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RESEARCH ARTICLE

Trace determination of triazine herbicides in fruit and vegetables using novel hydrophobic deep eutectic solvent-based dispersive liquid-liquid microextraction followed by high-performance liquid chromatography-ultraviolet

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In the present research, a novel hydrophobic deep eutectic solvent-based dispersive liquid-liquid microextraction technique was established and combined with high-performance liquid chromatography-ultraviolet for the determination of triazine herbicides in fruit and vegetable samples. A deep eutectic solvent was synthesized using *l*-menthol as a hydrogen bond acceptor and ethylene glycol as a hydrogen bond donor and used as a green extractant. The characterization of deep eutectic solvent was investigated by Fourier-transform infrared, nuclear magnetic resonance, and thermogravimetric analysis. Under the optimum conditions, relative standard deviation values for intra-day and inter-day of the method based on seven replicate measurements of 50.0 μg/kg of triazines were in the range of 2.8%–5.5% and 3.7%–7.2%, respectively. The calibration graphs were linear in the range of 3.0–500 μg/kg and the limits of detection were in the range of 1.0–2.0 μg/kg. The relative recoveries of different fruit and vegetable samples that have been spiked with two levels of target compounds were 91.5%–109.8%. The method has good linearity, sensitivity, accuracy, and precision. It is also environmentally friendly and was successfully used to determine the concentrations of triazines in fruit and vegetable samples.

Article Related Abbreviations: DES, deep eutectic solvent; DLLME, dispersive liquid-liquid microextraction; EG, ethylene glycol; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; LPME, liquid phase microextraction; MRL, maximum residue limit; TGA, thermogravimetric.

KEYWORDS
deep eutectic solvent, dispersive liquid-liquid microextraction, fruit, triazine herbicides, vegetables

1 | INTRODUCTION

Herbicides and pesticides are chemicals that are widely used in food production and in agriculture to protect crops from insects, fungi, bacteria, weeds, and other pests. Triazine herbicides are a group of chemical compounds that are used to kill weeds and pests in vegetable fields, greenhouses, and citrus and grape orchards around the world [1]. These compounds destroy pests by disrupting the photosynthesis process, especially in broad-leaved weeds [2]. Common triazine herbicides that are used the most in Iran are atrazine, simazine and propazine [3]. The release of these compounds in the environment causes the residues of these herbicides and their metabolites to enter the human body through the food chain and accumulate in the body [4]. The presence of these compounds in the body causes problems such as skin rashes, hormonal imbalances, birth defects, and types of cancer [5]. To protect consumers from the harmful effects of triazine herbicides, most countries have set maximum residue limits (MRLs) for triazine in agricultural products. The Environmental Protection Agency has declared the MRLs of triazines in most products to be 0.25 mg/kg, while the European Union has declared this limit to be 0.05 mg/kg in rice and oilseeds [6–8]. Although the European Union has set an MRL of 0.05 mg/kg for terbuthylazine in vegetables, MRLs for other triazines in fruits and vegetables have not been precisely defined [8]. Therefore, a simple, fast and highly sensitive analytical method is necessary to determine triazine residues in agricultural products. HPLC and GC equipped with different sensitive detectors have been used to measure and monitor triazines in different matrices [9–12]. However, due to the very low amounts of triazines in fruits and vegetables, as well as the complexity of the matrix, sample preparation, and preconcentration step is necessary before analysis. SPE [13], liquid-liquid extraction [14], SPME [15], and liquid phase microextraction (LPME) [16–18] techniques are most used in the extraction and preconcentration of triazine herbicides. These days, LPME has received more attention due to its simplicity, low cost, high preconcentration factor, and environmental friendliness.

Dispersive liquid-liquid microextraction (DLLME) is the latest version of LPME, which was presented by Asadi and co-workers in 2006 [19] and has been used for the extraction and preconcentration of various organic and inorganic compounds [20–22]. One of the major problems of the DLLME method in its early introduction was the high consumption of disperser solvents and the use of toxic and environmental pollutants organic solvents as extractants. That's why researchers have made many developments in the DLLME method to solve these problems. Recently, methods such as vortex, ultrasonic, pH, and temperature changes are used to increase the contact surface and dispersion of the extraction solvent, and the disperser solvent is no longer used [23–25]. Also, deep eutectic solvents (DESs) have been extensively developed as an extractant in the DLLME method. These solvents usually consist of two non-toxic components, one is the hydrogen bond acceptor (HBA) and the other is the hydrogen bond donor (HBD). Due to the formation of intramolecular hydrogen bonds, the melting point of the DES is lower than that of any of its components [26]. DES has good attributes including low toxicity, easy synthesis, non-flammability, and low vapor pressure [27]. These properties make these solvents superior to conventional organic solvents in extraction methods. In recent years, chemists have synthesized DESs whose hydrophilicity can be changed by changing environmental conditions. Limited studies have been reported in this field with changes in temperature and pH [24, 28].

In this research, based on the achievements in the field of DESs, the HDES consisting of *l*-menthol as HBA and ethylene glycol (EG) as HBD was used for extraction and preconcentration of triazine herbicides in fruits and vegetables. To the best of our knowledge, our work is the first report of EG:*l*-menthol as DES in the extraction of triazine herbicides in fruits and vegetables. The unique behavior of DES in the extraction of target analytes was investigated by optimization of different variables on analytical performance. The effect of various experimental parameters on extraction efficiency was investigated using one variable at a time.

2 | MATERIALS AND METHODS

2.1 | Reagents and materials

Standards of triazine herbicides including atrazine (1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine), simazine (6-chloro-2-N,4-N-diethyl-1,3,5-triazine-2,4-diamine) and propazine (6-chloro-N2,N4-diisopropyl-1,3,5-

triazine-2,4-diamine), *l*-menthol and EG were supplied from Sigma-Aldrich (Milwaukee, WI, USA). Acetonitrile, methanol, NaCl, KOH, and HCl (37%) (all reagents were either analytical or HPLC grade) were purchased from Merck (Darmstadt, Germany). Stock standard solution of triazines with 1000 mg/L concentration was prepared in methanol. Different fruits and vegetables were purchased from local supermarkets (Kermanshah, Iran).

2.2 | Instrumentation

The analysis of target triazines was done by a Knauer HPLC equipped with binary pumps Smartline-1000-1 and Smartline-1000-2, variable wavelength programmable detector Smartline-UV-2500 (Berlin, Germany), manual sample injector fitted with a 20 μl injection loop (model 7725i; Rheodyne, Cotati, CA, USA) and an on-line solvent vacuum degasser. An H5-ODS C18 column (15 cm × 4.6 mm, with 5 μm particle size) from Anachem (Luton, UK) was used for separation. ACN–water (70:30, v/v) was employed as the mobile phase with a flow rate of 1.0 ml/min in isocratic elution mode. The injection volume was 30.0 μl, with the detection wavelength at 224 nm. A Bruker PS-15 spectrometer (400–4000/cm) was used for the Fourier-transform infrared (FT-IR) spectrum of DES. The ^{1}H and ^{13}C NMR analysis of DES structure was performed by Bruker SP-400 Avance spectrometer. Analysis of thermogravimetric (TGA) was done on the DES and its components using TGA (Mettler Toledo Instrument Model TGA/SDTA 851 e, Switzerland)) purged with N_2, at the temperature range of 50–600°C with a heating rate of 10°C min^{-1}.

2.3 | Sampling and sample preparation

Kermanshah city was divided into five zones, North, South, East, West, and Center, and two fruit shop was randomly chosen in each of these zones. In each fruit shop, 10 different types of fruit and vegetables (mentioned in Table 2) were collected. As a whole, 100 samples were randomly chosen from the studied zones. Then, each sample was packaged and labeled separately, and it was kept away from the sun at 5°C prior to the experiment.

At first, about 100 grams of each sample was chopped with a knife and completely homogenized using a mixer (Buchi B 400; Flawil, Switzerland). Then, 1.00 g of this obtained sample (spiked or not with triazines) was transferred into a 10-ml screw cap test tube and 3.0 ml of acetone was added. The test tube was placed in an ultrasonic bath and sonicated for 20 min. After that 150.0 μl of DES was added to the test tube and then it was gently shaken for one minute. After centrifugation at 5000 rpm for 5 min, the supernatant was separated for further DES–DLLME.

2.4 | Preparation of DES

l-menthol and EG with the same molar ratio (1:1) were accurately weighed and mixed together in a 100-ml volumetric flask. The resulting mixture was stirred at 40°C for 30 min until a homogeneous and transparent liquid was obtained. The obtained clear liquid was cooled to room temperature and stored in a desiccator to be used as an extractant without any purification.

2.5 | Proposed procedure

For the DES–DLLME procedure, 1.00 ml of acetone extracted from Section 2.3, which contains 50.0 μl of DES and the desired analytes, is quickly injected into 5.00 ml of ultra-pure water. As a result of spreading very fine drops of DES in the aqueous solution, a cloudy solution is formed. Due to the high contact surface, the target analytes are immediately extracted into the DES. By centrifugation at 4000 rpm for 5 min, phase separation occurs and the extractant was collected on the top of the tube (30 ± 2 μl). The tube was transferred into an ice bath, and the DES was solidified after a few minutes. The resulting solidified DES was transferred into a conical vial, where it melted immediately at room temperature. Finally, the DES was injected into an HPLC–UV.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of DES

3.1.1 | FT-IR analysis

FT-IR spectra were employed to characterize the structure of the prepared DES and the interaction between its constituents (Figure 1A). The observed stretching vibration bands at 3263, 2959, 2929, and 1368/cm for *l*-menthol were assigned to the —OH, $—CH_3$, —CH, and $(CH_3)_2$ groups, respectively. Upon mixing the *l*-menthol with EG, the hydroxyl stretching band of *l*-menthol demonstrated a blue shift from 3263 to 3355/cm, which can reveal the formation of the intermolecular hydrogen bonding. It should also be mentioned that the broad hydroxyl stretching band from 3384/cm in EG has been shifted to 3355/cm, additionally suggesting the hydrogen bonding network between EG and *l*-menthol as HBD and HBA, respectively.

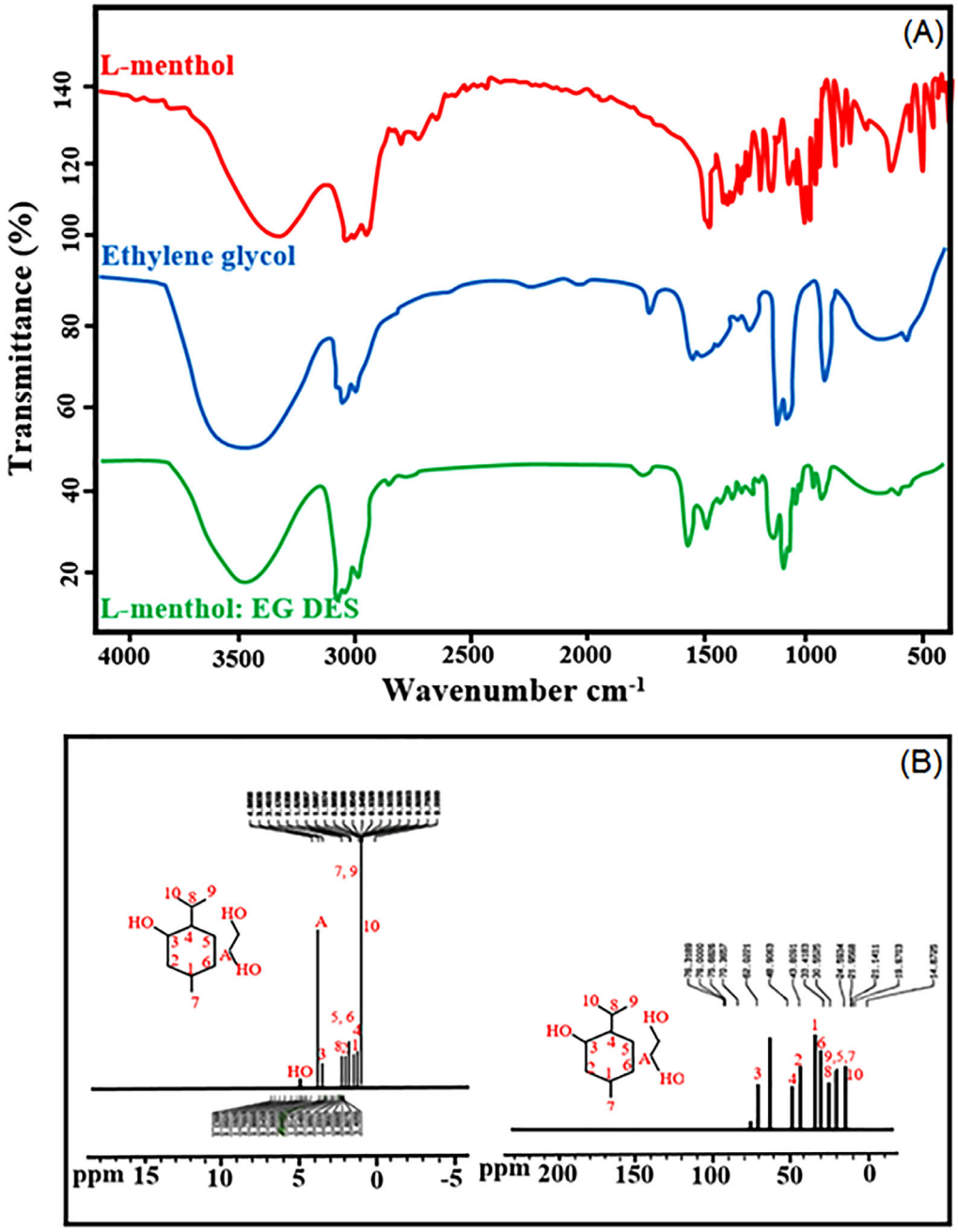

FIGURE 1 Fourier-transform infrared (FT-IR) spectra of the pure *l*-menthol, ethylene glycol (EG) and the DES mixture of *l*-menthol and EG (A), and NMR spectra of *l*-menthol:EG DES in $CDCl_3$ (B)

3.1.2 | NMR analysis

The purity and chemical structure of *l*-menthol:EG DES were also confirmed by NMR spectra (Figure 1B). All hydrogen peaks in the ^1H NMR spectrum were correlated with proton signals in starting materials, excluding the —OH peaks of the constituents. The existence of hydrogen bond interaction between the hydroxyl groups in *l*-menthol and EG as well as the hydroxyl hydrogen exchange process leads to the generation of a broad signal at 4.09 ppm. Furthermore, the ^{13}C NMR spectrum established that no additional signals were found, which indicated no chemical interaction except hydrogen bonding occurred between the components of the DES.

3.1.3 | Thermal analysis

Thermal behavior of *l*-menthol:EG DES was investigated using differential scanning calorimetry and TGA analysis,

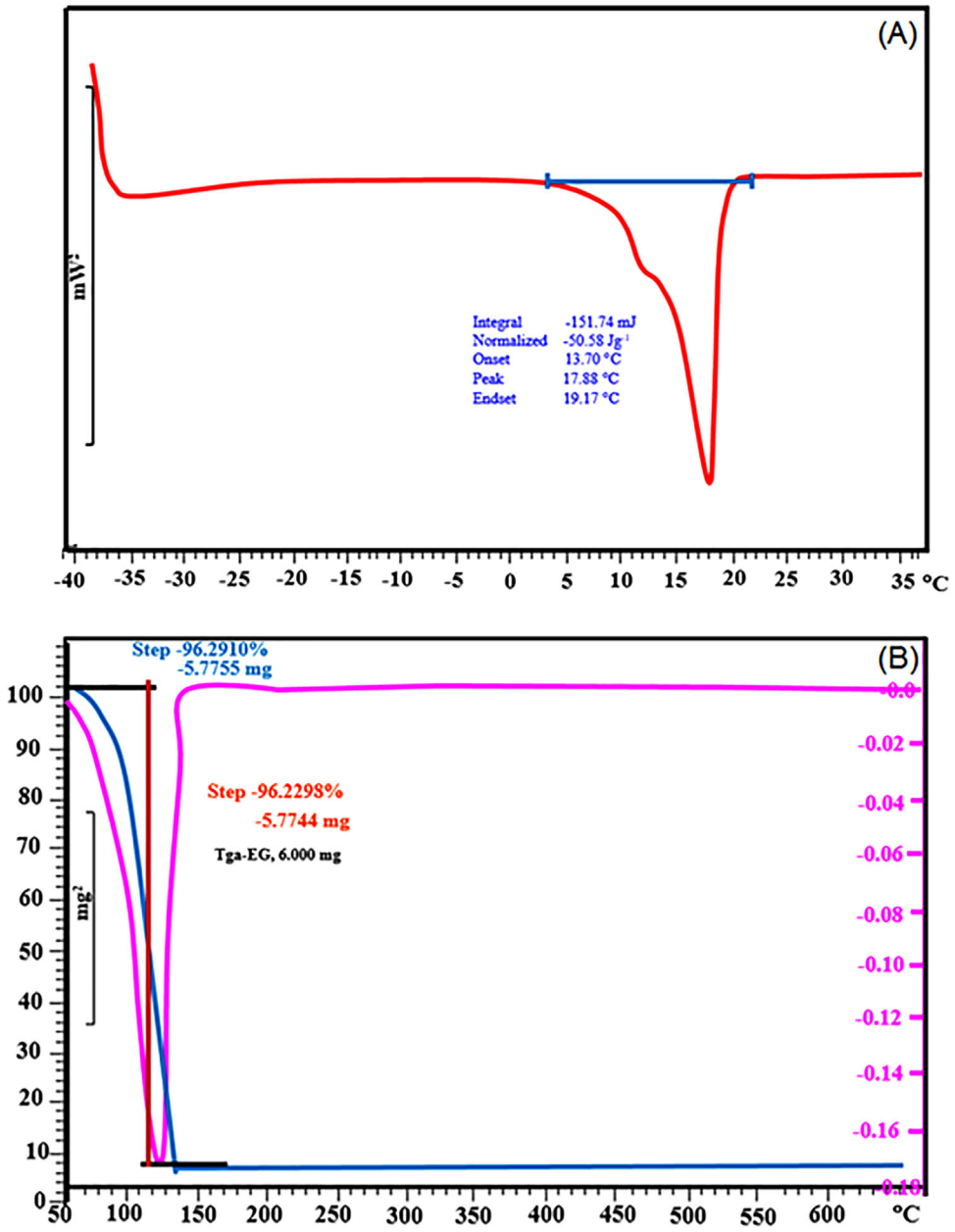

FIGURE 2 Differential scanning calorimetry (DSC) (A) and thermogravimetric analysis (TGA) of *l*-menthol:EG DES (B)

and their curves are shown in Figure 2A,B, respectively. The thermal decomposition pattern in the TGA thermogram of the DES was one step and is completed at 114°C. Therefore, DES indicated rapid weight loss of ≥96%. In the DTG curve, the maximum degradation temperature of the DES was revealed. The differential scanning calorimetry thermogram of *l*-menthol:EG presented a sharp endothermic peak at 17.9°C, which demonstrated the melting temperature (T_m) of the DES. The *l*-menthol:EG mixture showed a decrease in melting peak compared to *l*-menthol, which was probably owing to an asymmetrical system in the DES structure.

3.2 | Selection of HBA to HBD molar ratio

The molar ratio of HBA to HBD plays a very important role in the synthesis of DES and its physical properties, and it is also effective in extraction efficiency. For this purpose, *l*-menthol and EG were mixed in different molar ratios (4:1, 3:1, 2:1, 1:1, 1:2, and 1:3) to obtain DES with appropriate physical properties and effective extraction of analytes. Figure 3A shows that in a 1:1 molar ratio of *l*-menthol and EG, DES is obtained, which has the highest efficiency in the extraction of triazines. In other molar ratios, the extraction efficiency decreases, which is probably due to

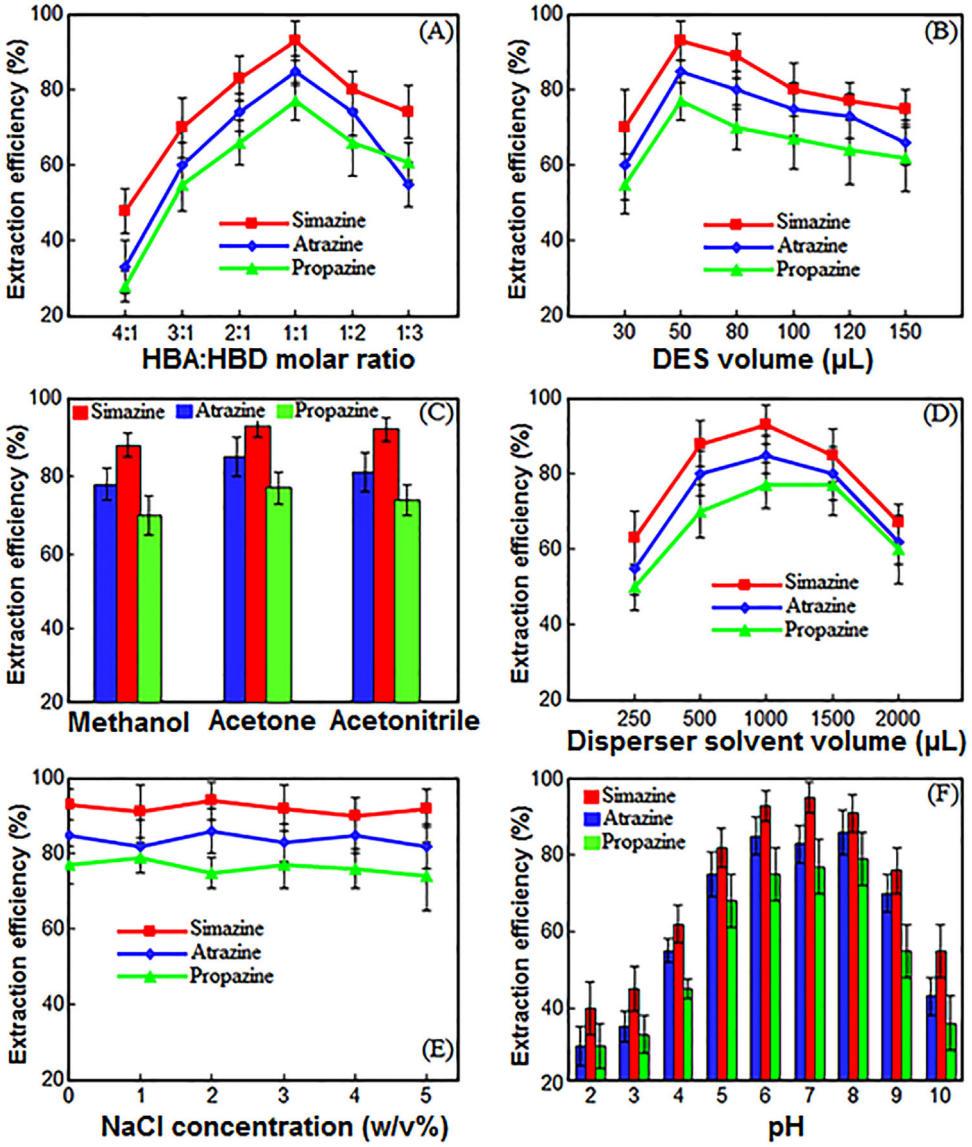

FIGURE 3 The effect of the molar ratio of HBA to HBD (A), the volume of DES (B), the type of disperser solvent (C), the volume of disperser solvent (D), salt addition (E), and sample solution pH (F) on the extraction efficiency of triazine herbicides obtained from DES–DLLME/HPLC–UV

the weakened interaction between the DES and the analytes. It should be noted that some molar ratios of HBA to HBD such as 4:1 and 5:1 translated into the formation of an opaque gelatinous mixture that could not be used as the extraction solvent. Therefore, the 1:1 molar ratio of *l*-menthol and EG was chosen as the best molar ratio in the synthesis of DES.

3.3 | Selection of extractant volume

The volume of DES plays a very important role in DLLME. In small volumes, the analytes are not extracted well, and in large volumes, the contact surface of the DES with the aqueous phase decreases because the disperser solvent cannot completely disperse the DES in the aqueous phase, as a result, the extraction efficiency decreases. Therefore, the optimal volume of DES should be selected. The effect of the DES volume on the extraction efficiency of the triazine herbicides was investigated in the range of 30–150 µl. The results in Figure 3B show that in the volume of 30 µl, the floated phase is not collected well and the reproducibility is not suitable. By increasing the DES volume up to 50 µl, the efficiency increases and the reproducibility improves. In volumes greater than 50 µl, the extraction efficiency decreases due to the reduction of the contact surface and the dilution effect. Therefore, 50 µl of DES was selected as the optimum volume.

TABLE 1 Analytical characteristics of the optimized method

Analyte	EE[a] (%)	EF[b]	RSD[c] % ($n = 5$, intra-day)	RSD% ($n = 5$, inter-day)	LR[d] (μg/kg)	r^2[e]	LOD[f] (μg/kg)	LOQ[g] (μg/kg)
Atrazine	85	141.6	4.1	6.3	5–500	0.9985	2	5
Simazine	93	155	2.8	3.7	3–500	0.9991	1	3
Propazine	77	128.3	5.5	7.2	5–500	0.9980	2	5

[a]EE, extraction efficiency.
[b]Enrichment factor.
[c]RSD at a concentration of 100 μg/kg of pesticides.
[d]LR, linear range.
[e]r^2, square of the correlation coefficient.
[f]LOD, limit of detection for S/N = 3.
[g]LOQ, limit of quantification for S/N = 10.

3.4 | Selection of disperser solvent

Considering that DLLME is for extracting analytes from aqueous samples, to use this method in extracting analytes from solid samples, a sample preparation step must be performed before DLLME. The extracting solvent in the sample preparation stage must play the role of disperser solvent in the DLLME stage. Therefore, the solvents that had this ability and were used in these experiments were acetone, methanol, and ACN. The results in Figure 3C show that the extraction efficiency of all analytes using acetone is slightly better than ACN and methanol. In addition, acetone is cheaper and less toxic than ACN and methanol. Therefore acetone was selected as the disperser solvent in DLLME.

3.5 | Selection of disperser solvent volume

The effect of disperser solvent volume on the extraction efficiency of triazines was investigated using different volumes of acetone in the range of 250–2000 μl. In order to keep the final extraction phase constant, the volume of the extraction solvent was changed at the same time as the volume of the disperser solvent was changed. The results in Figure 3D show that by increasing the volume of the disperser solvent from 250 to 1000 μl, the extraction efficiency of triazines increases, and with a further increase in the volume of the disperser solvent, the extraction efficiency decreases. As a result, 1000 μl was selected as the optimal volume of disperser solvent.

3.6 | Effect of salt addition

The effect of salt addition on the extraction and preconcentration of triazine herbicides was investigated by adding

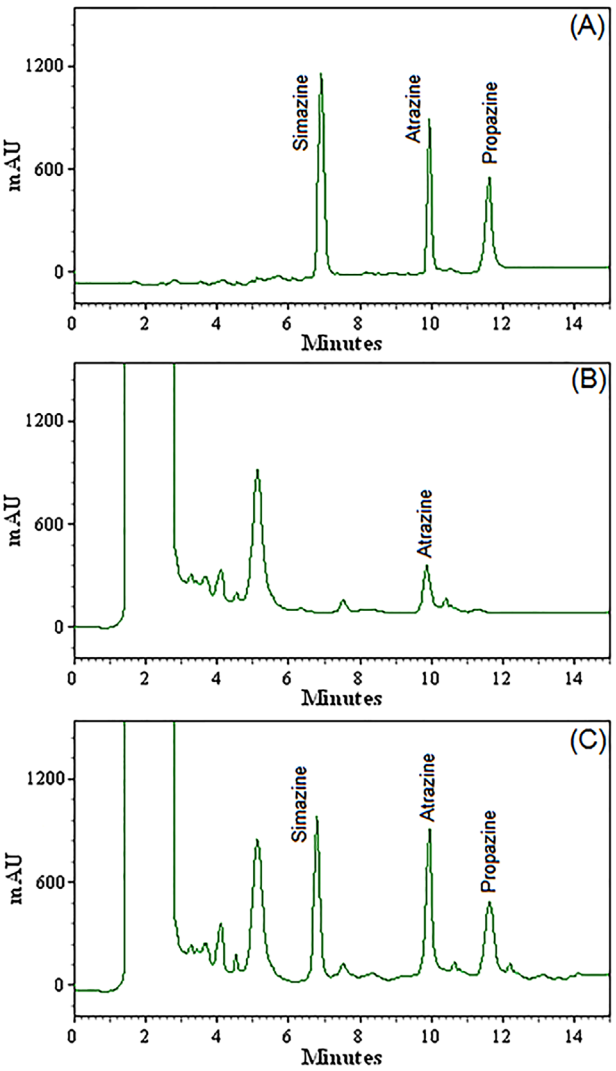

FIGURE 4 Chromatograms of direct injection of triazines standards at a concentration level of 20.0 mg/L (A), tomato sample (B), and the corresponding spiked ones at a concentration of 100.0 μg/kg for target triazines (C) obtained by using DES–DLLME combined HPLC–UV

TABLE 2 Concentrations and relative recoveries of triazines in fruit and vegetables with and without spiking of target analytes

Sample no.	Sample name	Analyte	Concentration mean ± SD[a)] (μg/kg)	Added (μg/kg)	Found mean ± SD (μg/kg)	Relative recovery (%)
1	Green vegetable	Atrazine	24.2 ± 3.5	10	34.5 ± 2.4	103.0
				100	127.4 ± 8.5	103.2
		Simazine	n.d.[b)]	10	9.3 ± 0.6	93.0
				100	98.2 ± 6.3	98.2
		Propazine	11.5 ± 1.6	10	22.3 ± 2.1	108.0
				100	116.6 ± 10.2	105.1
2	Green vegetable	Atrazine	n.d.	20	18.7 ± 1.6	93.5
				50	51.7 ± 3.5	103.4
		Simazine	41.5 ± 3.7	20	62.2 ± 5.2	103.5
				50	96.4 ± 6.7	109.8
		Propazine	n.d.	20	19.6 ± 1.2	98.0
				50	52.5 ± 4.6	105.0
3	Stewed vegetable	Atrazine	21.7 ± 2.4	30	54.1 ± 3.5	108.0
				60	83.1 ± 5.8	102.3
		Simazine	n.d.	30	28.5 ± 1.9	95.0
				60	62.3 ± 3.7	103.8
		Propazine	9.3 ± 0.5	30	41.4 ± 3.2	107.0
				60	71.1 ± 5.6	103.0
4	Tomato	Atrazine	33.1 ± 2.3	10	42.5 ± 3.3	94.0
				100	135.2 ± 10.4	102.1
		Simazine	n.d.	10	9.3 ± 0.5	93.0
				100	103.6 ± 8.2	103.6
		Propazine	n.d.	10	10.6 ± 0.7	106.0
				100	96.2 ± 6.7	96.2
5	Tomato	Atrazine	11.2 ± 1.3	40	53.0 ± 3.8	104.5
				80	90.5 ± 5.3	99.1
		Simazine	n.d.	40	41.7 ± 3.2	104.2
				80	83.1 ± 6.5	103.8
		Propazine	41.2 ± 3.7	40	84.5 ± 5.3	108.2
				80	124.5 ± 11.2	104.1
6	Potato	Atrazine	n.d.	5	5.3 ± 0.3	106.0
				50	50.8 ± 3.4	101.6
		Simazine	58.8 ± 4.1	5	64.0 ± 4.6	104.0
				50	110.6 ± 9.7	103.6
		Propazine	n.d.	5	4.9 ± 0.3	98.0
				50	52.9 ± 2.8	105.8
7	Apple	Atrazine	n.d.	10	10.2 ± 0.4	102.0
				100	95.7 ± 6.3	95.7
		Simazine	n.d.	10	9.6 ± 0.5	96.0
				100	99.2 ± 7.1	99.2
		Propazine	32.6 ± 2.9	10	43.0 ± 2.9	104.0
				100	135.8 ± 12.1	103.2

(Continues)

TABLE 2 (Continued)

Sample no.	Sample name	Analyte	Concentration mean ± SD[a)] (μg/kg)	Added (μg/kg)	Found mean ± SD (μg/kg)	Relative recovery (%)
8	Watermelon	Atrazine	14.4 ± 1.2	20	32.7 ± 3.0	91.5
				50	65.5 ± 5.2	102.2
		Simazine	n.d.	20	20.4 ± 1.3	102.0
				50	47.5 ± 2.6	95.0
		Propazine	n.d.	20	19.5 ± 1.7	97.5
				50	53.2 ± 4.0	106.4
9	Cucumber	Atrazine	n.d.	5	5.2 ± 0.2	104.0
				50	48.4 ± 2.6	96.8
		Simazine	n.d.	5	4.8 ± 0.3	96.0
				50	53.1 ± 3.2	106.2
		Propazine	n.d.	5	5.2 ± 0.4	104.0
				50	53.7 ± 4.7	107.4
10	Melon	Atrazine	n.d.	10	10.6 ± 0.5	106.0
				80	83.1 ± 5.4	103.8
		Simazine	n.d.	10	9.6 ± 0.6	96.0
				80	78.2 ± 6.5	97.7
		Propazine	n.d.	10	10.2 ± 0.7	102.0
				80	79.5 ± 5.8	99.3

[a)]SD, standard deviation ($n = 3$).
[b)]n.d., not detected.

different concentrations of NaCl ranging from 0% to 5% w/v into the sample solution. No obvious difference was found in the extraction efficiency of the triazines with increasing NaCl concentration (Figure 3E). Because on the one hand, the salting-out effect increases the extraction efficiency, and on the other hand, with the increase of NaCl, the solubility of the DES in the aqueous phase decreases, and the volume of the final extraction phase increases. As a result, the effect of dilution decreases the extraction efficiency. So, NaCl was not used in the subsequent experiments.

3.7 | Selection of sample solution pH

The pH of the sample solution is a very important factor that can be adjusted to bring the analytes into molecular form and increase extraction efficiency. The structure of triazines is such that they are easily hydrolyzed in strongly acidic and alkaline environments. It seems that they maintain their molecular form in a neutral environment or close to it. In the present procedure, the effect of sample solution pH on the extraction efficiency of triazine herbicides was studied within the pH range of 2.0–10.0. As can be seen in Figure 3F, the best extraction recoveries of triazine herbicides were obtained at a pH range of 6–8. As a result, the pH adjustment by using the acidic or alkaline solution, being the contamination source, was not necessary.

3.8 | Quantitative analysis

The analytical performance of the proposed DES–DLLME procedure under optimum conditions was validated through the determination of LODs, linear dynamic ranges, precision (RSDs), coefficients of determination (r^2), enrichment factor, and extraction efficiency for the triazine herbicides. The analytical performance data of our method is summarized in Table 1. The LODs, based on S/N of 3 ranged from 1.0 to 2.0 μg/kg. Linearity was observed over the range of 3.0–500 μg/kg with coefficients of determination better than 0.9980. The intra-day and inter-day repeatability values were studied by submitting seven replicates of triazine standards at a concentration level of 50.0 μg/L to the DES–DLLME method and reported as RSD%. Repeatability values were in the ranges of 2.8%–5.5% and 3.7%–7.2% for intra and inter-day evaluations, respectively. The enrichment factor and the extraction efficiency of triazine herbicides were from 128.3%–155% and 77%–93%, respectively.

TABLE 3 Comparison of DES–DLLME with other extraction methods for determination of triazines in fruit, vegetables, and food samples

Extraction methods	Instrument	LOD[a] (μg/kg)	LOQ[b] (μg/kg)	LR[c] (μg/kg)	RSD[d] %	Sample amount (gr)	Samples	Reference
ILFF–SPE[e]	HPLC–UV	1.3–2.7	4.5–9.2	3–160	1.44-5.21	5	vegetables	[8]
MSPD–MIL–DLLME[f]	UFLC–UV	1.2–2.72	3.99–9.06	8–1000	<7.7	1	Oilseeds	[12]
DMAE–SFO[g]	HPLC–UV	1.1–1.5	3.5–4.8	5–1000	7-8	1	Cereals	[16]
PLE[h]	Nonaqueous CE–UV	9–17	–	25–250	<10	7	Fruits and cereals	[29]
DSPE[i]	LC–MS	0.05–0.2	0.1–1	1–200	<10	10	Fruits and vegetables	[30]
DLLME–SFO	GC-MS	0.008–0.037	–	0.01–100	0.03–5.1	5 ml	Water and sugarcane	[10]
MA-LLME-SFO[j]	HPLC–DAD	0.95–1.39	3.15–4.63	5–250	<13.1	2	Honey	[11]
M-H-MIP[k]	HPLC–UV	0.16–0.39	–	0.5–200	<5.2	50	Corn, wheat, and soybean	[31]
MMLLE-MIP[l]	HPLC–UV	22–38	–	–	0.72-1.55	4-40	Lettuce and apple	[17]
MASE-MISPE[m]	HPLC–UV	1.3–3.3	–	–	2-20	18 ml	Cowpea and corn	[32]
UAE–DLLME–SFO[n]	HPLC–UV	1–2	3–6	5–800	3.6-5.4	1	Fruits and vegetables	[3]
CSDF–ME[o]	HPLC–UV	0.5–1	2–4	1.5–600	3.6-5.4	5 ml	Fruit juices	[5]
DES–DLLME	HPLC–UV	1–2	3–5	3–500	2.8-5.5	1	Fruits and vegetables	This work

[a] LOD, limit of detection.
[b] LOQ, limit of quantification.
[c] LR, linear range.
[d] RSD, relative standard deviation.
[e] Ionic liquid foam floatation solid phase extraction.
[f] Matrix solid-phase dispersion combined with magnetic ionic liquid dispersive liquid-liquid microextraction.
[g] Dynamic microwave-assisted extraction combined with solidification of floating organic drop.
[h] Pressurized liquid extraction.
[i] Dispersive solid-phase extraction.
[j] Microwave-assisted liquid-liquid microextraction based on solidification of floating organic droplets.
[k] Magnetic hollow molecularly imprinted polymer.
[l] Microporous membrane liquid-liquid extraction and molecularly imprinted polymer.
[m] Membrane-assisted solvent extraction and molecularly imprinted solid phase extraction.
[n] Ultrasound-assisted extraction-dispersive liquid-liquid microextraction with solidification of floating organic drop.
[o] Continuous sample drop flow-microextraction.

3.9 | Real samples analysis

To demonstrate the applicability of the developed DES–DLLME, it was applied to the extraction of triazine herbicides from different fruit and vegetables. One hundred samples of fruits and vegetables were subjected to the developed extraction method and each experiment was performed in triplicates. The results showed that triazines were detected in only eight samples out of 100 analyzed samples. In two samples of green vegetables, one sample of stewed vegetable, two samples of tomato, one sample of potato, one sample of apple, and one sample of watermelon, at least one of atrazine, simazine, or propazine compounds was found with different concentrations as shown in Table 2. To assess the matrix effect in real samples, eight samples in which triazines were found and 2 samples without triazines (10 samples in total) were spiked with triazine standard at two different concentration levels as represented in Table 2. The results in Table 2 showed that the relative recoveries of triazines in fruit and vegetable samples are in the range of 91.5%–109.8%, with RSD < 10. The reported results proved the suitability of our method in the extraction of triazine herbicides from fruit and vegetable samples. Chromatograms of triazine

standards, and the extracted target triazines in tomato samples before, and after spiking by standards of triazines are represented in Figure 4.

3.10 | Comparison of the present study with the previous literature

The performance of the proposed DES–DLLME method was compared with our previous research and also with the results of other methods for the extraction and determination of triazine herbicides from fruit and vegetables in Table 3. As can be seen from Table 3, in our previous research [5], the continuous sample drop flow-microextraction method was used to extract triazines from fruit juice samples. Although LODs and LOQs were slightly better compared to the present study, the extraction time was longer and the organic solvent was toxic. The extraction process reported by Zhang et al. [8] for the extraction of triazine herbicides from vegetables required an ionic liquid that is not only time-consuming but also required the consumption of organic solvents. However, our method provided better LODs, LOQs, and LR. Different research groups used DLLME for the extraction of triazine herbicides from fruit and vegetables [3], water and sugarcane [10], and oilseeds [12] using a variety of extraction solvents. According to the data reported in Table 3, our method provided comparable or lower LODs and RSDs. However, in some cases, toxic organic solvents were used. Although the DLLME reported by Sanagi et al. [10] exhibited lower LOD values, our method presented a wider concentration range by consuming only 1.0 gr of the sample. The LODs, LRs, and RSDs of the presented method are almost comparable with other methods and in some cases, it is better. From an environmental point of view, our DES–DLLME is more eco-friendly due to the application of cheap and safer components for the synthesis of the extraction solvent. The preparation of DES is simple and needs no consumption of harsh organic solvents.

4 | CONCLUDING REMARKS

In the present study, a DES-DLLME method combined with HPLC–UV was introduced for the extraction and determination of triazine herbicides in fruit and vegetable samples. A hydrophobic DES was synthesized using *l*-menthol as HBA and EG as HBD and used as a green extractant. This method is cheap, simple, and fast, and the whole process of DLLME takes less than a few minutes (regardless of the time required for sample preparation). The method has good accuracy, linearity, sensitivity, and precision. It was successfully employed for the determination of triazines in fruit and vegetables. The presented method can be easily used to extract analytes that can have a good interaction with the extractant.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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