Evaluation of low flow chromatography for sensitivity enhancement

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1. Aim and scope

This document reports the evaluation of the sensitivity using low flow methodologies for the analysis of pesticides included in the European Union Multi Annual Control Program (EU-MACP). The study was performed applying Supercritical fluid chromatography coupled to tandem mass spectrometry (SFC-MS/MS).

2. Short description

Limits of quantification and matrix effects were studied in 5 different matrices (tomato, orange, leek, cayenne, and black pepper). The samples were extracted using QuEChERS extraction methods. The obtained extracts were used to prepare matrix-matched calibration curves with concentrations ranging between 2 µg/Kg and 500 µg/Kg. To complete the study, pyrethroids pesticides were studied using SFC-MS/MS to evaluate the ionization benefits of the low flow chromatographic methods.

3. Apparatus and consumables

- Automatic pipettes, suitable for handling volumes from 10 µL to 5000 µL and from 1 mL to 5 mL.
- Graduated 10 mL pipette.
- 50 mL and 15 mL PTFE centrifuge tubes.
- Vortex Shaker IKATM 4 Basic.
- Axial shaker Agytax SR1 CP57.
- Centrifuge Orto Alresa Consul 21, suitable for the centrifuge tubes employed in the procedure and capable of achieving at least 4000 rpm.
- Injection vials, 2 mL, suitable for LC and GC auto-sampler.

4. Chemicals

- Acetonitrile ultra-gradient grade
- Trisodium citrate dihydrate
- Disodium hydrogenocitrate sesquihydrate
- Sodium chloride
- Anhydrous magnesium sulphate
- Anhydrous calcium chloride
- Primary secondary amine (PSA)
- Supel QuE Z-Sep
- Ammonium formate

- Ultra-pure water
- Methanol HPLC grade
- Formic acid
- Pesticide standards

5. Procedure

5.1. Sample preparation

Blank samples of tomato, orange and leek matrices were extracted following the QuEChERS extraction procedure including a clean-up step with PSA and magnesium sulphate. Cayenne and black pepper were prehydrated before the QuEChERS extraction. Enhanced matrix-removal (EMR) clean-up was employed as a clean-up step in the spice's extraction. The pyrethroids analyses were carried out using blank extracts from QuEChERS extraction applying PSA and magnesium sulphate as clean-up.

5.2. Pesticide stock solutions and working mix solutions

Individual pesticide stock solutions (1000–2000 mg/L) were prepared in acetonitrile or ethyl acetate and were stored in screw-capped glass vials in the dark at -20 °C. Working mixes were prepared in 10 mL volumetric flasks by pipetting the appropriate volume of each stock solution.

5.3. Extraction methods

5.3.1. Tomato, orange and leek

QuEChERS

1. Weigh 10 g of sample in a 50-mL PTFE centrifuge tube.
2. Add 10 mL acetonitrile.
3. Shake the sample in the Agytax® shaker for 4 min.
4. Add 4 g anhydrous magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate.
5. Shake the sample in the Agytax® shaker for 4 min.
6. Centrifuge the tubes at 4000 rpm for 5 min.
7. Transfer a 5 mL aliquot of the supernatant to a 15 mL PTFE tube containing 750 mg anhydrous magnesium sulphate and 125 mg PSA.
8. Vortex the tubes for 30 s.

9. Centrifuge at 4000 rpm for 5 min.
10. Take 4 mL and add 40 µL of a 5 % formic acid solution in I (v/v).

5.3.2. Spices (Cayenne and black pepper)

1. Weigh 2 g of sample in a 50-mL PTFE centrifuge tube.
2. Add 7 mL of milli-Q water.
3. Vortex tube for 30 s.
4. Wait 30 min.
5. Add 10 mL acetonitrile.
6. Shake the sample in the Agytax® shaker for 7 min.
7. Add 4 g anhydrous magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate.
8. Shake the sample in the Agytax® shaker for 7 min.
9. Centrifuge the tubes at 3700 rpm for 5 min.
10. Transfer a 5 mL aliquot of the supernatant to an EMR-Lipid tube preconditioned with 5 mL of Milli-Q water. The extracts were vortexed for 30 s and then centrifuged at 3700 rpm for 5 min.
11. A 5 mL extract was transferred to an EMR-polish tube containing 1 g of sodium chloride and 4 g of magnesium sulphate. The extracts were vortexed for 30 s and then centrifuged again at 3700 rpm for another 5 min.
12. Approximately 2mL of the final extract were collected.

5.4. Vial preparation

Extraction method	LC-QqQ-MS/MS
QuEChERS	5-fold dilution of the extract with ultrapure water
Modified QuEChERS with EMR for spices	2-fold dilution of the extract with ultrapure water
Pyrethroids analysis	No dilution

During the vial preparation, dimethoate-D_6 was added as an injection internal standard.

5.5. Methodology

A Shimadzu extended multireaction monitoring (MRM) library was used for the creation of the multiresidue method. This feature shows many transitions for each pesticide; three of them were selected following the sensitivity rank. Individual

standard solutions of the pesticides were injected to confirm the transition with a higher signal (quantifier) and the second most sensitive (qualifier). Some compounds, such as internal standards (dimethoate-d6, carbendazim-d3, malathion-d10, and dichlorvos-d6) and pyrethroids, were not present in the library and must be manually optimized using a precursor ion search. For a proper identification, two transitions must be detected with an ion ratio difference less than 30% and a retention time shift under 0.1 min. Acquisition windows of ±0.35 min were established for each pesticide in the multiresidue method.

5.6. Instrumentation and analytical conditions for the SFC- MS/MS system

5.6.1. Nexera UC (Shimadzu)

- Mobile phase A: Carbon dioxide (99.9993%)
- Mobile phase B (Modifier): Methanol (1 mM ammonium formate)
- Mobile phase C (Make-up): Methanol (0.1 % formic acid, 5 mM ammonium formate, 5 % water)
- Column temperature: 40 °C
- Flow rate: 1.3 mL/min
- Injection volume: 2 µL
- Column: Shimpack UC-X RP C18 2.1x250 mm and 3 µm particle size

Mobile phase gradient for pesticides analysis:

Time [min]	Mobile phase A	Mobile phase B
0	99 %	1 %
2	99 %	1 %
5	95 %	5 %
8	60 %	40 %
10	60 %	40 %
10,5	99 %	1 %

Re-equilibration time with initial mobile phase set for 2.5 minutes. The mobile phase C (Make-up) was introduced in the system isocratically at 0.080 mL/min.

5.6.2. 8060 triple quadrupole system (Shimadzu)

- Ionisation mode: Positive mode and negative mode
- Capillary (positive and negative): 4 kV
- Switching polarity: 5 ms
- Interface temperature: 300 °C

- Desolvation line temperature: 250 °C
- Heat block temperature: 400 °C.
- Nebulizer gas flow: 3 L/min
- Heating gas flow: 10 L/min
- Drying gas flow: 10 L/min

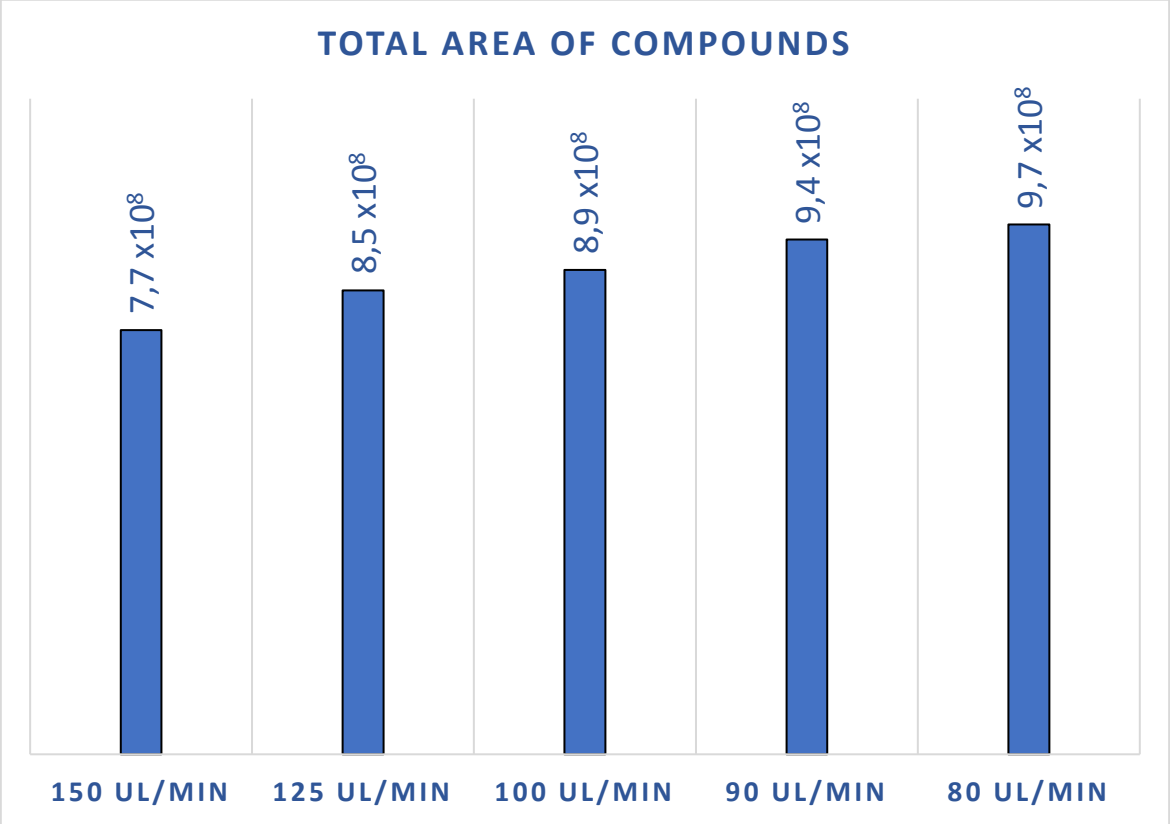
6. Results

The objective of this technical report is to evaluate low flow chromatography for sensitivity enhancement. When a low flow reaches the ionization chamber of the ESI source, improvement of the sampling efficiency is achieved [1]. The supercritical carbon dioxide employed as a mobile phase in SFC-MS/MS returns to gas state just before the ESI source. Accordingly, the compounds elute at the source only through the cosolvent (modifier) of the mobile phase and, mainly, the make-up flow. More than 90% of the compounds enter the source with a flow lower than 150 µL/min (being 300 µL/min the standard flow in liquid chromatography).

6.1. Make-up solvent optimization

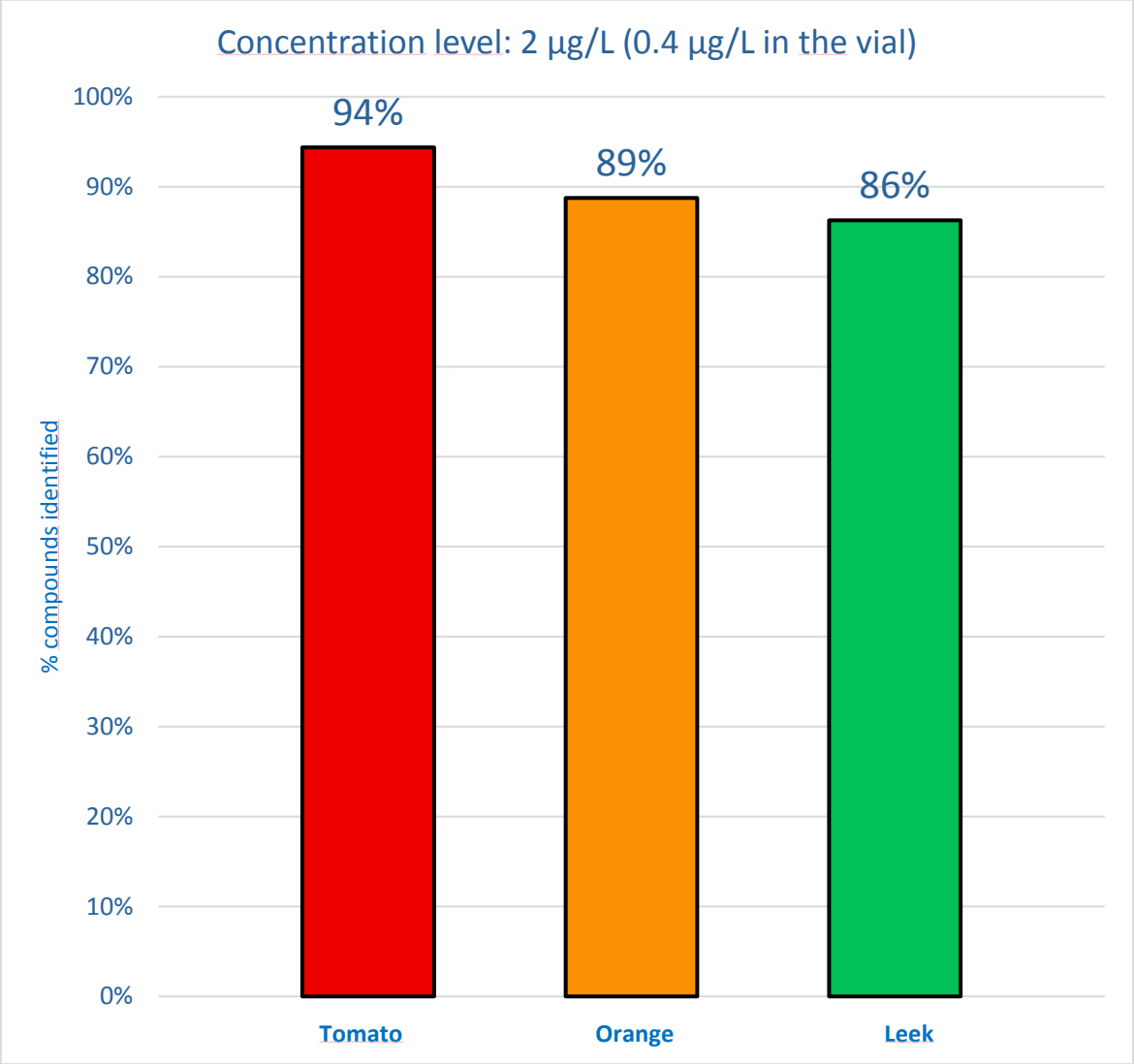
The SFC-MS/MS system is equipped with an auxiliary pump that provides a make-up solvent after the column isocratically. When carbon dioxide loses its supercritical state, most of the solvent that reaches the mass spectrometer source comes from the auxiliary pump. Therefore, an optimization of the post-column flow is necessary to increase the sensitivity without compromising reproducibility.

One hundred and sixty-four pesticides were analyzed at 10 µg/L in tomato matrix with different make-up flows ranging between 50 µL/min and 150 µL/min. The sensitivity was considered as the sum of the areas of the analytes studied. The lowest flows (50,60, 70 and 80 µL/min) showed a higher sensitivity for some compounds, However, with flow rates lower than 80 µL/min, 42 compounds gradually reduced their sensitivity (their reproducibility being also compromised). Furthermore, some compounds that eluted with flows below 80 µL/min presented bad peak shape or generated carry over between injections, which means that it is not enough volume for a proper elution. Using 80 µL/min as a post-column flow, the sensitivity increased by 25%, compared to 150 µL/min, without experiencing performance disadvantages (93% of the compounds showing RSD below 10%). After this evaluation, the auxiliary make-up pump was set at 80 µL/min for further analysis.



6.2. Instrumental limits of quantification

The evaluation of the instrumental LOQs was performed injecting matrix-matched calibration curves with ranges between 2-500 µg/L for fruits and vegetables, and 5-500 µg/L for spices. For the analysis of tomato, orange and leek there is a 5-fold dilution in the vial prior injection. The injection volume was set in 2 µL, being 0,4 mg the total amount of sample injected. The percentage of compounds identified at the concentration level of 2 µg/L (0,4 µg/L in the vial, after dilution) was 94%, 89% and 86% for tomato, orange, and leek, respectively. The compounds identified at 5 µg/L increased to 98% in tomato, 98% in orange and 94% in leek. At 10 µg/L, all the compounds were identified in tomato matrix and only one compound (Spiromesifen) was not identified in orange and leek.



This evaluation was also applied to spices: cayenne and black pepper. Most of the pesticides studied met the requirements to be identified at the lowest concentration level of 5 mg/L in both matrices. The extraction method applied a 5-fold dilution, and together with the 2-fold vial dilution, the total amount of sample injected was 0.1 mg.

Table 1. instrumental concentration ranges for spices matrices:

Compound	Instrumental concentration range (µg L^{-1})	
	Cayenne	Black pepper
2,4-D	5 – 500	5 – 500
Acephate	5 – 500	20 – 500
Acetamiprid	5 – 500	5 – 500
Aldicarb	20 – 500	20 – 500

Aldicarb-sulfone	5 – 500	5 – 500
Ametoctradin	5 – 500	5 – 500
Azinphos-methyl	5 – 500	5 – 500
Azoxystrobin	5 – 500	5 – 500
Bitertanol	5 – 500	5 – 500
Boscalid	5 – 500	5 – 500
Bromuconazole	5 – 500	5 – 500
Bupirimate	5 – 500	5 – 500
Buprofezin	5 – 500	5 – 500
Carbaryl	5 – 500	5 – 500
Carbendazim	5 – 500	20 – 500
Chlorantraniliprole	5 – 500	5 – 500
Chlorfenvinphos	5 – 500	5 – 500
Chlorpyrifos	5 – 500	5 – 500
Clofentezine	5 – 500	5 – 500
Clomazone	5 – 500	5 – 500
Coumaphos	5 – 500	5 – 500
Cyazofamid	5 – 500	5 – 500
Cymoxanil	5 – 500	10 – 500
Cyproconazole	5 – 500	10 – 500
Cyprodinil	5 – 500	5 – 500
Cyromazine	20 – 500	20 – 500
Deet	5 – 500	5 – 500
Demeton-S-methyl-sulfone	5 – 500	5 – 500
Diazinon	5 – 500	5 – 500
Dichlorvos	5 – 500	200-500
Dicrotophos	5 – 500	5 – 500
Diethofencarb	5 – 500	5 – 500
Difenoconazole	5 – 500	5 – 500
Diflubenzuron	5 – 500	5 – 500
Dimethoate	5 – 500	5 – 500
Dimethomorph	5 – 500	5 – 500
Diniconazole	5 – 500	5 – 500
Diuron	5 – 500	5 – 500
Emamectin B1a	5 – 500	5 – 500
EPN	5 – 500	5 – 500
Epoxiconazole	5 – 500	5 – 500
Ethion	5 – 500	5 – 500
Ethirimol	5 – 500	5 – 500
Ethoprophos	10 – 500	10 – 500
Etofenprox	5 – 500	5 – 500
Etoxazole	5 – 500	5 – 500
Famoxadone	5 – 500	5 – 500
Fenamidone	5 – 500	5 – 500

Fenamiphos	5 – 500	5 – 500
Fenamiphos-sulfone	5 – 500	5 – 500
Fenamiphos-sulfoxide	5 – 500	5 – 500
Fenarimol	5 – 500	5 – 500
Fenazaquin	5 – 500	5 – 500
Fenbuconazole	10 – 500	5 – 500
Fenhexamid	50 – 500	20 – 500
Fenoxycarb	5 – 500	5 – 500
Fenpropathrin	5 – 500	5 – 500
Fenpyroximate	5 – 500	5 – 500
Fenthion	5 – 500	5 – 500
Fenthion-sulfone	5 – 500	5 – 500
Fenthion-sulfoxide	5 – 500	5 – 500
Fipronil	5 – 500	5 – 500
Flonicamid	5 – 500	10 – 500
Fluazifop	5 – 500	5 – 500
Fludioxonil	5 – 500	10 – 500
Flufenacet	5 – 500	5 – 500
Flufenoxuron	5 – 500	5 – 500
Fluopyram	5 – 500	5 – 500
Fluquinconazole	5 – 500	5 – 500
Flusilazole	5 – 500	10 – 500
Flutriafol	5 – 500	5 – 500
Fluxapyroxad	5 – 500	5 – 500
Fosthiazate	5 – 500	5 – 500
Haloxyfop	5 – 500	5 – 500
Hexaconazole	5 – 500	50 – 500
Hexythiazox	5 – 500	5 – 500
Imazalil	5 – 500	20 – 500
Imidacloprid	5 – 500	5 – 500
Indoxacarb	5 – 500	5 – 500
Ioxynil	5 – 500	5 – 500
Iprodione	5 – 500	5 – 500
Iprovalicarb	5 – 500	5 – 500
Isoprocarb	5 – 500	50 – 500
Isoxaflutole	5 – 500	5 – 500
Kresoxim-methyl	5 – 500	5 – 500
Linuron	5 – 500	10 – 500
Lufenuron	5 – 500	5 – 500
Malathion	10 – 500	10 – 500
Mandipropamid	5 – 500	5 – 500
MCPA	5 – 500	5 – 500
Mepanipyrim	5 – 500	20 – 500
Meptyldinocap	5 – 500	5 – 500

Metaflumizone	5 – 500	5 – 500
Metalaxyl	5 – 500	5 – 500
Metconazole	5 – 500	5 – 500
Methamidophos	5 – 500	5 – 500
Methidathion	5 – 500	5 – 500
Methiocarb	5 – 500	5 – 500
Methiocarb-sulfone	5 – 500	5 – 500
Methiocarb-sulfoxide	5 – 500	5 – 500
Methomyl	5 – 500	5 – 500
Methoxyfenozide	5 – 500	5 – 500
Metobromuron	5 – 500	10 – 500
Monocrotophos	5 – 500	5 – 500
Myclobutanil	5 – 500	10 – 500
Nitenpyram	10 – 500	20-500
Omethoate	5 – 500	5 – 500
Oxadixyl	5 – 500	5 – 500
Oxamyl	5 – 500	5 – 500
Paclobutrazol	5 – 500	5 – 500
Paraoxon-methyl	5 – 500	5 – 500
Penconazole	5 – 500	5 – 500
Pencycuron	5 – 500	5 – 500
Pendimethalin	10 – 500	10 – 500
Phenthoate	5 – 500	20 – 500
Phosalone	5 – 500	5 – 500
Phosmet	5 – 500	5 – 500
Phoxim	5 – 500	5 – 500
Pirimicarb	5 – 500	20 – 500
Pirimicarb-desmethyl	5 – 500	5 – 500
Pirimiphos-methyl	5 – 500	5 – 500
Prochloraz	5 – 500	5 – 500
Profenofos	5 – 500	5 – 500
Propamocarb	5 – 500	5 – 500
Propaquizafop	5 – 500	5 – 500
Propargite	5 – 500	5 – 500
Propiconazole	5 – 500	5 – 500
Propoxur	5 – 500	10 – 500
Propyzamide	5 – 500	5 – 500
Proquinazid	5 – 500	5 – 500
Prothiophos	5 – 500	20 – 500
Pymetrozine	50 – 500	50 – 500
Pyraclostrobin	5 – 500	5 – 500
Pyridaben	5 – 500	5 – 500
Pyrimethanil	5 – 500	20 – 500
Pyriproxyfen	5 – 500	5 – 500

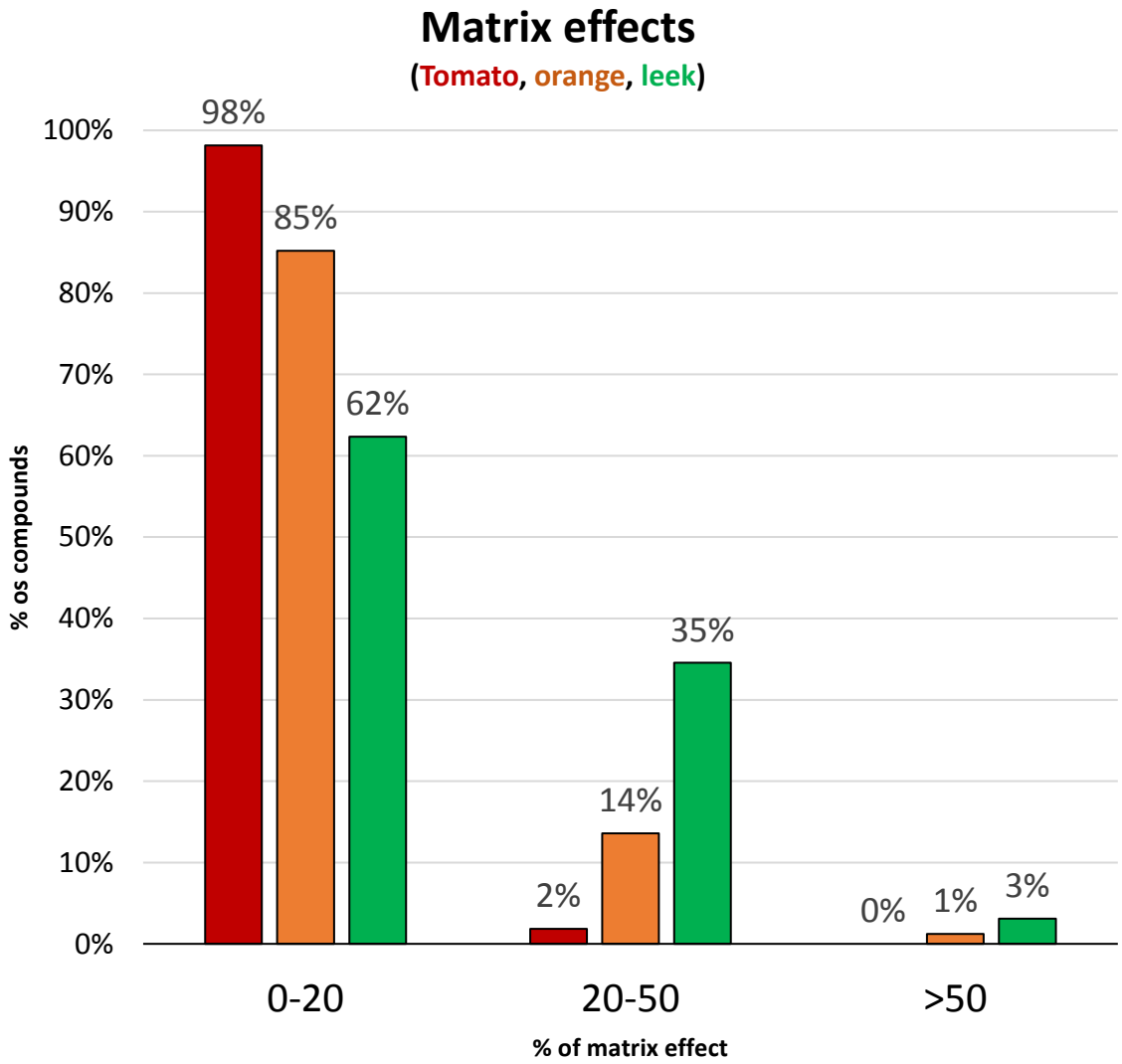
Quinoclamine	20 – 500	20 – 500
Quinoxyfen	50 – 500	50 – 500
Quizalofop-ethyl	5 – 500	5 – 500
Rotenone	5 – 500	5 – 500
Spinosad A	5 – 500	5 – 500
Spinosad D	10 – 500	10 – 500
Spirodiclofen	5 – 500	5 – 500
Spiromesifen	5 – 500	10 – 500
Spirotetramat	5 – 500	5 – 500
Tebuconazole	5 – 500	5 – 500
Tebufenozide	5 – 500	5 – 500
Tebufenpyrad	5 – 500	5 – 500
Teflubenzuron	5 – 500	5 – 500
Terbuthylazine	5 – 500	5 – 500
Tetraconazole	5 – 500	5 – 500
Thiabendazole	10 – 500	20 – 500
Thiacloprid	5 – 500	5 – 500
Thiamethoxam	20 – 500	10 – 500
Thiobencarb	5 – 500	5 – 500
Thiodicarb	5 – 500	5 – 500
Triazophos	5 – 500	5 – 500
Trichlorfon	5 – 500	10 – 500
Trifloxystrobin	5 – 500	5 – 500
Triflumuron	5 – 500	5 – 500
Triticonazole	5 – 500	5 – 500
Zoxamide	5 – 500	5 – 500

6.3 Matrix effects

The presence of other co-eluting compounds different from the analytes in the electrospray solution can strongly influence the signal. Signal suppression is more common than signal enhancement in ESI, as the competition for the charges available between the analytes and the co-eluting matrix compounds decreases ionization efficiency in the interface. The smaller the flow reaching the ion source, the better the sampling efficiency due to the production of smaller charged droplets, which results in increased ionization rates [1]. In supercritical fluid chromatography, the make-up and modifier flows are usually low and high efficiencies are easily achieved.

For the evaluation of the matrix effect, the slopes from the calibration curves obtained in extracts of the different matrices were compared with the ones

obtained from calibration curves built in solvent, considered as no suppression reference [2].In this way, it can be stated that zero matrix effect takes place when calibration graphs built in solvent and matrix have the same slope. Suppression of the signal between the range 0–20% was considered irrelevant matrix effect; between 20 and 50%, low signal suppression; and higher than 50%, a significant suppression of the signal. The matrix effect study for the 164 pesticides in fruits and vegetables showed very good results. Significant suppression was not found in tomato and only 1% of the compounds in orange and 3% in leek had an ion suppression above 50%.



Matrix effects are particularly higher when spices are analyzed by liquid chromatography. Spices are complex matrices that contain large amounts of

essential oils, plant nutrients and secondary metabolites such as flavonoids, terpenes and alkaloids. These interfering matrix components produce ion enhancement or suppression, which can be very strong and depend on the origin of the sample. However, using supercritical fluid chromatography, out of the 162 pesticides studied in spices, 132 (corresponding to 81%) showed weak matrix effect in cayenne and 91 (56%) in black pepper. Strong matrix effect, on the other hand, was only found in 10 (6%) pesticides in cayenne and 27 (17%) in black pepper. These results represent an improvement over most of the published literature using LC-MS/MS to analyse pesticide residues in spices.

Table 2. Matrix effects values for the spice matrices studied

Compound	Matrix effect	
	Cayenne	Black pepper
2,4-D	46%	-4%
Acephate	-12%	-25%
Acetamiprid	-73%	-51%
Aldicarb	7%	20%
Aldicarb-sulfone	-7%	-21%
Ametoctradin	-27%	-43%
Azinphos-methyl	3%	-11%
Azoxystrobin	-12%	-7%
Bitertanol	-16%	-46%
Boscalid	-93%	-56%
Bromuconazole	-21%	-45%
Bupirimate	0%	-8%
Buprofezin	2%	-12%
Carbaryl	0%	-17%
Carbendazim	-10%	-55%
Chlorantraniliprole	-54%	-83%
Chlorfenvinphos	-3%	-9%
Chlorpyrifos	9%	8%
Clofentezine	5%	-29%
Clomazone	4%	-1%
Coumaphos	-1%	-15%
Cyazofamid	2%	-10%
Cymoxanil	2%	-5%
Cyproconazole	-18%	-64%
Cyprodinil	12%	58%
Cyromazine	-48%	-63%
Deet	7%	-3%
Demeton-S-methyl-sulfone	-6%	-8%
Diazinon	1%	-9%
Dichlorvos	5%	-8%
Dicrotophos	-5%	-7%

Diethofencarb	-4%	-16%
Difenoconazole	-25%	-49%
Diflubenzuron	-11%	-47%
Dimethoate	0%	-10%
Dimethomorph	-13%	-24%
Diniconazole	-33%	-48%
Diuron	-37%	-61%
Emamectin B1a	-9%	-36%
EPN	5%	-9%
Epoxiconazole	-14%	-60%
Ethion	1%	6%
Ethirimol	-3%	-15%
Ethoprophos	3%	-4%
Etofenprox	0%	-13%
Etoxazole	-9%	-9%
Famoxadone	-52%	-61%
Fenamidone	-17%	-48%
Fenamiphos	-7%	-11%
Fenamiphos-sulfone	-5%	-16%
Fenamiphos-sulfoxide	-24%	-14%
Fenarimol	-25%	-68%
Fenazaquin	2%	-1%
Fenbuconazole	-21%	-50%
Fenhexamid	-54%	-77%
Fenoxycarb	0%	-12%
Fenpropathrin	-1%	-10%
Fenpyroximate	-11%	-26%
Fenthion	5%	-1%
Fenthion-sulfone	-1%	-14%
Fenthion-sulfoxide	-3%	-13%
Fipronil	-13%	-8%
Flonicamid	-15%	-56%
Fluazifop	-15%	-52%
Fludioxonil	-25%	-41%
Flufenacet	-1%	-3%
Flufenoxuron	-12%	-44%
Fluopyram	-5%	-21%
Fluquinconazole	-9%	-30%
Flusilazole	-17%	-81%
Flutriafol	-16%	-52%
Fluxapyroxad	-5%	-22%
Fosthiazate	-5%	-7%
Haloxyfop	-20%	-46%
Hexaconazole	-14%	-66%

Hexythiazox	-2%	-19%
Imazalil	8%	18%
Imidacloprid	-53%	-82%
Indoxacarb	-7%	-14%
Ioxynil	-20%	-49%
Iprodione	-16%	-22%
Iprovalicarb	-8%	-9%
Isoprocarb	-1%	-2%
Isoxaflutole	-3%	-7%
Kresoxim-methyl	8%	-1%
Linuron	5%	-1%
Lufenuron	1%	0%
Malathion	0%	-2%
Mandipropamid	2%	-21%
MCPA	-2%	6%
Mepanipyrim	2%	29%
Meptyldinocap	-4%	-4%
Metaflumizone	-12%	-46%
Metalaxyl	-3%	-5%
Metconazole	0%	-55%
Methamidophos	-10%	-37%
Methidathion	0%	0%
Methiocarb	4%	-7%
Methiocarb-sulfone	-10%	-36%
Methiocarb-sulfoxide	1%	-14%
Methomyl	4%	-18%
Methoxyfenozide	-53%	-26%
Metobromuron	7%	-3%
Monocrotophos	-8%	-16%
Myclobutanil	-9%	-41%
Nitenpyram	-45%	-72%
Omethoate	-9%	-27%
Oxadixyl	-5%	-9%
Oxamyl	1%	-22%
Paclobutrazol	-12%	-36%
Paraoxon-methyl	8%	-1%
Penconazole	-4%	-38%
Pencycuron	-84%	-36%
Pendimethalin	5%	4%
Phenthoate	9%	2%
Phosalone	-4%	-14%
Phosmet	-6%	-16%
Phoxim	1%	-8%
Pirimicarb	1%	1%

Pirimicarb-desmethyl	0%	-4%
Pirimiphos-methyl	3%	-1%
Prochloraz	-11%	-16%
Profenofos	-2%	-7%
Propamocarb	-20%	-81%
Propaquizafop	-5%	-24%
Propargite	1%	-4%
Propiconazole	-15%	-39%
Propoxur	1%	-3%
Propyzamide	8%	1%
Proquinazid	4%	-1%
Prothiophos	-2%	-9%
Pymetrozine	-38%	-67%
Pyraclostrobin	-8%	-18%
Pyridaben	-10%	-17%
Pyrimethanil	7%	-20%
Pyriproxyfen	4%	-2%
Quinoclamine	-37%	-26%
Quinoxyfen	11%	10%
Quizalofop-ethyl	-5%	-12%
Rotenone	-4%	-23%
Spinosad A	-41%	-11%
Spinosad D	-31%	2%
Spirodiclofen	-4%	-18%
Spiromesifen	-8%	-11%
Spirotetramat	-3%	9%
Tebuconazole	-26%	-55%
Tebufenozide	-1%	-33%
Tebufenpyrad	-3%	-10%
Teflubenzuron	-6%	-45%
Terbuthylazine	0%	-9%
Tetraconazole	-12%	-51%
Thiabendazole	-65%	-87%
Thiacloprid	-54%	-86%
Thiamethoxam	-47%	-85%
Thiobencarb	1%	-6%
Thiodicarb	11%	-24%
Triazophos	-5%	-9%
Trichlorfon	-4%	-15%
Trifloxystrobin	-4%	-8%
Triflumuron	-10%	-29%
Triticonazole	-14%	-47%
Zoxamide	-19%	-56%

6.4 Pyrethroids analysis

As a rule of thumb, liquid chromatography coupled to mass spectrometry with ESI source provides poor results for pyrethroid detection. Few studies have used LC for the analysis of pyrethroids, and in these cases, a very specific sample extraction method, including several clean up steps or preconcentration stages, is often applied to increase the sensitivity of the analysis. Fourteen pyrethroids (Acrinathrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, etofenprox, fenpropathrin, fenvalerate, flucythrinate, λ-cyhalothrin, permethrin, phenothrin, τ-fluvalinate, and tetramethrin) were studied using SFC-MS/MS. The ionization benefits of the low flow chromatography allowed to achieve an instrumental limit of quantification of 2 µg/kg for the majority of pyrethroids in the six matrices studied. Matrix effects were also studied for these compounds, being tea the only matrix with significant ion suppression for some pyrethroids. These results are comparable to those of gas chromatography. Therefore, supercritical fluid chromatography can be applied as an alternative for the analysis of pyrethroid pesticides.

Table 3. LOQs and matrix effects of pyrethroids in the six studied matrices:

	Tomato		**Pear**		**Zucchini**		**Orange**		**Onion**		**Tea**	
	LOQ	ME	LOQ	ME	LOQ	ME	LOQ	ME	LOQ	ME	LOQ	ME
Achrinathrin	2	-	2	5	2	5	2	0	2	0	2	-22
Bifenthrin	2	-	2	10	2	8	2	-7	2	-24	2	-3
Cyfluthrin	2	-	2	10	2	15	20	-4	20	-2	10	-57
Cypermethrin	2	-	2	-2	2	4	2	-8	2	-17	5	-32
Deltamethrin	2	-	2	1	2	3	2	-14	2	-63	2	-18
Etofenprox	2	-	2	0	2	-1	2	-8	2	-65	2	-10
Fenprotathrin	2	-	2	18	2	20	2	-7	2	-25	2	-1
Fenvalerate	2	-	2	0	2	4	5	-6	2	-40	5	-32
Flucythrinate	2	-	2	-5	2	-5	5	-8	5	-37	10	-86
Lambda-cyhalothrin	2	-	2	-7	2	-6	2	-9	2	-18	5	-19
Permethrin	2	-	2	2	2	8	2	2	2	-11	2	-10
Phenothrin	2	-	2	6	2	11	5	-8	2	-17	10	-75
Tau-Fluvalinate	2	-	2	6	2	10	2	-6	2	-12	2	-50
Tetramethrin	2	-	2	-3	2	3	2	-4	2	-2	2	-11

6.5. Conclusions

- The make-up flows were tested looking for a compromise between sensitivity increment and reproducibility. Finally, the make-up was set to be pumped isocratically to 80 µL/min.

- After post-column flow optimization, Supercritical fluid chromatography coupled to mass spectrometry allows the identification and quantification of pesticides at very low concentration levels (2 µg/kg), with low amount of total sample injected in the system.

- A decrement of the matrix effects was observed even in spices, considered one of the most complex matrices due to the large amount of coeluting compounds.

- The low flow reaching the ESI source provided ionization benefits that allowed the identification and quantification of pyrethroids at very low concentration levels.

For further information about the studies carried out in supercritical fluid chromatography for the analysis of spices matrices and pyrethroids pesticides check the following scientific publications:

-Supercritical fluid chromatography coupled to tandem mass spectrometry for the analysis of pesticide residues in dried spices. Benefits and drawbacks: https://www.sciencedirect.com/science/article/pii/S0003267019300492?via%3Dihub
- Supercritical Fluid Chromatography and Gas Chromatography Coupled to Tandem Mass Spectrometry for the Analysis of Pyrethroids in Vegetable Matrices: A Comparative Study:
https://pubs.acs.org/doi/abs/10.1021/acs.jafc.9b00732

7. References

[1] Page, J. S., et al. (2007). "Ionization and Transmission Efficiency in an Electrospray Ionization–Mass Spectrometry Interface." Journal of the American Society for Mass Spectrometry 18(9): 1582-1590.
[2] Gómez-Ramos, M. d. M. (2016). "The evaluation of matrix effects in pesticide multi-residue methods via matrix fingerprinting using liquid chromatography electrospray high-resolution mass spectrometry." Analytical methods v. 8(no. 23): pp. 4664-4673-2016 v.4668 no.4623.