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Application of deep eutectic solvents in bioanalysis

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ABSTRACT

The application of deep eutectic solvents (DESs) is sharply surging as a green alternative to conventional solvents due to their unique properties in terms of simplicity of preparation, designability and low cost. A great deal of attention has been paid to the application of these green solvents in analytical chemistry in recent years, and a lot of interesting work has been reported. This review summarizes the most relevant applications of DESs in bioanalysis related to both sample preparation and analyte quantification. Special attention is devoted to the discussion of practical applications for various types of biological samples (blood, plasma, serum, urine, saliva, hair, tear, sweat), with a focus on the advantages of DES-based methods over traditional ones. We cover the articles that were available online up to the end of January 2022. The applications of the reported techniques are summarized in tables.

1. Introduction

The analysis of biological samples is performed in various types of laboratories, such as clinical, toxicological and forensic labs, and the determination of pharmaceuticals is also of great importance for environmental and pharmacokinetic studies. Clinical laboratories provide information about a patient's condition, which is helpful for both diagnosis and therapy [1], and biomonitoring aimed at controlling and assessing health risks to workers as well as the general population should certainly be mentioned.

Ensuring sustainable development is today essential in all areas of human activity. To this end, there are various ways to achieve this aim in analytical chemistry, such as miniaturization and automation, as well as the replacement of hazardous chemicals with the use of environmentally friendly ones. This effort has led to the emergence of several novel microextraction techniques, such as dispersive liquid–liquid microextraction (DLLME) [2], single-drop microextraction (SDME) [3], hollow-fiber liquid-phase microextraction (HF-LPME) [4] and solidified floating organic drop microextraction (SFODME) [5], as well as the introduction of new types of solvents, such as switchable hydrophilicity solvents (SHS) [6] and deep eutectic solvents (DESs) [7], into analytical practice.

Deep eutectic solvents, which were introduced by the Abbott group about 20 years ago [8], were first adopted in materials science and chemical engineering, and only recently, in about the middle of the last decade, did they begin being used in analytical chemistry. However, we are currently seeing a significant increase in the number of research papers devoted to the use of DESs in analytical chemistry.

In this review, we discuss articles that use DESs in bioanalysis. Included are articles that were available online as of the end of January 2022 in the Scopus database when using the keywords *deep eutectic solvent*, *blood*, *plasma*, *serum*, *urine*, *saliva*, *hair*, *tear*, and *sweat*. The applications of DESs are summarized in Tables. The development in the number of publications devoted to the topic is shown in Fig. 1. To the best of our knowledge, only one review

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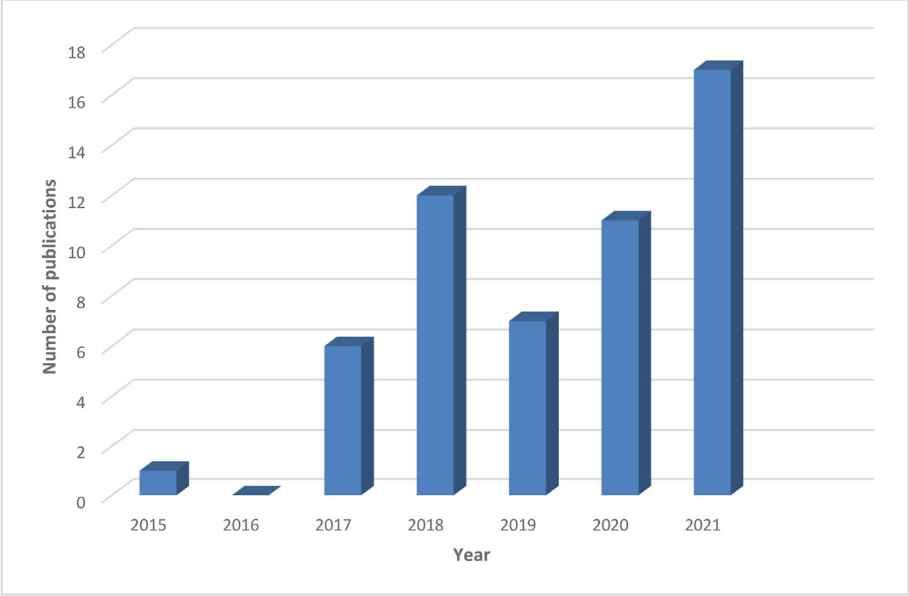

Fig. 1. Evolution in the number of publications devoted to the topic based on the Scopus (searched keywords: deep eutectic solvents and blood, plasma, serum, urine, saliva, hair, tear, sweat; accessed on April 2022).

article has been published on this topic thus far [9]. Other topics, such as the importance of DESs in various fields, methods of their preparation, their composition, the effect of the HBA-to-HBD ratio, the supramolecular structure, the role of water, the physical properties of DESs and methods of their investigation (FTIR, NMR, DSC), as well as their greenness, biodegradability, applications on analytical areas, and other general issues, are beyond the scope this review, whereas other authors have already dealt with them [10–14]. Therefore, we recommend that readers interested in these narrower topics turn to review articles devoted specifically to them.

2. Samples and analytes

DES-based procedures have been used for the analysis of biological samples, especially blood (whole blood, plasma and serum) and urine, and to a much lesser extent saliva (Fig. 2). Blood shows high matrix interferences, mainly due to the high protein concentration and possible analyte–protein interactions. Urine is a simpler matrix consisting mainly of water and a high content of urea and sodium chloride, as well as other minor components. The advantage of urine is the ability to take a large sample volume; however, problems can be caused by volume variability, which significantly affects the concentration of the analytes to be determined [15]. Saliva is considered a promising biological sample, but currently it is rarely analyzed. It should be emphasized that urine and saliva sampling is are low-cost and completely non-invasive procedures.

Whatever the purpose (clinical, toxicological, forensic, etc.), the analysis of biological samples is a challenge due mainly to the complexity of the matrices; the variety of the analytes of interest; the low concentration of target analytes, which are often below the limit of detection of the equipment used; and the possible

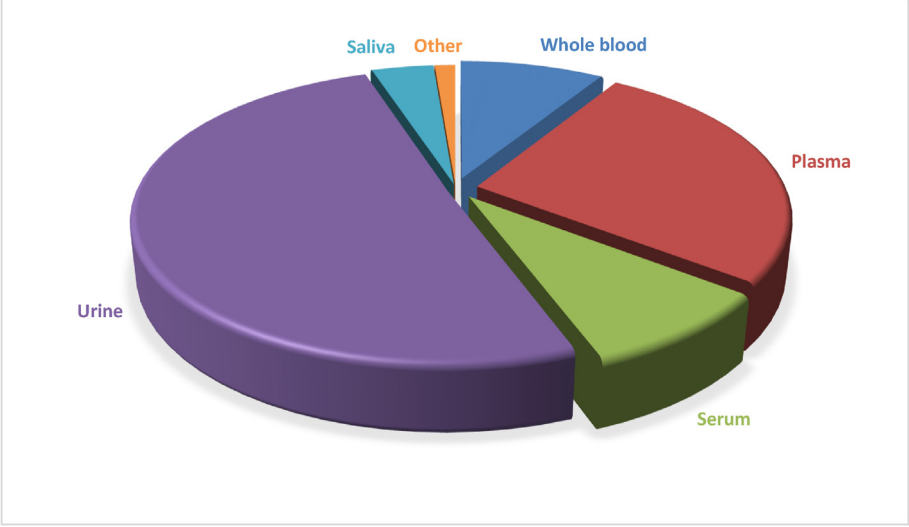

Fig. 2. Application of DES-based procedures for the analysis of biological samples. Data extracted from Tables 1–3.

Table 1
Selected examples of DES-based procedures for determining pharmaceuticals and biomarkers in biological samples.

Analyte	Sample	Microextraction[a]	Detection	Comments[b]	LOD	Ref.
Sulfonamides (*sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole*)	Urine samples	Microfluidic LPME	HPLC-UV	**DES**: coumarin and thymol, 1:2 **Sample pretreatment**: adjusted to pH 1.0 with HCl solution; filtered **Procedure**: Donor phase: acidic sample solution, pH 1.0; acceptor phase: alkaline solution, pH 12.0; flow rate, 1 μL min^{-1}; SLM 3 μL DES **Note**: biodegradable SLM based on a DES as the extraction phase and agarose as the support membrane	0.05–0.083 μg mL^{-1}	[18]
Sulfonamides (*sulfapyridine, sulfamethoxazole, sulfamethazine*)	Urine samples	Automated	UV–Vis	**DES:** vanillin and thymol, 1:1 **Sample pretreatment:** filtered **Procedure:** DES and sample thermostated at 50°C to reduce viscosity of the extraction solvent; 300 μL DES and then 4.5 mL sample aspirated into the syringe; stirring, 2 min, 200 rpm; waiting 5 min to phase separation	0.06–0.1 mg L^{-1}	[19]
Tetracycline antibiotics (*doxycycline*)	Urine, blood plasma and milk samples	VA-HDES-ferrofluid-DLLME	HPLC-UV	**HDES:** octanoic acid and DL-menthol, 1:1 **Sample pretreatment:** Urine: centrifuged, 4000 rpm, 30 min; filtered. Blood: 5 mL sample, sodium citrate, 4% w/v; centrifuged, 10 min, 4000 rpm; diluted to 10 mL **Procedure:** 10 mL sample solution, pH 3, 4% w/v NaCl; 150 μL ferrofluid; vortexed, 7 min; magnetic separation; 50 μL methanol; 20 μL injected	3.6 ng mL^{-1}	[20]
Quinolone antibiotics (*levofloxacin*)	Plasma samples	DES-MIP-MEPS	UHPLC-UV	**DES:** choline chloride and ethylene glycol, 1:2 **Sample pretreatment:** 100 μL plasma, 300 μL methanol and 10 μL internal standard; vortexed, 5 min; centrifuged, 12000 rpm, 5 min; supernatant filtered **Procedure:** MEPS syringe, 4 mg DES-MIP between two frits; activated with 1 mL methanol and 1 mL water; 400 μL pretreated sample solution aspirated 20 times, 40 μL s^{-1}; MEPS syringe washed with 200 μL water-methanol (50:50, v/v) and eluted with 400 μL acetonitrile-ammonia (95:5, v/v); eluate evaporated with N_2 and quantified to 50 μL with mobile phase and injected	0.012 μg mL^{-1}	[21]
Antiviral drugs (*acyclovir*)	Urine and blood samples	–	Electrochemical	**DES:** choline chloride and ethylene glycol **Sample pretreatment**: no pretreatment for urine and deproteinization for blood samples	0.008 μM	[22]
Antiviral drugs (*daclatasvir and sofosbuvir*)	Urine samples	UA-HLLME	HPLC-UV	**DES**: TBAC and *p*-aminophenol, 1:2 **Procedure**: 5 mL urine sample; 0.35 g (7% w/v) NaCl; thermostated in water bath, 50°C; 105 μL DES; 206 μL ammonia solution (1 M); sonicated, 4 min; centrifuged, 5 min, 2240×*g*; sedimented phase (19 ± 1 μL), diluted to 50 μL with mobile phase; injected	1.0 μg L^{-1} daclatasvir and 1.3 μg L^{-1} sofosbuvir	[23]
NSAIDs (*mefenamic acid, diclofenac, flurbiprofen and ketoprofen*)	Urine samples	LPME	Optical fiber sensor	**DES:** menthol and pivalic acid, 1:1 **Sample pretreatment:** 8 mL urine sample; 1.5 mL 1 mol L^{-1} NaOH, kept 30 min at ambient conditions; 1.5 mL 1 mol L^{-1} HCl; stored in a refrigerator; before analysis diluted 10-fold with buffer solution (1 mol L^{-1}, pH 3.8) **Procedure:** 500 μL urine sample; aspirated through HC-MOF, 8 min, 0.062 mL min^{-1}; sample excess removed with argon stream, 0.5 min. **Note**: optical fiber modified by DES	3 μg L^{-1}	[24]
NSAIDs (*meloxicam*)	Plasma and urine samples	MD-μSPE	HPLC-UV	**DES:** choline chloride and ethylene glycol, 1:2 **Sample pretreatment:** Plasma: 7% v/v $HClO_4$; centrifuged, 5000 rpm, 10 min. Urine and plasma samples filtered, and double diluted with water **Procedure:** DES-ferrofluid (2 mg SiO_2@Fe_3O_4 MNPs dispersed in 250 μL DES) injected into 10 mL aqueous solution, pH 4; magnetic separation; eluted with 200 μL ethanol, sonicated, 2 min; 20 μL injected	1.5–3.0 μg L^{-1}	[25]
NSAIDs (*mefenamic acid*)	Urine samples	VALLME-ferrofluid	HPLC-UV	**HDES:** DL-menthol and acetic acid, 1:1 **Sample pretreatment:** filtered **Procedure:** 10 mL solution, pH 4.0, containing 3% w/v NaCl; 60 μL ferrofluid; vortexed, 7 min; magnetic separation; 50 μL methanol; 20 μL injected	1.351 ng mL^{-1}	[26]
Antipyretics and NSAIDs (*acetaminophen and diclofenac*)	Synthetic urine and bovine serum	–	Electrochemical	**DES**: choline chloride and glycerol, 1:2	2.6×10^{-8} mol L^{-1} acetaminophen and 5.2×10^{-8} mol L^{-1} diclofenac	[27]

(*continued on next page*)

Table 1 (*continued*)

Analyte	Sample	Microextraction[a]	Detection	Comments[b]	LOD	Ref.
Anti-migration drugs (*paracetamol and rizatriptan*)	Urine samples	–	Electrochemical	**DES**: chloride and 4-methoxybenzyl alcohol, 1:4 **Sample pretreatment**: filtered	0.7 nM and 9.0 nM	[28]
Paracetamol and its metabolite 4-aminophenol	Plasma and urine samples	–	Electrochemical	**DES**: choline chloride and urea, 1:2 **Sample pretreatment:** Plasma: 1000 μL sample, 800 μL acetonitrile; centrifuged, 30 min; supernatant diluted with buffer solution (0.1 mol/L, pH 6.0). Urine: diluted.	0.010 and 0.017 μmol L^{-1}	[29]
Cardiovascular drugs (*aspirin, metformin, metoprolol, andatorvastatin*)	Plasma and urine samples	–	MLC	**DES:** choline chloride and ethylene glycol, 2:1 **Sample pretreatment:** 100 μL sample, 500 μL acetonitrile (plasma)/600 μL acetonitrile (urine); vortexed, 30 s; equilibration time, 10 min; centrifuged, 5 min; supernatant filtered **Note**: DES as mobile phase additive	0.05–25 μg mL^{-1}	[30]
Cardiovascular drugs (*hydrochlorothiazide, triamterene, losartan*)	Plasma samples	–	MLC	**DES**: choline chloride and ethylene glycol, 1:3 **Sample pretreatment:** diluted, 1:3 with the developed mobile phase; vortexed, 5 min; filtered **Note**: DES as mobile phase additive	0.04–0.60 μg mL^{-1}	[31]
β-blockers (*atenolol, propranolol, and metoprolol*)	Plasma samples	LLME	GC-MS	**HDES:** TMAC and alpha terpineol, 1:2 **Sample pretreatment**: diluted with water, 1:4 **Procedure**: 5 mL diluted sample; 0.20 g TMAC (HBA); 0.5 mL DMF (co-disperser) mixed with 610 μL alpha terpineol (HBD); MW radiation, 1 min; cooled with tap water; centrifuged, 5 min, 5000 rpm; 1 μL DES phase (7 ± 0.5 μL) injected	0.130–0.205 ng mL^{-1}	[32]
Antiarrhythmic drugs (*propranolol, carvedilol, verapamil, and amlodipine*)	Plasma and urine samples, pharmaceutical wastewater	HF(3)-LPME	HPLC-UV	**HDES**: choline chloride and 1-phenylethanol, 1:4 **Procedure**: 10 mL aqueous sample, pH ~12.25 (donor phase); ~40 μL DES (SLM solvent); 40 μL aqueous solution, pH 2.5 (acceptor phase, HF lumen); stirring, 1100 rpm, 40 min; acceptor solution was retracted into a microsyringe; 20 μL injected **Note**: DES as SLM solvent	0.3–0.8 ng mL^{-1}	[33]
Anticoagulant drugs (*warfarin*)	Plasma and urine samples	AALLME	HPLC-UV	**HDES**: borneol and decanoic acid; 1:3 **Sample pretreatment:** Plasma: 7% v/v $HClO_4$; centrifuged, 10 min, 5000 rpm; supernatant filtered, diluted, 1:2. Urine: filtered, diluted with water, 1:2 **Procedure:** 10 mL aqueous sample solution, pH 3.9; 60 μL DES; 15 aspiration-dispersion cycles; centrifuged, 10 min, 6000 rpm; DES-rich phase collected; 20 μL injected	0.5–2.7 μg L^{-1}	[34]
		DES-NF-MD-μSPE	HPLC-UV	**HDES:** TMAC and thymol, 1:4 **Sample pretreatment:** Plasma: 7% v/v $HClO_4$; centrifuged, 5000 rpm, 20 min; supernatant decanted; filtered; diluted, pH adjusted. Urine: centrifuged, filtered; diluted, pH adjusted **Procedure:** 15 mL solution, pH 5.0, 2% w/v NaCl; 300 μL NF injected; sonicated, 1 min; magnetic separation; 300 μL acetonitrile, 10 min to desorb the analyte; 40 μL supernatant injected	1.6 ng mL^{-1}	[35]
	Urine and plasma samples	LDH-MMM-DES	HPLC-UV	**DES**: aloe vera gel and urea and choline chloride, 1:2:1 **Sample pretreatment**: Urine: filtered and extracted without any dilution. Plasma: 7% v/v $HClO_4$; centrifuged, 5000 rpm, 10 min; filtered; clear solution diluted 2:3 with water, pH 3 adjusted **Procedure:** 25 μL DES injected into the HF lumen; assembly immersed into the sample; stirred, 1500 rpm, 30 min; extraction solvent from the lumen collected; 25 μL desorption solvent (acetonitrile), ultrasonic bath, 2 min; extraction and desorption solvent combined; 20 μL mixed solvent injected **Note**: layered double hydroxides mixed matrix membrane	0.14 μg L^{-1} (urine), 0.30 μg L^{-1} (plasma)	[36]
Proton-pump inhibitors (*omeprazole and lansoprazole*)	Serum and urine samples	–	Electrochemical	**DES:** choline chloride and urea **Sample pretreatment:** Plasma: 1 mL serum deproteinized by vortexed mixing with 0.9 mL methanol and 0.1 mL acetic acid; centrifuged, 12000 rpm, 5 min; filtered; supernatant diluted with buffer, pH 6.0. Urine: diluted ten-fold with buffer, pH 6.0	0.006–0.009 μM	[37]
Antiepileptic drugs (*lamotrigine*)	Plasma samples	USAEME-DES-BE	UV–Vis	**HDES:** choline chloride and 1-phenylethanol, 1:4 **Sample pretreatment:** 1.2 mL plasma sample; 3.6 mL acetonitrile; vortexed, 4 min; centrifuged, 10 min, 6000 rpm; supernatant separated; acetonitrile excess removed by gentle stream of N_2; 19.3% w/v NaCl added; sample diluted to 6 mL by water; pH adjusted to 11.34	0.15 μg mL^{-1}	[38]

Table 1 (*continued*)

Analyte	Sample	Microextraction[a]	Detection	Comments[b]	LOD	Ref.
				Procedure: USAEME: 126 μL DES; sonicated, 81 s; centrifuged, 15 min; BE: collected DES phase; 180 μL 0.1 M HCl; sonicated, 10 min; centrifuged, 10 min, 6000 rpm; HCl phase collected, neutralized by 20 μL 1 M NaOH; volume adjusted to 200 μL		
		VALLME	HPLC-UV	**DES**: choline chloride and ethylene glycol, 1:2 **Procedure**: 200 μL plasma; diluted to 1 mL with DES, vortexed, 3 min; centrifuged, 4000 rpm, 30 min; supernatant filtered, 20 μL injected **Note**: greenness of the method was evaluated using the AGREE metric approach	LOQ: 0.1 μg mL^{-1}	[39]
Addictive drugs (*amphetamine and methamphetamine*)	Human plasma samples and pharmaceutical wastewater	AALLME	HPLC-UV	**HDES:** choline chloride and phenylethanol, 1:4 **Procedure:** 6 mL pretreated real sample; adjusted to pH 12; homemade extraction cell; 250 μL DES; 8 air-agitation cycles; centrifuged, 5000 rpm, 2 min **Note**: homemade extraction cell with narrow neck	2–5 ng mL^{-1}	[40]
Opioid analgesics (*morphine and oxymorphone*)	Exhaled breath condensate	AALLME	GC-MS	**DES**: choline chloride and menthol and phenylacetic acid **Sample pretreatment**: diluted with water, 1:4 **Procedure**: 5 mL sample; 7.5% w/v Na_2SO_4; 15 μL picoline (catalyst); 80 μL DES mixed with 8 μL butyl chloroformate (derivatization agent); 6 aspiration-dispersion cycles; microwave irradiation, 10 s; centrifuged, 1118×*g*, 5 min; 1 μL sedimented phase (10 ± 0.5 μL) injected	2.1 ng mL^{-1} morphine and 1.5 ng mL^{-1} oxymorphone	[41]
Illicit drugs	Urine samples	DLLME	HPLC-MS	**DES**: choline chloride and sesamol, 1:3 **Sample pretreatment**: 3 mL urine sample; adjusted pH 10; centrifuged, 10000 rpm, 5 min; supernatant diluted with 2 mL water **Procedure**: 5 mL pretreated sample; 100 μL DES and 400 μL ethyl acetate; vortexed, 1 min; centrifuged, 10000 rpm, 10 min; 2 μL injected	0.042–0.072 μg L^{-1}	[42]
Opioid analgesics (*methadone*)	Urine and plasma samples	AALLME	GC-FID	**DES:** choline chloride and TNO, 1:2 **Sample pretreatment:** Urine: pH adjusted; diluted to 10 mL with water. Blood: 1 mL plasma; 0.5 mL zinc sulfate solution (0.7 mol L^{-1}) and 0.1 mL 1 mol L^{-1} sodium hydroxide; centrifuged, 8 min, 4000 rpm; supernatant diluted to 10 mL with water **Procedure:** 10 mL sample, pH 10; 100 μL DES; 100 μL THF; 10 aspiration-dispersion cycles; centrifuged 4000 rpm, 3 min; upper phase collected; 1 μL injected	0.7 μg L^{-1}	[43]
Opioid analgesics (*methadone*)	Plasma and urine samples, water	UA-DSPE	GC-FID and GC-MS	**DES:** choline chloride and TNO, 1:2 **Sample pretreatment:** Blood: 1 mL plasma; 0.1 mL 1 mol L^{-1} NaOH and 0.5 mL 0.7 mol L^{-1} zinc sulfate; centrifuged, 5 min, 4000 rpm; supernatant diluted to 10 mL with water. Urine: pH adjusted; diluted to 10 mL with water **Procedure:** sample solution, pH 10; 15 mg sorbent (Fe_3O_4@GO-DES); volume adjusted to 10 mL; sonicated, 2 min; magnetic separation; analyte desorbed by 500 μL ethanol and dried with stream of N_2; residue dissolved in 40 μL ethanol	0.03 μg L^{-1} (GC-MS); 0.8 μg L^{-1} (GC-FID)	[44]
Antidepressant drugs (*escitalopram, desipramine, and imipramine*)	Plasma samples and pharmaceutical wastewater	AALLME	HPLC-UV	**DES:** choline chloride and phenol, 1:4 **Sample pretreatment:** 1 mL plasma; 2 mL acetonitrile, shaken manually; centrifuged, 5000 rpm, 10 min; diluted; pH adjusted **Procedure:** 6 mL pretreated sample, pH 12; 200 μL DES; 430 μL THF; 14 air-agitation cycles; centrifuged, 4500 rpm, 5 min; ~60 μL extraction phase collected; 20 μL injected **Note**: special homemade extraction cell	3.0–4.5 ng mL^{-1}	[45]
Tri cyclic antidepressant drugs (*amitriptyline, nortriptyline, desipramine, clomipramine, imipramine*)	Urine and plasma samples	DSPE-AALLME	GC-MS	**DES:** choline chloride and 4-chlorophenol **Sample pretreatment:** Urine: diluted, 1:1 with ammoniacal buffer. Blood: 1 mL supernatant plasma diluted, 1:4 with ammoniacal buffer **Procedure:** DSPE step: 5 mL diluted urine or plasma samples; 75 mg C_{18} sorbent; vortexed, 3 min; centrifuged, 5 min, 5000 rpm; adsorbed analytes eluted with 150 μL DES under the sonication, 3 min. DES-based AALLME step: supernatant solution from previous step added to 5 mL ammoniacal buffer; 5 aspiration-dispersion cycles; centrifuged, 5 min, 5000 rpm; DES phase (10 ± 0.5 μL) removed; 1 μL injected	8–15 ng L^{-1} urine, 32–60 ng L^{-1} plasma	[46]

(*continued on next page*)

Table 1 (*continued*)

Analyte	Sample	Microextraction[a]	Detection	Comments[b]	LOD	Ref.
				Note: DES as elution/extraction solvent simultaneously		
Anticonvulsant drugs (*carbamazepine*)	Plasma samples	UA-LLME	HPLC-UV	**DES:** choline chloride and phenol, 1:2 **Sample pretreatment:** 1 mL sample; 3 mL acetonitrile; vortexed, 5 min; centrifuged, 10 min, 6000 rpm; supernatant separated; excess ofacetonitrile removed by stream of N_2; solution diluted to 6 mL by water, pH adjusted to 11.78 **Procedure:** 314 μL DES; 523 μL THF; sonicated, 9 min; centrifuged, 15 min, 6000 rpm; enriched phase collected; THF evaporated under a gentle stream of N_2; volume adjusted to 90 μL with acetonitrile and injected	1.17 ng mL^{-1}	[47]
Anti-seizure drugs (*carbamazepine, diazepam, and chlordiazepoxide*)	Urine samples	SI-LLE and DES-DLLME	GC-FID	**DES**: choline chloride and benzyl ethylenediamine, 1:2 **Procedure**: 5 mL urine sample, pH 10; 1.5 g NaCl; 2 mL *iso*-propanol; manually shaken, 2 min; centrifuged, 5 min, 4000 rpm; 0.9 mL *iso*-propanol phase removed and mixed with 85 μL DES; mixture injected into 5 mL water, 5% w/v NaCl, pH 10; centrifuged, 7 min, 5000 rpm; 10 μL separated, 1 μL injected **Note**: SI-LLE-DLLME combination	3.4–6.9 ng mL^{-1}	[48]
Benzodiazepines (*lorazepam and clonazepam*)	Urine and plasma samples	AA-MD-μSPE and AA-DLLME-DES	HPLC-UV	**DES:** choline chloride and phenol, 1:4 **Sample pretreatment:** Urine: filtered, 4 mL diluted to 8 mL with water. Plasma: filtered; 4 mL sample diluted to 8 mL with water; 0.1 g trichloroacetic acid; centrifuged, adjusted to pH 7 **Procedure:** mixture of methanol enriched with the target analytes (SPE step) and 250 μL DES injected into 6 mL neutral aqueous solution in 7 mL homemade extraction cell; 440 μL THF, 8 air-agitation cycles; centrifuged, 2 min, 1132×*g*; ~60 μL DES phase collected, 25 μL injected **Note**: DSPE-DLLME combination	0.7–1.0 ng mL^{-1}	[49]
Steroidal hormones (*dydrogesterone and cyproterone acetate*)	Urine and plasma samples	HF(3)-LPME	HPLC-UV	**DES:** methyltriphenylphosphonium iodide and ethylene glycol, 1:4 **Sample pretreatment:** Urine: centrifuged, 3000 rpm, 5 min; supernatant diluted 1:1 with water. Plasma: 2 mL sample; 100 μL trichloroacetic acid; centrifuged, 4000 rpm, 8 min; diluted with water to 20 mL **Procedure:** 20 mL aqueous sample as donor phase; *n*-dodecane (SLM solvent); 30 μL DES (acceptor phase, HF lumen); stirred, 40 min, 900 rpm; acceptor solution was retracted into a microsyringe; 20 μL extract injected	0.5–2 μg L^{-1}	[50]
Polar basic drugs (*tyramine, metaraminol, sotalol, ephedrine, atenolol, and metoprolol*)	Plasma samples	EME	UHPLC-MS/MS	**HDES**: coumarin and thymol, 2:1 **Sample pretreatment**: plasma diluted 1:1 with 250 mM phosphate buffer pH 2.0 **Procedure**: 96-well format; 100 μL samples; 96-well filter plate with polyvinylidene fluoride filter membranes (as the SLM support); 100 μL acceptor solution; extraction initiated by simultaneous application of voltage and 900 rpm shaking **Note**: DES as SLM solvent	–	[51]
Polar and non-polar acids (*hippuric acid, anthranilic acid, ketoprofen and naproxen*)	Urine samples	Microfluidic LPME	HPLC-UV	**DES:** camphor and menthol, 1:1 **Sample pretreatment:** diluted 1:1; adjusted to pH 2; filtered **Procedure:** 6 μL pretreated urine sample delivered at 1 μL min^{-1} across the DES SLM into pure aqueous phosphate buffer, pH 11.0, as acceptor phase **Note**: DES as SLM solvent	0.075–0.09 μg mL^{-1}	[52]
Clorprenaline (CLP) and bambuterol (*BAM*)	Urine samples	MIPFMR-SPE	HPLC-UV	**DES:** choline chloride and ethylene glycol, 1:2 **Sample pretreatment:** centrifuged and filtered **Procedure:** cartridge, 20 mg MIPFMR, pretreated with 2 mL methanol and 2 mL water; 1 mL urine sample loaded; washed with 0.8 mL acetonitrile-water (1:1, v/v); eluted with 0.8 mL methanol-ammonia (9:1, v/v); eluate evaporated under N_2; reconstituted with 0.2 mL mobile phase	3.3 ng mL^{-1} BAM, 3.9 ng mL^{-1} CLP	[53]
C-reactive protein	Human serum samples	–	Electrochemical	**DES**: choline chloride and itaconic acid	0.0003 ng mL^{-1}	[54]
Sympathomimetic agent(s) (*dopamine*)	Human serum samples	–	Electrochemical	**DES:** choline chloride and urea, 1:2	1.32 μM	[55]
	Urine samples	–	Electrochemical	**DES:** choline chloride and ethylene glycol, 1:2	288 nM	[56]
Sympathomimetic agent(s) (*dopamine, epinephrine and norepinephrine*)	Urine and serum samples	MD-μSPE	HPLC-UV	**DES:** choline chloride and urea, 1:2 **Sample pretreatment:** Urine: centrifuged, 2000 rpm, 10 min; diluted with water. Blood: 1 mL serum; 3 mL	0.22–0.36 μg L^{-1}	[57]

Table 1 (*continued*)

Analyte	Sample	Microextraction[a]	Detection	Comments[b]	LOD	Ref.
				acetonitrile; centrifuged, 2000 rpm, 10 min; supernatant collected and diluted with water **Procedure:** 10 mL diluted sample; 22 mg adsorbent; sonicated, few seconds; vortexed, 5 min; magnetic separation; 50 μL eluent (phosphate buffer-DES; 7:3); sonicated, 12.5 min; eluent solvent withdrawn, 20 μL injected **Note**: DES as eluent solvent		
Corticosteroids (*cortisol and cortisone*)	Saliva samples	UA-LLME	LC-UV	**HDES**: TAC and pentafluorophenol, 1:2 **Sample pretreatment**: centrifuged, 3000×*g*, 4°C, 15 min **Procedure**: 500 μL pretreated sample; 500 μL 10% NaCl, internal standard in 20 μL acetonitrile; 10 μL DES; vortexed, sonicated, 5 min; centrifuged, 14800×*g*, 4°C, 10 min; 40 μL methanol added to the obtained sediment phase; centrifuged, 14800×*g*, 4°C, 10 min; supernatant (5 μL) injected	2.1 pmol mL^{-1} cortisol, 1.8 pmol mL^{-1} cortisone	[58]
Glucose	Human serum, saliva and sweat samples	–	Electrochemical	**DES**: choline chloride and fructose, 1:1 **Sample pretreatment**: diluted with 0.1 M NaOH	0.86 nM	[59]
Glucose	Human serum samples	–	Electrochemical	**DES**: choline chloride and urea, 1:2	0.2 mM	[60]
Acetylcholine	Synthetic urine	–	Electrochemical	**DES**: choline chloride and ethylene glycol, 1:2	1.04 μM	[61]
Cardiac troponin I	Whole blood samples	–	Electrochemical	**DES**: choline chloride and urea	0.9 pg mL^{-1}	[62]

[a] We left the abbreviations used by the authors (apart from some exceptions), even if this is not in accordance with our previous recommendations [100].
[b] For a detailed description of the procedure, please see the original article.

incompatibility of biological samples with the analytical instruments used. Therefore, pretreatment of the sample is usually necessary before the analysis itself. Sample preparation most often involves protein precipitation, centrifugation, filtration, dilution and pH adjustment. Zhao et al. used a mixture of choline chloride-ethylene glycol (1:4) and a little acetonitrile to develop a one-step extraction procedure for ginsenosides from rat plasma [16]. Feng et al. reported a method for the determination of icarrin and icarisid II in rat plasma samples using DES extraction followed by UPLC-MS/MS quantification. The procedure involved application of a precipitation reagent consisting of L-proline–ethylene glycol and acetonitrile, where acetonitrile was used to precipitate proteins and DES to extract the target analytes [17].

Both organic [16–74] and inorganic [75–82] analytes have been determined, but with a clear predominance of organic analytes (87%). Therapeutic drug monitoring is very important element of treatment, particularly in the case of a narrow therapeutic window [34], as it allows setting an individual treatment strategy within the concept of personalized medicine and adjusting drug doses based on human metabolic phenotype [19] to achieve satisfactory efficacy and reduce side effects, thus achieving optimal treatment. The importance of forensic sciences, doping controls as well as the study of environmental impacts should also be mentioned. DES-based procedures have been used to determine different classes of drugs, such as civilization disease therapeutics, anti-infective drugs, antipyretics, anticoagulant drug, non-steroidal anti-inflammatory drugs (NSAIDs) and antipsychotics, as well as addictive substances in biological samples (Fig. 3). The determination of addictive and illicit substances is essential for the diagnosis of poisoning and for forensic purposes [40,41]. The determination of C-reactive protein [54], dopamine [55,57], cortisol and cortisone [58], glucose [59,60], acetylcholine [61] and cardiac troponin I [62] may be useful in the diagnosis of certain diseases.

For toxicology and occupational medicine, monitoring exposure to various xenobiotics, such as toluene and xylene [71], volatile aromatic hydrocarbons (benzene, toluene, ethylbenzene and xylene) [72], polycyclic aromatic hydrocarbons [65,66,70], pesticides and insecticides [68,69], formaldehyde [64,67], benzotriazole and benzothiazole derivatives [63], is important. The applications, selected experimental conditions and most important parameters of DES-based procedures for the determination of organic analytes in biological samples are summarized in Tables 1 and 2

Inorganic analysis mainly monitors metals (Table 3) that may have vital or toxic effects on living organisms, depending on their concentration and oxidation state. Low levels are essential for biological systems, but high concentrations can lead to health problems. Some metals are toxic regardless of their concentration and chemical form. Concentrations of heavy metals [79], cobalt [76] and chromium [75], were monitored in urine samples, while copper and nickel were examined in human urine and plasma samples [77]. The concentration of lead [78] as well as the speciation of arsenic, selenium [80] and mercury [80,81], were investigated in blood samples, while nitrite was determined in urine and saliva samples [82]. The distribution of articles among the individual inorganic analytes is shown in Fig. 4.

3. DES-based (micro)extraction approaches

Deep eutectic solvents have been used for bioanalytical purposes in both liquid-phase and sorbent-based microextraction procedures with approximately equal representation of both approaches. Both of these directions will be discussed in this review.

3.1. Application of DES in liquid-phase microextraction

Solvent microextraction (SME) techniques can be divided to several categories, such as single-drop microextraction (SDME), dispersive liquid-liquid extraction (DLLME), and hollow-fibre liquid-phase microextraction (HF-LPME). All of these techniques have been used in the analysis of biological samples.

3.1.1. Dispersive liquid-liquid extraction and derived techniques

One of the most popular microextraction techniques is DLLME. However, authors often comment on the disadvantages and limitations of this technique, such as the use of a dispersive solvent, which reduces the efficiency of polar analytes, and the need for

Table 2
Selected examples of DES-based procedures for the determination of organic analytes in biological samples.

Analyte	Sample	Microextraction[a]	Detection	Comments[b]	LOD	Ref.
Benzotriazole and benzothiazole derivatives	Urine samples	UA-LLME	UHPLC-QTOF-MS	**DES:** choline chloride and phenol, 1:2 **Sample pretreatment:** 1 mL urine sample; 100 μL enzyme solution; incubated at 40°C, 40 min **Procedure:** pretreated urine sample, 300 μL DES and 0.3 g NaCl; adjusted to pH 3; shaken manually, sonicated, 6 min; centrifuged, 7 min, 3000 rpm; DES phase (120 μL), adjusted to 500 μL by methanol	0.1–3.0 ng mL^{-1}	[63]
Formaldehyde	Biological samples (*pig and duck blood*) and indoor air	VALLME	HPLC-UV	**HDES:** TAC and cyanophenol, 1:1 **Sample pretreatment:** Pig and duck blood: 1.5 g blood sample and 30 mL acid water (pH 4.0) homogenized in lab blender, 1 min; centrifuged, 8000 rpm, 5 min; aqueous phase filtered **Procedure:** 5 mL sample (pH 4.0); 2,4-dinitrophenylhydrazine (600 mg L^{-1}, 0.4 mL); heated to 60°C, kept 10 min; 150 mg DES, vortexed, 120 s, 2500 rpm; centrifuged, 8000 rpm; DES phase rinsed and diluted by methanol	0.2 μg L^{-1}	[64]
Hydroxylated polycyclic aromatic hydrocarbons	Urine samples	DSPE-DES-DLLME	GC-MS	**DES**: choline chloride and dichloroacetic acid, 1:2 choline chloride and 3,3-dimethyl butyric acid **Procedure**: 10 mL urine sample; 175 mg polystyrene dissolved in 1.25 mL *iso*-propanol and injected into the solution; re-precipitated sorbent separated by centrifugation, 1120×*g*, 5 min; analytes eluted by 0.75 mL choline chloride –dichloroacetic acid DES under sonication, 3 min; supernatant phase removed after centrifugation, 1120×*g*, 1 min and mixed with 83 μL choline chloride–3,3-dimethyl butyric acid DES; mixture dispersed into 5 mL deionized water containing 8% NaCl; turbid solution centrifuged, 1120×*g*, 5 min; DES phase collected; 1 μL injected **Note**: DSPE-DLLME combination; greenness of the method was evaluated using the Analytical Eco–Scale approach	3.6–7.2 ng L^{-1}	[65]
Hydroxylated metabolites of polycyclic aromatic hydrocarbons	Urine samples	SI-DES-HSPE	HPLC-FLD	**DES**: choline chloride and butyric acid, 1:2 **Procedure**: 10 mL urine sample, pH 4; 63 mg PVP dissolved (*homogenous solution obtained*); 2 g NaCl (*PVP released from the sample solution as tiny particles and dispersed into the sample solution*); centrifuged, 5000 rpm, 2 min; sorbent sedimented; adsorbed analytes eluted by 87 μL DES; 20 μL injected **Note**: DES as eluent	14–28 ng L^{-1}	[66]
Malondialdehyde and formaldehyde	Urine samples, and apple juice, rain water	VALLME	HPLC-UV	**HDES:** decanoic acid and methyltrioctylammonium bromide, 2:1 **Sample pretreatment:** urine samples diluted 25-fold **Procedure:** 10 mL solution, 240 μL DNPH (1000 μg mL^{-1}), incubated, 62.15 min at 34 °C; 35 μL DES; vortexed, 3900 rpm, 2.12 min; centrifuged, 3900 rpm, 1 min; extraction phase three-fold diluted by ethanol	2 and 10 ng mL^{-1}	[67]
Pesticides	Urine samples	DLLME	HPLC-MS	**DES:** choline chloride and sesamol, 1:3 **Procedure:** 3 mL urine sample diluted with 2 mL water, 250 mg NaCl; 100 μL DES and 400 μL ethyl acetate; vortexed, 1 min; centrifuged, 10 min, 10000 rpm; 100 μL upper phase collected; 2 μL injected	LOQ: 0.02 –0.76 μg L^{-1}	[68]
Pesticides	Farmer urine and plasma samples	DLLME-SFO	GC-MS	**DES**: menthol and phenylacetic acid, 3:1 **Sample pretreatment:** Plasma: centrifuged, 2000×*g*, 10 min in citrate-treated tubes; supernatant centrifuged, 4000×*g*, 5 min; 2 mL plasma mixed with 3 mL water **Procedure:** 5 mL sample containing 0.25 g NaCl (5%, w/v), 41 μL DES placed in the narrow section of the device under a glass filter; extractant forced to pass through with N_2 stream, 2 min, 2 mL min^{-1}; formed droplets of the solvent solidified with cool water, collected and transferred to a microtube; melted, 1 μL injected **Note**: homemade extraction device; disperser-less, centrifuge-less, ice-bath-less procedure	2–17 ng L^{-1} (urine) and 4 –36 ng L^{-1} (plasma)	[69]
Polycyclic aromatic hydrocarbons	Saliva and urine samples	DESF-AALLME	GC-MS	**DES:** choline chloride and stearic acid, 1:2 **Sample pretreatment:** diluted with water, 1:3 **Procedure:** 5 mL diluted saliva or urine sample; 5% w/v NaCl; 90 μL ferrofluid; 5 aspiration-dispersion cycles; magnetic separation; ferrofluid mixed with 10 μL *n*-heptane; sonicated, 3 min; 1 μL injected **Note**: ferrofluid from toner powder	18–63 ng L^{-1}	[70]
Toluene and xylene exposure biomarkers (*hippuric acid and methylhippuric acid*)	Urine samples	DFG-MPT-SPE	HPLC-UV	**DES:** allyltriethylammonium bromide and ethylene glycol, 1:2 **Sample pretreatment:** diluted ten-fold with water, pH 3 **Procedure:** MPT-SPE cartridge, 2.00 mg DFG; activated with 0.50 mL methanol and water, pH 3; 0.5 mL diluted urine sample; cartridges rinsed with 0.50 mL water, pH 3; eluted with 0.40 mL methanol; concentrated by evaporation; 0.10 mL mobile phase **Note**: miniaturized pipette-tip procedure, MPT	1.66 –2.89 ng mL^{-1}	[71]

Table 2 (*continued*)

Analyte	Sample	Microextraction[a]	Detection	Comments[b]	LOD	Ref.
Volatile aromatic hydrocarbons (*benzene, toluene, ethylbenzene, and xylene isomers*)	Water and urine samples	HS-SDME	GC-FID	**DES:** choline chloride and chlorophenol, 1:2 **Procedure:** 30 mL sample with 20% NaCl; 20 μL DES-MBG placed on the bottom of rod magnet as acceptor phase; solution stirred, 1200 rpm, 30°C, 10 min; after extraction micro-droplet dissolved in 50 μL acetonitrile, sonicated, 1 min; magnetic separation; 1 μL injected **Note**: hydrophobic magnetic bucky gel, MBG	0.05 −0.90 ng mL^{-1}	[72]
Erythrosine (E127)	Blood and urine samples, pharmaceutical tablet, syrup	UA-LLME	UV−Vis	**HDES:** TBAB and 1-octanol, 1:2 **Sample pretreatment:** Blood: 1 mL sample; acetonitrile; centrifuged; supernatant collected. Urine: filtered **Procedure:** 40 mL aqueous sample; 1 mL buffer, 200 μL DES; sonicated, 2 min; centrifuged, 5 min, 4000 rpm; DES phase (~200 μL) collected; completed to 1 mL with ethanol	3.75 μg L^{-1}	[73]
Aristolochic acid I and II	Rat urine	SPE	HPLC-UV	**DES:** choline chloride and ethyl glycol, 1:2 **Sample pretreatment:** centrifuged, 8000 rpm, 4 min **Procedure:** 80 mg MMC@MIPs; 3 mL rat urine; sonicated, 3 min; stirred, 30 min; magnetic separation; clear supernatant decanted; sorbent washed using 2 mL ultrapure water, eluted with 3 mL methanol-DES under vibration, 10 min **Note**: DES as elution solvent	0.03 −0.17 μg mL^{-1}	[74]
Ginsenosides	Rat plasma	DES extraction	UPLC-MS/MS	**DES**: choline chloride and glycol, 1:4 **Sample pretreatment**: centrifuged, 4000 rpm, 10 min **Procedure**: 50 μL plasma; 300 μL DES−acetonitrile mixture, 2:3 **Note**: one-step sample pretreatment procedure	0.1−0.5 ng mL^{-1}	[16]
Icarrin and icarisid II	Rat plasma	DES extraction	UPLC-MS/MS	**DES:** L-proline and ethylene glycol, 1:4 **Procedure**: 50 μL plasma, 200 μL DES−acetonitrile, 3:7, v/v; vortexed, 5 min; centrifuged, 10 min, 13000 rpm **Note**: one-step sample pretreatment procedure	LOQ: 0.32 and 0.43 ng mL^{-1}	[17]

[a] We left the abbreviations used by the authors (apart from some exceptions), even if this is not in accordance with our previous recommendations [100].
[b] For a detailed description of the procedure, please see the original article.

centrifugation to separate the phases after extraction, which is a time-consuming step, as well as the need for cooling in an ice bath for modes based on the solidification of the solvent. Various ways have been proposed to overcome these limitations. For this reason, several researchers have proposed replacing the dispersive solvent with auxiliary energy to ensure the even distribution of the extraction solvent in the sample solution. Thus, vortex-assisted (VA-) [20,26,39,64,67,81,82], ultrasound-assisted (UA-) [23,38,47,63,73,75] or air-assisted (AA-) (based on the performing of several aspiration-dispersion cycles) [34,40,41,43,45,79] LPME techniques have been introduced and applied in bioanalysis. The distribution of articles among the liquid-phase microextraction techniques is shown in Fig. 5.

Jouyban et al. reported a disperser-less, centrifuge-less, ice-bath-less DLLME-SFO procedure based on the application of a deep eutectic solvent. The extraction was performed in a homemade device. The extraction solvent was dispersed in the solution by passing it through a glass filter under a nitrogen gas stream. After the extraction, the solvent droplets were solidified using cool water and collected at the top of the solution with the aid of nitrogen gas. The developed procedure was applied for the analysis of pesticides in plasma and urine samples using gas chromatography−mass spectrometry (GC-MS) [69].

3.1.2. Hollow-fiber liquid-phase microextraction and derived techniques

Hollow-fiber liquid-phase microextraction (HF-LPME) [83] is mainly performed in two ways, namely in a two-phase mode [84] and a three-phase mode [85]. In the two-phase mode, the analytes are extracted from an aqueous donor phase into the acceptor phase (extraction solvent) located in both the pores and lumen of the hollow fiber. In the three-phase mode, the analytes are extracted from an aqueous sample solution into a supported liquid membrane solvent fixed in the pores of the hollow fiber and then back-extracted into the acceptor phase situated in the lumen of the hollow fiber. Some other SLM-based approaches, such as electromembrane extraction (EME) or microfluidic device-based procedures, can also be considered as HF-LPME modalities. One significant advantage of this technique is that, due to the low cost of the hollow fiber, a new fiber can be used for each extraction, which prevents carryover and cross-contamination, thus ensuring high reproducibility and repeatability.

Very recently, deep eutectic solvents were introduced into HF-LPME techniques as new types of solvents, and up to now procedures have been reported in which a DES was used either as the acceptor phase [50,78] or as the SLM solvent [33]. Alavi et al. presented a three-phase hollow-fiber liquid phase microextraction (HF(3)-LPME) for the extraction and determination of lead content in the whole blood of addicts in an effort to find a correlation with the lead content in opium samples. Lead was extracted from 17 mL of pretreated acidic sample solution into 1-octanol containing CTAB and back extracted into 25 μL of a choline chloride−urea (1:2) DES containing potassium perchlorate as the acceptor phase. After extraction, the acceptor phase was diluted with water to reduce viscosity and to enable repeated injections into the ETAAS. The method showed a linearity in the range of 1−200 ng mL^{-1} with a limit of detection (LOD) of 0.1 ng mL^{-1}. Finally, control and test samples were compared with the lead content in the opium samples to determine the correlation between them [78].

A deep eutectic solvent was also used as the SLM solvent in an HF(3)-LPME for trace analyses of antiarrhythmic drugs in biological and environmental samples. The extraction solvent constituted of choline chloride and 1-phenylethanol was characterized by good compatibility with the pores of the hollow fiber. The final determination was performed using high performance liquid chromatography-ultraviolet detection (HPLC-UV) with enrichment factors in the range of 110−135 and low LODs of 0.3−0.8 ng mL^{-1} [33]. Different hydrophobic DESs based on mixtures of camphor−DL-menthol, camphor−thymol, menthol−thymol and coumarin−thymol in various molar ratios were tested as an SLM for EME. Finally, the mixture of coumarin and thymol was found to be a highly efficient SLM. The EME experiments were performed in a 96-

Table 3
Selected examples of DES-based procedures for the determination of inorganic analytes in biological samples.

Analyte	Sample	Microextraction[a]	Detection	Comments[b]	LOD	Ref.
Chromium	Urine samples	SDES	ETAAS	**DES:** BTPPB and phenol, 1:7 **Sample pretreatment:** 4 mL urine sample; centrifuged, 5000 rpm, 10 min; supernatant filtered; diluted to 10 mL by 10 mM sodium phosphate monobasic solution **Procedure**: 10 mL pretreated and diluted sample; 128 μL DES; sonicated, 1 min; ice bath, 3 min; centrifuged, 4 min, 5000 rpm; supernatant decanted; solidified droplets melted, diluted with methanol, 1:1 **Note**: urine samples first complexed using DPC and then extracted by the method; solidification of dispersed fine droplets	2 ng L^{-1}	[75]
Cobalt	Urine samples	DES-MCG-DSPE	SQT-FAAS	**DES:** choline chloride and phenol, 1:3 **Sample pretreatment:** microwave-assisted digestion using a mixture of nitric acid and hydrogen peroxide **Procedure:** 8 mL sample solution; 1 mL pH 8.0 buffer solution; 150 μL DES-MCG; sonicated, 60 s; centrifuged, 2 min, 6000 rpm; magnetic separation; 0.10 mL 2 M nitric acid as eluent, sonicated, 60 s	4.6 ng mL^{-1}	[76]
Copper and nickel	Urine and plasma samples	SPE	FAAS	**DES:** choline chloride and urea, 1:2 **Sample pretreatment:** Urine: centrifuged, 4000 rpm, 5 min; filtered; diluted 5-fold with water. Serum: small volume of acetonitrile; centrifuged, 4000 rpm, 10 min; supernatant decanted, diluted five-fold with water **Procedure:** 50 mL sample, pH 6.0; passed through microcolumn, 7.0 mL min^{-1}; adsorbed analytes eluted by 250 μL nitric acid (2 mol L^{-1}), 3.5 mL min^{-1} **Note**: DES modified cotton	0.05 μg L^{-1} Cu, 0.60 μg L^{-1} Ni	[77]
Lead	Whole blood samples	HF(3)-LPME	ETAAS	**DES**: choline chloride and urea, 1:2 **Sample pretreatment:** 4.5 mL blood sample; 7 mL methanol; vortexed, 10 min; cooled, ice bath, 10 min; stay in ambient temperature, 10 min; centrifuged, 10 min, 4000 rpm; 2 mL HCl 6.0 mol L^{-1}; diluted to 25 mL **Procedure:** 17 mL pretreated sample (donor phase); 1-octanol containing CTAB (SLM, HF pores); 25 μL DES containing $KClO_4$ (acceptor phase, HF lumen); stirring, 800 rpm, 30 min; extract removed by air-blowing using a medical syringe, diluted 1:1 with water **Note**: DES as acceptor phase	0.1 ng mL^{-1}	[78]
Heavy metals (*Cd, Ni, Pb and Cu*)	Black tea, urine and water samples	AALLME	FAAS	**DES:** choline chloride and TNO, 1:2 **Sample pretreatment:** Urine: sample; mixture of HNO_3–H_2O_2 (2:1 v/v), heated, 90–95°C, 1 h; cooled and filtered **Procedure:** 20 mL sample, pH 7; 100 μL triethylamine and DES mixture (1:1); 100 μL THF; 5 aspiration-dispersion cycles; centrifuged, 4000 rpm, 3 min; upper phase collected; 150 μL HNO_3 (63%) added and 100 μL aliquot injected	0.31–0.99 μg L^{-1}	[79]
Arsenic, selenium and mercury speciation	Blood samples	LPME-SDES	ETAAS	**HDES:** choline chloride and decanoic acid, 1:2 **Sample pretreatment:** 1 mL blood sample; 600 μL acetonitrile and 900 μL 15% w/v $ZnSO_4$; vortexed, 5 min; maintained, 5°C, 10 min; centrifuged, 5000 rpm, 3 min; supernatant collected, diluted to 10 mL with water **Procedure:** 10 mL pretreated/diluted blood sample; 60 μL DES containing 15 μL DDTP; vortexed, 5 min; centrifuged, 4 min, 5000 rpm; cooled, ice bath; solidified DES melted; 20 μL acidic ethanol; 30 μL injected **Note**: solidification of DES	0.05 μg L^{-1}, As, 0.015 μg L^{-1} Se, and 0.10 μg L^{-1} Hg	[80]
Mercury (*speciation of organic/inorganic mercury and total mercury*)	Blood samples	VA-DLLME-FDES	ETAAS	**HDES:** 1-decyl-3-methylimidazolium chloride and 1-undecanol, 1:2 **Sample pretreatment:** 2 mL blood sample; 700 μL acetonitrile and 1000 μL 15% w/v $ZnSO_4$; vortexed, 8 min, maintained at 4°C, 10 min; centrifuged, 4000 rpm, 5 min; supernatant collected and diluted to 10 mL by water **Procedure:** 10 mL sample solution; 55 μL DES containing 15 μL DDTP; maintained at 50°C in a water bath; 350 mg NaCl; vortexed, 3 min; centrifuged, 4 min, 5000 rpm; ice bath, 5 min; solidified DES melted; 25 μL injected **Note**: freezing of DES, FDES	0.10 μg L^{-1}	[81]
Nitrite	Water, urine and saliva samples	VALLME	HPLC-UV	**HDES:** TAC and oleic acid, 1:2 **Sample pretreatment:** Urine: diluted; 5 mL sample decolored by 0.5 g active carbon; mixed, 5 min; filtered. Saliva: diluted, filtered **Procedure:** 5 mL prepared sample solution, pH 0.8; 0.1 mL *p*-nitroaniline (40 mmol/L) and 0.1 mL diphenylamine (12 mmol/L); mixed, waiting time, 10 min; 150 mg DES; vortexed, 100 s, 1000 rpm; centrifuged, 4000 rpm, 5 min; HDES phase rinsed with appropriate amount of methanol; 20 μL injected	0.2 μg L^{-1}	[82]

[a] We left the abbreviations used by the authors (apart from some exceptions), even if this is not in accordance with our previous recommendations [100].
[b] For a detailed description of the procedure, please see the original article.

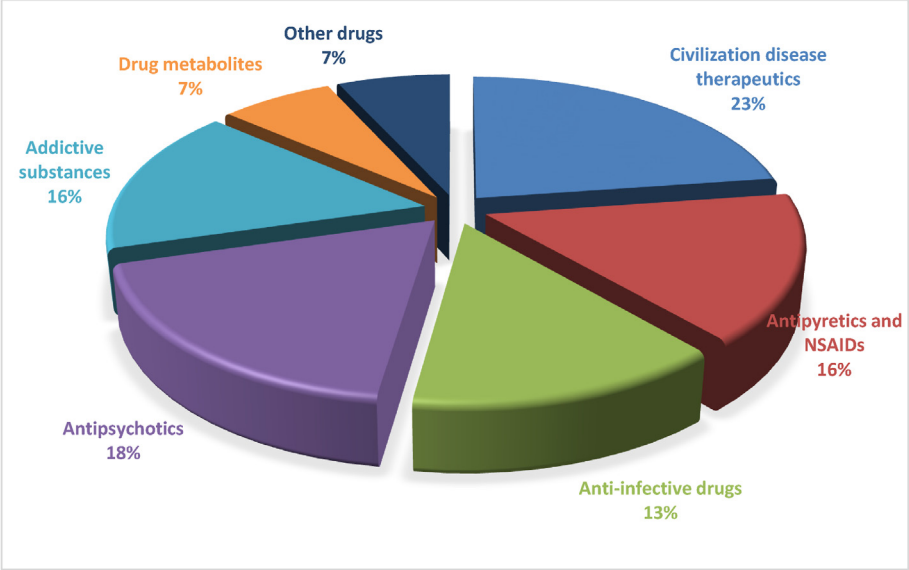

Fig. 3. Application of DES-based procedures to the determination of pharmaceuticals in biological samples. Data extracted from Table 1.

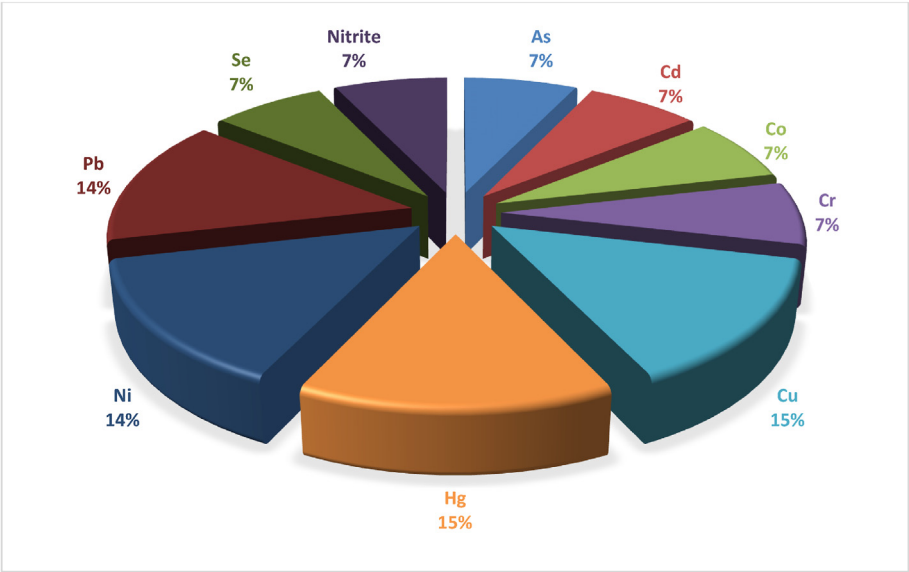

Fig. 4. Application of DES-based procedures for the determination of inorganics. Data extracted from Table 3.

well format, which allowed for high throughput. The procedure requires only 100 μL of sample solution, 4 μL of SLM solvent and 100 μL of acceptor solution for each sample. After the extraction, the acceptor solutions were transferred directly for UHPLC analysis [51].

Santigosa et al. [52] designed a microfluidic platform for simultaneous extraction of compounds of different polarity (hippuric acid, anthranilic acid, ketoprofen and naproxen) in a single step using only a microliter volume of green reagents. The donor and acceptor channels were separated by a flat polypropylene membrane impregnated with the DES prior to extraction. The diluted urine sample with the pH adjusted was delivered at 1 μL min^{-1} across the SLM into the aqueous phosphate buffer pH 11 using a microsyringe pump. Extraction was performed from only 6 μL of sample to only 6 μL of acceptor phase and using less than 1 μL of DES as the organic phase and was completed within 7 min. The pharmaceuticals were isolated from a matrix of human urine and detected using HPLC-UV. In addition, the total waste was less than 1 mg [52]. Only a bit later, Dowlatshah et al. reported a biodegradable SLM based on a coumarin–thymol DES as the extraction phase and agarose as the support membrane. The method was applied for the determination of sulfonamide pharmaceuticals as model analytes in urine samples with satisfactory recovery [18].

3.2. Application of DES in sorbent-based techniques

In addition to liquid-based techniques, DES can play an important role in sorbent-based techniques in several different scenarios: i) as a medium for sorbents synthesis; ii) as a carrier in dispersive solid phase extraction (DSPE); iii) as a sorbents modifier and iv) as an eluent. This section will cover these applications with specific examples in bioanalysis.

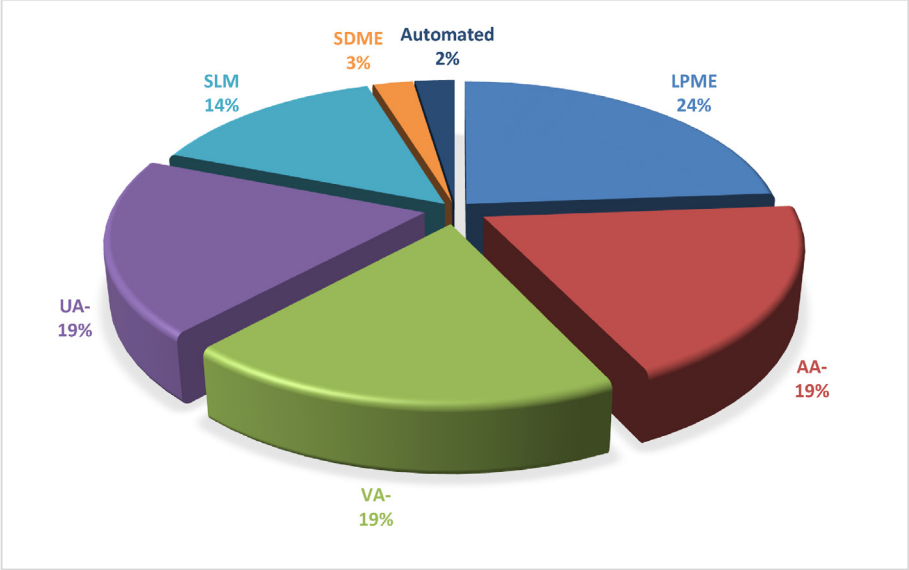

Fig. 5. The distribution of articles among various liquid-phase microextraction techniques. Data extracted from Tables 1–3.

3.2.1. DES as a medium for sorbents synthesis

The favorable properties of DESs, including designability and high thermal stability, have led to their currently playing multiple roles in the synthesis of advanced sorbents. They have been successfully used as a medium for the synthesis of, among others, silica and carbon-based materials, metal organic frameworks (MOFs) and molecular imprinted polymers (MIPs). The authors emphasize that the use of DESs at the stage of sorbent synthesis not only increased the greenness of the procedure by eliminating organic solvents but also improved their sorptive properties, which brings great advantages in separation and purification processes. A review of papers published in recent years in the field of analytical chemistry indicates that they are applied mainly in the synthesis of MIPs. DESs have been successfully used in molecular imprinting techniques, either as a functional monomer, an auxiliary solvent, an MIP modifier, or even as an MIP template [86]. Only a few studies have reported the application of DESs-MIPs in the analysis of biological samples. Liu et al. described the application of a choline chloride and methacrylic acid (1:2) DES as the functional monomer for MIP polymerization to the selective recognition and separation of bovine hemoglobin. Similar studies, in which DESs were used as a cross-linker and a functional monomer, were also provided by He et al. [87]. The authors stated that the interaction of the DES with the surface of the MIP, improved the affinity, selectivity and adsorption of the analyte [88]. Some DESs have been successfully used in molecular imprinting techniques, such as a functional monomer for the recognition of lysozyme [89], the isolation of transferin from human serum [90], and for selective recognition of clorprenaline and bambuterol in urine samples [53]. All of the subsequent studies demonstrated that DES-MIPs are highly efficient and selective in the recognition and isolation of analytes. Some authors also underlined the improved molecular recognition ability and adsorption capacity of the DES-based materials, as compared with other MIPs synthesized in traditional ways.

3.2.2. DES as a carrier in dispersive solid phase extraction

Dispersive solid phase extraction (DSPE) is one of the alternative approaches to conventional solid phase extraction (SPE) based on the dispersion of a sorbent through an aqueous solution. In this technique, a mixture of a sorbent and a carrier fluid is injected directly into a sample solution, followed by dispersion that favors the contact between the sorbent and the analytes. After dispersion, the sorbent containing the analytes on its surface, is separated by means of centrifugation, filtration or an external magnetic field when a magnetic sorbent is used. Currently, one of the main challenges in this field is the search for both appropriate sorbent materials and dispersion methods in order to increase the extraction recovery, as well as alternative carriers to increase the greenness of the method. To minimize the use of toxic organic solvents, DESs have recently been applied. In the literature are papers that describe the use of DESs in the synthesis of colloidal gels, which were then used to pretreat, among others, fruits, vegetables and water samples [91–94]. However, in the field of bioanalysis, the use of colloid gels is currently limited. Only a few papers concerning this area can be found.

Borahan et al. used a magnetic colloidal gel (MCG) in a DSPE method for the extraction/preconcentration of trace levels of cobalt ions in urine samples. The magnetic colloidal gel was prepared by mixing Fe_3O_4 nanoparticles used as the colloidal suspending agent and a choline chloride–phenol (1:3) DES. After the extraction, the magnetic nano particles (MNPs) were separated using a strong magnet, and the cobalt ions were eluted with 2 mol L^{-1} nitric acid, with subsequent determination using slotted quartz tube-flame atomic absorption spectrophotometry (SQT-FAAS). The authors compared the effectiveness of four different procedures, namely FAAS, SQT-FAAS, DES-MCG-dSPE-FAAS and DES-MCG-dSPE-SQT-FAAS. The enhancement factor in terms of detection power, calculated by comparing the LOD values of the developed procedure with the conventional FAAS system, was found to be 61.5 [76]. A similar study was also conducted for the determination of warfarin in human plasma and urine samples. In this approach, the MCG was prepared by mixing magnetic-activated charcoal and tetramethylammonium chloride–thymol (1:4) DES and injecting it into the sample. After sonication for 1 min at ambient temperature, the MNPs were gathered, eluted using acetonitrile, and injected into the HPLC-UV for analysis. The LOD values of the proposed method were in the range of 1.5–1.6 ng mL^{-1}, which was lower than in the other methods applied for preconcentration of warfarin, which may indicate that the application of a DES as a carrier improves the extraction efficiency [35]. In another paper, a colloidal gel of SiO_2@Fe_3O_4 nanoparticles and a choline chloride–ethylene glycol DES in a 1:2 M ratio was used as a sorbent for the magnetic-micro-

dSPE of the anti-inflammatory drug meloxicam from human fluid samples, followed by its determination using HPLC-UV. After optimization, the method achieved recovery values in the range of 93% and 98% and LODs of 1.5–3 μg L^{-1} [25].

3.2.3. DES as a sorbent modifier

Taking into account some of the exceptional properties of DESs, they appear to be suitable for modifying the surface of sorbents to increase extraction efficiency and to meet the requirements of green analytical chemistry. A few examples are presented below. A choline chloride–urea DES was immobilized on the surface of cotton fibers to improve the extraction capacity of the cotton. The modified cotton was packed into a microcolumn and then used to determine trace copper and nickel concentrations in biological samples, such as human urine and plasma, using flow-injection FAAS. The procedure offers huge potential to be applied as an environmentally friendly, fast, simple, cheap, ligandless, and effective method for the preconcentration and resolution of copper and nickel from complicated matrices [77].

Another example of applying DESs as sorbents modifiers was presented by Lamei et al., who used magnetic graphene nanoparticles coated with a Fe_3O_4@GO-DES for efficient preconcentration of methadone in biological and water samples followed by GC-FID and GC-MS. For synthesis of the sorbent, DES and Fe_3O_4@GO nanoparticles were added to a round-bottomed flask and stirred at room temperature for 1 h. In the extraction procedure, ultrasound was used to disperse the sorbent, which increased the contact area and the mass transfer. The developed method presented good linearity, with a detection limit of 0.8 μg L^{-1} for GC-FID and 0.03 μg L^{-1} for GC-MS [44].

In another work, a DES was used to functionalize a graphene oxide composite adsorbent (DFG) for the miniaturized pipette-tip solid-phase extraction (MPT-SPE) of toluene and xylene exposure biomarkers in urine prior to their determination with HPLC-UV. In a comparison to commercially available adsorbents, the DFG presented a superior adsorption ability due to the multiple adsorption interactions of the DFG for the three analytes. In addition, the consumption of organic reagents and adsorbents was reduced [71].

3.2.4. DES as eluent

Several studies reported the application of a DES as a green eluent in the SPE technique in order to elute target analytes from a sorbent [57,66], and underlined the DES's impact on increasing the extraction capacity via hydrogen bonding and dipole–dipole interaction with the analytes is of interest. Considering the field of bioanalysis, such an application was applied in order to extract hydroxylated metabolites of polycyclic aromatic hydrocarbons from urine samples using a DES as an elution solvent prior to HPLC-FLD analysis [66]. A homogenous DSPE based on an organic polymer was chosen as the sample preparation technique. The method showed some advantages, such as broad range of linearity, low LODs, acceptable RSDs and high extraction efficiency [66].

4. Quantification techniques

4.1. Chromatographic techniques

Following a DES-based separation/preconcentration step, target analytes are quantified using a variety of instrumental techniques. The most commonly used are chromatographic techniques (73% of the reviewed papers), atomic absorption techniques (11%), UV–Vis spectrophotometry (6%) and electrochemical methods (8%) (Fig. 6). Among the chromatographic techniques, liquid chromatography (LC) is the most represented (73%), while gas chromatography (GC) accounts for only 27%. In terms of detection, HPLC-UV (62%) predominates, while mass spectrometry (MS) accounts for 25%, followed by GC-MS (16%) and LC-MS (9%) (Fig. 7). Atomic spectrometry was used for the determination of inorganics in urine [75–77,79] and blood [77,78,80,81] samples, with flame atomization (FAAS) [76,77,79] and electrochemical atomization (ETAAS) [75,78,80,81] represented in approximately equal proportions.

Several interesting articles were found that address the possibility of using a DES as a mobile phase additive, highlighting the fact that DESs have high solubilizing capabilities for many compounds [95], such as proteins in plasma samples [31]. Such an approach can have a positive effect on eliminating band tailing, improving the chromatographic peak, as well as increasing the number of theoretical plates [96]. Tan et al. utilized a DES as mobile phase additive for improving the separation of bioactive quaternary alkaloids on a C_{18} column by RP-HPLC [97]. The results demonstrated that choline cations, as a part of the HBA, played a key role in the resolution performance, interacting with deprotonated silanols on a silica-based stationary phase surface, or making the access of basic compounds through adsorption to the chains of C_{18} more difficult. From the other site, ethylene glycol, as the HBD, had a positive effect on the reduction of the retention time of selected analytes. The authors stated that the importance of DESs as a mobile phase additive can be related to the combined effect of both the HBA and the HBD [97].

Ramezani et al. utilized a DES as a green modifier in micellar liquid chromatography (MLC) [30,31], developing an MLC procedure for simultaneous isocratic isolation of a basic-polar (hydrophilic) cardiovascular drug (hydrochlorothiazide) as well as acidic-nonpolar (hydrophobic) cardiovascular drugs (triamterene and losartan potassium) in human plasma [31]. It is worth noting that one of the serious issues connected with the uses of one of the most popular solvents, which is acetonitrile, and some routinely alcoholic modifiers, such as propanol, in the MLC system is the poor retention of polar compounds. The application of a DES as an additive can improve and overcome this limitation. In addition, the presented methodology has the advantage of using a salt-less mobile phase. Furthermore, a procedure based on MLC with a DES as a mobile phase additive has the ability to avoid matrix interference in complex medical samples without any sample preparation step [31]. However, the effect of the DES on the separation mechanism in MLC has not yet been discussed in detail.

4.2. Less common techniques

Less common techniques include UV–Vis spectrophotometry, optical and electrochemical sensors and automated systems.

4.2.1. UV–Vis spectrophotometry

Only a few papers reported on the coupling of DES microextraction procedures with UV–Vis spectrophotometric detection [38,73], probably because this combination encounters a problem in determining analytes that have an absorption maximum in the UV range, as most of the DESs used are not transparent in this area. To address this limitation, Heidari and Mammostafaei developed an ultrasound-assisted emulsification-microextraction procedure based on using a hydrophobic deep eutectic solvent followed by back-extraction (USAEME-DES-BE) for the determination of lamotrigine in human plasma samples. The using of a hydrophobic DES allows the emulsifier to be omitted and moreover facilitates the back-extraction of the analyte into another aqueous phase, which is compatible with spectrophotometric quantification in the UV region [38].

4.2.2. Automated procedures

Shakirova et al. presented an automated UV–Vis procedure

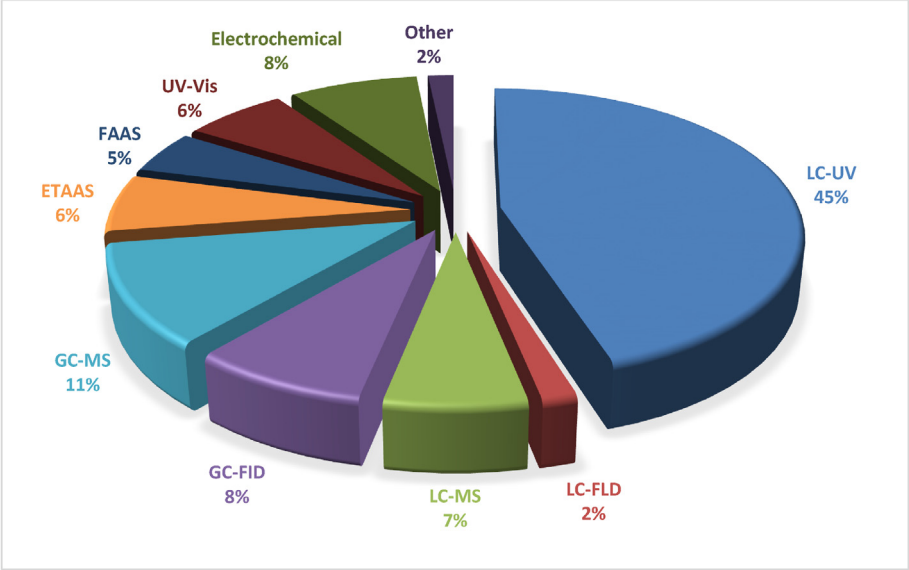

Fig. 6. Coupling of DES-based procedures to detection techniques. Data extracted from Tables 1–3.

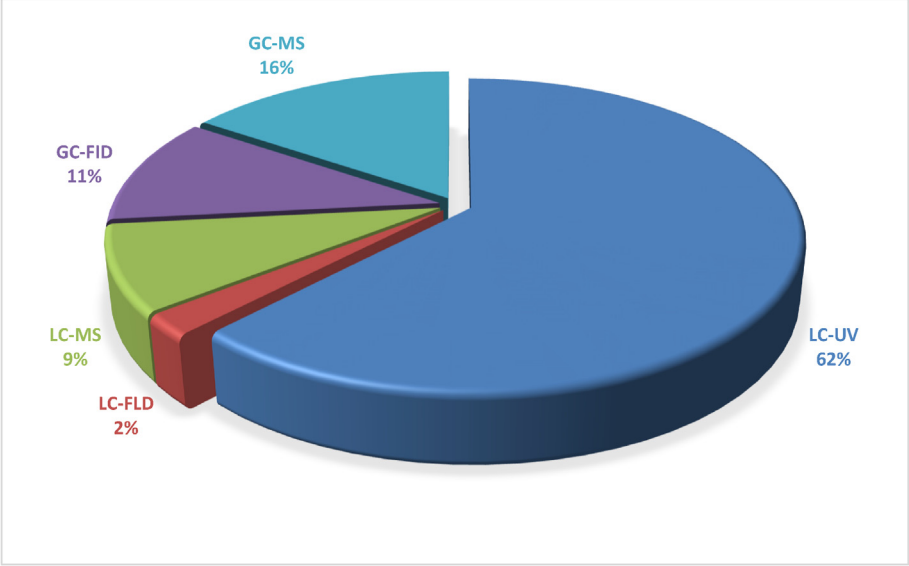

Fig. 7. Coupling of DES-based procedures to chromatographic techniques. Data extracted from Tables 1 and 2

based on the Lab-In-Syringe concept for the determination of one of the three sulfonamides (sulfamethoxazole, sulfamethazine, sulfapyridine) in urine samples. The extraction is based on the formation of colored Schiff bases in the presence of vanillin as a one component of the DES. The sensitivity of the developed procedure is sufficient to determine the concentration of analytes at therapeutic levels, which could be useful for adjusting the dosage of a drug based on acetylation by individual human phenotype [19].

4.2.3. Optical sensors

Optical sensors are described only rarely. Pidenko et al. developed an optical sensor based on hollow core microstructure optical fibers (HC-MOF) modified with DESs for the determination of non-steroidal anti-inflammatory drugs (NSAIDs) in human urine samples. Deep eutectic solvents based on two natural monoterpenoids and various fatty acids were investigated for glass surface modification, and a DES consisting of menthol and pivalic acid in a 1:1 M ratio was found to be the best. The liquid-phase microextraction step was performed in a DES phase supported on the inner surface of the HC-MOF, with subsequent measurement of the transmission spectrum in one analytical device. The preparation of the sensor, i.e. modification of the inner surface of the optical fiber, probably or almost certainly requires some skill on the part of the analyst. However, in the determination itself, the sensor is simply filled with sample by capillary forces and the excess sample is removed with a stream of argon. The authors mention the detection limit at the low level of 3 μg L^{-1}; however, they applied the designed sensor only for the analysis of spiked urine samples [24].

4.2.4. Electrochemical sensors

In comparison to articles focused on optical sensors, the number of publications on electrochemical sensors is significantly higher;

however, even this detection technique is not used very often. The principles and main areas of application of DESs in electrochemistry are discussed in detail in the papers [98,99]. Therefore, in this section, we focus only on the latest applications in the field of bioanalysis. Based on a literature review, it can be noted that electrodes modified in the DES environment have been applied mainly for biomolecular sensing, especially for the detection of monosaccharides and neurotransmitters. Rajaji et al. presented a high-sensitive amperometric platform for the sensing of glucose in human saliva, sweat, and blood samples. In their paper, a deep eutectic solvent prepared with a 1:1 M ratio of choline chloride and fructose was used for the synthesis of $CeAlO_3$. The polycrystalline powder was then mixed with carbon nitride to form the $CeAlO_3$@CN composite, which was used to modify a glassy carbon electrode. The electrode demonstrated excellent electrochemical performance, with a lower detection limit (0.86 nM) in comparison to other reported works [59]. In another paper, choline chloride and urea (1:2) were applied as a soft-template for the preparation of a Au@SiO_2-GO_x modified glassy carbon electrode. The electrode showed excellent selectivity and specificity towards glucose detection and good stability for applications using human serum samples. The authors emphasized that a high viscosity DES application assured stability for the SiO_2 NPs and enabled a controlled surface functionalization process [60]. A modified electrode containing acetylcholinesterase immobilized on poly(neutral red) films was synthesized in a choline chloride–ethylene glycol DES. To increase the ionic strength, different acid dopants with the anions NO_3^-, SO_4^{2-}, Cl^-, ClO_4^- were tested. The obtained results showed that the use of a DES containing NO_3^- to prepare the poly(neutral red) films contributed to a more uniform morphology and superior electrochemical parameters in comparison to those made in an aqueous solution. The biosensor was tested for acetylcholine determination in synthetic urine and demonstrated good selectivity, reproducibility, stability and high selectivity, as well as a rapid response and a low limit of detection [61]. Another approach, using a sensor based on La-doped magnesium titanate ceramics, was recently proposed for the determination of dopamine. In this strategy, a DES mixture of choline chloride and urea in a 1:2 ratio was used as a medium to render ceramics synthesized using a solid-state synthesis route to modify a glassy carbon electrode. It was shown that the presence of lanthanum dopant in the crystal lattice led to an increase in the active surface area (nearly five times greater than that of the pure magnesium titanate) as well as its porosity. The presence of a large number of surface electroactive sites contributed to the enhanced electrochemical sensing performance (LOD 1.32 μM), satisfying RSD values (2.04%) and a recovery rate of 97.95% in spiked human serum [55]. What is more, a highly selective tool for the quantification of dopamine in urine was manufactured by the polymerization of melamine in a DES prepared by mixing choline chloride and ethylene glycol (1:2) [56].

There are also many reports where researchers have successfully demonstrated the great utility of electrochemical sensors modified in a DES environment for the detection of C-reactive protein [54], biomarkers [62] and pharmaceuticals [22,28,29].

5. Concluding remarks

Green analytical chemistry is facing significant challenges due to different priorities for validating analytical methods, with sensitivity, selectivity, accuracy, precision and robustness being the most critical factors. In contrast, the time, cost (including purchase, operation, training and space) and environmental safety of an analytical procedure are often secondary considerations. Worldwide, huge amounts of organic and halogenated solvents are produced each year, and most of these organic solvents are volatile, flammable and toxic compounds (often carcinogenic and/or mutagenic). They cause adverse, long-term effects to both humans and the environments that are exposed to them. Limiting their use is important, as this will obviously result in limiting their production. In fact, the best solution is to replace them altogether with green solvents, and here DESs are coming to the rescue. DESs have been found to form proper media in the case of biological sample analysis, and many advantages, such as their simple and fast preparation, low-cost and simple analytical workflows, are linked to this potential. Most studies involving the application of DESs in the analytical management of biological samples are devoted to microextraction techniques in both liquid-phase and sorbent-based modes, with approximately equal representation of both approaches. In the first type of extraction, DESs play the role of solvent, while in sorbent-based extractions, DESs can perform several different and important roles, such as a medium for sorbent synthesis, as carriers in dispersive solid-phase extractions, as sorbent modifiers and as eluents. In addition to sample preparation, DESs are also used in the quantification stage with satisfactory results. In contrast, the complex composition of biological samples can generate certain limitations in some applications. Considering future directions for the application of DESs in the field of bioanalysis, the development and assessment of automated and semi-automated procedures is still necessary. In addition, new materials containing poly(DESs) and magnetic DESs are also demonstrating huge potential for use in the analysis of biological matrices. We hope to see more publications in this area in the near future.

CRediT authorship contribution statement

Vasil Andruch: Writing – Original Draft, Writing – Review & Editing. Alica Varfalvyová: Writing – Original Draft. Radoslav Halko: Writing – Original Draft. Natalia Jatkowska: Writing – Original Draft. Justyna Płotka-Wasylka: Writing – Original Draft, Writing – Review & Editing.

Declaration of competing interest

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

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Abbreviations

AA	Air-assisted
AALLME	Air-assisted liquid–liquid microextraction
BE	Back-extraction
BTPPB	Benzyltriphenylphosphonium bromide;
CTAB	N,N,N-cetyltrimethyl ammonium bromide;
DES	Deep eutectic solvent
DESF	Deep eutectic solvent based ferrofluid
DFG	Deep eutectic solvent functionalized graphene oxide composite adsorbent

DLLME Dispersive liquid–liquid microextraction
DMF Dimethylformamide;
DPC 1,5-Diphenylcarbazone
DSC Differential scanning calorimetry
DSPE Dispersive solid phase extraction
EME Electromembrane extraction
ETAAS Electrothermal atomic absorption spectrometry
FAAS Flame atomic absorption spectrometry
FDES Freezing of deep eutectic solvent
FTIR Fourier transform infrared spectroscopy
GC-FID Gas chromatography–flame ionization detection
GC-MS Gas chromatography–mass spectrometry
HBA Hydrogen bond acceptor
HBD Hydrogen bond donor
HC-MOF Hollow-core microstructure optical fibers
HDES Hydrophobic deep eutectic solvent
HF-LPME Hollow-fiber liquid-phase microextraction
HPLC-UV High performance liquid chromatography–ultraviolet detection
HSPE Homogenous solid phase extraction
HS-SDME Headspace single-drop microextraction
LDH-MMM Layered double hydroxides mixed matrix membrane
LLE Liquid–liquid extraction
LPME Liquid-phase microextraction
MBG Magnetic bucky gel
MCG Magnetic colloidal gel
MD-μSPE Magnetic dispersive micro-solid-phase extraction
MEPS Microextraction by packed sorbent
MIP Molecularly imprinted polymer
MIPFMR Molecularly imprinted phloroglucinol–formaldehyde–melamine resin
MLC Micellar liquid chromatography
MOF Metal organic framework
MPT-SPE Miniaturized pipette-tip solid-phase extraction
NF Nanofluids
NMR Nuclear magnetic resonance
NSAID Non-steroidal anti-inflammatory drugs
PVP Polyvinylpyrrolidone
SDES Solidification of deep eutectic solvent
SFODME Solidified floating organic drop microextraction
SI Salt-induced
SLM Supported liquid membrane
SLM Solvent microextraction (SME)
SPE Solid-phase extraction
SQT-FAAS Slotted quartz tube equipped flame atomic absorption spectrometry
TAC Trioctylmethylammonium chloride;
TBAB Tetrabutylammonium bromide;
TBAC Tetrabutylammonium chloride;
THF Tetrahydrofuran
TMAC Tetramethylammonium chloride;
TNO 5,6,7,8-Tetrahydro-5,5,8,8-tetramethylnaphthalen-2-ol
UA Ultrasound-assisted
UA-LLME Ultrasound-assisted liquid–liquid microextraction
UHPLC Ultrahigh performance liquid chromatography
UHPLC-QTOF-MS Ultrahigh performance liquid chromatography quadrupole-time-of-flight mass spectrometry
UPLC-MS/MS Ultra performance liquid chromatography–triple quadrupole tandem mass spectrometry
USAEME Ultrasound-assisted emulsification-microextraction
VA Vortex-assisted
VALLME Vortex-assisted liquid-liquid microextraction

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