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Salt-induced Homogeneous Liquid-liquid Microextraction as a Rapid Clean-up and Preconcentration Technique for Chromatographic Determination of Diazinon and Cypermethrin in Milk Samples

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Herein, a convenient, simple, rapid, labor efficient and economical salt-induced homogeneous liquid-liquid microextraction technique coupled with high-performance liquid chromatography-photo diode array detection system was developed for sample preparation, extraction and quantitative determination of diazinon and cypermethrin in milk samples. In a single step, a homogeneous solution containing acetone and dichloromethane was used for the extraction of analytes and precipitation of milk proteins. This solution was broken by the addition of sodium chloride as a phase separator agent. Several variables affecting the extraction efficiency such as composition of homogeneous solution, concentration of phase separator agent and extraction time were fully evaluated and optimized. Under optimal conditions for analysis of two pesticides, matrix-matched calibration curves with dynamic linear ranges of 0.065-1300 μg l^{-1}, limit of detection of 0.018-0.03 μg l^{-1}, and the limit of quantification of 0.065-0.1 μg l^{-1} were obtained. The enrichment factors and extraction recoveries were 158-174 and 79-87%, respectively. Precision and accuracy of the method based on RSDs and REs for three concentration levels of both pesticides were achieved between 2.7-12.3% and 6.1-8.3% (n = 5), respectively. The represented method was successfully applied for the simultaneous assay of diazinon and cypermethrin in different milk samples.

Keywords: Salt-induced homogeneous liquid-liquid microextraction**,** Diazinon, Cypermethrin high-performance liquid chromatography, Food analysis, Milk

INTRODUCTION

Pesticides composed of more than 1000 active substances including insecticides, herbicides, and fungicides are widely used for the control of diseases and pests in the plants. Most of the pesticides are stable under environmental conditions [1]. Pesticides have harmful effects on endocrine, respiratory, renal, cardiovascular, central nervous, and immune systems, leading to serious human diseases such as Parkinson's, Alzheimer, kidney failure and various types of malignancy [2-4]. Diazinon (DIZ) (0,0-diethyl O-[6-methyl-2-(-methyl)-4-pyrimidinyl] phosphorothioate) is an organophosphorus pesticide used as a contact insecticide, nematicide and acaricide [5]. Cypermethrin (CYP) ([cyano-(3-phenoxyphenyl)methyl] (1R,3R)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate) is photostable and the most important pyrethroid pesticides; synthetic or semisynthetic compounds allied to the natural insecticides pyrethrins. Because of relatively low toxicity, persistence, and broad-spectrum activity, this insecticide uses as a suitable replacement to other high toxic biocides; *e.g.*, organophosphates, organochlorines and carbamates [6].

Bovine milk is a rich source of proteins, fat, and minerals formulated in the diet of children and adults. Consequently, the contamination of milk with pesticide

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residues is a matter of global concern [7]. The European Union (EU) advice defines the maximum residue levels (MRLs) for DIZ and CYP in milk 20 and 50 ppb, respectively [8]. So, to ensure food safety, the monitoring of these pesticide residues in milk at a lower concentration level than the established MRLs is necessary. Among the various analytical methods, chromatography techniques are more acceptable and reliable than other methods to achieve this goal. In the literature, gas chromatography equipped with various detection systems, as well as high performance liquid chromatography with DAD and MS detectors, are extensively reported to detection and quantification of pesticides in different samples [9-11]. On the other hand, several technical limitations including the matrix complexity of the samples, trace content of analyte and some instrumental constraints caused the preconcentration steps are required prior to analysis. For this aim, various pre-concentration methods such as liquid-liquid extraction (LLE), dispersive liquid- liquid microextraction (DLLME), solid phase extraction (SPE) and solid phase microextraction (SPME) have been developed in the literature [12-15]. Although each of these methods has its advantages, they are often complex, time consuming, labor-intensive and not environmental-friendly. For example, the processes of SPE, SPME and MSPD need special devices and are relatively expensive. Furthermore, some of the methods require special procedures, such as centrifugation, vortexing, and ultrasonication, their utility is thus limited to laboratory conditions. On the other hand, despite the advantages reported for each of the above mentioned methods, they often suffer a series of shortcomings including need special devise, costly, time spending, labor-intensive, and not eco-friendly.

Homogeneous liquid-liquid microextraction (HLLME) is one of the liquid phase microextraction (LPME) modes known as a simple and powerful preconcentration technique. The method is based on the formation of a homogeneous solution between aqueous and organic solvents which provides a very large contact area. Therefore, the effective mass transform of analytes between aqueous solution and organic solvent will be achieved quickly [16-18]. In this method, the phase separation phenomena will be occurred by changes in temperature, ion strength and pH without any vigorous mechanical shaking or sonication. In recent years, several reports have been published on the successful use of this method for the extraction of organic and inorganic analytes from various matrices [19-26].

In the present work, a modified version of HLLME namely salt-induced homogeneous liquid-liquid microextraction (SI-HLLME) has been introduced for simultaneous sample clean-up and preconcentration of DIZ and CYP from milk as a complex matrix sample. The principle of SI-HLLME is based on the salting out and changing the order of the organic and aqueous phases after the salt addition. This phenomenon increases the two-phase contact and in addition to increasing the extraction efficiency of target molecules to the organic phase, the proteins present in milk precipitate in the high-density aqueous phase. The experimental conditions of the proposed sampling method coupled with HPLC were optimized for the determination of DIZ and CYP in milk samples. The obtained results revealed that this method is a rapid, simple and good performance in the preconcentration of DIZ and CYP residues in the selected matrix.

EXPERIMENTAL

Chemical and Solutions

Diazinon and cypermethrin were purchased from Supelco, Sigma (USA), respectively, and HPLC grade solvents acetonitrile, methanol, and water were from Dae-Jung (South Korea). Acetone, dichloromethane, ethyl acetate, n-hexane, cyclohexane, sodium chloride, sodium sulfate, sodium carbonate, and ammonium acetate were obtained from Merck (Darmstadt, Germany). The standard stock solutions containing 50 mg l^{-1} of DIZ and CYP were prepared separately in acetonitrile and stored at 4 °C being protected from light. Fresh mixture working solution was prepared daily by an appropriate dilution of stock solutions with acetonitrile.

Instruments and Chromatographic Conditions

A Knauer (Berlin, Germany) liquid chromatography system equipped with a smart line 1000 solvent pump unit, a Rheodyne 7725 injector of 10 µl loop volume (USA), and a K-2600 photodiode array detector operating at 210 nm was used. All analyses were performed on a reversed phase

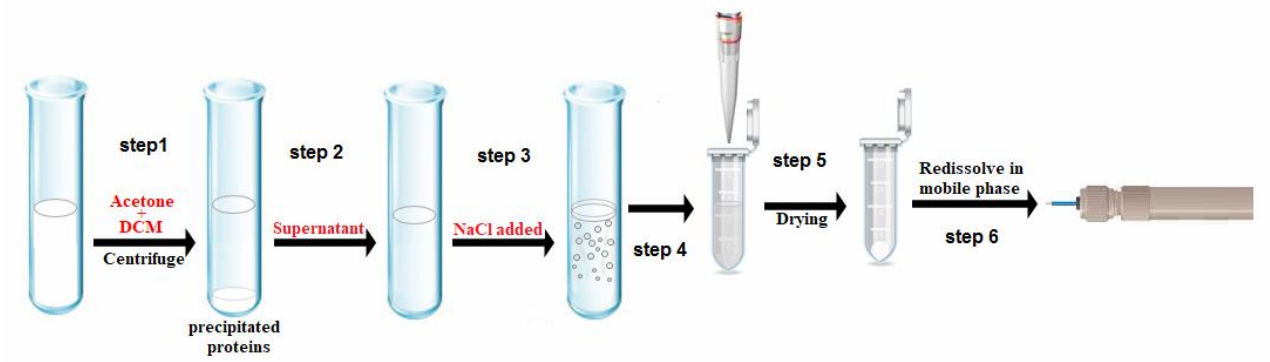

Fig. 1. Schematic display of the proposed SI-HLLME procedure for the milk sample clean-up and extraction of DIZ and CYP.

analytical column (C_{18}, 5 µm, 250 × 4.6 mm i.d.) and pre-column (Knauer, Germany). The column thermostate Jetstream Knauer oven was used for temperature maintenance at 25 °C. Displaying and acquisition of chromatograms as well as calculation of individual peak area were accomplished using Chromgate software. A vortex mixer (Dragon Lab, MX-S, Connecticut, USA) and Hettich centrifuge (Tuttlingen, Germany) were used for the homogenization of mixtures and protein precipitation step. Separation and quantification of analytes carried out an isocratic elution. The mobile phase was a mixture of acetonitrile 80% and water 20%. The flow rate was kept at 1 ml min^{-1}.

Milk Samples

A total of 40 milk samples including 20 raw milk, 10 pasteurized and 10 Ultra-high temperature processing (UHT) low fat milk (different brands) were obtained from fresh dairy local sources in Urmia, Iran.

SI-HLLME Procedure

The steps of proposed SI-HLLME procedure are schematically illustrated in Fig. 1. In the first step, 5 ml of milk sample was transferred into the 15 ml polytetrafluoroethylene (PTFE) tube. Then, 5 ml of acetone (as protein precipitation (PP) reagent and homogeneous solvent) containing 200 µl dichloromethane (as extracting solvent) was added to sample tube, vortexed for 30 s, and the resulting mixture was centrifuged at 25 °C for 5 min at 6000 rpm. So far, protein precipitation and microextraction steps are performed simultaneously. Subsequently, the supernatant was transferred into a 15 ml PTFE tube and 0.5 g sodium chloride (5% w/v) was added. In this step, a cloudy solution with very fine droplets of dichloromethane was formed. The mixture was then rested for 3 min, causing the fine droplets of the extraction phase are collected on the top of the tube. The upper phase (accepter phase) was carefully and quantitatively transferred to a conical bottom microtube and dried under a gentle stream of nitrogen at room temperature. Finally, the residue was dissolved in the 20 µl of the HPLC mobile phase and 10 µl of it was injected.

RESULTS AND DISCUSSION

In this research, SI-HLLME combined with HPLC-DAD was developed for the preconcentration and quantification of DIZ and CYP in milk samples and the optimization process executed using one variable at a time method. To achieve high extraction efficiency several effective parameters such as the type and volume of extraction and the homogeneous solvents, salt amount and extraction time were fully evaluated and optimized. For optimization experiments, working solutions with 50 µg l^{-1} concentration of analytes was chosen. All the experiments were performed triplicate.

Selection of Extraction and Homogeneous Solvents

The selection of extraction solvent is a critical and important step in the HLLME method. The extraction

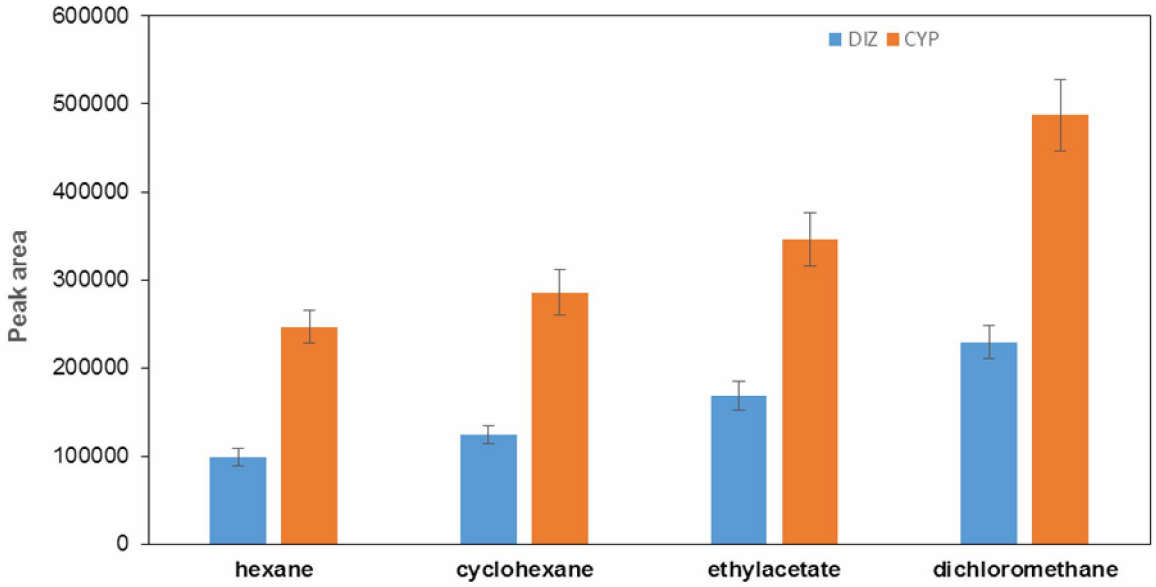

Fig. 2. Selection of extraction solvent type. Extraction conditions: sample volume, 5 ml spiked with 50 µg l^{-1} of each analyte; extraction solvent volume 350 µl of each solvent except ethylacetate (500 µl); homogeneous solvent volume (acetone), 5 ml; phase separator agent, NaCl 3% w/v; extraction time 3 min.

solvent must have some basic futures such as high extraction capability of the analytes, immiscibility with water, good stability and finally adaptability with the final analytical method (appropriate chromatographic behavior). Therefore, in this study, the suitable extraction phase was tested using several organic solvents such as hexane, cyclohexane, ethyl acetate, and dichloromethane. As shown in Fig. 2, the obtained peak area using dichloromethane had the highest extraction efficiency, so, it was selected as an organic extraction solvent. It is well demonstrated that in the HLLME technique, the consolute solvent should be miscible in both extraction solvent and water [17,18]. On the other hand, due to the simultaneous cleaning and extraction steps, the homogeneous solvent should be able to precipitate milk proteins. Hence, methanol, acetone, and acetonitrile were examined as a consolute solvent. The obtained results showed no significant change in extraction efficiency using these solvents. Therefore, due to low toxicity and the low cost of acetone, it was selected as the homogeneous and protein precipitant solvent.

Extraction Solvent Volume

Solvent extraction volume is one of the most important factors affecting extraction efficiency in HLLME. To find out the optimal value of the extraction solvent volume, the extraction outline was conducted by applying various volumes of dichloromethane at the range of 50-400 µl. As illustrated in Fig. 3, the highest peak areas of analytes were achieved at 200 µl of dichloromethane. Accordingly, 200 µl of dichloromethane was selected as an optimum volume of extraction solvent in the subsequent experiments. It should be noted that the decrease in extraction efficiency in dichloromethane volume greater than 200 µl may be related to the reduction of homogeneity and the formation of a separate phase of solvent extraction.

Homogeneous Solvent Volume

Homogeneous solvent volume is another effective factor on extraction efficiency. It should be noted that in this proposed method, acetone acts as a protein precipitant and homogeneous solvent. The study of the homogeneous solvent volume effect on extraction efficiency was carefully examined by a series of experiments using different volumes of acetone in the range of 3-8 ml (Fig. 4). At the lower volumes of acetone (< 3 ml), milk proteins did not completely precipitate and the homogeneous solution was

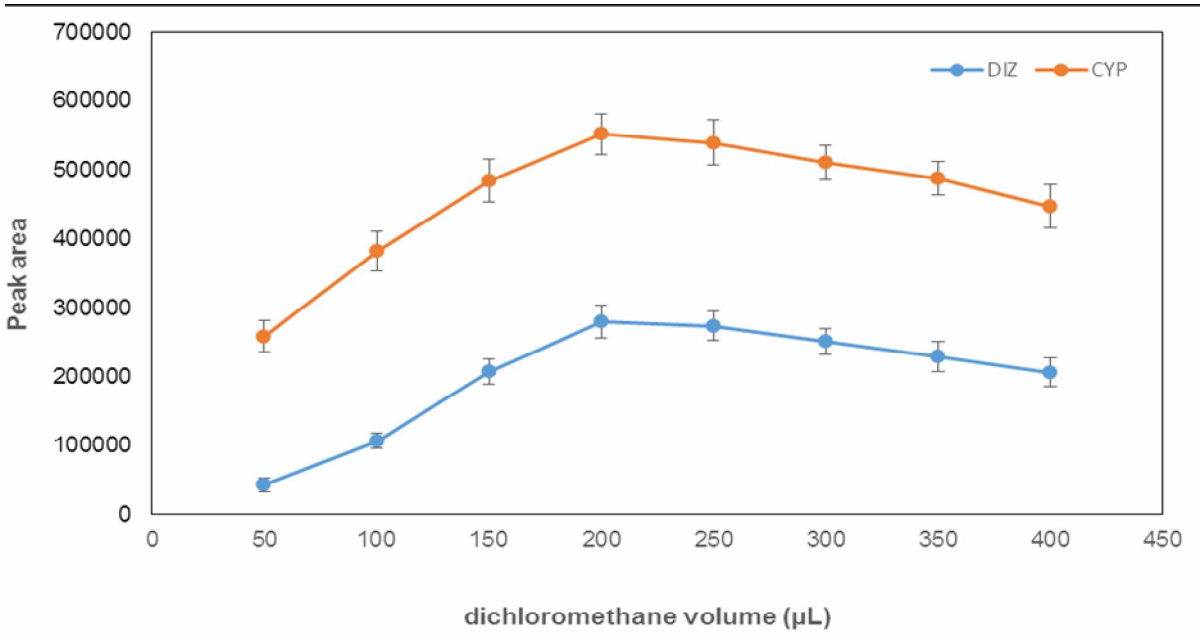

Fig. 3. Study of extraction solvent volume (dichloromethane) on the peak areas of selected pesticides. Conditions: sample volume, 5 ml spiked with 50 µg l^{-1} of each analyte; homogeneous solvent volume (acetone), 5 ml; phase separator agent, NaCl 3% w/v; extraction time 3 min.

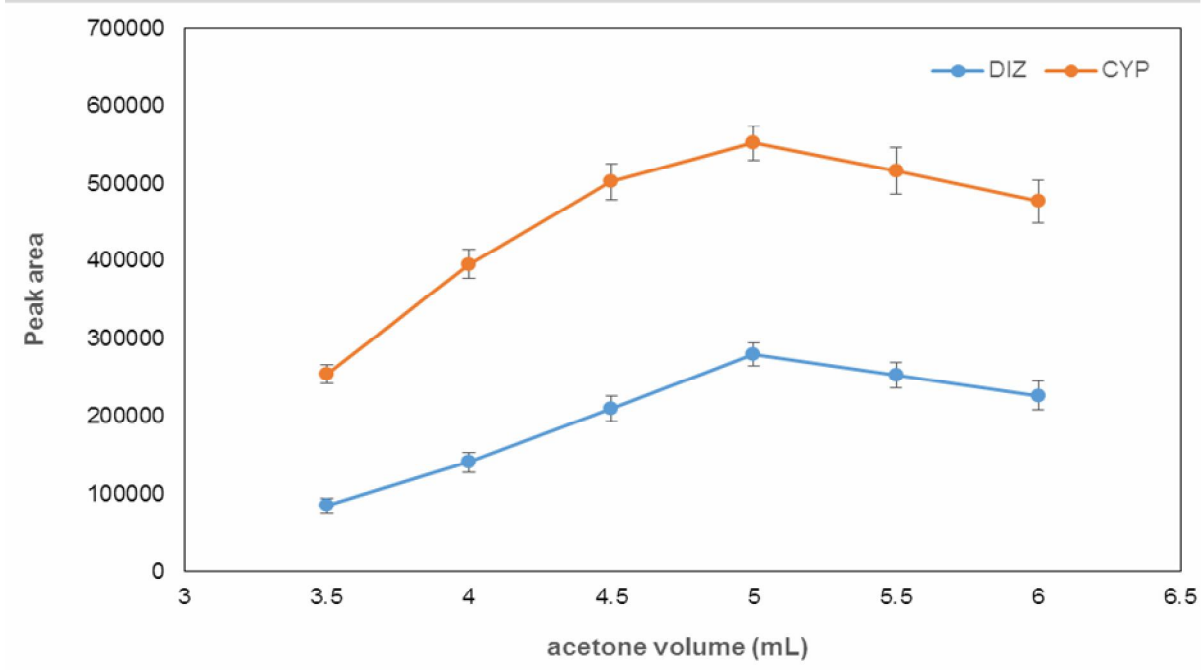

Fig. 4. Study of the homogeneous solvent volume (actone) on the peak areas of selected pesticides. Conditions: sample volume, 5 ml spiked with 50 µg l^{-1} of each analyte; extraction solvent volume (dichloromethane), 200 µl; phase separator agent, NaCl 3% w/v; extraction time 3 min.

not formed. At higher volumes of acetone (>6 ml) due to the increased solubility of dichloromethane in the aqueous phase, the acceptor phase was not collected on the top of the solution in the presence of phase separator agent. So, 5 ml of homogenous solvent was selected as an optimum volume of acetone in future experiments.

Selection of Phase Separation Agent and its Concentration

In HLLME methods, after homogeneous solution formation, the two-phase separation must be completed to ensure the efficiency of the extraction process. The phase separation can occur by changes in the temperature, ionic strength and pH [23,27,28]. Salt addition is a common method and usually used in HLLME, as salt reduces the solubility of the analytes in aqueous solution (salting-out), thereby increasing their distribution in the extraction solvent, which subsequently increases extraction efficiency. Moreover, the salt addition disturbs the homogeneity of the mixture and affects the phase separation rate. Therefore, several common salts such as sodium sulfate, sodium carbonate, sodium chloride, and ammonium acetate were examined. Among them, sodium chloride was shown maximum extraction efficiency (Fig. 5). In the next step, the amount of sodium chloride was optimized (in the range of 0.5-10% w/v) (Fig. 6). The low extraction efficiency at lower concentrations of sodium chloride (<2 wt%) is probably due to the low disturbance of the homogeneous solution making the phase separations incomplete. Further experiments showed that the extraction efficiency for both analytes reached to highest values at 5% w/v NaCl. So, 5% w/v of NaCl was selected as the optimal concentration for subsequent experiments.

Extraction Time

Extraction time is another effective parameter on the extraction procedures, especially in conventional LLE, LPME, and SPME. Therefore, in this extraction process, the effect of time on extraction efficiency at the range of 0-10 min was examined under optimal extraction conditions. The obtained results indicated that extraction efficiency is not dependent to the extraction time. This phenomenon was fully expected due to the existence of an extremely large contact surface between the extraction solvent and the aqueous phase. So, the analyte molecules are rapidly transferred from the aqueous phase to the extracting phase. This is the great advantage of homogeneous liquid-liquid microextraction technique [18,28]. In the developed method, 3 min was adopted as an extraction time to reach the maximum extraction efficiency of target analytes.

Quantitative Analysis

To evaluate the analytical figures of merit for the developed method under optimal conditions established above, several analytical parameters including enrichment factor (ER), linear range (LR), extraction recovery (ER), limit of detection (LOD), limit of quantification (LOQ), relative standard deviation (RSD), and determination coefficient (R^2) were estimated to validate the determination of target analytes in spiked sample (matrix-matched standard solution) by the proposed method. As summarized in Table 1, the obtained results show wide LRs with an appropriate linearity ($r^2 \geq 0.996$) for both analytes. The LODs and LOQs were calculated based on signal to noise ratio of 3 and 10, respectively. The LODs and LOQs values were from 0.018 to 0.03 μg l^{-1} and 0.065 to 0.1 μg l^{-1}, respectively. The enrichment factor is expressed as the concentration of target analyte in the extractant phase (C_e) divided to its concentration in the sample solution (C_0) (Eq. (1)):

$$EF = \frac{C_e}{C_0} \quad (1)$$

Also, extraction recovery is calculated as the percentage of the moles of an analyte (n_0) collected into the extractant phase (n_e) (Eq. (2)):

$$\%ER = \%EE = \frac{n_e}{n_0} \times 100 = EF \times \frac{V_a}{V_d} \times 100 \quad (2)$$

where V_a and V_d are volumes of the acceptor phase (extraction phase) and donor phase, respectively. As shown in Table 1, the obtained EFs and ERs for the selected analytes were ranged from 158-174 and 79-87, respectively. The precision of an analytical method is the closeness of agreement between a series of individual measurements

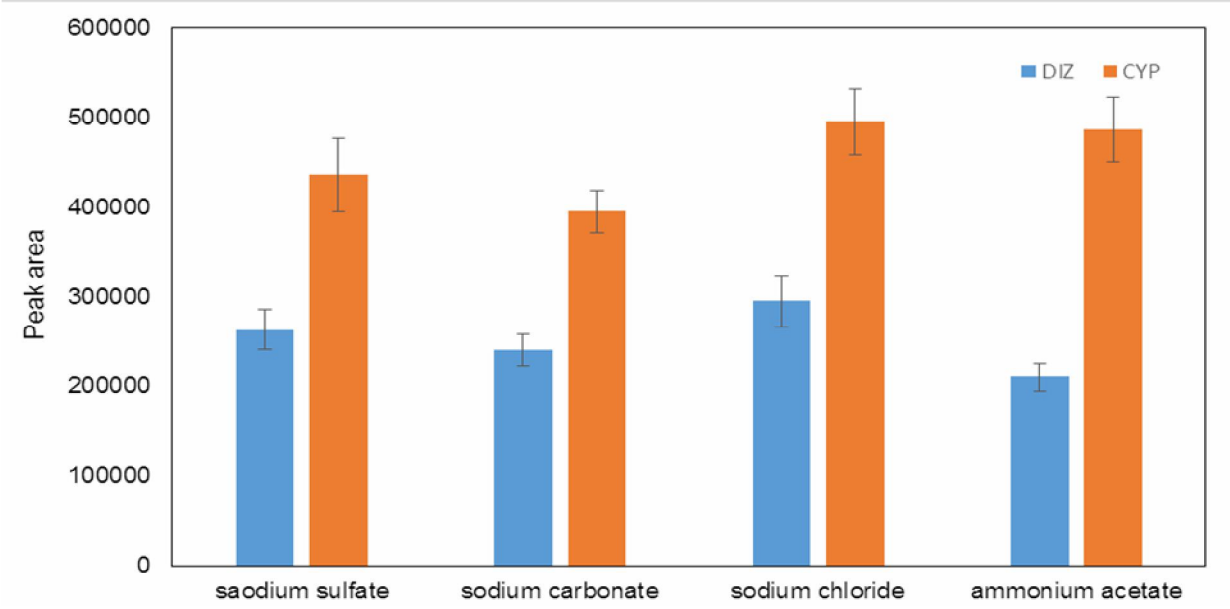

Fig. 5. Selection of phase separation agent type. Conditions: sample volume, 5 ml spiked with 50 µg l^{-1} of each analyte; extraction solvent volume (dichloromethane), 200 µl; homogeneous solvent volume (acetone), 5 ml; phase separator agent concentration 3% w/v of each salt; extraction time 3 min.

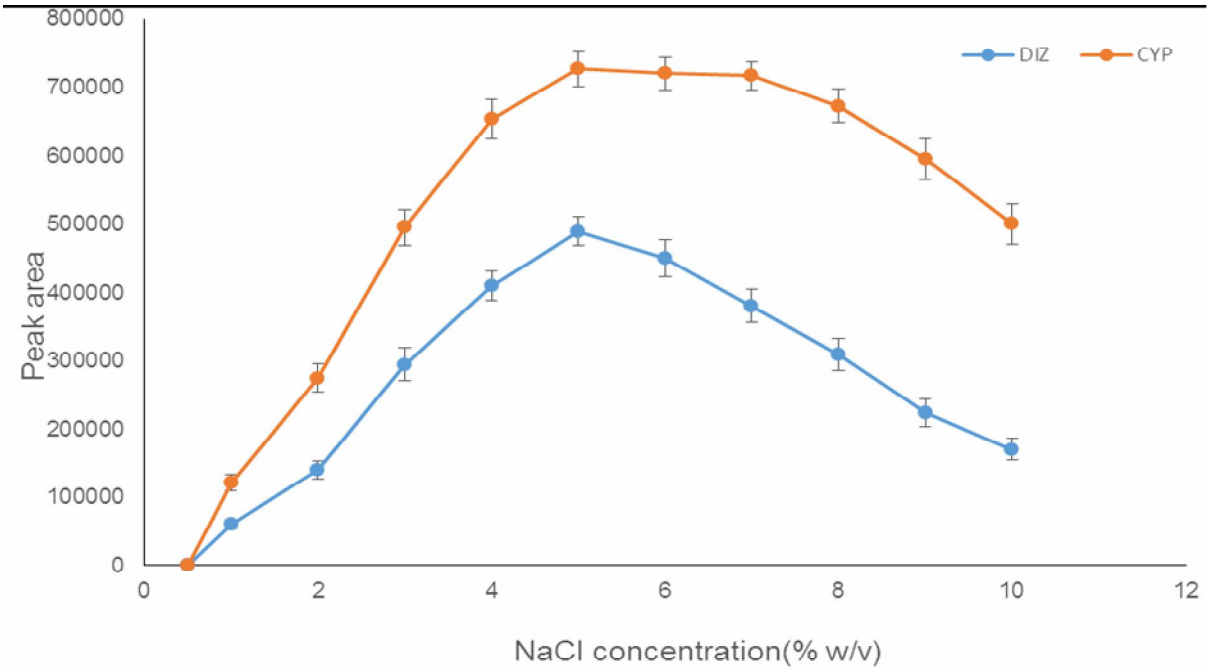

Fig. 6. Effect of phase separator agent (NaCl) concentration on the peak areas of target analytes. Conditions: sample volume, 5 ml spiked with 50 µg l^{-1} of each analyte; homogeneous solvent volume (acetone), 5 ml; extraction solvent (dichloromethane), 200 µl; extraction time 3 min.

Table 1. Detection Parameters for the Developed Method to Determine the Target Pesticides in Milk

Analyte	Slope ($\times 10^{-3}$) ± SD[a] (n = 3)	Intercept ($\times 10^{-3}$) ± SD[b] (n = 3)	r[c]	LOD[d] ($\mu g\ l^{-1}$)	LOQ[e] ($\mu g\ l^{-1}$)	LR[f] ($\mu g\ l^{-1}$)	EF ± SD[g]	ER ± SD[h]
Diazinon	5.769 ± 0.462	0.598 ± 0.0326	0.9963	0.03	0.1	0.1-1300	158 ± 13	79 ± 6
Cypermethrin	14.22 ± 0.835	0.682 ± 0.0562	0.9982	0.018	0.065	0.065-600	174 ± 16	87 ± 8

[a]Standard deviation of slope. [b]Standard deviation of intercept. [c]Correlation coefficient. [d]Limit of detection (S/N = 3). [E]Limit of quantification (S/N = 10). [f]Linear range. [g]Enrichment factor ± standard deviation (n = 3). [h]Extraction recovery ± standard deviation (n = 3).

Table 2. Precision and Accuracy of the Method for Determination of the Analytes in Pesticides Free Milk Samples

Analyte	Nominal concentration ($\mu g\ l^{-1}$)	Intra-day RSD%; n = 5[a1]	Accuracy (RE%)	Inter-day RSD%; n = 5[b1]	Accuracy (RE%)[c]
Diazinon	5	8.3	8.4	11.4	-5.2
	50	3.5	-4.3	6.8	-6.1
	250	5.1	-6	8.5	-4.5
Cypermethrin	5	7.6	3.2	12.3	5.4
	50	1.8	0	4.6	2.1
	250	2.7	2.6	5.8	3.8

[a]Number of replicates. [b]Number of days. [1]In each case the highest RSD was reported. [c]RE% = 100 × ((found value-nominal value)/nominal value).

results when the procedure is applied repeatedly to the same sample and expressed by %RSD. Since, the accuracy is described as the proximity of mean experimental results obtained (measured value) by the method to the actual concentration (nominal value) of the analyte, the accuracy of the suggested method was evaluated by computing relative errors (%RE) for each analyte after performing the optimized microextraction procedure using the following equation (Eq. (3));

$$\%RE = \frac{Measured\ value - \text{Nominal}\ value}{No\min al\ value} \times 100 \qquad (3)$$

The REs were found between -6.1% to 8.4%. On the other hand, the calculated RSDs at three concentration levels (low, medium and high) for each analyte at five times daily (intra-day) and at five different days (inter-day) are summarized in Table 2. The obtained values demonstrated that the inter- and intra-day precisions (RSDs) were between 1.8-8.3% and 4.6-2.3%, respectively.

Consequently, in accordance to the obtained results, it can be concluded that the developed method with the appropriate analytical figures of merit can be successfully utilized for quantification of the selected pesticides in milk. The main advantages of the suggested method are

Table 3. Comparison of the Method Proposed with other Methods Reported

Method	Analyte	Sample	LOD ($\mu g\ l^{-1}$)	LOQ ($\mu g\ l^{-1}$)	LR ($\mu g\ l^{-1}$)	%RSD	Ref.
UA-dSPE-HPLC-DAD[a]	Diazinon	Milk, urine,plasma	0.2	-	0.8-800	6.1-10.2	[29]
SPE-GC-MS[b]	Diazinon	Food commodities Milk	0.02	0.05	0.05-50	1.82	[30]
SPME-CD-IMS[c]	Diazinon	Water and juice	0.4	-	1.5-200	4-11.2	[31]
LPME-FDES-HPLC-UV[d]	Diazinon		0.5	1.6	2-500	1.3-2.8	[32]
UA-DLLME-HPLC-UV[e]	Cypermethrin	River water	0.3	-	0.6-1520	2.7	[33]
LLE-GC-MS/MS[f]	Cypermethrin	Milk Egg	- -	0.01 0.009	- -	<25	[34]
SPE-HPLC-DAD[g]	Cypermethrin	Vegtables oils	0.0290	0.089	0.2-5	3-10	[35]
SPE-GC-ECD[h]	Cypermethrin	Chiken, egg and meat	0.06-0.09	0.21-0.3	0.93-500	3-8	[36]
SI-HLLME-HPLC-DAD	Diazinon Cypermethrin	Milk	0.03 0.018	0.1 0.065	0.1-1300 0.065-600	3.5-11.4 1.8-12.3	This work

[a]Ultrasound assisted-dispersive solid phase extraction-high performance liquid chromatography-diode array detection. [b]Solid phase extraction-gas chromatography-mass spectrometry. [c]Solid phase microextraction-corona discharge-ion mobility spectrometry. [d]Liquid phase microextraction-freezing of deep eutectic solvent-high performance liquid chromatography-ultraviolet detection. [e]Ultrasound assisted-dispersive liquid- liquid microextraction-high performance liquid chromatography-ultraviolet detection. [f]Liquid liquid extraction-gas chromatography-tandem mass spectrometry. [g]Solid phase extraction-high performance liquid chromatography-diode array detection. [h]Solid phase extraction-gas chromatography-electron capture detection.

simultaneous cleanup and microextraction steps, no need for the centrifuge to separate the extraction phase from the aqueous phase, short extraction time, good repeatability, large linear range, and high extraction factors and extraction recoveries.

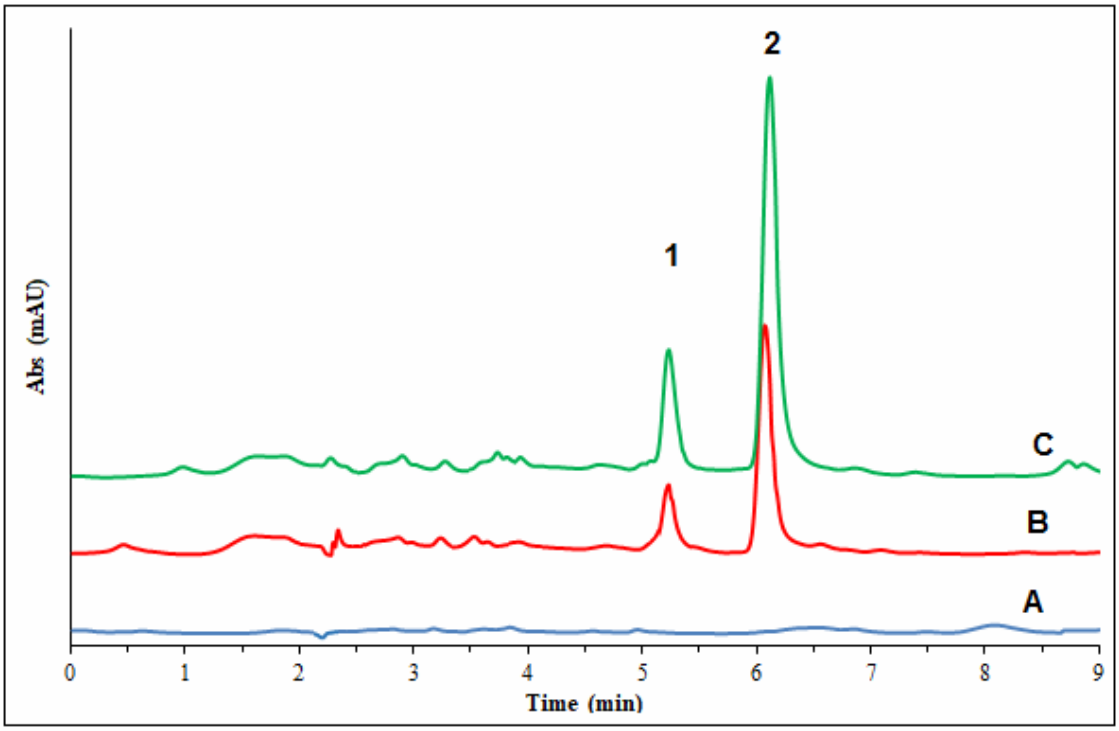

Fig. 7. Typical HPLC-DAD chromatogram of (A) unspiked milk sample after performing the developed method, (B) direct injection of standard solution (10 mg l^{-1} in acetonitrile, each pesticide), and (C) the milk sample spiked with 50 µg l^{-1} of each analyte. Peak identification: 1) diazinon, 2) cypermethrin.

Table 4. Recoveries of the Analytes Obtained when the Developed SI-HLLME-HPLC-DAD Method was Applied to Milk Samples Spiked with the Analytes at Three Concentrations

Sample	Analyte	Added concentration (µg l^{-1})	Found concentration (µg l^{-1})	Mean relative recovery ± SD (n = 3)
Raw milk	Diazinon (cypermethrin)	0	0 (0)	- (-)
		10	8.7 (9.1)	87 ± 3 (91 ± 4)
		50	45 (47)	90 ± 2 (94 ± 3)
		250	230 (240)	92 ± 3 (96 ± 2)
Pasteurized milk	Diazinon (cypermethrin)	0	0 (0)	- (-)
		10	8.4 (9.3)	84 ± 4 (93 ± 4)
		50	43 (46)	86 ± 3 (92 ± 4)
		250	238 (255)	95 ± 2 (102 ± 3)
UHT milk	Diazinon (cypermethrin)	0	0 (0)	- (-)
		10	8.8 (8.9)	88 ± 2 (89 ± 3)
		50	46 (45)	92 ± 3 (90 ± 2)
		250	235 (238)	94 ± 4 (95 ± 2)

Comparison of SI-HLLME with other Methods

To investigate the efficiency of the proposed method, the obtained results from the current method and several other previously reported methods concerning the extraction techniques, analytical instruments, sample matrix, LOD, LOQ, LR, RSDs and obtained recoveries have been compared (Table 3). As can be seen, the obtained LODs and LOQs are lower than those of most of the listed reports [29-36]. It has to be noted that techniques with better LODs and LOQs (SPE-GC-MS and LLE-GC-MS/MS) are equipped with mass detection systems which are inherently very sensitive and selective. Also, this method shows wider LRs than those of other methods and the calculated RSDs are comparable or even less than other studies mentioned. Therefore, it can be suggested that the SI-HLLME-HPLC-DAD technique is very suitable for simultaneous extraction and determination of DIZ and CYP residues in milk samples.

Analysis of Real Samples

The developed SI-HLLME method (overall analytical procedure) was applied for the determination of DIZ and CYP residues in 40 samples including raw milk (20 samples), pasteurized and UHT low-fat milk (20 samples). Collected milk samples were kept in PTFE tubes and stored at 4 °C before analysis. Sample analysis was carried out with the minimum possible lag. The results from all of the samples showed that they were not contaminated with DIZ (diazinon concentration was below LOD). CYP was found in 3 raw, and 1 pasteurized milk samples with concentrations 8, 13, 20 and 4 μg l^{-1}, respectively, obviously all of them were less than the MRL for CYP (50 ppb). The typical HPLC-DAD chromatograms of standard solution at the concentration of 10 mg l^{-1} of DIZ and CYP (direct injection), milk spiked with 50 μg l^{-1} of both analytes and unspiked milk after performing SI-HLLME are shown in Fig. 7. To evaluate the matrix effect, pesticide-free milk samples (raw, pasteurized and UHT) spiked by studied analytes at three concentration levels (low, medium and high) and relative recovery were calculated. As represented in Table 4, the obtained RRs were from 84% to 102% indicating that the matrices of analyzed milk samples had no significant effect on SI-HLLME procedure for simultaneous determination of DIZ and CYP.

CONCLUSIONS

In this study, a simple, rapid and sensitive method was developed based on SI-HLLME technique combined with HPLC-DAD for simultaneous determination of DIZ and CYP in milk samples. Several factors affecting the extraction efficiency were examined and optimized. Experimental results indicate that the method offered has numerous advantages such as simplicity, easy operating, less centrifuge, rapid operating, high EFs, ERs and RRs, low REs, RSDs, LODs, a wide LRs, and negligible matrix effect. Besides, due to the concurrent cleanup and microextraction steps in this technique, sample preparation is quicker than other previously reported methods. Based on the results obtained, this method is an appropriate approach for the quantification of target pesticides residues at the μg l^{-1} level in the milk samples. Finally, it seems the represented method can be extended for the determination of DIZ and CYP in other similar samples with complex matrices by varying the extraction conditions.

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