Development and validation of an automated extraction method for the analysis of high-water content commodities

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1. Aim and scope

This document reports the validation data for 244 LC-amenable pesticides, most of them included in the European Union Multi Annual Control Program (EU-MACP) and the Working Document SANCO/12745/2013, using a multiresidue method by liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS). An automatic extraction method based on automated pressurized liquid extraction employing the EDGE instrument has been developed for the extraction of these residues in high-water content matrices.

2. Short description

A new automated extraction method based on the use of an automated pressurised liquid extraction instrument (EDGE) has been validated for 244 pesticides in high water content matrices using an LC-MS/MS instrument. With this purpose, homogeneous samples have been spiked at 0.010 mg/kg concentration level and extracted automatically. The EDGE combines the process of pressurised fluid extraction (PFE) and dispersive solid phase extraction (dSPE) in one instrument. The obtained extracts have been analysed by LC-MS/MS and GC-MS/MS.

The validation of the extraction method has been performed in terms of accuracy (spiking concentrations 0.010 and 0.050 mg/kg) and repeatability (n = 5). Method results have been compared to those obtained by an extraction method based on AcN (QuEChERS citrate).

3. Apparatus and consumables

- Automatic pipettes, suitable for handling volumes from 1 µL 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 4000 rpm
- Concentration workstation
- Injection vials, 2 mL, suitable for LC auto-sampler
- Amber vials, 7 mL
- EDGE instrument supplied by CEM Corporation (Charlotte, North Carolina, United States of America)

- Q-Discs® (CEM Corporation) G1 and C9 models.
- Q-Cups (CEM Corporation)
- Q-Matrix Hydra (CEM Corporation)
- Q-Screens (CEM Corporation)

4. Chemicals

- Acetonitrile ultra-gradient grade (AcN)
- Primary secondary amine (PSA)
- Anhydrous magnesium sulphate
- Sodium chloride (reagent grade)
- Disodium citrate sesquihydrate (reagent grade)
- Trisodium citrate dihydrate (reagent greade)
- Ammonium formate
- Ultra-pure water
- Methanol HPLC grade (MeOH)
- Formic acid LC-MS grade
- Acetic acid glacial
- Pesticide analytical standards

5. Procedure

5.1. Recovery experiments for method validation

Individual pesticide stock solutions (1000–2000 mg/L) were prepared in acetonitrile and were stored in screw-capped glass vials in the dark at -20 °C.

For the spiking procedure, representative portions of the previously homogenised samples were spiked with the appropriate amount of a working standard solution in acetonitrile. Method validation was performed at spiking levels 0.010 and 0.050 mg/kg. Five replicates (n = 5) were analysed at each level.

5.2. Extraction methods

5.2.1. Automated sample extraction using EDGE

Plenty of modifications regarding method parameters were evaluated based on the modification of several key parameters, which will be thoroughly discussed below:

- Sample mass in the Q-Cup (grams)
- Q-Matrix Hydra water sorbent mass in the Q-Cup (grams)
- Magnesium sulphate in the Q-Cup (grams)
- Solvent (acetonitrile vs. acetonitrile acidified with acetic acid (99:1, V/V)
- Bubbling time (with nitrogen) (seconds)
- Hold time (seconds)

5.2.2 Manual extraction method based on QuEChERS citrate

Real samples were also extracted using QuEChERS citrate without a clean-up step:

1. Weigh 10 g of homogenate sample in a 50-mL PTFE centrifuge tube.
2. Add 10 mL of acetonitrile.
3. Shake the samples in an Agytax axial extractor for 4 min.
4. Add 0.5 g of disodium citrate sesquihydrate, 1.0 g of trisodium citrate dihydrate, 1.0 g sodium chloride and 4.0 g anhydrous magnesium sulphate.
5. Shake the samples in an Agytax axial extractor for 4 min.
6. Centrifuge at 4000 rpm for 5 min.
7. Transfer a 4 mL aliquot of the supernatant into a 4 mL glass vial.

5.3. Injection vial preparation

During the vial preparation, sample extracts were diluted 5-fold with ultrapure water of extracts and dimethoate-D_6 (LC) was added as an internal injection standard (0.050 mg/L). In the case of GC, an aliquot of the acetonitrile extract was gently evaporated using nitrogen, then dissolved again in ethyl acetate, and finally lindane-D_6 was added as an internal injection standard (0.050 mg/L).

5.4. Methodology

Both the LC-MS/MS and the GC-MS/MS instruments were operated in selected reaction monitoring (SRM). First, full scan (FS) analyses were carried out to select the most sensitive precursor ions. Then, product ion scans (PIS) were performed to select the most abundant product ions. Finally, two SRM transitions and the correct ratio between the abundances of the two optimised SRM transitions (SRM1/SRM2) were used, alongside retention time matching to obtain the maximum sensitivity for the detection of the target molecules. The instrumental responses produced by the different constituents and/or isomers of a single pesticide were

assessed and compared in terms of area and ratio between two transitions (quantitation and confirmation transitions). The mass transitions used are presented in the **Appendix** (**Table A1** for LC-MS/MS parameters).

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

5.5.1. UHPLC (Thermo Scientific™ Transcend™ DUO LX-2 LC)

- Column: Accucore C18 2.1x100 mm and 2.6 µm particle size (Thermo Scientific™)
- 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: 30 °C
- Flow rate: 0.35 ml/min
- Injection volume: 2.5 µL
- Autosampler temperature: 10 °C

Mobile phase gradient for pesticides analysis:

Time (min)	Mobile phase A
0.0	100 %
1.0	100 %
2.0	70 %
3.0	50 %
11.0	0 %
14.0	0 %
14.1	100 %
17.0	100 %
Data window (min)	1.1-11.55

5.5.2. Triple quadrupole system (Thermo Scientific™ TSQ Altis™)

- Ion source: Opta Max NG
- Positive ion spray voltage: 3500 V
- Negative ion spray voltage: 2500 V
- Sheath gas: 50 (arbitrary units)

- Aux gas: 10 (arbitrary units)
- Sweep gas: 1 (arbitrary units)
- Ion transfer tube temperature: 325 °C
- Vaporiser temperature: 350 °C

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

5.6.1. GC system (Agilent Intuvo 9000 GC)

- Column: 2 Planar columns HP-5MS UI (15 m long × 0.25 mm i.d. × 0.25 µm film thickness)
- Injection mode: Splitless
- Ultra-inert inlet liner with a glass wool frit from Agilent
- Injection volume: 1 µl
- Injector temperature: 80 °C hold for 0.1 min, then up to 300 °C at 600 °C/min, hold for 5 min and then to 250 °C at 100 °C/min
- Carrier gas: Helium at constant flow = 1.28 mL/min column 1, 1.48 mL/min column 2
- Carrier gas purity: 99.999 %
- Oven temperature: 60 °C for 0.5 min, up to 170 °C at 80 °C/min, and up to 310 °C at 20 °C/min

5.6.2. Triple quadrupole system (Agilent 7410)

- Ionisation mode: electron impact ionisation
- Temperature of the transfer line: 280 °C
- Temperature of ion source: 280 °C
- Collision gas: nitrogen
- Collision gas purity: 99.999 %
- Solvent delay: 2.6 min

6. Results

6.1. Method development

6.1.1. Evaluated parameters and initial method

The parameters to be optimised during method development, as indicated above, were: sample mass in the Q-Cup (grams), Q-Matrix Hydra water sorbent mass in the Q-Cup (grams), magnesium sulphate in the Q-Cup (grams), extraction

solvent (acetonitrile vs. acetonitrile acidified with acetic acid (99:1, V/V), bubbling time (with nitrogen) (seconds) and hold time (seconds). A thorough description of each parameter can be found in the literature, alongside a description of each step (Díaz-Galiano, Murcia-Morales et al., 2021; EURLFV 2019-M34; and EURLFV 2020-M39). In short, samples are extracted during the hold time, which is the length of time during the sample is mixed with the extraction solvent at a predefined temperature under pressurised conditions. Then, samples are rinsed with an additional aliquot of the solvent of choice, without any hold time. Before the hold time, samples can be mixed (agitated) using a nitrogen source, which is done at ambient pressure and room temperature. All extracts (hold and rinse steps) are collected in a PTFE tube, into which salts, e.g. magnesium sulphate, can be pre-weighed.

Based on prior experience, 244 LC-amenable pesticides were evaluated using four initial methods as a starting point (M01-M04), with a spiking level of 0.010 mg/kg:

- Sample preparation
- M01: 2.5 g Q-Matrix Hydra + 10 g sample
- M02: 2.5 g Q-Matrix Hydra + 1 g NaCl + 10 g sample
- M03: 2.5 g Q-Matrix Hydra + 10 g sample
- M04: 2.5 g Q-Matrix Hydra + 1 g NaCl + 10 g sample

- EDGE extraction
- 20 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)
- 90 s bubbling (nitrogen, 40 psi input pressure)
- 90 s hold (40 °C)
- Collection of the extract
- 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse)

- Post-extraction
- M01: -
- M02: -
- M03: 2 g of $MgSO_4$ in the PTFE collection tube
- M04: 2 g of $MgSO_4$ in the PTFE collection tube

After the evaluation of the recovery experiments (**Table 1**, **Figure 1**), M01 yielded marginally better results than M02, M03 or M04, with a lower use of reagents, so further method modifications were based on M01.

Table 1. Comparison of recovery results (0.010 mg/kg, LC) for M01, M02, M03 and M04.

	M01	M02	M03	M04
< 30 %	0.0 %	0.0 %	0.0 %	0.0 %
[30-40) %	0.0 %	0.0 %	0.0 %	0.0 %
[40-50) %	0.0 %	0.0 %	0.0 %	0.4 %
[50-60) %	1.6 %	0.8 %	0.4 %	0.0 %
[60-70) %	7.4 %	2.9 %	0.4 %	2.5 %
[70-80) %	11.1 %	7.4 %	1.2 %	4.5 %
[80-90) %	14.3 %	16.4 %	7.8 %	23.0 %
[90-100) %	15.6 %	19.7 %	13.1 %	15.6 %
[100-110) %	10.2 %	5.7 %	18.4 %	12.7 %
[110-120] %	11.5 %	6.1 %	19.7 %	10.7 %
(120-130) %	15.2 %	11.9 %	11.9 %	10.2 %
[130-140] %	7.4 %	6.1 %	8.6 %	6.6 %
> 140 %	4.5 %	20.9 %	16.4 %	11.5 %

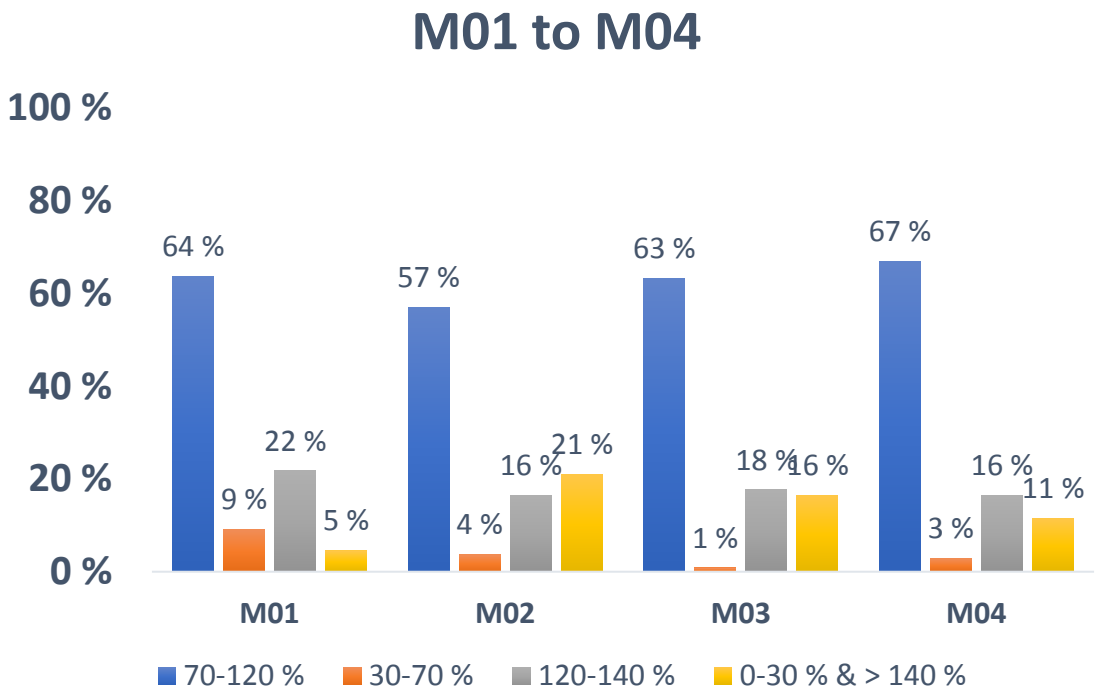

Figure 1. Comparison of recovery results (0.010 mg/kg, LC) for M01, M02, M03 and M04.

6.1.2. Method development

After tomato, several high-water content matrices were evaluated by LC-MS/MS (cucumber, carrot and onion) alongside matrices from different commodity groups (orange, avocado), to assess the initial method performance and select the high water content matrix with which to continue method development (**Table 2**, **Figure 2**).

Table 2. Comparison of recovery results (0.010 mg/kg, LC) for M01 in several matrices.

	Cucumber	Carrot	Onion	Orange	Avocado
< 30 %	0.4 %	0.0 %	0.0 %	0.4 %	0.0 %
[30-40) %	0.0 %	0.0 %	0.0 %	0.0 %	0.4 %
[40-50) %	0.4 %	0.0 %	0.0 %	0.0 %	0.4 %
[50-60) %	1.6 %	0.4 %	2.9 %	0.0 %	1.6 %
[60-70) %	10.2 %	0.0 %	6.6 %	4.1 %	25.0 %
[70-80) %	16.8 %	7.4 %	15.6 %	4.5 %	12.7 %
[80-90) %	33.2 %	5.7 %	25.8 %	11.9 %	7.4 %
[90-100) %	24.2 %	15.2 %	34.8 %	19.7 %	15.6 %
[100-110) %	6.6 %	18.4 %	7.4 %	18.9 %	11.1 %
[110-120] %	2.5 %	22.1 %	3.3 %	16.0 %	7.4 %
(120-130) %	1.6 %	17.6 %	0.4 %	7.4 %	8.6 %
[130-140] %	0.0 %	6.1 %	0.4 %	2.5 %	4.5 %
> 140 %	1.2 %	5.7 %	1.6 %	12.7 %	4.1 %

In the light of these results, cucumber was chosen as the representative matrix for further high-water content method development. Next, several parameters were changed and tested in methods M08 to M10.[1] In particular, all three methods swapped acetonitrile w/ acetic acid (1 %, V/V) for acetonitrile. Furthermore, in M09, the bubbling step was removed in favour of a longer hold time. Finally, M08 and M09 employed acetonitrile also in the rinse step, whereas M10 used acetonitrile w/ acetic acid (1 %, V/V). The experiments helped determine several key points:

1. A low volume of a different rinse solvent is less effective than outright modifying both extraction and rinse solvents.
2. The bubbling step helps increase extraction efficiency significantly.

[1] Although method numbering does not follow continuity at this point (M04 → M08), other experiments (M05, M06 and M07) were being carried out simultaneously. For internal purposes, method numbering is kept as shown here.

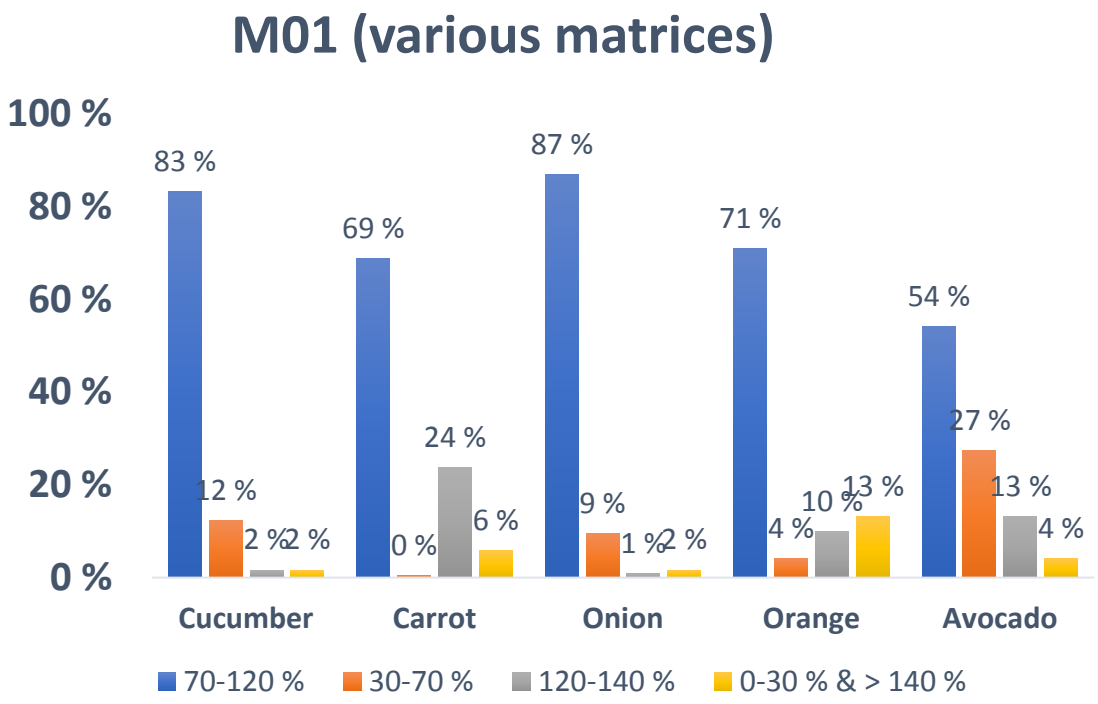

Figure 2. Comparison of recovery results (0.010 mg/kg, LC) for M01 in several matrices.

Results for M08, M09 and M10 are summarised in **Table 3** and **Figure** 3. The experimental conditions for methods M08 to M10 were:

- Sample preparation
- M08: 2.5 g Q-Matrix Hydra + 10 g cucumber
- M09: 2.5 g Q-Matrix Hydra + 10 g cucumber
- M10: 2.5 g Q-Matrix Hydra + 10 g cucumber

- EDGE extraction
- 20 mL acetonitrile (top)
- 90 s bubbling (nitrogen, 40 psi input pressure) (M08 & M10)
- 90 s hold (40 °C) (M08 & M10)
- 150 s hold (40 °C) (M09)
- Collection of the extract
- 5 mL acetonitrile (rinse) (M08 & M09)
- 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse) (M10)

- Post-extraction
- M08, M09 & M10: -

Table 3. Comparison of recovery results (0.010 mg/kg, LC) for M08 to M10 in cucumber.

	M08	M09	M10
< 30 %	0.0 %	0.0 %	0.0 %
[30-40) %	0.0 %	0.0 %	0.4 %
[40-50) %	0.0 %	0.4 %	0.0 %
[50-60) %	0.4 %	0.4 %	7.0 %
[60-70) %	0.8 %	1.6 %	44.3 %
[70-80) %	3.3 %	17.2 %	29.9 %
[80-90) %	34.4 %	41.4 %	9.4 %
[90-100) %	48.0 %	22.5 %	2.9 %
[100-110) %	9.0 %	5.7 %	0.8 %
[110-120] %	1.2 %	3.3 %	1.6 %
(120-130) %	0.8 %	0.8 %	0.4 %
[130-140] %	0.0 %	0.8 %	1.2 %
> 140 %	0.8 %	4.1 %	1.2 %

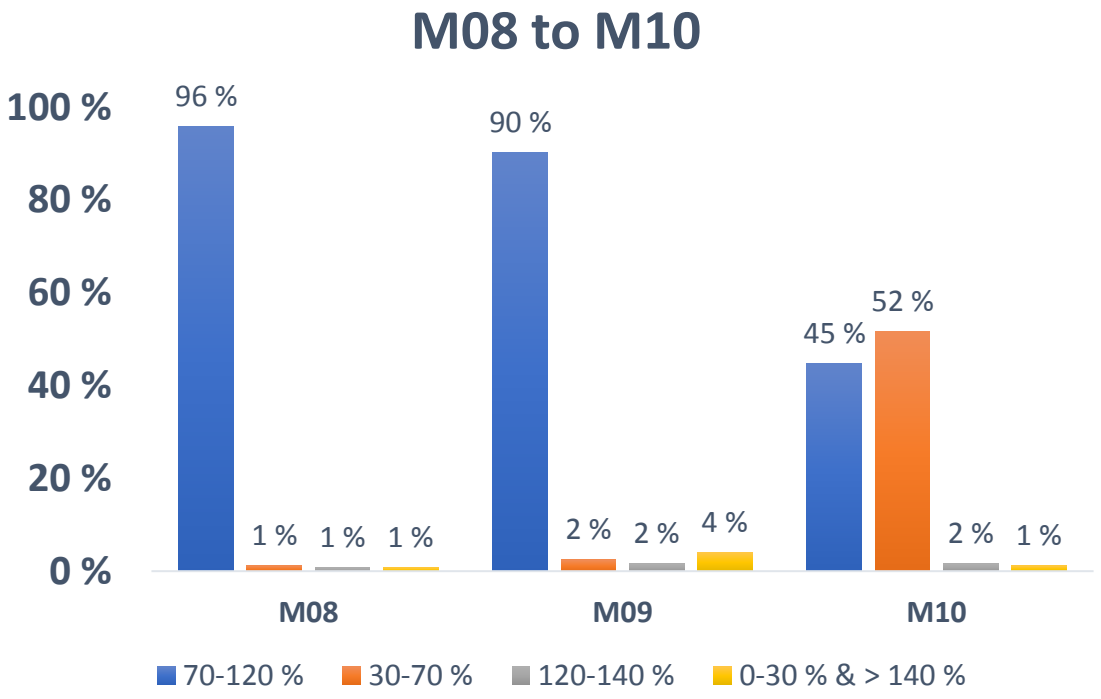

Figure 3. Comparison of recovery results (0.010 mg/kg, LC) for M08 to M10 in cucumber.

After the evaluation of the results, it was decided to continue method development based on M01 and M08, the best performing methods at the time, which differed in the extraction solvent (acetonitrile w/ acetic acid (1 %, V/V) and acetonitrile, respectively). The basic extraction parameters were unchanged in methods M11 to M14; however, different amounts of Q-Matrix Hydra were added to the Q-Cup: 2.50 g, 3.75 g and 5.00 g. Furthermore, to the same extracts (M11 to M14) magnesium sulphate was added to further ensure that no water was left on said extracts (M15-M20) (**Table 4**, **Figure 4**). Method parameters were as follows:

- Sample preparation
- M01 & M08: 2.5 g Q-Matrix Hydra + 10 g cucumber
- M11: M08 but 3.75 g Q-Matrix Hydra
- M12: M08 but 5.00 g Q-Matrix Hydra
- M13: M01 but 3.75 g Q-Matrix Hydra
- M14: M01 but 5.00 g Q-Matrix Hydra

- EDGE extraction
- M01: 20 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)
- M08: 20 mL Acetonitrile (top)
- 90 s bubbling (nitrogen, 40 psi input pressure)
- 90 s hold (40 °C)
- Collection of the extract
- M01: 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse)
- M08: 5 mL acetonitrile (rinse)

- Post-extraction
- M11: -
- M12: -
- M13: -
- M14: -
- M15: M08 and 0.9 g $MgSO_4$ to 4 mL of the extract
- M16: M11 and 0.9 g $MgSO_4$ to 4 mL of the extract
- M17: M12 and 0.9 g $MgSO_4$ to 4 mL of the extract
- M18: M01 and 0.9 g $MgSO_4$ to 4 mL of the extract
- M19: M13 and 0.9 g $MgSO_4$ to 4 mL of the extract
- M20: M14 and 0.9 g $MgSO_4$ to 4 mL of the extract

The decision to test the addition of magnesium sulphate to the PTFE collection tubes was made as a result of the visual aspect of the initial extracts: the acetonitrile extracts appeared cloudy, and unremoved water during the extraction step was suspected as the cause (**Figure 5**).

Table 4. Comparison of recovery results (0.010 mg/kg, LC) for M01, M08 and M11 to M20 in cucumber.

	M01	M08	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20
< 30 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.4 %	0.4 %	0.0 %
[30-40) %	0.4 %	0.0 %	0.0 %	0.4 %	0.4 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.8 %	0.4 %
[40-50) %	0.0 %	0.4 %	0.4 %	0.0 %	0.4 %	0.4 %	0.0 %	0.0 %	0.0 %	0.8 %	0.8 %	0.4 %
[50-60) %	0.8 %	0.0 %	0.0 %	1.6 %	2.5 %	0.8 %	0.0 %	0.8 %	0.4 %	0.8 %	2.0 %	0.4 %
[60-70) %	9.4 %	4.5 %	5.3 %	7.8 %	36.1 %	3.3 %	3.7 %	6.1 %	7.4 %	3.7 %	31.1 %	0.8 %
[70-80) %	64.3 %	44.3 %	54.1 %	45.5 %	51.6 %	41.4 %	33.2 %	34.8 %	19.7 %	53.7 %	50.8 %	27.5 %
[80-90) %	21.7 %	45.9 %	36.9 %	38.5 %	8.2 %	46.7 %	54.1 %	50.8 %	11.1 %	34.0 %	12.7 %	61.1 %
[90-100) %	2.0 %	3.3 %	2.5 %	4.9 %	0.8 %	6.1 %	7.0 %	5.3 %	12.7 %	6.6 %	1.2 %	7.0 %
[100-110) %	0.8 %	0.8 %	0.4 %	0.4 %	0.0 %	0.8 %	0.8 %	1.2 %	13.9 %	0.0 %	0.0 %	2.0 %
[110-120] %	0.0 %	0.4 %	0.0 %	0.4 %	0.0 %	0.0 %	0.0 %	0.0 %	16.8 %	0.0 %	0.0 %	0.0 %
(120-130) %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	13.9 %	0.0 %	0.0 %	0.0 %
[130-140] %	0.0 %	0.0 %	0.0 %	0.4 %	0.0 %	0.0 %	0.0 %	0.0 %	2.9 %	0.0 %	0.0 %	0.0 %
> 140 %	0.4 %	0.4 %	0.4 %	0.0 %	0.0 %	0.4 %	1.2 %	0.8 %	1.2 %	0.0 %	0.0 %	0.4 %

In green, methods based on M01; in blue, methods based on M08.

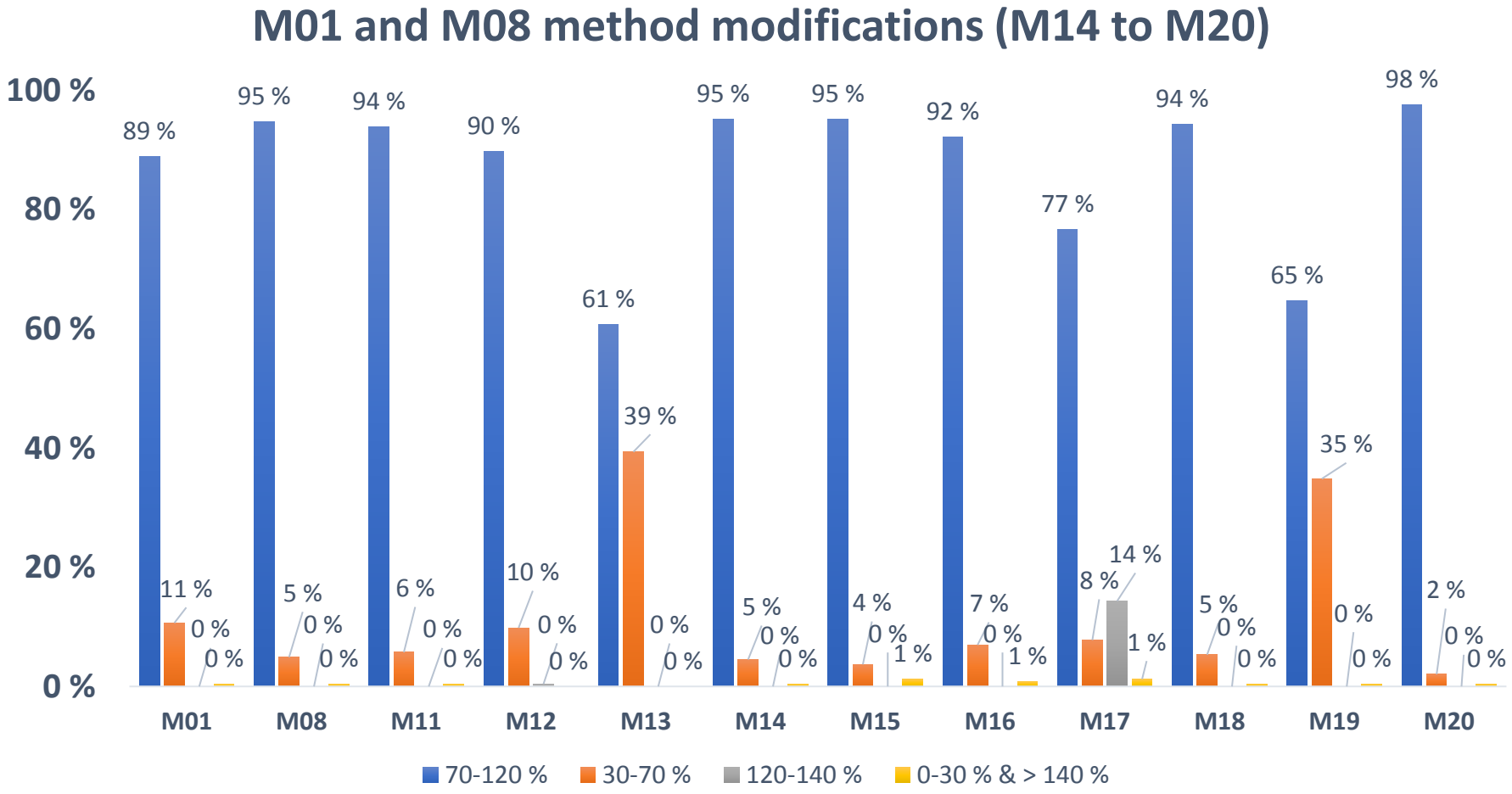

Figure 4. Comparison of recovery results (0.010 mg/kg, LC) for M01, M08 and M11 to M20 in cucumber.

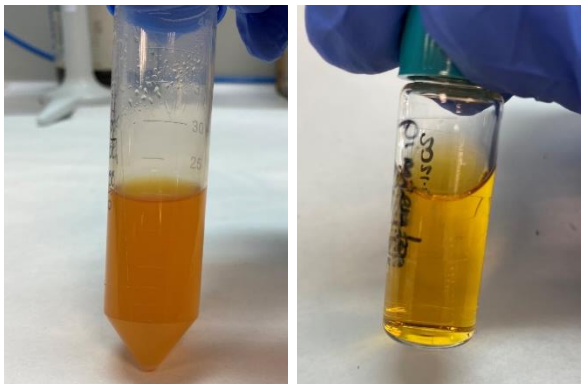

Figure 5. Visual comparison of a sweet pepper extract without a $MgSO_4$ clean-up (*left*) and with a $MgSO_4$ post-extraction clean-up (*right*).

An evaluation of the results on **Table 4** and **Figure 4** shows that M14 (M01 using 5.00 g of Q-Matrix Hydra instead of 2.50 g), based on an extraction with acidified acetonitrile, performs the best amongst the extraction methods without post-extraction magnesium sulphate addition. Most analytes presented recoveries between 70 and 80 % (41.4 %) and between 80 and 90 % (46.7 %). On a similar note, M20, which is M14 with a post-extraction magnesium sulphate addition step, performed similarly but with increased efficiency, as the recovery values between 70 and 80 % were reduced (27.5 %) in favour of recovery values between 80 and 90 % (61.1 %). In short, not all water seems to be removed during the evaluated extraction conditions, and an additional desiccant agent improves method efficiency.

To assess whether increasing the amount of Q-Matrix Hydra and/or increasing the amount of the post-extraction magnesium sulphate yielded even better results, even without the need of the post-extraction step, M21 to M30 were evaluated building off M14 and M20 (**Table 5**, **Figure 6**). Increasing the amount of Q-Matrix Hydra proved counterproductive, however, higher amounts of post-extraction magnesium sulphate significantly improved recovery values, with M28 performing far better than any of the previously evaluated methods. Method M28 is based on M01, with a modification of the Q-Matrix Hydra amount (5.00 g instead of 2.50 g) and a post-extraction step with magnesium sulphate (3.0 g to 5 mL of acetonitrile extract). Recovery values between 70 and 80 % dropped to 3.7 %, as did those between 80 to 90 %, whereas recovery values between 90 and 100 % rose to 54.5 %.

Different sample preparation steps were taken in M14, M21 and M22: Q-Matrix Hydra amounts were 5.00 g, 6.25 g and 7.50 g, respectively. Then, to each different extraction, three different clean-up strategies were applied: 1.0 g (M20, M23 and M24), 1.5 g (M25-M27) and 3.0 g (M28-M30) of $MgSO_4$ to 5 mL of extract:

Table 5. Comparison of recovery results (0.010 mg/kg, LC) for M14 and M20 to M30 in cucumber.

	M14	M20	M21	M22	M23	M24	M25	M26	M27	M28	M29	M30
< 30 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.4 %	0.4 %	0.0 %	0.4 %	0.4 %
[30-40) %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.4 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
[40-50) %	0.0 %	0.4 %	0.8 %	0.4 %	0.8 %	0.4 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
[50-60) %	1.6 %	0.4 %	0.0 %	0.8 %	0.4 %	0.4 %	0.4 %	2.5 %	0.8 %	0.4 %	0.4 %	0.4 %
[60-70) %	1.2 %	1.2 %	2.9 %	1.6 %	9.4 %	1.6 %	0.8 %	6.6 %	1.6 %	1.2 %	0.8 %	0.4 %
[70-80) %	23.4 %	7.4 %	19.7 %	29.5 %	20.5 %	17.2 %	5.7 %	23.8 %	19.7 %	3.7 %	9.0 %	6.1 %
[80-90) %	68.0 %	71.7 %	67.2 %	60.7 %	56.6 %	65.2 %	66.4 %	53.3 %	63.9 %	34.0 %	70.1 %	65.2 %
[90-100) %	4.5 %	16.8 %	8.6 %	6.1 %	9.4 %	12.7 %	23.8 %	11.5 %	11.1 %	54.5 %	16.4 %	25.0 %
[100-110) %	0.8 %	1.6 %	0.8 %	0.4 %	1.6 %	1.2 %	1.2 %	0.4 %	1.6 %	4.9 %	2.5 %	2.0 %
[110-120] %	0.4 %	0.4 %	0.0 %	0.0 %	0.4 %	0.4 %	0.8 %	0.4 %	0.4 %	1.2 %	0.0 %	0.0 %
(120-130) %	0.0 %	0.0 %	0.0 %	0.4 %	0.0 %	0.0 %	0.4 %	0.8 %	0.0 %	0.0 %	0.0 %	0.0 %
[130-140] %	0.0 %	0.0 %	0.0 %	0.0 %	0.8 %	0.4 %	0.4 %	0.4 %	0.4 %	0.0 %	0.4 %	0.4 %
> 140 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %

In black, methods based on M14; in purple, methods based on M21; in orange, methods based on M22.

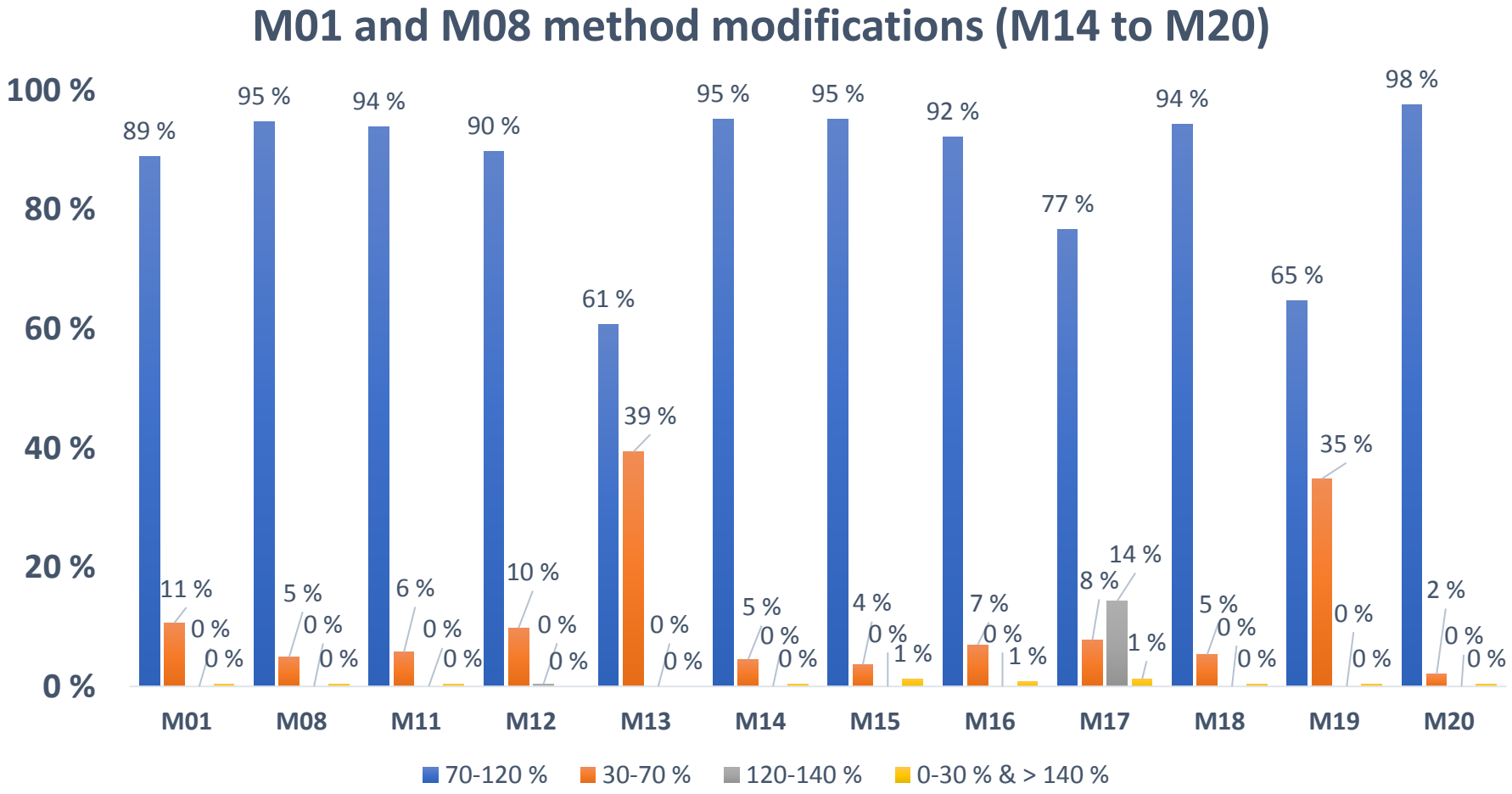

Figure 6. Comparison of recovery results (0.010 mg/kg, LC) for M01, M08 and M11 to M20 in cucumber.

- Sample preparation
- M14: 5.00 g Q-Matrix Hydra + 10 g cucumber
- M21: 6.25 g Q-Matrix Hydra + 10 g cucumber
- M22: 7.50 g Q-Matrix Hydra + 10 g cucumber

- EDGE extraction
- 20 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)
- 90 s bubbling (nitrogen, 40 psi input pressure)
- 90 s hold (40 °C)
- Collection of the extract
- 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse)

- Post-extraction
- M20 (*bis*): M14 and 1.0 g $MgSO_4$ to 5 mL of extract
- M23: M21 and 1.0 g $MgSO_4$ to 5 mL of extract
- M24: M22 and 1.0 g $MgSO_4$ to 5 mL of extract
- M25: M14 and 1.5 g $MgSO_4$ to 5 mL of extract
- M26: M21 and 1.5 g $MgSO_4$ to 5 mL of extract
- M27: M22 and 1.5 g $MgSO_4$ to 5 mL of extract
- M28: M14 and 3.0 g $MgSO_4$ to 5 mL of extract
- M29: M21 and 3.0 g $MgSO_4$ to 5 mL of extract
- M30: M22 and 3.0 g $MgSO_4$ to 5 mL of extract

During the post-extraction step, the magnesium sulphate formed a hard, rock-like aggregate. As 3.0 g of magnesium sulphate were seen unusually high, since during the extraction step 5.00 g of Q-Matrix Hydra (a water removal reagent) were already being added, the quality of the magnesium sulphate reagent being used was evaluated. Two different magnesium sulphate reagents were tested: one with a higher particle size ("coarse"), and another one with a smaller particle size ("fine") (**Figure 7**). The result was that the finer magnesium sulphate, after centrifugation, didn't present the formation of an aggregate and, instead, was collected at the bottom of the 15 mL PTFE tube (**Figure 7**).

To better assess the differences between both magnesium sulphate reagents, the median particle size was measured. In the case of the coarse magnesium sulphate, the median particle size was 225 µm, with a range of sizes from barely 10 µm to over 600 µm. In the case of the fine magnesium sulphate, median particle size was 75 µm, with a range of sizes from 1 µm to 150 µm (**Figure 7**). The median particle size, thus, was 300 % greater in the coarse magnesium sulphate compared to the fine magnesium sulphate.

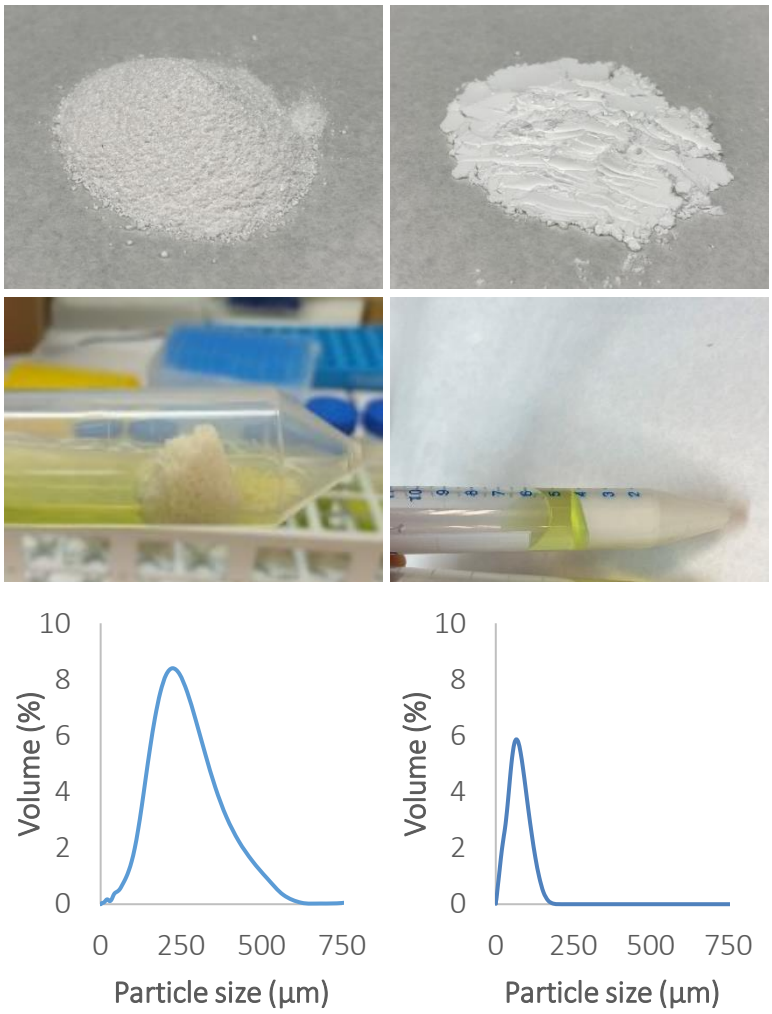

Figure 7. Visual comparison of the coarse magnesium sulphate (*top left*) and the fine magnesium sulphate (*top right*); the post-extraction step using 3.0 g of coarse magnesium sulphate (*centre left*) and 3.0 g of fine magnesium sulphate (*centre right*); and the particle size distribution for coarse magnesium sulphate (*bottom left*) and fine magnesium sulphate (*bottom right*).

In view of these results, a final method optimisation was performed to evaluate the necessary amount of the new, fine magnesium sulphate required in the post extraction step. Method parameters were:

- Sample preparation
- M14: 5.00 g Q-Matrix Hydra + 10 g cucumber

- EDGE extraction
- 20 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)

- 90 s bubbling (nitrogen, 40 psi input pressure)
- 90 s hold (40 °C)
- Collection of the extract
- 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse)

- Post-extraction
- M34: M14 and 0.2 g $MgSO_4$ to 3 mL of extract
- M33: M14 and 0.3 g $MgSO_4$ to 3 mL of extract
- M20: M14 and 0.6 g $MgSO_4$ to 3 mL of extract
- M25: M14 and 0.9 g $MgSO_4$ to 3 mL of extract
- M32: M14 and 1.2 g $MgSO_4$ to 3 mL of extract
- M31: M14 and 1.5 g $MgSO_4$ to 3 mL of extract

To evaluate the minimum amount of magnesium sulphate required, 0.1 mL aliquots of the final acetonitrile extract were evaporated under a nitrogen flow. The elapsed times were:

- M34: 24 min 41 s
- M33: 14 min 17 s
- M20: 5 min 40 s
- M25: 6 min 25 s
- M32: 5 min 5 s
- M31: 6 min 0 s

These results indicate that amounts lower than 0.2 g of magnesium sulphate per mL of acetonitrile extract are insufficient to completely remove any remaining water. In comparison with M28, which required 3.0 g of magnesium sulphate per 5 mL of the final extract (0.6 g/mL), M20, using the finer magnesium sulphate, required 0.2 g/mL only. Hence, based on the obtained data, there appears to be a direct correlation between median particle size and the required amount of magnesium sulphate (300 % greater particle size requires 300 % greater amount of magnesium sulphate); however, as this was not the focus of this work, this line of investigation was not further pursued.

Method results for this optimisation procedure can be found on **Table 6** and **Figure 8**. Slightly better results were found for M32 than M20, so M32 was the chosen method in this step.

Since the magnesium sulphate reagent had been changed, a final experiment was carried out which consisted in the addition of varying amounts of magnesium sulphate directly into the Q-Cup (5.00 g, 6.25 g and 7.50 g were tested) alongside 5.00 g of Q-Matrix Hydra and 10 g of sample blank.

Table 6. Comparison of recovery results (0.010 mg/kg, LC) for M20, M25 and M31 to M34 in cucumber.

	M34	M33	M20	M25	M32	M31
< 30 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
[30-40) %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
[40-50) %	0.0 %	0.0 %	0.4 %	0.0 %	0.4 %	0.0 %
[50-60) %	0.0 %	0.4 %	0.0 %	0.0 %	0.0 %	0.0 %
[60-70) %	0.8 %	0.4 %	0.0 %	0.8 %	0.8 %	0.8 %
[70-80) %	1.6 %	1.2 %	2.5 %	2.0 %	1.6 %	1.6 %
[80-90) %	18.4 %	22.1 %	15.6 %	16.0 %	13.9 %	12.7 %
[90-100) %	65.6 %	63.9 %	70.9 %	66.0 %	66.8 %	68.9 %
[100-110) %	11.5 %	9.8 %	9.0 %	13.1 %	13.9 %	14.3 %
[110-120] %	1.2 %	1.6 %	1.6 %	1.6 %	2.0 %	0.8 %
(120-130) %	0.8 %	0.4 %	0.0 %	0.0 %	0.0 %	0.4 %
[130-140] %	0.0 %	0.0 %	0.0 %	0.0 %	0.4 %	0.4 %
> 140 %	0.0 %	0.0 %	0.0 %	0.4 %	0.0 %	0.0 %

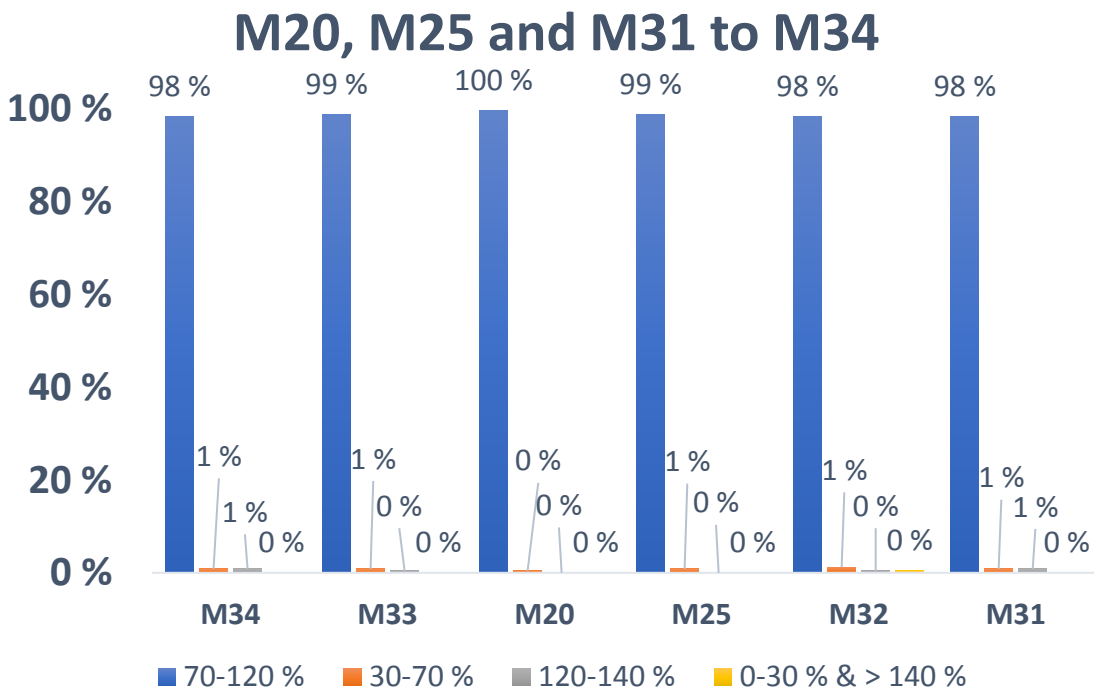

Figure 8. Comparison of recovery results (0.010 mg/kg, LC) for M20, M25 and M31 to M34 in cucumber.

Method parameters of this last experiment were:

- Sample preparation
- M35: 5.00 g Q-Matrix Hydra + 5.00 g MgSO4 + 10 g
- M36: 5.00 g Q-Matrix Hydra + 6.25 g MgSO4 + 10 g
- M37: 5.00 g Q-Matrix Hydra + 7.50 g MgSO4 + 10 g

- EDGE extraction
- 20 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)
- 90 s bubbling (nitrogen, 40 psi input pressure)
- 90 s hold (40 °C)
- Collection of the extract
- 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse)

- Post-extraction
- M35: -
- M36: -
- M37: -

Two more experiments were carried out, M38 (M35, but with the addition of magnesium sulphate before the Q-Matrix Hydra) and M39 (M35, but with the addition of magnesium sulphate on top of the sample). However, evaporation of a 0.1 mL aliquot took over 20 min, which was indicative of high water content. Thus, these experiments were discarded. The results for M35 to M37 are shown on **Table 7** and **Figure 9**.

Thus, the final developed method was M32, with the following parameters:

- Sample preparation
- M14: 5.00 g Q-Matrix Hydra + 10 g cucumber

- EDGE extraction
- 20 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)
- 90 s bubbling (nitrogen, 40 psi input pressure)
- 90 s hold (40 °C)
- Collection of the extract
- 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse)

- Post-extraction
- M32: M14 and 1.2 g MgSO4 to 3 mL of extract

Table 7. Comparison of recovery results (0.010 mg/kg, LC) for M35 to M37 in cucumber.

	M35	M36	M37
< 30 %	0.8 %	0.8 %	5.3 %
[30-40) %	2.5 %	4.9 %	52.9 %
[40-50) %	22.5 %	32.8 %	38.9 %
[50-60) %	69.7 %	56.1 %	2.5 %
[60-70) %	4.1 %	3.3 %	0.0 %
[70-80) %	0.4 %	0.0 %	0.0 %
[80-90) %	0.0 %	0.4 %	0.0 %
[90-100) %	0.0 %	0.8 %	0.4 %
[100-110) %	0.0 %	0.0 %	0.0 %
[110-120] %	0.0 %	0.4 %	0.0 %
(120-130) %	0.0 %	0.0 %	0.0 %
[130-140] %	0.0 %	0.0 %	0.0 %
> 140 %	0.0 %	0.4 %	0.0 %

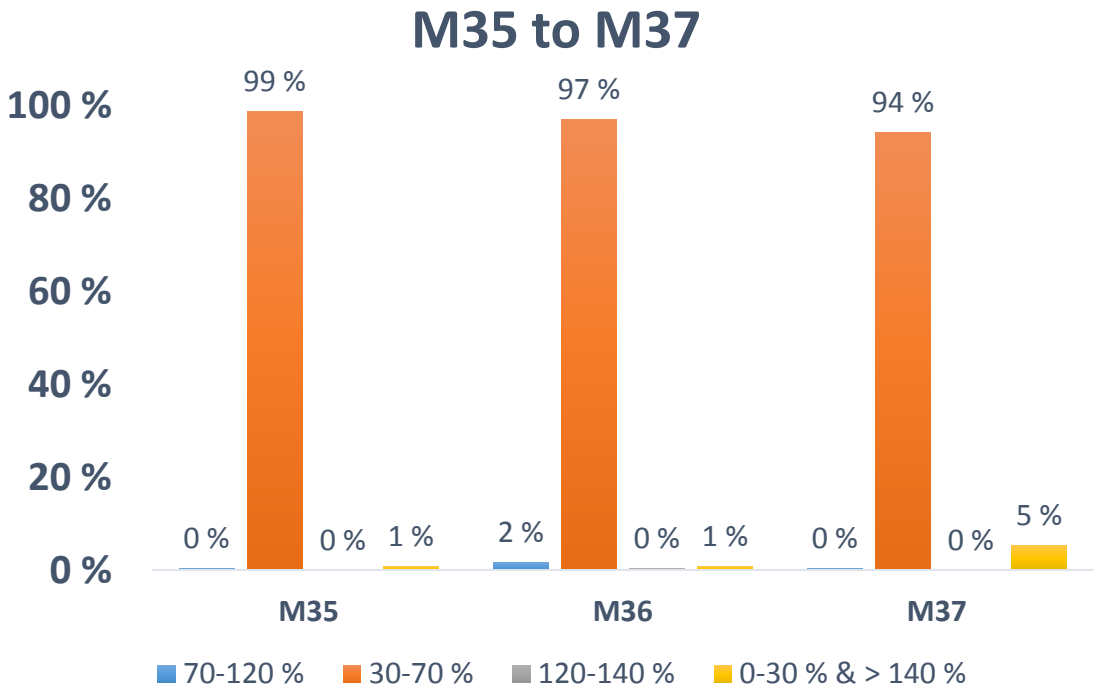

Figure 9. Comparison of recovery results (0.010 mg/kg, LC) for M35 to M37 in cucumber.

6.2. Method validation

6.2.1. Validation in cucumber for LC-amenable compounds

Once M32 was chosen as the final method, it was validated on the LC-MS/MS instrument at two spiking levels (0.010 mg/kg and 0.050 mg/kg, *n* = 5 per concentration level) in cucumber. Recovery values for most analytes were in the 80-110 % range (**Table 8**), with remarkably low relative standard deviations (RSD). At the 0.010 mg/kg level, the average RSD was 4.8 % for all 244 LC-amenable compounds, and at the 0.050 mg/kg level, the average RSD was even lower, 2.7 % (**Table 9**). These low values are indicative of the repeatability that can be achieved when using automated instrumentation.

Table 8. Recovery results for M32 validation at 0.010 and 0.050 mg/kg in cucumber.

	0.010 mg/kg (*n* = 5)	**0.050 mg/kg (*n* = 5)**	**Average**
< 30 %	0.0 %	0.0 %	0.0 %
[30-40) %	0.0 %	0.4 %	0.0 %
[40-50) %	0.8 %	0.4 %	0.8 %
[50-60) %	0.0 %	0.0 %	0.0 %
[60-70) %	0.0 %	0.0 %	0.0 %
[70-80) %	0.8 %	4.9 %	1.6 %
[80-90) %	14.3 %	27.9 %	19.7 %
[90-100) %	71.3 %	61.9 %	75.4 %
[100-110) %	12.7 %	4.1 %	2.5 %
[110-120] %	0.0 %	0.4 %	0.0 %
(120-130) %	0.0 %	0.0 %	0.0 %
[130-140] %	0.0 %	0.0 %	0.0 %
> 140 %	0.0 %	0.0 %	0.0 %

Table 9. RSD for M32 validation at 0.010 and 0.050 mg/kg in cucumber.

Relative standard deviation	**0.010 mg/kg (*n* = 5)**	**0.050 mg/kg (*n* = 5)**	**Average**
Mean	4.8 %	2.7 %	4.9 %
Median	4.3 %	2.4 %	4.4 %

6.2.2. *Extension to other high-water content matrices*

The validated method was also tested on five additional matrices at a 0.010 mg/kg spiking level: tomato, red pepper, pear, cabbage and carrot, with similarly positive results (**Table 10** and **Figure 10**). Red pepper exhibited the lowest average recovery values amongst the five matrices evaluated, but results were satisfactory, nonetheless.

Table 10. M32 performance on tomato, pepper, cabbage, pear and carrot.

	Tomato	Pepper	Cabbage	Pear	Carrot
< 30 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
[30-40) %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
[40-50) %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
[50-60) %	0.0 %	0.4 %	0.4 %	0.4 %	0.0 %
[60-70) %	0.8 %	0.8 %	0.4 %	0.0 %	0.4 %
[70-80) %	1.2 %	16.8 %	3.7 %	3.3 %	0.0 %
[80-90) %	20.9 %	71.7 %	31.1 %	68.4 %	2.0 %
[90-100) %	66.8 %	7.4 %	58.2 %	25.8 %	8.2 %
[100-110) %	7.4 %	2.5 %	4.5 %	2.0 %	73.4 %
[110-120] %	2.9 %	0.4 %	1.6 %	0.0 %	15.2 %
(120-130) %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
[130-140] %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
> 140 %	0.0 %	0.0 %	0.0 %	0.0 %	0.4 %

6.2.3. *Initial evaluation of GC-amenability of the developed method*

Complete method validation has not been performed by GC-MS/MS instrumentation; however, initial testing has provided promising -yet not definitive- results. Cucumber and carrot sample blanks were spiked at 0.010 mg/kg, and 190 GC-amenable were analysed (**Table 11** and **Figure 11**). These initial results show higher average recovery values for carrot than cucumber, with most analytes within the 90-110 % value range.

Nevertheless, complete method validation is necessary to accurately assess the viability of the developed method for GC-amenable compounds using a GC-MS/MS instrument, and the data presented herein should only be interpreted as an initial estimation of potential method performance only.

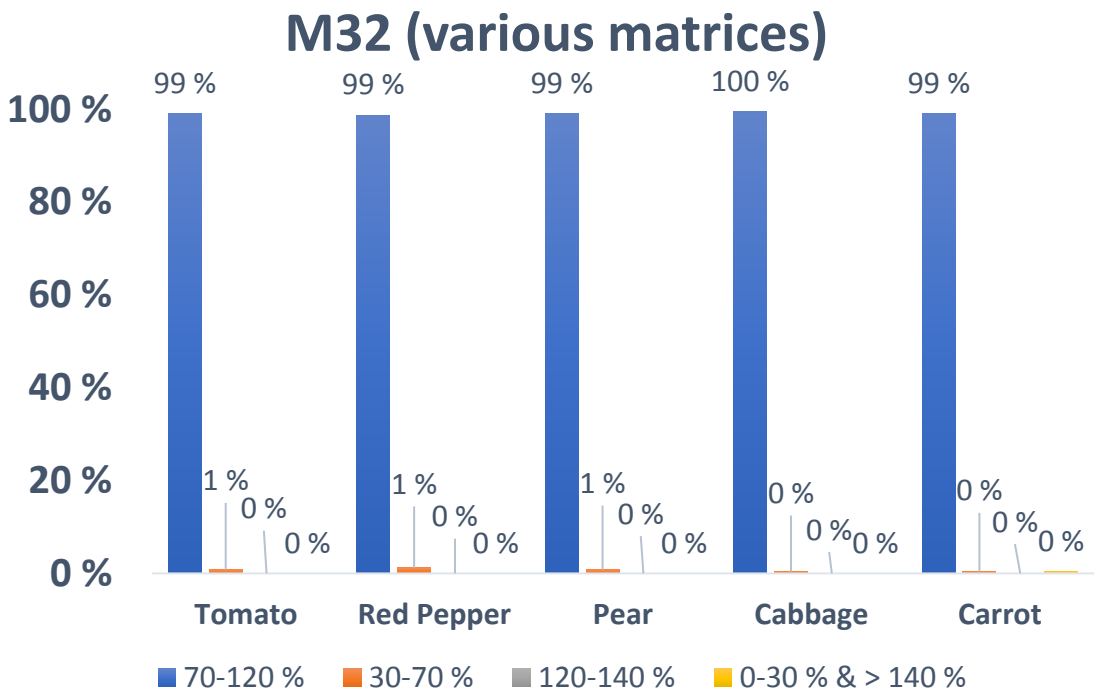

Figure 10. M32 performance on tomato, pepper, cabbage, pear and carrot.

Table 11. M32 testing for GC-amenable analytes.

	Carrot	Cucumber
< 30 %	0 %	0 %
[30-40) %	0 %	0 %
[40-50) %	0 %	0 %
[50-60) %	0 %	0 %
[60-70) %	0 %	0 %
[70-80) %	0 %	1 %
[80-90) %	1 %	10 %
[90-100) %	13 %	65 %
[100-110) %	71 %	21 %
[110-120) %	16 %	3 %
[120-130) %	0 %	1 %
[130-140) %	0 %	0 %
> 140 %	1 %	0 %

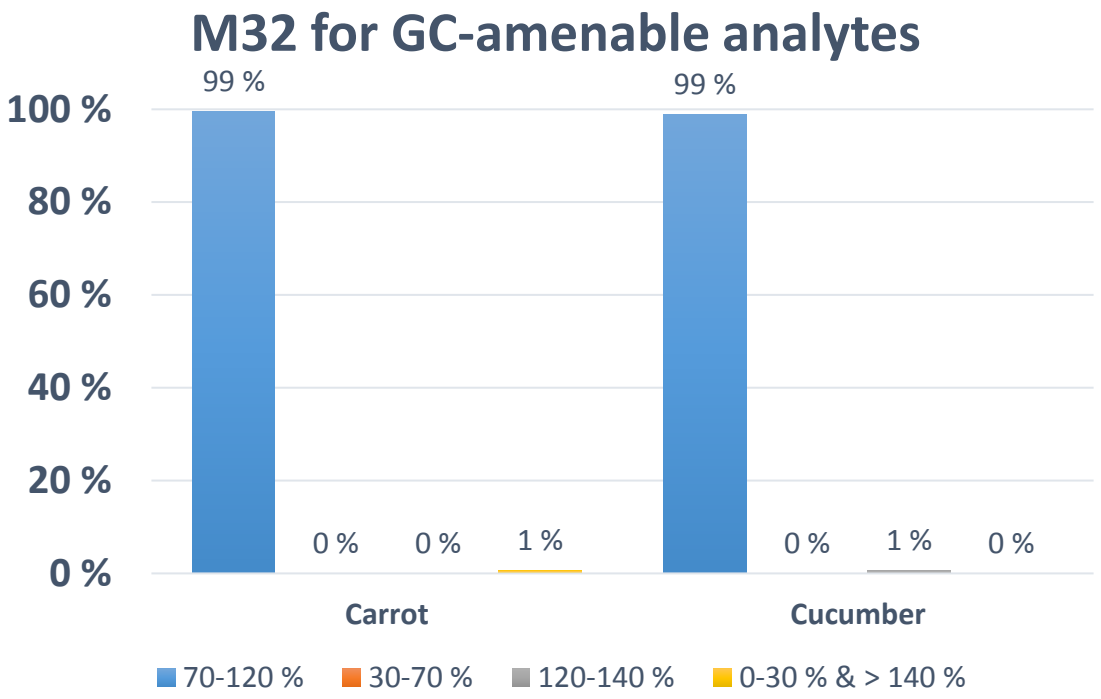

Figure 11. M32 testing for GC-amenable analytes.

6.3. Application to real samples and comparison with a manual extraction method

To evaluate the automated method performance compared to a QuEChERS extraction without a clean-up step (QC), both were applied to three real samples known to contain pesticide residues: apple, aubergine, cucumber, potato, tomato and zucchini samples. The evaluated pesticides were acetamiprid, chlorantraniliprole, fludioxonil, fluopyram, indoxacarb and linuron, covering a wide range of chemical families and physicochemical properties (**Table 12**). Out of the evaluated pesticides, acetamiprid presented virtually no difference in terms of concentration between QC and M32 methods. However, the concentrations for other pesticide residues were lower when samples were extracted using M32 compared to QC. Although sample spiking was performed as carefully as possible trying to mimic real pesticide presence in a sample, differences in the extraction procedure were nonetheless found.

The main differences between the methods are (*i*) pH control via acetic acid or buffer salts and sodium chloride and (*ii*) the extraction step, using nitrogen bubbling or axial, automated shaker. As no differences were found in acetamiprid, samples containing only this pesticide residue were not further evaluated.

Table 12. Comparison of M32 and QC results for several real samples (concentration values in mg/kg).

LC-MS/MS analytes	Sample 1 (zucchini)		Sample 2 (zucchini)		Sample 3 (cucumber)		Sample 4 (tomato)		Sample 5 (aubergine)		Sample 6 (apple)		Sample 7 (aubergine)		Sample 8 (potato)	
	QC	M32	QC	M32	QC	M32	QC	M32	QC	M32	QC	M32	QC	M32	QC	M32
Acetamiprid	9.0	9.2	18.0	15.7	-	-	22.0	19.9	-	-	18.6	16.1	21.1	18.5	-	-
Boscalid	-	-	-	-	56.0	30.0	-	-	-	-	-	-	-	-	5.2	3.6
Chlorantraniliprole	-	-	-	-	-	-	-	-	-	-	5.3	3.1	-	-	-	-
Flonicamid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fludioxonil	-	-	-	-	-	-	-	-	-	-	17.0	6.0	-	-	-	-
Fluopyram	-	-	-	-	32.0	21.4	11.0	5.5	-	-	-	-	-	-	-	-
Indoxacarb	-	-	-	-	-	-	18.0	5.0	7.9	4.7	-	-	-	-	-	-
Linuron	-	-	-	-	-	-	9.0	4.4	-	-	-	-	-	-	-	-

The final evaluated pesticides were acetamiprid (tomato and apple), boscalid (cucumber), chlorantraniliprole (apple), fludioxonil (apple), fluopyram (cucumber and tomato), indoxacarb (tomato) and linuron (tomato). Since the extraction step was suspected as the possible cause of differences in the obtained concentrations, different extraction conditions were tested during some minor method adjustments, the results of which are shown on **Table 13**. In short, the modifications consisted in adding the extraction solvent during two steps (2x10 mL) instead of in a single step (1x20 mL), to increase bubbling time (from 90 s to 150 s) or to increase the hold temperature (from 40 °C to 50 °C):

- Sample preparation
- M40, M41 & M42: 5.00 g Q-Matrix Hydra + 10 g sample

- EDGE extraction
- M40 & M41: 20 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)
- M42: 2 x 10 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)
- M40 & M42: 90 s bubbling (nitrogen, 40 psi input pressure)
- M41: 150 s bubbling
- M40: 90 s hold (50 °C)
- M41 & M42: 90 s hold (40 °C)
- Collection of the extract
- 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse)

- Post-extraction
- M40: 0.6 g $MgSO_4$ to 3 mL of extract
- M41: 0.6 g $MgSO_4$ to 3 mL of extract
- M42: 0.6 g $MgSO_4$ to 3 mL of extract
- M43: M40 and 1.2 g $MgSO_4$ to 3 mL of extract
- M44: M41 and 1.2 g $MgSO_4$ to 3 mL of extract
- M45: M42 and 1.2 g $MgSO_4$ to 3 mL of extract

As can be derived from the results, there was not any method from M40 to M45 which presented any significant advantage over M32. Two of them, however, did show decreased efficiency compared to the rest and to M32: M42 and M45. These methods were identical to one another except for the post-extraction step and differed from the rest in that solvent addition was performed in two steps (2x10 mL) instead of in a single step. Thus, it can be affirmed that solvent addition is preferably added in a single step. Increased bubbling time, from 90 to 150 s (M41 and M44) did not improve the results, either.

As the method parameters appeared to be sufficiently well optimised, the focus was changed to the sample mass to extraction solvent volume ratio. Initially, 5.00 g of Q-Matrix Hydra and 10 g of sample were extracted with 25 mL of solvent (0.4 g/mL, or 40 % dilution). In methods M46 to M47, different ratios were tested: 7.5 g of sample to 25 mL of solvent (0.3 g/mL, or 30 % dilution) and 5.0 g to 25 mL of solvent

(0.2 g/mL, or 20 % dilution). A modification named M50 was also tested (1.25 g of magnesium sulphate and 5.0 g of sample), however, recoveries were very low (40-60 %), and its results are not shown in this document. Method parameters are shown below, and the results are presented on **Table 14**:

- Sample preparation
- M46: 3.75 g Q-Matrix Hydra + 7.5 g sample
- M47: 2.50 g Q-Matrix Hydra + 5.0 g sample
- M48: 3.75 g $MgSO_4$ + 7.5 g sample
- M49: 2.50 g $MgSO_4$ + 5.0 g sample

- EDGE extraction (M32)
- 20 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)
- 90 s bubbling (nitrogen, 40 psi input pressure)
- 90 s hold (40 °C)
- Collection of the extract
- 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse)

- Post-extraction
- 0.6 g $MgSO_4$ to 3 mL of extract

The best results were obtained for M47, which was an identical extraction method to the validated M32, modifying the sample preparation step only (half of Q-Matrix Hydra and sample amounts). Results were comparable to those obtained by the QC method. Although very sensitive instrumentation was being used both in the case of LC-MS/MS and GC-MS/MS instruments, sample dilution was very significant at this point. A sample containing 0.010 mg/kg, when extracted with M32, would result in a concentration of 0.004 mg/L in the final extract; when extracted with M47, the final concentration would be 0.002 mg/L.

One final sample preparation modification was attempted, in M51 to M56. Lower amounts of Q-Matrix Hydra and combinations with magnesium sulphate in the Q-Cup were tested one last time (**Table 15**). To account for lower water sorbent reagents in the Q-Cup, two different amounts of magnesium sulphate were evaluated during the post-extraction procedure. However, no improvements were observed. Method parameters for methods M51 to M56 were:

- Sample preparation
- M51: 1.25 g Q-Matrix Hydra + 5.0 g sample
- M53: 1.25 g Q-Matrix Hydra + 1.25 g $MgSO_4$ + 5.0 g sample
- M55: 1.00 g Q-Matrix Hydra + 0.25 g $MgSO_4$ + 5.0 g sample

Table 13. Comparison of M40 to M45 and QC results for several real samples (concentration values in mg/kg).

LC-MS/MS analytes	Cucumber sample							Tomato sample							Apple sample						
	QC	M40	M43	M41	M44	M42	M45	QC	M40	M43	M41	M44	M42	M45	QC	M40	M43	M41	M44	M42	M45
Acetamiprid	**-**	-	-	-	-	-	-	**18.9**	19.5	20.8	20.9	20.9	11.5	11.9	**17.7**	15.0	15.5	16.7	16.6	9.5	10.0
Boscalid	**44.9**	27.9	28.7	25.9	26.1	15.5	16.3	**-**	-	-	-	-	-	-	**-**	-	-	-	-	-	-
Chlorantraniliprole	**-**	-	-	-	-	-	-	**-**	-	-	-	-	-	-	**4.8**	3.1	2.9	3.4	3.0	2.1	2.0
Fludioxonil	**-**	-	-	-	-	-	-	**-**	-	-	-	-	-	-	**13.1**	6.2	6.0	5.0	5.3	2.8	3.4
Fluopyram	**24.9**	17.3	18.2	17.3	17.8	12.1	13.3	**8.3**	5.8	4.6	5.5	5.3	3.2	3.4	**-**	-	-	-	-	-	-
Indoxacarb	**-**	-	-	-	-	-	-	**17.8**	7.9	7.4	6.3	6.7	3.0	3.2	**-**	-	-	-	-	-	-
Linuron	**-**	-	-	-	-	-	-	**6.7**	4.8	4.5	4.4	4.5	2.5	2.7	**-**	-	-	-	-	-	-

Table 14. Comparison of M46 to M49 and QC results for several real samples (concentration values in mg/kg).

LC-MS/MS analytes	Cucumber sample					Tomato sample					Apple sample				
	QC	M46	M47	M48	M49	QC	M46	M47	M48	M49	QC	M46	M47	M48	M49
Acetamiprid	**-**	-	-	-	-	**17.8**	13.9	15.7	11.8	10.8	**17.3**	11.9	13.8	7.0	8.4
Boscalid	35.8	29.3	31.5	14.1	14.1	**-**	-	-	-	-	**-**	-	-	-	-
Chlorantraniliprole	**-**	-	-	-	-	**-**	-	-	-	-	**4.5**	2.7	4.5	2.5	3.0
Fludioxonil	**-**	-	-	-	-	**-**	-	-	-	-	**12.2**	5.0	9.5	3.5	3.9
Fluopyram	**23.4**	17.5	18.0	8.8	8.9	**7.4**	4.3	6.5	2.9	3.6	**-**	-	-	-	-
Indoxacarb	**-**	-	-	-	-	**9.3**	6.7	9.9	4.2	5.2	**-**	-	-	-	-
Linuron	**-**	-	-	-	-	**6.2**	3.8	5.8	2.8	3.3	**-**	-	-	-	-

Table 15. Comparison of M51 to M56 and QC results for several real samples (concentration values in mg/kg).

LC-MS/MS analytes	Cucumber sample							Tomato sample							Apple Sample						
	QC	M51	M52	M53	M54	M55	M56	QC	M51	M52	M53	M54	M55	M56	QC	M51	M52	M53	M54	M55	M56
Acetamiprid	**-**	-	-	-	-	-	-	**19.3**	14.0	14.5	10.9	11.3	15.9	16.2	**18.6**	13.9	14.2	11.2	10.7	13.6	14.2
Boscalid	**47.4**	32.4	33.4	27.3	27.6	29.1	29.0	**-**	-	-	-	-	-	-	**-**	-	-	-	-	-	-
Chlorantraniliprole	**-**	-	-	-	-	-	-	**-**	-	-	-	-	-	-	**4.8**	2.9	2.3	2.1	2.2	3.1	3.2
Fludioxonil	**-**	-	-	-	-	-	-	**-**	-	-	-	-	-	-	**12.6**	8.0	6.4	3.5	4.6	6.7	7.0
Fluopyram	**24.5**	17.6	18.2	16.3	16.1	16.0	16.5	**7.8**	4.3	4.8	3.0	3.2	4.8	4.7	**-**	-	-	-	-	-	-
Indoxacarb	**-**	-	-	-	-	-	-	**15.3**	7.6	7.6	5.0	4.4	8.1	9.2	**-**	-	-	-	-	-	-
Linuron	**-**	-	-	-	-	-	-	**6.3**	3.7	3.8	2.8	3.0	4.3	4.6	**-**	-	-	-	-	-	-

- EDGE extraction (M32)
- 20 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)
- 90 s bubbling (nitrogen, 40 psi input pressure)
- 90 s hold (40 °C)
- Collection of the extract
- 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse)

- Post-extraction
- M51: 0.6 g $MgSO_4$ to 3 mL of extract
- M53: 0.6 g $MgSO_4$ to 3 mL of extract
- M55: 0.6 g $MgSO_4$ to 3 mL of extract
- M52: M51 but 1.2 g $MgSO_4$ to 3 mL of extract
- M54: M53 but 1.2 g $MgSO_4$ to 3 mL of extract
- M56: M55 but 1.2 g $MgSO_4$ to 3 mL of extract

7. Conclusions

A new automated extraction method based on pressurised liquid extraction and LC-MS/MS analysis has been developed for the analysis of high-water content samples. Counting both major and minor modifications, 53 different method modifications were evaluated, from M01 to M56.

Initially, M32, a method which extracted 10 g of sample mixed with 25 mL of acetonitrile (with 5.0 g of Q-Matrix Hydra, a water sorbent) was optimised and validated. When compared against a manual QuEChERS extraction, lower recoveries were observed for selected, incurred pesticides. One last method tuning step, in which sample amount and Q-Matrix Hydra were reduced to 5 g and 2.50 g, respectively, provided slightly lower, but comparable results to the QuEChERS extraction.

The validated automated method is promising, particularly, due to its great repeatability (average RSD < 5.0 % for 244 LC-amenable compounds, combining both experiments at 0.010 mg/kg and 0.050 mg/kg). Preliminary results indicate that the method performs correctly for 190 GC-amenable pesticides, however, the method should be completely validated prior to its implementation for GC analyses. The discrepancies found between QuEChERS and M47 in terms of concentration indicate that further evaluation would be needed prior to its implementation. Nevertheless, due to the high dilution factor the extraction procedure causes (0.010 mg/kg in a sample become 0.002 mg/L in an M47 extract), very sensitive instrumentation is needed for accurate measurements and quantitation.

The final automated method parameters (M47) are summarised below:

- Sample preparation
- 2.50 g Q-Matrix Hydra + 5.0 g sample

- EDGE extraction (M32)
- 20 mL Acetonitrile w/ acetic acid (1 %, V/V) (top)
- 90 s bubbling (nitrogen, 40 psi input pressure)
- 90 s hold (40 °C)
- Collection of the extract
- 5 mL acetonitrile w/ acetic acid (1 %, V/V) (rinse)

- Post-extraction
- 0.6 g $MgSO_4$ to 3 mL of extract

8. References

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APPENDIX I: MASS TRANSITIONS AND VALIDATION RESULTS

Table A1. Detection and chromatographic parameters for the compounds analysed by LC-MS/MS. Retention times are relative to the data window, i.e. 1.1 min must be added to the retention times below to obtain the absolute retention times.

Name	t_R (min)	Precursor ion 1 (m/z)	Product ion 1 (m/z)	CE 1 (eV)	Precursor ion 2 (m/z)	Product ion 2 (m/z)	CE 2 (eV)	Polarity
Acephate	1.42	184.019	48.845	20.58	184.019	142.863	10.23	Positive
Acetamiprid	2.74	223.074	55.786	16.52	223.074	125.702	21.18	Positive
Alachlor	6.30	270.125	162.000	20.35	270.125	238.000	10.23	Positive
Albendazole	4.79	266.095	190.845	32.86	266.095	233.929	19.67	Positive
Aldicarb	3.21	116.053	61.082	13.72	116.053	88.970	9.51	Positive
Aldicarb-sulfone	1.95	240.101	86.000	10.23	240.101	147.970	14.09	Positive
Aldicarb-sulfoxide	1.82	207.079	88.988	13.18	207.079	131.970	10.23	Positive
Ametoctradin	7.43	276.218	176.000	37.71	276.218	176.970	28.99	Positive
Anilofos	6.91	368.030	124.845	31.27	368.030	198.762	14.21	Positive
Atrazine	4.38	216.101	103.917	28.35	216.101	173.970	17.35	Positive
Azinphos-ethyl	6.07	346.044	131.917	16.07	346.044	260.887	10.23	Positive
Azinphos-methyl	5.02	318.013	131.804	14.30	318.013	167.016	11.30	Positive
Azoxystrobin	5.19	404.124	343.929	25.35	404.124	371.929	14.74	Positive
BAC-C10	5.79	276.268	91.054	27.96	276.268	184.292	19.45	Positive
BAC-C8	4.29	248.237	91.054	26.82	248.237	156.208	18.40	Positive
Benalaxyl	6.88	326.175	148.000	21.75	326.175	293.929	10.23	Positive
Bendiocarb	3.70	224.091	108.774	17.47	224.091	166.857	10.23	Positive
Bifenazate	5.98	301.154	170.000	19.36	301.154	197.857	10.23	Positive
Bitertanol	7.27	338.186	69.929	10.23	338.186	269.012	10.23	Positive
Boscalid	5.52	343.039	270.940	31.76	343.039	306.845	19.86	Positive
Bromacil	3.69	261.023	187.762	28.54	261.023	204.762	14.47	Positive
Bromuconazole	6.30	375.961	69.917	20.80	375.961	158.899	28.16	Positive
Bupirimate	5.98	317.164	165.929	24.67	317.164	237.012	19.89	Positive
Buprofezin	8.01	306.163	115.845	16.56	306.163	200.857	12.54	Positive
Butoxycarboxim	1.90	223.074	62.845	10.99	223.074	105.845	11.48	Positive
Carbaryl	3.96	202.086	126.929	28.96	202.086	144.929	10.23	Positive
Carbendazim	2.18	192.076	131.929	30.70	192.076	159.929	18.15	Positive
Carbofuran	3.69	222.112	122.845	22.02	222.112	164.929	12.43	Positive
Chlorantraniliprole	4.93	481.978	283.875	12.69	481.978	450.815	16.82	Positive
Chlorbromuron	5.52	292.968	181.845	16.79	292.968	203.690	19.71	Positive
Chlorfenvinphos	7.01	358.976	98.774	28.92	358.976	154.899	12.88	Positive
Chlorfluazuron	9.11	539.970	157.970	19.06	539.970	382.833	20.08	Positive
Chloridazon	2.79	222.042	76.929	33.73	222.042	103.845	23.16	Positive
Chlorotoluron	4.32	213.078	71.857	18.95	213.078	139.845	25.09	Positive
Chloroxuron	6.01	291.089	71.917	20.77	291.089	163.929	17.24	Positive
Chlorpyriphos	8.51	349.933	197.762	19.40	349.933	321.679	12.05	Positive

Chlorpyriphos-methyl	7.59	321.902	124.845	20.43	321.902	289.679	15.91	Positive
Chlorpyrifos-methyl	7.59	321.902	289.679	15.91	395.232	174.982	16.96	Positive
Chromafenozide	6.04	395.232	174.982	16.96	395.232	339.149	7.40	Positive
Clofentezine	7.27	303.019	101.845	34.41	303.019	137.845	14.97	Positive
Clomazone	4.97	240.078	88.899	46.28	240.078	124.845	21.11	Positive
Coumaphos	6.91	363.021	226.774	26.07	363.021	306.762	17.85	Positive
Cyazofamid	6.33	325.052	107.774	14.28	325.052	260.929	10.23	Positive
Cyflufenamid	7.24	413.128	240.917	23.19	413.128	294.899	15.50	Positive
Cymoxanil	2.92	199.082	110.917	18.49	199.082	127.970	10.23	Positive
Cyproconazole	5.95	292.121	88.970	52.57	292.121	124.899	29.87	Positive
Cyprodinil	6.24	226.133	92.857	34.98	226.133	107.857	26.68	Positive
Cyromazine	0.60	167.103	67.988	34.57	167.103	125.000	19.14	Positive
Dazomet	2.00	163.035	89.899	10.23	163.035	119.774	12.81	Positive
Demeton-S-methyl	3.77	231.027	60.917	30.36	231.027	88.899	10.23	Positive
Demeton-S-methylsulfone	2.19	263.017	124.845	23.50	263.017	168.845	16.37	Positive
Demeton-S-methylsulfoxide	2.10	247.000	109.042	27.07	247.000	169.054	14.27	Positive
Diazinon	6.93	305.110	153.208	21.26	305.110	169.137	21.09	Positive
Dichlorvos	3.65	220.938	108.774	17.85	220.938	144.845	14.02	Positive
Dicrotophos	2.41	238.080	112.125	12.71	238.080	126.970	18.35	Positive
Diethofencarb	5.18	268.154	179.929	18.26	268.154	225.929	10.23	Positive
Difenoconazole	7.51	406.071	250.845	25.70	406.071	336.845	17.62	Positive
Difenoxuron	4.66	287.000	71.929	20.35	287.000	122.899	19.89	Positive
Diflubenzuron	6.60	311.039	140.917	32.10	311.039	157.970	14.06	Positive
Dimethoate	2.77	230.006	124.845	21.75	230.006	198.762	10.23	Positive
Dimethomorph	5.50	388.131	164.929	31.61	388.131	300.845	20.73	Positive
Dimethylvinphos	5.93	331.000	127.042	13.51	331.000	170.042	36.34	Positive
Diniconazole	7.46	326.082	69.929	25.47	326.082	158.887	30.47	Positive
Diuron	4.70	233.024	71.917	18.87	233.024	159.815	26.95	Positive
DMF	3.41	150.091	106.125	33.14	150.091	107.125	21.43	Positive
DMPF	2.20	163.122	107.125	25.18	163.122	122.125	17.13	Positive
Edifenphos	6.76	311.032	108.845	32.18	311.032	282.845	13.79	Positive
Emamectin B1a	8.26	886.538	125.929	38.24	886.538	158.000	35.32	Positive
Emamectin B1b	8.09	872.495	82.279	46.00	872.495	158.178	37.00	Positive
Epoxiconazole	6.25	330.080	100.917	43.32	330.080	120.899	21.30	Positive
Ethiofencarb	4.14	226.089	106.845	15.72	226.089	164.000	10.23	Positive
Ethion	8.34	384.994	142.762	25.13	384.994	198.774	10.23	Positive
Ethiprole	5.51	394.975	261.845	28.80	394.975	330.917	10.23	Negative
Ethirimol	3.15	210.160	97.970	27.44	210.160	140.000	22.55	Positive
Ethoprofos	6.15	243.063	130.905	20.35	243.063	172.958	14.63	Positive
Etofenprox	9.87	394.240	135.156	26.00	394.240	359.174	12.00	Positive
Etoxazol	8.69	360.176	140.845	30.93	360.176	303.929	18.23	Positive
Famoxadone	7.11	392.160	237.911	17.28	392.160	330.911	10.23	Positive
Fenamidone	5.42	312.116	91.899	24.71	312.116	235.929	15.08	Positive
Fenamiphos	6.51	304.113	216.845	23.27	304.113	233.917	17.13	Positive

Fenamiphos-sulfone	3.87	336.102	265.917	20.12	336.102	307.917	15.72	Positive
Fenamiphos-sulfoxide	3.71	320.107	232.845	25.05	320.107	291.899	16.26	Positive
Fenarimol	6.14	331.039	188.845	47.19	331.039	267.917	22.93	Positive
Fenazaquin	9.27	307.180	146.917	19.74	307.180	161.054	16.98	Positive
Fenbuconazole	6.46	337.121	88.899	55.00	337.121	124.845	30.24	Positive
Fenhexamide	6.07	302.070	55.054	34.23	302.070	97.208	23.28	Positive
Fenobucarb	5.21	208.133	94.917	15.08	208.133	151.970	10.23	Positive
Fenoxycarb	6.60	302.138	87.970	18.98	302.138	115.857	10.68	Positive
Fenpropathrin	8.90	350.175	97.071	29.14	350.175	125.054	10.23	Positive
Fenpropidin	4.76	274.252	116.857	52.12	274.252	146.929	29.07	Positive
Fenpropimorph	4.95	304.263	130.054	25.43	304.263	147.054	28.88	Positive
Fenpyrazamine	5.85	332.142	230.083	19.25	332.142	231.054	18.11	Positive
Fenpyroximate	8.87	422.207	137.929	31.53	422.207	366.012	16.03	Positive
Fenthion	6.89	279.027	168.899	18.30	279.027	246.845	13.37	Positive
Fenthion-sulfone	4.07	311.017	124.917	20.05	311.017	278.917	17.92	Positive
Fenthion-sulfoxide	3.86	295.022	263.845	16.75	295.022	279.845	18.98	Positive
Fenuron	2.67	165.102	71.917	15.84	165.102	76.899	30.43	Positive
Fipronil	6.67	434.945	329.845	15.46	434.945	398.815	10.23	Negative
Flazasulfuron	5.02	408.058	139.000	39.12	408.058	181.970	18.42	Positive
Flonicamid	2.17	230.053	173.917	18.34	230.053	202.845	17.35	Positive
Fluacypirim	7.49	427.062	144.982	24.92	427.062	205.196	9.34	Positive
Fluazifop	5.44	328.079	253.929	26.83	328.079	281.929	19.10	Positive
Flubendiamide	6.79	683.030	273.833	31.38	683.030	407.887	10.23	Positive
Fludioxonil	5.57	247.032	168.857	32.78	247.032	179.857	28.69	Negative
Flufenacet	6.19	364.073	151.970	18.87	364.073	193.929	10.23	Positive
Flufenoxuron	8.84	489.043	140.917	42.83	489.043	157.970	18.95	Positive
Fluometuron	4.24	233.089	71.917	19.10	233.089	159.899	27.44	Positive
Fluopicolide	5.63	382.970	109.042	55.00	382.970	173.042	23.87	Positive
Fluopyram	6.03	397.053	172.845	28.69	397.053	207.845	21.90	Positive
Fluquinconazole	6.03	376.016	307.042	26.23	376.016	349.042	19.70	Positive
Flusilazole	6.56	316.107	164.929	27.25	316.107	246.929	18.34	Positive
Flutriafol	4.49	302.109	94.917	47.91	302.109	122.845	28.01	Positive
Fluxapyroxad	5.74	382.097	341.929	21.18	382.097	362.000	14.40	Positive
Formetanate-hydrochloride	1.62	222.123	92.845	35.44	222.123	165.000	15.57	Positive
Fosthiazate	4.17	284.053	103.845	21.71	284.053	227.845	10.23	Positive
Haloxyfop	6.74	362.040	287.845	27.25	362.040	315.649	18.53	Positive
Hexaconazole	7.16	314.082	69.899	20.77	314.082	158.845	31.15	Positive
Hexaflumuron	7.75	460.988	140.988	38.40	460.988	158.042	17.58	Positive
Hexythiazox	8.48	353.108	168.042	24.18	353.108	227.988	14.66	Positive
Imazalil	4.17	297.055	158.958	23.04	297.055	200.863	17.77	Positive
Imidacloprid	2.53	256.059	175.071	18.15	256.059	209.006	15.72	Positive
Indoxacarb	7.62	528.077	149.970	24.14	528.077	248.970	16.71	Positive
Ioxynil	4.84	369.823	126.958	33.00	369.823	214.958	32.00	Negative
Iprovalicarb	5.99	321.217	119.018	19.48	321.217	203.071	10.23	Positive

Isocarbofos	4.67	307.000	230.917	15.35	307.000	272.988	10.23	Positive
Isofenphos-methyl	6.73	332.107	120.899	33.96	332.107	230.917	14.44	Positive
Isoprocarb	4.45	194.117	94.970	14.63	194.117	137.071	10.23	Positive
Isoprothiolane	5.61	291.071	188.887	20.99	291.071	230.970	10.23	Positive
Isoproturon	4.57	207.149	71.988	18.42	207.149	165.071	14.09	Positive
Isoxaflutole	4.61	360.051	219.845	38.62	360.051	250.917	15.91	Positive
Kresoxim-methyl	6.68	314.138	222.071	10.23	314.138	267.071	10.23	Positive
Lenacil	4.51	235.144	135.988	31.72	235.144	153.000	15.31	Positive
Linuron	5.31	249.019	159.976	17.58	249.019	181.988	15.46	Positive
Lufenuron	8.38	508.971	326.042	18.65	508.971	339.113	11.32	Negative
Malathion	7.38	331.043	124.845	28.54	331.043	126.899	11.71	Positive
Malathion-D_{10}	5.75	341.106	100.042	23.28	341.106	132.042	12.46	Positive
Mandipropamid	5.56	412.131	328.018	14.28	412.131	356.018	10.23	Positive
Mepanipyrim	5.95	224.118	77.000	37.79	224.118	105.929	26.00	Positive
Meptyldinocap	9.25	295.150	134.042	55.00	295.150	194.083	25.39	Negative
Metaflumizone	8.28	507.125	177.970	25.24	507.125	287.000	25.09	Positive
Metalaxyl	4.54	280.154	220.054	14.40	280.154	248.054	10.23	Positive
Metamitron	2.72	203.092	103.917	23.34	203.092	175.000	17.09	Positive
Metconazole	7.20	320.152	69.970	24.03	320.152	124.970	38.85	Positive
Methamidophos	0.54	142.008	93.917	14.51	142.008	124.917	14.40	Positive
Methidathion	4.88	302.969	84.970	19.93	302.969	144.917	10.23	Positive
Methiocarb	5.41	226.089	106.917	37.64	226.089	121.000	10.23	Positive
Methiocarb-sulfone	2.83	258.079	122.071	18.98	258.079	200.970	10.23	Positive
Methiocarb-sulfoxide	2.60	242.084	122.000	29.03	242.084	184.970	13.68	Positive
Methomyl	2.12	163.053	87.970	10.23	163.053	105.917	10.23	Positive
Methoxyfenozide	5.76	369.217	149.000	17.28	369.217	312.982	10.23	Positive
Metobromuron	4.38	259.007	148.000	15.50	259.007	169.887	18.91	Positive
Metolachlor	6.26	284.141	176.054	25.85	284.141	252.054	15.19	Positive
Metolcarb	3.41	166.086	93.970	30.78	166.086	108.929	10.23	Positive
Metrafenone	7.31	409.064	208.929	13.98	409.064	226.845	20.46	Positive
Monocrotophos	2.31	224.068	126.899	15.80	224.068	192.845	10.23	Positive
Monolinuron	4.11	215.058	125.917	17.89	215.058	148.000	14.78	Positive
Monuron	3.58	199.063	71.970	16.71	199.063	125.970	26.11	Positive
Myclobutanil	5.88	289.121	89.000	54.73	289.121	124.899	32.59	Positive
Neburon	6.68	275.071	57.000	21.22	275.071	88.054	16.56	Positive
Nitenpyram	2.00	271.095	189.000	14.06	271.095	225.000	11.97	Positive
Novaluron	7.86	491.005	304.982	14.97	491.005	470.970	12.50	Negative
Omethoate	1.68	214.029	124.917	22.36	214.029	182.887	11.36	Positive
Oxadiargyl	7.15	341.045	222.917	16.07	341.045	229.958	14.81	Positive
Oxadixyl	3.31	279.133	132.000	31.15	279.133	219.054	10.23	Positive
Oxamyl	1.98	237.025	71.970	10.23	237.025	89.970	10.23	Positive
Oxasulfuron	3.43	407.000	107.196	43.25	407.000	150.196	18.44	Positive
Oxfendazole	3.35	316.075	159.071	33.81	316.075	191.196	20.84	Positive
Paclobutrazol	5.65	294.136	69.970	20.92	294.136	124.899	37.37	Positive

Paraoxon-methyl	3.31	248.030	109.071	19.49	248.030	202.125	19.07	Positive
Penconazole	6.81	284.071	122.917	49.28	284.071	158.917	29.60	Positive
Pencycuron	7.38	329.141	124.917	25.81	329.141	218.000	16.10	Positive
Pendimethalin	8.67	282.144	193.917	18.34	282.144	211.970	10.23	Positive
Penflufen	6.68	318.200	141.083	29.35	318.200	234.125	15.19	Positive
Penthiopyrad	6.89	360.140	177.125	34.82	360.140	276.125	14.60	Positive
Permethrin	9.70	408.112	183.125	19.79	408.112	355.125	8.71	Positive
Phenthoate	6.67	321.037	79.000	39.61	321.037	246.917	10.23	Positive
Phosalone	7.25	367.994	110.917	37.52	367.994	181.887	15.00	Positive
Phosmet	5.02	318.001	77.000	51.32	318.001	159.970	10.23	Positive
Phoxim	7.13	299.061	77.125	29.33	299.061	129.113	10.23	Positive
Pirimicarb	3.26	239.150	71.970	21.45	239.150	181.982	16.10	Positive
Pirimicarb-desmethyl	2.46	225.134	71.929	20.99	225.134	168.054	14.74	Positive
Pirimiphos-methyl	7.08	306.103	107.929	30.55	306.103	164.054	22.47	Positive
Prochloraz	7.01	376.038	69.929	25.77	376.038	307.815	11.71	Positive
Profenophos	7.89	372.942	302.720	18.68	372.942	344.833	13.34	Positive
Promecarb	5.57	208.133	108.929	16.48	208.133	151.054	10.23	Positive
Prometryn	5.30	242.143	157.970	23.65	242.143	199.929	18.76	Positive
Propamocarb	1.70	189.159	101.917	17.58	189.159	144.000	13.41	Positive
Propaquizafop	8.10	444.132	99.929	18.76	444.132	371.000	16.29	Positive
Propargite	8.70	368.000	175.054	16.03	368.000	231.065	10.23	Positive
Propazine	5.19	230.116	145.917	23.31	230.116	187.899	17.85	Positive
Propiconazole	7.02	342.077	69.000	20.12	342.077	158.917	29.14	Positive
Propoxur	3.64	210.112	110.917	14.28	210.112	168.054	10.23	Positive
Propyzamide	5.69	256.030	172.958	22.86	256.030	190.042	14.14	Positive
Proquinazid	9.05	373.040	288.851	23.46	373.040	330.774	14.47	Positive
Prosulfocarb	7.74	252.141	90.970	22.55	252.141	128.054	12.65	Positive
Pymetrozine	1.62	218.103	77.929	38.40	218.103	104.917	20.24	Positive
Pyraclostrobin	7.13	388.105	162.982	23.72	388.105	193.899	12.50	Positive
Pyridaben	9.20	365.144	147.054	24.41	365.144	308.988	12.09	Positive
Pyridalyl	10.26	489.975	108.887	27.21	489.975	182.917	17.35	Positive
Pyridaphenthion	5.87	341.071	188.970	21.41	341.071	204.899	21.03	Positive
Pyridate	9.60	379.124	206.845	17.17	379.124	350.982	10.23	Positive
Pyrifoenone	7.33	366.110	184.042	23.96	366.110	209.125	24.46	Positive
Pyrimethanil	4.84	200.118	106.917	24.18	200.118	183.042	24.03	Positive
Pyriproxyfen	8.34	322.143	95.988	15.61	322.143	227.071	14.51	Positive
Quinalphos	6.61	299.061	147.000	21.71	299.061	162.970	21.11	Positive
Quinoclamine	3.47	208.015	76.970	36.35	208.015	104.917	24.97	Positive
Quinoxyphen	8.45	308.003	161.988	45.29	308.003	196.833	31.87	Positive
Quizalfop	6.47	345.063	244.042	29.81	345.063	299.125	18.86	Positive
Quizalofop-P-ethyl	7.91	373.094	271.000	25.54	373.094	298.899	18.98	Positive
Rotenone	6.38	395.148	191.917	23.27	395.148	212.929	22.28	Positive
Simazine	3.63	202.090	124.125	18.56	202.090	132.054	19.53	Positive
Spinetoram J	7.50	748.499	97.946	42.38	748.499	142.000	28.80	Positive

Spinetoram L	7.93	760.499	97.946	42.38	760.499	142.000	28.80	Positive
Spinosyn A	7.06	732.460	97.940	42.19	732.460	141.982	29.49	Positive
Spinosyn D	7.46	746.483	97.940	42.19	746.483	141.982	29.49	Positive
Spirodiclofen	8.94	411.112	71.000	16.22	411.112	312.917	10.68	Positive
Spiromesifen	8.64	371.221	255.054	23.69	371.221	273.054	10.23	Positive
Spirotetramat	6.04	374.196	302.054	16.94	374.196	330.065	15.50	Positive
Spiroxamine	5.31	298.274	99.929	30.81	298.274	144.054	20.58	Positive
Sulfoxaflor	2.86	278.056	153.970	28.39	278.056	173.970	10.23	Positive
Tebuconazole	6.88	308.152	69.970	22.93	308.152	124.899	37.14	Positive
Tebufenozide	6.58	353.222	132.970	19.40	353.222	296.982	10.23	Positive
Tebufenpyrad	8.04	334.168	116.917	35.78	334.168	144.970	27.14	Positive
Teflubenzuron	8.40	379.000	196.042	21.26	379.000	339.042	9.72	Negative
Terbuthylazine	5.37	230.116	103.845	32.18	230.116	173.970	17.17	Positive
Terbuthylazine-desethyl	3.88	202.080	104.042	28.13	202.080	146.113	16.75	Positive
Terbutryn	5.41	242.143	90.988	40.52	242.143	185.988	18.34	Positive
Tetraconazole	6.29	372.028	69.970	22.28	372.028	158.887	29.79	Positive
Thiabendazole	2.43	202.043	130.970	32.82	202.043	174.970	25.54	Positive
Thiacloprid	2.98	253.030	89.970	35.63	253.030	125.917	20.92	Positive
Thiamethoxam	2.21	292.026	180.970	22.13	292.026	210.970	11.36	Positive
Thiobencarb	7.29	258.071	89.000	47.23	258.071	124.970	19.14	Positive
Tolfenpyrad	8.20	384.147	170.982	24.63	384.147	197.208	25.35	Positive
Triadimefon	5.77	294.100	196.899	15.35	294.100	224.929	13.07	Positive
Triallate	8.56	304.009	86.113	16.29	304.009	142.833	27.02	Positive
Triazophos	5.95	314.072	118.929	34.00	314.072	162.000	18.45	Positive
Trichlorfon	2.75	256.892	109.125	19.15	256.892	220.905	9.76	Positive
Triclocarban	7.81	314.985	126.857	32.75	314.985	161.887	18.83	Positive
Tricyclazole	3.11	190.043	135.917	28.50	190.043	162.970	22.59	Positive
Trifloxystrobin	7.60	409.136	144.917	43.06	409.136	185.917	17.39	Positive
Triflumizole	7.70	346.092	73.000	16.37	346.092	277.970	10.23	Positive
Triflumuron	7.28	359.040	138.917	30.47	359.040	155.988	15.46	Positive
Triticonazole	6.17	318.136	69.917	18.30	318.136	124.970	32.90	Positive
Tritosulfuron	5.09	446.035	194.917	18.64	446.035	220.929	18.72	Positive
XMC	4.15	180.101	94.929	20.01	180.101	122.929	12.16	Positive
Zoxamide	7.02	336.031	158.917	39.00	336.031	186.815	22.17	Positive

Table A2. Detection and chromatographic parameters for the compounds analysed by GC-MS/MS.

Name	t_R (min)	Precursor ion 1 (m/z)	Product ion 1 (m/z)	CE 1 (eV)	Precursor ion 2 (m/z)	Product ion 2 (m/z)	CE 2 (eV)
2-Phenylphenol	4.42	170	141	30	170	115	40
Acrinathrin	9.19	289	93	5	208	181	5
Azoxystrobin	11.65	344	329	10	344	156	40
Bifenthrin	8.59	181	166	10	181	115	50
Biphenyl	3.90	154	126	40	154	102	40
Boscalid	10.23	140	112	10	140	76	25
Bromopropylate	8.62	341	185	20	341	155	20
Bupirimate	7.49	273	193	5	273	108	15
Buprofezin	7.49	305	172	5	172	57	15
Captan	6.99	264	79	25	151	80	5
Chlorfenapyr	7.61	247	227	15	247	200	25
Chlorothalonil	5.83	266	231	20	266	133	40
Chlorpropham	4.92	213	171	5	213	127	5
Chlorpyrifos	6.51	314	286	5	314	258	15
Chlorpyrifos-methyl	6.07	288	93	26	286	271	16
Cyfluthrin	9.90	226	206	10	163	127	5
Cypermethrin	10.06	165	127	5	163	127	5
Cyproconazole	7.71	222	125	18	139	111	14
Cyprodinil	6.76	224	208	20	224	197	21
Deltamethrin	11.34	253	172	5	181	152.1	25
Diazinon	5.58	304	179	15	137	84	15
Dicloran	5.39	206	176	5	206	148	20
Dichlorvos	3.39	185	109	15	185	93	15
Dichlorvos-D_6	3.38	191	115	20	191	99	15
Dieldrin	7.49	345	263	8	279	243	8
Endosulfan sulfate	8.22	387	289	5	272	237	15
Endosulfan-alpha	7.25	239	204	15	195	160	5
Endosulfan-beta	7.79	207	172	15	195	159	10
EPN	8.64	157	110	15	157	77	25
Epoxiconazole	8.45	192	138	10	192	111	35
Ethion	7.83	231	175	5	231	129	25
Etofenprox	10.26	163	135	5	163	107	15
Fenamidone	8.74	268	180	20	238	103	20
Fenarimol	9.26	219	107	10	139	111	15
Fenazaquin	8.77	160	145	5	160	117	20
Fenbuconazole	10.00	198	129	5	129	102	15
Fenhexamid	8.21	177	113	10	177	78	20
Fenitrothion	6.31	277	260	5	277	109	20

Fenpropathrin	8.67	265	210	10	265	89	30
Fenpropidin	6.26	273	98	3	98	55	12
Fenpropimorph	6.47	128	110	10	128	70	12
Fenthion	6.49	278	169	20	278	109	20
Fenvalerate	10.80	167	125	12	125	89	20
Fludioxonil	7.45	248	154	25	248	127	30
Fluopicolide	8.17	209	182	20	173	109	25
Fluopyram	6.86	223	196	15	173	145	15
Fluquinconazole	9.69	340	298	20	340	286	30
Flusilazole	7.48	233	165	20	233	152	20
Flutriafol	7.25	219	123	12	219	95	20
Fluvalinate-tau	10.89	250	200	20	250	55	15
Folpet	7.01	260	130	15	147	103	5
Fosthiazate	6.69	195	139	5	195	103	5
Hexaconazole	7.33	214	172	20	214	159	20
Indoxacarb	11.29	264	148	25	203	134	10
Iprodione	8.41	244	187	5	187	124	25
Iprovalicarb	7.45	158	116	5	158	98	10
Isoprothiolane	7.33	162	134	5	162	85	15
Kresoxim-methyl	7.48	206	131	10	206	116	5
Lambda-Cyhalothrin	9.10	197	161	5	197	141	10
Lindane-d6	5.51	224	187	5	224	150	20
Malathion	6.38	173	99	15	158	125	8
Malathion-D_{10}	6.34	183	151	3	183	132	5
Mepanipyrim	7.17	222	207	30	222	158	30
Methidathion	7.08	145	85	5	145	58	15
Methiocarb	6.31	168	153	10	153	109	10
Myclobutanil	7.46	179	152	5	179	125	10
Oxadixyl	7.85	163	132	15	163	117	25
Paclobutrazol	7.16	236	132	15	236	125	10
Parathion-methyl	6.08	263	109	10	233	124	10
Penconazole	6.84	248	192	15	248	157	25
Pendimethalin	6.82	252	191	10	252	162	10
Permethrin	9.52	183	153	15	163	127	5
Phosmet	8.69	160	133	15	160	77	30
Phthalimide	4.17	147	76	30	104	76	10
Procymidone	6.99	283	255	8	283	96	8
Profenofos	7.37	337	309	5	337	267	15
Propiconazole	8.17	259	191	8	259	173	10
Propyzamide	5.53	173	145	15	173	109	30
Prosulfocarb	6.21	251	128	5	128	86	3
Pyridaben	9.63	147	132	10	147	117	20
Pyrimethanil	5.60	198	156	25	198	118	25
Pyriproxyfen	8.98	136	96	10	136	78	20

Quinoxyfen	8.11	307	272	5	307	237	25
Spirodiclofen	9.58	312	259	10	312	109	20
Spiromesifen	8.51	272	254	3	272	209	12
Tebuconazole	8.29	250	153	12	250	125	20
Tefluthrin	5.64	177	137	15	177	127	15
Tetraconazole	6.55	336	218	30	336	204	30
Tetradifon	8.89	356	229	10	356	159	10
Tetrahydrophthalimide	4.30	151	122	8	151	80	5
Tolclofos-methyl	6.13	265	250	15	265	220	25
Triadimefon	6.54	208	181	5	208	127	15
Triazophos	7.94	161	134	5	161	106	10
Trifloxystrobin	8.07	222	190	3	222	130	15
Vinclozolin	6.05	212	172	15	212	109	40