Talanta 119 (2014) 291–298

Contents lists available at ScienceDirect

Talanta

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

Rapid determination of phthalate esters in alcoholic beverages by conventional ionic liquid dispersive liquid–liquid microextraction coupled with high performance liquid chromatography

Yingying Fan [a], Shuhui Liu [a,b,*], Qilong Xie [a]

[a] College of Science, Northwest A&F University, No. 3 Taicheng Road, Yangling, Shaanxi 712100, China
[b] State Key Laboratory of Crop Stress Biology in Arid Areas, Yangling, China

ARTICLE INFO

Article history:
Received 13 August 2013
Received in revised form
6 November 2013
Accepted 7 November 2013
Available online 15 November 2013

Keywords:
Ionic liquid
Dispersive liquid–liquid mircoextraction
Phthalate esters
White spirit

ABSTRACT

A very simple, fast and environmentally friendly sample extraction method was proposed for the analysis of phthalate esters (PAEs, di-isobutyl phthalate (DIBP), dibutylphthalate (DBP), butylbenzylphthalate (BBP) and bis(2-ethylhexyl)phthalate (DEHP)) in alcoholic beverages by using conventional ionic liquid dispersive liquid–liquid microextraction. The samples were extracted by 160 μL 1-octyl-3-methylimidazolium hexafluorophosphate in the presence of appropriate amount of ethanol and 10% (w/v) sodium chloride solution; the enriched analytes in sedimented phases were analyzed by high performance liquid chromatography-diode array detector (HPLC-DAD). Under the optimum conditions, a satisfactory linearity (in the range of 0.02–1 μg mL^{-1} for white spirits and 0.01–0.5 μg mL^{-1} for red wines with the correlation coefficients (r) varying from 0.9983 to 1), acceptable recovery rates (88.5–103.5% for white spirits and 91.6–104.6% for red wines), good repeatability (RSD ≤ 8.0%) and low detection limits (3.1–4.2 ng mL^{-1} for white spirits and 1.5–2.2 ng mL^{-1} for red wines) were obtained. The developed method was successfully applied for the determination of the four PAEs in 30 white spirits and 11 red wines collected locally, and the DBP content in 63% (19:30) white spirits exceeded the specific migration limit of 0.3 mg kg^{-1} established by international regulation.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Phthalate esters (PAEs) are the most common plasticizers used for polymers, such as polyvinyl chloride (PVC), polyethylene (PE), polyethylene terephthalate (PET) and polyvinyl acetates (PVA), primarily to improve their extensibility, elasticity and workability [1]. Thus, they are widely present in products like building materials, clothing, cosmetics, medical devices, pharmaceuticals, flooring and wall-covering, electric cables, packaging materials, printing inks, etc. [2] Being physically bound to the polymer structure, PAEs can easily migrate from plastic materials to surrounding medium, which gives rise to their ubiquitous presence in soil [3], water [4], indoor dust [5] and foodstuff [6].

To date, the results of numerous animal studies have suggested that certain PAEs, as well as their main metabolites and degradation products, can cause toxic effects in multiple organ systems including the liver, reproductive tract, kidneys, lungs and heart [7,8], which have raised a great concern about the possibility of PAEs as contributors to reproductive and developmental adverse effects in humans [9,10]. In general, human exposed to PAEs occurs via dermal absorption from cosmetics, inhaling from air and, above all, ingesting from contaminated foods. Due to their potential risks to human health, several PAEs including dimethylphthalate (DMP), diethylphthalate (DEP), butylbenzylphthalate (BBP), dibutylphthalate (DBP), bis(2-ethylhexyl)phthalate (DEHP) and dioctylphthalate (DOP) have been listed as Priority Toxic Pollutants by the United States Environmental Protection Agency (US EPA) [11]. To guarantee human health, the European Union established Specific Migration Limits (SMLs) for PAEs using food simulants. According to the Directive 2007/19/EC [12], these values are in particular 0.3 mg kg^{-1} food simulant (fs) for DBP, 30 mg kg^{-1} for BBP, 1.5 mg kg^{-1} for DEHP and 18 mg kg^{-1} for bis(2-ethylhexyl) adipate (DEHA). In addition, Tolerable Daily Intakes (TDI) for several PAEs have been specified by the European Food Safety Authority (EFSA), and they are 0.01, 0.5, 0.05, 0.15 and 0.15 mg/kg body weight/day for DBP [13], BBP [14], DEHP [15], di-isononylphthalate (DINP) [16] and di-isodecylphthalate (DIDP) [17], respectively.

The food contamination with PAEs has become a matter of public concern in recent years. Beverages, milk and milk products, meat and meat products, cooking oils, cereals, vegetables, fruit, etc., have been reported the occurrence of PAEs [18]. Owing to the lipophilic property of PAEs, alcoholic products with high alcoholic

* Corresponding authors at: College of Science, Northwest A&F University, No. 3 Taicheng Road, Yangling, Shaanxi 712100, China. Tel.: +86 29 87092226; fax: +86 29 87092226.
E-mail address: shliu815@126.com (S. Liu).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.11.023

content are prone to accelerating the migration of PAEs from plastic contact material during processing, transport and storage [19]. Average values of 34 ng g^{-1} for DBP and 32 ng g^{-1} for DEHP were detected by Yano et al. [20] in Japanese red wine and beer. Guo et al. [21] reported that DMP, DIBP, DBP and DEHP were detected at the corresponding range of 0.25–97, 0.37–107, 2.03–557 and 0.2–7.03 ng g^{-1} in wine and beer from China. In 36 white and red wines from Italy, DIBP, DBP, BBP and DEHP were found at the average level of 45–115 ng mL^{-1} with a detection frequency of 100%, 89%, 47% and 100%, respectively [22].

Up to now, to the authors' knowledge, there have not been any reports specifically toward the sample pretreatment in the determination of PAEs in white spirits. As to wine and other low-alcoholic beverages, various pretreatment techniques developed to extract PAEs were reported. Liquid–liquid extraction (LLE) was used to extract PAEs from alcoholic drinks [20,21]. However, this procedure is tedious and consumes large amounts of toxic organic solvents. Solid-phase extraction (SPE), as an alternative to LLE owing to its low consumption of organic solvent, was developed to determine PAEs using C18 [22] and Carbograph I [23] as adsorbents. Whereas, the SPE procedures are comprised of activation of SPE columns, sample elution and elute evaporation steps, resulting in being labor-intensive and toxic solvent-consuming to some extent. To address these drawbacks, a number of studies were directed toward the development of efficient, miniaturized and environmentally benign sample pretreatment methods. Carrillo et al. [24,25] proposed a headspace solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME-GC–MS) method, in which PAEs were extracted onto a solid porous hollow fiber coated with a stationary phase. SPME procedures using sol–gel calixarene-contained fiber [26] and multi-walled carbon nanotubes (MWCNTs)/SiO_2-reinforced fiber [27] were also designed to determine PAEs in beer. From the practical point of view, the coated fibers are generally expensive, fragile and have limited lifetimes.

Recently, a modified liquid phase microextraction (LPME) technique termed as dispersive liquid–liquid microextraction (DLLME) was developed by Rezaee et al. in 2006 [28]. It is based on the fast injection of a mixture of extraction solvent and disperser solvent into the aqueous solution to form a cloudy ternary component solvent (aqueous solution: extraction solvent: disperser solvent) system; after centrifugation, the enriched analytes in the sedimented phase are withdrawn using a micro-syringe and determined by chromatography or spectrometry. This technique was successfully applied for the determination of α-tocopherol in cereal grains [29], bisphenol A in edible oils [30] and the migration of bisphenol A from polycarbonate water bottles [31] in our previous studies. Several very recent reviews [19,32,33] have summarized a good number of works on rapid development and wide applications of DLLME. Cinelli et al. [34] proposed an ultrasound-vortex-assisted DLLME (USVA-DLLME) procedure to extract six PAEs in wine samples with 200 μL dichloromethane. Although this method has the merits of the simplicity of operation, low cost, high recovery, high enrichment factor and very short extraction time, it consumed highly toxic chloro-solvent.

Ionic liquids (ILs) are a group of new organic salts that exist as liquids at a low temperature (<100 °C) [35]. In comparison with traditional organic solvents, ILs have a variety of unique physicochemical properties [36] including negligible vapor pressures, good thermal stabilities and good solubility for organic and inorganic compounds. Over the past few years, ILs have been used as green extractants in DLLME replacing chloro-solvents, which is termed as IL-based DLLME (IL-DLLME). This technique has been successfully applied for the analysis of polycyclic aromatic hydrocarbons [37], pesticides [38], antibiotics [39] and trace metal ions [40] in water, bananas, milk and environmental samples as well as the determination of PAEs in water samples [41–43]. However, the analysis of PAEs in alcoholic beverages based on IL-DLLME has remained unexplored.

The aim of this study was to develop a simple, fast, inexpensive and environmentally friendly sample preparation method for the determination of PAEs (DIBP, DBP, BBP and DEHP) in alcoholic beverages prior to HPLC-DAD by using IL-DLLME. Additionally, given the fact that white spirit is one of the most traditional alcoholic beverages in China and has been maintained at a high consumption level, it would be an imperative and important work to conduct a preliminary survey of the PAEs contamination in alcoholic products for consumers' health and confidence.

2. Experimental

2.1. Reagents

Di-isobutylphthalate (DIBP, 99%), dibutylphthalate (DBP, 99%), benzylbutylphthalate (BBP, 99%) and bis(2-ethylhexyl)phthalate (DEHP, 99%) were purchased from Aladdin Chemistry Co. (Shanghai, China). 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4MIM][PF_6]$), 1-hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM][PF_6]$) and 1-octyl-3-methylimidazolium hexafluorophosphate ($[C_8MIM][PF_6]$) were purchased from Cheng Jie Chemical Co. (Shanghai, China). HPLC-grade methanol (≥ 99.9% pure) and ethanol (≥ 99.8% pure) were obtained from Kermel Chemical Reagent Co. (Tianjin, China). Sodium chloride (≥ 96%), hexane (≥ 99%) and acetone (≥ 99%) were provided by BODI Chemical Reagent Co. (Tianjin, China).

The stock standard solutions of each PAE were prepared at 1 mg mL^{-1} in ethanol and stored at −20 °C in darkness. The working mixed standard solution (10 μg mL^{-1}) was prepared by suitable dilution with methanol from the stock standard solutions weekly and stored at 4 °C.

2.2. Instrumentation

The quantitative analysis was performed on a Shimadzu LC-20 A (Kyoto, Japan) equipped with two LC-20AT pumps, a 7725i manual sample injector and a SPD-M20A photodiode array detector (190–800 nm). 226 nm, 240 nm and 260 nm were used to evaluate the purity of the PAEs in real samples, 240 nm for quantitative analysis. Separations were carried out on a Waters Xterra C18 column (15 cm × 4.6 mm, with 5 μm particle size). With methanol/water as the mobile phase at a flow rate of 1.0 mL min^{-1}, the gradient elution programmed as follows: 64% methanol was initially used and linearly increased to 74% within 10 min, then to 82% within 4 min and to 88% next within 9 min. Total run-time was 35 min. The injection volume was 20 μL.

An ultrasonic cleaning machine (Model KQ-5200E, Kunshan City Ultrasonic Instruments Co., China) was used to exhaust air from the mobile phases. Centrifugation was done with a Beckman (Allegra X-12) system (Beckman Coulter Inc., USA). A microsyringe (Hamilton L) was used to collect and measure the volume of extraction solvent. Water was purified using a Millipore Direct-Q 3 system (Millipore Corporation, Bedford, MA, USA).

2.3. Glassware and reagent control

To avoid PAE contamination, all glassware used in this study were soaked in acetone for at least 30 min, then washed with acetone, rinsed with hexane and dried at 120 ºC for at least 4 h before using. Three reagent blanks were prepared per extraction batch by using 5.0 mL 50% ethanol solution in place of sample

matrix. The concentrations of PAEs found in the reagent blanks were averaged and subtracted from sample assay results.

2.4. Samples

30 white spirits (numbered from W-1 to W-30), mainly made by sorghum, maize, wheat and rice with different flavor types, and 11 red wines (numbered from R-1 to R-11) were purchased from the local supermarkets. All samples were stored in glass bottles, with the alcoholic content were in the range of 42–56% (v/v) for white spirits and 11.5–12% (v/v) for red wines. Considering 50% (v/v) alcohol as the average content for white spirits, we used 50% ethanol solution as a good simulated sample.

2.5. Sample treatment

An aliquot of 5 mL white spirit sample (or simulated sample) mixed with 160 μL [C_8MIM][PF_6] was placed in a 10 mL screw cap glass tube with conical bottom. Subsequently, 5 mL 20% (w/v) NaCl solution was added into the sample solution. The glass tube was gently shaken for 5 min and then centrifuged at 4000 rpm for 4 min. The dispersed fine droplets of the extractant were sedimented at the bottom of tube, which was transferred to a 1.5 mL sample vial using a microsyringe. The volume of the sedimented phase was precisely measured (100 ± 2 μL). After dilution with 100 μL methanol, the sample was subjected to HPLC analysis.

An aliquot of 10 mL red wine was placed in a 10 mL screw cap glass tube with conical bottom. After adding 1 g NaCl, the mixture was shaken vigorously to dissolve NaCl. Subsequently, a mixture of 160 μL [C_8MIM][PF_6] and 1 mL ethanol were injected into the sample solution. The following DLLME procedure was identical with the treatment for white spirit described above.

3. Results and discussion

3.1. Optimization of DLLME for white spirit

In the previously reported IL-DLLME procedures for the analysis of PAEs in water samples, the extractant used was [C_8MIM][PF_6], with acetone [42] and acetonitrile [41] as dispersers. Also, a temperature controlled IL-DLLME method was designed, in which the IL was dispersed in the sample solution by the drive force of elevated extraction temperature instead of using a disperser [43]. In this study, as white spirit samples have high alcohol content, which can play a dispersive effect, no an additional disperser is needed; besides, owing to the high-volatility of alcohol, the temperature controlled IL-DLLME procedure [43] can alter the content of alcohol in white spirits, thus affecting the accuracy and precision of the analysis. In this context, the IL-DLLME procedures mentioned above are not appropriate for the extraction of PAEs in white spirit samples.

Preliminary experiment showed that if adopting 10 mL white spirit directly for DLLME procedure, the sedimented IL phase could not form after centrifugation with the IL volume added < 500 μL. Thus, a 5 mL aliquot of white spirit sample (spiked at 0.4 μg mL^{-1} each PAE) diluted at the ratio of 1:1 with deionized water was used to optimize various parameters influencing the extraction recovery (ER%) and enrichment factor (EF) of the analytes and the performance of IL-DLLME. All experiments were conducted in triplicate and the average of the results was used for plotting curves or tables.

3.1.1. Selection of ionic liquids

The selection of an appropriate IL is a pivotal step for developing an IL-DLLME method. Some special characteristics are required such as a higher density than water, low solubility in water, strong extraction capability for analytes and good chromatographic behavior. For these reasons, the imidazolium-ILs containing $[PF_6]^{6-}$ and side hydrophobic alkyl chain, such as [C_4MIM][PF_6], [C_6MIM][PF_6] and [C_8MIM][PF_6], drew our attention and were tested in this study.

In order to compare the affinity of the ILs to the analytes, we managed to keep the volumes of the sedimented IL phase constant (about 70 μL) by adjusting their initially added volumes. 140 μL of [C_8MIM][PF_6] and 230 μL of [C_6MIM][PF_6] were adopted. With regard to [C_4MIM][PF_6], owing to its relatively high solubility in water, this IL could not form the sedimented phase at the bottom of test tube after centrifugation even with a volume of 400 μL. Thus, [C_4MIM][PF_6] was not examined further. As shown in Fig. 1, [C_8MIM][PF_6] provided higher the ERs% for all of the analytes than [C_6MIM][PF_6]. This phenomenon could be attributed to its structure characteristics with longer alkyl chain, which decreases its solubility in water and meanwhile increases its affinity to the hydrophobic analytes. In addition, in comparison with [C_6MIM][PF_6], the volume of [C_8MIM][PF_6] consumed was much less. Hence the latter was selected for subsequent experiments.

3.1.2. Volume of ionic liquid

The effect of the IL volume on the ERs% and EFs was evaluated in the range of 100–200 μL in 20 μL intervals with other experimental conditions constant. Fig. 2(A) showed that the ERs% of each PAE tested increased gradually when the IL volume was increased from 100 to 160 μL; however, with the volume exceeding 160 μL, the ERs% almost leveled off at a high level, which was due to the completed extraction equilibrium. From Fig. 2(B) we can see that an increase in the volume of IL from 100 to 200 μL reduced the EFs from 91–119 to 36–39 folds. This was resulted from the accordingly increased volumes of sedimented IL phase. Considering acceptable ERs% and higher EFs of each PAE, 160 μL [C_8MIM][PF_6] was therefore utilized.

3.1.3. Influence of ionic strength

For liquid–liquid extraction procedures, changing the ionic strength of an aqueous solution can alter the solubility of analytes (salting out effect) and thus affecting extraction efficiency. In this work, the different concentrations of NaCl ranging from 0 to 15% (w/v) were evaluated in the IL-DLLME. The results revealed that the ERs% of each analyte increased slowly as the salt concentration

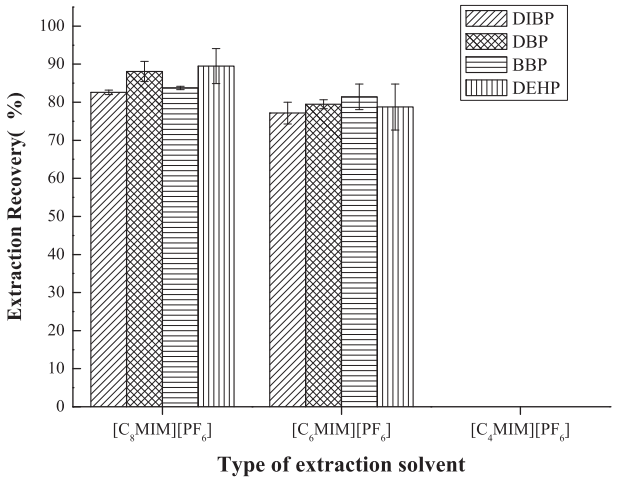

Fig. 1. Effect of the type of ionic liquids on the extraction recovery of PAEs in a white spirit sample (alcohol content, 50% v/v). Extraction conditions: sample, 5.0 mL spiked white spirit diluted with 5.0 mL water (0.4 μg mL^{-1} each PAE); salt addition, 10% (w/v); extraction time, 5 min; centrifugation time, 4 min.

was increased up to 10%. However, when the amount of NaCl exceeded 15%, the volume of the sedimented phase declined significantly, giving rise to the decreased ERs%. This might be explained by the fact that large amount of salt produced a high Cl^- concentration in the extraction mixture, which could accelerate the generation of water-soluble $[C_8MIM]Cl$, thus reducing the $[C_8MIM][PF_6]$ volume. Based on the results, 10% NaCl was adopted in the following study.

3.1.4. Influence of pH

In DLLME procedure, pH value of the sample solution usually plays an important role because sample pH determines the degree of dissociation of polar analytes and affects the stability of apolar ones, thus influencing extraction performance. In this experiment, a series of pH of sample solutions including 2.0, 4.3 (unadjusted) and 11.0 were investigated. The results demonstrated that the ERs % were nearly constant in this pH range examined. In the following DLLME experiment the sample solutions without adjusting the pH value were adopted for convenience.

3.1.5. Range of alcohol content

Generally speaking, the alcohol content in alcoholic beverages is 0.5–60% (v/v) and the distilled spirits' alcohol content is 18–60% (v/v) [44]. To determine the alcohol range of real samples in which the developed DLLME procedure could be applied, the effect of the alcohol content on the performance of the proposed DLLME was investigated using a series of simulated samples. As demonstrated in Table 1, when the alcohol content was increased from 10% to 25%, the ERs% of the analytes were not statistically significant different. By increasing the alcohol content from 25% to 28%, the ERs% of DIBP, DBP and BBP decreased due to the much less volume of sedimented IL phase. When the sample contained alcohol content above 30% (v/v), scarcely the sedimented IL phase could be found at the bottom of the tube after centrifugation. Thus, this proposed DLLME method could be applied for white spirits containing 10–25% alcohols. For the alcohol content out of the range, samples require appropriate dilution.

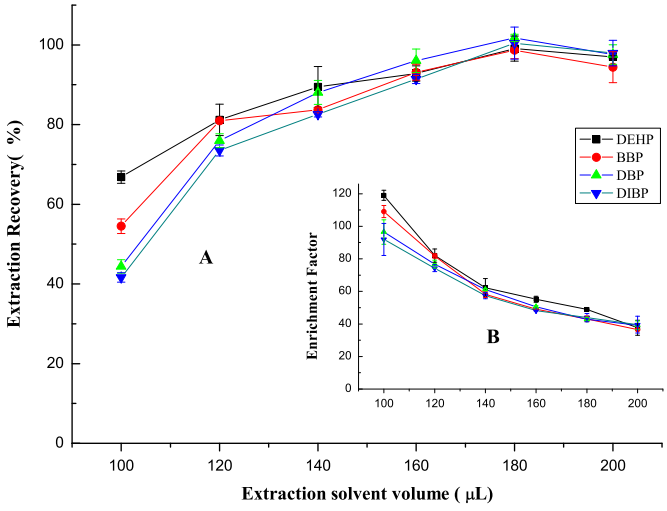

Fig. 2. Effect of the volume of $[C_8MIM][PF_6]$ on the extraction recovery and enrichment factor of PAEs in a white spirit sample (alcohol content, 50% v/v). Other extraction conditions are the same as those for Fig. 1.

3.1.6. Influence of extraction time and centrifugal time

Extraction time is one of the key parameters affecting the extraction capability. In this study, extraction time in the range of 3–10 min was examined. When the time was increased from 3 to 5 min, the ERs% increased; with the period being longer, the ERs% did not increase accordingly. Therefore, a shaking time of 5 min was optimal.

Centrifugation is an important step in this proposed method and the final performance would benefit from a full phase separation. Generally, a shorter centrifugation time results in the incomplete sedimentation of dispersive extractant drops and a longer centrifugation time generates heat effect, which leads to the slight re-dissolving of the sedimented phase. Herein the effect of centrifugation time was investigated in the range of 2–10 min at 4000 rpm. The results indicated that 4 min was optimum and no appreciable improvement was observed for a longer time. Thus a centrifugation time of 4 min at 4000 rpm was chosen.

3.2. Optimization of DLLME for red wine

Red wines generally have 10–14% till 17% alcohol content [34], which is in the optimal alcohol content range for white spirits obtained above. Thus, 10 mL spiked red wine (free of PAEs) was assayed directly using the proposed DLLME for white spirits. The results showed the ERs% (Fig. 3(A)) for the four analytes were rather low, which seems reasonable considering that red wines have diversified compositions and are not miscible well with the IL. To improve the ERs%, disperser solvents was therefore taken into account to dissolve ILs in advance. Under the other optimized conditions obtained for white spirit, a series of 10 mL red wine samples were subjected to the DLLME procedure using 500 μL different dispersers including acetonitrile, methanol and ethanol. The results in Fig. 3(A) showed that all of the organic solvents achieved acceptable ERs% and they were not significant different. However, using acetonitrile obtained a lower EF than using methanol or ethanol (Fig. 3(B)). Since ethanol is less toxicity and had a higher EF in the DLLME, it was selected as the disperser solvent in the subsequent experiments for red wines.

To obtain a suitable alcohol content range achieving good extraction efficiency, different volumes of ethanol ranging from 0.5 to 2 mL in 0.5 mL intervals were added to a red wine sample (free of PAEs), which corresponded to 16%, 20%, 23% and 27% alcohol content (including the alcohol in red wine itself) in the sample solution. Table 2 showed that no significant difference in ERs% was observed by increasing ethanol content from 16% to 23%. However, lower ERs% for DIBP, DBP and BBP were obtained when

Table 1
Effect of alcohol content on the extraction recovery of PAEs in simulated samples spiked with 0.2 μg mL^{-1} each of the analytes.

Analytes	Extraction recovery (%) ± SD				
	10%	15%	20%	25%	28%
DIBP	$101.16^{b} \pm 3.69$	$109.72^{b} \pm 4.80$	$109.21^{b} \pm 2.82$	$103.86^{b} \pm 7.14$	$81.32^{a} \pm 5.06$
DBP	$94.04^{b} \pm 3.63$	$102.55^{bc} \pm 3.37$	$106.07^{c} \pm 4.05$	$97.81^{bc} \pm 1.11$	$84.17^{a} \pm 4.80$
BBP	$93.52^{b} \pm 4.10$	$99.40^{bc} \pm 5.09$	$103.41^{c} \pm 5.23$	$97.70^{bc} \pm 1.97$	$80.12^{a} \pm 1.70$
DEHP	$94.54^{a} \pm 7.44$	$104.75^{b} \pm 5.44$	$103.61^{ab} \pm 2.45$	$103.21^{ab} \pm 5.73$	$102.97^{ab} \pm 0.17$

[a-c]On the basis of Duncan's multiple range test, the different letters within the same row indicate statistically significant difference at the 5% probability level.

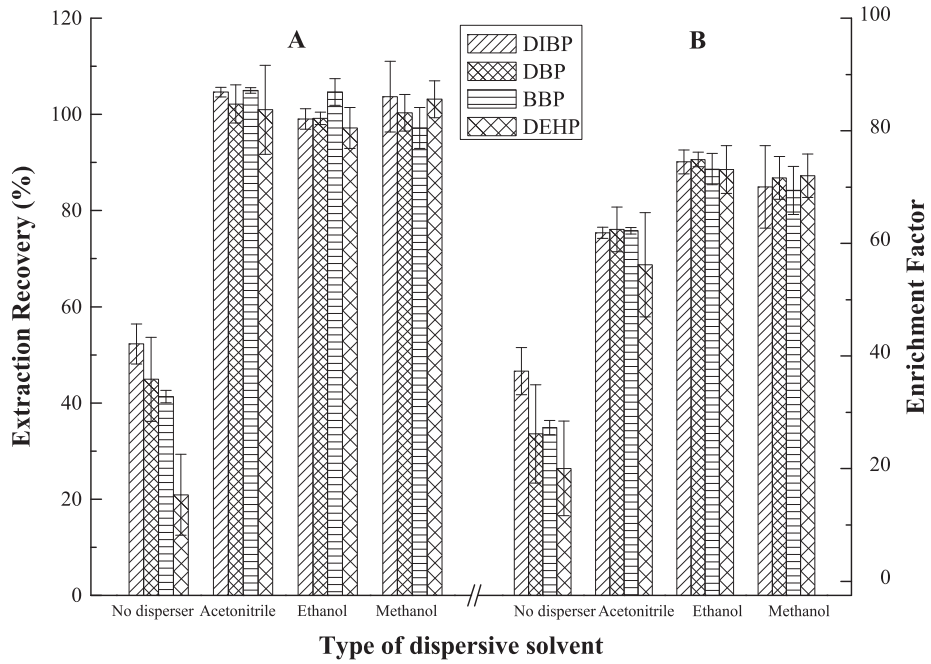

Fig. 3. Effect of the type of disperser solvents on the extraction recovery and enrichment factor of PAEs in a red wine sample (alcohol content, 12% v/v). Extraction conditions: sample, 10.0 mL spiked red wine (0.2 μg mL^{-1} each PAE); extraction solvent, 160 μL [C_8MIM][PF_6]; salt addition, 10% (w/v); extraction time, 5 min; centrifugation time, 4 min.

Table 2
Effect of resultant alcohol content on the extraction recovery after adding different volume of ethanol to a red wine sample (alcohol content, 12% v/v).

Analytes	Extraction Recovery (%) ± SD			
	16%	20%	23%	27%
DIBP	$99.06^{b} \pm 2.12$	$106.21^{c} \pm 5.15$	$102.66^{bc} \pm 1.54$	$88.47^{a} \pm 0.12$
DBP	$99.16^{b} \pm 1.29$	$99.89^{b} \pm 4.31$	$103.40^{b} \pm 2.51$	$82.49^{a} \pm 0.38$
BBP	$104.63^{b} \pm 2.78$	$99.23^{b} \pm 3.03$	$102.29^{b} \pm 1.23$	$86.69^{a} \pm 0.70$
DEHP	$97.20^{a} \pm 4.25$	$105.31^{ab} \pm 2.93$	$106.09^{b} \pm 5.52$	$104.79^{ab} \pm 3.83$

[a-c]On the basis of Duncan's multiple range test, the different letters within the same row indicate statistically significant difference at the 5% probability level.

the alcohol content was 27%, which resulted from the reduced volume of sedimented IL phase. Therefore, for red wine's analysis, appropriate amount of ethanol was required to add and the alcohol content should be in the range of 16–23%.

3.3. Method validation

3.3.1. Calibration curve and analytical performance characteristics of the method

Three calibration curves corresponding to a simulated sample, white spirit and red wine treated with the IL-DLLME procedure were constructed in order to evaluate the matrix effect. The curves were established at different concentration levels corresponding to 20, 50, 100, 200, 400 and 1000 ng mL^{-1} for simulated and white spirit samples, and 10, 20, 50, 100, 200 and 500 ng mL^{-1} for red wine sample. The linearity obtained for each of the calibration curves was satisfactory with correlation coefficients (r) ranging from 0.9983 to 1 and the slope values are shown in Table 3. The results obtained from Duncan's multiple range test results indicated that there was no significant difference at a confidence level of 95% for each of the PAEs among simulated, white spirit and red wine samples. Therefore, the calibration curve obtained for the simulated sample can be used for white spirits and red wines.

Limits of detection (LODs) and quantification (LOQs) were calculated at signal-to-noise ratio of 3 and 10, respectively. The obtained values were summarized in Table 3. The LODs were 3.1–4.2 ng mL^{-1} in white spirits and 1.5–2.2 ng mL^{-1} in red wines, which were comparable with 4 ng mL^{-1} achieved by LLE–HPLC–UV [20]. The calculated LOQs were 10.3–14.0 ng mL^{-1} in white spirits and 5.0–7.3 ng mL^{-1} in red wines, which were lower than the results obtained by SPE–GC–MS method [22]. As the injection volume for HPLC was 20 μL, the LODs shown as mass data were 0.062–0.084 ng for white spirits and 0.030–0.044 ng for red wines, respectively. LOQs shown as mass data were 0.206–0.280 ng for white spirits and 0.100–0.146 ng for red wines, respectively.

3.3.2. Precision study

The precision of the method was evaluated in terms of intra-day and inter-day repeatability. Intra-day repeatability was assessed by application of the proposed IL-DLLME–HPLC method to a white spirit and a red wine spiked at 0.05 μg mL^{-1} each of the analytes, respectively, and all the experiments were carried out five times on the same day. Inter-day repeatability was evaluated with the same procedure, but samples were treated and analyzed for three continuous days. The results obtained, expressed as the relative standard deviation (%RSD) of peak areas, were $\leq 2.0\%$ and $\leq 8.0\%$, respectively.

3.3.3. Trueness assessment

In order to check the trueness of the proposed method for the analysis of alcoholic beverages, recovery experiments were carried out at three different concentration levels of PAEs for two white spirits and a red wine (Table 4). Satisfactory recoveries of all the analytes were obtained in the range of 88.5–103.5% (RSD $\leq 7.6\%$) for white spirits and 91.6–104.6% (RSD $\leq 9.5\%$) for the red wine. These results demonstrated that the proposed method was reliable for the analysis of PAEs in alcoholic beverages.

Table 3
Analytical and statistical parameters of the proposed IL-DLLME–HPLC method for the determination of PAEs.

	Analytes	Linearity (ng mL^{-1})	Slope ± SD	r	EF	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
DLLME-simulate sample	DIBP	20–1000	93.90 ± 1.05	0.9998	51	1.5	5.0
	DBP	20–1000	84.78 ± 1.01	0.9999	52	1.8	6.0
	BBP	20–1000	77.71 ± 2.08	0.9994	53	1.9	6.3
	DEHP	20–1000	56.24 ± 0.49	0.9983	52	2.0	6.7
DLLME-white spirit	DIBP	20–1000	92.62 ± 1.66	0.9990	51	3.1	10.3
	DBP	20–1000	83.51 ± 1.27	0.9986	51	4.0	13.3
	BBP	20–1000	76.35 ± 2.45	0.9989	51	4.2	14.0
	DEHP	20–1000	55.83 ± 1.24	0.9995	52	4.1	13.7
DLLME-red wine	DIBP	10–500	90.24 ± 0.82	1	104	1.5	5.0
	DBP	10–500	83.24 ± 1.38	0.9996	104	2.2	7.3
	BBP	10–500	76.53 ± 0.87	0.9994	102	2.1	7.0
	DEHP	10–500	54.55 ± 0.58	0.9992	107	2.0	6.7

Table 4
Spiked recoveries of PAEs in real samples by the proposed method

Samples	Added (μg mL^{-1})	Analytes							
		DIBP		DBP		BBP		DEHP	
		Total found (μg mL^{-1})	Average recovery ± RSD% (n=3)	Total found (μg mL^{-1})	Average recovery ± RSD% (n=3)	Total found (μg mL^{-1})	Average recovery ± RSD% (n=3)	Total found (μg mL^{-1})	Average recovery ± RSD% (n=3)
W-2	0	0		0.035		0		0.011	
	0.1	0.095	95.0 ± 7.6	0.124	89.0 ± 0.5	0.0935	93.5 ± 3.1	0.113	102.0 ± 1.4
	0.2	0.177	88.5 ± 1.4	0.218	91.5 ± 0.7	0.207	103.5 ± 0.8	0.213	101.0 ± 0.9
	0.4	0.360	90.0 ± 2.6	0.405	92.5 ± 3.8	0.408	102.0 ± 2.9	0.424	103.2 ± 4.8
W-23	0	0.155		0.126		0		0.022	
	0.05	0.202	94.0 ± 0.8	0.177	102.0 ± 2.8	0.044	88.0 ± 1.8	0.07	96.0 ± 2.6
	0.1	0.245	90.0 ± 1.1	0.226	100.0 ± 1.3	0.092	92.0 ± 0.9	0.114	92.0 ± 0.2
	0.2	0.332	88.5 ± 4.0	0.322	98.0 ± 2.8	0.178	89.0 ± 1.6	0.207	92.5 ± 1.0
R-11	0	0		0		0		0	
	0.05	0.046	91.6 ± 5.3	0.049	97.9 ± 8.5	0.047	94.2 ± 7.3	0.048	95.9 ± 9.5
	0.1	0.985	98.5 ± 7.0	0.935	93.5 ± 1.8	0.944	94.4 ± 1.2	0.983	98.3 ± 0.7
	0.2	0.198	99.0 ± 2.1	0.198	99.2 ± 1.3	0.209	104.6 ± 2.8	0.194	97.2 ± 4.2

Table 5
Comparison of the proposed method with other previously reported methods for the determination of PAEs.

Methods	Sample amout	Matrix	Organic solvent consumption	Extraction time (min)	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	RSD %	Ref
LLE–HPLC/UV	5 g	Beverage, red and white wines	Hexane and CH_2Cl_2 (10:1, 5mL)	> 30	4	–	–	[20]
LLE-GC–MS	10–20 g	Wine and beer	Hexane (30 mL)	> 30	–	2	–	[21]
SPE–GC–MS	5 mL	Wine	CH_2Cl_2 (25mL)	> 30	15–18	24–29	10–21	[22]
HS-SPME–GC–MS	4 mL	Wine	–	120	0.09–1.3	0.15–2.2	0.3–20	[25]
HF-SPME–GC–MS	1 mL	Juice, wine, milk	Diethyl ether (300 μL)	50	0.006–0.03	0.02–0.1	2.6–4.1	[27]
DLLME–GC/FID or GC–IT/MS	10 mL	Wine	CH_2Cl_2 (200 μL)	10	0.022–0.1	0.075–0.335	4.9–8.2	[34]
IL-DLLME–HPLC	5–10 mL	White spirit and red wine	[C_8MIM][PF_6] (160 μL)	5	1.5–4.2	5.0–14.0	0.7–8.0	This work

3.4. Comparison with other methods

Comparison of the IL-DLLME–HPLC method with previously reported procedures for the analysis of PAEs in alcoholic beverages was shown in Table 5, illustrating the former offered some advantages over the latter. The IL used in this study was lower consumption (160 μL) and environmental friendly than hexane (30 mL) [21], diethyl ether (300 μL) [27] and dichloromethane (25 mL) [22] used in other methods. Besides, the extraction time (5 min) in the current procedure was much shorter than in LLE (> 30 min) [20,21], SPE (> 30 min) [22] and HS-SPME (120 min) [25]. Additionally, the LODs, LOQs and RSDs obtained in this study were comparable or better than in LLE–HPLC–UV [20] and SPE–GC–MS [22], respectively. Also this method did not require special instrumentations. In conclusion, the proposed IL-DLLME–HPLC method proved to be an alternative approach for the extraction and determination of PAEs in alcoholic beverages.

3.5. Analysis of real samples

The proposed IL-DLLME–HPLC method was used to survey the PAEs contamination in different samples including 30 white spirits

and 11 red wines. Fig. 4 showed the chromatograms of the real samples extracted by the IL-DLLME. The purity of the PAEs peaks was evaluated by comparing the ratio of the heights of the PAE peaks at three wavelengths (226 nm, 240 nm and 260 nm) [45].

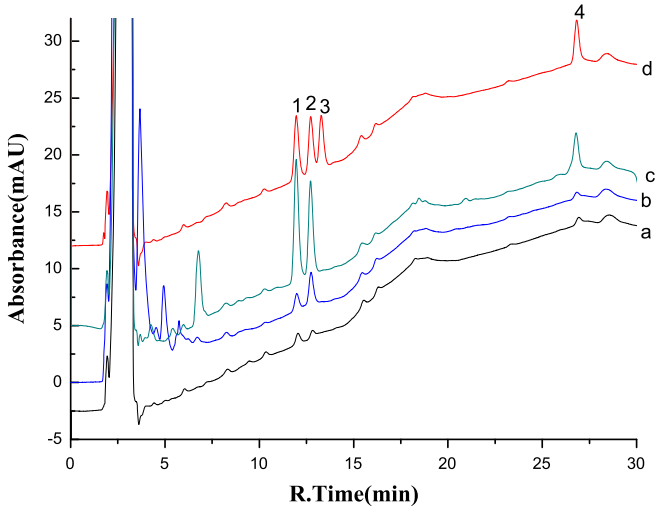

Fig. 4. HPLC chromatograms of the extracted samples with the IL-DLLME procedure. (a) simulated sample, (b) red wine, (c) white spirit, (d) simulated sample spiked at 0.4 μg mL^{-1} each PAE. Peak identification: (1) DIBP; (2) DBP; (3) BBP; and (4) DEHP.

Identical peak height ratios of 226/240, 240/260 and 260/226 for standard solutions and samples were obtained (Table S1, shown in the supplementary material), indicating the peaks of the analytes were free from impurities. In order to avoid false positive results, it is necessary to refer to the PAEs laboratory contamination once again. The reagent blank values found were from ethanol and [C_8MIM][PF_6], being 0.018 ± 0.001, 0.020 ± 0.001 and 0.024 ± 0.002 μg mL^{-1} for DIBP, DBP and DEHP, respectively (n=3) by HPLC-DAD analysis, which were subtracted from the real sample assay results.

Table 6 summarized the PAEs concentrations in the real samples and the corresponding median, average value and detection frequency. DBP was the PAE detected at the highest levels and the content (assuming all phthalates in samples were from migration during production process) in 63% white spirit samples exceeded the established SML (0.3 mg kg^{-1}) in Directive 2007/19/EC [12], with the maximum (6.426 μg mL^{-1}) being about 21 times the SML. DIBP and DEHP were detected at the range of < LOQ ~3.997 μg mL^{-1} and < LOQ~0.667 μg mL^{-1} with the detection frequency of 97% and 93%, respectively. BBP was found in a much lower level and frequency. In red wine samples (n=11), only DIBP and DBP were found at rather low concentrations, which were comparable or lower than those reported in other surveys on wine [20–23].

Considering the TDI value for DBP established by EFSA is the lowest (0.01 mg/kg body weight/day) [13], the daily intake of DBP for adult males by drinking white spirit was estimated based on the maximum concentrations of DBP (6.426 μg mL^{-1}) found in this

Table 6
PAEs content (μg mL^{-1}) in different alcoholic beverages and median, average value, detection frequency for each of the PAEs.

Samples	DIBP	DBP	BBP	DEHP	Samples	DIBP	DBP	BBP	DEHP
White spirit					Red wine				
W-1	0.016 ± 1.55[a]	0.029 ± 6.29	0.017 ± 7.78	0.390 ± 2.34	R−1	< ql	nd	nd	nd
W-2	< ql[b]	0.035 ± 3.12	nd[c]	< ql	R−2	nd	nd	nd	nd
W-3	0.555 ± 5.73	**0.993**[d] ± 7.26	nd	0.148 ± 5.57	R−3	0.032 ± 0.81	< ql	nd	nd
W-4	0.040 ± 0.84	0.025 ± 2.54	nd	< ql	R−4	< ql	0.036 ± 1.98	nd	nd
W-5	0.022 ± 3.71	0.043 ± 3.82	< ql	0.0120 ± 6.30	R−5	0.042 ± 6.29	0.122 ± 5.94	nd	< ql
W-6	0.129 ± 2.25	**0.379** ± 0.27	nd	0.030 ± 6.93	R−6	< ql	nd	nd	nd
W-7	0.190 ± 2.75	0.204 ± 2.11	0.014 ± 5.02	0.078 ± 1.21	R−7	0.024 ± 3.90	0.062 ± 3.44	nd	< ql
W-8	0.103 ± 2.92	**0.340** ± 3.86	nd	0.226 ± 1.51	R−8	< ql	< ql	nd	nd
W-9	0.987 ± 1.53	**6.426** ± 1.29	< ql	0.123 ± 3.04	R−9	0.018 ± 4.07	0.040 ± 5.93	nd	< ql
W-10	0.520 ± 1.66	**0.503** ± 0.62	nd	0.183 ± 3.04	R−10	nd	nd	nd	< ql
W-11	0.020 ± 2.51	**0.649** ± 7.23	nd	0.030 ± 7.00	R−11	< ql	nd	nd	nd
W-12	0.142 ± 5.27	**0.353** ± 4.46	nd	0.150 ± 8.52					
W-13	0.114 ± 2.03	**0.363** ± 1.33	nd	0.149 ± 2.62					
W-14	0.425 ± 3.29	**0.871** ± 2.49	nd	0.174 ± 4.25					
W-15	0.809 ± 1.66	**4.871** ± 0.35	< ql	0.119 ± 4.43					
W-16	0.030 ± 0.56	**0.773** ± 2.57	nd	0.124 ± 0.47					
W-17	1.362 ± 0.82	**1.886** ± 0.17	nd	0.642 ± 4.09					
W-18	3.997 ± 2.05	**1.528** ± 0.92	< ql	0.257 ± 1.75					
W-19	0.351 ± 1.07	**1.230** ± 2.14	< ql	0.080 ± 4.08					
W-20	2.078 ± 1.38	**1.786** ± 2.10	nd	0.122 ± 0.47					
W-21	0.430 ± 1.82	0.103 ± 4.17	< ql	0.122 ± 0.87					
W-22	0.691 ± 2.92	**1.035** ± 3.36	nd	0.324 ± 4.10					
W-23	0.155 ± 1.84	0.126 ± 0.57	nd	0.022 ± 0.57					
W-24	0.411 ± 1.78	0.157 ± 2.04	nd	0.074 ± 2.54					
W-25	1.904 ± 4.80	**0.444** ± 0.85	Nd	0.016 ± 4.66					
W-26	0.015 ± 5.97	0.053 ± 5.64	nd	0.042 ± 0.90					
W-27	0.841 ± 0.40	**1.790** ± 1.37	nd	0.667 ± 0.70					
W-28	0.767 ± 6.64	**0.352** ± 6.49	nd	0.437 ± 2.40					
W-29	0.684 ± 1.51	0.207 ± 1.65	nd	0.099 ± 1.95					
W-30	0.019 ± 5.90	0.015 ± 0.72	nd	0.035 ± 1.51					
Median	0.379	0.336	< ql	0.09	Median	< ql	< ql	< ql	< ql
Average	0.592	0.917	< ql	0.15	Average	< ql	< ql	< ql	< ql
Detection frequency (%)	97	100	7	93	Detection frequency (%)	36	36	0	0

[a] Mean ± %RSD, n=3;
[b] below the quantification limit;
[c] not detected;
[d] (shown in bold) exceeding the SML.

study. Assuming that a 60 kg Chinese adult consumes 100 mL white spirit, the maximum DBP intake calculated is 0.642 mg, which is higher than the TDI (0.60 mg). The result suggested that the exposure to PAEs (especially to DBP) by drinking white spirit should not be neglected. The DBP concentrations in all the investigated red wine samples were so low that they were not of any concern for human exposure.

As shown in Table 6, the contents of PAEs in white spirits and red wines were significantly different. This may be caused by their different production processes. Plastic piping, tanks, stoppers and other materials are widely used in the production of white spirits, while the wine-making prefers to apply the traditional oak barrels or stainless steel tanks. Indeed, sometimes in order to improve organoleptic properties (i.e. mouthfeel and flavor) of white spirits, it cannot be denied that some white spirit producers may add the maturingagent, which might contain certain amount of PAEs.

4. Conclusions

A new method based on IL-DLLME–HPLC has been successfully developed for the determination of PAEs in alcoholic beverages. The proposed method is simple, low cost, environmental benign and less time-consuming, and especially it can be applied for samples containing high alcohol percentage. In addition, it was found no matrix effect existed among white spirits, red wines and simulated samples. The suitable alcohol content ranges in both white spirits and red wines were optimized, which made this proposed method become a global procedure of PAEs analysis in alcoholic beverages with different alcohol contents.

The survey of the real alcoholic beverages (11 red wines and 30 white spirits) indicated the PAEs contamination is ubiquitous in white spirits; especially the DBP content in more than half of the investigated samples exceeded the SML. More attention should be paid to human exposure to PAEs via drinking white spirits.

Acknowledgment

The authors acknowledge with gratitude and appreciation financial support from Northwest A&F University.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.11.023.

References

[1] Z.Y. Guo, S. Wang, D.Y. Wei, M.L. Wang, H.N. Zhang, P.P. Gai, J. Duan, Meat Sci. 84 (2010) 484–490.
[2] J. Antian, Environ. Health. Perspect. (1973)3–26.
[3] F. Zeng, K.Y. Cui, Z.Y. Xie, L.N. Wu, M. Liu, G.Q. Sun, Y.J. Lin, D.L. Luo, Z.X. Zeng, Environ. Pollut. 156 (2008) 425–434.
[4] P. Liang, J. Xu, Q. Li, Anal. Chim. Acta 609 (2008) 53–58.
[5] M. Abb, T. Heinrich, E. Sorkau, W. Lorenz, Environ. Int. 35 (2009) 965–970.
[6] Y. Guo, K. Kannan, Anal. Bioanal. Chem. 404 (2012) 2539–2554.
[7] F.A. Arcadi, C. Costa, C. Imperatore, A. Marchese, A. Rapisarda, M. Salemi, G.R. Trimarchi, G. Costa, Food Chem. Toxicol. 36 (1998) 963–970.
[8] M. Petrovic, E. Eljarrat, M.J. López de Alda, D. Barceló, TrAC, Trends. Anal. Chem. 20 (2001) 637–648.
[9] A.J. Martino-Andrade, I. Chahoud, Mol. Nutr. Food Res. 54 (2010) 148–157.
[10] J.S. Fisher, Reproduction 127 (2004) 305–315.
[11] US Environmental Protection Agency, Washington, DC, 1980.
[12] Commission Directive 2007/19/EC, Off. J. Eur. Commun. L91 (2007) 17.
[13] European Food Safety Authority, EFSA J. 242 (2005) 1–17.
[14] European Food Safety Authority, EFSA J. 241 (2005) 1–14.
[15] European Food Safety Authority, EFSA J. 243 (2005) 1–20.
[16] European Food Safety Authority, EFSA J. 244 (2005) 1–18.
[17] European Food Safety Authority, EFSA J. 245 (2005) 1–14.
[18] H.Y. Yan, H. Wang, J. Chromatogr. A 1295 (2013) 1–15.
[19] X.L. Cao, Compr. Rev. Food Sci. Food Saf. 9 (2010) 21–43.
[20] K. Yano, N. Hirosawa, Y. Sakamoto, H. Katayama, T. Moriguchi, K.E. Joung, Y.Y. Sheen, K. Asaoka, Bull. Environ. Contam. Toxicol. 68 (2002) 463–469.
[21] Y. Guo, Z.F. Zhang, L.Y. Liu, Y.F. Li, N.Q. Ren, K. Kannan, J. Agric. Food Chem. 60 (2012) 6913–6919.
[22] M. Del Carlo, A. Pepe, G. Sacchetti, D. Compagnone, D. Mastrocola, A. Cichelli, Food Chem. 111 (2008) 771–777.
[23] M.V. Russo, I. Notardonato, G. Cinelli, P. Avino, Anal. Bioanal. Chem. 402 (2012) 1373–1381.
[24] J.D. Carrillo, C. Salazar, C. Moreta, M.T. Tena, J. Chromatogr. A 1164 (2007) 248–261.
[25] J.D. Carrillo, M.P. Martinez, M.T. Tena, J. Chromatogr. A 1181 (2008) 125–130.
[26] C.W. Ye, J. Gao, C. Yang, X.J. Liu, X.J. Li, S.Y. Pan, Anal. Chim. Acta 641 (2009) 64–74.
[27] J. Li, Q. Su, K.Y. Li, C.F. Sun, W.B. Zhang, Food Chem. 141 (2013) 3714–3720.
[28] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1–9.
[29] S.H. Liu, Q.L. Xie, J.P. Cao, P.P. Song, J. Chen, W.W. Bai, J. Sep. Sci. 36 (2013) 1135–1141.
[30] S.H. Liu, Q.L. Xie, J. Chen, J.Z. Sun, H. He, X.K. Zhang, J. Chromatogr. A 1295 (2013) 16–23.
[31] J.P. Cao, S.H. Liu, W.W. Bai, J. Chen, Q.L. Xie, Anal. Lett. 46 (2013) 1342–1354.
[32] M. Rezaee, Y. Yamini, M. Faraji, J. Chromatogr. A 1217 (2010) 2342–2357.
[33] C. Mahugo-Santana, Z. Sosa-Ferrera, M.E. Torres-Padrón, J.J. Santana -Rodríguez, TrAC, Trends Anal. Chem. 30 (2011) 731–748.
[34] G. Cinelli, P. Avino, I. Notardonato, A. Centola, M.V. Russo, Anal. Chim. Acta 769 (2013) 72–78.
[35] X.W. Chen, J.W. Liu, J.H. Wang, Anal. Methods 2 (2010) 1222–1226.
[36] S. Pandey, Anal. Chim. Acta 556 (2006) 38–45.
[37] M.T. Pena, M.C. Casais, M.C. Mejuto, R. Cela, J. Chromatogr. A 1216 (2009) 6356–6364.
[38] L.M. Ravelo-Pérez, J. Hernández-Borges, M. Asensio-Ramos, M.A. Rodríguez -Delgado, J. Chromatogr. A 1216 (2009) 7336–7345.
[39] S.Q. Gao, H.Y. Jin, J.Y. You, Y. Ding, N. Zhang, Y. Wang, R.B. Ren, R. Zhang, H.Q. Zhang, J. Chromatogr. A 1218 (2011) 7254–7263.
[40] H. Abdolmohammad-Zadeh, G.H. Sadeghi, Talanta 81 (2010) 778–785.
[41] H. Zhang, X.Q. Chen, X.Y. Jiang, Anal. Chim. Acta 689 (2011) 137–142.
[42] S. Chen, Y.S. Zhong, S.Y. Cheng, T. Qian, H. Sun, J. Sep. Sci. 34 (2011) 1503–1507.
[43] Q.X. Zhou, X.G. Zhang, G.H. Xie, Anal. Methods 3 (2011) 1815–1820.
[44] National Standards of the People's Republic of China, GB/T 17204-1998.
[45] Y. Haroon, D.S. Bacon, J.A. Sadowski, Clin. Chem. 32 (1986) 1925–1929.