Acta Chimica Slovaca, Vol.1, No. 1, 2008, 112 – 123

Fast GC, GC-MS and sample preparation methods for pesticide residue analysis in non-fatty baby food

Andrea Hercegová*, Eva Matisová

Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia

**andrea.hercegova@stuba.sk*

Abstract

Possibilities and limitations of a bench top mass spectrometric detector (MSD) in selective ion monitoring mode (SIM) and electron capture detector (ECD) in fast gas chromatography (GC) with narrow bore columns of samples with complex matrices at ultra trace levels of pesticide residues were studied and compared.. Various injection systems were searched (splitless, on-column, programmable temperature vaporiser).

Two narrow-bore capillary columns with the same stationary phase, phase ratio and separation power but different I.D. 0.15 mm and 0.10 mm were compared with regards to their advantages, practical limitations and applicability in fast GC under the conditions of separation/speed trade-off.

Fast GC-MS detection was applied to determination of selected pesticides in apples, the common raw material for baby food production in Slovakia, at the concentration level ≤10 μg kg^{-1} - maximum residual limit (MRL). Four sample preparation methods were compared for the ultratrace analysis of pesticide residues in baby food. The effectiveness of clean-up of the final extract was determined. Optimization of extraction and GC measurements as well as the simplifying of the whole process of sample preparation were carried out.

Keywords: baby food, fast GC, GC-MS, sample preparation

Introduction

Pesticide is a general term that includes a variety of chemical and biological products used to kill or control living organisms such as rodents, insects, fungi and plants (The Pesticides Safety Directorate, UK 2008b). Adverse effects on human health of pesticide residues remaining in food after they are applied to food crops are generally known: acute

neurologic toxicity, chronic neurodevelopment impairment, possibly dysfunction of the immune, reproductive and endocrine systems or cancer and many other. In the European Union (EU) approximately 320 000 tonnes of active substances are sold every year, which accounts for one quarter of the world market (PAHO/WHO 2005).

Residues in fruit and vegetables, cereals and foodstuffs of animal origin (and processed baby food) are controlled through a system of statutory Maximum Residue Limits (MRLs). MRLs defined as: 'The maximum concentration of pesticide residue (expressed as milligrams of residue per kilogram of commodity (mg kg^{-1})) likely to occur in or on food commodities and animal feeds after the use of pesticides according to Good Agricultural Practice (GAP)' (The Pesticides Safety Directorate, UK 2008a) are being set through a long-term European Commision (EC) programme establishing individual limits for different active substance/food commodity combinations. The lower values of MRLs are set for baby food - EC specified the MRL of 0.010 mg kg^{-1} (Commission Directive 2003/13/EC 2003). Scientifically valid methods of analysis at low concentration levels – currently still often very close to limits of quantification (LOQs) are essential for surveillance/compliance programs established with the terminal goal to minimize the hazards and the risks to health and achieving more sustainable use of pesticides.

The most suitable approaches in the determination of the pesticide residues contents in food samples are chromatographic methods with various sample preparation methods. In the field of GC there are obvious advantages of fast GC compared to conventional capillary GC: increased laboratory throughput; improved precision and accuracy; improved sensitivity; reduced costs (Matisová and Dömötörová 2003). Trends in GC are the ever increasing need for positive identification and the need for more flexible systems that allow the analysis of a wide variety of samples in one system. These trends clearly result in the need of mass spectrometric (MS) detection. In fast GC mostly time of flight (TOF) mass spectrometers are preferred due to their fast data acquisition rates. Quadrupole instruments have been most widely used in conventional capillary GC. Proving their abilities for adequate detection of narrow peaks without the loss of sensitivity would therefore help to extend the use of the fast GC to routine laboratories.

The most common row material for baby food production in Slovakia is apple. The matrix of apple is complex; therefore, the sample preparation represents one of the most critical parts of the whole analysis. In general sample preparation methods for non-fatty foods include liquid extractions followed by some clean-up method e.g. solid phase extraction (SPE) (an example is the Schenck's method published in 2002 (Schenck et al. 2002)), or

several alternative methods developed with the goal of solvent consumption reduction (e.g. matrix solid phase dispersion (MSPD), which includes homogenization, extraction and clean-up in one step (Berrueta 1995)). The research in the field of sample preparation methods of plant matrix nowadays heads towards faster, less laborious, less harmful and cost-effective procedures, preserving high recoveries, good precision and are multiresidual and rugged. In 2003, Anastassiades et al. (Anastassiades et al. 2003) developed quick, easy, cheap, effective, rugged and safe method (QuEChERS) aimed to overcome critical flaws and practical limitations of existing methods. The method is based on acetonitrile extraction followed by dispersive SPE (DSPE),that means, that the sorbent for clean-up is added directly to the extract.

The main aims were to explore possibilities of splitless injection system in fast GC with narrow-bore columns in trace and ultra-trace analysis of pesticides; compare two narrow-bore column I.D.s, 0.10 mm and 0.15 mm with the focus on the explanation and quantification of differences in speeding up the analysis, sample capacity, ruggedness and flexibility with respect to the practical operation; modify, evaluate and compare the different sample preparation methods combined with fast GC-MS for determination of pesticide residues in baby food.

Experimental

Chemicals

Standards of *n*-alkanes and pesticides were obtained from different sources and were of purity >95 %. Stock solution of *n*-alkanes C16, C18, C20, C22, C24, C26, C28 (Fluka, Buchs, Switzerland) and separately of *n*-alkanes C16, C22 and C28 were prepared in *n*-hexane (Suprasolv, Merck, Darmstadt, Germany) at approximate concentration 1 mg mL^{-1}. 21 pesticides belonging to different chemical classes were obtained from various sources and were of purity >95 %. Stock solution of pesticides was prepared in toluene (Suprasolv, Merck, Darmstadt, Germany) with approximate concentration 0.5 mg mL^{-1}. Stock solutions were stored at −18 °C and were diluted in toluene to get working standards. Standards were weighted on Sartorius Analytic MC1 scales (Sartorius, Göttingen, Germany).

Sample preparation

The apples with peel were homogenously mixed with blender Braun MX 2050 (Kronberg, Germany). The apples used for validation purposes were checked by GC-MS for studied pesticide residues and none of the selected ions were found at the corresponding retention times of selected pesticides.

Modified Shenck's method

25 g of apple sample was extracted with 50 mL of acetonitrile using sonication sonda of the pulsed ultrasonic cell disrupter VibraCell (Sonics and Materials Inc., Danbury, CT. USA, CVX 400, frequency 20 kHz). The ultrasonic pulses at 80 % amplitude with duration of 3 s paused for 3 s were applied for 1–5 min. The extract was filtered through glass fibre paper (Papírna Perštejn, Czech Republic), and the filtrate was transferred into an Erlenmayer flask with a tap. NaCl (2.5 g) was added and the mixture was shaken for 1 min. Phases were allowed to separate for 15 min. The upper acetonitrile phase was transferred into an Erlenmayer flask, anhydrous magnesium sulfate (2 g) was added and the mixture was shaken for 1 min. 25 mL of the dried extract were evaporated to less than 1 mL in a vacuum evaporator and transferred into SPE-NH2 column. Magnesium sulfate (1 cm layer) was always added to the top of SPE column; column was previously conditioned with acetone. The eluates were collected into 20 mL vials. Analytes were eluted with 15 mL of acetone and eluates were evaporated to dryness under a stream of nitrogen. The final volumes of the extracts were adjusted with toluene to 5 mL and analyzed by GC-MS. Preconcentration factor of the method is 2.5.

MSPD

5 g of a sample and 8 g of Florisil were blended in glass mortar, transferred into a I.D. glass column (250 × 15 mm) plugged with glass wool and containing a layer of 2.5 g anhydrous magnesium sulphate. The column head was covered with a second 2 mm layer of anhydrous magnesium sulphate. The column was eluted with 60 mL of ethyl acetate by gravitational flow. The eluate was collected, concentrated to dryness using a rotary vacuum evaporator, than the dry rest was dissolved in 1 mL of toluene (preconcentration factor 5).

QuEChERS

10 g of apple sample weighed into the 40 mL centrifuge tube was extracted with 10 mL of acetonitrile using Ultra-Turrax (IKA, Germany) homogenizer at 19000 min^{-1} for 3 min. Then liquid-liquid partitioning (LLP) followed: 1 g of NaCl and 4 g of $MgSO_4$ were added and the mixture was shaken by hand for 1 min. The mixture was than centrifuged at 3000 min^{-1} for 5 min. Portion of the upper layer was transferred into a 10 mL centrifuge tube containing 25 mg of PSA sorbent and 125 mg of $MgSO_4$ per 1 mL of the cleaned extract. The mixture was shaken by hand for 1 min, than centrifuged for 5 min at 3000 min^{-1} to separate solids from solution. Extract of minimum 1 mL was transferred into the vial evaporated under N_2 to dryness to perform solvent exchange to toluene at preconcentration factor 1.

QuEChERS modified in clean-up step. After the first centrifugation of the original QuEChERS method, 5 mL of the upper layer was transferred on SPE column filled with acetone conditioned 0.5 g of NH_2 sorbent covered with 1 cm layer of $MgSO_4$. SPE column was eluted with 10 mL of acetone. The cleaned extract was evaporated under N_2 to dryness, and the solvent exchange to 2 mL of toluene was performed (preconcentration factor 2.5).

Chromatographic instrumentation and conditions

Two narrow bore capillary columns CP-Sil 8 CB (Varian, Middelburg, The Netherlands) with poly(5 % diphenyl–95 % dimethylsiloxane) stationary phase (a) 15 m × 0.15 mm I.D. × 0.15 μm and (b) 10 m × 0.10 mm I.D. × 0.10 μm were tested. In all cases they were connected to a non-polar deactivated pre-column (1m long, 0.32 mm I.D., Supelco, Bellefonte, USA) via a press-fit connector 0.32–0.2 (0.1) mm (Agilent Technologies, Switzerland) and sealed with a polyimide resin (Supelco, Bellefonte, USA).

GC-FID/ECD

GC measurements were performed on a HP 6890 gas chromatograph (Hewlett-Packard, Little Falls, DE, USA) equipped with a split/splitless injector, an autosampler HP 7683, FID and ECD both operated at 320 °C with the rate of data acquisition of 50 Hz. Single tapered liner of 4 mm I.D. and direct liner of 2 mm I.D. (Agilent Technologies, Switzerland) were used for splitless injection, purge flow was set 160 mL min^{-1}. Split injector operated at 300 °C, split liner with cup (Agilent Technologies, Switzerland) was used. Bleed and temperature optimized septa (BTO, Agilent Technologies, Switzerland) were used. As a

carrier gas hydrogen (purity 99.99 %) was used (Linde, Technoplyn, Bratislava, Slovak Republic).

Other conditions on 0.15 mm I.D. column: Constant pressure mode was used, 260 kPa. Chromatographic separation was performed under a temperature program, 118 °C (1.25 min), 41.20 °C min^{-1}, 290 °C (0.6 min). Other conditions on 0.10 mm I.D. column: Constant pressure mode was used, 437 kPa. Chromatographic separation was performed under a temperature program, 138 °C (0.5 min), 64.20 °C min^{-1}, 290 °C (0.1 min).

GC-MSD

GC-MS measurements were performed on an Agilent 6890N GC coupled to 5973 MSD (Agilent Technologies, Little Falls, DE, USA) equipped with PTV and autoinjector Agilent 7683. MS with electron impact ionization (EI) mode (70 eV) was operated in SIM mode; for each pesticide two specific ions were selected and sorted into groups; the used dwell time was 10 ms. PTV was operated in cold splitless mode. The injection volume was 2 μL. Helium with purity 5.0 (Linde Technoplyn, Bratislava, Slovak Republic) was used as the carrier gas.

Other conditions on 0.15 mm I.D. column: Constant pressure mode 363.5 kPa was used until the elution of the last analyte (ethofenprox, 7.90 min), additional pressure ramp (1000 kPa min^{-1}, 685 kPa) was used to speed-up the elution of higher boiling matrix co-extractives. Chromatographic separation was performed under a temperature program, 130 °C (1.13 min), 27.25 °C min^{-1}, 290 °C (6 min). PTV conditions: temperature program, 150 °C, 400 °C min^{-1}, 300 °C (2 min), 400 °C min^{-1}, 350 °C (5 min); split vent open time 1.13 min; flow rate 160 mL min^{-1}. Other conditions on 0.10 mm I.D. column: Carrier gas flow programming was used: 0.5 mL min^{-1} (9.0 min), 5.0 mL min^{-1}, 0.8 mL min^{-1}. Chromatographic separation was performed under a temperature program, 115 °C (1.88 min), 27.60 °C min^{-1}, 290 °C (5.78 min). PTV conditions: temperature program, 149 °C, 400 °Cmin^{-1}, 300 °C (2 min), 400 °C min^{-1}, 350 (5 min); split vent open time 1.88 min; flow rate 100 mL min^{-1}.

Results and Disccusion

For ultra-trace analysis of pesticides non-splitting injection sample inlets were chosen and tested. Possibilities of increasing injection volumes in fast GC of pesticides with on-column injection were investigated with the combination of a normal-bore retention-gap and a narrow-bore analytical column (Dömötörová et al. 2005). A 1 m long retention-gap of inner

diameter of 0.32 mm allowed the introduction of 3 μL without any peak distortion. A retention-gap with internal diameter of 0.53 mm allowed the introduction of 8 μL of the sample with perfect symmetry of n-alkane peaks. However, the peaks of pesticides exhibited tailing.

Studies concerning splitless injection (Kirchner et al. 2004) proved that the most important parameter influencing maximal injection volume was found to be the retention-gap length; it must be capable to retain whole volume of recondensed solvent. There exists a certain possibility to "tune" its capacity by changing the initial oven temperature. Single tapered liner with internal diameter 4 mm allowed injection up to 8 μL, while direct liner with internal diameter of 2 mm allowed injection up to 5 μL. Peak shapes and peak broadening were not affected by injection volume. However, with not sufficiently clean extracts there is a problem with ruggedness. Programmable temperature vaporiser (PTV) injector enables large volume injection (>10 μL); it provides improved resistance to dirty samples. For fast GC, there is an important factor – small internal volume what results to faster sample transport (Kirchner et al. 2005).

Two narrow-bore capillary columns with the same stationary phase, phase ratio and separation power but different internal diameters 0.15 mm and 0.10 mm were compared with regards to their advantages, practical limitations and applicability in fast GC on commercially available instrumentation under the conditions of separation/speed trade-off (Dömötörová et al. 2006) If splitless injection is utilized, difficulties regarding focusation of solutes can occur. The main problem in method development is the different inlet pressure required to obtain speed optimised flow (SOF) conditions for columns with different I.D. and different carrier gases used (H_2 and He). For 0.10 mm I.D. column and He the required inlet pressure increased the boiling point of the solvent used (toluene) and thus its elimination from the retention gap was not effective enough what caused that the flooded zone moved into the analytical column and subsequent peak splitting occurred. Therefore, during the fast GC or fast GC-MS method development, it may be necessary to consider the change of the solvent and/or the carrier gas to H_2, if applicable. 0.10 mm I.D. versus 0.15 mm I.D. column provides speed gain of 1.74 with about 10 % decreased peak capacity in GC-FID analysis utilising splitless injection. For 0.15 mm I.D. column, the transfer of solutes from the inlet was more efficient then for 0.10 mm I.D. column resulting in higher responses, better measurements repeatability for splitless inlet and lower discrimination effects for split inlet. For determination of column capacity, symmetry factors provided more realistic results than peak widths. For 0.15 and 0.10 mm I.D. column capacity was determined to be approximately 0.5

and 1.5 ng, respectively. Narrow-bore capillary column with 0.15 mm I.D. showed significantly better ruggedness in real samples analysis (pesticide residues analysis in apple matrix) than column with 0.1 mm I.D. with regards to changes of peak areas and their repeatability during sequence consisted of 170 injections. While for 0.15 mm I.D. column no significant changes in peak areas occur, for 0.10 mm I.D. column, responses particularly of polar compounds significantly change. Also higher shifts of retention times were observed for 0.10 mm I.D. column. All detection searched with FID, ECD and MSD with quadrupole analyzer operating in SIM mode provide sufficient number of data point for proper peak reconstruction. The quality of quantitative data obtained is much more dependent on the injection, sample transfer process, than on the data acquisition rate of the used detector.

Efforts were given to study chromatographic induced response enhancement and ruggedness of the proposed sample preparation and GC-MS method (Kirchner et al. 2005). Responses of calibration standards of 18 pesticides (n = 5) in a neat toluene and matrix matched calibration standards were compared at a wide range of concentrations (0.025–2.5 ng μL^{-1}, corresponding to 0.01–1.0 mg kg^{-1} in apples). Response enhancement was found to be strongly dependent on the pesticide concentration. For the lowest concentration, response enhancement was in the range of 150 % for terbuthylazine to 750 % for bitertanol. With increasing concentration, it decreased. Another studied parameter was ruggedness of the GC-MS method. Responses of matrix matched calibration standards and standard solutions prepared in a neat toluene (n = 3) were assessed with regards to the number of real sample injections performed. The decrease of peak areas of pesticides caused by the deposit of non-volatile matrix constituents in the inlet liner was generally less than 25 % (for methidathion) within 130 injections. However, the exchange of the liner and the retention gap restored the responses to the initial values. The peak area repeatability of the matrix matched calibration standards injected in triplicate during the experiment was generally better than 6.6 %. Shift of the retention times was of the order of 1–2 s for 200 injections. Approximately 460 injections of real samples were performed with only mild deterioration of the GC column performance. The proposed fast GC-MS method proved good ruggedness with regards to the number of injections performed. The GC maintenance (inlet liner and retention gap exchange) should be performed after approximately 150 runs.

The possibilities of a bench top quadrupole mass spectrometer were evaluated for the qualitative and quantitative measurement of pesticides with fast GC separations using 0.15 mm I.D. narrow-bore capillary columns (Hercegová et al. 2005). It was found that the spectra acquisition rate had a great impact on sensitivity (peak areas and peak shapes). The quality of

the spectra obtained was not significantly influenced in the full scan-monitoring mode for the fastest acquisition rates. For quantitative analysis, the selected ion monitoring mode was fully able to acquire the sufficient number of data-points for the proper peak shape reconstruction and good repeatability of peak areas expressed by RSD (<5 %) for all tested dwell times. However, for shorter dwell times, the sensitivity was slightly lower. Utilization of 0.15 mm I.D. columns was found to be an ease way to gain fast GC separation while MS parameters method development for "slow scanning" instruments is not complicated.

Four sample preparation techniques representing different cleaning efficiencies were compared: a) Shenck's method; modified with the sample/solvent amount reduction and procedure simplifying related to the original method; b) QuEChERS method; c) QuEChERS modified with substitution of DSPE with cartridge based SPE; d) MSPD method. The methods were combined with fast GC/MS analysis. The effectiveness of clean-up of the final extract (expressed by those co-extractants which elute from the column under the given chromatographic conditions) was determined by comparison of chromatograms of unspiked apple samples in the full scan mode. Similar chromatograms were obtained for the modified Shenck`s and the modified QuEChERS method (both with concentration 2.5 g mL^{-1} of the final extract). Two times concentrated MSPD final extract (5 g mL^{-1}) shows different co-extractants, which with their intensity represent the worst burden on the chromatographic system. For all methods no significant interfering peaks were observed at the higher ions masses *m*/*z*. For lower, non-specific *m*/*z* ions (pesticides methidathion *m*/*z* 145, kresoxim-methyl 131, and etofenprox 163) more impurities were detected in the areas of interest in the case of the original and modified QuEChERS and MSPD methods; however, 15 m narrow-bore column with 0.15 mm I.D. provides satisfactory resolution. Modified Shenck`s method provides satisfactory cleaning also from interferences giving lower *m*/*z* ions. Time consumption, laboriousness, demands on glassware and working place and consumption of chemicals, especially solvents increase in the following order QuEChERS < modified QuEChERS < MSPD < modified Shenck`s method. All methods offer satisfactory analytical characteristics at the concentration levels of 0.005 mg kg^{-1}, 0.01 mg kg^{-1} and 0.1 mg kg^{-1} in terms of recoveries (method tested met commonly accepted validation requirements 70–110 % (Council Directives 94/43/EC 1994), the vast majority of recovery results exceed 80 %) and repeatability RSDs were <20 % (16)). Data are presented in Fig. 1 (Dömötörová et al. 2006). Differences due to analyte concentration were minimal in terms of recovery for all methods and nearly all analytes. Recoveries obtained for the modified QuEChERS method were lower than for the original QuEChERS. In general the best LOQs were obtained for the

modified Shenck`s method, Table 1. Notably for non-specific masses <170 *m*/*z* LOQs obtained for modified Shenck`s method were lower than for other three methods. Modified QuEChERS method provides by 21–72 % better LOQs than the original method for all pesticides analysed (except myclobutanil). LOQs of MSPD in some cases exceeds the LOQs obtained for the modified Shenck`s method by more than one order of magnitude. All methods reached the requirements of EU legislation on LOQs also for pesticides which shall not be used in agricultural production intended for the production of processed cereal-based foods and baby foods (Commission Directive 2003/13/EC 2003).

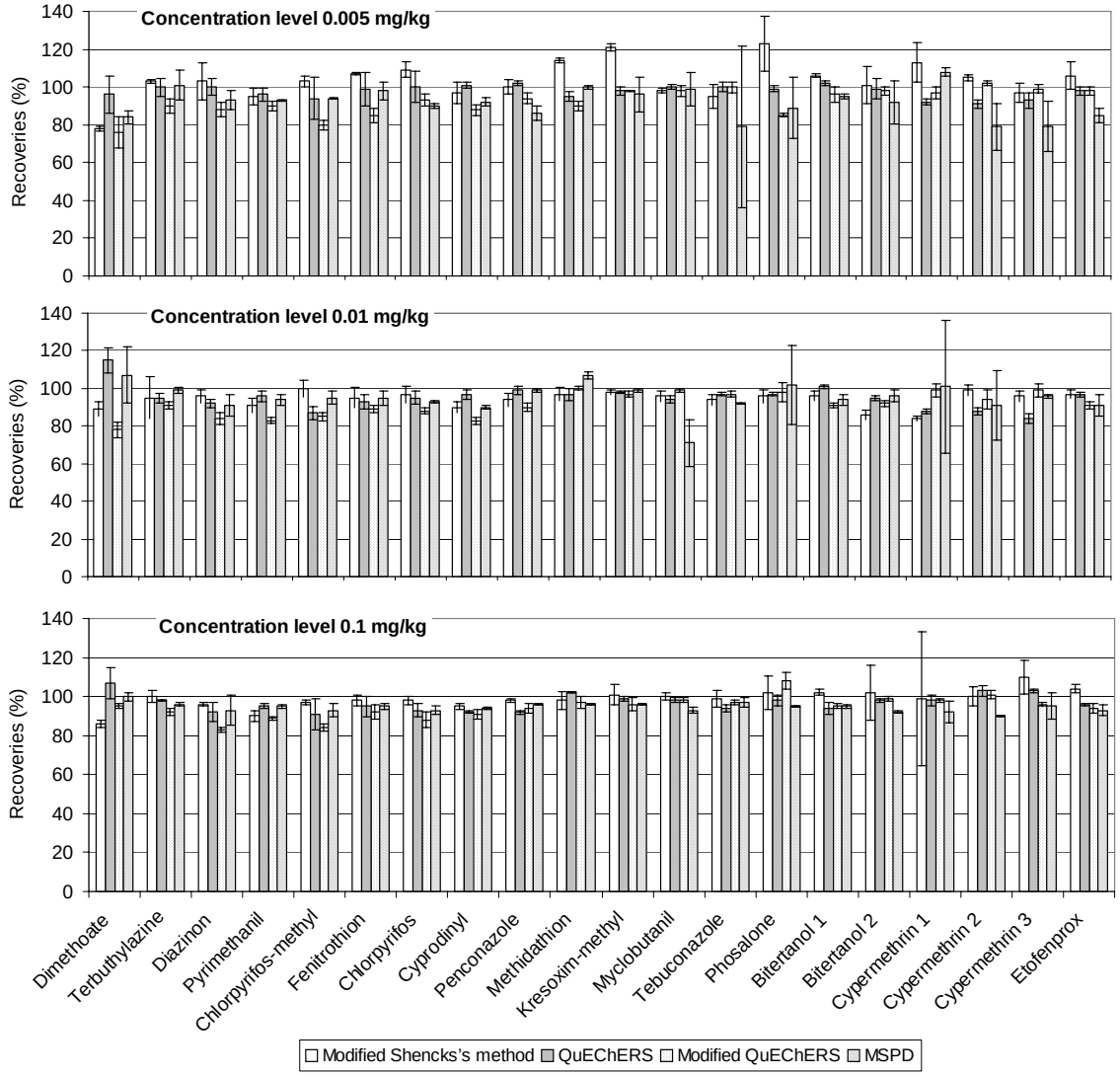

Fig. 1. Results of the recovery (R) experiments of pesticide residues from apples at spiking level 0.005 mg kg^{-1}, 0.01 mg kg^{-1} and 0.1 mg kg^{-1} (Hercegová et al. 2006)

Table 1. Limits of quantification (LOQs) (mg kg^{-1}) × 10^3 (Dömötörová et al. 2006)

Pesticide	Modified Shenck's method	Original QuEChERS	Modified QuEChERS method	MSPD
Dimethoate	0.38	0.54	0.30	2.67
Terbuthylazine	0.17	0.44	0.16	0.25
Diazinon	0.50	0.54	0.32	0.36
Pyrimethanil	0.07	0.15	0.07	0.07
Chlorpyrifos-methyl	0.18	0.19	0.08	0.06
Fenitrothion	0.33	0.40	0.22	0.16
Chlorpyrifos	0.46	0.69	0.34	0.30
Cyprodinyl	0.11	0.33	0.18	0.65
Penconazole	0.17	0.40	0.22	0.60
Methidathion	0.15	1.90	1.01	2.76
Kresoxim-methyl	0.22	1.05	0.65	0.68
Myclobutanil	0.14	1.15	1.44	2.00
Tebuconazole	0.29	1.52	0.43	0.59
Phosalone	0.73	1.52	1.20	0.57
Bitertanol 1	0.60	0.49	0.21	0.24
Bitertanol 2	1.44	2.31	1.02	2.25
Cypermethrin 1	0.08	3.22	2.33	1.42
Cypermethrin 2	0.38	2.04	1.31	2.64
Cypermethrin 3	0.17	3.97	3.43	5.03
Etofenprox	0.50	1.23	0.69	1.17

Acknowledgement

This work was supported by courtesy of NATO project No. SfP 977 983 and the Slovak Research and Development Agency under the contract No. 20-000705.

References

Anastassiades M, Lehotay J, Štajnbaher D, Schenck FJ (2003) Journal of AOAC International 86: 412-431.

Berrueta LA, Gallo B, Vicente F (1995) Chromatographia 40: 474–483.

Commission Directive 2003/13/EC (2003) Official Journal of the European Communities L41: 33–36.

Council Directives 94/43/EC (1994) Official Journal of the European Communities L227: 31.

Dömötörová M, Kirchner M, Matisová E, de Zeeuw J (2006) Journal of Separation Science 29: 1051–1063.

Dömötörová M, Matisová E, Hrouzková S, Hercegová A (2005) Chemia Analityczna (Warsaw) 50: 365–375.

Hercegová A, Dömötörová M, Kružlicová D, Matisová E (2006) Journal of Separation Science 29: 1102–1109.

Hercegová A, Dömötörová M,. Matisová E, Kirchner M, Otrekal R, Štefuca V (2005) Journal of Chromatography A 1084: 46–53.

Kirchner M, Matisová E, Dömötörová M, de Zeeuw J (2004) Journal of Chromatography A 1055: 159–168.

Kirchner M, Matisová E, Otrekal R, Hercegová A, de Zeeuw J (2005) Journal of Chromatography A 1084: 63–70.

Matisová E, Dömötörová M (2003) Journal of Chromatography A 1000: 199–221.

PAHO/WHO (2005) Proposed PAHO/WHO Plan of Action for Technical Cooperation in Food Safety, 2006–2007. In: FAO/WHO Regional Conference on Food Safety for the Americas and the Caribbean San José, Costa Rica, 6–9 December 2005. Available at ftp://ftp.fao.org/docrep/fao/meeting/010/af272e.pdf.

Schenck J, Lehotay SJ, Vega V (2002) Journal of Separation Science 25: 883–890.

The Pesticides Safety Directorate, UK (2008a) The Applicant Guide: Maximum Residue Levels. Available at http://www.pesticides.gov.uk/au_environment.asp?id=1251.

The Pesticides Safety Directorate, UK (2008b) The Background And The Need For Change. Available at http://www.pesticides.gov.uk/environment.asp?id=1523.