Chemistry: Bulgarian Journal of Science Education *Volume 24 Number 3, 2015* *Природните науки в образованието*

Sixth International Conference
South-West University
Faculty of Mathematics & Natural Sciences
Blagoevgrad, Bulgaria 10 - 14 June, 2015

MODIFIED QUECHERS METHOD FOR DETERMINATION OF METHOMYL, ALDICARB, CARBOFURAN AND PROPOXUR IN LIVER

[1,2]**I. Stoykova,** [1]**T. Yankovska-Stefenova,** [2]**L.Yotova,** [2]**D. Danalev**
[1]*Bulgarian Food Safety Agency, Sofia, Bulgaria*
[2]*University of Chemical Technology and Metallurgy, Sofia, Bulgaria*

Abstract. N-Methyl carbamates insecticides are widely used in homes, gardens, and agriculture. Unfortunately, traces of them can further be found into animal tissues, milk, honey, eggs, etc. Therefore, food safety is an integral part of the EU policy for protection of consumer's health and maximum residue levels for pesticides are defined in specific Regulations. A modified QuEchERS method was developed and followed by selective analysis using a high performance liquid chromatography with fluorescence detector. This method was successfully validated for liver samples according to the recommendations in the document SANCO/12571/2013 and is proved to be reliable, accurate, precise and sensitive.

Keywords: QuEchERS, pesticides, N-methyl carbamates, HPLC

Introduction

Pesticides are an integral part of agriculture today, but their use can lead to residues in agricultural products. Because of their potential adverse human health effects, the Federal Government sets limits on allowable levels of pesticide residues in food and animal feed and monitors these products to enforce those levels.

Aldicarb, Methomyl, Propoxur and Carbofuran are insecticides that belong to N-Methyl carbamates group. Their effect to the organism is in inhibiting cholinesterase enzymes. Unfortunately, traces of them can further be found into animal tissues, milk, honey, eggs, etc.

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was originally developed for extracting a wide range of pesticides in fruit and vegetables has become very popular since it was introduced in 2003 by Anastassiades and coworkers . The method is characterized by using the polar solvent acetonitrile for extraction of water containing matrices with addition of salts in order to get phase separation.

Since its introduction, this method has been readily accepted by many pesticide residue analysts because of its low cost, fast, the accurate procedures are no time consuming. Evaporation steps or clean-up using traditional SPE in cartridges no are required required (Sack et al., 2011 ; Kolberg et al., 2010 ; Payá et al., 2007). The original QuEChERS method version used neutral extraction conditions by single phase extraction of multiple analytes with a small volume (10 mL) of acetonitrile followed by liquid -liquid partitioning with the addition of 4 g of anhydrous MgSO4 plus 1 g of NaCl. Removal of residual water and clean-up of polar residues are performed simulta neously using a dispersive solid -phase (d-SPE) clean-up. The d-SPE clean-up carried out by just adding a primary secondary amine sorbent (PSA) (Anastassiades et al., 2003), C-18 material (Lehotay et al., 2005) or other adsorbents directly to the acetonitrile extract. A quick shaking followed by centrifugation removes several interferences.

Analysis of pesticide residues in foods from animal origin includes many different methods for extraction and clean-up steps. Basically these methods need specific equipment as Soxtec system, Accelerate solvent extractor etc., that are expensive, moreover time consuming and solvent intensive (Beyer et al., 2008).

The QuEChERS approach is very flexible and it serves as a template for modification depending on the analyte properties, matrix composition, equipment and analytical technique available in the laboratory. The ruggedness characteristics of the QuEChERS approach have been thoroughly evaluated in the original (Anastassiades et al., 2003) and subsequent publications (Lehotay et al., 2010; Aguilera-Luiz et al., 2008; Cunha et al ., 2007; Lehotay et al., 2005). Therefore, a modified QuEChERS method that can be applied for liver would be appropriate alternative for laboratory's routine work, to save time and money.

Materials and methods

Reagents and chemicals

Acetonitrile and methanol (for HPLC gradient grade) were obtained from VWR, water was purified through a Milli-Q system from Millipore (USA). Anhydrous sodium sulphate was supplied by Supelco (USA) and was heated at least 6 hours at 600°C. Sodium chloride, PSA (primary and secondary amine), Bulk, from Supelco (USA).

Standard solutions

Five certified standards solutions were used: Aldicarb, 99,9% purity, Propoxur, 99,8% purity, Carbofuran, 99,0% purity, Methomyl, 100% purity and 4-Bromo-3,5-dimethylphenyl-N-methylcarbamate (BDMC), 99,5% purity. All of them were purchased from Dr. Ehrenstorfer (Germany). The working standard solutions were prepared at 3 levels-50%, 100% and 150% from the maximum residue level (MRL), for each compound according to its MRL, published in Regulation 396/2005 (Table 1). Standard solution for the internal standard (BDMC) was prepared only in concentration 1µg/ml. All standards were diluted with acetonitrile.

Table 1. MRLs for the target pesticides, according to Regulation 396/2005

Analytes	Carbofuran	Methomyl	Aldicarb	Propoxur
MRL [mg/kg]	0,01	0,02	0,01	0,05

Sample preparation

The liver was homogenized with mixer to generate representative sample of the product. 10 g of the homogenized product was measured into a clean 50 mL centrifuge tube. Further, 10 mL of 85:15 water and methanol (v/v) mixture, 10 mL acetonitrile and 100µl of an internal standard solution (1µg/ml) BDMC were added. The extraction salts (4g MgSO4 and 1g NaCl) were added and immediately sample was vortex or shake vigorously for 1 minute. Than the sample was centrifuge for 5 minute at 3000 U/min to separate the solid material. Further, aliquot of the the upper organic layer was transfer into another tube for cleanup and 50 mg PSA, 150 mg $MgSO_4$, and 50 mg C18 per mL extract were added. The sample was again vortex or shakes vigorously by hand for 1 minute and centrifuges for 3 minute at 3000 U/min. Final aliquot for analysis was transfer, evaporate under gentle stream of nitrogen, re-solve in 0,5ml acetonitril and put into vial for further HPLC determination.

HPLC-fluorescence analysis

Residue levels in liver samples were determined by reversed-phase high-performance liquid chromatography with fluorescence detection after postcolumn derivatization. The separation of the 4 aim carbamates is achieved with the Pickering 5µm, C_{18} column, maintained at constant temperature and with water-acetonitrile gradient as mobile phase (Table 2).

Table 2. Gradient of mobile phase

Time	Flow Rate (ml/min)	% A Water	% B Acetonitrile
Initial	0.80	85	15
2.00	0.80	85	15
20.00	0.80	59	41

20.10	0.60	59	41
23.00	0.60	59	41
24.00	0.80	0	100
25.00	0.80	0	100

The separated carbamates are first saponified by treatment with sodium hydroxide at 100°C. Further, the released methylamine reacts in post-column reactionaccording to Fig. 1, to form a highly fluorescent 1-methyl-2-dimethyl-ethylamine thioisoindole derivate.

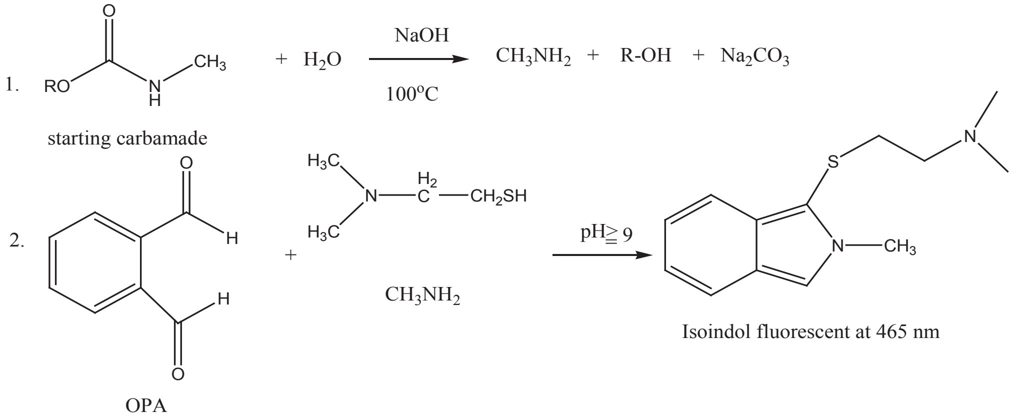

Fig. 1. The Principle of post-column derivatization reaction

Validation

The calibration curve was used for quantitative analysis. For the evaluation of recovery and precision (repeatability and within-laboratory reproducibility), blank liver samples spiked with mixed standard solution at three concentration levels of 50%, 100% and 150% of the MRL values for each compound were used. Spiked samples at each concentration level were analyzed in three series, each on the different day, and each in six replicates. The recoveries were calculated by comparing the determined concentrations of spiked samples to their target level. The precision was determined by calculating the coefficient of variation (CV). The working range was determined in relation to matrix-matched calibration curve of mixed standard solutions prepared on five levels. The linearity was checked by calculation of regression curve and correlation coefficients for each compound of mixed standard solution. The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated on the basis of blank sample analyses.

Results

The method was validated using liver samples, spiked at three concentration levels, according to the MRL values for each compound in mg/kg. Performance characteristics, such as linearity, LOD, LOQ, precision and recovery were studied. All validation parameters obtained for N-methyl carbamates determination in spiked samples are listed in Table 3. Estimated validation parameters of the method were satisfactory. The accuracy of the method was expressed as mean recoveries, and they were all higher than 75% for all spiked levels and all carbamates. The method showed good linearity and the LODs for the pesticides studied ranged from 0.0007 to 0.009 mg/kg.

Conclusions

A modified Quick Easy Cheap Effective Rugged and Safe (QuEchERS) extraction was developed and followed by selective analysis using a high performance liquid chromatography with fluorescence detector for the quantification of four N-methyl carbamates. This method was successfully validated for liver samples and is proved to be reliable, accurate, precise and sensitive.

The validation was performed with the recommendations of the document SANCO/12571/2013.[1)] This guidance document describes the method validation and analytical control requirements to support the validity of data used for checking compliance with maximum residue limits, enforcement actions, or assessment of consumer exposure to pesticides in the EU.

Table 3. Validation parameters obtained for liver matrix

Pesticide	Correlation coefficient R^2	Spike level [mg/kg]	Recovery % (n=18)	Repeatability CV, % (n=6)	Within-laboratory reproducibility CV, % (n=18)	Uncertainty (%)
Aldicarb	0,986	0,005	94,7	5,6	9,9	13,8
		0,010	101,4	5,5	10,5	
		0,015	97,2	2,9	7,3	
Methomyl	0,989	0,010	95,2	5,8	10,1	18,1
		0,020	98,1	4,6	13,4	
		0,030	96,7	3,9	8,3	
Carbofuran	0,985	0,005	75,6	5,0	9,4	14,1
		0,010	96,6	4,2	10,7	
		0,015	88,3	3,8	5,9	
Propoxur	0,998	0,025	90,9	4,8	9,2	16,3
		0,050	93,8	5,6	9,2	
		0,075	87,9	5,2	7,9	

Acknowledgment. This work was supported by the grant No. BG051PO001-3.3.06-0059, financed by the European Social Fund and Operational Program Human Resources Development (2007-2013) and Co-financed by Bulgarian Ministry and Education.

NOTES

1. http://ec.europa.eu/food/plant/pesticides/guidance_documents/docs/qualcontrol_en.pdf

REFERENCES

Aguilera-Luiz, M.M., Vidal, J.L.M., Romero-González, R. & Frenich, A.G. (2008). Multi-residue determination of veterinary drugs in milk by ultra high pressure liquid chromatograph- tandem mass spectrometry. *J. Chromatogr. A, 1205*, 10-16.

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. *J. AOAC Int., 86*, 412-431.

Beyer, A. & Biziuk, M. (2008). Applications of sample preparation techniques in the analysis of pesticides and PCBs in food. *Food Chem., 108*, 669–680.

Cunha, S.C., Lehotay, S.J., Mastovska, K. , Fernandes, J.O. , Beatriz, M. & Oliveira, P.P. (2007). Evaluation of the QuECHERS sample preparation approach for the analysis of pesticide residues in olives. *J. Sep. Sci., 30,* 620 -632.

Kolberg, D.I., Prestes, O.D., Adaime, M.B. & Zanella, R. (2010). Development of a fast multiresidue method for the determination of pesticides in dry samples (wheat grains, flour and bran) using QuEChERS based method and GC– MS. *Food Chem, 125*, 1436–1442.

Lehotay, S.J., Mastvoska, K. & Yun, S.J. (2005). Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. *J. AOAC Int., 88*, 630 -638.

Lehotay, S.J., Son, K.A., Kwon, H., Koesukwiwat, U., Fu, W., Mastovska, K., Hoh, E. & Leepipatpiboon, N. (2010) Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *J. Chromatogr. A, 1217*, 2548 -2560.

Payá, P., Anastassiades, M., Mack, D., Sigalova, I., Tasdelen, B., Oliva, J. & Barba, A. (2007). Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Anal. Bioanal. Chem., 89*, 1697 –1714.

Sack, C., Smoker, M., Chamkasem, N., Thompson, R., Satterfield, G., Masse, C., Mercer, G., Neuhaus, B., Cassias, I., Chang, E., Lin, Y., MacMahon, S., Wong, J., Zhang, K. & Smith, R.E. (2011). Collaborative validation of the QuEChERS procedure for the determination of pesticides in food by LC-MS/MS. *J. Agric. Food Chem*., *59*, 6383 – 6411.

🖂 **Ms. Iskra Stoykova** (corresponding author)
Central Laboratory of Veterinary Control and Ecology,
Bulgarian Food Safety Agency,
Sofia 1528, 5, Iskarsko Shousse, Blvd.
1528 Sofia, Bulgaria
E-mail: stoykova.iskra@abv.bg

.