

DEVELOPMENT OF THE ANALYTICAL METHOD FOR DETERMINING 397 PESTICIDES IN THE FORM OF RESIDUES IN PRODUCTS OF NON-ANIMAL ORIGIN AND THE IMPACT OF 7 TYPES OF VEGETABLE MATRICES ON THE QUALITY OF THE RESULTS, USING THE LC MS MS TECHNIQUE

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Abstract. *Given the large number of plant food samples that will be analyzed annually for pesticide residues, it was necessary to develop a fast and easy method for analyzing multiple residues using a system composed of a liquid chromatograph coupled to a mass spectrometer. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe Extraction) extraction technique combined with the liquid chromatography-tandem mass spectrometry analysis technique was used to optimize a multiresidue analysis method for 397 pesticide residues from matrices with high water content and high acid content, as well as other matrix groups. The performance parameters studied for the validation of the method were selectivity, limit of quantification (LOQ), linearity, repeatability, reproductibility, recovery, robustness and matrix effect. All compounds identified at very low levels (1, 5, or 10 mg/kg) could be quantified at LOQs lower than 0.01 mg/kg. The linearity of the calibration curve for each compound is in the range of 1–500 µg/L, and its coefficient of determination (R²) is >0.97. This method is applied within the Laboratory for determination of pesticide residues by LC-MS/MS technique for the following groups of food products of vegetable origin.*

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

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Introduction

The determination of pesticide residues in products of plant origin, aiming at ensuring compliance with maximum permitted limits and assessing consumer exposure to these substances, has as its fundamental objective the development of studies and research to investigate the accumulation of pesticides in fruits and vegetables, by developing and implementing an experimental and validation

program that will use ultramodern methodologies, techniques and equipment for the analysis and interpretation of results [9].

Experimental

The development and research study began with the validation of the multi-residue pesticide method with 74 compounds using the SCIEX LC-MS/MS technique, and then in 2023, we validated a new multi-residue method with 397 compounds using the LC MS MS technique with Shimadzu 8060 Nexera equipment [7].

The extraction for the first group of matrices (matrices with high water content) is performed as follows: Weigh 10 g of the sample to the nearest $\pm 0,1$ 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 μ L internal standard TFP (10 μ g / ml), vortex for 30 seconds.

Then add 10 ml of acetonitrile solvent 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 μ m), into 10 ml test tubes. Take 500 μ L 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 [8].

Preparation and homogenization of samples for extraction and analysis



Materials and methods

Blank samples of apple, lemon, avocado, wheat, almonds, figs, cocoa were fortified at LOQ level, at 0.010 mg/Kg and at 2 x LOQ, 0.020 mg/Kg for the apple, lemon, avocado, wheat, almonds, figs matrices.

- experiments were performed for repeatability (set 1 and set 2 of validation);

- samples were analyzed and the mean concentration, standard deviation and RSDr (%) were determined for set 1 and set 2 of validation.

- 5 repetitions were performed for the compounds: carbofuran and 3-OH carbofuran by fortification at MRL level=1µg/ml;

- The determinations were performed on both Shimadzu equipment (1 analyst, different days)

- Experiments were performed for fortification at the LOQ level, 0.010 mg/Kg, 2 repetitions for oilseeds - rapeseeds

- Several extraction methods were tried for these types of oilseed matrices, and finally it was established as the extraction method, the method for matrix group 4a - matrices with high fat content and very low water content of which nuts and oilseeds are part and this being the group validated by the almond matrix, respectively E3b+C2.

- For matrices with high fat content and very low water content, respectively rapeseeds, only 391 compounds could be detected and quantified.

Analysis instrument:

UHPLC MS/MS Shimadzu 8060 Nexera which is a coupling between:

- UHPLC – Ultra High Performance Liquid Chromatograph and

- Triple quadrupole mass spectrometer UHPLC is an equipment for separating the components of a mixture at ultra-high pressure (up to 1300 bars) used for injecting the sample extract.

It is modular and consists of a pumping system, autosampler, column oven in which a column filled with various materials (stationary phase) is installed, depending on the nature of the analytes.

The mobile phase is made up of a more or less complex mixture of solvent.

The separation takes place based on the analyte/mobile phase/stationary phase interaction. Once separated, the compounds are transported to the mass spectrometer.

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 397 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, or does not somehow influence the pesticides used in the method (397 substances), experiments were performed: preparation of the calibration curve by solvent and then by organic extract.

Experiments:

- The calibration range was chosen, 0.001-0.500 mg / L, and the curve will be drawn by 11 points, having the following concentrations: 0.001 mg / L, 0.005 mg / L, 0.01 mg / L, 0.02 mg / L, 0.04 mg / L, 0.08 mg / L, 0.100 mg / L, 0.200 mg / L, 0.300 mg / L, 0.400 mg / L, 0.500 mg / L for all analyzes.

- The regression coefficient is calculated automatically by the software. A coefficient ≥ 0.97 is accepted.

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

Certified analytical standards are used (standard pesticides) from CPA Chem. A blank samples 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.

List of 397 pesticides substances in the method:

(E)-Fenpyroximate [CAS:134098-61-6] ; Ferimzone [CAS:89269-64-7]; 1-(3,4-Dichlorophenyl)-3-methylurea [CAS:3567-62-2]; N,Nr-Diphenylurea [CAS:102-07-8]; 2,3,5-Trimethacarb [CAS:2655-15-4]; 2,4-Dimethylaniline [CAS:95-68-1]; Acephate [CAS:30560-19-1]; Acetamiprid [CAS:135410-20-7]; Aclonifen [CAS:74070-46-5]; Alachlor [CAS:15972-60-8]; Aldicarb [CAS:11606-3]; Aldicarb-sulfone [CAS:1646-88-4]; Aldicarb-sulfoxide [CAS:1646-87-3]; Allidochlor [CAS:93-71-0]; Ametoctradin [CAS:865318-974]; Ametryn [CAS:834-12-8]; Ancymidol [CAS:12771-68-5]; Anilofos [CAS:64249-01-0]; Atraton [CAS:1610-17-9]; Atrazine [CAS:1912-24-9]; Atrazine-desisopropyl [CAS:1007-28-9]; Azaconazole [CAS:60207-31-0]; Azadirachtin [CAS:11141-17-6]; Aziprotryne [CAS:4658-28-0]; Azoxystrobin [CAS:131860-33-8]; Beflubutamid [CAS:113614-08-7]; Benalaxyl [CAS:71626-11-4]; Bendiocarb [CAS:22781-23-3]; Benodanil [CAS:15310-01-7]; Bensulide [CAS:741-58-2]; Benzoximate [CAS:29104-30-1]; Benzthiazuron [CAS:1929-88-0]; Bioresmethrin [CAS:28434-01-7]; Bixafen [CAS:581809-46-3]; Boscalid [CAS:188425-85-6]; Bromacil [CAS:314-40-9]; Bufencarb [CAS:8065-36-9]; Bupirimate [CAS:41483-43-6]; Buprofezin [CAS:69327-76-0]; Butocarboxim [CAS:34681-10-2]; Butocarboxim-sulfoxide [CAS:34681-24-8]; Butoxydim [CAS:138164-12-2]; Buturon [CAS:3766-60-7]; Butylate [CAS:2008-41-5]; Cadusafos [CAS:95465-99-9]; Carbaryl [CAS:63-25-2]; Carbenfentimid [CAS:10605-21-7]; Carbofuran [CAS:1563-66-2]; Carbofuran-3-hydroxy [CAS:16655-82-6]; Carbofuran-3-keto [CAS:16709-30-1]; Carboxin [CAS:5234-68-4]; Carfentrazone-ethyl [CAS:128639-02-1]; Carpropamid [CAS:104030-54-8]; Chlorantraniliprole [CAS:500008-45-7]; Chlorbromuron [CAS:13360-45-7]; Chlorfenvinphos [CAS:470-90-6]; Chloridazon [CAS:1698-60-8]; Chloroxuron [CAS:1982-47-4]; Chlorthiamid [CAS:1918-13-4]; Chromafenozide [CAS:143807-66-3]; Clethodim [CAS:99129-21-2]; Climbazole [CAS:38083-17-9]; Clodinafop-propargyl ester [CAS:105512-06-9]; Clofentezine [CAS:74115-24-5]; Clomazone [CAS:81777-89-1]; Clomeprop [CAS:84496-56-0]; Cloquintocet-1-methylhexyl ester [CAS:99607-70-2]; Clothianidin [CAS:210880-92-5]; Crimidine [CAS:535-89-7]; Crotoxyphos [CAS:7700-17-6]; Cumyluron [CAS:99485-76-4]; Cyanofenphos [CAS:13067-93-1]; Cyazofamid [CAS:120116-88-3]; Cycloate [CAS:1134-23-2]; Cycloheximide [CAS:66-81-9]; Cycloxydim [CAS:101205-02-1]; Cycluron [CAS:2163-69-1]; Cyflufenamid [CAS:180409-60-3]; Cymiazole [CAS:61676-87-7]; Cymoxanil [CAS:57966-95-7]; Cyproconazole [CAS:94361-06-5]; Cyprodinil [CAS:121552-61-2]; Daimuron [CAS:42609-52-9]; Dazomet [CAS:533-74-4]; Demeton- [CAS:298-03-3]; Demeton-S-methyl [CAS:919-86-8]; Demeton-S-methyl-sulfone [CAS:17040-19-6]; Norflurazondesmethyl [CAS:23576-24-1]; Desmetryn [CAS:1014-69-3]; Dichlofenthion [CAS:97-176]; Dichlormid [CAS:37764-25-3]; Diclobutrazol

[CAS:75736-33-3]; Diclocymet [CAS:139920-32-4]; Dicrotophos [CAS:141-66-2]; Diethofencarb [CAS:87130-20-9]; Difenoconazole [CAS:119446-68-3]; Diflubenzuron [CAS:35367-38-5]; Diflufenican [CAS:83164-33-4]; Dimethachlor [CAS:50563-36]; Dimethametryn [CAS:22936-75-0]; Dimethoate [CAS:60-51-5]; Dimethomorph [CAS:110488-70-5]; Dimetilan [CAS:644-64-4]; Dimoxystrobin [CAS:149961-52-4]; Diniconazole [CAS:83657-24-3]; Dinotefuran [CAS:165252-70-0]; Dioxacarb [CAS:6988-21-2]; Diphenamid [CAS:957-51-7]; Dipropetryn [CAS:4147-51-7]; Disulfoton-sulfone [CAS:2497-06-5]; Disulfoton sulfoxide [CAS:2497-07-6]; Ditalimfos [CAS:5131-24-8]; Dithiopyr [CAS:97886-45-8]; Diuron [CAS:330-54-1]; DMST [CAS:66840-71-9]; Dodemorph [CAS:1593-77-7]; Dodine [CAS:2439-10-3]; Edifenphos [CAS:17109-49-8]; Epoxiconazole [CAS:133855-98-8]; EPTC [CAS:759-94-4]; Esprocarb [CAS:85785-20-2]; Etaconazole [CAS:60207-93]; Ethiofencarb [CAS:29973135]; Ethiofencarbsulfone [CAS:53380-23-7]; Ethiofencarb-sulfoxide [CAS:53380-226]; Ethiprole [CAS:181587-01-9]; Ethirimol [CAS:23947-06]; Ethoprophos [CAS:13194484]; Ethoxyquin [CAS:91-53-2]; Ethychlozate [CAS:27512-727]; Etofenprox [CAS:80844071]; Famphur [CAS:52-85-7]; Fenamidone [CAS:161326-34-7]; Fenamiphos [CAS:22224-92-6]; Fenamiphos sulfone [CAS:31972-44-8]; Fenamiphos-sulfoxide [CAS:31972-43-7]; Fenazaquin [CAS:120928-09-8]; Fenazox [CAS:495-48-7]; Fenbuconazole [CAS:114369-43-6]; Fenchlorazol-ethyl [CAS:103112-35-2]; Fenfuram [CAS:24691-80-3]; Fenhexamid [CAS:126833-17-8]; Fenobucarb [CAS:3766-81-2]; Fenothiocarb [CAS:62850-32-2]; Fenoxanil [CAS:115852-48-7]; Fenoxycarb [CAS:72490-01-8]; Fenpropimorph [CAS:67564-91-4]; Fensulfothion [CAS:115-90-2]; Fensulfothion-oxon [CAS:6552-21-2]; Fensulfothion-oxon-sulfone [CAS:6132-17-8]; Fensulfothion-sulfone [CAS:14255-72-2]; Fenthion [CAS:55-38-9]; Fenthion-oxon [CAS:6552-12-1]; Fenthion-oxon-sulfoxide [CAS:6552-13-2]; Fenthion-sulfoxide [CAS:3761-41-9]; Fenuron [CAS:101-42-8]; Fipronil [CAS:120068-37-3]; Fipronil-desulfinyl [CAS:205650-65-3]; Fipronil-sulfide [CAS:120067-83-6]; Flamprop-isopropyl [CAS:52756-22-6]; Flamprop-methyl [CAS:52756-25-9]; Flamprop-M-isopropyl [CAS:63782-90-1]; Flonicamid [CAS:158062-67-0]; Florasulam [CAS:145701-23-1]; Fluacrypyrim [CAS:229977-93-9]; Fluazifop-butyl [CAS:69806-50-4]; Fluazifop-P-butyl [CAS:79241-46-6]; Fluazinam [CAS:79622-59-6]; Flucycloxiuron - mixture of (E) and (Z) isomers [CAS:113036-88-7]; Fludioxonil [CAS:131341-86-1]; Flufenacet [CAS:142459-58-3]; Flufenoxuron [CAS:101463-69-8]; Flumetralin [CAS:62924-70-3]; Flumiclorac-pentyl [CAS:87546-18-7]; Flumorph [CAS:211867-47-9]; Fluoroglycofen-ethyl [CAS:77501-90-7]; Fluoxastrobin [CAS:361377-29-9]; Fluquinconazole [CAS:136426-54-5]; Fluridone [CAS:59756-60-4]; Flurochloridone [CAS:61213-25-0]; Fluroxypyr [CAS:69377-81-7]; Fluroxypyr-1-methylheptylester [CAS:81406-37-3]; Flurprimidol

[CAS:56425-91-3]; Flutolanil [CAS:66332-96-5]; Flutriafol [CAS:76674-21-0]; Fluvalinate [CAS:102851-06-9]; Fomesafen [CAS:72178-02-0]; Formetanate hydrochloride [CAS:23422-53-9]; Fosthiazate [CAS:98886-44-3]; Fuberidazole [CAS:3878-19-1]; Furalaxyl [CAS:57646-30-7]; Furathiocarb [CAS:65907-30-4]; Furmecyclox [CAS:60568-05-0]; Heptenophos [CAS:23560-59-0]; Hexaconazole [CAS:79983-71-4]; Hexaflumuron [CAS:86479-06-3]; Hexazinone [CAS:51235-04-2]; Hexythiazox [CAS:78587-05-0]; Imazalil [CAS:35554-44-0]; Imazamethabenz-methyl [CAS:81405-85-8]; Imibenconazole [CAS:86598-92-7]; Imidacloprid [CAS:138261-41-3]; Inabenfide [CAS:82211-24-3]; S-Indoxacarb [CAS:173584-44-6]; Iaconazole [CAS:125225-28-7]; Iprobenfos [CAS:26087-47-8]; Iprovalicarb [CAS:140923-17-7]; Irgarol 1051 [CAS:28159-98-0]; Isazofos [CAS:42509-80-8]; Isafenphos [CAS:25311-71-1]; Isafenphos-oxon [CAS:31120-85-1]; Isoprocarb [CAS:2631-40-5]; Isoprothiolane [CAS:50512-35-1]; Isouron [CAS:55861-78-4]; Isoxaben [CAS:82558-50-7]; Isoxadifen-ethyl [CAS:163520-33-0]; Isoxathion [CAS:18854-01-8]; Karbutilate [CAS:4849-32-5]; Lactofen [CAS:77501-63-4]; Lenacil [CAS:2164-08-1]; Linuron [CAS:330-55-2]; Lufenuron [CAS:103055-07-8]; Mandipropamid [CAS:374726-62-2]; Mecarbam [CAS:2595-54-2]; Mefenacet [CAS:73250-68-7]; Mefenpyr-diethyl [CAS:135590-91-9]; Mepanipyrim [CAS:110235-47-7]; Mephosfolan [CAS:950-10-7]; Mepronil [CAS:55814-41-0]; Metalaxyl [CAS:57837-19-1]; Metalaxyl-M [CAS:70630-17-0]; Metazachlor [CAS:67129-08-2]; Metconazole [CAS:125116-23-6]; Methabenzthiazuron [CAS:18691-97-9]; Methacrifos [CAS:62610-77-9]; Methfuroxam [CAS:28730-17-8]; Methiocarb [CAS:2032-65-7]; Methiocarb-sulfone [CAS:2179-25-1]; Methiocarb-sulfoxide [CAS:2635-10-1]; Methomyl [CAS:16752-77-5]; Methoprotryne [CAS:841-06-5]; Methoxyfenozide [CAS:161050-58-4]; Metobromuron [CAS:3060-89-7]; Metolachlor [CAS:51218-45-2]; Metolcarb [CAS:1129-41-5]; (E)-Metominostrobin [CAS:133408-50-1]; Metoxuron [CAS:19937-59-8]; Metrafenone [CAS:220899-03-6]; Mexacarbate [CAS:315-18-4]; MGK 264 [CAS:113-48-4]; Molinate [CAS:2212-67-1]; Monocrotophos [CAS:6923-22-4]; Monolinuron [CAS:1746-81-2]; Monuron [CAS:150-68-5]; Napropamide [CAS:15299-99-7]; Neburon [CAS:555-37-3]; Nitenpyram [CAS:150824-47-8]; Norflurazon [CAS:27314-13-2]; Nuarimol [CAS:63284-71-9]; Omethoate [CAS:1113-02-6]; Orbencarb [CAS:34622-58-7]; Oxadiargyl [CAS:39807-15-3]; Oxadiazon [CAS:19666-30-9]; Oxadixyl [CAS:77732-09-3]; Oxamyl [CAS:23135-22-0]; Oxaziclomefone [CAS:153197-14-9]; Oxycarboxin [CAS:5259-88-1]; Demeton-S-methyl-sulfoxide [CAS:301-12-2]; Paclbutrazol [CAS:76738-62-0]; Paraoxon-ethyl [CAS:311-45-5]; Paraoxon-methyl [CAS:950-35-6]; Parathion-methyl [CAS:298-00-0]; Pebulate [CAS:1114-71-2]; Penconazole [CAS:66246-88-6]; Penthiopyrad [CAS:183675-82-3]; Pethoxamid [CAS:106700-29-2]; Phenothrin [CAS:26002-80-2]; Phenthoate [CAS:2597-03-7]; Phorate [CAS:298-02-2]; Phorate-oxon

[CAS:2600-69-3]; Phorate-sulfone [CAS:2588-04-7]; Phorate-sulfoxide [CAS:2588-03-6]; Phosalone [CAS:2310-17-0]; Phosfolan [CAS:947-02-4]; Phosmet [CAS:732-11-6]; Phosmet oxon [CAS:3735-33-9]; Phosphamidon [CAS:13171-21-6]; Phoxim [CAS:14816-18-3]; Picolinafen [CAS:137641-05-5]; Picoxystrobin [CAS:117428-22-5]; Piperonyl butoxide [CAS:51-03-6]; Piperophos [CAS:24151-93-7]; Pirimicarb [CAS:23103-98-2]; Pirimicarb-desmethyl [CAS:30614-22-3]; Pirimiphos-ethyl [CAS:23505-41-1]; Pirimiphos-methyl [CAS:29232-93-7]; Prallethrin [CAS:23031-36-9]; Pretilachlor [CAS:51218-49-6]; Prochloraz [CAS:67747-09-5]; Profoxydim lithium salt [CAS:281664-76-4]; Promecarb [CAS:2631-37-0]; Prometon [CAS:1610-18-0]; Prometryn [CAS:7287-19-6]; Propachlor [CAS:1918-16-7]; Propamocarb [CAS:25606-41-1]; Propanil [CAS:709-98-8]; Propaphos [CAS:7292-16-2]; Propaquizafop [CAS:111479-05-1]; Propargite [CAS:2312-35-8]; Propazine [CAS:139-40-2]; Propham [CAS:122-42-9]; Propiconazole [CAS:60207-90-1]; Propisochlor [CAS:86763-47-5]; Propoxur [CAS:114-26-1]; Proquinazid [CAS:189278-12-4]; Prothioconazole-desthio [CAS:120983-64-4]; Pyracarbolid [CAS:24691-76-7]; Pyraclifos [CAS:89784-60-1]; Pyraclostrobin [CAS:175013-18-0]; Pyrazophos [CAS:13457-18-6]; Pyrazoxyfen [CAS:71561-11-0]; Pyridalyl [CAS:179101-81-6]; Pyridaphenthion [CAS:119-12-0]; Pyrifenox [CAS:88283-41-4]; Pyrimethanil [CAS:53112-28-0]; Pyriproxyfen [CAS:95737-68-1]; Quinalphos [CAS:13593-03-8]; Quinoclamine [CAS:2797-51-5]; Quizalofop-ethyl [CAS:76578-14-8]; Quizalofop-P-ethyl [CAS:100646-51-3]; Resmethrin [CAS:10453-86-8]; Rotenone [CAS:83-79-4]; Sebumeton [CAS:26259-45-0]; Sethoxydim [CAS:74051-80-2]; Siduron [CAS:1982-49-6]; Silthiofam [CAS:175217-20-6]; Simazine [CAS:122-34-9]; Simeconazole [CAS:149508-90-7]; Simetryn [CAS:1014-70-6]; Spinetoram [CAS:935545-74-7]; Spinosad [CAS:168316-95-8]; Spirotetramat [CAS:203313-25-1]; Spiroxamine [CAS:118134-30-8]; Sulfoxaflor [CAS:946578-00-3]; Tebufenozide [CAS:112410-23-8]; Tebufenpyrad [CAS:119168-77-3]; Tebupirimfos [CAS:96182-53-5]; Tebutam [CAS:35256-85-0]; Tebuthiuron [CAS:34014-18-1]; Temephos [CAS:3383-96-8]; Terbucarb [CAS:1918-11-2]; Terbufos-sulfone [CAS:56070-16-7]; Terbufos-sulfoxide [CAS:10548-10-4]; Terbumeton [CAS:33693-04-8]; Terbumeton-desethyl [CAS:30125-64-5]; Terbutylazine [CAS:5915-41-3]; Terbutryn [CAS:886-50-0]; cis-Tetrachlorvinphos [CAS:22248-79-9]; Tetraethylpyrophosphate [CAS:107-49-3]; Tetramethrin [CAS:7696-12-0]; Thenylchlor [CAS:96491-05-3]; Thiabendazole [CAS:148-79-8]; Thiacloprid [CAS:111988-49-9]; Thiamethoxam [CAS:153719-23-4]; Thiazafurion [CAS:25366-23-8]; Thiazopyr [CAS:117718-60-2]; Thidiazuron [CAS:51707-55-2]; Thifluzamide [CAS:130000-40-7]; Thiobencarb [CAS:28249-77-6]; Thiodicarb [CAS:59669-26-0]; Thiofanox [CAS:39196-18-4]; Thiofanox-sulfone [CAS:39184-59-3]; Thiofanox-sulfoxide [CAS:39184-27-5]; Thionazin

[CAS:297-97-2]; Thiophanate-methyl [CAS:23564-05-8]; Thiophanate ethyl [CAS:23564-06-9]; Tolfenpyrad [CAS:129558-76-5]; Triadimefon [CAS:43121-43-3]; Triadimenol [CAS:55219-65-3]; Tri-allate [CAS:2303-17-5]; Triazamate [CAS:112143-82-5]; Tribufos [CAS:78-48-8]; Tricyclazole [CAS:41814-78-2]; Tridemorph [CAS:81412-43-3]; Trietazine [CAS:1912-26-1]; Trifloxystrobin [CAS:141517-21-7]; Triflumizole [CAS:68694-11-1]; Triflumuron [CAS:64628-44-0]; Triforine [CAS:26644-46-2]; Triticonazole [CAS:131983-72-7]; Uniconazole [CAS:83657-22-1]; Vamidothion [CAS:2275-23-2]; XMC (3,5-xylyl methylcarbamate) [CAS:2655-14-3]; Zoxamide [CAS:156052-68-5].

Study of the impact of 7 types of vegetable groups of matrices on the quality of the results and about the number of pesticides identified for each group of matrices.

The validated method has been successfully applied in the implementation of the National Monitoring Control Program for the analysis of fruits and vegetables and each type of matrix is extracted and purified differently, and also behaves differently in compound separation analysis [3], [4], [5].

1. Matrices with high water content $\geq 80\%$ (e.g. vegetables, fruits and their processed products); Validation of this group of matrices was carried out for 397 pesticides, with experiments performed on the apple matrix.



Matrices with high water content of which fresh fruits and vegetables are : pome fruits (apples, pears), stone fruits (apricots, cherries, peaches), other fruits (bananas), Allium vegetables (onions, leeks), cucurbits (tomatoes, peppers, cucumbers, melons), Brassica vegetables (cauliflower, Brussels sprouts, cabbage, broccoli), leafy vegetables and fresh herbs (lettuce, spinach, basil), vegetable stems (celery stem, asparagus), fresh legumes (fresh peas with pods, peas, beans, French beans), fresh mushrooms, root vegetables and tubers (sugar beets, carrots, potatoes, sweet potatoes); processed products – juices [6].

Some of the matrix groups

Correspondence of fruit and vegetable groups correlated According to DG Sante 12682/2019, Reg 396 /2005 and European Standard 15662/2018 FS-L-208-15						
SANTE 11312/2021		396/2005				
Group No.	General groups of fruits and vegetables validated according to Sante12682/2019 ANNEX A	Product categories from the previous group	Example of product from the product categories	Examples of related varieties or other hybrid products according to Annex I from 396	Examples of common names / synonyms according to part B of Annex 1 of Reg 396/2005, to which the same MRL apply	Parts of the product that are processed for extraction
1	High water content High water content	Pome fruits	Apples	Cranberry	forest apples, wild apples, tejocote	Whole product after removing the tails
			Pears	Quince nashi (asia)	oriental pears, wild pears, "YA" pears, Chinese white pears	
			Quince		Chinese quinces, Japanese quinces	
			Others	Muslims, Japanese Muslims		
		Stone fruits	Apricots		Japanese apricots, Umes, apricot-peach hybrids	Whole product after removing the tails and stones
			Cherry	Sweet, bitter, sour cherries	black cherries, mulberries, Naking cherries, sour cherries	
			Peaches	Nectarines and similar hybrids	Paraguay peaches, nectarines, other <i>Persica vulgaris</i>	
			Plums	Corcoduse, Goldan (yellow plums), reglota (large reddish plums)	American plums, ornamental plums, Chickasaw plums, Chinese joboba, red dates, Chinese dates, Japanese plums, Klamath plums, gogonette plums, pigeon plums	

1	High water content	Various fruits with edible peel	Fresh figs		Acai fruit, Awara palm fruit, Dum palm fruit	Whole product after removing the tails	
			figs				
			Kumquat (mini orange with edible peel)		Limequat, marumi kumquat, round kumquat, nagami kumquat, oval kumquat, other species of the genus Fortunella not elsewhere mentioned		
	High water content	High water content	Kaki (Plaqueminier)	kaki de Virginia	sapote negre, sapote verzi, sapote albe, sapote galbene	Whole product after removing the tails	
			Various fruits with inedible peel, small	Kiwi			
				Litchi			
	Passion fruits						
			Indian figs (the fruit of the mother-in-law's tongue cactus)		Pitaya/dragon fruit, Red Pitaya, Saguaro fruit, Yellow Pitaya	Whole product after removing the tails	
	High water content	High water content	Bananas		bananas, dwarf bananas, cooking bananas	Whole product after removing the stems or without the crown (pineapple)	
			Mango				
			Papaya		Akee apples, feijoa fruits, langsat, mangosteen, naranjila, Asimina triloba fruits, tamarillo		
			Pomegranate				
			pineapple				

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1	High water content	Bulb vegetables	Onion		pearl onion, Chinese onion, silver onion	Whole product after removal of easily removable skins and soil (dehydrated product) or roots and soil (fresh product)
			Garlic			
			Green onion		spring onion, winter onion and similar varieties	
			Leek			
		Solanaeous fruiting vegetables	Tomatoes		cherry tomatoes, tibetan goji, chinese goji, lychee tomatoes, pear- ched tomatoes	Whole product after removing the tails
			Green and yellow peppers		sweet peppers, bell peppers, chili	
			Red peppers		sweet peppers, bell peppers, chili	
			Eggplants		pepino	
	Other					
	Legume fructoase cucurbitacee cu coaja comestibila	Castraveti			Whole product after removing the tails	
		Cornisoni				
		Dovlecei				
	High water content	Cucurbitaceous fruit vegetables with inedible peel	Watermelons			Whole product after removing the tails
			Yellow Melons			
Pumpkin						
Fruity vegetables - cobs without skin		Fresh sweet corn			Boabe si stiulete fara panusa	
Brassicaceae vegetables (cruciferous vegetables wrapped in 4 leaves of 4, arranged cruciferously)		Cauliflower			Whole product after removal of roots, wilted outer leaves and soil, as appropriate	
		Broccoli				
		Brussels sprouts				
		Head cabbage				
		Red cabbage				
		Chinese cabbage				
		Headless cabbage				
Kuoli						

High water content	High water content	Leafy vegetables and herbs	Lettuce	salad, green lettuce, iceberg lettuce, leaf		Whole product after removal of roots, wilted outer leaves and soil, as appropriate				
			Arugula							
			Spinach		joboda					
			Lemon grass							
			Beetroot for petioles and leaves							
			Vine leaves		climbing mimosa buds, Malabar spinach					
			Beef leaves							
			Chives		wild garlic, leurda					
			Celery leaves		sorrel, lovage leaves, fenugreek leaves, fennel leaves, dill leaves, coriander leaves, cumin leaves, angelica					
			Parsley leaves							
			Fresh sage							
			Fresh sage							
			Mint							
			Rosemary							
			Thyme	Marjoram, oregano	thyme					
			High water content	High water content	Fresh vegetables with stems		Basil	Lomentine leaves, thyme	marigolds (edible flowers), spearmint, other species and hybrids of the genus Mentha not elsewhere mentioned	Whole product after removal of roots, wilted outer leaves and soil, as appropriate
							Bay Leaves		curry leaves	
Tarragon		nettle, sweet stevia								
Asparagus										
High water content	High water content	Fresh legumes	Celery			Whole product				
			Fennel							
			Artichoke							
			Rhubarb							
			Green beans							
High water content	High water content	Fresh mushrooms	Fresh beans			Whole product after removal of soil				
			Pea pods		garden peas, green peas, sweet peas, peeled peas, chickpeas					
			Peas beans							
			Lentils		lupine/ lupine beans					
High water content	High water content	Fresh mushrooms	Cultivated mushrooms			Whole product after removal of soil				
			Wild mushrooms	Manatarchs, yellowtails	porcini mushrooms, black winter truffle, white truffle, ceps					

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1	Root and tuber vegetables	New potatoes			Whole product after removal of soil by washing or brushing
		Potatoes			
		Sweet potatoes			
		Beets			
		Carrots		colored carrot varieties, dwarf carrots	
		Celery root			
		Horseradish		dandelion roots, gentian roots	
		Parsnips			
		Parsley root		lovage roots, nettle roots	
		Radishes		black radishes, winter radishes	
		Turnips			
		Forage cabbage			

a. The extraction is performed as follows:

- weigh 10g sample (for modes E1, E2a, E2b) with an accuracy of ± 0.1 g on an analytical balance in a 50mL centrifuge tube, over which a ceramic stopper is added.
- adjust the pH to pH=4.5-5.5 if necessary, as follows:
 - In the case of samples with a high water content ($\geq 80\%$) and very acidic (pH<3) (mode E2a), the pH is corrected with 5M NaOH solution, (neutralization with 0.6mL 5M NaOH) to pH=4.5-5.5 (from close to close, homogenizing and checking the pH each time).
 - In the case of samples with high water content ($\geq 80\%$) and less acidic (pH ≥ 3) (E2b mode), the pH correction is performed with 5M NaOH solution, (neutralization with 0.2mL 5M NaOH) up to pH=4.5-5.5 (from close to close, homogenizing and checking the pH each time).
- add 50 μ L TFP internal standard (10 μ g/mL), vortex for 30 seconds.
- add 10mL acetonitrile and homogenize for 20 minutes, then freeze the samples for 15 minutes.
- add the saline mixture consisting of: 4g MgSO₄, 1g NaCl, 1g sodium citrate dihydrate and 0.5g sodium citrate hexahydrate, then shake vigorously for one minute.
- centrifuge for 5 minutes at 5000 rpm.

- keep in the freezer for one hour (for modes E2a and E2b).

b. Purification is performed as follows:

- take 6mL of supernatant and transfer it into 15 mL centrifuge tubes containing the saline mixture consisting of: 900mg MgSO₄ and 150mg PSA (mode C2) for mode E1.

For pigmented matrices, different amounts of GCB (graphitized activated carbon) are added to the saline mixture, which already contains 900mg MgSO₄ and 150mg PSA, for depigmentation, depending on the matrix:

- 15mg GCB (graphitized activated carbon) for mode C5a;
- 45mg GCB (graphitized activated carbon) for mode C5b.
- vigorously homogenize for one minute;
- centrifuge for 5 minutes at 5000 rpm;
- filter through PTFE filters (0.2µm), into 10mL test tubes;

For the SHIMADZU LC MS/MS equipment, proceed as follows:

- from the filtrate obtained, take 495µL with a micropipette and add it to the 2mL vial;
- add 5 µL of 5% formic acid solution in ACN;
- vortex for 1 minute;
- the 2mL vial, containing the final solution, is inserted into the autosampler and the sample is read by LC-MS/MS.

2. Matrices with high acid content and high water content (Ex. citrus fruits, small fruits and berries and their processed products);

The validation of this group of matrices was carried out for a number of 397 pesticides, with experiments performed on the lemon matrix.

Matrices with high acid content and high water content, which include the citrus fruit group (e.g.: lemons, mandarins, tangerines, oranges, etc.) and the small fruit and berry group (e.g.: strawberries, blueberries, raspberries, blackberries, blackcurrants, redcurrants, whitecurrants, grapes, etc.); processed products: juices and wines.

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2	High acid content and high water content	Citrus fruits	Grapefruit	Pomelo, including giant citrus, sweeties, tangelo, ugli and other hybrids	other hybrids of Citrus Paradisi not elsewhere mentioned	Whole product
			Oranges	Bergamot, bitter oranges, blood oranges, chinotes, trifoliolate oranges	other hybrids of Citrus Sinensis not elsewhere mentioned	
			Lemons		chitre,	
			Limes			
			Tangerines	Clementine, tangerine	clementines, minoles, other hybrids of Citrus Reticulata not mentioned elsewhere	
		Berries and small fruits	Table grapes			Produs intreg dupa indepartarea varfului/bazei si a pedunculului, cu exceptia coacazelor:fructe cu peduncul
			Wine grapes			
			Strawberries		alpine strawberries, strawberries	
			Blackberries			
			Raspberries			
			Blueberries		sea buckthorn	
			Cranberries			
			Blackcurrants			
			Redcurrants			
Whitecurrants						
Gooseberries						
Rose hips						
Mulberries						
Elderberries		hawthorn				

3. Matrices with high sugar content and low water content (Ex. dried fruits).

The validation of this group of matrices was carried out for a number of 392 pesticides, with experiments carried out on the candied fig matrix.

Matrices with high sugar content and low water content of which dried fruits are part (raisins, dried apricots, figs, prunes and jam fruits).

a. The extraction is performed as follows:

Homogenize 500g of frozen dried fruits with 850g of cold water;

- weigh 13.5g of the homogenized sample (for E4 modes, with a water content of 15%-40%) with an accuracy of ± 0.1 g on an analytical balance in a 50mL centrifuge tube, over which a ceramic stopper is added; 1111

- add 25 μL of TFP internal standard (10 $\mu\text{g}/\text{mL}$), vortex for 30 seconds;
- add 10mL of acetonitrile and homogenize for 20 minutes, then freeze the samples for 15 minutes.
- add the saline mixture, consisting of: 4g MgSO_4 , 1g NaCl , 1g sodium citrate dihydrate and 0.5g sodium citrate hexahydrate, then shake vigorously for one minute;
- centrifuge for 5 minutes at 5000 rpm.

b. Purification is performed as follows:

- take 6mL of supernatant and transfer it into 15mL centrifuge tubes containing:
 - saline mixture consisting of 900mg MgSO_4 and 150mg PSA (mode C2)
 - homogenize vigorously for one minute;
 - centrifuge for 5 minutes at 5000 rpm;
 - filter through PTFE filters (0.2 μm) into 10mL test tubes

For Shimadzu LC MS/MS equipment, proceed as follows:

- take 495 μL of the filtrate obtained with a micropipette and add it to the 2mL vial;
- add 5 μL of 5% formic acid solution in ACN;
- vortex for 1 minute;
- the 2mL vial, containing the final solution, is inserted into the autosampler and the sample is read by LC-MS/MS;

4. Matrices with high fat content and very low water content (e.g. nuts, oilseeds, nut pastes, peanuts);



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4a	High fat content and very low water content	nuts (tree fruits) with or without shell	Commun nuts			Whole product after removal of the shell (except chestnuts)
			Almonds		apricot kernels, bitter almonds, "Canarium" or "Pili" type nuts, okari nuts	
			Brazilian nuts			
			Cashews			
			Chestnuts			
			Coconuts		areca nuts (or betel)	
			Hazelnuts		acorns, hazelnuts	
			Macadamia Nuts			
			Pecan Nuts		American walnut nuts	
			Pine Nuts		other species of the genus Pinus, not elsewhere specified	
		Pistachios				
		Oilseeds	Rapeseed Seeds		seminte de ridiche, seminte de naveta	Whole product after removal of the peel, the stone and the skin, if possible
			Sunflower Seeds			
			Cotton Seeds			
			Soybean Seeds		seminte de arborele vietii	
			Peanuts			
			Sesame Seeds			
			Flax Seeds			
			Poppy Seeds			
			Soybeans			
			Mustard Seeds			
			Cotton Seeds			
			Pumpkin Seeds	watermelon seeds	other seeds of species of the family Curcubitaceae, not elsewhere specified	
			Safflower			
			Lamb's tongue		field marigold, primrose, wild sesame seeds	
		Camelina				
		Hemp				
Castor		grape seeds, sea buckthorn seeds				
Pastas and purees obtained from nuts and oil seeds	Peanut butter					
	Hazelnut paste					
	Chestnut puree					
	Tahini (sesame paste)					

The validation of this group of matrices was carried out for a number of 391 pesticides, with experiments performed on the almond kernel matrix.

Matrices with high fat content and very low water content including nuts and oil seeds (walnuts, peanuts, almonds, hazelnuts, chestnuts, rapeseed, sunflower seeds, cottonseed, soybeans, sesame, peanut butter, tahini, peanut paste).

5. Matrices with high fat content and intermediate water content 40%-80% (e.g. fruits and oilseed products);

4 b	Intermediate water content and high fat content	Oil fruits and derived products	Fresh table olives and paste obtained from them		black olives from China, white olives from China	
			Avocado and avocado paste/puree		avocado for oil	

The validation of this group of matrices was carried out for a number of 341 pesticides, with experiments performed on the avocado matrix.

Matrices with high fat content and intermediate water content include fruits and oil products (olives, avocados and their products).

The extraction is performed as follows:

- weigh:
 - 5g sample (for E6 mode - with intermediate water content 40%-80% and high fat content >5%) with an accuracy of ± 0.1 g on an analytical balance in a 50mL centrifuge tube, on top of which add a ceramic stopper.
 - add water depending on the matrix, as follows:
 - in the case of samples with intermediate water content 40%-80% and high fat content >5% (for E6 mode), add 6 mL water.
 - add 25 μ L TFP internal standard (10 μ g/mL), vortex for 30 seconds;
 - add 10mL acetonitrile and homogenize for 20 minutes, then freeze the samples for 15 minutes.
 - add the saline mixture consisting of: 4g MgSO₄, 1g NaCl, 1g sodium citrate dihydrate and 0.5g sodium citrate hexahydrate, then shake vigorously for one minute;
 - centrifuge for 5 minutes at 5000 rpm.

Purification is performed as follows:

- take 6mL of supernatant and transfer it into 15mL centrifuge tubes containing:

- saline mixture consisting of 900mg MgSO₄ and 150mg PSA (mode C2)– in the case of samples with an intermediate water content of 40%-80% (for modules E3a and E3b);

- saline mixture consisting of 900mg MgSO₄ and 150mg PSA (mode C2)– in the case of leafy vegetables and aromatic herbs (for module E6);

- 900mg MgSO₄, 150mg PSA and 150mg C18 (C4 mode)-in case of samples with intermediate water content 40%-80% and high fat content >5% (for E6 mode);

- homogenize vigorously for one minute;

- centrifuge for 5 minutes at 5000 rpm;

- filter, through PTFE filters (0.2µm), into 10mL tubes;

For the Shimadzu LC MS/MS equipment, proceed as follows:

- from the filtrate obtained, take 495µL with a micropipette and add to the 2mL vial;

- add 5 µL of 5% formic acid solution in ACN;

- vortex for 1 minute;

- the 2mL vial, containing the final solution, is inserted into the autosampler and the sample is read by LC MS MS.



6. Matrices with high starch content and/or low water <15% and fat content (e.g. dried vegetables and legumes, cereals and derived/processed products);

The validation of this group of matrices was carried out for a number of 354 pesticides, with experiments performed on the wheat matrix.

Matrices with high starch content and/or low water and fat content (dried vegetables and legumes, cereals and derived products (beans, lentils, wheat, rice, barley, oats, rye, corn, flour);

5	High starch and protein content and low water and fat content	Dried vegetables/legumes	Field beans			
			Dried beans (Vicia Faba-garden bean)			
			Dried small bean, (yellow, white/navy, brown, spotted color varieties)			
			Lentils			
		Cereale si produse derivate	Whole product	Wheat	durum wheat, small alac, emmer wheat, Khorasan wheat, triticale	alte specii din genul <i>Triticum</i> care nu sunt mentionate in alta parte
				Rye		
				Barley		
				Oatmeal		
				Buckwheat		amaranth, kaniwa, quinoa, chia seeds
				Millet		Digitaria iburua plant, common millet, Job's tear
				Corn kernels		Indian/glassy corn
				Rice		African rice, Nerica hybrid, Indian/wild rice
				Rye		
				Sorghum	Sorghum bicolor plant, Sudan grass	other species of the genus Sorghum not mentioned elsewhere
				Wholegrain bread		
				White bread		
				Cookies		
				Breakfast cereals		
				Pasta		
Flour						

a. The extraction is performed as follows:

- weigh 5g of homogenized sample (for E5 modes, with water content <15%) with an accuracy of ± 0.05 g on an analytical balance in a 50mL centrifuge tube, on top of which add a ceramic stopper;

- add 25 μ L TFP internal standard (10 μ g/mL), vortex for 30 seconds;

- add 10 ml of ultrapure water;

- add 10mL of acetonitrile and homogenize for 20 minutes, then freeze the samples for 15 minutes.

- add the saline mixture, consisting of: 4g MgSO₄, 1g NaCl, 1g sodium citrate dihydrate and 0.5g sodium citrate hexahydrate, then shake vigorously for one minute;

- centrifuge for 5 minutes at 5000 rpm

b. Purification is performed as follows:

- take 6mL of supernatant and transfer it into 15mL centrifuge tubes containing:

- saline mixture consisting of 900mg MgSO₄ and 300mg PSA (mode C1 and C3a)

- vigorously homogenize for one minute;

- centrifuge for 5 minutes at 5000 rpm;

- filter through PTFE filters (0.2µm) into 10mL tubes.

For Shimadzu LC MS/MS equipment, proceed as follows:

- take 495µL of the filtrate obtained with a micropipette and add it to the 2mL vial;

- add 5µL of 5% formic acid solution in ACN;

- vortex for 1 minute;

- the 2mL vial, containing the final solution, is inserted into the autosampler and the sample is read by LC-MS/MS;



7. Other matrices considered difficult or unique (e.g. coffee, cocoa, spices, hops, tea etc);

The validation of this group of matrices was carried out for a number of 350 pesticides, with experiments performed on the cocoa matrix.



6	Matrici dificile sau unice		Hops (dehydrated), including hop granules and non-concentrated powder			Whole product
			Cocoa beans and derived products			
			Coffe			
			Carob		Archidendron pauciflorum tree	Whole product after removal of the peduncle or the crown
			Tea (dried infusions)	Flowers	Chamomile flowers	Whole product
					Hibiscus flowers	
					Rose petals	
					Jasmine flowers	
					Lime tree	
				Leaves	Strawberry leaves	
			Rooibos leaves	Roots	Valerian	
					Ginseng	
			Others			
			Spices	from seeds	Anise	Dried whole, ground or powdered product
				Black cumin		
				Celery seeds		
				Coriander seeds		
				Cummin seeds		
				Dill seeds		
				Fennel seeds		
	Fenugreek					
	Nutmeg					
	from fruits	Pepper (white, black and green)				
	Allspice					
	Sichuan pepper					
	Cardamom					
	Vanilla pods					
	Juniper berries					
	from the bark	Cinnamon				
	from roots or rhizomes	Ginger				
	Licorice					
	Turmeric/Indian saffron					
	Horseradish					
	from buds	Cloves				
	Capers					
	from flower pistils	Saffron				
	Other					

a. The extraction is performed as follows:

- weigh 2g of sample, add 10 ml of water (for E7 modes, with water content <15%) with an accuracy of ± 0.02 g on an analytical balance in a 50mL centrifuge tube, on top of which add a ceramic stopper;

- add 10 μ L of TFP internal standard (10 μ g/mL), vortex for 30 seconds;

- add 10mL of acetonitrile and homogenize for 20 minutes, then freeze the samples for 15 minutes.

- add the saline mixture, consisting of: 4g MgSO₄, 1g NaCl, 1g sodium citrate dihydrate and 0.5g sodium citrate hexahydrate, then shake vigorously for one minute;

- centrifuge for 5 minutes at 5000 rpm.

b. Purification is performed as follows:

- take 6mL of supernatant and transfer it into 15mL centrifuge tubes containing:

- saline mixture consisting of 900mg MgSO₄ and 150mg PSA (mode C2)

- vigorously homogenize for one minute;

- centrifuge for 5 minutes at 5000 rpm;

- filter through PTFE filters (0.2 μ m) into 10mL test tubes;

- For Shimadzu LC MS/MS equipment, proceed as follows:

- take 495 μ L of the filtrate obtained with a micropipette and add it to the 2mL vial;

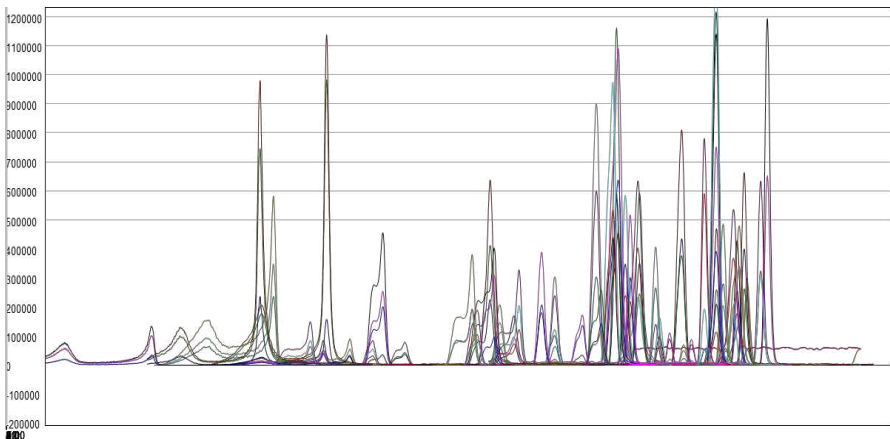
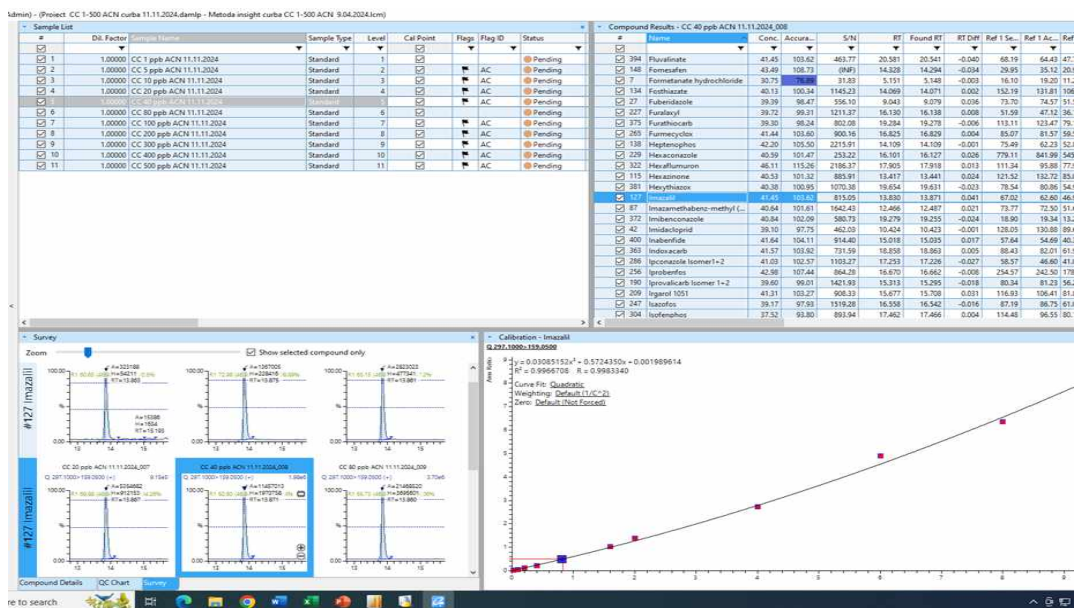
- add 5 μ L of 5% formic acid solution in ACN;

- vortex for 1 minute;

- insert the 2mL vial containing the final solution into the autosampler and read the sample by LC-MS/MS;

Interpretation of quantitative data is done using LabSolution Insight integration software, automatically marking retention times and integrating chromatographic peaks, directly displaying their area and extract concentration, for each compound of mix 397 pesticides substances.

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Ingrid Alina Coman, Marilena Canuta, Natalia Roșoiu



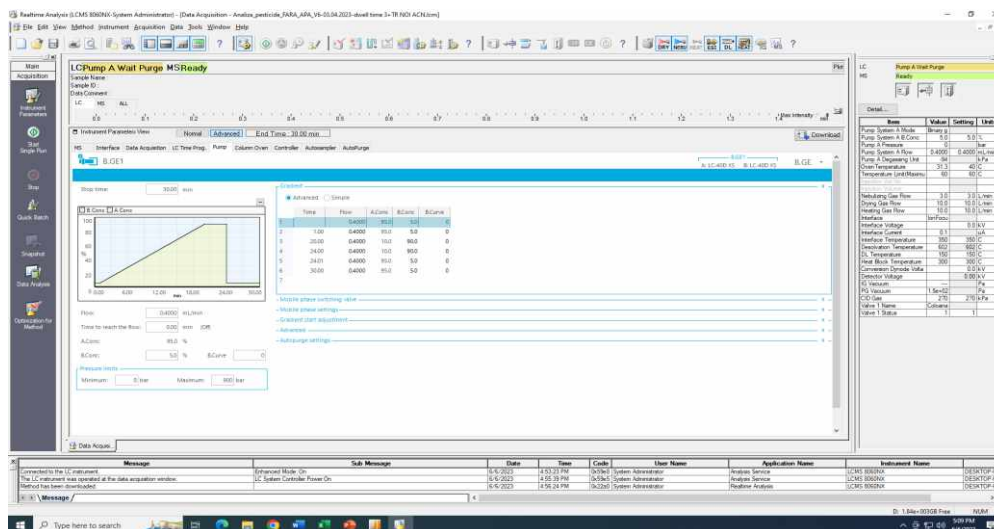
Analytical and Chromatographic Conditions for LC-MS/MS

Shimadzu 8060 Nexera Liquid Chromatograph Settings:

- Column: LC Column Shim-pack Velox Biphenyl, 1.8 μ m, 100 x 2.1 mm;
- Run Time: 30 min;
- Injection volume: 1 μ L;
- Column temperature: 40 $^{\circ}$ C; Flow 0,4 ml/min.

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Shimadzu 8060 Nexera gradient



Shimadzu 8060 Nexera gradient

Nr crt	Time	Flux	A conc	B conc
1		0.400	95	5
2	1.00	0.400	95	5
3	20.00	0.400	10	90
4	24.00	0.400	10	90
5	24.01	0.400	95	5
6	30.00	0.400	95	5

Demonstrated results for performance requirements

1. Specificity

Detection limit – the analyte concentration that produces a peak with a height of 3 times the noise level (signal/noise ratio > 3/1);

Ionic ratio: the ionic ratio of the analyte must fall within ±30% of the average of the ion ratios of the concentration points in the calibration curve. This parameter is automatically generated by the equipment software.

Retention time: the time elapsed from the injection of the sample into the equipment until the appearance of the maximum of the chromatographic peak (the time the compound was retained in the chromatographic column); its variation during the analysis must fall within ±0.1min compared to the time established in the method when scanning the compound.

2. Quantification limit LOQ ≤MRL (maximum permissible limit) in compliance with the recovery and precision criteria; signal/noise ratio > 3/1;

3. Recovery

The percent recovery for fortified samples used in validation should be in the range of 70-120%.

In exceptional cases, average recovery rates outside the range of 70-120% may be accepted if the data are constant over time ($RSD \leq 20\%$), but the average recovery should not be less than 30% or more than 140% during validation.

4. Fidelity (Accuracy): the result obtained from the use of MR / MRC must fall within the certified value taking into account the measurement uncertainty.

5. Repeatability (Precision) - The coefficient of variation must be $\leq 20\%$ and the percentage of recovery must be within the range 70-120 $RSD_r < RSD_{Horwitz}$, $HORRAT_r$ values < 2

6. Reproducibility within the laboratory (Precision) - The coefficient of variation must be $\leq 20\%$ and the percentage of recovery must be within the range 70-120. $RSD_R < RSD_{Horwitz}$, $HORRAT$ values < 2

7. Linearity: the standard deviation of the concentration obtained must be $\leq \pm 20\%$ of the theoretical concentrations and the regression coefficient, r^2 of the curve must be ≥ 0.97 ;

8. Matrix effect demonstrated by comparing (difference) the analytical response of the compounds on the calibration curve drawn by solvent to the analytical response of the compounds on the calibration curve drawn by matrix extract. For a standard deviation of the analytical response $\leq \pm 20\%$ the matrix effect does not influence the result and the curve drawn by solvent can be used. For a standard deviation of the analytical response $> \pm 20\%$ the matrix effect influences the result and the curve drawn by matrix extract is used.

9. Robustness/Stability: the matrix/component must be stable over time and at temperature changes of 5°C .

Stability – the stability of the analyte in the matrix over time. The results must fall within the assured range. The stability of the matrix and the component is analyzed over a period of 1 year (EURL test).

Robustness – the stability of the analyte in the matrix to temperature variations in the laboratory. The temperature variation of 5°C must not influence the measurement results.

10. Measurement margin of error: Measurement uncertainty, $U_c \leq 50\%$ [5];

The standard deviation of the concentration obtained should be $\leq \pm 20\%$ of the theoretical concentrations; All readings should be performed in a single sequence.

The percentage recovery should be within the range of 70–120%, with an associated repeatability, $RSD_r \leq 20\%$, $RSD < RSD_{Horwitz}$, for all analytes within a method. In exceptional cases, according to DG Sante 11312/2021, average recovery rates outside the range of 70-120% can be accepted if justified ($RSD \leq 20\%$ but the average recovery should not be less than 30% or more than 140%). The acceptability conditions for the difference between the initial QC and the final QC should be within $\pm 20\%$ of the average QC value [1], [2].

Limit of quantification: in the present situation, of a multi-residue method, with a great diversity of plant matrices and very different values for MRLs, the values obtained/demonstrated for LOQ are:

- 0.001 mg/Kg for carbofuran; 0.005 mg/kg for fipronil, and 0.01 mg/kg for all other analytes in this validation protocol.

The LOQ values are considered lower limits of analytical determination, for most pesticides in the list of validated compounds, according to Reg. EC 396/2005 with subsequent amendments and completions.

Conclusions

The results obtained from the tests on the goods as classified according to Document N0 DG/SANTE, namely food products of non-animal origin and animal feed, having as reference matrix apple, lemon, avocado, wheat, almonds, figs, cocoa for the determination of pesticides by LC-MS/MS, indicate that the method is correctly applied, is efficient and adequate for the purpose.

The validation was carried out on blank samples.

The performance requirements according to Document N0 DG/SANTE are met for the performance parameters mentioned in this validation

Human exposure to pesticides can be the consequence of consuming fruits and vegetables that contain residues from pesticides sprayed on plants.

This study for the validation of the presented method, carried out within the Constanta Sanitary Veterinary and Food Safety Laboratory, is of particular importance and involved, throughout the validation period, countless material and human resources.

The analysis of fruits and vegetables for the evaluation of pesticide residues is an important quality control procedure, established to reduce the abusive use of pesticides and to ensure the quality and safety of food intended for human consumption.

Every year, reports are made with the results obtained in the laboratory to the European Food Safety Authority (EFSA), so that the maximum permissible

limits of pesticide substances can be reduced annually or certain substances used abusively can even be banned from the market.

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 approve or not to use of plant protection product. If pesticides are applied 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.

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