LARGE SCALE MULTI-RESIDUE METHODSBY GAS AND LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY FOR THE DETERMINATION OF PESTICIDE RESIDUES IN FOOD MATRICES

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Abstract– Nowadays, more than 1600 pesticides belonging to more than 100 chemical classes are in use worldwide for food production. In the EU, Regulation (EC) 396/2005 sets maximum pesticide residue limits in food (MRLs), the majority of which ranges over the concentration range 0.01-1 mg / kg. Large scale multi-residue methods based on general extraction procedures in combination with hyphenated instrumental analysis techniques such as gas and liquid chromatography with mass spectrometry are currently used in analytical laboratories working in the field of Pesticide Residues Analysis. In our laboratory, a multi-residue method based on QuEChERS method in combination with LC-MS/MS and GC-MS/MS techniques has been developed for the detection and quantification of pesticide residues, parent compounds and their metabolites, in various food matrices, fulfilling the performance criteria established in the European Union guidelines (SANTE Doc. No 11945/2015). A LC-MS/MS method using QqQ-MS technology in the SRM acquisition mode has been developed for the analysis of more than 250 compounds. Additionally, a GC-MS/MS method using Ion-Trap MSn technology has been applied for the determination of more than 80 GC-amenable compounds. More than 2000 pesticide/food commodity combinations have been validated using the LC- and GC-MS/MS methods, achieving recoveries in the range 70-120% with reproducibility values in terms of relative standard deviations less than 20%; limits of quantification of 0.01mg/Kg were established in most of the cases. Both methods have been successfully applied for the monitoring of multiclass pesticide residues in food samples in the frame of National and EU - Multi Annual Coordinated Control Programs.

Keywords: *pesticides, LC-MS/MS, GC-MSn*

1. INTRODUCTION

The health impact of intake of pesticides from foods of plant and animal origin is the subject of on-going research and monitoring studies implemented, in the countries members of European Union, in the framework of Community and National coordinated annual monitoring programs on residues. The maximum permitted residues levels of pesticides in foodstuffs, set in the Regulation (EC) 396/2005 [1], are varying in the concentration range 0.01-10 mg/kg. Multiple and chemically distinct groups of pesticides- triazoles, strobilurins, benzoïmidazoles, carbamates, pyrethrins, organoSOIL phorous compounds, sulfonylureas, acidic pesticides, triazines and others -have been approved for use in agricultural practice according to the requirements opposed in the Regulation (EC) 1107/2009 [2].

Control of pesticide residues - parent compounds and metabolites -requires the development of dynamic multi- residue methods with inherent capability of continuous expansion of their analytical field to new compounds. Currently, the multi-residue methods rely on generic extraction methods [3] and on instrumental analytical techniques capable to identify multiple compounds such as mass spectrometry hyphenated with gas or liquid chromatography, like GC-MS, GC-MS/MS [4] and LC-MS/MS [5].

The challenge for analytical laboratories working in the field of pesticide residues, is the continuous expansion of their scope to new compounds and new substrates in a rational way of saving time and resources.

In this study, the experimental design of extension and modification of the multi-residue method used in our laboratory, is presented, aiming to maximize the performance, both in number of target compounds and substrate, to reduce the time and cost of analysis of samples with no sacrifice on detectability and sensitivity. The number of compounds included in the scope of our laboratory was significantly increased to 350, analyzed with GC-MS/MS and LC -MS/MS methods. A significant number of pesticide/commodity combinations have been validated according to the requirements set by European guidelines in the SANTE Doc. No 11945/2015[6]. The methods have been successfully applied in numerous samples of official control and of proficiency testing schemes.

2. EXPERIMENTAL

2.1 Materials.

Certified pesticides standards used were purchased from Dr. Ehrenstorfer, Sigma-Aldrich and ChemService in the highest purity available. Pesticide stock solutions standard substances at concentration levels > 100-500 μg mL-1 were prepared in pestiscan grade acetonitrile or acetone and kept at-20 °C. Pesticides mixtures were prepared from the stock solutions at the concentration level of 10 μg mL-1 in acetonitrile and kept at-20 °C. These mixtures were used for the preparation of the calibration solutions and for spiking samples used in the validation experiments. Ready to use QuEChERS citrated salts mixtures were purchased from Supelco, Sigma-Aldrich Co,LLC. Acetonitrile and methanol of LC-MS grade, ammonium formate of LC-MS grade additive from Merck (Darmstadt, Germany) and formic acid from Sigma-Aldrich House (Steinheim, Germany) were used for the preparation of LC-MS mobile phases. All solvents were of pesticide grade purity and.

2.2.Methods

2.2.1. Sample preparation

An amount of 10-g homogenized sample was extracted with 10 ml of acetonitrile, acidified with 1% acetic acid, under mechanical shaking for 30 min. In the case of dried samples like cereals, 5 g of the sample was soaked with water before extraction. The first salt mixture as described in the QuEChERS method EN 15662 [7] was added followed by shaking and centrifugation. A 5 ml portion of the supernatant solution was further cleaned up with PSA and MgSO4 before subjected to LC-MS/MS and GC-MS analysis/MS.

2.2.2. LC-MS/MS analysis.

A TSQ Quantum Ultra equipped with Max API Source from Thermo Scientific USA, coupled to a liquid chromatography system consists of a Surveyor LC pump and an automatic sampler Surveyor (ThermoElectron, Usa) used for the LC-MS/MS analysis. Separation of target compounds was performed on the Synergi Fusion RP-18 LC column (4μm, 50mmx2mm) with a gradient of two mobile phase, one of water and the other of methanol both containing 0.1% formic acid and ammonium formate 10mM, at a flow rate 0.35-0.4 ml/min. The LC run time was 12 minutes.The software used for data collection and processing is the Xcalibur 2.0 version.

2.2.3. GC-MSn analysis.

The GC-MS system consists of a gas-chromatograph Trace GC Ultra, with a PTV injector (ThermoScientific., USA) and of an ion trap mass detector Polaris Q, (ThermoScientific., USA) equipped with an ion source with electron ionization. The autosampler is a TriPlus-AS from ThermoScientific, USA. Software for data collection and processing is the Xcalibur (version 2.07). The separation of compounds was performed on a DB-35MS capillary column, (0.25 mm internal diameter, length 30 m, film thickness 0, 25μm). The ionization
mode is electron ionization , EI, 70 eV.

2.3 Method Validation

Accuracy (trueness and precision) was studied by recovery tests using “blank” samples free of residues. Samples were spiked with pesticide standard solutions at two concentration levels, usually 0.010-0.050 mg Kg-1. Five to six replicates at each concentration level were analyzed using the above procedures, on different days, according to the requirements of the SANTE No 11945/2015, for validation of methods used for the determination of pesticide residues in food.

3. RESULTS AND DISCUSSION

The accuracy of the multi-residue methods was studied in recovery experiments at the concentrations, 0.010 and 0.050 mg Kg-1 of sample. The recovery rates were between 70 and 120% and precision in terms of Relative Standard deviation of the inter-laboratory reproducibility was less than 20% for the majority of the studied compounds. Method performance is considered satisfactory according to the performance criteria of the SANTE 11945/2015. Based on the method validation data, the quantification limit can be set at the concentration of 0.010 mg Kg-1 while the limits of detection were ranging at lower concentrations.

The method performance was tested by participation in Proficiency tests such as the EUPTs organized by the European Union Reference Laboratories, and others like SCHEMA PTs, COI PTs. The majority of z-scores were in the acceptable range (| 0-2 |).

The methods was applied in real samples, processed fruits and vegetables, baby foods, cereals, juices as well as in foodstuffs of animal origin and oils, slightly modified.

4. CONCLUSIONS

The multi-residue method with LC-MS/MS and GC-MS/MS is capable of reliable detection and quantification of a large number of pesticides at low concentrations. The developed method increased the scope of the laboratory and the effectiveness of controls while saving time and resources. The method reliability was demonstrated through successful participations in numerous proficiency testing schemes and (EU-PTs, SCHEMA-PTs, COI-PTs).

REFERENCES

[1]. Regulation (EC) 396/2005. REGULATION (EC) NO 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC Official Journal of the European Union, 16-3-2005, L70, 1-16.

[2].Regulation (EC) 1107/200. REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union, 24-11-2009, L309, 1-50.

[3]Y. Picó (2015). Advanced Sample Preparation Techniques for MS Analysis, in Mass Spectrometry for the Analysis of Pesticide Residues and their Metabolites. D. Tsipi, H. Botitsi, A. Economou (eds) John Wiley & Sons, Inc., Hoboken, New Jersey, pp. 53-89.

[4] K. Banerjee, S. Utture (2015). Recent Developments in Gas Chromatography-Mass Spectrometry, in Mass Spectrometry for the Analysis of Pesticide Residues and their Metabolites. D. Tsipi, H. Botitsi, A. Economou (eds) John Wiley & Sons, Inc., Hoboken, New Jersey, pp. 91-112.

[5] A. Lozano, L. Rajski, M. del Mar Gomez-Ramos, C. Ferrer, M.D. Hernando, A.R. Fernandez-Alba (2015). Liquid Chromatography-Mass Spectrometry Analysis of Pesticide Residues and their Metabolites in Food Samples, in Mass Spectrometry for the Analysis of Pesticide Residues and their Metabolites. D. Tsipi, H. Botitsi, A. Economou (eds) John Wiley & Sons, Inc., Hoboken, New Jersey, pp.187-205.

[6] "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed" Document No SANCO 11945/2015. http://www.eurl-pesticides.eu

[7] European Standard EN 15662: "Foods of Plant Origin - Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE (QuEChERS method)" (www.cen.eu).