Cost-benefit analysis of not applying a clean-up step and an evaluation of the negative effects of clean-up sorbents on the analytes

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1. Aim and scope

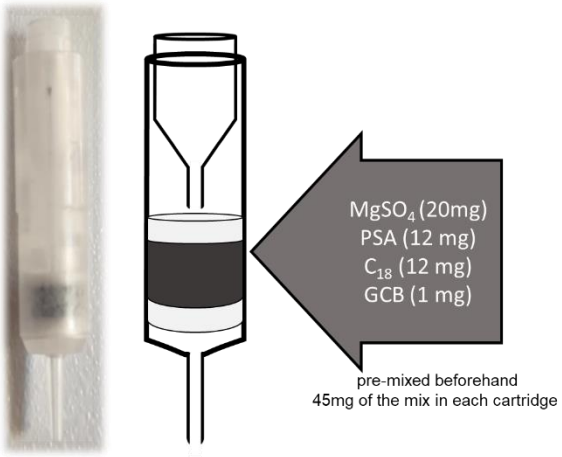
This report evaluates the effects of the sorbents used during clean-up compared to the possibility of not including this step or using an automated clean-up to analyze pesticide residues in different matrices (spinach, onion, garlic, celery, Brussel sprouts and orange) by liquid chromatography coupled to mass spectrometry.

2. Short description

The QuEChERs method for pesticide residue analysis introduced the use of dispersive solid-phase extraction clean-up (dSPE). This step aims to remove undesired matrix components (such as coextracted matrix compounds) that may cause analytical interferences. Nevertheless, this additional step is time-consuming and needs to be optimized depending on the kind of matrix (e.g., matrices with a high oil content might need a freezing-out step to remove the lipids, whilst others only need a solid sorbent).

In the present work, six different matrices (spinach, onion, garlic, celery, Brussel sprouts, and orange) have been extracted following manual dispersive clean-up in parallel with an automated µSPE clean-up workflow, in both cases based on QuEChERS extraction. The automated technique employs clean-up cartridges containing a mixture of sorbent materials suitable for multiple matrices (**Figure 1**). All the samples (with dSPE, µSPE, or no clean-up step) were analyzed by liquid chromatography coupled to mass spectrometry and the results have been compared in terms of how clean the extracts are, troublesome compounds, and matrix effects.

Figure 1: Real cartridge and sketch.



3. Experimental

3.1. Sample treatment

The samples were extracted using the QuEChERS method (one extraction of blank matrix and three extractions of a sample spiked with a mixture of 230 LC-amenable compounds at a concentration of 10 µg/kg. The general experimental procedure was as follows:

1. Weighing 10 g of sample in a 50-mL PTFE centrifuge tube.
2. Adding 10 mL acetonitrile.
3. Shaking the sample in an axial agitator (Agitax) for 6 minutes.

4. Adding 4 g anhydrous magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate and shaking manually (3 sec).
5. Shaking the sample in an axial agitator (Agitax) for 6 minutes.
6. Centrifuging the tubes at 4000 rpm for 5 min.
7. Taking an aliquot of the supernatant for the µSPE experiments.

The following **dSPE clean-up** procedure was carried out:

1. Transferring 5 mL of the supernatant to a 15-mL PTFE centrifuge tube containing 750 mg anhydrous magnesium sulphate and 125 mg PSA (primary secondary amine) and vortexing for 30 sec.
2. Centrifuging the tubes at 4000 rpm for 5 min.
3. Transferring the supernatant to a 4-mL vial and adding 10 µL of a formic acid solution in acetonitrile (5 % volume) per mL of extract.

For the injection vial preparation, samples that had undergone the clean-up step (**dSPE extracts**) followed the same procedure as those samples that had not. The samples were diluted with acetonitrile and the calibration curves were prepared using the blank extract with the pesticide mix in acetonitrile. The blank sample and recovery had the same dilution as the calibration curve with the addition of acetonitrile. Finally, all the vials were diluted (1:5) with Optima® water (using dimethoate-d6 as the injection standard) (**Figure 2**).

The sample aliquots that had not undergone the clean-up (for **µSPE**) were diluted with acetonitrile and directly injected into the automatic robot while the calibration curves were prepared using the blank extract (with no clean-up) and the pesticide mix in acetonitrile, which was directly injected into the automatic robot. The resulting µSPE extracts were diluted (1:5) with Optima® water (using dimethoate-d6 as the injection standard) (**Figure 2**).

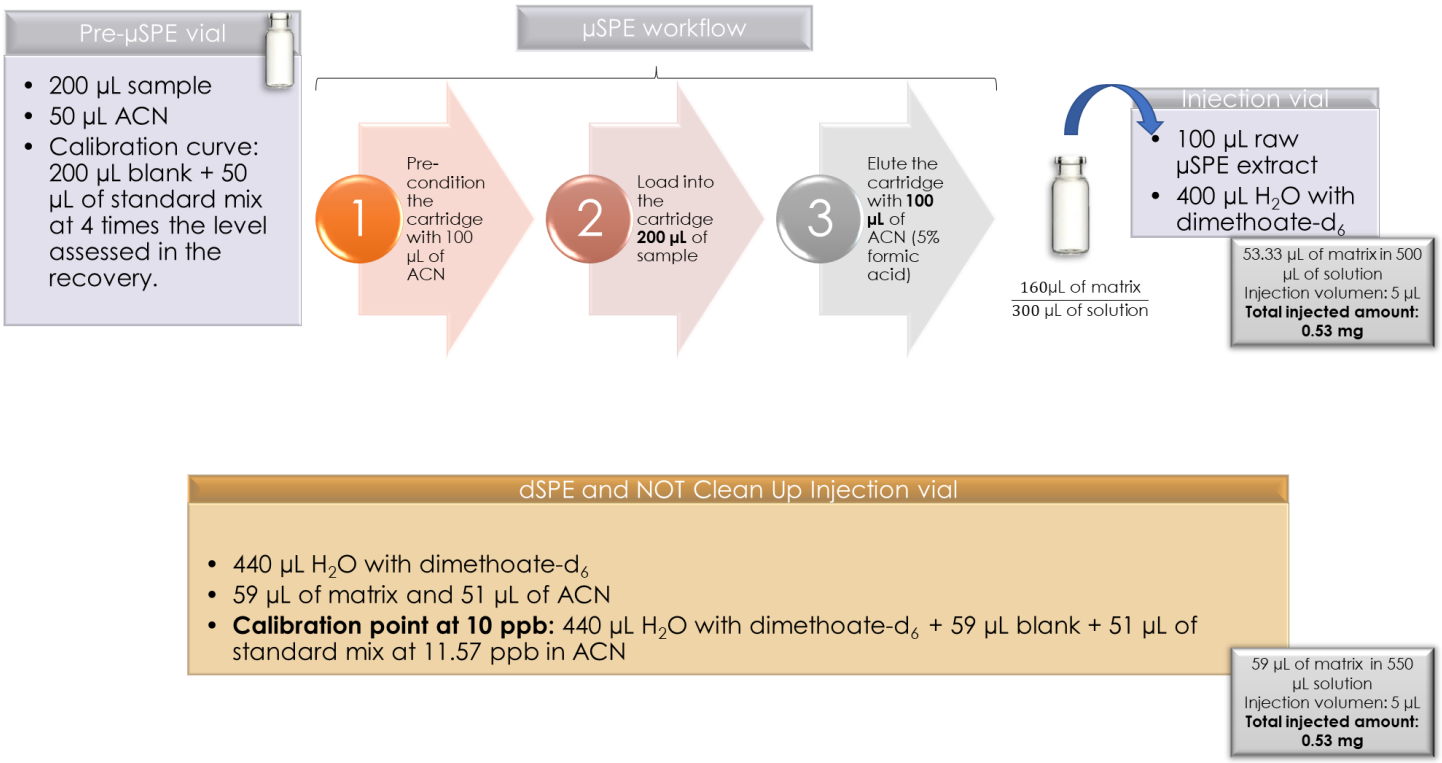


Figure 2. Injection vials

3.2. Automated µSPE

µSPE cartridges (p/n µSPE-GCQuE1-45 provided by CTC Analytics) were employed to perform an automated clean-up procedure which was then compared to the manual dispersive clean-up methods. The cartridge contained 45 mg of sorbent with the following composition (weight percentage): 20/12/12/1 anhydrous MgSO4/PSA/C18/CarbonX. The following sample workflow was optimized based on the method developed by Lehotay *et al.* [1]: first, the cartridges were pre-conditioned with 100 µL acetonitrile prior to sample loading. Then, 200 µL of each raw extract sample was loaded into the cartridge at 100 µL/sec and the clean extract was collected in a 2-mL vial with a pre-cut septum cap. Then, the cartridges were eluted with 100 µL acetonitrile (5% formic acid). Finally, 100 µL of the total extract was mixed with 400 µL of Optima® water (dilution 1:5) and injected into the LC-QqQ-MS/MS.

The entire automated sample treatment was performed using a robotic module with different racks (**Figure 3)**

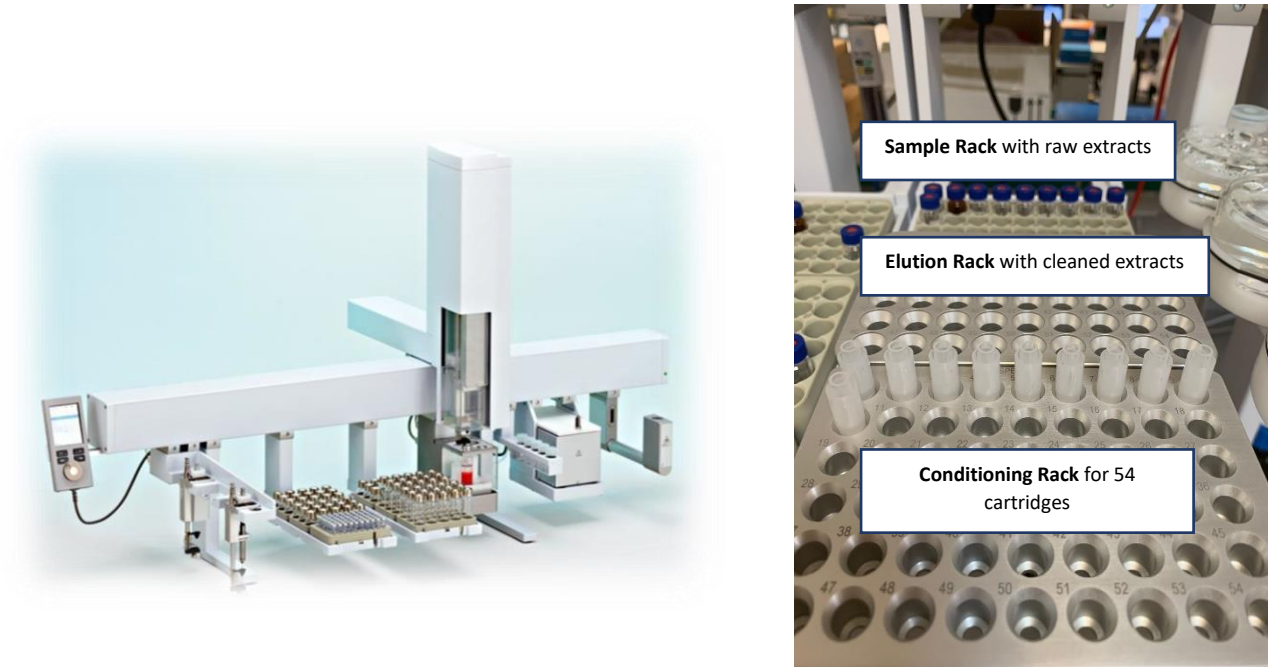


Figure 3: Robot used for automated sample treatment.

3.3. Analysis by LC-QqQ-MS/MS

All the samples were analyzed using the LC system operated in multiple-reaction-monitoring mode (MRM). Selected reaction monitoring (SRM) experiments were carried out to obtain the maximum sensitivity for detecting the target molecules. To confirm the studied compounds, two SRM transitions and a correct ratio between the abundances of the two optimised SRM transitions (SRM2/SRM1) were used along with retention time matching. The mass transitions are presented in **Appendix I (Table 1).**

Instrumentation and analytical conditions for the LC- MS/MS system

- Column: Zorbax Eclipse Plus C8 2.1x100 mm and a 1.8 µm particle size
- Mobile phase A: Water (0.1 % formic acid, 5 mM ammonium formate, 2 % MeOH)
- Mobile phase B: Methanol (0.1 % formic acid, 5 mM ammonium formate, 2 % water)
- Column temperature: 35 °C
- Flow rate: 0.3 ml/min
- Injection volume: 5 µL
- Autosampler temperature: 12 °C

Mobile phase gradient for the pesticide analysis (**Figure 4**):

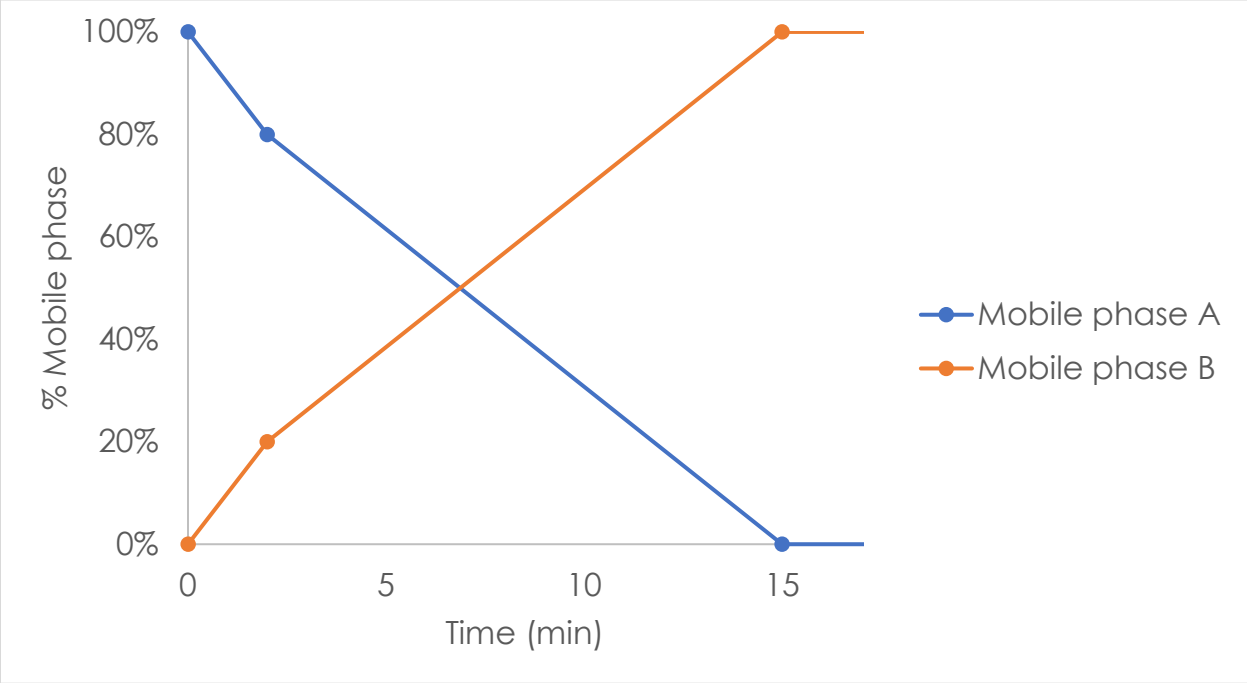


Figure 4: Mobile phase gradient used. A (Water [0.1 % formic acid, 5 mM ammonium formate, 2 % MeOH]) and B (Methanol [0.1 % formic acid, 5 mM ammonium formate, 2 % water])

Triple quadrupole system

- Ionisation mode: Positive mode and negative mode
- Capillary (positive and negative): 3000 V
- Nebulizer: 45 psi
- Nozzle: 400 V
- Drying gas flow: 13 L/min
- Drying gas temperature: 120°C
- Sheath gas flow: 10 L/min
- Sheath gas temperature: 375°C
- High Pressure RF (positive): 150 V
- High Pressure RF (negative): 110 V
- Low Pressure RF (positive): 60 V
- Low Pressure RF (negative): 60 V

4. Results and discussion

4.1 Automated µSPE workflow optimization

The following workflows were evaluated:

- Method 1 (**M1**): Cartridges were pre-conditioned with 100 µL acetonitrile prior to sample loading. Then, 200 µL of each raw extract sample was loaded into the cartridge at 100 µL/sec and the clean extract was collected in a 2-mL vial with a pre-cut septum cap.

- Method 2 (**M2**): Raw QuEChERS extracts without clean-up were acidified with 5% formic acid in acetonitrile in the same proportion as the dSPE method (10 µL/mL extract) followed by the M1 step.
- Method 3 (**M3**): Cartridges were pre-conditioned with 100 µL acetonitrile prior to sample loading. Then, 200 µL of each raw extract sample was loaded into the cartridge at 100 µL/sec and the clean extract was collected in a 2-mL vial with a pre-cut septum cap. After this, the cartridges were eluted with 100 µL acetonitrile.
- Method 4 (**M4**): Cartridges were pre-conditioned with 100 µL acetonitrile prior to sample loading. Then, 200 µL of each raw extract sample was loaded into the cartridge at 100 µL/sec and the clean extract was collected in a 2-mL vial with a pre-cut septum cap. After this, the cartridges were eluted with 100 µL acetonitrile (5% formic acid).
- Method 5 (**M5**): Cartridges were pre-conditioned with 100 µL acetonitrile prior to sample loading. Then, 200 µL of each raw extract sample was loaded into the cartridge at 100 µL/sec and the clean extract was collected in a 2-mL vial with a pre-cut septum cap. After this, the cartridges were eluted with 200 µL acetonitrile (5% formic acid).
- Method 6 (**M6**): Cartridges were pre-conditioned with 100 µL acetonitrile prior to sample loading. Then, 200 µL of each raw extract sample was loaded into the cartridge at 100 µL/sec and the clean extract was collected in a 2-mL vial with a pre-cut septum cap. After this, the cartridges were eluted with 600 µL acetonitrile (5% formic acid).

The optimized method was M4 since the acidified acetonitrile elution step allows the recovery of acidic compounds such as haloxyfop, quizalofop and fluazifop by breaking their interaction with the PSA. In M1 and M2, these compounds were not detected (**Figure 5**) while in M5 and M6, the sample dilution increased thus leading to a decrease in sensitivity.

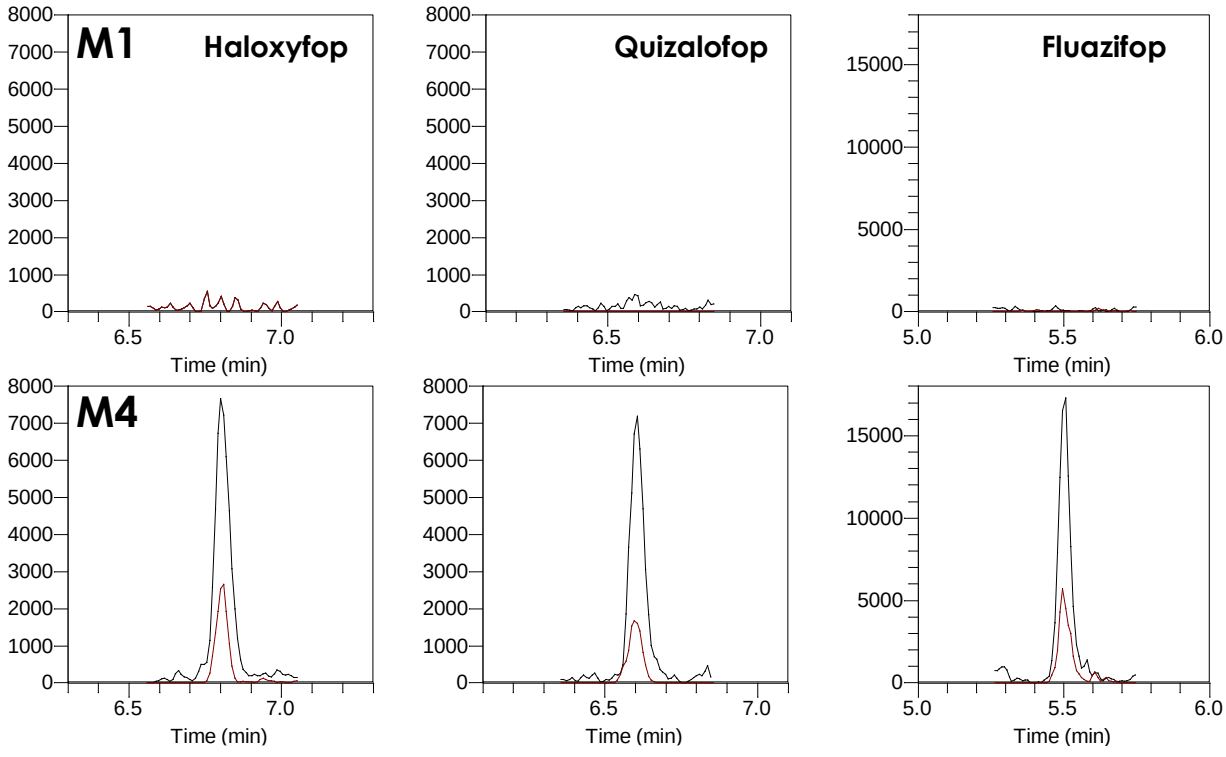
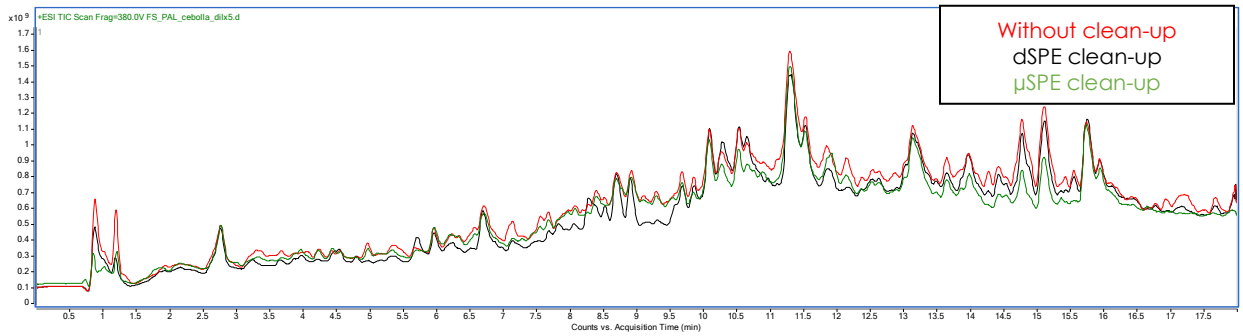


Figure 5: Comparative µSPE chromatograms with M1 and M4 of the calibration curve point at 10 µg/kg pesticide mix standard in tomato.

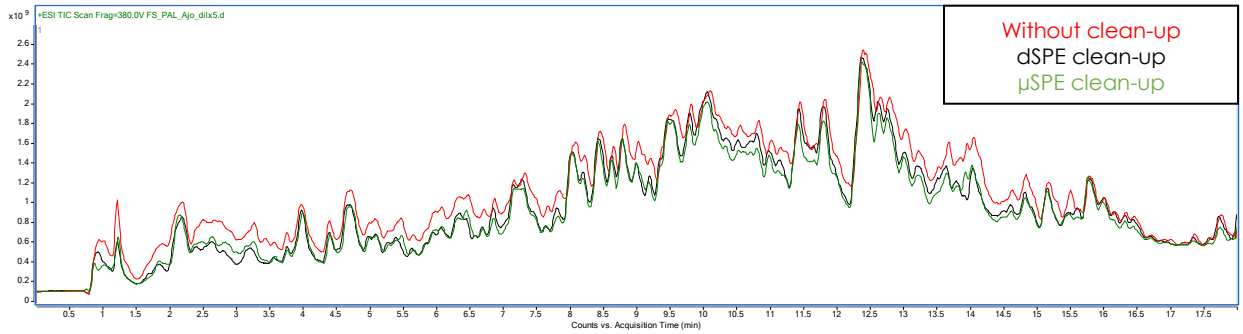
4.2 TICs and extract appearance

To compare the Total Ion Current (TIC), the raw QuEChERS extracts without clean-up, the dSPE blank extracts, and the µSPE blank extracts were injected in *full scan* mode.

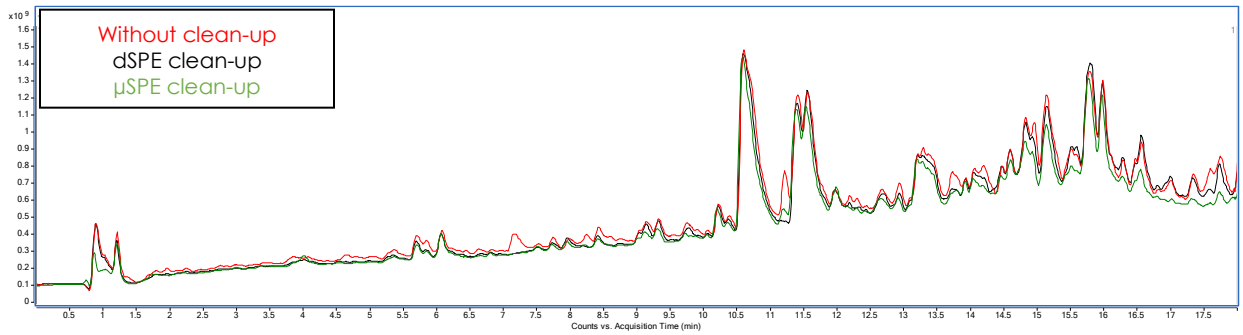
Onion



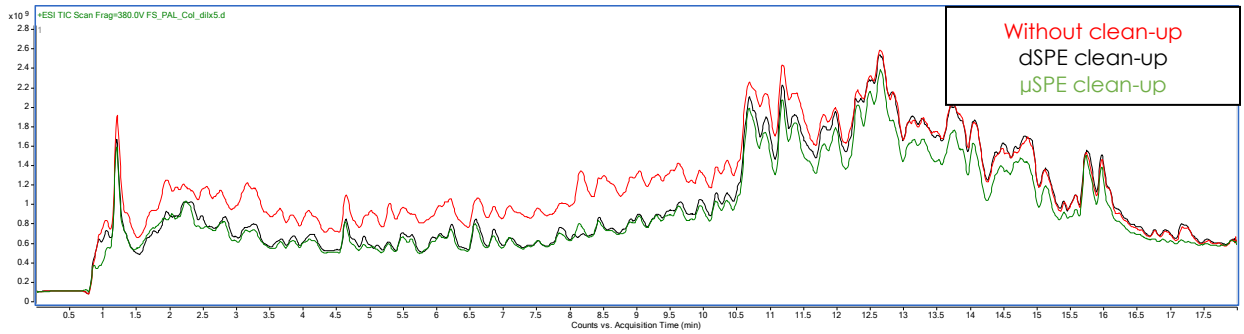
Garlic



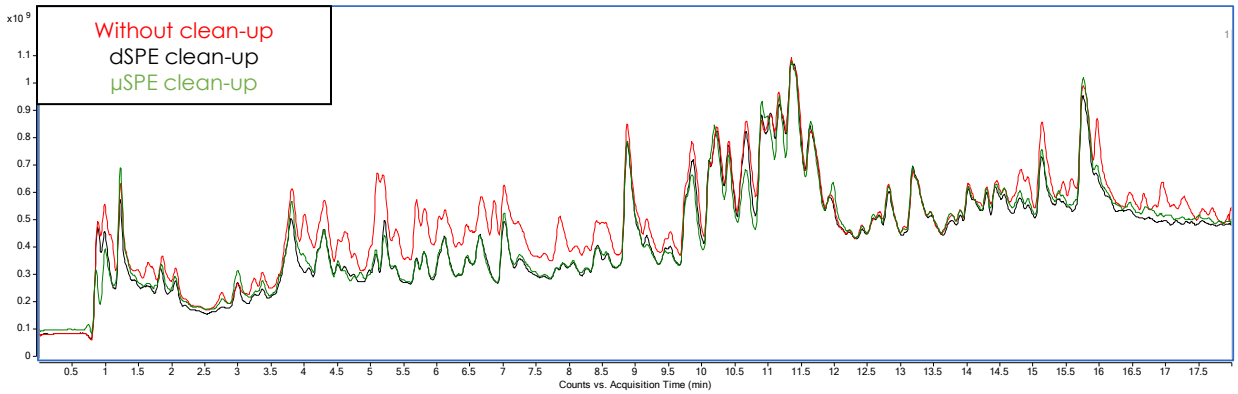
Celery



Brussel sprouts



Orange



Spinach

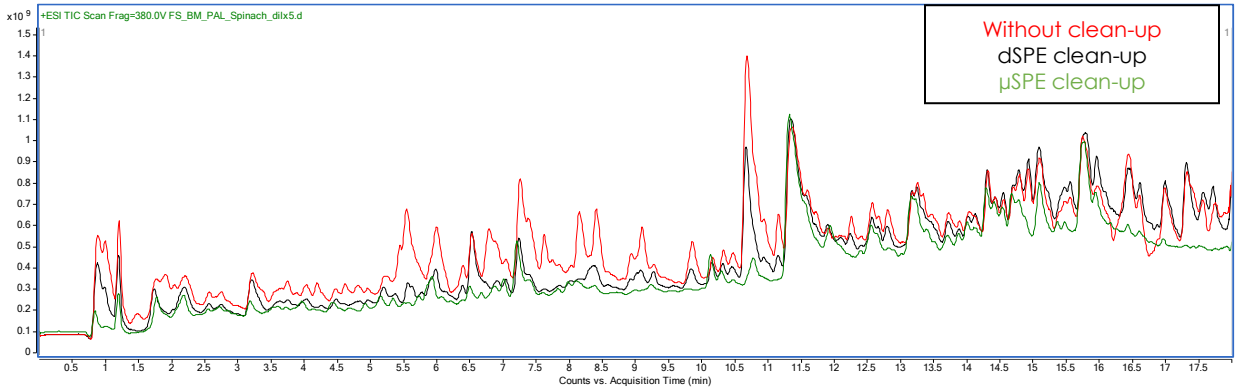


Figure 6: TIC chromatograms obtained with an LC-MS/MS instrument for the blank extracts from the dSPE method (black color), µSPE method (green color) and without clean-up (red color).

It is important to broaden and focus on the elution zone of compounds where interferents are found, an important example being the interferent found with the pesticide neburon. **Figure 7** shows an interference in the 275.1>57.1 ion transition. The minimum calibration level (5 ppb) for neburon in spinach does not meet the ion ratio acceptance criteria when the clean-up step is not included. It is possible to see how the interference co-elutes with the neburon.

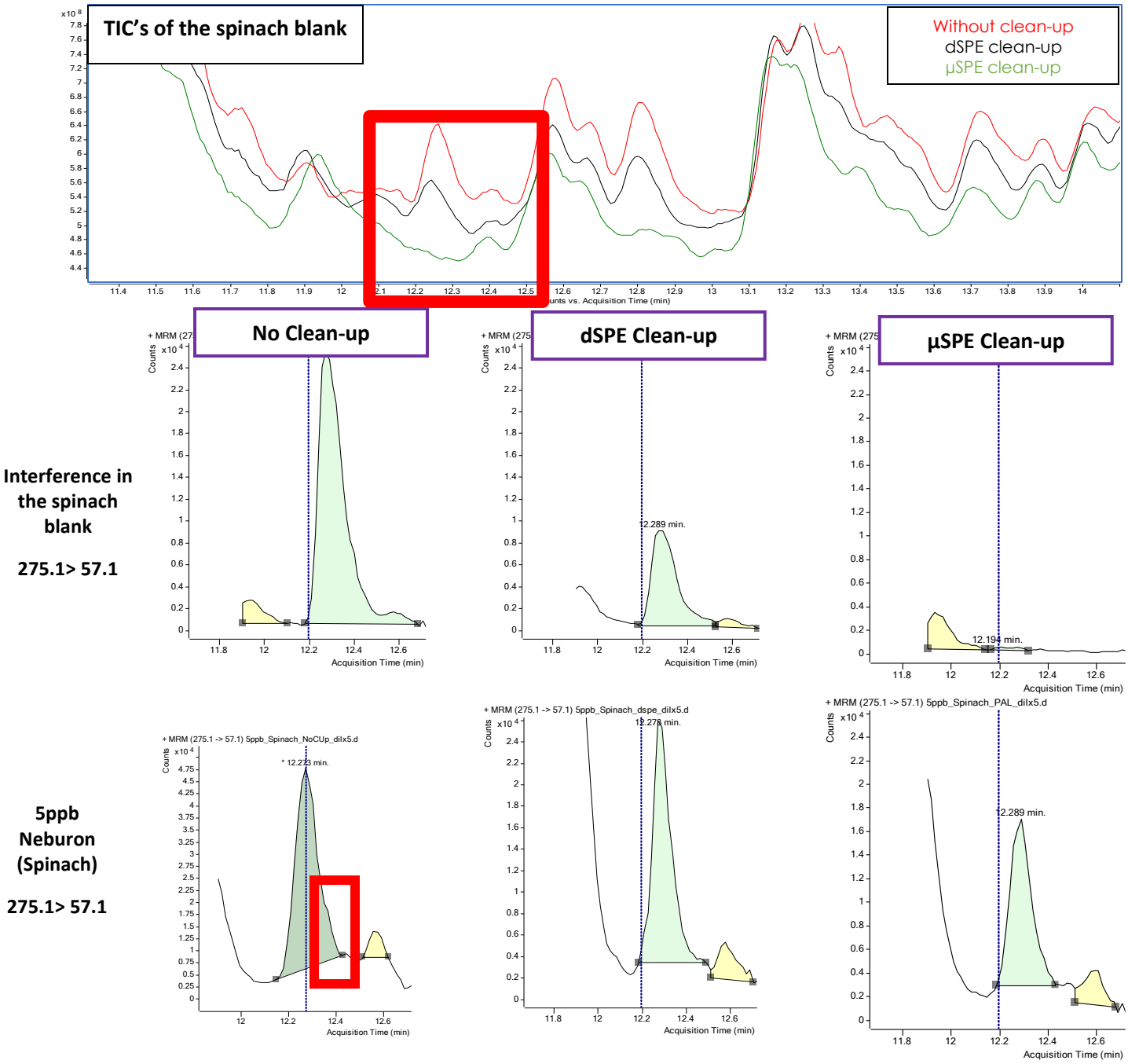


Figure 7. TIC's of the spinach blank analysis without a clean-up step, with dSPE, and with µSPE clean-up. Example of neburon interference.

Figure 8 shows three vials of each sample: the QuEChERS extract without clean-up, the final extract after manual dispersive clean-up and the final extract after µSPE clean-up. Only minor differences can be seen between the raw extracts and the QC extracts (with dispersive clean-up) whereas the µSPE extracts were clear and colorless in all cases.

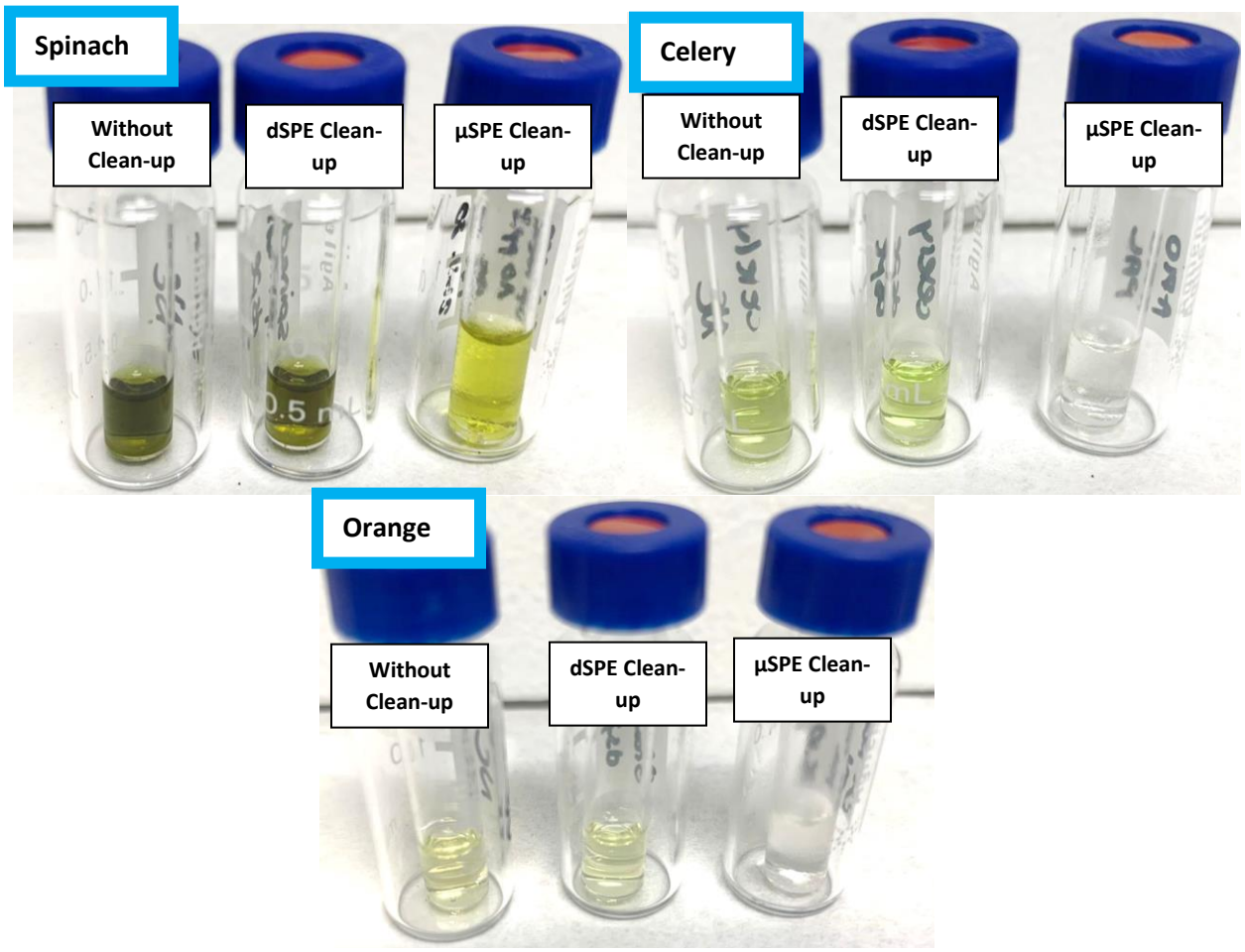
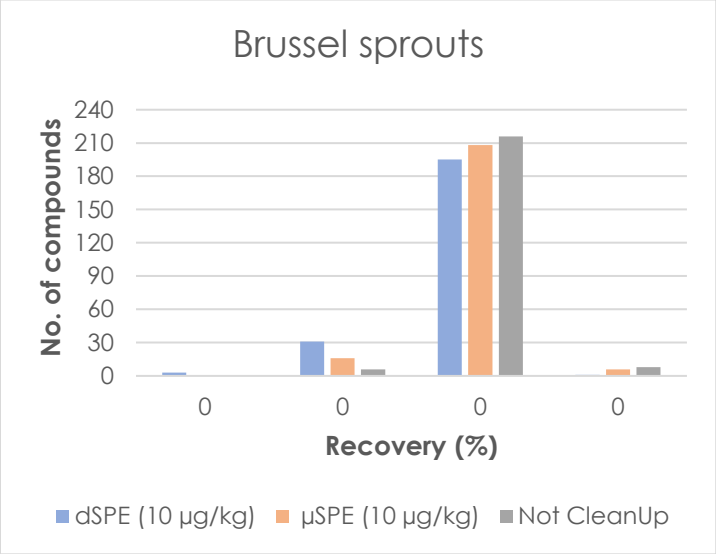
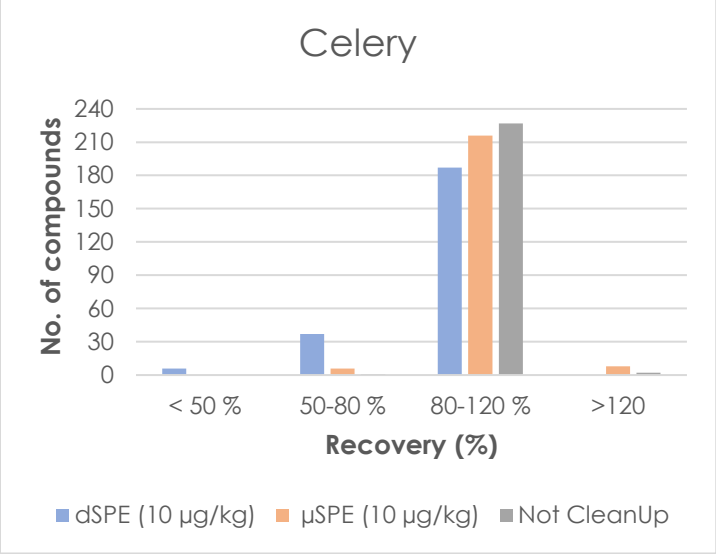


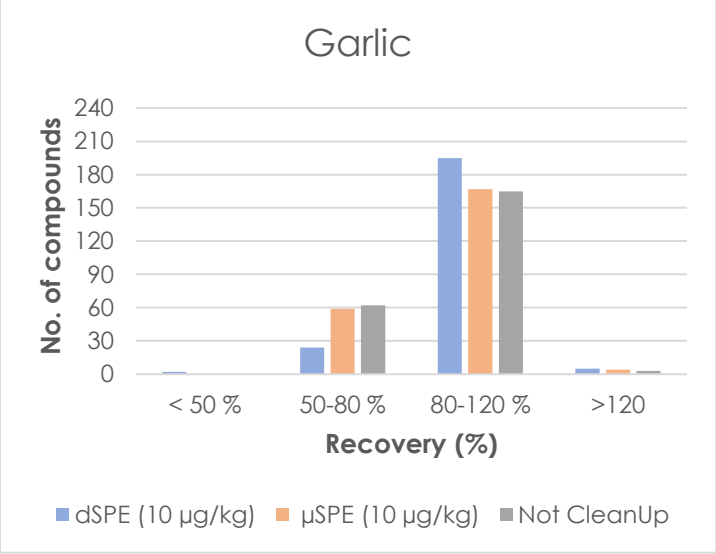
Figure 8. Extracts of spinach, celery, and orange. From left to right: raw QuEChERS extracts without clean-up, QC extracts (dispersive clean-up) and µSPE extracts (cartridges)

4.3 Recoveries

Recoveries were studied for each matrix at 10 µg/kg. **Figure 9** shows the number of compounds present in the different recovery ranges for the matrices tested in this study - very similar results were obtained for the vast majority of compounds.


Brussel sprouts
No. of compounds
240
210
180
150
120
90
60
30
0
0
0
0
0
Recovery (%)
dSPE (10 µg/kg)
µSPE (10 µg/kg)
Not CleanUp


Celery
No. of compounds
240
210
180
150
120
90
60
30
0
< 50 %
50-80 %
80-120 %
>120
Recovery (%)
dSPE (10 µg/kg)
µSPE (10 µg/kg)
Not CleanUp


Garlic
No. of compounds
240
210
180
150
120
90
60
30
0
< 50 %
50-80 %
80-120 %
>120
Recovery (%)
dSPE (10 µg/kg)
µSPE (10 µg/kg)
Not CleanUp

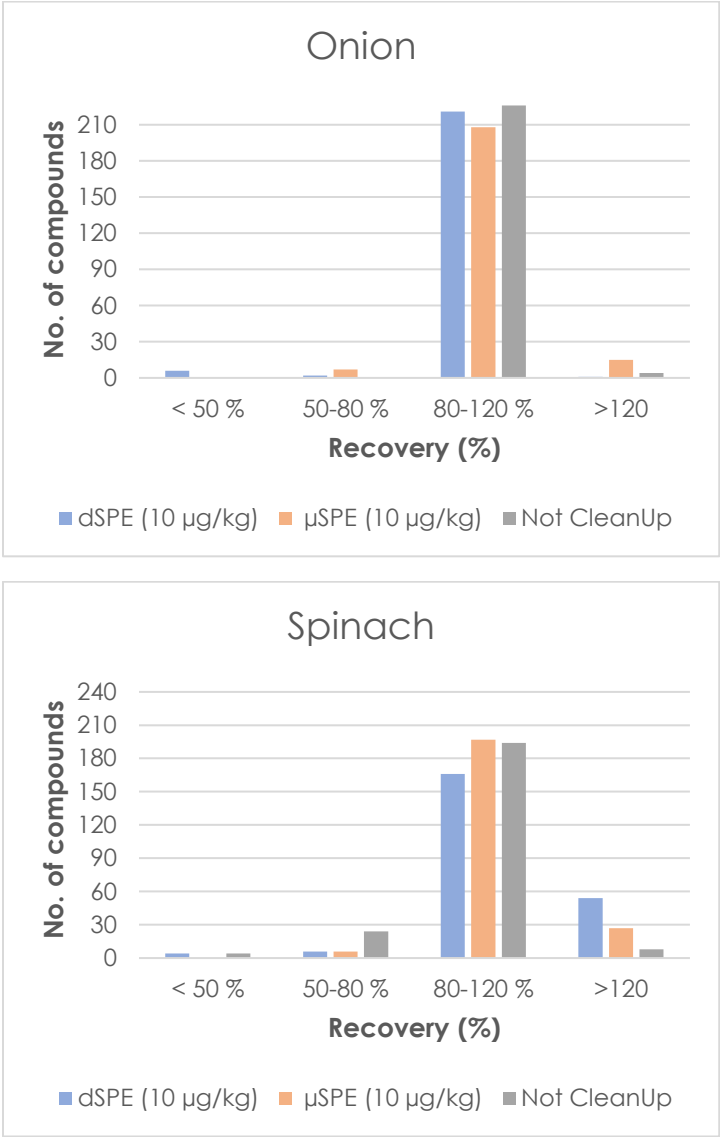


Figure 9. Recoveries of 230 compounds in the different matrices with dSPE (blue), µSPE (orange) and with no clean-up (grey)

The most notable differences were observed for acidic compounds such as quizalofop, fluazifop and haloxyfop when the dispersive clean-up step included PSA; these were due to ion-pairs between the molecules. However, in the absence of PSA (without clean-up) or with the addition of the acid elution step (the µspe workflow), acceptable recovery values were obtained. (**Table 1**).

Table 1: Acidic compound recoveries.

Matrix	Compound	R 10(%)	R 10(%)	R 10(%)
Spinach	Quizalofop	100	ND	101
	Fluazifop	63	ND	140
	Haloxyfop	62	ND	114
Onion	Quizalofop	111	ND	89
	Fluazifop	119	ND	115
	Haloxyfop	106	ND	101
Garlic	Quizalofop	92	ND	118
	Fluazifop	90	ND	100
	Haloxyfop	100	ND	138
Celery	Quizalofop	98	ND	115
	Fluazifop	116	ND	130
	Haloxyfop	129	ND	90
Brussel sprouts	Quizalofop	103	ND	72
	Fluazifop	93	ND	80
	Haloxyfop	138	ND	86
Orange	Quizalofop	126	ND	92
	Fluazifop	94	ND	101
	Haloxyfop	117	ND	78

ND: Not detected

However, for pyrimidinylsulfonylureas such as flazasulfuron and orthosulfamuron, recoveries were < 59 % in dSPE for high water content matrices, while in µSPE and with no clean-up step, they were in the 70 – 120 % range. These compounds interact with the PSA presents in the dispersive clean-up salts, whereas it is more likely to be eluted from the µSPE cartridges. (**Table 2**)

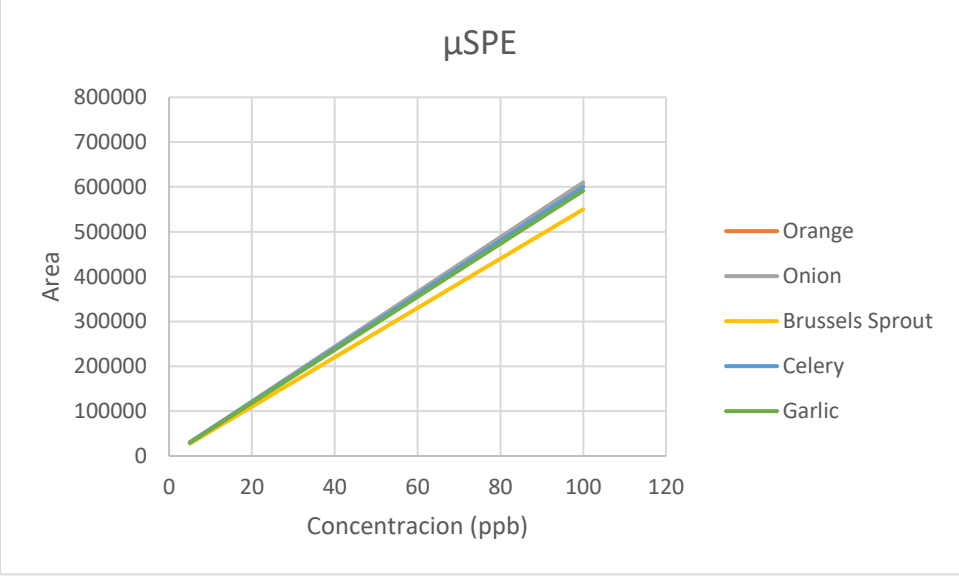
Table 2: Recoveries of pyrimidinylsulfonylureas

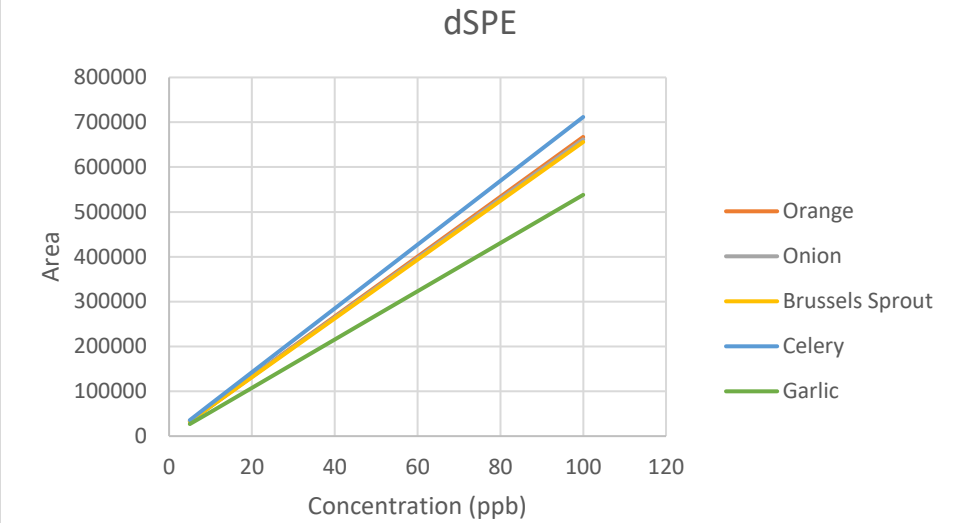
		µSPE	dSPE	No clean-up
Matrix	**Compound**	**R 10(%)**	**R 10(%)**	**R 10(%)**
Spinach	**Flazasulfuron**	94	59	71
	Orthosulfamuron	80	43	72
Onion	**Flazasulfuron**	99	50	104
	Orthosulfamuron	140	50	95
Garlic	**Flazasulfuron**	86	49	85
	Orthosulfamuron	87	46	70
Celery	**Flazasulfuron**	92	49	96
	Orthosulfamuron	87	46	100
Brussel Sprouts	**Flazasulfuron**	85	52	91
	Orthosulfamuron	78	56	100

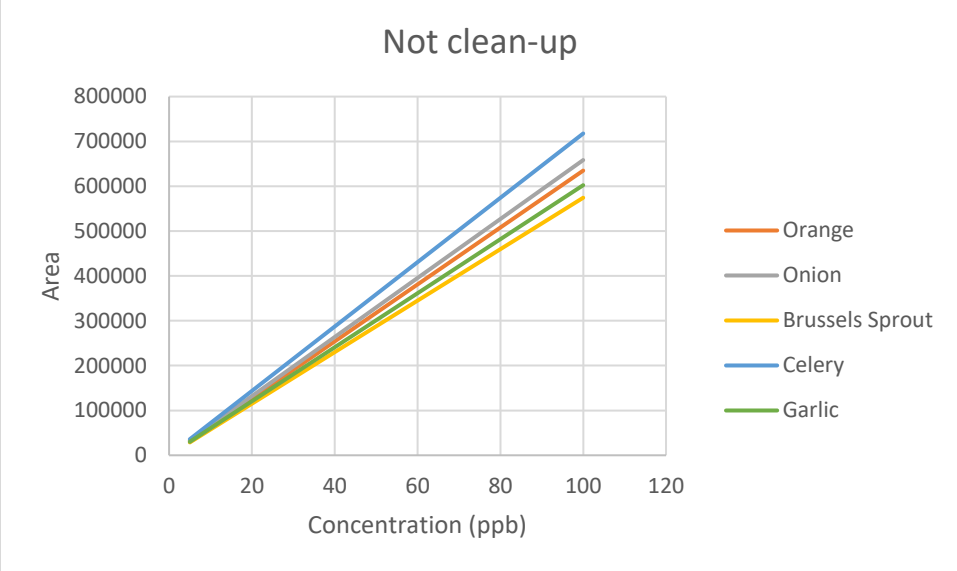
4.4 Linearity.

A comparison between the calibration curves for orange, onion, Brussel sprouts, celery and garlic was carried out. All linearities were graphed in **Figure 10** for two compounds (methomyl and buprofezin).

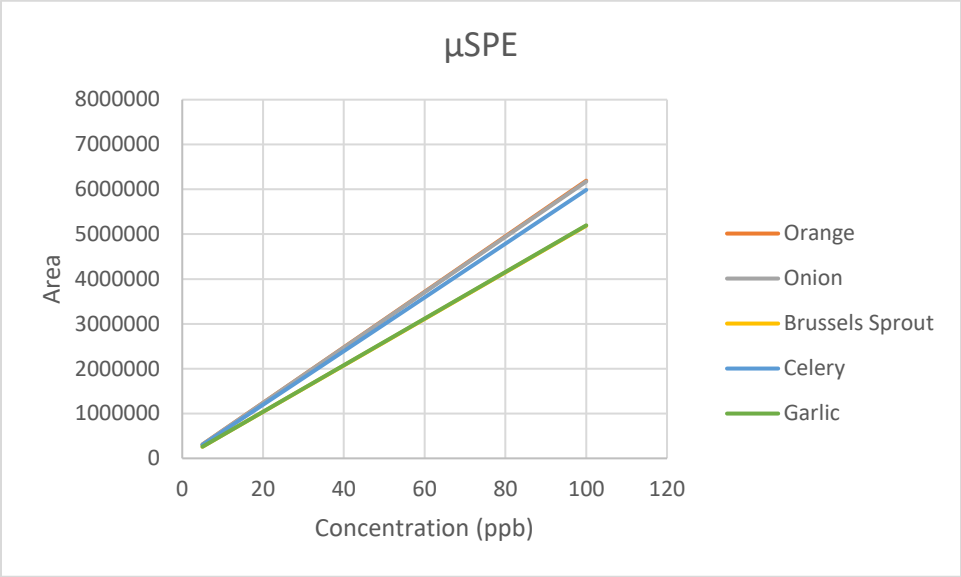
Methomyl

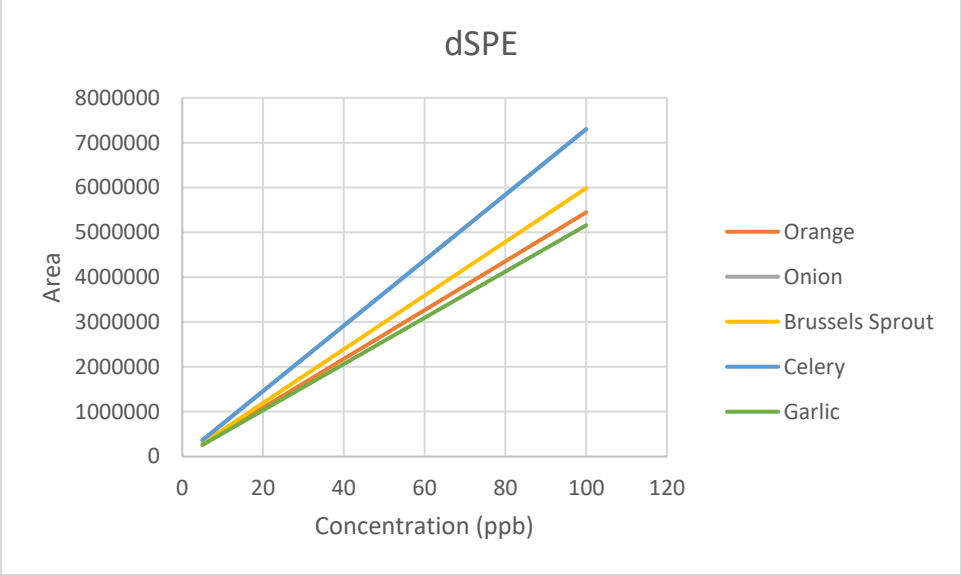



dSPE
800000
700000
600000
500000
400000
300000
200000
100000
0
Area
0
20
40
60
80
100
120
Concentration (ppb)
Orange
Onion
Brussels Sprout
Celery
Garlic


Not clean-up
800000
700000
600000
500000
400000
300000
200000
100000
0
Area
0
20
40
60
80
100
120
Concentration (ppb)
Orange
Onion
Brussels Sprout
Celery
Garlic

Buprofezin


µSPE
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
Area
0
20
40
60
80
100
120
Concentration (ppb)
Orange
Onion
Brussels Sprout
Celery
Garlic


dSPE
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
Area
0
20
40
60
80
100
120
Concentration (ppb)
Orange
Onion
Brussels Sprout
Celery
Garlic

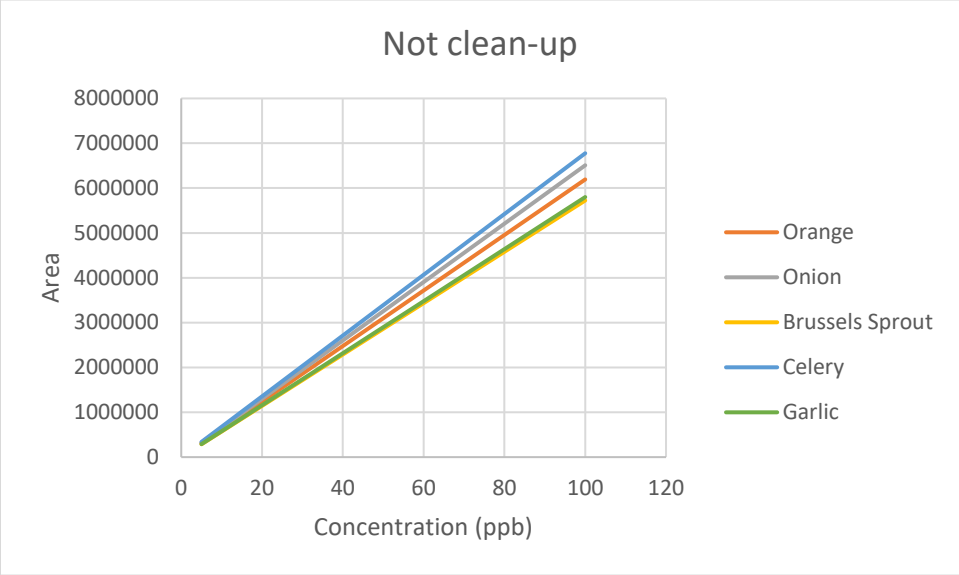


Figure 10: Linearity graphs

The calibration curves have slopes that are close enough to quantify the compounds in different matrices with a single calibration curve when µSPE is used, considering that the total injected amount is the same in all the evaluated experiments (0.53 mg of matrix).

As a consequence of the coeluting sample components, the analyte signal may be enhanced or suppressed, compared to the signal from the same analyte when injected into the solvent (ACN). To evaluate the percentage of matrix effects (ME) for each analyte, the slope of the calibration curves prepared in solvent at the same concentration levels were used, and the ME levels were determined by comparing the solvent and the matrix-matched calibration curves in terms of slope ratios (Equation 1).

Equation 1:

$$\mathrm{ME} = \left(\frac{Slope_{matrix}}{Slope_{\ solvent}} - 1\right) \mathrm{x}\ 100$$

Signal suppression would occur if the percentage difference between these slopes were negative. If it were positive, it would indicate signal enhancement. Ion suppression is a far more common phenomenon, especially with electrospray ionization. The matrix effects were classified into different categories based on this percentage value. A strong matrix effect would occur when the absolute matrix effect was > 50%, a medium matrix effect when the value was between 20 % - 50%, and a low or negligible matrix effect when it was < 20%.

The matrix that had the highest percentage of compounds with a high matrix effect was garlic when there was no clean-up step or when a dSPE clean-up step was used - with around 50 % of compounds presenting a strong matrix effect. However, when µSPE clean-up was used, this percentage decreased to 30%. Another notable example was celery, where 67% of the compounds presented a low matrix effect when the µSPE clean-up was used; in contrast, when using dSPE or when no clean-up step was included, 26% and 31% of the compounds presented a low matrix effect, respectively.

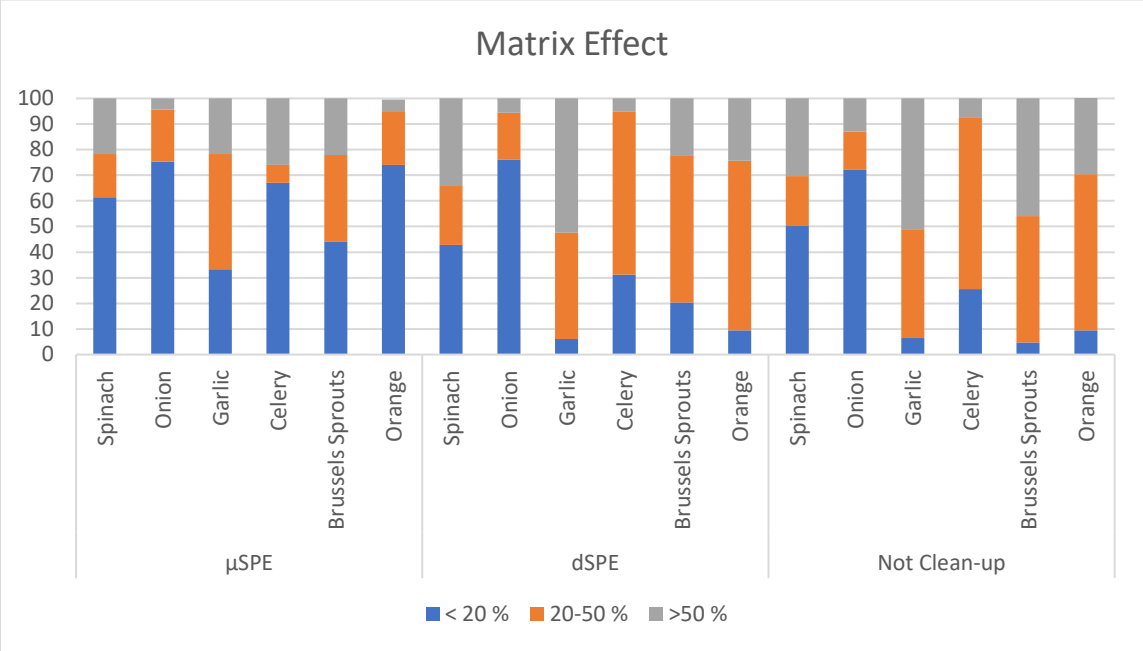


Figure 10: Matrix effect

5. Conclusions

Not including a cleaning stage can lead to matrix co-extracts. At the lower concentration points of the linear range, these interferences may affect the signal obtained and also the ion ratio. Furthermore, to improve the maintenance of the equipment and extend its lifetime, a clean-up step is recommended.

Using an automated µSPE clean-up reduces the laboratory workflow and allows increased sample throughput in routine analysis by eliminating tedious manual steps (the dSPE clean-up). Instrument maintenance is also positively affected because, generally, cleaner extracts are obtained and so the lifespan of certain instrument parts (such as the ion source and columns) increase.

In general, the µSPE method provides recoveries that are very similar to those obtained with manual clean-up because the extraction step is the same for both approaches. However, some compounds are positively affected when using µSPE cartridges, such as acidic compounds (fluazifop, haloxyfop and quizalofop), which are not detected when manual dispersive clean-up is employed (they are retained by the PSA sorbent). For these compounds, the automated clean-up step was able to obtain recoveries from 70 % -120 % because a final elution step of the cartridge (with acidified acetonitrile) was included. In addition, some compounds, such as sulfonylureas (flazasulfuron and orthosulfamuron), have better recoveries when using the automatic method because it is based on chromatography and is therefore more effective at avoiding inconvenient trapping processes. In conclusion, automatic µSPE avoids the qualitative and quantitative errors that are produced when dSPE is applied.

6. References

[1] Lehotay, S. J., Han, L., Sapozhnikova, Y. Automated Mini-Column Solid-Phase Extraction Cleanup for High-Throughput Analysis of Chemical Contaminants in Foods by Low-Pressure Gas Chromatography—Tandem Mass Spectrometry, Chromatographia (79), 2016,1113–1130.

APPENDIX I: MASS TRANSITIONS AND VALIDATION RESULTS

Table 1. Detection and chromatographic parameters for the compounds analyzed by LC-MS/MS.

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Ret Time (min)	Fragmentor (V)	Collision Energy (eV)	Polarity
Acephate	184	143	2.816	380	5	Positive
Acephate	184	125	2.816	380	15	Positive
Acetamiprid	223	126	6.041	380	20	Positive
Acetamiprid	223	56	6.041	380	15	Positive
Albendazole	266.2	234.1	10.104	380	15	Positive
Albendazole	266.2	191	10.104	380	20	Positive
Aldicarb	213	116	7.525	380	10	Positive
Aldicarb	213	89	7.525	380	15	Positive
Aldicarb-sulfone	239.9	223	3.653	380	5	Positive
Aldicarb-sulfone	239.9	86	3.653	380	20	Positive
Aldicarb-sulfoxide	207	132	3.346	380	5	Positive
Aldicarb-sulfoxide	207	89	3.346	380	10	Positive
Ametoctradin	276.2	176.1	12.991	380	35	Positive
Ametoctradin	276.2	149	12.991	380	35	Positive
Anilofos	368.1	198.7	12.521	380	10	Positive
Anilofos	368.1	170.9	12.521	380	20	Positive
Atrazine	216.2	173.8	9.766	380	15	Positive
Atrazine	216.2	131.9	9.766	380	20	Positive
Azinphos-ethyl	368	160.1	11.63	380	10	Positive
Azinphos-ethyl	368	131.9	11.63	380	15	Positive
Azinphos-methyl	318	261	10.364	380	0	Positive
Azinphos-methyl	318	132.1	10.364	380	8	Positive
Azoxystrobin	404	372	10.749	380	10	Positive
Azoxystrobin	404	344	10.749	380	20	Positive
Benalaxyl	326.2	208	12.579	380	15	Positive
Benalaxyl	326.2	148	12.579	380	15	Positive
Bendiocarb	224.1	166.7	8.717	380	5	Positive
Bendiocarb	224.1	109.1	8.717	380	20	Positive
Benzovindiflupyr	398	377.9	12.483	380	10	Positive
Benzovindiflupyr	398	342	12.483	380	15	Positive
Bitertanol	338.2	269.2	12.711	380	5	Positive
Bitertanol	338.2	99.1	12.711	380	10	Positive
Boscalid	343	307.1	11.057	380	16	Positive
Boscalid	343	272.1	11.057	380	32	Positive
Bupirimate	317	272	11.739	380	20	Positive

Bupirimate	317	166	11.739	380	20	Positive
Buprofezin	306	201	13.649	380	10	Positive
Buprofezin	306	116	13.649	380	15	Positive
Butoxycarboxim	240.1	222.7	3.595	380	5	Positive
Butoxycarboxim	240.1	165.9	3.595	380	5	Positive
Carbaryl	202	145	9.027	380	10	Positive
Carbaryl	202	127	9.027	380	20	Positive
Carbendazim	192	160	4.166	380	15	Positive
Carbendazim	192	132	4.166	380	20	Positive
Carbendazim-D3	195.1	159.8	4.155	380	20	Positive
Carbendazim-D3	195.1	131.9	4.155	380	20	Positive
Carbofuran	222	165	8.717	380	10	Positive
Carbofuran	222	123	8.717	380	15	Positive
Chlorantraniliprole	483.9	452.9	10.415	380	16	Positive
Chlorantraniliprole	483.9	285.9	10.415	380	8	Positive
Chlorbromuron	292.9	203.9	11.002	380	20	Positive
Chlorbromuron	292.9	181.9	11.002	380	15	Positive
Chlorfenvinphos	358.9	155	12.78	380	8	Positive
Chlorfenvinphos	358.9	99.2	12.78	380	28	Positive
Chloridazon	222.1	104.1	5.978	380	20	Positive
Chloridazon	222.1	92	5.978	380	20	Positive
Chlorotoluron	213.1	140	9.512	380	20	Positive
Chlorotoluron	213.1	72	9.512	380	20	Positive
Chloroxuron	291.2	217.8	11.476	380	20	Positive
Chloroxuron	291.2	71.9	11.476	380	20	Positive
Chlorpropham	214	123.9	8.58	380	15	Positive
Chlorpropham	214	96	8.58	380	25	Positive
Chlorpyrifos	352	200	13.791	380	20	Positive
Chlorpyrifos	349.93	198	13.791	380	20	Positive
Chlorpyrifos-methyl	321.9	289.9	12.86	380	14	Positive
Chlorpyrifos-methyl	321.9	125	12.86	380	16	Positive
Chromafenozide	395.2	339.1	11.873	380	5	Positive
Chromafenozide	395.2	174.9	11.873	380	10	Positive
Clofentezine	303	138	12.48	380	12	Positive
Clofentezine	303	102	12.48	380	40	Positive
Clofentezine-hydrazine	303	138	8.95	380	12	Positive
Clofentezine-hydrazine	303	102	8.95	380	40	Positive
Clomazone	240.1	127.8	10.544	380	10	Positive
Clomazone	240.1	124.9	10.544	380	20	Positive
Coumaphos	363	307	12.366	380	20	Positive
Coumaphos	363	227	12.366	380	28	Positive
Cyazofamid	325	261.2	11.915	380	10	Positive

Cyazofamid	325	108.1	11.915	380	15	Positive
Cyflufenamid	413	294.9	12.892	380	15	Positive
Cyflufenamid	413	240.8	12.892	380	15	Positive
Cymoxanil	199.1	128	6.437	380	4	Positive
Cymoxanil	199.1	110.9	6.437	380	12	Positive
Cyproconazole	292.1	125	11.52	380	32	Positive
Cyproconazole	292.1	70	11.52	380	16	Positive
Cyprodinil	226.2	92.9	11.666	380	40	Positive
Cyprodinil	226.2	76.9	11.666	380	40	Positive
Demeton-S-methyl	230.9	89.1	8.764	380	5	Positive
Demeton-S-methyl	230.9	61.1	8.764	380	20	Positive
Demeton-S-methylsulfone	263.02	169	4.401	380	12	Positive
Demeton-S-methylsulfone	263.02	109	4.401	380	24	Positive
Demeton-S-methylsulfoxide (Oxydemeton-methyl)	247	169	4.131	380	8	Positive
Demeton-S-methylsulfoxide (Oxydemeton-methyl)	247	109	4.131	380	24	Positive
Desethylterbuthylazine	202.1	146.1	9.06	380	15	Positive
Desethylterbuthylazine	202.1	110.1	9.06	380	20	Positive
Diazinon	305	169	12.598	380	15	Positive
Diazinon	305	153	12.598	380	20	Positive
Dichlorvos	220.8	108.8	8.563	380	15	Positive
Dichlorvos	220.8	78.9	8.563	380	30	Positive
Dichlorvos-D6	226.9	132.9	8.511	380	20	Positive
Dichlorvos-D6	226.9	115	8.511	380	20	Positive
Dicrotophos	238.09	112.1	5.154	380	8	Positive
Dicrotophos	238.09	72.1	5.154	380	28	Positive
Diethofencarb	268	226	10.703	380	5	Positive
Diethofencarb	268	180	10.703	380	15	Positive
Difenoconazole	406	337	12.936	380	15	Positive
Difenoconazole	406	251	12.936	380	20	Positive
Difenoxuron	287.2	123.1	9.939	380	15	Positive
Difenoxuron	287.2	72.1	9.939	380	15	Positive
Diflubenzuron	311	158	11.941	380	8	Positive
Diflubenzuron	311	141	11.941	380	32	Positive
Dimethoate	230	199	6.065	380	5	Positive
Dimethoate	230	171	6.065	380	10	Positive
Dimethoate-D6	236	205	6	380	4	Positive
Dimethoate-D6	236	131	6	380	16	Positive
Dimethomorph	388	301	11	380	20	Positive
Dimethomorph	388	165	11	380	20	Positive
Dimethylvinphos	331	204.8	11.56	380	10	Positive

Dimethylvinphos	331	127	11.56	380	10	Positive
Diniconazole	326.1	159	13.049	380	28	Positive
Diniconazole	326.1	70	13.049	380	28	Positive
Dinotefuran	203.1	129.1	3.28	380	9	Positive
Dinotefuran	203.1	114.1	3.28	380	9	Positive
Diuron	233.03	160	10.092	380	20	Positive
Diuron	233.03	72.1	10.092	380	20	Positive
Diuron	233.03	46.1	10.092	380	16	Positive
DMA	122	106.9	6.357	380	15	Positive
DMA	122	79.1	6.357	380	20	Positive
DMA	122	77.1	6.357	380	20	Positive
Dodine	228.2	60.1	12.617	380	20	Positive
Dodine	228.2	57.2	12.617	380	20	Positive
Edifenphos	311.1	282.8	12.401	380	10	Positive
Edifenphos	311.1	110.9	12.401	380	20	Positive
Epoxiconazole	330.1	121	11.804	380	16	Positive
Epoxiconazole	330.1	101.2	11.804	380	52	Positive
Ethiofencarb	226.1	163.8	9.392	380	5	Positive
Ethiofencarb	226.1	107.2	9.392	380	10	Positive
Ethion	385.1	199	13.8	380	5	Positive
Ethion	385.1	171	13.8	380	10	Positive
Ethiprole	397	351	11.055	380	20	Positive
Ethiprole	397	254.8	11.055	380	40	Positive
Ethirimol	210.16	140.1	7.351	380	20	Positive
Ethirimol	210.16	43.1	7.351	380	52	Positive
Ethoprophos	243.1	130.9	11.905	380	15	Positive
Ethoprophos	243.1	97	11.905	380	30	Positive
Etofenprox	394.2	359.1	14.98	380	10	Positive
Etofenprox	394.2	177.3	14.98	380	8	Positive
Etoxazole	360	304	14.114	380	20	Positive
Etoxazole	360	140.9	14.114	380	30	Positive
Famoxadone	392	331	12.536	380	10	Positive
Famoxadone	392	238	12.536	380	20	Positive
Fenamidone	312	92.2	11.056	380	28	Positive
Fenamidone	312	65.1	11.056	380	56	Positive
Fenamiphos	304.1	234	12.121	380	12	Positive
Fenamiphos	304.1	217.1	12.121	380	20	Positive
Fenamiphos-sulfone	336.1	266	9.029	380	16	Positive
Fenamiphos-sulfone	336.1	188	9.029	380	24	Positive
Fenamiphos-sulfoxide	320.11	292.1	8.808	380	8	Positive
Fenamiphos-sulfoxide	320.11	108.1	8.808	380	44	Positive
Fenarimol	331	268	11.788	380	20	Positive
Fenarimol	331	259	11.788	380	20	Positive

Fenazaquin	307.3	161.3	14.419	380	15	Positive
Fenazaquin	307.3	147.2	14.419	380	15	Positive
Fenbendazole	300.1	268	11.196	380	20	Positive
Fenbendazole	300.1	158.9	11.196	380	35	Positive
Fenbuconazole	337.1	125.1	11.97	380	40	Positive
Fenbuconazole	337.1	70	11.97	380	33	Positive
Fenhexamid	302	97	11.698	380	25	Positive
Fenhexamid	302	55	11.698	380	30	Positive
Fenoxycarb	302.2	116.2	12.073	380	5	Positive
Fenoxycarb	302.2	88.2	12.073	380	20	Positive
Fenpicoxamid	615.3	515	13.348	380	13	Positive
Fenpicoxamid	615.3	238.9	13.348	380	25	Positive
Fenpropidin	274.3	147.1	10.38	380	30	Positive
Fenpropidin	274.3	85.8	10.38	380	25	Positive
Fenpropimorph	304.3	147.1	10.661	380	30	Positive
Fenpropimorph	304.3	130	10.661	380	25	Positive
Fenpyrazamine	332.2	272.1	11.553	380	10	Positive
Fenpyrazamine	332.2	230.2	11.553	380	20	Positive
Fenpyroximate	422.21	366.2	13.97	380	12	Positive
Fenpyroximate	422.21	107	13.97	380	64	Positive
Fensulfothion	309	252.8	10.009	380	17	Positive
Fensulfothion	309	157	10.009	380	29	Positive
Fenthion	279	247.1	12.326	380	8	Positive
Fenthion	279	169.1	12.326	380	12	Positive
Fenthion-sulfone	310.7	125	9.286	380	15	Positive
Fenthion-sulfone	310.7	108.8	9.286	380	15	Positive
Fenthion-sulfoxide	295.02	280	8.986	380	16	Positive
Fenthion-sulfoxide	295.02	109	8.986	380	32	Positive
Fenuron	165.2	92.1	5.674	380	20	Positive
Fenuron	165.2	71.8	5.674	380	20	Positive
Fipronil	434.9	329.9	12.28	380	12	Negative
Fipronil	434.9	249.9	12.28	380	28	Negative
Flazasulfuron	408	227	10.457	380	20	Positive
Flazasulfuron	408	182.1	10.457	380	20	Positive
Flonicamid	230.1	202.6	4.38	380	10	Positive
Flonicamid	230.1	173.9	4.38	380	10	Positive
Fluacrypyrim	427.1	205	13.118	380	10	Positive
Fluacrypyrim	427.1	145.1	13.118	380	15	Positive
Fluazifop	328.2	282.2	10.969	380	15	Positive
Fluazifop	328.2	254.2	10.969	380	20	Positive
Flubendiamide	680.9	273.9	12.457	380	15	Negative
Flubendiamide	680.9	254	12.457	380	20	Negative
Fludioxonil	265.9	228.9	11.131	380	5	Positive

Fludioxonil	265.9	158	11.131	380	20	Positive
Flufenacet	364.1	194.1	11.912	380	15	Positive
Flufenacet	364.1	152	11.912	380	15	Positive
Flufenoxuron	489.1	158	14.07	380	20	Positive
Flufenoxuron	489.1	140.9	14.07	380	56	Positive
Fluometuron	233.2	187.9	9.461	380	20	Positive
Fluometuron	233.2	72.2	9.461	380	20	Positive
Fluopicolide	382.9	172.9	11.329	380	20	Positive
Fluopicolide	382.9	144.8	11.329	380	20	Positive
Fluopyram	397.1	208	11.765	380	20	Positive
Fluopyram	397.1	173.1	11.765	380	20	Positive
Fluquinconazole	376	307.1	11.55	380	24	Positive
Fluquinconazole	376	108	11.55	380	56	Positive
Flusilazole	316.1	247.1	12.159	380	12	Positive
Flusilazole	316.1	165	12.159	380	24	Positive
Flutriafol	302.1	95	9.897	380	56	Positive
Flutriafol	302.1	70.1	9.897	380	16	Positive
Fluxapyroxad	381.9	362	11.302	380	10	Positive
Fluxapyroxad	381.9	342	11.302	380	15	Positive
Formetanate Hydrochloride	222.13	165.1	2.89	380	8	Positive
Formetanate Hydrochloride	222.13	65.1	2.89	380	52	Positive
Fosthiazate	284	227.8	9.52	380	10	Positive
Fosthiazate	284	103.8	9.52	380	20	Positive
Haloxyfop	362.1	316.2	12.22	380	12	Positive
Haloxyfop	362.1	288.1	12.22	380	24	Positive
Hexaconazole	314.1	159	12.775	380	30	Positive
Hexaconazole	314.1	70.1	12.775	380	20	Positive
Hexaflumuron	459	439	13.121	380	5	Negative
Hexaflumuron	459	276.1	13.121	380	20	Negative
Hexythiazox	353.1	228.2	13.98	380	10	Positive
Hexythiazox	353.1	168.2	13.98	380	20	Positive
Imazalil	297	255	9.508	380	15	Positive
Imazalil	297	159	9.508	380	20	Positive
Imidacloprid	256	209	5.287	380	15	Positive
Imidacloprid	256	175	5.287	380	15	Positive
Indoxacarb	528.1	218	13.131	380	20	Positive
Indoxacarb	528.1	203	13.131	380	45	Positive
Iprovalicarb	321.2	202.9	11.883	380	0	Positive
Iprovalicarb	321.2	119	11.883	380	16	Positive
Isofenfos-methyl	231	199	12.39	380	15	Positive
Isofenfos-methyl	231	121	12.39	380	15	Positive
Isoprocarb	194.1	152	9.917	380	5	Positive

Isoprocarb	194.1	95.1	9.917	380	15	Positive
Isoprothiolane	291	230.7	11.248	380	10	Positive
Isoprothiolane	291	189.1	11.248	380	15	Positive
Isoproturon	207.15	165.1	9.984	380	20	Positive
Isoproturon	207.15	72.1	9.984	380	10	Positive
Isoxaflutole	360	250.9	10.168	380	15	Positive
Isoxaflutole	360	219.7	10.168	380	50	Positive
Kresoxim-methyl	314.1	267	12.26	380	0	Positive
Kresoxim-methyl	314.1	222.1	12.26	380	10	Positive
Linuron	249.02	160.1	10.784	380	20	Positive
Linuron	249.02	133	10.784	380	36	Positive
Lufenuron	508.9	339	13.742	380	10	Negative
Lufenuron	508.9	325.9	13.742	380	10	Negative
Malathion	331	285	11.283	380	5	Positive
Malathion	331	127.1	11.283	380	15	Positive
Malathion-D10	341.11	132	11.315	380	12	Positive
Malathion-D10	341.11	100	11.315	380	24	Positive
Mandipropamid	412.13	356.1	11.153	380	4	Positive
Mandipropamid	412.13	328.1	11.153	380	8	Positive
Mebendazole	296.1	263.9	9.227	380	21	Positive
Mebendazole	296.1	105	9.227	380	37	Positive
Mebendazole	296.1	77	9.227	380	55	Positive
Metaflumizone	505	328	13.377	380	10	Negative
Metaflumizone	505	302	13.377	380	10	Negative
Metalaxyl	280.3	220	10.103	380	5	Positive
Metalaxyl	280.3	192.4	10.103	380	10	Positive
Metamitron	203.2	174.9	5.662	380	15	Positive
Metamitron	203.2	104.1	5.662	380	15	Positive
Metconazole	320.1	125	12.728	380	48	Positive
Metconazole	320.1	70.1	12.728	380	24	Positive
Methamidophos	142.1	125	2.305	380	10	Positive
Methamidophos	142.1	94.1	2.305	380	10	Positive
Methidathion	302.9	145	10.294	380	0	Positive
Methidathion	302.9	85.1	10.294	380	15	Positive
Methiocarb	226.1	121.1	10.961	380	12	Positive
Methiocarb	226	169	10.961	380	5	Positive
Methiocarb-sulfone	275	201.1	6.353	380	5	Positive
Methiocarb-sulfone	275	122	6.353	380	15	Positive
Methiocarb-sulfoxide	242	185	5.792	380	10	Positive
Methiocarb-sulfoxide	242	170	5.792	380	20	Positive
Methomyl	163.1	106	4.114	380	4	Positive
Methomyl	163.1	88	4.114	380	0	Positive
Methoxyfenozide	369.3	149	11.567	380	15	Positive

Methoxyfenozide	369.3	133	11.567	380	20	Positive
Metobromuron	259	170	9.576	380	15	Positive
Metobromuron	259	148	9.576	380	10	Positive
Metolachlor	284.2	252.1	12.013	380	15	Positive
Metolachlor	284.2	175.9	12.013	380	20	Positive
Metolcarb	166	109.1	7.964	380	5	Positive
Metolcarb	166	91	7.964	380	20	Positive
Metrafenone	409.1	226.9	12.83	380	16	Positive
Metrafenone	409.1	209.1	12.83	380	8	Positive
Monocrotophos	224.2	193.1	4.725	380	5	Positive
Monocrotophos	224.2	127	4.725	380	10	Positive
Monolinuron	215.06	148.1	9.175	380	8	Positive
Monolinuron	215.06	126	9.175	380	16	Positive
Monuron	199.1	125.8	8.197	380	20	Positive
Monuron	199.1	71.9	8.197	380	15	Positive
Myclobutanil	289.2	125.1	11.522	380	20	Positive
Myclobutanil	289.2	70.2	11.522	380	15	Positive
Neburon	275.1	113.9	12.294	380	10	Positive
Neburon	275.07	88.1	12.294	380	12	Positive
Neburon	275.07	57.1	12.294	380	20	Positive
Nitenpyram	271	225	3.833	380	10	Positive
Nitenpyram	271	99	3.833	380	10	Positive
Novaluron	490.8	470.7	13.29	380	5	Negative
Novaluron	490.8	305.1	13.29	380	15	Negative
Omethoate	214.1	183	3.118	380	5	Positive
Omethoate	214.1	125	3.118	380	20	Positive
Orthosulfamuron	425	226.9	10.03	380	15	Positive
Orthosulfamuron	425	199.1	10.03	380	15	Positive
Oxamyl	237	90	3.821	380	5	Positive
Oxamyl	237	72	3.821	380	10	Positive
Oxasulfuron	407.1	209.7	8.143	380	24	Positive
Oxasulfuron	407.1	150.1	8.143	380	16	Positive
Oxathiapipronil	540.2	522	11.217	380	29	Positive
Oxathiapipronil	540.2	500	11.217	380	29	Positive
Oxfendazole	316.1	284.1	7.979	380	20	Positive
Oxfendazole	316.1	159.1	7.979	380	35	Positive
Paclobutrazol	294.1	125.2	11.322	380	36	Positive
Paclobutrazol	294.1	70.1	11.322	380	16	Positive
Penconazole	284	159	12.435	380	20	Positive
Penconazole	284	70	12.435	380	15	Positive
Pencycuron	329.1	125.1	12.972	380	24	Positive
Pencycuron	329.1	89.1	12.972	380	60	Positive
Pendimethalin	282.1	212.1	13.889	380	4	Positive

Pendimethalin	282.1	194.1	13.889	380	16	Positive
Penflufen	318.1	234	12.369	380	10	Positive
Penflufen	318.1	141	12.369	380	20	Positive
Penthiopyrad	357.9	207.6	12.538	380	20	Negative
Penthiopyrad	357.9	149	12.538	380	25	Negative
Phenthoate	321	247.1	12.29	380	4	Positive
Phenthoate	321	79.1	12.29	380	44	Positive
Phosalone	368	182	12.739	380	8	Positive
Phosalone	368	110.9	12.739	380	44	Positive
Phosmet	317.99	160	10.458	380	8	Positive
Phosmet	317.99	133	10.458	380	36	Positive
Phoxim	299	129.1	12.668	380	4	Positive
Phoxim	299	77.1	12.668	380	24	Positive
Pirimicarb	239.2	182.1	7.618	380	15	Positive
Pirimicarb	239.2	72.2	7.618	380	20	Positive
Pirimiphos-methyl	306.2	164.2	12.664	380	20	Positive
Pirimiphos-methyl	306.2	108.2	12.664	380	20	Positive
Prochloraz	376	308	12.493	380	10	Positive
Prochloraz	376	266	12.493	380	15	Positive
Profenofos	374.9	347	13.417	380	5	Positive
Profenofos	374.9	304.9	13.417	380	15	Positive
Promecarb	208.2	150.9	11.247	380	5	Positive
Promecarb	208.2	108.8	11.247	380	10	Positive
Prometryn	242.2	201	11.033	380	20	Positive
Prometryn	242.2	157.8	11.033	380	20	Positive
Propamocarb	189.2	144.1	3.271	380	10	Positive
Propamocarb	189.2	102.1	3.271	380	15	Positive
Propaquizafop	444.1	371	13.454	380	15	Positive
Propaquizafop	444.1	99.9	13.454	380	20	Positive
Propargite	368.1	231.2	14.126	380	0	Positive
Propargite	368.1	175.2	14.126	380	8	Positive
Propazine	230.2	187.9	10.868	380	15	Positive
Propazine	230.2	146	10.868	380	20	Positive
Propiconazole	342.1	159	12.48	380	32	Positive
Propiconazole	342.1	69.1	12.48	380	16	Positive
Propoxur	210.11	168.1	8.595	380	5	Positive
Propoxur	210.11	111.1	8.595	380	10	Positive
Propyzamide	256	190	11.31	380	10	Positive
Propyzamide	256	173	11.31	380	20	Positive
Proquinazid	373	331	14.152	380	20	Positive
Proquinazid	373	289.1	14.152	380	20	Positive
Prosulfocarb	252.1	128	13.319	380	10	Positive
Prosulfocarb	252.1	90.9	13.319	380	20	Positive

Pyraclostrobin	388.11	193.8	12.536	380	8	Positive
Pyraclostrobin	388.11	163.1	12.536	380	20	Positive
Pyridaben	365.2	309.2	14.536	380	10	Positive
Pyridaben	365.2	147.3	14.536	380	20	Positive
Pyridaphenthion	341.1	205	11.502	380	20	Positive
Pyridaphenthion	341.1	189	11.502	380	15	Positive
Pyrimethanil	200	183	10.068	380	20	Positive
Pyrimethanil	200	107	10.068	380	20	Positive
Pyriofenone	366.1	209	12.863	380	20	Positive
Pyriofenone	366.1	183.9	12.863	380	20	Positive
Pyriproxyfen	322	185	13.633	380	20	Positive
Pyriproxyfen	322	96	13.633	380	10	Positive
Quinalphos	299.1	270.8	12.122	380	10	Positive
Quinalphos	299.1	242.8	12.122	380	10	Positive
Quinoclamine	208	105.1	7.697	380	25	Positive
Quinoclamine	208	77	7.697	380	40	Positive
Quinoxyfen	308.1	271.9	13.705	380	25	Positive
Quinoxyfen	308.1	196.9	13.705	380	35	Positive
Quizalofop	345	299	11.834	380	20	Positive
Quizalofop	345	254.9	11.834	380	35	Positive
Quizalofop-ethyl	373.09	271.2	13.254	380	24	Positive
Quizalofop-ethyl	373.09	255.1	13.254	380	36	Positive
Rotenone	395	213.1	11.894	380	20	Positive
Rotenone	395	192.1	11.894	380	20	Positive
Simazine	202.2	131.8	8.447	380	15	Positive
Simazine	202.2	124	8.447	380	15	Positive
Spinetoram J	748.3	203	13.075	380	30	Positive
Spinetoram J	748.3	142	13.075	380	25	Positive
Spinetoram L	760.4	203	13.393	380	35	Positive
Spinetoram L	760.4	142.1	13.393	380	35	Positive
Spinosyn A	732.5	142.1	12.589	380	30	Positive
Spinosyn A	732.5	98.1	12.589	380	40	Positive
Spinosyn D	746.5	142	12.95	380	25	Positive
Spinosyn D	746.5	98	12.95	380	40	Positive
Spiromesifen	371	273	14.218	380	5	Positive
Spiromesifen	371	255	14.218	380	20	Positive
Spirotetramat	374.2	330.3	11.723	380	15	Positive
Spirotetramat	374.2	270.1	11.723	380	20	Positive
Spiroxamine	298	144	11.015	380	20	Positive
Spiroxamine	298	100	11.015	380	20	Positive
Sulfoxaflor	278	153.9	6.49	380	20	Positive
Sulfoxaflor	278	105.1	6.49	380	10	Positive
Tebuconazole	308	125	12.448	380	20	Positive

Tebuconazole	308	70	12.448	380	20	Positive
Tebufenozide	353.2	296.9	12.345	380	5	Positive
Tebufenozide	353.2	133.1	12.345	380	15	Positive
Tebufenpyrad	334.2	145.1	13.576	380	20	Positive
Tebufenpyrad	334.2	117	13.576	380	47	Positive
Teflubenzuron	379	359	13.573	380	0	Negative
Teflubenzuron	379	339	13.573	380	4	Negative
Terbutryn	242.2	186.2	11.146	380	15	Positive
Terbutryn	242.2	91	11.146	380	20	Positive
Terbutylazine	230	174	11.065	380	15	Positive
Terbutylazine	230	146	11.065	380	20	Positive
Tetraconazole	372	159	11.921	380	36	Positive
Tetraconazole	372	70	11.921	380	20	Positive
Thiabendazole	202	175	4.83	380	30	Positive
Thiabendazole	202	131	4.83	380	40	Positive
Thiacloprid	253	186	6.754	380	10	Positive
Thiacloprid	253	126	6.754	380	20	Positive
Thiamethoxam	292	211	4.378	380	10	Positive
Thiamethoxam	292	181	4.378	380	20	Positive
Thiobencarb	258	124.7	12.824	380	15	Positive
Thiobencarb	258	99.9	12.824	380	10	Positive
Tolclofos-methyl	300.9	269	12.621	380	10	Positive
Tolclofos-methyl	300.9	125	12.621	380	15	Positive
Tolfenpyrad	384.1	197	13.534	380	25	Positive
Tolfenpyrad	384.1	170.9	13.534	380	20	Positive
Triadimefon	294.2	225	11.475	380	10	Positive
Triadimefon	294.2	197.1	11.475	380	10	Positive
Triallate	306.01	145	13.935	380	25	Positive
Triallate	306.01	86	13.935	380	15	Positive
Triazophos	314.1	286.2	11.523	380	10	Positive
Triazophos	314.1	162.2	11.523	380	20	Positive
Trichlorfon	258.9	222.5	5.961	380	5	Positive
Trichlorfon	258.9	108.8	5.961	380	20	Positive
Triclorcarban	313	160	13.103	380	20	Negative
Triclorcarban	313	126	13.103	380	20	Negative
Tricyclazole	190.1	163	7.025	380	25	Positive
Tricyclazole	190.1	136.1	7.025	380	35	Positive
Trifloxystrobin	409.2	206.2	13.199	380	10	Positive
Trifloxystrobin	409.2	186.2	13.199	380	20	Positive
Triflumizole	346.1	277.8	13.234	380	5	Positive
Triflumizole	346.1	72.9	13.234	380	15	Positive
Triflumuron	359	156	12.709	380	8	Positive
Triflumuron	359	139	12.709	380	32	Positive

Triticonazole	318.1	125.2	11.785	380	20	Positive
Triticonazole	318.1	70.2	11.785	380	20	Positive
Valifenalate	399	313	11.58	380	10	Positive
Valifenalate	399	143.7	11.58	380	15	Positive
XMC	180.1	123.1	9.04	380	10	Positive
XMC	180.1	95.1	9.04	380	20	Positive
Zoxamide	336	187	12.594	380	16	Positive
Zoxamide	336	159	12.594	380	44	Positive