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Trace-Level Determination of Triazole Fungicides Using Effervescence-Assisted Liquid−Liquid Microextraction Based on Ternary Deep Eutectic Solvent Prior to High-Performance Liquid Chromatography

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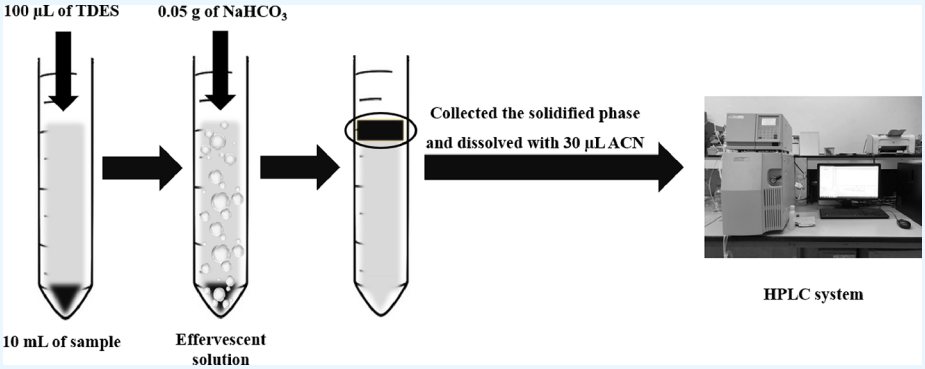

ABSTRACT: A simple and sensitive preconcentration method, namely, effervescence-assisted liquid−liquid microextraction based on the ternary deep eutectic solvent method, was developed for enrichment of triazole fungicide residues prior to their determination by high-performance liquid chromatography coupled with UV detection. In this method, a ternary deep eutectic solvent (as extractant) was prepared by combination of octanoic acid, decanoic acid, and dodecanoic acid. The solution was well dispersed with sodium bicarbonate (as effervescence powder) without using auxiliary devices. In order to obtain relatively high extraction efficiency, analytical parameters were investigated and optimized. Under optimum conditions, the proposed method showed good linearity within the range of 1−1000 $\mu g\ L^{-1}$ with a coefficient for determination (R^2) greater than 0.997. The low limits of detection (LODs) were in the range of 0.3−1.0 $\mu g\ L^{-1}$. The precisions were assessed from the relative standard deviations (RSDs) of retention time and peak area obtained from intra- (n = 3) and inter-day (n = 5 × 5) experiments, which were greater than 1.21 and 4.79%, respectively. Moreover, the proposed method provided high enrichment factors ranging from 112 to 142 folds. A matrix-match calibration method was used for analysis of real samples. Finally, the developed method was successfully applied for determination of the triazole fungicide in environmental water (near agricultural area), honey, and bean samples, and it represents a promising alternative method for analysis of triazoles. The recoveries of the studied triazoles were obtained in the range of 82−106% with an RSD less than 4.89.

1. INTRODUCTION

Pesticides (fungicide, herbicide, insecticide, acaricide, and rodenticide) have been widely applied during cultivation and post-harvest for storage of crops.[1] Therefore, their degradation products are often found at low concentrations and in different complex matrices.[2] Triazole fungicides (TFs) are a class of highly effective systemic fungicides containing a hydroxyl group (ketone group), a substituted phenyl group, and a 1,2,4-triazole group in the main chain.[3] However, because of their high chemical stability and low biodegradability, TFs may persist longer in the environment.[4] Their residues can cause harm to the environment and human beings who are using TFs.[5] In order to decrease the harm of TF residues, the allowable level in food is usually defined by the maximum residue limit (MRL). The MRLs of hexaconazole and triadimefon established by the EU are 0.01−0.02 mg kg^{-1}; that of tebuconazole is 0.02−5.0 mg kg^{-1}; and that of

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myclobutanil is 0.05−3.0 mg kg^{-1}.[6] Due to the low concentration of the analytes in real samples, performing a sample pretreatment step for extraction/preconcentration of the analytes from sample matrices is therefore of great importance.[7] To date, a variety of analytical methods for determination of triazoles in apple, peach, wheat, flour, juice, coffee, honey, water, and soil have been reported.

Owing to their extremely low concentration level and real sample matrices, it is therefore difficult to detect triazoles directly by analysis using various instruments.[8] Consequently, sample preparation is required. Up to now, several sample preparation methods have been developed for the extraction of TFs, which can be divided into adsorbent-based extraction[9−12] and solvent-based extraction.[6,13−16]

In 2000, green analytical chemistry (GAC) was introduced to reduce the side effects of analytical methods on the environment or operators[17] and several principles have been declared from the point of view of GAC.[18,19] Therefore, developing an environmentally friendly and cost-effective solvent is of the most importance in chemical manufacturing. The demand of low cost, environmentally friendly, less toxic, and biodegradable solvents has led to a gradual growth of new alternative solvents. In the past years, several green solvents have been developed, namely, ionic liquids (ILs), as an extraction medium due to their unique properties. Besides the mentioned advantages for ILs, however, their high price and tedious synthesis routes, the need for further purification of synthesized ILs, and toxicity of some cases have led to a limitation of their extensive applications.[20,21] Deep eutectic solvents (DESs) are known as cheap analogs of ILs. They are green chemicals that can be used as extraction solvents instead of the common toxic and expensive organic solvents in the extraction methods.[22] DESs, as green solvents, are composed of safe, cheap, renewable, and biodegradable compounds that make them more suitable than ILs.[23] Synthesis of a DES is carried out by hydrogen bond formation between a hydrogen bond acceptor and a functional group of hydrogen bond donors. It has a melting point lower than those of its individual components.[24] Therefore, DESs have properties such as ease of synthesis, low vapor pressure, low toxicity, high thermal stability, inexpensiveness, biocompatibility, and renewability.[25−27] Recently, the preparation and application of hydrophobic DESs were reported, such as using decanoic acid and various quaternary ammonium salts,[28] menthol-based hydrophobic low-viscosity solvents,[29] and indium extracts from hydrochloric and oxalic acids using hydrophobic DESs and low-transition-temperature mixtures,[28] thus greatly enlarging the possibilities of DESs.

Various agitators such as ultrasound,[30] vortex,[31] magnetic stirrer,[32] in-syringe,[33] and air agitator[34] were used to increase the dispersion and mass transfer of fine droplets of extraction solvent into an aqueous solution. Effervescence is a novel-assisted method that has been used very recently for assisting the dispersion of solvents or adsorbents.[35] The mechanism is that it produces gas of carbon dioxide by reaction of acid and carbonate/bicarbonate. Compared with conventional methods such as ultrasound and vortex, it is simple, effective, and energy-free.

In the current study, a cheap, simple, selective, and green analytical method was developed for trace determination of TF residues using effervescence-assisted liquid−liquid microextraction based on ternary DESs prior to high-performance liquid chromatography. Single-chain fatty acids (octanoic acid, decanoic acid, and dodecanoic acid) and double-chain fatty acids (octanoic acid−decanoic acid, octanoic acid−dodecanoic acid, decanoic acid−dodecanoic acid) were investigated since they exhibited a lower price. Effervescence powder was used for assisting the dispersion of solvents without auxiliary devices. The parameters affecting the extraction efficiency were investigated, and the proposed method was applied to analyze complex sample matrices. The proposed method was compared with other extraction methods previously reported. Moreover, to our knowledge, this is the first time that hyphenation of a simple effervescence method with ternary DESs without using auxiliary devices was performed for analysis of TFs.

2. RESULTS AND DISCUSSION

2.1. Characterization of Ternary DES (TDES). FTIR spectra (as shown in Figure 1) were used for characterization

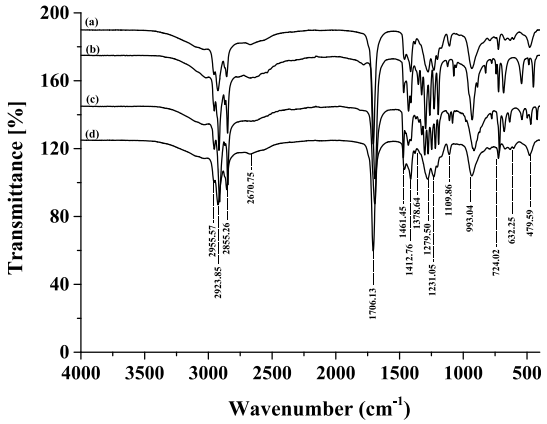

Figure 1. FTIR spectra of (a) octanoic acid, (b) decanoic acid, (c) dodecanoic acid, and (d) ternary deep eutectic solvent when a hydrogen bond was formed.

of the synthesized TDES. The spectra were obtained in the range of 500−4500 cm^{-1}. In the spectrum in Figure 1a, the bands located at 3031.03, 2955.81, 2925.13, 2856.54, and 2671.11 cm^{-1} correspond to the vibration bonds of the broad peak due to the O−H stretch superimposed on the sharp band due to the C−H stretch, that at 1705.74 cm^{-1} corresponds to the vibration bonds of the C═O stretch, those 1460.74, 1412.72, and 1378.99 cm^{-1} correspond to the vibration bonds of the C−O stretch and O−H bend, and that at 932.79 cm^{-1} corresponds to the vibration bonds of the C−H bend, which are indicators of octanoic acid. In the spectrum in Figure 1b, the bands located in the areas of 3017.23, 2953.32, 2916.47, 2849.11, 2767.87, 2669.98, and 2636.34 cm^{-1} correspond to the vibration bonds of the O−H and C−H stretch, that at 1692.33 cm^{-1} corresponds to the vibration bonds of the C═O stretch, those at 1467.38, 1429.19, 1410.98, 1352.41, 1326.51, and 1295.60 cm^{-1} correspond to the vibration bonds of the C−O stretch and O−H bend, and that at 930.11 cm^{-1} corresponds to the vibrations of the O−H bend, which are the indicators of decanoic acid. In the spectrum in Figure 1c, the bands located at 3031.90, 2953.45, 2911.72, 2847.81, 2666.02, and 2640.71 cm^{-1} correspond to the vibration bonds of the O−H and C−H stretches, that at 1694.50 cm^{-1} corresponds to the vibration bonds of the C═O stretch, those at 1470.02, 1431.06, 1302.50, 1275.79, 1247.84, 1219.17, and 1193.30 cm^{-1} correspond to the vibration bonds of the

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C−O stretch and O−H bend, and that at 915.94 cm^{-1} corresponds to the vibrations of the O−H bend, which are the indicators of dodecanoic acid; these wavenumbers of the three components show the unique FTIR spectra of carboxylic acids.

The FTIR spectra in Figure 1d shows the spectrum of solvent-synthesized TDES. One of the significant changes was a decrease in the vibration signal found in the 2955.57−2670.75 cm^{-1} region, possibly related to the displacement of the O−H and C−H bands in forming hydrogen bonds between octanoic acid, decanoic acid, and dodecanoic acid. This combination can be accredited to the slight modification in the force constant generated by the reduced electron cloud density, which is a strong indication of hydrogen binding in the TDES. Moreover, the FTIR spectrum of TDES was obtained after the extraction (Figure 2). The results showed that the structure of TDES did not change after extraction.

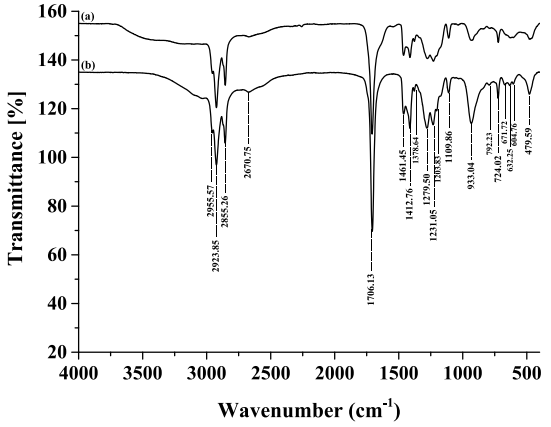

Figure 2. FTIR spectra of (a) standard with TDES and (b) solvent-synthesized TDES.

2.2. Optimization of Effervescence-Assisted Liquid−Liquid Microextraction Based on TDES (EA-LLME-TDES) Conditions. In order to obtain high extraction efficiencies of studied triazoles, several experimental parameters were investigated and optimized via the univariate method. A mixed standard solution containing 100 $\mu g\ L^{-1}$ of each standard was used to examine the extraction performance of the proposed microextraction method under different experimental conditions. All optimization experiments were carried out in triplicate (n = 3). Peak areas were used to evaluate the extraction efficiency of the developed procedure.

The appropriate extracting solvent is important because this is a significant parameter in the proposed method. Extracting solvents should have low viscosity, high hydrophobicity, and melting point below room temperature.[36] TDESs as extracting solvent were synthesized through mixing various molar ratios of the hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). The fatty acids with a shorter alkyl chain (octanoic acid or decanoic acid) act as HBA, while those with the longest one (dodecanoic acid) act as HBD. It is worth noting that use of acids smaller than octanoic acid (hydrophilic acids) was not considered due to the chemical instability of the formed DES upon contacting with water (acids leach to water).[36] The results are shown in Figure 3. As a conclusion, TDES with the mole ratio of 4:1:1 (octanoic acid:decanoic acid:dodecanoic acid) gave the highest extraction efficiency. All the eutectic solvents gave a recovery higher than that of their

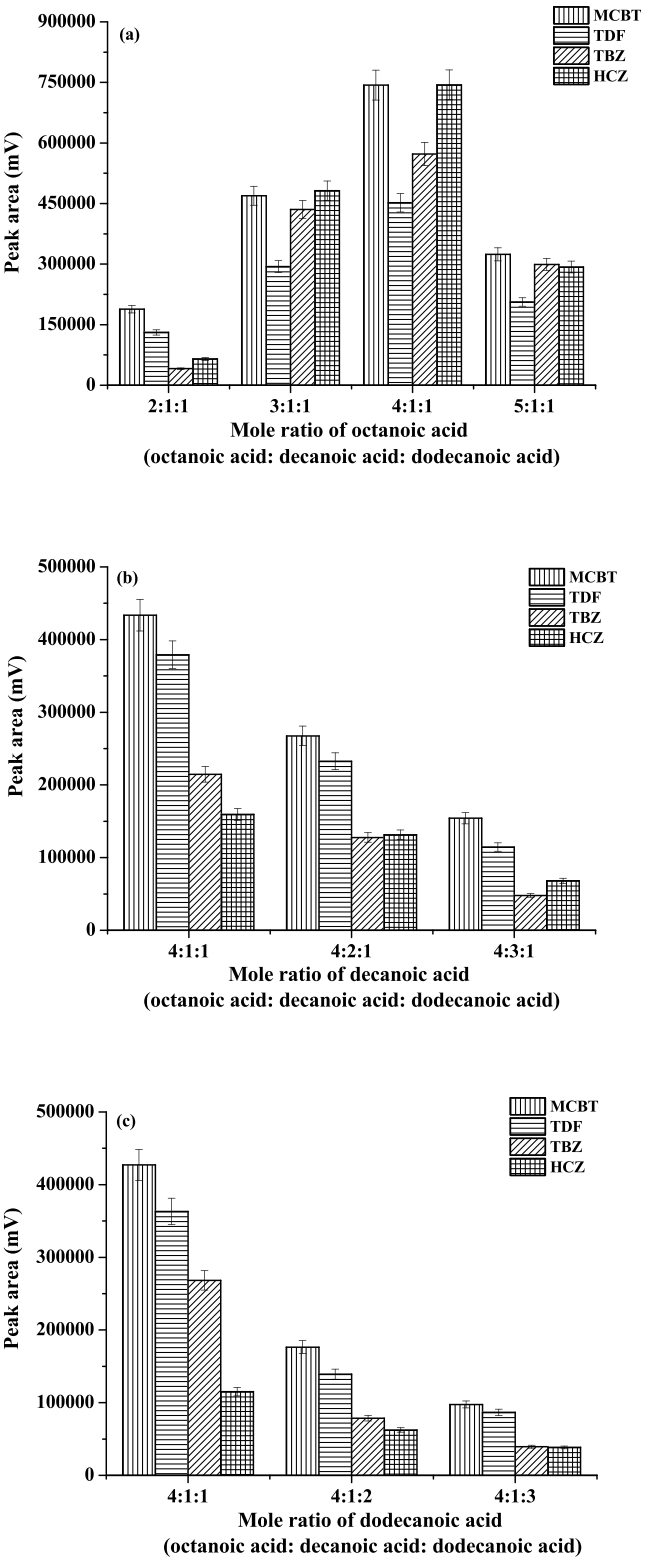

Figure 3. Effect of mole ratio of (a) octanoic acid, (b) decanoic acid, and (c) dodecanoic acid.

own constituents (octanoic acid and decanoic acid) owing to the higher affinity of the analytes to the hydrophobic TDES. Finally, TDES with the mole ratio of 4:1:1 (octanoic acid:decanoic acid:dodecanoic acid) was selected as the best extracting solvent for separation of the studied compounds.

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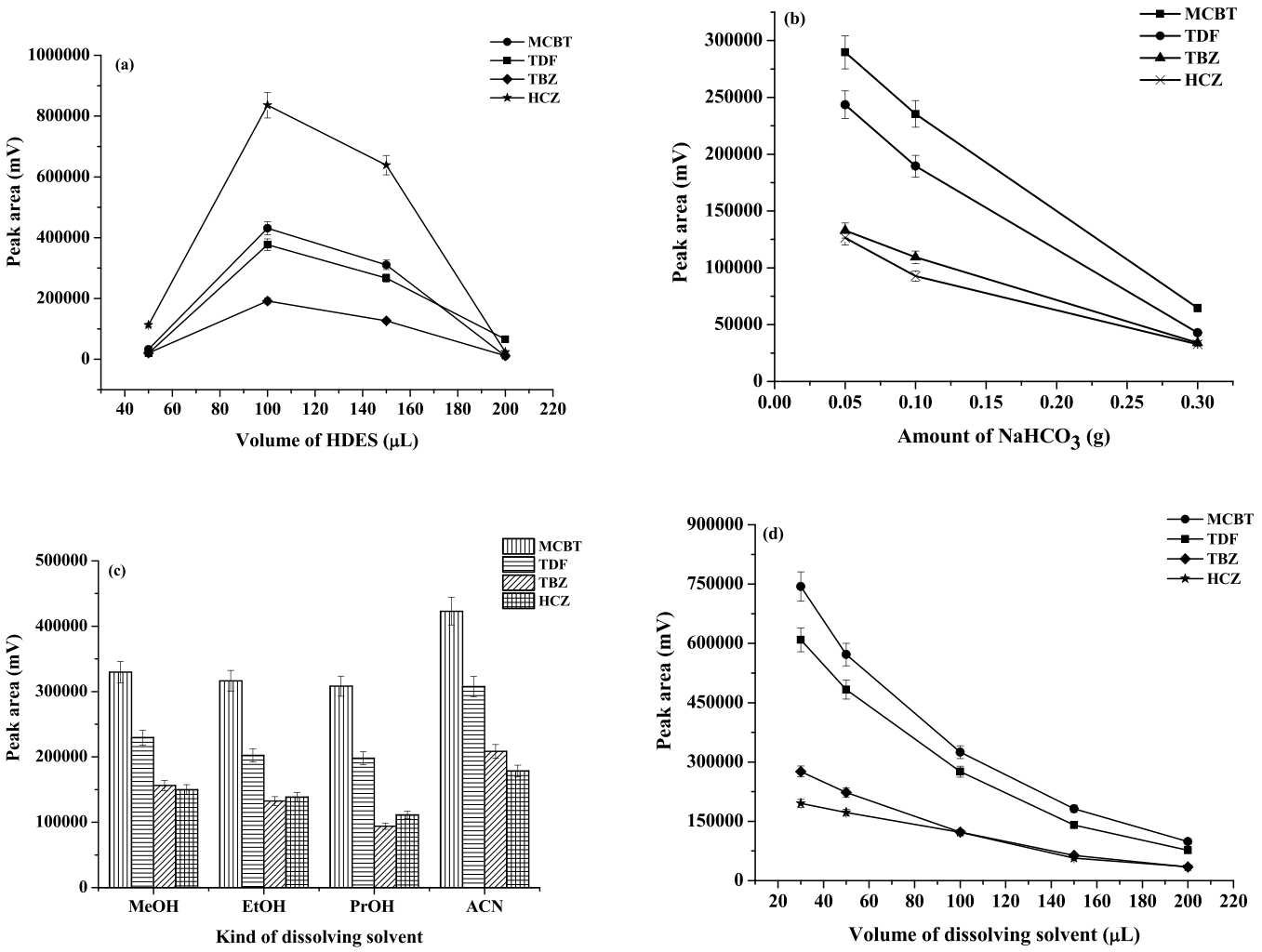

Figure 4. Effect of extraction condition on the extraction efficiency: (a) volume of TDES (μL), (b) amount of $NaHCO_3$, (c) kind of dissolving solvent, (d) volume of dissolving solvent (μL).

Table 1. Analytical Performance of the Proposed Method for Triazole Fungicides

analyte	linear range ($\mu g\ L^{-1}$)	linear equation	r^2	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	intra-day precision (n = 5), RSD (%)		inter-day precision (n = 5 × 5), RSD (%)		EF
						t_R	peak area	t_R	peak area	
myclobutanil	1−1000	$y = (1 \times 10^6)x + 56{,}742$	0.9979	0.3	3.0	1.06	4.35	1.21	4.08	142.04
triadimefon	1−1000	$y = (2 \times 10^6)x + 59{,}918$	0.9989	0.3	3.0	0.84	2.65	0.95	4.02	139.13
tebuconazole	1−1000	$y = (2 \times 10^6)x + 5323.4$	0.9979	0.3	3.0	0.39	4.02	0.43	4.79	112.60
hexaconazole	1−1000	$y = 924{,}257x + 5964.9$	0.9988	1.0	10.0	0.15	4.70	0.31	4.69	134.70

Moreover, volumes of TDES were studied (50, 100, 150, and 200 μL). The results in Figure 4a demonstrate that the extraction efficiency increased with the volume of TDES from 50 to 100 μL and then decreased due to the dilution effect. As a result, 100 μL of TDES was found to be sufficient for successful extraction of the analytes at the concentration test and it was used for the subsequent experiment.

To induce the mass transfer (without another agitator), the main necessities of an effective effervescent process are an effervescency agent (CO_2 source) and a proton donor agent, which can be less-alkali compounds (sodium carbonate, sodium bicarbonate, etc.).[37] In this work, sodium bicarbonate ($NaHCO_3$) was used. The amount of $NaHCO_3$ was investigated (0.02, 0.05, 0.1, 0.3 g); the results are shown in Figure 4b. When 0.02 g of $NaHCO_3$ was added, the final phase was not obtained. A high extraction efficiency for all analytes was obtained when 0.05 g of $NaHCO_3$ was added. Therefore, further studies were done using 0.05 g of $NaHCO_3$.

In order to reduce the viscosity of the extract before injecting it to the chromatographic system, different dissolving solvents were studied including methanol, ethanol, isopropanol, and acetonitrile (shown in Figure 4c). It was found that acetonitrile gave a high extraction efficiency in terms of peak area. Therefore, acetonitrile was used and the volume of acetonitrile was evaluated in the range of 25, 50, 75, 100, 150, and 200 μL (shown in Figure 4d). It was found that 25 μL of

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acetonitrile could not dissolve the TDES phase. The highest response was obtained when 50 μL of acetonitrile was added. Therefore, 50 μL of acetonitrile was used.

2.3. Analytical Performance and Method Validation. In order to evaluate the proposed method, some analytical parameters such as linearity, limit of detection (LOD), limit of quantitation (LOQ), recovery, and precision were determined under optimal conditions. The analytical performances of the proposed method are summarized in Table 1. After preconcentration by the proposed microextraction method, the linearity ranged from 1 to 1000 μg L^{-1} for all triazoles, with the coefficient for determination (R^2) greater than 0.99. The LODs and LOQs were evaluated based on the signal-to-noise ratios of 3 and 10, respectively. The LODs and LOQs were in the range of 0.3−1.0 and 3−10 μg L^{-1}, respectively. The precisions were calculated from the relative standard deviations (RSDs) of retention time and peak area obtained from intra- (n = 3) and inter-day (n = 3 × 5) experiments, which were greater than 1.66 and 13.52%, respectively. Moreover, enrichment factors (EFs) were calculated from the analyte concentration in the final phase (C_{sed}) and the initial concentration in the analyte in the aqueous sample solution (C_0), which ranged from 112 to 142 folds. Chromatograms of the studied triazoles obtained by direct HPLC and the proposed microextraction method are shown in Figure 5. After the microextraction process, the chromatographic signals were increased.

A matrix-match calibration method was used to study the matrix effect of real-sample analysis. The matrix-match calibration was investigated by spiking each target compound in real samples in the range of 50−500 μg L^{-1}. All compounds showed good linearity with R^2 greater than 0.9. In addition, the matrix effect (ME, %) of each calibration graph in the soil sample was calculated using eq 1.

$$\mathrm{ME}\ (\%) = (S_m/S_s) \times 100 \quad (1)$$

where S_m and S_s are the slopes of the calibration curve in the matrix and solvent, respectively. In general, an ME between 80 and 120% shows no matrix effects, an ME between 50 and 80% or between 120 and 150% shows minor matrix effects, and an ME <50 or >150% indicates major matrix effects.[38] ME values were in the range of −65.1 to −75.6% in water samples, −68.1 to −78.6% in honey samples, and −41.1 to −70.6% in bean samples. The results indicate that the honey and bean samples showed major effects while the water samples showed minor effects for triazole analysis.

2.4. Application to Real Samples. The proposed microextraction procedure was then applied for analysis of TF in water, honey, and bean samples. Prior to their analysis, each sample was prepared and extracted by the proposed microextraction. On the other hand, matrix-match calibration was used for determination of the studied compounds in real samples to compensate for the matrix effect. It was found that no residues of the investigated TFs were detected in all studied samples (as shown in Table 2). In order to investigate the accuracy of the proposed method, the water, honey, and bean samples were spiked with 50 μg L^{-1} of each triazole before applying the proposed microextraction procedure. The recoveries of the studied triazoles (as shown in Table 2) were obtained in the range of 82−106% with an RSD less than 4.89.

2.5. Comparison of the Proposed Method with Other Previously Reported Methods. To highlight the emphasizing purposes of the developed method, some major characteristics were compared with those obtained from other reported methods,[39−43] as listed in Table 3. In comparison with other methods, the investigated method has various advantages, such as the use of a green extraction solvent (TDES), less amount of solvent (100 μL), and avoidance of the use of a disperser solvent. The sensitivity of the present method is greater than that of previous approaches with a short extraction time (<1 min). Additionally, the dispersion of extractant and extraction of analytes was performed by using the proposed technique without the need for extraction equipment. Moreover, the proposed method exhibits a favorable linear range, a low LOD, acceptable recovery, and a high EF, demonstrating that the sample preparation in this work is substantially more efficient and selective, followed by the relatively simple and inexpensive HPLC-DAD technique. In addition, the precision and accuracy of the proposed method fit for purpose adhered to the AOAC acceptability criteria.[44] The comparison clearly highlights the advantages of the proposed microextraction method.

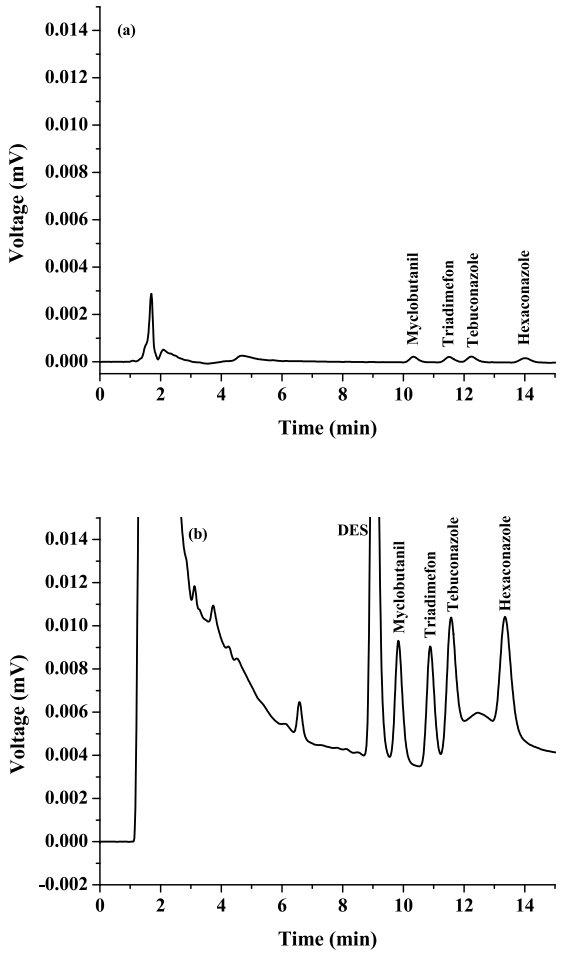

Figure 5. Chromatograms of the studied triazole fungicides obtained (a) by direct HPLC and (b) after extraction using effervescence-assisted liquid−liquid microextraction based on the ternary deep eutectic solvent (EA-LLME-TDES)-HPLC method: the concentration of all standards is 100 μg L^{-1}.

3. CONCLUSIONS

In the present study, a green, rapid, and efficient sample pretreatment method based on effervescence-assisted liquid−liquid microextraction based on TDES for trace-level

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Table 2. Determination of Triazole Fungicides and Recovery in Studied Samples ($N = 3$)

analyte	spiked ($\mu g\ L^{-1}$)	environmental water sample I			environmental water sample II			soybean sample			mung bean sample			red bean sample			honey sample		
		found ($\mu g\ L^{-1}$)	%R	% RSD	found ($\mu g\ L^{-1}$)	%R	% RSD	found ($\mu g\ L^{-1}$)	%R	% RSD	found ($\mu g\ L^{-1}$)	%R	% RSD	found ($\mu g\ L^{-1}$)	%R	% RSD	found ($\mu g\ L^{-1}$)	%R	% RSD
myclobutanil	0	ND[a]			ND[a]			ND[a]			ND[a]			ND[a]			ND[a]		
	50	43.7	87.4	1.05	51.8	103.7	1.89	47.6	103.7	1.97	47.9	95.8	1.39	44.1	88.2	0.11	47.7	95.4	0.99
triadimefon	0	ND[a]			ND[a]			ND[a]			ND[a]			ND[a]			ND[a]		
	50	41.32	82.7	0.88	44.4	88.8	2.36	46.2	92.3	1.71	45.8	91.6	0.59	42.6	85.1	0.67	41.7	83.5	0.78
tebuconazole	0	ND[a]			ND[a]			ND[a]			ND[a]			ND[a]			ND[a]		
	50	53.25	106.5	1.35	49.4	98.8	2.07	48.9	97.8	1.92	50.8	101.6	1.36	48.9	97.7	1.70	47.5	94.9	1.58
hexaconazole	0	ND[a]			ND[a]			ND[a]			ND[a]			ND[a]			ND[a]		
	50	41.5	82.9	4.89	49.1	98.2	4.13	49.3	98.5	2.01	47.5	95.0	0.78	46.4	92.8	4.58	47.5	95.1	2.70

[a]ND: not detected.

determination of TFs was used prior to high-performance liquid chromatography. TDESs (as extractant) were prepared by combination of octanoic acid, decanoic acid, and decanoic acid. The solution was well dispersed with effervescence powder without using auxiliary devices, which could be applied for on-site extraction in the field. The proposed preconcentration method exhibited good linearity, high sensitivity, and satisfactory accuracy and precision. In addition, this method was used as an alternative green extraction method for determination of TFs in water, honey, and bean samples.

4. EXPERIMENTAL SECTION

4.1. Chemicals and Reagents. TF standards, including myclobutanil (MCBT), triadimefon (TDF), tebuconazole (TBZ), and hexaconazole (HCZ), were obtained from Dr. Ehrenstorfer GmbH (Germany). Stock standard solutions (1000 mg L^{-1}) were prepared using methanol and stored in a refrigerator at 4 °C. Working solutions of analytes were prepared by dilution of the stock solution of triazoles with water. Type I deionized water (18.2 MΩ cm) used throughout this work was prepared by a RiOs Simplicity 185 water purification system (Merck, Darmstadt, Germany). Methanol and acetonitrile of HPLC grade were obtained from Merck (Darmstadt, Germany). Octanoic acid (Sigma-Aldrich, China), decanoic acid (Sigma-Aldrich, Malaysia), and dodecanoic acid (Sigma-Aldrich, Malaysia) were used for preparation of TDES. Sodium hydrogen carbonate was obtained from KEMAUS (Australia). Before being subjected to the HPLC system, all reagents were filtered through a 0.45 μm membrane filter.

4.2. Instrumentation. Chromatographic separation of the analytes was accomplished on a Waters 1525 Binary HPLC pump (Water, Massachusetts, USA) equipped with an in-line degasser and a Waters 2489 UV/visible detector. A Rheodyne injector with an injection volume of 20 μL was equipped. Empower 3 software was used as the data acquisition system. Separation was performed on a Purospher STAR RP-18 endcapped (4.6 × 150 mm, 5 μm) column (Merck, Germany) at an ambient temperature with a mobile phase composed of acetonitrile and water (50:50, v/v) at a flow rate of 1.0 mL min^{-1}. The detection wavelength was set at a wavelength of 220 nm.

Fourier transformed infrared spectroscopy (FTIR) (Bruker INVENIO-S FTIR; Bruker Corp., Massachusetts, USA) was acquired using diamond lens attenuated total resistance (ATR). Furthermore, a centrifuge (Centurion, England) was used for phase separation.

4.3. Preparation of TDESs. TDESs were synthesized through mixing various molar ratios of the HBD (the longest alkyl chain of fatty acids such as dodecanoic acid) and HBA (the shorter alkyl chain of fatty acids such as octanoic acid or decanoic acid) in glass vials and then placed in an ultrasonic bath at 65 °C until homogeneous clear liquids were formed.

4.4. Effervescence-Assisted Liquid−Liquid Microextraction Based on TDES. A mixed standard/sample solution (10 mL) was transferred into a centrifuge tube. Then, 100 μL of TDES (extracting solvent) was added. After that, 0.05 g of sodium hydrogen carbonate (effervescence precursors) was added to the centrifuge tube. The dispersion of the organic phase by carbon dioxide bubbles and phase separation was observed (without agitators) within 20 s. The floating TDES droplets were collected and dissolved in 30 μL of acetonitrile to decrease viscosity before being injected into HPLC. The

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Table 3. Comparisons of the Proposed Method with Previous Reported Methods for the Analysis of Triazole Fungicides[a]

method	linear range	extraction solvents	solvent usage	extraction time	LOD	%recovery	ref.
SDES-HLLME-HPLC-DAD	0.001−10 μg mL^{-1}	SDES	110 μL	3.45 min	0.089−0.351 ng mL^{-1}	90.6−110.9	39
DES-UALPME-UHPLC-QTOF-MS	5−1000 ng mL^{-1}	DES	800 μL	15 min	0.5−4.0 ng mL^{-1}	65−107	40
DES-DLLME-liquid polymer-GC-μECD	0.004−100 μg L^{-1}	DES	450 μL	4 min	0.001−100 μg L^{-1}	60.5−105.0	41
DES-HS-SDME-GC-FID	0.01−100 mg L^{-1}	DES	2 μL	30 min	0.08−1.0 μg L^{-1}	93−97	42
DES-UAE-MSPE-HPLC-DAD	0.1−50 μg mL^{-1}	DES	2 mL	11 min	0.02−0.05 μg mL^{-1}	76.09−97.96	43
EA-LLME-TDES-HPLC	1−1000 μg L^{-1}	TDES	100 μL	<1 min	0.3−1.0 μg L^{-1}	70.3−106.5	this work

[a]SDES-HLLME-HPLC-DAD: switchable deep eutectic solvents-homogeneous liquid−liquid microextraction-high-performance liquid chromatography-diode array detector, DES-UALPME-UHPLC-QTOF-MS: deep eutectic solvent-ultrasound-assisted liquid-phase microextraction-quadrupole time-of-flight mass spectrometry, DES-DLLME-liquid polymer-GC-μECD: deep eutectic solvent-dispersive liquid−liquid microextraction-based liquid polymer-gas chromatography-micro-electron capture detector, DES-HS-SDME-GC-FID: deep eutectic solvent-head-space single-drop microextraction- gas chromatography-flame ionization detector, DES-UAE-MSPE-HPLC-DAD: deep eutectic solvent-ultrasound-assisted extraction-magnetic solid-phase extraction-high-performance liquid chromatography-diode array detector, EA-LLME-TDES: effervescence-assisted liquid−liquid microextraction based on ternary deep eutectic solvent-high performance liquid chromatography-diode array detector.

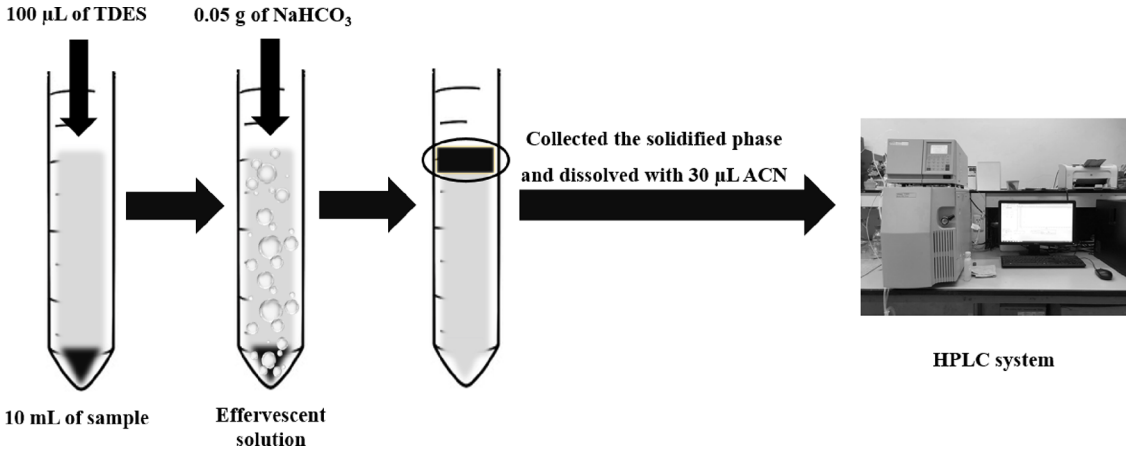

Figure 6. Schematic of the proposed effervescence assisted liquid−liquid microextraction based on the ternary deep eutectic solvent method (photograph courtesy of Rawikan Kachangoon. Copyright 2023).

microextraction method is demonstrated schematically in Figure 6.

4.5. Sample Preparation. *4.5.1. Environmental Water Samples.* Environmental water samples were collected near an agricultural area in Kantharawichai District, Maha Sarakham Province, northeast Thailand. After the environmental water samples were taken to the laboratory, they were filtered by a 0.45 μm membrane filter and kept at 4 °C in a refrigerator until extraction (see Section 4.4).

4.5.2. Honey Samples. Honey samples were bought from a supermarket in Kantharawichai District, Maha Sarakham Province, northeast Thailand. For the honey samples, 5 g of honey was diluted to 50 mL using water. The sample solution was mixed using hand shaking for 30 s and then filtered through Whatman (no. 1) filter paper to remove particulate matter. The diluted honey was passed through a 0.45 μm nylon membrane filter and then extracted using the developed method (see Section 4.4).

4.5.3. Bean Samples. Bean samples including soybean, red bean, and mung bean were bought from a local market in Maha Sarakham Province, northeast Thailand. The beans were washed and soaked in water and left to dry in atmosphere. After that, the dried seeds were placed in an oven at 65 °C for 8.0 h before grinding into fine powder. The powdered bean sample was weighed (0.10 g) into centrifuge tubes and extracted with 5.0 mL of acetonitrile on a mechanical shaker for 15 min. Then, the supernatant was adjusted to 10 mL with deionized water, passed through a 0.45 μm nylon membrane filter, and then extracted using the developed method.

4.6. Evaluation of EF and Relative Recovery (RR). In order to study the effect of experimental extraction conditions on the extraction efficiency, EF was evaluated between the analyte concentration in the final phase (C_{sed}) and the initial concentration in the analyte in the aqueous sample solution (C_0), according to eq 2:

$$EF = C_{sed}/C_0 \quad (2)$$

The percentage relative recovery (RR, %) was calculated as the % amount of analyte recovered from the matrix with reference to the extracted standard (standard spiked into the same matrix), according to eq 3:

$$RR\ (\%) = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (3)$$

where C_{found} is the concentration of analyte after adding a known amount of working standard to real samples, C_{real} is the analyte concentration in real samples, and C_{added} represents the concentration of a known amount of working standard that was spiked into the real samples.

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Notes

The authors declare no competing financial interest.

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