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Combination of dispersive liquid–liquid microextraction and solid–phase microextraction: An efficient hyphenated sample preparation method

Mohammad T. Jafari *, Mohammad Saraji, Mehdi Mossaddegh

Department of Chemistry, Isfahan University of Technology, Isfahan 84156-83111, Iran

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ABSTRACT

Two well-known microextraction methods, dispersive liquid–liquid microextraction (DLLME) and solid–phase microextraction (SPME), were combined, resulting in as an encouraging method. The method, named DLLME–SPME, was performed based on total vaporization technique. For the DLLME step, 1,1,2,2-tetrachloroethane and acetonitrile were used as extraction and disperser solvents, respectively. Halloysite nanotubes–titanium dioxide was used as the fiber coating in the SPME step. The method was applied for the extraction of diazinon and parathion (as the test compounds) in environmental water samples and fruit juices, and gas chromatography–corona discharge ion mobility spectrometry was used as the determination apparatus. Desorption temperature and time, extraction temperature and time, and the volume of the extracting solvent in the DLLME step were optimized as the effective parameters on the extraction efficiency. The relative standard deviations (RSDs) of intra-day were found to be 4–7% and 6–8% for diazinon and parathion, respectively. Also, the RSDs of inter-day were 7–9% and 8–10% for diazinon and parathion, respectively. The limits of quantification and detection were obtained to be 0.015 and 0.005 $\mu g\,L^{-1}$ for diazinon, and 0.020 and 0.007 $\mu g\,L^{-1}$ for parathion. A good linearity range (r^2 □ 0.993) was obtained in the range of 0.015–3.000 and 0.020–3.000 $\mu g\,L^{-1}$ for diazinon and parathion, respectively. The high enrichment factors were obtained as 3150 and 2965 for diazinon and parathion, respectively. This method showed high sensitivity with good recovery values (between 87 and 99%) for the extraction of target analytes in the real samples. Overall, the results revealed that the developed DLLME–SPME method had better extraction efficiency than DLLME and SPME alone.

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1. Introduction

The analysis of compounds at ultra-trace level encouraged the researchers to develop new sample preparation methods in the separation science. In the last two decades, several microextraction methods have been introduced for the sample preparation and preconcentration of various organic and inorganic analytes. Basically, microextraction methods are divided in two general categories: i) sorbent-based techniques such as solid–phase extraction (SPE) [1], micro solid–phase extraction (μ-SPE) [2], stir–bar sorptive extraction [3], and solid–phase microextraction (SPME) [4]; and ii) solvent-based methods such as single–drop microextraction [5], hollow–fiber liquid–phase microextraction [6], and dispersive liquid–liquid microextraction (DLLME) [7].

SPME was introduced by Pawliszyn and co-workers in 1990 [4]. In this method, the analyte is extracted by a sorbent coated on a fiber. Based on the vapor pressure of the target analytes, SPME can be applied in the headspace or immersion mode. The main advantages of SPME include simplicity, the feature of being solvent-free, high enrichment factor, capability of the analysis of analytes in different types of matrices (gas, liquid and solid), in vivo sampling and easy automation. The polarity of the fiber coating can be a quasi-selective parameter for the extraction of polar, semi-polar and non-polar compounds. Therefore, the choice of sorbent phase can offer selectivity in this method. On the other hand, SPME has a few considerable limitations; for example, there are limited polar-sorbent coatings for the extraction of polar analytes [8]; also the addition of salt, existence of non-volatile particles, the use of the organic solvent, and acidic or basic solution may damage the fiber coating. Further, the partitioning of the analyte among the sample, headspace and fiber coating can affect the extraction efficiency [9,10]. In complex matrices (e.g. foodstuff and biological samples),

* Corresponding author.
E-mail address: jafari@cc.iut.ac.ir (M.T. Jafari).

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the macro molecules and other particles are immobilized on the surface of the fiber coating. This effect lead to damaging the coating structure and/or loss of some adsorption sites on the fiber coating, finally decreasing the extraction efficiency. Despite the above mentioned limitations, the high recovery in complex matrices with low relative standard deviation (RSD) can be regarded as the important features that make SPME a favorable method in analytical chemistry.

Dispersive liquid–liquid microextraction as a high-performance technique was introduced by Assadi and co-workers in 2006 [7]. Briefly, in this method, a low volume of extraction and disperser solvents is mixed and rapidly injected into an aqueous sample. A cloudy solution is formed and then the solution is centrifuged. So, analytes are extracted with small volume of the extraction solvent. High enrichment factor and clean-up efficiency, short extraction time and easy performance are the main advantages of this method. Based on the density of the solvent extraction, DLLME can be performed using both low-density and high-density solvents [11,12]. Besides the good advantages, DLLME is suffering from some limitations. The matrix effect and the use of toxic solvents are the main drawbacks of DLLME; the particles or non-volatile compounds can be introduced into the analytical instrument by liquid injection, resulting in instrumental malefaction. Additionally, after the centrifuge step, the collected extraction solvent is about 10–50 μL and just a portion of it (~1 μL) can be injected to the detection system. This results in a considerable reduction of sensitivity, thereby making analysis more challenging, especially in ultra-trace scales in complex samples. Generally, most studies in the field of sample preparation have been published annually with the subjects of DLLME and SPME. According to the privileges and capabilities of the two described methods, it can be of interest to have the advantages of both methods together, as a novel designed method, while their drawbacks can be lessened. To promote the extraction and clean-up capability of the sample preparation step, a few combined methods such as solid–liquid phase microextraction (SLME) and combination of DLLME with SPE and μ-SPE as sorbent-based extraction techniques have been previously reported for the extraction of different compounds in various samples [13–15]. SLME has advantages like high enrichment factor, easy performance, feature of being no memory effect and no stripping of the coating. However, this method suffers from the limitations for the selection of solid sorbent and analysis of high volatile compounds. Also, the main benefits of DLLME with SPE and μ-SPE are high enrichment factor and high clean up capability. But, the challenges of two mentioned methods are use of large sample and solvent volumes, multi-step procedure and need the vacuum. More importantly, using the large volumes of toxic organic solvents are risky and unfriendly for the environment. Therefore, it is desirable to develop a combined method to obtain a better extraction efficiency with green aspects.

The aim of this study was combining the DLLME and SPME techniques as a new powerful hyphenated sample preparation method to improve the extraction efficiency. The combined method (DLLME–SPME) has a higher selectivity (related to DLLME) because of using the solid-sorbent in SPME step, and higher clean-up capability (related to SPME) by performing the DLLME procedure before the SPME step. By total vaporization procedure, the partitioning between the liquid sample and headspace is eliminated, and analyte will be totally existence at the headspace. Also, we have no problematic liquid direct injection. Diazinon and parathion as organophosphorus pesticides (OPPs) were selected as the model compounds. Halloysite nanotubes–titanium dioxide (HNT–TiO_2) fiber was used for SPME experiments. Gas chromatography–corona discharge ion mobility spectrometry (GC–CD–IMS) was also applied for the separation and quantification of the extracted analytes. The effective parameters on the extraction efficiency, such as collected solvent volume in the DLLME procedure, extraction temperature and extraction time in SPME step, were investigated and optimized. The feasibility and performance of the present method were evaluated in environmental and wastewater samples.

Table 1
Instrumental parameters for CD–IMS.

Parameter	Setting
Needle voltage	11.70 kV
Target electrode voltage	9.00 kV
Drift field	500 V cm^{-1}
Drift gas flow (N_2)	700 mL min^{-1}
Make-up gas flow (N_2)	20 mL min^{-1}
Drift tube temperature	200 °C
Shutter grid pulse	0.2 ms
Number of IMS averages	25
Number of points per ion mobility spectrum	500

2. Experimental

2.1. Chemicals and materials

Diazinon was purchased from Accustandard, Inc. (New Haven, USA). Parathion, halloysite nanotubes and titanium isopropoxide (TTIP) were obtained from Sigma-Aldrich (St. Louis, USA). 1,1,2,2-tetrachloroethane (1,1,2,2-TCE) (99%), tetraethoxysilane (TEOS), isopropyl alcohol, nitric acid (HNO_3), hydrochloric acid (HCl), methanol (HPLC grade) and sodium chloride (NaCl) (99.5%) were purchased from Merck (Darmstadt, Germany). Methyltrimethoxysilane (MTMOS) was supplied by Fluka (Buchs, Switzerland). Acetonitrile (ACN) was purchased from Caledon Laboratories (Georgetown, ON, Canada). Ethanol was purchased from Bidestan Co. (Qazvin, Iran). Pure water was prepared by OES (Overseas Equipment & Services) water purification system (OK, USA). Stock standard solutions of diazinon and parathion (1000 mg L^{-1}) were produced in methanol. A mixture of standard working solutions with the concentration of 10 mg L^{-1} was prepared. Working standard solutions were prepared by appropriate stepwise dilution of the standard mixture solution using pure water daily.

2.2. Instrumentation

The GC–CD–IMS used for this research was designed and constructed at Isfahan University of Technology. The instrumental details of CD–IMS have been described previously [16]. The main parts of CD–IMS are a cell equipped with the corona discharge needle, two high voltage power supplies, a pulse generator, an analog to digital converter and a computer. The instrumental conditions of the IMS in this research are tabulated in Table 1.

The GC was carried out using a Shimadzu (model 14A, Kyoto, Japan) fitted with a split/splitless injector. GC separation was performed with a capillary column (Agilent, HP-5, 30 m by 0.32 mm i.d., and 0.5-μm film thickness, Palo Alto, CA, USA). Nitrogen was used as the carrier gas and set at 1 mL min^{-1}. The temperatures of the injector and detector (IMS) were set at 260 and 200 °C, respectively. The column was held at the initial temperature of 70 °C for 1 min, and this was followed by a linear thermal gradient of 15 °C min^{-1} to 220 °C (held for 1 min), resulting in a run time of 12 min.

2.3. SPME fiber preparation

The SPME fiber used in this research had been developed previously at our research group [17]. In the first step, for the preparation of HNTs–TiO_2 heteroarchitecture, a solution of 0.5-mL of TTIP, 7.5-mL of isopropyl alcohol and 22.5-mL of HNO_3 2 mol L^{-1} was prepared and stirred at room temperature for 1 h to form a homogenous solution. After that, the solution was diluted to 125 mL by

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adding the pure water. The amount of 0.5 g of HNTs was added to the prepared solution and stirred for 2 h. The produced mixture was held on the heater/stirrer at 65 °C for 24 h to form a HNTs–TiO_2 heteroarchitecture. Then, the mixture including the heterogeneous HNTs–TiO_2 was centrifuged at 2500 rpm for 10 min. The precipitate was washed with pure water and dried in oven at 120 °C for one day.

In the second step, for the production of SPME fiber, the 3-cm long fused-silica fiber (0.34 mm o.d.) was cut and the surface of the tip end (1 cm) was burned to remove the elastic polyimide layer. The fiber was rinsed with acetone and water, and then located into the NaOH solution (1 mol L^{-1}) to active the silanol groups on the surface of the fused-silica fiber. After 1 h, the fiber was inserted into the 0.1 mol L^{-1} HCl solution for 30 min to neutralize the remaining NaOH on the fiber surface. Then, it was rinsed with pure water and dried in a desiccator. The HNTs–TiO_2 fiber was prepared using the sol–gel technique as follows: 100-μL TEOS, 200-μL MTMOS, 150-μL ethanol, 50-μL water, 25-μL HCl, and 20 mg HNTs–TiO_2 were mixed in a 2-mL vial and stirred for 2 h. To form the gel on the activated surface of the fiber, the treated fused silica was dipped vertically into the sol solution. After 1 min, the coated-fiber was withdrawn from the produced gel for further re-coating cycles (three times). Then, the fiber was placed in a desiccator for 1 day to form a silica-based network. In order to accomplish the thermal conditioning, the prepared fiber was inserted into the injection port of GC under a mild flow of nitrogen gas at 100 and 280 °C for 1 and 2 h, respectively. The characterization of the HNTs–TiO_2 (scanning electron microscopy and thermo-gravimetric graph) has been reported previously [17].

2.4. DLLME–SPME procedure

The DLLME step was performed according the research previously reported [18]. Briefly, 5-mL of sample solution at the concentration of 2 μg L^{-1} containing 0.25 g NaCl was transferred into a 10-mL home-made glass tube with a conical bottom. 30 μL 1,1,2,2-TCE, as the extraction solvent, was added to 1.5-mL of ACN as the disperser solvent, and the final mixture was rapidly injected into the aqueous sample to form a cloudy solution. The resulted mixture was centrifuged at 5000 rpm for 5 min. The sediment organic phase containing the extracted OPPs was withdrawn by a 50-μL microsyringe, and transferred to a 25-mL glass vial equipped with the septum to start the SPME step. For the headspace SPME procedure, the HNTs–TiO_2 fiber was inserted into the vial, helping us to extract the analyte inside the vial. In order to ensure the total vaporization of the analyte, the vial was placed in an oil-bath at 120 °C. In this condition, the solvents and the extracted OPPs were totally vaporized, before performing the SPME. After 30 min, the fiber was withdrawn from the vial and immediately inserted into the injection port of GC–IMS at 260 °C for desorption and subsequent analysis.

2.5. Real samples

River and lake waters were collected from Zayandeh-Rood, Isfahan, Iran. Agricultural wastewater was collected from Dourcheh area, near the Isfahan city. Well water was taken from Asgharabad village (Khomeini-Shahr, Iran). The water samples were filtered through a 0.45 μm nylon membrane filter (Millipore, Bedford, MA, USA) and stored at room temperature. Apple and grape juices were purchased from local supermarkets (Isfahan, Iran). The fruit juice samples were diluted at a ratio of 1:2 with pure water before the analysis.

3. Results and discussion

3.1. Total vaporization

In the total vaporization, as a headspace-based procedure, the solution containing the analytes was completely vaporized and transferred into the headspace. This step is necessary for obtaining the satisfactory results for the RSD and the extraction efficiency. Recently, the volume of some organic solvents can be completely vaporized as a function of temperature, as calculated by Rainey et al. [19]. They used the combination of ideal gas law and Antoine equation as:

$$V_0 = \left(\frac{(10^{A-B/T+C})V_V}{RT}\right)\left(\frac{M}{\rho}\right) \quad (1)$$

In this equation, V_0 is the volume of sample (mL), V_v is the volume of the vial (L), R is the ideal gas constant (8.3145×10^{-2} L bar K^{-1} mol^{-1}), T is the temperature (K), M is the molar mass of the solvent (g mol^{-1}), and ρ is the density of the solvent (g mL^{-1}) at the temperature at which it is placed in the vial. A, B, and C are Antoine constants for the solvent. Their results showed that the solvents with high molar mass and low density could be more vaporized in the defined volume. Although 1,1,2,2-TCE (as the extraction solvent used in this study) is a high-density solvent, it can be an appropriate organic solvent according to the Eq. (1), due to its high molecular weight (167.848 g mol^{-1}). By replacing the experimental condition in Eq. (1) and obtaining the Antoine constant (the NIST Chemistry WebBook), V_0 was calculated over 75 μL. By considering the 30 μL solvent as a volume extracting solvent in this study, it could be concluded that the total vaporization occurred for 1,1,2,2-TCE in the SPME step.

3.2. Improving method efficiency

Very recently, George et al. reported that the volatility of analytes (especially for semi and/or non-volatile analytes) was improved by adding a little μL of the organic solvent into the aqueous media [20]. In other words, the headspace sampling for analytes at the conditions used in this study (higher temperature and the presence of organic solvent) would be simpler and more effective. Consequently, the partitioning of sample solution and headspace could be eliminated using this combination, thereby enhancing the extraction efficiency. Moreover, when the solvent was totally evaporated, some amount of solvent molecules would be adsorbed on the fiber coating and could act as an adsorption site on the fiber surface, in addition to the sorptive coating [19].

One of the most important parameters for each separation technique is selectivity. In the DLLME–SPME method, it is possible to select different types of both SPME fiber and DLLME extraction solvent. This can lead to a better selectivity in the extraction of analytes, especially in a complex matrix. In this proposed method, there is no direct liquid injection to detection instrument, so all the non-volatile compounds will remain in SPME vial. Finally, DLLME–SPME approach can keep the advantages of both methods, such as simplicity, inexpensiveness, low extraction time and high recovery.

3.3. Optimization of DLLME–SPME method

Some effective parameters on the extraction efficiency of DLLME–SPME method, such as the volume of the solvent in the DLLME step (SPME sample volume), extraction temperature and time (in SPME step), desorption temperature and time, were evaluated and optimized.

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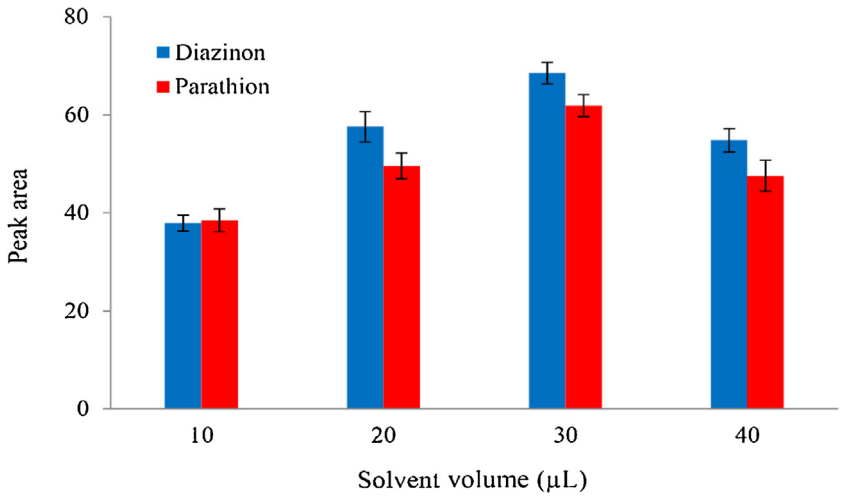

Fig. 1. The Effect of extraction solvent volume on the extraction efficiency of OPPs. (concentration of analytes, 5.0 μg L^{-1}; extraction temperature, 75 °C; extraction time, 20 min; desorption temperature, 260 °C and desorption time, 5 min).

3.3.1. Extraction solvent volume

Generally, in the DLLME procedure, the volume of extraction solvent is an important effective parameter on the extraction efficiency. At the low solvent volume, the capability of disperser solvent is decreased and the cloudy solution cannot be formed. On the other hand, at high solvent volume, the extracted analyte is diluted and therefore, the enrichment factor is decreased [21]. In this method, the extraction solvent volumes of 10, 20, 30, and 40 μL were evaluated for the SPME step. As can be seen in Fig. 1, the extraction efficiency was enhanced when the solvent volume was increased up to 30 μL. This considerable enhancement of peak area was probably due to the immobilization of the solvent on the coated surface, acting as the analyte adsorbent. This is known as the solvent effect, as in gas chromatography technique. By using an extraction solvent volume higher than 30 μL, the extraction efficiency was decreased, possibly due to the decrease of enrichment factor in the DLLME step.

3.3.2. Extraction temperature

The extraction temperature is a key parameter in the optimization of DLLME–SPME method. Herein, the temperature should be high enough so that total vaporization would occur. Furthermore, increasing the temperature leads to reducing the partition constant of the analyte between the headspace and fiber coating [22]. Therefore, the extraction temperature as a contrasting factor was investigated from 60 to 150 °C. Based on the obtained results (data not shown), the maximum peak area was obtained at 120 °C and therefore, 120 °C was selected as the optimum extraction temperature.

3.3.3. Extraction time

SPME is an equilibrium-based method that has been explained by Nernst's partition Law [23]. The extraction time means the time required for extracting the maximum amount of analytes by the coated fiber while there is no significant variation in the extraction efficiency. Accordingly, the extraction time was investigated from 10 to 40 min. Based on the obtained results (data not shown), the peak area of diazinon was increased up to 30 min and then reached to an almost constant value. Although the extraction time was obtained to be 40 min for parathion, 30 min was selected as the optimum extraction time for this compound due to the lower standard deviation value, in addition to the shorter time related to 40 min.

3.3.4. Desorption condition

Desorption time and temperature on the analyte response in GC–CD–IMS were also investigated. In the constant desorption time of 5 min, the temperature of the injection port was changed in the range of 200 to 280 °C. The responses of analytes were increased by enhancing the temperature up to 260 °C, without observing any carry–over. In order to optimize the desorption time, the fiber was held inside the injection port of GC in the range of 1–6 min. Based on the obtained results (data not shown), the peak area was increased and reached to its maximum value in 5 min. Therefore, the desorption procedure was performed at 260 °C for 5 min for further experiments.

3.4. Comparison of DLLME–SPME with DLLME and SPME alone

In order to evaluate the propriety of the proposed method, the extraction efficiency of method was compared with single DLLME and SPME methods at the concentration of 2.0 μg L^{-1}. The results shows (data not shown) the peak areas of DLLME, SPME and DLLME–SPME methods as obtained for diazinon 34 (±5), 70 (±5) and 138 (±3), respectively. Also, the peak area values for parathion were calculated 29 (±3), 59 (±5) and 108 (±4) for DLLME, SPME and DLLME–SPME methods, respectively. The DLLME extraction conditions for OPPs were applied according to that previously reported by Farajzadeh et al. [18]. The SPME extraction conditions were also applied based on our recently published research [17]. Based on these results, the extraction efficiency values of the proposed method (DLLME–SPME) were two and three times better than those obtained by SPME and DLLME alone, respectively. In addition, comparison of the proposed method (DLLME-SPME) with other related combination methods such as DLLME-μSPE, SLME, and SPE-DLLME are tabulated in Table 2.

3.5. Method validation

To evaluate the capability of the method, the figures of merit, including the limit of detection (LOD), the limit of quantification (LOQ), linear dynamic range (LDR) and precision, were calculated for the analysis of OPPs compounds under the optimized extraction conditions (Table 3). The ion mobility spectra of diazinon and parathion are shown in Fig. 2a. The typical GC–CD–IMS chromatogram obtained after the extraction of a water sample spiked with the OPPs (at 0.020 μg L^{-1}) is also shown in Fig. 2b. To obtain the chromatogram of analytes, the drift time range between 8.80

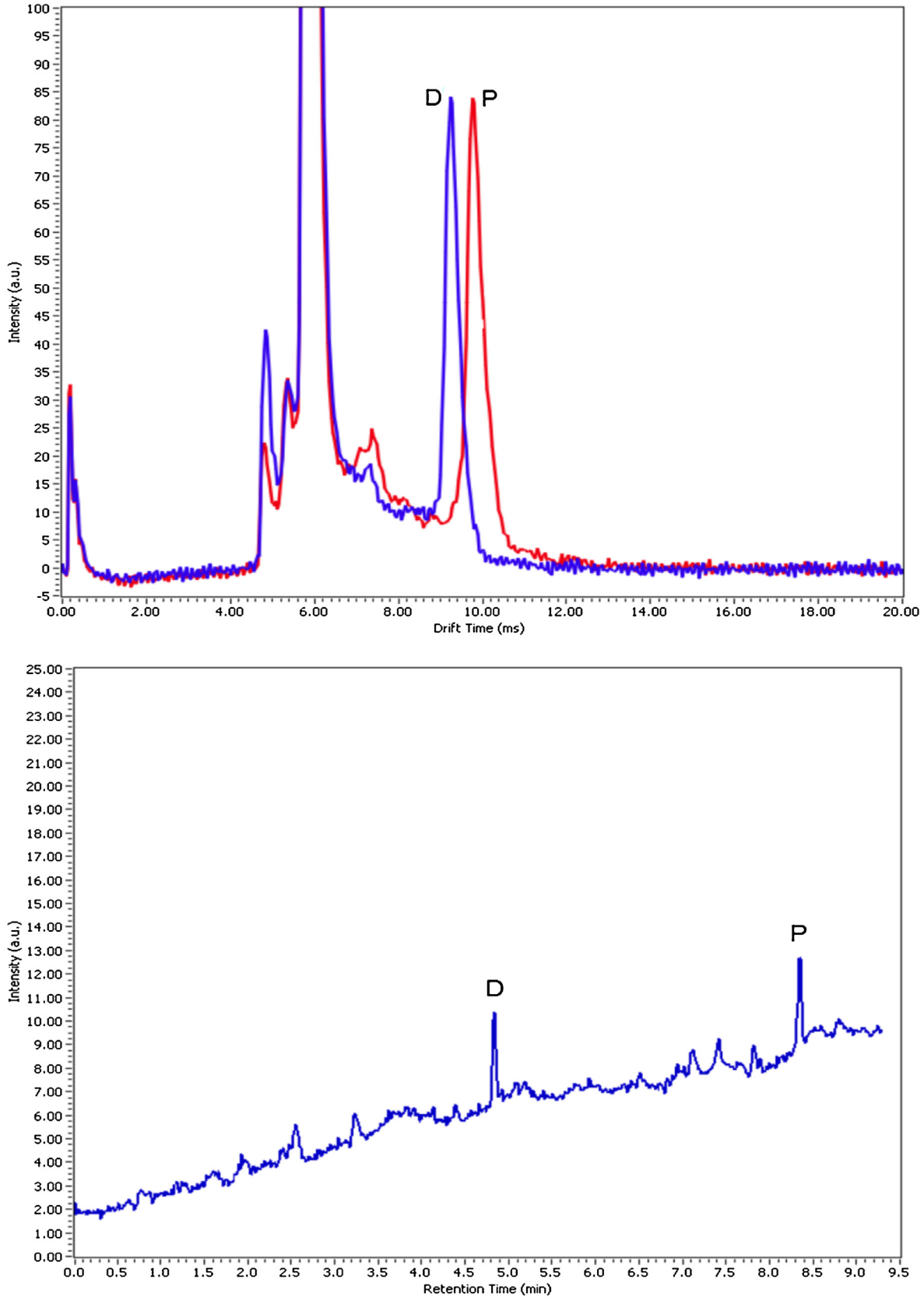

Fig. 2. The ion mobility spectra obtained after injection of the standard solutions of OPPs. (D) diazinon, (P) parathion (above). Typical GC–CD–IMS chromatogram of extracted OPPs from aqueous solutions (0.020 $\mu g\,L^{-1}$) obtained by DLLME–SPME method (bottom).

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Table 2
Comparison of DLLME–SPME method with other related combination methods.

Combined Methods	Advantages	Drawbacks	Reference
Dispersive μ-SPE[a]	High enrichment factor	Use of large sample volume Multi-step procedure	[32]
SPE–DLLME	High enrichment factor High clean up capability	Use of large sample volume Use of large solvent volume Multi-step procedure Need the vacuum	[29]
SLME[b]	High enrichment factor Easy performance No memory effect No stripping of the coating	Limitation from the solid sorbent selection Limitation from the analysis of high volatile compounds	[15]
DLLME–SPME	High enrichment factor Easy performance Low solvent volume Use of vast variety of solid sorbent No direct liquid injection No damage the solid sorbent by sample matrix Total vaporization procedure Use of solvent on the coating surface as an excess sorbent	Limitation from the analysis of nonvolatile analytes Stripping of the SPME fiber coating	This study

[a] Micro solid phase extraction.
[b] Solid–liquid phase microextraction.

Table 3
Analytical parameters obtained for analyzing the pure water spiked with selected OPPs.

Compound	Linear range ($\mu g L^{-1}$)	Determination coefficient (r^2)	LOQ[a] ($\mu g L^{-1}$)	LOD[b] ($\mu g L^{-1}$)	Enrichment factor	Repeatability (RSD%)[c]							
						Intra-day[d]				Inter-day[d]			
						LOQ[e]	0.1	0.5	3.0	LOQ	0.1	0.5	3.0
Diazinon	0.015-3.000	0.994	0.015	0.005	3150	4	5	5	7	8	8	7	9
Parathion	0.020-3.000	0.995	0.020	0.007	2965	6	8	7	8	9	10	9	8

[a] Limit of quantification.
[b] Limit of detection.
[c] Relative standard deviation.
[d] Intra-day and inter-day precision were calculated by analyzing water samples within one day ($n = 3$) and over a period of three days ($n = 3$), respectively.
[e] Concentration of analytes spiked in pure water ($\mu g L^{-1}$).

and 10.80 ms was selected. To evaluate the LDR of the method, the spiked water samples at different concentrations of OPPs were prepared and the extraction was carried out based on the procedure mentioned above. The linear dynamic range was obtained with good linearity ($r^2 > 0.993$) in the range of 0.015–3.000 and 0.020–3.000 $\mu g L^{-1}$ for diazinon and parathion, respectively. The LOQs and LODs were calculated based on the signal-to-noise ratio (S/N) of 10:1 and 3:1, respectively. The LODs for diazinon and parathion were calculated to be 0.005 and 0.007 $\mu g L^{-1}$, respectively (Table 3). By using a single fiber, the repeatability values within one day (intra-day) and over a period of three days (inter-day) were evaluated for the spiking solution, which was LOQ, low, medium, and high concentrations (LOQ, 0.1, 0.5 and 3.0 $\mu g L^{-1}$) in each real samples ($n = 3$). The intra-day RSDs were calculated to be 4–7% for diazinon, and 6–8% for parathion. Also, the inter-day RSDs were obtained to be 7–9% and 8–10% for diazinon and parathion, respectively. The enrichment factor (EF), was defined as the ratio of the concentration of analyte after extraction to the initial analyte concentration spiked in the solution. The enrichment factor values were obtained to be 3150 and 2965 for diazinon and parathion, respectively.

3.6. Real sample analysis

In order to evaluate the method trueness, the OPPs were extracted and analyzed in environmental waters as real samples. Diazinon and parathion were not detected in the water and juice samples; however, these samples were spiked with the analytes at two concentration levels of 0.1 and 1.0 $\mu g L^{-1}$. For comparison, the chromatograms obtained for the spiked and un-spiked analytes related to agricultural wastewater sample are shown in Fig. 3. In order to investigate the method validation, the spiking recovery values were calculated according the following equation:

$$\text{Spiking recovery}(\%) = (C_{found} - C_{real})/C_{added} \quad (2)$$

Herein, C_{found}, C_{real} and C_{added} are the concentration of analyte after the addition of a known amount of standard in the real sample, the concentration of the analyte in the real sample, and the concentration of a known amount of the standard spiked to the real sample, respectively. It must be noted that $C_{real} = 0$ for all the water samples. The summarized quantitative results are listed in Table 4. The spiking recovery values were obtained to be between 87 (±6) to 99% (±6) for water and wastewater samples, and between 88 (±7) to 95% (±8) for juice samples. The high spiking recovery values revealing that no significant matrix effect was observed in the real samples. Consequently, the proposed method showed satisfactory accuracy and good capability in the analysis of OPPs in water and juice samples.

3.7. Quality control and quality assurance

To verify the reliability of analytical data, a proper quality assurance and quality control (QA/QC) procedures are applied for the measurement results. One of the approaches of QA/QC procedures is the estimation of analytical measurement uncertainty. The main sources of uncertainty in the chromatographic analysis are

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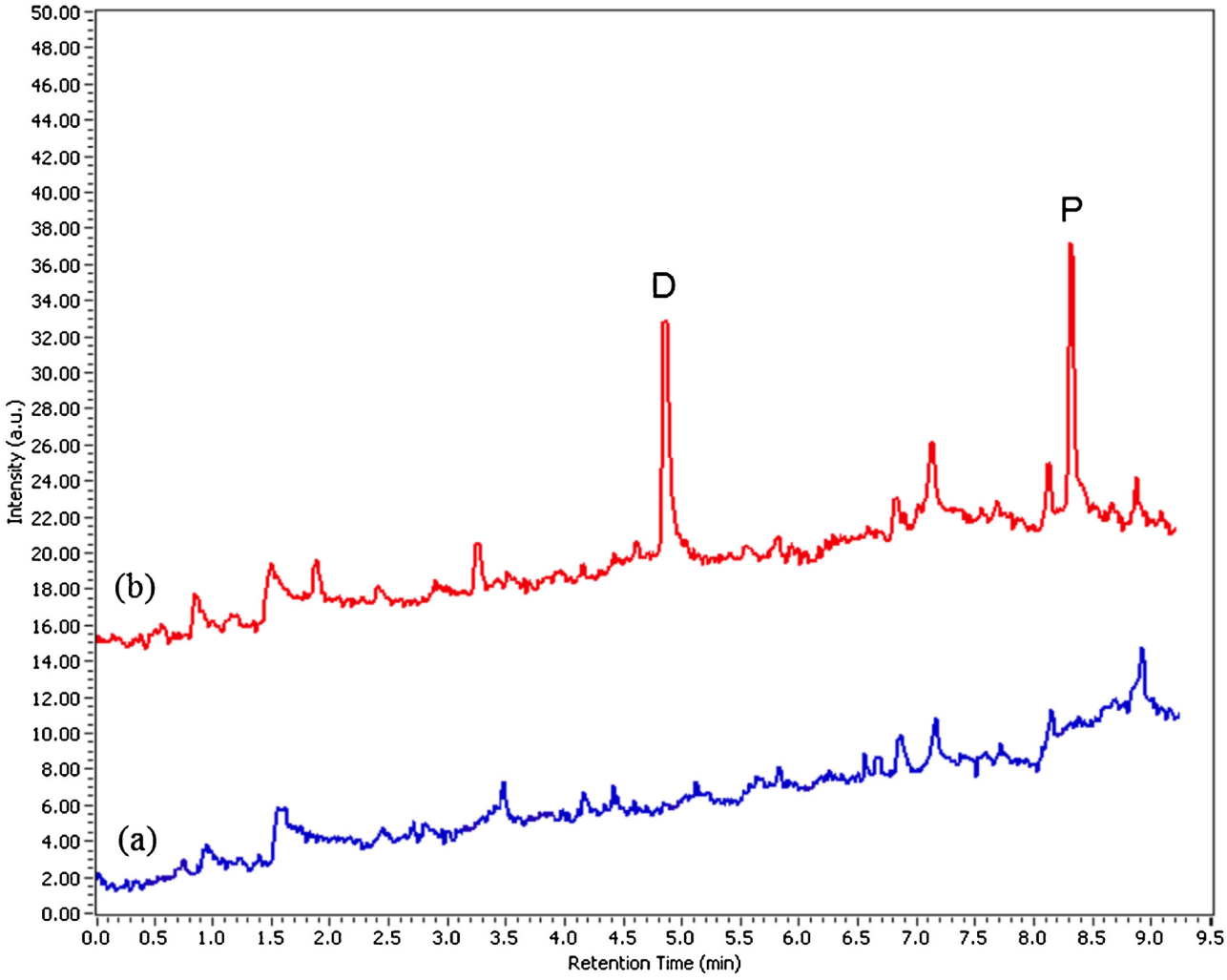

Fig. 3. The GC–CD–IMS chromatograms obtained after extraction of OPPs from agricultural wastewater; (a) blank and (b) spiked sample (0.1 μg L^{-1}). (D) diazinon (P) parathion.

including the sample amount, recovery values, repeatability, and concentration related to the upper than LOD [24,25]. The amount of sample term is related to pick up the volumes of 5 mL (for DLLME step) and 30 μL (for SPME step). The relative uncertainty of sample volume is calculated by the following equation:

$$u_r(v_s) = \frac{\left(\frac{Tol_{vol}}{\sqrt{3}}\right)}{v} \quad (3)$$

where Tol_{vol} is the tolerance obtained from the literature supplied with the catalogues of the pipette and microsyringe and V is the sample volume.

The relative uncertainty corresponded to recovery was assessed from three replicated experiments for the spiked solution at selected concentration levels (0.1 and 1.0 μg L^{-1}) and was evaluated by following equation:

$$u_r(c_{recovery}) = \frac{\left(\frac{SD_{rec}}{\sqrt{n_{rec}}}\right)}{R_{rec}} \quad (4)$$

where SD_{rec} is the standard deviation of recoveries evaluated by three replicate experiments, n_{rec} is the number of replicated analyses, and R_{rec} is the mean of the recovery obtained.

The relative uncertainty associated to the repeatability $u_r(rep)$ can be calculated by:

$$u_r(rep) = \frac{\left(\frac{SD_{rep}}{\sqrt{n_{rep}}}\right)}{R_{rep}} \quad (5)$$

where SD_{rep} is the standard deviation of recoveries evaluated by three replicate experiments, n_{rep} is the number of replicated analyses, and R_{rep} is the mean of the recovery obtained.

The uncertainty associated with analyte at LOD concentration were estimated as follow:

$$u_r(LOD) = \frac{LOD}{C_{det}} \quad (6)$$

C_{det} is the concentration evaluated for obtaining the LOD.

Finally, based on the separate mentioned uncertainties, the relative combined uncertainty of results can be formulated by:

$$u_r = \sqrt{(u_r(v_s))^2 + (u_r(c_{recovery}))^2 + (u_r(rep))^2 + (u_r(LOD))^2} \quad (7)$$

Also, the expanded uncertainty, U, is calculated by:

$$U = kxu_r \quad (8)$$

U is the expanded uncertainty, factor k is the normal distribution of the measurement which provides an approximate confidence level of 95% (usually $k = 2$) [24,25]. As can be seen in Table 4, the expanded uncertainty (U) values determined by DLLME–SPME–GC–IMS were

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Table 4
The analytical results obtained for real samples using DLLME-SPME–GC–CD–IMS.

Sample	Compound	Amount added ($\mu g\,L^{-1}$)	Amount found ($\mu g\,L^{-1}$)	Spiking recovery [a] (%)	Uncertainty					
					u_r (sample)[c]	u_r (recovery)[d]	u_r (rep)[e]	u_r (LOD)[f]	u_r[g] (%)	U[h] (%)
River water	Diazinon	0.10	0.09 (5)[c]	98 (4)[b]	0.020	0.023	0.030	0.020	4.7	9.4
	Parathion	0.10	0.09 (6)	97 (5)	0.020	0.029	0.036	0.028	5.7	11.5
	Diazinon	1.00	0.99 (7)	99 (6)	0.020	0.036	0.043	0.020	6.2	12.5
	Parathion	1.00	0.98 (6)	98 (5)	0.020	0.031	0.032	0.028	5.6	11.2
Lake water	Diazinon	0.10	0.09 (4)	93 (3)	0.020	0.019	0.021	0.020	4.0	8.0
	Parathion	0.10	0.09 (6)	90 (4)	0.020	0.024	0.033	0.028	5.3	10.6
	Diazinon	1.00	0.95 (7)	95 (4)	0.020	0.026	0.039	0.020	5.4	10.9
	Parathion	1.00	0.89 (8)	89 (6)	0.020	0.037	0.044	0.028	6.7	13.4
Well water	Diazinon	0.10	0.09 (6)	99 (4)	0.020	0.028	0.032	0.020	5.1	10.2
	Parathion	0.10	0.09 (7)	98 (5)	0.020	0.032	0.039	0.028	6.1	12.2
	Diazinon	1.00	0.98 (4)	98 (3)	0.020	0.020	0.021	0.020	4.0	8.1
	Parathion	1.00	0.97 (4)	97 (5)	0.020	0.028	0.022	0.028	4.9	9.9
Agricultural wastewater	Diazinon	0.10	0.08 (7)	88 (5)	0.020	0.030	0.039	0.020	5.6	11.3
	Parathion	0.10	0.09 (5)	90 (7)	0.020	0.041	0.027	0.028	5.9	11.9
	Diazinon	1.00	0.87 (8)	87 (6)	0.020	0.036	0.044	0.020	6.3	12.6
	Parathion	1.00	0.89 (6)	89 (5)	0.020	0.028	0.034	0.028	6.2	12.5
Apple juice	Diazinon	0.10	0.09 (4)	95 (8)	0.025	0.046	0.022	0.020	6.0	12.0
	Parathion	0.10	0.09 (6)	94 (8)	0.025	0.045	0.033	0.030	6.8	13.6
	Diazinon	1.00	0.94 (7)	94 (6)	0.025	0.036	0.038	0.020	6.1	12.2
	Parathion	1.00	0.93 (6)	93 (4)	0.025	0.025	0.033	0.030	5.6	11.2
Grape juice	Diazinon	0.10	0.09 (4)	91 (5)	0.025	0.030	0.022	0.020	4.9	9.8
	Parathion	0.10	0.09 (7)	90 (6)	0.025	0.037	0.039	0.030	6.6	13.2
	Diazinon	1.00	0.89 (4)	89 (9)	0.025	0.053	0.021	0.020	6.5	13.0
	Parathion	1.00	0.88 (5)	88 (7)	0.025	0.043	0.028	0.030	6.4	12.8

[a] Spiking recovery was calculated by analyzing real samples spiked with 0.10 and 1.00 $\mu g\,L^{-1}$.
[b] Relative standard deviation, RSD (%).
[c] Relative standard uncertainty of sample volumes.
[d] Relative standard uncertainty of recovery.
[e] Relative standard uncertainty of repeatability.
[f] Relative standard uncertainty of limit of detection.
[g] Relative combined uncertainty.
[h] Expanded uncertainty (k = 2).

Table 5
Comparison of DLLME–SPME method with other methods used for the determination of OPPs.

Method	Sample type	Dynamic range ($\mu g\,L^{-1}$)	LOD ($\mu g\,L^{-1}$)	RSD (%)	Recovery (%)	Reference
DI–SPME–GC–MS	Groundwater	0.05–250	0.02	4.6–7.3	77–95	[26]
HS–SPME–GC–MS	River water and natural water	0.05–1	0.010–0.035	8–10	83–124	[27]
SBSE[a]–GC–FPD[b]	Lake water and pond water	0.2–100	0.047	5.9–8.9	94–115	[28]
SPE[c]–DLLME–GC–FPD	Well water and farm water	0.001–10	0.0003	3.6–6.5	98–106	[29]
DLLME–GC-FID	Water and fruit juice	2.60–1000	0.85	4.4	84–98	[30]
SDME[d]–GC–FPD	Orange juice	10–500	1.20–1.48	5–11.8	76–107	[31]
μSPE–HPLC–UV	Tea drinks	1–200	0.01	6.6–10.4	86–109	[32]
DLLME–SPME–GC–CD–IMS	Water, wastewaterFruit juices	0.015–3.0000.030–3.000	0.005–0.0070.010–0.015	5–9 4–9	87–9988–95	This study

[a] Stir bar sorptive extraction.
[b] Flame photometric detector.
[c] Solid phase extraction.
[d] Single drop micro extraction.

obtained between 8.0 and 13.0% for diazinon, and in the range of 9.9 to 13.6% for parathion. The satisfactory uncertainty values revealed the reliability of the analytical information obtained by the method.

3.8. Comparison of DLLME–SPME method with other methods

The analytical parameters of the method were compared with other previously published studies for the determination of OPPs in water and juice samples (Table 5). The LODs and the linear dynamic ranges are lower than those obtained by the most methods. The RSD values are comparable with the RSDs reported by other studies. Therefore, it can be said that the DLLME–SPME method is a suitable method for the preconcentration and microextraction of OPPs in targeted real samples.

4. Conclusions

In this work, two of the most applicable microextraction methods, namely, DLLME and SPME, were satisfactory coupled. Gas chromatography corona discharge–ion mobility spectrometry was carried out as a hyphenated instrument for the separation and determination of OPPs. The proposed method, named DLLME–SPME, was performed based on total vaporization procedure. HNTs–TiO_2 was prepared by the sol–gel technique and used for the SPME coating. The proposed method was compared with DLLME and SPME methods separately. The method was used for the extraction of diazinon and parathion in river water, well water, lake water and agricultural wastewater. The extraction efficiency of method was two and three times better than that of SPME and DLLME alone, respectively. Since the DLLME step had a short extrac-

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tion time (generally 5–10 min), there was no significant increase in the procedure time in the new method (compared to the SPME alone). The analysis time from receiving the sample to the final data analysis was less than 1 h. By combining the DLLME with SPME, The enrichment factors would be dramatically enhanced. The method was easy to performance. The satisfactory precision make the DLLME–SPME as a reliable analytical technique. In the proposed method, we have no direct liquid injection difficulties, resulting an analysis with lower interferences. The DLLME–SPME could be regarded as a capable and high-effective extraction method that could be developed for a wide range of analytes with volatile and semi-volatile solvents.

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