



A green miniaturized aqueous biphasic system prepared with cholinium chloride and a phosphate salt to extract and preconcentrate personal care products in wastewater samples

Jakub Šulc^{a,b}, Idaira Pacheco-Fernández^{b,c,*}, Juan H. Ayala^b, Petra Bajerová^a, Verónica Pino^{b,c,*}

^a Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, 53210 Pardubice, Czech Republic

^b Laboratorio de Materiales para Análisis Químico (MAT4LL), Departamento de Química, Unidad Departamental de Química Analítica, Universidad de La Laguna (ULL), Tenerife 38206, Spain

^c Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias, Universidad de La Laguna (ULL), Tenerife 38206, Spain

ARTICLE INFO

Article history:

Received 19 February 2021

Revised 23 April 2021

Accepted 27 April 2021

Available online 1 May 2021

Keywords:

aqueous biphasic system
extraction
personal care products
cholinium chloride
high-performance liquid chromatography
wastewater

ABSTRACT

A miniaturized extraction/preconcentration method based on an aqueous biphasic system (μ -ABS) was developed with reagents commonly used as food additives: cholinium chloride (ChCl) as main extraction phase, K_2HPO_4 as salting-out agent, and water as the main component (being the sample for analyses). With the aim of obtaining high enrichment factors, miniaturization, and adequate analytical performance, a point in the biphasic region with the lowest amount of ChCl was selected, corresponding to 1.55% (w/w) of ChCl, 59.5% (w/w) of K_2HPO_4 , and 38.95% (w/w) of water. The green μ -ABS (attending to its main elements and performance mode) was used in combination with high-performance liquid chromatography with diode-array detection (HPLC-DAD) for the determination of 9 personal care products in wastewater samples. The μ -ABS-HPLC-DAD method showed high enrichment factors (up to 100), and quantitative extraction efficiencies for those compounds containing OH groups in their structure, which can undergo hydrogen bonding with ChCl. Thus, limits of quantification down to $0.8 \mu\text{g}\cdot\text{L}^{-1}$ and extraction efficiencies between 66.4 and 108% (concentration levels of 1.3 and $13 \mu\text{g}\cdot\text{L}^{-1}$) were reached for the group of parabens and the UV-filter benzophenone-3. The method is characterized by the use of non-harmful reagents and the absence of organic solvents in the entire sample preparation procedure, while being simple, low-cost, easily compatible with HPLC, and highly efficient.

© 2021 The Author(s). Published by Elsevier B.V.

This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

1. Introduction

Nowadays, the development of analytical methods in accordance with the Green Analytical Chemistry (GAC) guidelines is one of the most important research lines within the Analytical Chemistry scientific community [1]. Among all the stages of the analytical procedure, the unavoidable sample preparation is still the most challenging step since it not only affects the sensitivity and selectivity of the resulting method, but also has a negative impact on its sustainability [2]. Thus, most efforts are shifted to

the development of innovative microextraction techniques to reduce or even eliminate the use of toxic solvents and reagents in sample preparation [3,4], and to the substitution of conventional extraction phases by more environmentally friendly alternatives [5,6].

Given their biocompatibility and high extraction efficiency, aqueous biphasic systems (ABSs) fulfill all these conditions [7]. ABSs are ternary systems composed of water and two water-miscible solutes that can separate in two coexisting water-rich phases (each of them enriched in one of the solutes) at a certain composition [8]. Depending on the composition of the ABSs, analytes present a specific partition behavior, and can be concentrated in one of the solute-rich phases, leading to quantitative extractions in some cases [9,10]. Despite the attractive features of ABS, there are a few studies that work with ABS compositions in which the

* Corresponding authors.

E-mail addresses: jakub.sulc@student.upce.cz (J. Šulc), ipacheco@ull.edu.es (I. Pacheco-Fernández), jayala@ull.edu.es (J.H. Ayala), petra.bajerova@upce.cz (P. Bajerová), veropino@ull.edu.es (V. Pino).

amount (relative composition, % w/w) of the solute that acts as extraction phase is the lowest, and thus, their preconcentration capabilities to develop microextraction strategies are scarcely exploited [7,11,12].

With respect to the composition of ABS intended for extraction or analytical sample preparation, polymers, ionic liquids (ILs), and deep eutectic solvents (DESSs), have been used as solutes to form the extraction phase, while different inorganic or organic salts are commonly used as salting-out agents to induce the phases separation [7]. Due to their impressive tunability and low viscosity, ILs and DESSs have displaced polymers in the application of ABSs as extraction and microextraction systems in most of the recent applications [13]. However, they also present certain limitations.

Thus, there is an increasing concern on the toxicity of ILs, which may compromise the claimed sustainability and biocompatibility of ABSs [14,15]. In this sense, conventional imidazolium-based ILs are substituted by other cations with safer toxicological profiles, such as cholinium [16,17] and guanidinium [11]. Nevertheless, it is still important to take into account the IL synthetic procedure when evaluating the sustainability of the overall extraction method, which, in general, still requires the use of organic solvents [14].

In the case of DESSs, these new solvents decompose once they are added to water [18] and therefore, ABSs containing DESSs present a more complex behavior [19]. Indeed, it has been demonstrated that the interactions between the DESSs components (hydrogen bond donor (HBD) and acceptor (HBA)) are disrupted, and there is a partition of HBD and HBA between the coexisting phases [20,21]. Thus, these ABSs must be considered as quaternary systems in which, depending on their hydrophobicity, the HBA or HBD is enriched in one of the phases, and the HBD or HBA acts as an additive, respectively [19,20,22].

Among all DESSs that have been described and widely used in analytical sample preparation applications, those containing cholinium chloride (ChCl) as HBA are the most commonly used [7]. ChCl shows excellent solubility, low volatility, good biodegradability, low toxicity, and low cost as it is produced in industrial scale as an important additive in animal feed [23]. Taking into account the decomposition issue of DESSs in water, it is possible to form an ABS by mixing water, a salting-out agent, and only one of the components of the DES, such as ChCl. Indeed, the phase diagrams of ABSs composed of ChCl, water and K_2HPO_4 [21,24] or K_3PO_4 [25] as salting-out agents have been already described, demonstrating the feasibility of ChCl-rich phases to act as extraction sustainable "solvents". These systems have been exhaustively characterized, the tie-lines were determined, and their extraction efficiency was assessed only with model target molecules, by the calculation of the partition coefficients of gallic acid [21], stevioside [25], and penicillin G [24]. However, these ABSs have never been used for the development of analytical monitoring methods, including multi-analyte determinations and thorough validation studies, neither for preconcentration schemes.

Thus, this study aims to develop a truly green (free of organic solvents as it only requires food additives produced at large scale) and highly simple (only requiring mixing two food additives – ChCl and K_2HPO_4 – in water) microextraction and preconcentration strategy based on a μ -ABS for the extraction and preconcentration of pollutants (specifically personal care products) in environmental waters. The preconcentration is achieved by preparing the μ -ABS with a composition that ensures a high-volume ratio between sample and ChCl-rich phase, which corresponds to the extraction phase in which the analytes will be preconcentrated. The final low volume of the ChCl-rich phase is totally compatible with typical high-performance liquid chromatographic (HPLC) mobile phases, thus the method is combined with HPLC and diode array detection (DAD).

2. Experimental

2.1. Chemicals, reagents and samples

A group of 9 personal care products (PCPs) including preservatives, UV-filters, and a disinfectant, was selected as target analytes. Methylparaben (MPb), ethylparaben (EPb), propylparaben (PPb), and triclosan (Tr), were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), while benzylparaben (BzPb), benzophenone (BP), benzophenone-3 (BP3), 3-(4-methylbenzylidene) camphor (MBC), and octocrylene (OCR), were purchased from Sigma-Aldrich (Steinheim, Germany). The purity of the standards was higher than 98% for all the analytes. The chemical structure and several physicochemical properties of these analytes are included in Table S1 of the Supplementary Material (SM). HiPerSolv Chromanorm® acetonitrile grade LC-MS (VWR, Llinars del Vallès, Spain) was used for preparation of standard solutions at the following concentrations: 4124 mg·L⁻¹ for MPb, 4064 mg·L⁻¹ for EPb, 4224 mg·L⁻¹ for PPb, 4256 mg·L⁻¹ for BzPb, 1270 mg·L⁻¹ for BP, 1060 mg·L⁻¹ for BP3, 4136 mg·L⁻¹ for Tr, 1500 mg·L⁻¹ for MBC, and 1009 mg·L⁻¹ for OCR. All solutions were protected from the light and refrigerated at 4 °C.

Potassium phosphate dibasic (K_2HPO_4 , ≥ 98%) and cholinium chloride (ChCl, ≥ 98%) were purchased from Honeywell-Fluka™ (Seelze, Germany) and Sigma-Aldrich, respectively. Ultrapure water (resistivity 18.2 MΩ·cm) was obtained from a Milli-Q water purification system (Bedford, MA, USA). Glacial acetic acid was acquired from Merck (Darmstadt, Germany).

Wastewater effluent samples from two different wastewater treatment plants in Tenerife (Canary Islands, Spain) were analyzed. They were provided by an environmental analysis laboratory (SEMALL), which carried out the sampling in 200 mL bottles, while avoiding the formation of bubbles. The samples were kept in a portable fridge until they reached the laboratory, where they were stored protecting from light at 4 °C until the analysis. Before analysis, the samples were left at room temperature and their pH was measured.

2.2. Instrumentation and equipment

An analytical balance from Sartorius (Madrid, Spain) with readability of 0.1 mg was used to weigh all reagents. 50 mL polypropylene centrifuge tubes from Corning® (New York, NY, USA) and 20 mL polypropylene/polyethylene syringes purchased from Sigma-Aldrich were used to perform the microextraction method. A vortex agitation system from Velp® Scientifica (Usmate, Italy), and a centrifuge 5702 from Eppendorf™ (Hamburg, Germany) were also used during the microextraction procedure.

The separation and identification of the target PCPs were performed by HPLC-DAD using a Varian ProStar 230 solvent delivery (Palo Alto, CA, USA) and a Varian ProStar 330 DAD. An Infinity-Lab Poroshell 120 EC-C18 column (3 mm × 150 mm, 2.7 μm) from Agilent Technologies (Santa Clara, CA, USA) was used for the chromatographic separation. The column was protected by a Pelliguard LC-18 guard column (4.6 mm × 20 mm) from Supelco (Bellefonte, PA, USA). The HPLC-DAD system was equipped with a manual injection system consisting of a Rheodyne 7725i valve and a 5 μL loop from Supelco. A 50 μL glass syringe from Hamilton (Reno, NV, USA) was used for the manual injection in the chromatographic system.

2.3. Procedures

2.3.1. HPLC-DAD method

The chromatographic separation of the target PCPs was performed at a constant flow rate of 0.5 mL·min⁻¹ using a binary mo-

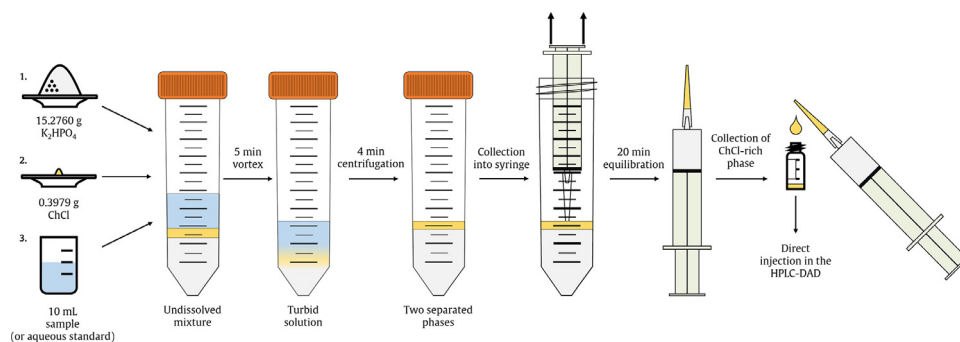


Fig. 1. Scheme of the μ -ABS-HPLC-DAD method under optimum conditions.

bile phase composed of acetonitrile and ultrapure water with 0.1% (v/v) of acetic acid. The elution gradient started at 35% (v/v) of acetonitrile and was kept at this composition for 2 min. Then, it was increased to 39% (v/v) in 2 min and increased again to 100% (v/v) of acetonitrile in 21 min, being finally held at this composition for 5 additional min. For column re-equilibration, the acetonitrile content was decreased to 35% (v/v) in 7 min and kept at this composition for 3 additional minutes before the next injection. The wavelengths used for detection and quantification were: 254 nm for MPb, EPb, PPb, BzPb, BP, and 289 nm for BP3, Tr, MBC, and OCR. The identification of the analytes in the ChCl-rich extracts was performed by comparing their retention time and UV spectra with those of the respective standards.

2.3.2. μ -ABS-based microextraction method

The optimum microextraction method consisted of a μ -ABS formed by ChCl + K_2HPO_4 + water with the following composition: 1.55% (w/w) of ChCl, 59.5% (w/w) of K_2HPO_4 and 38.95% (w/w) of ultrapure water, which corresponds to a point of the biphasic region of the phase diagram [21,24]. Thus, 15.2760 g of K_2HPO_4 was weighted in the 50 mL centrifuge tube, followed by the addition of 0.3979 g of ChCl as extraction phase. Subsequently, 10 mL (which corresponds to ~10 g, considering the density of water) of an aqueous standard in ultrapure water, a non-spiked or a spiked sample (depending on the experiment), were added to the tube. The mixture was briefly and intensively hand shaken to prevent the formation of poorly soluble aggregates and immediately stirred for 5 min using a vortex. Next, the tube was centrifuged at $2509 \times g$ for 4 min to speed up the phase separation. The whole mixture was then collected using a 20 mL syringe with a 200 μ L pipette tip attached to its plain tip. The syringe containing the mixture was left for 20 min for equilibration. After this time, the top ChCl-rich phase containing the analytes was separated by pushing the plunger and collected into a vial. Finally, the extract was directly injected in the HPLC-DAD system. Fig. 1 shows a scheme of the optimum μ -ABS microextraction procedure.

3. Results and discussion

3.1. Selection of μ -ABS composition and region

The composition of an ABS plays a crucial role in phases formation while it also affects the extraction ability of the system [7]. With the aim of demonstrating the potential of ABSs to develop simple, low cost and environmentally friendly extraction and pre-concentration methods, cheap, readily accessible, and truly green reagents were selected as ABS components. Therefore, ChCl is used as extraction phase, while K_2HPO_4 was selected as salting-out inducing agent. Despite other salts have been used in ChCl-based ABS, this phosphate salt was used since it exhibits a relatively

high ability to induce salting-out effect given its capability to create high-charged density inorganic ions and strong interacts with water [26]. A higher salting-out effect of the salt means smaller amount of the salt to achieve the formation of two phases, thus a reduction of the reagent consumption [27]. Moreover, this salt is commonly used as food additive (E340) in dairy products and nutritional supplements [28].

The ABS composed of ChCl, K_2HPO_4 and water has been already described in the literature by different authors, which reported congruential phase diagrams [21,24]. As it is observed in Fig. 2, the phase diagrams were obtained in a range between 1.6 and 59.2% (w/w) of ChCl, and from 3 to 59.3% (w/w) of K_2HPO_4 . In this study, the purpose is to achieve a high pre-concentration rather than getting the maximum extraction efficiency. This can be attained by decreasing the final volume of the extraction phase and by ensuring a high volume ratio between the initial sample and the final extract.

Given these considerations, the sample volume was fixed to 10 mL (~10 g), the K_2HPO_4 amount was fixed at 59.5% (w/w), and the ChCl composition was varied between 1.50 and 1.65% (w/w) to obtain the ABS according to the data from the phase diagrams. As shown in Fig. 2, this corresponds to the bottom right region of the phases diagram where maximum pre-concentration is obtained. The mixtures were vortex stirred for 5 min to initially dissolve all the reagents, while 4 min of centrifugation at $2509 \times g$ were fixed to speed up the phases separation. The mixture composition that yielded two defined phases with a reproducible and easy to handle amount of ChCl-rich phase was the following: 1.55% (w/w) of ChCl, 59.5% (w/w) of K_2HPO_4 and 38.95% (w/w) of ultrapure water, which corresponds to 0.3979 g of ChCl and 15.2760 g of K_2HPO_4 . It is important mentioning that the cost for the extraction step for each sample is less than 1 €, if considering the amounts of reagents required and the prices at which they were purchased (0.06 $\text{€}\cdot\text{g}^{-1}$ and 0.06 $\text{€}\cdot\text{g}^{-1}$ for ChCl and K_2HPO_4 , respectively).

In any case, it is also certain that one of the main drawbacks of working with ABSs with such low amounts (μ -ABS shifted to pre-concentration) is the collection of the top phase that contains the extracted compounds, since it normally forms a thin flat layer on the surface of the salt-rich phase [7]. Different tubes and containers have been designed to overcome this issue, in a manner similar to solve similar problems commonly found in dispersive liquid-liquid microextraction methods that use extraction solvents less dense than water [29,30]. However, it requires the acquisition of custom glass material that is not commercially available, which increases the costs of the method. In this sense, and with the aim of reducing costs while facilitating the sampling of the ChCl-rich phase, the obtained ABS was transferred to a plastic syringe with a pipette tip as needle. As it is observed in Fig. 2, the device allowed the easy collection of the ChCl-rich phase formed, which has a volume of $108 \pm 6 \mu\text{L}$ ($n = 20$) under the ABS composition selected.

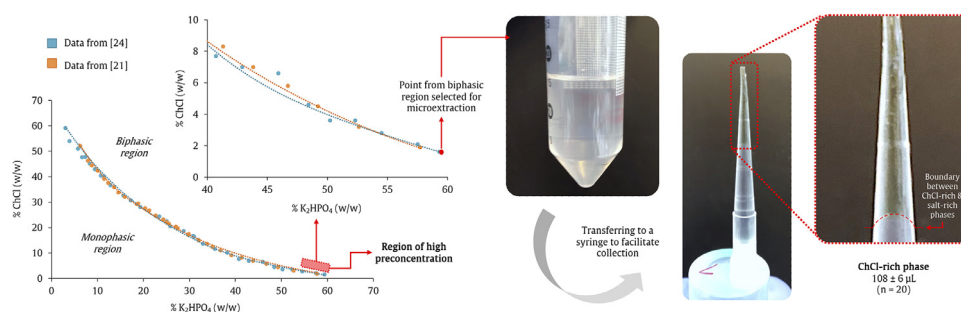


Fig. 2. Phase diagrams of the ABS formed by ChCl, K_2HPO_4 and water, according to the data reported in the literature [21,24], together with photos of the μ -ABS obtained at the selected point from the biphasic region in which the preconcentration is higher: 1.55% (w/w) of ChCl, 59.5% (w/w) of K_2HPO_4 , and 38.95% (w/w) of water.

3.2. Application of ChCl-based μ -ABS for the determination of PCPs

3.2.1. Coupling the μ -ABS with HPLC-DAD

Despite the ABS formed of ChCl, K_2HPO_4 and water has been thoroughly characterized, their analytical applicability only refers to the extraction of penicillin G [24] and gallic acid [21], while other ABSs composed of K_2HPO_4 and DESs containing ChCl as HBA have been applied for the extraction of bovine serum albumin [31], and to evaluate the partition behavior of phenolic compounds, alkaloids, and amino acids [26]. Nevertheless, these studies mainly focus on determining the phase diagrams and assessing the partition of the analytes at different ABS compositions, using an UV-Vis spectrophotometer. Thus, exhaustively validated analytical extraction/preconcentration methods have not been developed with this ABS, and certainly not under the μ -ABS mode.

Therefore, with the purpose of expanding the application of this extraction system, the determination of a group of PCPs in water samples was selected as target application given their categorization as contaminants of emerging concern [32]. Only one study has reported the partition of 4 PCPs (i.e., parabens) in a polymer-based ABS and using an UV-Vis spectrophotometer as detection system [33]. The mix of analytes selected included different groups of PCPs (parabens, UV-filters, and a disinfectant) to cover analytes with different structures and characteristics, as shown in Table S1 of the SM. The determination was carried out by HPLC-DAD, which is a more affordable instrument, and also taking advantage of the hydrophilicity of the ChCl-rich phase obtained with the μ -ABS to ensure compatibility with the chromatographic system. However, the injection of the ChCl-rich phase may affect the chromatographic separation and hamper the correct identification and quantification of the target analytes. Hence, the ChCl-rich phase (without dilution) was directly injected in the HPLC-DAD system, observing a signal at the beginning of the chromatogram, indicating the ChCl is not retained in the column. A small signal was also observed at around 8.3 min, but any of those signals interfered in the determination of the analytes. Fig. 3 shows representative chromatograms obtained after the injection of a PCPs standard in acetonitrile, the ChCl-rich phase after performing the μ -ABS method using 10 mL of ultrapure water, and the ChCl-rich phase of the μ -ABS obtained with 10 mL of a PCPs aqueous standard.

Once the HPLC-DAD separation was optimized, external calibration was carried out. Table S2 of the SM lists several analytical quality parameters of the instrumental method, including the calibration slopes obtained, which will be used to evaluate the preconcentration achieved with the μ -ABS-HPLC-DAD method.

3.2.2. Optimization of the μ -ABS-based microextraction procedure

The elaboration of the phase diagram of an ABS and the selection of the point in the biphasic region directly leads to the amounts of reagents required to reach the desired preconcentration levels. This directly leads to the optimum amounts of reagents

required for the μ -ABS-based microextraction procedure, clearly simplifying the method optimization.

However, there are other experimental conditions that need to be considered since they may affect the μ -ABS extraction performance for the target application. Thus, the vortex stirring time required for the initial solubilization of all the reagents in the water sample, further facilitating the mass transfer of the analytes from the aqueous phase to the ChCl-rich phase, was studied at two levels: 3 and 5 min. The study was performed by preparing the μ -ABS using aqueous standards containing the analytes at $100 \mu\text{g}\cdot\text{L}^{-1}$ (in the water component), followed by the vortex stirring at these times, and 4 min of centrifugation at $2509 \times g$. As it is observed in Fig. 4 A), the peak areas of the entire group of PCPs in the ChCl-rich phase increases with the stirring time (but without observing statistically significant differences for several PCPs), thus 5 min were selected for subsequent experiments. Longer times were not considered to avoid a tedious method and to safeguard the operator's health (vortex stirring involved).

Another variable that can exert and influence is related with the equilibration time of ABSs, which is the time required for the complete phase separation (which ultimately ensures an adequate partition of the analytes between the phases). It has been pointed out that it may be a drawback since times up to 12 h have been reported in several cases [34]. Despite this, many recent studies do not present an optimization of the equilibration time [12,35–37], or do not address this parameter at all [38,39]. In any case, despite long equilibration times are employed in certain applications, the use of times between 10 min [40] and 30 min [11,41] have also been reported as successful.

To address this issue, the equilibration time for the μ -ABS was assessed between 10 and 60 min, intending to obtain the highest extraction efficiency in the shortest time possible. Aqueous standards containing the PCPs at $100 \mu\text{g}\cdot\text{L}^{-1}$ were extracted by the proposed μ -ABS method, and then left to equilibrate at different times. Afterwards, the ChCl-rich phase was collected and directly injected in the HPLC-DAD system, yielding the results shown in Fig. 4 B). It is observed that the peak area for all the analytes initially increases as the time increases, reaching the equilibrium at 20 min. After this time, the peak area remains practically the same for all the analytes, except for OCR, for which the affinity towards the ChCl-rich phase is the lowest. With the aim of benefiting most analytes and avoiding long analysis times, while considering that each chromatographic run takes 30 min, 20 min was selected as optimum equilibration time.

With respect to the pH of the sample, according to the data shown in Table S1 of the SM, the target analytes present pK_a values ranging from 7.56 and 8.31. Previous studies in the literature have pointed out that the pH is not a significant factor when determining these families of analytes as long as it is kept below the pK_a [42]. The ultrapure water used during the optimization and validation of the method had a pH of 6.0–6.5, while the pH of the

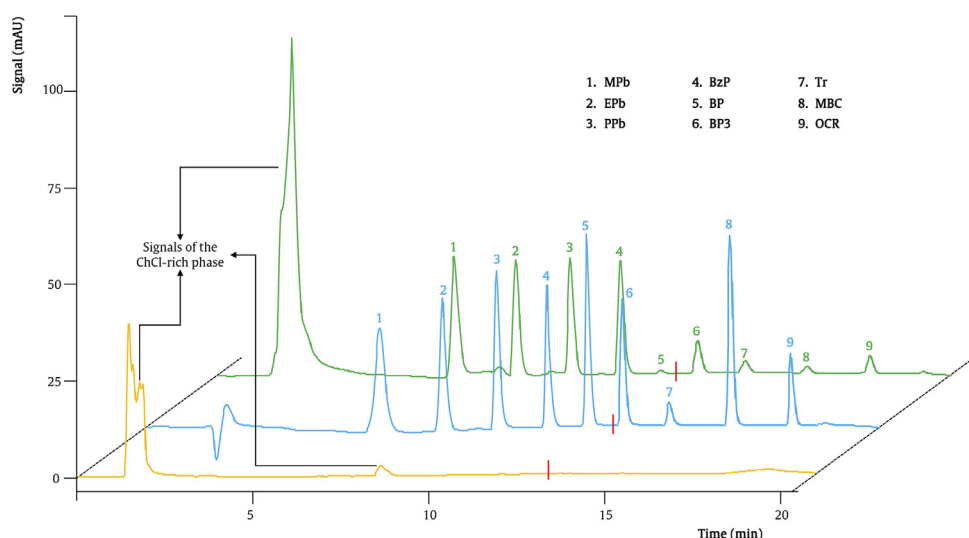


Fig. 3. Representative chromatograms obtained after the injection of: A) a PCPs standard in acetonitrile containing each analyte at $3 \text{ mg}\cdot\text{L}^{-1}$ (blue line), B) the ChCl-rich phase of the μ -ABS obtained using 10 mL of ultrapure water (yellow line), and C) the ChCl-rich phase of the μ -ABS obtained using 10 mL of an aqueous standard containing each PCP at $34 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ (green line). There are offsets of 10% in the signal axis and 7% in the retention times between the different chromatograms. The different red lines indicate a different wavelength channel: 254 nm for analytes 1 to 5, 289 nm for the remaining analytes.

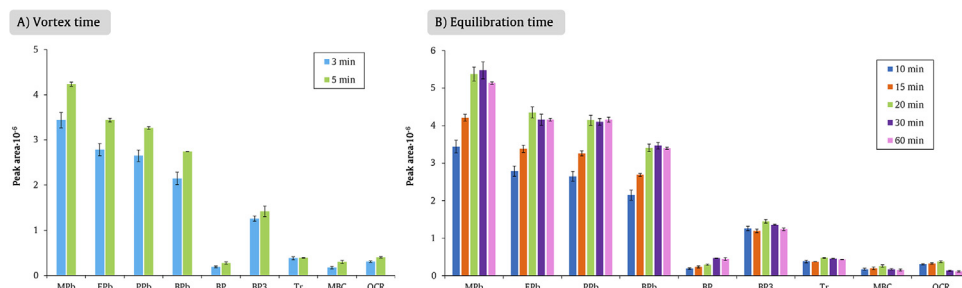


Fig. 4. Influence of A) vortex time and, B) equilibration time on the extraction performance of the μ -ABS-HPLC-DAD method. Experimental conditions: 10 mL of aqueous standard containing each PCP at $100 \text{ }\mu\text{g}\cdot\text{L}^{-1}$, 1.55% (w/w) of ChCl, 59.5% (w/w) of K_2HPO_4 , 4 min of centrifugation at $2509 \times g$, 15 min of equilibration time during the vortex time optimization, and 5 min of vortex time during the equilibration time optimization.

samples analyzed were around 7.3 ± 0.2 ($n = 3$). Thus, as the analytes are non-ionized at these pH values, this parameter was not considered during the optimization of the method. However, this parameter should be taken into account when the samples to be analyzed present a pH higher than the pK_a values of the target analytes.

3.2.3. Analytical performance of the ChCl-based μ -ABS-HPLC-DAD method

Calibration curves of the μ -ABS-HPLC-DAD method were obtained at different concentration ranges depending on the analyte: from 0.80 to $50 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ for the parabens and BP3, and from 3.0 to $50 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ for the remaining PCPs. Several quality analytical parameters are shown in Table 1, including the calibration slope, determination coefficients (R^2), limits of detection (LODs), and limits of quantification (LOQs). LODs were experimentally determined by decreasing the concentration of PCPs in the aqueous standard subjected to the extraction method until a signal-to-noise ratio (S/N) of 3 was obtained. LOQs were first estimated as $10/3$ the LODs and then experimentally verified by preparing standards at the predicted concentrations and performing the microextraction-HPLC-DAD procedure.

In all cases, R^2 values higher than 0.994 were obtained for the evaluated calibration range. The sensitivity, evaluated as the calibration slope, was higher for the parabens. Thus, the LODs and LOQs for this group of analytes were 0.24 and $0.80 \text{ }\mu\text{g}\cdot\text{L}^{-1}$, respec-

tively. With respect to the UV filters and the disinfectant, BP3 exhibited the highest calibration slope, while BP and MBC presented the lowest values. Therefore, LODs of $0.90 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ and LOQs of $3.0 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ were obtained, except for BP3, for which they were $0.24 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ and $0.80 \text{ }\mu\text{g}\cdot\text{L}^{-1}$, respectively.

The analytical performance of the μ -ABS-HPLC-DAD method was evaluated in terms of precision (measured as relative standard deviation, RSD), relative recovery (RR), and extraction performance, at a concentration level of $13 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ for each PCP. The results of these studies are included in Table 2. Intra-day RSDs ranged from 0.42% for BPb to 18% for OCR, while the inter-day RSD values (evaluated at 3 non-consecutive days) were always lower than 16% for the entire group of analytes. Considering their higher sensitivity, the precision of parabens was also evaluated at a concentration level of $1.3 \text{ }\mu\text{g}\cdot\text{L}^{-1}$, obtaining intra-day RSD ranges between 1.8 and 9.9% for PPb, and from 9.2 to 15% for MPb. The RR values were calculated as the ratio between the concentration obtained after applying the μ -ABS-HPLC-DAD method and the initial concentration of the aqueous standard. They ranged from 90.9% for BPb to 122% for MBC.

Regarding the extraction performance, the enrichment factors (E_f) were first calculated as the ratio between the calibration slopes of the entire μ -ABS-HPLC-DAD method (Table 1) and those of the chromatographic method (Table S2 of the SM) [11]. This way, the preconcentration capacity of the method is evaluated at the entire range of concentrations. The E_f values shown in Table 2 indicate that the higher preconcentration was achieved for parabens

Table 1
Quality analytical parameters of the μ -ABS-HPLC-DAD method using aqueous standards of the PCPs.

Analyte	Linear range ($\mu\text{g}\cdot\text{L}^{-1}$)	(Slope \pm tSD ^a) $\cdot 10^{-4}$	$S_{y/x}$ ^b $\cdot 10^{-4}$	R ²	LOD ^c ($\mu\text{g}\cdot\text{L}^{-1}$)	LOQ ^d ($\mu\text{g}\cdot\text{L}^{-1}$)
MPb	0.80 – 50	6.89 \pm 0.13	2.89	0.999	0.24	0.80
EPb	0.80 – 50	5.53 \pm 0.13	2.73	0.999	0.24	0.80
PPb	0.80 – 50	5.23 \pm 0.13	2.90	0.999	0.24	0.80
BPb	0.80 – 50	4.65 \pm 0.15	3.34	0.998	0.24	0.80
BP	3.0 – 50	0.18 \pm 0.01	0.21	0.996	0.90	3.0
BP3	0.80 – 50	1.57 \pm 0.08	1.75	0.996	0.24	0.80
Tr	3.0 – 50	0.64 \pm 0.03	0.55	0.998	0.90	3.0
MBC	3.0 – 50	0.36 \pm 0.03	0.55	0.994	0.90	3.0
OCR	3.0 – 50	0.84 \pm 0.08	1.29	0.994	0.90	3.0

^a 95% confidence limits for $n = 10$ calibration levels ($n - 2$ degrees of freedom) within the calibration range, except for BP, Tr, MBC and OCR, for which $n = 7$ calibration levels.

^b Error of the estimate or standard deviation of the residuals.

^c Limit of detection, experimentally determined by decreasing the concentration until a signal-to-noise ratio of 3 was obtained.

^d Limit of quantification, estimated as 10/3 times the LOD, and experimentally verified with standards prepared at these levels.

Table 2
Precision, relative recovery, and extraction performance of the μ -ABS-HPLC-DAD method evaluated using aqueous standards containing each PCP at 13 $\mu\text{g}\cdot\text{L}^{-1}$.

Analyte	E_F ^a	Intra-day RSD range ^b (%)	Inter-day RSD ^c (%)	RR ^d (%)	E_F ^e	E_R ^f (%)
MPb	86.3	1.1 – 3.2	4.5	100	77.2	83.2
EPb	85.4	1.5 – 4.5	4.8	102	77.0	82.9
PPb	83.6	1.2 – 4.1	4.8	100	74.8	80.6
BPb	88.8	0.42 – 7.7	6.1	90.9	100	108
BP	3.02	2.5 – 7.5	5.8	117	14.5	15.6
BP3	33.5	5.7 – 7.3	6.9	117	40.3	43.4
Tr	76.3	1.4 – 4.3	2.7	105	61.6	66.4
MBC	5.43	5.9 – 9.4	7.1	122	3.44	3.71
OCR	35.2	8.3 – 18	16	110	31.1	33.5

^a Enrichment factor, calculated as the ratio between the calibration slopes.

^b Range of relative standard deviation for intra-day precision ($n = 3$, day 1 to day 3).

^c Relative standard deviation for inter-day precision in 3 non-consecutive days ($n = 9$).

^d Relative recovery.

^e Enrichment factor, calculated using the data obtained with the μ -ABS at 13 $\mu\text{g}\cdot\text{L}^{-1}$ and the chromatographic calibration curves.

^f Extraction efficiency, calculated considering a maximum enrichment factor of 93.

and Tr, ranging from 76.3 for Tr to 88.8 for BPb. E_F values around 30 were obtained for BP3 and OCR, while BP and MBC presented low E_F values, of 2.58 and 5.43, respectively. The E_F values were also determined at 13 $\mu\text{g}\cdot\text{L}^{-1}$ as the ratio between the concentration obtained after applying the μ -ABS-HPLC-DAD method but using the chromatographic calibration curves (Table S2 of the SM) and the initial concentration of the aqueous standard [42]. The values obtained are also included in Table 2 and were in agreement with those calculated using the calibration slopes. Thus, they ranged from 3.44 for MBC to 100 for BPb.

The extraction efficiency (E_R) for the μ -ABS-HPLC-DAD method was calculated as the ratio between the E_F values (obtained at 13 $\mu\text{g}\cdot\text{L}^{-1}$) and the theoretical maximum enrichment factor ($E_{F_{\text{max}}}$) [42]. In this case, $E_{F_{\text{max}}}$ was estimated as a ratio between the volume of the initial aqueous sample (10 mL) and the volume of the ChCl-rich phase that contains the preconcentrated analytes (around 108 \pm 6 μL), obtaining a value of 92.9. The E_R values are shown in Table 2, observing quantitative results for the group of parabens with E_R values higher than 80%. For the remaining PCPs, the E_R varied from 3.71% for MBC to 66.4% for Tr.

Given the results shown in Table 2 and considering the physicochemical characteristics of the analytes (see Table S1 of the SM), it can be observed that there is not a correlation considering the polarity of the molecules. However, it is obvious that significant higher extraction efficiencies are obtained for the analytes containing an OH group in their structure. Thus, parabens, BP3 and Tr exhibit higher affinity towards the ChCl-rich phase used as extraction “solvent” in the proposed method, with E_R values from 43.4%

to 108%. This behavior may be due to the formation of hydrogen bonding between the ChCl as HBA and these analytes, for which the OH group can act as HBD [21,24]. Moreover, it is interesting to mention the possible steric effects of BP3 and Tr due to the position of their OH group, which may hinder the formation of hydrogen bonding and explain the better results obtained for the parabens in comparison with BP3 and Tr. With respect to OCR, the relatively good results obtained (E_R of 33.5%) compared to BP and MBC (15.6% and 3.71%, respectively) may be due to the hydrogen bonding between the nitrile group of OCR (HBA) and the OH group of ChCl (HBD) [21,24].

The analytical features of the μ -ABS-HPLC-DAD method were compared with other methods reported in the literature for the same analytical application and using HPLC with DAD or UV-Vis detection, as shown in Table S3 of the SM [43–51]. It can be observed that a wide variety of sample preparation techniques have been used, including dispersive liquid-liquid microextraction (DLLME) using ILs, DESs or surfactants, and sorbent-based microextraction approaches using polymers or metal-organic frameworks as extraction phases. With respect to the sensitivity, the method proposed in this study presents similar LOQs and good E_F values for the entire group of analytes, and even lower LOQs values for Tr and the target parabens. Moreover, despite the relatively long equilibration time required in the μ -ABS procedure, other methods report sample preparation time longer than 60 min [45,51].

Apart from the adequate analytical features, the main advantage of the proposed method is the absence of organic solvents and toxic reagents during all the sample preparation procedure (from

Table 3

Several quality analytical parameters of the μ -ABS-HPLC-DAD method in wastewater samples obtained by the standard addition calibration method, together with the concentrations in the samples predicted by extrapolation.

Sample 1	Sample 2			Predicted concentration $\pm S_{XE}^d$ ($\mu\text{g}\cdot\text{L}^{-1}$)	(Slope $\pm t\text{-SD}^a$) $\cdot 10^{-4}$	$S_{y/x}^b \cdot 10^{-4}$	R^2	Predicted concentration $\pm S_{XE}^c$ ($\mu\text{g}\cdot\text{L}^{-1}$)
	Analyte	(Slope $\pm t\text{-SD}^a$) $\cdot 10^{-4}$	$S_{y/x}^b \cdot 10^{-4}$					
MPb	6.12 \pm 0.47	2.76	0.998	n.d.	6.05 \pm 0.23	1.45	0.999	0.4 \pm 0.1
EPb	4.94 \pm 0.13	0.77	0.999	n.d.	4.32 \pm 0.30	1.83	0.998	0.8 \pm 0.2
PPb	4.66 \pm 0.23	1.27	0.999	0.8 \pm 0.1	4.43 \pm 0.39	2.22	0.998	n.d.
BPb	4.07 \pm 0.26	1.54	0.998	3.3 \pm 0.2	3.42 \pm 0.55	3.40	0.987	4.6 \pm 0.6
BP	0.50 \pm 0.11	0.65	0.981	9 \pm 1	0.64 \pm 0.15	0.90	0.979	12 \pm 1
BP3	1.97 \pm 0.25	1.49	0.994	n.d.	2.06 \pm 0.14	0.84	0.998	n.d.
Tr	0.59 \pm 0.02	0.13	0.999	0.5 \pm 0.1	0.51 \pm 0.02	0.14	0.999	0.4 \pm 0.1
MBC	0.69 \pm 0.16	0.97	0.978	n.d.	0.73 \pm 0.21	1.16	0.977	n.d.
OCR	1.18 \pm 0.46	2.67	0.994	n.d.	0.81 \pm 0.16	1.00	0.981	1.5 \pm 0.7

^a 95% confidence limits for $n = 6$ calibration levels ($n - 2$ degrees of freedom) within the calibration range of 0–22 $\mu\text{g}\cdot\text{L}^{-1}$.

^b Error of the estimate or standard deviation of the residuals.

^c Uncertainty in the prediction of the concentration, calculated using the following equation: $S_{XE} = \frac{S_{y/x}}{b} \cdot \sqrt{\frac{1}{n} + \frac{y^2}{b^2 \cdot \sum_{i=1}^n (x_i - \bar{x})^2}}$

the synthesis of the extraction phase to the extraction phase itself). Thus, despite a DLLME method using a DES and without requiring organic solvents has also been reported [49], the components of the DES (i.e., menthol and decanoic acid) have been labelled as irritating and harmful to aquatic environment according to the European Chemicals Agency [52,53], while hazardous effects have not been reported for ChCl and K_2HPO_4 [54,55]. Therefore, the μ -ABS-HPLC-DAD method is characterized by using easily accessible, cheap, and environmentally friendly reagents, while still providing good extraction performance and selectivity for specific groups of analytes.

3.2.4. Analysis of wastewater samples

Wastewater samples from two different wastewater treatment plants were analyzed by the standard addition method (considering the complexity of these samples) using the μ -ABS-HPLC-DAD procedure. Thus, the calibration curves were obtained by spiking the samples with the PCPs in a concentration range of 0 – 22 $\mu\text{g}\cdot\text{L}^{-1}$. Several analytical parameters of these calibrations are included in Table 3, showing that R^2 values ranging from 0.977 to 0.999 were obtained.

The calibration slopes obtained with the standard addition method (Table 3) were compared with those obtained when using standards in ultrapure water (Table 1) to evaluate the matrix effects. Figure S1 of the SM shows that the sensitivity decreases for the group of parabens and Tr in the samples, while a slight enhancement of the signal is observed for the remaining analytes (i.e., BP, BP3, MBC and OCR) when the method is performed in the wastewater samples. This can be also observed in Figure S2, with representative chromatograms obtained with the μ -ABS-HPLC-DAD method for a standard in ultrapure water (34 $\mu\text{g}\cdot\text{L}^{-1}$) and for the spiked wastewater samples (22 $\mu\text{g}\cdot\text{L}^{-1}$).

Matrix effects have been mainly associated to alterations of the ionization efficiency when using mass spectrometry detection [56]. In this particular case using UV-Vis detection, this behavior may be due to (i) the extraction of other substances that are present in the wastewater samples and present higher affinity towards the ChCl-rich phase, which leads to a suppressing matrix effect, and (ii) the co-extraction and co-elution of interfering compounds from the wastewater samples, which may lead to an enhancement of the signal. A statistical test was carried out to compare the calibration slopes according to Andrade and Estévez-Pérez [57], and the results are included in Tables S5 and S6 of the SM. The Student's t test revealed there is matrix effects for all the analytes in both samples, except for OCR in the sample 2, for which the calibration slopes present statistically the same value: $(0.84 \pm 0.08) \cdot 10^4$ versus $(0.81 \pm 0.16) \cdot 10^4$ for ultrapure water and wastewater, respectively.

Therefore, it is advisable the use of internal standards, the standard addition method or other types of calibrations that consider the matrix effects with the proposed μ -ABS-HPLC-DAD, for compensation of matrix effects when analyzing complex water samples.

In order to carry out the determination of the analytes in the sample, the P values were calculated to establish whether the intercepts of the calibration curves of the standard addition method were zero. According to the results of this statistical test, which are included in Table S4 of the SM, the intercepts for several PCPs were different to zero and their concentration could be calculated by extrapolation of the calibration curves. Thus, the predicted concentrations for the detected PCPs are shown in Table 3. In general, most analytes could be quantified in the wastewater sample 2, with concentrations ranging from 0.4 ± 0.1 $\mu\text{g}\cdot\text{L}^{-1}$ for MPb and Tr to 12 ± 1 $\mu\text{g}\cdot\text{L}^{-1}$ for BP. In the case of wastewater sample 1, only PPb, BPb, BP and Tr could be quantified at concentrations up to 9 ± 1 $\mu\text{g}\cdot\text{L}^{-1}$ for BP. It is important to point out that BP was the analyte determined with the highest concentration in both samples, but it is also the analyte for which enhancing matrix effect was observed. This indicated the possible co-elution of other interfering compounds, which was confirmed by comparing the UV-Vis spectra of the sample and a standard solution at the retention time for BP. Thus, the quantification data for this analyte should be confirmed with other detection techniques. (i.e., mass spectrometry). Moreover, in the case of MBC and BP3, which are the analytes with the lowest E_F and E_R values, they could not be detected in the samples.

4. Conclusions

A μ -ABS composed of ChCl, K_2HPO_4 and water was used for the first time for the development of a microextraction procedure with environmentally friendly and high enrichment characteristics. Thus, a point of the biphasic region with the lowest amount of ChCl was selected to ensure preconcentration, while still facilitating the collection of the ChCl-rich phase that acts as extraction phase where the analytes are enriched.

The μ -ABS procedure only required less than 0.4 g of ChCl, around 15 g of K_2HPO_4 , few minutes of vortex stirring, and only 20 min of equilibration time. It was easily combined with HPLC-DAD for the determination of 9 contaminants of emerging concern with different chemical structures, including parabens, UV filters and a disinfectant, being the first time this type of μ -ABSs is used for a multi-residue determination. The method was validated, showing good precision and high enrichment factors for most analytes. Indeed, quantitative extraction efficiencies were achieved for the group of parabens, while E_R values higher than 40% were obtained

for BP3 and Tr. The ChCl-rich phase demonstrated certain selectivity towards the analytes with OH groups in their structures due to the formation of hydrogen bonds that enhance the affinity and partition to the ChCl-extraction phase.

Thanks to the high enrichment factors, and despite DAD being used as detection technique, the proposed method provided low LOQs and several of the target PCPs could be quantified in two different wastewater samples by the standard addition method.

As advantages, the μ -ABS-HPLC-DAD method is characterized for its greenness given the absence of organic solvents in the entire sample preparation procedure and the use of non-toxic reagents in comparison with the methods reported in the literature dealing with the same application. This study demonstrates the feasibility of exploiting ABS characteristics to develop sustainable and highly efficient monitoring methods by selecting the adequate components and composition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Jakub Šulc: Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Idaira Pacheco-Fernández:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing. **Juan H. Ayala:** Investigation, Supervision, Writing - review & editing. **Petra Bajerová:** Investigation, Supervision, Writing - review & editing. **Verónica Pino:** Conceptualization, Methodology, Validation, Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.

Acknowledgments

J.Š. thanks the Erasmus+ Programme. I. P.-F. thanks the Canary Agency of Research and Innovation (ACIISI), co-funded by the European Social Fund, for her FPI PhD fellowship. V.P. thanks the Spanish Ministry of Economy and Competitiveness (MINECO) for the project MAT2017-89207-R, and the ACIISI for the project [ProID2020010089](https://doi.org/10.1016/j.chroma.2021.462219).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.chroma.2021.462219](https://doi.org/10.1016/j.chroma.2021.462219).

References

- [1] P.M. Nowak, R. Wietecha-Postuszyn, J. Pawliszyn, White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality, *Trac-Trends Anal. Chem.* 138 (2021) 116223, doi:[10.1016/j.trac.2021.116223](https://doi.org/10.1016/j.trac.2021.116223).
- [2] S. Armenta, S. Garrigues, F.A. Esteve-Turrillas, M. de la Guardia, Green extraction techniques in green analytical chemistry, *Trac-Trends Anal. Chem.* 116 (2019) 248–253, doi:[10.1016/j.trac.2019.03.016](https://doi.org/10.1016/j.trac.2019.03.016).
- [3] Y. Yamini, M. Rezaeizadeh, S. Seidi, Liquid-phase microextraction – the different principles and configurations, *Trac-Trends Anal. Chem.* 112 (2019) 264–272, doi:[10.1016/j.trac.2018.06.010](https://doi.org/10.1016/j.trac.2018.06.010).
- [4] M.J. Trujillo-Rodríguez, I. Pacheco-Fernández, I. Taima-Mancera, J.H. Ayala, V. Pino, Evolution and current advances in sorbent-based microextraction configurations, *J. Chromatogr. A* 1634 (2020) 461670, doi:[10.1016/j.chroma.2020.461670](https://doi.org/10.1016/j.chroma.2020.461670).
- [5] I. Pacheco-Fernández, V. Pino, Green solvents in analytical chemistry, *Curr. Opin. Green Sustain. Chem.* 18 (2019) 42–50, doi:[10.1016/j.cogsc.2018.12.010](https://doi.org/10.1016/j.cogsc.2018.12.010).
- [6] F. Pena-Pereira, A. Kloskowski, J. Namieśnik, Perspectives on the replacement of harmful organic solvents in analytical methodologies: a framework toward the implementation of a generation of eco-friendly alternatives, *Green Chem.* 17 (2015) 3687–3705, doi:[10.1039/c5gc00611b](https://doi.org/10.1039/c5gc00611b).
- [7] I. Pacheco-Fernández, R. González-Martín, F.A. e Silva, M.G. Freire, V. Pino, Insights into coextractive and dispersive liquid-phase microextraction strategies with hydrophilic media – a review, *Anal. Chim. Acta* 1143 (2021) 225–249, doi:[10.1016/j.aca.2020.08.022](https://doi.org/10.1016/j.aca.2020.08.022).
- [8] M. Iqbal, Y. Tao, S. Xie, Y. Zhu, D. Chen, X. Wang, L. Huang, D. Peng, A. Sattar, M.A. Shabbir, H.I. Hussain, S. Ahmed, Z. Yuan, Aqueous two-phase system (ATPS): an overview and advances in its applications, *Biol. Proced. Online* 18 (2016) 18, doi:[10.1186/s12575-016-0048-8](https://doi.org/10.1186/s12575-016-0048-8).
- [9] R.C. Assis, B.A. de Araújo Faria, C.L. Caldeira, A.B. Mageste, L.R. de Lemos, G.D. Rodrigues, Extraction of arsenic (III) in aqueous two-phase systems: a new methodology for determination and speciation analysis of inorganic arsenic, *Microchem. J.* 147 (2019) 429–436, doi:[10.1016/j.microc.2019.03.058](https://doi.org/10.1016/j.microc.2019.03.058).
- [10] H.F.D. Almeida, M.G. Freire, I.M. Marrucho, Improved monitoring of aqueous samples by the preconcentration of active pharmaceutical ingredients using ionic-liquid-based systems, *Green Chem.* 19 (2017) 4651–4659, doi:[10.1039/C7GC01954H](https://doi.org/10.1039/C7GC01954H).
- [11] R. González-Martín, I. Pacheco-Fernandez, J.H. Ayala, A.M. Afonso, V. Pino, Ionic liquid-based miniaturized aqueous biphasic system to develop an environmental-friendly analytical preconcentration method, *Talanta* 203 (2019) 305–313, doi:[10.1016/j.talanta.2019.05.083](https://doi.org/10.1016/j.talanta.2019.05.083).
- [12] T.B.V. Dinis, H. Passos, D.L.D. Lima, A.C.A. Sousa, J.A.P. Coutinho, V.I. Esteves, M.G. Freire, Simultaneous extraction and concentration of water pollution tracers using ionic-liquid-based systems, *J. Chromatogr. A* 1559 (2018) 69–77, doi:[10.1016/j.chroma.2017.07.084](https://doi.org/10.1016/j.chroma.2017.07.084).
- [13] I.S. Cardoso, A.Q. Pedro, A.J.D. Silvestre, M.G. Freire, *Green Analytical Chemistry, Green Chemistry and Sustainable Technology*, Springer, Singapore (2019) 203–240, doi:[10.1007/978-981-13-9105-7_8](https://doi.org/10.1007/978-981-13-9105-7_8).
- [14] M. Sivapragasam, M. Moniruzzaman, M. Goto, An overview on the toxicological properties of ionic liquids towards microorganisms, *Biotechnol. J.* 15 (2020) 1900073, doi:[10.1002/biot.201900073](https://doi.org/10.1002/biot.201900073).
- [15] P. Kumari, V.V.S. Pillai, A. Benedetto, Mechanisms of action of ionic liquids on living cells: the state of the art, *Biophys. Rev.* 12 (2020) 1187–1215, doi:[10.1007/s12551-020-00754-w](https://doi.org/10.1007/s12551-020-00754-w).
- [16] C.M.S.S. Neves, M. Figueiredo, P.M. Reis, A.C.A. Sousa, A.C. Cristóvão, M.B. Fideiro, L.P.N. Rebelo, J.A.P. Coutinho, J.M.S.S. Esperança, M.G. Freire, Simultaneous separation of antioxidants and carbohydrates from food wastes using aqueous biphasic systems formed by cholinium-derived ionic liquids, *Front. Chem.* 7 (2019) 459, doi:[10.3389/fchem.2019.00459](https://doi.org/10.3389/fchem.2019.00459).
- [17] M.R. Almeida, D.C.V. Belchior, P.J. Carvalho, M.G. Freire, Liquid-liquid equilibrium and extraction performance of aqueous biphasic systems composed of water, cholinium carboxylate ionic liquids and K₂CO₃, *J. Chem. Eng. Data* 64 (2019) 4946–4955, doi:[10.1021/acs.jced.9b00452](https://doi.org/10.1021/acs.jced.9b00452).
- [18] A. Shishov, A. Gorbunov, L. Moskin, A. Bulatov, Decomposition of deep eutectic solvents based on choline chloride and phenol in aqueous phase, *J. Mol. Liq.* 301 (2020) 112380, doi:[10.1016/j.molliq.2019.112380](https://doi.org/10.1016/j.molliq.2019.112380).
- [19] H. Passos, D.J.P. Tavares, A.M. Ferreira, M.G. Freire, J.A.P. Coutinho, Are aqueous biphasic systems composed of deep eutectic solvents ternary or quaternary systems? *ACS Sustain. Chem. Eng.* 4 (2016) 2881–2886, doi:[10.1021/acssuschemeng.6b00485](https://doi.org/10.1021/acssuschemeng.6b00485).
- [20] F.O. Farias, J.F.B. Pereira, J.A.P. Coutinho, L. Igarashi-Mafra, M.R. Mafra, Understanding the role of the hydrogen bond donor of the deep eutectic solvents in the formation of the aqueous biphasic systems, *Fluid Ph. Equilibria* 503 (2020) 112319, doi:[10.1016/j.fluid.2019.112319](https://doi.org/10.1016/j.fluid.2019.112319).
- [21] F.O. Farias, F.H.B. Sosa, L. Igarashi-Mafra, J.A.P. Coutinho, M.R. Mafra, Study of the pseudo-ternary aqueous two-phase systems of deep eutectic solvent (choline chloride:sugars) + K₂HPO₄ + water, *Fluid Ph. Equilibria* 448 (2017) 143–151, doi:[10.1016/j.fluid.2017.05.018](https://doi.org/10.1016/j.fluid.2017.05.018).
- [22] F.O. Farias, H. Passos, A.S. Lima, M.R. Mafra, J.A.P. Coutinho, Is it possible to create ternary-like aqueous biphasic systems with deep eutectic solvents? *ACS Sustain. Chem. Eng.* 5 (2017) 9402–9411, doi:[10.1021/acssuschemeng.7b02514](https://doi.org/10.1021/acssuschemeng.7b02514).
- [23] P. Panas, C. Lopes, M.O. Cerri, S.P.M. Ventura, V.C. Santos-Ebinuma, J.F.B. Pereira, Purification of clavulanic acid produced by *Streptomyces clavuligerus* via submerged fermentation using polyethylene glycol/cholinium chloride aqueous two-phase systems, *Fluid Ph. Equilibria* 150 (2017) 42–50, doi:[10.1016/j.fluid.2017.07.005](https://doi.org/10.1016/j.fluid.2017.07.005).
- [24] M. Osloob, A. Roosta, Experimental study of choline chloride and K₂HPO₄ aqueous two-phase system, and its application in the partitioning of penicillin G, *J. Mol. Liq.* 279 (2019) 171–176, doi:[10.1016/j.molliq.2019.01.134](https://doi.org/10.1016/j.molliq.2019.01.134).
- [25] T. Abolghasembeyk, S. Shahriari, M. Salehifar, Extraction of stevioside using aqueous two-phase systems formed by choline chloride and K₃PO₄, *Food. Bioproc. Process* 102 (2017) 102–115, doi:[10.1016/j.fbp.2016.12.011](https://doi.org/10.1016/j.fbp.2016.12.011).
- [26] F.O. Farias, H. Passos, M.G. Sanglard, L. Igarashi-Mafra, J.A.P. Coutinho, M.R. Mafra, Designer solvent ability of alcohols in aqueous biphasic systems composed of deep eutectic solvents and potassium phosphate, *Sep. Purif. Technol.* 200 (2018) 84–93, doi:[10.1016/j.seppur.2018.02.029](https://doi.org/10.1016/j.seppur.2018.02.029).
- [27] M.G. Freire, A.F.M. Cláudio, J.M.M. Araújo, J.A.P. Coutinho, I.M. Marrucho, J.N.C. Lopes, L.P.B. Rebelo, Aqueous biphasic systems: a boost brought about by using ionic liquids, *Chem. Soc. Rev.* 41 (2012) 4966–4995, doi:[10.1039/C2CS35151J](https://doi.org/10.1039/C2CS35151J).
- [28] CODEX alimentarius, Food and agriculture organization of the United Nations/World Health Organization, 2020. <http://www.fao.org/gsaonline/additives/index.html?lang=en>.
- [29] M. Saraji, M.K. Boroujeni, Recent development in dispersive liquid-liquid microextraction, *Anal. Bioanal. Chem.* 406 (2014) 2027–2066, doi:[10.1007/s00216-013-7467-z](https://doi.org/10.1007/s00216-013-7467-z).

- [30] M. Martinefski, N. Feizi, M.L. Lunar, S. Rubio, Supramolecular solvent-based high-throughput sample treatment platform for the biomonitoring of PAH metabolites in urine by liquid chromatography-tandem mass spectrometry, *Chemosphere* 237 (2019) 124525, doi:10.1016/j.chemosphere.2019.124525.
- [31] K. Xu, Y. Wang, Y. Huang, N. Li, Q. Wen, A green deep eutectic solvent-based aqueous two-phase system for protein extracting, *Anal. Chim. Acta* 864 (2015) 9–20, doi:10.1016/j.aca.2015.01.026.
- [32] I.Y. López-Pacheco, A. Silva-Núñez, C. Salinas-Salazar, A. Arévalo-Gallegos, L.A. Lizarazo-Holguin, D. Barceló, H.M.N. Iqbal, R. Parra-Saldívar, Anthropogenic contaminants of high concern: existence in water resources and their adverse effects, *Sci. Total Environ.* 690 (2019) 1068–1088, doi:10.1016/j.scitotenv.2019.07.052.
- [33] R.T. Gonsalves, F.O. Farias, M.R. Mafra, L. Igarashi-Mafra, Aqueous biphasic systems as a suitable route to remove and concentrate Parabens from aqueous media, *Ind. Eng. Chem. Res* 59 (2020) 21882–21893, doi:10.1021/acs.iecr.0c04102.
- [34] J. An, M.J. Trujillo-Rodríguez, V. Pino, J.L. Anderson, Non-conventional solvents in liquid phase microextraction and aqueous biphasic systems, *J. Chromatogr. A* 1500 (2017) 1–23, doi:10.1016/j.chroma.2017.04.012.
- [35] F.O. Farias, G. Oliveira, F.C. Leal, J.P. Wojeicchowski, C.I. Yamamoto, L. Igarashi-Mafra, M.R. Mafra, Cholinium chloride effect on ethanol-based aqueous biphasic systems: liquid-liquid equilibrium and biomolecules partition behavior, *Fluid Ph. Equilibria* 505 (2020) 112363, doi:10.1016/j.fluid.2019.112363.
- [36] M.O. Toledo, F.O. Farias, L. Igarashi-Mafra, M.R. Mafra, Salt effect on ethanol-based aqueous biphasic systems applied to alkaloids partition: an experimental and theoretical approach, *J. Chem. Eng. Data* 64 (2019) 2018–2026, doi:10.1021/acs.jced.8b01024.
- [37] T. Trtič-Petrović, A. Dimitrijević, N. Zdošek, Jelena Đorđević, A. Tot, M. Vraneš, S. Gadžurić, New sample preparation method based on task-specific ionic liquids for extraction and determination of copper in urine and wastewater, *Anal. Bioanal. Chem.* 410 (2018) 155–166, doi:10.1007/s00216-017-0705-z.
- [38] S. Ji, Y. Wang, X. Shao, C. Zhu, Y. Lin, S. Gao, D. Tang, Extraction and purification of triterpenoid saponins from licorice by ionic liquid based extraction combined with in situ alkaline aqueous biphasic systems, *Sep. Purif. Technol.* 247 (2020) 116953, doi:10.1016/j.seppur.2020.116953.
- [39] K.Y. Chong, R. Stefanova, J. Zhang, M.S. Brooks, Aqueous two-phase extraction of bioactive compounds from haskap leaves (*Lonicera caerulea*): comparison of salt/ethanol and sugar/propanol systems, *Sep. Purif. Technol.* 252 (2020) 117399, doi:10.1016/j.seppur.2020.117399.
- [40] D.C.V. Belchior, M.V. Quental, M.M. Pereira, C.M.N. Mendonça, I.F. Duarte, M.G. Freire, Performance of tetraalkylammonium-based ionic liquids as constituents of aqueous biphasic systems in the extraction of ovalbumin and lysozyme, *Sep. Purif. Technol.* 233 (2020) 116019, doi:10.1016/j.seppur.2019.116019.
- [41] Q. Zeng, Y. Wang, N. Li, X. Huang, X. Ding, X. Lin, S. Huang, X. Liu, Extraction of proteins with ionic liquid aqueous two-phase system based on guanidine ionic liquid, *Talanta* 116 (2013) 209–416, doi:10.1016/j.talanta.2013.06.011.
- [42] I. Pacheco-Fernández, V. Pino, J.H. Ayala, A.M. Afonso, Guanidinium ionic liquid-based surfactants as low cytotoxic extractants: analytical performance in an in-situ dispersive liquid-liquid microextraction method for determining personal care products, *J. Chromatogr. A* 1559 (2018) 102–111, doi:10.1016/j.chroma.2017.04.061.
- [43] K.Z. Mousavi, Y. Yamini, S. Seidi, Dispersive liquid-liquid microextraction using magnetic room temperature ionic liquid for extraction of ultra-trace amounts of parabens, *New. J. Chem.* 42 (2018) 9735–9743, doi:10.1039/c8nj01154k.
- [44] Y. Jiang, B. Zhang, J. Li, Y. Sun, X. Wang, P. Ma, D. Song, One-step fabrication of hydrophilic MIL-68(Al)/Chitosan-coated melamine sponge for vortex-assisted solid-phase extraction of parabens in water samples, *Talanta* 224 (2021) 121799, doi:10.1016/j.talanta.2020.121799.
- [45] D.C. Morrelli, G. Mafra, A.V. Santos, J. Merib, E. Carasek, Designing a green device to B_APE: recycled cork pellet as extraction phase for the determination of parabens in river water samples, *Talanta* 219 (2020) 121369, doi:10.1016/j.talanta.2020.121369.
- [46] N. Feizi, Y. Yamini, M. Moradi, M. Karimi, Q. Salamat, H. Amanzadeh, A new generation of nano-structured supramolecular solvents based on propanol/gemini surfactant for liquid phase microextraction, *Anal. Chim. Acta* 953 (2017) 1–9, doi:10.1016/j.aca.2016.11.007.
- [47] P. González-Hernández, A.B. Lago, J. Pasán, C. Ruiz-Pérez, J.H. Ayala, A.M. Afonso, V. Pino, Application of a pillared-layer Zn-triazolate metal-organic framework in the dispersive miniaturized solid-phase extraction of personal care products from wastewater samples, *Molecules* 24 (2019) 690, doi:10.3390/molecules24040690.
- [48] H. Wang, L. Hu, X. Liu, S. Yin, R. Lu, S. Zhang, W. Zhou, H. Gao, Deep eutectic solvent-based ultrasound-assisted dispersive liquid-liquid microextraction coupled with high-performance liquid chromatography for the determination of ultraviolet filters in water samples, *J. Chromatogr. A* 1516 (2017) 1–8, doi:10.1016/j.chroma.2017.07.073.
- [49] D. Ge, Y. Zhang, Y. Dai, S. Yang, Air-assisted dispersive liquid-liquid microextraction based on a new hydrophobic deep eutectic solvent for the preconcentration of benzophenone-type UV filters from aqueous samples, *J. Sep. Sci.* 41 (2018) 1635–1643, doi:10.1002/jssc.201701282.
- [50] R. Suárez, S. Clavijo, J. Avivar, V. Cerdà, On-line in-syringe magnetic stirring assisted dispersive liquid-liquid microextraction HPLC-UV method for UV filters determination using 1-hexyl-3-methylimidazolium hexafluorophosphate as extractant, *Talanta* 148 (2016) 589–595, doi:10.1016/j.talanta.2015.10.031.
- [51] Y. Lin, Y. Liu, Z. Liu, X. Hu, Z. Xu, β -Cyclodextrin molecularly imprinted solid-phase microextraction coatings for selective recognition of polychlorophenols in water samples, *Anal. Bioanal. Chem.* 410 (2018) 509–519, doi:10.1007/s00216-017-0746-3.
- [52] Decanoic acid substance infocard, European chemicals agency, 2020. <https://echa.europa.eu/substance-information/-/substanceinfo/100.005.798>.
- [53] DL-menthol substance infocard, European chemicals agency, 2020. <https://echa.europa.eu/substance-information/-/substanceinfo/100.014.614>.
- [54] Choline chloride substance infocard, European chemicals agency, 2020. <https://echa.europa.eu/substance-information/-/substanceinfo/100.000.596>.
- [55] Dipotassium hydrogen phosphate trihydrate substance infocard, European chemicals agency, 2020. <https://echa.europa.eu/substance-information/-/substanceinfo/100.117.661>.
- [56] M. Cortese, M.R. Gigliobianco, F. Magnoni, R. Censi, P. Di Martino, Compensate for or minimize matrix effects? Strategies for overcoming matrix effects in liquid chromatography-mass spectrometry technique: a tutorial review, *Molecules* 25 (13) (2020) 3047, doi:10.3390/molecules25133047.
- [57] J.M. Andrade, M.G. Estévez-Pérez, Statistical comparison of the slopes of two regression lines: a tutorial, *Anal. Chim. Acta* 838 (2014) 1–12, doi:10.1016/j.aca.2014.04.057.