Talanta 99 (2012) 758–766

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Preconcentration and speciation of trace amounts of chromium in saline samples using temperature-controlled microextraction based on ionic liquid as extraction solvent and determination by electrothermal atomic absorption spectrometry

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ARTICLE INFO

Article history:
Received 1 November 2011
Received in revised form
5 July 2012
Accepted 6 July 2012
Available online 16 July 2012

Keywords:
Speciation
Chromium
Temperature-controlled microextraction
Ionic liquid
Electrothermal atomic absorption spectrometry

ABSTRACT

A sensitive and selective method for the preconcentration and speciation of sub ng L^{-1} levels of chromium species in aqueous solutions with high salt contents is described. The developed method is based on temperature-controlled microextraction of chromium species using the 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF_6]) ionic liquid as an extractant followed by electrothermal atomic absorption spectrometry (ETAAS) determination. The extraction of chromium species from aqueous solution into the fine droplets of [HMIM][PF_6] was performed with ammonium pyrrolidine dithiocarbamate (APDC) as the chelating agent. Some predominant factors affecting the preconcentration and speciation of both Cr(III) and Cr(VI) species were evaluated and optimized. Under the optimum conditions, the calibration graphs were linear over the concentration ranges from 50 to 200 ng L^{-1} for Cr(III) and from 25 to 150 ng L^{-1} for Cr(VI). The limits of detection (LOD) of the developed method were 5.40 ng L^{-1} and 2.45 ng L^{-1} for Cr(III) and Cr(VI) ions, respectively. The enrichment factor for chromium species was found to be 42. The relative standard deviations for six replicate determinations of 100 ng L^{-1} of either Cr(VI) or Cr(III) were 4.24% and 3.05%, respectively. The developed method was successfully applied to the speciation and determination of chromium species in water and urine samples.

1. Introduction

Chromium is one of the most abundant elements, having the potential to use in the chemical industries. Although chromium exists in several oxidation states, but it is in mainly two oxidation states in the environment, Cr(III) and Cr(VI), which have different physiological effects [1]. Cr(III) is considered as an essential nutrient element for living organisms and is necessary for glucose, lipid, and protein metabolism. In contrast, Cr(VI) is a suspected carcinogen that can penetrate inside the cell and react with protein components. Moreover, it has the ability to oxidize other species and has the potential to contaminate soil, surface water, and groundwater. Therefore, development of methods for separate determination of the chromium species is great important for analytical chemists.

Many of the available methods are based on determination of the total chromium, but it does not provide sufficient information to understand its toxicity and bioavailability in the environment. Only a few analytical methods have enough sensitivity and selectivity for the direct determination and speciation of trace amounts of each chromium species in environmental samples. Generally, techniques such as liquid–liquid extraction [2,3], solid phase extraction [4–6], ion exchange [7,8] and coprecipitation [9,10] have been used for separation and speciation of chromium. The employed methods are based on two steps: separation of one or both of the chromium species from the original matrix, and then determination of the separated species. In these methods, the detection limits for determination of chromium species in coupling with flame and/or electrothermal atomic absorption spectroscopy have been improved, but the obtained results often have inadequate sensitivity for determination of trace concentrations of chromium in real samples.

The most frequently chelating agents have been employed for extraction and preconcentration of chromium species into organic solvents in liquid–liquid and solid phase extraction methods are dithiocarbamates [11–14], dithizone [15], hydrazones [16], and β-diketones [17].

Ammonium pyrrolidine dithiocarbamate (APDC) is known to be one of the effective chelating agents and quite stable chelate in acidic media for batch liquid/liquid extraction and preconcentration of several trace metal ions [2,3]. The chemical structures of

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http://dx.doi.org/10.1016/j.talanta.2012.07.018

individual complexes of Cr(III) and Cr(VI) with APDC are different: Cr(III) forms a complex formulated as $Cr(PDC)_3$, whereas Cr(VI) is reduced to Cr(III) by APDC which leads to two different chromium complexes; $Cr(PDC)_3$ and $Cr(PDC)_2(OPDC)$ [3,13,14].

Bergman et al. [14] reported Cr(VI) can be extracted into methyl isobutyl keton (MIBK) by APDC; whereas the extraction of Cr(III) by APDC occurs at higher pH with low efficiency at room temperature because of strong hydration of Cr(III) ions and difficult displacement of those coordinated water molecules by APDC. However, they have also reported that elevated temperatures (50–80 °C) could facilitate the extraction of Cr(III) by APDC. The simultaneous extraction of Cr(III) and Cr(VI) from water samples was reported by Subramanian [3], who used high concentrations of APDC to extract both chromium species into MIBK for FAAS in the presence of a potassium hydrogen phthalate (PHP) buffer. Wang and Chiu [13] used an elevated temperature (50 °C) and a high concentration of APDC in phthalate buffer to increase the extraction efficiency of chromium, so that both Cr(III) and Cr(VI) could be simultaneously extracted by APDC. They found that whilst the extraction of Cr(VI) by APDC was independent of phthalate buffer concentration, the extraction of Cr(III) depended on the buffer concentration. It seems that the APDC and PHP buffer concentrations have significant roles on the effective extraction of chromium species.

Recently, room-temperature ionic liquids (RTILs) are being considered as an environmentally benign alternate for traditional solvents due to their unique chemical and physical properties. These materials have negligible vapor pressure, non-flammability, good extractability for various organic compounds and metal ions as charged or neutral complexes, tunable viscosity and miscibility with water and organic solvents [18]. They have been attracted as new solvents in green chemistry.

Due to the specific properties of RTILs, coupling of metal ions extraction with appropriate ionic liquids have been utilized in analytical chemistry. Conventional liquid–liquid extraction based on ILs has been reported by several researchers [19–21]. However, this method requires to a large amount of expensive ILs for extraction of the analyte. Several liquid–liquid microextraction techniques such as single drop microextraction [22–24], cold-induced aggregation microextraction [25], in situ solvent formation microextraction [26], dispersive liquid–liquid microextraction [27,28], temperature-controlled dispersive liquid–liquid microextraction [29], and ultrasonic probe-assisted ionic liquid dispersive liquid–liquid microextraction [30] have been employed ILs as extraction solvents. The extraction process is accomplished after the formation of the fine droplets of the extractant phase [25]. In liquid–liquid microextraction based on ILs, there is no interface between the water and the extracting phases; as a result, mass transfer from aqueous phase into IL phase has no considerable effect on the extraction efficiency.

The aim of this work was to examine the possibility of using APDC and $[HMIM][PF_6]$ as the chelating agent and extraction solvent, respectively, for the sequential extraction and determination of chromium species in saline samples by using the temperature-controlled microextraction method followed by determination with ETAAS. The possible factors affecting the chelating and extraction of both Cr(VI) and Cr(III) were investigated in details. The developed method was applied to the determination of chromium species in water and urine samples.

2. Experimental

2.1. Apparatus

The determination of chromium was carried out by using atomic absorption spectrometer (Shimadzu AA6300G, Kyoto, Japan) equipped with pyrolitic platform graphite (GFA-EX7i). A Deuterium background correction was employed to correct non-specific absorbance and a chromium hallow cathode lamp (Hamamatsu Photonics, Japan) was used as the radiation source for the measurements. The optimum operating parameters for ETAAS are given in Table 1. The pH measurements were carried out using a Corning pH meter 125 equipped with a combined glass electrode. Phase separation was assisted by a centrifuge (EBA 20, Andreas Hettich GmbH& Co. KG, Zentrifugen, Germany). Deionized water was obtained from an AquaMax system (Young-Lin, Korea).

Table 1
ETAAS operating conditions for determination of chromium.

Parameters	
Wavelength/nm	357.9
Slit width/nm	0.7
Lamp current/mA (Low)	10.0
Lamp mode	BGC-D_2
Drying temperature (°C)	150 (Ramp 10 s, hold 20 s) 250 (Ramp 10 s, hold 10 s)
Ashing temperature (°C)	800 (Ramp 10 s, hold 10 s) 800 (Step, hold 10 s) 800 (Step, hold 3 s), Sensitive
Atomization temperature (°C)	2300 (Step, hold 2 s), Sensitive
Cleaning temperature (°C)	2500 (Step, hold 2 s)
Cold	Hold 50 s
Sample volume/μL	20
Integration mode	Peak height

2.2. Standard solutions and reagents

All reagents used were of the analytical grade, and all solutions were prepared in deionized doubly distilled water. The nonionic surfactant Triton X-114 and Triton X-100 were obtained from Sigma (St. Louis, Mo, USA) and used without further purification. Stock solutions of Cr(III) and Cr(VI) at a concentration of 1000 μg mL^{-1} were prepared by dissolving appropriate amounts of $Cr(NO_3)_3.6H_2O$ and $K_2Cr_2O_7$ in 100 mL of deionized water. Working standard solutions were obtained by appropriate dilution of the stock standard solutions.

A solution of 5 (%w/v) ammonium pyrrolidine dithiocarbamate (APDC) was prepared by dissolving appropriate amount of APDC, (Merck, Darmstadt, Germany), in deionized water.

Potassium hydrogen phthalate (PHP) was used to prepare buffers and the pH adjustment was carried out with 1.0 mol L^{-1} NaOH solution.

The ionic liquids 1-butyl-3-methylimidazolium hexafluorophosphate $[BMIM][PF_6]$, 1-hexyl-3-methylimidazolium hexaflourophosphate $[HMIM][PF_6]$ and 1-octyl-3-methylimidazolium hexafluorophosphate $[OMIM][PF_6]$ were purchased from Merck (Darmstadt, Germany) and used as obtained.

2.3. Recommended procedure for preconcentration and speciation of chromium species

For preconcentration of pure Cr(VI) species into IL, 5 mL of aqueous solution containing Cr(VI), 0.1 (%w/v) APDC, 0.04 (%w/v) Triton X-114, PHP buffer (pH=5, 0.45 mol L^{-1}), and 25 μL [HMIM][Br] was transferred to a 10 mL conical bottom centrifuge tube. Then, 25 μL of $[HMIM][PF_6]$ was added quickly to the sample solution, and the tube was put in a water bath at 45 °C for 5 min until a homogeneous phase was formed. Subsequently, the tube was placed in an ice bath for 10 min until a cloudy solution was formed. Afterwards, the mixture was centrifuged for 10 min at 3000 rpm. About 7 μL of the fine droplets of $[HMIM][PF_6]$ containing hydrophobic chromium complex settled at the bottom of the

centrifuge tube. Bulk aqueous phase was removed simply by inverting the tube. Due to high viscosity of the ionic liquid, it was dissolved in 100 μL of ethanol, and a volume of 20 μL of this solution was used for direct analysis by ETAAS. Cr(III) was extracted by the same procedure as described above for the Cr(VI) species except for APDC concentration and heating time that adopted at 0.45 (%w/v) and 25 min, respectively.

For sequential extraction of the chromium species, 5 mL of standard solution or real sample containing both Cr(VI) and Cr(III) ions was subjected to the extraction process as described above. The APDC and PHP buffer were added to the aqueous solution at the concentrations of 0.1 (%w/v) and 0.45 mol L^{-1}, respectively. By heating the solution at 45 °C for 5 min, only Cr(VI) was extracted. In the second step, the remaining aqueous phase including Cr(III) ions was transferred into another centrifuge tube simply by inverting the tube and the APDC concentration was elevated to 0.45 (%w/v), while no change in buffer concentration was made. This ensured complete complex formation between Cr(III) and APDC and the extraction efficiency of Cr(III) was enhanced. Then, 25 μL of [HMIM][PF_6] was quickly added to the solution. Afterwards the tube was heated in water bath at 45 °C for 25 min and the extraction procedure was continued. In this step, the quantity of the chromium species extracted is attributed to Cr(III) species.

2.4. Sample preparations

Two different water samples i.e. underground water and tap water (Shokat Abad, Birjand) were investigated. Before the analysis, the water samples were filtered through a 0.45 μm membrane filter to remove suspended particulate matters.

Urine samples were stored in refrigerator at 4 °C for less than three days until their analysis. In some cases, an acidification of samples is effective for preservation. For the determination of Cr(VI), however, the natural samples should not be acidified because Cr(VI) reduces in the presence of natural organic matters [18].

3. Results and discussion

3.1. Optimization of extraction conditions

The influence of various parameters affecting the complex formation and extraction conditions such as the type and the amount of ionic liquid (IL) and anti-sticking agent, APDC concentration, sample pH, salt concentration, time and temperature of heating and cooling, and centrifugation time were studied and optimized thoroughly in order perform a selective extraction method for speciation of chromium with high extraction efficiency and enrichment factor. All optimization steps were done at 100 ng L^{-1} concentration level of chromium.

3.1.1. Selection of ionic liquid

As an extraction solvent, ILs with good extraction ability, low solubility in water and higher density than water should undertake, so that fine particles of IL can be formed and settled in saline solutions. In this study, three ILs containing imidazolium cations were employed. Imidazolium-ILs containing PF_6^- anion are in the liquid state, water immiscible, and relatively inexpensive. According to some physicochemical properties like density, viscosity, and water solubility of the ILs listed in Table 2, [BMIM][PF_6] shows an acceptable viscosity and high density but high water miscibility in comparison with the [OMIM][PF_6] and [HMIM][PF_6]; while, [OMIM][PF_6] has high viscosity and water immiscibility but too low density to form fine particles to settle in saline solutions. Selection of proper IL with low solubility and viscosity reduces the required volume of the extracant phase for complete extraction of chromium species, because high viscosity decreases the mass transfer between two phases and leads to problems during the microextraction process. For rapid assessment of the potential of the investigated ILs, 50 μL of [BMIM][PF_6], [HMIM][PF_6] or [OMIM][PF_6] was added to 5 mL aqueous solutions containing 100 ng L^{-1} chromium species, and the solutions were subjected to the extraction process. The results are presented in Table 3. [HMIM][PF_6] was found to give the best extraction efficiencies of Cr(III) and Cr(VI) and was chosen as the extraction solvent.

Table 2
Physicochemical properties of ILs used in this work at 25 °C.

IL	Density (g mL^{-1})	Viscosity (mPa s)	Melting point (°C)	Water solubility (g 100 mL^{-1})
[BMIM][PF_6]	1.36–1.37	148–450	−8	1.88
[HMIM][PF_6]	1.29–1.31	560–586	−61	0.75
[OMIM][PF_6]	1.20–1.23	682–710	–	0.20

Table 3
Extraction efficiency of Cr(VI) into several imidazolium-ILs containing PF_6^- anion.

IL	Sedimented volume of IL (μL)	Extraction (%)[a] of Cr(VI)	Extraction (%) of Cr(III)
[BMIM][PF_6]	10	81.9 ± 4.4[b]	61.7 ± 2.9
[HMIM][PF_6]	16	90.3 ± 3.3	87.3 ± 5.2
[OMIM][PF_6]	12	86.3 ± 5.2	73.7 ± 3.8

[a] Extraction conditions: 5.0 mL chromium at 100 ng L^{-1} conc.; volume of ILs, 25 μL; Triton X-114, 0.04 (%w/v); PHP buffer, pH 5, 0.2 mol L^{-1}; APDC, 0.2 (%w/v); Heating, 45 °C, 25 min; time of cooling, 10 min; Extraction conditions of Cr(VI) was the same as with Cr(III) except for: heating time of 5 min; APDC, 0.1 (%w/v).
[b] Mean value ± standard deviation, $n=3$.

3.1.2. Effect of ionic liquid volume

The influence of the IL volume on the extraction efficiency and enrichment factor of chromium species was investigated. The enrichment factor was defined as the following Eq. (1):

$$EF = C_{IL}/C_0 \quad (1)$$

where EF, C_{IL} and C_0 are enrichment factor, concentration of chromium in the IL settled phase, and initial concentration of chromium in the aqueous sample, respectively. In this study, different volumes of [HMIM][PF_6] ranged from 10 to 100 μL, were added to 5 mL aqueous solutions containing 100 ng L^{-1} chromium, and the extraction procedure was performed according to Section 2.3. When the volume of [HMIM][PF_6] was increased from 10 to 100 μL, the settled volume of IL phase increased from 3 to 48 μL. As it can be seen from Fig. 1, the extraction efficiencies of Cr(III) and Cr(VI) increased with increase of the initial [HMIM][PF_6] volume from 10 to 25 μL and then reached to a plateau. Higher volumes of the [HMIM][PF_6] did not improve the extraction efficiency, but lead to increase in background signal. Moreover, the enrichment factor increased along with increase in the [HMIM][PF_6] volume up to 25 μL and then significantly decreased. The minimum volume of IL that satisfies high enrichment factor and improved analytical sensitivity, was 25 μL. Thereby, the initial volume of 25 μL for [HMIM][PF_6] was selected for the subsequent experiments. Before the analysis, the final IL phase was diluted with 100 μL of ethanol, and the amount of chromium was determined.

3.1.3. Effect of anti-sticking agent

It was observed that some of the IL-phase sticks on the centrifuge tube wall and couldn't settle. It was supposed that in

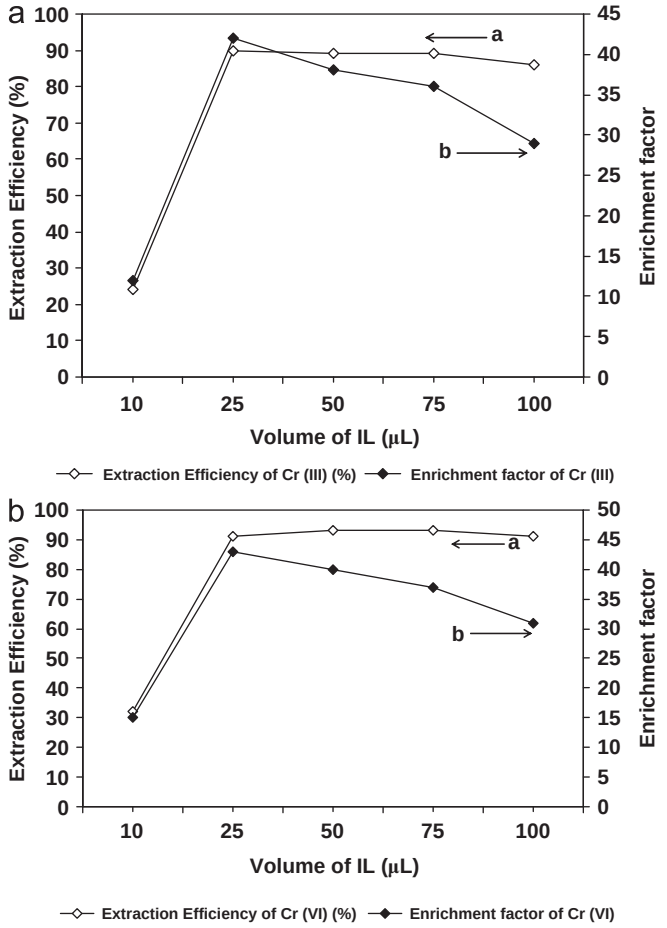

Fig. 1. Effect of [HMIM][PF_6] volume on extraction efficiency (a) and enrichment factor (b) for Cr(III) (A) and Cr(VI) (B). Extraction conditions for Cr(III): 5.0 mL 100 ng L^{-1} Triton X-114, 0.1 (%w/v); PHP buffer, 0.1 mol L^{-1}; APDC, 0.2 (%w/v); Heating step, 45 °C, 25 min; cooling step, 10 min. The same conditions were used for Cr(VI) except for the time of heating, 25 min.

Table 4
Effect of nonionic anti-sticking agents on the extraction efficiency of Cr(III) and Cr(VI).

Non-ionic anti sticking	Extraction (%)[a] of Cr(III)	Extraction (%) of Cr(VI)
Triton X-100	61.1 ± 3.0	52.7 ± 3.2[b]
Triton X-114	88.0 ± 4.8	91.2 ± 5.4

[a] Extraction conditions of 100 ng L^{-1} Cr(III): sample volume, 5.0 mL; [HMIM][PF_6], 25 μL; [HMIM][Br], 25 μL; nonionic anti-sticking, 0.04 (%w/v); pH, 5; PHP buffer, 0.2 mol L^{-1}; APDC, 0.2 (%w/v); Heating, 45 °C, 25 min; time of cooling, 10 min. Extraction conditions of Cr(VI) was the same as Cr(III) except for: heating time of 5 min; APDC, 0.1 (%w/v).

[b] Mean value ± standard deviation, $n=3$.

the presence of non ionic surfactant the interactions of IL with the inner wall of the tube are reduced through surrounding the fine droplets of the ILs and the adherence of the IL to the tube walls is diminished. Therefore, the effect of two nonionic surfactants Triton X-100 and Triton X-114 were examined. As shown in Table 4, in the presence of Triton X-114, higher extraction efficiencies were obtained for both Cr(VI) and Cr(III). So, Triton X-114 was chosen as the anti-sticking agent, and the effect of its concentration was investigated. As shown in Fig. 2, the extraction efficiencies of chromium species was enhanced by an increase in the anti sticking concentration up to 0.04 (%w/v), and no

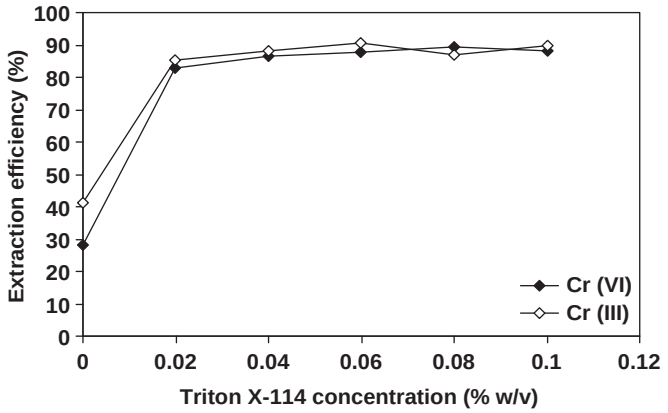

Fig. 2. Effect of Triton X-114 concentration on extraction efficiency of Cr(III) and Cr(VI). Extraction conditions are the same as Fig. 1 except for [HMIM][PF_6] volume, 25 μL.

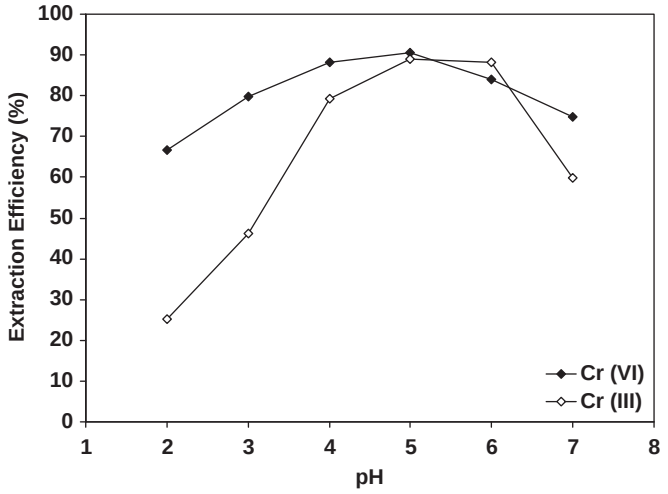

Fig. 3. Effect of pH on extraction efficiency of Cr(III) and Cr(VI): Extraction conditions: 5.0 mL 100 ng L^{-1} chromium; 25 μL [HMIM][PF_6]; Triton X-114, 0.04 (%w/v); PHP buffer, 0.1 mol L^{-1}; APDC, 0.2 (%w/v); Heating step, 45 °C, 25 min; cooling step, 10 min. The same conditions were used for Cr(VI) except for time of heating, 5 min.

considerable effects on the extraction efficiencies were observed at higher concentrations. As a result, a concentration of 0.04 (%w/v) of Triton X-114 was chosen for the following experiments.

3.1.4. Effect of pH

The sample pH plays a critical role on the formation of hydrophobic complex between the APDC and chromium species [18,25]. According to results of former experiments, PHP buffer was chosen for the pH adjustment. The effect of sample pH on the formation of chromium complexes was investigated in the pH range of 2–7. The extraction efficiencies of Cr(III) and Cr(VI) drastically decreased at pH < 4 and at pH > 6, whereas the extraction efficiencies remained constant at pH values of 5–6 for Cr(III) and 4–5 for Cr(VI). The reason for the decrease may be explained by the presence of other soluble forms of chromium and the inability of chromium to form complex with APDC (Fig. 3). Thus, a pH value of 5 was selected as the working pH to extract Cr(III) and Cr(VI) with high extraction efficiency.

3.1.5. Effect of buffer concentration

To study the influence of buffer concentration on the performance of microextraction of chromium species, the effect of

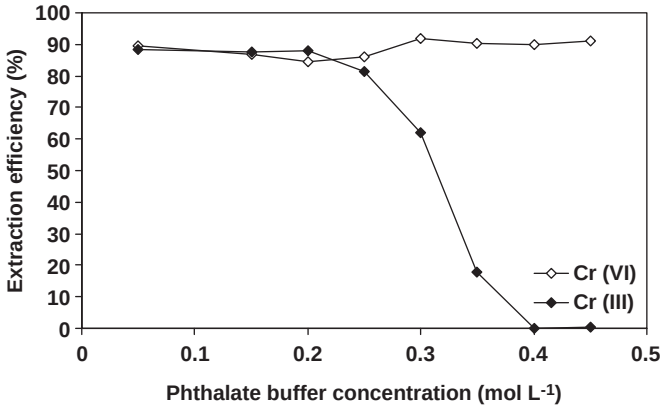

Fig. 4. Effect of PHP buffer concentration on the extraction efficiency of Cr(III) and Cr(VI) at pH, 5. Other conditions are the same as Fig. 3.

buffer concentrations in the range of 0.1–0.5 mol L^{-1}, by keeping the concentration of APDC constant at 0.1 (%w/v), on the extraction efficiency of Cr(III) and Cr(VI) was considered. As illustrated in Fig. 4, high concentration of phthalate buffer (above 0.25 mol L^{-1}) suppress the extraction of Cr(III) species, while the extraction efficiency of Cr(VI) was independent of the buffer concentration. This likely resulted from the competition of phthalate ion with APDC to form complex with Cr(III). Meanwhile, such decreasing did not observe for Cr(VI) due to inability of Cr(VI) to complex formation with phthalate anion. When the buffer concentration was increased to 0.45 mol L^{-1}, the difference in extraction efficiencies of Cr(III) and Cr(VI) reached to the maximum value and then remained constant. The above results clearly showed that the buffer concentration exerted great influence on the speciation performance of chromium. Therefore, a concentration of 0.45 mol L^{-1} PHP buffer was chosen for speciation experiments.

3.1.6. Effect of APDC concentration

Extraction efficiency of Cr(III) and Cr(VI) as a function of APDC concentration in the range of 0.1–0.8 (%w/v) was studied. The results indicate that not only the buffer concentration affect on speciation of chromium, but also the APDC concentration exhibits a remarkable impact on the extraction efficiency. For instance, at buffer concentrations of 0.2, 0.35 and 0.45 mol L^{-1}, complete formation of Cr(III)–APDC complex was occurred at 0.15, 0.3, and 0.45 (%w/v) of APDC concentration, respectively. APDC concentration of 0.1% (w/v) was enough for complete formation of Cr(VI)–APDC complex at pH 5 of 0.45 mol L^{-1} buffer concentration. However, at this buffer concentration, the extraction of Cr(III) by APDC was incomplete. Consequently, a high concentration of APDC was required (Fig. 5A and B). Thus, at buffer concentration of 0.45 mol L^{-1} (pH 5.0), 0.1 and 0.45 (%w/w) of APDC concentrations were used for complete extraction of Cr(VI) and Cr(III), respectively.

3.1.7. Effect of temperature

For a complete extraction, IL must be dissolved in aqueous phase giving a homogeneous phase with a low viscosity. The complete solubility of IL into the aqueous solution depends on temperature. High temperature is in favor of dispersion of [HMIM][PF_6] into the aqueous solution and shortening the partition equilibrium time between IL and aqueous phases. At room temperature, the extraction efficiency of Cr(III) and Cr(VI) were 15% and 30%, respectively. Elevating temperature to 45 °C, improve the extraction efficiencies of Cr(III), so that in the range

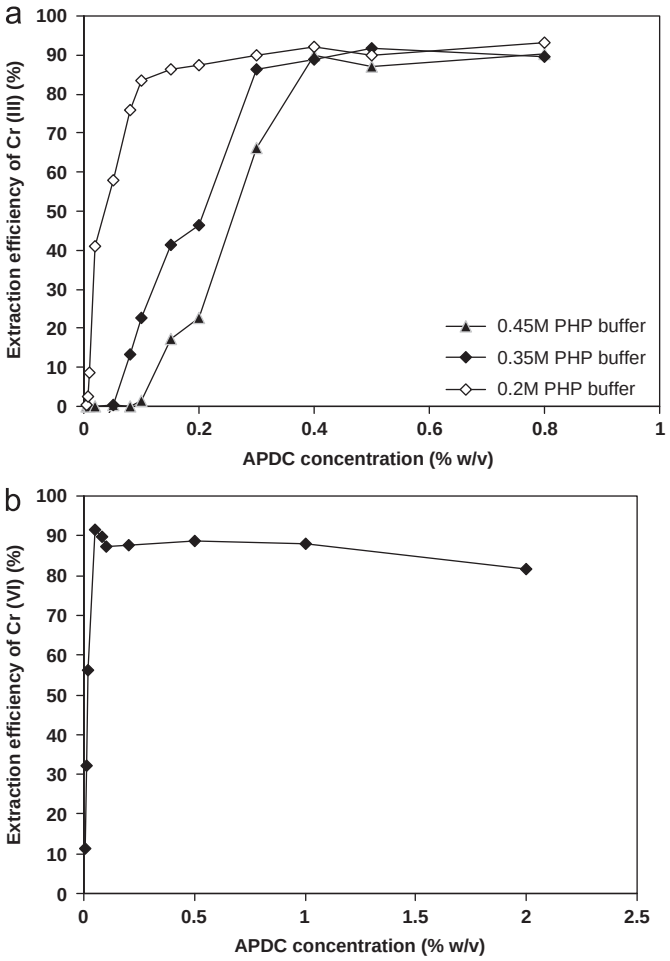

Fig. 5. Effect of APDC concentration on the extraction efficiency of Cr(III) (a) and Cr(VI) (b); Extraction conditions for Cr(III): 5.0 mL 100 ng L^{-1} Cr(III) at pH 5; [HMIM][Br], 25 μL; Triton X-114, 0.04 (%w/v); PHP buffer, 0.2, 0.35, 0.45 mol L^{-1}; Heating step, 45 °C, 25 min; cooling step, 10 min. The same conditions were used for Cr(VI) except for heating time, 5 min.

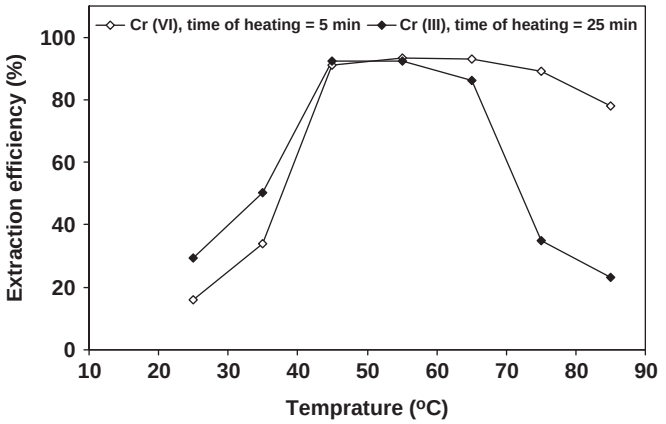

Fig. 6. Effect of heating temperature on the extraction efficiency of Cr(III) and Cr(VI). Heating time, 5 min and 25 min for Cr(VI) and Cr(III), APDC concentration, 0.1 (%w/v) and 0.45 (%w/v) for Cr(VI) and Cr(III), respectively. Other conditions are the same as Fig. 4.

of 45–65 °C, the extraction efficiencies raised to 90%; suggesting that more chromium–APDC complexes were generated. At 45 °C, the extraction of Cr(III) was almost completed in 25 min, while Cr(VI) could be extracted at this temperature in 5 min.

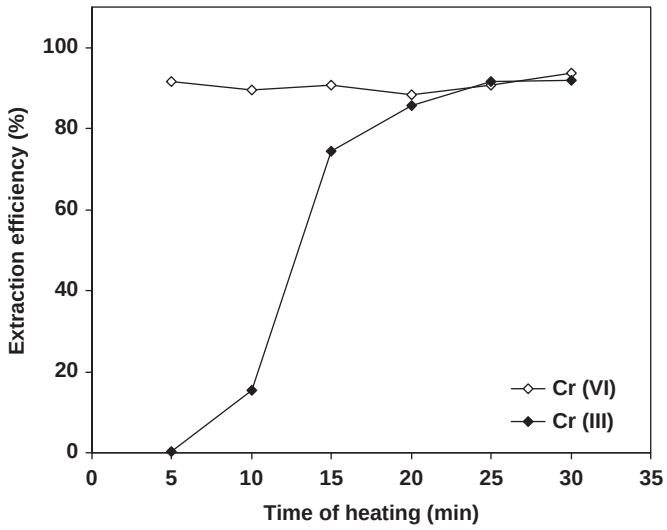

Fig.7. Effect of time of heating on the extraction efficiency of Cr(III) and Cr(VI). PHP buffer, 0.45 mol L^{-1}. Other conditions are the same as Fig. 6.

At temperatures of less than 45 °C, the incomplete dissolution of IL was occurred. At higher temperatures than 65 °C, a water soluble form of IL was obtained due to hydrolyze the PF_6^- anion of the ionic liquid to F^- [31–33] and the extraction efficiencies were diminished. Thus, the optimum extraction conditions were set at a temperature of 45 °C.

Heating time has also considerable effect on the extraction efficiency of Cr(III) species, so the heating time of less than 25 min caused a drastic decrease in extraction efficiency (Fig. 7). With increasing the extraction time to 25 min, the maximum yield of Cr(III)–APDC complex was generated, whereas the extraction rate of the Cr(VI) species had no changed after 5 min.

After dissolving the IL, the sample solution should be cooled to decrease the solubility of IL, so that IL-phase is seen again. Cooling temperature was also studied in the range of 0–25 °C. It was observed that with decreasing the temperature, extraction efficiencies of both Cr(VI) and Cr(III) species were increased. Hence, cooling the solution at 0 °C for 10 min was sufficient for the reformation of IL-phase.

3.1.8. Effect of centrifugation time

To ensure the separation of the IL and water phases, the effect of centrifugation time was studied in the time interval of 1–20 min at 3000 rpm. It was found that, over a centrifugation time of 10 min at 3000 rpm, extraction efficiencies for Cr(III) and Cr(VI) were 88.6 ± 2.4% and 92.1 ± 3.7%, respectively; indicating the complete separation of the IL phase was occurred.

3.1.9. Effect of salt

It is noteworthy to mention that the solubility of IL in the presence of high concentration of salts, due to ion exchange ability of IL with anions like NO_3^-, Cl^-, Br^-, … increases. As a result, the phase separation does not occur. The salt effect was studied in the concentration range 0–25 (%w/v) of $NaNO_3$. As shown in Fig. 8, $NaNO_3$ salt concentration had no significant effect on the extraction efficiency of both Cr(III) and Cr(VI) species up to 3 (%w/v) and then decreased. According to the common ion effect, solubility of the [HMIM][PF_6] in the presence of ionic liquid [HMIM][Br] decreases. Hence, the effect of presence and absence of [HMIM][Br] on the extraction efficiency of chromium species was also investigated. Obviously, in the presence of water soluble [HMIM][Br] IL, the solubility of [HMIM][PF_6] decreased and the

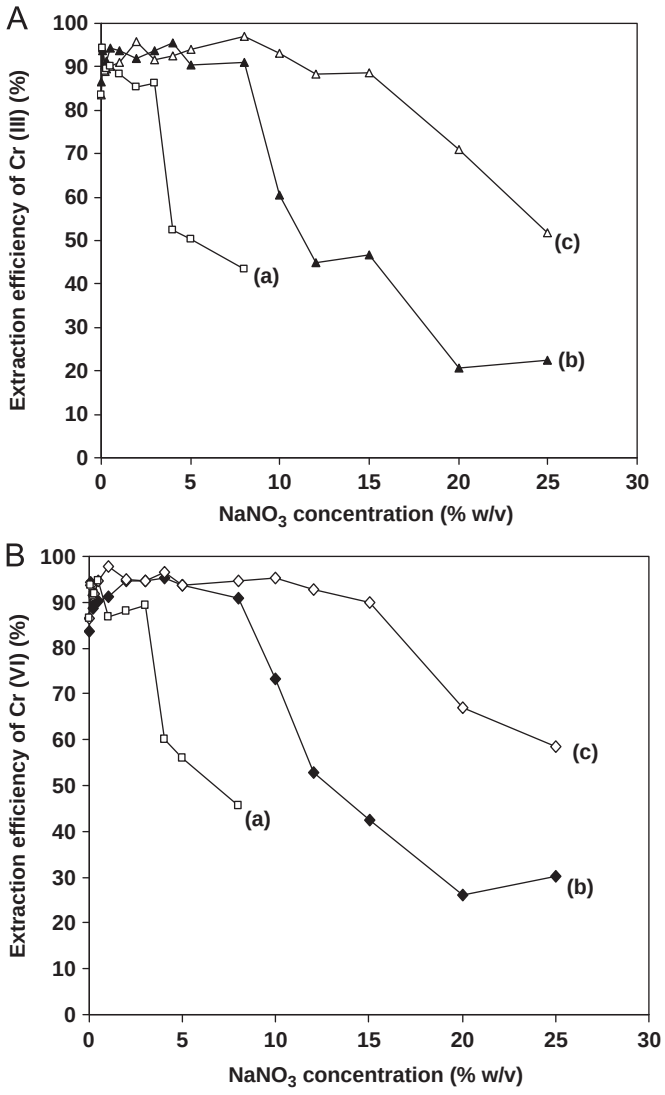

Fig. 8. Salt effect on the extraction efficiency of Cr(III) (A) and Cr(VI) (B) in the absence (a) and presence of 5 μL (b) and 25 μL(c) of [HMIM][Br]. Heating temperature, 45 °C. Other conditions are the same as Fig. 7.

extraction efficiencies of Cr(III) and Cr(VI) were unchanged up to 8 (%w/v) of $NaNO_3$. Further experiments showed that an increase in [HMIM][Br] volume over 25 μL caused no decrease in extraction efficiency of chromium species up to 15 (%w/v) $NaNO_3$ concentration. An advantage of this method is that Cr(III) and Cr(VI) can be subsequently extracted from high saline samples like underground water and urine

3.2. Effect of coexisting ions

In our method, most possible interferences might arise during the preconcentration step. Therefore, in order to investigate the selectivity of the developed microextraction method for speciation of chromium, the effect of coexisting ions on the extraction efficiency of chromium species was evaluated. For this purpose, 5 mL of sample solutions containing 100 ng L^{-1} of either Cr(III) or Cr(VI) and different quantities of coexisting ions were treated in accordance with the recommended procedure in Section 2.3. The tolerance limit was considered as the interferent concentration making a relative error less than 5% in the absorbance of the analyte. The results of this investigation are summarized in Table 5. Most frequent matrix constituents of real samples such as alkali

Table 5
Effect of interferents on the extraction efficiencies of 100 ng L^{-1} chromium species in aqueous solutions.

Tolerance limit (w/w)	Ion/Cr(III)	Ion/Cr(VI)
10,000	NO_3^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-, Cl^-, ClO_3^-, Br^-, I^-	NO_3^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-, Cl^-, ClO_3^-, Br^-, I^-
10,000	K^+, Na^+, Mg^{2+}, Ba^{2+}, Li^+, Ca^{2+}	K^+, Na^+, Mg^{2+}, Ba^{2+}, Li^+, Ca^{2+}
1000	As^{5+}	As^{5+}
500	Al^{3+}	As^{3+}, Al^{3+}
300	–	Zn^{2+}
250	–	Cd^{2+}, Sn^{2+}
200	–	Hg^{2+}
100	As^{3+}, Sn^{2+}, Zn^{2+}, Hg^{2+}	Fe^{3+}, Ni^{2+}
75	–	Pb^{2+}
50	Fe^{3+}, Mn^{2+}	Co^{2+}, Mn^{2+}, Cu^{2+}
25	Cd^{2+}	–
10	Ni^{2+}, Cu^{2+}, Pb^{2+}, Co^{2+}	–

and alkaline earth elements do not form a stable complex with APDC, hence these elements did not extract from the aqueous solution into the IL phase and have no significant interference in the extraction efficiency of chromium species at the given concentration level. However, Cu^{2+}, Ni^{2+}, Pb^{2+} and Co^{2+} ions that react with APDC, interfere in the speciation of Cr(III) at the concentration of ten times excess of Cr(III). Moreover, the effect of some common anions like NO_3^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-, Cl^-, ClO_3^-, Br^- and I^- on the extraction efficiency of chromium species was investigated. The results showed that the selected ions had no adverse effect on the extraction of Cr(III) and Cr(VI) ions at the concentrations up to 10000 μg L^{-1}. This suggested that the method would be suitable to determine the chromium species in high saline solutions.

3.3. Speciation of chromium

There are three strategies in simultaneous determination of Cr(III) and Cr(VI) in aqueous solutions by ETAAS:

(1) Simultaneous extraction of both Cr(III) and Cr(VI) species by using different chelating agents before quantification.
(2) Analysis of the sample for determination of either Cr(III) or Cr(VI) and the total chromium and calculation of the other species concentration by subtraction the two concentrations.
(3) Sequential extraction of Cr(III) and Cr(VI) by one chelating agent in one aqueous sample as in this work.

It is difficult to find a suitable chelating agent for the first method. The second method has inability to be employed on a single sample and a minimum of two samples is required for determination. In our method, sequential extraction and determination of both chromium species are performed in one sample.

The procedure for the sequential extraction of Cr(III) and Cr(VI) mixture was the same as mentioned in Section 2.3. In the first step, the APDC and PHP buffer were added to the sample at the concentrations of 0.1 (%w/v) and 0.45 mol L^{-1}, respectively. By heating the solution at 45 °C for 5 min, Cr(VI) was only extracted. In the second step, the APDC concentration and heating time of this sample were elevated to 0.45 (%w/v) and 25 min, respectively, under the constant buffer concentration. This providing a complete complex formation of Cr(III) with APDC and distinguishing the two chromium species. Under the specified conditions, mixtures of Cr(VI) and Cr(III) in different ratios were also examined and the satisfactory extraction efficiencies for Cr(III) and Cr(VI) were obtained (Table 6).

Table 6
Analytical results for sequential extraction of Cr(III) and Cr(VI) in mixture.

Cr(VI)/Cr(III) ratio (ng L^{-1})	Extraction (%) of Cr(III)	Extraction (%)[a] of Cr(VI)
200/0	0.0	92.3 ± 2.2
150/50	89.1 ± 4.6	102.1 ± 4.5
100/100	104.0 ± 3.5	94.5 ± 5.8
50/150	97.3 ± 4.1	90.4 ± 3.9
0/200	75.8 ± 6.1	20.7 ± 6.7

[a] Mean value ± standard deviation, $n=3$.

Table 7
Analytical characteristics of the developed method.

Parameters	Cr(III)	Cr(VI)
Linear range (ng L^{-1})	50–200	25–150
Correlation coefficient (R)	0.9971	0.9979
Slope	0.0014	0.0035
Intercept	0.0126	0.0028
Limit of detection (ng L^{-1}) ($3S_B/m$, $n=11$)	5.40	2.45
Precision (RSD, %)(100 ng L^{-1}, $n=6$)	4.24	3.05
Enrichment factor	42	43

3.4. Analytical figures of merit

Under the optimized conditions, the analytical characteristics of the developed method for speciation of standard solutions of Cr(III) and Cr(VI) are summarized in Table 7. In the concentration range 50–200 ng L^{-1}, the calibration curve for Cr(III) was linear with a regression equation of $Abs.=0.0014C_{Cr(III)}+0.0126$ and correlation coefficient (R) of 0.9971, where Abs. and C are the absorbance and chromium concentration in ng L^{-1}, respectively. Also, in the concentration range 25–150 ng L^{-1}, the calibration curve for Cr(VI) was linear with a regression equation of $Abs.=0.0035C_{Cr(VI)}-0.0028$ and correlation coefficient of 0.9979. The limit of detection, defined as the ratio of three times of the standard deviation of the blank signal ($n=11$) to the slope of calibration graph after preconcetration, was 5.40 and 2.45 ng L^{-1} for Cr(III) and Cr(VI) species, respectively. The relative standard deviation (RSD %) resulted from the analysis of six replicate extractions and determinations of aqueous solutions containing 100 ng L^{-1} Cr(VI) and Cr(III) were 4.24% and 3.05%. The enrichment factor, defined as the ratio of concentration of chromium species in the settled phase to the initial concentration of Cr species in the aqueous sample, was about 42.

Table 8
Speciation and determination of Cr(III) and Cr(VI) in real samples.

Samples	Added (ng L^{-1})		Found[a] (ng L^{-1})		Recovery (%)	
	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)
Tape water	–	–	128.3 ± 3.7	42.3 ± 5.6	–	–
	50	–	180.6 ± 1.5	–	101.3 ± 0.8	–
	–	50	–	88.0 ± 2.6	–	95.3 ± 2.8
	100	–	227.9 ± 3.4	–	99.8 ± 1.5	–
	–	100	–	138.2 ± 2.7	–	97.1 ± 1.9
	50	50	181.3 ± 2.1	87.6 ± 3.1	101.7 ± 1.2	94.9 ± 3.4
	100	100	226.1 ± 2.6	131.1 ± 4.1	99.0 ± 1.1	92.1 ± 2.9
Underground water	–		34.7 ± 1.3	20.5 ± 6.3	–	–
	50	–	81.2 ± 2.0	–	95.8 ± 2.3	
	–	50	–	65.2 ± 1.0	–	92.5 ± 1.4
	100	–	125.0 ± 4.5	–	92.8 ± 3.3	–
	–	100	–	111.2 ± 1.1	–	92.3 ± 0.9
	50	50	83.9 ± 3.3	62.5 ± 3.0	99.1 ± 3.9	88.7 ± 4.3
	100	100	137.2 ± 3.2	107.5 ± 2.5	101.8 ± 2.4	89.2 ± 2.1
Urine	–	–	0.0	0.0	0.0	0.0
	50	–	45.1 ± 2.1	0.0	90.2 ± 4.2	0.0
	–	50	–	42.7 ± 2.3	0.0	85.4 ± 4.6
	100	–	–	88.9 ± 3.8	90.5 ± 3.5	0.0
	–	100	90.5 ± 3.5	–	0.0	88.9 ± 3.8
	50	50	47.4 ± 2.4	40.7 ± 2.5	94.8 ± 4.9	81.4 ± 5.0
	100	100	94.2 ± 3.4	84.1 ± 4.0	94.2 ± 3.4	84.1 ± 4.0

[a] Mean value ± standard deviation, $n=3$

Table 9
Comparison of analytical characteristics of the developed method with other methods for extraction and speciation of chromium species.

Method	LOD (ng L^{-1})	[a]EF	Sample volume (mL)	Dynamic range (ng L^{-1})	Ionic liquid	Ref.
Direct speciation-ETAAS	700	[g] NR	4	< 20,000	NR	[34]
[b]DLLME-FAAS	70 for Cr(III), 80 for total Cr	275 for Cr(VI), 262 for total Cr	25	300–20,000	NR	[35]
[c]SPE-ETAAS	3.0	35	10	< 50,000	NR	[36]
Coprecipitation-ETAAS	780 for Cr(VI) 650 for Cr(III)	50	25	NR	NR	[37]
[d]CPE-ETAAS	10 for Cr(VI)	50 for Cr(VI)	10	500–10,000 for Cr(VI)	NR	[38]
[e]SFODME-ETAAS	6 for Cr(III)	333 for Cr(III)	10	30–130 for Cr(III)	NR	[39]
[f]TCME-ETAAS	2.45 for Cr(VI) 5.40 for Cr(III)	43 42	5	25–150 for Cr(VI) 50–200 for Cr(III)	$[HMIM][PF_6]$	This work

[a] Enrichment factor.
[b] Dispersive liquid–liquid microextraction.
[c] Solid phase microextraction.
[d] Cloud point extraction.
[e] Solidified floating organic drop microextraction.
[f] Temperature-controlled microextraction.
[g] NR; Not reported.

3.5. Application

The developed method was applied for the speciation and determination of chromium in tap water, underground water and urine samples. Before any treatment, the pH of 1.0 mL of the real samples was adjusted to 5.0 and then appropriate amounts of APDC, Triton X-114, and [HMIM][Br] were added to the sample. After five fold dilution, the water samples were analyzed according to the recommended procedures for sequential extraction of the chromium species. The concentrations of chromium species in tap water and underground water samples were determined as 42.3 ± 5.6 ng L^{-1} and 20.5 ± 6.3 ng L^{-1} for Cr(III) and 128.3 ± 3.8 ng and 34.7 ± 4.2 ng$L^{-1}$for Cr(VI) L^{-1}, respectively. No Cr(III) and Cr(VI) were detected in the urine samples. The obtained recoveries of chromium species at the spiked concentration levels for water and urine samples were in the ranges of 92–101% for Cr(III) and 81–95% for Cr(VI). The results indicate that the matrices of the examined real samples have no considerable effects on the speciation and determination of both chromium species (Table 8).

3.6. Comparison with other speciation methods

The developed method was compared with other reported methods [34–39] and the results are given in Table 9. As it can be seen, the developed method shows a comparatively low detection limit and an appropriate enrichment factor by using low volume of sample (5 mL). As well, this method is free of volatile organic solvents by using the ionic liquid as the green extraction solvent. Except for SPE-ETAAS method (on-line preconcentration; throughput sample=31 sample h^{-1}) [36], other methods were based on batch wise extraction mode and the overall time required for preconcentration of the samples were about one hour or less. The developed method is reproducible and inexpensive approach for sequential preconcentration and speciation of chromium species on a single saline samples.

4. Conclusion

In this study, a temperature-controlled microextraction method based on ionic liquid as extraction solvent combined with ETAAS

was developed for the speciation and determination of ultra trace amounts of chromium species. The predominant advantages of the developed method are low consumption of reagents, eco-friendly separation and speciation of chromium, and robust against high content of salt. Moreover, the matrix effects in the ETAAS analysis step are eliminated. This method provides high recoveries and good enrichment factors for preconcentration and determination of trace amounts of chromium species in water and urine samples.

Acknowledgement

Authors are grateful to the Research Council of the Birjand University for funding this work.

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