ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LIX 13 Number 1, 2011

DETERMINATION OF SOME SELECTED PESTICIDE RESIDUES IN APPLE JUICE BY SOLID - PHASE MICROEXTRACTION COUPLED TO GAS CHROMATOGRAPHY – MASS SPECTROMETRY

A. Hercegová, M. Mőder

Received: September 23, 2010

Abstract

HERCEGOVÁ, A., MŐDER, M.: *Determination of some selected pesticide residues in apple juice by solid-phase microextraction coupled to gas chromatography – mass spectrometry.* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 1, pp. 121–128

The performance of solid phase microextraction (SPME) for enrichment of pesticides from apple juice was investigated. Samples were diluted with water, extracted by solid-phase microextraction and analysed by gas chromatography using mass-spectrometry detector (MSD) in selected ion monitoring mode (SIM). The method was tested for the following pesticides used mostly in fruit culturing at Slovakia: tebuthylazine, fenitrothion, chlorpyrifos, myclobutanil, cyprodinil, phosalone, pyrimethanil, tebuconazole, kresoxim-methyl, methidathion, penconazole. All pesticides were extracted with polydimethylsiloxane fibre 100μm thickness. The linear concentration range of application was 0.05 μg dm^{-3}–10 μg dm^{-3}. The method described provides detectabilities complying with the maximum residue levels (MRLs) set by regulatory organizations for pesticides in apple juice matrices. The solvent – free SPME procedure was found to be quicker and more cost effective then the solvent extraction methods commonly used.

solid phase microextraction, pesticide residues, apple juice, gas chromatography-mass spectrometry

The analysis of nutrients, flavour and contaminated chemicals in food has been a challenge to many researches for a long time. Production of safe and healthy food is a key priority of EU and problem of contamination of food commodities from pesticide use of is a great social importance for Partner countries. Fresh and processed apples are mostly used as a baby food or for production of apple juice in Slovakia. The pesticides to be studied were chosen on the basis of certain criteria. The basis of criterion was their importance for local market, availability on the market, and especially their use on crops (apples) producing baby food or apple juice Determination of some selected pesticide residues in apple juice by solid – phase microextraction coupled to gas chromatography – mass spectrometry detector. Studied pesticides were chosen in accordance with the consumption of pesticides applied on apple trees in south-west part of Slovakia (http://www.uksup.sk/index.php?n=25), which are amenable to GC analysis and also represent pesticides of a wide range of polarity and other physico-chemical properties. The model mixture of 11 pesticides belonging to variety groups (anilinopyrimidins, organophosphorus pesticides, triazines, oximinoacetate, triazol) of pesticides in apple juice was studied. The existence of MRL (10 $\mu g.kg^{-1}$ for baby food) established by Codex Alimentarius Commission of FAO/WHO was checked. Analysis of real – life samples of apples might leads to problems with tolerance of GC system.

Separation of target analytes from the matrix is the primary and often the most difficult step in analysis. The currently used protocols for the determination of pesticides from liquid samples involve liquid-liquid extraction (LLE), or solid-phase ex-

traction (SPE). Standard pesticide-multiresidue protocols licensed by the European Community for non-fatty foods [EN 1528. 1996] use overall solvent extractions of fruits and GC-ECD or GC-MS analysis (Schenck *et al*., 2002; Lacassie *et al*., 1998; Anastassiades *et al*., 2003; Ripley et al., 2000; Navickiene *et al*., 2001). They all require a further time and solvent consuming clean up by column chromatography (Schenck *et al*., 2002), or gel permeation chromatography. Actual method development strives to reduce or even avoid any toxic solvent for sample preparation. Solvent – free sample preparation techniques base on the sorptive extraction of analytes by polymer coating on solid surface such as solid phase microextraction, and stir bar sorptive extraction (SBSE). Both procedures have been demonstrated as fast, efficient and environmental-friendly alternatives to LLE and SPE in pesticide determination from liquid matrix.

Solid phase microextraction introduced by Pawliszyn and co-workers allows simultaneous extraction and pre-concentration of analytes from sample matrix, eliminating some disadvantages of conventional techniques. The principles and applications of novel introduced sorptive extraction methods have been reviewed recently (Baltussen *et al*., 2002). Baltussen described and compared their new approaches of sorptive extraction. The most of the techniques presented such as SBSE or open tubular trapping (OTT), and gum-phase extraction (GPE) requires additional equipment for thermodesorption. In previous investigations concerning multiresidue screening of pesticides in vegetables and fruits the sorptive extraction techniques proved to be very sensitive and feasible in practice, also suited for automatic processing and high throughput analyses (Sandra *et al*., 2003). Solid phase microextraction has been applied to the analysis of a range of pesticides and can offer solution to the above issue at a low cost. SPME has been widely used for the analysis of pesticides in water (Lambropoulou *et al*., 2002; Goncalves *et al*. 2004; Beceiro-Gonzalez *et al*., 2007; Xu *et al*., 2007), fewer data exist on its application to the analysis in other matrices such as soil (Zambonin *et al*., 2000), sediment, food (Zambonin *et al*., 2000; Navalon *et al*., 2002; Kataoka *et al*., 2000; Chaia *et al*., 2009), and beverages (Zambonin *et al*., 2002; Zambonin *et al*., 2004). In this work we describe a method, proposed for the analysis of pesticide residues in apple juice, using a sample preparation step by SPME.

MATERIALS AND METHODS

Materials

Pesticide standards were supplied by different sources: cyprodinil, methidathion, penconazole, terbuthylazine (Ciba-Geigy / Basel, Switzerland), fenitrothion (Sumimoto Chemical Co. /Japan), chlorpyrifos (Dow Chemical Company / USA), kresoxim-methyl (BASF), myclobutanil (Dow Agro Science / USA), tebuconazole (Argovita), phosalone (Dr. Ehrenstorfer / Germany). All pesticide standards were used without any further purification.

For preparation of standard stock solutions, hexane from Merck (Darmstadt, Germany) was used. Working solutions of pesticides were prepared daily with bidistilled water or 1% (2%) apple juice. NaCl used was of analytical grade from Merck. 100% apple juice commercially available was used (Fructop, Ostratice, Slovakia).

Equipment

A manual fibre holder for SPME was purchased from Supelco (Bellefonte, Pa, USA). Two types of fibre, 100μm polydimethylsiloxane (PDMS) and 85μm polyacrylate (PA) were obtained from the same manufacturer. The fibres were conditioned as recommended by manufacturer. Magnetic stirrer (Heidoloph MR 3001) was used for stirring the sample during extraction. Chromatographic analysis was performed using a GC-MS system (Agilent Technologies, San José, CA, USA) with HP 6890 gas chromatograph equipped with an HP-5MS column (30 m x 0.25 mm I. D., film thickness 0.25μm) and a 5973 MSD for mass analysis.

Conditions

Working solutions were prepared by spiking 4 ml of bidistilled water and 1g of NaCl into 5 ml vials (Supelco). The extraction was carried out at room temperature for 30 min under magnetic stirring (1000 rpm). After extraction, the fibre was directly introduced into the hot injector of the gas chromatograph for 2 min. Thermal desorption was performed at 280 °C. Transfer line temperature was 280 °C. The oven temperature started at 50 °C and was increased to 280 °C at a rate of 15 °C min^{-1}. It was held for 10 min. Helium was used as carrier gas at a 55 kPa.

The detection of analytes was accomplished in electron impact ionisation mode (70 eV) by single ion monitoring, using the target ions in Table I. The assignment of analytes peaks in the GC-MS chromatograms based on the comparison of mass spectra and GC retention times of the reference pesticides used for method optimization.

I: *Typical fragment ions (m/z), retention times of the selected pesticides using GC-MS in SIM mode*

Pesticide	Molecular mass	Water solubility(a) (mg dm^{-3})	log Kow (b)	Retention time (min)	m /z
terbuthylazine	229.7	8.5 (20°C)	3.21	11.29	214, 229
pyrimethanil	199.3	0.121 (25°C)	2.84	11.41	198, 77
fenitrothion	227.2	14 (30°C)	3.43	12.51	277, 125
chlorpyrifos	350	1.4 (25°C)	4.7	12.79	314, 197
cyprodinil	225	20–13(pH) (25°C)	3.9	13.11	224, 210
penconazole	284	73 (20°C)	3.72	13.25	248, 159
methidathion	302.3	200 (25°C)	2.2	13.58	145, 85
myclobutanil	288	142 (25°C)	2.94	14.07	288, 179
kresoxim-methyl	313.4	2 (20°C)	3.4	14.15	206, 116
tebuconazole	307.8	36 (20°C)	3.7	15.15	250, 125
phosalone	367.8	3.05 (25°C)	4.01	16.13	367, 182

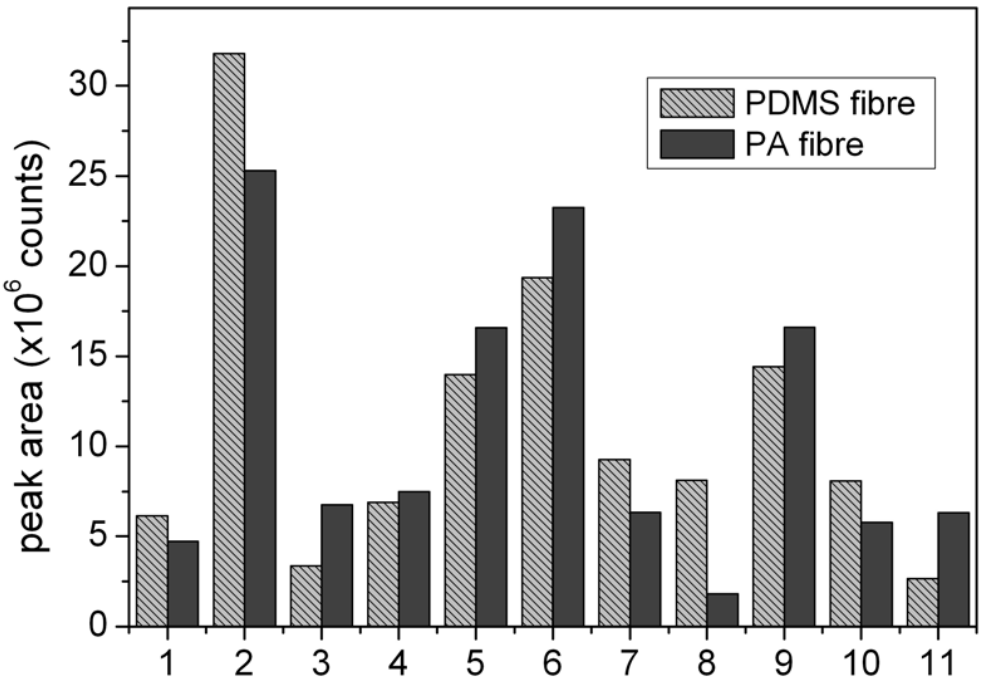
a – water solubility (The e-Pesticide Manual, Twelth Edition, Version 2.1, 2001–2002)
b – log of n-octanol-water partition coefficient (The e-Pesticide Manual, Twelth Edition, Version 2.1, 2001–2002

Preparation of standards

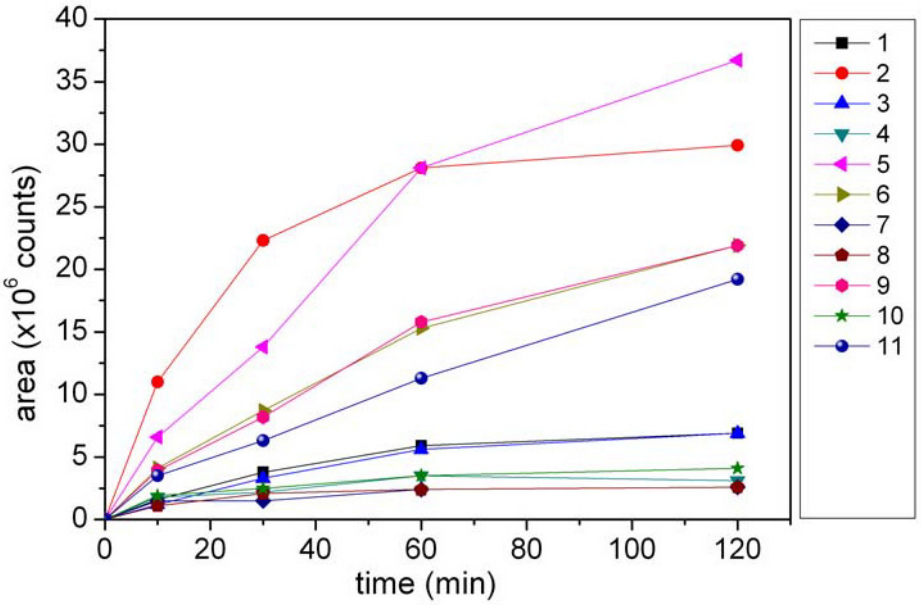
An individual solutions about of each pesticide were prepared in hexane with concentration 100 mg dm^{-3}. Stocks standard mixture (in hexane) containing each pesticide with the concentrations of 10 mg dm^{-3} was used. Working solutions with concentration of 0.05, 0.1, 0.25, 0.5, 1, 5 and 10 µg dm^{-3} were prepared daily by spiking 4 ml of bidistilled water and 1g of NaCl into 5 ml vials was add. 100% apple juice was diluted in water to 1% or 2% apple juice, 1g of NaCl into 5 ml vials was add and concentration levels of 0.1 and 10 µg dm^{-3} were tested.

RESULTS AND DISCUSSION

Conditions for SPME were tested using standard solutions of 5 µg dm^{-3}. Among the coating materials that are available from Supelco the 85µm PA fibre and the 100µm PDMS fibre are most suitable for the extraction of OPP (organophosphorus pesticides) and tiazols from water (Tomkins and Ilgner, 2002). Preliminary experiments were carried out to select the optimum fibre coating and extraction parameters affecting the SPME-GC-MS detection of the target pesticides. For comparison of the extraction efficiency of the different fibre coatings, a standard



1: *Comparison of PDMS and PA fibre at concentration level 0.01 ng mm^{-3}. 1-terbuthylazine, 2-pyrimethanil, 3-fenitrothion, 4-chlorpyriphos, 5-cyprodinyl, 6-penconazole, 7-methidathion, 8-myclobutanil, 9- kresoxym-methyl, 10-tebuconazole, 11-phosalone (Three parallel extractions were performed with three repeated GC measurements.)*



2: *Absorption time profile of 5 µg dm⁻³ of the pesticides in bidestillated water, c = (NaCl) = 250g. dm⁻³.* 1-terbuthylazine, 2-pyrimethanil, 3-fenitrothion, 4-chlorpyriphos, 5-cyprodinyl, 6-penconazole, 7-methidathion, 8-myclobutanil, 9-kresoxym-methyl, 10-tebuconazole, 11-phosalone (Two parallel sample extractions were performed with three repeated GC measurements).

solution of 5 µg dm⁻³ of each pesticide in water was sampled with PA (85µm) and PDMS (100µm) fibres. The fibre coating was directly exposed to the aqueous phase at room temperature. Due to the rather low vapour pressures and volatilities of the target pesticides headspace sampling would not be so successful. Both fibre coatings showed sufficient extraction yields to reach the trace concentration needed for the detection in apples and derived juice (Fig. 1). Finally, the PDMS fibre was preferred for the analyses in this work due to its robustness and known absorption phenomena those can be related to physico-chemical properties of the analytes and allows some mechanistical interpretation of data.

Extraction time

After the selection of the suitable fibre coating the next step in method development is addressed to the optimisation of the extraction time. Figure 2 shows the profiles of the extraction amounts versus extraction time. The absorption profiles for all selected pesticides were studied between 5 and 120 min in combination with 0.25 g cm⁻³ NaCl.

As shown in Fig. 1, 60 minutes extraction by PDMS is enough to reach the partition equilibrium of terbuthylazine, fenitrothion, tebuconazole, chlorpyrifos, methidathion, pyrimethanil and myclobutanil. Figure 2 shows too, that some compounds (cyprodinil, penconazole, kresoxim-methyl, phosalone) have not reached equilibrium even after 2h of absorption. Although maximum recommendable for practical work and large series of samples. In this case to fit for work the sensitivity is obtained by allowing the analytes to reach equilibrium, long extraction times are not analytical method should keep a reasonable extraction time, and operation under non-equilibrium conditions is acceptable. 30 min extraction time was found to be a good compromise between an adequate extraction time and a good response of the pesticide at acceptable reproducibility.

Carryover

To study carry over effect, blanks were run after every extraction of pesticides on all concentration levels used. No significant signals were obtained, which ensure a complete desorption of studied pesticides.

Linearity

After completing the method development, the method linearity was examined using PDMS sampling with GC-MS analysis. Aqueous standards were spiked with increasing concentrations of the pesticides over a range from 0.05 µg dm⁻³ to 10 µg dm⁻³ (working solutions with concentrations of 0.05 µg dm⁻³, 0.1 µg dm⁻³, 0.25 µg dm⁻³, 0.5 µg dm⁻³, 1 µg dm⁻³, 5 µg dm⁻³, 10 µg dm⁻³) and following conditions were chosen for the analytical method: 4 ml of solution with 1g of NaCl were extracted with PDMS with stirring at 1000 rpm and room temperature for 30 min. Regression analysis was performed to generate the linear equation of the calibration curve and the correlation coefficients were 0.988-0.9978.

Repeatability

To evaluate the precision of the measurements, analyses of standard solutions with concentration

II: *Limit of detection, limit of quantification, and precision of measurements with standard solution*
(0.5 μg dm^{-3}, n = 3)

Pesticide	LOD	LOQ	RSD
	(μg dm^{-3})	(μg dm^{-3})	(%)
terbuthylazine	0.007	0.025	2.8
pyrimethanil	0.001	0.002	4.5
fenitrothion	0.007	0.025	7.7
chlorpyrifos	0.008	0.026	7.4
cyprodinil	0.001	0.003	5.8
penconazole	0.005	0.016	5.9
methidathion	0.006	0.02	10.7
myclobutanil	0.025	0.08	3.6
kresoxim-methyl	0.002	0.008	3.8
tebuconazole	0.02	0.07	10.2
phosalone	0.009	0.03	8.6

LOQ= S/N ration = 10; calculated by MS software.
LOD= S/N ration = 3; calculated by MS software.
n = 3, RSD is the relative standard deviation for triplicate GC–MS analysis

0.5 μg dm^{-3} were performed in triplicate. The analyses led to RSDs of the peak areas to 20% (max.). Results for the 0.5 μg dm^{-3} standard solutions are presented in Table II. Spiked juice samples were analysed triplicate to evaluate the precision of the method: RSDs for apple juice are in Table III.

Spiked samples

In order to study the method with real samples, pure 100% apple juice was analysed under the same conditions as working standard solutions. The recoveries were found of about 30% in the analysis of pure apple juice of a concentration level of 0.5 μg dm^{-3} prepared by spiking. It leads to the conclusion that suspended matter or the high viscosity of the juice caused by saccharide content could interfere with the analysis. Perhaps the analytes diffusion to the fibre is delayed or reduced by competitive absorption or adsorption processes. The recoveries could be much improved by diluting the 100% apple juice. In the following experiments, 1% and 2% apple juice (appropriately diluted 100% apple juice in bidistilled water) spiked by representative pesticides at concentration levels 10 μg dm^{-3} and 0.1 μg dm^{-3} were analysed by GC-MS to evaluate the recoveries of the method.

In GC pesticide residues analysis in food matrices is well known matrix effects and mainly matrix-induced chromatographic response enhancement. In the injection port there is presence of various active sites (and also in a separation column) that can be responsible for irreversible adsorption and/or catalytic (thermo) decomposition of susceptible analytes. Besides free silanol groups and metals potentially present in a surface of even high quality glass injection liner declared by producers as "deactivated". The mentioned two effects can induce the recoveries of some pesticides higher than 100% as it shows Table III.

Limits of detection and limits of quantification

The limits of detection were estimated as the concentration of analytes that produce 3 times higher signal than the noise level. The LOQ is defined as the concentration of analytes that produce a signal equal 10 times the noise level. The calculated LOD and LOQ are listed in detail in Table II. The detection and quantification levels are low enough to guarantee a reliable detection of all pesticides in real apples and juices by a very simple, fast and reproducible multiresidue screening for quality control of food.

III: *Recoveries over fortified apple juice (spiked level 10 μg.L^{-3})*

Pesticides	1 % juice		2 % juice	
	recovery (%)	RSD (%)	recovery (%)	RSD(%)
terbuthylazine	98.1	7.2	102.1	9.1
pyrimethanil	19.5	6.3	16.4	9.1
fenitrothion	97.2	8.1	104.1	11.2
chlorpyrifos	81.3	3.3	76.2	12.1
cyprodinil	60.2	2.7	52.3	3.4
penconazole	101.1	7.2	105.2	11.1
methidathion	107.1	4.6	113.2	1.6
myclobutanil	94.4	6.2	114.4	2.1
kresoxim-methyl	94.5	8.1	95.3	8.3
tebuconazole	96.3	5.2	108.2	3.4
phosalone	96.8	9.1	87.7	11.1

Three parallel sample extractions were performed with three repeated GC measurements. n = 3, RSD is the relative standard deviation for triplicate GC–MS analysis.

SUMMARY

A practical GC-MS method in combination with direct SPME for the determination of selected pesticides in 100% apple juice is presented. The obtained LOQs are below the maximum residue limits fixed by European legislation (Council Directives 90/642/EEC, 1990). Sensitive responses were obtained using 100µm PDMS fibre, 0.25g cm^{-3} NaCl, 30min extraction time and room temperature in combination. Non-equilibrium conditions were adopted in order to reduce the extraction time. The complexity of pure apple juice matrix makes it difficult to obtain reproducible quantitative results of pesticides amounts. A dilution of the juice reduces the concentration of interfering components and a quantification of pesticides in the range between 0.05 µg dm^{-3}–10 µg dm^{-3} is solid.

Acknowledgement

This work was supported by the Slovak Grant Agency under the project No. 1/0647/11.

REFERENCES

ANASTASSIADES, M., LEHOTAY, S. J., STAJNBAHER, D., SCHENCK, F. J., 2003: Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International,* 86, 2: 412–431. ISSN: 1060-3271.

BALTUSSEN, E., CRAMERS, C. A., SANDRA, P. J. F, 2002: Sorptive sample preparation – a review. *Analytical and Bioanalytical Chemistry*, 373, 1–2: 3–22. ISSN: 1618-2642.

BECEIRO-GONZALEZ, E., CONCHA-GRANA, E., GUIMARAES, A., GONCALVES, C., MUNIATEGUI-LORENZO, S., ALPENDURADA, M. F. 2007: Optimisation and validation of a solid-phase microextraction method for simultaneous determination of different types of pesticides in water by gas chromatography-mass spectrometry. *Journal of Chromatography* A, 1141, 2: 165–173. ISSN: 0021-9673.

CORTES-AGUADO, S. SANCHEZ-MORITO, N., ARREBOLA, F. J., FRENICH, A. G., VIDAL, J. L. M., 2008: Fast screening of pesticide residues in fruit juice by solid-phase microextraction and gas chromatography-mass spectrometry. *Food Chemistry*, 107, 3: 1314–1325. ISSN: 0308-8146.

COUNCIL DIRECTIVES 90/642/EEC, 1990.

GONCALVES, C., ALPENDURADA, M. F. 2004: Solid-phase micro-extraction-gas chromatography-(tandem) mass spectrometry as a tool for pesticide residue analysis in water samples at high sensitivity and selectivity with confirmation capabilities. *Journal of Chromatography* A, 1026, 1–2: 239–250. ISSN: 0021-9673.

http://www.uksup.sk/index.php?n=25

KATAOKA, H., LORD, H. L., PAWLISZYN, J., 2000: Applications of solid-phase microextraction in food analysis. *Journal of Chromatography* A, 880, 1–2: 35–62. ISSN: 0021-9673.

LACASSIE, E., DREYFUSS, M. F., DAGUET, J. L., VIGNAUD, M., MARGUET, P., LACHATRE, G., 1998**:** Multiresidue determination of pesticides in apples and pears by gas chromatography mass spectrometry. *Journal of Chromatography* A, 805, 1–2: 319–326. ISSN: 0021-9673.

LAMBROPOULOU, D. A., SAKKAS, V. A., HELA, D. G., ALBANIS, T. A., 2002*:* Application of solid-phase microextraction in the monitoring of priority pesticides in the Kalamas River (NW Greece). *Journal of Chromatography* A, 963, 1–2: 107–116. ISSN: 0021-9673.

CHAIA, M. K., Tanb, G. H., 2009: Validation of a headspace solid-phase microextraction procedure with gas chromatography-electron capture detection of pesticide residues in fruits and vegetables. *Food Chemistry,* 117, 3: 561–567: ISSN: 0308-8146.

NAVALON, A., PRIETO, A., ARAUJO, L., VÍLCHEZ, J. L., 2002: Determination of pyrimethanil and kresoxim-methyl in green groceries by headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Journal of Chromatography* A, 975, 2: 355–360. ISSN: 0021-9673.

NAVICKIENE, S., RIBEIRO, M. L., 2001*:* A simplified method for the gas chromatographic determination of pyrimethanil residues in fruits. *Journal of Separation Sciences,* 24, 6: 470–472. ISSN: 1615-9314.

RIPLEY, B. D., LISSEMORE, L. I., LEISHMAN, P. D., DENOMME, M. A., RITTER, L., 2000: Pesticide residues on fruits and vegetables from Ontario, Canada, 1991–1995. *Journal of AOAC International,* 83, 1: 196–213. ISSN: 1060-3271.

SANDRA, P., TIENPONT, B., DAVID, F., 2003*:* Multiresidue screening of pesticides in vegetables, fruits and baby food by stir bar sorptive extraction-thermal desorption-capillary gas chromatography-mass spectrometry. *Journal of Chromatography* A, 1000, 1–2: 299–309. ISSN: 0021-9673.

SCHENCK, F. J., LEHOTAY, S. J., VEGA, V., 2002: Comparison of solid-phase extraction sorbents for cleanup in pesticide residue analysis of fresh fruits and vegetables. *Journal of Separation Sciences*, 25, 14: 883–890. ISSN: 1615-9314.

TOMKINS, B. A., ILGNER, R. H., 2002: Determination of atrazine and four organophosphorus pesticides in ground water using solid phase microextraction (SPME) followed by gas chromatography

with selected-ion monitoring. *Journal of Chromatography* A, 972, 2: 183–194. ISSN: 0021-9673.

XU, X., YANG, H., WANG, L., HAN, B., WANG, X., SEN-CHUN LEE, F., 2007: Analysis of chloroacetanilide herbicides in water samples by solid-phase microextraction coupled with gas chromatography-mass spectrometry. *Analytica. Chimica Acta*, 591, 1: 87–96. ISSN: 0003-2670.

ZAMBONIN, C.G, CILENTI, A., PALMISANO, F., 2002: Solid-phase microextraction and gas chromatography-mass spectrometry for the rapid screening of triazole residues in wine and strawberries. *Journal of Chromatography* A, 967, 2: 255–260. ISSN: 0021-9673.

ZAMBONIN, C. G., PALMISANO, F., 2000: Determination of triazines in soil leachates by solid-phase microextraction coupled to gas chromatography-mass spectrometry. *Journal of Chromatography* A, 874, 2: 247–255. ISSN: 0021-9673.

ZAMBONIN, C. G., QUINTO, M., DE VIETRO, N., PALMISANO, F. 2004: Solid-phase microextraction - gas chromatography mass spectrometry: A fast and simple screening method for the assessment of organophosphorus pesticides residues in wine and fruit juices. *Food Chemistry*, 86, 2: 269–274. ISSN: 0308-8146.

Address

Dipl. Eng. Andrea Hercegová, PhD., Department of Analytical Chemistry, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia, e-mail: andrea.hercegova@stuba.sk, Dipl. Eng. Monika Móder, PhD., Umweltforschungszentrum Leipzig-Halle, Permoserstraße 15, D-04318 Leipzig, Germany, e-mail: monika.moeder@ufz.de