Corona discharge ionization ion mobility spectrometry for ultra-trace determination of methamphetamine extracted from urine and plasma samples by dispersive liquid–liquid microextraction

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Abstract
In this work, dispersive liquid–liquid microextraction (DLLME) based on high-density extraction solvent was applied as a simple, fast and sensitive method for extraction and preconcentration of methamphetamine from human plasma and urine samples. The efficiency of positive corona discharge ionization ion mobility spectrometry was investigated for direct analysis of the extracted analyte. Effective parameters on the extraction efficiency, such as type and volume of the extraction and disperser solvents, centrifugation time, and sample solution pH were optimized. Trichloromethane and isopropanol were selected as the extracting and disperser solvents, respectively. Under the optimized conditions, the linear dynamic range (R² = 0.9969) was found to be 0.5–18 µg/L, and 0.15 µg/L was calculated as the limit of detection. The relative standard deviations of intra- and inter-day were obtained 4 and 10%, respectively, and finally, in the analysis of human plasma and urine samples, the extraction recovery was obtained 104%.

Keywords Methamphetamine · Ion mobility spectrometry · Biological samples · Sample extraction method · DLLME

Introduction
Amphetamine and methamphetamine, as the most common central nervous system exciters, release excess dopamine into the synaptic clefts of dopaminergic neurons. Amphetamines may be prescribed to treat narcolepsy and Parkinson’s disease, and methamphetamine may be used in bronchial inhalers and nasal decongestants. However, these drugs increase the level of consciousness, satisfaction, and the desire to be socialized; and methamphetamine as a more favorite drug causes many social and psychological problems [1, 2]. Therefore, researchers have been reported a variety of analytical methods for the identification and quantitative analysis of these compounds in different biological samples. Some of the commonly techniques used for the extraction of amphetamine and methamphetamine are solid-phase extraction (SPE) [3], liquid–liquid extraction (LLE) [4], solid-phase microextraction (SPME) [5], liquid-phase microextraction (LPME) [6–8], molecular imprinted polymer (MIP) [9], thin film microextraction (TFME)-aptamer carbon materials [10], and stir-bar sorptive extraction (SBSE) [11]. These techniques are often time-consuming, expensive, the short lifetime of the fibers, and consume a large solvent volume [12]. In 2006, Assadi et al. [13] introduced dispersive liquid–liquid microextraction (DLLME) as an extraction technique, using a very low volume of organic solvents. This technique is simple, fast, inexpensive, with a high extraction recovery and enrichment factor [14, 15]. Briefly, in the DLLME technique, the mixture of a disperser organic solvent (fully miscible with the water and extraction solvent) and an extracting solvent (only miscible with the disperser solvent) is rapidly injected into the aqueous sample solution containing the analyte. After forming the cloudy solution, the surface area between the aqueous sample and extraction organic solvent would be increased by creating the extraction solvent droplets in an aqueous sample. The cloudy solution is centrifuged and, the sedimented phase is separated with a microsyringe, to be analyzed by an analytical instrument such as gas chromatography (GC), high-performance liquid chromatography (HPLC), and ion
mobility spectrometry (IMS) [16, 17]. Since derivatization procedure and other sample preparation steps for GC methods are time-consuming, so, these methods are limited to volatile compounds. Also, HPLC methods require expensive pure solvents in addition to their long run times.

IMS, introduced by Karasek and Cohen in 1970 [18], could separate chemical compounds based on the mobility of their gas-phase ions in the weak electric field. The evaporated compound was ionized by an ionization mechanism such as corona discharge. Gas-phase ions are introduced into the drift tube utilizing an ion gate. The transfer of ions toward the detector depends on their mass, charge, and shape. Some advantages of this technique are high sensitivity, fast response time, no need to vacuum, portability, and low cost. So, IMS has been grown as a popular technique in analytical methods, for 2 decades [19]. Methamphetamine has relatively high proton affinities, and so, the positive mode of IMS could be more suitable for its determination. However, to determine the analyte in the complex matrix by IMS, the matrix interferences are serious because of the competitive gas-phase reactions [20]. To overcome this problem, a novel sample preparation method based on the slug-flow microextraction (SFME) was used for the extraction of methamphetamine before detection by IMS [21]. Additionally, Liu et al. [22] used pyridine compound as a dopant to improve the selectivity of the methamphetamine and interference elimination in the IMS apparatus.

In this work, the DLLME method was coupled with corona discharge ionization (CD)-IMS as a high-speed, simple, and sensitive technique for sample preparation and detection of ultra-trace amounts of methamphetamine. Some experimental parameters affecting the extraction efficiency such as type and volume of extraction and disperser solvents, centrifugation time, and sample solution pH were optimized. To validate the applicability, the mentioned method was used for the analysis of methamphetamine in urine and plasma samples.

### Experimental

#### Reagents and standards

Methamphetamine (97% purity) was obtained from Anti-narcotics Police (Isfahan, Iran). Methanol (HPLC grade), isopropanol, acetonitrile, tetrachloromethane (CCl4), trichloromethane (CHCl3), dichloromethane (CH2Cl2), trichloroacetic acid (TCA), acetic acid (CH3COOH), boric acid (H3BO3), and phosphoric acid (H3PO4) (100, 99.8, and 85% purity) were purchased from Merck Co. (Darmstadt, Germany). Deionized water was produced by OES (Overseas Equipment & Services) water purification system (OK, USA). Stock standard solutions of methamphetamine were provided in methanol with the concentration of 1000 mg/L, and the required standard aqueous solutions were provided daily by dilution of the stock standard solution using deionized water.

#### Instrumentation

In this research, all materials were weighed with the Shimadzu Libror scale (0.1 mg precision scale, model AEU 210, Japan). Also, Iran’s RST 16 centrifuge device, which has a centrifugal capacity of up to 4000 rpm, was used for the extraction procedure. The CD-IMS used for the analysis of methamphetamine, was designed and manufactured by Teif Azmon Espadana Co. (Isfahan, Iran). The instrument consists of the IMS cell (corona discharge ionization source, ion gate, aperture grid, and faraday cup detector), two high voltage power supplies (4 and 10 kV for ionization and drift region, respectively), an injection port, a pulse generator, amplifier, an analog to digital converter and a computer. The IMS cell was housed in an oven 22-L (22-Bahman company, Iran) for heating and setting the temperature of input drift and carrier gases. The oven temperature was adjustable from 25 to 200 °C. The instrumental conditions of CD-IMS in the positive mode of operation are reported in Table S1.

#### Sample preparation

In this study, drug-free human urine and plasma samples were assayed. To prepare the human plasma sample, 4 mL of aqueous trichloroacetic acid (10%, w/v) was added to 2 mL of the plasma, and the resulted mixture was stirred for 20 s. The obtained mixture was cooled for 20 min at the temperature of 4 °C and then, it was centrifuged for 10 min (3000 rpm). To reduce the matrix effects and increase the relative recovery, the supernatant solution was transferred into a 50-mL volumetric flask, and then diluted with a universal buffer solution (pH = 12). After dilution, different concentrations of methamphetamine were spiked. After precipitation of proteins, the supernatant was transferred into an extraction cell and the extraction and disperser solvents were added for extracting of analyte, under the optimal conditions. For urine, 5 mL of the sample was transferred into a 50-mL volumetric flask and diluted with a universal buffer solution. Afterward, the methamphetamine solution was spiked and centrifuged for 10 min (3000 rpm), to separate proteins. Finally, the extraction and disperser solvents were added to the supernatant solution in an extraction cell [23], and the extraction was performed in the optimal conditions.

#### DLLME procedure

To extract the analyte, 750 µL isopropanol (as disperser solvent) and 65 µL trichloromethane (as extraction solvent)
were quickly injected into a 5-mL methamphetamine solution with the concentration of 10 µg/L (pH = 12). The obtained cloudy solution was then centrifuged for 2 min at 3000 rpm to be extracted the target analyte into the fine droplets of trichloromethane. Finally, 5 µL of the extraction phase collected at the vial bottom was directly injected into the CD-IMS for determining the analyte.

Results and discussion

After injecting the analyte extracted by the proposed method, the originated ion cluster peak was appeared at the drift time of 8.80 ms (Fig. S1). The corresponding reduced mobility value \( K_0 \) of this peak was calculated 1.63 cm²/V s which is similar to 1.61 cm²/V s reported previously [24]. The total area under this peak was calculated during the acquisition time and considered as the IMS signal for all the experiments.

Optimization

To determine the trace amount of methamphetamine and increase the extraction efficiency, different parameters such as type and volume of extraction and disperser solvents, pH of the sample solution, and the centrifugation time were investigated. Some parameters such as type of the extraction and disperser solvents, that could affect the IMS signal, are dependent on the IMS apparatus due to the competitive effect between the analyte and solvent molecules to capture proton in the ionization source of IMS. All experiments are repeated three times, and the average values are used in the tables and charts.

Extraction solvent

Selecting a proper organic extraction solvent is one of the main steps for the DLLME method. The extraction solvent is effective on the enrichment factor, extraction recovery, and selectivity. Due to the evaporation problem of light solvents, which deteriorates the repeatability [25], in this work, some extraction solvents with higher density than water were investigated. To this end, three types of water-immiscible organic solvents, including dichloromethane (density, 1.32 g/mL), trichloromethane (density, 1.48 g/mL), and tetrachloromethane (density, 1.58 g/mL) as well as 0.5 mL isopropanol (disperser solvent) were studied. To achieve the same volume of the sedimented phase (about 15 µL), 100, 50, and 30 µL of dichloromethane, trichloromethane, and tetrachloromethane were used, respectively. As can be seen in Fig. 1A, the maximum extraction efficiency is attributed to trichloromethane, due to the lower solubility than dichloromethane in the aqueous phase and more polarization than tetrachloromethane.

Disperser solvent

In DLLME, disperser solvent must be miscible in both aqueous and organic extraction solvents. To select the best type of disperser solvent, 0.5 mL of isopropanol, methanol, acetone, and acetonitrile as well as 50 µL trichloromethane (extraction solvent) were investigated, and the results are depicted in Fig. 1B. This figure shows that isopropanol increases the extraction efficiency by increasing the contact area by emulsification of extraction solvent; therefore, it was used as the disperser solvent.

Volume of extraction solvent

To study the effect of the extraction solvent volume on the extraction efficiency, 0.5 mL of isopropanol and different volumes of trichloromethane (40–80 µL at 10 µL interval) were added to the aqueous sample solution. According to the Fig. 2A, the peak area was increased when the volume of trichloromethane increased from 40 to 65 µL, and then the IMS signal decreased by risen the extraction solvent volume up to 80 µL. In fact, with the lower volumes than 65 µL, the extraction efficiency was reduced due to the dissolution of a large volume of the extracting solvent in an aqueous sample and finally decreasing the formation of extraction solvent droplets. With the volumes higher than 65 µL, the extraction efficiency was reduced because of the dilution effect. Therefore, 65 µL was chosen as the best extraction solvent volume.

Volume of disperser solvent

To investigate the effect of disperser solvent volume on the extraction efficiency, the various volume of disperser solvent (250–1000 µL, at 250 µL interval) were tested. Based on the results shown in Fig. 2B, 750 µL of disperser solvent volume has the highest extraction efficiency. At the low volumes (<750 µL), the cloudy state was not formed completely, and thus, the extraction efficiency decreased. On the other hand, at the high volumes (>750 µL), the analyte solubility in the aqueous solution was increased, and so, the extraction efficiency was decreased.

Sample pH

The effect of solution pH on the extraction efficiency was investigated in the range of 5–12. The pKa of methamphetamine is about 10 [26], thus this compound is an alkaline compound, and methamphetamine is in its protonated form at lower pH values, reducing the extraction efficiency. At the pH values higher than pKa, the analyte is in its molecular
form; so, the extraction efficiency may be improved. The effect of the sample solution pH on the extraction efficiency is illustrated in Fig. S2-A. Based on these results, pH = 12 was selected as the optimum pH value for sample solutions.

Centrifugation time

In the DLLME process, the extraction time is considered as the interval time between the formation of cloudy solution and the end of centrifugation. Since there is a high contact area between the extraction solvent and the aqueous sample solution, the equilibrium state would be obtained quickly [27]. To that end, the centrifugation time was investigated in the range of 1–4 min. According to the results shown in Fig. S2-B, the peak area of methamphetamine signal was increased up to 2 min and then decreased slightly due to further increases extraction phase volume and dilution effect. Consequently, 2 min was chosen as the equilibrium time based on the obtained result.

Evaluation of the method performance

To evaluate the method performance for the analytical applications, the limits of detection (LOD) and
quantification (LOQ), linearity, and the enrichment factor (EF) were calculated. Under the optimized conditions, the linear dynamic range (LDR) was obtained 0.5–18 µg/L with a good determination coefficient ($R^2 = 0.9969$). According to the analytical results, LOD and LOQ were calculated 0.15 and 0.5 µg/L, based on the signal-to-noise ratio of 3 and 10, respectively. To investigate the precision, the intra- and inter-day standard deviation values were calculated at the methamphetamine concentration of 10 µg/L, within 1 day and 3 consecutive days, respectively. The calculated intra- and inter-day standard deviation values were 4 and 10%, respectively. Based on the following equation, the EF parameter was obtained 15.

$$EF = \frac{C_{sed}}{C_o}.$$  \hspace{1cm} (1)

where $C_{sed}$ and $C_o$ are the concentration of methamphetamine in sediments phase and initial concentration in the aqueous sample solution, respectively.

**Field sample analysis**

To study the capability of the proposed methods in field sample analysis, human urine and plasma were studied as the field samples. To remove the effect of the matrix, multiple standard addition method was applied. The ion mobility
Fig. 3 The CD-IMS spectra obtained after injecting the extracted samples from A spiked plasma (25 µg/L), and B spiked urine (10 µg/L) samples.
spectra of a spiked urine sample (10 µg/L), plasma sample (25 µg/L), and blank samples are indicated in Fig. 3, and Table 1 exhibits the analytical parameters. The LOD and LOQ for human urine sample were 0.15 and 0.5 µg/L, and for human plasma sample were obtained 0.6 and 2.0 µg/L, respectively. Additionally, the relative spiking recovery values were calculated by the following equation:

\[
\text{Spikingrecovery(\%)} = \frac{C_{\text{found}} - C_{\text{real}}}{C_{\text{added}}} \times 100, \tag{2}
\]

where \(C_{\text{found}}\), \(C_{\text{real}}\), and \(C_{\text{added}}\) are the concentrations of the analyte after spiking standard sample to the field sample, the concentration of analyte that exists in the field sample, and the spiked standard solution to the field sample, respectively. The spiking recovery values were about 104%; so, no significant matrix effect was indicated in the analysis of methamphetamine in human urine and plasma samples. In this study, methamphetamine was not detected in any investigated field samples.

**Comparison of DLLME-IMS with other methods**

Table 2 shows some figures of merit obtained by the proposed method in comparison with the other methods used for determining methamphetamine. In most reports, GC and HPLC were used for methamphetamine analysis; however,

| References | Recovery (%) | RSDb (%) | LODa (µg/L) | Dynamic range (µg/L) | Sample type | Method |
|-----------|-------------|---------|-------------|----------------------|-------------|--------|
| [2]       | 80          | 5.2     | 1.7         | 10–1000              | Urine       | IL-DLLME-HPLC |
| [3]       | 97–87       | 3.8     | 20          | 100–2500             | Urine       | MSPEc-HPLC-UV |
| [28]      | 97.8        | 7.2     | –           | –                    | Hair        | GOd-EMEe-GC |
| [29]      | 93.9–94.8   | 1.6–2.1 | 0.5         | 2–100                | Urine       | SPDEf-LC/TOF-MS |
| [30]      | 89–103      | 5.2–18.2| 1.5         | 5–100                | Oral fluid  | SPME-DI-MS/MS |
| [31]      | 107–119.3   | 2.4–5.9 | 1           | 5–200                | Oral fluid  | SPME-TMh-DARTi-MS/MS |
| [32]      | 95.5–102.3  | 3.4–5   | 2.5         | 25–200               | Urine       |       |
| [33]      | 86.9        | 6.1     | 0.25        | –                    | Urine       | EEj-SPME-GC-MS |
| [34]      | 107.9       | 1.1     | 0.5         | 1–1500               | Urine       | LLLMEk-HPLC-UV |
| [35]      | 88          | 5.5     | 14          | 50–3500              | Saliva      | MIPl-SPME-GC-FID |
| This study| 104         | 4       | 0.6         | 2.0–15.0             | Plasma      | DLLME-CD-IMS |
|           | 7           | 0.15    | 0.5–18.0    |                      | Urine       |       |

\(a\) Limit of detection  
\(b\) Relative standard deviation  
\(c\) Magnetic solid-phase extraction  
\(d\) Graphene oxide  
\(e\) Electromembrane extraction  
\(f\) Solid-phase dispersive extraction  
\(g\) Direct infusion mass spectrometry  
\(h\) Direct analysis in real-time mass spectrometry  
\(i\) Direct analysis in real-time  
\(j\) Electroenhanced  
\(k\) Liquid–liquid–liquid microextraction  
\(l\) Molecularly imprinted polymer  
\(m\) Microextraction by packed sorbent
these methods have some drawbacks such as long analysis time, used expensive solvent, and derivatization procedures. The time that is consumed for sample pretreatment and detection of analyte was assumed as analysis time and was about 2 min. In addition to rapidity of IMS relative to GC and HPLC techniques, this time is shorter than that for DLLME-GC or DLLME-HPLC due to the lack of derivatization and water removal from the extracted sample. On the other hand, IMS has some advantages such as very fast analysis (≈5 s) with high sensitivity (~pg), portability, low cost, and no need for expensive solvent. Also, at comparing with other extraction methods, DLLME is an inexpensive, simple, and fast method with a relatively high enrichment factor and recovery value. The analytical parameters reported in this work are acceptable, promising a successful determination of ultra-trace amount of methamphetamine in urine and plasma matrix.

Conclusions

The combination of DLLME with CD-IMS appears to be suitable for preconcentration and identification of methamphetamine in the human urine and plasma samples. Comparisons of the method presented here with two widely used chromatographic methods (GC and HPLC) show that the DLLME-CD-IMS combination is good comparable in analytical parameters, but better in response time, cost, portability, and simplicity. In fact, CD-IMS showed an amazing sensitivity for methamphetamine molecules because of high proton affinity by the amine functional group in its structure. But, the dynamic range obtained in this work is lower than that reported by some other methods, might be due to matrix interferences in the ionization source. The described method may be advantageous for rapid field investigations of methamphetamine in biological samples, without any tedious derivatization process.

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