSD) was calculated by performing triplicates of extractions using the 20 wt% β-CD:LcA SUPRADES, the $[N_{4444}^+][Cl^-]$:2LcA DES, the solution of 20 wt% β-CD in $[Ch^+][Cl^-]$:2Urea DES, 1.7% (w/v) β-CD in aqueous solution, and $[Ch^+][Cl^-]$:2Urea DES, as shown in Table 3.

The reproducibility of method, determined by RSD, ranged from 2.3% to 9.9% for the 20 wt% β-CD:LcA SUPRADES, 2.1%-7.2% for the $[N_{4444}^+][Cl^-]$:2LcA DES, 4.2%-11.6% for the solution of 20 wt% β-CD in $[Ch^+][Cl^-]$:2Urea DES, 2.8%-16.5% for the 1.7% (w/v) β-CD in aqueous solution, and 0.6%-14.5% for the $[Ch^+][Cl^-]$:2Urea DES. The enrichment factor (E_F) was used to evaluate and compare the extraction performance of different solvents and was calculated using equation 1:

$$E_F = \frac{\text{Concentration of analyte in the solvent microdroplet}}{\text{Initial concentration of analyte in the sample}} \qquad (1)$$

where the concentration of analyte in the solvent microdroplet was calculated from the calibration curve obtained by direct injection of analyte standard solutions in acetonitrile (Tables S4 and S5) and the solvent microdroplet volume of 6.5 μL.

As shown in Table 3, the $[N_{4444}^+][Cl^-]$:2LcA DES provided the highest E_F for most analytes including AP, 2-NiPh, BFB, CNB, EB, BP, 2-NiNap, Nap, BiPh, Fl, Phe, Ant, DPP, and ES. The 20 wt% β-CD:LcA SUPRADES provided the highest E_F for 2-Cla, 3-tBP, 4-OP, and Py. Overall, under the optimized conditions, the 20 wt% β-CD:LcA SUPRADES, $[N_{4444}^+][Cl^-]$:2LcA DES, and solution of 20 wt% β-CD in $[Ch^+][Cl^-]$:2Urea DES provided higher enrichment factors than previously employed solvents for HS-SDME, namely, 1.7% (w/v) β-CD in aqueous solution and the $[Ch^+][Cl^-]$:2Urea DES [25], [44].

3.3 Analysis of real matrices

The optimized HS-SDME method was employed for the analysis of real water samples, including lake water and tap water. The NaCl content of the real matrices was adjusted to 30% (w/v) based upon the aforementioned experimental results. No analytes were detected in the lake and tap water samples. Therefore, spiked samples were analyzed to examine the presence of any matrix effects for the determination of analytes. Matrix effects originate from components of the sample other than the target analytes that can interfere in the quantitative determination of analytes [56]. Relative recoveries of analytes are used to evaluate the effect of sample matrix on the extraction method. Relative recoveries were calculated as the ratio of the peak area of extracted analyte from the sample matrix and predicted peak area obtained from the calibration curves of the overall method (Table 2 and 3**)**. Generally, relative recoveries within the range of 80%-120% are deemed acceptable and indicate that matrix effects do not give rise to significant error in analyte quantification [57]**,** [58].

Table 4 shows the obtained reproducibility (expressed as RSD) and relative recoveries (RR) of the target analytes in the lake and tap water using the 20 wt% β-CD:LcA SUPRADES and the $[N_{4444}^{+}][Cl^{-}]$:2LcA DES. The relative recoveries ranged from 95.1%-119.7% and 92.4%-112.7% for the lake and tap water, respectively, using the 20 wt% β-CD:LcA SUPRADES. The RSD values were observed to be lower than 19.2% and 16.1% for all analytes in the lake water and tap water, respectively, using this solvent. Employing the $[N_{4444}^{+}][Cl^{-}]$:2LcA DES extraction solvent, the relative recoveries ranged from 83.7%-113.9% and 95.2%-114.6% in the lake water and tap water, respectively. RSD values lower than 13.6% were obtained for both real matrices using the $[N_{4444}^{+}][Cl^{-}]$:2LcA DES. The results show that the SUPRADES and DES employed in this study are tolerant to the real matrices and can be used for extraction of analytes in real samples.

Conclusions

For the first time, SUPRADES comprised of β-CD and its derivatives were employed as extraction solvents in HS-SDME for the extraction of organic pollutants. The extraction performance of SUPRADES was compared with neat DESs ($[N_{4444}^{+}][Cl^{-}]$:2LcA and $[Ch^{+}][Cl^{-}]$:2Urea) as well as with 1.7% (w/v) β-CD in aqueous solution. Experimental parameters including microdroplet volume, stir rate, salt concentration, extraction temperature, and extraction time were optimized to attain the maximum extraction efficiencies for target analytes. The addition of native β-CD and its derivatives to the neat DESs was also studied.

Among the evaluated extraction solvents, the 20 wt% β-CD:LcA SUPRADES and the $[N_{4444}^{+}][Cl^{-}]$:2LcA DES provided the highest enrichment factors for the target analytes. The extraction efficiency of the $[Ch^{+}][Cl^{-}]$:2Urea DES increased upon addition of the 20 wt% native β-CD. The developed HS-SDME method was validated for the 20 wt% β-CD:LcA SUPRADES and the $[N_{4444}^{+}][Cl^{-}]$:2LcA DES. High linearity (R>0.983) and low LODs (<14.6 μg L^{-1}) were attained for all analytes. The developed method was also applied for analysis of real matrices including lake water and tap water. RSD values lower than 19.2% were obtained, in all cases. No matrix effects were observed in the lake water and the tap water, with relative recovery values ranging from 83.7%-119.7%.

This study demonstrates that SUPRADES comprised β-CD-based HBAs can be easily employed as selective solvents in HS-SDME. No significant variation in analyte extraction efficiencies were observed when SUPRADES comprised of different derivatives of β-CD (possessing varying degree of substitution) were employed as extraction solvents. This study shows that the extraction behavior of DESs can be modulated by tailoring their HBAs and HBDs and that derivatized CDs can serve as beneficial HBAs for imparting selectivity into the solvent.

In future studies, the effect of CD cavity size on the extraction selectivity of SUPRADESs can be evaluated by using α-CD and γ-CDs in the design and preparation of SUPRADESs. Moreover, the application of SUPRADESs to other microextraction techniques that can exploit their physico-chemical and solvent properties will be explored.

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Declaration of competing Interest

The authors declare no conflicts of interest.

Supporting Information. Water content, viscosity of DESs, acronyms of all chemicals, figures of merit of the calibration curves, chemical structures of CDs and DESs, results of the optimization of experimental parameters for all extraction solvents, ^{1}H and ^{13}C NMR spectra for $[N_{4444}^+][Cl^-]$:2LcA and $[Ch^+][Cl^-]$:2Urea DESs.

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Figure Legends:

Figure 1. Schematic diagram of HS-SDME experimental setup, where a microsyringe needle equipped with a pipette tip is inserted into the sample headspace of the vial septum. The analyte solution is stirred continuously using a magnetic stirrer. A solvent microdroplet is exposed to the headspace of sample vial by completely depressing the plunger. After extraction, the solvent microdroplet is withdrawn into the microsyringe and injected into the sample loop of the HPLC.

Figure 2. Effect of microdroplet volume on the extraction performance of studied analytes using the 20 wt% β-CD:LcA SUPRADES extraction solvent. Experimental conditions: volume of analyte solution:10 mL; stir rate: 1000 rpm; temperature: 20 °C; and extraction time: 10 min. Spiked analyte concentrations: 500 μg L^{-1} for AP, BFB, 2-NiNap, 600 μg L^{-1} for 2-NiPh, CNB, BP, 1200 μg L^{-1} for 2-Cla, 4-OP, ES, 2000 μg L^{-1} for 3-tBP, 250 μg L^{-1} for EB, 100 μg L^{-1} for Nap, Fl, Phe, Ant, 40 μg L^{-1} for BiPh, 1000 μg L^{-1} for Py, and 3000 μg L^{-1} for DPP. All extractions were performed in triplicate.

Figure 3. Comparison of the stir rate for the HS-SDME method using the 20 wt% β-CD:LcA SUPRADES extraction solvent. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 μL; temperature: 20 °C; and extraction time: 10 min. Spiked analyte concentrations: 500 μg L^{-1} for AP, BFB, 2-NiNap, 600 μg L^{-1} for 2-NiPh, CNB, BP, 1200 μg L^{-1} for 2-Cla, 4-OP, ES, 2000 μg L^{-1} for 3-tBP, 250 μg L^{-1} for EB, 100 μg L^{-1} for Nap, Fl, Phe, Ant, 40 μg L^{-1} for BiPh, 1000 μg L^{-1} for Py, and 3000 μg L^{-1} for DPP. All extractions were performed in triplicate.

Figure 4. Influence of salt concentration on the amount of extracted analytes using the 20 wt% β-CD:LcA SUPRADES extraction solvent. The inset within the figure shows the enlarged peak areas for 4-OP, Py, DPP, and ES. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 μL; stir rate: 1000 rpm; temperature: 20 °C; and extraction time: 10 min. Spiked analyte concentrations: 500 μg L^{-1} for AP, BFB, 2-NiNap, 600 μg L^{-1} for 2-NiPh, CNB, BP, 1200 μg L^{-1} for 2-Cla, 4-OP, ES, 2000 μg L^{-1} for 3-tBP, 250 μg L^{-1} for EB, 100 μg L^{-1} for Nap, Fl, Phe, Ant, 40 μg L^{-1} for BiPh, 1000 μg L^{-1} for Py, and 3000 μg L^{-1} for DPP. All extractions were performed in triplicate.

Figure 5. Comparison of peak areas for target analytes using the SUPRADESs comprised of native β-CD or its derivatives (RAMEB, HP-β-CD, and TM-β-CD) as HBA and LcA HBD as extraction solvent. The inset represents the magnified peak areas for the last four analytes. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 μL; stir rate: 1000 rpm; temperature: 20 °C; salt concentration: 30% (w/v); and extraction time: 120 min. Spiked analyte concentrations: 500 μg L^{-1} for AP, BFB, 2-NiNap, 600 μg L^{-1} for 2-NiPh, CNB, BP, 1200 μg L^{-1} for 2-Cla, 4-OP, ES, 2000 μg L^{-1} for 3-tBP, 250 μg L^{-1} for EB, 100 μg L^{-1} for Nap, Fl, Phe, Ant, 40 μg L^{-1} for BiPh, 1000 μg L^{-1} for Py, and 3000 μg L^{-1} for DPP. All extractions were performed in triplicate.

Figure 6. Effect of native β-CD concentration in the $[Ch^+][Cl^-]$:2Urea DES on the extraction performance using HS-SMDE. The inset within the figure shows the enlarged peak areas for 4-

OP, Py, DPP, and ES. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 μL; stir rate: 1000 rpm; temperature: 20 °C; salt concentration: 30% (w/v); and extraction time: 120 min. Spiked analyte concentrations: 500 μg L^{-1} for AP, BFB, 2-NiNap, 600 μg L^{-1} for 2-NiPh, CNB, BP, 1200 μg L^{-1} for 2-Cla, 4-OP, ES, 2000 μg L^{-1} for 3-tBP, 250 μg L^{-1} for EB, 100 μg L^{-1} for Nap, Fl, Phe, Ant, 40 μg L^{-1} for BiPh, 1000 μg L^{-1} for Py, and 3000 μg L^{-1} for DPP. All extractions were performed in triplicate.

Figure 7. Comparison of the peak areas for studied analytes at an extraction temperature 20 °C and 40 °C using the 20 wt% β-CD:LcA SUPRADES as extraction solvent. The enlarged peak areas for 4-OP, Py, DPP, and ES are shown in the figure inset. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 μL; stir rate: 1000 rpm; salt concentration: 30% (w/v); and extraction time: 90 min. Spiked analyte concentrations: 500 μg L^{-1} for AP, BFB, 2-NiNap, 600 μg L^{-1} for 2-NiPh, CNB, BP, 1200 μg L^{-1} for 2-Cla, 4-OP, ES, 2000 μg L^{-1} for 3-tBP, 250 μg L^{-1} for EB, 100 μg L^{-1} for Nap, Fl, Phe, Ant, 40 μg L^{-1} for BiPh, 1000 μg L^{-1} for Py, and 3000 μg L^{-1} for DPP. All extractions were performed in triplicate.

Figure 8. Sorption-time profile of the studied analytes using the 20 wt% β-CD:LcA SUPRADES as extraction solvent at 40 °C. (a) (◆) AP; (●) 2-Cla; (△) BP; and (■) 2-NiNap, (b) (X) BFB; (○) CNB; (◇) Nap; (△) BiPh; (□) Fl; (+) Phe; and (—) Ant, (c) (X) 2-NiPh; (◉) 4-OP; (◇) Py; (▲) DPP; and (■) ES, (d) (■) 3-tBP and (◆) EB. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 μL; stir rate: 1000 rpm; temperature: 40 °C; and salt concentration: 30% (w/v). Spiked analyte concentrations: 500 μg L^{-1} for AP, BFB, 2-NiNap, 600 μg L^{-1} for 2-NiPh, CNB, BP, 1200 μg L^{-1} for 2-Cla, 4-OP, ES, 2000 μg L^{-1} for 3-tBP, 250 μg L^{-1} for EB, 100 μg L^{-1} for Nap, Fl, Phe, Ant, 40 μg L^{-1} for BiPh, 1000 μg L^{-1} for Py, and 3000 μg L^{-1} for DPP. Triplicate extractions were performed at 10, 60, 120, and 150 min and duplicates were carried out at 30 and 90 min.

Table 1. Analytical performance of the overall HS-SDME method using the 20 wt% β-CD:LcA SUPRADES extraction solvent

Analytes	**Linear range ($\mu g\ L^{-1}$)**	**Slope ± SD ($x10^2$)**	$S_{y/x}$ [a]	**R** [b]	**LOD** [c] **($\mu g\ L^{-1}$)**	**LOQ** [d] **($\mu g\ L^{-1}$)**
AP	5-500	275.0±5.4	2372	0.9990	0.7	2.3
2-NiPh	12.5-1250	59.6±1.0	1156	0.9992	3.2	10.5
2-Cla	5-500	144.5±1.9	820	0.9996	1.3	4.3
3-tBP	5-500	316.9±8.1	3558	0.9984	0.6	2.0
BFB	5-500	188.5±3.8	1690	0.9990	1.0	3.3
CNB	5-500	187.8±4.0	1735	0.9989	1.0	3.3
EB	5-500	251.9±6.4	2809	0.9984	0.7	2.5
BP	5-500	559.9±8.0	3532	0.9995	0.3	1.1
2-NiNap	5-500	416.7±6.0	2663	0.9995	0.5	1.5
Nap	5-500	149.4±3.2	1389	0.9989	1.3	4.2
BiPh	2-200	267.7±4.3	760	0.9993	0.7	2.3
Fl	5-500	320.7±9.1	4012	0.9980	0.6	2.0
Phe	2-200	1077.7±25.5	4486	0.9986	0.2	0.6
Ant	2-200	1843.2±32.7	5694	0.9992	0.1	0.3
4-OP	25-2500	78.2±2.5	5514	0.9974	2.4	8.0
Py	10-2500	163.1±6.7	14765	0.9958	1.2	3.9
DPP	50-2500	12.9±0.5	1061	0.9965	14.6	48.6
ES	25-2500	35.5±0.9	2000	0.9984	5.3	17.7

[a] Standard deviation of the regression

[b] Correlation coefficient

[c] Calculated as three times the signal-to-noise ratio

[d] Calculated as ten times the signal-to-noise ratio

[e] Conditions: Microdroplet volume: 6.5 μL; sample volume: 10 μL; extraction time: 90 min; extraction temperature: 40 °C; stir rate: 1000 rpm; salt concentration: 30 % (w/v)

Table 2. Figures of merit of the calibration curves, limit of detection, and limit of quantification of the HS-SMDE method using the $[N_{4444}^{+}][Cl^{-}]$:2LcA DES extraction solvent

Analytes	**Linear range (μg L^{-1})**	**Slope ± SD (x10^{2})**	$S_{y/x}$ [a]	**R** [b]	**LOD** [c] **(μg L^{-1})**	**LOQ** [d] **(μg L^{-1})**
AP	5-500	403.4±10.1	4454	0.9984	0.4	1.3
2-NiPh	12.5-1250	111.1±1.7	1882	0.9994	1.4	4.6
2-Cla	5-500	141.7±2.7	1187	0.9991	1.1	3.6
3-tBP	5-500	276.8±7.0	3080	0.9984	0.6	1.9
BFB	5-500	286.7±8.4	3717	0.9978	0.5	1.8
CNB	5-500	381.9±12.1	5333	0.9975	0.4	1.3
EB	5-500	683.1±23.6	10401	0.9970	0.2	0.8
BP	5-500	577.4±9.0	3940	0.9994	0.3	0.9
2-NiNap	5-500	473.3±8.7	3815	0.9992	0.3	1.1
Nap	5-500	230.7±4.7	2068	0.9990	0.7	2.2
BiPh	2-200	1095.3±15.3	2698	0.9995	0.1	0.5
Fl	5-500	808.3±20.4	8990	0.9984	0.2	0.6
Phe	2-200	1638.2±44.4	7817	0.9982	0.1	0.3
Ant	2-200	2919.7±81.2	14281	0.9981	0.1	0.2
4-OP	25-2500	54.3±4.5	9908	0.9832	2.9	9.5
Py	10-2500	208.4±2.8	3868	0.9996	0.7	2.5
DPP	50-2500	24.8±0.7	9547	0.9985	6.2	20.8
ES	25-2500	83.0±1.8	4050	0.9988	1.9	6.2

[a] Standard deviation of the regression

[b] Correlation coefficient

[c] Calculated as three times the signal-to-noise ratio

[d] Calculated as ten times the signal-to-noise ratio

[e] Conditions: Microdroplet volume: 6.5 μL; sample volume: 10 μL; extraction time: 90 min; extraction temperature: 40 °C; stir rate: 1000 rpm; salt concentration: 30 % (w/v)

Table 3. Enrichment factors and relative standard deviations of the target analytes obtained by HS-SDME using 20 wt% β-CD:LcA SUPRADES, $[N_{4444}^+][Cl^-]$:2LcA DES, solution of 20 wt% β-CD in $[Ch^+][Cl^-]$:2Urea DES, 1.7% (w/v) β-CD in aqueous solution, and $[Ch^+][Cl^-]$:2Urea DES extraction solvents

Analytes	20 wt% β-CD:LcA SUPRADES		$[N_{4444}^+][Cl^-]$:2LcA DES		20 wt% β-CD in $[Ch^+][Cl^-]$:2Urea DES		1.7% (w/v) β-CD in aqueous solution		$[Ch^+][Cl^-]$:2Urea DES	
	E_F [a]	RSD[b] (%)	E_F [a]	RSD[b] (%)	E_F [a]	RSD[b] (%)	E_F [a]	RSD[b] (%)	E_F [a]	RSD[b] (%)
AP	369.7	3.6	584.0	4.6	241.9	5.1	44.1	11.7	74.1	4.0
2-NiPh	171.5	8.3	425.8	7.2	85.8	11.6	43.2	10.9	44.4	4.0
2-Cla	961.1	6.2	854.6	2.0	362.0	4.4	50.4	12.4	167.0	5.0
3-tBP	715.0	7.5	643.3	5.8	469.3	5.2	552.7	2.8	329.1	4.4
BFB	365.5	3.2	671.0	2.1	253.6	4.2	85.7	12.4	53.0	0.6
CNB	345.2	2.9	797.9	3.8	273.5	4.5	84.2	3.7	71.5	3.1
EB	366.2	5.0	1121.8	3.3	340.9	9.2	69.9	10.1	32.9	1.4
BP	338.3	8.6	358.5	4.2	182.5	4.7	222.2	11.2	82.5	10.0
2-NiNap	283.1	9.9	299.2	4.1	153.2	4.7	151.2	12.8	90.1	10.8
Nap	523.2	2.3	883.3	2.9	283.7	9.8	540.1	13.0	26.9	4.2
BiPh	193.9	3.8	767.1	4.9	356.8	7.8	157.2	15.8	14.1	8.2
Fl	274.1	6.1	546.0	6.9	245.7	8.5	153.0	10.3	22.9	14.5
Phe	221.5	10.5	262.0	6.1	126.0	6.9	116.2	8.8	33.7	9.7
Ant	148.4	11.1	173.0	5.8	95.8	8.5	59.3	10.5	16.6	5.5
4-OP	42.9	10.1	33.9	8.6	16.8	5.4	17.9	5.4	6.4	8.9
Py	34.7	8.2	30.5	4.3	13.4	2.5	16.0	14.4	6.7	6.9
DPP	2.1	10.4	2.5	8.6	1.3	1.9	0.6	3.0	0.4	12.5
ES	74.1	11.4	142.4	6.3	63.4	7.8	33.3	16.5	1.5	11.1

[a] Enrichment factor calculated by Equation 1 in the text

[b] Relative standard deviation

[c] Enrichment factor is calculated by using microdroplet volume of 6.5 µL. Although the microdroplet volume increases overtime during extraction, it does not affect the extraction of target analytes. Using the 6.5 µL neat ultrapure water microdroplet as extraction solvent did not result in the extraction of any analytes

[d] Spiked analyte concentrations: 500 µg L^{-1} for AP, BFB, 2-NiNap, 600 µg L^{-1} for 2-NiPh, CNB, BP, 1200 µg L^{-1} for 2-Cla, 4-OP, ES, 2000 µg L^{-1} for 3-tBP, 250 µg L^{-1} for EB, 100 µg L^{-1} Nap, Fl, Phe, Ant, 40 µg L^{-1} for BiPh, 1000 µg L^{-1} for Py, and 3000 µg L^{-1} for DPP

[e] All extractions were performed in triplicate

Table 4. Analysis of real samples by HS-SDME using 20 wt% β-CD:LcA SUPRADES and $[N_{4444}^{+}][Cl^{-}]$:2LcA DES extraction solvents

Analytes	**20 wt% β-CD:LcA SUPRADES**				**$[N_{4444}^{+}][Cl^{-}]$:2LcA DES**			
	Lake water		**Tap water**		**Lake water**		**Tap water**	
	RR [a] (%)	**RSD [b] (%)**	**RR [a] (%)**	**RSD [b] (%)**	**RR [a] (%)**	**RSD [b] (%)**	**RR [a] (%)**	**RSD [b] (%)**
AP	114.1	9.9	96.6	8.4	100.1	1.6	105.5	7.6
2-NiPh	95.1	8.9	94.6	8.1	83.7	7.2	110.6	11.2
2-Cla	119.7	10.7	104.3	6.4	106.8	0.7	96.7	7.0
3-tBP	113.9	11.9	96.3	6.0	99.4	4.0	96.4	6.7
BFB	115.4	6.6	99.8	8.0	102.3	4.9	101.6	4.2
CNB	116.6	11.6	98.0	5.9	101.0	3.3	99.5	5.0
EB	119.4	8.8	111.1	8.6	102.1	6.5	110.7	2.6
BP	112.9	17.1	103.3	13.0	98.3	3.8	97.9	2.1
2-NiNap	112.2	16.3	92.4	6.7	89.8	4.2	95.2	5.3
Nap	109.6	5.5	103.1	10.7	103.2	8.5	112.3	4.8
BiPh	115.0	16.6	95.7	13.6	108.4	8.9	114.6	8.1
Fl	114.1	17.0	102.7	8.7	104.1	6.5	109.3	3.7
Phe	105.5	17.5	100.3	12.4	96.8	5.6	103.2	10.4
Ant	105.9	18.0	100.5	12.3	99.9	5.8	97.9	7.6
4-OP	111.5	19.2	112.7	9.0	113.9	11.1	107.6	3.0
Py	110.4	12.1	110.0	9.0	106.5	8.7	97.3	13.6
DPP	102.7	17.7	111.4	8.7	110.1	13.4	99.8	16.1
ES	95.4	17.9	102.6	8.8	98.6	3.6	94.8	11.1

[a] Relative recovery

[b] Relative standard deviation

[c] Spiked analyte concentrations: 150 $\mu g\ L^{-1}$ for analytes AP, 2-Cla, 3-tBP, BFB, CNB, EB, BP, 2-NiNap, Nap, Fl, and Ant; 375 $\mu g\ L^{-1}$ for 2-NiPh; 60 $\mu g\ L^{-1}$ for BiPh and Phe; 300 $\mu g\ L^{-1}$ for Py; 750 $\mu g\ L^{-1}$ for 4-OP, DPP, and ES

[d] Four replicates of all extractions were performed

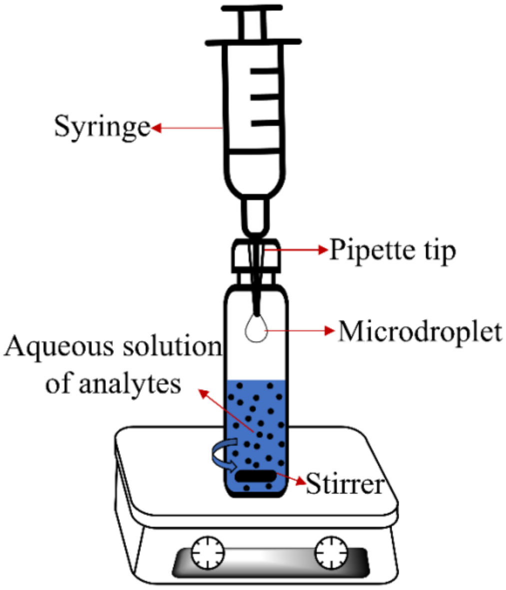
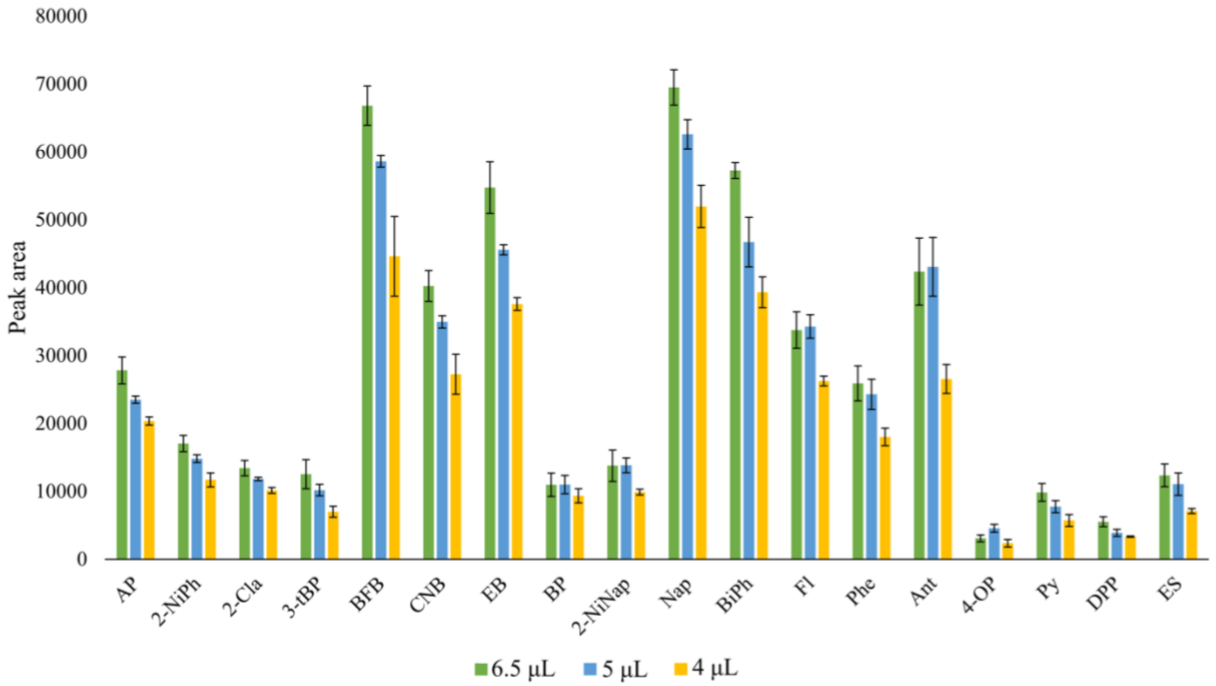


Figure 1. Schematic diagram of HS-SDME experimental setup, where a microsyringe needle equipped with a pipette tip is inserted into the sample headspace through the vial septum. The analyte solution is stirred continuously using a magnetic stirrer. A solvent microdroplet is exposed to the headspace of sample vial by completely depressing the plunger. After extraction, the solvent microdroplet is withdrawn into the microsyringe and injected into the sample loop of the HPLC.



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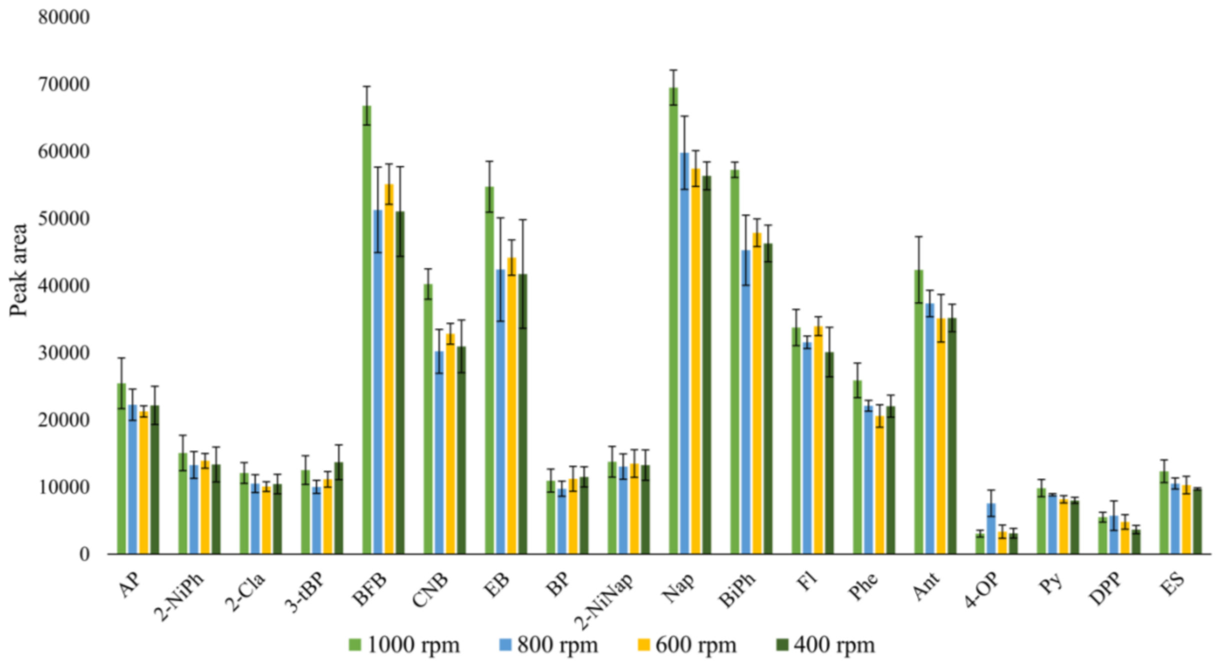
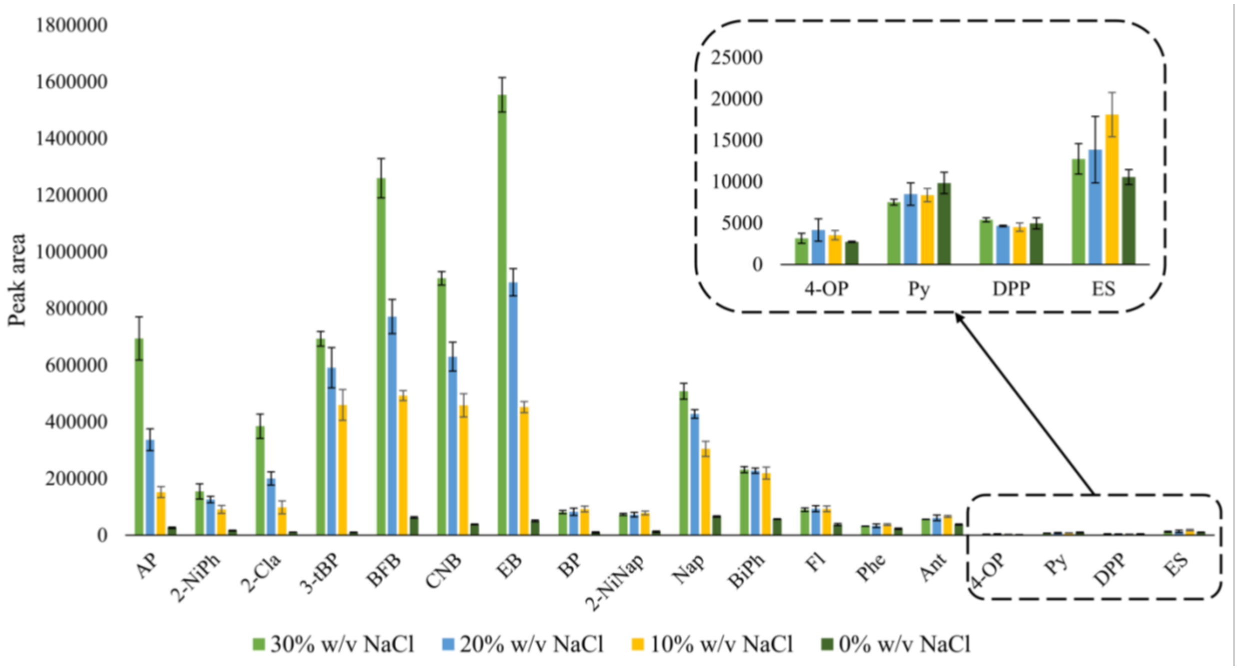


Figure 3. Comparison of the stir rate for the HS-SDME method using the 20 wt% β-CD:LcA SUPRADES extraction solvent. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 µL; temperature: 20 °C; and extraction time: 10 min. Spiked analyte concentrations: 500 µg L^{-1} for AP, BFB, 2-NiNap, 600 µg L^{-1} for 2-NiPh, CNB, BP, 1200 µg L^{-1} for 2-Cla, 4-OP, ES, 2000 µg L^{-1} for 3-tBP, 250 µg L^{-1} for EB, 100 µg L^{-1} for Nap, Fl, Phe, Ant, 40 µg L^{-1} for BiPh, 1000 µg L^{-1} for Py, and 3000 µg L^{-1} for DPP. All extractions were performed in triplicate.



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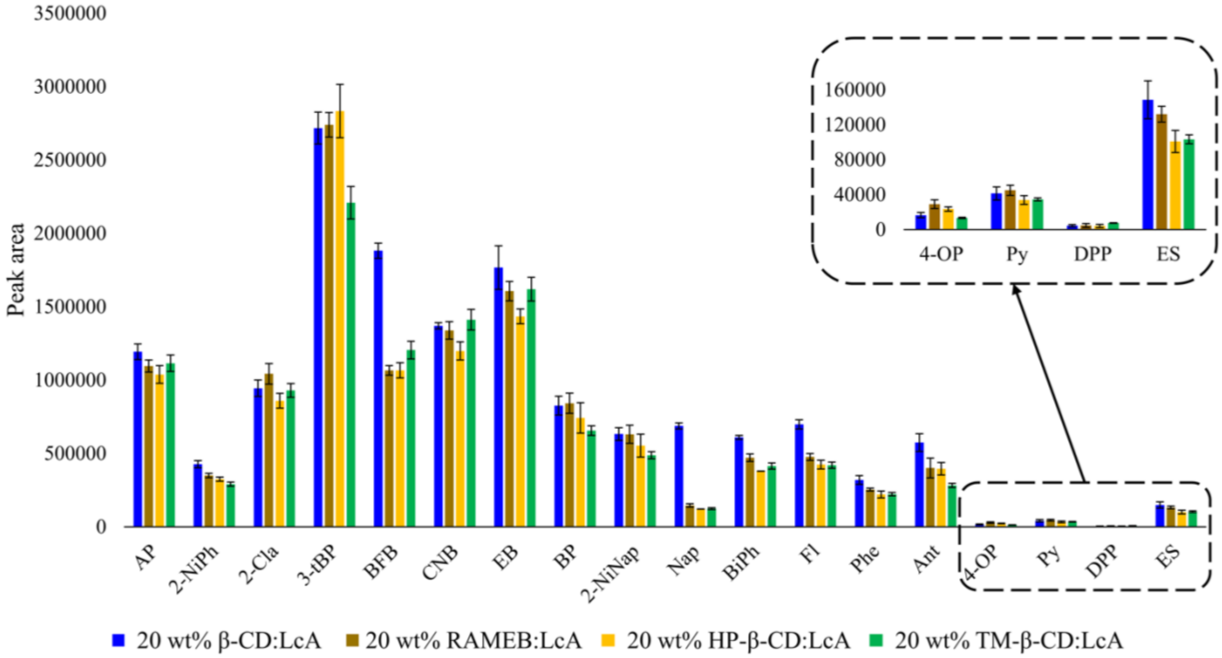

Figure 5. Comparison of peak areas for target analytes using the SUPRADESs comprised of native β-CD or its derivatives (RAMEB, HP-β-CD, and TM-β-CD) as HBA and LcA HBD as extraction solvent. The inset represents the magnified peak areas for the last four analytes. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 µL; stir rate: 1000 rpm; temperature: 20 °C; salt concentration: 30% (w/v); and extraction time: 120 min. Spiked analyte concentrations: 500 µg L^{-1} for AP, BFB, 2-NiNap, 600 µg L^{-1} for 2-NiPh, CNB, BP, 1200 µg L^{-1} for 2-Cla, 4-OP, ES, 2000 µg L^{-1} for 3-tBP, 250 µg L^{-1} for EB, 100 µg L^{-1} for Nap, Fl, Phe, Ant, 40 µg L^{-1} for BiPh, 1000 µg L^{-1} for Py, and 3000 µg L^{-1} for DPP. All extractions were performed in triplicate.

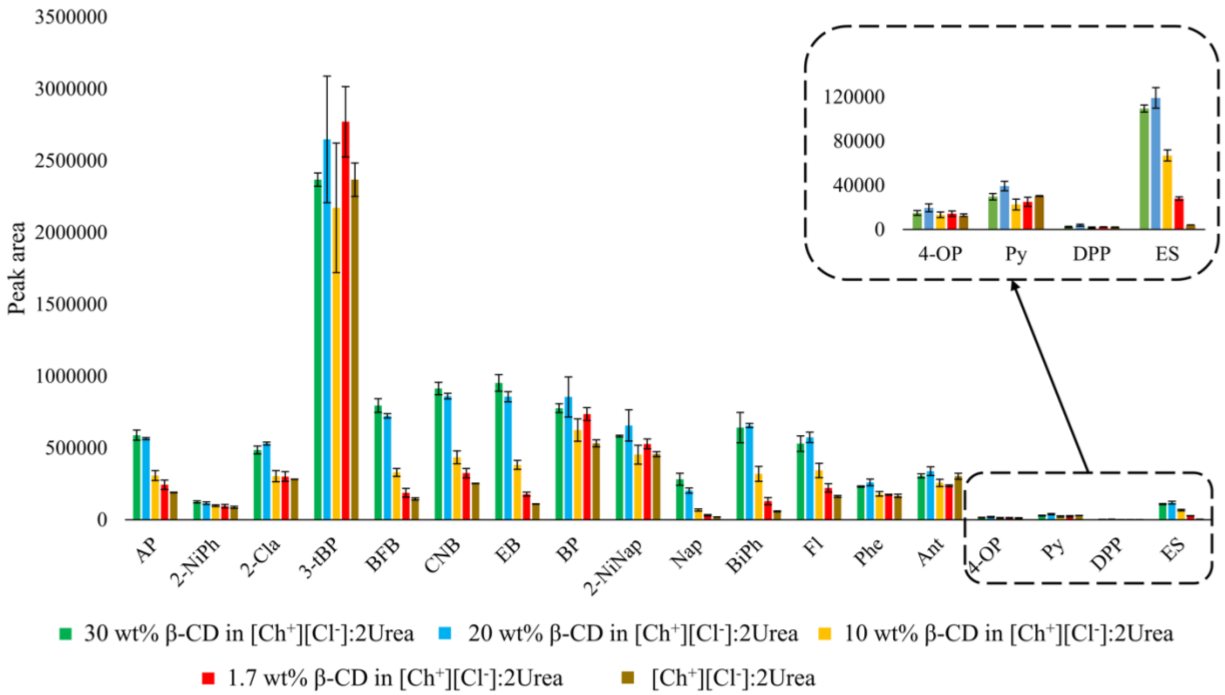

Figure 6. Effect of native β-CD concentration in the $[Ch^+][Cl^-]$:2Urea DES on the extraction performance using HS-SMDE. The inset within the figure shows the enlarged peak areas for 4-OP, Py, DPP, and ES. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 µL; stir rate: 1000 rpm; temperature: 20 °C; salt concentration: 30% (w/v); and extraction time: 120 min. Spiked analyte concentrations: 500 µg L^{-1} for AP, BFB, 2-NiNap, 600 µg L^{-1} for 2-NiPh, CNB, BP, 1200 µg L^{-1} for 2-Cla, 4-OP, ES, 2000 µg L^{-1} for 3-tBP, 250 µg L^{-1} for EB, 100 µg L^{-1} for Nap, Fl, Phe, Ant, 40 µg L^{-1} for BiPh, 1000 µg L^{-1} for Py, and 3000 µg L^{-1} for DPP. All extractions were performed in triplicate.

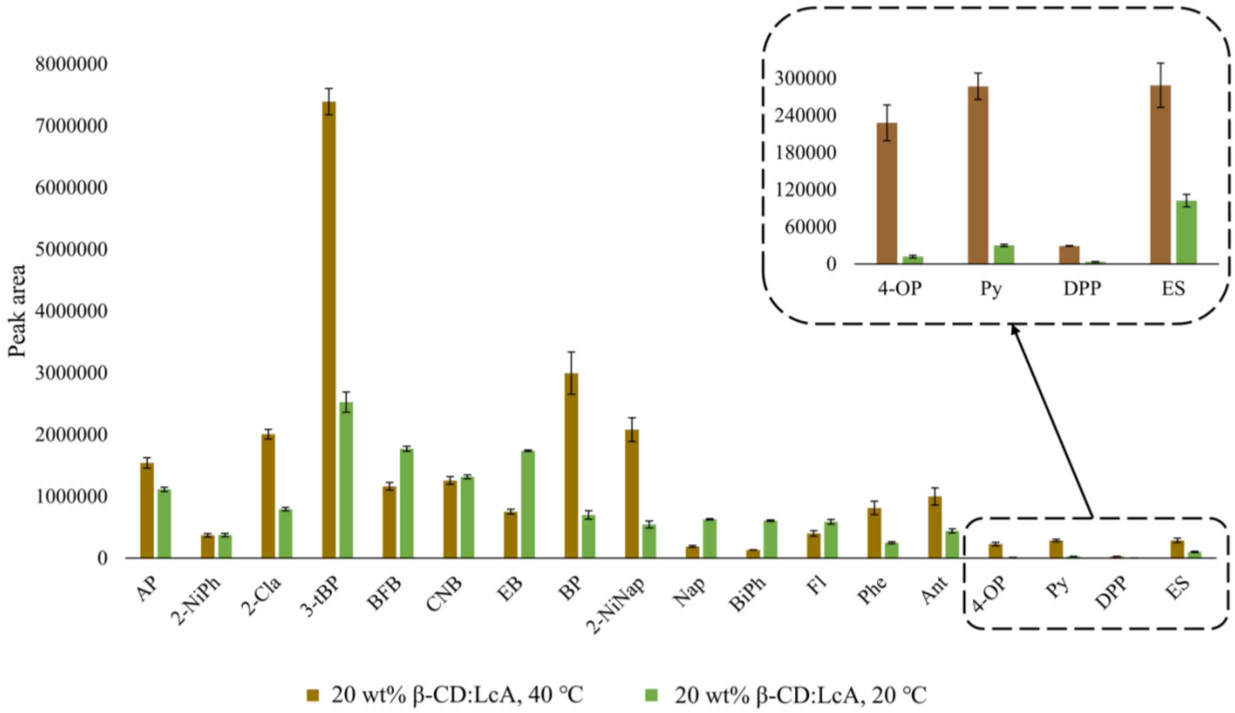

Figure 7. Comparison of the peak areas for studied analytes at an extraction temperature 20 °C and 40 °C using the 20 wt% β-CD:LcA SUPRADES as extraction solvent. The enlarged peak areas for 4-OP, Py, DPP, and ES are shown in the figure inset. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 µL; stir rate: 1000 rpm; salt concentration: 30% (w/v); and extraction time: 90 min. Spiked analyte concentrations: 500 µg L^{-1} for AP, BFB, 2-NiNap, 600 µg L^{-1} for 2-NiPh, CNB, BP, 1200 µg L^{-1} for 2-Cla, 4-OP, ES, 2000 µg L^{-1} for 3-tBP, 250 µg L^{-1} for EB, 100 µg L^{-1} for Nap, Fl, Phe, Ant, 40 µg L^{-1} for BiPh, 1000 µg L^{-1} for Py, and 3000 µg L^{-1} for DPP. All extractions were performed in triplicate.

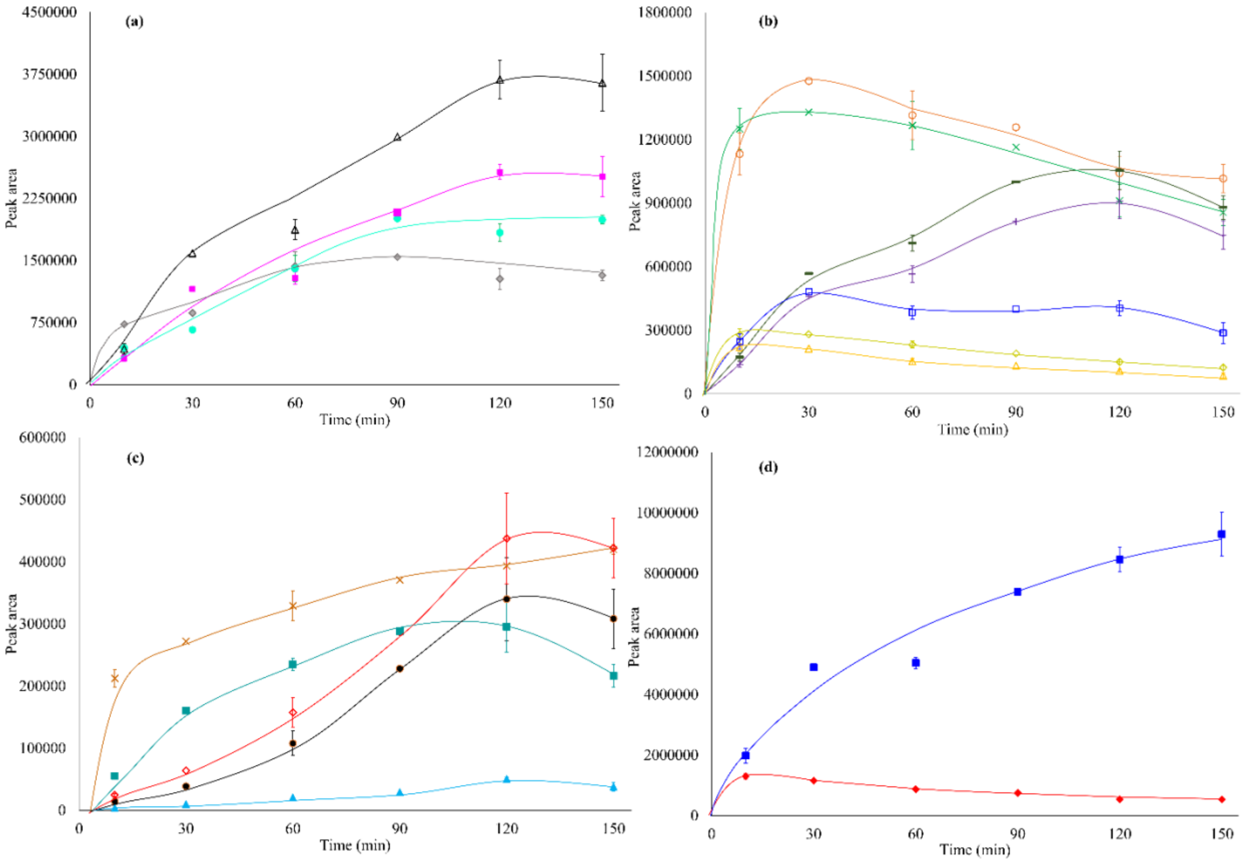

Figure 8. Sorption-time profile of the studied analytes using the 20 wt% β-CD:LcA SUPRADES as extraction solvent at 40 °C. (a) (◈) AP; (●) 2-Cla; (△) BP; and (■) 2-NiNap, (b) (X) BFB; (○) CNB; (◇) Nap; (△) BiPh; (□) Fl; (+) Phe; and (−) Ant, (c) (X) 2-NiPh; (●) 4-OP; (◇) Py; (▲) DPP; and (■) ES, (d) (■) 3-tBP and (◆) EB. Experimental conditions: volume of analyte solution: 10 mL; microdroplet volume: 6.5 µL; stir rate: 1000 rpm; temperature: 40 °C; and salt concentration: 30% (w/v). Spiked analyte concentrations: 500 µg L^{-1} for AP, BFB, 2-NiNap, 600 µg L^{-1} for 2-NiPh, CNB, BP, 1200 µg L^{-1} for 2-Cla, 4-OP, ES, 2000 µg L^{-1} for 3-tBP, 250 µg L^{-1} for EB, 100 µg L^{-1} for Nap, Fl, Phe, Ant, 40 µg L^{-1} for BiPh, 1000 µg L^{-1} for Py, and 3000 µg L^{-1} for DPP. Triplicate extractions were performed at 10, 60, 120, and 150 min and duplicates were carried out at 30 and 90 min.