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Extraction and determination of strobilurin fungicides residues in apple samples using ultrasound-assisted dispersive liquid-liquid microextraction based on a novel hydrophobic deep eutectic solvent followed by H.P.L.C-U.V

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ABSTRACT

In this study, several novel and natural hydrophobic deep eutectic solvents (D.E.Ss) were prepared using methyl trioctylammonium chloride (M.T.O.A.C) as H.B.A and different types of straight chain alcohols as H.B.Ds. One of the D.E.Ss composed of M.T.O.A.C and n-butanol was advantageously used to develop an ultrasound-assisted dispersive liquid-liquid microextraction (U.A–D.L.L.M.E) method combined with high-performance liquid chromatography-ultraviolet detection (H.P.L.C–U.V) for the determination of some strobilurin fungicides in apple samples. Several important parameters influencing extraction efficiency were investigated and optimised, including the type and volume of extractant in ultrasound stage, sonication time, the type and volume of D.E.S, sample solution pH and effect of salt addition. Under optimal experimental conditions, the method showed good linearity with correlation coefficients (R^2) of 0.9985 – 0.9991 in the linear range of 4–1500 µg kg^{-1}, low limits of detection of 1.5–2 µg kg^{-1} and acceptable extraction recoveries in the range of 76–92%. Enrichment factor was in the range of 95–115. The proposed method was successfully applied for the extraction and preconcentration of trace fungicides in apple samples, and the results demonstrated the potential of the synthesised D.E.S for the extraction and determination of contaminants in aqueous samples.

Introduction

Apple is a fruit with high nutritional value and is very beneficial for human health. Kermanshah Province is located in the western part of Iran with a temperate climate, where most of the orchards are apple and, apple consumption is high in this province. Nowadays, many efforts have been made to improve and increase agricultural products. Part of this improvement is related to the use of fungicides to increase agricultural and food production in the world (Nogueira et al. 2020). Strobilurins, which are used as a protective or preventive agent in agriculture, are natural fungicides extracted from mushrooms. These compounds are more effective in plants before infection and the onset of the disease cycle (Balba 2007). Azoxystrobin, pyrimethanil and kresoxim-methyl are the most important new generation strobilurin fungicides that have different function and higher efficiency compared to traditional fungicides

(Liang et al. 2013a). Azoxystrobin kills the fungus by disrupting its respiratory system and kresoxim-methyl is a strobilurin that acts on the respiration process by blocking the transport of electrons within the mitochondria (Cabras et al. 1998). Pyrimethanil is an anilinopyrimidine that acts by inhibiting the biosynthesis of methionine by the pathogen (Sun et al. 2010). The use of strobilurin fungicides is an effective method to control the population of fungus and thus increase agricultural products, but the widespread use of these fungicides has led to direct contamination of agricultural and food products and the residue of these toxins in agricultural products and foods, especially fruits and vegetables, have caused public health concerns due to their high consumption in daily life (Liang et al. 2013b).

Methods including micellar electrokinetic capillary chromatography (MEKC) (Guo et al. 2017) and various electrochemical techniques such as

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voltammetry (Pacheco et al. 2010), amperometry (Dornellas et al. 2014), etc., have been used to analyse strobilurin fungicide residues, but the most common methods for separation and quantification of these compounds are high performance liquid chromatography (H.P.L.C) (Campillo et al. 2010; Fontana et al. 2011; Noh et al. 2019) and gas chromatography (G.C) (Vinas et al. 2010; Xue et al. 2015; Rodrigues et al. 2017). Isolation and extraction of strobilurin fungicide residues are an important stage for their determination in food samples. However, despite the use of a suitable analytical instrument, an extraction procedure is required before the strobilurin fungicide residues analysis. It was well known that with the continuous development of technology, in addition to the common solid-phase extraction (S.P.E) and solid-phase microextraction (S.P.M.E) methods, liquid-phase extraction (L.P.E) and liquid-phase microextraction (L.P.M.E) methods have been popular among researchers. In these methods the analytes are extracted into a proper solvent at millilitre or microlitre level (Farajzadeh et al. 2014). Dispersive liquid-liquid microextraction (D.L.L.M.E) is a rising sample pre-treatment technique first introduced by Assadi and co-workers in 2006 (Rezaee et al. 2006). One of the most important disadvantages of this method was the use of toxic organic solvents as the extracting solvent (Ahmadi-Jouibari & Fattahi 2015; Birjandi et al. 2008; Taheri et al. 2015a). Therefore, it is important to exploit the greener and more eco-friendly extractants. Different organic, ionic, and deep eutectic solvents (D.E.Ss) have been used in liquid phase extraction and microextraction procedures as the extractive media (Shamsipur and Fattahi 2011; Pirsaheb et al. 2013a, 2013b; Ahmadi-Jouibari et al. 2014; Sadeghi et al. 2015; Taheri et al. 2015b; Ataee et al. 2016; Yilmaz and Soylak 2016; Karimaei et al. 2017; Aydin et al. 2018; Tuzen et al. 2021a). In the recent years D.E.Ss have attracted many attentions due to their cheapness, easy preparation, high extraction capability, and lesser toxicity. D.E.S are homogeneous and clear solutions formed by combining hydrogen bond donor (H.B.D) with hydrogen bond acceptor (H.B.A) in a certain ratio at the suitable temperature. Currently, D.L.L.M.E combined with hydrophobic D.E.S as extractant has been suitable for the extraction of organic and inorganic analytes from food and environmental samples (Akramipour et al. 2018; Faraji 2019; Habibollahi et al. 2019; Davoodi et al. 2020; Erbas and Soylak 2020; Afshar Mogaddam et al. 2021; Tuzen et al. 2021b).

In this study, several novel hydrophobic D.E.Ss were synthesised and investigated for the U.A−D.L.L.M.E extraction of three common strobilurin fungicides (azoxystrobin, pyrimethanil and kresoxim-methyl) from apple samples in combination with high-performance liquid chromatography coupled with an ultraviolet detection (H.P.L.C−U.V). Methyltrioctylammonium chloride (M.T.O.A.C) was used as a H.B.A and ethylene glycol, n-butanol, glycerol, n-heptanol and n-nonanol were used as H.B.Ds. Ultrasound assistance was used to extract the analytes from the solid sample and to prepare the samples for the D.L.L.M.E step. The extraction efficiency of the obtained D.E.Ss was compared to select the optimum D.E.S, and the main parameters affecting the extraction efficiency were optimised, including the volume of the D.E.S, sample solution pH and the amount of salt addition. Finally, the proposed U.A−D.L.L.M.E−H.D.E.S method was validated under the optimised conditions and employed for the determination of common strobilurin fungicides in real apple samples.

Materials and methods

Reagents and solutions

Azoxystrobin (95%), pyrimethanil (97%) and kresoxim-methyl (99%) were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Stock standard solutions of fungicides were prepared in methanol (5.0 mL), with concentration of 1000 mg L^{-1} and stored at −20°C. The working standard solutions were prepared daily by diluting the stock solutions with deionised water to the required concentrations. The ultra-pure water was purchased from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran). Methanol, acetonitrile, phosphate salt (analytical grade), ethylene glycol, n-butanol, glycerol, n-heptanol, n-nonanol and NaCl were obtained from Merck (Darmstadt, Germany). M.T.O.A.C with purity higher than 97% were purchased by Aladdin Biochemical Co., Ltd. (Shanghai, China).

Instrumentation

The analysis of target fungicides was achieved on a H.P.L.C Knauer (Berlin, Germany) equipped with a Knauer, Azura UVD 2.1 L U.V detector, Azura P 6.1 L pump and a 20 μL injection loop injector (model 7725i, Rheodyne, Cotati, CA, USA). The separation was carried out on an Anachem C_{18} analytical column (15 cm × 4.6 mm, with 5 μm particle size), preceded by a Security Guard Cartridge C_{18} (both from Anachem, Luton, UK). The mobile phase consisted of 70% methanol and 30% water at a flow rate of 1 mL min^{-1}, and the column temperature was maintained at 30°C. The U.V detection wavelength was set to 210 nm for target compounds.

Sampling and preparation of sample

To collect samples, Kermanshah Province was first zoned into 4 regions: north, south, east and west. Two orchards were randomly selected in each zone and a sample was taken from each orchard. Generally speaking eight samples were chosen and each sample was packaged and labelled separately, and was kept in the dark at 5°C before analysis.

For preparation of sample, a representative portion of apple sample was chopped using a knife and homogenised by Buchi Mixer B 400 (Flawil, Switzerland). Analytical portions of 1.00 g homogenised apple sample (spiked or not with target fungicides) was exactly weighted into a 10-mL test tube and 5.0 mL of acetone as extractant was added and extracted in an ultrasonic bath for 30 min. The test tube was then centrifuged at 5000 rpm for 3 min and the supernatant was transferred to another test tube for further D.L.L.M.E–H.D.E.S procedure.

Preparation of deep eutectic solvents

Preparation of the D.E.Ss was performed by mixing one of the five organic reagents (ethylene glycol, *n*-butanol, glycerol, *n*-heptanol and *n*-nonanol) as H.B.D and M.T.O.A.C as H.B.A with a molar ratio of 1:1 and the mixtures were heated at 75°C under magnetic stirring until a transparent and uniform liquid formed. Other molar ratios (3:1, 2:1, 1:2, 1:3, 1:4 and 1:5) of the best D.E.S (M.T.O.A.C:*n*-butanol) were also obtained in the same way.

D.L.L.M.E–H.D.E.S procedure

Before D.L.L.M.E procedure, about 250 μL of D.E.S was added to the acetone collected in section 2.3 (50 μL of D.E.S per 1 mL of acetone). For D.L.L.M.E–H.D.E.S, 1.0 mL of this mixture was rapidly injected into 5.0 mL distilled water which was placed in a 10-mL test tube using a Hamilton 1.00 mL syringe (gastight, Nevada, USA). By injecting the mentioned mixture in water, dispersed fine droplets of D.E.S form a cloudy solution. In this step, target fungicides were extracted into these fine droplets of D.E.S, in a few seconds. After centrifugation for 3 min at 5000 rpm, the D.E.S floated at the top of the test tube. The test tube was put into an ice bath (3 min); where the D.E.S layer solidified. The solidified D.E.S was transferred into a another test tube where it was melted at room temperature and diluted with the same volume of methanol to reduce viscosity. Finally, 25 μL of the mixture was injected into the H.P.L.C–U.V.

Results and discussion

Optimisation of ultrasonic parameters

Selection of extraction solvent

In the U.A–D.L.L.M.E method, the extractant in the ultrasonic step must also play the role of the dispersant at the D.L.L.M.E step. Accordingly, solvents of acetone, acetonitrile, T.H.F and methanol, which have the required ability, were used as extractant in the ultrasonic step and the efficacy of these solvents on the extraction recovery of target compounds from apple sample were investigated. The results in Figure 1(a) show that using acetone as an extractant in the ultrasonic stage, the maximum extraction efficiency for the desired analytes is obtained. Therefore, acetone was selected as the extractant at this stage.

Selection of sonication time

To study the efficacy of sonication time on the extraction of target fungicides, some experiments were performed using different sonication times (10, 20, 30, 40, 50 and 60 min) using an ultrasonic instrument with fixed power of 350 W. The results in Figure 1(b) show that by increasing the sonication time from 10 to 30 min, the extraction efficiency increases due to mass transfer of analyte
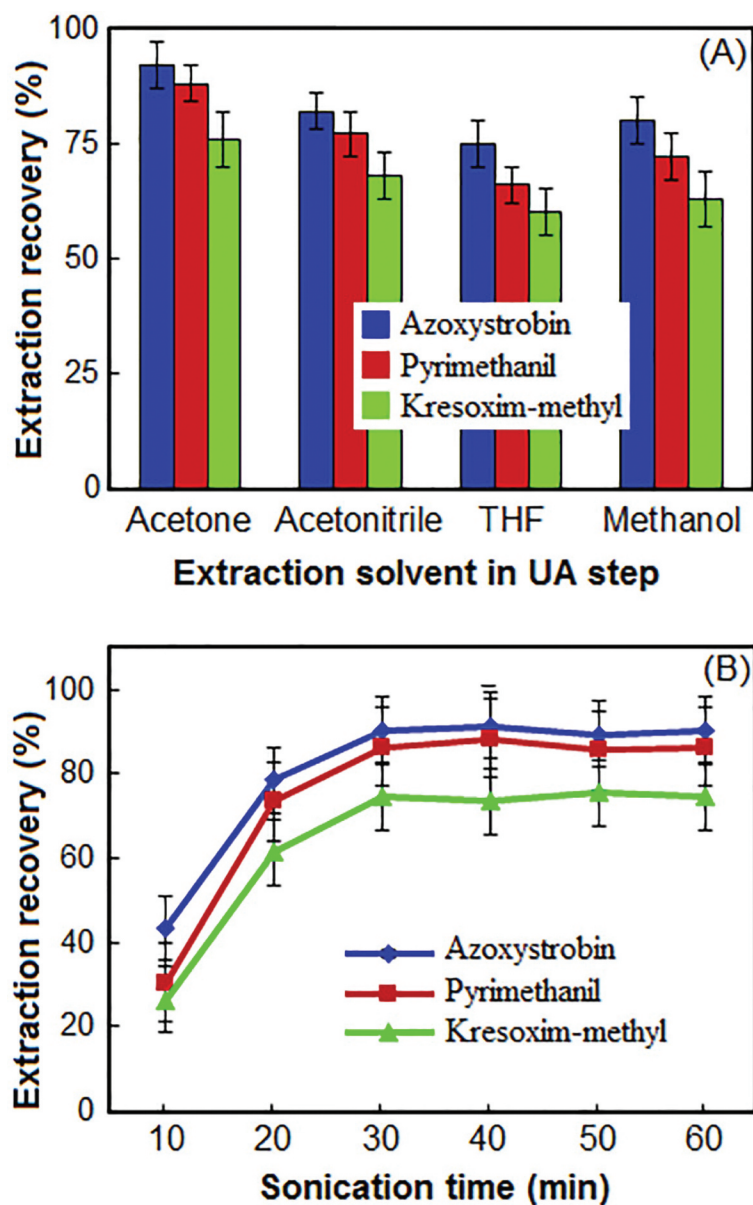
Figure 1. Effect of type of extraction solvent (a) and sonication time (b) in ultrasonic step on the recovery of three strobilurin fungicides from apple samples. Extraction conditions: types of D.E.S, T.O.M.A.C:*n*-butanol; molar ratio of M.T.O.A.C to n-butanol, 1:3; D.E.S volume, 50 μL; disperser solvent volume, 1.0 mL; sample solution pH, 7; concentration of NaCl, without salt addition; and room temperature.

from cellular material to acetone by diffusion and osmosis (Bidari et al. 2011). As the sonication time increases further, there is no significant change in the analytes extraction efficiency. Thus, 30 min was chosen as the best time of sonication.

Optimisation of D.L.L.M.E–H.D.E.S parameters

Select the type of D.E.S

For effective extraction of the strobilurin fungicides in D.L.L.M.E–H.D.E.S, D.E.S as an extractant must have a high partition coefficient for the analytes in the extractant, be non-volatile and insoluble in water, and not interfere with the subsequent determination of the analytes. Based on these parameters, five D.E.Ss including M.T.O.A.C:ethylene glycol, M.T.O.A.C:*n*-butanol, M.T.O.A.C:glycerol, M.T.O.A.C:*n*-heptanol and M.T.O.A.C:*n*-nonanol were tested as possible extraction solvent. As shown in Figure 2(a), the D.E.S composed of M.T.O.A.C and *n*-butanol has a better extraction efficiency than other D.E.Ss. So, it was preferred to use in the next steps.

Select the molar ratio of M.T.O.A.C:n-butanol

In this study, the most suitable molar ratio of M.T.O.A.C:n-butanol was obtained to achieve best extraction efficiency. For this purpose, the D.E.Ss were obtained by using M.T.O.A.C and *n*-butanol with different ratios of 3:1, 2:1, 1:1, 1:2, 1:3, 1:4 and 1:5. The results in Figure 2(b) display that M.T.O.A.C and *n*-butanol at a 3:1 and 2:1 molar ratios could not form D.E.S. The mixture of M.T.O.A.C and *n*-butanol in other molar ratios has a positive effect on the extraction efficiency of the target fungicides. However, D.E.S obtained from a mixture of M.T.O.A.C and *n*-butanol in a 1:3 molar ratio, has higher extraction efficiency. So, the 1:3 molar ratio of M.T.O.A.C and *n*-butanol was chosen for subsequent experiments.
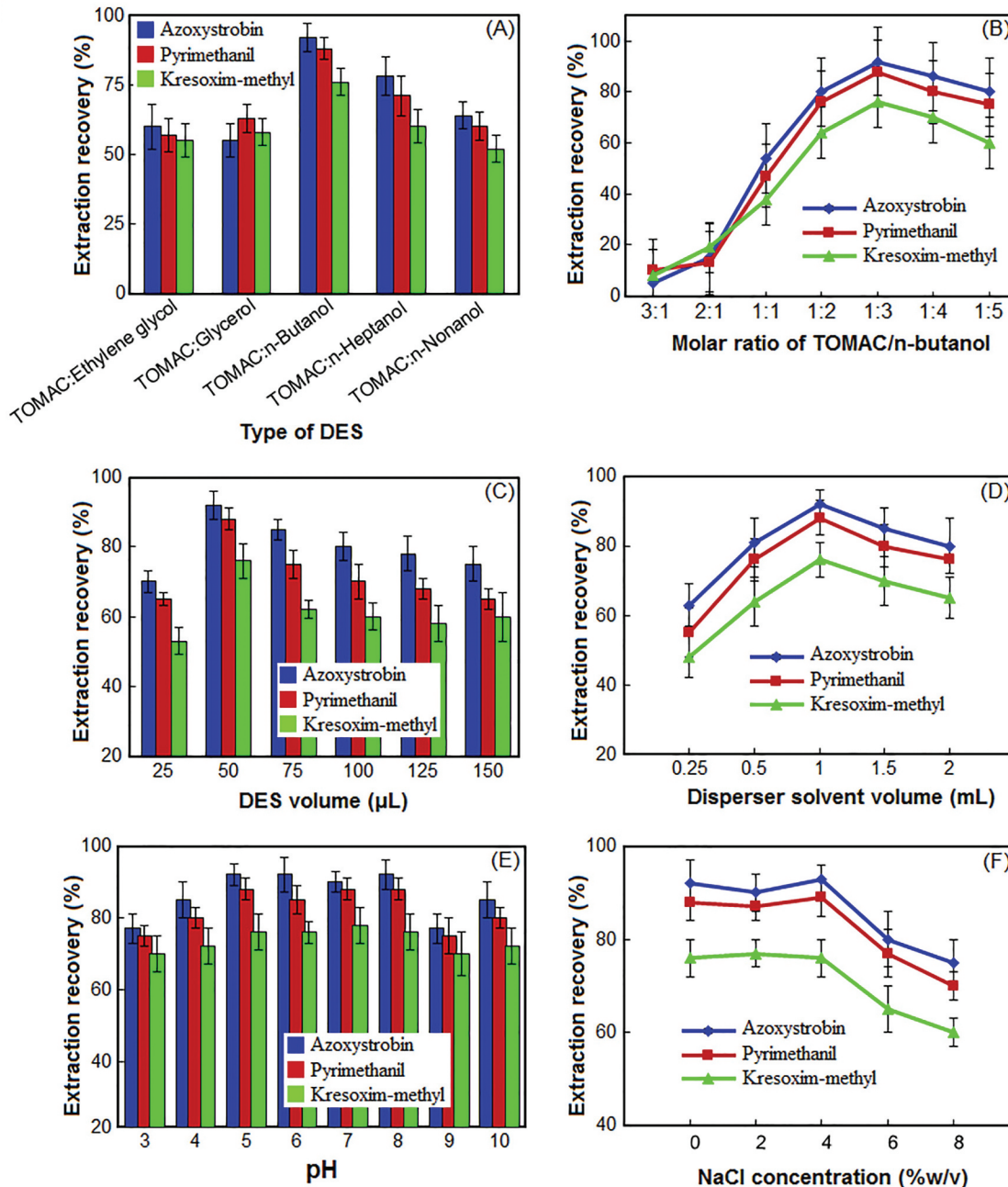
Figure 2. Effect of the different types of D.E.S (a), molar ratio of M.T.O.A.C to *n*-butanol (b), D.E.S volume (c), disperser solvent volume (d), sample solution pH (e) and concentration of NaCl (f) on the extraction recovery of three strobilurin fungicides from apple samples. Extraction conditions: similar to those in Figure 1, except for a extraction solvent in U.A step, acetone; sonication time, 30 min.

Table 1. Quantitative result of U.A−D.L.L.M.E−HD.E.S and H.P.L.C−U.V of strobilurin fungicides from apple.

Analyte	E.R%	E.F	R.S.D% (Intra-day, n = 7)	R.S.D% (Inter-day, n = 7)	L.R (µg kg^{-1})	r^2	L.O.D (µg kg^{-1})	(µg kg^{-1})
Azoxystrobin	92	115	4.6	6.3	4 − 1500	0.9991	1.5	4
Pyrimethanil	88	110	3.8	5.6	4 − 1500	0.9988	1.5	4
Kresoxim-methyl	76	95	5.2	7.3	6 − 1000	0.9985	2	6
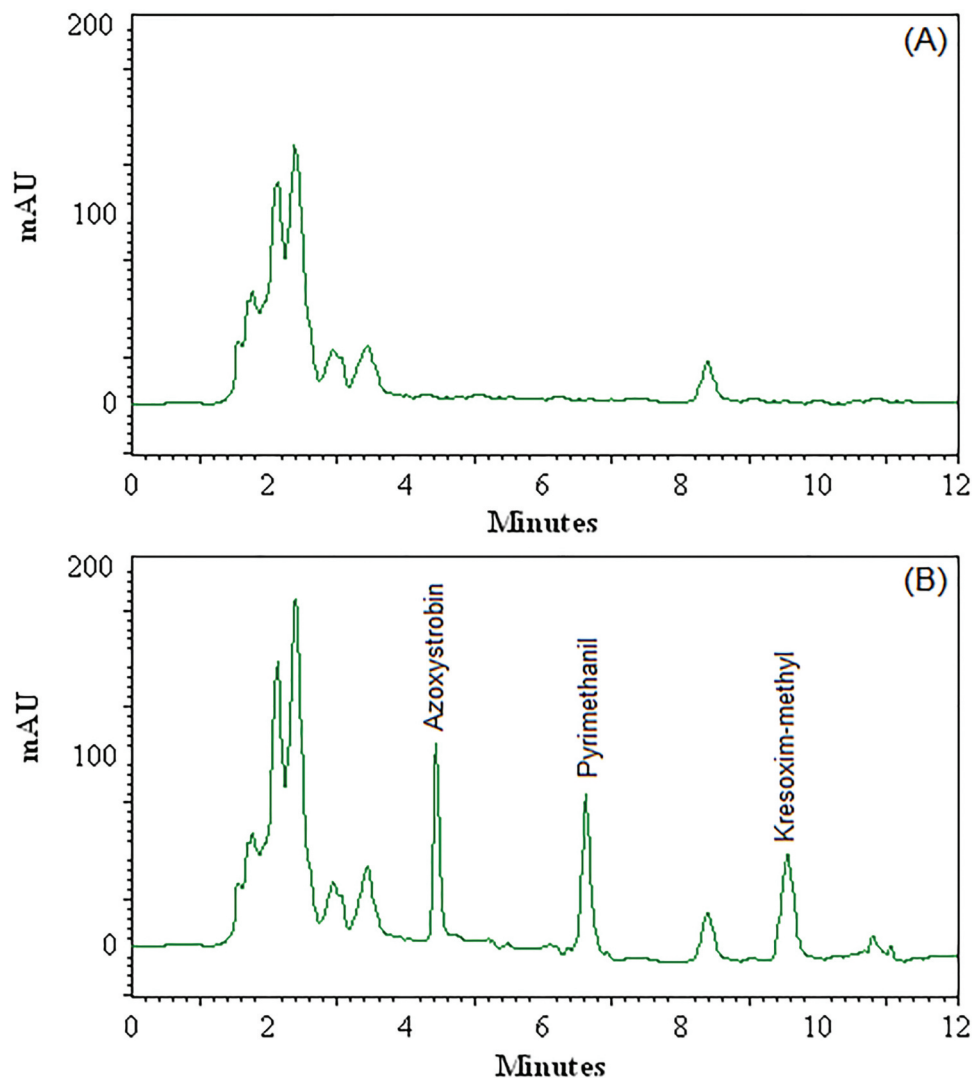
Figure 3. Chromatograms of apple sample (a) and the corresponding spiked ones at concentration of 100 µg kg^{-1} for target fungicides (b) obtained by using U.A−D.L.L.M.E−HD.E.S combined H.P.L.C−U.V. Extraction conditions: similar to those in Figures 1 and 2.

Select the volume of D.E.S

The volume of extractant is a crucial parameter that directly affected on the extraction efficiency by enriching or diluting the concentration of analytes. On the one hand, insufficient D.E.S volume can lead to incomplete extraction of analytes. On the other hand, an excess volume of D.E.S will reduce the extraction efficiency due to dilution effect. To investigate the effect of the D.E.S volume on extraction efficiency of target analytes, different volumes of D.E.S (25, 50, 75, 100, 125 and 150 µL) were used to perform the experiments under the same conditions. As shown in Figure 2(c), the extraction recovery of the target fungicides increased when the D.E.S volume changed from 25 to 50 µL. With further increase in the volume of D.E.S, the extraction efficiency decreases slightly, which is probably due to the dilution effect. Therefore, 50 µL of D.E.S was selected in order to obtain higher extraction efficiency.

Select the type of disperser solvent and its volume

As mentioned in Section 3.1.1, the extraction solvent in the ultrasonic stage should act as the disperser solvent in D.L.L.M.E stage. Therefore, acetone, acetonitrile, T.H.F and methanol were selected for this purpose. According to the results in Section 3.1.1, acetone was selected as disperser solvent.

For obtaining optimised volume of disperser solvent, different experiments were done by using volumes 0.25, 0.50, 1.00, 1.50 and 2.00 mL of acetone containing different volume of D.E.S to obtain the same floating volume of extractant. In order to attain a constant volume of the floated phase, acetone and D.E.S volumes were changed simultaneously. Figure 2(d) shows that at low volumes (0.25 and 0.50 mL), acetone cannot disperse the D.E.S properly and the cloudy solution is not formed completely. By using more than 1.00 mL acetone, the solubility of target analytes in aqueous solution increases and it causes a decrease in the extraction efficiency. According to the results, 1.00 mL of acetone was chosen as the optimum volume.

Select the sample solution pH

The pH value of sample solutions would change the degree of ionisation and speciation of analytes, and further influence the partition coefficient and extraction efficiency of the target compounds. The effect of sample solution pH on the extraction recovery of the target analytes was investigated in the range of 3–10. As show in the Figure 2(e), the highest extraction recovery was obtained when the pH of the sample phase were between 5 and 8. However, acceptable efficiencies were obtained for target analytes at pH less than 5 or greater than 8. The results demonstrated that the pH value of the samples need not be adjusted for further steps.

Salt effect

To find the optimum amount of salt, different amounts of NaCl including 0, 2, 4, 6 and 8% (w/v) were investigated. As shown in Figure 2(f), with increasing NaCl from 0 to 4%, the extraction recoveries of strobilurin fungicides remain nearly constant, because on the one hand, the salting-out effect increases the extraction recoveries, while on the other hand the D.E.S solubility in the sample solution decreases and the volume of the floated phase increases and due to the dilution effect, the

extraction recoveries of analytes decreases. As a result, these two contrasting effects neutralise each other and the efficiency of target analytes remains almost constant. At concentrations higher than 4%, the dilution effect prevails on salting-out effect and the extraction recoveries decreases. Therefore, the experiments were carried out in the absence of any salt.

Table 2. Relative recoveries and standard deviations of target fungicides from spiked apple samples.

Sample No.	Analyte	Added (µg kg^{-1})	Found (Mean ± SD) (µg kg^{-1})	Relative Recovery (%)
1	Azoxystrobin	50	49.6 ± 3.2	99
		100	103.8 ± 6.5	104
	Pyrimethanil	50	50.3 ± 2.8	101
		100	96.6 ± 6.7	97
	Kresoxim-methyl	50	47.2 ± 3.6	94
		100	91.9 ± 6.1	92
2	Azoxystrobin	50	51.2 ± 3.3	102
		100	97.8 ± 5.5	98
	Pyrimethanil	50	52.9 ± 4.8	106
		100	93.6 ± 8.5	94
	Kresoxim-methyl	50	50.7 ± 4.3	101
		100	99.0 ± 6.5	99
3	Azoxystrobin	50	53.0 ± 2.5	106
		100	93.1 ± 4.8	93
	Pyrimethanil	50	46.8 ± 2.6	94
		100	97.5 ± 5.5	97
	Kresoxim-methyl	50	52.5 ± 3.7	105
		100	99.0 ± 7.5	99
4	Azoxystrobin	50	48.5 ± 3.5	97
		100	100.8 ± 4.2	101
	Pyrimethanil	50	51.7 ± 3.1	104
		100	94.2 ± 6.3	94
	Kresoxim-methyl	50	51.4 ± 4.2	103
		100	98.2 ± 8.1	98
5	Azoxystrobin	50	53.5 ± 2.5	107
		100	103.4 ± 8.2	103
	Pyrimethanil	50	49.2 ± 3.1	98
		100	91.6 ± 4.9	92
	Kresoxim-methyl	50	48.5 ± 3.7	97
		100	102.8 ± 6.3	103
6	Azoxystrobin	50	46.5 ± 2.2	93
		100	98.0 ± 5.9	98
	Pyrimethanil	50	52.8 ± 4.3	106
		100	97.6 ± 7.3	98
	Kresoxim-methyl	50	52.0 ± 3.8	104
		100	101.6 ± 8.7	102
7	Azoxystrobin	50	50.5 ± 2.5	101
		100	93.4 ± 7.2	93
	Pyrimethanil	50	45.5 ± 3.6	91
		100	96.6 ± 7.0	97
	Kresoxim-methyl	50	48.5 ± 2.7	97
		100	102.8 ± 4.3	103
8	Azoxystrobin	50	47.3 ± 3.2	95
		100	99.0 ± 6.9	99
	Pyrimethanil	50	48.8 ± 3.3	98
		100	105.6 ± 8.3	106
	Kresoxim-methyl	50	46.2 ± 2.8	92
		100	101.6 ± 5.7	102

Table 3. Comparison of U.A–D.L.L.M.E–HD.E.S with other extraction methods for determination of strobilurin fungicides in different samples.

Methods	L.O.D[a] (μg kg^{-1} or L^{-1})	L.R[b] (μg kg^{-1} or L^{-1})	R.S.D[c] %	Extraction solvent volume	Extraction time (min)	Samples	Reference
B.D.D–H.P.L.C–U.V–D.A[d]	1.33–1.57	5–15	<10	35 mL	22	Bean	Nogueira et al. (2020)
I.L-U.S.A.E.M.E–H.P.L.C–V.W.D[e]	0.73–2.2	5–5000	3.1–5.4	40 μL	<30	Water samples	Liang et al. (2013b)
U.A.S.E.M.E–S.F.O.D–H.P.L.C–V.W.D[f]	2–4	5–10000	3.2–4.9	45 μL	20	Fruit juice	Liang et al. (2013a)
S.P.E–L.C–M.S/M.S[g]	0.07–0.6	0.2 – 2000	1.5–9.5	2 mL	<30	Wine	Fontana et al. (2011)
S.B.S.E–L.C–D.A.D[h]	5–10	10–5000	<11	2 mL	50	Fruit	Campillo et al. (2010)
U.S.A.E.M.E/S.D.M.E–G.C–M.S[i]	0.006–0.21	0.06–300	<10	10 & 20 μL	<35	Juices & fruits	Vinas et al. (2010)
U.A–D.L.L.M.E–HD.E.S–H.P.L.C–U.V	1.5–2	4 – 1500	3.8 – 5.2	50 μL	5	Apple	This work

[a]L.O.D, limit of detection.
[b]L.R, linear range.
[c]R.S.D, relative standard deviation.
[d]Boron-doped diamond–high-performance liquid chromatography–ultraviolet and amperometric detection.
[e]Ionic liquid-based ultrasound-assisted emulsification microextraction–high-performance liquid chromatography–variable wavelength detector.
[f]Ultrasound-assisted surfactant-enhanced emulsification microextraction with solidification of floating organic droplet–high-performance liquid chromatography–variable wavelength detector.
[g]Solid-phase extraction–liquid chromatography quadrupole time-of-flight tandem mass spectrometry.
[h]Stir bar microextraction–liquid chromatography–diode array detection.
[i]Ultrasound-assisted emulsification and single-drop liquid–liquid microextraction–gas chromatography–mass spectrometry.

Quantitative analysis

The U.A–D.L.L.M.E–H.D.E.S method was validated with respect to linearity (L.R), limit of quantification (L.O.Q), limit of detection (L.O.D), precision including repeatability (intra–day) and reproducibility (inter–day), extraction recovery (E.R) and enrichment factor (E.F). The characteristics of the calibration curve are summarised in Table 1. The linear range was 4–1500 μg kg^{-1} with r^2 ranging from 0.9985 to 0.9991, which showed a good linearity. The L.O.Ds (signal-to-noise ratio of 3) and L.O.Qs (signal-to-noise ratio of 10) for the three strobilurin fungicides were in the range of 1.5–2 μg kg^{-1} and 4–6 μg kg^{-1}, respectively. The repeatability (intra-day) and reproducibility (inter-day) of the present method were calculated by using R.S.D (n = 7) having 100 μg kg^{-1} of target analytes and were in the range of 3.8–5.2 and 5.6–7.3%, respectively. The E.F and E.R% of the method were 95–115 and 76–92%, respectively, at the concentration level of 100 μg kg^{-1} of target strobilurin fungicides.

Analysis of real samples

To confirm the method applicability in determination of the strobilurin fungicides in apple, different apple samples were analysed by the developed method. The results showed that the analysed apple samples were free of fungicides contamination. All apple samples were spiked with the standards of three strobilurin fungicides at two concentrations (50 and 100 μg kg^{-1}, each fungicide) to assess matrix effects. Figure 3 shows the chromatograms of apple sample (A) and the corresponding spiked ones at concentration of 100 μg kg^{-1} for target fungicides (B). The results of relative recovery of apple samples are shown in Table 2. Relative recoveries for all fungicides in different apple samples are between 91 and 107. These results demonstrate that the apple matrices, in our present context, have no significant effect on U.A–D.L.L.M.E–H.D.E.S for determination of strobilurin fungicides.

Comparison of U.A–D.L.L.M.E–H.D.E.S with other methods

The U.A–D.L.L.M.E–H.D.E.S combined with H.P.L.C–U.V is compared with other procedures for determination of strobilurin fungicides in different samples and the results are summarised in Table 3. As shown in Table 3, the method has the advantage of lower limits of detection as well as a lower extraction time compared to other methods. The consumption of toxic and expensive organic solvents is greatly reduced. The R.S.D of the presented method are superior to those reported before and the linear range is comparable to other methods and in some

cases is better. All these results indicate that U.A–D.L. L.M.E–H.D.E.S is a simple, inexpensive and reproducible technique that can be used for the extraction of strobilurin fungicides in apple samples.

Conclusions

In this work, for the first time, a novel hydrophobic deep eutectic solvent (H.D.E.S) as extractant for U.A−D.L.L.M.E combined with H.P.L.C −U.V has been applied for the determination of three strobilurin fungicides in apple samples. In the procedure, a hydrophobic D.E.S consisting of T.O.M.A.C as H.B.D and *n*-butanol as H.B.A with molar ratio of 1:3, was highly effective for extraction and preconcentration of the fungicides. The proposed method can reach equivalent or even higher extraction recovery than the previous methods using conventional organic solvents as extracting agents. This method is simple, low cost, and provides good repeatability and high extraction efficiency for the selected fungicides. The application of this technique in the determination of fungicides in real apple samples indicates that the proposed method is reliable and suitable for the determination of fungicides in trace levels. Furthermore, The method could be extended to other analytes and other types of fruits and vegetables samples.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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