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Air-assisted Liquid-liquid Microextraction *vs.* Dispersive Liquid-liquid Microextraction; A Comparative Study for the Analysis of Multiclass Pesticides

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Two convenient sample preparation methods, air-assisted liquid-liquid microextraction (AALLME) and dispersive liquid-liquid microextraction (DLLME) have been developed for the simultaneous determination of multiclass pesticide residues in vegetable and fruit juice samples with gas chromatography-flame ionization detection and the advantages of each method were investigated. In AALLME, fine droplets of an extraction solvent were immediately formed by suction with a syringe and injection of the mixture of an aqueous sample solution and an extraction solvent into a test tube for several times. In DLLME, the cloudy solution was formed with the aid of a disperser solvent. The effect of main factors, such as type and volume of extraction solvent, salt addition, pH, *etc*. was studied. Under the optimum conditions, enrichment factors and extraction recoveries were obtained in the ranges of 262-515, 52-103% and 45-438, 9.2-88% in AALLME and DLLME methods, respectively. Both methods are inexpensive, simple, fast, efficient, reliable and sensitive. Therefore, the proposed methods are suitable for determination of trace levels of multiclass pesticide residues in fruit juice and vegetable samples.

Keywords: Air-assisted liquid-liquid microextraction, Dispersive liquid-liquid microextraction, Fruit and vegetable samples, Gas chromatography; Multiclass pesticides

Abbreviations: AALLME, Air-assisted liquid-liquid microextraction; DLLME, Dispersive liquid-liquid microextraction; EF, Enrichment factor; ER, Extraction recovery; FID, Flame ionization detection; LPME, Liquid phase microextraction; LR, Linear range; QuEChERS, Quick, Easy, Cheap, Effective, Rugged, and Safe; RSD, Relative standard deviation; SDME, Single drop microextraction; SPME, Solid phase microextraction

INTRODUCTION

Along with a dramatic increase in population, the demand for food production has increased. Around 20 to 30% of whole agriculture products are lost by pests every year. To overcome this problem, pesticides are used for plagues extermination [1]. Due to pesticides mobility, capability of bioaccumulation and ability to take part in various physical, chemical and biological processes, they remain in surface and ground waters, and agricultural products [2]. According to some studies, they have unfavorable effects on human health, such as developmental, immune, and neuropsychological disorders, increase in endocrine, and high probability of neurodegenerative diseases, especially Parkinson's and Alzheimer diseases as well as different kinds of cancer [3,4]. Therefore, presence of these toxic compounds in food chain is an absolute risk for general health, and therefore, development of the efficient methods for determination of these compounds is necessary. Due to presence of matrix interferences in real samples, a sample preparation step is usually required in most analytical procedures. An ideal

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sample preparation method should be fast, accurate, precise, economic, easy to use, environmentally friendly, compatible with a wide range of analytical instruments, and easy to automate [5,6]. Numerous methods, such as liquid-liquid extraction (LLE) [7,8], solid phase extraction (SPE) [9,10], QuEChERS [11], single drop microextraction (SDME) [12,13], and solid phase microextraction (SPME) [14,15] were previously developed for the extraction and preconcentration of pesticides in different matrices. Although they are useful methods for pesticides analysis, a number of disadvantages are also identified for them. LLE needs large amounts of toxic organic solvents and SPE is time-consuming. In addition, SPE can be expensive because of single use of disposable sorbents. The organic drop instability and long extraction time limit the application of SDME. The fibers used in SPME are expensive and fragile. Sample carryover is also another problem in SPME [16-18]. Low preconcentration factor (nearly 2-5 times) is the main disadvantage of the QuEChERS method compared to other common methods [19]. Dispersive liquid–liquid microextraction (DLLME) was introduced in 2006 as an efficient microextraction method [20]. It has many benefits and eliminates most disadvantages of the above-mentioned sample preparation methods. It is based on a ternary component solvent system involving an aqueous phase, a disperser solvent, and an extraction solvent. In DLLME, a cloudy solution is formed by dispersing the mixture of extraction and disperser solvents into the aqueous sample. In this way, the analytes are rapidly extracted into the fine droplets of the extraction solvent [21-23]. DLLME has many advantages, such as rapidity, low cost, short extraction time, simple operation, environmental friendliness, and high enrichment factors [24,25]. However, the presence of disperser solvent in the conventional DLLME increases solubility of the analytes in the aqueous phase and results in relatively low extraction efficiency [26]. In order to solve this problem, a new version of DLLME (disperser solvent-free method), air-assisted liquid-liquid microextraction (AALLME) was developed [27]. In AALLME, an extraction solvent is added into the aqueous sample solution and fine droplets of the extraction solvent are dispersed into the aqueous sample during suction/injection cycles with a glass syringe [28-30].

This study was performed to compare DLLME and AALLME methods for the analysis of 14 multiclass pesticide residues (common pesticides used in Iran) with gas chromatography-flame ionization detection (GC-FID). Some important parameters, such as type and volume of extraction solvent, salt addition, pH, *etc.* were optimized. Ultimately, the performance of both methods was evaluated for the analysis of the selected pesticides in grape, cucumber, tomato, onion, and apple juices.

EXPERIMENTAL

Apparatus

The analysis of the analytes was performed using a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with an FID and a split/splitless injection operated in a splitless/split mode (sampling time 1 min and split ratio 1:10). Helium (99.999%, Gulf Cryo, United Arab Emirates) was used as the carrier gas at a linear velocity of 30 cm s^{-1}. The FID temperature was maintained at 300 ºC. Hydrogen gas was generated with a hydrogen generator (OPGU-1500S, Shimadzu, Japan) for FID at a flow rate of 30 ml min^{-1}. The flow rate of air for FID was 300 ml min^{-1}. The analytes were separated on an HP-5 (5% poly diphenyl 95% poly dimethyl siloxane) capillary column (30 m × 0.25 mm i.d., and film thickness of 0.25 μm) (Agilent Technologies, USA). The initial column oven temperature was set at 70 ºC and held for 2 min, then programmed to ramp to 300 ºC at a rate of 10 ºC min^{-1} and held for 3 min. Gas chromatography-mass spectrometry (GC-MS) analysis was carried out using an Agilent 7890A gas chromatograph equipped with a 5975C mass-selective detector (Agilent Technologies, CA, USA). Separation was carried out by 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 oven temperature programming was the same as that used in GC-FID analysis mentioned above. The pH measurements were performed with a Metrohm pH meter model 654 (Herisau, Switzerland). A Hettich centrifuge model D-7200 (Germany) was used to accelerate phase separation.

Materials and Methods

Penconazole, hexaconazole, diniconazole, tebuconazole, triticonazole, diazinon, chloropyriphos, fenazaquin,

clodinafop-propargyl, fenoxaprop-P-ethyl, and haloxyfop-R-methyl were kindly provided by GYAH Corporation (Karaj, Iran). Bromopropylate, ametryn, and atrazine were from Dr. Ehrenstorfer GmbH (Agsburg, Germany). All pesticide standards were of high purity grade (purity higher than 98%). Analytical grade methanol, acetone, *iso*-propanol, and acetonitrile (as disperser solvents in DLLME), carbon tetrachloride (CCl_4), 1,1,2,2-tetrachloroethane (1,1,2,2-TCE), 1,2-dibromoethane (1,2-DBE), and 1,1,2-trichloroethane (1,1,2-TCE) (as extraction solvents in both methods), hydrochloric acid, sodium hydroxide, and sodium chloride were purchased from Merck (Darmstadt, Germany). Deionized water was from Ghazi Company (Tabriz, Iran). A stock solution of the pesticides (1000 mg l^{-1} of each pesticide) was prepared in methanol. An aqueous working solution with a concentration of 0.5 mg l^{-1} of each pesticide was prepared daily by diluting the stock solution with deionized water. Another standard solution of the analytes (200 mg l^{-1}, each analyte) was prepared in 1,1,2-TCE. This solution was directly injected into the GC-FID each day (three times) for quality control of the separation system and the obtained peak areas were used in calculation of extraction recoveries (ERs) and enrichment factors (EFs).

Samples

The packaged grape juice, fresh apples, onions, cucumbers, and tomatoes were purchased from local supermarkets (Tabriz, Iran). The fruit and vegetable samples were squeezed with a commercial juice squeezer (Kenwood Electronic, England) and then centrifuged at 4000 rpm for 7 min. The obtained juices were used in the extraction procedure. Cucumber, onion, tomato, and apple juices were diluted with deionized water at a ratio of 1:4 in both methods. The grape juice was used without dilution in DLLME. It was diluted at a ratio of 1:3 with deionized water in AALLME.

DLLME Procedure

5 ml sample solution (see section Samples) or standard solution with a concentration of 0.5 mg l^{-1} of each pesticide containing 0.125 g NaCl (2.5%, *w/v*) was placed into a 10-ml glass test tube with conical bottom. Then, a mixture of 1,1,2-TCE (35 µl) as an extraction solvent and acetone (2 ml) as a disperser solvent was immediately injected into the solution with a 5-ml glass syringe and a cloudy solution was obtained. This cloudy solution was centrifuged for 5 min at 7000 rpm. The fine droplets of the 1,1,2-TCE containing the extracted analytes were sedimented at the bottom of the tube (10 ± 1 µl). An aliquot of the sedimented phase (1 µl) was removed with a 1-µl GC microsyringe (zero dead volume, Hamilton, Switzerland) and injected into the separation system for analysis.

AALLME Procedure

5 ml sample solution (see section Samples) or standard solution with a concentration of 0.5 mg l^{-1} of each pesticide was placed into a 10-ml glass test tube with conical bottom. Then, 30 µl 1,1,2-TCE (extraction solvent) was added. The fine droplets of the extraction solvent were formed by aspirating and injecting the mixture with a 5-ml syringe into the test tube for 10 times. This step was performed in less than 1 min. Then, 0.125 g (2.5%, *w/v*) NaCl was added and shaken to dissolve completely. The cloudy solution was centrifuged for 5 min at 8000 rpm and the extraction solvent was settled at the bottom of the tube (10 ± 1 µl). Ultimately, 1 µl of the sedimented phase was removed and injected into the GC-FID system for analysis.

Calculation of Analytical Parameters

Two main parameters, EF and ER, were used to evaluate the proposed extraction methods. The EF is defined as the ratio of the analyte concentration in the sedimented phase (C_{sed}) to the initial concentration of the analyte (C_0) in the aqueous sample:

$$EF = \frac{C_{sed}}{C_0} \qquad (1)$$

C_{sed} was obtained by comparing the peak areas of the analytes in two cases: direct injection of the standard solution of the pesticides (200 mg l^{-1} of each analyte prepared in 1,1,2-TCE), and injection of the sedimented phase containing the enriched analytes into the separation system.

The ER is defined as the percentage of total analyte amount (n_0) extracted into the sedimented phase (n_{sed}):

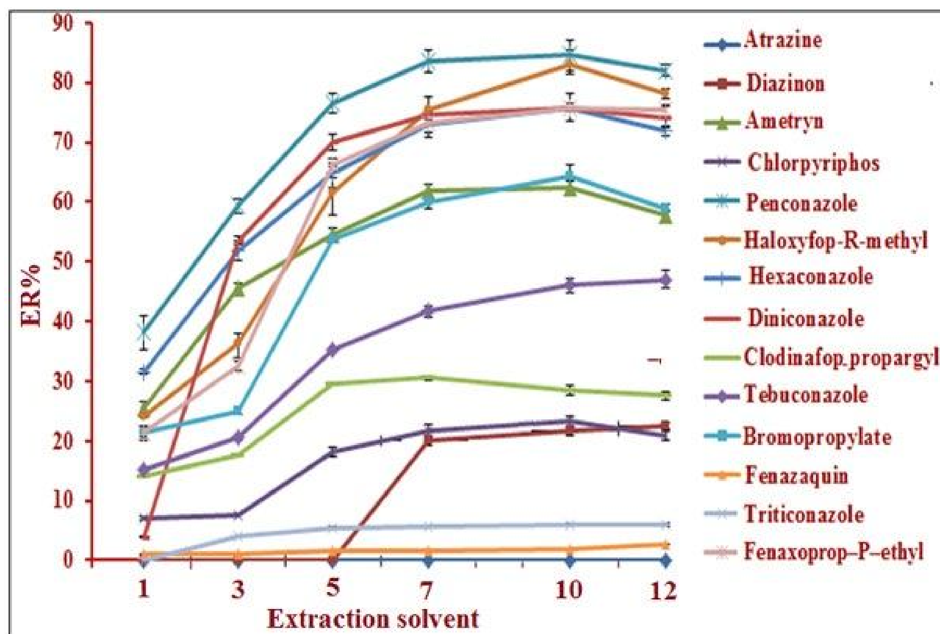

Fig. 1. Optimization of number of extraction cycles. Extraction conditions: 5 ml of aqueous solution containing 0.5 mg l^{-1} of each analyte without pH adjusting and salt addition; extraction solvent, 1,2-DBE, (50 µl); and centrifuging time and rate, 5 min and 7000 rpm, respectively. The error bars indicate standard deviation of three repeated determinations.

$$ER = (\frac{n_{sed}}{n_0}) \times 100 = (\frac{C_{sed} \times V_{sed}}{C_0 \times V_{aq}}) \times 100$$

$$ER = (\frac{V_{sed}}{V_{aq}}) \times EF \times 100 \qquad (2)$$

where V_{sed} and V_{aq} are the volumes of the sedimented phase and aqueous solution, respectively.

RESULTS AND DISCUSSION

In this study, the performance of DLLME and AALLME procedures was compared for the extraction of multiclass pesticides. To obtain the optimum experimental conditions, some important parameters affecting the extraction efficiency, such as type and volume of extraction solvent, pH, and ionic strength in both methods were investigated. Furthermore, type and volume of disperser solvent in DLLME and number of aspiration/injection cycles in AALLME were optimized.

Optimization of AALLME and DLLME Parameters

Study of aspiration/injection cycles in AALLME. The number of aspiration/injection cycles is considered as the number of the repeated operation of suction and injection of the mixture of the aqueous sample solution and extraction solvent with a glass syringe. 1,2-DBE (50 µl) was used as the extraction solvent for the extraction of the target analytes from 5 ml aqueous sample solution and the number of extraction was studied in the range of 1-12 times. As it can be seen from Fig. 1, by increasing the extraction cycles, ERs increase up to 10 times for most of the analytes and then remain nearly constant. Thus, 10 times of extraction cycles was selected for the further studies.

Selection of Disperser Solvent Type and its Volume in DLLME

In DLLME, the disperser solvent must be miscible with extraction solvent and aqueous phase. Accordingly, four organic solvents including *iso*-propanol, acetone,

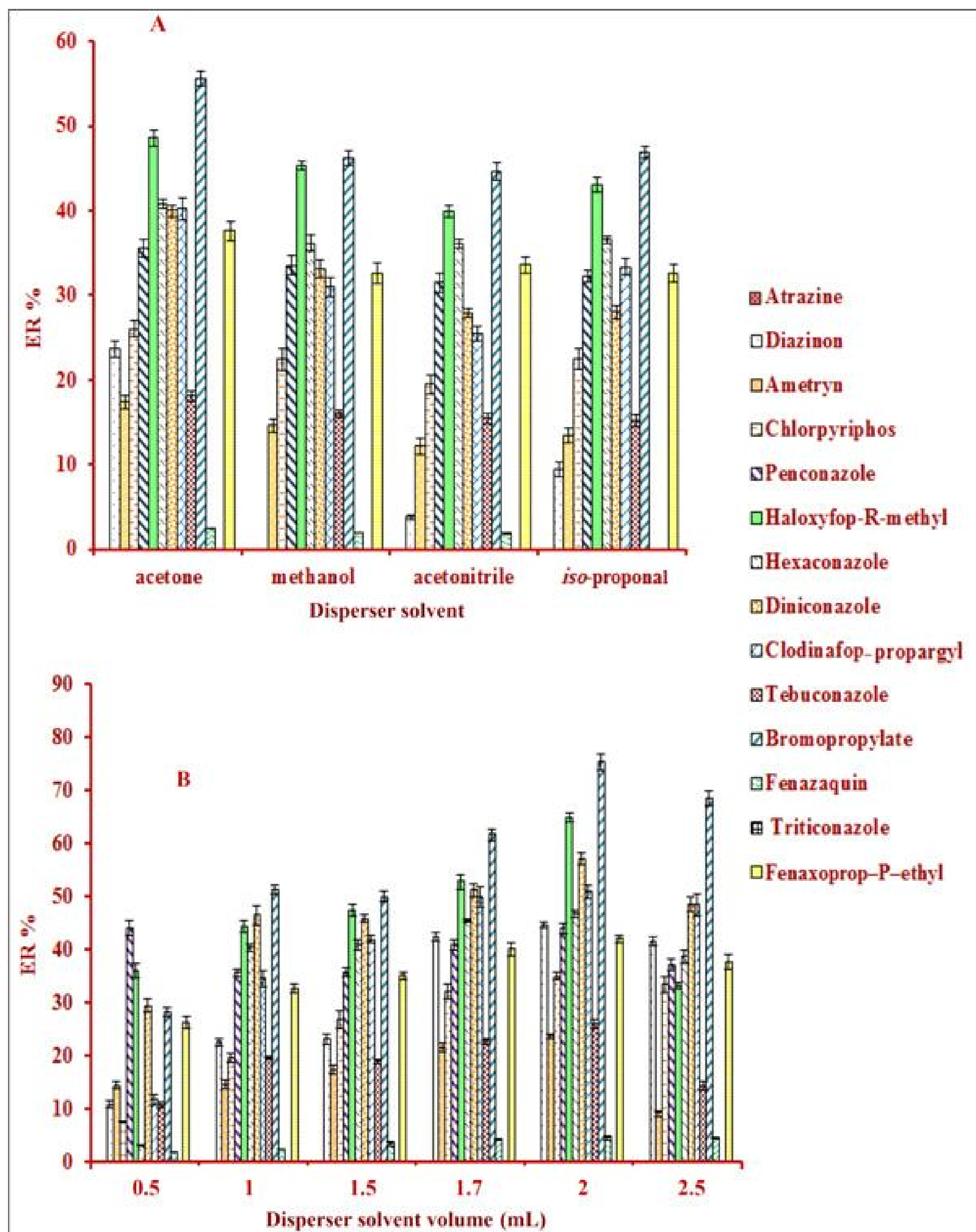

Fig. 2. Selection of disperser solvent type (A) and its volume (B). Extraction conditions: 5 ml of aqueous solution containing 0.5 mg l^{-1} of each analyte without pH adjusting and salt addition; extraction solvent, 1,2-DBE (50 µl); disperser solvent volume in Fig. 2 (A) 1 ml; and centrifuging time and rate, 5 min and 7000 rpm, respectively.

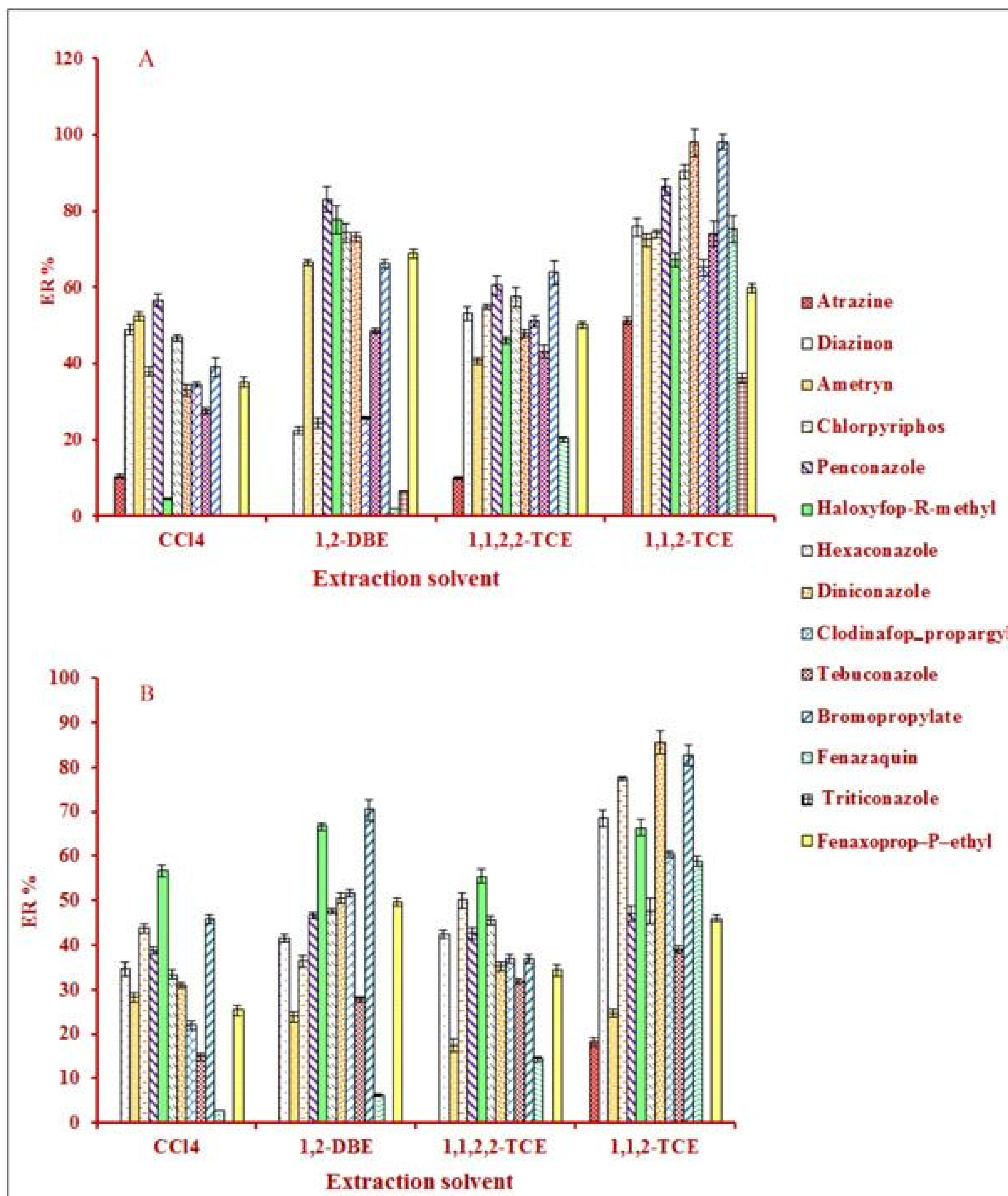

Fig. 3. Selection of extraction solvent type in AALLME (A) and DLLME (B). Extraction conditions; number of extraction cycles in AALLME, 10; volume and kind of the disperser solvent in DLLME, 2 ml acetone. The other conditions are the same as those used in Fig. 2.

acetonitrile, and methanol were tested. Using 1 ml of each disperser and 50 µl of 1,2-DBE, acetone offers high ERs compared to those in most of the other analytes (Fig. 2A). Therefore, it was selected as the optimum disperser solvent for the subsequent experiments. As mentioned, atrazine and triticonazole are not extracted in the above-mentioned disperser solvents. Also, diazinon and fenazaquin are not extracted in methanol and *iso*-propanol, respectively. At low volumes of the disperser solvent (acetone), the extraction solvent was not dispersed well into the aqueous solution and the organic extractant droplets were not formed properly. Therefore, low volumes of the disperser solvent led to low ERs. On the other hand, at high volumes of the disperser solvent, the polarity of the aqueous phase was reduced, thereby, the solubility of the analytes increased in the aqueous phase and extraction efficiency decreased. To evaluate the effect of the disperser solvent volume, the experiments were performed with different volumes of acetone (0.50, 1.0, 1.5, 1.7, 2.0 and 2.5 ml). According to the obtained results shown in Fig. 2B, the ERs increase for all analytes by increasing the disperser volume from 0.50 to 2.0 ml and then decrease. Therefore, 2.0 ml acetone was selected for the next optimization steps.

Selection of Extraction Solvent

The selection of an appropriate extraction solvent is very important for both AALLME and DLLME procedures. The extraction solvent should have good chromatographic behaviour and high capability to extract the analytes. It should also be insoluble in water. Based on these requirements and considering the fact that denser extraction solvents than water can be easily collected after extraction, carbon tetrachloride, 1,1,2-TCE, 1,1,2,2-TCE and 1,2-DBE were selected and compared. Considering the obtained ERs for the target analytes (Figs. 3A and 3B), 1,1,2-TCE was selected as a suitable extraction solvent in both methods.

pH Optimization

The sample pH should be optimized to ensure that all analytes are in their molecular states and can be extracted into the extraction solvent. In addition, some analytes may be hydrolyzed at highly acidic or alkaline conditions. To investigate the effect of aqueous solution pH, 1 M HCl or NaOH solution was used for pH adjustment in the range of 2-12. The results were similar in both methods. In the pH range of 4-6, the ERs were higher than those for other pHs in the cases of most analytes (Fig. 4). The extraction efficiency decreased at pH = 2 and pHs higher than 8 in the cases of most studied pesticides. The results indicated that high extraction efficiency is obtained at pH = 6 in the cases of most analytes. Therefore, it was selected for the subsequent experiments. It is noted that the pH of samples used in this study was 6 ± 1 and the pH adjustment was not necessary.

Study of Salt Addition

Salt addition can have different effects as follows: (1) increasing viscosity of the aqueous phase which leads to decrease in diffusion coefficients of the analytes, EFs, and ERs, (2) decreasing the solubility of the extraction solvent in the aqueous phase which leads to increase in volume of the sedimented organic phase, and (3) improving extraction efficiency and enhancing the partitioning of the analytes into the organic phase by decreasing the solubility of the analytes in the aqueous phase (salting-out effect). In order to evaluate the effect of ionic strength of aqueous phase on the extraction efficiency of AALLME, different concentrations of NaCl (0-15%, *w/v*) before, and (0-10%, *w/v*) after performing the suction/injection cycles (extraction cycles) were added into the aqueous sample solution, separately. The results in Fig. 5 indicate that the ERs increase with increasing NaCl concentration up to 5% and 2.5% (*w/v*) before and after the extraction cycles, respectively, and then decrease gradually by increasing the extra salt. In addition, high ERs are obtained by salt addition after performing the extraction cycles (Fig. 5B) compared to its addition before the extraction cycles (Fig. 5A). Therefore, the subsequent experiments were conducted using 2.5% *w/v* NaCl after performing the extraction cycles.

To evaluate the ionic strength effect of the aqueous phase on the extraction efficiency in DLLME, different concentrations of NaCl were added into the aqueous phase in the range of 0-10%, *w/v*, and 0-7.5%, *w/v*, before and after dispersion of the extraction solvent into the aqueous phase, respectively. By adding the salt in both cases, extraction efficiencies increased up to 2.5%, *w/v*, NaCl and after that decreased gradually (Fig. 6). Through comparing the obtained ERs, the subsequent experiments were conducted using 2.5%, *w/v*, NaCl before dispersion of

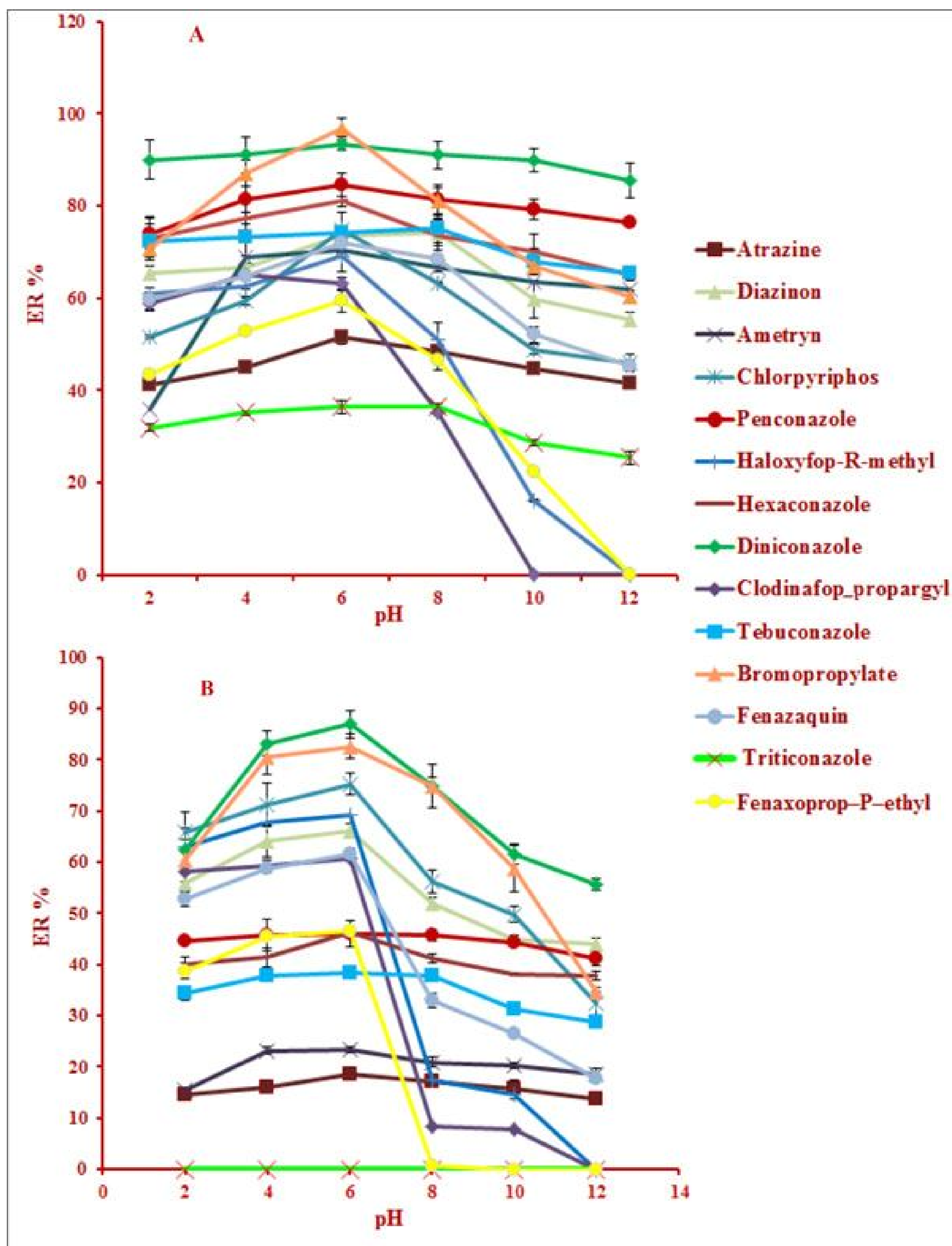

Fig. 4. Effect of sample solution pH in AALLME (A) and DLLME (B). Extraction conditions are the same as those used in Fig. 3, except 1,1,2-TCE was used as the extraction solvent.

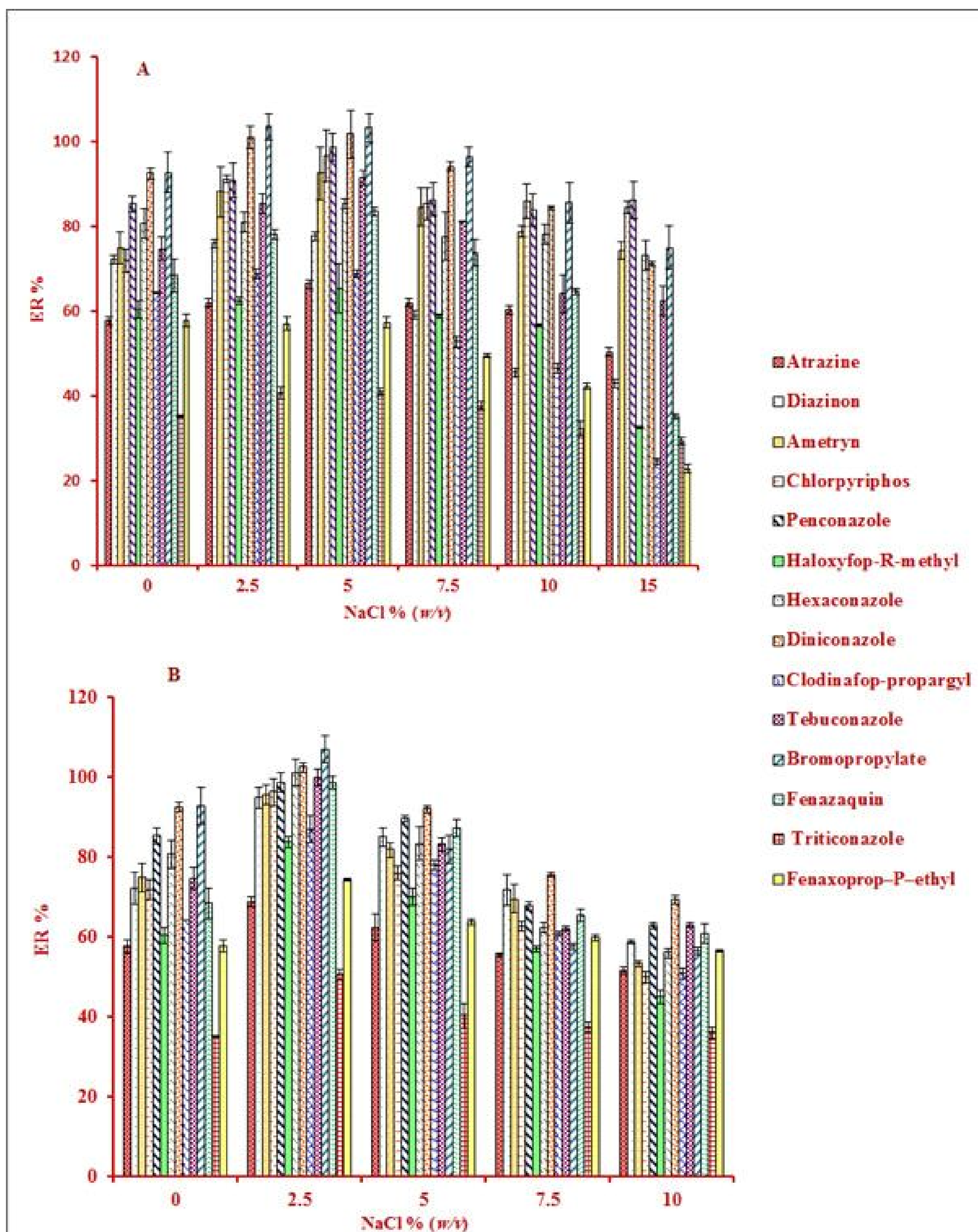

Fig. 5. Effect of NaCl concentration on the ERs of AALLME procedure before (A) and after (B) performing suction/injection cycles. Extraction conditions are the same as those used in Fig. 4.

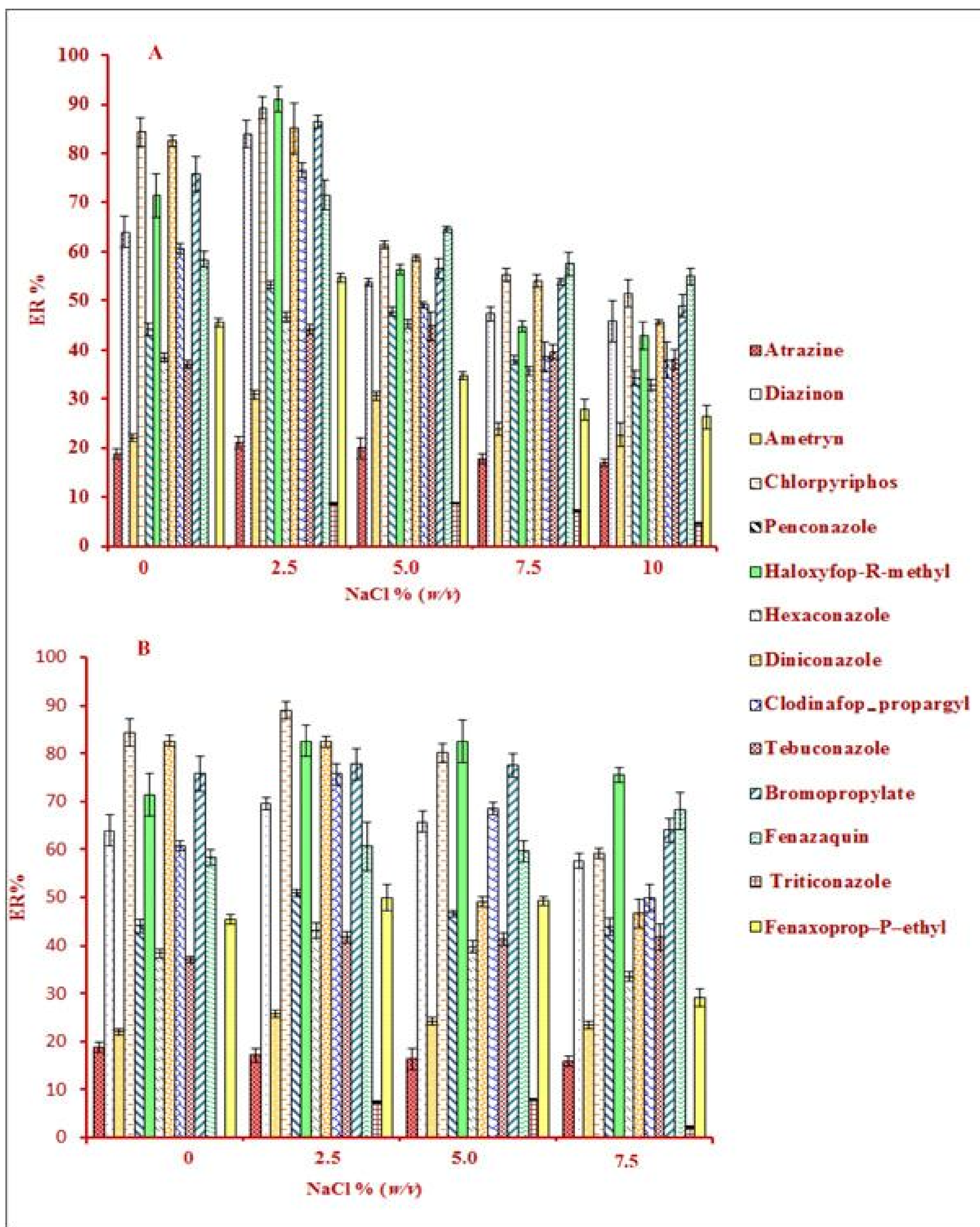

Fig. 6. Effect of NaCl concentration before (A) and after (B) dispersion of the extraction solvent into the aqueous phase in DLLME. Extraction conditions are the same as those used in Fig. 4.

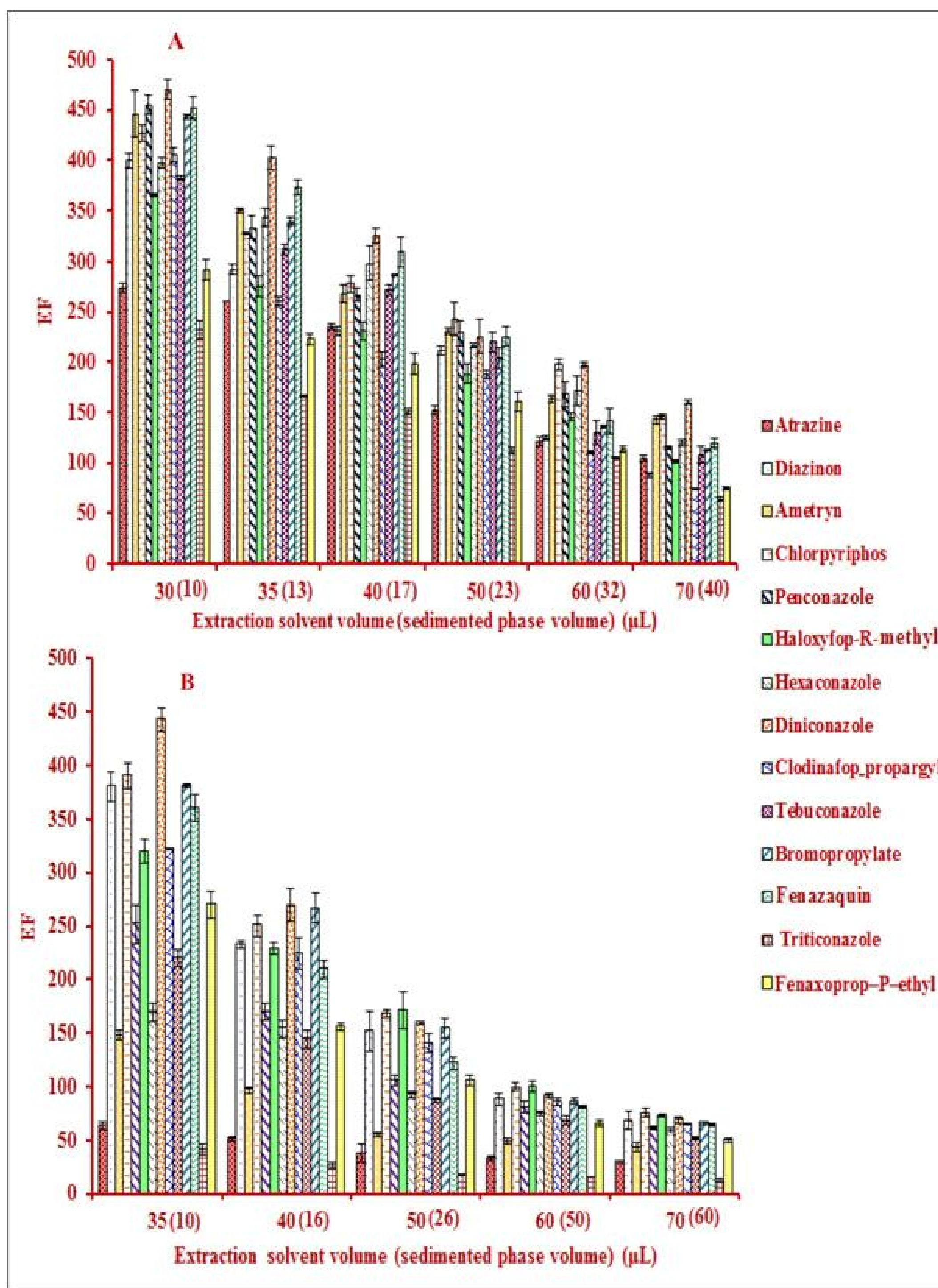

Fig. 7. Effect of the extraction solvent volume on the extraction efficiency in AALLME (A) and DLLME (B). Extraction conditions: NaCl concentration, 2.5% (*w/v*) added after performing the extraction cycles in AALLME, and 2.5% (*w/v*) added before dispersion step in DLLME. Other extraction conditions are the same as those used in Fig. 6.

the extraction solvent into the aqueous phase.

Selection of Extraction Solvent Volume

Due to effect of the extraction solvent volume on the extraction efficiency, repeatability, and limits of detection (LODs), selection of the suitable extraction solvent volume is critical for both AALLME and DLLME methods. To this end, different volumes of 1,1,2-TCE (30-70 µl in AALLME and 35-70 µl in DLLME) were used in these procedures (Figs. 7A and 7B, respectively). The results show that EFs decrease by increasing the volume of 1,1,2-TCE in both extraction methods. In the case of low volumes of the extraction solvent (<30 µl in AALLME and <35 µl in DLLME), removing the sedimented phase was difficult. Therefore, 30 µl in AALLME and 35 µl in DLLME leading to 10 ± 1 µl of the sedimented phase volume in both methods, were selected for the further studies.

Analytical Characteristics of the Proposed Methods

To assess the quantitative characteristics of the proposed methods under the above-mentioned conditions, some quantitative parameters including linear range (LR) of the calibration graph, coefficient of determination (R^2), LOD, limit of quantification (LOQ), relative standard deviation (RSD), EFs, and ERs were evaluated (Table 1). Under the optimum conditions, the EFs and ERs were in the ranges of 262-515 and 52-103% in AALLME, and 45-438 and 9.2-88% in DLLME, respectively. The LODs were in the ranges of 0.21-4.3 and 0.53-8.5 µg l^{-1} and LOQs in the ranges of 0.71-14 and 1.7-28 µg l^{-1} for AALLME and DLLME, respectively. In AALLME and DLLME, the RSDs for the extraction of the target analytes (50 µg l^{-1} of each pesticide) were in the ranges of 2-5 and 3-7% for intra- (n = 6) and 3-6 and 3-8% (n = 5) for inter-day precisions, respectively. It is mentioned that at a concentration of 100 µg l^{-1} of each pesticide, the RSDs were in the ranges of 2-4 and 2-5% for intra- (n = 6) and 2-4 and 3-6% for inter-day (n = 5) precisions for AALLME and DLLME, respectively. The calibration graphs linearity ranges were completely broad for all target analytes in both methods. Good linearity was obtained for all selected pesticides (R^2 > 0.990). Considering the results, both methods are rapid, sensitive, efficient, reliable, and easy to use procedures for the extraction of multiclass pesticides. The AALLME method has some priorities over the DLLME method, including low LODs and LOQs, high EFs and ERs, and good repeatability.

Investigation of Matrix Effect

The standard addition method was used to evaluate the matrix effect in real samples. The samples were spiked at three concentrations (50, 100 and 200 µg l^{-1} of each analyte). The relative recoveries were calculated by performing the proposed methods on the samples and comparing the obtained results with those of deionized water spiked at the same concentrations. The results are listed in Table 2. The relative recoveries between 80 and 106% show that there is no significant matrix effect for most studied samples.

Samples Analysis

The performance of the proposed methods were evaluated by the extraction and determination of the target analytes in fruit juice and vegetable samples, including apple, grape, onion, cucumber, and tomato under the optimum conditions. Figure 8 shows the GC-FID chromatograms of deionized water, aqueous solution of the analytes (50 µg l^{-1} of each pesticide), apple juice, onion juice, cucumber juice, tomato juice, grape juice, and the grape juice spiked with 20 µg l^{-1} of each analyte after performing the above-mentioned AALLME procedure. There are suspected peaks in the retention times of penconazole and tebuconazole in the grape juice. Therefore, this sample was injected into the GC-MS to confirm the obtained results with GC-FID. The typical GC-total ions current-MS chromatogram obtained for the grape juice and mass data are given in Fig. 9. The results certified the presence of penconazole (14.2 ± 0.2 µg l^{-1}, n = 3) and tebuconazole (13.6 ± 0.3 µg l^{-1}, n = 3) in this sample. Other studied samples were free of analytes.

Comparison of AALLME and DLLME Methods

In comparison to DLLME, AALLME needs low extraction solvent volume (30 *vs.* 35 µl) and no disperser solvent. Therefore, AALLME is more environmentally friendly. Furthermore, the poor extraction efficiency of DLLME is eliminated by the AALLME method. Despite these advantages, high matrix effect was observed in AALLME for the studied samples in comparison to

Table 1. Analytical Features of the AALLME (1) and DLLME (2) Methods

Analytes	LR (μg l^{-1})[a]		R^{2b}		LOD (μg l^{-1})[c]		LOQ (μg l^{-1})[d]		EF ± SD[e]		ER ± SD[f]		RSD%[g] (Intra-day)		RSD%[h] (Inter-day)	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Atrazine	15-10000	30-10000	0.995	0.994	4.3	8.5	14	28	340±19	66.4±5.3	68.1±4	13±1	3	5	3	5
Diazinon	5-10000	15-10000	0.998	0.997	0.81	1.7	2.7	5.6	467±11	376±15	93.2±2	75±3	3	4	3	3
Ametryn	15-10000	30-10000	0.993	0.992	1.9	6.4	6.3	21	515±6.2	146±3.3	103±1	30±1	3	5	3	6
Chlorpyriphos	5-10000	15-10000	0.998	0.999	1.4	1.7	4.7	5.7	487±7.1	395±10	98.5±1	80±2	4	5	5	5
Penconazole	5-10000	15-10000	0.999	0.998	0.92	3.0	3.0	10	515±15	252±15	103±3	50±3	2	5	4	6
Haloxyfop-R-methyl	5-10000	15-10000	0.999	0.999	0.84	1.3	2.7	4.3	447±2.9	322±7.1	89.4±1	64±1	3	5	4	5
Hexaconazole	5-10000	15-10000	0.998	0.999	1.0	2.9	3.3	9.6	448±8.0	170±14	90.0±2	34±3	3	5	4	6
Diniconazole	5-10000	15-10000	0.997	0.999	1.2	1.9	4.0	6.3	500±11	438±13	100±2	88±3	4	5	5	6
Clodinafop-propargyl	5-10000	15-10000	0.996	0.998	1.0	2.1	3.0	7.0	462±14	315±16	92.2±3	63±3	3	3	4	7
Tebuconazole	5-10000	15-10000	0.998	0.999	0.71	2.0	2.3	6.6	435±10	218±12	87.4±2	43±2	5	4	5	7
Bromopropylate	5-10000	15-10000	0.999	0.998	0.52	0.83	1.7	2.7	515±14	384±16	103±3	77±3	4	4	4	7
Fenazaquin	5-10000	15-10000	0.995	0.998	0.21	0.53	0.71	1.7	478±11	367±13	96.4±2	73±2	4	6	5	8
Triticonazole	15-10000	30-10000	0.995	0.990	2.6	8.4	8.7	28	262±17	45.3±4.1	52.1±3	9.2±1	4	6	4	6
Fenaxoprop-P-ethyl	5-10000	15-10000	0.997	0.996	1.2	2.4	4.0	8.0	366±6.3	276±11	73.2±1	55±2	2	7	6	7

[a]Linear range. [b]Coefficient of determination. [c]LOD, (S/N = 3). [d]LOQ, (S/N = 10). [e]Enrichment factor ± standard deviation (n = 3). [f]Extraction recovery ± standard deviation (n = 3). [g]Relative standard deviation (n = 6, C = 50 μg l^{-1} of each analyte). [h]Relative standard deviation (n = 5, C =50 μg l^{-1} of each analyte).

Table 2. Matrix Effect Study in the Samples Spiked at Different Concentrations. The Apple, Cucumber, Onion and Tomato Juices were Analyzed after Dilution with Deionized Water at a Ratio of 1:4. The Commercial Grape Juice was Analyzed without Dilution in DLLME and by Dilution at a Ratio of 1:3 with Deionized water in AALLME. Analytes' Contents of the Samples were Subtracted

Analytes	Mean relative recovery (%) ± standard deviation (n = 3)									
	Apple juice		Grape juice		Cucumber juice		Tomato juice		Onion juice	
	AALLME	DLLME	AALLME	DLLME	AALLME	DLLME	AALLME	DLLME	AALLME	DLLME
	All samples were spiked with each analyte at a concentration of 50 µg l^{-1}									
Atrazine	82 ± 2	99 ± 1	90 ± 4	100 ± 4	81 ± 4	99 ± 5	85 ± 5	105 ± 3	101 ±2	104 ±2
Diazinon	82 ± 2	98 ± 6	84 ± 3	104 ± 1	85 ± 5	98 ± 4	89 ± 4	102 ± 4	89 ± 3	95 ± 2
Ametryn	89 ± 5	97 ± 3	85 ± 4	102 ± 1	85 ± 1	96 ± 2	90 ± 5	102 ± 3	90 ± 3	98 ± 3
Chlorpyriphos	84 ± 2	98 ± 5	91 ± 3	99 ± 5	85 ± 4	99 ± 3	87 ± 2	103 ± 3	90 ± 4	95 ± 3
Penconazole	85 ± 3	103 ± 4	92 ± 3	103 ± 4	85 ± 1	99 ± 5	86 ± 4	106 ± 5	88 ± 5	96 ± 4
Haloxyfop-R-methyl	81 ± 4	99 ± 3	93 ± 5	103 ± 6	89 ± 2	101 ± 3	85 ± 6	104 ± 3	89 ± 2	97 ± 5
Hexaconazole	81 ± 4	98 ± 2	94 ± 4	102 ± 3	91 ± 3	102 ± 3	86 ± 4	105 ± 2	88 ± 4	97 ± 2
Diniconazole	93 ± 4	96 ± 5	88 ± 2	100 ± 4	84 ± 4	104 ± 4	85 ± 2	100 ± 5	90 ± 4	103 ± 3
Clodinafoppropargyl	80 ± 5	99 ± 5	81 ± 5	95 ± 3	86 ± 4	103 ± 5	90 ± 2	101 ± 6	95 ± 4	95 ± 4
Tebuconazole	86 ± 4	100 ± 5	85 ± 5	93 ± 5	89 ± 4	104 ± 4	88 ± 5	102 ± 3	90 ± 3	96 ± 2
Bromopropylate	85 ± 5	94 ±5	86 ± 5	102 ± 2	90 ± 3	90 ± 5	89 ± 5	99 ± 4	94 ± 4	94 ± 3
Fenazaquin	80 ± 3	94 ± 3	87 ± 5	100 ± 5	89 ± 4	94 ± 2	92 ± 3	103 ± 2	93 ± 3	99 ± 3
Triticonazole	81 ± 4	97 ± 4	87 ± 5	101 ± 5	88 ± 3	95 ± 4	88 ± 4	100 ± 3	91 ± 3	97 ± 4
Fenaxoprop-P-ethyl	86 ± 2	95 ± 2	88 ± 5	101 ± 3	86 ± 5	96 ± 4	86 ± 3	98 ± 3	90 ± 5	100 ± 3
	All samples were spiked with each analyte at a concentration of 100 µg l^{-1}									
Atrazine	83 ± 2	100 ± 3	90 ± 4	101 ± 3	89 ± 3	102 ± 4	89 ± 4	106 ± 4	102 ± 3	105 ± 4
Diazinon	86 ± 3	98 ± 5	89 ± 6	104 ± 4	90 ± 4	100 ± 3	90 ± 3	103 ± 3	91 ± 4	99 ± 3
Ametryn	90 ± 4	98 ± 2	86 ± 4	104 ± 4	85 ± 4	103 ± 3	91 ± 4	102 ± 2	91 ± 2	106 ± 2
Chlorpyriphos	93 ± 1	97 ± 1	92 ± 4	100 ± 1	89 ± 1	104 ± 5	89 ± 3	101 ± 4	92 ± 5	99 ± 3
Penconazole	95 ± 2	103 ± 4	93 ± 5	99 ± 3	90 ± 4	103 ± 6	91 ± 4	106 ± 4	90 ± 4	98 ± 2
Haloxyfop-R-methyl	92 ± 3	100 ± 5	92 ± 4	103 ± 1	92 ± 5	101 ± 6	90 ± 4	105 ± 6	92 ± 3	96 ± 3
Hexaconazole	85 ± 2	101 ± 3	94 ± 4	101 ± 4	92 ± 4	103 ± 3	90 ± 5	104 ± 5	93 ± 4	99 ± 4
Diniconazole	94 ± 4	99 ± 2	90 ± 4	101 ± 5	89 ± 2	101 ± 5	88 ± 3	103 ± 4	91 ± 3	104 ± 1

Table 2. Continued

Clodinafoppropargyl	82 ± 4	102 ± 5	83 ± 3	96 ± 6	88 ± 5	100 ± 4	91 ± 4	103 ± 2	96 ± 2	96 ± 3
Tebuconazole	87 ± 3	100 ± 4	85 ± 3	96 ± 4	90 ± 2	103 ± 4	87 ± 2	104 ± 5	92 ± 4	97 ± 3
Bromopropylate	87 ± 2	96 ± 5	87 ± 5	104 ± 3	91 ± 4	95 ± 6	91 ± 3	100 ± 2	94 ± 5	95 ± 2
Fenazaquin	82 ± 2	95 ± 3	88 ± 5	103 ± 4	91 ± 5	93 ± 4	94 ± 4	104 ± 4	94 ± 4	100 ± 1
Triticonazole	81 ± 2	97 ± 4	88 ± 4	99 ± 3	89 ± 3	99 ± 5	94 ± 5	101 ± 2	92± 3	103 ± 4
Fenaxoprop-P-ethyl	89 ± 3	97 ± 5	88 ± 3	99 ± 3	89 ± 2	103 ± 3	89 ± 3	100 ± 3	91 ± 4	100 ± 3
All samples were spiked with each analyte at a concentration of 200 $\mu g\ l^{-1}$										
Atrazine	89 ± 1	101 ± 4	91 ± 4	105 ± 4	90 ± 2	103 ± 3	85 ± 5	105 ± 5	102 ± 5	105 ± 2
Diazinon	88 ± 4	101 ± 3	92 ± 4	105 ± 4	91 ± 6	102 ± 3	89 ± 4	105 ± 4	92 ± 4	102 ± 3
Ametryn	89 ± 4	99 ± 4	87 ± 3	103 ± 4	95 ± 5	100 ± 4	90 ± 3	103 ± 3	92 ± 3	106 ± 2
Chlorpyriphos	94 ± 3	101 ± 3	94 ± 5	105 ± 2	94 ± 3	100 ± 4	87 ± 2	103 ± 3	90 ± 3	100 ± 3
Penconazole	96 ± 3	102 ± 2	94 ± 4	100 ± 4	92 ± 6	102 ± 3	86 ± 2	103 ± 2	93 ± 4	101 ± 1
Haloxyfop-R-methyl	95 ± 3	100 ± 2	94 ± 4	103 ± 2	93 ± 5	99 ± 2	81 ± 4	105 ± 3	94 ± 1	103 ± 4
Hexaconazole	82 ± 5	103 ± 1	94 ± 5	105 ± 4	95 ± 5	102 ± 1	86 ± 6	105 ± 2	94 ± 2	100 ± 3
Diniconazole	94 ± 3	100 ± 2	92 ± 4	104 ± 5	95 ± 3	104 ± 7	83 ± 3	102 ± 4	92 ± 3	104 ± 3
Clodinafoppropargyl	84 ± 3	103 ± 4	84 ± 3	95 ± 4	91 ± 5	106 ± 2	89 ± 3	104 ± 5	97 ± 5	99 ± 3
Tebuconazole	91 ± 3	101 ± 2	86 ± 2	100 ± 4	92 ± 4	101 ± 5	85 ± 3	104 ± 3	93 ± 4	98 ± 4
Bromopropylate	87 ± 2	100 ± 3	88 ± 4	104 ± 4	92 ± 6	97 ± 5	88 ± 2	101 ± 2	95 ± 1	98 ± 4
Fenazaquin	88 ± 3	99 ± 2	90 ± 4	105 ± 3	92 ± 4	93 ± 1	89 ± 3	105 ± 3	90 ± 4	101 ± 3
Triticonazole	83 ± 4	99 ± 4	90 ± 3	103 ± 4	90 ± 2	100 ± 6	90 ± 3	102 ± 5	91 ± 3	102 ± 3
Fenaxoprop-P-ethyl	88 ± 1	98 ± 2	91 ± 4	102 ± 8	91 ± 1	106 ± 5	87 ± 3	101 ± 3	93 ± 2	103 ± 4

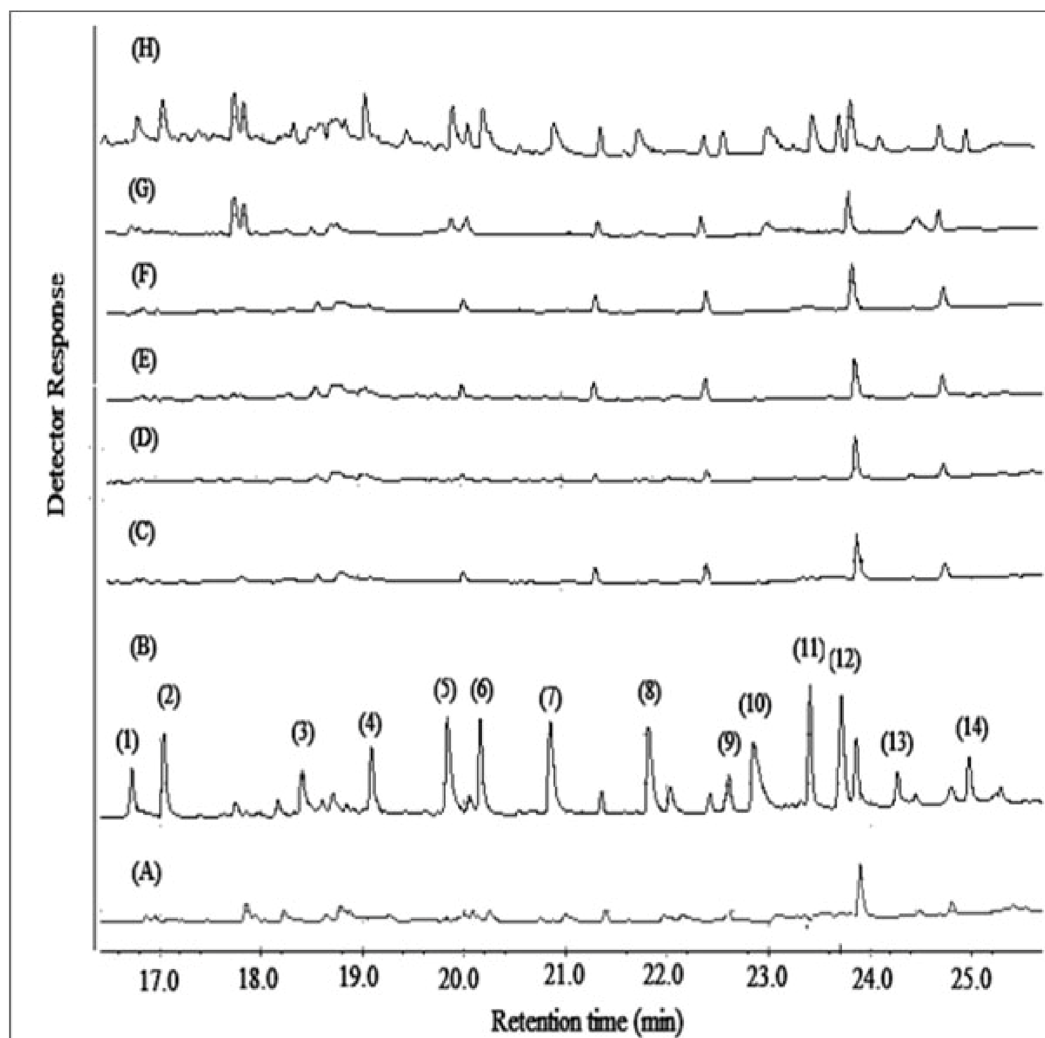

Fig. 8. GC-FID chromatograms of (A) blank, (B) aqueous solution of the analytes (50 µg l^{-1} each pesticide), (C) apple juice, (D) onion juice, (E) cucumber juice, (F) tomato juice, (G) grape juice, and (H) the grape juice spiked with 20 µg l^{-1} of each analyte. In all cases, the proposed AALLME method was performed on the sample and 1 µl of the sedimented phase was injected into the separation system. Peaks identification: (1) atrazine, (2) diazinon, (3) ametryn, (4) chlorpyriphos, (5) penconazole, (6) haloxyfop-R-methyl, (7) hexaconazole, (8) diniconazole, (9) clodinafop-propargyl (10) tebuconazole, (11) bromopropylate, (12) fenazaquin, (13) triticonazole, and (14) fenaxoprop-P-ethyl.

DLLME. The results show that both AALLME and DLLME methods are simple, repeatable, inexpensive, and reliable. They are also useful for determination of the pesticide residues in vegetable and fruit matrices. Low LODs and LOQs, wide LRs, good RSDs, and high EFs and ERs in AALLME and low matrix effect in DLLME make the difference in selection of the suitable method in different matrices.

CONCLUSIONS

In this study, two liquid phase microextraction methods, AALLME and DLLME, have been developed and compared. The proposed methods were employed for the analysis of multiclass pesticides in grape, cucumber, tomato, onion, and apple juices with GC-FID. Each of both methods has own advantages and disadvantages *vs.* the other

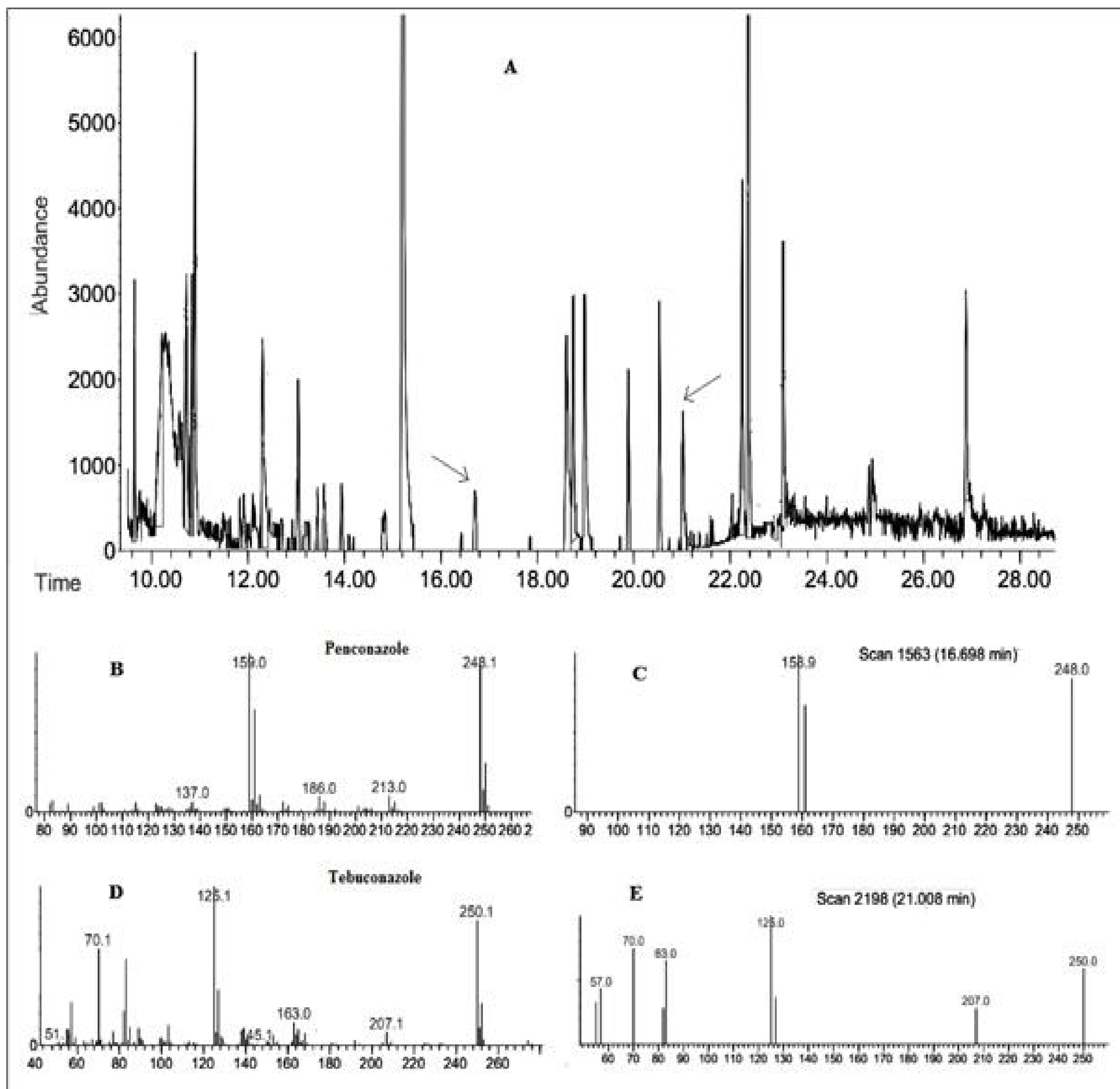

Fig. 9. GC-total ions current-MS chromatogram of the grape juice after performing the proposed AALLME method (A), mass spectrum of penconazole (B), scan 1563 (retention time 16.698 min) (C), mass spectrum of tebuconazole (D), and scan of 2198 (retention time 21.008 min) (E).

one, such as better repeatability, lower solvent consumption, lower LODs and LOQs, and higher EFs and ERs in AALLME and lower matrix effect in DLLME. Application of both methods for the simultaneous extraction and preconcentration of multiclass pesticide residues can be considered as the high performance methods due to their low cost, rapidity, and high extraction efficiency.

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