Caspian J. Chem. 2(2013) 37-44

Caspian Journal of Chemistry

http://caschemistry.journals.umz.ac.ir

Determination of Malathion and α-Endosulfan Residue in Khazar Rice Using Matrix Solid Phase Dispersion and HPLC

Mohammad Reza Hadjmohammadi *, Hosein Asri, S. Saman S.J. Nazari

Department of Chemistry, University of Mazandaran, P.O. Box 453, Babolsar, Iran

* Corresponding author phone and fax number: +98 1125342350.

E-mail address: hadjmr@umz.ac.ir

Received 20 August 2012|Received in revised form 9 March 2013|Accepted 7 April 2013

Abstract: An extraction method based on matrix solid phase dispersion (MSPD) and high performance liquid chromatography with UV detection for quantification of two widely used pesticides (Malathion and α-Endosulfan) in Khazar rice from north of Iran were investigated. The ratio of solid phase (adsorbent) to sample, types of eluting solvent and its volume were optimized to achieve the highest recoveries for the analytes. Under the appropriate condition, MSPD extraction of target pesticides from rice sample was carried out through mixing of neutral alumina to rice sample in the ratio of 3:1 and elution of analytes from sample mixture using 16 ml of acetone as eluting solvent. Limit of detection, mean recoveries (n=3) and relative standard deviation for malathion and α-endosulfan were 2.31, 2.27 ppm, 99.04%, 71.70% and 0.73%, 1.89% respectively. Linear dynamic range (LDR) for malathion and α-endosulfan were 7.7 – 142.2 ppm and 7.6 – 176.5 ppm, respectively. Standard addition method was used to determine the amount of pesticides residue in the marketed Khazar rice. Malathion was not detected in the marketed rice sample but the amount of α-endosulfan residue was 0.04 μg/g which is lower than the maximum residue limit (MRL) of this pesticide in rice samples.

Key words: Pesticides residue, Malathion, α-endosulfan, MSPD, HPLC, Rice

1. Introduction

Rice is an important alimentary source throughout the world and the quality of it's grain can be judged by the presence of pesticide residues in this product. The presence of high levels of pesticide in the food supply threatens human health. Also, due to the potential hazards of existence of pesticides residues foods, monitorization of pesticides residue levels in the food is important for the protection of human health. Determination of a high number of pesticides in a high number of foodstuff samples can guarantee the fulfillment of maximum residue level (MRL)

legislations [1]. Therefore, the required analytical methodology for high throughput analysis of pesticides in routine laboratories should be fast, robust and simple. It can be applied, after appropriate validation of mentioned quality criteria.

Conventional extraction of pesticide from food samples usually begins with a homogenization step, followed by tedious liquid–liquid extraction (LLE) procedures with one or more several clean-up steps, and purification of the extract to remove co-extractants before the sample is subjected to chromatographic separation [2,3].

Methods used to determine pesticide residues in fruits and vegetables are mainly based on liquid partitioning with organic solvents such as ethyl acetate, chloroform and dichloromethane, etc., usually followed by a solid-phase extraction cleanup step [4-9].

In the last years, new extraction procedures have been developed to overcome the drawbacks caused by using high amounts of toxic solvents in the classical liquid – liquid extraction methods, so several procedures based on solid-phase microextraction (SPME) [10], supercritical fluid extraction (SFE) [11-13], pressurized liquid extraction (PLE) [14], and microwave-assisted extraction (MAE) [15] have been used.

Matrix solid-phase dispersion (MSPD) as a suitable extraction procedure for the simultaneous disruption and extraction of analytes from semi-solid and solid samples. It is introduced in 1989 [16] and extensively applied to the analysis of many solid matrices. MSPD is based on the dispersion of solid sample to a solid adsorbent, such as Florisil, alkyl bonded silica (C18), alumina, silica etc. and allows the extraction and cleanup of analytes in one single step.

Dispersion of solid samples in solid adsorbent is previously done in a mortar and then the mixture is transferred to the extraction columns [17]. In the case of liquid samples, the dispersion of the sample in the adsorbent can be done directly in the extraction columns [18, 19]. MSPD has been applied to extract pesticide residues from fruit [20], vegetables [21], milk [22, 23], muscle tissues [24, 25], fish [26- 28] and biota [29].

In this work, matrix solid phase dispersion (MSPD) and high performance liquid chromatography (HPLC) with UV detection were used for extraction and determination of organophosphorus pesticides (Malathion) and the organochlorine pesticide (α-Endosulfan) in Khazar rice from north of Iran. Chemical structure of Malathion and α-Endosulfan are shown in Fig. 1.

In order to achieve high recoveries for the analytes, some important experimental parameters in MSPD such as the ratio of adsorbent to rice sample, type and volume of eluting solvent were investigated.

2. Experimental

2.1. Chemicals and solutions

Sodium dihydrogen phosphate, aluminum oxide (particle size 50-150 μm), HPLC-grade methanol, acetonitrile and acetone were purchased from Fluka (Buches, Switzerland). Ethyl acetate, standards of malathion (97.3%) and α-endosulfan (99.6%) were from Riedel-deHean (Seelze, Germany). Water was double distilled deionized and filtered through a 0.45 μm Millipore filter (Millipore, Bedford, MA, USA).

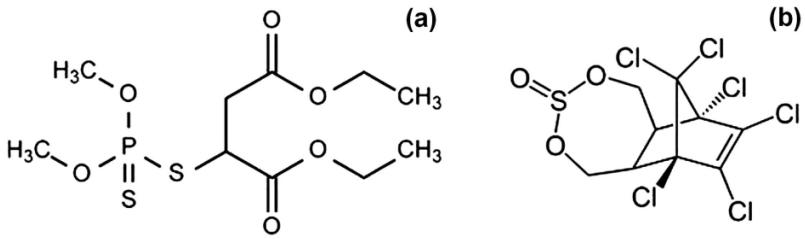

Figure 1. Structure of a) malathion b) α-endosulfan.

Blank rice sample (without using any pesticides) was taken from Rice Research Center (Rasht, Iran). The stock solutions were prepared by dissolving of malathion and α-endosulfan in HPLC grade methanol to obtain 500 μg/ml solution for each standard. Working solutions containing 20-300 μg/ml of each pesticide in methanol were prepared and used to fortify the blank Khazar rice samples. Working solutions were prepared each week and stored at 4˚C.

2.2. Apparatus

HPLC system equipped with the series 10-Liquid chromatograph pump (Norwalk, CT, USA), a model LC- 95 UV Detector (Norwalk, CT, USA), a AR-55 linear recorder (Pye Unicam, Holland) and Spherisorb column C18, 250 × 4.6mm, 5μm (Milford, MA, USA) were used. A Jenway pH meter 3030 (Leeds, UK) was used for adjustment of pH.

2.3. Sampling

The Khazar rice was seeded on March 2006 in open field and transplanted on May 2006. In north of Iran, solutions of 50% malathion and 37% endosulfan have been used for spraying. The first step of spraying with a mixture of malathion and α-endosulfan at recommended normal doses (0.140 ppm/m^2 and 0.051 ppm/m^2 for malathion and α – endosulfan respectively) was performed on June 2006 (45 days after transplant). The second step of spraying was performed on late of July 2006 (30 days before harvesting time). We did extra step of spraying on August 2006 (two weeks after second step). Sampling was done 2 hours and 1, 2, 3, 4, 5, 7, 9, 11 and 13 days after the last treatment, by collecting ~500 g of rice with random sampling.

2.4. Extraction procedure

Blank rice sample (2.0 g) placed into a mortar, blended and pulverized with 6.0 g of neutral alumina as solid adsorbent for 15 minutes (for spiked samples, a mixture of standard solutions of malathion and α-endosulfan was added to the blank rice sample) and transferred into a glass syringe. In order to hold and fix the sample mixture (alumina powder and powdered rice sample) in the glass syringe, glass wool was used as frits at bottom and top of syringe. The sample mixture was pressed with syringe plunger and then 16 ml of acetone was passed through the syringe to elute the pesticides form sample mixture. The effluent was collected and evaporated using rotary evaporator under the vacuum in the water bath (40-45˚C). The residue was quantitatively transferred to a 5 mL volumetric flask and diluted to the mark with methanol, filtered using 0.45 μm syringe filter and injected to HPLC.

2.5. HPLC conditions

HPLC conditions were optimized for separation and determination of malathion and α-endosulfan in Khazar rice of north of Iran. Suitable volume percentage of acetonitril in water, mobile phase pH (using phosphate buffer) and temperature for separation of malathion and α-endosulfan from matrix peaks were 80% (v/v), 5.0 and 25˚C, respectively. The flow rate was 1 ml/min. Detection was performed at a wavelength of 230 nm.

3. Results and discussion

Blank rice sample was placed in oven (35˚C) for 2 hours, to remove the moisture, and then powdered in a blender. Powdered blank sample was fortified with 200 µl of standard solutions containing 20- 300 µg/ml of each pesticide in methanol.

Table 1. Effect of the ratio of adsorbent to rice sample, type and volume of eluting solvent on the malathion and α-endosulfan recoveries.

volume of eluting solvent	*adsorbent :rice*	*Ethylacetate*		*Acetone*	
		R% malathion	R% α-endosulfan	R% malathion	R% α-endosulfan
8	1:1	15.1	3.2	3.4	10.4
12	1:1	42.2	13.7	4.0	10.4
16	1:1	43.5	23.4	5.6	17.9
20	1:1	21.1	36.3	5.3	16.0
8	2:1	67.8	26.6	5.0	16.0
12	2:1	26.1	28.2	75.0	26.4
16	2:1	49.5	18.6	85.8	21.7
20	2:1	47.6	30.2	91.4	21.7
8	3:1	20.7	23.6	65.4	14.2
12	3:1	11.5	73.6	57.7	21.7
16	3:1	15.4	106.6	99.0	71.7
20	3:1	15.4	75.1	72.5	59.8
8	4:1	6.2	15.1	34.1	21.7
12	4:1	11.1	23.6	63.9	19.8
16	4:1	13.9	33.9	39.2	30.2
20	4:1	15.4	42.4	47.3	35.8

R%: Mean recovery (n=3).

This fortified sample were mixed with neutral alumina as solid adsorbent (particle size: 50-150μm) in adsorbent : rice ratios 1:1 to 4:1 and blended for 15 minutes. The resulting homogeneous sample mixture was transferred into a glass syringe. Acetone and ethyl acetate were examined as eluting solvent in volumes of 8-20 ml.All extractions were performed as mentioned in section 2.4 and replicated 3 times. The final extract of each extraction condition was injected to HPLC system and the recoveries of malathion and α-endosulfan were calculated against standard solutions. The effects of the ratio of adsorbent to rice sample, type and volume of eluting solvent on the recoveries of malathion and α-endosulfan are shown in Table 1. As can be observed, the highest recovery for the analytes was obtained using the adsorbent : rice ratios 3:1 and 16 ml of acetone as eluting solvent. The mean recoveries (n = 3) for malathion and α-endosulfan were 99.04 % ± 0.73% and 71.70%±1.89% respectively. For comparison purpose, the recoveries of malathion and α-endosulfan were evaluated in the extraction procedure same as the section 2.4 but, without addition of neutral alumina to rice powder. Analysis of the effluent and calculation of recoveries showed that the malathion and α- endosulfan were not properly removed from rice powder and low recovery values were obtaned (5.48% and 37.73% for malathion and α- endosulfan, respectively). Fig. 2(a) shows the chromatogram of extracted rice sample using MSPD without addition of alumina.

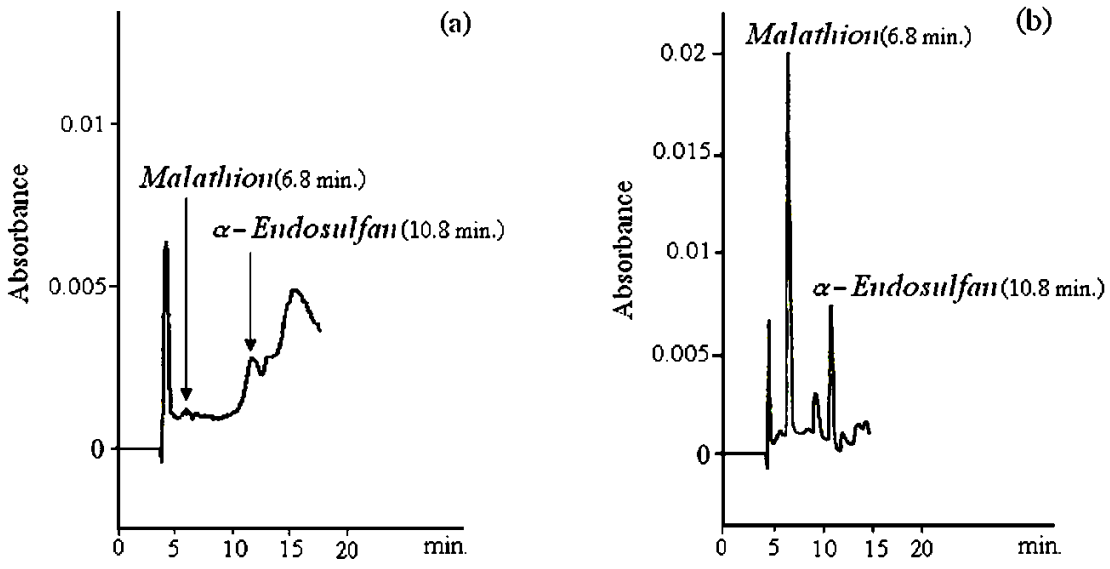

Figure 2. Chromatogram of marketed Khazar rice sample (a): extracted without alumina; (b): spiked with malathion and α-endosulfa (10 μg/g) and extracted at optimized MSPD-HPLC conditions. See sections 2.4 and 2.5 for experimental details.

These results showed the significant effect of solid support in improvement of extraction yield in MSPD. The limits of detection (LOD) and the limits of quantification (LOQ) for malathion and α-endosulfan were 2.31, 7.7 ppm and 2.27, 7.57 ppm respectively. Linear dynamic range (LDR) for Malathion and α-Endosulfan were 7.7 – 142.2 ppm and 7.6 – 176.5 ppm, respectively. LOD and LOQ were determined considering three times and ten times of the baseline noise, respectively. For determination of pesticides residue in marketed rice, the standard addition method was used. The calibration curves were obtained after injection of each extract (from spiked rice samples) to HPLC system under the optimized extraction and separation conditions. Fig. 3 shows the calibration curves for malathion and α-Endosulfan. Representative chromatogram of spiked Khazar rice sample (10 µg/g), extracted under optimum MSPD is shown in Fig 2 (b). Malathion was not detected in studied Khazar rice sample but, the results showed that 0.04 µg/g of α - endosulfan was remained in the rice sample which is lower than maximum residue level (MRL) for this pesticide (Table2).

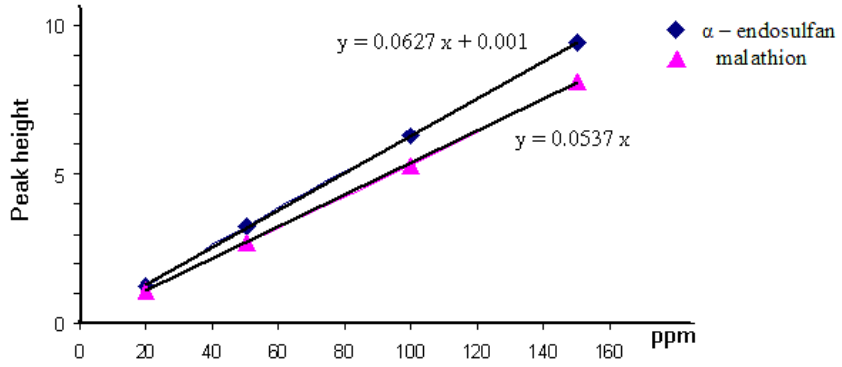

Figure 3. Calibration curves for malathion and α-endosulfan using standard addition method.

Table 2. Pesticides residue in marketed Khazar rice

Pesticide	Measured pesticide residue (µg/g)	Tolerance limit in codex alimentarius (µg/g)	MRL in rice (µg/g)
Malathion	-	8.0	4.0
- endosulfanα	0.04	0.1	0.2

(−): not detected

An extra spraying step of malathion and α-endosulfan was done in open field two weeks after the second step of pesticide spraying (see section 2.3). Sampling was done 2 hour and 1, 2, 3, 4, 5, 7, 9, 11 and 13 days after the last treatment, by collecting ~500 g of rice with random sampling. The amount of malathion and α-endosulfan residue in each collected sample for each time period was determined using represented MSPD-HPLC method.

The results are given in Table 3. As observed, malathion and α-endosulfan was not detected after 3 and 13 days after the last treatment.

Table 3. Pesticides residue in Khazar rice after treatment.

Time after spraying	$\alpha-$endosulfan (μg/g)	Malathion (μg/g)
2 hours	366	627
1days	281	245
2 days	205	24
3 days	155	-
4 days	105	-
5 days	67	-
7 days	30	-
9 days	15	-
11 days	10	-
13 days	-	-

(−): not detected

4. Conclusion

A simple and sensitive multiresidue analysis method with high recoveries based on MSPD and HPLC was developed for determination of organophosphorus pesticides (Malathion) and the organochlorine pesticide (α-Endosulfan) in Khazar rice from north of Iran. The analytes extraction and matrix cleanup were carried out in a single step without additional purification. The analysis time and cost were substantially reduced as compared to other extraction methods.

5. References

[1] Codex Alimentarius Commission (WHO/ FAO), Codex maximum Residue Limits for Pesticides, Vol. 2, Rome, 1993.

[2] R. Carabias-Martinez, E. Rodríguez-Gonzalo, P.H. Paniagua-Marcos, J. Hernández-Méndezet, J. Chromatogr. A, 869 (2000) 427.

[3] P. Hess, J. Boer, W.P. Cofino, P.E.G. Leonards, D.E. Wells, J. Chromatogr. A, 703 (1995) 417.

[4] R.S. Sheridan, J.R. Meola, J. AOAC Int., 82 (1999) 982.

[5] W. Liao, J. Assoc. Off. Anal. Chem., 74 (1991) 554.

[6] A. Sannino, J. AOAC Int., 81 (1998) 1048.

[7] C. Sorce, N. Ceccarelli, R. Lorenzi, Agric. Med., 124 (1994) 132.

[8] J. Fillion, R. Hindle, M. Lacroix, J. Selwyn, J. AOAC Int., 78 (1995) 1252.

[9] J. Engebretson, G. Hall, M. Hengel, T. Shibamoto, J. Agric. Food Chem., 49 (2001) 2198.

[10] M. Volante, M. Cattaneo, M. Bianchi, G. Zoccola, J. Environ. Sci. Health B, 33 (1998) 279.

[11] K. Yoshii, M. Okada, Y. Tsumura, Y. Nakamura, I. Susumu, T. Yashahide, J. AOAC Int., 82 (1999) 1239.

[12] A. Kaihara, K. Yoshii, Y. Tsumura, Y. Nakamura, S. Ishimitsu, Y. Tonogai, J. Health Sci., 46 (2000) 336.

[13] K. Adou, W. R. Bontoyan, P. J. Sweeney, J. Agric. Food Chem., 49 (2001) 4153.

[14] A. Bould, A. Martin Esteban, P. Fernan dez, C. Camara, J. Anal. Chem., 367 (2000) 291.

[15] L. Kadenczki, J. AOAC Int., 75 (1992) 53.

[16] S.A. Barker, A.R. Long, C.R. Short, J. Chromatogr., 475 (1989) 353.

[17] J.L. Tadeo, C. Sánchez-Brunete, Chromato graphia, 57 (2003) 793.

[18] B. Albero, C. Sánchez-Brunete, J.L. Tadeo; J. Agric. Food Chem., 51 (2003) 6915.

[19] S.A. Barker, J. Chromatogr. A, 880 (2000) 63.

[20] Q.H. Zou, Y. Liu, M.X. Xie, J. Han, L. Zhang, Anal. Chim. Acta, 551 (2005) 184.

[21] S. Topuz, G. Ozhan, B. Alpertunga, Food Control, 16 (2005) 87.

[22] H. Kubala-Drincic, D. Bazulic, J. Sapunar-Postruznik, M. Grubelic, G. Stuhne, J. Agric. Food Chem., 51 (2003) 871.

[23] A.M. Carro, R.A. Lorenzo, F. Fernández, R. Rodil, R. Cela, J. Chromatogr. A, 1071 (2005) 93.

[24] M.S.S. Curren, J.W. King, J. Chromatogr. A, 954 (2002) 41.

[25] S.A. Barker, Chem. Tech., 23 (1993) 42.

[26] Y.C. Ling, M.Y. Chang, I.P. Huang, J. Chromatogr. A, 669 (1994) 119.

[27] J. Tolls, M. Haller, D.T. Sijm, Anal. Chem., 71 (1999) 5242.

[28] J. Tolls, M. Haller, D.T. Sijm, J. Chromatogr. A, 839 (1999) 109.

[29] A. Martinez, M. Ramil, R. Montes, D. Hernanz, E. Rubí, I. Rodríguez, R. C.Torrijos, J. Chromatogr. A, 1072 (2005) 83.