Ionic liquid-based dispersive liquid–liquid micro-extraction combined with ultra performance liquid chromatography for the determination of metal ions

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A novel, strongly hydrophobic 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)tri-fluorophosphate ([HMIM][FAP]) ionic liquid (IL) was investigated as an extraction solvent for dispersive liquid–liquid microextraction (DLLME) of Cr(VI), Co(II), Cu(II) and Ni(II) chelates with ammonium pyrrolidinedithiocarbamate (APDC) and a subsequent analysis of the extracts by ultra performance liquid chromatography. Compared with other commonly used ILs containing hexafluorophosphate and bis(trifluoromethylsulfonyl)imide anions, [HMIM][FAP] exhibited the highest extraction efficiency. Several factors influencing the DLLME performance, such as sample pH, APDC concentration, IL volume, sample volume and the effect of potential interferents were established. Under the optimized conditions, up to 78-fold enrichment factors of analytes and acceptable extraction recoveries (>80%) were obtained. The reliability of the method was evaluated by analysing two water certified reference materials. The measured concentrations were in good agreement with certified ones.

Key words: dispersive liquid–liquid microextraction, ionic liquids, metal ions, ultra performance liquid chromatography

INTRODUCTION

Sample preparation is regarded as a bottleneck in the analytical measurement process, especially when isolating and preconcentrating trace analytes from a large number of samples or complex matrices. Although a variety of separation / preconcentration methods has been proposed for the determination of metal species in various matrices, liquid-liquid extraction [1, 2] and solid-phase extraction [3, 4] techniques are most popular. However, these methods are time-consuming, tedious and require relatively large amounts of high-purity organic solvents, which are expensive and toxic. At present, there is an increasing demand of miniaturized and simplified sample preparation methods that ensure reduction of the amount of organic solvents or even their complete elimination, and also keep the number of stages to a minimum [5]. In 2006, Assadi and co-workers [6, 7] introduced a novel microextraction technique called dispersive liquid–liquid microextraction (DLLME). In this method, a water-immiscible extraction solvent dissolved in a water-miscible dispersive solvent is rapidly injected into an aqueous sample with a syringe. A cloudy solution containing fine droplets of an extraction solvent completely dispersed in the aqueous phase is formed. Extraction of the analytes from the aqueous sample into the dispersed organic droplets takes place. After centrifugation, the sedimented organic phase is removed with a microsyringe and analysed. The simplicity of the operation, speed, low solvent volume, low cost, a high recovery and high enrichment factor are the advantages of DLLME. Over the recent years, the DLLME technique has been successfully applied for the isolation / preconcentration of organic [8, 9] and inorganic [10, 11] compounds from aqueous samples. However, organic solvents required for DLLME should meet the requirements

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such as low solubility in water, good extraction ability for the target analytes, and a higher than water density. Therefore, only a few solvents, mainly chlorinated hydrocarbons, have been extensively used in DLLME.

Ionic liquids (ILs), resulting from the combination of organic cations (e. g., imidazolium, pyridinium, pyrrolidinium) and various anions, can be defined as organic salts that are liquid at or close to room temperature, taking 100 °C as the arbitrary reference [12]. The scientific interest in these liquids has started to grow exponentially upon discovering air- and moisture-stable imidazolium salts in 1992 [13]. As a group of novel solvents, ILs possess many significant advantages that make them ideal candidates as extraction solvents for DLLME. ILs exhibit "structural tuneability" in that their properties (e. g., density, viscosity, hydrophobicity) can be easily tuned and manipulated by simply changing the combination of cations and anions. Furthermore, compared with commonly used chlorinated hydrocarbons, ILs are more compatible with conventional chromatographic separations. Until now, the ILs most often employed in DLLME contain hexafluorophosphate (PF_6^-) or bis[(trifluoromethyl)sulfonyl] imide (NTf_2^-) anions [14, 15]. Recently, a new class of ILs containing tris(perfluoroalkyl)trifluorophosphate (FAP) anions was developed by Merck KGaA [16–18]. One of the most peculiar properties exhibited by these ILs is their exceptionally strong hydrophobic nature. It has been shown that the water uptake of these ILs is much less than that of ILs containing NTf_2^- and PF_6^- anions. Furthermore, replacement of three fluorine atoms in the PF_6^- anion by pentafluoroethyl groups decreases drastically (~7 times) the viscosity of ILs [17]. These properties make them ideal candidates as extraction solvents in DLLME.

In the present work, a novel, strongly hydrophobic 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate (Fig. 1) ionic liquid was investigated as an extraction solvent for DLLME of Cr(VI), Co(II), Cu(II) and Ni(II) chelates with ammonium pyrrolidinedithiocarbamate (APDC) and by a subsequent analysis of the extracts by ultra performance liquid chromatography (UPLC).
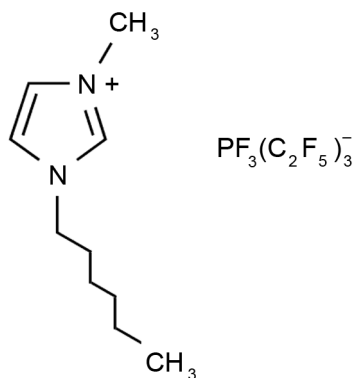

Fig. 1. Structure of 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate ([HMIM][FAP]) ionic liquid

EXPERIMENTAL

UPLC separations were performed on the Waters Acquity UPLC system (Waters, Milford MA) equipped with an Acquity UPLC PDA detector. The following columns, maintained at 30 °C, were used in the experiments: Acquity UPLC BEH C18, Acquity UPLC BEH C8, and Acquity UPLC BEH Phenyl (100 mm × 2.1 mm i. d., 1.7 μm, Waters). Separations were performed under isocratic conditions at a flow rate of 0.5 mL/min using mobile phase composed of acetonitrile–water (70 : 30, v/v). The injection volume was 5 μL; a partial loop with needle overfill injection mode was used. Absorbance data were collected at 256 nm. Data collection and management were performed with Empower 2 build 2154 software (Waters).

1-Hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([HMIM][NTf$_2$]), 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF$_6$]) and 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate ([HMIM][FAP]) were purchased from Merck (Darmstadt, Germany). HPLC grade acetonitrile was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade, obtained from Sigma-Aldrich.

Stock standard solutions (100 mg/L) of Cr(VI), Co(II), Cu(II) and Ni(II) were prepared from appropriate salts in 0.001 mol/L HCl solution. Working standard solutions were prepared immediately before use by a stepwise dilution of a stock standard solution. Ammonium pyrrolidinedithiocarbamate (Merck) 4% (w/v) solutions were prepared daily in water. Appropriate amounts of ILs were weighed and dissolved in methanol.

For the optimized DLLME procedure, an aliquot of 10.0 mL of a standard or sample solution was placed into a 15-mL glass test tube with a conical bottom and 0.5 mL of 1 mol/L HCOOH buffer (pH 3.0 with NaOH) containing 0.02 mmol/L of phenyl-2-acetyloxybenzoate (internal standard), and 0.5 mL of 4% (w/v) APDC was added. Then the mixture was gently shaken for about 1 min, and 0.5 mL of methanol (dispersive solvent) containing 80 μL of [HMIM][FAP] (extraction solvent) was injected rapidly into the sample solution with a 2-mL syringe. A cloudy solution was formed in a test tube. The mixture was then centrifuged for 3 min at 5000 rpm. The upper aqueous phase was removed with a syringe, and the IL phase was dissolved with 100 μL of acetonitrile and analysed.

RESULTS AND DISCUSSION

UPLC separation

Ammonium pyrrolidinedithiocarbamate is one of a few chelating agents that can form very stable neutral chelates with Cu(II), Co(II), Ni(II) and some other metal cations. In addition, Cr(VI) is reduced easily by APDC to Cr(III) and also produces uncharged complexes [1]. The complexation of Cr(III) ions, initially present in the sample, has been

found to be inefficient due to the difficulty of displacing the coordinated water from the strongly hydrated Cr(III) by the APDC ligand. This complexation reaction with the subsequent liquid–liquid extraction of the chelates formed has been widely used for the separation / preconcentration of metal ions.

Initially, the chromatographic separation of four metal–APDC complexes was optimized. In view of the nature of the analytes, reversed-phase separation mode was selected for separation. In the first attempt, the retention properties of three UPLC columns (C18, C8 and phenyl) were compared for a mixture of four complexes in the isocratic elution mode with a 70 : 30 (*v/v*) acetonitrile / water mobile phase at a flow rate of 0.5 mL/min. Comparable trends in elution orders and peak shapes were obtained on all three phases, but separation selectivity was slightly better for the C18 phase.

Next, the pH of the mobile phase was investigated in the pH range 3.0–7.0, using appropriate phosphoric acid / phosphate and acetic acid / acetate buffer solutions. It was found that this parameter did not generate retention and / or selectivity shifts to a relevant extent: for all analytes, the retention time and peak efficiency fluctuated only slightly (less than 10% variation) with pH, most likely due to the lack of change in the ionization state of the analytes in the examined pH range. Based on these results, the buffer-free mobile phase was selected for further experiments.

Finally, the effect of mobile phase flow-rate (from 0.2 to 0.8 mL/min) on the efficiency was studied. However, since no significant improvement in peak efficiency was noted for all compounds, the further work was carried out at 0.5 mL/min mobile phase flow-rate. The experiments indicated that the mobile phase of acetonitrile–water (70 : 30, v/v) showed the best separation performance. As is shown in Fig. 2, an excellent separation of the analytes was achieved isocratically within less than 3 min.

Comparison of ILs

To compare the extraction performance among different hydrophobic ILs, studies were performed on 1-hexyl-3-methylimidazolium ILs containing the NTf_2^-, PF_6^-, and FAP^- anions. The density, viscosity and water solubility of these ILs are listed in Table 1. However, water solubility data for [HMIM][FAP] were not available. Nonetheless, the much less water uptake of [HMIM][FAP] than that of ILs containing NTf_2^- and PF_6^- anions [17] indicates its stronger hydrophobicity.
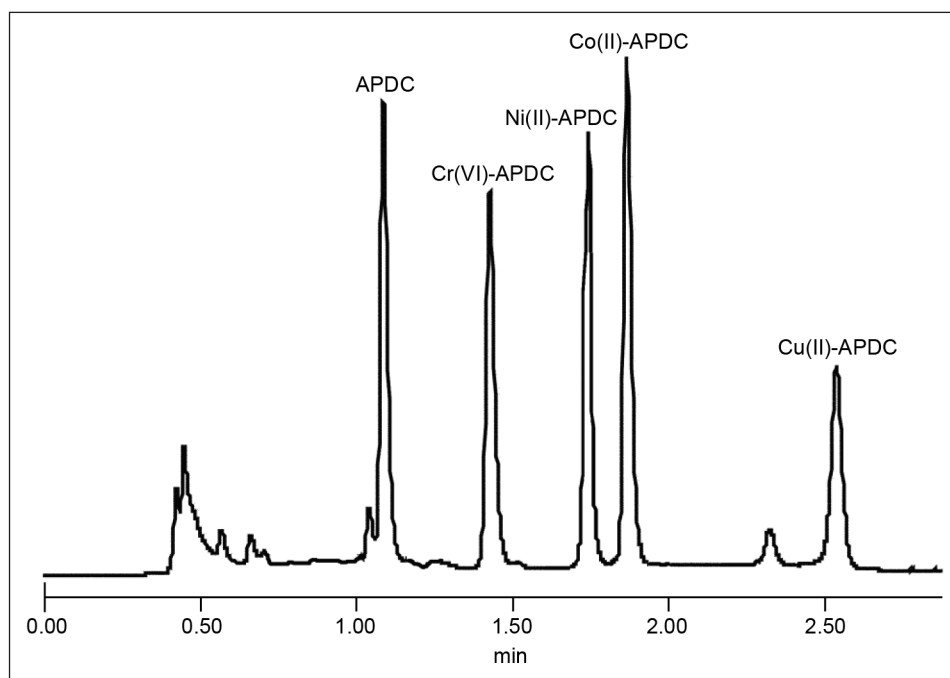

Fig. 2. UPLC separation of metal–APDC complexes under optimized conditions. Mobile phase: acetonitrile / water 70 : 30 (v/v). Flow rate 0.5 mL/min. UV detection at 256 nm

Table 1. **Some physicochemical properties (at 25 °C) of ILs used in this study [17, 19]**

IL	Density, g/mL	Viscosity, mPa · s	Water solubility, g/100 mL
[HMIM][PF$_6$]	1.29–1.31	560–586	0.75
[HMIM][NTf$_2$]	1.33	70	0.34
[HMIM][FAP]	1.56	74	–[a]

[a] Data not available.

Initial DLLME experiments were performed with 5 mL of aqueous standard solutions, using 0.5 mL of methanol containing 50 μL of the appropriate IL as an extraction mixture. However, due to different water solubility of the Ils, the volume of the sedimented phase also differed. In addition, for more viscous [HMIM][PF$_6$] it was difficult to sorb up the IL phase into the microsyringe for the subsequent analysis. In order to overcome these problems, all sedimented phases were dissolved with 100 μL of acetonitrile. The results (Fig. 3) show that the extraction efficiency of metal-APDC complexes increased in the order [HMIM][PF$_6$] < [HMIM][NTf$_2$] < [HMIM][FAP], which is consistent with the decrease in water solubility of these ILs.

Optimization of DLLME conditions

In order to optimize the extraction conditions, the effects of sample pH, the amount of APDC, the nature of disperser solvent, [HMIM][FAP] volume, and sample volume on the extraction efficiency of metal–APDC complexes were investigated. The analyte concentrations used for these experiments were 10, 50 and 100 μg/L.

The pH of the sample solution is one of the important factors affecting the formation of complexes and subsequent extraction. A series of standard metal solutions were prepared in a HCOOH + CH$_3$COOH mixture (0.05 mol/L of each acid) adjusted with NaOH to a desired pH (from 2 to 6), treated with APDC (5 min), then extracted and analyzed. The complexation / extraction of Cr(III) under the same conditions was also investigated. Figure 4 depicts the effect of pH on the peak areas of metal–APDC complexes. The maximum and almost constant signal intensities for all four complexes were observed in the pH range 2.5–4.0,
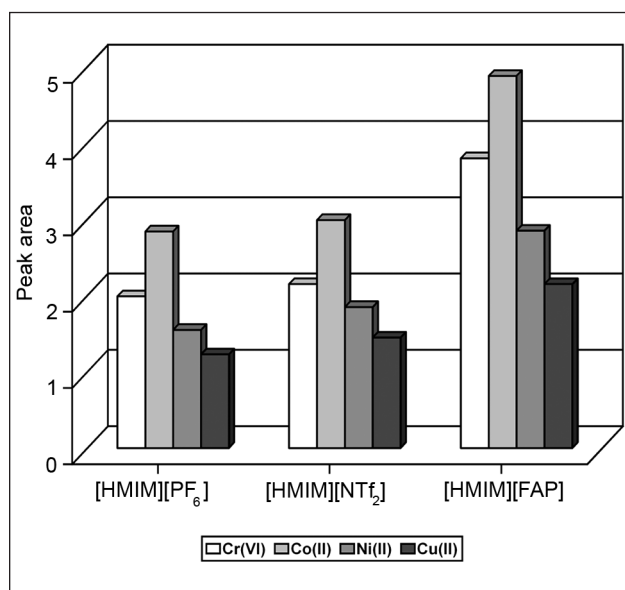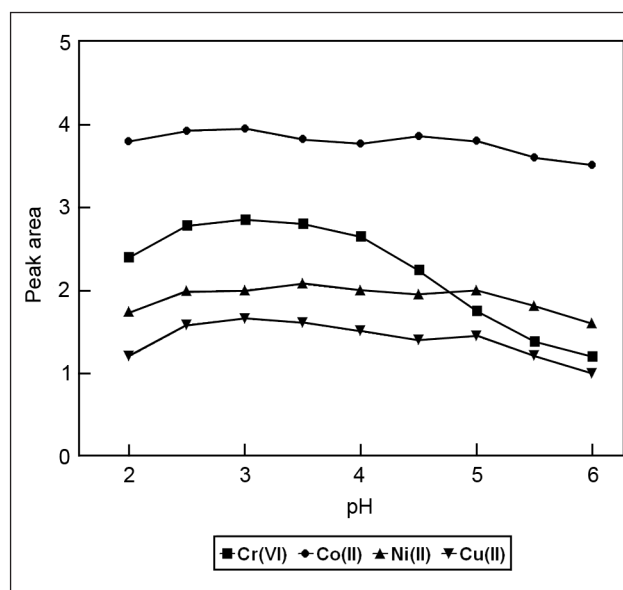
confirming that the complexation required a weakly acidic solution [1]. When the sample pH was higher than 4, the peak area of the Cr(VI) complex considerably decreased, most likely, because of a slower reduction of Cr(VI) ions and, consequently, incomplete complexation. As expected, no Cr(III)–APDC complex was detected in the whole range of the pH values tested.

In the next experiment, the effect of the APDC amount in the range 0.01–0.5% (w/v) on the complexation / extraction performance of the analytes at pH 3.0 was investigated. For the highest analyte concentration studied (100 μg/L), the signal intensity increased up to about 0.02–0.03% APDC, reaching a plateau which was considered as the complete complexation / extraction. Hence, APDC concentration of 0.1% was used to optimize the other parameters. The time required for a complete complexation of metal cations was also evaluated. The results showed that one minute was enough for a complete formation of the metal–APDC complexes studied.

Four common disperser solvents (acetonitrile, acetone, ethanol and methanol) were tested using 0.5 mL of each solvent containing 50 μL of [HMIM][FAP] as an extraction mixture. However, there was no statistically significant difference in the extraction performance obtained with these solvents.

In order to evaluate the effect of [HMIM][FAP] volume on the extraction performance, extraction mixtures containing 0.5 mL of methanol and different volumes (from 20 to 100 μL) of [HMIM][FAP] were examined for standard analyte solutions. Figure 5 shows the effect of IL volume on the recoveries (percentage of the total extracted amount of the analyte) of the analytes studied. The results indicate that

Fig. 3. Effect of IL nature on the peak areas of metal–APDC complexes (50 μg/L of each cation). Sample: 5 mL, pH 3.0. APDC concentration 0.05% (w/v). Extraction mixture: 0.5 mL of methanol containing 50 μL of appropriate IL

Fig. 4. Effect of sample pH on the peak areas of metal–APDC complexes. Extraction mixture: 0.5 mL of methanol containing 50 μL of [HMIM][FAP]. Other conditions as in Fig. 3

40 μL was the lowest amount of [HMIM][FAP] required to achieve satisfactory recoveries (>80%) for all four analytes. Higher amounts of IL did not improve extraction efficiency. Nevertheless, in order to obtain a higher extraction capacity for real samples, 80 μL of [HMIM][FAP] was selected as the optimum volume for all subsequent experiments.

Sample volume is another factor that affects the extraction efficiencies in microextraction techniques. It should be noted that the effects of sample volume are not only a function of the amount of analytes in a solution, but also of the solubility of the IL phase. Figure 6 shows the effect of sample volume on the amount of analytes extracted with 80 μL [HMIM][FAP].
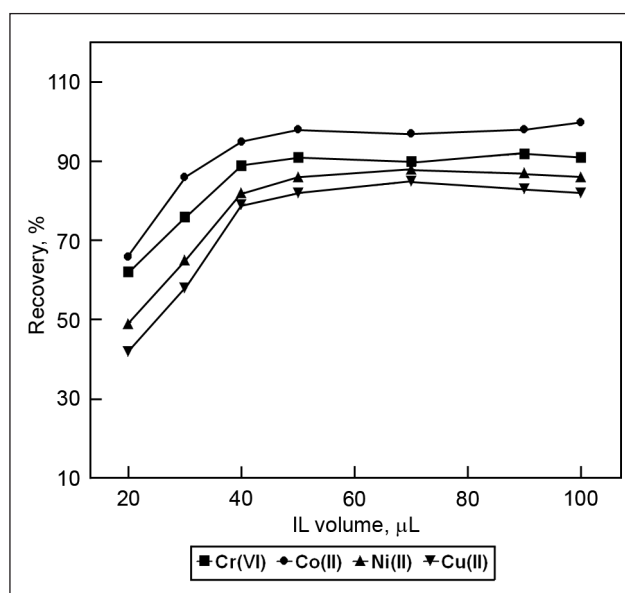

Fig. 5. Effect of [HMIM][FAP] volume on the recoveries of metal–APDC complexes. APDC concentration 0.1% (w/v). Other conditions as in Fig. 3
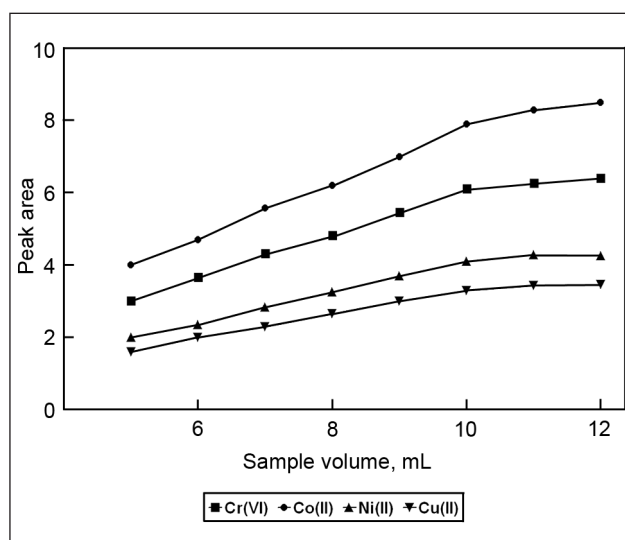

Fig. 6. Effect of sample volume on the peak areas of metal–APDC complexes. APDC concentration 0.1% (w/v). Extraction mixture: 0.5 mL of methanol containing 80 μL of [HMIM][FAP]. Other conditions as in Fig. 3

A gradual increase in the peak area was observed for all of the studied analytes with an increase in the sample volume up to 10 mL. At higher sample volumes, a reduction of extraction efficiency was observed.

Finally, the effect of potential interferents on the extraction and determination of Cr(VI), Co(II), Cu(II) and Ni(II) was assessed. The effect of K^+, Na^+, Ca^{2+}, Mg^{2+}, Cd^{2+}, Cr^{3+}, Fe^{3+}, Pb^{2+}, and Zn^{2+} was studied. In these experiments, solutions containing 10 μg/L of the appropriate analyte and the interfering ions were extracted and analysed. The interference was defined as significant if a variation of more than ±10% in the analyte signal was observed. The results showed that at least 0.5 g/L alkali and alkaline earth metal cations (K^+, Na^+, Ca^{2+} and Mg^{2+}) had no remarkable interferences with the determination of Cr(VI). Only higher concentrations of transition metals caused significant depressive effects due to a competitive reaction with APDC: Cd^{2+}, Cr^{3+}, Pb^{2+} and Zn^{2+} could be tolerated up to about 5 mg/L, and Fe^{3+} could be tolerated up to 1 mg/L. These data show that the methodology is suitable for natural waters in which the levels of these elements (except Fe) are usually lower than those necessary to cause an interference. The interfering effect of Fe^{3+} could be reduced by increasing the amount of APDC or by masking Fe^{3+} ions with an appropriate reagent prior to complexation with APDC. In this work, the first approach was adopted. We found that by using a twice higher APDC amount (0.2% instead of 0.1%), the tolerance limit of Fe^{+3} ions was increased at least to about 10 mg/L.

Analytical performance
It should be noted that, after centrifugation, part of sedimented phase always remains on the wall of a centrifuge tube. Therefore, the measured recovery values are lower than the true ones. The use of an internal standard is required to enhance the recoveries and to improve the accuracy of measurements. Phenyl-2-acetyloxybenzoate was selected for standardization purposes because, under optimized UPLC conditions, it is well separated from the analytes and well extracted into organic solvents. Moreover, common water samples do not contain measurable levels of this compound. In an additional experiment, the extraction performance of phenyl-2-acetyloxybenzoate was checked, and the obtained results showed that under the above optimized DLLME conditions its extraction was quite reproducible.

The main analytical performance characteristics of the method are summarized in Table 2. Detection limits were obtained based on a signal-to-noise ratio of 3. The repeatability of the method, expressed as relative standard deviations (RSDs), was calculated for five replicates of the standard at an intermediate concentration of the calibration curve. The enrichment factors were calculated as the ratio of the analyte concentration in the final extract to that of the initial aqueous phase.

Finally, the accuracy of the method was evaluated by analysing two certified reference materials (CRM): natural water

Table 2. **Analytical performance characteristics**

Analyte	Linear range, µg/L	Correlation coefficient, *r*	Limit of detection, µg/L	Enrichment factor	RSD, %
Cr(VI)	1.5–200	0.9989	0.5	75	2.0
Co(II)	1.0–100	0.9992	0.3	78	2.6
Cu(II)	5.0–150	0.9975	2.0	68	2.8
Ni(II)	5.0–150	0.9958	1.5	71	3.3

Table 3. **Results of metal ion determination in certified reference materials (n = 3)**

CRM	Analyte	Certified concentration, µg/L	Measured concentration, µg/L
NIST SRM 1643e	Co	27.1 ± 0.3	26.2 ± 2.4
	Ni	62.4 ± 0.7	64.8 ± 2.8
BCR-544	Cr(VI)	22.8 ± 1.0	21.5 ± 2.1

CRM NIST SRM 1643e with certified Co and Ni concentrations and lyophilized water CRM BCR-544 with a certified Cr(VI) concentration. These CRMs contain also other ions commonly present in water samples. The obtained results are summarized in Table 3. It is evident that the measured values are in good agreement with certified ones.

CONCLUSIONS

A novel, strongly hydrophobic [HMIM][FAP] ionic liquid was applied for the first time as an extraction solvent for DLLME of Cr(VI), Co(II), Cu(II) and Ni(II) chelates with APDC. Compared with other commonly used ILs, [HMIM][FAP] exhibited a considerably higher extraction efficiency. The extraction efficiency of metal-APDC complexes was shown to increase in the order $[HMIM][PF_6] < [HMIM][NTf_2] < [HMIM][FAP]$, which is consistent with the decrease in water solubility of these ILs. The obtained results indicate that the optimized IL-based DLLME-UPLC method is very fast, robust, and sufficiently sensitive. It is worth noting that the detection limits obtained in this study could potentially be improved by using a more powerful detection system, such as inductively coupled plasma mass spectrometry or atomic absorption spectrometry.

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**DISPERSINĖ SKYSČIŲ–SKYSČIŲ MIKROEKSTRAK-
CIJA JONINIU SKYSČIU IR ULTRAEFEKTYVIOJI
SKYSČIŲ CHROMATOGRAFIJA METALŲ JONAMS
NUSTATYTI**

Santrauka

Naujas, labai hidrofobiškas 1-heksil-3-metilimidazolio tris(penta-fluoretil)trifluorfosfato ([HMIM][FAP]) joninis skystis panaudotas kai kurių metalų (Cr(VI), Co(II), Cu(II) ir Ni(II)) kompleksų su amonio pirolidinditiokarbamatu (APDC) koncentravimui disper-sinės skysčių–skysčių mikroekstrakcijos (DSSME) metodu ir jų nustatymui ultraefektyviąja skysčių chromatografija. Palyginus su kitais DSSME metode naudojamais hidrofobiniais joniniais skys-čiais, [HMIM][FAP] pasižymi efektyvesnėmis ekstrakcinėmis sa-vybėmis. Ištirta mėginio pH, APDC koncentracijos, [HMIM][FAP] tūrio, mėginio tūrio bei pašalinių metalų jonų įtaka ekstrakcijos efektyvumui. Optimizuotomis sąlygomis analitės sukoncentruoja-mos iki 78 kartų, o ekstrakcijos išgavos didesnės nei 80 %. Metodo patikimumas buvo įvertintas išanalizavus dvi paliudytąsias pama-tines medžiagas. Nustatytos metalų koncentracijos gerai sutapo su tikrosiomis vertėmis.