VALIDATION OF A SCREENING METHOD FOR ANALYSIS OF 49 PESTICIDES IN FOODS OF PLANT ORIGIN

Ivan Lj. Milovanović[1*], Aleksandra ć . Mišan[1], Bojana M. Beljkaš[1]

[1]Institute for Food Technology, Novi Sad, Serbia

UDC 543.632 : 632.95

Abstract: Since pesticides are potentially harmful to the environment and consequently to human beings through the consumption of pesticide contaminated food and water, food commodities have to be controlled to assure the non-violation of the maximum residue levels (MRLs) set by domestic and European regulations. In this work, a multiresidue GC-MS method for screening of 49 common pesticide residues in various foods of plant origin, which uses the QuEChERS method for sample extraction and cleanup, has been validated. Recovery values, linearity and reporting limits (RL) in various food commodities were determined for the analyzed compounds, and instrument performance was assessed by the use of mixture of internal standards. Eight pesticides did not show satisfactory recovery values or colud not be detected in levels required by the regulations, and were therefore excluded from the final scope of validation.

Key words: *Pesticides, Validation, QuEChERS, recovery, reporting limits*

INTRODUCTION

More and more different pesticides are used nowadays in agriculture. Since pesticides are potentially harmful to the environment and consequently to human beings through the consumption of pesticide contaminated food and water, the European Community established maximum residue levels (MRLs), based on the assumption that good agricultural practice is applied at the use of pesticides in farming, for pesticide residues in water (Commission of the European Communities, 2000) and foodstuff (Commission of the European Communities, 1990). As a consequence, food commodities have to be controlled to assure the non-violation of the MRLs. For apolar and middle polar pesticides, the detection of pesticide residues is commonly achieved through analysis with gas chromatography (GC) coupled to single quadropole (SQ) and, less frequently, triple quadropole (QQQ) mass spectrometers

*Corresponding author:
e-mail: ivan.milovanovic@fins.uns.ac.rs:
Tel: +381 21 485 3837: Fax: +381 21 450725

(MS) (Lesueur et al, 2008). The determination of pesticides in fruits and vegetables has been simplified by a new sample preparation method, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), and published recently as an AOAC Method 2007.01 (Lehotay, 2007).

In this work, a multiresidue GC-MS method for screening of 49 common pesticide residues in various foods of plant origin, which uses the QuEChERS method for sample extraction and cleanup (Anastassiades, 2006), has been validated. Recovery values, linearity and reporting limits (RL) in various food commodities were determined for the analyzed compounds, and instrument performance was assessed by the use of mixture of internal standards.

2. EXPERIMENTAL

2.1. Materials

Pesticide standards were purchased either from Dr. Ehrenstorfer or from Sigma–Aldrich with the highest available purity. QUECHERS kits for sample extraction and dispersive cleanup were purchased from Agilent Technologies. Ultra-residue reagent n-heptane and HPLC grade acetonitrile were purchased from Sigma-Aldrich, and p.a. grade sodium-hydroxide was purchased from J.T. Baker. Internal standards were purchased from Sigma-Aldrich with the highest available purity.

Standard mixes and single pesticide standards were used for preparation of standard solutions. Standard stock solutions of pesticides were prepared by dissolving the standard mixes with acetonitrile and diluting them to obtain 20 µg/ml concentrations. Stock solutions of single standards were prepared by dissolving 10 mg of each standard in 10 ml of acetonitrile and further diluted with acetonitrile to get 20 µg/ml concentrations. Working standard solutions were prepared by diluting the stock solutions with acetonitrile to get 1, 0.5, 0.25, 0.125, 0.0625 and 0.05 µg/ml concentrations. Internal standard solutions were prepared by dissolving 11.8 mg of antracene, 5.50 mg of tris (1,3-dichloroizopropyl) phosphate and 10 mg of PCB138 with acetonitrile to get 118. 5 and 100 µg/ml of each compound, respectively (ISTD 1). 100 µl of ISTD 1 was further diluted with n-heptane to obtain 1.18, 0.55 and 1.00 µg/ml concentrations of antracene, tris (1,3-dichloroizopropyl)phosphate and PCB138, respecttively (ISTD 2).

Blank samples used for fortification experiments were fruits, vegetables and grains, bought at local supermarkets, and presented no pesticide residues when analyzed by GC-MS.

2.2. Sample extraction and cleanup

The fruit and vegetable samples were prepared with the modified QuEChERS method (CEN/TC 275, N 236, 2006). Around 10.0 g of previously homogenized sample was weighed into the extraction tube, and distilled water was added so that the total amount of water (water in sample plus added water) was about 10 ml. A prepackaged mixture of $MgSO_4$ and sodiumcytrate buffers was added and the extraction tube was shaken vigorously for few seconds to dissolve the salts. pH of the mixture was checked and, if needed, set to 5-5.5 value with 20% NaOH. After that, 10 ml of acetonitrile was added to the extraction tube, and shaken vigorously during one minute, after which it was centrifuged at 3000 rpm for 10 minutes. After centrifuging, 6 ml of the acetonitrile layer was transferred to the cleanup tube containing the needed sorbents for each comodity type, shaken vigorously during one minute, and centrifuged at 3000 rpm for 10 minutes. 1 ml of extract was then transferred into 2 ml glass vial and the solvent was exchanged by evaporation under a gentle stream of nitrogen to dryness and dissolving the dry residue in 200 µl of n-heptane. The extract dissolved in n-heptane was then ready for analysis by GC-MS.

2.3. Analyses

The GC/MS analyses were performed on Agilent Technologies GC-MS Model 7890 A Series gas chromatograph coupled to 5975 C mass selective detector. A HP 5 MS (30 m × 0.25 mm i.d.) (J & W Scientific, USA) fused silica capillary column with a 0.25 µm film thickness was used with helium as carrier gas at a constant pressure (chlorpyrifos-methyl RT relocked to 16.596 min). 2.0 µl of the sample was injected in the splitless mode at 280 ^{o}C. The GC oven was operated with the following temperature

program: initial temperature 70 °C held for 2 min, ramped at 25 °C /min to 150 °C not held, followed by a ramp of 3 °C /min to 200 °C not hold, followed by another ramp of 8 °C /min to 280 °C held for 10 min. The total run time was 41.86 min. The interface was kept at 250 °C, the quadropole at 150 °C and the mass spectra were obtained at an electron energy of 70 eV. The Agilent Chemstation Software version D.02.00 was used for data analysis and the analyses were operated on the principle of a simultaneous full scan/SIM mode method presented elsewhere (Wylie, 2006). Several distinct scan/SIM methods were derived for each group of pesticides by modifying the principal analysis method (TRI_PEST.M), to increase the sensitivity of the pesticide screening and to minimize the effects of matrix.

2.4. Method validation

We selected 57 pesticides for the GC/MS analysis, based on their relevance in food samples and chromatographic properties, for the validation of the QuEChERS method according to the European SANCO Guideline (Commission of the European Communities, 2006) in 5 reference matrices: wheat (high starch content), rapeseed (high oil content), lettuce (high water content), tangerine (high acidity) and green tea ("difficult or unique commodities"). Due to a lack of immediate availability of all pesticide standards, the mentioned commodities were spiked only with organochlorine pesticide standard mix in three replicates at 0.1 and 1 mg/kg levels, and their recovery was determined. Linearity was determined from the calibration curve constructed for single standards. Reporting limits were determined by spiking lemon sample with mixture of the standards at 0.01 mg/kg levels, and 0.005 mg/kg level for *cis*- and *trans*-chlordane, as required by domestic and European regulations. Since SANCO Guideline also outlines requirements for GC-MS instrument performance, mixture of internal standards was used to assess the stability and repeatability of retention times and peak areas (Commission of the European Communities, 2006).

3. RESULTS AND DISCUSSION

According to the European SANCO Guidline, recovery of pesticides in quantitative analytical methods should be between 70-120%, although lower and higher values might be acceptable if the analyzed compound shows a good repeatability (Commission of the European Communities, 2006). Organochlorine pesticides in standard mix all showed a satisfactory values of recovery by the above mentioned criteria. When different matrices, spiked with organochlorine pesticides mix at 0.1 and 1 mg/kg levels were analyzed, recovery values were generally satisfactory, with most values above 70%. Lower recovery values were shown for endrin-aldehyde (35% in green tea, 44% in lettuce, and 44% in tangerine). Higher than recommended recovery values were found for ć-BHC (189% in green tea). The obtained recovery values fulfll the purpose of showing that the QuEChERS extraction and cleanup procedure gives satisfactory recovery values for different pesticides, and does not cause significant losses of desired compounds. This is in accordance with other published works which assessed the suitability of the QuEChERS method for pesticide analysis (Butler et al, 2008; Lesueur et al, 2008; Anastassiades, 2006).

Although the scope of the work vas to validate the analysis method for pesticide screening, it is possible to validate the same procedure as a confirmatory quantitative method for pesticide analysis, employing selected ion monitoring (SIM) GC-MS methods for quantification (Butler et al, 2008; Lesueur et al, 2008). This was also shown in results of spiking different food commodities with organochlorine pesticides at various levels (results not shown in this paper).

Linearity was calculated from calibration curves made for single pesticides, and the results are shown in Table 1. All pesticides showed a good linearity, with R^2 value above 0.97, except 4,4'-DDT which showed R^2 value of 0.95.

Table 1.
Linearity of pesticide calibration curves

method	compound	range (mg/kg)	equation	R^2
TRI_PESTSIMOP.M	dichlorvos	0.05-1	y=815408.84x+1294.73	0.98
TRI_PESTSIMOP.M	etoprophos	0.05-1	y=1052437.82x-31402.06	0.99
TRI_PESTSIMOP.M	disulfoton	0.05-1	y=2454390.68x-90075.89	1
TRI_PESTSIMOP.M	parathion-methyl	0.25-1	y=333993.84x-57029.07	0.98
TRI_PESTSIMOP.M	fenchlorphos	0.05-1	y=1835925.44x-87540.35	0.99
TRI_PESTSIMOP.M	chlorpyrifos	0.05-1	y=958808.29x-40622.64	0.99
TRI_PESTSIMOP.M	prothiofos	0.05-1	y=1264454.52x-39839.8	1
TRI_PESTSIMOCP.M	alpha-BHC	0.05-0.5	y=2428088.97x-63199.63	0.98
TRI_PESTSIMOCP.M	beta-BHC	0.05-0.5	y=658968.53x-19685.74	0.99
TRI_PESTSIMOCP.M	lindane	0.05-0.5	y=788128.61x-17003.63	0.99
TRI_PESTSIMOCP.M	heptachlor	0.05-0.5	y=432559.09x-10924.27	0.97
TRI_PESTSIMOCP.M	delta-BHC	0.05-0.5	y=222655.29x-10902.67	0.97
TRI_PESTSIMOCP.M	aldrin	0.05-0.5	y=987587.84x-2275.66	0.99
TRI_PESTSIMOCP.M	heptachlor-epoxyde	0.05-0.5	y=1211214.56x-21075.22	1
TRI_PESTSIMOCP.M	gamma-chlordane	0.05-0.5	y=1841353.57x-25764.88	1
TRI_PESTSIMOCP.M	alpha-chlordane	0.05-0.5	y=1893775.33x-31127.97	1
TRI_PESTSIMOCP.M	endosulfan I	0.0625-0.5	y=389662.34x-6791.99	1
TRI_PESTSIMOCP.M	4,4'-DDE	0.05-0.5	y=3816183.14x-68457.48	1
TRI_PESTSIMOCP.M	dieldrin	0.05-0.5	y=1864006.53x-30407.08	1
TRI_PESTSIMOCP.M	endrin	0.125-0.5	y=256920.99x-15041.87	0.99
TRI_PESTSIMOCP.M	4,4'-DDD	0.05-0.5	y=4431876.45x-153368.77	0.98
TRI_PESTSIMOCP.M	endosulfan II	0.05-0.5	y=403778.11x-1102.61	1
TRI_PESTSIMOCP.M	4,4'-DDT	0.05-0.5	y=723885.17x-37441.72	0.95
TRI_PESTSIMOCP.M	endrin-aldehyde	0.05-0.5	y=1050819.35x-24969.61	1
TRI_PESTSIMOCP.M	endrin-ketone	0.05-0.5	y=546932.25x+370.47	0.98
TRI_PESTSIMOCP.M	metoxychlor	0.05-0.5	y=1253695.08x-64067.56	0.97
TRI_PESTSIMTRIAZIN.M	simazine	0.05-1	y=1435149.27x-33292.71	0.99
TRI_PESTSIMTRIAZIN.M	prometon	0.05-1	y=1422293.67x-28250.21	0.99
TRI_PESTSIMTRIAZIN.M	atrazine	0.05-1	y=2558749.57x-73638.6	0.99
TRI_PESTSIMTRIAZIN.M	propazine	0.05-1	y=2439743.42x-92297.1	1
TRI_PESTSIMTRIAZIN.M	ametryn	0.05-1	y=3020996.61x-73263.13	1
TRI_PESTSIMTRIAZIN.M	prometryn	0.05-1	y=3208392.7x-98344.21	0.99
TRI_PESTSIMTRIAZIN.M	terbutryn	0.05-1	y=3075216.91x-106111.61	0.99
TRI_PESTSIMTRIAZIN.M	propiconazole	0.05-1	y=806332.19x-17901.88	0.98
TRI_PESTSIMPIRET.M	cyfluthrin	0.05-1	y=2747945.85x-11293.45	0.99
TRI_PESTSIMPIRET.M	cypermethrin	0.05-1	y=1373394.75x-78039.87	1
TRI_PESTMIX1.M	pirimiphos-methyl	0.25-1	y=2025807.55x-194942.79	0,99
TRI_PESTMIX1.M	malathion	0.5-1	y=1324626.09x-191174.49	1
TRI_PESTMIX1.M	dimethoate	0.5-1	y=1339398.05x-275515.27	1
TRI_PESTMIX2.M	difenoconazole	0.05-1	y=5458032.21x-378786.55	0.98
TRI_PESTMIX2.M	vinclozolin	0.05-1	y=728810.39x-24575.08	1
TRI_PESTMIX2.M	flutriafol	0.05-1	y=3648430.64x-300878.61	0.99
TRI_PESTMIX2.M	fenoxycarb	0.05-1	y=1992001.62x-126621.71	0.99
TRI_PESTMIX3.M	metalaxyl	0.25-1	y=1457691.4x-329830.59	0.99
TRI_PESTMIX3.M	hexaconazole	0.25-1	y=2423345.71x-679576.47	0.97
TRI_PESTMIX3.M	fenarimol	0.25-1	y=2782758.82x-678786.92	0.98

Reporting limit represents the lowest calibration point at which an analyzed compound can be determined with high certainty. Reporting limits were determined by spiking lemon sample with mixture of the standards at 0.01 mg/kg levels, and 0.005 mg/kg level for *cis*- and *trans*-chlordane, as required by domestic and European regulations, and the determined values are shown in Table 2. The obtained values were in accordance with the lowest maximum residue levels (MRLs) permitted by domestic

and European regulations. Of the 57 pesticides analyzed, eight compounds (azinphos-methyl, parathion-methyl, indoxacarb, acetampirid, cymoxanil, chlorothalonil, folpet and captafol) showed either very low responses, or could not be detected by GC-MS at the concentration levels required by the regulations.

Table 2.
Reporting limits determined for single pesticide standards

pesticide	reporting limit (mg/kg)	MRL in Serbia (mg/kg)	EU MRL (mg/kg)
aldrin	0.01	0.01	0.01
α-BHC	0.01	0.01	0.01
β-BHC	0.01	0.01	0.01
lindane	0.01	0.05	0.01
δ-BHC	0.01	0.01	0.01
α-chlordane	0.005	0.005	0.01
γ-chlordane	0.005	0.005	0.01
4,4'-DDD	0.01	0.05	0.05
4,4''-DDE	0.01	0.05	0.05
4,4′-DDT	0.01	0.05	0.05
dieldrin	0.01	0.01	0.01
α-endosulfan	0.05	0.1	0.05
β-endosulfan	0.05	0.1	0.05
endosulfan sulfate	0.05	0.1	0.05
endrin	0.01	0.01	0.01
endrin aldehyde	0.05	-	-
endrin ketone	0.01	-	-
heptachlor	0.01	0.01	0.01
heptachlor epoxide isomer B	0.01	0.01	0.01
methoxychlor	0.01	-	0.01
chlorpyrifos (Dursban)	0.01	0.05	0.05
dichlorvos	0.01	0.05	0.01
disulfoton	0.01	-	0.02
ethoprophos (MOCAP)	0.01	0.02	0.02
fenchlorphos (Ronnel)	0.01	-	0.01
prothiofos (Tokuthion)	0.01	-	-
pirimiphos-methyl	0.05	0.05	0.05
malathion	0.01	0.5	0.02
dimethoate	0.05	0.05	0.02
fenitrothion	0.01	0.05	0.01
ametryn	0.01	0.05	-
atrazine	0.05	0.1	0.05
prometon	0.01	-	-
prometryn	0.01	0.05	-
propazine	0.01	-	-
simazine	0.01	0.05	0.05
terbutryn	0.01	0.05	-
propiconazole	0.05	0.05	0.05
difenoconazol	0.01	-	0.05
flutriafol	0.01	0.02	0.05
cyfluthrin	0.02	20	0.02
α-cypermethrin	0.02	0.05	0.05
deltamethrin	0.05	0.01	0.05
fenoxycarb	0.01	-	0.05
vinclozolin	0.01	0.1	0.05
metalaxyl	0.01	0.05	0.05
azoxystrobin	0.01	-	0.1
fenarimol	0.01	0.1	0.02
hexaconazol	0.01	0.05	0.02

The mixture of internal standards was analysed in triplicates on a monthly basis, to assess the the stability and repeatability of retention times and peak areas. The obtained results showed less than 5% of difference in retention times, as well as less than 10% of difference in peak areas for single compounds, which is in accordance with the values required by SANCO for ensuring the correct instrument perfor-mance (Commission of the European Communities, 2006). Since a single internal stan-

dard compound represents the chromatographic properties of a larger group of pesticides, we have also spiked the mixture of internal standards into a representative matrix (wheat flour), which was then extracted and cleaned up by the QuEChERS procedure and analyzed by GC-MS. The results of the analysis of internal standards mixture and the spiked sample are shown in Table 3. The calculated recovery values were 93.49% for antracene, 120.64% for tris (1,3-dichloroizopropyl) phosphate and 95.02% for PCB138, which showed very good extraction and cleanup efficiency of the QuEChERS procedure. Chromatogram of the spiked sample is shown in the Fig. 1.

Table 3.
Retention times and responses of internal standards mixture and the spiked sample

	antracene	tris(1,3-dichloroizopropyl) phosphate	PCB138
ISTD2			
R.T.	14.070	26.798	27.110
QIon	178	75	360
Response	20347458	1589948	5173550
ISTD2 spike			
R.T.	14.071	26.799	27.112
QIon	178	75	360
Response	19023634	1918181	4915737

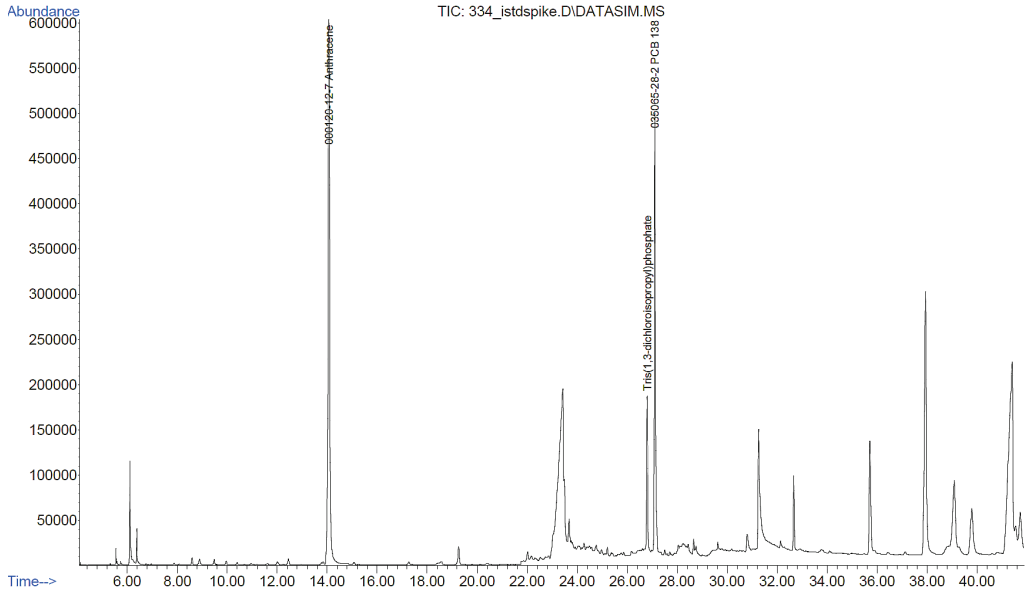

Fig. 1. Selected ion monitoring (SIM) chromatogram of the sample spiked with internal standards mixture

4. CONCLUSION

In this work, a screening method for determination of 49 different pesticides in various food commodities of plant origin has been validated. The obtained results fulfill the European SANCO Guideline requirements for screening analytical methods of pesticide traces in foods. Most of the analyzed pesticides showed good recovery values between 70-120%, which indicates that the QuEChERS extraction and cleanup method are well suited for analysis of pesticides in various, often difficult to analyze food commodities. Eight compounds (azinphos-methyl, parathion-methyl, indoxacarb, acetampirid, cymoxanil, chloro-thalonil, folpet and captafol) did not show satisfactory recovery values or colud not be detected in levels required by the regulations. These pesticides were excluded from the final scope of validation, and further possibility of

analyzing them by high performance liquid chromatography coupled to triple quadricpole mass detector (LC-QQQ) should be investigated.

ACKNOWLEDGEMENT

Original scientific paper was written as a result of work on a project TR20068 "Prehrambeni proizvodi za grupe potrošaća sa specijalnim zahtevima i potrebama", funded by Ministry of Science and Technological Development, Republic of Serbia.

5. REFERENCES

1. Anastassiades, M. (2006), QuEChERS - Mini-multiresidue method for the analysis of pesticides in low-fat products, *Application note (http://www.quechers.com)*.
2. Butler, J., Steiniger, D., Phillips, E. (2008), Analysis of pesticide residues in lettuce using a modified QuEChERS extraction technique and single quadripole GC/MS, *Technical note:10222, Thermo Fisher Scientific*, Texas, USA.
3. CEN/TC 275, N 236, Draft. (2006), Food of plant origin – Determination of pesticide residues using GC–MS and/or LC-MS/(MS) following acetonitrile extraction/partitioning and cleanup by dispersive SPE – QuECheRS-method, *European Comitte for Standardisation*.
4. Commission of the European Communities (1990), Directive 90/642/EEC on the fixing of maximum levels for pesticide residues in and on certain products of plant origin, including fruit and vegetables, *Directorate General for Agriculture*, DG VI B II-1, Brussels, Belgium.
5. Commission of the European Communities (2000), Directive 2000/60/EC establishing a framework for Community action in the field of water policy, *Directorate General for Agriculture*, DG VI B II-1, Brussels, Belgium.
6. Commission of the European Communities (2006), Directive SANCO/10232/2006 on the quality control procedures for the pesticide residues analysis, *Directorate of General Health and Consumer Protection*, Brussels, Belgium.
7. Lehotay, S. (2007), AOAC Official Method 2007.01, Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate, *Journal of AOAC International, 90 (2)*, 485-520.
8. Lesueur, C., Knittl, P., Gartner, M., Mentler, A., Fuerhacker, M. (2008), Analysis of 140 pesticides from conventional farming foodstuff samples after extraction with the modified QuECheRS method, *Food Control, 19*, 906-914.
9. Wylie, P., L. (2006), Screening for 926 pesticides and endocrine disruptors by GC/MS with Deconvolution Reporting Software and a new pesticide library, *Application note, Agilent Technologies*, Wilmington, USA.