



Supramolecular solvent microextraction and ultra-performance liquid chromatography-tandem mass spectrometry combination for the preconcentration and determination of malathion in environmental samples

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ABSTRACT

A supramolecular solvent microextraction method was used for the fast and selective preconcentration separation of malathion, and then ultra-performance liquid chromatography-tandem mass spectrometry was applied for the detection of malathion. The supramolecular solvent is a suitable medium for malathion collection from the sample extract. The results showed that the quantitative recovery of malathion was obtained at pH 4 with heptanol-tetrahydrofuran as the supramolecular solvent. The preconcentration of malathion by the developed microextraction method was established within 10 min. The limit of detection and the quantification limit were 1.4 and 4.2 µg/L, respectively. A preconcentration factor of 40 was obtained, and the relative standard deviation was <7%. The developed supramolecular solvent microextraction method was applied for different real samples, including fruits, vegetables, and water samples.

Keywords: Supramolecular solvent; Microextraction; Malathion; Heptanol; Tetrahydrofuran; Ultra-performance liquid chromatography-tandem mass spectrometry

1. Introduction

The wide application of pesticides in the agricultural field is due to the benefit of preventing crop damage by insects. These activities have been increased in the last 50 y, leading to some negative impacts on the environment worldwide due to soil contamination and water pollution as well as traces of these pesticides remaining in the crops and going through the food chain, thus causing many health problems to humans. The hazards of pesticides are due to

their ability to inhibit the activity of cholinesterase as well as their harmful effects on the immune system of the human body [1,2]. Furthermore, widespread pesticide use causes losses in the biodiversity in the ecosystem, thus leading to instability of the natural habitat. Among these pesticides, malathion (S-1,2-bis(ethoxycarbonyl)ethyl-O,O-dimethyl-phosphorodithioate) is considered to be the most used pesticide worldwide. Therefore, the analysis of malathion in our surrounding environmental areas, including crops and water, is an important task to maintain safety for a better life [3,4].

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The most commonly used techniques to analyze malathion are spectrophotometric and chromatographic instruments, which have the advantage of fast determination; however, the interfering impurities in the sample solution reduce the sensitivity [5,6]. To solve this problem, sample pretreatment methods to extract malathion from other impurities have been used, including solid-phase extraction (SPE) [7] and solid-phase microextraction [8].

Lofty et al. [9] developed a sensitive gas chromatographic (GC) method to detect some insecticides including malathion, and their process was based on extraction with acetone, clean-up with an SPE cartridge, and detection by GC. The obtained recovery was greater than 90%, and the limit of detection (LOD) was 0.001 ppm. Pandey et al. [10] applied a spectrophotometric method based on the oxidation of malathion with *N*-bromosuccinimide (NBS) and determined the unreacted NBS spectrophotometrically. The process showed good results for the determination of malathion in various samples with results comparable with GC–mass spectrometry (GC–MS) methods. Shamsipur et al. [11] optimized a preconcentration method of multiresidue pesticides by SPE coupled with dispersive liquid–liquid microextraction and GC–MS. This process was suitable for application for food samples.

Supramolecular-based solvent microextraction has been applied for the separation and detection of some pollutants [12–14]. Supramolecular solvents are formed by the assembly of long-chain alcohols or carboxylic acids in a mixture of tetrahydrofuran and water, which results in the formation of micelles with immiscible character in the water phase. These supramolecular solvents allow the presence of the organic phase in the aqueous sample solution phase. This facilitates the transfer of malathion to the organic immiscible supramolecular phase, which has a lower density than water, thus resulting in phase separation. Thus, the aim of this work is to apply supramolecular solvent microextraction for malathion and combine it with ultra-performance liquid chromatography (UPLC)-tandem MS for the separation, preconcentration, and determination of malathion in food and water samples. Various experimental parameters such as the pH, extraction solvent type, supramolecular solvent type, vortex time, and sample volume were studied to find the optimum conditions for malathion extraction.

2. Experimental

2.1. Reagents and solutions

High-performance liquid chromatography (HPLC) grade reagents and solvents were used in this work. The malathion standard (HPLC grade) and acetonitrile (ACN) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q water (Millipore, Bedford, USA) was used after filtration with 0.45 μm nylon filter paper. A stock solution of 10 mg/L malathion was prepared in a 1:2 (v/v) mixture of ACN: water and stored at 4°C. The rest of the standard solutions were prepared by dilution of the stock solution.

2.2. Instruments

The malathion standard and samples were analyzed by a Waters ACQUITY UPLC instrument. The dimensions of the C_{18} column were 100 mm \times 2.1 mm i.d. and a 1.7 μm particle size (Waters, Milford, MA, USA). The mobile phase

was applied (ACN and water with 0.1% formic acid) with a gradient of 40%–50% ACN (v/v) (linear model) starting from 0 to 8 min, and the flow rate was 0.45 mL/min. A Quattro Premier triple quadrupole mass spectrometer (Micromass, Milford, MA, USA) using a Z-spray electrospray ionization source was applied for malathion detection. The process was applied in the positive mode. The working conditions were as follows: cone voltage, 20 V; capillary voltage, 3 kV; source temperature, 120°C; desolvation temperature, 300°C; cone gas flow rate, 60 L/h; desolvation gas flow rate, 600 L/h; collision cell entrance potential, 1 V; collision energy, 20; collision cell exit potential, 0.5 V; ionspray voltage, 3,000 V; curtain gas, nitrogen; temperature, 120°C; ion source gas, nitrogen; collisionally activated dissociation gas, argon; quantifier of malathion, 330 > 127; and qualifier of malathion, 330 > 99. Nitrogen (99.99% purity, Peak Scientific, model NM30L LA nitrogen generator, Inchinann, UK) and high-purity argon (99.99%, Specialty Gas Centre, Jeddah, Saudi Arabia) were used as the cone and collision gases, respectively. An Oerlikon rotary pump (model SOGEVAC SV40 BI, France) provided the primary vacuum to the mass spectrometer. The data acquisition and processing were carried out by MassLynx V4.1 software.

2.3. Preconcentration process

For the separation of malathion from aqueous samples, 10 mL of the sample solution was taken in a 50-mL centrifuge tube. The supramolecular solvent mixture was injected, and then, the mixture was vortexed for 1 min. A cloudy solution was formed, which allowed the transfer of malathion from the aqueous phase to the organic layer. The complete separation was achieved by centrifuging the mixture at 4,000 rpm for 10 min. The malathion molecules were obtained in the organic phase, dissolved in ethanol, and finally detected by UPLC-MS. Figs. 1 and 2 show the chromatogram and mass fragments of malathion, respectively. The standard calibration curve in the range of 0.05–1.0 mg/L and the related peak areas are presented in the supplementary figure (Fig. S1). The linearity was investigated by spiking malathion

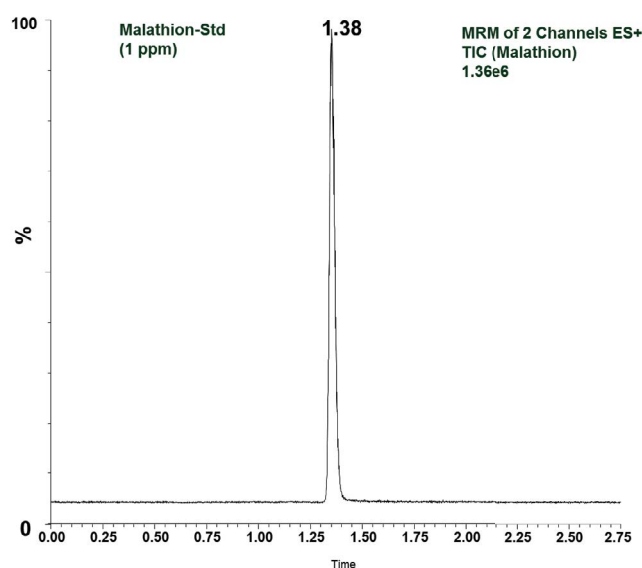


Fig. 1. The chromatogram of malathion.

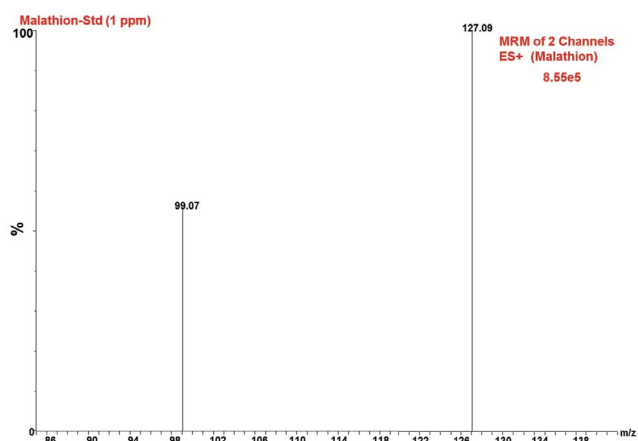


Fig. 2. The mass fragments of malathion.

concentrations in different real matrix samples and recording the recovered malathion concentrations, as shown in Table 1.

For the determination of malathion in fruit and vegetable samples, the fresh samples were collected from Riyadh city markets. The samples were washed with reverse osmosis water and then cut into small pieces. Thirty grams of each fresh sample was ground in a kitchen mixer, and the final volume was adjusted to 100 mL. Then, 10 mL was applied for the previously described supramolecular solvent microextraction procedure.

3. Results and discussion

Different parameters that have a noticeable effect on the extraction of malathion by supramolecular liquid-phase microextraction (Ss-LPME) were optimized by selecting the peak area as the response. These parameters include the pH of the solution, the type of supramolecular formula, and the sample volume.

3.1. Effect of the pH

The extraction behavior of malathion from the aqueous phase to the extraction phase is governed by its polar groups. Therefore, the pH value of the aqueous phase is quite important for the quantitative recovery of malathion during the Ss-LPME procedure by affecting the interactions including the possibility of hydrogen bonding between the hetero atoms such as O or S in the malathion structure and the OH groups in the extraction phase, which is the micelles of long-chain organic acids (heptanol in tetrahydrofuran/water). Therefore, the effect of the aqueous phase pH on the extraction efficiency was studied in the range of 2.0–6.0 (Fig. 3). Malathion was quantitatively extracted in the pH range of 4.0–5.0. Hence, further studies for Ss-LPME were performed at pH 4.

3.2. Effect of the type of supramolecular solvent

The interactions between the analyte and supramolecular solvent are important to provide an effective extraction medium. Hence, the selection of the right supramolecular solvent phase as the extraction solvent is an important issue. The supramolecular solvent is selected based on its ability to extract malathion in an efficient way and give a quantitative

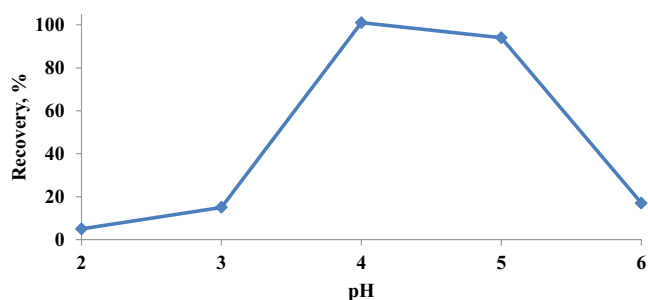


Fig. 3. Effect of the aqueous phase pH on the recovery of malathion ($N = 3$).

recovery from aqueous samples. In this study, different types of supramolecular solvents including THF were tested. The results given in Fig. 4 show that a quantitative recovery was obtained by using heptanol for the formation of the supramolecular solvent phase. As a consequence, the supramolecular solvent phase used was prepared by heptanol reverse micelles dispersed in a water:THF continuous phase. The formation of reverse micelles is based on the presence of tetrahydrofuran, which increases the nonpolar character of the sample medium and allows the heptanol molecules to reverse their direction making their polar part inside the micelle, thus enhancing the malathion separation.

3.3. Effect of the heptanol volume

The other important parameter that affects the extraction efficiency is the volume of the extraction solvent. The effect of the heptanol volume as a supramolecular solvent component was evaluated in the range of 50–400 μL . It can be observed that the extraction efficiency increased with increasing heptanol volumes up to 100 μL and then reached quantitative recovery. Considering the results, a heptanol volume of 100 μL was selected as optimal condition (Fig. 5).

3.4. Effect of the THF volume

The ratio between heptanol and THF is important for the quantitative recovery of malathion. The effect of the volume of THF on the recovery of malathion was studied by varying it from 200 to 1,000 μL . This is because the total

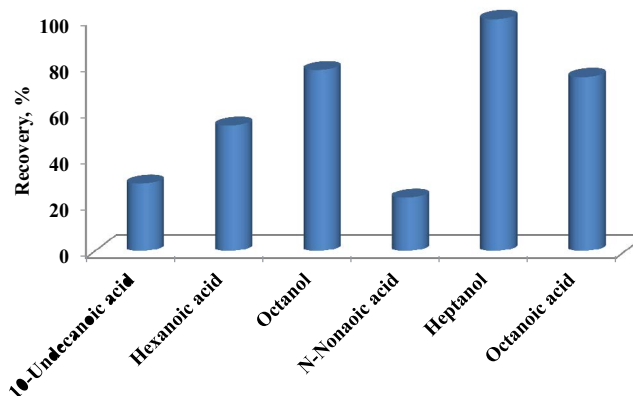


Fig. 4. Effect of the type of supramolecular solvent on the recovery of malathion ($N = 3$).

sample volume was between 10 and 20 mL, and at the same time, the amount of tetrahydrofuran should be suitable to make the reverse micelles of heptanol. Quantitative results were obtained between 400 and 1,000 μL (Fig. 6), and thus, 400 μL of THF was used for further steps. The results depicted in Figs. 5 and 6 indicate that the best and quantitative extraction conditions for malathion can be achieved when the heptanol/THF volume ratio was set at 0.25.

3.5. Optimization of the primary sample volume

The sample volume is a critical parameter in the separation and preconcentration studies [14–17]. The initial volume of the malathion sample solution before applying the supramolecular extraction method was investigated. The effect of the initial malathion sample solution volume was studied from 10 to 40 mL. Fig. 7 shows that the quantitative recovery percentage was obtained with a starting sample volume up to 20 mL. The preconcentration factor was calculated considering that the last volume after extraction is 500 μL . It is usually calculated as the ratio between the initial malathion sample volume and the last volume after extraction.

3.6. Analytical features and application to fruits, vegetables, and water samples

The supramolecular microextraction procedure developed in this work was validated by additional recovery

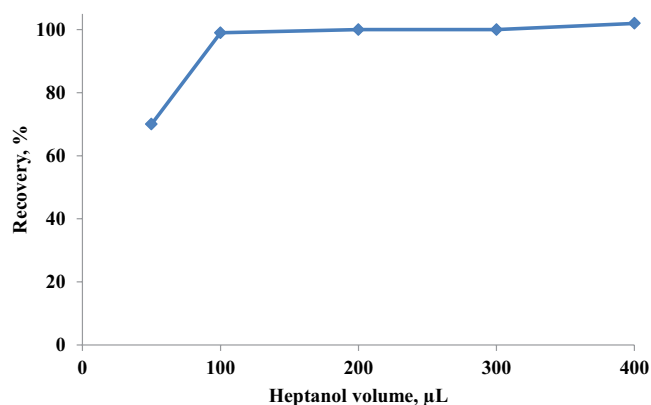


Fig. 5. Effect of the heptanol volume on the recovery of malathion ($N = 3$).

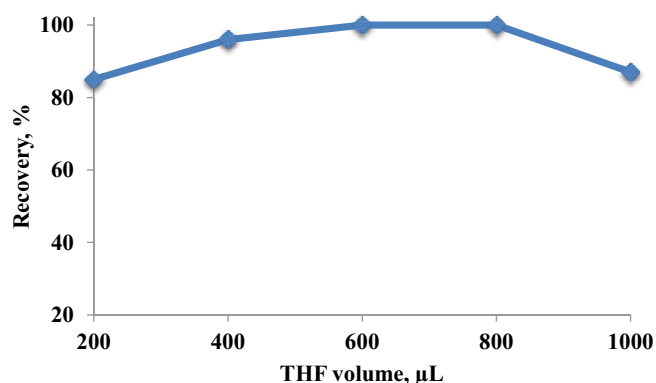


Fig. 6. Effect of the THF volume on the recovery of malathion ($N = 3$).

investigations from various samples with different matrices [18–20]. These samples include guava, pear, eggplant, tomatoes, and water. Different spiked concentrations were studied (0.0, 0.2, 0.4, and 0.8 mg/L). Table 1 shows the detected concentrations and recovery percentage in each case. The obtained recoveries confirm that the developed method was applicable with different concentrations of malathion.

The LOD of the developed supramolecular microextraction procedure was calculated based on 9 determinations of the

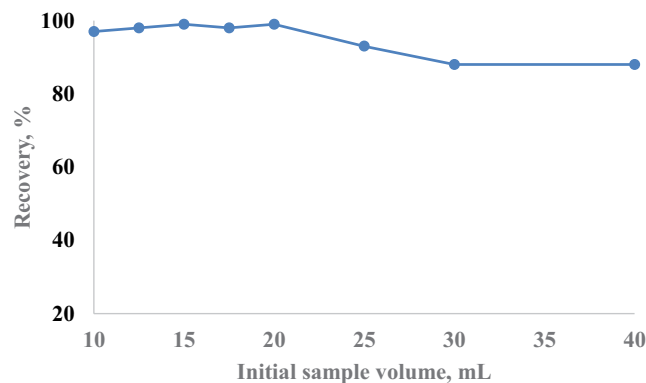


Fig. 7. Effect of the aqueous sample volume on the recovery of malathion ($N = 3$).

Table 1
The recovery percentage of different concentrations of malathion from water and food samples

Spiked malathion concentrations (mg/L)	Samples	Detected malathion concentrations (mg/L)	Recovery, %
0.0	Guava	0.0	–
	Pear	0.0	–
	Eggplant	0.0	–
	Tomatoes	0.0	–
	Water	0.0	–
0.2	Guava	0.20 ± 0.04	99
	Pear	0.20 ± 0.08	102
	Eggplant	0.19 ± 0.09	97
	Tomatoes	0.19 ± 0.06	96
	Water	0.20 ± 0.05	101
0.4	Guava	0.40 ± 0.07	101
	Pear	0.40 ± 0.06	101
	Eggplant	0.40 ± 0.07	101
	Tomatoes	0.35 ± 0.02	89
	Water	0.38 ± 0.09	95
0.8	Guava	0.84 ± 0.06	104
	Pear	0.76 ± 0.06	95
	Eggplant	0.82 ± 0.06	103
	Tomatoes	0.83 ± 0.07	103
	Water	0.77 ± 0.07	96

standard deviation of the blank readings, as shown in the following equation:

$$\text{LOD} = \frac{3 \times \text{STD}}{\text{PF}} \quad (1)$$

In Eq. (1), the LOD is the limit of detection, STD is the standard deviation of the seven blank readings, and PF is the preconcentration factor.

The LOD was found to be 1.4 µg/L, and the quantification limit was 4.2 µg/L. Meanwhile, the relative standard deviation determined from eleven tests of the malathion standard solution (0.2 mg/L) was 6.9%.

4. Conclusions

The application of supramolecular solvent with malathion leads to good interaction and the separation of malathion from aqueous solutions. At the same time, the combination of this supramolecular microextraction process with UPLC-MS allows the accurate determination of malathion at very low levels. The quantitative recovery percentage is achieved by variation of the volume of the starting sample solution up to a volume of 20 mL. The proposed supramolecular-based microextraction procedures are recommended for future application in the analysis of malathion in fruits, vegetables, and water samples.

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References

- [1] X. Wang, X. Zhao, X. Liu, Y. Li, L. Fu, J. Hu, C. Huang, Homogeneous liquid-liquid extraction combined with gas chromatography-electron capture detector for the determination of three pesticide residues in soil, *Anal. Chim. Acta*, 620 (2008) 162–169.
- [2] M.A. Habila, Z.A. ALOthman, S.A. Al-Tamrah, A. Ghafar, M. Soylak, Activated carbon from waste as an efficient adsorbent for malathion for detection and removal purposes, *J. Ind. Eng. Chem.*, 32 (2015) 336–344.
- [3] G. Giordano, Z. Afsharinejad, M. Guizzetti, A. Vitalone, T.G. Kavanagh, L.G. Costa, Organophosphorus insecticides chlorpyrifos and diazinon and oxidative stress in neuronal cells in a genetic model of glutathione deficiency, *Toxicol. Appl. Pharmacol.*, 219 (2007) 181–189.
- [4] M.I. Giron-Perez, A. Santerre, F. Gonzalez-Jaime, J. Casas-Solis, M. Hernandez-Coronado, J. Peregrina-Sandoval, A. Takemura, G. Zaitseva, Immunotoxicity and hepatic function evaluation in Nile tilapia (*Oreochromis niloticus*) exposed to diazinon, *Fish Shellfish Immunol.*, 23 (2007) 760–769.
- [5] D. Rodrigues, T. Carvalho, A. Sousa, V.S. Neto, P. Fachine, R. Nascimento, Determination of insecticide residues in vegetal fruits, *Chromatogr. Res. Int.*, 2011 (2011) 1–6.
- [6] N.V.S. Venugopal, B. Sumalatha, Syedabano, Spectrophotometric determination of malathion in environmental samples, *E-J. Chem.*, 9 (2012) 857–862.
- [7] D. Stajnbaher, L. Zupancic-Kralj, Multiresidue method for determination of 90 pesticides in fresh fruits and vegetables using solid phase extraction and gas chromatography-mass spectrometry, *J. Chromatogr. A*, 1015 (2003) 185–198.
- [8] P.P. Vazquez, A.R. Mughari, M.M. Galera, Application of solid phase microextraction for determination of pyrethroids in groundwater using liquid chromatography with postcolumn photochemically induced fluorimetry derivatization and fluorescence detection, *J. Chromatogr. A*, 1188 (2008) 61–68.
- [9] H.M. Lofty, A.A. Abd El-Aleem, H.H. Monir, Determination of insecticides malathion and lambda-cyhalothrin residues in zucchini by gas chromatography, *Bull. Fac. Pharm. Cairo Univ.*, 51 (2013) 255–260.
- [10] G.P. Pandey, A.K. Singh, L. Deshmukh, S. Prasad, L.J. Paliwal, A. Asthana, S.B. Mathew, A novel and sensitive kinetic method for the determination of malathion using chromogenic reagent, *Microchem. J.*, 113 (2014) 83–89.
- [11] M. Shamsipur, N. Yazdanfar, M. Ghambarian, Combination of solid-phase extraction with dispersive liquid-liquid microextraction followed by GC-MS for determination of pesticide residues from water, milk, honey and fruit juice, *Food Chem.*, 204 (2016) 289–297.
- [12] E. Yilmaz, M. Soylak, Latest trends, green aspects and innovations in liquid-phase-based microextraction techniques: a review, *Turk. J. Chem.*, 40 (2016) 868–893.
- [13] E. Yilmaz, M. Soylak, Development a novel supramolecular solvent microextraction procedure for copper in environmental samples and its determination by microsampling flame atomic absorption spectrometry, *Talanta*, 126 (2014) 191–195.
- [14] E. Yilmaz, M. Soylak, Supramolecular solvent microextraction of gold prior to its determination by microsample injection system coupled with flame atomic absorption spectrometry, *RSC Adv.*, 4 (2014) 47396–47401.
- [15] D.S. Chormey, S. Bodur, D. Baskin, M. Firat, S. Bakirdere, Accurate and sensitive determination of selected hormones, endocrine disruptors, and pesticides by GC-MS after the multivariate optimization of switchable solvent liquid phase microextraction, *J. Sep. Sci.*, 41 (2018) 2895–2902.
- [16] Y. Hamid, M.R. Fa'hi, A fast and green preconcentration method based on surfactant ion pair-switchable solvent dispersive liquid-liquid microextraction for determination of phenazopyridine in pharmaceutical and biological samples, *J. Iran. Chem. Soc.*, 15 (2018) 1813–1820.
- [17] M. Soylak, E. Yilmaz, Ionic liquid dispersive liquid-liquid microextraction of lead as pyrrolidinedithiocarbamate chelate prior to its flame atomic absorption spectrometric determination, *Desalination*, 275 (2011) 297–301.
- [18] M.A. Habila, Z.A. ALOthman, A.M. El-Toni, J.P. Labis, X. Li, F. Zhang, M. Soylak, Mercaptobenzothiazole-functionalized magnetic carbon nanospheres of type Fe₃O₄@SiO₂@C for the preconcentration of nickel, copper and lead prior to their determination by ICP-MS, *Microchim. Acta*, 183 (2016) 2377–2384.
- [19] M.A. Habila, Z.A. ALOthman, A.M. El-Toni, J.P. Labis, M. Soylak, Synthesis and application of Fe₃O₄@SiO₂@TiO₂ for photocatalytic decomposition of organic matrix simultaneously with magnetic solid phase extraction of heavy metals prior to ICP-MS analysis, *Talanta*, 154 (2016) 539–547.
- [20] Z.A. ALOthman, M. Habila, E. Yilmaz, M. Soylak, Solid phase extraction of Cd(II), Pb(II), Zn(II) and Ni(II) from food samples using multiwalled carbon nanotubes impregnated with 4-(2-thiazolylazo) resorcinol, *Microchim. Acta*, 177 (2012) 397–403.

Supplementary Information

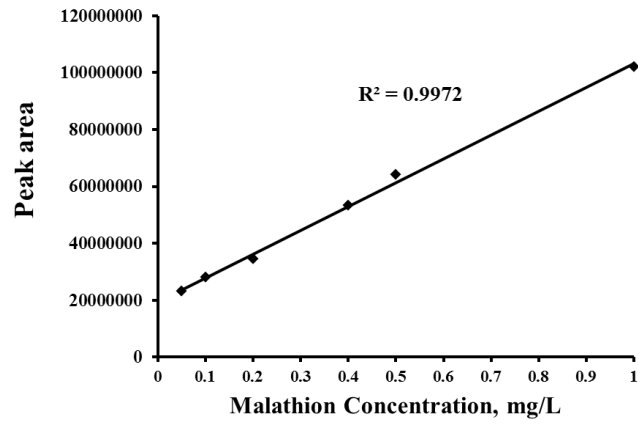


Fig. S1. The standard calibration curve in the range of 0.05–1.0 mg/L.