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Development of a new microextraction method based on a dynamic single drop in a narrow-bore tube: Application in extraction and preconcentration of some organic pollutants in well water and grape juice samples

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ABSTRACT

A novel sample preparation technique, the microextraction method based on a dynamic single drop in a narrow-bore tube, coupled with gas chromatography–flame ionization detection (GC–FID) is presented in this paper. The most important features of this method are simplicity and high enrichment factors. In this method, a microdrop of an extraction solvent assisted by an air bubble was repeatedly passed through a narrow-bore closed end tube containing aqueous sample. It has been successfully used for the analysis of some pesticides as model analytes in aqueous samples. Parameters affecting the method's performance such as selection of extraction solvent type and volume, number of extractions, volume of aqueous sample (tube length), and salt effect were studied and optimized. Under the optimal conditions, the enrichment factors (EFs) for triazole pesticides were in the range of 141–214 and the limits of detection (LODs) were between 2 and 112 $\mu g\,L^{-1}$. The relative standard deviations ($C = 1000\,\mu g\,L^{-1}$, $n = 6$) were obtained in the range of 2.9–4.5%. The recoveries obtained for the spiked well water and grape juice samples were between 71 and 106%. Low cost, relatively short sample preparation time and less solvent consumption are other advantages of the proposed method.

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1. Introduction

In recent years, the development of fast, precise, accurate, and sensitive methodologies has become an important issue. Despite notable technological advances in the analytical field, most instruments cannot handle sample matrices directly and, as a result, a sample preparation step is commonly involved in an analytical procedure. There are various methods of sample preparation which depend on the sample type. For organic trace analysis, this step mainly comprises extractions, which serve to isolate and enrich compounds of interest from a sample matrix. Liquid–liquid extraction (LLE) is a classic sample preparation technique that has been applied to a wide range of compounds [1–6]. However, LLE is time-consuming, tedious, and uses large amounts of potentially toxic organic solvents that are usually expensive because of their high purity (a necessity for analytical applications). Emulsion formation and low enrichment factor are other disadvantages of LLE. Solid-phase extraction (SPE) [7–14] is the most common and popular method. Although it has higher enrichment factors than LLE, but the SPE cartridges need conditioning and require further toxic organic solvents for washing and elution steps. Therefore, analytical researchers have been searching for solvent-free methods or other approaches which require low consumption of toxic organic solvents in order to minimize their negative effects. Solid-phase microextraction (SPME) is a solvent-free microextraction technique which was introduced in 1990 by Pawliszyn [15,16]. In spite of the method advantages, most commercial extractive fibers used in SPME are expensive and fragile (in SPME experiments using fibers as extractants), furthermore, sample carry-over is also a problem [17,18]. To overcome the drawbacks of LLE, microextraction techniques have been developed, resulting in simple, fast, and green procedures. Liquid phase microextraction (LPME) as a miniaturized sample preparation approach emerged in the mid-to-late 1990s [19,20]. The LPME methods are generally divided into three main groups: 1) single-drop microextraction (SDME); 2) membrane liquid phase microextraction (MLPME); and 3) dispersive liquid–liquid microextraction (DLLME). Their differences rely on the way the solvent contacts the aqueous phase. Among the LPME-based techniques, SDME and MLPME were first developed. SDME is inexpensive, and since very little solvent is used, there is minimal exposure to toxic organic solvents [21,22]. However, some disadvantages of this method are as follows: fast stirring would tend to break up the organic drop; air bubble formation is possible [23]; extraction is time-consuming and equilibrium cannot be attained after a long time in most cases [22]. Also, membrane methodologies

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proved to be more attractive as alternatives to microdrop techniques because they are simple, inexpensive, and fast (high level of stirring is possible). Despite the great advantages of membrane techniques, some drawbacks, such as memory effects caused by the online configuration and poor reproducibility because of manual cutting and/or sealing of the membrane in laboratory have been reported [24,25]. DLLME as an alternative method was introduced by Assadi [26] in 2006. It is a miniaturized liquid–liquid extraction that uses μL volumes of an extraction solvent and mL volumes of a dispersive solvent [27–29]. It is a simple and fast microextraction technique. However, in a common DLLME technique, the toxic chlorinated solvents were often used as extraction solvent.

In the present work a new sample preparation technique was proposed based on a single drop of a safe solvent. The extraction device involved a narrow-bore tube in which aqueous sample containing analytes was filled. A single drop of an organic solvent was used to extract analytes. To accelerate mass transfer, extractive phase was passed repeatedly through the tube. The practicability of the procedure was tested for trace analysis of the selected pesticides in water and fruit juice samples after optimization of parameters affecting the extraction. For this purpose, a well water sample was picked up from a well in the vicinity of a unit which produces the studied pesticides. As a fruit juice sample, the grape juice was selected, because the studied triazole pesticides, especially penconazole, have fungicidal property and are used for the control of grape pests. Contrary to LLE the proposed method does not require large volume of toxic extraction solvent. In addition, unlike SDME, there is no instability of microdrop in this technique. Other advantages of this method are simplicity of operation and low cost similar to DLLME. The main disadvantage of this method is relatively high sample preparation time compared to LLE and DLLME and comparable with those of SPE and SDME.

2. Experimental

2.1. Reagents and chemicals

All pesticides (penconazole, hexaconazole, diniconazole, tebuconazole, triticonazole, and difenconazole) were provided kindly by Gyah Corporation (Karadj, Iran). *n*-Octanol and *n*-hexane as extraction solvents and other chemicals such as methanol and sodium chloride were purchased from Merck (Darmstadt, Germany). *n*-Hexanol, toluene, and 1-hexadecanol (cetyl alcohol) were from Fluka (Switzerland). De-ionized water (Zakaria Company, Tabriz, Iran) was used for preparation of aqueous solutions.

2.2. Standard solutions

Each pesticide (10 mg) was dissolved in 10.0 mL mixture of *n*-hexanol and *n*-hexane (50:50, v/v) (extraction solvent) to obtain a standard solution with a concentration of 1000 mg L^{-1} (each analyte). This solution was injected into the separation system each day (three times) for quality control and the obtained peak areas were used to calculate enrichment factors and extraction recoveries of analytes. A 1000 mg L^{-1} stock solution of five pesticides (except diniconazole) was prepared in methanol. Working solutions were prepared daily by appropriate dilutions of this stock solution with de-ionized water. Diniconazole was used as internal standard (IS). Solution of IS in *n*-hexanol and *n*-hexane (50:50, v/v) mixture was prepared at a concentration of 500 mg L^{-1} and used for optimization of experimental conditions. Cetyl alcohol (1-hexadecanol) solution was prepared in n-hexanol and *n*-hexane (50:50, v/v) mixture at a concentration of 250 mg L^{-1} in order to determine the volume of extraction solvent drop after extraction.

2.3. Samples

A well water sample was picked up from a well located in the zone of a unit which produces the studied pesticides (Karadj, Iran). Various grape juice samples were purchased from local supermarkets (Tabriz, Iran). The pH of all samples ranged 6–8. These samples were used for development of the method and analysis of target pesticides within them.

2.4. Instrumentation

Separation and determination of the selected pesticides were carried out on a Shimadzu 15A gas chromatograph (Kyoto, Japan) equipped with a split/splitless injector and a flame ionization detector (FID). Pure helium (99.999%, Gulf Cryo, United Arab Emirates) at a constant linear velocity of 30 cm s^{-1} was used as the carrier gas. Injections (1 μL) were carried out in the splitless mode with a 30 s purge time and injector temperature of 250 °C. The separation was carried out on a PT-5 capillary column (30 m × 0.25 mm i.d. with a 0.25 μm stationary film thickness). Samples were analyzed using the following oven temperature programming: initial temperature 100 °C (held for 2 min), increased by 15 °C min^{-1} to 250 °C, and held at 250 °C for 5 min. The total time for one GC run was 17 min. The FID temperature was maintained at 250 °C. Hydrogen gas was generated with a hydrogen generator (OPGU-1500S, Shimadzu, Japan) for FID at a flow rate of 40 mL min^{-1}. The flow rate of air for FID was 300 mL min^{-1}. Gas chromatography-mass spectrometry (GC–MS) analysis was carried out on an Agilent 6890N gas chromatograph with a 5973 mass-selective detector (Agilent Technologies, CA, USA). The separation was performed on an HP-5 MS capillary column (30 m × 0.25 mm i.d. and film thickness of 0.25 μm). Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The injector temperature and oven temperature programming were the same as GC–FID analysis mentioned above.

2.5. Microextraction procedure

The set-up included a narrow-bore glass tube (120 cm × 5 mm i.d.). One end of tube was closed with a septum. Initially by using a 50-mL syringe, 23 mL aqueous sample of target analytes, the well water or the grape juice sample, without dilution (containing 1% NaCl, w/v), was filled into the tube (Fig. 1a). A 50-μL microsyringe was used to transfer 30 μL mixture of *n*-hexanol: *n*-hexane (50:50, v/v) as an extractive solvent (lighter than water) into the tube through the septum. It formed a single drop (Fig. 1b). It was noted that the needle of microsyringe should be contacted with interior surface of the tube while extraction solvent was transferred into the tube. By this action, drop adhered on the interior surface of the tube and did not go up. Movement of drop within the tube was assisted by an air bubble (50 μL) injected by the microsyringe (Fig. 1c). After injection of the air bubble, solvent drop started to go up into the tube along with air bubble. Indeed by injection of an air bubble, speed of the organic drop into tube can be controlled. When it reached near to the last two centimeters in the top of the tube, the status of tube was reversed upside down manually (Fig. 1d). By this action the drop passed within the tube another time. After 21 times of passing within the tube (extraction number), a portion of the gathered drop on the surface of aqueous phase in the open end of tube was rapidly collected by a capillary tube (glass capillary tube, 100 mm length and 1.5 mm o.d., Electrothermal, Denmark) (Fig. 1e). This was performed by entering one end of the capillary tube into the organic drop and, therefore, a portion of the organic solvent was transferred into the capillary tube by capillary action. Total sample preparation time was about 17 min. Finally, 5 μL of the collected phase into the capillary tube was mixed with 5 μL of

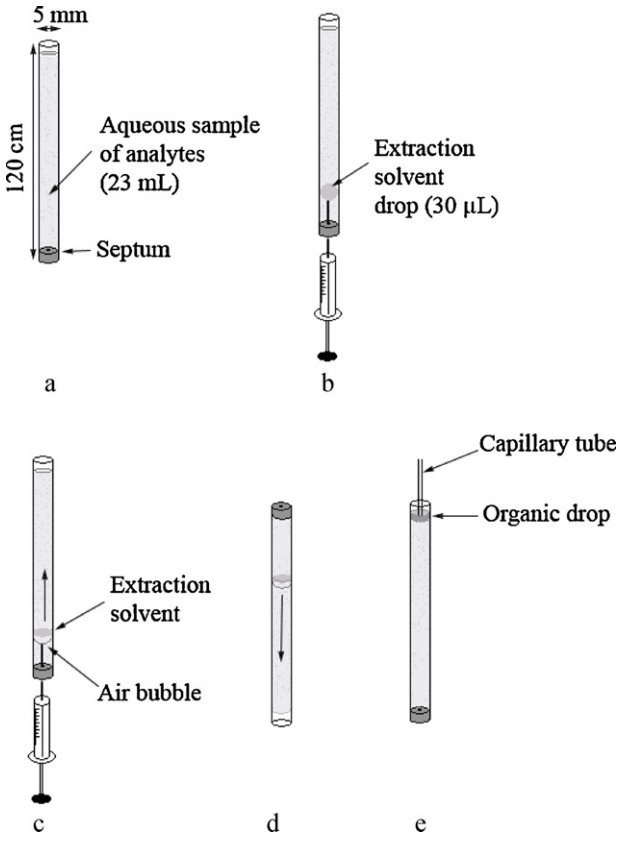

Fig. 1. Microextraction procedure. (a) A narrow-bore tube filled with aqueous sample of analytes; (b) introducing the drop of extraction solvent; (c) introducing an air bubble; (d) reversing the tube (upside down); and (e) removal of a portion of organic drop by a capillary tube. It is mentioned that due to narrow-bore nature of the tube, no solution spilled out after reversing the tube.

IS solution and 1 μL of the mixture was injected into the GC–FID for analysis.

2.6. Calculation of enrichment factor and extraction recovery

The enrichment factor (EF) was defined as the ratio of the analyte concentration in the collected phase (C_{coll}) and the initial concentration of analyte (C_0) within the sample:

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

C_{coll} was obtained from the calibration graph.

The extraction recovery (ER) was defined as the percentage of the total analyte (n_0) which was extracted into the collected phase (n_{coll}):

$$ER = \left(\frac{n_{coll}}{n_0}\right) \times 100 = \left[\frac{C_{coll}V_{coll}}{C_0V_{aq}}\right] \times 100$$

$$ER = \left(\frac{V_{coll}}{V_{aq}}\right) EF \times 100 \quad (2)$$

where V_{coll} and V_{aq} were the volumes of collected phase and aqueous solution, respectively. It is noted that removal of the whole organic phase collected on the aqueous phase into the narrow-bore tube is difficult. To calculate volume of the collected phase, a compound (cetyl alcohol) which was freely soluble in the extraction solvent but was practically insoluble in aqueous phase ($\log K_{o/w} = 6.65$) [30] was used (see Section 3.6).

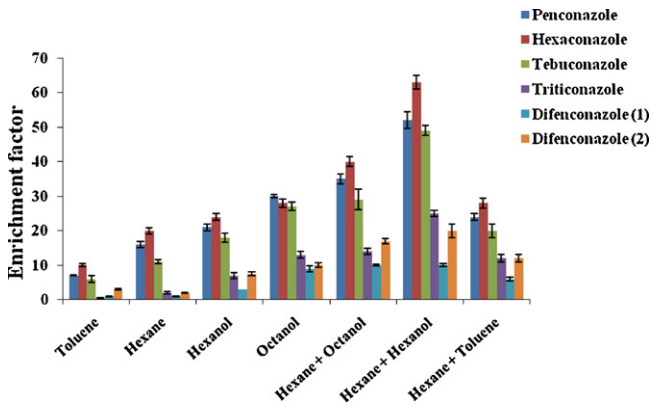

Fig. 2. Effect of extraction solvent kind on the extraction efficiency. Extraction conditions: aqueous sample volume, 16.5 mL (narrow-bore tube dimension, 85 cm × 5 mm i.d.); analytes concentrations, 5 μg mL^{-1} of each pesticide; extraction solvent volume, 50 μL; and number of extraction, 11. The binary mixtures of solvents were at a ratio of 50:50 (v/v). The bars indicate the maximum and minimum of three determinations.

2.7. Performance of the analytical method

To test the performance of the analytical method, its precision was evaluated on six similar solutions at two concentrations (1 and 5 μg mL^{-1}, for each pesticide). Effect of the matrix and accuracy of the method were assessed by the added-found method at three levels (0.5, 1 and 2 μg mL^{-1}, for each pesticide). Robustness of the method was tested by performing extraction numbers of 19, 21, and 23, tube lengths of 115, 120, and 125 cm and extraction solvent volumes of 28, 30, and 32 μL.

3. Results and discussion

In this study a new microextraction method using a dynamic single drop of an extraction solvent was presented for preconcentration of some triazole pesticides from aqueous samples. Before its application in analysis of samples, it was necessary to optimize experimental conditions which affect the extraction efficiency. Extraction solvent type and volume, ionic strength of aqueous phase, sample volume, etc. were some of the studied parameters.

3.1. Selection of extraction solvent

Selecting a suitable extraction solvent is critical in all extraction methods. The solvent selection was carried out regarding its solubility in water and its performance in analytes extraction. Moreover, the extraction solvent has to possess high drop stability, low level of toxicity, and good chromatographic behavior. For this purpose, *n*-hexane, toluene, *n*-hexanol, *n*-octanol, and binary mixtures of them were tested. According to Fig. 2 (enrichment factor vs. solvent kind), high analytical signals were obtained when the mixture of *n*-hexanol:*n*-hexane (50:50, v/v) was used as the extraction solvent. It seems that the presence of hexane in the composition of extraction solvent is important from two aspects: (1) it adjusts the polarity of extraction solvent to enhance distribution coefficients of analytes and (2) it is a low-viscose solvent which improves diffusion coefficients of analytes into the extractive phase. Both of them increase extraction efficiency. It was noted that difenconazole gave split peaks in the chromatograms for its *cis* and *trans* isomers [31,32] which were indicated as difenconazole (1) and difenconazole (2) in this study. To evaluate the effect of hexane percentage in extraction solvent composition on performance of the method, a series of experiments were performed using different mixtures of hexanol: hexane as extractive phase. The results showed that

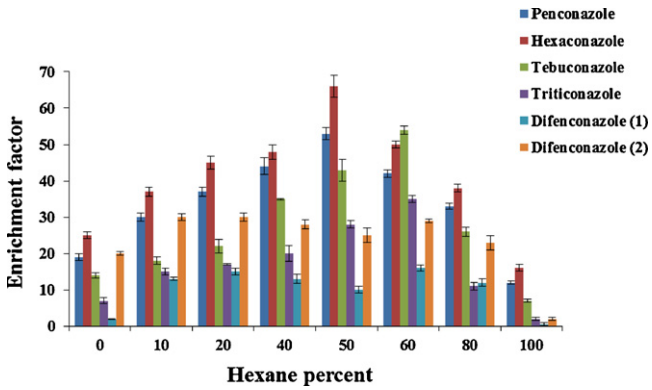

Fig. 3. Effect of hexane percent on the extraction efficiency. Extraction conditions: the same as Fig. 2. The bars indicate the maximum and minimum of three determinations.

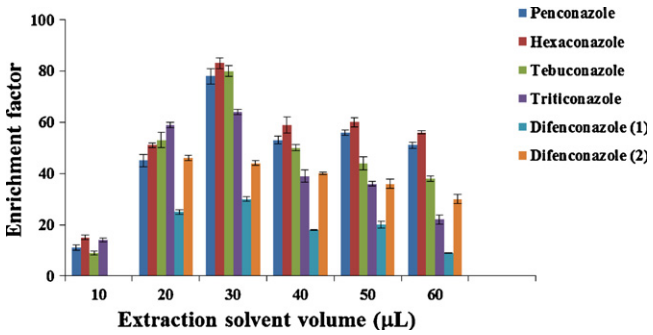

Fig. 4. Effect of extraction solvent volume on the extraction efficiency. Extraction conditions: the same as Fig. 3. The bars indicate the maximum and minimum of three determinations.

by increasing hexane content of extraction solvent, analytical signals increased till 50% and then decreased (Fig. 3). Therefore, the mixture of hexanol:hexane (50:50, v/v) was selected as extraction solvent for further studies.

3.2. Optimization of extraction solvent volume

The selection of extraction solvent volume depends on solvent gathering ability on the aqueous phase after extraction, repeatability of results, and extraction efficiency. Large volumes of organic solvent are difficult to manipulate and form numerous drops instead of a single drop. On the other hand, small volumes of organic solvent do not form drops due to its solubility into aqueous phase and/or vaporization, or form a very small drop which is not easy to remove it from the tube. Therefore, extraction solvent volume was studied in the range of 10–60 μL. Enrichment factors as a function of organic solvent volume are shown in Fig. 4. By increasing the volume of extraction solvent from 10 to 30 μL, analytical signals increased and then decreased at higher volumes. By increasing extraction solvent volume, the ratio of extractant to sample volume was improved which led to increase the extraction efficiency. But another factor, i.e. dilution, decreased enrichment factors (analytical signals) at high volumes of extractant. By considering the results in Fig. 4, 30 μL was selected as extraction solvent volume in this study.

3.3. Optimization of extraction numbers

In this study, numbers of extractions were defined as the number of repeatedly movements of organic drop within the tube. Similar to multiple batch extraction, it was predictable that by increasing extractions number, recoveries should be increased. Unlike batch extraction, it was impossible to reach the equilibrium after one extraction in this method. Therefore, to reach the equilibrium status, the number of extractions was studied in the range of 1–31 times. The results in Fig. 5 show that by increasing extractions number, analytical signals were also increased until 21th extraction and then remained constant till 31th extraction. Hence, 21 times of extraction was selected for further studies. In higher than 21 times, repeatability of the method decreased. This was concluded by the comparison of error bars in the cases of 21, 25, and 31 times. It was noted that in the case of high extractions number, vaporization of extraction solvent was significant.

3.4. Salt and pH effects

In some papers it has been reported that adding a salt to the aqueous solution can reduce the amount of water available to dissolved analyte molecules due to the formation of hydration spheres around the ions formed from salt molecules [33]. This can improve the extraction efficiency for the target analytes. The variations of analytical signals vs. salt amount were investigated using sodium chloride at different concentrations between 0 and 30% (w/v). The opposite results (salting in effect) were observed in the present study. Similar effect was reported in other papers [34]. The pres-

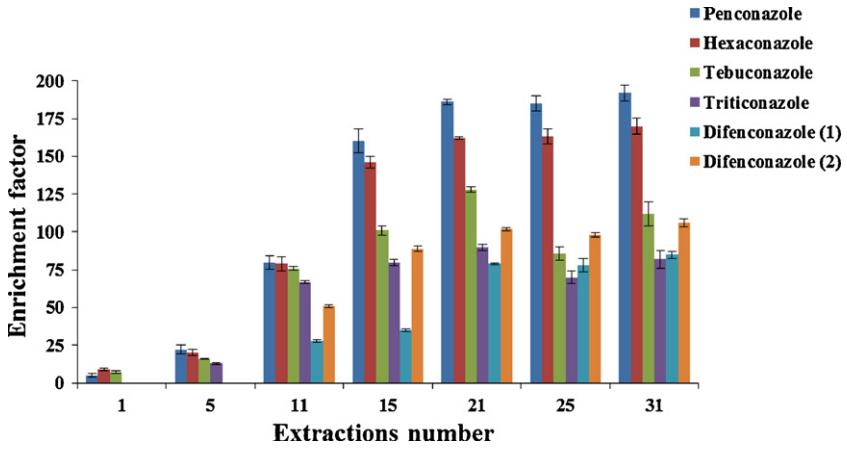

Fig. 5. Study of extractions number. Extraction conditions: the same as Fig. 4, except 30 μL mixture of hexanol:hexane (50:50, v/v) was used as extraction solvent. The bars indicate the maximum and minimum of three determinations.

Table 1
Quantitative features of the method for the selected pesticides.

Analyte	LR[a] ($\mu g\,L^{-1}$)	R^2 [b]	LOD[c] ($\mu g\,L^{-1}$)	LOQ[d] ($\mu g\,L^{-1}$)	EF ± SD[e]
Penconazole	10–10000	0.997	2	8	214 ± 20
Hexaconazole	10–10000	0.994	6	10	205 ± 17
Tebuconazole	100–10000	0.995	12	40	141 ± 17
Triticonazole	300–10000	0.994	27	90	152 ± 21
Difenconazole (1)	500–10000	0.994	102	340	155 ± 8
Difenconazole (2)	500–10000	0.994	112	375	175 ± 20

[a] Linear range.
[b] Square of correlation coefficient.
[c] Limit of detection, S/N = 3.
[d] Limit of quantification, S/N = 10.
[e] Mean enrichment factor ± standard deviation, $n = 3$.

ence of a salt may increase the viscosity of solution which, in turn, decreases the mass transfer in aqueous phase. The obtained data proposed that the extraction efficiency in the absence of NaCl was high. However, salts were found in all aqueous samples such as water, wastewater, and fruit juice samples and to match the matrices of samples and standard solutions, the following experiments were performed in the presence of 1% (w/v) NaCl. This amount of salt agreed with the matrices of aqueous samples and no significant decrease of the method efficiency was observed. For tested values of 0 and 0.25% (w/v) NaCl the efficiency of extraction was higher but these amounts of salt were lower compared with the aqueous samples.

The effect of pH was studied in the range of 2–12. The results agreed well with those of the previous experiment [35]. The efficiency of the method was pH-independent in the pH range of 6–8. pH of all samples was between 6 and 8. Therefore, there was no need to adjust pH in this study.

3.5. Optimization of the aqueous sample volume

The effect of sample size on the extraction efficiency was investigated with the tubes having different lengths varying from 30 to 120 cm (constant i.d.) or with the tubes having different internal diameters (3, 5 and 7 mm) and constant length (85 cm). Performing extractions in the tubes with i.d. 3 and 7 mm were not practical. In the case of 3 mm i.d. movement of organic drop into the tube was not accomplished. On the other hand, in the case of 7 mm i.d., sample solution spilled out by turning the tube upside down and the method became useless. Therefore, i.d. of the tube was selected as 5 mm and its length varied for testing different samples. The experimental observations showed that the extraction efficiency was significantly influenced by the sample volume. Increasing sample volume leads to increase of the analytical signals. However, because of some difficulties in using very long tubes, such as some problems in their handling and also dissolving extraction solvent into the aqueous sample, tubes with the lengths higher than 120 cm were not tested. The following experiments were performed by using the tube (120 cm × 5 mm i.d.) which had a capacity of 23 mL.

3.6. Determination of extraction solvent drop volume after extraction

To determine the volume of organic solvent collected on the surface of aqueous sample after extraction, a solution of 1-hexadecanol was prepared in a mixture of *n*-hexanol:*n*-hexane (50:50, v/v) at a concentration of 250 $\mu g\,mL^{-1}$. Then, in order to determine the remained extraction solvent volume after extraction, the following equation was used:

$$V_{coll} = \left(\frac{A_{dir}}{A_{ext}}\right) V_{added} \quad (3)$$

Table 2
Study of matrix effect in samples spiked at different concentrations.

Analyte	Mean recovery (%) ± standard deviation ($n = 3$) in			
	Grape juice			Well water
	Sample 1	Sample 2	Sample 3	
All samples were spiked with each analyte at a concentration of 0.5 $\mu g\,mL^{-1}$.				
Penconazole	82 ± 1	99 ± 4	87 ± 3	98 ± 2[a]
Hexaconazole	81 ± 4	93 ± 3	75 ± 7	96 ± 5
Tebuconazole	77 ± 5	72 ± 3	74 ± 6	85 ± 3
Triticonazole	88 ± 4	86 ± 2	85 ± 4	80 ± 6
Difenconazole (1)	75 ± 7	75 ± 7	85 ± 7	80 ± 1
Difenconazole (2)	74 ± 4	86 ± 1	86 ± 1	86 ± 1
All samples were spiked with each analyte at a concentration of 1 $\mu g\,mL^{-1}$.				
Penconazole	85 ± 1	72 ± 2	93 ± 4	106 ± 1[a]
Hexaconazole	82 ± 2	73 ± 6	92 ± 4	97 ± 3
Tebuconazole	87 ± 4	75 ± 4	81 ± 1	98 ± 1
Triticonazole	91 ± 5	78 ± 6	71 ± 3	96 ± 1
Difenconazole (1)	97 ± 4	75 ± 1	99 ± 4	84 ± 4
Difenconazole (2)	97 ± 4	92 ± 4	95 ± 1	92 ± 4
All samples were spiked with each analyte at a concentration of 2 $\mu g\,mL^{-1}$.				
Penconazole	99 ± 1	97 ± 1	96 ± 2	104 ± 4[a]
Hexaconazole	97 ± 2	96 ± 1	94 ± 5	97 ± 2
Tebuconazole	95 ± 4	89 ± 2	85 ± 6	94 ± 6
Triticonazole	96 ± 1	97 ± 1	91 ± 5	88 ± 6
Difenconazole (1)	82 ± 3	78 ± 1	80 ± 3	97 ± 4
Difenconazole (2)	99 ± 1	94 ± 1	91 ± 3	91 ± 3

[a] Penconazole content of sample was subtracted.

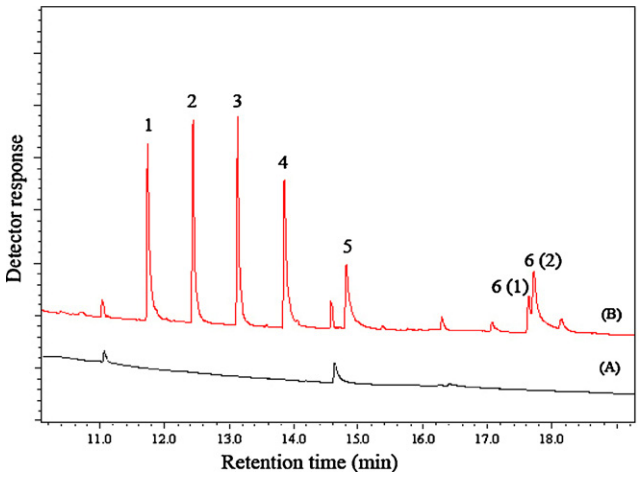

Fig. 6. Typical GC–FID chromatograms of grape juice sample. (A) Chromatogram of unspiked sample and (B) chromatogram of spiked sample with pesticides (each 1 μg mL^{-1}). The preconcentration technique under the optimized conditions was performed on them and 1 μL of the collected phase was injected into GC. Peaks identification: 1, penconazole; 2, hexaconazole; 3, diniconazole; 4, tebuconazole; 5, triticonazole, and 6 (1) and, 6 (2), difenconazole.

where A_{dir} is the peak area of 1-hexadecanol after direct injection of 1 μL of its standard solution (250 μg mL^{-1}) into GC. In another experiment, 30 μL (V_{added}) of 250 μg mL^{-1} 1-hexadecanol in extraction solvent was subjected to the proposed extraction method and 1 μL of the collected organic phase was injected into the separation system. A_{ext} is the peak area of 1-hexadecanol in the chromatogram. The difference in peak area of 1-hexadecanol in two cases can be attributed to the decrease of extraction solvent volume during extraction due to its solubility in aqueous phase or evaporation. The V_{coll} was 17 ± 1 for the three determinations.

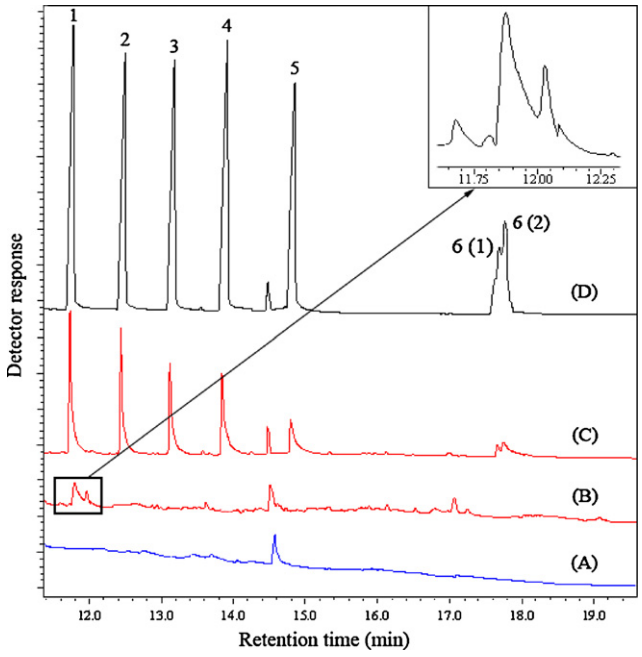

Fig. 7. GC–FID chromatograms of (A) blank, (B) well water, (C) spiked well water (1 μg mL^{-1}, for each pesticide), and (D) standard solution of pesticides in the mixture of hexanol:hexane (50:50, v/v) (1000 μg mL^{-1}, for each pesticide). In all cases except chromatogram (D), the extraction method was performed and 1 μL of the collected organic phase was injected into GC. In the case of chromatogram (D), 1 μL was directly injected into the separation system. Peaks identification: (1), penconazole; (2) hexaconazole; (3) diniconazole; (4) tebuconazole; (5) triticonazole; and 6 (1) and 6 (2) difenconazole.

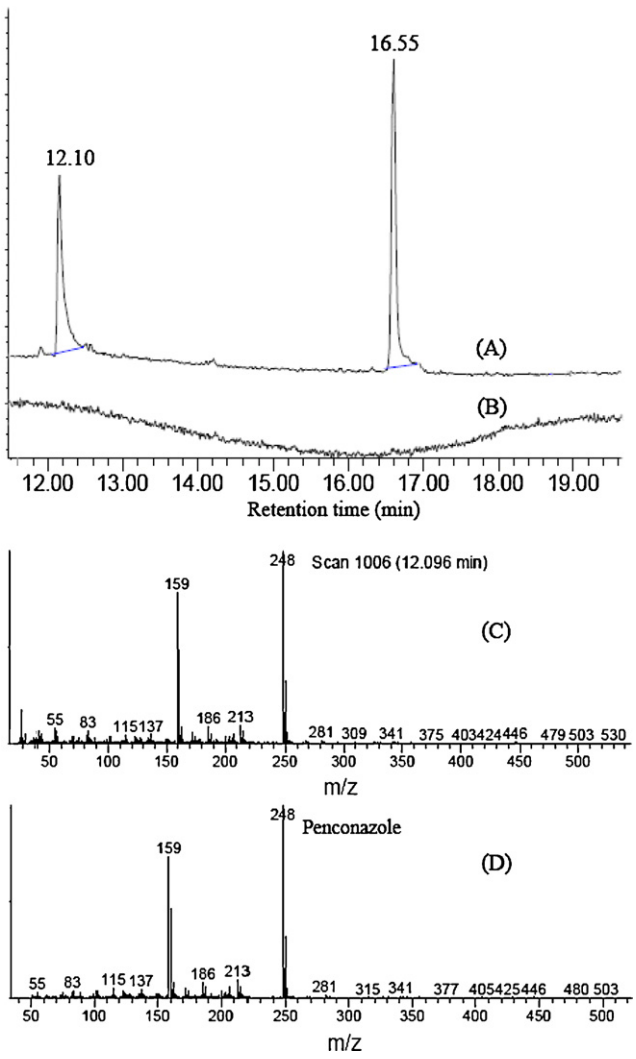

Fig. 8. Total ions chromatograms (TIC) of (A) well water sample, and (B) blank after performing the proposed method (top) and mass spectra of (C) scan 1006, retention time 12.096 min, and (D) penconazole (bottom).

3.7. Quantitative features of the method

The analytical performance of the presented method was validated by calculation of linear range, square of correlation coefficient, limit of detection (LOD), limit of quantification (LOQ), and enrichment factor (EF) for the target analytes and was summarized in Table 1. The results showed that wide linear ranges with good linearity (R^2 > 0.994) were achievable for the selected pesticides by the proposed method. In most cases, low detection and quantification limits in the range of 2–27 and 8–90 μg L^{-1}, respectively, were obtained by GC–FID. High enrichment factors ranging from 141 to 214 were another advantage of the method. Repeatability of the method was assessed by its application on the six similar standard solutions at two concentrations (1 and 5 μg mL^{-1}, for each pesticide) and the relative standard deviations (RSD) were in the range of 2.9–4.5% which indicated the repeatability of the method. Robustness of the method was evaluated by different volumes of the extraction solvent (28, 30, and 32 μL), extraction numbers (19, 21, and 23) and lengths of the tube (115, 120, and 125 cm). The results were comparable and there were no remarkable differences among them.

Table 3
Comparison of the presented method with other methods used in preconcentration and determination of the target analytes.

Analytes	Sample	RSD (%)[a]	LR[b]	LOD[c]	LOQ[d]	EF[e]	Method	Ref
Penconazle	Soya grain	2.2	0.25–10 ng mL^{-1}	–	100 μg kg^{-1}	–	QuEChERS–LC–MS/MS[f]	[36]
Hexaconazole		12.1		–	–	–		
Diniconazole		7.2		–	50 μg kg^{-1}	–		
Tebuconazole		5.4		–	100 μg kg^{-1}	–		
Triticonazole		10.8		–	100 μg kg^{-1}	–		
Difenconazole		6.3		–	100 μg kg^{-1}	–		
Penconazole	Wine	–	0.25–20 ng mL^{-1}	–	0.040 ng mL^{-1}	190	SPE–DLLME–GC-ECD[g]	[10]
Diniconazle		–		–	0.060 ng mL^{-1}	188		
Difenconazole		–		–	0.250 ng mL^{-1}	254		
Penconazole	Wine	–	0.25–50 ng mL^{-1}	–	0.020 ng mL^{-1}	190	SPE-DLLME–GC–MS[h]	[10]
Diniconazle		–		–	0.030 ng mL^{-1}	188		
Difenconazole		–		–	0.100 ng mL^{-1}	254		
Hexaconazole	Crops	–	10–200 μg kg^{-1}	3.0 μg kg^{-1}	10.0 μg kg^{-1}	–	SPE–GC–MS[i]	[9]
Tebuconazole		–	10–200 μg kg^{-1}	5.0 μg kg^{-1}	10.0 μg kg^{-1}	–		
Hexaconazole	Crops	–	5–100 μg kg^{-1}	0.5 μg kg^{-1}	5.0 μg kg^{-1}	–	SPE–GC–ECD[j]	[9]
Tebuconazole		–	10–200 μg kg^{-1}	4.0 μg kg^{-1}	10.0 μg kg^{-1}	–		
Penconazle	Aqueous samples	3.4	0.01–10 μg mL^{-1}	0.002 μg mL^{-1}	0.008 μg mL^{-1}	214	Microextraction method based on a dynamic single drop in a narrow-bore tube-GC–FID	This method
Hexaconazole		3.9	0.01–10 μg mL^{-1}	0.006 μg mL^{-1}	0.010 μg mL^{-1}	205		
Tebuconazole		4.5	0.10–10 μg mL^{-1}	0.012 μg mL^{-1}	0.040 μg mL^{-1}	141		
Triticonazole		3.5	0.30–10 μg mL^{-1}	0.027 μg mL^{-1}	0.090 μg mL^{-1}	152		
Difenconazole (1)		3.3	0.50–10 μg mL^{-1}	0.102 μg mL^{-1}	0.340 μg mL^{-1}	155		
Difenconazole (2)		2.9	0.50–10 μg mL^{-1}	0.112 μg mL^{-1}	0.375 μg mL^{-1}	175		

[a] Relative standard deviation.
[b] Linear range.
[c] Limit of detection, S/N = 3.
[d] Limit of quantification, S/N = 10.
[e] Enrichment factor.
[f] Quick, easy, cheap, effective, rugged and safe-liquid chromatography–tandem mass spectrometry.
[g] Solid-phase extraction-dispersive liquid–liquid microextraction–gas chromatography–electron capture detector.
[h] Solid-phase extraction–dispersive liquid–liquid microextraction–gas chromatography–mass spectrometry.
[i] Solid-phase extraction–gas chromatography–mass spectrometry.
[j] Solid-phase extraction–gas chromatography–electron capture detector.

3.8. Samples analysis

The proposed method was applied for determining the selected triazole pesticides in well water sample and three grape juice samples without dilution. Well water sample was picked up from a well located in the zone of a unit which produces the pesticides (Karadj, Iran) and grape juice samples were purchased from local supermarkets. None of the pesticides were detected in the grape juice samples. Penconazole was found (30 ng mL^{-1}) in the well water sample. Typical GC–FID chromatograms of a grape juice sample and spiked grape juice sample (with 1 μg mL^{-1} of each pesticide), after performing the proposed microextraction method on them, are shown in Fig. 6. As it can be seen from the chromatograms, there was no interfering peak in the retention times belonging to target analytes. The GC–FID chromatograms of blank, well water and spiked well water (1 μg mL^{-1} of each pesticide) are also shown in Fig. 7. In the chromatogram of preconcentrated well water sample there was a peak in the retention time of penconazole. To identify the compound, this sample (after performing the proposed method) was injected into the GC–MS. Total ions chromatogram along with mass spectra of scan 1006 (retention time 12.096 min) and penconazole are given in Fig. 8. From the mass data, the presence of penconazole in well water was confirmed. Like many other sample preparation approaches for trace analysis, the efficiency of method may be affected by the complexity of the matrix involved. In order to evaluate the matrix effect, the samples were spiked with analytes at three levels (0.5, 1, and 2 μg mL^{-1} of each pesticide) and the proposed method was applied to them (three times for each concentration). The recoveries in comparison with those obtained for standard solutions at the same concentrations are summarized in Table 2. In the case of well water, good recoveries were obtained in the range of 80 to 106% which indicated that the matrix effect was negligible. For grape juice samples the recoveries were in the range of 71–99%. These results demonstrate that well water and grape juice matrices, have little effect on the efficiency of the proposed method. Therefore, there was no need for their dilution or performing other treatments.

3.9. Comparison of the proposed method with other methods

To assess the performance of method, its analytical parameters were compared with those of other methods used in the analysis of target analytes. For this purpose, relative standard deviation, linear range, limit of detection, limit of quantification, and enrichment factor of the reported methods along with those of the presented method were listed in Table 3. Repeatability of the method reported as RSDs was very good compared to the first method mentioned in the table. Quantification limits obtained by the method presented in this study were lower or comparable with those of other methods. It was noted that in the reported meth-

ods in Table 3, high sensitive methods such as gas chromatography coupled with mass spectrometry or electron capture detection which are inherently more sensitive than GC-FID, were used. Higher or comparable enrichment factor was another advantage of the present method compared to others. Generally the presented method can be considered as a cheap, simple, and reliable analytical technique in determination of analytes of interest in aqueous samples.

4. Conclusion

The present analytical approach offers the opportunity of a new practical and reliable methodology for the extraction and preconcentration of organic pollutants from aqueous samples. The optimized technique in conjunction with GC–FID was considered to be a facile, economical procedure, and nearly fast for analysis of some pesticides in water and fruit juice samples. This method does not require any preliminary sample preparation step and also, reduces the volume of solvents used. The method is precise, repeatable, and has a high level of linearity over a wide range of analyte concentrations. In comparison with other methods, there are not any drawbacks such as consumption of relatively high volumes of organic solvent, i.e. mL volume of disperser solvent in DLLME and instability of microdrop in SDME. On the other hand, safe solvents, i.e. hexane and hexanol, were used instead of chlorinated solvents (used in DLLME) in this study. Finally, the method was successfully applied in determination of triazole pesticides at $\mu g\,L^{-1}$ level in aqueous samples such as well water and grape juice samples.

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