Academy of Romanian Scientists *Volume 10, No. 2, 2021, pp. 94 - 141*
Annals Series on Biological Sciences Online ISSN 2285 – 4177

ORIGINAL PAPERS

Use of Chromatography and Mass Spectrometry in Quality Control of some Vegetables and Fruits sold on the Romanian Market

Drd. ing. Corina Mihaela OPRITA[1], ing. Nela LUPU[2],
Ch. Marilena CANUTA[3], Prof.Univ. Emerit Dr. CS I Natalia ROȘOIU[4]

[1] Doctoral school institute of Ovidius University, SD Applied Sciences Biology / Biochemistry, specialist engineer at the Veterinary Sanitary Laboratory and for Food Safety Constanţa, email cioara.corina-ct@ansvsa.ro;
[2] Specialist engineer at the Veterinary Sanitary Laboratory and for Food Safety Constanţa;
[3] Chemical Engineer at the Sanitary Veterinary and Food Safety Laboratory Constanta, Manager of the quality system;
[4] Faculty of Medicine, Ovidius University of Constanta, IOSUD Univ. Ovidius Constanta, SD Applied Sciences Biology / Biochemistry, Full Member of the Romanian Academy of Scientists.

Abstract. The study aims to optimize and validate the QuEChERS extraction method and develop a multi-residue separation method for the detection of the 74 pesticides studied (frequently used in spraying treatment on vegetables and fruits).
The study presents the experimental results regarding the dynamics of pesticides in apples on the Romanian market, both in the organic and in the ordinary Romanian ones.
The optimized QuEChERS extraction method was used to determine the pesticide residues and the final extract was analyzed using an EXION LC - Sciex chromatograph liquid coupled to a SCIEX QTRAP 4500 mass spectrometer.

Keywords: *pesticides in fruits and vegetables, chromatographic liquid analysis, mass spectrometry, food safety*

1. Introduction

The study entitled Determination of pesticide residues from plant products aimed at ensuring compliance with the maximum permitted limits and assessing consumer exposure to these substances has as a fundamental objective the development of studies and research to investigate the accumulation of pesticides in fruits and vegetables, by developing and implementing an experimental program that will use advanced methodologies, techniques and equipment for analysis and interpretation of results.

Ideally, a pesticide should be lethal only to targeted pests, but not to non-target species, including humans. Unfortunately, this is not possible, so there has been an ongoing controversy over the use and abuse of pesticides. The rampant

use of these chemicals has often brought ecological disasters to human health and other life forms.

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.

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.

Behavior of the matrix (organic apple purchased from Kaufland), during processing in order to validate the extraction method and its analysis using the LC-MS / MS equipment, Sciex Q trap 4500.

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.

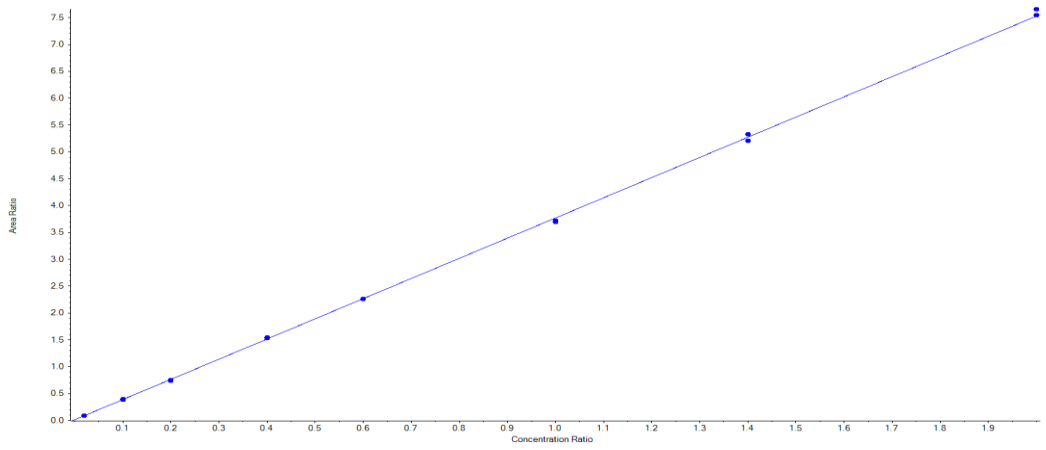
-LC-MS / MS was used, EXION LC coupled with AB SCIEX 4500 QTRAP mass spectrometer.

-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.

Pesticide: **Acephate.**
Internal Standard: **TPP. (Triphenylphosphat)**

Expected Concentration	*Number of Values*	*Mean Calculated Concentration*	*% Accuracy*	*Std. Deviation*	*%CV*
1.00	2 of 2	1.0	101.7	0.00	1.0
5.00	2 of 2	5.0	100.8	0.20	3.6
10.00	2 of 2	9.7	97.2	0.10	1.1
20.00	2 of 2	20.3	101.6	0.00	0.2
30.00	2 of 2	29.9	99.6	0.00	0.1
50.00	2 of 2	49.2	98.3	0.10	0.3
70.00	2 of 2	69.9	99.9	1.20	1.7
100.00	2 of 2	101.0	101.0	1.10	1.1



In order to demonstrate 2 of the most important performance criteria (conditions) of the multi-residue analysis method used on this type of LC-MS / MS equipment (repeatability and repeatability), the following were demonstrated:

Experiments: 6 samples are fortified[1] at 2 concentration levels: 0.01mg / Kg, 0.10 mg / Kg.

Appropriate amounts of the standard working solution of 1 mg / l are added to the blank samples to obtain the pesticide levels indicated above:

- in order to obtain a fortified sample at the level of 0.01 mg / kg, add 100 µl standard working solution (from the stock of 1 mg / l) and 50 l internal standard TFP solution (from the stock of 10 mg / L) to the sample white;
- in order to obtain a fortified sample at the level of 0.100 mg / kg, add 1000 µl standard working solution (from the stock of 1 mg / l) and 50 l internal standard TFP solution (from the stock of 10 mg / L) to the sample blank.

Proba:Mar	Ziua 1 validare						Ziua 2 validare					
	Fortificare 10 ppb	Fortificare 0.01 ppm	Recuperare %	Fortificare 100 ppb	Fortificare 0.1 ppm	Recuperare %	Fortificare 10 ppb	Fortificare 0.01 ppm	Recuperare %	Fortificare 100 ppb	Fortificare 0.1 ppm	Recuperare %
R1	8.600000	0.008600	86.000000	92.900000	0.092900	92.900000	7.200000	0.007200	72.000000	83.700000	0.083700	83.700000
R2	7.600000	0.007600	76.000000	81.400000	0.081400	81.400000	8.100000	0.008100	81.000000	85.600000	0.085600	85.600000
R3	7.600000	0.007600	76.000000	89.600000	0.089600	89.600000	8.000000	0.008000	80.000000	87.300000	0.087300	87.300000
R4	8.600000	0.008600	86.000000	86.000000	0.086000	86.000000	7.400000	0.007400	74.000000	90.300000	0.090300	90.300000
R5	8.600000	0.008600	86.000000	86.100000	0.086100	86.100000	8.300000	0.008300	83.000000	83.500000	0.083500	83.500000
R6	8.500000	0.008500	85.000000	97.700000	0.097700	97.700000	8.000000	0.008000	80.000000	87.600000	0.087600	87.600000
media	8.250000	0.008250	82.500000	88.950000	0.088950	88.950000	7.833333	0.007833	78.333333	86.333333	0.086333	86.333333
Sr	0.504975	0.000505	5.049752	5.769142	0.005769	5.769142	0.432049	0.000432	4.320494	2.598974	0.002599	2.598974
RSDr%	6.120912	6.120912	6.120912	6.485826	6.485826	6.485826	5.515524	5.515524	5.515524	3.010395	3.010395	3.010395
r=Sr*2.8	1.413931	0.001414	14.139307	16.153598	0.016154	16.153598	1.209738	0.001210	12.097383	7.277128	0.007277	7.277128
ur=Sr		0.000505	5.049752		0.005769	5.769142		0.000432	4.320494		0.002599	2.598974
RSDHorwitz		32.000000	32.000000		22.627417	22.627417		32.000000	32.000000		22.627417	22.627417
RSDr < RSD Horwitz		OK	OK		OK	OK		OK	OK		OK	OK
Horrat r = RSDr / RSD Horwitz		0.191279	0.191279		0.286636	0.286636		0.172360	0.172360		0.133042	0.133042
Horrat r < 2		OK	OK		OK	OK		OK	OK		OK	OK

Substanta	Acephat

TABEL REPRODUCTIBILITATE Substanta: Acephat				
Proba:Mar	Fortificare 0.01 ppm	Recuperare %	Fortificare 0.1 ppm	Recuperare %
R1	**0.008600**	86.000000	**0.092900**	92.900000
R2	**0.007600**	76.000000	**0.081400**	81.400000
R3	**0.007600**	76.000000	**0.089600**	89.600000
R4	**0.008600**	86.000000	**0.086000**	86.000000
R5	**0.008600**	86.000000	**0.086100**	86.100000
R6	**0.008500**	85.000000	**0.097700**	97.700000
R7	**0.007200**	72.000000	**0.083700**	83.700000
R8	**0.008100**	81.000000	**0.085600**	85.600000
R9	**0.008000**	80.000000	**0.087300**	87.300000
R10	**0.007400**	74.000000	**0.090300**	90.300000
R11	**0.008300**	83.000000	**0.083500**	83.500000
R12	**0.008000**	80.000000	**0.087600**	87.600000
media	0.008042	80.416667	0.087642	87.641667
SR	0.000588	5.880760	0.004424	4.423705
RSD R%	7.312862		5.047491	
R=SR*2,8	0.001647			
u R=S R	0.000588			
RSDHorwitz	32.000000		22.627417	
RSD R < RSD Horwitz	OK		OK	
Horrat R < 2	OK		OK	
u etalonare LC- MS/MS	0.001202			
u recuperare = [(SR recuperare%/100)/√12]/ (Recuperare%/100)		0.021110		0.014571
u solutie=√ u pip^2+u purit^2+u masa^2+u vol bal cot^2	0.000823			
u balanta=√ u repetabilitate^2+u specifica bal^2	0.000040			
uc=√UR^2+uechip^2+u sol^2+u balanta^2	0.001571			
urec=(SR/100)/sqrt6	0.024008		0.018060	
Uf = SQRT((LOD/2)^2+(α*C)^2)	0.005385		0.020616	
U c=u cx2	0.003142			
U %=(Uc/Media)x100	39.072594			
U % < 50%	OK			

The maximum allowed LMA / MRL limits for the apple matrix, as regulated in reg 396/2005[2], for each substance in the analysis method, are the following:

Pesticidul	**MRL (mg/Kg)**	**LOQ (mg/Kg)**	**Pesticidul**	**MRL (mg/Kg)**	**LOQ (mg/Kg)**
Acephate	0.01	0.01	Indoxacarb	0.5	0.01
Acetamiprid	0.40	0.01	Iprovalicarb	0.01	0.01
Aldicarb sulfone	0.02	0.01	Isoprothiolane	0.01	0.01
Aldicarb sulfoxide	0.02	0.01	Linuron	0.01	0.01
Aldicarb	0.02	0.01	Lufenuron (suma de izomeri)	1.0	0.01
Azoxystrobin	0.01	0.01	Mandipropamid	0.01	0.01
Boscalid	2.00	0.01	Mepanipyrim	0.01	0.01
Bupirimate	0.3	0.01	Metalaxyl si Metalaxyl M	1.0	0.01
Buprofezin	0.01	0.01	Methiocarb	0.1	0.01
Carbaryl	0.01	0.01	Methiocarb-Sulfoxide	0.1	0.01
Carbendazim	0.20	0.01	Methomyl	0.01	0.01
Carbofuran	0.001	0.005	Methoxyfenozide	2.0	0.01
3-Hydroxy-carbofuran	0.001	0.005	Monocrotophos	0.01	0.01
Clofentezin	0.5	0.01	Ometoate	0.01	0.01
Clothianidin	0.4	0.01	Oxadixyl	0.01	0.01
Cymoxanil	0.01	0.01	Oxamyl	0.01	0.01
Cyproconazole (suma de izomeri)	0.1	0.01	Oxydemeton-methyl (Demeton-S-methyl-sulfoxide)	0.01	0.01
Cyprodinil	2.0	0.01	Paclobutrazol (suma de izomeri)	0.05	0.01
Dietofencarb	0.01	0.01	Penconazol	0.15	0.01
Diflubenzuron	0.01	0.01	Pirimicarb	0.5	0.01
Difenoconazole	0.8	0.01	Propargite	0.01	0.01
Dimethoate	0.01	0.01	Propiconazole (suma de izomeri)	0.15	0.01
Dimethomorph (suma de izomeri)	0.01	0.01	Pymetrozin	0.02	0.01
Epoxiconazole	0.05	0.01	Pyraclostrobin	0.5	0.01
Etofenprox	0.7	0.01	Pyrimethanil	15	0.01
Fenamidone	0.01	0.01	Pyriproxyfen	0.2	0.01
Fenamiphos	0.02	0.01	Tau-Fluvalinate	0.3	0.01
Fenhexamid	0.01	0.01	Tebufenozide	1.0	0.01
Fenoxycarb	0.7	0.01	Tebufenpyrad	0.3	0.01
Fenpyroximate	0.3	0.01	Terbuthylazine	0.1	0.01
Flufenoxuron	0.01	0.01	Thiacloprid	0.3	0.01
Fluquiconazole	0.01	0.01	Thiamethoxam	0.3	0.01
Flutriafol	0.4	0.01	Thiophanate-methyl	0.5	0.01
Formetanate	0.01	0.01	Triadimefon	0.01	0.01
Hexaconazole	0.01	0.01	Triflumuron	0.5	0.01
Hexythiazox	1.0	0.01	Trifloxystrobin	0.7	0.01
Imazalil	0.01	0.01	Thiodicarb	0.01	0.01
Imidacloprid	0.5	0.01			

3. Conclusions

- 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.
- The content of pesticides in organic apples was determined, as well as in Romanian ones, resulting in data of the existence of traces of residues, but not quantifiable in order to report numerical values.

R E F E R E N C E

[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.