Research Article

Development of Dispersive Liquid-Liquid Microextraction method combined with UV spectrophotometry for the Determination of Malathion Pesticide

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Article Info

Received
19/10/2016

Accepted
18/01/2017

Abstract

A simple and novel method was developed by combination of dispersive liquid-liquid microextraction with UV spectrophotometry for the preconcentartion and determination of trace amount of malathion. The presented method is based on using a small volume of ethylenechloride as the extraction solvent was dissolved in ethanol as the dispersive solvent, then the binary solution was rapidly injected by a syringe into the water sample containing malathion. The important parameters, such the type and volume of extraction solvent and disperser solvent, the effect of extraction time and rate, the effect of salt addition and reaction conditions were studied. At the optimum conditions, the calibration graph was linear in the range of 2-100 ng mL-1 of malathion with a limit of detection of 0.8 ng L-1. In addition, the enrichment factor was 30. The developed method was successfully applied for the determination of malathion pesticide in water samples.

Keywords: Malathion, Spectrophotometry, Pesticide, Microextraction, Sample preparation.

الخلاصة

يهدف البحث الى تطوير طريقه جديدة وبسيطة وذلك بأقتران طريقه تشتت سائل-سائل المايكرويه مع مطياف الاشعه فوق البنفسجية وذلك لتقدير تراكيز ضئيلة جدا من مبيد الملاثايون. تعتمد الطريقة على الحقن السريع لمزيج المذيبات الثنائي والمتكون من حجوم صغيرة من مذيب كلوريد المثيلين (كمذيب استخلاص) والممتزج مع حجوم صغيره جدا من الايثانول المستخدم كمذيب تشتت الى محلول مائي يحتوي على الملاثايون. تمت دراسة الظروف المثلى للأستخلاص والمتضمنه ظروف التفاعل ونوع وحجم كل من المذيب المشتت والمذيب المستخلص وتاثير سرعة وزمن الاستخلاص وتأثير أضافة الملح. عند الظروف تم الحصول على خطيه جيدة تراوحت بين 2-100 نانوغرام لكل مل مع حد كشف قيمته 0.8 نانوغرام لكل مل. بالإضافة الى ذلك تم الحصول على عامل تركيز جيد قيمته 30. تم تطبيق الطريقة المطورة بنجاح على لتقدير مبيد المالاثايون في عينات مياه ملوثة.

Introduction

Pesticides are one of the most potentially harmful chemicals introduced into the environment. Worldwide using pesticides increased enormously since the 1960s. However, using pesticides helped to significantly reduce crop losses and to improve the yield of crops such as corn, maize, vegetables, potatoes, and cotton. Organophosphate pesticides are commonly used pesticides in the world and require more awareness because of their possible toxic effects on-target animals [1]. Malathion (*S*-1,2-bis(ethoxycarbonyl) ethyl-*O,O*-dimethyl-phosphorodithioate) Figure 1 is an organophosphorus pesticide that used in both agriculture and households to control insect pests affecting a number of crops, stored grains and livestock feed via ground and aerial sprays and aerosols. Malathion rapidly converts to its metabolism malaoxon either after absorption or ingestion into the human body or in untreated water during the chlorination phase of water treatment. Therefore, malathion should not be used in the drinking waters or any upstream waters. In addition, the toxic exposure effect to high level of malathion considers a source of body-wide symptoms such skin and eye irritation, cramps, nausea, diarrhea, excessive sweating, seizures and even death [2] [3] [4].

Various analytical methods have been reported for the determination of malathion such gas chromatography [3] [5] HPLC [6] [7] thin layer chromatography [8], capillary electrophoresis [9], spectrophotometry [2] [3], atomic absorption spectrometry [10], fourier transform infrared (FTIR) spectrometry [11] [12] [13] [14].

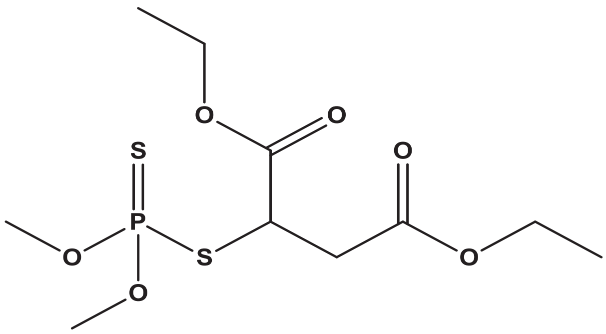

Figure 1: Chemical structure of malathion.

In the analytical process, sample preparation is the first necessary and crucial step since it provides reliable and accurate results at low detection limits. Recently, there is considerable awareness about the environment; therefore sample preparation techniques using solvents with low toxicity that are more environmentally friendly are important.

Most of traditional sample preparation techniques are time consuming and tedious procedure. Moreover, they demand using large amounts of hazardous high-purity organic solvents, that resulting in the production of toxic laboratory wastes. Microextraction technique effectively overcomes these problems by reducing the amount of organic solvent. In addition, extraction, preconcentration and sample introduction are done in one step. Various microextraction techniques have been widely used for the pesticide analysis such as solid-phase microextraction [15] [16] liquid-phase microextraction [17] [18], stir-bar sorptive extraction [19] [20] combination of dispersive liquid-liquid microextraction (DLLME) with HPLC-MS/MS [21] and micro-solid phase extraction [22] [23]. Most of microextraction methods are simple, rapid and consume smaller amounts of toxic solvents than conventional sample-preparation methods.

DLLME is one of the effective microextraction techniques that have been developed by Rezaee *et al.* in 2006 [24]. It based on formation a cloudy solution after injection of appropriate mixture of extraction and disperser solvents rapidly into the aqueous solution. The simplicity of operation, rapidity, low time, high recovery and high enrichment factor are among the main advantages of DLLME [25]. In this work, reliable, simple, inexpensive method for the determination of malathion in water samples by spectrophotometric determination after preconcentration by DLLME technique. Moreover, some parameters such as extraction solvent, dispersive solvent, effect of salt addition, and extraction time were investigated and optimized.

Materials and Methods

A Shimadzu-160 double beam UV-Vis spectrophotometer with 1 cm quartz micro cell was used for the determination and analysis of Malathion. Phase separation was accelerated using Hettich centrifuge (EBA 20) with 10 mL calibrated centrifuge tubes was used.

All chemical reagents used were of analytical grade and double distilled water was used. Stock solution (1000 μg mL^{-1}) was prepared by dissolving the appropriate amount of malathion in methanol while working standard solutions were prepared freshly by appropriate dilution of the standard stock solution. Methanol, ethanol, chloroform, carbon tetrachloride, 1, 2-dichloroethane, dichloromethane, and ethylene chloride were obtained from Merck (Darmstadt, Germany).

Dispersive liquid–liquid microextraction procedure

To a glass test tube with a conical bottom, an aliquot amount of the sample solution containing 10 ng/mL^{-1} was added. After a few minutes, a binary solution containing 1.5 mL of ethanol (disperser solvent) and 300 μL of ethylene chloride (extraction solvent) was injected rapidly into the sample solution using a syringe and a stable cloudy solution (water, ethanol and ethylene chloride) was rapidly produced, resulting in fine droplets. Thereafter, malathion was extracted into fine droplets of ethylene chloride. Later, the mixture was centrifuged at 4000 rpm for 3 min, and the fine droplets of ethylene chloride were joined together and sedimented at the bottom of the conical test tube. After removing of the aqueous phase, the remained organic phase was diluted to 400 μL with ethanol and transferred to a 500 μL cell the absorbance measured at 226 nm against blank. A blank solution was prepared as for the sample solution without adding any malathion.

Results and Discussion

It is necessary to investigate the maximum absorption wavelength for the quantitative analysis of malathion. Therefore, sample solution containing different concentrations of malathion was examined according to the recommended procedure of DLLME and corresponding spectra of sedimented phase were recorded in the range of 200-650 nm.

Figure 2 shows maximum absorbance wavelength at 226 nm. Therefore, this wavelength was selected for measuring the absorbance of the extracted phase throughout this work. In order to achieve a high recovery and enrichment factor, all the parameters that can probably influence the extraction performance such as; type and volume of extraction solvent, kind and volume of disperser solvent, extraction time and salt concentration that were investigated and optimized.

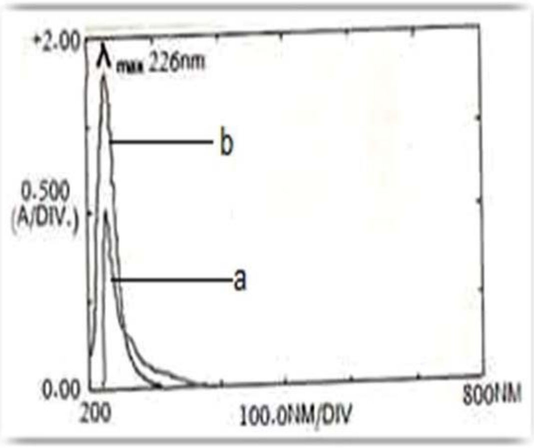

Figure 2: UV-Spectrum of malathion a) 500 ng mL^{-1} before DLLME and b) 50 ng mL^{-1} after DLLME.

Effect of type and volume of the extraction solvent

In DLLME, the type of extraction solvent is an important factor for efficient extraction and should meet three requirements. The solvent should be denser than water, low solubility in water and it capable to extract the interested compounds [26]. Due to high density of halogenated hydrocarbons, usually they are examined as an extraction solvent. Thus, chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), 1,2-dichloroethane ($C_2H_4Cl_2$), dichloromethane (CH_2Cl_2), and ethylene chloride were examined for this purpose. In spite of a cloudy solution and two-phase system was formed using the selected solvents but ethylene chloride shows higher signal than others. Therefore, ethylene chloride was selected as extraction solvent. The effect of the extraction solvent volume was performed using 1.5 mL of ethanol different volumes of ethylene chloride in the range of 200–800 µL. According to Figure 3, the absorbance increased by increasing the volume of ethylene chloride to 300 µL and then remained approximately constant with further increasing of its volume. Therefore, 300 µL was selected as optimum volume in other experiments.

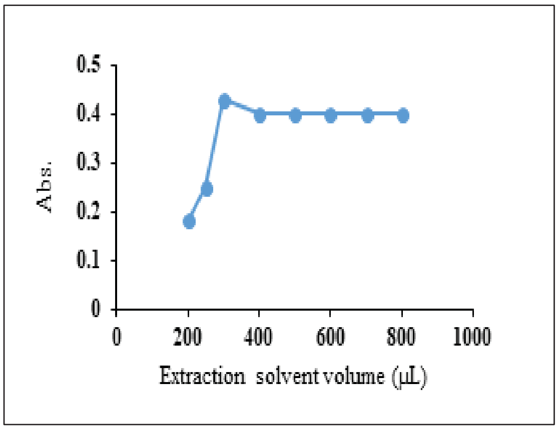

Figure 3: Effect of the extraction solvent (ethylene chloride) volume on the analytical responses after DLLME

Effect of type and volume of the disperser solvent

Selection of the disperser solvent is based on its miscibility in the extraction solvent and aqueous solution, which enables the extraction solvent to be dispersed as fine droplets in the aqueous phase to form a cloudy solution. Therefore, it is limited to some solvents such as methanol, ethanol, acetonitrile, and acetone. A series of sample solutions were studied using 1.5 mL of each disperser solvent with 400 µL of ethylene chloride as the extraction solvent. The results show that high enrichment factors were obtained with ethanol. The effect of different volume of ethanol ranged from1000–3000 µL using 400 µL of chloroform on the extraction recovery was examined. As it is shown in Figure 4 the absorbance reached its maximum value at 1500 µL of ethanol to disperser solvent volume and after that, the absorbance decreases slowly. Thus, 1500 µL of ethanol was chosen as the proper amount.

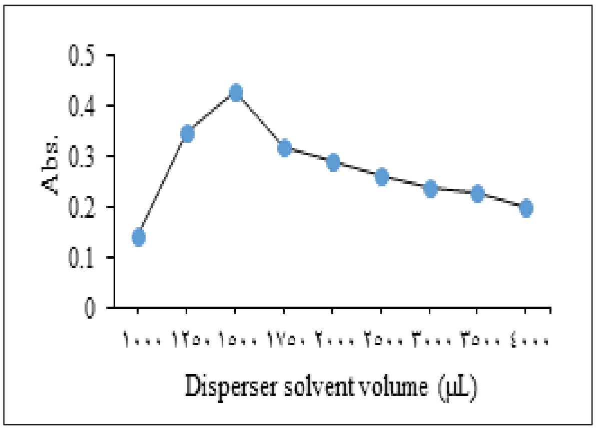

Figure 4: Effect of the disperser solvent (ethanol) volume on the analytical responses after DLLME.

Effect of extraction time and extraction rate

Extraction time is the important factor in most extraction techniques. It defined as the interval time between the injection of the solvents mixture and starting to centrifuge. The effect of extraction time was studied over the range of 1 to 15 min under the constant experimental conditions. Due to the infinitely large surface area between extraction solvent and aqueous phase after the formation of cloudy solution, malathion diffuses quickly into the extraction solvent. Therefore, equilibrium state is achieved quickly and the extraction time is very short about 3 min. Extraction rate (centrifuge rate) was also examined in the range of 1000-5000 rpm for 5 min. Higher absorbance value was obtained at 4000 rpm. Subsequently, it was selected as the best centrifuge rate.

Effect of salt addition

In general, addition of salt decreases the solubility of analytes in the sample solution by increasing the ionic strength (salting out effect) and enhances their partitioning into the organic phase. The influence of ionic strength on the performance of DLLME, was investigated by adding 0, 2.5, 5 and 10% (w/v) sodium chloride, respectively to sample that was spiked with malathion. However, it was found that salt addition did not result in an increase in extraction efficiency while addition of large amounts of salt to the aqueous phase increases its viscosity. Therefore, the velocity of the mass transfer processes of the analytes from the aqueous layer to the organic layer is diminished. Thus, no salt was added in further experiments.

Analytical features of the proposed method

After optimization all the experimental conditions, analytical parameters of the proposed method such regression equation, linear range, limit of detection, reproducibility, preconcentration, and improvement factors were investigated and the obtained data were summarized in Table 1. The calibration graph was linear in the range of 2-100 ng/mL^{-1} with a correlation coefficient (r) of 0.9996 while, the limit of detection (LOD) was calculated based on $LOD = {}^{3Sb}/_{m}$ and limit of quantification (LOQ) based on ${}^{10Sb}/_{m}$ (where Sb, and m are standard deviation of the blank, and slope of the calibration equation, respectively). However, LOD and LOQ values were 0.8 ng/mL^{-1} and 2.56 ng/mL$^{-1,}$ respectively. The preconcentration factor for the proposed DLLME method is calculated by the ratio of the highest sample volume (10 mL) and the final volume of nearly 300 µL. Therefore, the solution is concentrated by a factor of 33.3. The enrichment improvement factor was obtained from the slope ratio of the calibration graph after and before the extraction. According to this concept, enrichment improvement factor of 29 was obtained.

Table 1: Analytical parameters of the proposed method.

Parameters	**Data**
Regression equation using DLLME	A = 0.0088C + 0.012, R^2= 0.9996
Regression equation before DLLME	A = 0.0003C + 0.02, R^2= 0.9993
Linear range (ng mL^{-1})	2-100 (200-5000)[a]
Limit of detection (ng mL^{-1})	0.8 (6)[a]
Repeatability (RSD%) (n=5)	1.74 (1.34)[a]
Preconcentration factor	33.3
Enrichment factor	29

[a] The results before DLLME

Application to real samples

To evaluate the performance and validation of the suggested method, it was applied to determine the malathion in two water samples, which were selected from Tap and river water (Baghdad, Iraq). The tested water samples were found to be free from malathion according to the optimized procedure. The results are presented in Table 2. The recoveries for the addition of different concentrations of malathion to samples are in the range of 98.8–102.1% and indicate that the proposed method is suitable for determination of trace amounts of malathion in the real samples.

Table 2: Determination of malathion in river and tap waters samples by proposed method..

Sample	Added (ng mL^{-1})	Found^{a} (ng mL^{-1})	Recovery %
River water	0	ND^{b}	-
	10	10.21	102.10
	50	50.80	101.60
	80	79.99	99.98
Tap water	0	ND^{b}	-
	10	9.98	99.8
	50	49.40	98.8
	80	80.12	100.12

^{a} Standard deviation (n= 5); ^{b} Not detected

Conclusions

Spectrophotometric method by using DLLME technique for the extraction, preconcentration and determination of trace amount of malathion in water was developed. Besides employment of usual laboratory equipment, the proposed DLLME method provides advantages such as simplicity of operation, low sample volume, low cost and high preconcentration factor.

Moreover, due to the large surface area between the organic droplets and the aqueous sample solution, it is much shorter since the extraction equilibrium is reached quickly. Finally, in comparison to solvent extraction methods, it is much safer, since only a small amount of the solvent is used.

References

[1] W. Aktar, D. Sengupta and A. Chowdhury, "Impact of pesticides use in agriculture: their benefits and hazards," *Interdisciplinary toxicology,* vol.2, no.1, pp.1-12, 2009.

[2] B. Sumalatha and N. V. S. Venugopal, "Spectrophotometric determination of Malathion (an organo phosphorous insecticide) with Potassium bromate," *Eurasian Journal of Analytical Chemistry,* vol.8, pp.131-135, 2013.

[3] N. V. S. Venugopal,, B. Sumalatha and S. Bonthula, "Spectrophotometric determination of Malathion (an organo phosphorous insecticide) with Potassium bromate," *Eurasian Journal of Analytical Chemistry,* vol.8, pp.131-135, 2013.

[4] A. Rauf, "Acute toxicity and effects of malathion exposure on behavior and hematological indices in Indian carp, Cirrhinus mrigala (Hamilton)," *International Journal of Aquatic Biology,* vol.3, no.4, pp.199-207, 2015.

[5] H. M. Lofty,. A. E. -A. A. Abd El-Aleem and H. H. Monir, "Determination of insecticides malathion and lambda-cyhalothrin residues in zucchini by gas chromatography," *Bulletin of Faculty of Pharmacy, Cairo University,* vol.51, pp.255-260, 2013.

[6] M. Khuhawar, A. Channar and S. Lanjwani, "Indirect liquid chromatographic determination of malathion based on Copper (II) dimethyldithiophosphate complex formation," *Chromatographia,* vol.42, pp.680-682, 1996.

[7] A. W. Abu-Qare and M. B. Abou-Donia, "Simultaneous determination of malathion, permethrin, DEET (N,N-diethyl-m-toluamide), and their metabolites in rat plasma and urine using high performance liquid chromatography," *Journal of Pharmaceutical and Biomedical Analysis,* vol.26, pp.291-299, 2001.

[8] . K. Futagami, C. Narazaki, Y. Kataoka,. H. Shuto and R. Oishi, "Application of high-performance thin-layer chromatography for the detection of organophosphorus

insecticides in human serum after acute poisoning," *Journal of Chromatography B: Biomedical Sciences and Applications,* vol.704, pp.369-373, 1997.

[9] . C. García-Ruiz, G. Alvarez-Llamas, Á. Puerta,. E. Blanco,. A. Sanz-Medel and M. L. Marina, "Enantiomeric separation of organophosphorus pesticides by capillary electrophoresis: Application to the determination of malathion in water samples after preconcentration by off-line solid-phase extraction," *Analytica chimica acta,* vol.543, pp.77-83, 2005.

[10] J. H. Méndez,. O. J. De Bias,. V. R. Martín and E. S. López, "Indirect Determination of the Pesticide Malathion by Atomic Absorption Spectrophotometry," *Analytical Letters,* vol.18, pp.2069-2081, 1985.

[11] M. Khanmohammadi,. M. A. Karimi,. K. Ghasemi, M. Jabbari and A. B. Garmarudi, "Quantitative determination of Malathion in pesticide by modified attenuated total reflectance-Fourier transform infrared spectrometry applying genetic algorithm wavelength selection method," *Talanta,* vol.72, pp.620-625, 2007.

[12] A. K. Sharma, U. Tiwari, M. S. Gaur and R. K. Tiwari, "Assessment of malathion and its effects on leukocytes in human blood samples," *Journal of biomedical research,* vol.30, pp.52-59, 2015.

[13] G. Quintás, S. Garrigues and M. de la Guardia, "FT–Raman spectrometry determination of Malathion in pesticide formulations," *Talanta,* vol.63, pp.345-350, 2004.

[14] G. Quintás, A. Morales-Noé, S. Armenta, S. Garrigues and de la Guardia, Miguel, "Fourier transform infrared spectrometric determination of Malathion in pesticide formulations," *Analytica Chimica Acta,* vol.502, pp.213-220, 2004.

[15] S. Magdic and J. B. Pawliszyn, "Analysis of organochlorine pesticides using solid-phase microextraction," *Journal of Chromatography A,* vol.723, pp.111-122, 1996.

[16] J. Beltran, F. J. López and. F. Hernández, "Solid-phase microextraction in pesticide residue analysis," *Journal of chromatography A,* vol.885, pp.389-404, 2000.

[17] M. Andraščíková, E. Matisová and S. Hrouzková, "Liquid phase microextraction techniques as a sample preparation step for analysis of pesticide residues in food," *Separation & Purification Reviews,* vol.44, pp.1-18, 2015.

[18] M. A. Farajzadeh, S. M. Sorouraddin and M. R. A. Mogaddam, "Liquid phase microextraction of pesticides: a review on current methods," *icrochimica Acta,* vol.181, pp.829-851, 2014.

[19] W. Liu, Y. Hu, J. Zhao, Y. Xu and Y. Guan, "Determination of organophosphorus pesticides in cucumber and potato by stir bar sorptive extraction," *Journal of Chromatography A,* vol.1095, pp.1-7, 2005.

[20] A. Juan-García, Y. Picó and G. Font, "Capillary electrophoresis for analyzing pesticides in fruits and vegetables using solid-phase extraction and stir-bar sorptive extraction," *Journal of Chromatography A,* Vols.229-236, p.1073, 2005.

[21] M. Pastor-Belda, I. Garrido, N. Campillo, P. Vinas,. P. Hellín, P. Flores and. J. Fenoll, "Dispersive liquid–liquid microextraction for the determination of new generation pesticides in soils by liquid chromatography and tandem mass spectrometry," *Journal of Chromatography A,* vol.1394, pp.1-8, 2015.

[22] C. Basheer,. A. A. Alnedhary, B. S. M. Rao and H. K. Lee, "Determination of carbamate pesticides using micro-solid-phase extraction combined with high-performance liquid chromatography," *Journal of Chromatography A,* vol.1216, pp.211-216, 2009.

[23] Basheer, Chanbasha, A. A. Alnedhary,. B. M. Rao, S. Valliyaveettil and H. K. Lee, "Development and application of porous membrane-protected carbon nanotube micro-solid-phase extraction combined with gas chromatography/mass spectrometry," *Analytical Chemistry,* vol.78, pp.2853-2858, 2006.

[24] M. Rezaee,. Y. Assadi, M. -R. Milani Hosseini,. E. Aghaee, F. Ahmadi and. S. Berijani, "Determination of organic compounds in water using dispersive

liquid–liquid microextraction," *Journal of Chromatography A,* vol.1116, pp.1-9, 2006.

[25] . H. Ebrahimzadeh,. Y. Yamini and F. Kamarei, "Optimization of dispersive liquid–liquid microextraction combined with gas chromatography for the analysis of nitroaromatic compounds in water," *Talanta,* vol.79, pp.1472-1477, 2009.

[26] A. R. Zarei and F. Gholamian, "Development of a dispersive liquid–liquid microextraction method for spectrophotometric determination of barbituric acid in pharmaceutical formulation and biological samples," *Analytical biochemistry,* vol.412, pp.224-228, 2011.