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ORIGINAL RESEARCH

Analysis of Malathion pesticide residues in rice samples using ultrasound-assisted emulsification-microextraction coupled to UV photoionization source ion mobility spectrometry

Hamideh Kalhor[1] · **Mohsen Motamedi**[2] · **S. Hossein Mousavi**[3] · **A. Froozan Shokri**[4]

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Abstract The present paper describes the validation of ultrasound-assisted emulsification-microextraction method followed by ion mobility spectrometry (IMS) for determination malathion pesticides. Ultrasound radiation was applied for accelerating the emulsification of microliter organic solvent in aqueous solutions and enhancing the microextraction efficiency. This preconcentration step combined with IMS detection provided a precise and accurate method for determination of trace amounts of malathion pesticides. The effect of parameters influencing the extraction efficiency such as sonication time, type of extraction solvent, extraction solvent volume, and salt concentration were investigated and discussed. Under the optimum conditions, enrichment factors was 270 with corresponding LOD of 4 μg/L. Linearity with a coefficient of estimation (r^2) were >0.99 in the concentration level range of 6–750 μg/L for extraction of Malathion in water samples. The applicability of the proposed method was evaluated by determination of the residues of the investigated pesticide in rice paddy water gathered from four stations during 60 days after spraying (June 2014), and in storage rice samples in Mazandaran province, Iran.

Keywords Malathion · USAEME · IMS · Rice

Introduction

Organophosphorus pesticides are one of the major classes of the pesticides, which are widely used in Iran agricultural lands because of their low price and broad biological activities. Rice consumption has increased over recent decades, as it has become one of the most common foods worldwide. Rice, as the major staple of the Iranian people, has always been attacked by the various pests during cultivation and harvest. Thus, farmers in the rice paddies of northern Iran use different pesticides such as Malathion to protect their crops. Due to the vast consumption of these phosphorous pesticides, excessive amounts can be released into the water system through permeation and scouring. The water system, including underground and surface water, is often the source of drinking water. Therefore, it is crucial to develop rapid, sensitive and easily operated field methods to detect OPPs (Diazinon, Malathion, Parathion) in environmental water [1–6].

Gas chromatography (GC) and high performance liquid chromatography (HPLC) are the common used techniques for the separation and quantification of organophosphorus residues in different matrices [7–10].

Recently ion mobility spectrometry (IMS) has been developed as an instrumental analytical technique for detecting and identifying various compounds based upon the mobility of gas phase ions in an electric field [11–13]. The ions are detected at the distal end of the drift tube with a Faraday-plate detector. Ionic species with different mass, cross-sections and charges will be separated by differing velocities and arrive at the detector at different times. The mobility of an ion (K, $cm^2\ s^{-1}\ V^{-1}$) is determined by the

✉ Hamideh Kalhor
Kalhor1363.h@gmail.com

[1] University of Applied Science and Technology, Qazvin, Iran

[2] Physic Department, Islamic Azad University, Science and Research Branch, Tehran, Iran

[3] Islamic Azad University, Zanjan Branch, Zanjan, Iran

[4] Chemistry Department, Payame Noor University, Eslam Abad Gharb Branch, Eslamabad-E-Gharb, Iran

velocity (υ, $cm^2\ s^{-1}$) attained under the influence of an electric field gradient (E, $V\ cm^{-1}$) in the presence of a drift gas, given by:

$$\upsilon = KE \tag{1}$$

If the time taken to traverse a drift cell of length d (cm) is td (s), then:

$$K = \frac{d}{t_d E} \tag{2}$$

Ion mobilities are usually expressed as reduced mobility (K0) corrected to standard conditions of temperature (T in Kelvin) and pressure (P in Torr):

$$K0 = \frac{273}{T} \times \frac{P}{760} \tag{3}$$

The increasing demands for faster, more cost-effective and more environmental friendly analytical methods are a major incentive to improve the classical procedures used for sampling treatments in environmental analysis. In these approaches, miniaturization has been a key factor in designing integrated analytical systems to provide higher sample throughput and/or unattended operation.

Application of the conventional liquid–liquid extraction (LLE) and solid-phase extraction (SPE) methods were limited due to the disadvantages such as time-consumption, labor-intensive and higher need to the large amounts of organic solvents [14–16].

Recent efforts are being placed on the development of miniaturized, efficient and environment-friendly extraction techniques for the analysis of Organophosphorus pesticides in complex matrix. Recent developments in microextraction procedures resulted in some extraction methods based on the extension of the contact surface between both liquid phases to reduce the extraction time. Ultrasound-assisted emulsification microextraction (USAEME) is an efficient, simple, rapid and inexpensive extraction technique which was first introduced in 2008 [17]. In USAEME, only a small volume of extraction solvent is used without any water-miscible organic solvents that are needed as dispersers in dispersive liquid – liquid- microextraction (DLLME). That is because the extraction solvent is emulsified by the use of ultrasound waves that boost mass-transfer from the aqueous phase to the organic phase by promoting the formation of a large surface area [18–21].

IMS which is coupled to extraction techniques has recently been used to detect various compounds such as pesticide, fungicide, volatile organic compound and biomolecules. The method combines both high sensitivity and relatively low technical expenditure with high speed data achievement [22–26].

In this paper, the combination of USAEME and ion mobility spectrometry (USAEME -IMS) was evaluated for monitoring of Malathion in rice paddy's samples. Some parameters affecting the extraction efficiency such as the type and volume of extracting solvent, salt addition, pH and centrifuge time were studied.

Experimental

Chemicals and reagents

The sampling stations were located between 36° 25' 49" to 36° 27' 32" N and 52° 16' 16" to 52° 18' 50" E, south west of the Amol in the north of the Iran (Fig. 1). the mean annual precipitation of the study area is 829 mm and the mean annual temperature is 15.9 °C.

All solvents (HPLC-grade) were purchased from Merck (Darmstadt, Germany). Commercial-grade Malathion pesticides were obtained from Plant Protection Organization in Tehran. Stock standard solutions (1000 mg/L) of these compounds were prepared in methanol and then working solutions were made by successive dilution of the stock solutions.

Apparatus

Deionized water with a resistivity of at least 18.2 MΩ was produced by a M-UV-3^+ Zolalan water purification system (Iran). An ElmaSonic ultrasonic bath (frequency of 37 kHz and power of 0.138 kW) was used for emulsification of extraction organic solvent. Detection and quantification of malathion was carried out using a laboratory designed ion mobility spectrometer with a UV-light ionization source (vacuum–UV Krypton lamp powered by a 1.2 kV DC power supply) working in positive mode. A GC injection port was equipped with a heating element and a digital temperature controller was used for introducing the sample in the IMS. The carrier gas was passed through the port and carries the analyte vapor to the IMS cell. The drift length was 10 cm and the applied electric field was adjusted at 550 $V\ cm^{-1}$. The shutter grid was made of two series of parallel wires biased to a potential, creating an orthogonal field relative to the drift field, to block ion passage to the drift tube. The grid potential was removed for a short period of time by the pulse generator, to admit an ion pulse to the drift region. In this work, the period of time was selected 120 μs. The IMS cell was held in a thermostated oven in which temperature was controlled within ±5 °C. The optimized experimental conditions for obtaining the ion mobility spectra of the compounds are listed in Table 1.

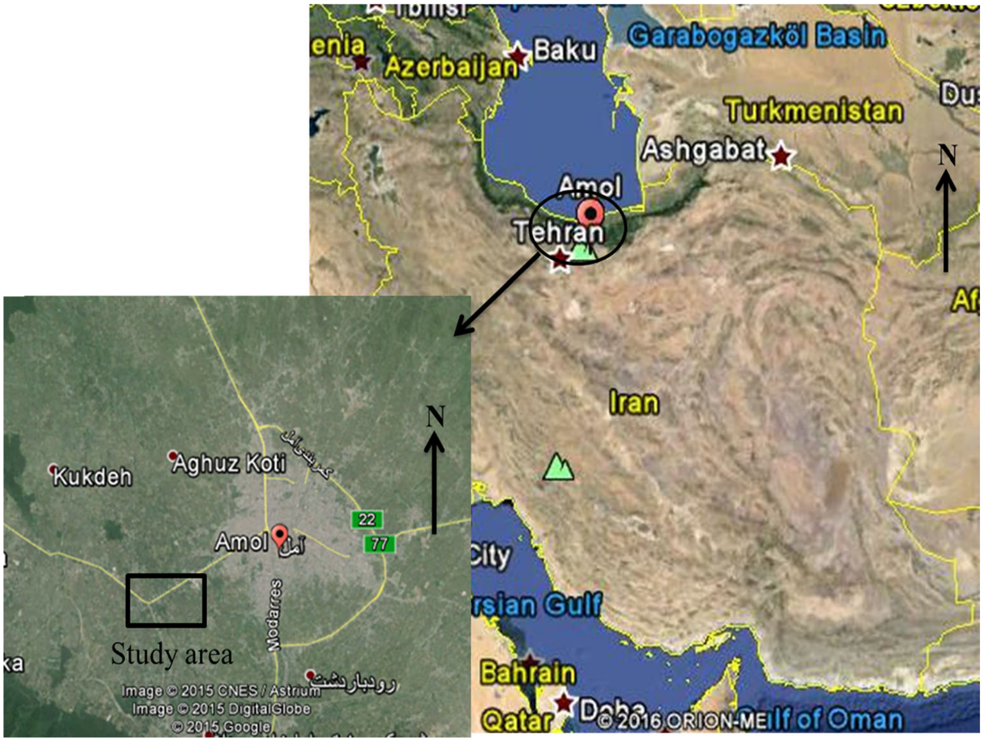

Fig. 1 The study area in Mazandaran-Amol **a**) google map **b**) google earth

Ultrasound-Assisted Emulsification Microextraction (USAEME) procedure

A centrifuge glass vial was filled with 10 mL aqueous sample up to the middle of the conic head of the vial. Appropriation volume of 0.5 µg/mL solution of Malathion was added to the vial by a microsyringe (Hamilton). A volume of 50 µL of organic solvent (chloroform) was injected into the water sample. This mixture was irradiated in an ultrasonic water bath, which operated at 37 kHz with an output power of 138 W for 3 min, at 25 ± 3 °C. Then, the emulsion was centrifuged at 3500 rpm for 5 min to separate the phases. After separation of the phases, 2 µL of the extraction solvent was collected with a microsyringe and injected into the IMS system for subsequent pesticides determinations.

Table 1 The optimized experimental ion mobility spectrometry (IMS) conditions for Malathion

Parameter	Value
Drift field voltage (V)	4700
Drift gas flow, N_2 (mL min^{-1})	380
Carrier gas flow, N_2 (mL min^{-1})	120
Drift tube temperature (°C)	180
Injection temperature (°C)	200
Drift tube length (cm)	10
Shutter grid pulse (µs)	120
Dopant	Toluene

Results and discussion

Optimization of USAEME conditions for Malathion determination

Different factors affect the emulsification and extraction processes. Some of these factors come from the selection of the suitable extraction solvent, the duration of centrifugation time, the ionic strength and the volume of extraction solvent. The study and optimization of the above mentioned variables were performed using one variable at a time (OVAT) method. The ion mobility spectrometry peak height was the parameter used to evaluate the influence of the factors on the extraction efficiency of Malathion.

Selection of an appropriate extraction solvent is one of the most important parameters for developing an efficient USAEME procedure. For its selection, some criteria should be taken into account. It must have low solubility in water, high extraction capability of the target analytes, and in addition, it should be compatible with IMS. Therefore, three types of solvent immiscible with water including dichloromethane, chloroform and carbon tetrachloride were investigated in preliminary experiment. The experiments were carried out using 10 mL water solution containing 0.5 mg/L analytes. in the earlier experiment, all the solvents could easily form an emulsion after US of 4 min. The effects of different solvent on the recovery were shown in Fig. 2. The results showed that chloroform has the highest recovery for the target analyte. Based on the above considerations, chloroform was selected as the most suitable solvent in the following experiment.

To study the effect of extraction solvent volume, water solutions containing different volumes of chloroform ranging from 30 to 80 μL were examined with the same USAEME procedure. The obtained results depicted in Fig. 3 and revealed that, lower volume than 40 μL lead to lower extraction efficiency, due to the insufficient volume of chloroform in the extraction of Malathion effectively. With increasing the volume of chloroform, the analytical signal increased up to 50 μL and after that, with further increment in solvent volume, a decrease in extraction efficiency was observed. This is due to the decrease in final concentration of Malathion in the extraction phase and the lower enrichment factor. Therefore, the optimum volume of 50 μL of chloroform was selected for further studies.

The influence of sonication time on the extraction recovery was investigated by varying the sonication time in the range of 1–10 min. The results of different ultrasonic time versus peak areas were shown in Fig. 4. It was found that extraction recovery was increased when sonication time was increased from 1 to 4 min. After 4 min, the extraction recovery decreased. This may be explained by the fact that the numbers of droplets increased as the sonication time increased from 1 to 4 min. Hence, it improves the interfacial area between the water phase and extraction solvent phase. Therefore, a 4 min sonication was used in the following studies.

The salting out effect has been universally used in liquid extraction methods. The effect of the ionic strength of aqueous sample was evaluated by adding NaCl in the range of 0–5 % *W*/*V* into the water sample. USAEME experimental conditions were the same as those described former. The results revealed that the extraction efficiency was increased with increasing NaCl concentration up to 3 % (*w*/*v*). At higher concentrations, a decrease on the extraction efficiency occurred. The salting-out effect can decrease the solubility of analytes in the aqueous phase and promote the transfer of the analytes towards the organic phase, thus improving the extraction efficiency. On the other hand, as the ionic strength of the medium increases, both the viscosity and density of the aqueous solution are enhanced, leading to a reduction of the efficiency of the mass transfer process and thus the extraction efficiency of the procedure. Based on the experimental results, 3 % *w*/*v* NaCl was added in the sample solution (Fig. 5).

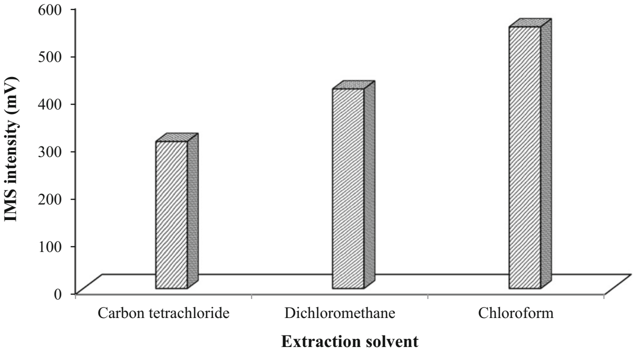

Fig. 2 Selection of extracting solvent: pH 7, 60 μL solvent extraction, sonication time 3 min, centrifuge time 5 min for 0.5 μg/mL of Malathion in USAEME

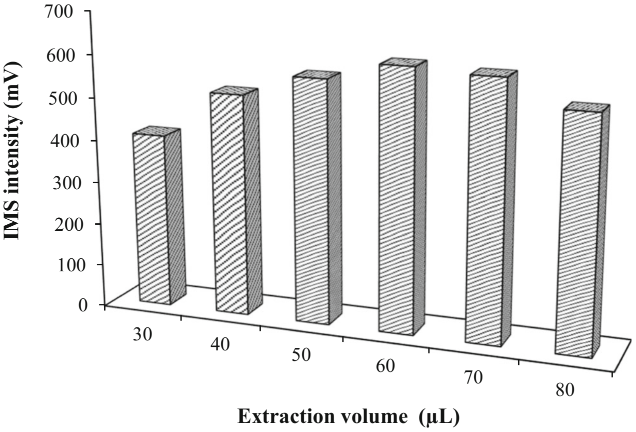

Fig. 3 Effect of the volume of chloroform: pH 7, sonication time 3 min, centrifuge time 5 min for 0.5 μg/mL of Malathion in USAEME

The influence of pH in the range of 2–9 was also studied. The extraction recovery was nearly constant in the range of 2–9. this might be due to the fact that under optimized conditions the analyte is mostly neutral.

Rice paddy's water and rice analysis

Farmers in Mazandaran province in the north of Iran use Malathion insecticide in large amounts and this study aimed to

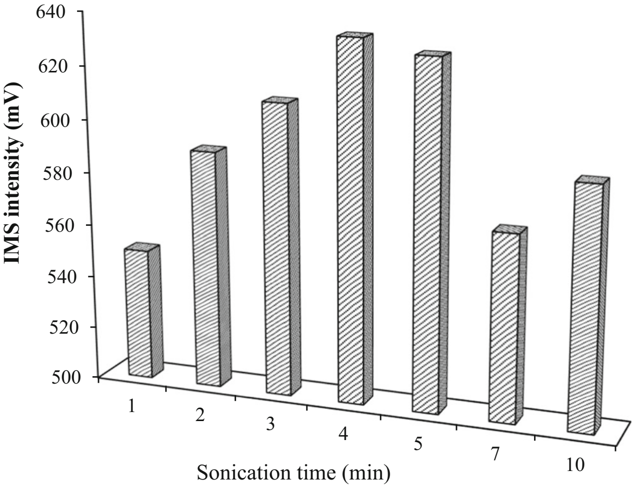

Fig. 4 Effect of sonication time: pH 7, 50 μL chloroform, centrifuge time 5 min for 0.5 μg/mL of Malathion in USAEME

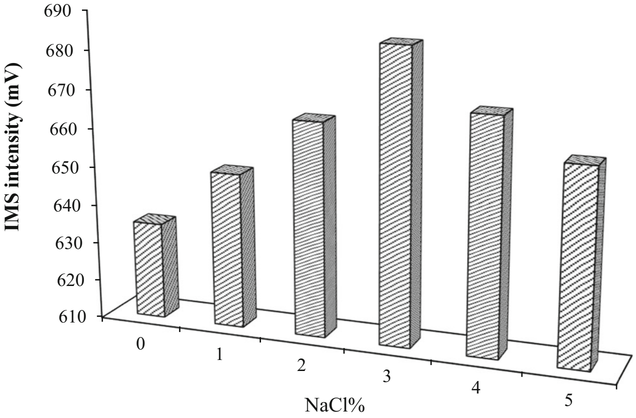

Fig. 5 Effect of the NaCl%: pH 7, sonication time 4 min, centrifuge time 5 min for 0.5 μg/mL of Malathion in USAEME

Table 2 Analytical results for determination of Malathion in water sample by USAEME-IMS

Sample	LDR (μg/L)	Linearity	LOD (μg/L)	ER	RSD (*n* = 5)
Malathion	6–750	0.998	4	270	8 %

investigate the level of this insecticide residue in rice paddy's water and rice samples produced in Amol, north of Iran. To evaluate the reliability of the proposed method, USAEME following IMS was applied to the quantification of trace amounts of Malathion in rice paddy. Rice paddy's water and rice samples were collected from agriculture sites located near Amol. Before the spraying, samples were taken from two selected stations in order to ensure that the water is free of Malathion insecticide. In order to do water sampling, 5 samples (100 ml) from a depth of 10–15 cm were collected. The collected samples were mixed together in a 2 L container. To prevent the decomposition of Malathion present in the water samples, samples were kept in a refrigerator and immediately ready to measure. The sampling from two stations were taken at least 5 times with the interval times of 1, 10, 20, 40 and 60 days. Sampling was done at least 2 months after spraying at the end of spring and summer of 2014 (Fig. 6). This exactly coincided with the commencement of the spraying season which continued until 20 August, when the residues of the insecticides were hardly detectable in the water. In order to determine Malathion content in rice, individually 5 samples (100 g) from two storages were collected. The collected samples were mixed together. Then 500 g of rice sample was submitted to the laboratory. 20 g of grounded rice seeds were placed in a vial and 25 mL of distilled water was added to the vial and stirred by a magnet for 15 min at room temperature. For USAEME-IMS analysis 10 mL of this solution was placed in a conical tube and USAEME was applied on it.

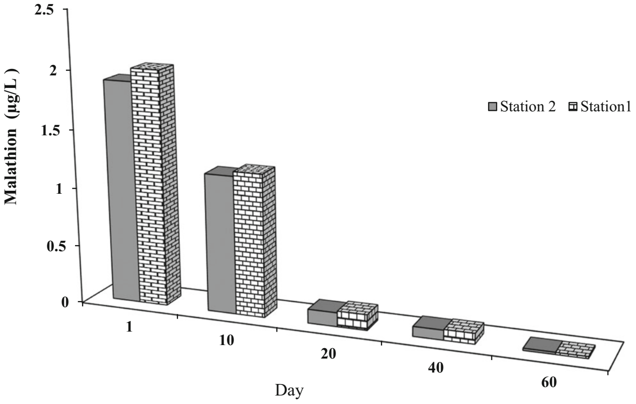

Fig. 6 The comparison of average residues of Malathion in various times in rice paddy

Method applicability

Figures of merit of the proposed method including linear dynamic range (LDR), repeatability and reproducibility, enrichment factor (PF) and limit of detection (LOD) were investigated under optimized conditions to evaluate its applicability for quantification of Malathion in spiked water samples. In order to assess repeatability, the peak height of five replicate analyses for 500 μg/L Malathion were used and expressed as percentage relative standard deviation (RSD%). The RSD% for Malathion was ≤8 % (*n* = 5). The calibration graphs showed linear relationship in the range of 6–750 μg/L for Malathion with $R^2 \geq 0.998$ in aqueous solution. The limit of detection was determined as 4 μg/L at a signal-to-noise ratio (S/N) of 3. Results of the USAEME analysis for spiked water samples are displayed in Table 2.

Real sample analysis

To evaluate the reliability of the proposed method, USAEME–IMS was applied to the quantification of trace amounts of Malathionin in rice paddy's water during 2 month (samples that were collected in first day and 10 days were diluted 3 times by distillated water). In order to validate

Table 3 Residues of Malathion in rice paddy's water

	Day after spraying	Malathion (μg/L) *n* = 4
Rice paddy water sample Station -1	1	2012 ± 8
	10	1220 ± 7
	20	135 ± 8
	40	91 ± 9
	60	17 ± 8
Rice paddy water sample Station -2	1	1900 ± 9
	10	1180 ± 8
	20	120 ± 7
	40	85 ± 6
	60	18 ± 8

Table 4 Residues of Malathion in rice

Sample	Add (μg Kg^{-1})	Found (μg Kg^{-1}) $n = 4$
Rice –storage 1	0	12 ± 7
	10	19 ± 6
	20	31 ± 6
Rice –storage 2	0	10 ± 5
	10	12 ± 4
	20	23 ± 5

reliability and accuracy of the method, an add–found method ($n = 4$) was considered for Malathion. The summarized results in Tables 3 and 4 showed that the USAEME supports an effective sample clean-up for the monitoring Malathion in rice paddy's water and rice. The RSD% ($n = 4$) values were demonstrating the acceptable reproducibility of this method. The amounts of Malathion in the stations 1 and 2 were detectable hardly until 60 days after the spraying time; this is probably due to the insecticide's degradation in the ambient environment. Malathion concentration in storages 1 and 2 was remained in the rice sample which is lower than maximum residue level (MRL) for this insecticide (0.4 μg/g) [27]. Ion mobility spectrum of a spiked water sample, rice paddy's water sample before spraying, and 20 day after spraying were shown in Fig. 7.

Conclusion

A method based on ultrasound-assisted emulsion liquid-liquid microextraction combined with ion mobility spectrometry (USAEME-IMS) for determination of Malathion was developed. The presented method was very simple, rapid, inexpensive, and accurate. The relatively wide linear range for determination of the studied analytes was another advantage of the presented method. The proposed USAEME-IMS method was successfully used for monitoring the concentration variation of Malathion in rice samples and water samples collected from rice paddies located in Mazandaran province (North Iran).

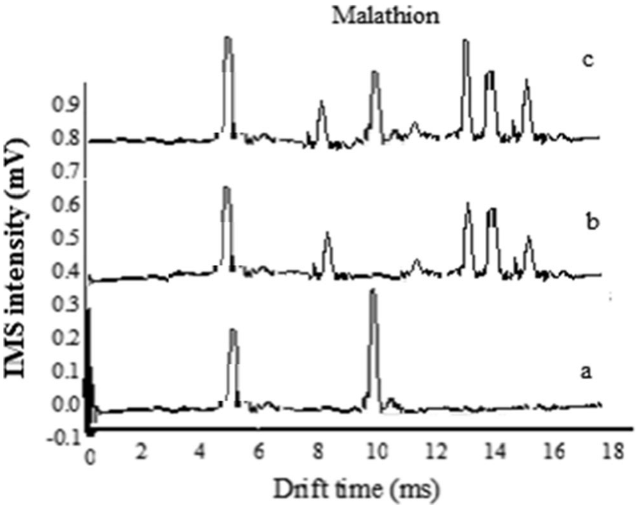

Fig. 7 **a** The ion mobility spectrum of Malathion 0.3 μg/mL obtained by the USAEME–IMS in optimized condition. **b** The ion mobility spectrum of rice paddy water in station 1 before spry and **c** 20th day after spray by the USAEME–IMS in optimized condition

DLLME, dispersive liquid–liquid microextraction; IMS, ion mobility spectrometry; LLE, liquid–liquid extraction; SPE, solid-phase extraction; USAEME, ultrasound-assisted emulsification–microextraction.

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