Determination of parabens and bisphenol A in sludge samples using hydrophobic deep eutectic solvents by gas chromatography coupled to mass spectrometry and sample introduction via pyrolizer.

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ABSTRACT

This study reports on the development and use of hydrophobic deep eutectic solvents (HDES) as environmentally-friendly solvents to address the demand for low-toxicity materials for analysts and the environment. The study involved the application of HDES in environmental matrices for extracting emerging contaminants. In this case, HDESs were used as extraction solvents in dispersive liquid-liquid microextraction (DLLME) to determine parabens and bisphenol A from sludge samples. Gas chromatography-mass spectrometry (GC-MS) featuring a pyrolizer was used for sample introduction. Chemometric tools were utilized to optimize analysis conditions, considering the sample introduction and DLLME parameters. HDESs were characterized using Fourier-Transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and thermogravimetric analysis (TG). Univariate and multivariate strategies were employed to determine the optimal experimental conditions. In this case, the injection volume was fixed as 20 µL, with a temperature of 320 °C and a pyrolizer duration time of 0.5 minutes. For DLLME, full factorial design permitted identification of the best condition of each experimental variable, including the salting-out effect, pH, volume of dispersing solvent, and volume of extraction solvent. Optimal conditions for DLLME in sludge samples were determined as 300 µL of HDES, 350 µL of ACN, pH = 10, and the addition of NaCl at 27% m/v. Following optimization studies, a HDES was successfully applied as an extraction solvent to determine endocrine disruptors in sludge samples. Future work involves the analysis of additional sludge samples (both liquid and solid phases) using standard addition to quantify analytes using the developed methodology. The utilization of HDES within environmental samples facilitated the identification of emerging contaminants, aligning with the tenets of sustainable analytical chemistry. This was substantiated by applying the GREEnness Analytical (AGREE) evaluation metric system, yielding values of 0.72 and 0.76 for the employed methodology and sample preparation, respectively.

Keywords: Sample preparation; Hydrophobic Deep Eutectic Solvents; pyrolizer; gas chromatography

1. INTRODUCTION

Over the years, human activities have been responsible for introducing contaminants into various ecosystems, and especially in the last decade, studies on emerging contaminants (ECs) in aquatic matrices have become a global concern due to the demand for clean water. ECs originate from domestic, industrial, and agricultural waste and are present at concentration levels ranging from µg L^{-1} to ng L^{-1} and have been monitored in various research studies [1–9]. Generally, wastewater treatment plants (WWTPs) are inefficient in removing these contaminants from water. Therefore, the harmful effects of these substances are imminent in the short and long term. Among the harmful substances, endocrine-disrupting compounds (EDCs) can be highlighted. Exogenous chemicals can affect the synthesis and release of substances in the body, compromising essential functions. Bisphenol A (BPA), methylparaben (MeP), propylparaben (PrP), and butylparaben (BuP) are examples of these substances [1,10]. Endocrine-disrupting compounds can be found in effluents at very low concentration levels; however, their incidence is increasing worldwide. Table 1 shows the concentrations found in aqueous matrices for MeP, PrP, BuP, and BPA that have been recently reported.

Please insert Table 1 here

The identification and tracking of these substances are relevant, and environmentally sustainable analytical methods have become increasingly valuable due to their sustainability and reduced reliance on toxic solvents. Harnessing hydrophobic deep eutectic solvents (HDES) in microextraction techniques can provide numerous advantages over traditional methods [1,10]. HDESs generally consist of two or three precursors that can form intermolecular hydrogen bonds involving hydrogen bond donors (HBD) and hydrogen bond acceptor (HBA) components. These interactions, along with van der Waals forces, reduce the melting temperature of the mixture compared to the melting temperature of individual components [11–15].

HDESs are widely used in aqueous two-phase systems, and provide high-efficiency in the extraction of analytes even in low-concentration levels such as ng L^{-1} to µg L^{-1}. HDESs provide essential characteristics such as viscosity below 100 mPa.s, density differences from the aqueous phase, and limited pH change after introduction in aqueous samples [11,16]. HDESs were used to extract Cd from sewage sludge [17] and riboflavin from water [11], caffeine, tryptophan, isophthalic acid, and vanillin. Moreover, they were found to recover furfural (FF) and hydroxymethylfurfural (HMF) in aqueous solutions through hydrophobic membranes impregnated with HDES [18]. These solvents offer advantages due to their low toxicity, biodegradability, stability of density or viscosity, and ease of production.

The sample preparation stage can directly impact the precision and accuracy of the proposed method. Among microextraction techniques, DLLME offers critical advantages over traditional methods like liquid-liquid extraction (LLE), including reduced organic solvent volume, low cost, high extraction efficiency, and a simple experimental workflow [14,15,19]. Following the sample preparation stage, an instrumental technique is generally adopted to separate and determine EDCs. Gas chromatography-mass spectrometry (GC-MS) is a vital separation technique that can be used when thermally stable compounds are analyzed [19,20].

It is important to note that studies applying HDES using Py-GC-MS, particularly when pyrolysis is not applied directly. This gap in the literature indicates that optimizing the technique remains a promising opportunity to enhance the analytical performance of the developed methods. Furthermore, it is essential to highlight that the use of a pyrolyzer represents an innovative approach for introducing the eluate, which consists solely of the organic phase, primarily a viscous eluate. This type of eluate introduces certain complexities due to the presence of HDES. In this study, it is essential to emphasize that the compatibility of employing a pyrolyzer with viscous solvents like HDES enables the transfer of analytes from the sample to the chromatography column. During the analyte volatilization process by Py-GC-MS, it is essential to emphasize that there is no degradation of the compounds, as the temperature used does not correspond to the temperatures applied in commonly used pyrolysis processes. This method has proven to be innovative because it injects HDES into the system without requiring extensive dilution of the sample. The dry eluate in the crucible (HDES + analytes) means that liquid portions are not injected directly into the system. Although HDES based on DL-menthol exhibits lower viscosity than other eutectic solvents, these solvents should not be injected directly into the system, thereby avoiding chromatographic column saturation. Therefore, the use of the pyrolyzer for this purpose becomes efficient.

A recent trend in analytical chemistry involves creating methods that reduce environmental impact by minimizing the use of harmful substances. Modern concepts, such as Green Analytical Chemistry (GAC), have emerged to evaluate the environmental friendliness of analytical methodologies. GAC encompasses 12 principles aimed at addressing the requirements of analytical chemistry methods. The GREEnness Analytical (AGREE) metric system evaluates the ecological aspects of analytical processes based on established principles [21–23].

The aim of this study was to prepare, characterize, and apply HDESs in the sample preparation step of domestic sludge samples using DLLME in the extraction of methylparaben (MeP), propylparaben (PrP), butylparaben (BuP), and bisphenol A (BPA) and quantification by GC-MS. A pyrolysis system was used as a sample introduction system and green metrics were used to assess the proposed method's sustainability. This study stands out from others due to its innovative approach that combines established solid, semi-solid and viscous samples, such as the pyrolizer, with DLLME and HDESs used as an extraction solvent. The approach allows for a comprehensive exploration of the technique, making it suitable for analyzing various complex samples including domestic sewage sludge.

2. MATERIALS AND METHODS

2.1. Materials

DL-menthol (Sigma-Aldrich, purity ≥ 98%); decanoic acid (Sigma-Aldrich, purity ≥ 98%); dodecanoic acid (Biosynth, purity ≥ 98%); acetic acid (Sigma-Aldrich, purity ≥ 99%) were used for the preparation of HDES. The analytes methylparaben (Sigma-Aldrich, purity ≥ 98%); propylparaben (Sigma-Aldrich, purity ≥ 98%); butylparaben (Sigma-Aldrich, purity ≥ 98%); bisphenol A (Sigma-Aldrich, purity ≥ 98%) were used for method development. The sample consisted of domestic sludge from an office materials industry in São Paulo State, Brazil.

2.2. Preparation of HDES

DL-menthol was used as the hydrogen bond acceptor (HBA) in all solvents, where it was combined with different hydrogen bond donors (HBDs), such as acetic acid (AcA), decanoic acid (DecA), and dodecanoic acid (DoDecA). The proportions used for preparation were DL-menthol:AcA (1:1), DL-menthol:DecA (1:1), and DL-menthol:DoDecA (2:1). The structures of these compounds and their physicochemical properties are provided in Table S1 of the supplementary material (SI). HDES was prepared using a mixture of the two precursors (HBA and HBD) based on the processes described by Ribeiro, Dwamena, and Caldeirão [14,15,24]. In this case, agitation of 60 rpm with heating at 60 ºC was employed for 25 min. Masses of the precursors were measured according to the molar ratios reported in the literature [13–15], with 5.00 g for DL-menthol in all cases, and 5.50 g for DecA, 3.20 for DoDecA, and 1.92 g for AcA.

2.3. Density and viscosity measurements

HDES density (ρ) measurements were conducted using a pycnometer (5.00 mL) calibrated with Milli-Q water at 25 °C for 30 minutes to stabilize the system temperature and an analytical balance. Calibration determined the pycnometer's actual volume, facilitating subsequent analysis with the HDES. Viscosity measurements were obtained using an automatic vibrational viscometer (SV-10 A&D Company) with a precision of ± 3% (1 to 1000 mPa·s), operating at a vibration frequency of 30 Hz at 25 °C. The viscosity and density were the parameters used to assess the stability of HDES over one year, and to find answers regarding the possibility of storing HDES for extended periods. For the DL-menthol:AcA mixture, data collection occurred over 20 consecutive weeks and then monthly for the next eight months, completing a one-year duration.

2.4. Fourier transform infrared spectroscopy (FTIR)

This analysis provides information about the formation of the HDES. It was performed from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} and 64 scans (VERTEX 70, BRUKER), equipped with a DLaTGS detector at room temperature.

2.5. Thermal analysis

The melting points of each HDES and their precursors were determined using DSC (Mettler-Toledo). In DSC, approximately 8 mg of the sample was added to aluminum crucibles with a 40.0 µL capacity and analyzed under conditions involving cooling and heating in a range of -40 to 60 °C at a rate of 5 °C min^{-1} in an N_2 flow of 50.0 mL min^{-1}. Mass loss steps were assessed using TG in conjunction with the DTA (TG/DTA model SDT 2960, TA Instruments) in a dry air atmosphere with a flow rate of 50.0 mL min^{-1}, heated in a temperature range of 30 to 800 ºC at a heating rate of 10 ºC min^{-1}. The HDES samples were placed in α-Al_2O_3 crucibles with a volume of 90 µL. For Evolved Gas Analysis (EGA), a TG/DSC (Mettler Toledo) coupled with FTIR (Nicolet IS10) was used, with a gas analysis component heated to 175 ºC. HDES samples were added to α-Al_2O_3 crucibles with a capacity of 70.0 µL, with a heating rate of 10 ºC min^{-1} in a dry air atmosphere with a flow rate of 50.0 mL min^{-1}. The gases released in the first stage were transferred to the gas analysis accessory through a 3 mm diameter stainless steel transfer line heated to 150 ºC. For FTIR analysis, 16 scans per spectrum were performed with a resolution of 4 cm^{-1}.

2.6. Sludge sample measurements

The raw sludge collection was carried out in an industrial area located in the city of São Carlos (22° 00′ 00″ S, 47° 53′ 27″ W), in the state of São Paulo (Brazil). The sample was vacuum filtered, allowing particulate materials to be removed. The aqueous phase of the collected sludge was analyzed to determine the sample pH, measured with a pH meter (Meter Tec-2, Tecnal), conductivity with an S30 SevenEasy conductivity meter (Mettler Toledo), and chemical oxygen demand (COD) and total organic carbon (TOC), as detailed in Table S2 of the supplementary information.

2.7. Dispersive liquid-liquid microextraction (DLLME)

In the initial stage of DLLME, 300 µL of the extraction solvent (HDES) and 350 µL of the disperser solvent (ACN) were used. Subsequently, this mixture was added to a falcon tube containing 4.00 mL of previously spiked filtered sludge sample with MeP, PrP, BuP, and BPA each at concentrations of 3.00 mg L^{-1} for the factorial design stage. Later, concentrations ranged from 0.0830 mg L^{-1} to 1.00 mg L^{-1} for the method validation step. After this process, the tube was vortexed for 20 seconds. At this stage, dispersion of the extraction solvent into microdroplets in the aqueous phase was observed due to the action of the disperser solvent. The system was then centrifuged at 8000 rpm with cooling at 25 °C for 20 minutes, allowing for equilibrium between the organic phase, rich in HDES, and the aqueous phase. A 20.0 µL volume of the organic phase was collected and diluted in 50.0 µL of acetonitrile (ACN) for subsequent Py-GC-MS analysis.

2.8. Pyrolysis Gas Chromatography-Mass Spectrometry (Py-GC-MS)

Analyses were performed using the pyrolizer (EGA/PY-3030D, Frontier Laboratories, Japan), and the ceramic furnace temperature was programmed to 320 °C with a hold time of 0.5 minutes. Chromatographic separation employed a capillary metal column coated with a 5% diphenyl dimethylpolysiloxane stationary phase (Ultra ALLOY®-Frontier Laboratories), with helium (99.99%) used as the carrier gas. The pyrolizer was coupled to a gas chromatograph (GC-2010 plus Shimadzu) with a mass spectrometry detector (GCMS-QP2020, Shimadzu). It was operated at 40 °C for 1 minute, then ramped to 250 °C at a rate of 30 °C min^{-1} for 4 minutes, followed by an increase to 320 °C at a rate of 40 °C min^{-1} and held for 4 minutes. For electron ionization (EI), the mass spectrometer was operated at a temperature of 230 °C in the ion chamber and 320 °C in the detector interface. Analysis in SCAN mode was conducted in the range of 50 to 300 *m/z*, with compound identification based on the NIST library and the instrument's software library. Retention times were determined experimentally by injecting standards. SIM mode involved selecting specific ions for each analyte based on ions observed in SCAN mode and literature data.

2.9. Design of experiments (DOE)

DOE was employed using a full factorial design for optimizing injection conditions and a fractional factorial design for optimizing DLLME. Table S3 in the supplementary material outlines the variables and levels for the fractional factorial design in the sample preparation step. Univariate optimization refined the injection study, followed by fine-tuning the best DLLME conditions using a central composite design, as detailed in Table S4 of the supplementary material.

2.10. Evaluation of the analytical figures of merit.

Validation of the proposed method was conducted based on the guidelines of regulatory agencies [19,20]. The method's precision of the method was measured through repeatability, utilizing the same operating conditions (equipment, analyst, reagents, and environmental conditions). Nine experiments were carried out at different concentration levels (3 repetitions at low concentration, three repetitions at medium concentration, and three repetitions at high concentration). The method selectivity was determined by the comparison to identify or quantify the analyte in a sample against a blank. The limits of detection (LOD, S/N = 3), and limit of quantification (LOQ, S/N = 10), were determined by evaluating the signal-to-noise ratio in the chromatogram of the sample blank. Linearity was assessed by preparing solutions with varying concentrations and evaluating if the analytical responses were proportional to the analyte concentration, typically using at least five concentration points. Recovery (Rec %) was analyzed to determine the pre-concentration parameters of the analytes in the studied matrix, and the experiments were conducted in triplicate [9–11]. Initially, DLLME was performed without spiking analytes to the sample. The resulting eluate was fortified (B) with 415 µg L^{-1} of MeP and 830 µg L^{-1} of PrP, BuP, and BPA concentrations. Subsequently, the sample was fortified (C) under the same conditions mentioned earlier, and a new DLLME step was performed, with the eluate also collected and analyzed following Matuszewski et al. [25]. The results regarding the obtained area were applied in Equation 1 below.

$$Rec\ (\%) = \frac{C}{B}\ x\ 100 \qquad (1)$$

Enrichment factor (EF) was defined as the ratio of analyte concentration present in the organic phase (C_{org}) to the initial concentration (C_0) present in the aqueous phase. This value was obtained from the calibration curve and the initial concentration in the aqueous phase, respectively. The concentrations employed were 415 µg L^{-1} for MeP and 830 µg L^{-1} for PrP, BuP, and BPA. The results were then inserted into Equation 2 [26,27].

$$EF = \frac{C_{org}}{C_0} \qquad (2)$$

2.11. Application of green metrics for the developed method and sample preparation.

Based on the AGREE metric system, each of the 12 input variables was assessed on a standard scale ranging from 0 to 1 [22,28,29]. The outcome of the final evaluation was represented by creating a clock-like chart with a color scale ranging from red to yellow and green. Calculations were performed using the Analytical Greenness Calculator software provided by Pena-Pereira and colleagues [23]. The inputs used for evaluating the method and sample preparation are described in Table S5 of the supplementary material.

3. RESULTS AND DISCUSSION

3.1. Preparation of hydrophobic deep eutectic solvents (HDES)

Eutectic mixtures using DL-menthol, as the HBA species, emerge as an alternative to replace toxic precursors and are widely studied for sample preparation applications. This precursor, extracted from the leaves of *Mentha sp*. species, is abundant and relatively inexpensive compared to other precursors that serve the same purpose, making it an advantageous option [15,30,31]. After preparation of the HDESs, the system was cooled to room temperature, where the formation of transparent liquids could be observed. This characteristic is one of the initial indicators of HDES formation [12,15]. The final volumes of each produced HDES were approximately 12.3 mL (DL-menthol:DecA), 10.7 mL (DL-menthol:DoDecA), and 8.00 mL (DL-menthol:AcA). Only the mixture containing DoDecA showed a yellowish color, which is highly characteristic of the precursor.

According to Florindo [12], in the case of short-chain carboxylic acids such as acetic acid, butyric acid, levulinic acid, hexanoic acid, and pyruvic acid, it is important to use HBAs, such as DL-menthol or N_{4444}-Cl, to vary the alkyl chain, and thus adjust the polarity of the HDES for a defined application. Ribeiro and Florindo [12,15] reported that some solvents exhibit less interference from their precursors in water. NMR analyses of the aqueous phase described by Florindo (2017) confirmed some of these claims for DL-menthol:DecA and DL-menthol:DoDecA. In contrast, for DL-menthol:AcA, a portion of the HBD precursor (acetic acid) was observed in the aqueous phase. For the present study, even if one of the HBDs is present in the phase, this factor does not directly interfere with the analyzing the compounds of interest. With techniques such as mass spectrometry, only ion fragments corresponding to the analytes are identified (SIM mode), and in this case, none of the ions related to the precursors exhibit similarities with the analytes, thereby avoiding any interference in the analytical response.

3.2. Density, viscosity, and measurements

The study investigated the properties of HDESs regarding density and viscosity and their implications in extraction processes. All tested HDESs exhibited density values lower than that of water (0.990 g cm^{-3}), potentially resulting in longer separation times during extraction due to having densities close to water. However, the optimal density values for HDESs depend on the extraction method used. Viscosity also plays a crucial role, with many HDESs showing higher viscosities than water thereby affecting their mass transport during extraction [32].

The recorded densities for DL-menthol:DecA, DL-menthol:DoDecA, and DL-menthol:AcA were 0.896 g cm^{-3}, 0.895 g cm^{-3}, and 0.917 g cm^{-3}, respectively, with corresponding viscosity values of 12.4 mPa·s, 20.4 mPa·s, and 7.88 mPa·s. Despite the higher viscosity compared to water (0.890 mPa·s), these values were lower than typical reported viscosities for HDESs, which can reach 100 mPa s. Lower viscosity values are preferred for HDES as extractants to enhance dispersion in the aqueous medium [32,33]. This study also investigated the stability of HDES over one year, showing insignificant changes in density and viscosity parameters. A stability study revealed minimal variation in density (CV% = 0.42%), and viscosity (CV% = 2.5%) over one year, suggesting the suitability of HDES for long-term applications in extraction processes, as demonstrated in Fig 1. The dispersion of HDESs in the sample, combined with sample agitation, facilitates mass transfer processes by reducing the distance between phases and enhancing diffusion of analyte molecules [32,34–36].

Please insert Figure 1 here

Although viscosity and density values exhibit slight variation, other characterization techniques were used to assess the mixture's stability. FTIR served as another technique used to characterize HDESs. Intermolecular interactions primarily determined the formation of bands corresponding to hydrogen bonding between DL-menthol and organic acids. Extremely broad O─H absorption bands appeared from 3400 to 2400 cm^{-1}, originating from strong hydrogen bonding. Generally, at this wavelength, the O─H absorption obscures the vibrations of C─H sp^3 stretching (alkanes) found in the same region [12,37]. All hydrogen bond donors used in eutectic mixtures possess a carboxylic acid group in their structure, observed in the spectra by the representative band (ketone or carbonyl group) around 1700 cm$^{−1}$. In the FTIR spectra of the hydrogen bond acceptor (DL-menthol), only a band corresponding to the hydroxyl group, at around 3300 cm$^{−1}$, can be observed.

In the eutectic mixture containing DL-menthol:AcA, the most pronounced characteristic in the spectrum that characterizes it as a carboxylic acid, in addition to the O─H absorption, is the presence of the C═O bond. Acids in concentrated liquid solutions tend to dimerize. Dimerization weakens the C═O bond, decreasing the frequency of the carbonyl of saturated acids to approximately 1710-1700 cm^{-1} [17]. The stretching absorption of the carbonyl (C═O) is broader and more intense compared to an aldehyde or a ketone. The C─O stretching vibrations in acids appear with medium intensity between 1300-1000 cm^{-1}, as depicted in Figure S2 of the supplementary material [11,12,14,37]. Results from FTIR provided significant information for the DL-menthol:AcA mixture, where alterations in the characteristic functional groups of HDES were observed. These alterations indicate possible modifications in the solvent composition and suggest the formation of methyl acetate, a product of the esterification reaction. In the observed case, a reduction in the amplitude of the O─H band was noted, accompanied by an increase in the intensity of the C─H sp3 stretching absorption bands. As a complementary technique to confirm compound formation, EGA was conducted by heating the sample at a controlled temperature using TG. FTIR detected volatilized compounds, and the corresponding spectrum of the supplementary material can be observed in Figure S1. Comparison with literature spectra supports the possibility of methyl acetate formation. However, the influence of this compound on the extraction process using the solvent after storage was not evaluated.

Understanding the long-term stability of HDES has led to optimized storage processes, ensuring its availability and enhancing the overall efficiency and sustainability of analytical procedures. Investigations into HDES stability also offers valuable insights for improving its formulation and application, driving significant advancements in analytical chemistry and practical applications.

3.4. Thermal analysis characterization

Thermal analysis was performed as a complementary method to FTIR to confirm the preparation of HDES. From DSC analyses, it was possible to assign the endothermic events to the corresponding melting points of each HDES. Evaluation of the data suggested that eutectic mixtures were formed in all three cases, as evidenced by the lower melting points obtained compared to their individual precursors, which corroborates the studies described in the literature [12,14,15]. The values obtained for DL-menthol:AcA were 12.3 °C; for DL-menthol:DecA, it was 1.15 °C; and for DL-menthol:DoDecA, it was 11.5 °C, as illustrated in Figure S3.

Other analyses were employed for HDES characterization, such as thermogravimetric analysis, allowing for the investigation of the HDESs and analytes' thermal stability at extreme temperature. This facilitated the determination of temperature values for the pyrolyzer to completely volatilize the extract after DLLME. Mass loss stages and endothermic events related to these losses occurred between 140 and 210 °C.

A temperature of 320 °C was selected in the developed method, as it corresponds to the complete volatilization of the analytes in the crucible, which, according to the analyses conducted, undergoes this process within the temperature range of 200 to 300 °C. Therefore, the chosen temperature proved to be suitable for the proposed analysis, as illustrated in Figure S4 of the SI.

3.5. Sludge sample measurements

The collected domestic sludge sample was analyzed to provide important information capable of influencing the sample preparation step, and an obtained pH value of 7 complied with the standards established by official regulatory agencies. According to Makós [38], information regarding the acidity properties of HDES is still limited. This limitation is because the pH of the aqueous sample is more significant than the pH of the extraction solvent in sample preparation methods. The optimal pH of the aqueous sample needs to be determined for the group of analytes being studied. Solution pH can profoundly impact extraction by influencing compounds' ionization degree, affecting the analyte distribution coefficient (Kd) [39,40]. The sample's conductive capacity was analyzed, and a value corresponding to the reference values in official documents was obtained (Table S2). Generally, molecules of organic compounds do not dissociate in aqueous solutions and, in most cases, exhibit low conductivity [19,41,42].

3.6. DOE applied to sample introduction and DLLME

In preliminary tests regarding optimization of the sample preparation step, challenges arose regarding the use of solvents containing DecA and DoDecA. This occurred mainly due to the ease of DL-menthol volatilization during the Py-GC-MS analysis, whose intensity surpassed that of the analytes, as illustrated in Figure S5 and Figure S6 of the SI. This limitation hindered clear conclusions in the chromatograms when these two solvents were used. However, for the DL-menthol:AcA mixture, this problem was not observed, as the signals corresponding to the eutectic mixture did not appear at the same retention time as the analytes, thus not interfering with the analytical response. Due to this factor, this HDES was chosen for optimizations in DLLME.

The full factorial design (2^k) was initially conducted to assess the influence of parameters such as time, temperature, and injection volume on sample introduction through the pyrolizer. At this stage, a MeOH solution containing analytical standards of parabens and bisphenol A at a concentration of 3.00 mg L^{-1} was used. After eight experiments, it was observed that the analytical response tends to be more efficient with an increase in the injection volume, with this variable being the most significant, indicating an optimal volume of 10.0 µL in this initial evaluation.

Univariate optimization was carried out to confirm the response from the previous analysis. Therefore, variables corresponding to temperatures (320 °C) and times (0.50 min) remained fixed, and the values of volumes varied from 2.00 µL to 20.0 µL, generating a total of ten experiments. After this analysis, the influence of volume was again observed, proving that 20.0 µL offered the best response, used for all subsequent tests. For DLLME, the sludge sample was fortified at concentration of 3.00 mg L^{-1} of analytes, and variables that could influence the analytical responses of the factorial design were evaluated, such as pH, salting-out, volume of the disperser solvent, volume of the extracting solvent, and sludge dilution. Firstly, a fractional factorial design (2^{5-1}) was carried out in triplicate at the central point.

A central composite design was applied for optimize DLLME, and the study range of each variable was altered. This allowed for a precise analysis of the optimal working conditions corresponding to critical points of 300 µL volume for HDES, 350 µL volume for ACN, and 27% m/v of NaCl. The response surfaces generated in this stage are depicted in Fig. 2.

Please insert Figure 2 here

The volume of the collected organic phase (20.0 µL) was diluted in 50.0 µL of ACN to eliminate aqueous phase residues that may be present in the organic phase, thus minimizing the chances of potential contamination in the Py-GC-MS system. The volume added after dilution in the crucible was 20.0 µL, corresponding to the optimized injection value.

3.7. Py-GC-MS

After injecting the analytical standards, the chromatograms obtained in SCAN mode were compared with the literature to identify corresponding fragment ions for each analyte. Additionally, retention times, fragment ions, and their relative intensities were used in subsequent SIM mode analyses to aid in identifying each analyte in a fortified sample. Fragment ions for MeP (*m/z* 121, 152, 93, and 65), PrP (*m/z* 121, 180, 93, and 65), BuP (*m/z* 121, 194, 93, and 65), and BPA (*m/z* 213, 119, and 138) were chosen based on literature data and experimental considerations (data not shown). The obtained retention times were 8.31 min for MeP, 9.05 min for PrP, 9.54 min for BuP, and 12.7 min for BPA. In Fig. 3, chromatograms are provided for the mixture containing analytes at a concentration of 3.00 mg L^{-1} in MeOH in SIM mode of analytical standards (Fig. 3a) and under the optimized conditions for both injection and DLLME in the sludge samples (Fig. 3b).

Please insert Figure 3 here

3.8. Analytical performance

The application of HDESs in the sample preparation step using Py-GC-MS was validated in filtered domestic sludge samples, considering the performance parameters described by the Food and Drug Administration (FDA) [20]. Linearity was assessed in the solvent (MeOH) and filtered sludge samples were fortified with the analytes. Five calibrants points were used to generate the analytical curve in triplicate, with the analytical response being the signal intensity of the *m/z* = 121 ion for parabens and the *m/z* = 213 ion for bisphenol A. The concentration range used for MeP was from 83 to 500 µg L^{-1}, and for the other analytes, it was from 166 to 1000 µg L^{-1}. Results for the analytical curve after DLLME and with MeOH are presented in Table 2 and Table 3.

For the intra-day and inter-day precision tests, low, medium, and high concentrations were used at different times in triplicate. The figures of merit for the applying of the DLLME analytical method to samples of domestic sludge spiked with MeP, PrP, BuP, and BPA. The coefficient of determination (R^2) resulted in values between 0.961 and 0.996 indicating suitable linearity following the FDA validation guideline [19]. The LOD between 25.0 and 50.0 µg L^{-1}, with recovery % between 92.4 – 99.3 values, and variances were between 3.07 to 10.5 as described in Table 2.

Please insert Table 2 here

Please insert Table 3 here

Although the inter-day precision for bisphenol A (BPA) showed a variation of 15%, which was higher compared to other analytes, as shown in Table S6 of the supplementary material, this value is within the limits established by official guidelines, which can range from 15% to 20%. However, the HDES (DL-menthol:AcA) used in this study is considered a low-viscosity solvent, according to Ribeiro [15], and is an effective alternative for the extraction and quantification of compounds of interest in the sample, as its volatilization temperature is higher than that of methanol and ACN. Therefore, the use of HDES in this study favored the analytes' transport from the crucible to GC column, consequently, a more reliable analytical response was produced. Hence, knowledge about solvent and analytes volatility and the HDES thermal characteristics and chemical properties is crucial to optimize and obtain the best efficient using the pyrolizer during the sample introduction.

Selectivity assessment confirmed that only signals corresponding to the analytes with specific ions were detected for each analyte (Fig. 4a), suggesting the absence of significant interferents during the analysis compared with the same raw sludge sample chromatogram on the same specific ions (Fig. 4b). This indicates the method's selectivity for the compounds of interest, with minimal interference from other substances. This is supported by different retention times of these compounds compared to the analytes. Comparison between the chromatograms obtained after DLLME and from the sludge affirmed the method's selectivity exclusively for the compounds of interest.

The efficiency of HDES (DL-menthol:AcA 1:1) as an extraction solvent in DLLME was tested after 24 months of storage in a desiccator. The diluted sludge sample was spiked with analytes at concentration levels of 415 μg L-1 for MeP, and 830 μg L-1 for PrP, BuP, and BPA, under the conditions optimized by the experimental design. The data showed that HDES, in addition to providing high stability as demonstrated in Figure 1, also exhibited excellent performance in the efficiency of DLLME after storage for 24 months, showing an intra-day CV% between 2.82 and 7.48 compared to those obtained in the first extraction with 1.05 to 8.22 for the different analytes (Table S8). These findings, combined with stability data related to density and viscosity, supports the use of these solvents due to their capability for long-term applications.

Please insert Figure 4 here

The analysis provided EF values of 183, 102, 93, and 92 for MeP, PrP, BuP, and BPA, respectively. The obtained values are consistent with the data described in the literature for parabens and bisphenol A [4,43–45]. EF values were considered high considering the notable reduction in the volume of solvent used (μL). These results demonstrate the efficiency of the HDES preconcentration method, associated with the versatility of the Py-GC-MS technique used, with excellent LOD, LOQ, extraction recoveries, and stability for parabens and bisphenol A, present in low concentrations in aquatic matrices.

3.9. Analytical GREEnness Assessment (AGREE) for the proposed method

The final score of the metric indicates that a predominantly green color suggests the assigned score of 0.72 for the developed method aligns with environmentally sustainable expectations. Lower scores in criteria 3 and 10 were attributed to the use of solvents, such as ACN, to dilute the HDES in the stage preceding the GC-MS analysis. Although ACN is necessary, the volume used (in µL) does not significantly compromise the method's sustainability, which is considered green. Principle 9 evaluated the energy consumption of the GC-MS analysis, revealing a higher value compared to GC using other types of detectors.

A score of 0.76 was achieved in the sample preparation step, indicating a relatively low environmental impact. However, criteria 2, 3, 7, and 10, highlighted in yellow, received lower scores due to the sample amount, waste generation after extraction, and the absence of renewable reagents throughout the procedure. These assessments are shown in Fig. 5.

Please insert Figure 5 here

Both processes effectively minimized environmental impacts, as assessed by the AGREE method. This result emphasizes the importance of considering the overall picture of evaluating the 12 principles, as optimizing these criteria can offset any individual disagreements, aiming to increase the method's efficiency and sustainability over time. In this context, using HDES with miniaturized sample preparation techniques, such as DLLME, emerges as a valuable alternative to promote more sustainable analytical practices.

3.10. Comparative analysis of the proposed method and other approaches mentioned in the literature

In other studies employing DLLME, an evaluation of recovery was conducted on various samples, including biological samples and food, as illustrated in Table S7 of the SI. Evaluating the recovery (Rec %) from these studies, the effectiveness of DLLME in biological and food samples is evident, reflecting the behavior observed in sludge with high recovery values obtained. One of the crucial parameters for implementing DLLME is the use of reduced volumes throughout the process. Table 4 details the specific values used for DLLME in the literature. These values are considerably low, reflecting the fundamental premise of this method. In this study, the employed volumes were smaller than those described in existing literature.

Please insert Table 4 here

In all studies used in the comparison, the absence of tools related to the green metric was observed. Applying this assessment is essential as it promotes using methods to make experiments more sustainable in current practices. Many of these works employed some planning or optimization in the extraction step. Overall, the method developed in this study proved efficient in using smaller volumes of solvents, aligning with the principles of Green Analytical Chemistry (GAC). Although the limits of quantification (LOQ) are higher than those evaluated in the literature, it is essential to consider the innovative proposal of using the pyrolizer, which can influence potential losses of analytes during sample introduction.

4. CONCLUSIONS

The study confirmed the stability of eutectic mixture formation in HDES solvents for over a year using thermoanalytical and FTIR analyses. Challenges arose in sample preparation optimization due to DL-menthol volatilization, especially with DecA and DoDecA. However, DL-menthol:AcA demonstrated notable extraction efficiency in DLLME, with recoveries exceeding 90%, validating its selectivity and robustness. Overall, the study underscores the potential of the proposed HDES and Py-GC-MS method for monitoring contaminants in sludge samples, contributing significantly to the sustainable extractor and analytical methods. Using a pyrolizer for sample introduction is innovative, mitigating contamination risks associated with the continuous use of viscous liquids like HDES. Its effectiveness in injecting small liquid sample quantities minimizes contamination issues, enhancing its appeal for such sample types.

The developed approach in this study has the potential to advance more effective and sensitive analytical methods for detecting contaminants in environmental samples. It also opens avenues for creating new HDES capable of monitoring other contaminants, thereby enhancing environmental management and monitoring practices. The use of AGREE to assess method sustainability directly facilitates the exploration of new approaches and optimization of the analytical method to meet the demands of GAC. Future perspectives include applying HDES, DL-menthol:DecA, and DL-menthol:DoDecA in the DLLME technique and explore the development of new magnetic solvents for extracting different compounds from various samples.

Credit authorship contribution statement

Karen C. Ferreira: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. Thais Rodrigues: Review & editing. Guilherme Isquibola: Review & editing. Saidy Cristina Ayala-Durán: Writing – review & editing. Josias de Oliveira Merib: Writing – review & editing. Jared L. Anderson: Writing – review & editing. Mario H. Gonzalez: Writing – review & editing. Paulo C. F. Lima Gomes: Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Conflicts of 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.

Acknowledgments

The authors wish to acknowledge the financial support from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grants #2022/02991-3 and INCT-DATREM #2014/50945-4), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant #465571/2014-0), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES #88887136426/2017/00), and São Paulo State University, Institute of Chemistry (Araraquara, Brazil). S.C.A.D. for the scholarship granted in the scope of the Program CAPES-PrInt (#88887.891984/2023-00). J.L.A. acknowledges support from the Chemical Measurement and Imaging Program at the National Science Foundation (Grant number CHE-2203891).

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