

Headspace Solid Phase Microextraction in Pesticide Residues Analysis: 1. Optimisation of Extraction Conditions

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SUMMARY

The method of headspace solid phase microextraction (HS/SPME) was successfully used in a simultaneous multicomponent analysis of hexachlorobenzene (HCB), tefluthrin, heptachlor, aldrin, chlorpyrifos, fenthion and bifenthrin in aqueous medium. Measurements were performed using a nonpolar polydimethyl siloxane (PDMS) fiber. Detection and quantification were done by gas chromatography/mass spectrometry (GC/MS).

Optimal conditions for HS/SPME were determined both by performing extraction at different temperatures and examining extraction time profiles at constant temperature. Optimal extraction temperature for each pesticide studied was determined as follows: 60°C for HCB and for heptachlor, 80°C for aldrin and for chlorpyrifos, fenthion and tefluthrin, and temperature exceeding 80°C for bifenthrin. For the pesticide mixture studied, 60°C was identified as the optimum extraction temperature.

Based on the time profiles obtained, it was confirmed that satisfactory extraction sensitivity can be obtained even for extraction times shorter than the time required to reach a sorption equilibrium. This conclusion was confirmed by linear concentration profiles obtained for the following ranges: 0.05-10 ng/ml (HCB), 0.05-25 ng/ml (tefluthrin), 0.05-40 ng/ml (heptachlor), 0.05-40 ng/ml (aldrin), 0.05-25 ng/ml (chlorpyrifos), 0.05-25 ng/ml (fenthion) and 0.05-25 ng/ml (bifenthrin).

Relative standard deviation (RSD) values for triplicate measurements did not exceed 15%.

Keywords: HS/SPME; Optimisation; Pesticide residues

INTRODUCTION

Detailed investigation of pesticide residues began in mid-20th century in reaction to early uses of organic pesticides. Numerous methods of analysis were developed, mostly using a traditional liquid-liquid extrac-

tion (LLE). However, considering the long duration of such analyses and use of high amounts of hazardous organic solvents, the analyst's task is evidently not only to use the existing methods but also to develop new and reliable ones. Up-to-date methods should be cost-efficient and should considerably lower the risk of

affecting the analyst's health and causing environmental contamination. Consequently, the method of solid phase extraction (SPE) expanded significantly in early 1980s, while the innovative solid phase microextraction (SPME) was introduced in late 1990s.

The basic postulate of the SPME technique was given by Belardi and Pawliszyn in 1989 (Belardi and Pawliszyn, 1989). More than 3000 papers relating to SPME applications were published by the end of 2005 (Dietz et al., 2006). The SPME is a simple, selective and efficient sorption/desorption method, based on analytes distribution between the sample matrix and extraction medium. This technique can be used for extracting organic compounds from a solution (direct mode – DM) or a gas phase (headspace mode – HS) without using organic solvents. Extraction is performed in a thin polymer film coating a fused silica fiber, which is either immersed in a liquid sample or exposed to a headspace above the sample avoiding direct contact of fibre and solution. After extraction, the fiber carrying sorbed analytes is introduced into a gas chromatograph injector for thermal desorption (GC), while in the case of liquid chromatography (LC) analytes are desorbed by solvent elution.

Of some 400 papers on SPME applications published until 2000, 60 related to determination of pesticide residues by using the direct mode (Beltran et al., 2000). Although headspace mode is more appropriate for pesticide residues extraction from samples with complex matrix, its application is far less investigated. Besides water analysis (Lambropoulou and Allbanis, 2001; Sakamoto and Tsutsumi, 2004), the HS/SPME method has been used in pesticide residues analysis of wine (Correia et al., 2000; Bellavia et al., 2000; Navalón et al., 2002), strawberry fruit and cherry juice samples (Lambropoulou and Allbanis, 2002).

It is obvious that the HS/SPME process has not been thoroughly investigated and the method is not widely used in pesticide residue analyses. Hence, the aim of this work was to study the headspace extraction of selected pesticides from aqueous solutions and to optimise extraction conditions in simultaneous multiresidue analysis of simple water matrix. Compounds of different chemical structure, belonging to different pesticide groups, were investigated. Aldrin, heptachlor and hexachlorobenzene (HCB) were chosen as representatives of organochlorines, chlorpyrifos and fenitrothion as organophosphorous pesticides, and bifenthrin and tefluthrin as members of the pyrethroid group. The pesticides studied were chosen based on their hav-

ing vapour pressure values high enough to allow analyte concentration in the gas phase and on their widespread use for crop protection. Although not in use any more, organochlorine pesticides were included in this study because, being markedly persistent, they can still be detected in the environment.

MATERIAL AND METHODS

Reagents and standards

Pesticide standards were of 96-99.5% purity (Dr Ehrenstorfer, Germany). Stock standard solutions of 1 mg/ml of each pesticide were prepared in acetone (J. T. Baker, USA). Working mixed standard solutions were prepared by diluting the stock solution with acetone.

Mixed water standard solutions were used for all SPME measurements. Highly purified deionized water (Purelab Option – R7, Elga, UK) was used for the dilution of acetone standard solutions.

Materials and instruments

A fused silica fiber coated with 100 μm (PDMS) film (Supelco) was used. Before use, the fiber was conditioned in a gas chromatograph injection port as recommended by the manufacturer. A magnetic stirrer (Roth RCT Basic, Germany) and 8×3 mm stirring bars were used to mix the samples during extraction. Extraction was performed in 4 ml vials (Supelco).

A gas chromatograph/mass spectrometer (GC/MS) was used as a detection device (CP-3800/Saturn 2200, Varian, Australia). A 30 m × 0.25 mm × 0.25 μm , VF-5ms column (Varian) was used. The injection port (1079 Universal capillary injector) temperature was set at 270°C. After desorption time of 9 minutes, the injector was set to split mode (1 : 60). By measuring blank fiber, it was confirmed that the temperature of 270°C was high enough for a complete desorption of analytes from fiber.

The GC was programmed as follows: initial temperature was 120°C, then increased to 170°C at 10°C min⁻¹ and held for 20 minutes, increased to 280°C at 15°C min⁻¹ and held for 2 minutes, increased to 290°C at 10°C min⁻¹ and held for 10 minutes. Helium was used as the carrier gas and its flow rate was 1.1 ml/min.

The ion trap mass spectrometer was operated in the electron impact/selected ion monitoring (EI/SIM)

mode. The ion trap and transferline temperatures were set to 220°C and 250°C, respectively. One specific pesticide ion was selected for detection and quantification, while a second one was used for confirmation. The ions inspected were as follows: 284 (214) for HCB, 177 (141) for tefluthrin, 274 (272) for heptachlor, 66 (293) for aldrin, 314 (286) for chlorpyrifos, 278 (109) for fenthion, and 181 (165) for bifenthrin.

Sample preparation and analysis

In order to confirm the HS/SPME method as suitable for simultaneous multicomponent pesticide analysis, measurements with individual and mixed standard solutions were performed. One hour extraction procedure was done at 60°C with water solution fortified at concentration level of 15 ng/ml of each pesticide.

As described previously (Đurović et al., 2006), temperature profiles (temperature dependence of the analyte amount extracted) for every pesticide studied were obtained by HS/SPME measurements of mixed aqueous solutions in a temperature range from 23 to 90°C. One hour extraction procedure was performed with water solutions at concentration level of 15 ng/ml of each pesticide.

Time profiles (time dependence of the amount extracted) were obtained by HS/SPME measurements of mixed water solutions at concentration level of 10 ng/ml of each pesticide. Extraction at 60°C was performed, and the extraction time was varied in time intervals from 2 to 120 minutes. Linearity tests were performed in a concentration range from 0.05 to 40 ng/ml, performing one hour HS/SPME analysis at 60°C.

Water standard solutions were prepared with acetone content not higher than 1 % v/v, thus not affecting the extraction procedure (Arthur et al., 1992; Eisert and Levsen, 1995a, 1995b; 1995; Urruty and Montury, 1996).

In all experiments, 4 ml vials were filled with 2 ml of sample. Each sample was analysed in triplicate.

RESULTS

Figure 1 shows the GC/MS peak areas related to HS/SPME measurements of both individual and mixed standard solutions of the pesticides studied. Comparing the values of peak areas obtained in

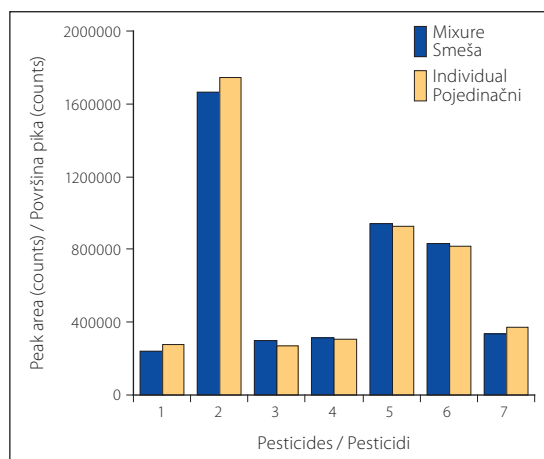


Figure 1. HS/SPME – GC/MS peak areas for various pesticides studied - single and multicomponent analysis (1 – HCB, 2 – tefluthrin, 3 – heptachlor, 4 – aldrin, 5 – chlorpyrifos, 6 – fenthion i 7 – bifenthrin)

Slika 1. Površine HS/SPME – GC/MS pikova ispitivanih pesticida - jednokomponentna i višekomponentna analiza (1 – HCB, 2 – teflutrin, 3 – heptahlor, 4 – aldrin, 5 – hlorpirifos, 6 – fention i 7 – bifentrin)

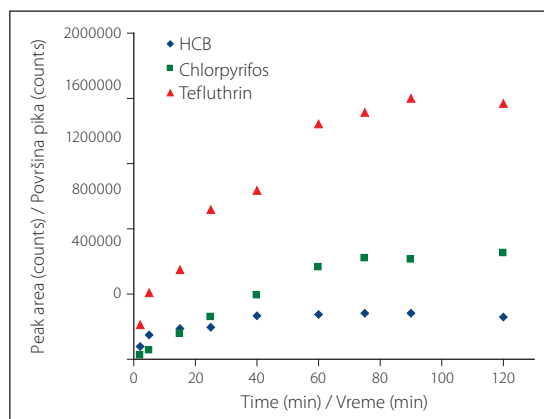


Figure 2. Time profiles for HS/SPME – GC/MS analysis of HCB, chlorpyrifos and tefluthrin

Slika 2. Vremenski profili za HS/SPME – GC/MS analizu HCB-a, hlorpirifosa i teflutrina

two sets of measurements, the calculated differences were less than 7% (6.85%) for HCB (1), 2.47% for tefluthrin (2), 5.27% for heptachlor (3), 1.51% for aldrin (4), 0.91% for chlorpyrifos (5), 0.79% for fenthion (6) and 4.51% for bifenthrin (7).

In order to determine the optimum extraction temperature, temperature profiles for each pesticide were obtained, as explained in detail in a previous paper (Đurović et al., 2006). The presented dependences clearly show that there is an optimum extraction tem-

Table 1. Linearity ranges obtained from concentration profiles, with relevant correlation coefficient values (R)**Tabela 1.** Opsezi linearnosti dobijeni na osnovu koncentracijskih profila i odgovarajući korelacioni koeficijenti (R)

Pesticide Pesticid	Linearity range Opseg linearnosti (ng/ml)	R
HCB	0.05-10	0.9996
Tefluthrin	0.05-25	0.9977
Heptachlor	0.05-40	0.9967
Aldrin	0.05-40	0.9988
Chlorpyrifos	0.05-25	0.9966
Fenthion	0.05-25	0.9953
Bifenthrin	0.05-25	0.9914

perature (temperature of maximum extraction rate) for each pesticide studied, and it is: 60°C for HCB and heptachlor, 80°C for aldrin, chlorpyrifos, fenthion and tefluthrin, and temperature higher than 90°C for bifenthrin. For the pesticide mixture studied, extraction temperature of 60°C was identified as optimal.

The extracted amounts of HCB, chlorpyrifos and tefluthrin are related to extraction times and presented in Figure 2. It is obvious that sorption equilibrium was attained for extraction time below 90 minutes. The time profiles reveal the same pattern for all pesticides studied.

Linear dependence of the pesticide amount sorbed as a function of the initial analyte concentration in the sample during one-hour extraction time was investigated in concentration range from 0.05 to 40 ng/ml. Linearity ranges and regression coefficients (R) obtained are presented in Table 1.

Relative standard deviation values for the triplicate measurements were not higher than 15%.

DISCUSSION

Inspecting the results obtained for individual and mixed standard solutions (Figure 1), it is evident that the HS/SPME method applied can be successfully used for simultaneous analysis of various pesticides present in the sample. As the SPME technique is usually applied to highly diluted solutions (ppb level concentrations), we may assume that the solvent (matrix) and solutes obey Raoult's and Henry's laws, respectively (Atkins and De Paula, 2002). Consequently, Henry's law would be valid for each analyte independently during the SPME analysis, i. e. the concentration of each analyte in the gas phase would be proportional

to its vapour pressure in the mixture regardless of the presence of other analytes. Hence, there is no competition for the fibre between the pesticides in the system, enabling this technique to be suitable for simultaneous multicomponent analysis.

As already concluded in our previous paper (Đurović et al., 2006), the dependence of extraction efficiency on temperature clearly indicates that the amount of analytes sorbed by the fiber increases with rising temperature as a consequence of higher analyte vapour pressure and its higher concentration in the gas phase. Maximum extraction efficiency temperature is identified for each pesticide studied, and the efficiency decreases at temperatures exceeding the maximum. This effect can be explained by an increased analyte desorption from fiber at elevated temperatures. Since the maximum amount extracted was achieved in the 60-80°C temperature range for most pesticides studied, 60°C was identified as the optimum extraction temperature.

Proportional relation between the extracted amount of analyte and extraction time, already confirmed by theoretical models describing the HS/SPME process (Ai, 1997; Ai, 1998; Đurović et al., 2006), indicates that quantification is possible before sorption equilibrium is reached. It is evident from the time profiles obtained (Figure 2) that for all pesticides analysed equilibrium was attained within the extraction time of up to 90 minutes. Practical application is confirmed by the linearity of concentration profiles in the one-hour extraction procedure with correlation coefficients in the 0.9914-0.9996 range (Table 1).

The results presented in this paper verify the HS/SPME method applied as being suitable for simultaneous multiresidue pesticide analysis of water solutions. Since complex matrix effects can be eliminated using the HS/SPME method, optimisation of the most important extraction parameters done for simple water matrix can be used as a basis for pesticide residue analysis of more complex matrix samples.

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Mikroekstrakcija u čvrstoj fazi - uzorkovanje iz gasovite faze u određivanju ostataka pesticida: 1. Optimizacija uslova ekstrakcije

REZIME

Metoda mikroekstrakcije u čvrstoj fazi – uzorkovanje iz gasovite faze (HS/SPME) je uspešno primenjena za istovremenu višekomponentnu analizu heksahlorobenzena (HCB), te flutrina, heptahlor, aldrina, hlorpirifosa, fentiona i bifentrina u vodenoj sredini. U radu je korišćeno nepolarano polidimetilsiloksansko vlakno (PDMS). Detekcija i kvantifikacija analita je vršena metodom gasno-masene spektrometrije (GC/MS).

Optimalni uslovi HS/SPME metode su određeni praćenjem efikasnosti ekstrakcije na različitim temperaturama, odnosno u funkciji vremena na konstantnoj temperaturi. Za svaki ispitivani pesticid, određena je optimalna temperatura ekstrakcije: 60°C za HCB i heptahlor,

80°C za aldrin, hlörpirifos, fention i teflutrin, temperatura veća od 80°C za bifentrin. Za ispitivanu smešu, temperatura od 60°C je izabrana za praktičan rad. Na osnovu dobijenih vremenskih profila, utvrđeno je da se dobra osetljivost metode postiže i kada se radi sa vremenom ekstrakcije kraćim od ravnotežnog. Ovaj zaključak je potvrđen i eksperimentalno dobijenim koncentracionim profilima, pri čemu su dobijeni sledeći opsezi linearnosti: 0.05-10 ng/ml (HCB), 0.05-25 ng/ml (teflutrin), 0.05-40 ng/ml (heptahlor), 0.05-40 ng/ml (aldrin), 0.05-25 ng/ml (hlörpirifos), 0.05-25 ng/ml (fention) i 0.05-25 ng/ml (bifentrin).

Relativne standardne devijacije (RSD) računane na osnovu tri ponovljena merenja su uvek bile manje od 15%.

Ključne reči: HS/SPME; optimizacija; ostaci pesticida