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Multi-residue analysis for the determination of pesticide residues in fruit and vegetables using liquid chromatography coupled with mass spectrometry

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Abstract. *This article summarizes the results of validation studies of a multi-residue method for the determination of pesticide residues in fruits and vegetables. The results of some of the monitoring control programs carried out in Romania are also presented. Liquid chromatography coupled with mass spectrometry (LC-MS / MS) was used to assess pesticide residue levels. The QUECHERS extraction method was used to extract the compounds. To validate the method, the control samples were fortified with a solution of 74 pesticides for LC-MS / MS analyzes, at 3 levels.The validation study is based on the regulations in Document DG Nº SANTE / 12682/2019. The scope of validation included the following performance requirements: Limit of detection; Quantification limit; Recovery; Fidelity; Repeatability; Reproducibility; Recovery; Selectivity / Specificity; Stability / Robustness; Linearity; Matrix effect; Measuring the margin of error.*

Key words: pesticides in fruits and vegetables, chromatographic liquid analysis, mass spectrometry, food safety

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1. Introduction

In recent decades, the growing interest in this direction has stimulated research into the risks associated with the consumption of fruits and vegetables, which are an important part of human nutrition. Therefore, the fact that pesticide residues could affect final consumers, especially when these products are consumed fresh, has amplified research to investigate the distribution of pesticide mass in plant products both during growth and development and in ripening and harvesting. , as well as in the post-harvest stages.

2. Experimental

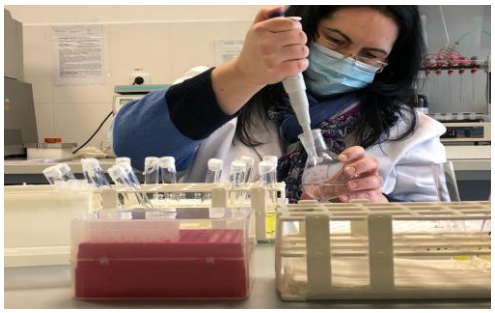
My research study began with the validation of the multiresidue method of pesticides by the determination technique using liquid chromatograph coupled to a mass spectrometer.(LC-MS/MS).Validation of the LC MS / MS multi-residue method was performed for samples of lemon (product with a high acid and water content) and apples (product with a high water content), according to DG SANTE / 12682/2019, categories of which are part and other fruits and vegetables, such as oranges, grafts, mandarins, grapes,

tomatoes, cucumbers, eggplants, asparagus, zucchini, lettuce, onions, melons, cabbage, peppers and others.

The extraction is performed as follows: Weigh 10 g of the sample to the nearest ± 0,1 g on an analytical balance into a 50 ml centrifuge tube over which to add a ceramic stopper. PH correction is performed with 5 M NaOH solution, up to pH = 4.5-5.5 (from close to close, homogenizing and checking the pH each time). Add 50 µL internal standard TFP (10µg / ml), vortex for 30 seconds. Add 10 ml of acetonitrile and mix for 10 minutes.

Add the mixture, then shake vigorously for one minute. Take 6 ml of supernatant and transfer to 15 ml centrifuge tubes, in which there is the saline mixture (QuEChERS 5982-5056CH), consisting of: 900mg MgSO4, 150 mg PSA. Homogenize vigorously for one minute. Centrifuge for 5 minutes at 5000 rpm. Filter, through PTFE filters (0.2 µm), into 10 ml test tubes. Take 500 µL of the obtained filtrate with a micropipette and add to the 2 ml vial. Swirl for 1 minute. This 2 ml vial, containing the final solution, is inserted into the autosampler and the sample is read by LC-MS / MS.

Preparation and homogenization of samples for extraction and analysis

Liquid chromatograph (LC) is the first component part of the analysis system and is a separation and qualitative chromatographic analysis equipment. Here we inject the sample extract.

With its help, the 74 pesticides in the method are separated and identified. The liquid chromatograph contains a column loaded with different materials (stationary phase), a pump that pushes the mobile phase through the column and a detector that indicates the retention times of the molecules. The retention time depends on the interaction between the stationary phase, the molecules to be analyzed and the solvents used.

The mass spectrometer (MS) is the second component part of the system and represents the technique by which the pesticide substances identified in the Chromatograph Liquid are ionized and sorted based on the mass / charge ratio (m / z) and quantified. In other words, a mass spectrometer measures the masses in a sample, resulting in the values of the pesticide concentrations in the sample.

To demonstrate that the matrix effect does not somehow influence the pesticides used in the method (74 substances), experiments were performed: preparation of the calibration curve by solvent and then by organic apple extract.

Experiments:

-The calibration range was chosen, 0.001-0.100 mg / L, and the curve will be drawn by 8 points, having the following concentrations: 0.001, 0.005, 0.01, 0.02, 0.03, 0.05, 0.07, 0.100 mg / L, for all analyzes .

-The regression coefficient is calculated automatically by the software. A coefficient ≥ 0.99 is accepted.

-LC-MS / MS was used, EXION LC coupled with AB SCIEX 4500 QTRAP mass spectrometer.

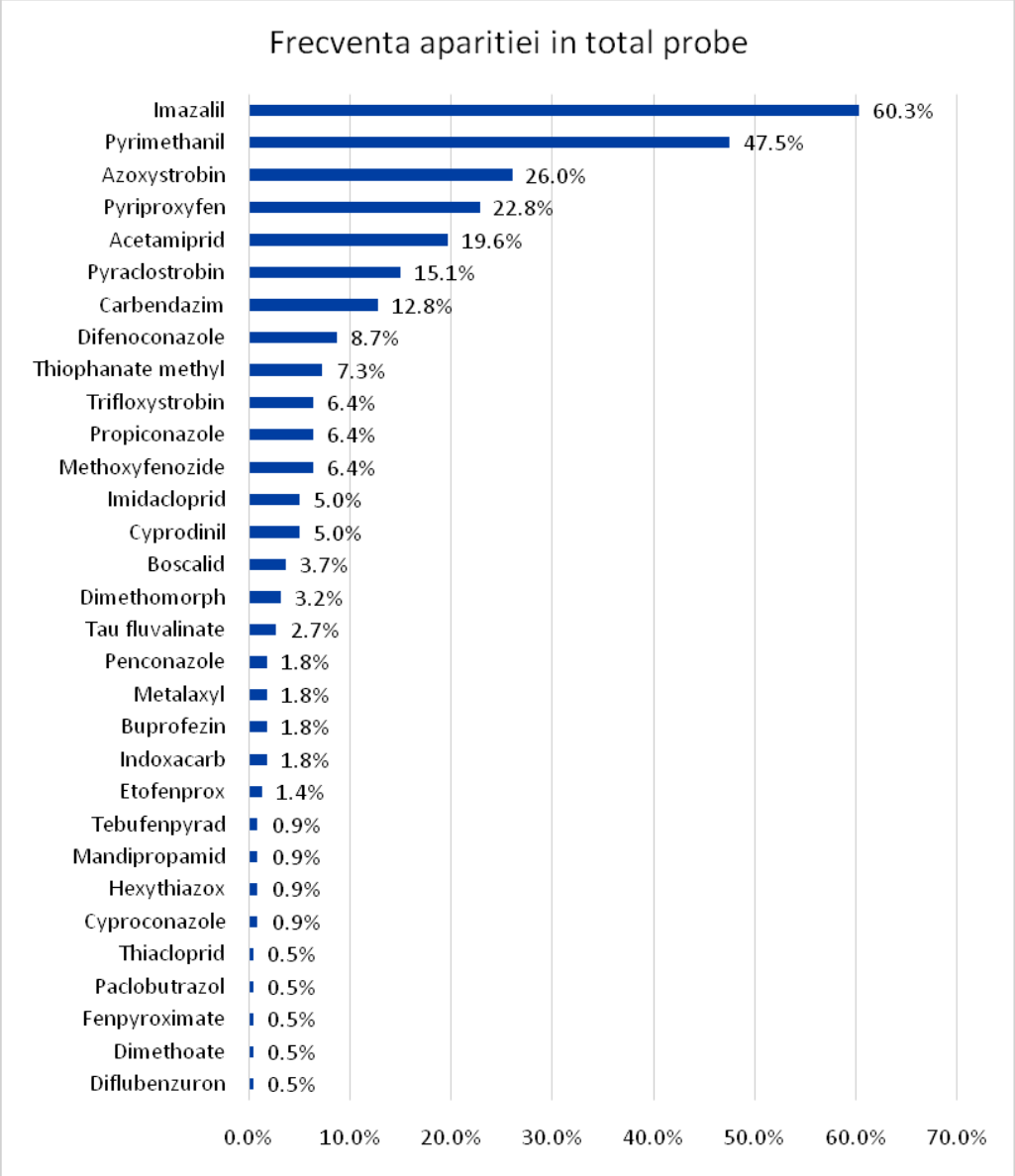
-For each set of readings a new calibration curve is drawn.

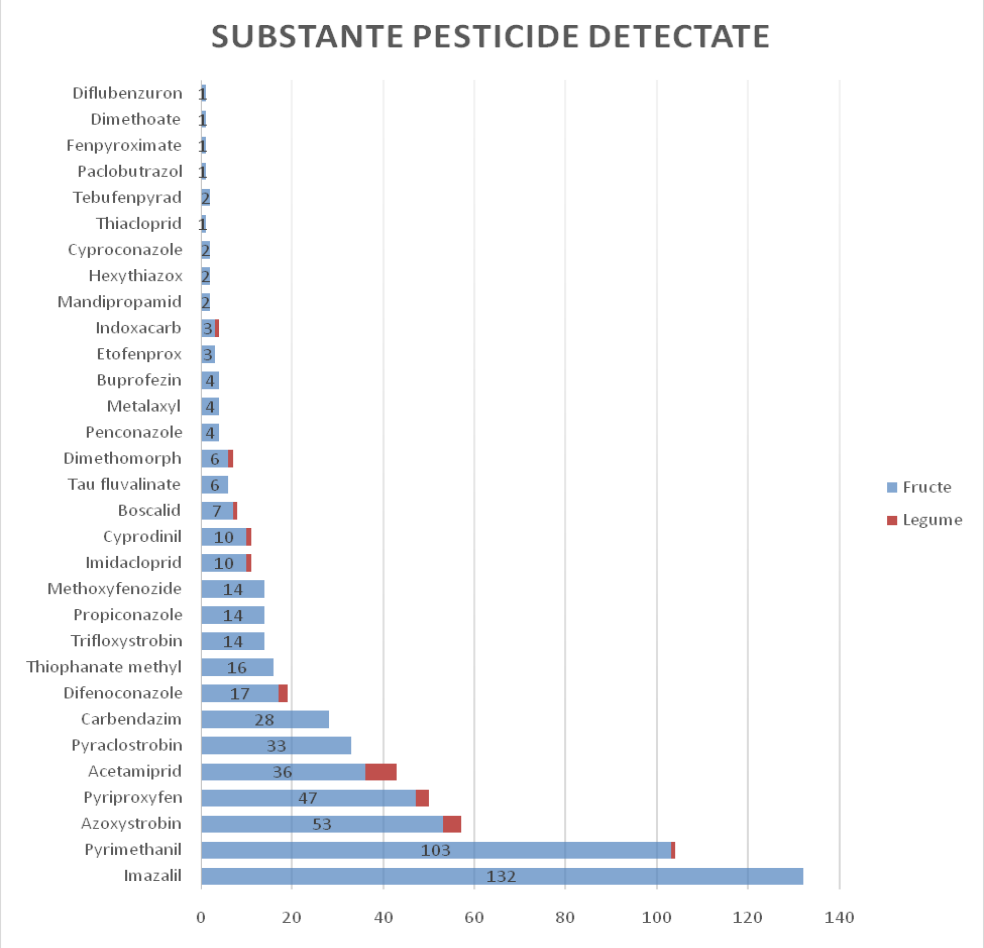
Certified analytical standards are used (standard pesticides) A blank apple sample was fortified at LOQ (0.01 mg / Kg) and was interpreted on the 2 curves (solvent and matrix) and the resulting CV was <20%. Calibration curves in solvent and matrix were overlapped for all substances and the CV condition <20% was met.

STUDY OF THE BEHAVIOR OF PESTICIDES IN VEGETABLES AND FRUITS ANALYZED IN 2021

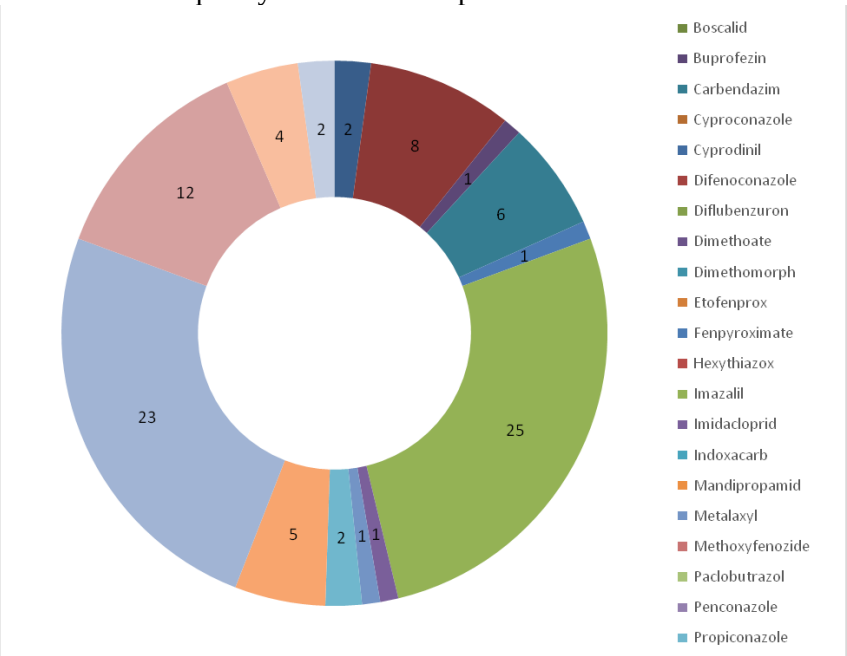
The validated method has been successfully a aplied in the implementation of the National Monitoring Control Program of 2021 for the analysis of fruits and vegetables.

In total, 255 fruit and vegetable samples were analyzed during 2021, of which 12 samples exceeded the maximum limits allowed by EU Regulation 396/2005. The most commonly and frequently detected pesticides were imazalil (60,3%), pyrimethalin (47,5%), pyriproxyfen (22,8%), azoxystrobin (26%), pyraclostrobin (15,1%).

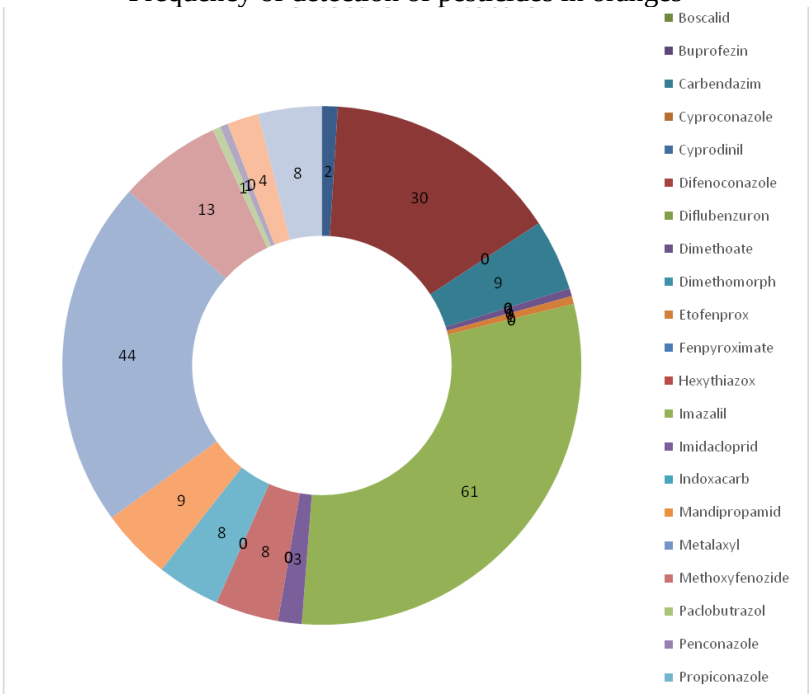

Frecventa aparitiei in total probe
Imazalil 60.3%
Pyrimethanil 47.5%
Azoxystrobin 26.0%
Pyriproxyfen 22.8%
Acetamiprid 19.6%
Pyraclostrobin 15.1%
Carbendazim 12.8%
Difenoconazole 8.7%
Thiophanate methyl 7.3%
Trifloxystrobin 6.4%
Propiconazole 6.4%
Methoxyfenozide 6.4%
Imidacloprid 5.0%
Cyprodinil 5.0%
Boscalid 3.7%
Dimethomorph 3.2%
Tau fluvalinate 2.7%
Penconazole 1.8%
Metalaxyl 1.8%
Buprofezin 1.8%
Indoxacarb 1.8%
Etofenprox 1.4%
Tebufenpyrad 0.9%
Mandipropamid 0.9%
Hexythiazox 0.9%
Cyproconazole 0.9%
Thiacloprid 0.5%
Paclobutrazol 0.5%
Fenpyroximate 0.5%
Dimethoate 0.5%
Diflubenzuron 0.5%
0.0% 10.0% 20.0% 30.0% 40.0% 50.0% 60.0% 70.0%


SUBSTANTE PESTICIDE DETECTATE
Diflubenzuron 1
Dimethoate 1
Fenpyroximate 1
Paclobutrazol 1
Tebufenpyrad 2
Thiacloprid 1
Cyproconazole 2
Hexythiazox 2
Mandipropamid 2
Indoxacarb 3
Etofenprox 3
Buprofezin 4
Metalaxyl 4
Penconazole 4
Dimethomorph 6
Tau fluvalinate 6
Boscalid 7
Cyprodinil 10
Imidacloprid 10
Methoxyfenozide 14
Propiconazole 14
Trifloxystrobin 14
Thiophanate methyl 16
Difenoconazole 17
Carbendazim 28
Pyraclostrobin 33
Acetamiprid 36
Pyriproxyfen 47
Azoxystrobin 53
Pyrimethanil 103
Imazalil 132
0 20 40 60 80 100 120 140
Fructe
Legume

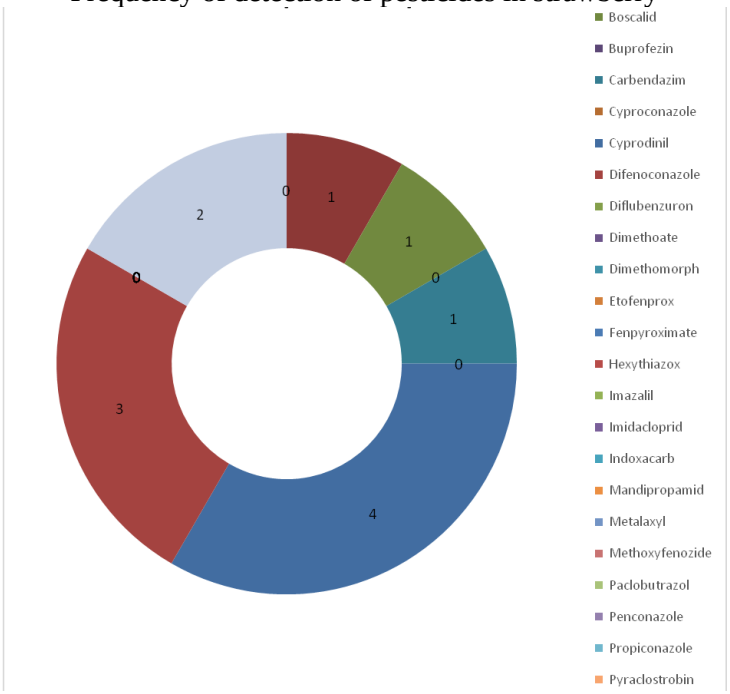
Frequency of detection of pesticides in lemons



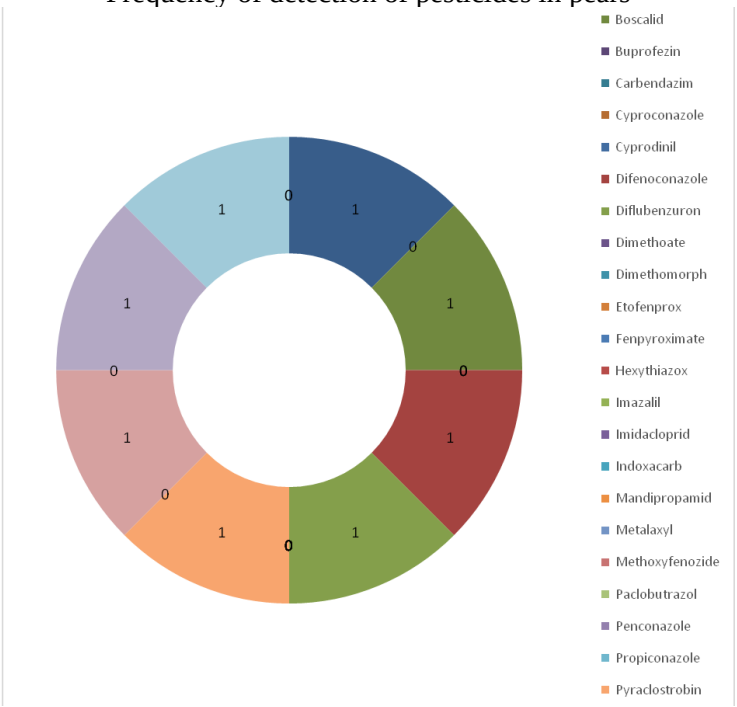
Frequency of detection of pesticides in oranges



Frequency of detection of pesticides in strawberry



Frequency of detection of pesticides in pears



3. Conclusions

The amount of pesticides consumed and the risk analysis are of great interest as they are used by the regulators for their decisions on setting legally enforceable limits for pesticides, Maximum Residue Levels MRL, and also to support regulatory decisions to aprove or not to use of plant protection product.If pesticides are aplied in accordance with good agricultural practices, by the time fresh products reach the markets or retail outlets, residue levels on crops should be below legal limits.

The analysis of fruit and vegetables for the assessment of pesticide residues is an important quality control procedure, established to reduce the misuse of pesticides and to ensure the quality and safety of food for human consumption.

R E F E R E N C E S

[1] SR EN ISO 17025, 2018 General requirements for the competence of testing and calibration laboratories.

[2] European Regulation 396/2005 on establishing the maximum permissible limits for pesticide residues in and on fruits, vegetables, cereals and other products of plant origin, with subsequent additions.

[3] Document N0 DG/SANTE/11945/2015*Method validation and Quality control procedures for pesticide residues analysis in foood and feed.

[4] Document No. SANTE/12682/2019 Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed