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Ionic liquid-based single drop as a simple and efficient microextraction method for simultaneous determination of aminophenol isomers in human urine, hair dye and water samples using HPLC

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Abstract

A user-friendly and inexpensive ionic liquid-based single-drop microextraction (IL-SDME) method was developed to preconcentrate trace amounts of aminophenol isomers (APs) from human urine, hair dye and water samples prior to analysis by high performance liquid chromatography-ultraviolet spectrophotometry detection (HPLC-UV). Under optimum conditions (i.e., 3.5 µL of 1-hexyl-3-methylimidazolium hexafluorophosphate [C_6MIM][PF_6], 10 mL of sample, NaCl free adjusted to pH 5.5, 15 min extraction time and 800 rpm agitation speed at room temperature) enrichment factors and limits of detection were ranged from 348 to 364 and 0.06 to 0.14 depending on the target analytes. The method gave good levels of repeatability with relative standard deviations varying between 3.6 and 4.2 % (n = 5). Recoveries of the analytes were ranged between 87.3 and 99.3 %, showing that the matrix had a negligible effect upon extraction. Finally, the proposed method was successfully applied to the analysis of different real samples such as human urine, hair dye and water samples.

Keywords: Ionic liquid-based single drop microextraction, aminophenol isomers, human urine, hair dye, water samples, HPLC

1. Introduction

The ortho-, meta- and para-aminophenol (2-, 3-, 4-AP) isomers are important industrial raw and processed materials that are widely used in the dyestuffs, chemical inhibitors, petroleum additives and synthetic intermediates in chemical and pharmaceutical industries. As an example, some of these isomers such as 4- AP is used in the production of paracetamol and could be formed by the degradation of paracetamol containing medicines in the human body [1, 2]. However, despite this extensive applicability, due to their significant nephrotoxicity and teratogenic effect for human, animals, and plants, APs are deemed to be typical environmental pollutants [3, 4].

Amount of the APs in environmental and biological samples is low and in order to determine these compounds by a suitable technique, an extraction or a preconcentration step is necessary. In the past few years, with the developing interest in much simpler miniaturized configuration of extraction procedures, a novel liquid–liquid microextraction system named liquid-phase microextraction (LPME) was developed [5]. Because of rapidness, simplicity and minimum use of solvent, LPME has attracted increasing attention and has successfully been applied to different types of sample [6-8].

One of the most popular two-phase LPME methods is Single drop microextraction (SDME). In a typical SDME method the extraction phase is a drop of water-immiscible solvent suspended in the stirred aqueous solution. This technique has several advantages such as significant reduction in the amount of organic solvent used, simplicity, cost-effectiveness and higher analytical frequency. Also, it uses inexpensive apparatus and minimizes solvent consumption, and it combines extraction, preconcentration and sample introduction in

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one step [9-12]. However, the demerits of this technology such as instability of drop, relatively low precision and sensitivity [13] are often encountered. One of the main reasons for these demerits is the use of organic solvents (such as octanol, cyclohexane, toluene, etc.) as extractant phases in SDME. Nevertheless, in recent years, ionic liquids (IL), which are organic salts that are liquids at room temperature and have high boiling points, have been proposed for use in SDME [14–20]. IL have various advantages over traditional organic solvents, such as low vapor pressure, high stability, high viscosity, moderate dissolvability of organic compounds, adjustable miscibility and polarity, good extractability for different organic compounds, as well as the possibility of using longer sampling time, larger droplet volume, and compatibility with High-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). Nevertheless, despite these advantages, there are still few IL-based SDME applications but there is growing interest.

The aim of this paper was to develop a user-friendly, inexpensive, sensitive and environmentally friendly analytical method able to determine APs isomers in human urine, hair dye and water samples, simultaneously. In this way, Ionic liquid-based single drop microextraction (IL-SDME) method, which can possess these characteristics, was investigated and developed. Furthermore, due to their similar structures and characteristics, APs usually coexist and interfere with each other, so the simultaneous separation of aminophenol isomers was also examined and a simple HPLC-UV method was developed. The results showed that IL-SDME –as well as its easy operation, short extraction time, high sensitivity and use of no hazardous extraction solvent– is an effective method, providing good enrichment factor of the analytes under the optimum conditions. To the best of our knowledge, there are no published methods based on IL-SDME focusing on APs determination in human urine, hair dye and water samples.

2. Experimental

2.1. Reagents and solutions

1-hexyl-3-methylimidazolium hexafluorophosphate, 1-butyl-3-methylimidazolium hexafluorophosphate, 1-Hexyl-3-methylimidazolium bis (trifluoromethylsulfonylimide), sodium chloride (NaCl), and ultra-pure water were all from Merck (Darmstadt, Germany). Sodium hydroxide and concentrated hydrochloric acid, bought from Merck (Darmstadt, Germany) were used to adjust the pH of the samples. Commercial permanent hair dyes were collected from retail stores locally.

A mixture of stock solution containing APs at 1000 µg mL-1 was prepared in HPLC grade methanol. A series of standard solutions were prepared by mixing an appropriate amount of the stock solution with ultra-pure water in a 10 mL volumetric flask. The aqueous solutions were prepared daily by diluting the standard mixture with ultra-pure water. All the standard solutions were stored at 4 ◦C in the dark.

2.2. Sample preparation

Urine: Two kind of human urine samples were prepared from a healthy volunteer in our lab: i) Blank urine samples (APs-free) and ii) Model urine samples. Blank urine samples (APs-free) were prepared from the volunteer not intaken to any drug at least for 1 week, whereas Model urine samples were prepared 3, 6 and 9h after the administration of paracetamol (750 mg single dose) and were stored one week below 0 ◦C in plastic containers. Before use, the samples were thawed to room temperature and working solutions were prepared by dilution of 4 mL of the urine sample in a 100 mL volume flask. The resulting solutions were filtered and subjected to the IL-SDME method.

Hair dye: 200 mg (± 0.1 mg) of the hair dye sample was dissolved by ultra-pure water in a 200 mL volumetric flask. Working solutions of hair dye samples were freshly prepared by dilution of 2 mL of the stock sample in a 10 mL volume flask. The resulting solutions were filtered and subjected to the IL-SDME method.

Real water: Real water samples including tap, river and waste water samples were collected from different locations of Iran. 5 mL of water sample was subjected to the IL-SDME method, without any pretreatment.

All the standard solutions, solvents and samples were filtered through a 0.45 µm membrane to eliminate particulate matters before analysis.

2.3. Apparatus

A Knauer HPLC system (Berlin, Germany), equipped with a K-1001 HPLC pump, D-14163 degasser, and a K-2600 UV detector was used. Chromgate software (version 3.1) for HPLC system was employed to acquire and process chromatographic data. The analytical column was ODS III (250 mm × ID 4.6 mm, 5 µm) from MZ-Analysentechnik (Mainz, Germany). The pH of the solutions was measured by a PHS-3BW model pH-meter (Bell, Italy).

A mobile phase comprised of water/acetonitrile (70:30, v/v) at a flow rate of 0.80 mL min-1 was found to be optimum. Prior to use, the mobile phases were filtered through a 0.45 µm membrane filter and degassed under vacuum. The sample injection volume was 20 µL and the analytes were monitored at 235 nm (at room temperature).

2.4. IL-SDME method

10 mL of sample solution was added into a 15 mL vial and the vial was then placed on a magnetic stirrer with a stirring rate of 800 rpm. 3.5 µL of 1-hexyl-3-methylimidazolium hexafluorophosphate was suspended from the tip of the 20 µl HPLC syringe, which was fixed above the vial into the sample solution. The micro-drop was left for 15 min under constant stirring rate, and after

extraction was drown back into the syringe and injected into the HPLC system.

3. Results and discussion

3.1.1. Optimization of IL-SDME method

SDME has been successfully employed to face a wide variety of analytical problems. However, properties of the organic solvents commonly used such as low viscosity and high tendency to evaporation, result in drop instability and poor precision levels. The use of ILs as alternative to these conventional solvents increases the drop stability, thanks to their higher viscosity, which also facilitates the formation of larger-volume drops. Moreover, the lower vapor pressure of ILs minimizes the drop evaporation, which results in better reproducibility of the measurements.

3.1.2. Type and volume of extraction solvent

The ILs studied for the extraction of the analytes were 1-hexyl-3-methylimidazolium hexafluorophosphate [C_6MIM][PF_6], 1-butyl-3-methylimidazolium hexafluorophosphate [C_4MIM][PF_6], 1-Hexyl-3-methylimidazolium bis (trifluoromethylsulfonylimide) [C_6MIM][$N(SO_2CF_3)_2$]). The three ILs led to high extraction efficiencies. Since [C6MIM][PF6] was cheaper and led to cleaner chromatograms, it was selected for subsequent experiments for the extraction of APs (Fig. 1).

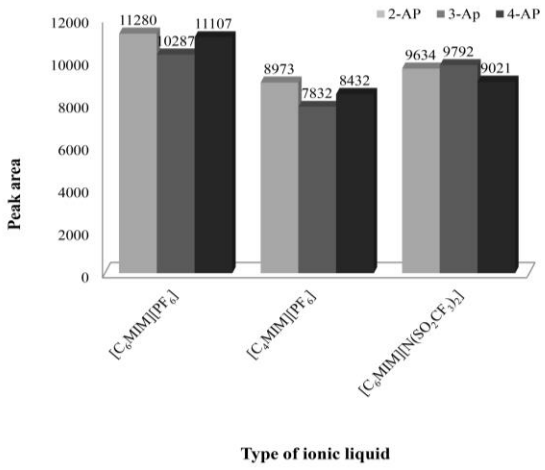

Figure 1. Effect of the type of ionic liquid on the extraction efficiency of Aps. Conditions: micro-drop volume: 2 µL, sample 10 mL, without pH adjustment and salt addition, stirring rate: 800 rpm, extraction time: 20 min, at room temperature.

One of the important factors in SDME, which has great influence on the extraction efficiency, is the volume of the micro-drop. The effect of [C_6MIM][PF_6] drop size on the peak area of the APs was investigated in the range of 1 to 3.5 µL. As can be seen in Fig. 2, the peak area of the analytes was increased with the increasing of the micro-drop volume. The surface area increases with an increase in the drop volume, resulting in a more efficient mass transfer from the bulk aqueous solution to IL drop. However, when volumes larger than 3.5 µL are used, the micro-drop becomes unstable and can be easily released from the tip of the syringe needle. So, this volume was selected as appropriate drop size.

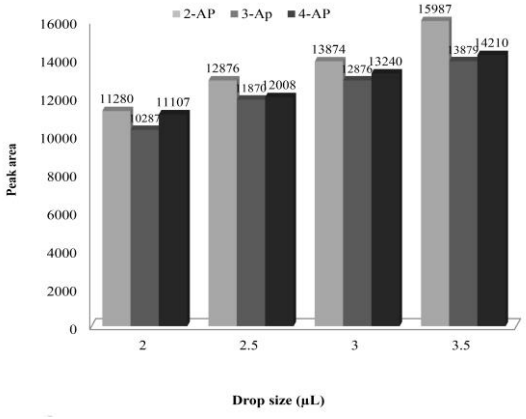

Figure 2. Effect of micro-drop volume on the extraction efficiency of Aps Conditions: ionic liquid: [C_6MIM][PF_6], sample 10 mL, without pH adjustment and salt addition, stirring rate: 800 rpm, extraction time: 20 min, at room temperature

3.1.3. Extraction temperature and time

Temperature might have an important influence on extraction of the analytes, the viscosity of the ionic liquid and, therefore, the mass-transfer process. Its effect on the extraction of APs was investigated different temperatures. Analytes extraction decreases with increase of temperature higher room temperature. In principle, higher temperatures causes to increase of mass transfer and extraction efficiency, but it also leads to increase of IL solubility in sample solution. Therefore, further experiments were investigated at room temperature (data were not shown).

In general, mass transfer is a time-dependent process and the maximum peak area is attained when the system is at equilibrium. However, as long as extraction condition is reproducible, complete equilibrium needs not to attain to obtain accurate and precise analysis. The effect of extraction time has been studied by varying the exposure time of the micro-drop to the aqueous solution from 5 to 25 min and the results are shown in Fig. 3. As could be seen, the peak area increased with the increase of extraction time. In order to achieve a higher analytical frequency, the extraction time of 15 min was selected for all subsequent experiments.

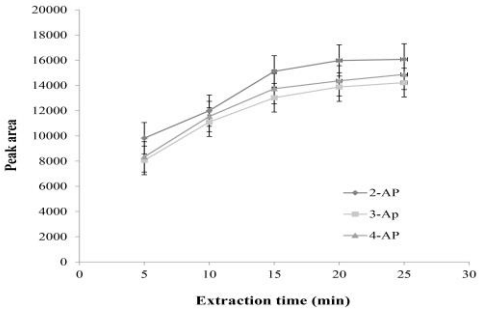

Figure 3. Effect of extraction time on the extraction efficiency of Aps. Conditions: ionic liquid: 3 µL of [C6MIM][PF6], sample 10 mL,

without pH adjustment and salt addition, stirring rate: 800 rpm, at room temperature.

3.1.4. Stirring rate, ionic strength and pH

Agitation of the sample is assumed to reduce the time required for extraction, because stirring induces convection in the sample, facilitating also the mass transference towards the micro-drop. The effect of this parameter on the extraction was studied by varying the stirring rate from 0 to 800 rpm using a magnetic stirrer. The maximum speed of the agitator offered the best results. Higher stirring rate causes to dislodge of the IL micro-drop, therefore, 800 rpm was the rate used for subsequent experiments (Fig. 4).

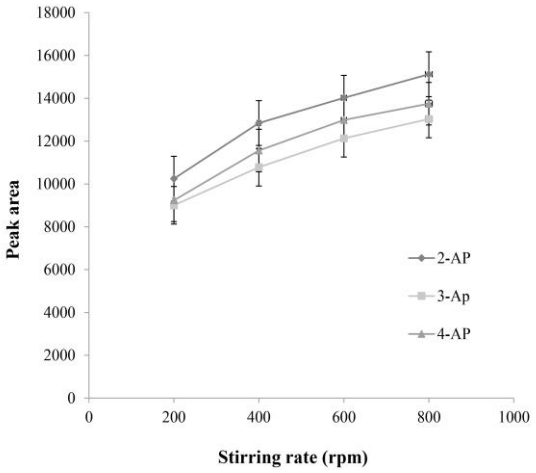

Figure 4. Effect of stirring rate on the extraction efficiency of APs
Conditions: ionic liquid: 3 µL of [C_6MIM][PF_6], sample 10 mL, without pH adjustment and salt addition, extraction time: 15 min, at room temperature

In general, ionic strength can affect the affinity of the analytes for the extraction phase since less water molecules are available for the solubilization of the analytes, which facilitates its transference towards the extractant (salting-out effect). Besides, it can also causes to the increase of IL solubility in the aqueous solution. The salting-out effect was examined by monitoring the variation of peak areas with a salt concentration ranging from 0 to 10 mg mL^{-1}. According to observed results, the peak area of the analytes decreases with NaCl concentration. Hence, no salt was added for further experiments.

The effect of pH of the sample solution on the extraction efficiency, ranging from 2 to 10, was studied. The extraction efficiency increased with pH increase from 2 to 5.5, and then decreased. Hence, the subsequent studies were carried out at pH 5.5 (data were not shown).

3.2. Analytical figures of merit

Under the optimized conditions, linearity, limits of detection (LODs), repeatability, enrichment factor and recovery of the analytes were determined; the results are shown in Table 1. The linearity of the method was evaluated using mixed working solution with analytes over the concentration range of 0.21- 91.4 µg mL^{-1}. The calculated calibration curves gave a good linearity for all analytes with correlation coefficients (R^2) higher than 0.991. LODs (calculated as 3 times of the standard deviation divided on the calibration slope), after IL-SDME, were ranged from 0.6 to 0.14. To assess the precision of the proposed method, the repeatability was determined by performing five experiments and the RSDs were found to be <4.2.

After optimization, the spiked blank urine sample was used for calculation of enrichment factor and recovery. The following equation was used to calculate the enrichment factor.

$$EF = C_i / C_0 \quad (1)$$

where EF, C_i, and C_0 are the enrichment factor, the analyte concentration after preconcentration obtained from the calibration graph of direct injection of standard solution, and the initial concentration of analyte within the sample, respectively.

The recoveries were calculated based on following equation .

$$\%R = EF \times V_{sed}/V_0 \quad (2)$$

where, V_i and V_0 are the volume of extraction phase and the volume of initial phase (volume of the sample).

Table 1. LOD, LOQ, Linear range (LR), intra-day precision (%RSD), squared correlation coefficient (r^2), enrichment factor (EF) for the proposed method, under the optimum conditions Peak area was used for quantification.

EF	R^2	(%RSD)[a]	LR (µg mL^{-1})	LOD (µg mL^{-1})	Analyte
348	0.991	3.7	0.44-84.1	0.14	2-AP
364	0.998	3.6	0.21-91.4	0.06	3-AP
360	0.995	4.2	0.29-82.3	0.09	4-AP

Conditions: mobile phase water/acetonitrile (70:30, *v/v*); flow rate = 0.8 mL min−1; column ODS III (250 mm × ID 4.6 mm, 5 µm); injection volume = 20 µL; λ= 235 nm an=5

Table 2. Recovery of the analytes under the optimum conditions.

Sample	Recovery (%) (spiked at 5, 10 µg mL^{-1})		
	2-AP	3-AP	4-AP
Urine 1	89.5-95.7	91.8-93.4	89.2-95.9
Hair dye 1	91.9-97.8	93.1-98.4	87.3-93.9
Waste water (Semnan)	90.4-96.4	91.6-96.8	90.4-96.1
Tap water (Semnan)	91.7-95.1	90.7-94.5	93.5-99.3

Conditions: see table 1.

3.3. Analysis of real samples

Due to the biological and environmental significance of APs, several samples of human urine, hair dye and water were analyzed to validate the accuracy and applicability of the proposed method. In order to eliminate possible matrix effects, the standard addition method was adopted for the quantitative determination of APs in the real samples. The concentrations of APs are shown in Table 3. The typical chromatograms for urine samples areshown in Fig. 8.

The presence of 4-AP in the urine sample 1 (peak 1 in chromatogram C) indicated that the 4-AP is the only isomer produced by the metabolism of paracetamol. Regarding to the chromatograms, it can be seen that the sample matrices had no significant interferences for the determination of APs in the real samples.

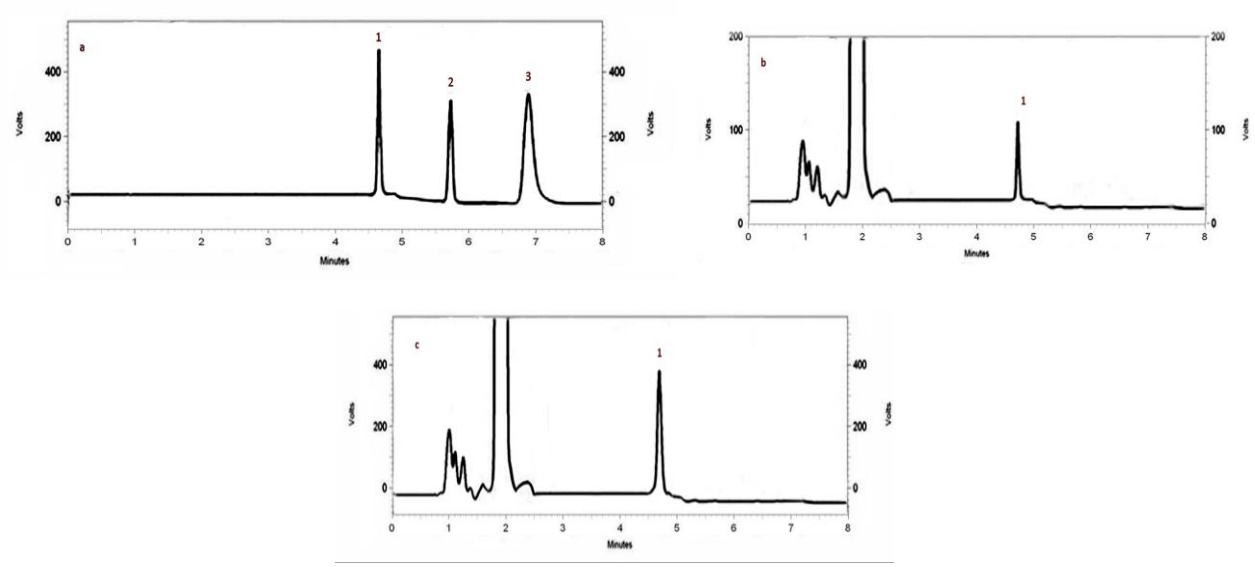

Figure 5. HPLC chromatograms of (a) standards (10 µg mL-1), (b) urine sample, and (c) model urine sample (5 µg mL^{-1} of 4-AP) after IL-SDME. Peak identification: (1) 4-AP, (2) 3-AP, (3) 2-AP

Table 3. Determination of aminophenol isomers under optimum conditions (n=3)

Sample	Concentration[a] ± RSD					
	2- aminophenol (spiked at)		3- aminophenol (spiked at)		4- aminophenol (spiked at)	
	10.0 µg mL^{-1}	20.0 µg mL^{-1}	10.0 µg mL^{-1}	20.0 µg mL^{-1}	10.0 µg mL^{-1}	20.0 µg mL^{-1}
Urine 1 (After 3 h)	10.21±2.4	20.26±2.6	10.33±2.9	19.75±2.8	12.39±3.2	22.84±3.1
Urine 2 (After 6 h)	10.54± 3.1	19.74±2.9	9.21±3.3	19.28±3.1	11.36±2.9	21.69±3.3
Urine 3 (After 9 h)	9.89± 2.8	19.81± 3.2	9.75± 3.2	20.32±3.0	10.12±3.1	20.25±3.2
Waste water (Semnan)	12.94±3.1	23.14±2.8	10.19±3.0	20.41±2.9	13.31±3.2	23.28±2.9
Tap water (Semnan)	10.74±3.3	20.45±2.9	10.37±2.9	20.23±2.6	10.29±2.9	20.37±2.8
Hair dye 1	10.36±3.2	20.69±3.0	9.88±2.9	20.11±2.7	9.57±2.8	20.26±2.9
Hair dye 2	11.59±3.1	21.78±3.4	10.67±3.1	20.41±3.2	10.16±2.9	19.83±2.8
Hair dye 3	9.97±2.6	19.71±2.9	9.74±3.2	19.87±2.9	14.98±3.1	25.14±3.2

[a] Peak area was used for quantification. Conditions: See Table 1

4. Conclusion

In this study, a simple and efficient method for separation and preconcentration of aminophenol isomers before simultaneous HPLC determination was developed. The widely and commonly used HPLC was coupled with IL-SDME, which largely minimized organic solvents consumption and greatly increased the sensitivity for the determination of APs. Good figures of merit have been obtained, although the limits of detection could be improved by employing more sensitive detectors. With the application of ionic liquid as extractant for SDME, the proposed method showed the characteristic of relatively green and environmentally friendly chemistry.

Acknowledgements

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