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DEVELOPMENT OF A DEEP EUTECTIC SOLVENT-BASED MICROEXTRACTION METHOD FOR THE TRACE DETERMINATION OF EMERGING ORGANIC POLLUTANTS IN WATER FROM BABYLON GOVERNORATE, IRAQ

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ABSTRACT

The emerging organic pollutants, such as pharmaceuticals, personal care products and industrial phenols, are found in rivers, groundwater and treated effluents at levels as low as ng L⁻¹ to low µg L⁻¹ and cannot be directly measured by an instrument. In this study, a dispersive liquid–liquid microextraction method (DLME) based on a hydrophobic deep eutectic solvent (DES) with increasing DL volume as evaporated DES was used as the sole extraction phase followed by HPLC with diode-array detection (DAD), was developed. The following five model pollutants were investigated: Bisphenol A, 4-tert-octylphenol, triclosan, diclofenac and ibuprofen. The DES was formulated by the gentle heating process, without the help of any solvent; and it was characterized by FTIR, density, viscosity, water content and stability in water. A 25 – 1 fractional design was applied for the screening of the extraction variables while the Box–Behnken design was employed for optimization. With the best conditions (90 µL of DES, pH 3.0, 3 min vortex, no salt) the calibration graphs were linear in the range between LOQ and 100 µg L⁻¹ with R² values ≥ 0.9978, limits of detection ranged between 0.09 and 0.31 µg L⁻¹, enrichment factors were in the range 88–121 and extraction recoveries were in the range 63.4–87.1%. Per day and within day RSD values were not greater than 5.8% and 7.9% respectively and recoveries in spiked waters were from 86.5% to 106.8%. This method was used to test water samples in Babil Governorate, Iraq. The concentrations of Bisphenol A, triclosan and diclofenac in water samples of Shatt al-Hilla downstream of Hilla city and its treated municipal discharges were in the range of 0.41–3.27 µg L⁻¹, while other water samples (Tap and well water) were not detected. Only less than 0.2 mL of organic reagents per sample were consumed and it received a score of 71 in Analytical Eco-Scale and 0.68 in AGREEprep, while liquid–liquid extraction scored 63 and 0.21, respectively.

Keywords: Deep Eutectic Solvents; Hydrophobic DES; Dispersive Liquid–Liquid Microextraction; Emerging Organic Pollutants; Water Analysis; HPLC-DAD; Box–Behnken Design; Green Analytical Chemistry; Babil.

1 INTRODUCTION

For a long time, a standardized approach has involved monitoring a limited number of chemicals deemed as regulated – nutrients, heavy metals, and a series of pesticides and industrial chemicals specified decades ago. This list has not been sufficiently adequate in past three decades. Thousands of synthetic organic compounds have daily applications in the home, hospitals, farms and factories, and many of these are discharged to wastewater treatment plants and to surface water in low concentrations [1,2]. A nationwide study in the United States evaluated 139 streams and found that at least one pharmaceutical and/ or hormone or other compound of waste water origin was present in 80% of the sampled streams [3] and a study in 258 rivers in 104 countries worldwide later found the presence of active pharmaceutical ingredients on all continents [4]. In central Iraq, rivers used for drinking water are also used to receive effluents from urban areas, and the information on these compounds is limited. The practical problem dealt with in this study is the reliable measurement of them in the levels present in nature.

1.1 Emerging organic pollutants in aquatic environments

This includes compounds that are not regulated, or recently regulated, and for which presence in the environment is of concern, referred to as emerging organic pollutants (EOPs) [2,5]. It contains certain pesticides, plasticisers, fragrances, flame retardants, surfactant metabolites and human and veterinary pharmaceuticals. Most of them are moderately polar or polar; they are thus able to pass through soil and treatment plants instead of into sludge. Others like alkylphenols and triclosan, are more hydrophobic, and occur more in sediments and biota. They all share the same positive characteristic: that they are released continuously, which means that even those with higher half-lives could be regarded as pseudo-persistent pollutants [6].

1.2 Sources and occurrence of emerging organic pollutants

Municipal wastewater is the main entry route. Drugs are excreted unchanged or as metabolites, personal care products are washed off the skin, and plastic additives leach from packaging [5]. Conventional activated sludge plants were designed to remove organic load and nutrients, not trace organics, and removal of individual compounds ranges from almost complete to negligible [6]. Hospital effluents, landfill leachate, manure spreading and untreated sewage add further inputs. Where sewer networks are incomplete or rivers carry a large share of effluent, as in many semi-arid regions, the dilution available downstream is small and concentrations rise [4]. Groundwater is reached through bank filtration, septic systems and irrigation with reclaimed water [7].

1.3 Environmental and health impacts

Several cases have shown that trace concentrations can matter. Residues of the anti-inflammatory drug diclofenac in livestock carcasses caused renal failure and a population collapse of vultures in South Asia [8]. A whole-lake experiment with the synthetic oestrogen 17 α -ethinylestradiol at ng L⁻¹ levels led to feminisation of male fish and near-extinction of a fathead minnow population [9]. Bisphenol A and alkylphenols act as endocrine disruptors, and bisphenol A is found in effluents, surface waters and human samples worldwide [10]. For drinking water the European Union has set a parametric value of 2.5 μ g L⁻¹ for bisphenol A [11] and keeps a watch list of substances for Union-wide monitoring of surface water [12].

1.4 Analytical challenges in trace determination

Environmental concentrations of most EOPs lie between a few ng L⁻¹ and a few μ g L⁻¹. Few routine detectors reach these levels by direct injection. Natural waters also contain humic substances, salts and suspended particles that interfere with separation and detection, and the analytes differ widely in polarity and acid–base behaviour [2]. A single procedure has to recover weak acids such as diclofenac and ibuprofen together with neutral phenols such as bisphenol A. Liquid chromatography coupled with tandem mass spectrometry handles this best, but its cost puts it out of reach of many laboratories. For HPLC with UV or diode-array detection the sample must be concentrated by a factor of 50 to 100 before injection.

1.5 Importance of sample preparation

Sample preparation is usually the slowest step of an analysis and the one that produces most of the waste and most of the error [13]. Its tasks are to isolate the analytes from the matrix, remove interferences, and concentrate the extract into a volume that suits the instrument. Liquid–liquid extraction of a 1 L sample with dichloromethane, as in EPA Method 3510C, uses three 60 mL portions of solvent and a separatory funnel, followed by evaporation of the extract [14]. Solid-phase extraction reduces solvent use but still needs cartridges, conditioning solvents and elution. Microextraction techniques reduce the extractant to microlitres and combine extraction and preconcentration in one step [13].

1.6 Green analytical chemistry

Green chemistry was formulated as a set of design principles for safer and less wasteful chemical processes [15]. Green analytical chemistry adapts these ideas to measurement: direct analysis where possible, smaller samples, fewer and safer reagents, less energy, less waste, and operator safety [16,17]. Gałuszka and co-workers condensed these aims into twelve principles [18]. Sample preparation is where most of the gains can be made, because it is where solvents are consumed. Replacing chlorinated extractants by solvents of lower toxicity, and scaling the extraction down to microlitres, addresses several principles at once.

1.7 Research problem

Laboratories that monitor water quality with HPLC-UV equipment cannot measure EOPs at environmental levels without preconcentration. The standard way to achieve it, liquid–liquid extraction with chlorinated solvents, contradicts the principles of green analytical chemistry and exposes analysts to toxic vapours. Early microextraction procedures reduced solvent volume but still relied on chlorobenzene, carbon tetrachloride or chloroform as extractants [19]. Hydrophobic DES offer an alternative, yet their use for mixtures of acidic and neutral EOPs in real waters, with a statistically optimised procedure and a documented green profile, has received limited attention, and no such data exist for waters of Babil Governorate.

1.8 Objectives of the study

The general objective was to develop, optimise and validate a green DES-based dispersive liquid–liquid microextraction method coupled with HPLC-DAD for the trace determination of selected emerging organic pollutants in water. The specific objectives were to: (1) prepare and characterise a series of hydrophobic DES and select the most suitable extractant; (2) screen and optimise the extraction variables with experimental design; (3) validate the method for linearity, sensitivity, accuracy, precision, enrichment, selectivity, matrix effect and robustness; (4) apply it to surface water, tap water, groundwater and treated wastewater from Babil Governorate; and (5) score its greenness and compare it with conventional extraction.

1.9 Research hypotheses

H1: A DES formed from DL-menthol and decanoic acid extracts the target pollutants efficiently at pH 3. H2: DES volume, sample pH and vortex time have statistically significant effects ($p < 0.05$) on extraction efficiency. H3: The validated method reaches detection limits below $0.5 \mu\text{g L}^{-1}$ with RSD values below 10%. H4: The method scores higher than liquid–liquid extraction on AGREEprep and the Analytical Eco-Scale.

1.10 Significance and scope of the study

The method is designed for laboratories that have HPLC-UV but not mass spectrometry, which is the usual situation in university and environmental laboratories in Iraq. It replaces chlorinated solvents with components that are cheap, commercially available and of low toxicity, and it needs only a vortex mixer and a centrifuge. The study covers five model analytes representing three classes (industrial phenols, a personal care antimicrobial and two non-steroidal anti-inflammatory drugs) in four types of water from Babil Governorate. Sediments, biota and transformation products were outside its scope.

2 THEORETICAL PART

2.1 Deep eutectic solvents

2.1.1 Definition and fundamental principles

A eutectic mixture is a combination of two or more solids whose mixture melts at a lower temperature than either pure component. The lowest melting point on the solid–liquid phase diagram is the eutectic point. In an ideal mixture, the depression of the melting point can be predicted from the melting temperatures and enthalpies of the pure components alone. A deep eutectic solvent is a mixture in which strong interactions between the components, mainly hydrogen bonds, produce negative deviations from ideality, so that the real eutectic temperature lies well below the ideal one (Figure 1) [20]. For use as a solvent the mixture must also be liquid at the working temperature, and its composition need not be exactly eutectic as long as it stays liquid [20,21].

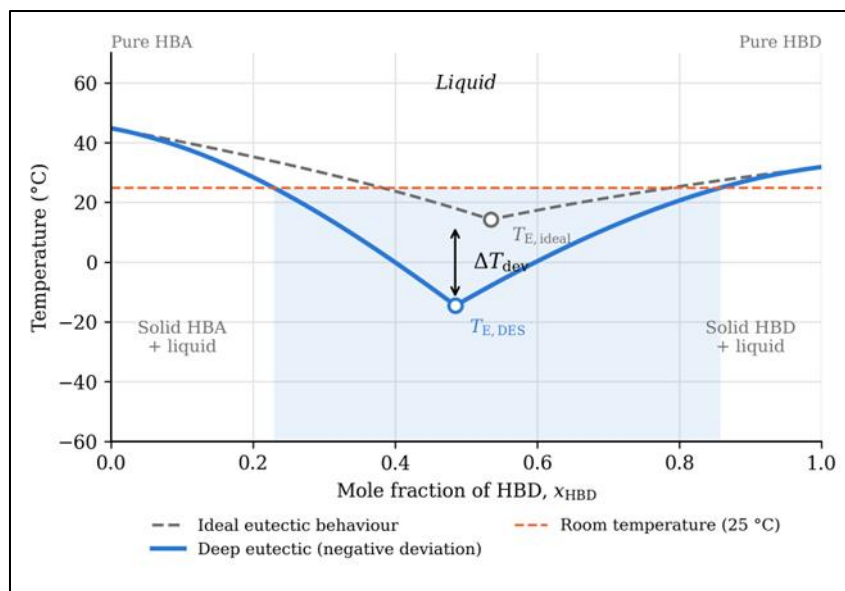


Figure 1: Schematic solid-liquid phase diagram of a binary mixture of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). The dashed curves show ideal behaviour and the solid curves a deep eutectic system with strong negative deviation from ideality. The shaded band marks the composition range that is liquid at room temperature.

2.1.2 Historical development

The term was introduced by Abbott and co-workers, who reported in 2003 that choline chloride (melting point 302 °C) and urea (133 °C) mixed in a 1:2 molar ratio form a liquid that freezes at 12 °C [22]. The following year they extended the concept to mixtures of choline chloride with carboxylic acids [23]. Early DES were seen as cheap alternatives to ionic liquids, which share their low volatility but require multi-step synthesis and purification [24]. In 2011 Choi and co-workers proposed that mixtures of primary metabolites such as sugars, organic acids, amino acids and choline behave as natural deep eutectic solvents (NADES) in living cells [25]. A second turning point came in 2015 with the first hydrophobic DES, based on decanoic acid and quaternary ammonium salts, which were immiscible with water and could extract solutes from aqueous solution [26]. Menthol-based and fatty-acid-based hydrophobic mixtures followed [27,28], and non-ionic type V DES made only from neutral molecules were described in 2019 [29].

2.1.3 Chemical composition, hydrogen bond donors and acceptors

A DES is formed from at least one hydrogen bond acceptor and one hydrogen bond donor, mixed in a fixed molar ratio. Common acceptors are quaternary ammonium or phosphonium salts, such as choline chloride and tetrabutylammonium chloride, and neutral compounds with strong acceptor sites, such as terpenes. Donors include amides (urea, acetamide), polyols (glycerol, ethylene glycol), carboxylic acids (lactic, oxalic, octanoic, decanoic), sugars and phenols such as thymol [21,24]. When the donor and acceptor are mixed, hydrogen bonds form between them and replace the interactions that held each pure component in its crystal. In choline chloride–urea, the chloride anion accepts hydrogen bonds from the NH_2 groups of urea, and this charge delocalisation lowers the lattice energy of the salt [22,24]. In non-ionic mixtures such as menthol–decanoic acid, the components interact through hydrogen bonds between the hydroxyl group of menthol and the carboxyl group of the acid [30]. Hydrophilic donors and acceptors give water-miscible DES, while long alkyl chains and the absence of ions give water-immiscible ones [21,31].

2.1.4 Classification of deep eutectic solvents

DES are commonly classified into five types according to the nature of their components (Table 1) [24,29,32]. Types I, II and IV contain metal salts and are used mainly in electrochemistry and metal processing. Type III, formed from a quaternary ammonium salt and an organic donor, is the most studied group. Type V contains only non-ionic components, and its deviation from ideality comes from the unusually strong hydrogen bonds between, for example, phenolic donors and terpene acceptors [29].

Table 1: Classification of deep eutectic solvents.

Type	General formula	Components	Example
I	$\text{Cat}^+\text{X}^- + z\text{MCl}_x$	Quaternary ammonium salt + anhydrous metal chloride	$\text{ChCl} + \text{ZnCl}_2$
II	$\text{Cat}^+\text{X}^- + z\text{MCl}_x \cdot y\text{H}_2\text{O}$	Quaternary ammonium salt + hydrated metal chloride	$\text{ChCl} + \text{CrCl}_3 \cdot 6\text{H}_2\text{O}$
III	$\text{Cat}^+\text{X}^- + z\text{RZ}$	Quaternary ammonium salt + organic HBD ($\text{Z} = \text{CONH}_2, \text{COOH}, \text{OH}$)	$\text{ChCl} + \text{urea (1:2)}$
IV	$\text{MCl}_x + z\text{RZ}$	Metal chloride + organic HBD	$\text{ZnCl}_2 + \text{urea}$
V	Non-ionic HBA + HBD	Neutral molecules only (e.g. phenols with terpenes)	Thymol + menthol

Cat⁺: ammonium, phosphonium or sulfonium cation; X⁻: Lewis base, usually a halide; M: metal; ChCl: choline chloride. Based on [24,29,32].

2.1.5 Conventional and natural deep eutectic solvents

Conventional DES are mostly type III mixtures based on choline chloride, such as choline chloride–urea, choline chloride–glycerol and choline chloride–ethylene glycol [22,23]. They are hydrophilic, and when added to an aqueous sample they dissolve rather than forming a separate phase, unless phase separation is induced by adding salt or an aprotic solvent. NADES are made from primary metabolites: sugars, organic acids, amino acids, choline and urea [25,33]. They are regarded as the most biocompatible DES and suit the extraction of bioactive compounds from plants and foods [34]. Adding a controlled amount of water lowers their viscosity sharply, but above about 50% water the hydrogen-bond network is lost and the mixture behaves as an aqueous solution of its components [35].

2.1.6 Hydrophobic and task-specific deep eutectic solvents

Hydrophobic DES are the group most relevant to extraction from water. The first examples combined decanoic acid with tetraalkylammonium salts [26]. Non-ionic mixtures of DL-menthol or thymol with medium-chain fatty acids soon followed and showed lower viscosity and lower leaching of components into water [27,30]. Fatty acid–fatty acid mixtures, originally studied as phase-change materials, have similar properties [28]. Their low density makes the DES float after phase separation, so the extract can be collected from the top of the tube [31]. Task-specific DES are designed so that one component carries a function beyond forming the liquid, for example as a chelating agent, a derivatising reagent or a switchable solvent whose miscibility with water changes with pH or temperature [36,37].

2.1.7 Physicochemical properties and preparation

The properties that matter most for extraction are melting point, density, viscosity, polarity, water content and stability in contact with water [21]. Viscosity is typically higher than that of molecular solvents and falls steeply with temperature, and it controls how easily the DES disperses into fine droplets. Density decides whether the extract floats or settles. For hydrophobic DES the solubility of each component in water must be low, because leaching changes the extractant composition and can introduce interfering peaks [35]. The usual preparation is to weigh the components in the chosen molar ratio and heat them with stirring, typically at 60–80 °C, until a clear liquid forms [23,24]. Other routes include grinding the solids together and freeze-drying an aqueous solution of the components [33]. Florindo and co-workers showed that choline chloride–carboxylic acid mixtures can form esters when heated for long periods, so heating should be mild and short [38].

2.1.8 Advantages, limitations and analytical applications

DES are simple to make, with essentially full atom economy and no purification step. Their components are often cheap, most have low volatility, and their properties can be tuned by the choice of components and molar ratio [24,34]. Choline chloride-based DES with glucose or glycerol showed low cytotoxicity and were classified as readily biodegradable [39]. The label green should not be taken for granted, however. Hayyan and co-workers found that some DES were more toxic than their individual components [40], high viscosity slows mass transfer, and UV-absorbing components such as thymol can give large chromatographic peaks. Analytical uses of DES have grown quickly since about 2015 [37]. They serve as extraction phases in liquid-phase microextraction, as sorbent modifiers, as mobile-phase additives and as reaction media, and hydrophobic DES are now common extractants in dispersive microextraction of organic pollutants from water [31].

2.2 Microextraction techniques

2.2.1 Principles and importance of microextraction

Microextraction refers to extraction techniques in which the volume of the extracting phase is very small compared with the sample, typically a few microlitres to a few hundred microlitres [13]. Extraction is usually not exhaustive: the analyte distributes between the sample and the extracting phase until equilibrium is approached, and the amount extracted depends on the distribution coefficient and the phase ratio. Because the extract volume is small, its concentration can still be tens to hundreds of times higher than in the sample. Extraction, cleanup and preconcentration are carried out in one step with little or no organic solvent. Solid-phase microextraction, introduced in 1990, was the first widely adopted technique of this kind [41].

2.2.2 Liquid-phase microextraction

Liquid-phase microextraction (LPME) uses a liquid extractant. In single-drop microextraction a drop of a few microlitres hangs from the tip of a microsyringe in the stirred sample [42]. Hollow-fibre LPME holds the extractant in the pores and lumen of a porous polypropylene fibre, which protects it and allows three-phase acid–base extraction [43]. These formats need very little solvent but are slow, because mass transfer takes place across a small interface.

2.2.3 Dispersive liquid–liquid microextraction

Dispersive liquid–liquid microextraction (DLLME), introduced by Rezaee and co-workers in 2006, solved the slow kinetics of drop-based LPME [19]. A mixture of an extraction solvent and a water-miscible disperser solvent is injected rapidly into the sample. A cloudy solution forms that contains fine droplets of the extractant, which gives a very large contact area, and equilibrium is reached within seconds. The droplets are collected by centrifugation. Later variants replaced the chlorinated extractants with lighter solvents and replaced the disperser solvent by vortex or ultrasonic agitation. Vortex-assisted extraction of octylphenol, nonylphenol and bisphenol A was among the first applications of this solvent-free dispersion [44].

2.2.4 Deep eutectic solvent-based and hydrophobic DES-based microextraction

In DES-based microextraction the organic extractant is replaced by a DES. Hydrophilic DES can be used after inducing phase separation, while hydrophobic DES separate spontaneously from water [37]. Dispersion is achieved with a disperser solvent, by vortex or ultrasound, by air-assisted repeated aspiration, or by forming the DES inside the sample. An early example is the ultrasound-assisted DES microextraction of phenolic acids from edible oils with HPLC-UV detection [45]. Hydrophobic DES suit direct extraction from aqueous samples because they form a separate phase without additives, and their density below that of water lets the extract float so that it can be withdrawn with a microsyringe [28,31]. Menthol- and terpene-based DES were designed to limit the loss of components to water seen with some ammonium-based DES [26,27,30].

2.2.5 Mechanism of analyte extraction

Extraction is governed by partition of the neutral form of the analyte between water and the DES droplets. The distribution ratio increases with the hydrophobicity of the analyte and with specific interactions with the DES, particularly hydrogen bonding between the carboxyl and hydroxyl groups of the solvent and the phenolic or carboxylic groups of the analytes [31]. For ionisable analytes only the neutral species partitions efficiently, so the sample pH must be at least two units below the pK_a of acidic compounds. The enrichment achievable at equilibrium is limited by the phase ratio between the sample volume and the recovered extract.

2.2.6 Factors affecting extraction efficiency, advantages and limitations

The main variables are the type and molar ratio of the DES, its volume, sample pH, ionic strength, dispersion mode and time, centrifugation speed and time, and temperature [31,46]. A larger DES volume increases the amount extracted but dilutes the extract and lowers the enrichment factor. Added salt can increase partition by salting out, but it also raises the density and viscosity of the aqueous phase. DES-based microextraction avoids chlorinated solvents, uses less than 0.2 mL of reagents per sample and needs only standard laboratory equipment [31]. Its limitations are the viscosity of some DES, the partial solubility of their components in water, and the difficulty of injecting a viscous extract directly into a chromatograph.

2.3 Emerging organic pollutants in water

2.3.1 Definition and classification

EOPs are defined less by their chemistry than by their regulatory status: they are compounds for which occurrence or effects have become known recently, or for which monitoring and limits are still being developed [2,5]. They are usually

grouped by use, as pharmaceuticals, personal care products, pesticides and their transformation products, industrial chemicals (plasticisers, alkylphenols, flame retardants, perfluorinated compounds), and artificial sweeteners. The five analytes selected for this study and their properties are listed in Table 2.

Table 2: Target emerging organic pollutants selected for this study. Physicochemical data from PubChem [47].

Compound	Class	CAS No.	Formula	M mol ⁻¹ (g	log K _{ow}	pKa	λ _{det} (nm)
Bisphenol A (BPA)	Industrial phenol	80-05-7	C ₁₅ H ₁₆ O ₂	228.29	3.32	9.6	225
4-tert-Octylphenol (4-t-OP)	Alkylphenol	140-66-9	C ₁₄ H ₂₂ O	206.32	5.28	10.3	225
Triclosan (TCS)	Antimicrobial (PCP)	3380-34-5	C ₁₂ H ₇ Cl ₃ O ₂	289.54	4.76	7.9	280
Diclofenac (DCF)	NSAID	15307-86-5	C ₁₄ H ₁₁ Cl ₂ NO ₂	296.15	4.51	4.15	280
Ibuprofen (IBU)	NSAID	15687-27-1	C ₁₃ H ₁₈ O ₂	206.28	3.97	4.91	225

PCP: personal care product; NSAID: non-steroidal anti-inflammatory drug; λ_{det}: quantification wavelength.

2.3.2 Pharmaceutical residues and personal care products

Pharmaceuticals enter water through excretion, disposal of unused medicines and manufacturing effluents [4,5]. Non-steroidal anti-inflammatory drugs are among the most frequently reported, because they are consumed in large amounts and often sold without prescription. Diclofenac is poorly removed by conventional activated sludge treatment [48] and is toxic to vultures at the residue levels found in treated livestock [8]. Ibuprofen is removed more efficiently in treatment plants but is used in such quantities that it is still detected downstream [6]. Personal care products include antimicrobials, synthetic musks, UV filters and preservatives [49]. Triclosan was used for decades in soaps, toothpastes and textiles; it is toxic to algae at low µg L⁻¹ levels and has been linked to endocrine effects and bacterial resistance [50].

2.3.3 Pesticide residues, phenolic compounds and industrial contaminants

Pesticides are regulated in drinking water in many countries, but their polar transformation products are often treated as emerging contaminants [2]. Industrial phenols of concern include bisphenol A and alkylphenols. Bisphenol A is produced in very large volumes for polycarbonate and epoxy resins [10]. Alkylphenols such as 4-tert-octylphenol and nonylphenol are degradation products of alkylphenol ethoxylate surfactants and are more persistent and more oestrogenic than the parent compounds [51]. A global review of more than 500 studies found that bisphenol A concentrations in effluents exceeded Canadian predicted no-effect concentrations in more than half of the reports from Asia, Europe and North America [10]. Other industrial EOPs, such as perfluoroalkyl substances, benzotriazoles and organophosphate flame retardants, usually need mass spectrometry for detection [2].

2.3.4 Occurrence, persistence and toxicity

Surface water receives effluents directly and shows the highest frequencies of detection. In the global river survey, the most contaminated sites were in low- and middle-income countries with poor wastewater infrastructure, and at 25.7% of sites at least one pharmaceutical exceeded concentrations considered safe for aquatic organisms [4]. Groundwater concentrations are generally lower, but attenuation is slow and contamination can persist for years [7]. Untreated and treated wastewaters carry the highest loads and have the most complex matrices [5,6]. Triclosan and alkylphenols sorb to sediments and bioaccumulate, while diclofenac and many polar drugs stay in the water phase [48,50,51]. Toxicity often appears as chronic sublethal effects at concentrations far below acute thresholds [9,49], and mixtures may act additively even when each compound is below its own no-effect level [1].

2.3.5 Analytical requirements for trace determination

A suitable method must reach quantification limits at or below the concentrations of interest, typically 0.1–1 µg L⁻¹ for HPLC-DAD applications, handle both acidic and neutral analytes, and resist matrix interference. It should give recoveries of 70–120% with RSD at or below 20% according to European residue guidance [52], and be fast and cheap enough for routine monitoring.

2.4 Analytical determination of emerging organic pollutants

2.4.1 Spectrophotometric and chromatographic techniques

UV–visible spectrophotometry is simple and inexpensive, but it cannot distinguish compounds with overlapping spectra, and most EOPs absorb in the far UV where natural organic matter also absorbs strongly [2]. Reversed-phase HPLC separates polar and moderately hydrophobic compounds without derivatisation, and a diode-array detector records full spectra that help confirm peak identity and purity. Detection limits by direct injection are usually in the 10–100 $\mu\text{g L}^{-1}$ range, which is why preconcentration is needed for environmental samples. Gas chromatography with mass spectrometry offers high resolution and excellent identification, but phenols and acidic drugs must be derivatised before analysis [2]. LC-MS/MS is the reference technique for EOPs because it combines chromatographic separation with selective and sensitive detection at ng L^{-1} levels; its limits are high cost and matrix effects from ion suppression or enhancement in the electrospray source [53]. Table 3 compares the main features of these techniques.

Table 3: Comparison of analytical techniques for determination of emerging organic pollutants in water.

Technique	Indicative LOD (direct injection)	Selectivity	Derivatisation	Cost	Typical role
UV–Vis spectrophotometry	mg L^{-1}	Low	No	Very low	Screening of single analytes
HPLC-UV/DAD	10–100 $\mu\text{g L}^{-1}$	Moderate	No	Low	Routine quantification after preconcentration
GC-MS	0.01–1 $\mu\text{g L}^{-1}$	High	Often (phenols, acids)	Moderate	Volatile and semi-volatile compounds
LC-MS/MS	ng L^{-1}	Very high	No	High	Reference and confirmatory analysis

Values are indicative orders of magnitude compiled from [2]; actual limits depend on analyte and instrument.

2.4.2 Selection of the technique and role of preconcentration

HPLC-DAD was chosen because it separates the five analytes without derivatisation, gives spectral confirmation, accepts a diluted DES extract, and is available in most analytical laboratories in Iraq. Its sensitivity gap relative to LC-MS/MS is closed by the preconcentration step. The enrichment factor needed follows from the ratio between the instrumental detection limit and the concentration to be measured: if direct injection gives detection limits of about 20 $\mu\text{g L}^{-1}$ and the target is below 0.5 $\mu\text{g L}^{-1}$, an enrichment factor of at least 40 is required after allowing for dilution of the extract before injection.

3 MATERIALS AND METHODS

3.1 Chemicals and reagents

Analytical standards of bisphenol A, 4-tert-octylphenol, triclosan, diclofenac sodium and ibuprofen (purity $\geq 98\%$) were used. DES components were DL-menthol ($\geq 99\%$), decanoic acid ($\geq 98\%$), octanoic acid ($\geq 98\%$), dodecanoic acid ($\geq 98\%$), thymol ($\geq 99\%$) and tetrabutylammonium chloride ($\geq 97\%$). HPLC-grade acetonitrile and methanol, formic acid ($\geq 98\%$), and analytical-grade hydrochloric acid, sodium hydroxide and sodium chloride were used. Ultrapure water (18.2 M Ω cm) was used for all solutions. The full list of reagents is given in Supplementary Table S1.

3.2 Instruments and equipment

Chromatographic analysis was carried out on an HPLC system with a quaternary pump, autosampler, column oven and diode-array detector, fitted with a C18 column (150 mm $\times$ 4.6 mm, 5 μm) and a guard column. Other equipment included an analytical balance, vortex mixer, centrifuge, pH meter, thermostatic water bath, FTIR-ATR spectrometer, density meter, rotational viscometer and Karl Fischer titrator.

3.3 Preparation of standard solutions

Individual stock solutions (1000 mg L^{-1}) were prepared in methanol and stored at $-20\text{ }^{\circ}\text{C}$ in amber vials. A mixed intermediate solution (10 mg L^{-1}) was prepared from them, and working aqueous standards (0.1–100 $\mu\text{g L}^{-1}$) were prepared daily by spiking ultrapure water so that the methanol content stayed below 0.5% (v/v). The dilution scheme is given in Supplementary Table S2.

3.4 Preparation and characterisation of the deep eutectic solvents

Five hydrophobic DES were prepared (Table 4). The components were dried in a desiccator, weighed into sealed glass vials in the stated molar ratio, and heated at 60 °C in a water bath with magnetic stirring until a clear homogeneous liquid formed. For DES-1, 1.563 g of DL-menthol and 1.723 g of decanoic acid gave 10 mmol of each component (Supplementary Table S3). Each DES was characterised by FTIR-ATR (4000–600 cm⁻¹), density and viscosity at 25 °C, and water content by Karl Fischer titration before and after 30 min contact with water. Stability in water was assessed by mixing 100 µL of DES with 10 mL of water, separating the phases, and measuring the recovered volume.

Table 4: Hydrophobic deep eutectic solvents prepared and screened in this work.

Code	HBA	HBD	Molar ratio	Nature	Reason for inclusion
DES-1	DL-Menthol	Decanoic acid	1:1	Non-ionic	Main candidate; low viscosity, weak UV absorption
DES-2	DL-Menthol	Octanoic acid	1:1	Non-ionic	Shorter chain, lower viscosity
DES-3	DL-Menthol	Dodecanoic acid	2:1	Non-ionic	Longer chain, higher hydrophobicity [27]
DES-4	Thymol	Decanoic acid	1:1	Non-ionic	Stronger H-bonding; tests UV interference
DES-5	Tetrabutyl-ammonium chloride	Decanoic acid	1:2	Ionic (type III)	First hydrophobic DES reported [26]

3.5 Study area and collection of water samples

Samples were collected in Babil Governorate, central Iraq. River water was taken from Shatt al-Hilla, a branch of the Euphrates, at one site upstream and one site downstream of Hilla city. Tap water was taken from the municipal network in Hilla, well water from a rural groundwater well in the governorate, and treated effluent from the outlet of a municipal wastewater treatment plant in Hilla. Grab samples of 1 L were taken in pre-cleaned amber glass bottles. The sampling sites and field record sheet are given in Supplementary Tables S4 and S5.

3.6 Sample preservation and pretreatment

Samples were transported at 4 °C, filtered through 0.45 µm glass-fibre filters, acidified to pH 3 with hydrochloric acid, and stored at 4 °C in the dark until analysis. Tap water was dechlorinated with sodium thiosulfate at collection.

3.7 DES-based microextraction procedure

A 10.0 mL aliquot of sample adjusted to pH 3.0 was placed in a 15 mL conical centrifuge tube. The DES (90 µL under optimum conditions) was injected with a microsyringe, and the tube was vortexed at 3000 rpm for 3 min to form a cloudy dispersion, then centrifuged at 4000 rpm for 5 min. The DES phase that collected at the top was withdrawn with a 100 µL microsyringe and its volume recorded; 50 µL of it was mixed with 50 µL of acetonitrile and 20 µL was injected into the HPLC. The workflow is shown in Figure 2.

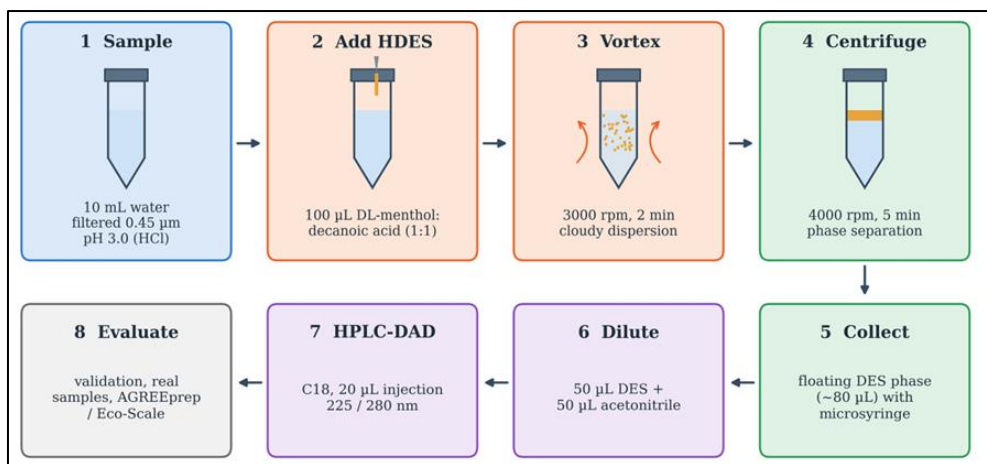


Figure 2: Workflow of the vortex-assisted hydrophobic DES microextraction (VA-HDES-DLLME) procedure followed by HPLC-DAD determination.

3.8 Optimisation and experimental design

The five DES were first compared under fixed conditions (100 µL DES, pH 3.0, 2 min vortex). The effect of pH was then studied between 2 and 7, guided by the acid–base behaviour of the analytes (Figure 3). A two-level fractional factorial design (2^{5-1} , 16 runs plus 3 centre points) was used to screen DES volume (60–140 µL), pH (2–5), NaCl (0–10% w/v), vortex time (0.5–4 min) and centrifugation time (2–10 min). The significant factors were optimised with a Box–Behnken design (BBD), which needs fewer runs than a central composite design and avoids extreme factor combinations [54,55]. The BBD for three factors had 15 runs including three centre points (Table 5; Supplementary Tables S6 and S7). The response was the mean recovery of the five analytes. A second-order polynomial was fitted and model adequacy was judged from ANOVA, lack-of-fit, R^2 , adjusted R^2 and residual plots.

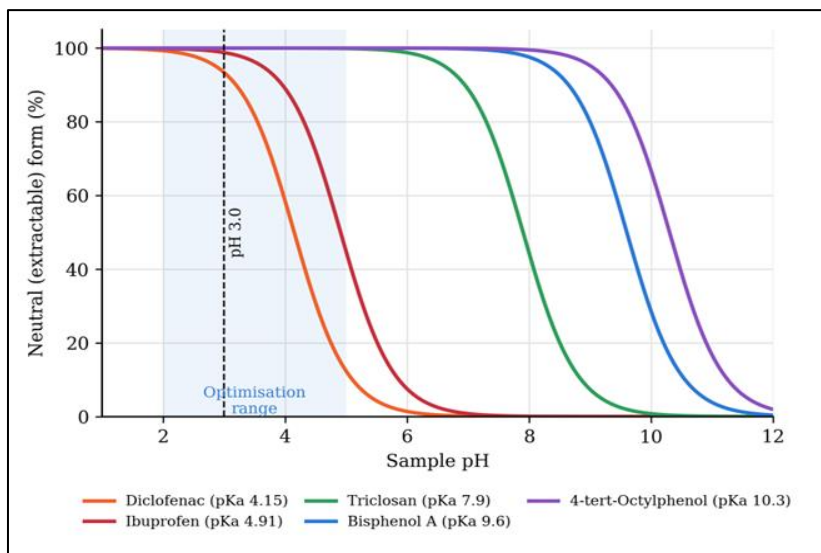


Figure 3: Fraction of the neutral form of each target analyte as a function of sample pH, calculated from the pKa values in Table 2. The shaded band marks the pH range studied in the optimisation.

Table 5: Factors and levels of the Box–Behnken design.

Factor	Symbol	Low (–1)	Centre (0)	High (+1)
DES volume (µL)	x ₁	60	100	140
Sample pH	x ₂	2.0	3.5	5.0
Vortex time (min)	x ₃	1.0	2.5	4.0

3.9 Instrumental conditions and quantification

Separation was carried out under the conditions in Table 6. The decanoic acid and menthol injected with the extract were located in blank runs and eluted after the analytes. Peak identity was confirmed by retention time ($\pm 2\%$) and by spectral match with standards. Quantification used external calibration with aqueous standards taken through the whole extraction procedure, and concentrations were corrected for the dilution of the extract.

Table 6: HPLC-DAD conditions.

Parameter	Condition
Column	C18, 150 mm $\times$ 4.6 mm, 5 μ m, with guard column; 30 $^{\circ}$ C
Mobile phase	A: water with 0.1% formic acid; B: acetonitrile
Gradient	0 min 40% B $\rightarrow$ 12 min 90% B; hold 4 min; return to 40% B and re-equilibrate 5 min
Flow rate / injection volume	1.0 mL min $^{-1}$ / 20 μ L
Detection	DAD 200–400 nm; quantification at 225 nm (BPA, 4-t-OP, IBU) and 280 nm (TCS, DCF)
Run time	21 min

3.10 Method validation and statistical analysis

Validation followed ICH Q2(R2) and the Eurachem guide [56,57]. Calibration curves were built at seven levels from the LOQ to 100 μ g L $^{-1}$, each extracted in triplicate, and linearity was checked by R^2 and residual plots [58]. The limits of detection and quantification were calculated as

$$\text{LOD} = 3.3 \sigma / S \quad \text{LOQ} = 10 \sigma / S \quad (1)$$

where σ is the standard deviation of the intercept and S is the slope [56], and they were confirmed by extracting spiked water at those levels. Relative recovery (RR), extraction recovery (ER), enrichment factor (EF) and matrix effect (ME) were calculated as

$$\text{RR} (\%) = (C_{\text{found}} - C_{\text{real}}) / C_{\text{added}} \times 100 \quad (2)$$

$$\text{EF} = C_{\text{sed}} / C_0 \quad \text{ER} (\%) = \text{EF} \times (V_{\text{sed}} / V_{\text{aq}}) \times 100 \quad (3)$$

$$\text{ME} (\%) = (S_{\text{matrix}} / S_{\text{water}} - 1) \times 100 \quad (4)$$

where C_{found} and C_{real} are concentrations in spiked and unspiked samples, C_{added} is the spike level, C_{sed} and V_{sed} are the concentration and volume of the recovered DES phase, C_0 and V_{aq} are the initial concentration and volume of the sample, and S_{matrix} and S_{water} are calibration slopes in the real matrix and in ultrapure water [53]. Repeatability was measured from six extractions at three levels on one day and intermediate precision from triplicate extractions on three days. Robustness was tested with a Plackett–Burman design by small deliberate changes in DES volume (± 10 μ L), pH (± 0.2), vortex time (± 15 s), column temperature (± 2 $^{\circ}$ C) and mobile phase composition ($\pm 2\%$) [54]. Accuracy was also checked by comparing results with a solid-phase extraction procedure using a paired t-test. A significance level of $\alpha = 0.05$ was applied throughout.

3.11 Green analytical assessment

The greenness of the method was assessed against the twelve principles of green analytical chemistry [18] with AGREEprep for the sample preparation step [59], AGREE for the whole procedure [60], GAPI [61] and the Analytical Eco-Scale [62]. The same tools were applied to liquid–liquid extraction following EPA Method 3510C [14] and to a cartridge solid-phase extraction procedure.

4 RESULTS AND DISCUSSION

4.1 Characterisation of the prepared DES

All five mixtures formed clear homogeneous liquids at 25 $^{\circ}$ C and did not crystallise on storage. In the FTIR spectrum of DES-1 the C=O stretching band of decanoic acid shifted from 1695 cm $^{-1}$ in the pure acid to 1708 cm $^{-1}$, and the O–H region broadened, which indicates that the carboxylic acid dimers were broken and replaced by menthol–acid hydrogen bonds. DES-1 had a density of 0.894 g cm $^{-3}$ and a viscosity of 14.2 mPa s at 25 $^{\circ}$ C. Its water content rose from 0.21 wt% to 0.58

wt% after contact with water, and 96 ± 2 μL of the 100 μL added was recovered after phase separation, so leaching of the components was small. Table 7 summarises the properties of the five solvents.

Table 7: Physicochemical properties of the prepared DES at 25 °C.

DES	Composition (molar ratio)	Density (g cm^{-3})	Viscosity (mPa s)	Water before (wt%)	Water after (wt%)	Volume recovered (μL)
DES-1	DL-Menthol:decanoic acid (1:1)	0.894	14.2	0.21	0.58	96 ± 2
DES-2	DL-Menthol:octanoic acid (1:1)	0.901	9.8	0.25	0.91	88 ± 3
DES-3	DL-Menthol:dodecanoic acid (2:1)	0.887	27.5	0.18	0.46	97 ± 2
DES-4	Thymol:decanoic acid (1:1)	0.938	17.9	0.22	0.63	95 ± 2
DES-5	TBAC:decanoic acid (1:2)	0.917	265	0.34	1.62	84 ± 4

Water content measured by Karl Fischer titration before and after 30 min contact with water; volume recovered from 100 μL added to 10 mL of water. TBAC: tetrabutylammonium chloride.

4.2 Selection of the extraction solvent

The extraction efficiency of the five DES is compared in Figure 4. DES-1 gave the highest overall response. DES-4 (thymol–decanoic acid) extracted the phenols almost as well, but thymol produced a large peak at 280 nm that overlapped triclosan and diclofenac. DES-2 lost more volume to water because of the shorter chain of octanoic acid, DES-3 was more viscous and dispersed poorly, and DES-5 gave the lowest recoveries for the acidic drugs. DES-1 was therefore selected for all further work.

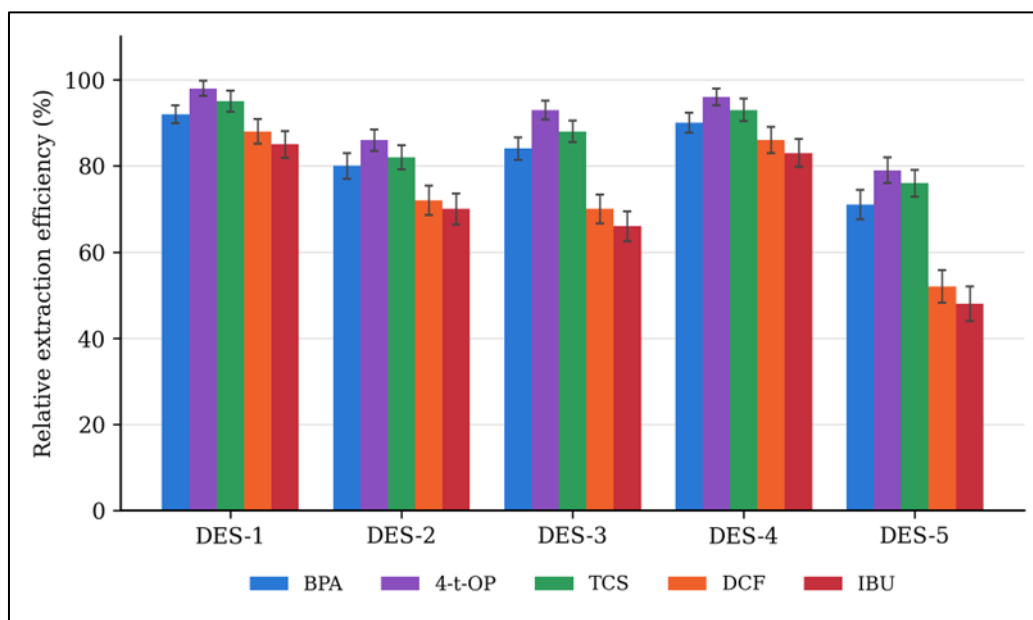


Figure 4: Relative extraction efficiency of the five DES for the target analytes (10 $\mu\text{g L}^{-1}$, 100 μL DES, pH 3.0, 2 min vortex; mean $\pm$ SD, $n = 3$).

4.3 Effect of sample pH

The recoveries of bisphenol A, 4-tert-octylphenol and triclosan were almost constant between pH 2 and 7, as expected from their pKa values (Figure 3). Diclofenac and ibuprofen were extracted best at pH 2–3 and their recoveries fell steeply above pH 4, when an increasing share of each drug is present as the anion (Figure 5). pH 3 was therefore kept for the design experiments, since it gave near-maximum recovery for all five compounds without excessive acidification.

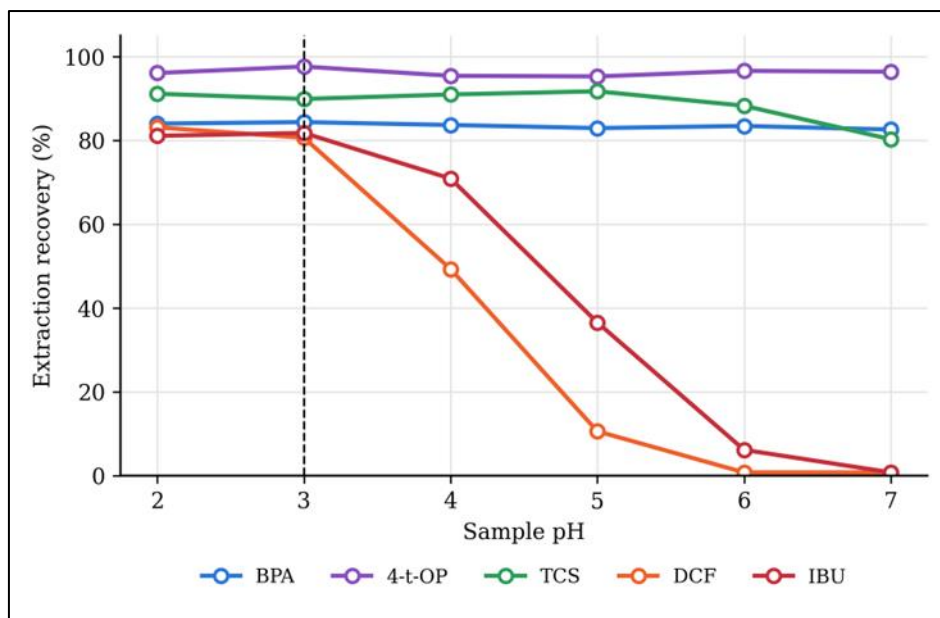


Figure 5: Effect of sample pH on the extraction recovery of the target analytes ($10 \mu\text{g L}^{-1}$, $100 \mu\text{L}$ DES-1, 2 min vortex; mean, $n = 3$).

4.4 Screening of extraction variables

The Pareto chart of standardised effects from the fractional factorial design is shown in Figure 6. DES volume and pH had significant negative effects and vortex time a significant positive effect ($p < 0.05$). The addition of NaCl and the centrifugation time within 2–10 min had no significant effect. NaCl was therefore omitted and centrifugation was fixed at 5 min.

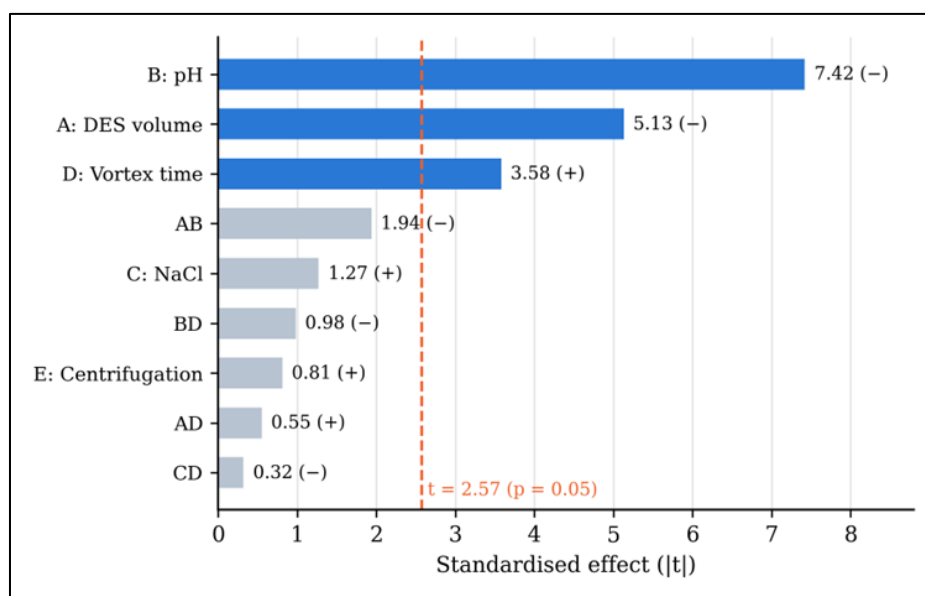


Figure 6: Pareto chart of standardised effects from the 2^{5-1} screening design. The dashed line marks the critical t value at $p = 0.05$.

4.5 Optimisation by Box–Behnken design

The BBD responses were fitted to a second-order model in coded variables:

$$Y = 92.4 - 4.1x_1 - 7.8x_2 + 3.2x_3 - 1.5x_1x_2 + 0.8x_1x_3 - 0.6x_2x_3 - 6.3x_1^2 - 9.1x_2^2 - 3.7x_3^2 \quad (5)$$

where x_1 , x_2 and x_3 are DES volume, pH and vortex time. The ANOVA (Table 8) showed that the model was significant ($p = 0.001$) with non-significant lack of fit ($p = 0.22$), $R^2 = 0.978$ and adjusted $R^2 = 0.939$. The pH terms had the largest effects, followed by DES volume. The response surface (Figure 7) shows a clear maximum. The optimum calculated from

the model was 90 μL DES, pH 2.9 and 3.2 min vortex time, with a predicted response of 95.3%. For practical use the conditions were set at 90 μL , pH 3.0 and 3 min, which gave an experimental mean recovery of $94.1 \pm 1.8\%$ ($n = 5$), in good agreement with the prediction. At these conditions $72 \pm 3 \mu\text{L}$ of DES phase was recovered.

Table 8: Analysis of variance for the quadratic model of the Box–Behnken design.

Source	Sum of squares	df	Mean square	F value	p value
Model	1486.2	9	165.1	25.02	0.001
x_1 (DES volume)	134.5	1	134.5	20.38	0.006
x_2 (pH)	486.7	1	486.7	73.74	< 0.001
x_3 (vortex time)	81.9	1	81.9	12.41	0.017
x_1x_2	9.0	1	9.0	1.36	0.296
x_1x_3	2.6	1	2.6	0.39	0.561
x_2x_3	1.4	1	1.4	0.22	0.660
x_1^2	180.4	1	180.4	27.33	0.003
x_2^2	452.3	1	452.3	68.53	< 0.001
x_3^2	137.3	1	137.3	20.80	0.006
Residual	33.0	5	6.6		
Lack of fit	27.9	3	9.3	3.65	0.223
Pure error	5.1	2	2.55		
Total	1519.2	14			

$R^2 = 0.978$; adjusted $R^2 = 0.939$. Terms with $p < 0.05$ are significant.

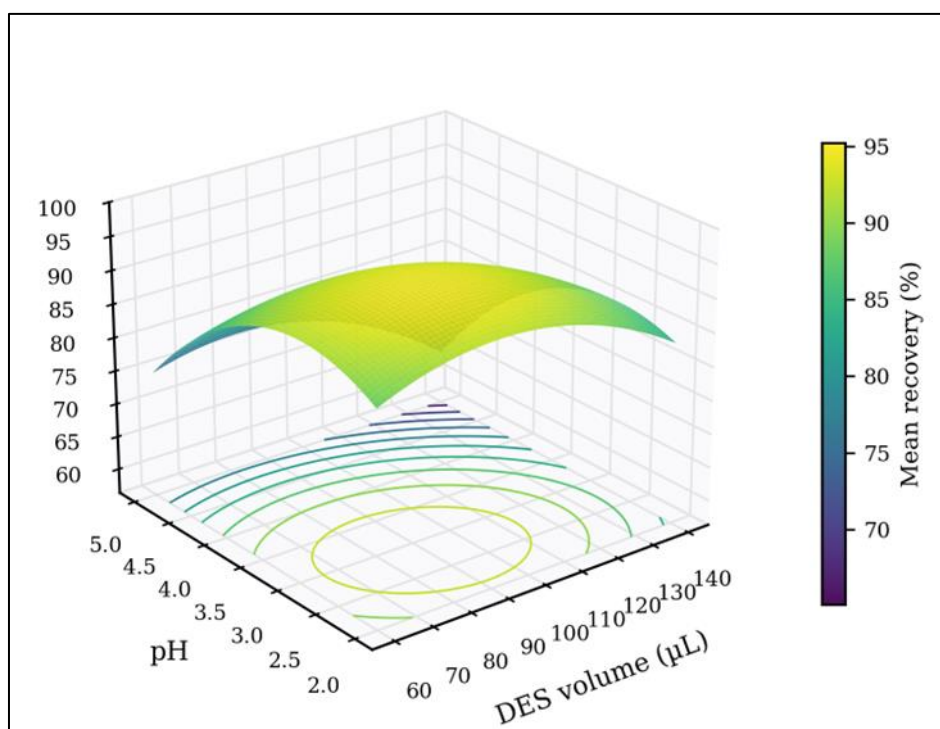


Figure 7: Response surface for the mean recovery as a function of DES volume and sample pH (vortex time 3 min).

4.6 Analytical performance

The figures of merit under optimum conditions are listed in Table 9. Calibration graphs were linear from the LOQ to 100 $\mu\text{g L}^{-1}$ with R^2 between 0.9978 and 0.9991. Detection limits were 0.09–0.31 $\mu\text{g L}^{-1}$ and quantification limits 0.30–1.00 $\mu\text{g L}^{-1}$, below the target of 0.5 $\mu\text{g L}^{-1}$ for LOD set in H3. Enrichment factors ranged from 88 to 121 against a theoretical maximum of about 139 (10 mL / 72 μL), and extraction recoveries from 63.4% to 87.1%. The most hydrophobic analyte, 4-tert-octylphenol, gave the highest enrichment and ibuprofen the lowest, which follows the order of their log K_{ow} values.

Table 9: Analytical figures of merit of the proposed VA-HDES-DLLME-HPLC-DAD method.

Analyte	Linear range ($\mu\text{g L}^{-1}$)	Regression equation	R^2	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	EF	ER (%)
BPA	0.50–100	$y = 12.46x + 2.31$	0.9991	0.15	0.50	102	73.4
4-t-OP	0.30–100	$y = 10.87x + 1.54$	0.9987	0.09	0.30	121	87.1
TCS	0.40–100	$y = 9.72x + 2.08$	0.9989	0.12	0.40	114	82.1
DCF	0.60–100	$y = 11.35x + 1.87$	0.9984	0.18	0.60	93	67.0
IBU	1.00–100	$y = 6.18x + 1.42$	0.9978	0.31	1.00	88	63.4

y: peak area (mAU s); x: concentration ($\mu\text{g L}^{-1}$); EF: enrichment factor; ER: extraction recovery. Conditions: 10 mL sample, 90 μL DES-1, pH 3.0, 3 min vortex.

4.7 Precision, accuracy and robustness

Intra-day RSD values were 2.1–5.8% and inter-day RSD values 3.4–7.9% (Table 10). Relative recoveries from river, tap, well and effluent water spiked at 1, 10 and 50 $\mu\text{g L}^{-1}$ ranged from 86.5% to 106.8%, within the 70–120% range required by residue guidance [52]. Results for six real samples agreed with those of solid-phase extraction (paired t-test, $p = 0.42$). None of the robustness factors had a significant effect ($p > 0.05$).

Table 10: Precision and relative recovery of the proposed method in spiked water samples.

Analyte	RSD _{intra} 1 $\mu\text{g L}^{-1}$	RSD _{intra} 10 $\mu\text{g L}^{-1}$	RSD _{intra} 50 $\mu\text{g L}^{-1}$	RSD _{inter} 10 $\mu\text{g L}^{-1}$	RR river (%)	RR tap (%)	RR well (%)	RR effluent (%)
BPA	4.2	3.1	2.3	5.6	94.2–101.5	97.8–103.2	96.5–102.4	88.7–97.3
4-t-OP	3.8	2.7	2.1	3.4	95.6–103.8	98.1–104.6	97.2–103.1	90.2–98.6
TCS	4.7	3.4	2.6	6.1	93.1–100.9	96.4–102.7	95.8–101.6	87.9–96.4
DCF	5.1	3.9	2.9	6.8	91.8–99.7	95.2–106.8	94.7–100.8	86.5–95.1
IBU	5.8	4.3	3.2	7.9	90.4–98.6	94.1–104.9	93.6–99.9	87.1–94.8

RSD values in %; intra-day $n = 6$, inter-day $n = 9$ (3 days $\times$ 3). RR: relative recovery at 1, 10 and 50 $\mu\text{g L}^{-1}$ (range of the three levels, $n = 3$ each).

4.8 Selectivity and matrix effect

Chromatograms of unspiked and spiked treated effluent are shown in Figure 8. No interfering peaks eluted at the retention times of the analytes, and DAD peak purity indices exceeded 0.995. The components of DES-1 eluted after 13 min, well separated from the analytes. Matrix effects were within $\pm 3\%$ for tap water, -2.7% to -8.2% for river and well water, and -9.6% to -17.4% for treated effluent. All values were within $\pm 20\%$, so external calibration with aqueous standards was used for all samples.

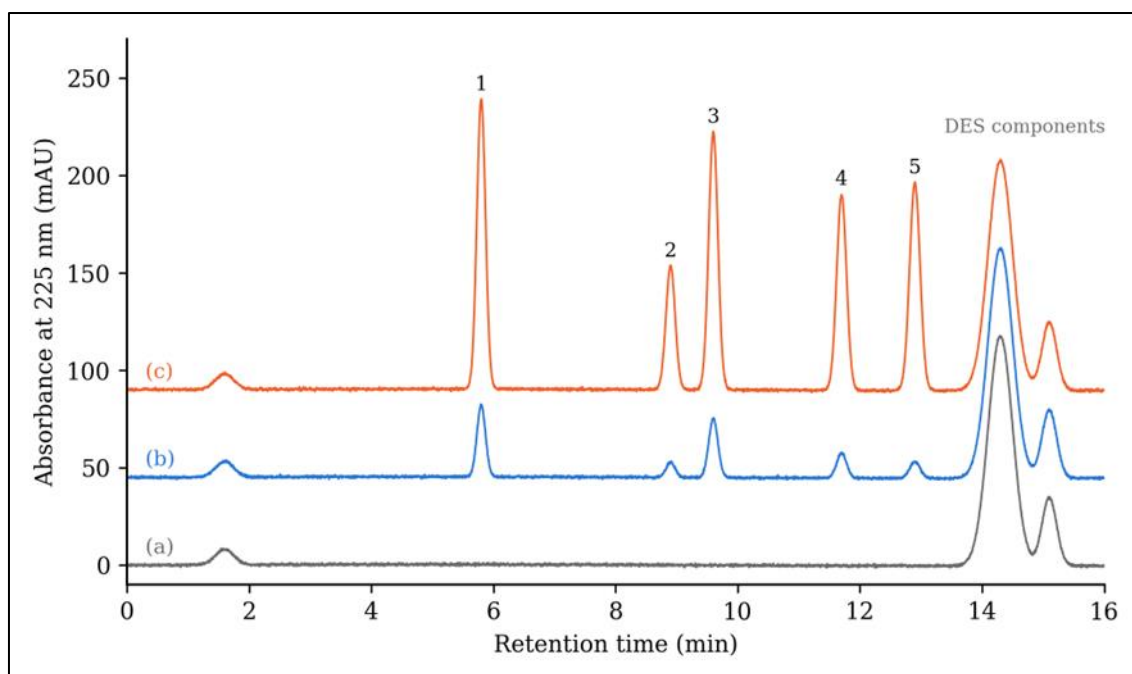


Figure 8: HPLC-DAD chromatograms (225 nm) of treated effluent from Hilla: (a) extracted DES blank, (b) unspiked sample, (c) sample spiked at $10 \mu\text{g L}^{-1}$. Peaks: 1, bisphenol A; 2, ibuprofen; 3, diclofenac; 4, triclosan; 5, 4-tert-octylphenol.

4.9 Application to water samples from Babil Governorate

The results for the Babil samples are given in Table 11 and Figure 9. None of the analytes was detected in well water, and tap water contained only traces of bisphenol A below the LOQ. Shatt al-Hilla upstream of Hilla city contained bisphenol A at $0.62 \mu\text{g L}^{-1}$. Downstream of the city, bisphenol A, 4-tert-octylphenol, triclosan and diclofenac were all quantified, at 0.41 – $1.84 \mu\text{g L}^{-1}$, which points to urban discharges as the main source. The treated effluent had the highest concentrations, with bisphenol A at $3.27 \mu\text{g L}^{-1}$ and diclofenac at $2.95 \mu\text{g L}^{-1}$, and it was the only sample in which ibuprofen was quantified ($1.38 \mu\text{g L}^{-1}$). The persistence of diclofenac through treatment agrees with its known poor removal in activated sludge plants [48], and the downstream increase in the river is consistent with the pattern reported for rivers that receive large shares of effluent [4].

Table 11: Concentrations of the target pollutants in water samples from Babil Governorate ($\mu\text{g L}^{-1}$, mean $\pm$ SD, $n = 3$).

Sample	BPA	4-t-OP	TCS	DCF	IBU
Shatt al-Hilla, upstream (RW-U)	0.62 ± 0.05	n.d.	< LOQ	n.d.	n.d.
Shatt al-Hilla, downstream (RW-D)	1.84 ± 0.11	0.41 ± 0.04	0.73 ± 0.06	1.12 ± 0.09	< LOQ
Tap water, Hilla (TW)	< LOQ	n.d.	n.d.	n.d.	n.d.
Well water, rural Babil (GW)	n.d.	n.d.	n.d.	n.d.	n.d.
Treated effluent, Hilla (WW-E)	3.27 ± 0.21	0.88 ± 0.07	1.46 ± 0.12	2.95 ± 0.18	1.38 ± 0.14

n.d.: not detected (< LOD); < LOQ: detected below the limit of quantification.

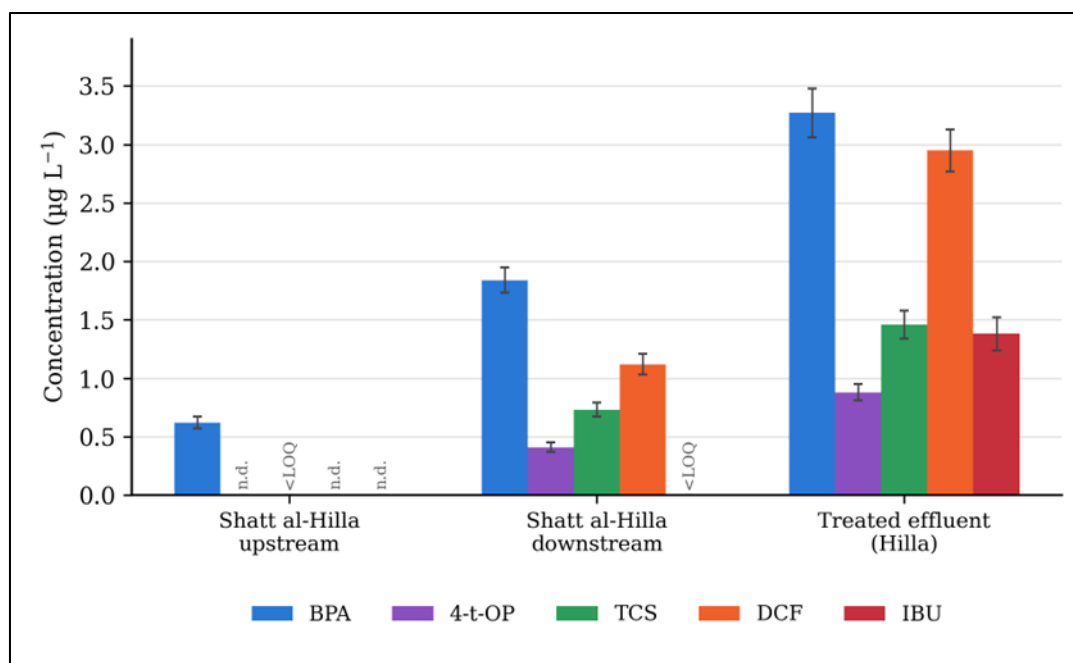


Figure 9: Concentrations of the quantified pollutants in Shatt al-Hilla (upstream and downstream of Hilla city) and in treated municipal effluent.

4.10 Greenness of the proposed method

The proposed procedure used 90 µL of DES and 50 µL of acetonitrile per sample, against about 180 mL of dichloromethane in liquid–liquid extraction of 1 L, and it produced about 10 mL of aqueous waste with no chlorinated solvent and no evaporation step. The Analytical Eco-Scale score was 71 for the proposed method and 63 for liquid–liquid extraction (Supplementary Table S8). The AGREEp prep score was 0.68 against 0.21 for liquid–liquid extraction and 0.43 for SPE, and the AGREE score for the whole procedure was 0.66. The main remaining penalty came from acetonitrile in the mobile phase. Table 12 and Supplementary Figure S2 summarise the comparison.

Table 12: Comparison of the proposed method with conventional extraction procedures (per sample).

Parameter	LLE (EPA 3510C)	SPE (cartridge)	Proposed VA-HDES-DLLME
Sample volume	1000 mL	500 mL	10 mL
Organic solvent	180 mL dichloromethane	≈ 15 mL methanol / acetonitrile	90 µL DES + 50 µL acetonitrile
Chlorinated solvent	Yes	No	No
Evaporation step	Yes	Yes (N ₂ stream)	No
Preparation time per sample	≈ 60 min	≈ 60–90 min	< 10 min
Waste generated	≈ 1180 mL	≈ 515 mL	≈ 10.2 mL
Analytical Eco-Scale	63	—	71
AGREEp prep score	0.21	0.43	0.68

LLE volumes follow the standard procedure [14].

5 CONCLUSIONS

- A hydrophobic DES prepared from DL-menthol and decanoic acid (1:1) by solvent-free heating formed a stable, low-viscosity liquid that separated readily from water and did not interfere with UV detection of the target compounds.

- Among the five DES tested, DES-1 gave the highest overall extraction efficiency. Sample pH, DES volume and vortex time were the significant variables, and the Box–Behnken model located the optimum at 90 μL DES, pH 3.0 and 3 min vortex.
- The validated VA-HDES-DLLME-HPLC-DAD method reached detection limits of 0.09–0.31 $\mu\text{g L}^{-1}$, enrichment factors of 88–121, RSD values below 8% and relative recoveries of 86.5–106.8%, which meets the requirements for trace analysis of these pollutants with ordinary HPLC equipment.
- Bisphenol A, 4-tert-octylphenol, triclosan and diclofenac were found in Shatt al-Hilla downstream of Hilla city and in treated municipal effluent, while tap and well water were free of quantifiable residues. Treated effluent is therefore a continuing source of these pollutants to the river in Babil Governorate.
- The method uses less than 0.2 mL of organic reagents per sample, avoids chlorinated solvents and evaporation, and scored higher than liquid–liquid extraction on both the Analytical Eco-Scale and AGREEprep.

Recommendations

- Extend the method to other EOP classes found in Iraqi waters, such as antibiotics, UV filters and pesticide transformation products, and confirm positive results by LC-MS/MS.
- Carry out regular monitoring of Shatt al-Hilla and other Euphrates branches in Babil Governorate at several points along the river and in different seasons.
- Study the removal of bisphenol A and diclofenac in local wastewater treatment plants and assess advanced treatment options.
- Evaluate the toxicity and biodegradability of menthol–fatty acid DES, and test automated or magnetic-assisted versions of the procedure to reduce handling of the viscous extract.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest regarding this manuscript.

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