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Evaluation of 11 ionic liquids as potential extraction solvents for benzodiazepines from whole blood using liquid-liquid microextraction combined with LC-MS/MS

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ABSTRACT

Ionic liquids (ILs) are becoming a trending topic in sample preparation technology, due to their low volatility, low flammability and tunable physiochemical properties. The latter affords the opportunity for creating task-specific solvents. In toxicology, these tailored ILs were not introduced yet; however, they can be significant for drug extraction purposes. This study screens 11 commercially available ILs as potential extraction media for benzodiazepines from blood samples via liquid-liquid microextraction, coupled to liquid chromatography – tandem mass spectrometry (LC-MS/MS). Structure-extraction relationships were deducted from recovery and matrix effect results (n = 5), allowing us to devise some of the favorable features in a tailored IL for benzodiazepine extraction. Overall, long alkyl chains are to be avoided, as they may sterically hinder desirable π-stacking interactions. For the same reason, aromatic planar cations, as imidazolium and pyridinium, were more effective in comparison with ammonium and pyrrolidinium. Furthermore, viscosity negatively impacted both recoveries and matrix effects. High viscosities limited mass transfer during dispersion and impeded electrospray ionization processes. In conclusion, this study evaluates which physicochemical features an IL should possess to efficiently extract benzodiazepines from whole blood.

1. Introduction

The quest for novel extraction solvents is an important topic in analytical chemistry. Current research strives to develop more environmentally friendly and safe solvents, which is closely related to recent green trends of sample preparation miniaturization [1]. Volatile organic compounds (VOCs) are still conventionally used. VOCs are known to be volatile, flammable and toxic; it is clear they should be replaced by safer and greener alternatives. In this regard, deep eutectic solvents (DESs) and ionic liquids (ILs) are studied [1,2].

DESs are mixtures of two components that form strong hydrogen bonds. This translates in significantly lower melting points, when compared to the individual components. Strong intermolecular interactions are also present in ILs and result in low volatility, which makes both DESs and ILs attractive solvents in green analytical chemistry [1,3]. ILs are salts that melt below 100 °C [1], relatively easy to synthesize by combining the desired cation and anion. They generally display low vapor pressures, low flammability, high chemical and thermal stability and their physicochemical features can be tailored for a specific application, obtaining task-specific ionic liquids (TSILs) [1,4–8]. Thanks to this multitude of attractive features, ILs are applied in diverse scientific fields, such as electrochemistry [9], organic synthesis and catalysis [10], medicine [11] and sample preparation technology [12,13]. Currently, ILs are frequently applied in liquid-liquid microextractions, more specifically IL-based dispersive liquid-liquid microextractions (IL-DLLME). This technique belongs to green

Abbreviation: BZD, benzodiazepine; IL, ionic liquid; IL_A, butyltrimethylammonium bis(trifluoromethylsulfonyl)imide; IL_B, 1-butyl-3-methylimidazolium hexafluorophosphate; IL_C, 1-hexyl-3-methylimidazolium hexafluorophosphate; IL_D, 1-octyl-3-methylimidazolium hexafluorophosphate; IL_E, 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; IL_F, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; IL_G, 1-benzyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; IL_H, 1-butylpyridinium bis(trifluoromethylsulfonyl) imide; IL_I, 1-butyl-2-methylpyridinium bis(trifluoromethylsulfonyl)imide; IL_J, 1-butyl-4-methylpyridinium bis(trifluoromethylsulfonyl)imide; IL_K, 1-butyl-1-methylpyrrolidinium bis (trifluoromethylsulfonyl)imide; IL-DLLME, IL-based dispersive liquid-liquid microextraction; ME, matrix effect; RE, recovery; RTIL, room temperature ionic liquid; TSIL, task-specific ionic liquid

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chemistry as it consumes only small volumes ($\leq$ 100 µL) of non-volatile solvents. Furthermore, IL-DLLME is an easy and fast procedure [2,14]. It consists in the addition of an IL to the aqueous sample, creating a fine dispersion by means of a disperser solvent, followed by phase separation and collection of the IL phase for analysis. However, the disperser solvent can also be replaced by proper physical mixing [2,15,16].

Room temperature ionic liquids (RTILs) are particularly interesting for IL-DLLME. Their liquid state at room temperature (RT) is often indicative of low viscosity, high density and hydrophobicity; all desirable features for extraction applications. Promising RTILs can be found within five cation-based IL classes: ammonium, imidazolium, pyridinium, pyrrolidinium and phosphonium [1,17,18]. Especially imidazolium and pyridinium cations are frequently used in the extraction of metal ions, organic compounds and biomolecules from different sorts of matrices [1,13,16,19]. Ideally, a TSIL could be designed for each IL-DLLME application, but IL extraction behavior is still hard to predict a priori [17]. Thus, it is of great value to screen commercially available ILs on their extraction capabilities and draw conclusions from the observed relationship between physicochemical structure of the IL and obtained extraction yields.

In clinical and forensic toxicology, ILs are also expected to play an important role as alternative extraction solvents. The last 10 years, publications have reported the use of IL-DLLME for the extraction of toxicologically relevant molecules in biological samples, such as methamphetamine [20,21], benzodiazepines (BZDs) [22] and antidepressants [23]. Despite risen interest, the number of IL publications in toxicology is scarce. More research is needed to understand IL extraction behavior, thereby making ILs more attractive solvents to use. BZDs are a cohesive class of compounds (5-aryl-1,4-benzodiazepine skeleton), widely prescribed and frequently analyzed in clinical and forensic laboratories [24]. Their structural homogeneity makes them a good target for IL design optimization, to maximize IL-BZD interactions. Not only recoveries (REs) need to be considered when optimizing IL-DLLME procedures in toxicology. Also matrix effects (MEs) need to be evaluated as they can be an issue when IL extracts are analyzed using liquid chromatography – electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS).

In this study, we aim to investigate the applicability of 11 commercially available ILs for the extraction of a broad group of 26 BZDs and 2 BZD-like hypnotics from whole blood. The optimized and validated IL-DLLME-LC-MS/MS method, described by De Boeck et al. [22], was used to evaluate RE and ME for the selected ILs. To corroborate the last parameter, chromatographic elution of the tested ILs was studied. Overall, the general goal of this study is to gain insight into IL extraction mechanisms - based on RE and ME results – and determine desirable physicochemical features for the design of a novel BZD-specific IL.

2. Materials and methods

2.1. Chemicals and reagents

BZD analytical reference standards (1 mg/mL) were purchased from Cerilliant (Round Rock, Texas, USA): alprazolam, clobazam, clonazepam, diazepam, flurazepam, lorazepam, lormetazepam, midazolam, nitrazepam, nordiazepam, oxazepam, prazepam, temazepam and zopiclone. 7-aminoflunitrazepam, bromazepam, chlordiazepoxide, estazolam, etizolam, flunitrazepam, triazolam and zolpidem tartrate were purchased from LGC (Molsheim, France) (1 mg/mL). Brotizolam, ethyl loflazepate and loprazolam were obtained as powder reference standards from, respectively, EDQM Council of Europe (Strasbourg, France), Sanofi-Aventis (Diegem, Belgium) and the British Pharmacopoeia Commission Laboratory (Teddington, UK). Powder standards were dissolved to a final concentration of 1 mg/mL in methanol. Clorazepate, clotiazepam and cloxazolam were obtained from the hospital pharmacy (UZ Leuven, Belgium) as commercially available tablets; Tranxene®, Clozan® and Akton®, respectively. Tablets were extracted and diluted to a final concentration of 1 mg/mL in methanol. All 26 BZDs and 2 BZD-like hypnotics were combined in a methanolic stock solution of 10 µg/mL. Analytical reference standards and stock solutions were stored at − 20 °C. As extraction solvents, 11 ILs were purchased from IOLITEC Ionic Liquids Technologies GmbH (Heilbronn, Germany): butyltrimethylammonium bis(trifluoromethylsulfonyl)imide (IL_A), 1-butyl-3-methylimidazolium hexafluorophosphate (IL_B), 1-hexyl-3-methylimidazolium hexafluorophosphate (IL_C), 1-octyl-3-methylimidazolium hexafluorophosphate (IL_D), 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (IL_E), 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (IL_F), 1-benzyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (IL_G), 1-butylpyridinium bis(trifluoromethylsulfonyl)imide (IL_H), 1-butyl-2-methylpyridinium bis(trifluoromethylsulfonyl)imide (IL_I), 1-butyl-4-methylpyridinium bis(trifluoromethylsulfonyl)imide (IL_J), 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide (IL_K). All ILs were presaturated with Milli-Q water, obtained from a Millipore Purification System (Brussels, Belgium). LC-MS grade methanol was obtained from Biosolve (Valkenswaard, The Netherlands). LC-MS grade ammonium bicarbonate and ammonium hydroxide were purchased from Sigma-Aldrich (Bornem, Belgium). Aqueous buffer was prepared by dissolving ammonium bicarbonate in Milli-Q water (10 mM) and adjusting to pH 8.0 with ammonium hydroxide.

2.2. Biosamples

Blank donor whole blood was obtained from the blood transfusion centre (Gasthuisberg, Leuven, Belgium). Blood samples were stored at − 20 °C, with the addition of 1% sodium fluoride (Merck, Darmstadt, Germany). To avoid including positive samples in the study, all donors were screened for the presence of BZDs and BZD-like hypnotics using a validated SPE-LC-MS/MS method [25]. The use of blank whole blood was approved by the Committee for Medical Ethics UZ Leuven.

3. IL-DLLME-LC-MS/MS

A validated IL-DLLME procedure coupled to LC-MS/MS, described by De Boeck et al. [22], was used to determine BZDs and BZD-like hypnotics in whole blood. The method uses 1.0 mL of whole blood. One mL aqueous buffer pH 8.0 and 60 µL of IL were added. Thereafter, a 5-min rotation step was performed, followed by centrifugation and collection of 10 µL IL. The IL extract was diluted 1:10 v/v in methanol and analyzed using LC-MS/MS. A Kinetex® Biphenyl LC Column (100 mm × 2.1 mm, 2.6 mm) (Phenomenex, Utrecht, The Netherlands) was used in combination with aqueous buffer pH 8.0 and methanol as mobile phases A and B, respectively. The following gradient elution program was applied: 0–9 min: 20–90% B; 9–11 min: 90% B; 11–12 min: 90–20% B; 12–14 min: 20% B. The MS was operated in scheduled multiple reaction monitoring (sMRM) mode, using positive electrospray ionization. Further details on sample preparation, LC-MS analysis, data acquisition and statistical processing were described by De Boeck et al. [22].

To effectively monitor the selected ILs, LC-MS/MS settings were adapted by adding one MRM transition for each IL cation. Transitions were manually determined by direct infusion. Optimized compound-dependent settings can be found in Table 1.

3.1. Recovery and matrix effect

For each IL, RE and ME were evaluated for the extraction of BZDs at 100 ng/mL in whole blood. All tests were performed in quintuplet. RE was calculated as a percentage, by dividing area under the curve (AUC) of pre-extraction spiked samples by AUC of post-extraction spiked samples. ME was calculated as a percentage, by dividing AUC of post-extraction spiked samples by AUC of methanolic standard solutions. RE and ME results for each IL were graphically presented as box plots. Each

Table 1
MRM transitions and other compound-dependent MS settings for each IL cation.

	Q1 mass (Da)	Q3 mass (Da)	CE (V)	DP (V)	EP (V)	CEP (V)
[Butyltrimethylammonium] $^+$	116.1	60.0	25.0	36.0	7.5	14.0
[1-Ethyl-3-methylimidazolium] $^+$	111.1	83.0	21.0	31.0	10.5	16.0
[1-Butyl-3-methylimidazolium] $^+$	139.1	83.0	21.0	26.0	10.0	10.0
[1-Hexyl-3-methylimidazolium] $^+$	167.1	83.0	25.0	36.0	6.0	12.0
[1-Octyl-3-methylimidazolium] $^+$	195.1	83.0	25.0	36.0	9.5	14.0
[1-Benzyl-3-methylimidazolium] $^+$	173.1	91.0	23.0	31.0	9.5	18.0
[1-Butylpyridinium] $^+$	136.1	80.0	21.0	26.0	11.0	10.0
[1-Butyl-2-methylpyridinium] $^+$	150.1	94.0	21.0	31.0	9.0	12.0
[1-Butyl-4-methylpyridinium] $^+$	150.1	94.0	21.0	36.0	6.5	12.0
[1-Butyl-1-methylpyrrolidinium] $^+$	142.2	86.0	27.0	36.0	8.5	12.0

CE: collision energy; DP: declustering potential; EP: entrance potential; CEP: collision cell entry potential. The collision cell exit potential (CXP) was set at 4.0 V. MS settings were determined by direct infusion of an IL dilution 1/100,000 v/v in methanol.

box plot represents the results for 28 extracted analytes, using a certain IL. Additionally, relative standard deviations (RSDs) (n = 5 types of whole blood) were calculated as a measure of repeatability.

4. Results and discussion

4.1. Selection of ILs

A wide range of anions and cations can be combined to generate an almost infinite number of ILs with different physicochemical properties: melting point, solubility, viscosity, density, etc. Extraction yield is directly affected by these properties; therefore, they were taken into consideration when selecting the test batch of ILs for this study. Five inclusion criteria were listed. **1)** A first selection was made on melting points. For this extraction application, ILs molten at RT were selected. This feature is commonly seen in ILs with an asymmetric, bulky and charge dispersed cation, as this disrupts the formation of a crystalline lattice [17,26]. In literature, four IL classes are frequently used in extraction applications: ammonium-, imidazolium-, pyridinium- and pyrrolidinium-based ILs [1,17,18]. Table S1 (Supplementary material) shows all 11 selected RTILs; at least one RTIL was selected for each of the above-named classes. The selected ILs have melting points ranging from − 61 to 26 °C. Most are imidazolium-based ILs, as this class dominates the list of commercially available molten salts [27]. **2)** In IL-DLLME, a hydrophobic IL is used to form a separate layer from the aqueous sample. To obtain hydrophobic solvents, fluorinated anions were selected. Despite their favorable impact on physiochemical features of the IL, it is important to bear in mind that fluorine-containing anions induce solvent toxicity, as they have been reported to obtain lower EC_{50} values on bacteria, compared to other anions [28]. Additionally, they can be potentially dangerous in case of hydrolysis and the formation of hydrofluoric acid [28,29]. Less toxic and more environmentally friendly alternatives are amino acid-based, sugar-based (lactate) or methanesulfonate anions [28]. However, these were not considered in this study due to limited availability, limited knowledge of physicochemical features and/or unfavorable features. In general, it can be stated that decreasing IL lipophilicity, limits its interaction with cellular membranes and decreases toxicity [7]. **3)** A third requirement is for the IL to have a low viscosity. For this study, low viscosity was defined as a viscosity that allows accurate pipetting (< 600 cP at RT). As is shown in Table S1, the selection of ILs, with a wide range of viscosities (33–608 cP at RT), enables a thorough evaluation of the impact of viscosity on RE and ME. It should be noted that viscosity is highly dependent on the anion choice. Generally, small symmetric anions as hexafluorophosphate (PF_6) result in more viscous ILs, compared to bis(trifluoromethylsulfonyl)imide (Tf_2N) [30]. **4)** Another important physiochemical parameter is density. A higher density than water is favorable, as this allows the IL to form the lower phase. If a conical extraction tube is used, small amounts of IL are easier to collect from the bottom of the tube. For this reason, phosphonium-based ILs were not included in the test batch. Table S1 shows that selected ILs have a density higher than water, in a range of 1.24–1.52 g/mL. **5)** Finally, a high affinity toward the 5-aryl-1,4-benzodiazepine skeleton is needed to obtain high extraction yields. To evaluate which non-covalent interactions are dominant in IL-BZD binding, several tests were performed. Four different cation classes were compared with similar substituents. Planar sp^2 hybridized cations (imidazolium and pyridinium) were compared to sp^3 hybridized cations (ammonium and pyrrolidinium). In addition, substituents with varying alkyl chain length were selected to evaluate the importance of the London dispersion forces with respect to BZD binding; $IL_{B\text{-}F}$ were compared. Previous research already provided preliminary indications that shorter alkyl chain lengths resulted in higher process efficiencies [22]. Furthermore, arene substitution patterns were evaluated with the selection of $IL_{H\text{-}J}$. Additionally, IL_G was added to the test set, as a potential extraction boost could be expected thanks to additional pi-stacking of the benzyl substituent. An overview of the selected ILs and their physicochemical properties is given in Table S1.

4.2. Recovery

Fig. 1 shows box plots of the obtained RE results for each IL. In general, RE values were within 60–100% with the highest REs for imidazolium- and pyridinium-based ILs. This was seen when comparing analogues of each IL class: IL_A, IL_F, IL_J and IL_K. A possible explanation could be the aromaticity of imidazolium and pyridinium cations, which allows for π-stacking with the aryl substituent or fused benzene ring of

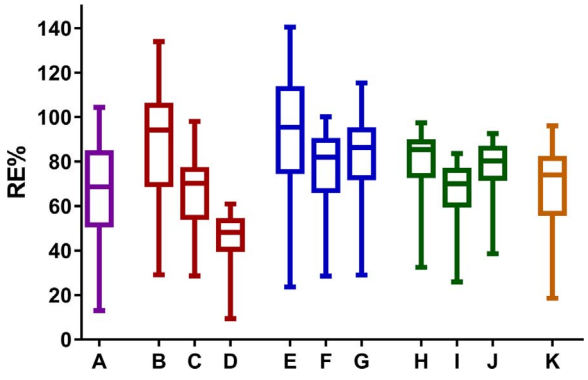

Fig. 1. Recoveries of 11 tested ILs, presented as box plots. RE: recovery; IL_A: butyltrimethylammonium bis(trifluoromethylsulfonyl)imide; IL_B: 1-butyl-3-methylimidazolium hexafluorophosphate; IL_C: 1-hexyl-3-methylimidazolium hexafluorophosphate; IL_D: 1-octyl-3-methylimidazolium hexafluorophosphate; IL_E: 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; IL_F: 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide; IL_G: 1-benzyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; IL_H: 1-butylpyridinium bis(trifluoromethylsulfonyl)imide; IL_I: 1-butyl-2-methylpyridinium bis(trifluoromethylsulfonyl)imide; IL_J: 1-butyl-4-methylpyridinium bis(trifluoromethylsulfonyl) imide; IL_K: 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide.

the 5-aryl-1,4-benzodiazepines. This π-stacking interaction has also been reported to play a crucial role in BZD binding in the $GABA_A$ pocket. Moreover, both phenylalanine (γ_2F77) and tyrosine (α_1Y209) residues are believed to interact with the aryl substituent and benzene ring, respectively [31]. The less successful results for ammonium- and pyrrolidinium-based ILs can be explained by their comparable 3D structure, both having tetrahedral ammonium ions. When considering the imidazolium group, a clear trend was observed with the elongation of the alkyl chain. Chain length was inversely proportional to extraction rates. This could be explained from several points of view. First, as most BZDs have both polar and polarizable aromatic groups in their structure, they are likely to better interact by forming π-stacking, dipole-dipole, or dipole-dipole induced interactions with the most polar part of the ILs. Therefore, shortening the alkyl chain, increases the interacting area of the IL and favors interactions with BZDs. Second, long alkyl chains may result in higher viscosities, as can be clearly seen from Table S1. High viscosity may result in hindered mass transfer and thus inefficient dispersion and phase separation. Eventually, this will result in reduced extraction yields [32]. Finally, a long alkyl chain may sterically hinder the crucial π-stacking, previously discussed. No additional London dispersion interactions will be present, as BZDs do not have long alkyl chains themselves. Moreover, long alkyl chains are known to enhance IL toxicity, as they can easily interfere with cell membranes [7,29,33]. Next to the alkyl chain length, arene substitution patterns were evaluated. When comparing IL_H, IL_I and IL_J, it is seen that the absence of the methyl function increases RE values. As discussed before, we assume that less steric hindrance results in more efficient pi-stacking interactions between pyridinium and BZDs. The absence of a methyl nearby the nitrogen atom, frees the area for possible interactions. This may also explain why a methyl in para-substitution is more favorable compared to ortho-substitution. Furthermore, high viscosity can also have an impact on the observed RE drop of IL_I. The impact of a benzyl-substituent on extraction yield was evaluated by testing IL_G. Intermediate extraction yields were observed, indicating no particular "extraction boost" was obtained. However, it is important to bear in mind that IL_G has a high viscosity, which may limit extraction yields [32]. The potential of benzyl substituents should be further evaluated by creating IL analogues with lower viscosities. When comparing Tf_2N and PF_6 analogues, Tf_2N gives better RE values. Again, this may be explained by favorable viscosities.

Table S2 (Supplementary material) gives a more detailed description of RE and RSD values for each tested BZD and BZD-like hypnotic. From the table, it can be concluded that the most polar analytes (bromazepam, chlordiazepoxide, lorazepam, oxazepam) can be found in the lower quartile of Fig. 1 box plots. This group of poorly extracted drugs should be targeted in upcoming research. Extraction could be enhanced by incorporating hydrogen bond donors and acceptors in the IL structure, to form stronger interactions and favor transfer toward the IL phase. For instance, the incorporation of hydroxyl functional groups in the IL structure could potentially form hydrogen bonds with carbonyl groups (position 2) of 1,4-benzodiazepines [31].

Overall, based on the obtained RE results, it is difficult to select a best choice IL for the extraction of benzodiazepines, because of similar yields. It is clear that IL_B, IL_E, IL_G and IL_H are the top performing ILs, thanks to their short or absent alkyl side chains, aromatic functionalities and/or lower viscosities.

4.3. Matrix effect

Fig. 2 shows box plots of the obtained ME results for each IL. Substantial ion suppression was observed for ILs in general. Most of the tested ILs show ME results within a range of 40–60%. Notwithstanding the green assets of the IL's low volatility, this may be a huge drawback when it comes to ionization. Nonvolatile and viscous compounds may limit ionization, as analytes are impeded of transforming into the gas phase, which can explain high ion suppression results [34]. IL_C, IL_D and IL_G, show even higher suppression. Viscosity may play a vital role, as the indicated ILs are significantly more viscous compared to the others (Table S1). As literature confirms, high viscosity may result in a hindered solvent evaporation during ionization, which leads to an insufficient fission as Rayleigh limits cannot be reached [34,35]. Furthermore, high viscosity results in high RSD values and thus low repeatability, as can be seen in Table S3 (Supplementary material).

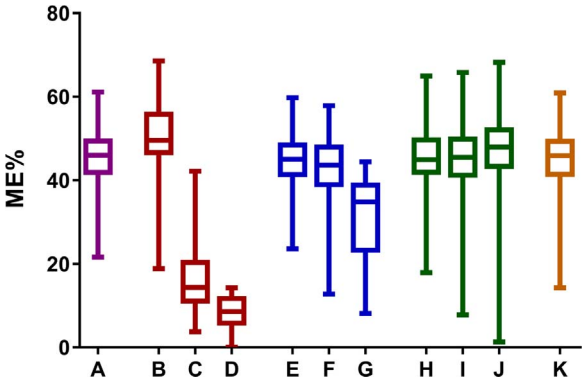

Fig. 2. Matrix effects of 11 tested ILs, presented as box plots. ME: matrix effect; IL_A: butyltrimethylammonium bis(trifluoromethylsulfonyl)imide; IL_B: 1-butyl-3-methylimidazolium hexafluorophosphate; IL_C: 1-hexyl-3-methylimidazolium hexafluorophosphate; IL_D: 1-octyl-3-methylimidazolium hexafluorophosphate; IL_E: 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; IL_F: 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide; IL_G: 1-benzyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; IL_H: 1-butylpyridinium bis(trifluoromethylsulfonyl)imide; IL_I: 1-butyl-2-methylpyridinium bis(trifluoromethylsulfonyl)imide; IL_J: 1-butyl-4-methylpyridinium bis(trifluoromethylsulfonyl) imide; IL_K: 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide.

A second general conclusion can be drawn when looking at the most severely suppressed analytes: the shorter analyte retention time (RT), the higher ion suppression. Table S3 clearly shows that early eluting compounds (7-aminoflunitrazepam, bromazepam and lorazepam) have much lower ME values and thus higher ion suppression, when compared to the last eluting compounds (flurazepam, loprazolam, prazepam). This can be explained by the chromatographic elution profile of the tested ILs. As can be seen from Fig. 3, they all show a wide descending signal across the chromatogram, as the IL sticks to the LC column. This shows that early eluting compounds (± 5.6 min) are severely affected by the IL. Again, IL_C, IL_D and IL_G show wide and high signals during BZD eluting windows, which may be a direct result of their high viscosities. Less viscous ILs, such as IL_E, show faster declining IL signals, which benefits the ionization of the first eluting – and thus polar – compounds. This can be seen from the IL_E box plot minimum that is higher compared to other ILs (Fig. 2). Overall, chromatographic separation of IL and BZDs seems an effective solution to reduce ionization problems. Unfortunately, high IL concentrations in the final extract give broad signals, which are difficult to separate. An extra diluting step of the final extract is not desirable, as sensitivity will no longer be sufficient. A desalting step is another possible solution; however, this will enhance the risk of analyte losses and lead to tedious procedures. Next to chromatographic solutions, an alternative approach for lowering ion suppression can be the use of atmospheric pressure chemical ionization (APCI), as it is less prone to matrix interference [36]. Furthermore, spectrophotometric detectors can solve ME issues, however, selectivity and sensitivity should be carefully monitored. Finally, future IL structural design should focus on creating low-viscosity solvents, for instance by including large asymmetric ions [30].

Overall, based on the obtained ME quantitative results, it can be concluded that IL_A, IL_B and IL_E showed the least ion suppression. This could be explained by the favorable elution profiles that were obtained for IL_A and IL_E, however, IL_B is a more surprising finding, as the obtained elution profile was average and its viscosity rather high.

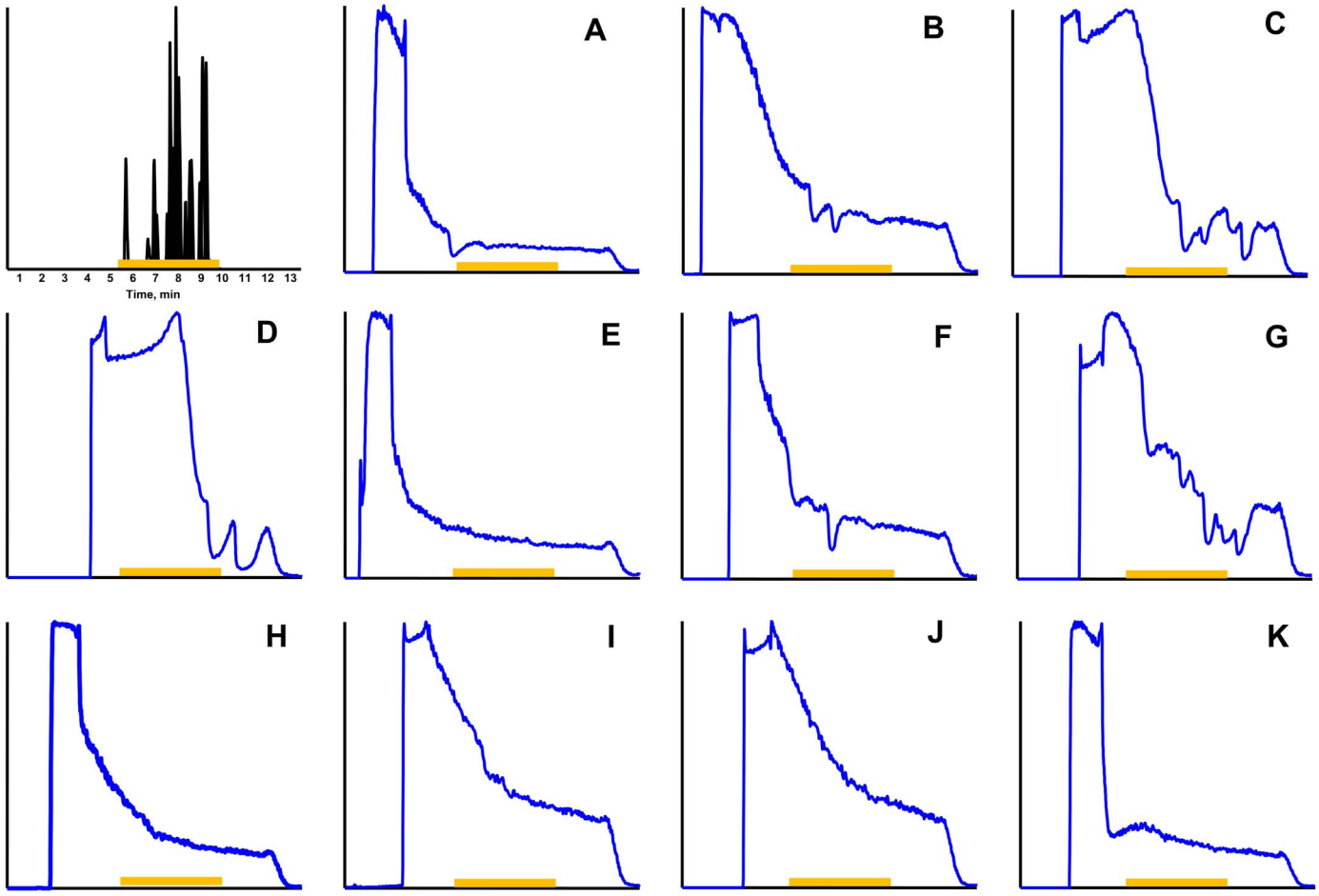

Fig. 3. Chromatographic elution profile of 11 tested ILs. Top left: elution profile of 28 benzodiazepines (BZDs). Yellow box: elution window of BZDs, first eluting BZD at 5.6 (± 0.10) min, last eluting BZD at 9.5 (± 0.05) min; IL_A: butyltrimethylammonium bis(trifluoromethylsulfonyl)imide; IL_B: 1-butyl-3-methylimidazolium hexafluorophosphate; IL_C: 1-hexyl-3-methylimidazolium hexafluorophosphate; IL_D: 1-octyl-3-methylimidazolium hexafluorophosphate; IL_E: 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; IL_F: 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; IL_G: 1-benzyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; IL_H: 1-butylpyridinium bis(trifluoromethylsulfonyl)imide; IL_I: 1-butyl-2-methylpyridinium bis(trifluoromethylsulfonyl)imide; IL_J: 1-butyl-4-methylpyridinium bis(trifluoromethylsulfonyl)imide; IL_K: 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide.

5. Conclusion

In this study, 11 ILs were evaluated for their potential as BZD extraction solvents in whole blood. ILs were selected based on specific required physicochemical properties: melting points lower than RT, immiscibility with water, low viscosity and higher density than water. One ammonium-, six imidazolium-, three pyridinium- and one pyrrolidinium-based ILs were included in the test batch. For each IL, RE and ME were determined by a previously validated IL-DLLME-LC-MS/MS method [22]. Based on RE results, several trends were observed and hypotheses formulated with regard to IL-BZD binding. The ideal TSIL should have sp^2 hybridization to effectuate π-staking with the BZD skeleton. Moreover, viscosity should be as low as possible, to enhance dispersion and accurate pipetting. In this perspective, Tf_2N seems to be the most interesting anion. Arene substitution should be avoided and alkyl chain lengths should be kept to a minimum. It was assumed that a high level of alkyl substitution would sterically hinder π-stacking interactions and thus lower extraction yields. However, a certain degree of alkyl substitution ensures liquid state ILs with hydrophobic properties. When considering ME results, one general conclusion was drawn: avoid ILs with high viscosity ($>$ 300 cP) as they severely suppress ionization. Furthermore, it should be noted that even for ILs with low viscosity, substantial ion suppression was observed due to the non-volatile nature of ILs. A complete chromatographic separation of IL and analyte is the most feasible solution for ME problems, yet hard to obtain. Chromatographic separation was not addressed in this study and needs further research to enhance sensitivity of the first eluting compounds. Taken together, this study was able to pinpoint several requirements for the design of a TSIL for BZD extraction. Next to extraction yields and LC-MS/MS matrix effects, extra attention should be paid to toxicity and biodegradability of the tailored IL. Doing so, the incorporation of naturally occurring anions, as amino acids, is a first step in a greener direction [37].

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Conflicts of interest

None.

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Author contributions

Experimental design: MDB, GD, WD, JT, EC. Conduct experiments: MDB, EC. Data analysis: MDB, GD, EC. Manuscript writing: MDB. Manuscript revision: GD, WD, JT, EC.

Declarations of interest

None.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/http://dx.doi.org/10.1016/j.talanta.2018.03.001.

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