# PESTICIDE RESIDUES IN CHICKEN EGGS - A SAMPLE PREPARATION METHODOLOGY FOR ANALYSIS BY GAS CHROMATOGRAPHY / MASS SPECTROMETRY

**Aleksandra M. Tasić[1]**

[1] *Institute of Veterinary Medicine of Serbia, Belgrade, Republic of Serbia*

## 1. Introduction

•The aim of this research was to adapt the QuEChERS method for routine pesticide multiresidue analysis in chicken eggs samples using gas chromatography coupled to mass spectrometry (GC/MS). The simultaneously analysis of a large number of pesticides in fatty foods has become a major challenge in analytics today ( Zanella et al, 2012). Fast analysis, consumption of small amounts of samples and reagents, high sensitivity and modern automation are some of most important goals during determination of pesticides. By using the QuEChERS method, the stated goals are successfully achieved.

•The difficulties of pesticide residue analysis in egg samples are caused by needed of remove of chemically non-related main components (e.g., organic matter, lipids, protein). The novel sorbent mixture, which are primarily present as a sorbent for fatty matrices, containing C18, some special kind of polymers, known as EMR – Lipid (Enhanced Matrix Removal – Lipid) and Florisil were applied in this study. Pesticide residues were extracted using the modified QuEChERS technique with acetonitrile and then purified using the dispersive solid phase extraction (d-SPE) clean-up. A sample preparation methods were developed for the analyses and validation in line with SANTE/12682/2019 guideline.

## 2. Materials and methods

### *Pesticide standards solution*

Stock standard solution (10 mg $L^{-1}$) was supplied by DR Ehrenstorfer (LGC, Germany). A stock standard solution was prepared in acetonitrile and stored at -18°C. Matrix-matched solutions were also prepared by serially diluting the intermediate solution with blank eggs sample extracts containing none of the tested analytes to perform matrix-matched calibration with the same concentrations as in the solvent. The use of matrix matched calibration solution is necessary to minimize errors associated with matrix induced enhancement or suppression effects during GC-determination.

### *GC/MS Analysis*

Quantification and quantification of analytes was carried out using a GC Clarus 680 PerkinElmer system comprising an autosampler and a gas chromatograph interfaced with an MS Clarus SQ8T instrument under the following conditions: capillary column Elite-5MS (30 x 0.25 mm ID x 0.25 µm df, composed of 95% dimethylpolysiloxane and 5% phenyl), operating in the electron impact mode at 70 eV.

### *Extraction procedure*

Eggs was obtained from a local market. The samples were homogenised and weighed at 5g each in a 50-mL centrifuge tube, 10 ml of acetonitrile was added and vortex for 3 min. Then 1 g NaCl and 4 g anhydrous $MgSO_4$ were added to mix, after vortexing for 3 min, the tube was centrifuged at 4000 rpm for 5 min. The supernatant was clean up using three different clean up procedures.

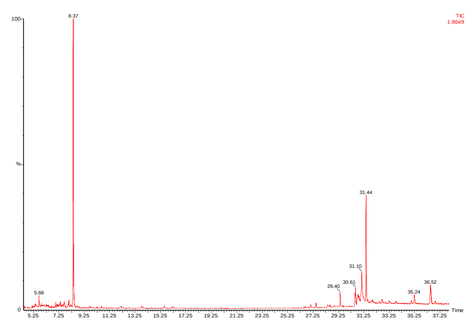
Three different clean-up approaches were tested: d-SPE with Enhanced Matrix Removal-Lipid (EMR-Lipid), combination of primary secondary amine (PSA) and C18 sorbents, and for the third preparation used florisil cartridge (tubes, 170 µm, 80 Å).

## 3. Results and discussion

Compared to the currently established EN 1528 method, this validated method uses much less solvent, it is quick and easy to prepare a sample for analysis. The linearity of the analytical response across the studied range of concentrations (0.010 - 0.10 mg/kg) was excellent, obtaining correlation coefficients higher than 0.99.

The effect of purifying agents on the recovery of pesticide were investigated by recovery experiment (fortified level 0.02 mg/kg), and the results are listed in Table 1. The recoveries of pesticides after purifying by PSA, $MgSO_4$, C18 and EMR-Lipid were relatively close, and both were better than Florisil cartridge. The matrix effect is another important parameter to evaluate purification effect (Fig.1 and Fig. 2). The matrix effect after using EMR-Lipid was much lower (Fig. 2.) than using PSA and C18. By comparing the chromatograms, it was found that the peak at 36.76 min from Cholesterol almost disappeared after EMR-Lipid purification. In conclusion, EMR-Lipid had better purification effect, and more suitable for the detection of all pesticides residues except acephate and propoxur.

The average recoveries of the pesticide for Florisil ranged from 51.6 to 101.1%, for fortification levels of 0.02 mg $kg^{-1}$. This results of recovery is not satisfactory (70-120%) for the prescribed guide (SANTE, 2019). The precision values associated with the analytical method, expressed as RSD values, were less than 20 % for the pesticide in the egg matrixes. Limit of quantification were 0.01 mg $kg^{-1}$ for the majority of the pesticides.


*GC-MS chromatograms of chicken egg samples in the full scan*

***Fig. 1. using*** **PSA, C18, $MgSO_4$** ***Fig. 2.*** *using* **EMR – Lipid**

Table 1. Average recovery (RSD), % using three different purifying agents

| | PSA, C18, $MgSO_4$ | EMR – Lipid | Florisil |
|---|---|---|---|
| Acephate | 71.2 (6.8) | Nd* | Nd* |
| Propoxur | 70.8 (9.2) | Nd* | Nd* |
| Ethoprophos | 76.4 (7.5) | 73.2 (8.5) | Nd* |
| Alpha HCH | 117 (7.9) | 71.6 (3.9) | 51.6 (11.8) |
| Dimethoate | 103.6 (9.5) | 84.0 (5.7) | 87.1 (9.1) |
| Carbofuran | 84.4 (5.2) | 81.1 (5.6) | 71.9 (5.2) |
| Beta HCH | 81.2 (7.1) | 92.5 (8.9) | 72.5 (7.7) |
| Lindan | 107 (4.9) | 107.1 (3.3) | 63.8 (10.8) |
| Diazinon | 80.4 (3.2) | 88.8 (4.9) | 65.5 (10.0) |
| Delta HCH | 76.4 (8.1) | 79.4 (5.2) | 75.5 (6.8) |
| Methyl Parathion | 75 (5.5) | 119.9 (4.5) | 59.9 (11.9) |
| Spiroxamine | 107.2 (6.4) | 99.1 (8.0) | 87.5 (8.1) |
| Heptachlor | 80.6 (9.0) | 119.4 (7.9) | 89.4 (15.2) |
| Carbaryl | 74.4 (8.6) | 99.8 (6.6) | 59.8 (11.5) |
| Metalaxyl | 73 (9.3) | 94.6 (11.9) | Nd* |
| Methiocarb | 108.8 (5.2) | 118.9 (5.2) | 101.1 (13.2) |
| Aldrin | 84 (8.2) | 98.9 (7.4) | 72.5 (5.5) |
| Malathion | 103.4 (9.4) | 82.8 (11.5) | 82.8 (6.8) |
| Chlorpyrifos | 90.6 (9.1) | 105.4 (12.4) | Nd* |

Nd* = not detected

### *Real samples*

The validated QuEChERS method with GC/MS determination in the SIR mode was applied to the 15 egg samples. In the two of the samples amount of Chlorpyrifos were between 10 and 30 µg/kg. This result is above the MRL (maximum residue level) which is 10 µg/kg from the COMMISSION REGULATION (EU) 2018/686 of 4 May 2018 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament.

## 4. Conclusion

A simple and rapid method was developed to determine pesticide residue in chicken eggs. The method which consist of a QuEChERS fast sample preparation, PSA, C18, $MgSO_4$ clean up and GC/MS analysis showed a high sensitivity and enough low matrix effect for routine determination. During analysis, the matrix-matched calibration curves were used because that minimizes the matrix interferences leading to higher accuracy for pesticides analyzed. The use of PSA, $MgSO_4$, C18 in combination with freezing-out showed important advantages such as pesticides recovery between 70-120% range and no pesticides losses when compered with other clean-up procedures evaluated in this study such as EMR-Lipid and Florisil sorbent.

## ***Reference***

1. *Zanella R, Prestes O.D., Friggi C.A., Martins M. L., Adaime M.B., An Overview About Recent Advances in Sample Preparation Techniques for Pesticides Residues Analysis in Cereals and Feedstuffs, Doi: 10.5772/46846*
2. *Anastassiades, M., Lehotay, S. 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 2003, 86(2), 412-431. DOI: 10.1093/JAOAC%2F86.2.412*