

Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol (QuPPE-Method)

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Note: Changes from V9.2 to V9.3 are highlighted in **yellow**

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1. Scope and Short Description

A method is described for the residue analysis of very polar, non-QuEChERS-amenable, pesticides in foods of plant origin such as fruits (including dried fruits), vegetables, cereals and processed products thereof as well as honey.

Residues are extracted from the test portion following water adjustment and the addition of acidified methanol. The mixture is centrifuged, filtered and directly analyzed by LC-MS/MS. Various options for the simultaneous LC-MS/MS analysis of different combinations of pesticides are provided. Quantification is in most cases performed with the help of isotopically labeled analogues of the target analytes, which are used as internal standards (ILISs). So far available, these ILISs are added directly to the test portion at the beginning of the procedure to compensate for any factors having an influence on the recovery-rates such as volume-deviations, analyte losses during sample preparation as well as matrix-effects during measurement.

2. Apparatus and Consumables

2.1. Powerful sample processing equipment,

e.g. Stephan UM 5 or Retsch Grindomix GM 300.

2.2. 50 mL centrifuge tubes with screw caps,

e.g.: a) reusable 50 mL Teflon® centrifuge tubes with screw caps (e.g. Nalgene/Rochester, USA; Oakridge, article-no. 3114-0050) or b) disposable 50 mL centrifuge tubes (e.g. Sarstedt / Nümbrecht, Germany, 114x28 mm, PP, article-no. 62.548.004).

2.3. Automatic pipettes,

suitable for handling volumes of 10 to 100 µL, 200 to 1000 µL and 1 to 10 mL.

2.4. 10 mL solvent-dispenser,

for the acidified methanol (3.6).

2.5. Centrifuge,

suitable for the centrifuge tubes employed in the procedure (2.2) and capable of achieving > 4000 rpm.

For the cleaning of extracts of commod that pose difficulties during filtration centrifugation at $\geq 10,000$ g are needed. In this case the tubes should be checked if they are suitable for higher velocities.

2.6. Syringe filters,

e.g. Cellulose mixed esters filters 0.45 µm pore size, Polyester filters 0.45 µm pore size (both from Macherey-Nagel, Düren, Germany).

Note: Significant levels of Perchlorate and Chlorate were detected in the above mentioned polyester filters. Cellulose mixed esters filters were found to be appropriate for these two compounds. For this suitability test take the worst case scenario into account where the filters are clogged by the extracts, not allowing large volumes (e.g. 200 µL) to pass. Thus elute only small volumes through the filters (e.g. 200 µL). Such clogging was observed when filtering extracts of industrially milled cereals, pears and pineapples. Furthermore, special attention is required if filters are used for diluted extracts as any detected levels in the extracts will have to be multiplied accordingly when calculating the levels in the sample. To avoid clogging in many cases centrifugation at higher speeds (>10,000 g for 5 min) is helpful.

2.7. Syringes,

e.g. 2 or 5 mL disposable polypropylene syringes suitable for the above mentioned filters 0.

2.8. Autosampler vials,

suitable for LC auto-samplers,

Use plastic vials if pesticides that tend to interact with glass-surfaces are present (e.g. Phosphonate, Paraquat, Diquat, Streptomycin and Glyphosate)¹.

2.9. Volumetric flask with stoppers,

for the preparation of stock and working solutions. E.g. 20 mL; 25 mL; 50 mL, 100 mL glass flasks.

Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. Phosphonate, Paraquat, Diquat, Streptomycin and Glyphosate).

2.10. LC-MS/MS instrumentation,

equipped with ESI source and appropriate columns, see details in chapters 5.7.1 till 5.7.11.

¹The list of compounds requiring plastic vessels is not necessarily comprehensive (this remark applies to the entire document). Such interactions with glass surfaces are typically more pronounced in solutions consisting of aprotic solvents (e.g. acetonitrile). Increasing water content and/or acidity typically reduce such interactions.

3. Chemicals

Unless otherwise specified, use reagents of recognized analytical grade. Take every precaution to avoid possible contamination of water, solvents, sorbents, inorganic salts, etc.

3.1. Water (deionized)

3.2. Methanol (HPLC quality)

3.3. Acetonitrile (HPLC quality)

3.4. Formic acid (concentrated; > 95%)

3.5. Acetic acid (concentrated; >98%)

3.6. Acidified methanol

Acidified methanol, pipette 10 mL formic acid (3.4) in a 1000 mL volumetric flask and fill up to volume with methanol (3.2).

3.7. Citric acid-monohydrate (p.a.)

3.8. Dimethylamine

Dimethylamine, e.g. 40 % by Fluka (article-no. 38940).

3.9. Ammonium formate (p.a.)

3.10. Ammonium citrate-tribasic, anhydrous (p.a.)

3.11. Sodium hydroxide (p.a.)

3.12. Di-Sodiumtetraborate-decahydrate (p.a.)

3.13. Dry ice

Dry ice, technical grade can be used, it should be periodically checked not to contain pesticides at relevant levels.

3.14. Pesticide standards

Pesticide standards, of known purity.

3.15. Pesticide stock solutions

Pesticide stock solutions, e.g. 1 mg/mL solutions of pesticide standards (3.14) in a water miscible solvent (e.g. water (3.1), methanol (3.2), acidified methanol (3.6), acetonitrile (3.3) or mixtures thereof). See **Table 20** for the conversion factors to be applied between typical purchased standards and analytes and **Table 21** for suggested solvents for the preparation of the stock solutions.

Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. **Phosphonate**, Paraquat, Diquat, Streptomycin and Glyphosate). Keep in mind that some standards are sold as salts or hydrates. Some exemplary conversion factors are shown in **Table 20**.

3.16. Pesticide working solutions / mixtures

Pesticide working solutions/mixtures, prepared at appropriate concentrations by diluting pesticide stock solutions (3.15) of one or more pesticides with water-miscible solvents as required for the spiking of samples in recovery experiments (5.4) or for the preparation of calibration standards (5.5). See suggestions in Table 21 in the Annex.

Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. **Phosphonate**, Paraquat, Diquat, Streptomycin and Glyphosate).

3.17. Internal standards

Internal standards (ISs) Exemplary sources are shown in Table 22.

Notes:

Due to the strong matrix effect assessment with this method. The use of isotopically-labeled IS is highly recommendable and in most cases indispensable for achieving sufficient recoveries. In general the absolute concentrations of the ILIS-solutions are not important as long as the ILIS-concentration in the final extract is high enough to produce a well measurable signal that is not disturbed by co-eluting matrix components. Important is furthermore that any content of the native analyte within the ILIS-standard (irrespective whether it was present as an impurity of the purchased standard or whether it was generated in the lab during storage of the ILIS-solution or during sample preparation) is low enough to exclude false positive results or significant influence on quantification. For quantification purposes it is of foremost importance that the ratio between the absolute ILIS amount added to the sample prior to extraction (or to the isolated aliquot of the sample extract) and the absolute amount of ILIS added to the calibration standard solutions is known as it is used in calculations.

Check whether the IS contain the respective native compounds at levels, which would lead to false positives or quantification errors.

IS Stock solutions,

e.g. 1 mg/mL solutions of ISs (3.17) in a water miscible solvent (e.g. methanol, acetonitrile, water or mixtures thereof). For solvent-suggestions see Table 21 in the Annex.

Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. ILISs of **Phosphonate**, Paraquat, Diquat and Glyphosate as well as Dihydrostreptomycin). Keep in mind that some standards are sold as salts or hydrates. Exemplary conversion factors are shown in Table 20.

3.18. IS-working solution I (IS-WS I)

IS-working solution I (IS-WS I) for spiking samples prior to extraction, prepared at appropriate concentrations by diluting IS stock solutions (3.17) of one or more ISs with water-miscible solvents. Suggestions for solvents are shown in Table 21 and suggestions for the concentrations in Table 23.

Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. ILIS of **Phosphonate**, Paraquat, Diquat and Glyphosate as well as Dihydrostreptomycin). In presence of water and especially at high pH levels, Phosphonic acid $^{18}\text{O}_3$ will gradually convert to $^{18}\text{O}_2^{16}\text{O}_1$, $^{18}\text{O}_1^{16}\text{O}_2$ and

eventually of $^{16}\text{O}_3$ (native) phosphonic acid. The $^{16}\text{O}_3$ phosphonic acid standard solution provided by the EURLs should be preferably diluted in acetonitrile, where it was shown to be stable for long periods.

3.19. IS-working solution II (IS-WS II)

IS-working solution II (IS-WS II) for preparation of calibration standards, prepared at appropriate concentrations by diluting IS-WS-I (3.18) with water-miscible solvents. Suggestions for solvents are shown in Table 21 and for concentrations in Table 23.

Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. ILIS of Phosphonate, Paraquat, Diquat and Glyphosate as well as Dihydrostreptomycin). For short term usage (e.g. up to one month) the ILIS of Phosphonic acid can be diluted in acidified methanol (3.6).

LC-MS/MS mobile phases, see details in chapter 5.7.

4. Disclaimer

This method refers to several trade name of products and instruments which are commercially available and suitable for the described procedure. This information is given for the convenience of the users of this method and does not constitute an endorsement by the EURL-SRM of the products named. The application of this method may involve hazardous materials, operations and equipment. It is the responsibility of the users of this method to establish appropriate safety and health practices prior to use. Any consumables and chemicals used in the procedure should be periodically checked, e.g. through reagent blank tests, for any relevant levels of the analytes of interest.

5. Procedure

5.1. Sample preparation

To obtain representative test-portions from the laboratory sample, proceed as required by the respective regulations and guidelines. For fruits and vegetables cryogenic milling (e.g. using dry ice) is to be preferred to minimize degradations, reduce particle size which leads to improved homogeneity and residue accessibility.

For dry commodities (e.g. cereals, pulses) small particle sizes improve the accessibility of residues enclosed in the interior of the materials. Thus fine grinding (e.g. particle size $<500\ \mu\text{m}$) is preferable. The larger the particles are the longer extraction times are required to achieve quantitative extraction.

For dried fruits and similar commodities ($< 30\%$ water content) the following procedure is proposed: Add 850 g of cold Water to 500 g frozen dried fruits and homogenize the mixture using a strong mixer (2.1), if possible with addition of dry ice to prevent or slow down any chemical and enzymatic reactions (3.13). 13.5 g of this homogenate will correspond to 5 g sample.

5.2. Extraction / Centrifugation / Filtration

The extraction procedure is shown at a glance in chapter 0.

5.2.1. Weighing of sample homogenate

Weigh a representative portion (ma) of the sample homogenate (5.1) into a 50 mL centrifuge tube (2.2). In case of fresh fruits and vegetables as well as juices take $10\ \text{g} \pm 0.1\ \text{g}$ of the homogenized sample. In case of dried fruits, dried vegetables, dried mushrooms take $5\ \text{g} \pm 0.05\ \text{g}$ or $13.5\ \text{g} \pm 0.1\ \text{g}$ of the re-hydrated and homogenized material according to 5.1 (corresponding to 5 g sample). In case of cereals, dried pulses and honey also take $5\ \text{g} \pm 0.05\ \text{g}$ of the homogenate. Smaller sample portions may have to be used for extract-rich commodities, such as spices or fermented products, or commodities with very high Water absorbing capacity not allowing proper extraction.

5.2.2. Adjustment of water content

Add water (3.1) to a total content of approx. 10 g according to the indications in **Table 23**.

No further water adjustment is needed where re-hydrated commodities (see 5.1) are employed. Where no ISs are used or where they are added after extract aliquotation, water adjustment to 10g is essential. Where the appropriate ISs are employed before any aliquotation has taken place water adjustment is less critical and may be skipped for commodities containing $\geq 80\%$ water (see **Table 23**)

5.2.3. Addition of extraction solvent and Isotopically Labeled Internal Standard

Add 10 mL acidified methanol (3.6) and 100 μL of the IS-WS I (3.18) containing isotopically labeled analogues of one or more of the analytes of interest (added IS mass = m_{ISsample}).

The resulting extract volume, taking into account the natural water content of the sample and the water added in 5.2.2 sums up to ca. 20 mL (corresponds to approx 0.5 g sample per mL extract if 10 g sample is employed for extraction). Where no ISs are used the aim should be to reach a total volume of the liquid phase that is as close as possible to 20 mL. Keep in mind that the water volume adjustments in **Table 23** are approximate and that there is an approximately 2.5% volume contraction occurring when methanol is mixed with water. In any case water adjustment will help to reduce the bias related to the volume deviation from 20 mL to an acceptable level.

For screening purposes the IS can be alternatively added to a sample extract aliquot (e.g. 1 mL, see 5.2.6), assuming that 1 mL extract corresponds to exactly 0.5 g sample equivalents. This way the added amount of IS per sample can be drastically reduced (e.g. 20-fold if added to 1 mL extract). The IS added at this step will compensate for matrix effects including retention-time shifts. The quantitative result should however be considered as tentative. For more accuracy samples should be re-analyzed with the IS being added in step 5.2.3.

Extraction solvent for Paraquat and Diquat

For Paraquat and Diquat the extraction solvent of acid. MeOH (3.6) is not strong enough in some commodities e.g. lentils for incurred residues

5.2.4. Shake

Close the tube and shake vigorously by a mechanical shaker. Shake between 1 min in the case of fresh products and 15 min in the case of dry commodities. Alternatively, in case of dry products the 1 min shaking by hand may be followed by a soaking period between 15-30 minutes and a subsequent second 1 min vigorous shaking by hand. The particle size plays an important role for dry products (e.g. cereals, pulses) as far as extractability is concerned. If a considerable fraction of the particles exceed 500 μm shaking or soaking times may have to be extended.

Extraction procedure for Paraquat and Diquat

For Paraquat and Diquat the 1 minute shaking is followed by a thermal treatment of 15 minutes at 80 °C in a water bath. Then shake again for 1 minute and wait for the sample to cool down to room temperature before centrifuging.

Note:

Placing the tubes in cold water will speed up cooling down. 1 minute extractions at room temperature with methanol containing 1% formic acid are well suitable Paraquat and Diquat screening. 15 minute extractions at 80 °C using the same extraction solvent were shown to provide quantitative extraction yields of incurred Diquat and Paraquat residues in wheat and potatoes. In an experiment on Lentils containing in-

curred Diquat residues a stronger extraction solvent was necessary (methanol/aqueous HCl 0.1M (1:1)) using the same volume, extraction temperature and extraction time as described above².

5.2.5. Centrifugation

Centrifuge (e.g. for 5 min at $\geq 4,000$ rpm).

The use of high speed centrifuge at 10,000 rpm was shown to have advantages in the separation of matrix-components causing clogging of filters (e.g. when dealing with extracts of finely milled cereals, pine-apples).

5.2.6. Filtration step

Filter an aliquot of the extract (e.g. 3 mL) through a syringe filter (2.6) into a sealable storage vessel.

The extracts of some commodity types (e.g. finely milled cereals) pose difficulties in filtration. To avoid this, place the extraction tubes from (5.2.4) or (5.2.5) for a few hours into the freezer, centrifuge and filter.

Check the filters for any cross-contamination of Perchlorate and Chlorate. Cellulose mixed-ester filters were found to be suitable for Chlorate and Perchlorate (see also chapter 2.6. for further information).

Transfer, as required, one or more aliquots (e.g. 1 mL each) of the filtered extract into auto-sampler vials (2.8)

5.3. Blank extracts

Using suitable blank commodities (not containing any detectable residues of the analytes of interest), proceed sample preparation exactly as described in 5.2 but SKIP THE ADDITION OF ISs.

5.4. Recovery experiments

Weigh an appropriate portion (see 5.2.1) of a blank commodity homogenate into a 50 mL centrifuge tube (2.2) and spike it with a suitable pesticide working solution (0 and Table 21).

Spike directly to the matrix, prior to any water or solvent addition. Use small volumes of pesticide working solutions (e.g. 50-300 μ L), to avoid too strong dilution. Conduct sample preparation exactly as described in 5.2.

² Kolberg DI, Mack D, Anastassiades M, Hetmanski MT, Fussell RJ, Meijer T, Mol HG. Anal Bioanal Chem. 404(8):2465-74 (2012); Development and independent laboratory validation of a simple method for the determination of paraquat and diquat in potato, cereals and pulses.

5.5. Preparation of calibration standards

5.5.1. Solvent-based calibration standards

An exemplary pipetting scheme for preparing solvent-based calibration standards is shown in Table 1. The calculation of the mass-fraction WR of the pesticide in the sample, when IS is used, is shown in 5.8.1. Where solvent-based calibrations are used the use of ILISs for quantification is essential as the IS compensates for any matrix-related signal suppressions / enhancements.

5.5.2. Matrix based calibration standards

Transfer suitable aliquots of the blank extract (5.3) to auto-sampler vials and proceed as shown in Table 1. The calculation of the mass-fraction WR of the pesticide in the sample using matrix-matched calibration standards, with and without the use of ILIS, is shown in 5.8.1 and 5.8.2 respectively.

Table 1: Exemplary pipetting scheme for the preparation of calibration standards

		Calibration standards								
		Solvent based (5.5.1)			Matrix-based (5.5.2)					
		using IS ⁴			without IS ⁵			using IS ⁴		
Calibration levels in µg pesticide /mL OR in µg pesticide/ "IS-portion" ¹		0.056	0.1	0.25	0.05	0.1	0.25	0.05	0.1	0.25
Blank extract (5.3)		-	-	-	850 µL	850 µL	850 µL	800 µL	800 µL	800 µL
1:1 (v/v) mix of water (3.1) and acidified methanol (3.6)		850 µL	800 µL	850 µL	100 µL	50 µL	100 µL	50 µL	-	50 µL
Pesticide working solutions (0) ²	1 µg/mL	50 µL	100 µL	-	50 µL	100 µL	-	50 µL	100 µL	-
	5 µg/mL	-	-	50 µL	-	-	50 µL	-	-	50 µL
IS-WS II (3.19) ^{1,3}		100 µL	100 µL	100 µL	-	-	-	100 µL	100 µL	100 µL
Total volume		1000 µL	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL

¹ One IS portion would correspond to the IS mass contained in 100 µL IS-WS II (which in the particular example is added to each calibration standard).

² The concentration of the pesticide working solution(s) should be sufficiently high to avoid excessive dilution of the blank extract which would result in matrix effect deviations.

³ For calibration standards of 1 mL it is recommended to prepare the IS-WS II (3.19) by diluting 20-fold the IS-WS I (3.18). The same volume and pipette as in 5.2.3 can then be used for the preparation of the calibration standards.

⁴ When employing IL-ISs matrix-matching and volume adjustments are of less importance as the IS compensates for any matrix-related signal suppressions / enhancements. Also solvent-based calibrations can be used here. Important is that a) the mass ratio of pesticide and IS in the respective calibration standards and b) the ratio between the IS mass added to the sample (5.2.3) and the IS mass added to the calibration standard(s) (5.5.1 and 5.5.2) is known and recorded. For convenience the latter mass ratio should be kept constant throughout all calibration levels (e.g. at 20:1 when preparing calibration standards of 1 mL).

⁵ Where ILISs are not available/employed, matrix-matching via matrix-matched standards (Table 1) or via the standard additions approach (0) are particularly important to compensate for matrix effects in measurement. In both cases the total volume of the sample extracts is assumed to be exactly 20 mL, which translates into 0.5 g sample equivalents per mL.

⁶ The calibration level of 0.05 µg/mL corresponds to 0.1 mg pesticide /kg sample, when using 10 g test portions, or to 0.2 mg/kg sample when using 5 g test portions.

5.5.3. Standard-Additions-Approach

Where no appropriate ISs are available the method of standard additions is a very effective approach for compensating matrix-induced enhancement or suppression phenomena. As this procedure involves a linear extrapolation it is mandatory that pesticide concentrations and detection signals show a linear relationship throughout the relevant concentration range. The procedure furthermore requires knowledge of the approximate (estimated) residue level in the sample ($w_R(\text{approx})$). This info is derived from a preliminary analysis.

Prepare 4 equal portions of the final extract and spike 3 of them with increasing amounts of analyte. The amounts to be added should be chosen in such a way to remain within the linear range. It should be avoided that the added levels are too close to the expected analyte level to avoid that measurement variability will influence too much the slope, which is used to calculate the analyte level. In case the concentrations are outside the linear range a dilution of all 4 extracts with the extraction solvent is indicated.

Prepare a working solution (0) of the analyte at a concentration level where 50 or 100 μL of the solution contain the lowest amount of analyte to be added.

Example A: Vial 1) no addition; vial 2) $0.5 \times w_R(\text{approx})$, vial 3) $1 \times w_R(\text{approx})$, and vial 4) $1.5 \times w_R(\text{approx})$,

Example B: Vial 1) no addition; vial 2) $1 \times w_R(\text{approx})$, vial 3) $2 \times w_R(\text{approx})$, and vial 4) $3 \times w_R(\text{approx})$.

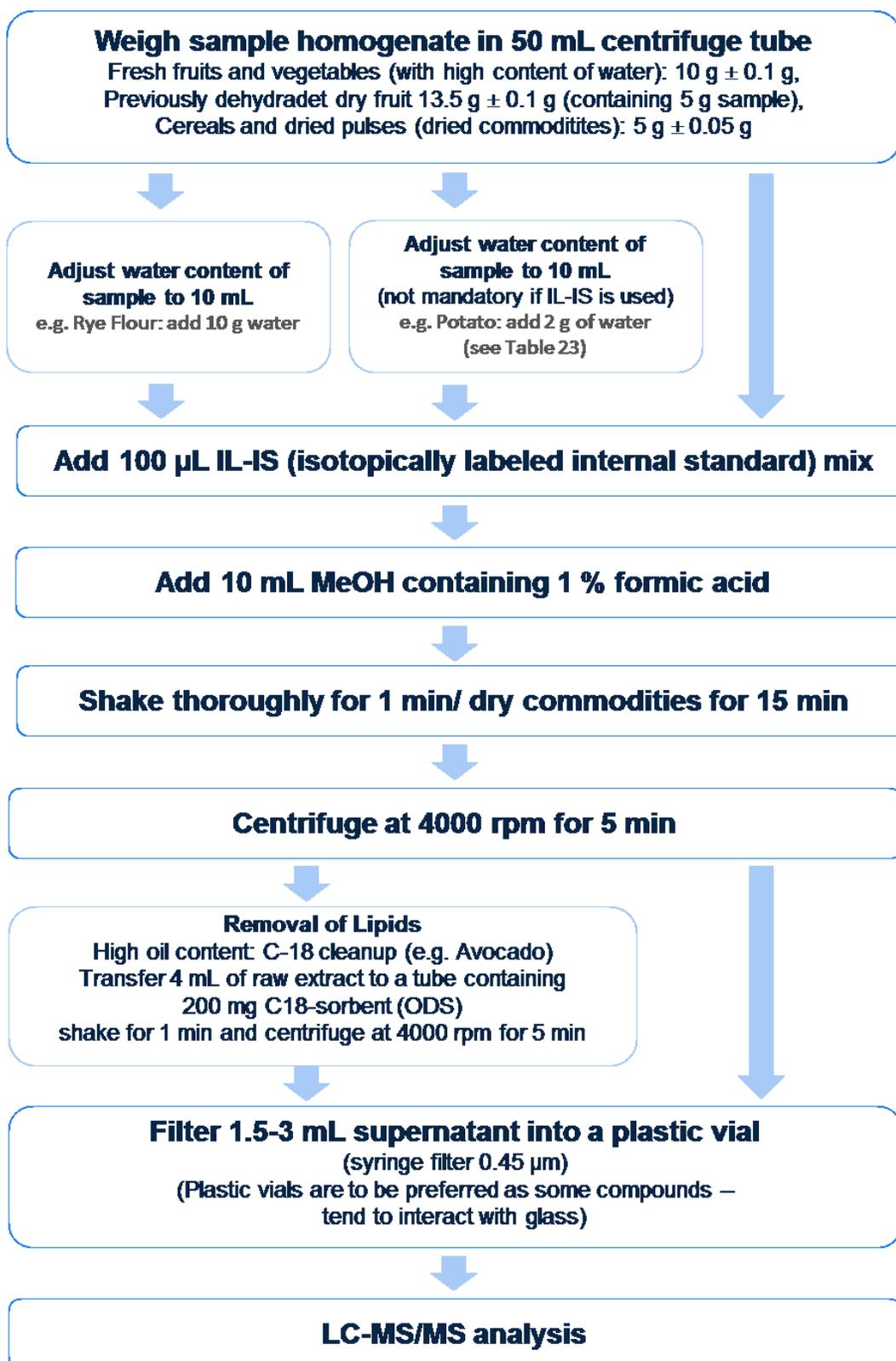
Adjust the volume within all vials by adding the corresponding solvent amounts.

Table 2 shows an example according to Example B. The calculation of the mass fraction of the pesticide in the sample w^R is shown in 5.8.2.

Table 2: Exemplary pipetting scheme of a standard additions approach (for a sample extract containing 0.5 g sample equivalents per mL and an estimated residue level ($w_R(\text{approx})$) of 0.5 mg/kg = 0.25 $\mu\text{g}/1000 \mu\text{l}$)

Additions	Vial 1	Vial 2	Vial 3	Vial 4
Volume of sample extract	1000 μL (= 0.5 g sample)			
IS	none	none	none	none
Added volume of pesticide working solution containing 5 $\mu\text{g}/\text{mL}$ (0)	-	50 μL	100 μL	150 μL
Resulting mass ($m_{pest}^{std\ add}$) of pesticide added to each vial		0.25 μg	0.5 μg	0.75 μg
Volume of solvent	150 μL	100 μL	50 μL	-
Final volume	1150 μL	1150 μL	1150 μL	1150 μL

5.6. QuPPE-PO-Method at a glance



5.7. LC-MS/MS Measurement

Any suitable LC-MS/MS conditions may be used. Some exemplary instrument measurement conditions are given below. An overview of LC-MS/MS conditions proposed within this document is given in Table 3:

Table 3: Overview and scope of the methods proposed within this document for the QuPPe method:

	M 1.1	M 1.2	M 1.3	M 1.4	M 2	M 3	M 4.1	M 4.2	M 5	M 6	M 7	M8	M 9
ESI-mode	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.
Separation principle	Anion Exch.	Anion Exch.	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon	HILIC
Column type	AS 11	AS 11-HC	Hypercarb	Hypercarb	Obe-lisc-R	Obe-lisc-R	Obe-lisc-R	BEH-Amide	PFP	Obe-lisc-R	Trinity P1	Hypercarb	Trinity P1
NEGATIVE MODE													
Ethephon	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT	NT
HEPA	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT	NT
Glufosinate	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT	NT
N-Acetyl-glufosinate	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT	NT
MPPA	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT	NT
Glyphosate	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT	NT
AMPA	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT	NT
Phosphonic acid	(✓)	(✓)	✓	✓	NT	NT	NT	NT	NT	NT	-	NT	NT
N-Acetyl-AMPA	NT	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT	NT
Fosetyl-Al	-	✓	✓	NT	✓	NT	NT	NT	NT	NT	✓*	NT	NT
Maleic hydrazide	-	-	✓	NT	✓	NT	NT	NT	NT	NT	✓*	NT	NT
Perchlorate	NT	-	✓	✓	✓	NT	NT	NT	NT	NT	✓*	NT	NT
Chlorate	NT	-	✓	✓	NT	NT	NT	NT	NT	NT	✓*	NT	NT
Bialaphos	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	-	NT	NT
Cyanuric acid	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	✓*	NT	NT
Bromide	NT	NT	-	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT
Bromate	NT	NT	(✓)	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT
N-Acetylglyphosate	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Difluoroacetic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓
Trifluoroacetic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓
POSITIVE MODE													
Amitrole	NT	NT	-	NT	NT	✓	-	✓	NT	NT	NT	NT	NT
ETU	NT	NT	✓	NT	NT	✓	-	✓	✓	NT	NT	NT	NT
PTU	NT	NT	✓	NT	NT	✓	-	✓	✓	NT	NT	NT	NT
Cyromazine	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT	NT
Trimesium	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT	NT
Daminozide	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT	NT
Chlormequat	NT	NT	✓	NT	NT	✓	✓	✓	✓	NT	NT	NT	NT
Mepiquat	NT	NT	✓	NT	NT	✓	✓	✓	✓	NT	NT	NT	NT
Difenzoquat	NT	NT	-	NT	NT	✓	✓	✓	✓	NT	NT	NT	NT
Propamocarb	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT	NT
Melamine	NT	NT	NT	NT	NT	NT	✓	✓	NT	NT	NT	NT	NT
Diquat	NT	NT	-	NT	NT	NT	✓	-	NT	NT	NT	NT	NT
Paraquat	NT	NT	-	NT	NT	NT	✓	-	NT	NT	NT	NT	NT
N,N-Dimethylhydrazine	NT	NT	-	NT	NT	NT	✓	-	NT	NT	NT	NT	NT
Nereistoxin	NT	NT	✓	NT	NT	NT	✓	✓	NT	NT	NT	NT	NT
Streptomycin	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT
Kasugamycin	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT
Morpholine	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT	✓
Diethanolamine	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT	NT
Triethanolamine	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT	NT
1,2,4-Triazole	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓	NT
Triazole-alanine	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓	NT
Triazole-acetic acid	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓	NT
Triazole-lactic acid	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓	NT
Aminocyclopyrachlor	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT

	M 1.1	M 1.2	M 1.3	M 1.4	M 2	M 3	M 4.1	M 4.2	M 5	M 6	M 7	M8	M 9
ESI-mode	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.
Separation principle	Anion Exch.	Anion Exch.	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon	HILIC
Column type	AS 11	AS 11-HC	Hypercarb	Hypercarb	Obe-lisc-R	Obe-lisc-R	Obe-lisc-R	BEH-Amide	PFP	Obe-lisc-R	Trinity P1	Hypercarb	Trinity P1
Chloridazon-desphenyl	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Mepiquat-4-hydroxy	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Propamocarb-N-desmethyl	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Propamocarb-N-	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT

✓ = satisfactory chromatography and detection sensitivity achieved,

NT = Not tested under the conditions shown in the respective sections,

(✓) = possible but compromised due to matrix effects or lacking separation or limited sensitivity or limited information for proper identification,

“-“ analysis was tested and found to be poor under the described conditions,

* Using a gradient (98% B -> 60% B in 5 min, hold 2 min)

Table 4: Practical Information: Methods mainly used by CVUA Stuttgart

Method	Special remarks on Substances	LC-MS/MS	Comments
Method 1.3 “Glyphosate & Co. Hypercarb” (see 5.7.3)	Glyphosate AMPA N-Acetyl-AMPA N-Acetylglyphosate Ethephon HEPA Glufosinate N-Acetyl-Glufosinate MPPA Fosetyl-Al Phosphonic acid (option: first screening) Maleic hydrazide Perchlorate (option: first screening) Chlorate (option: first screening) Cyanuric acid Bialaphos	Agilent 1200 Sciex QTRAP 5500	Evaluation via solvent-based calibration and ILISs except for Bialaphos and N-Acetyl-AMPA
Method 1.4 “PerChlo-Phos” (see 5.7.4)	Perchlorate (quantitative) Chlorate (quantitative) Phosphonic acid (quantitative) Bromide (Screening, quantitative) Bromate (quantitative)	Agilent 1200 Sciex QTRAP 5500	Mostly employed directly (option: screening by 1.3, if positive -> 1.4) Dilution 1:5-fold Evaluation via solvent-based calibration and ILISs
Method 4.1 “Quats & Co Obelisc R” (see 5.7.7)	Paraquat (for specific commodities) Diquat (for specific commodities)	Waters Acquity UPLC I-Class Sciex QTRAP 5500	Evaluation via matrix-based calibration and ILISs
Method 4.2 “Quats & Co BEH Amide” (see 5.7.8)	Amitrole ETU Chlormequat Mepiquat Daminozide PTU Cyromazine Trimethylsulfonium Nereistoxin Difenzoquat Melamine Propamocarb Morpholine (first screening) Diethanolamine (first screening) Triethanolamine (first screening) Aminocyclopyrachlor Chloridazon-desphenyl Mepiquat-4-hydroxy Propamocarb-N-desmethyl Propamocarb-N-oxide	Waters Acquity UPLC I-Class Sciex QTRAP 5500	Evaluation via matrix-based calibration and ILISs (except for Difenzoquat, Aminocyclopyrachlor, Mepiquat-4-hydroxy, Propamocarb-N-desmethyl, Propamocarb-N-oxide)
Method 6 “Streptomycin and Kasugamycin” (see 5.7.10)	Streptomycin Kasugamycin	Agilent 1200 Sciex QTRAP 5500	Seasonal analyses of selected commodities Evaluation via solvent-based calibration (using Dihydrostreptomycin as IS for Streptomycin)
Method 7 “Morpholine, Diethanolamine and Triethanolamine” (see 5.7.11)	Morpholine (quantitative) Diethanolamine (quantitative) Triethanolamine (quantitative)	Waters Acquity UPLC I-Class Sciex QTRAP 5500	Employed if screening by 4.2 was positive Employed if DEA was false negative, by 4.2 e.g. in cereals, dried mushrooms Evaluation via solvent-based calibration and ILISs
Method 8 “Triazole derivative metabolites (TDMs)” (see 5.7.12)	1,2,4-Triazole Triazol-alanine Triazole-acetic acid Triazole-lactic acid	Waters Acquity UPLC I-Class Sciex SeleXION Q-Trap® 5500	Method employed to collect data on residue situation Quantification via solvent-based calibration and ILISs
Method 9 “Difluoroacetic acid and Trifluoroacetic acid” (see 5.7.13)	Difluoroacetic acid Trifluoroacetic acid	Waters Acquity UPLC I-Class Sciex SeleXION Q-Trap® 5500	Method employed to collect data on residue situation Quantification via matrix-based calibration and ILISs

5.7.1. Method 1.1 “Glyphosate & Co. AS 11”

Table 5: Proposed LC-MS/MS conditions for Ethephon, HEPA (Ethephon metabolite), Glyphosat, AMPA (Glyphosate metabolite), Glufosinate, MPPA (Glufosinate metabolite), N-Acetyl-Glufosinate (Glufosinate metabolite), Phosphonic acid.

Instrument parameters	Conditions																														
Ionization mode	ESI neg																														
Column/temperature (see notes)	Dionex IonPac AS 11 2 x 250 mm (P/N 44077); 40°C																														
Pre-column	Dionex IonPac AG 11 2 x 50 mm (P/N 44079)																														
Pre-filters	e.g. Supelco column saver 2.0 µm Filter (optional)																														
Eluent A	Water (3.1)																														
Eluent B	1 mM Citric acid in water adjusted to pH 11 with Dimethylamine (DMA) Note: You will need approx 0.5 mL DMA solution for 500 mL 1 mM citric acid in water Make sure your eluent filters can handle alkaline solvents (see notes)!!																														
Gradient	<table border="1"> <thead> <tr> <th>%A</th> <th>Flow [mL/min]</th> <th>Time [min]</th> </tr> </thead> <tbody> <tr> <td>100</td> <td>0.3</td> <td>0</td> </tr> <tr> <td>50</td> <td>0.3</td> <td>8</td> </tr> <tr> <td>50</td> <td>0.3</td> <td>15</td> </tr> <tr> <td>100</td> <td>0.3</td> <td>15.1</td> </tr> <tr> <td>100</td> <td>0.3</td> <td>23</td> </tr> </tbody> </table>	%A	Flow [mL/min]	Time [min]	100	0.3	0	50	0.3	8	50	0.3	15	100	0.3	15.1	100	0.3	23												
	%A	Flow [mL/min]	Time [min]																												
	100	0.3	0																												
	50	0.3	8																												
	50	0.3	15																												
100	0.3	15.1																													
100	0.3	23																													
Injection volume	10-20 µL (Note: in case of analyzing only Ethephon 5 µL may be enough - depending on the instrument)																														
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS-portion* + one level at the reporting limit																														
Acquired mass transitions (m/z)	<table border="1"> <thead> <tr> <th>Compound</th> <th>Mass Transitions (m/z)</th> </tr> </thead> <tbody> <tr> <td>Glyphosate:</td> <td>168/63, 168/124, 168/150, 168/81</td> </tr> <tr> <td>Glyphosate-¹³C₂, ¹⁵N₁ (ILIS):</td> <td>171/63</td> </tr> <tr> <td>AMPA:</td> <td>110/63, 110/79, 110/81**</td> </tr> <tr> <td>AMPA-¹³C₁ ¹⁵N₁ (ILIS):</td> <td>112/63</td> </tr> <tr> <td>Ethephon:</td> <td>143/107, 143/79, 145/107</td> </tr> <tr> <td>Ethephon-D₄ (ILIS):</td> <td>147/111</td> </tr> <tr> <td>HEPA:</td> <td>125/79, 125/95, 125/63</td> </tr> <tr> <td>HEPA-D₄ (ILIS):</td> <td>129/79</td> </tr> <tr> <td>Glufosinate:</td> <td>180/63, 180/136, 180/85, 180/95</td> </tr> <tr> <td>Glufosinate-D₃ (ILIS):</td> <td>183/63</td> </tr> <tr> <td>N-Acetyl-glufosinate:</td> <td>222/63, 222/59, 222/136</td> </tr> <tr> <td>N-Acetyl-glufosinate-D₃ (ILIS):</td> <td>225/63</td> </tr> <tr> <td>MPPA:</td> <td>151/63, 151/107, 151/133</td> </tr> <tr> <td>MPPA-D₃ (ILIS):</td> <td>154/63</td> </tr> </tbody> </table>	Compound	Mass Transitions (m/z)	Glyphosate:	168/63, 168/124, 168/150, 168/81	Glyphosate- ¹³ C ₂ , ¹⁵ N ₁ (ILIS):	171/63	AMPA:	110/63, 110/79, 110/81**	AMPA- ¹³ C ₁ ¹⁵ N ₁ (ILIS):	112/63	Ethephon:	143/107, 143/79, 145/107	Ethephon-D ₄ (ILIS):	147/111	HEPA:	125/79, 125/95, 125/63	HEPA-D ₄ (ILIS):	129/79	Glufosinate:	180/63, 180/136, 180/85, 180/95	Glufosinate-D ₃ (ILIS):	183/63	N-Acetyl-glufosinate:	222/63, 222/59, 222/136	N-Acetyl-glufosinate-D ₃ (ILIS):	225/63	MPPA:	151/63, 151/107, 151/133	MPPA-D ₃ (ILIS):	154/63
	Compound	Mass Transitions (m/z)																													
	Glyphosate:	168/63, 168/124, 168/150, 168/81																													
	Glyphosate- ¹³ C ₂ , ¹⁵ N ₁ (ILIS):	171/63																													
	AMPA:	110/63, 110/79, 110/81**																													
	AMPA- ¹³ C ₁ ¹⁵ N ₁ (ILIS):	112/63																													
	Ethephon:	143/107, 143/79, 145/107																													
	Ethephon-D ₄ (ILIS):	147/111																													
	HEPA:	125/79, 125/95, 125/63																													
	HEPA-D ₄ (ILIS):	129/79																													
	Glufosinate:	180/63, 180/136, 180/85, 180/95																													
	Glufosinate-D ₃ (ILIS):	183/63																													
	N-Acetyl-glufosinate:	222/63, 222/59, 222/136																													
N-Acetyl-glufosinate-D ₃ (ILIS):	225/63																														
MPPA:	151/63, 151/107, 151/133																														
MPPA-D ₃ (ILIS):	154/63																														

AMPA: Aminomethylphosphonic acid; MPPA: 3-Methylphosphinopropionic acid; HEPA: 2-Hydroxyethylphosphonic acid (= hydroxy-ethephon),

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

** See comment 1 under 5.7.1a) concerning potential interference of AMPA by Fosetyl.

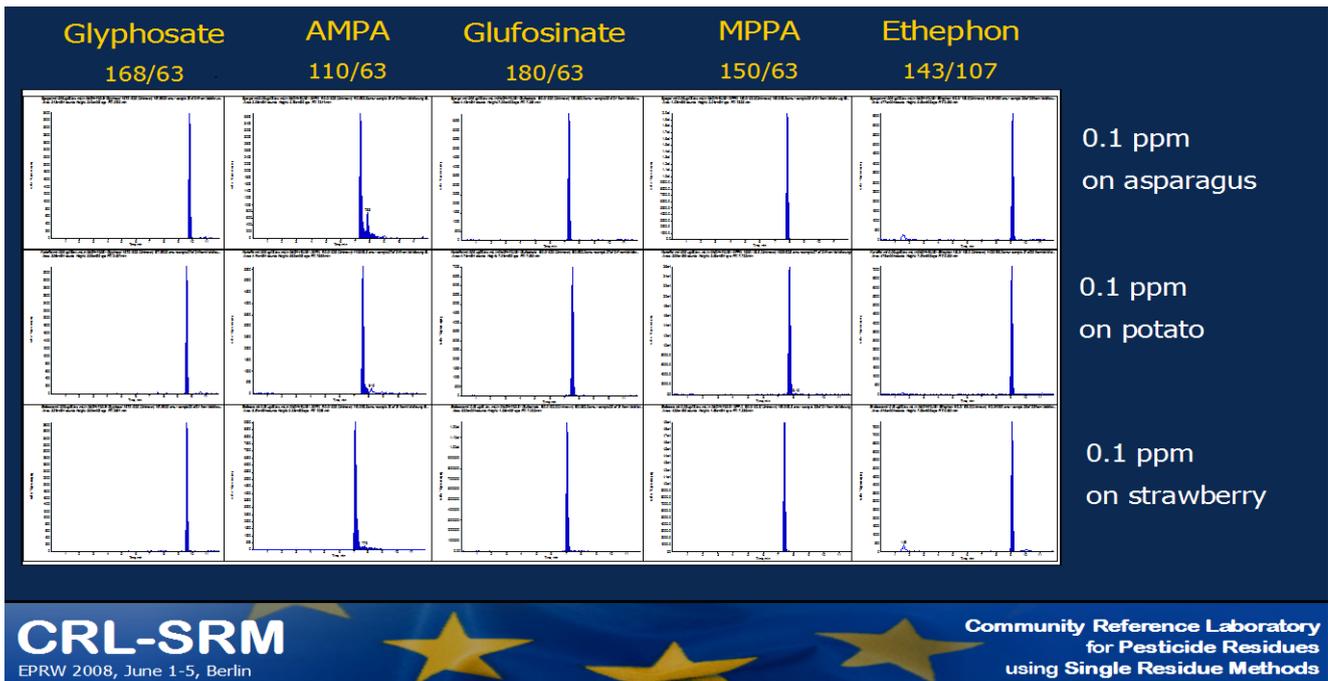


Figure 1: Typical chromatograms of HEPA in real samples

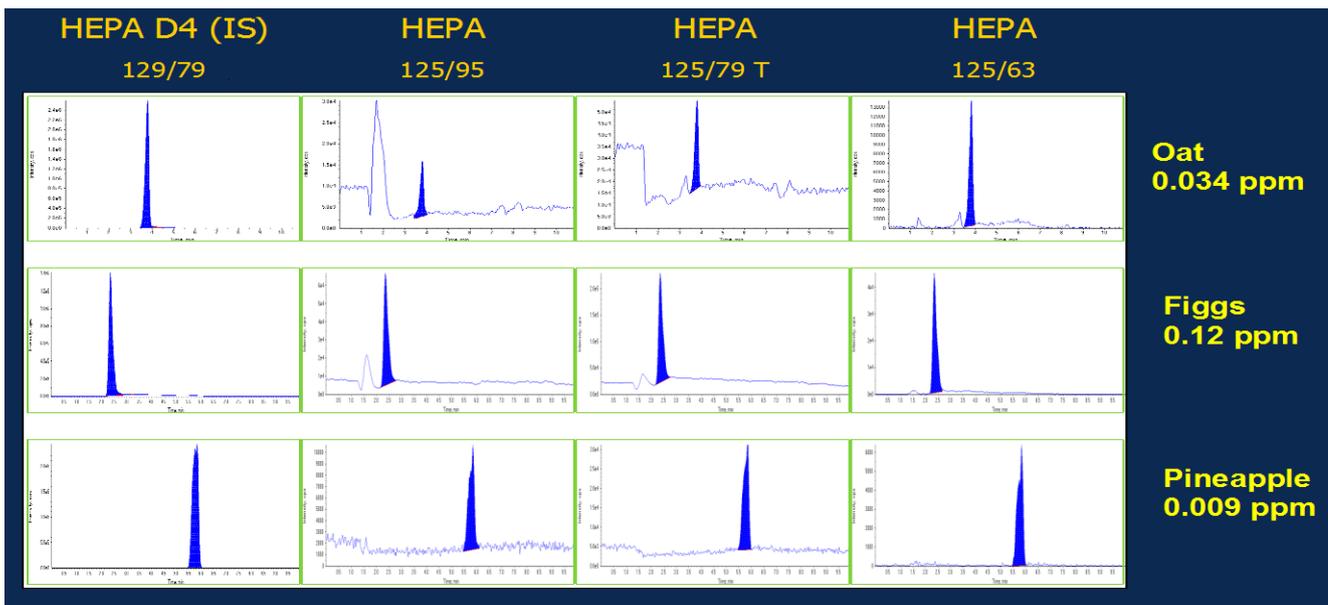


Figure 2: Typical chromatograms of Glyphosate, AMPA, Glufosinate, MPPA and Ethephon spiked on blank-QuPPE extracts of various commodities

Hints on Method 1.1

- a) AMPA and Fosetyl share the mass-transition 110/81. Chromatographic separation is thus needed.
- b) As the pH of the mobile phase is quite high, it is recommendable to **use alkali-compatible components**, e.g. metal frits instead of silica frits in the Eluent B reservoir; borosilicate 3.3 bottles instead of glass bottles for eluent B; rotor-seals from alkali-persistent materials, such as PEEK (polyetherketone) or Tefzel, rather than Vespel.
- c) **Priming and reconditioning of column:** before first use, after long storage (e.g. >2 weeks), after injection of 50-100 sample extracts for column A or 100-200 extracts for column B):
 - a. Flush column for 30 minutes with **100 mmol aqueous Borax solution** (7,62 g di-sodium tetraborate decahydrate in 200 mL water) at 0.3 mL/min **OR** Flush for 1 hour with 30 mM NaOH (240 mg NaOH in 200 mL water) at 0.3 mL/min
 - b. Flush column for 30 minutes with **Eluent A** (water) at 0.3 mL/min
 - c. Run system 3-4 times with full gradient (inject standards in matrix)
NOTE: When flushing NaOH or Borax solution through the column make sure that it will go directly into waste and not to the MS ion source!.
- d) **Storage of column:** If to be stored for short periods (<2 weeks), columns can be put aside after any normal sequence/run (full gradient). Run system 3-4 times with full gradient to reactivate the column (inject standards in matrix) before starting the sequence. If to be stored for longer periods (e.g. >2 months) recondition the column as described under 1.1.a-c
- e) **Pre-filters:** If pre-filters are used exchange them as soon as backpressure increases significantly. For practical and convenience reasons it is recommended to exchange pre-filters when performing other maintenance operations such as reconditioning or pre-column exchange.
NOTE: Losses of glyphosate, that could be clearly linked to interactions with a dirty pre-filter, have been once observed.
- f) **Pre-columns (guard columns):** The pre-column should be exchanged as soon as a clear deterioration of the separation performance (worsening of peak-shape) is noticed. The pre-column of method 1.1. needs to be exchanged more often than that of 1.2 and 1.3. If after pre-filter exchange (see above) the pressure does not come back to normal levels, the frit of the pre-column should be exchanged.

For further information on the storage and cleanup of column B see: <http://www.dionex.com/en-us/webdocs/113497-Man-065463-03-IonPac-AS11-HC-4um-Nov12.pdf>

5.7.2. Method 1.2 “Glyphosate & Co. AS 11-HC”

Table 6: Proposed LC-MS/MS conditions for Ethephon, HEPA (Ethephon metabolite), Glyphosat, AMPA (Glyphosate metabolite), Glufosinate, MPPA (Glufosinate metabolite), N-Acetyl-glufosinate (Glufosinate metabolite), Fosetyl-Al, N-Acetyl-AMPA and Phosphonic acid.

Instrument parameters	Conditions		
Ionization mode	ESI neg		
Column/temperature (see also notes below)	Dionex IonPac AS 11-HC 2 x 250 mm (P/N 052961); 40°C		
Pre-column	Dionex IonPac AG11-HC 2 x 50 mm (P/N 052963)		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter (optional)		
Eluent A	water (3.1)		
Eluent B	1 mM tribasic ammonium citrate in water		
Gradient	%A	Flow [mL/min]	Time [min]
	100	0.3	0
	0	0.3	8
	0	0.3	16
	100	0.3	16.1
	100	0.3	23
Injection volume	10 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS-portion* + one level at the reporting limit		
Acquired mass transitions (m/z)	Compound	Mass Transitions (m/z)	
	Glyphosate:	168/63, 168/124, 168/150, 168/81	
	Glyphosate- ¹³ C ₂ , ¹⁵ N (ILIS):	171/63	
	AMPA:	110/63, 110/79, 110/81**	
	AMPA- ¹³ C, ¹⁵ N (ILIS):	112/63	
	N-Acetyl-AMPA:	152/63, 152/79, 152/110	
	Ethephon:	143/107, 143/79, 145/107	
	Ethephon-D ₄ (ILIS):	147/111	
	HEPA:	125/79, 125/95, 125/63	
	HEPA-D ₄ (ILIS):	129/79	
	Glufosinate:	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D ₃ (ILIS):	183/63	
	N-Acetyl-glufosinate:	222/63, 222/59, 222/136	
	N-Acetyl-glufosinate-D ₃ (ILIS):	225/63	
	MPPA:	151/63, 151/107, 151/133	
	MPPA-D ₃ (ILIS):	154/63	
Fosetyl-Al:	109/81, 109/63 (Fosetyl)		
Fosetyl-Al-D ₁₅ (ILIS):	114/82 (Fosetyl-D5)		
Phosphonic acid***:	81/79, 81/63		
Phosphonic acid- ¹⁸ O ₃ (ILIS):	87/85		

AMPA: Aminomethylphosphonic acid; MPPA: 3-Methylphosphinicopropionic acid; HEPA: 2-Hydroxyethylphosphonic acid (=hydroxy-ethephon)

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

** See comment 1 under 5.7.1a) concerning potential interference of AMPA by Fosetyl.

*** See comment 3 on Phosphonic acid under 5.7.2c)

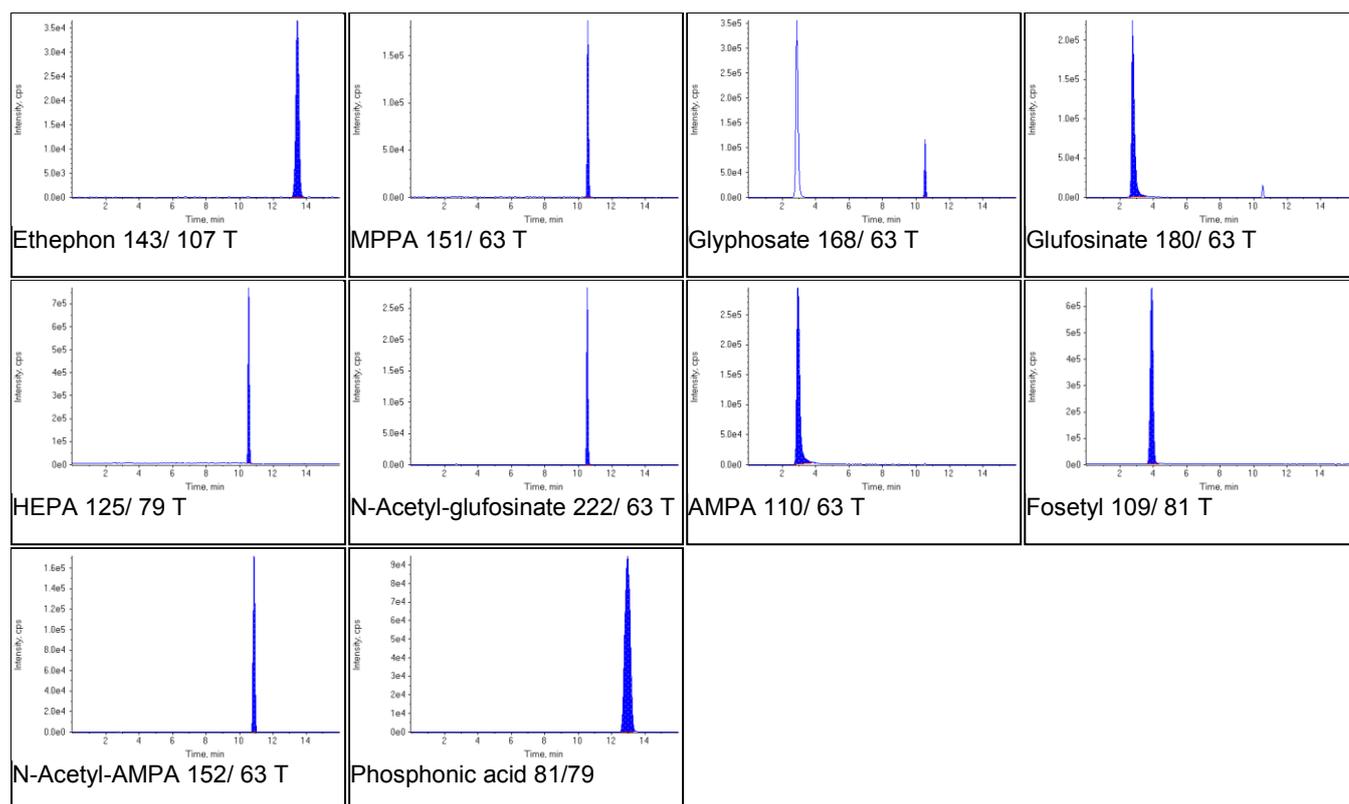


Figure 3: Typical chromatograms of Ethephon, HEPA, Glyphosat, AMPA, Glufosinate, MPPA, N-Acetyl-AMPA, N-Acetyl-glufosinate, Fosetyl-Al and Phosphonic acid at 0.1 mg/L in methanol with 1% formic acid.

Hints on Method 1.2

- a) Using this M1.2 some compounds (e.g. Glyphosate) in some commodities tend to give two sharp peaks. The corresponding ILIS typically behaves equally, so that quantification with any of the two peaks remains accurate
- b) AMPA and Fosetyl share the mass-transition (110/81). Chromatographic separation is thus needed (typically the case).
- c) Fosetyl and Fosetyl-D₅ tend to degrade to Phosphonic acid both in solutions and in the LC-MS/MS via in-source fragmentation. A good chromatographic separation between the two is thus necessary (typically the case).
- d) When extracts containing high levels of Phosphoric acid (which is naturally contained at high concentrations in many samples) are injected the chromatographic separation of Phosphoric and Phosphonic acid is compromised. This often results in a suppression of the Phosphonic acid signal and in some cases even leads to false negative results. **The most important qualifier mass-transition of Phosphonic acid (81/63) also occurs as a minor transition of Phosphoric acid**, but as the latter is often present at much higher levels than Phosphonic acid its interference on this mass transition can still be significant, especially if these two elute in close vicinity. The chromatographic separation of Phosphoric and Phosphonic acid considerably improves following dilution of the extracts typically allowing proper detection, identification and quantification of Phosphonic acid next to high levels of phosphoric acid. It is thus beneficial to inject smaller volumes of sample extract (e.g. 1-2 µL) **or to dilute QuPPE extracts 5-10-fold before injection**. Fortunately both, **Phosphoric and Phosphonic acid have at least 1 proper mass-transition (97/63 and 81/79 respectively), which in the case of Phosphonic acid can be used for quantitation and to improve identification certainty**. The elution time and peak shape of the Phosphonic acid ILIS can also be used to distinguish it from Phosphoric acid and to avoid false positives. Using signals on the 81/63 mass trace it was calculated that 20 mg/kg Phosphoric acid would simulate 0.1 mg/kg Phosphonic acid if this mass transition was used for quantification.
- e) **Priming and reconditioning of column:** before first use, after long storage (e.g. >2 weeks), after injection of 50-100 sample extracts for column A or 100-200 extracts for column B):
 - a. Flush column for 30 minutes with **100 mmol aqueous Borax solution** (7,62 g di-sodium tetraborate decahydrate in 200 mL water) at 0.3 mL/min **OR** Flush for 1 hour with 30 mM NaOH (240 mg NaOH in 200 mL water) at 0.3 mL/min
 - b. Flush column for 30 minutes with **Eluent A** (water) at 0.3 mL/min
 - c. Run system 3-4 times with full gradient (inject standards in matrix)
NOTE: When flushing NaOH or Borax solution through the column make sure that it will go directly into waste and not to the MS ion source!.
- f) **Storage of column:** If to be stored for short periods (<2 weeks), columns can be put aside after any normal sequence/run (full gradient). Run system 3-4 times with full gradient to reactivate the

column (inject standards in matrix) before starting the sequence. If to be stored for longer periods (e.g. >2 months) recondition the column as described under I.1.a-c

- g) **Pre-filters:** If pre-filters are used exchange them as soon as backpressure increases significantly. For practical and convenience reasons it is recommended to exchange pre-filters when performing other maintenance operations such as reconditioning or pre-column exchange.

NOTE: Losses of glyphosate, that could be clearly linked to interactions with a dirty pre-filter, have been once observed.

- h) **Pre-columns (guard columns):** The pre-column should be exchanged as soon as a clear deterioration of the separation performance (worsening of peak-shape) is noticed. The pre-column of method 1.2. needs to be exchanged less often than that of 1.1. If after pre-filter exchange (see above) the pressure does not come back to normal levels, the frit of the pre-column should be exchanged

5.7.3. Method 1.3 “Glyphosate & Co. Hypercarb”

Table 7: Proposed LC-MS/MS conditions for Ethephon, HEPA (Ethephon metabolite), Glyphosat, AMPA (Glyphosate metabolite), N-Acetylglyphosate (Glyphosate metabolite), N-Acetyl-AMPA (Glyphosate metabolite), Glufosinate, MPPA (Glufosinate metabolite), N-Acetyl-glufosinate (Glufosinate metabolite), Fosetyl-Al, Maleic hydrazide, Cyanuric acid and Bialaphos.

Instrument parameters	Conditions		
Ionization mode	ESI neg		
Column/temperature	Hypercarb 2.1 x 100 mm 5 µm (P/N 35005-102130); 40°C		
Pre-column	Hypercarb Guard 2.1 x 10 mm 5 µm (P/N 35005-102101)		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter (optional)		
Eluent A	1% acetic acid in water + 5% methanol		
Eluent B	1% acetic acid in methanol		
Gradient	%A	Flow [mL/min]	Time [min]
	100	0.2	0
	70	0.2	10
	70	0.4	11
	70	0.4	18
	10	0.4	19
	10	0.4	22
	100	0.2	22.1
	100	0.2	30
Injection volume	5 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS-portion* + one level at the reporting limit		
Acquired mass transitions (m/z)	Compound	Mass Transitions (m/z)	
	Glyphosate:	168/63, 168/124, 168/150, 168/81	
	Glyphosate- ¹³ C ₂ , ¹⁵ N (ILIS):	171/63	
	AMPA**:	110/63, 110/79, 110/81**	
	AMPA- ¹³ C, ¹⁵ N (ILIS):	112/63	
	N-Acetyl-AMPA:	152/63, 152/79, 152/110	
	N-Acetylglyphosate	210/63, 210/150, 210/79, 210/148	
	N-Acetylglyphosate-D ₃ (ILIS)	213/63	
	Ethephon:	143/107, 143/79, 145/107	
	Ethephon-D ₄ (ILIS):	147/111, 147/79 (optional, in case of interferences)	
	HEPA:	125/79, 125/95, 125/63	
	HEPA-D ₄ (ILIS):	129/79	
	Glufosinate:	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D ₃ (ILIS):	183/63	
	N-Acetyl-glufosinate:	222/63, 222/59, 222/136	
	N-Acetyl-glufosinate-D ₃ (ILIS):	225/63	
	MPPA:	151/63, 151/107, 151/133	
	MPPA-D ₃ (ILIS):	154/63	
	Fosetyl-Al:	109/81, 109/63 (detected as Fosetyl)	
	Fosetyl-Al-D15 (ILIS):	114/82 (detected as Fosetyl- D5)	
	Maleic hydrazide:	111/82, 111/42, 111/55, 111/83	
	Maleic hydrazide-D ₂ (ILIS):	113/42	
	Cyanuric acid:	128/42, 128/85	
Cyanuric acid- ¹³ C ₃ :	131/43		
Bialaphos:	322/88, 322/94, 322/134		

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

** See comment 1 under 5.7.1a) concerning potential interference of AMPA by Fosetyl.

*** See comment 3 on Phosphonic acid under 5.7.3f)

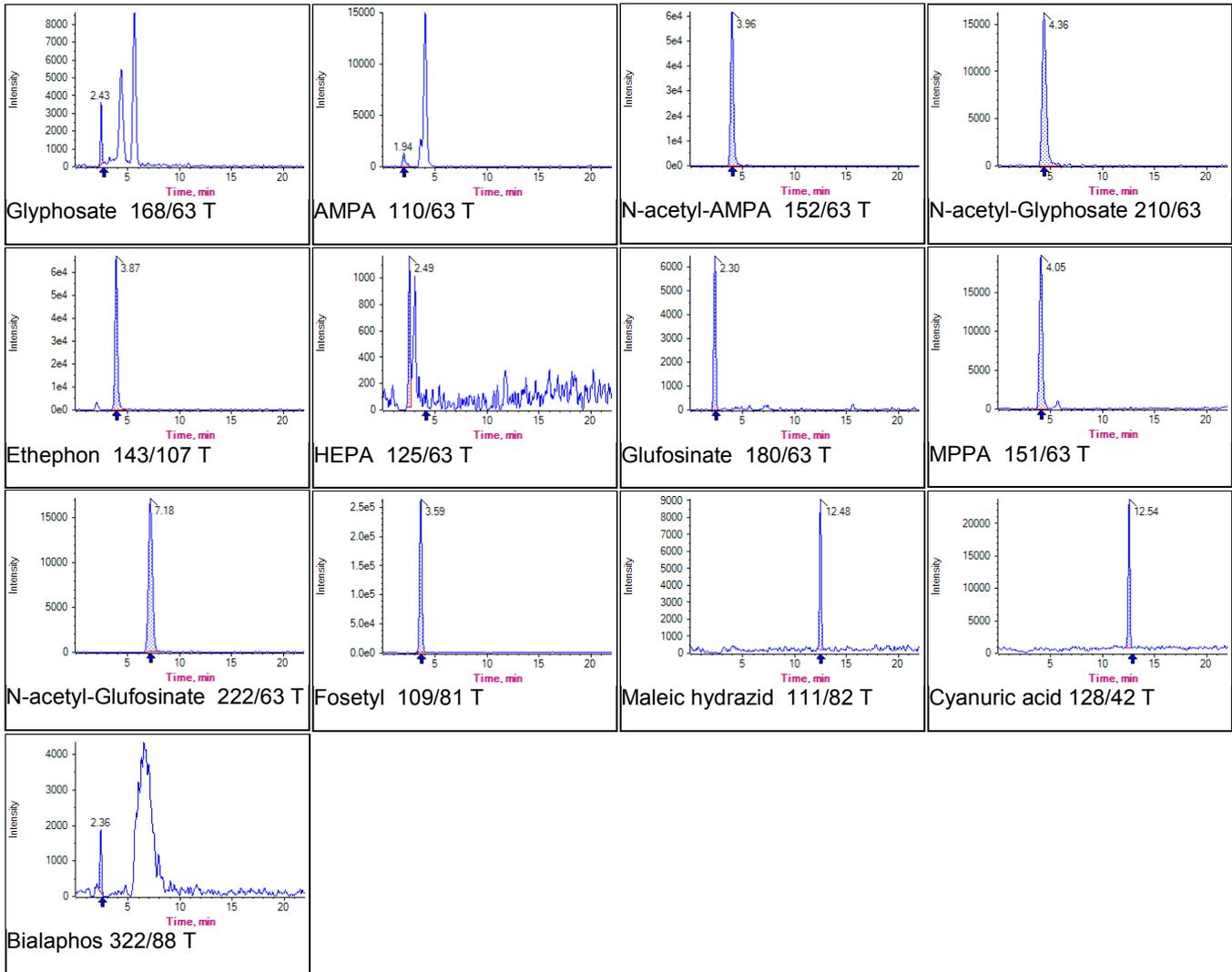


Figure 4: Chromatograms of Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetylgllyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetylglufosinate, Fosetyl, Maleic hydrazide, Cyanuric acid and Bialaphos at 0.02 ppm on apple extract.

Hints on Method 1.3

a) Priming and reconditioning of the column:

before the first use, the Hypercarb columns and pre-columns have to be thoroughly primed to cover certain active sites on the surface. Priming with solutions containing planar molecules such as chlorophyll and anthocyanins accelerates the priming period. A recommendable procedure for priming is the injection of QuPPE extract of spinach (for equilibration of the pre-column inject 10-15 injections spinach extracts, for column and pre-column inject 50 injections spinach extracts, if possible inject 50 µL) or the injection grape skin extract solution, prepared by dissolving 100 mg grape skin extract in 20 mL methanol + 1% FA-H₂O 1:1. This masking of the active sites is temporary and the activity of the column gradually increases with the injection of solvent or diluted extracts. Following a sequence of injections with low or no matrix load will typically raise the need for intermediate conditioning with extracts to restore the column. The impact of priming on the chromatographic properties of the column is exemplary shown in Figures 10, 11 and 12.

Table 8: Proposed LC-MS/MS conditions for priming and reconditioning of the Hypercarb column.

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Hypercarb 2.1 x 100 mm 5 µm (P/N 35005-102130); 40°C		
Pre-column	Hypercarb Guard 2.1 x 10 mm 5 µm (P/N 35005-102101)		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter (optional)		
Eluent A	1% acetic acid in water + 5% methanol		
Eluent B	1% acetic acid in methanol		
Gradient	%A	Flow [mL/min]	Time [min]
	100	0.3	0
	70	0.3	7
	100	0.3	7.1
	100	0.3	12
Injection volume	50 µL		
MS-System	If possible disconnect the MS-System to prevent contamination of the MS.		

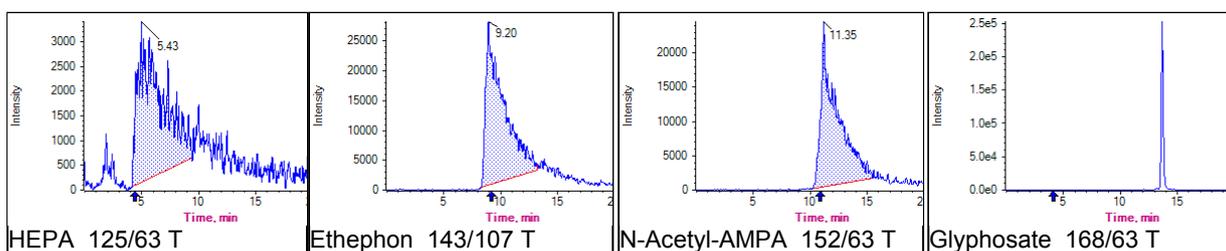


Figure 5: Chromatograms obtained using a new Hypercarb column, poor chromatographic behavior due to strong interactions of analytes with active sites. Same behavior is observed when the pre-column is new.

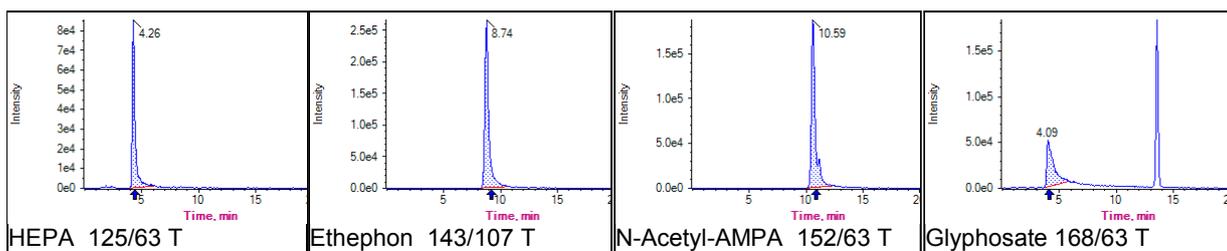


Figure 6: Chromatograms following priming with 25 injections (50 μ L) of Spinach QuPPE extracts.

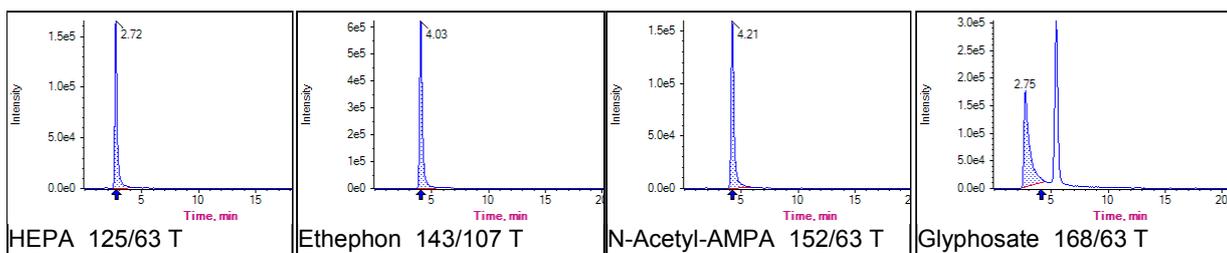


Figure 7: Chromatograms after additional injection of approx 100 QuPPE-extracts of various fruit and vegetables during normal routine use.

- b) Pre-columns (guard columns): The pre-column should be exchanged as soon as a clear deterioration of the separation performance (worsening of peak-shape) is noticed. The pre-column of method 1.3 needs to be clearly less often exchanged compared to the pre-columns of methods 1.1 and 1.2. Any exchange of the pre-column requires priming as described above. For this the pre-column does not have to be attached to the column. Connecting several pre-columns in a row and priming them simultaneously is also an option.
- c) Storage of columns: Following normal operation the column can be stored directly after any normal sequence/run (full gradient). Run system 3-4 times with full gradient to reactivate the column (inject standards in matrix) before starting the sequence. If to be stored for longer periods (e.g. >2 months) it is recommended to recondition the column as described above.
- d) Pre-filters: If pre-filters are used exchange them as soon as backpressure increases significantly. For practical and convenience reasons it is recommended to exchange pre-filters when performing other maintenance operations such as reconditioning or pre-column exchange. If after pre-filter exchange (see above) the pressure does not come back to normal levels, the frit of the pre-column may need to be exchanged.
 Note: Losses of Glyphosate, that could be clearly linked to interactions with a dirty pre-filter, have been once observed.
- e) AMPA and Fosetyl share the mass-transition m/z 110/81. Chromatographic separation is thus needed (typically the case).
- f) Fosetyl and its D_5 -analogon tend to degrade to Phosphonic acid both in solutions and via in-source fragmentation in LC-MS/MS. A good chromatographic separation between Fosetyl and Phosphonic acid is thus necessary (and is typically the case). Figure 8 shows an example of this in-source

fragmentation. Upon injection of 0.1 µg/mL Fosetyl a peak showed up on the mass traces of Phosphonic acid at the retention time of Fosetyl. The signal intensity of this peak corresponded to 0.04 µg/mL Phosphonic acid. When injecting Fosetyl-D₅ at 0.1 µg/kg the in-source fragmentation was less abundant (corresponding to approx. 0.001 µg/mL Phosphonic acid) but Phosphonic acid as impurity showed up at its proper retention time at a concentration corresponding to approx. 0.007 µg/mL. To be on the safe side, Fosetyl-ILIS should thus not be added to calibration solutions or samples or sample extracts intended to be used for the analysis of native Phosphonic acid. Furthermore calibration solutions used for the analysis of Phosphonic acid should better not contain any native Fosetyl.

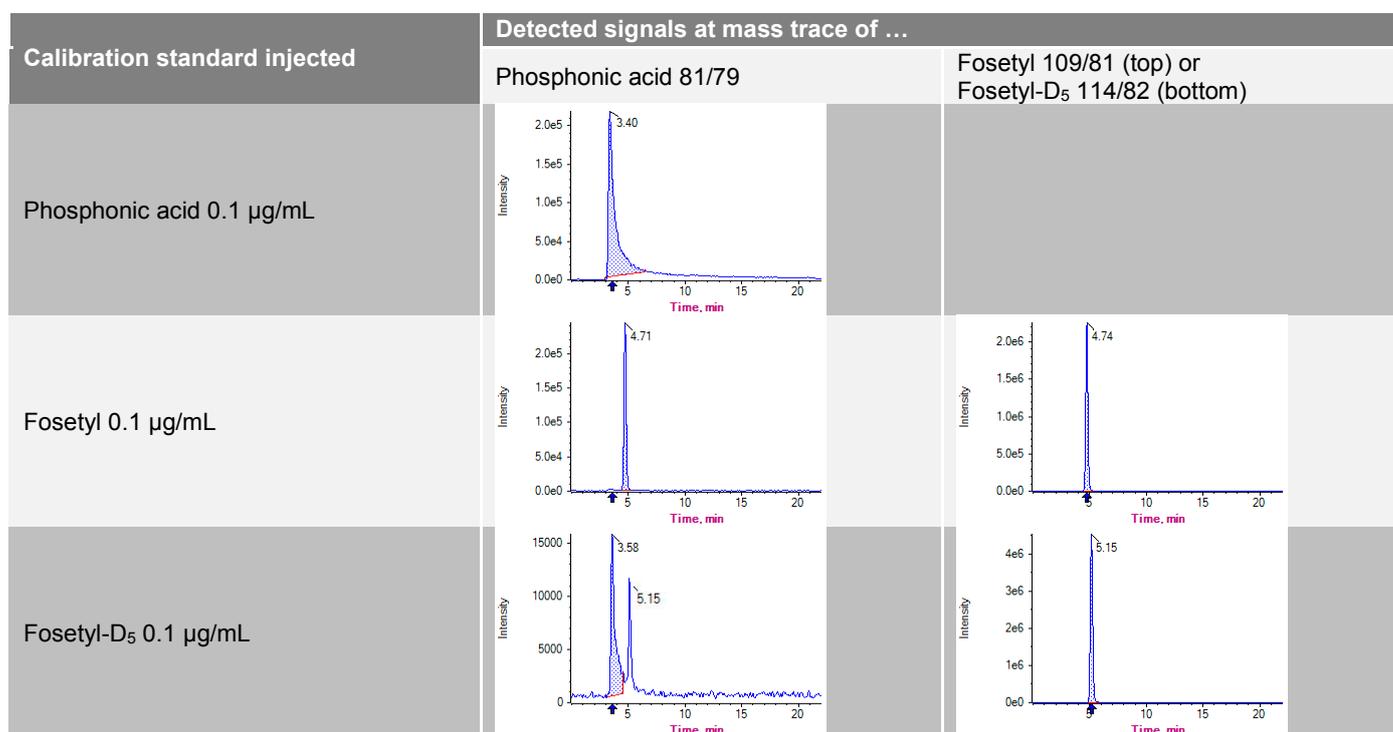


Figure 8: Chromatograms of Phosphonic acid, Fosetyl and Fosetyl-D₅ (each at 0.1 µg/mL). In addition to the proper mass-traces of Fosetyl and Fosetyl-D₅ the mass trace of Phosphonic acid is also shown to demonstrate the occurrence of in-source fragmentation of Fosetyl and Fosetyl-D₅ towards Phosphonic acid as well as the presence of Phosphonic acid as an impurity of the Fosetyl-D₅ standard solution.

A degradation of Ethephon to Phosphonic acid in solution is also observed. **Figure 9** shows a small peak of Phosphonic acid (corresponding to 0.002 µg/mL) that showed up when Ethephon standard at 1 µg/mL was injected. This contamination is considered negligible. However **Figure 9** also shows chromatograms of an unsuitable Ethephon-D₄ standard containing only ca. 0.08 µg/mL instead of the expected 1 µg/mL Ethephon-D₄ and ca. 0.8 µg/mL Phosphonic acid. The use of such an ILIS would contaminate the sample with Phosphonic acid leading to false positive results. To be on the safe side Ethephon-ILIS should thus not be added to calibration solutions, samples or sample extracts intended for the analysis of native phosphonic acid. Furthermore calibration solutions used to analyse phosphonic acid should better not contain any native Ethephon.

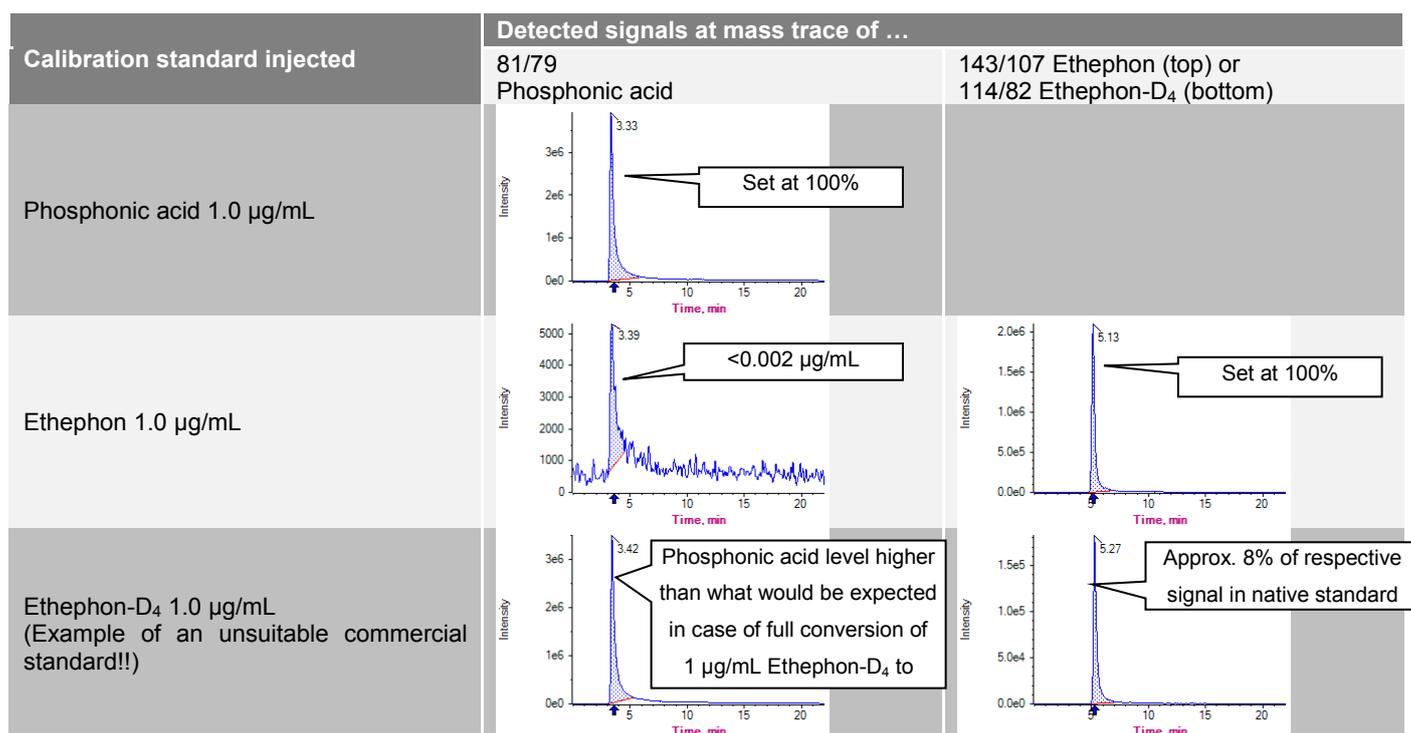


Figure 9: Chromatograms of Phosphonic acid, Ethephon and an unsuitable Ethephon-D₄ standard (each at 1.0 µg/mL). Whereas Phosphonic acid is only present at very low concentrations in the Ethephon standard the amount of Phosphonic acid in the Ethephon-D₄ standard is unacceptably high. That is caused by the Phosphonic acid having already been present at high amounts in the purchased standard.

- g) In case of the determination of Fosetyl and Phosphonic acid on the Hypercarb-column, we refer to the patent of D. Rosati and C. Venet from Bayer CropScience (Patent-No. WO 2006079566 A1).
- h) **Compound stability:**

A general overview regarding the stability of Glyphosate & Co. compounds in stock solutions is given in Table 9. For the compounds of this method (Maleic hydrazide and Cyanuric acid excluded) the use of 10% acetonitrile in water was shown to be a suitable solvent, see also Table 21. In case of Ethephon (native compound or ILIS), which is sensitive towards neutral and alkaline pH, we recommend acidifying the stock solution with hydrochloric acid. The addition of 0.1% (v/v) of concentrated HCl (37%) is proposed. This acid content will also sufficiently stabilize 100-fold diluted working solutions (of e.g. 10 µg/mL) without adding further acid. Other compounds of this method are not markedly compromised in their stability by this acid content. The previously recommended solvent of methanol/water+1% formic acid 1/1 proved to be less suitable in the long run with methylations, formylations as well as dehydrations being observed.

To some extent degradation also takes place in QuPPE extracts. AMPA and N-Acetylglyphosate are most affected. In general degradation is negligible if extracts are analyzed within 14 days as the degradation rate does not exceed 20% except of AMPA in dry commodities during this period. In any case such losses can be effectively corrected by the respective ILISs.

Table 9: Overview of the tested solvent and acid combinations regarding the standard stability of native compounds of method Glyphosate & Co. (✓= sufficiently stable (< 10%) ; ✗=not stable)

	Water*				Methanol (≤ 50 %)***			Acetonitrile (≤ 50 %)***		
	w/o acid	1% Formic acid	1% Acetic acid	0.1% Hydrochloric acid**	w/o acid	1% Formic acid	1% Acetic acid	w/o acid	1% Formic acid	1% Acetic acid
AMPA	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Bialaphos	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Cyanuric acid	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Ethephon [#]	✗	✓	✓	✓	NT	✓ in 100% MeOH	NT	NT	NT	NT
Fosetyl-Al	✓	✗	✓	✗	✓	NT	NT	NT	NT	NT
Glufosinate				NT	✓	NT	NT	NT	NT	NT
Glyphosate	✓	✗	✗	NT	✗	✗	✗	✗	✗	✗
HEPA	NT	NT	NT	NT	✓ in 100% MeOH	NT	NT	NT	NT	NT
Maleic hydrazide	NT	NT	NT	NT	✓ in 100% MeOH	NT	NT	NT	NT	NT
MPPA	✓	✓	✓	NT	✗	✗	✗	✓	✓	✓
N-Acetyl-AMPA	✓	✓	✓	NT	✗	✗	✗	✓	✓	✓
N-Acetyl-Glufosinate	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
N-Acetyl-Glyphosate	✓	✓	✓	NT	✗	✗	✗	✓	✓	✓

* or Deuteriumoxide (D₂O), in case of deuterated ILIS's

** 0.1% HCl-conc (37%) in water (v/v)

*** Solutions of 25% and 50% of organic solvent have been tested.

[#] also tested with 0.001% HCl and shown to be stable

5.7.4.Method 1.4 “PerChloPhos”

Table 10: Proposed LC-MS/MS conditions for Phosphonic acid (Fosetyl metabolite), Perchlorate, Chlorate, Bromide and Bromate.

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Hypercarb 2.1 x 100 mm 5 µm (P/N 35005-102130); 40°C		
Pre-column	Hypercarb Guard 2.1 x 10 mm 5 µm (P/N 35005-102101)		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter (optional)		
Eluent A	1% acetic acid in water + 5% methanol		
Eluent B	1% acetic acid in methanol		
Gradient	%A	Flow [mL/min]	Time [min]
	100	0.4	0
	70	0.4	10
	100	0.4	10.1
	100	0.4	15
Injection volume	5 µL		
Dilution	1:5 dilution methanol + 1% formic acid (1 µL sample extract + 4 µL methanol + 1% formic acid)		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion* + one level at the reporting limit		
Acquired mass transitions	Compound	Mass Transitions (m/z)	
	Bromate:	127/95, 129/113, 127/111, 129/97	
	Bromate- ¹⁸ O ₃ (ILIS):	135/117	
	Bromide*:	81/81, 79/79	
	Chlorate:	83/67, 85/69	
	Chlorate- ¹⁸ O ₃ (ILIS):	89/71	
	Perchlorate:	99/83, 101/85	
	Perchlorate- ¹⁸ O ₄ (ILIS):	107/89	
Phosphonic acid:	81/79, 81/63		
Phosphonic acid- ¹⁸ O ₃ (ILIS):	87/85		

* The 5-fold dilution was shown to be suitable for Bromide screening purposes. For quantification purposes where Bromide exceeds approx. 1 mg/kg, the sample extracts should be diluted e.g. 250-fold (50-fold manually and 5-fold by the HPLC).

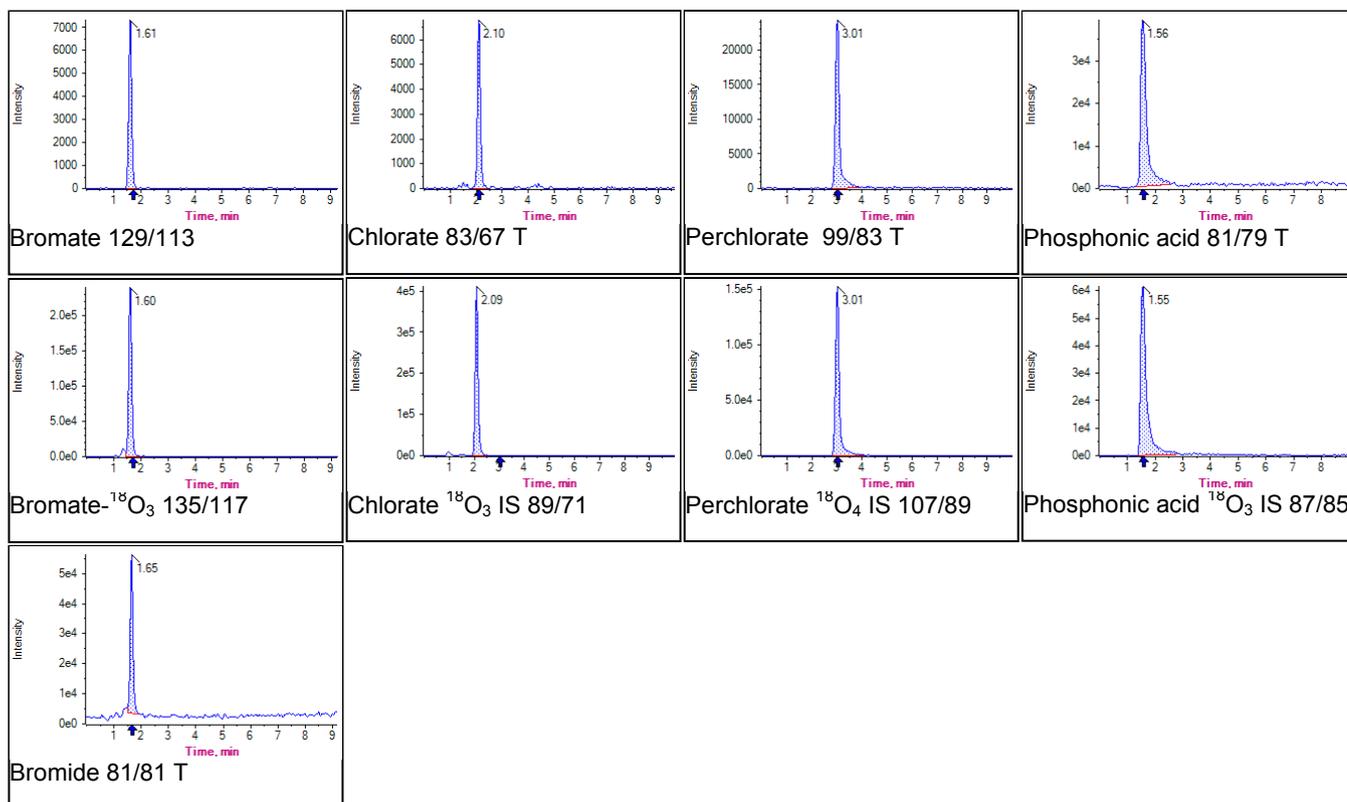


Figure 10: Chromatograms of Bromate (0.02 mg/kg in currant), Bromide (1 mg/kg in currant), Phosphonic acid (0.05 mg/kg in currant), Perchlorate (0.01 mg/kg in currant) and Chlorate (0.01 mg/kg in currant).

Hints on Method 1.4

- a) The hypercarb column and its pre-column should be thoroughly primed before usage, see hint on Method 1.3.
- b) Check the filters for any cross-contamination of Perchlorate and Chlorate. See comments under 2.6 Cellulose mixed ester filters were found to be suitable for this application!
- c) Fosetyl and Ethepon as well as their respective ILIS's degrade to Phosphonic acid. **To be on the safe side Fosetyl, Ethepon and their respective ILIS's should thus not be added to calibration solutions or samples or sample extracts intended to be used for the analysis of native phosphonic acid. Furthermore calibration solutions used for the analysis of phosphonic acid should better not contain any native Fosetyl.** See also hints on method 1.3.
- d) When extracts containing high levels of Phosphoric acid (which is naturally contained at high concentrations in many samples) are injected, the chromatographic separation of Phosphoric and Phosphonic acid is compromised. This often results in a suppression of the Phosphonic acid signal and in some cases even leads to false negative results. The most important qualifier mass-transition of Phosphonic acid (m/z 81/63) also occurs as a minor transition of the in-source fragment of Phosphoric acid, but as the latter is often present at much higher levels than Phosphonic acid the interference on this mass transition can still be significant, especially if these two elute in close vicinity (exemplarily shown at the chromatograms in Figure 11). The chromatographic separation of Phosphoric and Phosphonic acid considerably improves following dilution of the extracts typically allowing proper detection, identification and quantification of Phosphonic acid next to high levels of phosphoric acid. It is thus beneficial to inject smaller volumes of sample extract (e.g. 1-2 µL) or to dilute QuPPE extracts 5-10-fold before injection. Fortunately both, Phosphoric and Phosphonic acid have at least one proper mass-transition (m/z 97/63 and 81/79 respectively, shown in Figure 11 which in the case of Phosphonic acid can be used for quantitation and to improve identification certainty. The elution time and peak shape of the Phosphonic acid ILIS can also be used to distinguish it from Phosphoric acid and to avoid false positives. Using signals on the m/z 81/63 mass trace it was calculated that approx. 200 mg/kg Phosphoric acid would fake 0.1 mg/kg Phosphonic acid if this mass transition was used for quantification. In an experiment using Differential Mobility Separation (DMS) technique (see Figure 8 and Figure 9) a separation of Phosphoric acid and Phosphonic acid at the mass trace m/z 81/63 was achieved.

Sample	Detected signals at mass trace of		
	97/63 unique to Phosphoric acid	81/63 common to Phosphoric and Phosphonic acid	81/79 unique to Phosphonic acid

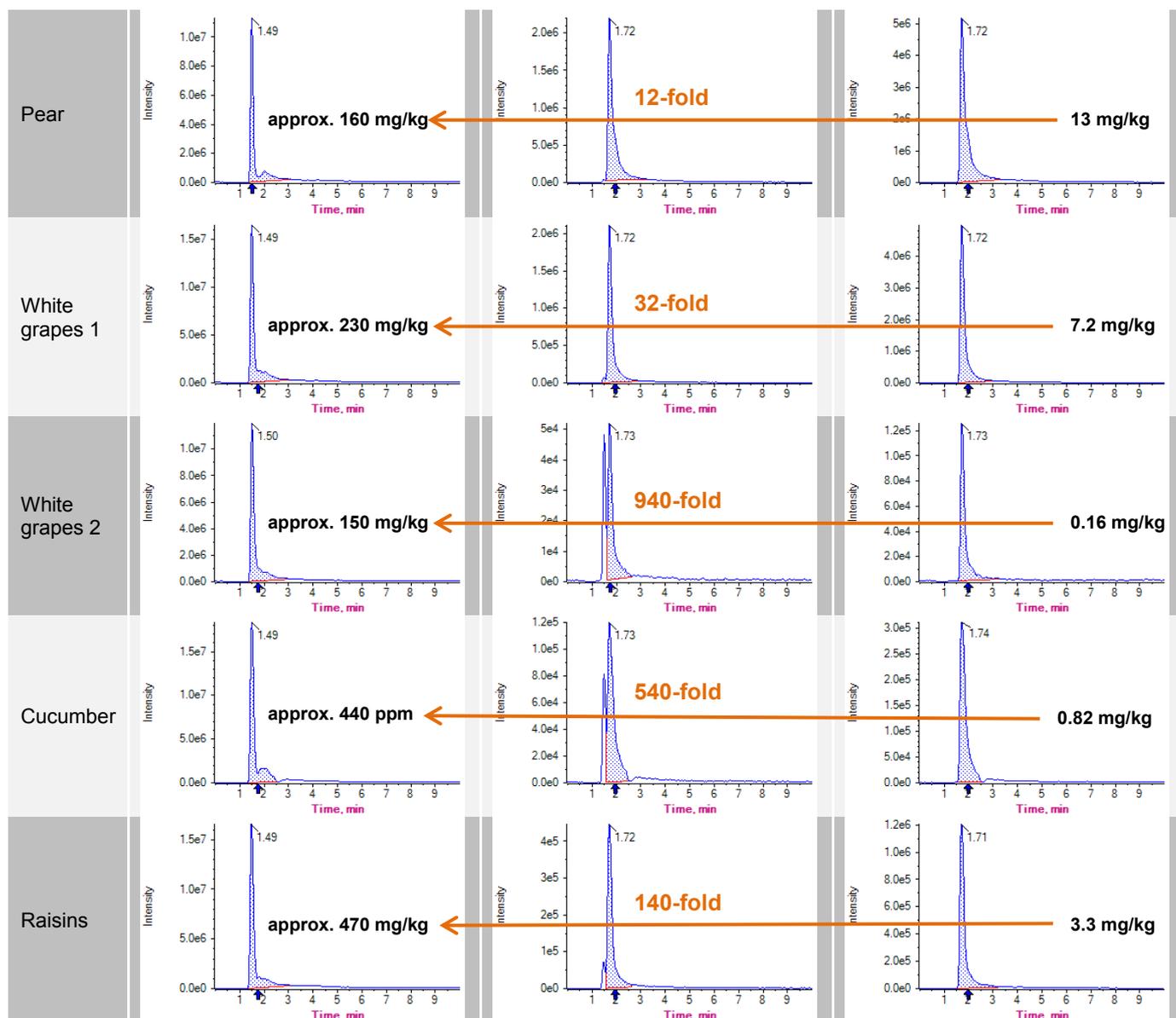


Figure 11: Chromatographic and mass-spectrometric separation of Phosphoric and Phosphonic acid.

e) High levels of Phosphoric acid (which is naturally contained at high concentrations in many samples) or Phosphonic acid (that is used as fungicide) could affect the determination of Bromide. Depending on the condition of the column, the separation of these three compounds could be insufficient, resulting in compromised identification and quantification. Bromide is mainly composed of two naturally occurring stable isotopes, that are almost equally frequent ($^{79}\text{Br}^-$ and $^{81}\text{Br}^-$). Being an element, no MS/MS fragmentation is possible so that MS/MS analysis has to rely on “parent/parent” analysis. The mass trace m/z 81/81 is recommended for quantifications whereas m/z 79/79 can be used as a qualifier.

The mass trace m/z 81/81 is interfered by Phosphonic acid (m/z of $[\text{H}_2\text{PO}_3]^- = 81$) whereas m/z 79/79 is highly affected by Phosphoric acid due to in-source fragmentation (Figure 12, the two columns declared as “CE -5 V”), the two left columns). At the mass trace m/z 81/81, 10 mg/kg Phosphonic acid simulated 7 mg/kg Bromide. At the mass trace m/z 79/79, 10 mg/kg Phosphoric acid simulated

aprox. 2.5 mg/kg bromide. In practice the interference by Phosphoric acid is more critical as it is naturally contained at high levels (e.g. 100-2000 mg/kg) in various samples. A 50-fold dilution of QuPPE extracts typically allows better identification and quantification of bromide next to high levels of Phosphoric and Phosphonic acid as chromatographic separation is improved and matrix-effects reduced.

To improve selectivity and increase quantification accuracy and identification certainty, the interferences caused by Phosphoric and Phosphonic acid can be further reduced by increasing the Collision Energy (CE) for the m/z 81 and 79 (Figure 12, the two columns declared as "CE -70 V"). While Bromide cannot be fragmented, the interfering quasi-molecular ion of Phosphonic acid (m/z 81) as well as the interfering in-source fragments of Phosphoric and Phosphonic acid (m/z 79) are largely destroyed by increased collision induced dissociation. While losing up to a 100-fold of absolute sensitivity, the interferences were largely decreased resulting in a better signal-to-noise ratio.

f)

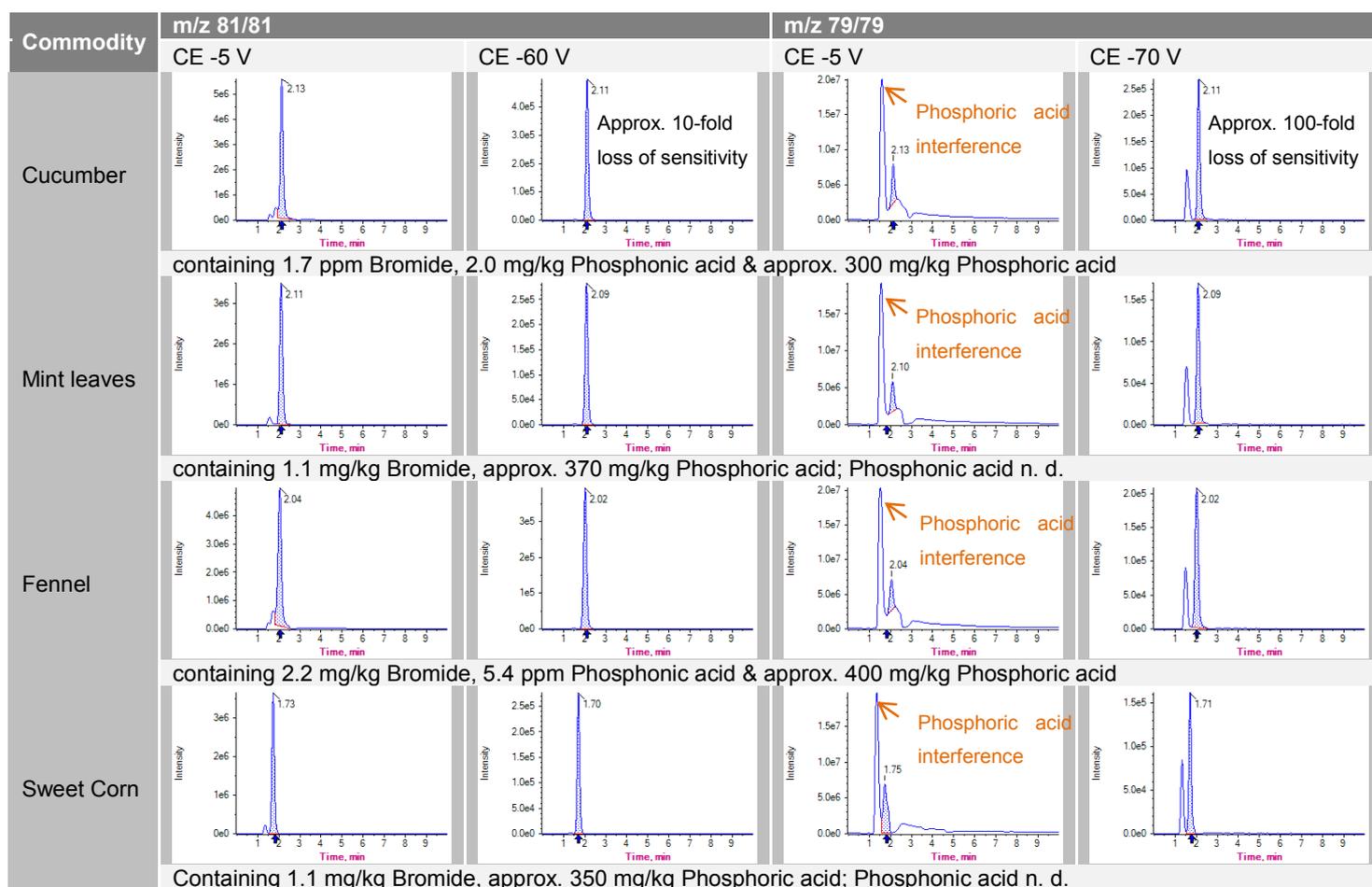


Figure 12: Chromatograms of Bromide using non-optimized collision energies (CE -5 V) showing the interference by Phosphoric acid and Phosphonic acid as well as optimized collision energies (CE -60 V and -70 V, the) showing reduced interferences.

g) Chlorate can be a minor contaminant of Perchlorate solutions and is also a minor in-source fragment of Perchlorate. In the experiment shown below Perchlorate standard at 0.2 µg/mL was injected resulting in two peaks on the mass traces of Chlorate (see Table 5). One originating from Chlorate contained as impurity in the Perchlorate solution (at approx. 0.35%) and one originating from in-source fragmentation at the retention time of Perchlorate, corresponding to a Chlorate amount of 0.001 µg/mL. This means that calibration solutions containing both chlorate and perchlorate at the same level the chlorate signal will be overestimated by approx. 0.5% which is negligible. Also samples containing perchlorate may fake the presence of chlorate at very low levels normally well below the reporting level of chlorate. When chlorate ILIS is co-injected misidentification is unlikely as the two compounds typically separate well chromatographically.

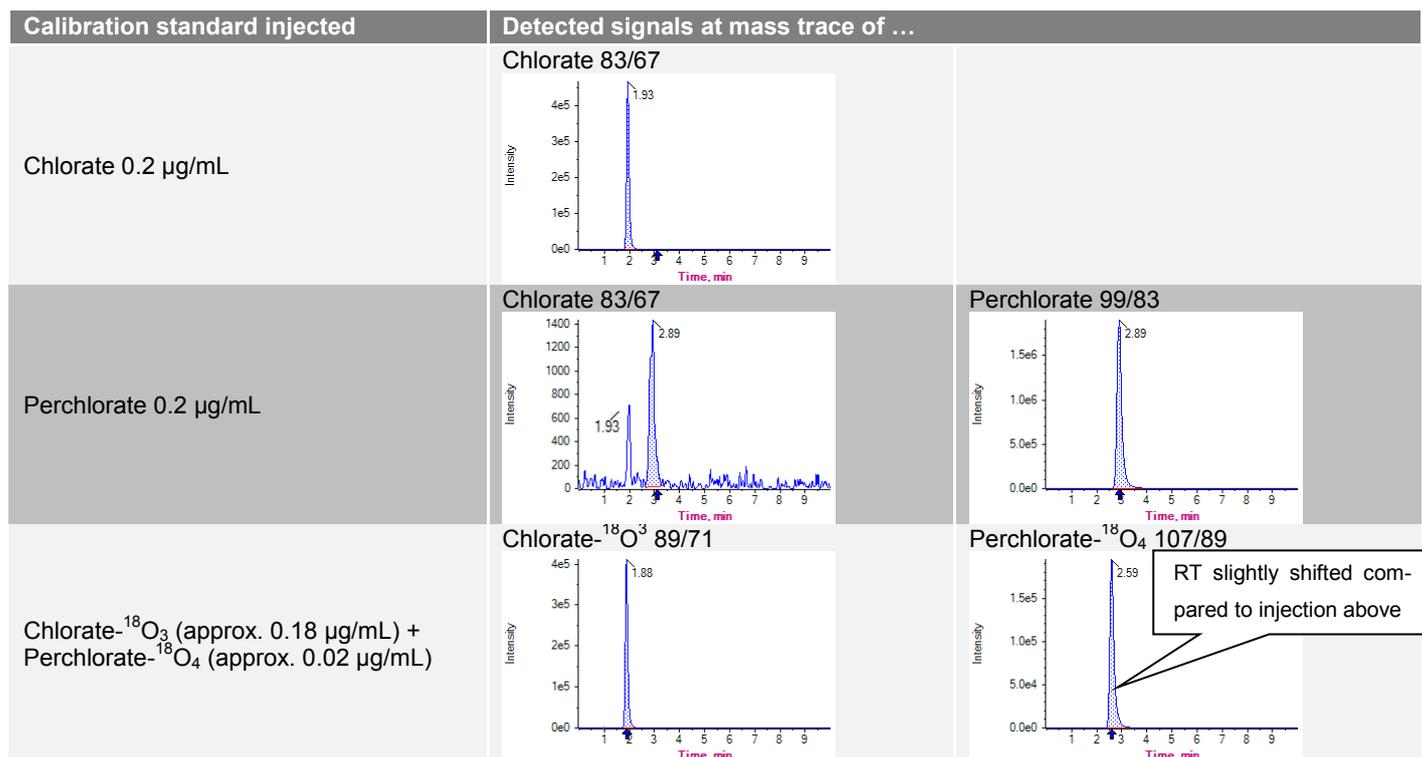


Figure 13: Chromatograms of Chlorate and Perchlorate at 0.2 µg/mL and of a mixture of Chlorate-¹⁸O₃ and Perchlorate-¹⁸O₄, containing approx. 0.2 µg/mL Chlorate-¹⁸O₃ and approx. 0.02 µg/mL Perchlorate-¹⁸O₄.

5.7.5. Method 2 “Fosetyl and Maleic Hydrazide”

Table 11: Proposed LC-MS/MS conditions for Fosetyl-Al, Maleic hydrazide and Perchlorate

Instrument parameters			
Ionization mode	ESI neg		
Column/temperature	Obelisc R 2.1 x 150 mm 5 µm 100 Å; (SIELC; OR-21.150.0510)		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter		
Pre-column	Obelisc R 2.1 x 10mm 5 µm (SIELC; OR-21.G.0510)		
Eluent A	50 mmol ammonium-formate in water + 0.1 % formic acid use brown glass bottles		
Eluent B	Acetonitrile		
Gradient	%A	Flow [mL/min]	Time [min]
	3	0.3	0
	10	0.3	6
	70	0.5	15
	70	0.5	18
	3	0.5	18.1
	3	0.5	28
Injection volume	5 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion*, + one level at the reporting limit For Maleic hydrazide (MH) an additional level at 1 or 2 µg/mL may be useful as well, due to high residue levels; consider that MH is typically only relevant for potatoes and crops of the leek family (onions etc.)		
Acquired mass transitions	Compound	Mass Transitions (m/z)	
	Fosetyl-Al:	109/81, 109/63 (detected as fosetyl)	
	Fosetyl-Al-D ₁₅ (ILIS):	114/82 (detected as fosetyl-D ₅)	
	Maleic hydrazide:	111/82, 111/42, 111/55, 111/83	
	Maleic hydrazide-D ₂ (ILIS):	113/42	
	Perchlorate:	99/83, 101/85	
Perchlorate- ¹⁸ O ₄ (ILIS):	107/89		

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

Note: It should be kept in mind that standards of isotopically labeled pesticides may contain small amounts of native (unlabelled) compounds as impurities. Typically these impurities are at low levels, so that the added amounts of native-pesticides, resulting from the addition of ISs, are insignificant. In the case of Maleic hydrazide (MH), however, the amount of IS added is comparably high due to the low detection sensitivity achieved for this compound. Assuming native MH being contained as impurity in D₂-MH at 0.25% the resulting concentration of native MH following the addition of 20 µg D₂-MH to 10 g sample will be at 0.005 mg /kg sample. This aspect is to be considered when setting the Reporting Limits of MH as well as when judging residue levels in samples having low MRLs (e.g. baby food) or organic food.

For Perchlorate better run Method 1.3 or 1.4

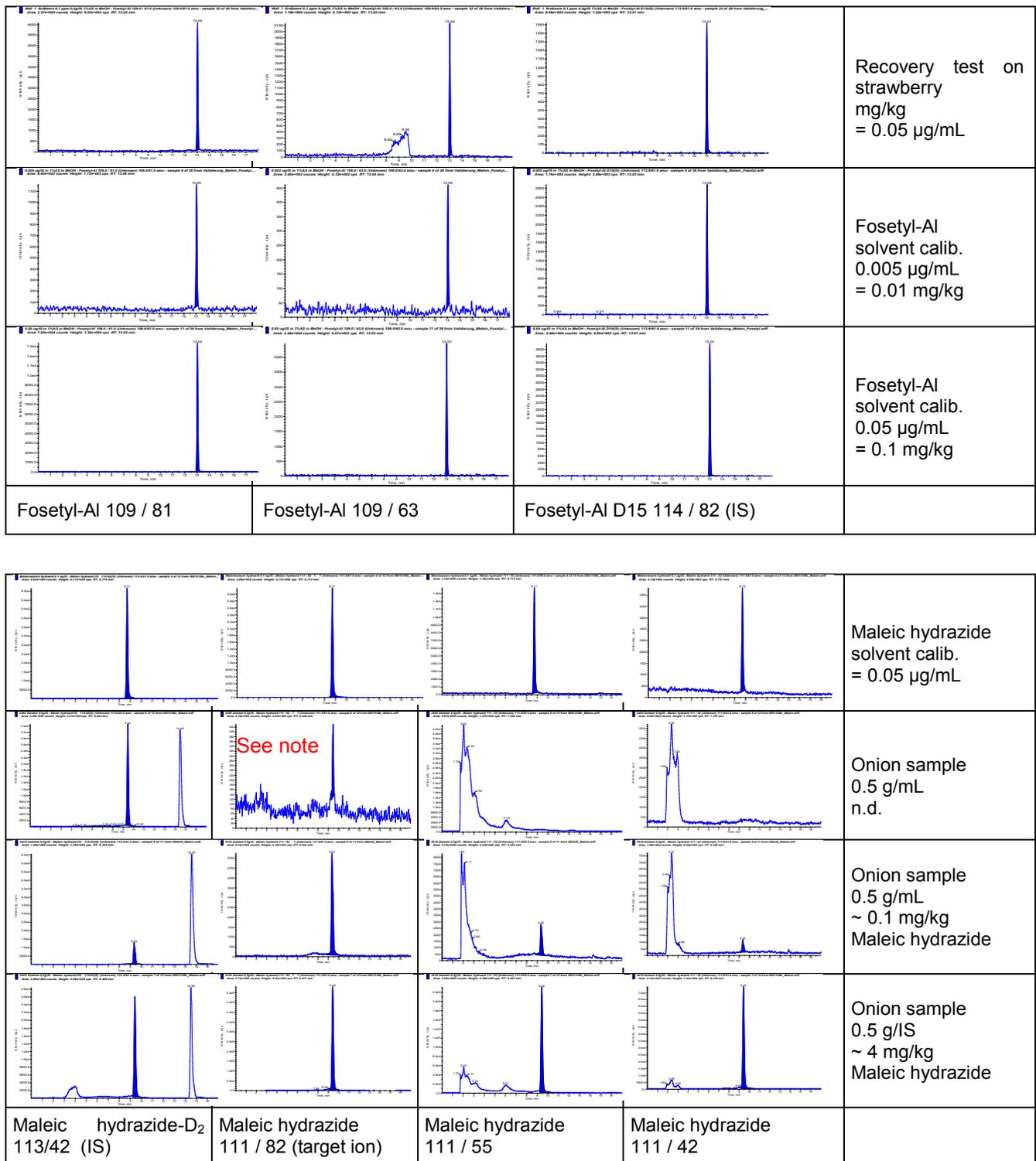


Figure 14: Typical chromatograms of Fosetyl-AI and Maleic hydrazide in various types of extracts and in pure solvent

5.7.6. Method 3 “Amitrole & Co”

Table 12: Proposed LC-MS/MS conditions for Amitrole, Chlormequat, Mepiquat, Daminozide, ETU, PTU, Trimesium, Difenzoquat and Cyromazine.

Instrument parameters	Conditions		
Ionisation mode	ESI pos		
Column/temperature	Obelisc R 2.1 x 150 mm 5 µm 100 Å (SIELC; OR-21.150.0510); 40°C		
Pre-column	Obelisc R 2.1 x 10 mm 5 µm (SIELC; OR-21.G.0510)		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter		
Eluent A	5 mmol NH ₄ -formate in water Use brown glass bottles		
Eluent B	5 mmol NH ₄ -formate acetonitrile/water 95 :5 (v/v)		
Gradient	%A	Flow [mL/min]	Time [min]
	2	0.4	0
	2	0.4	2.5
	80	0.4	5
	80	0.4	11
	2	0.4	11.1
	2	0.4	18
Injection volume	5 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion* + one level at the reporting limit		
Acquired mass transitions	Compound	Mass Transitions (m/z)	
	Amitrole:	85/43, 85/57, 85/58	
	Amitrole- ¹⁵ N (ILIS):	86/43	
	Amitrole- ¹⁵ N ₂ , ¹³ C ₂ (ILIS):	89/44	
	Chlormequat:	122/58, 122/63, 124/58	
	Chlormequat-D ₄ (ILIS):	126/58	
	Mepiquat:	114/98, 114/58	
	Mepiquat-D ₃ (ILIS):	117/101	
	Daminozide:	161/143, 161/61, 161/101, 161/115, 161/44	
	Daminozide- ¹³ C ₄ (ILIS):	165/147	
	Daminozide-D ₆ (ILIS):	167/149	
	Cyromazine:	167/68, 167/125, 167/85, 167/108,	
	Cyromazine-D ₄ (ILIS):	171/86	
	ETU (Ethylenethiourea):	103/44, 103/60, 103/86	
	ETU-D ₄ (ILIS):	107/48	
	PTU (4-Methyl-2-imidazolidinethione)**:	117/100, 117/58, 117/60, 117/72	
PTU-D ₆ (N,N'-Propylenethiourea-D ₆):	(123/64)		
Trimethylsulfonium:	77/62, 77/47		
Trimethylsulfonium-D ₉ (ILIS):	86/68		
Difenzoquat:	249/77, 249/130, 249/193		
	No ILIS currently available		
	-		

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

** The acronym PTU, commonly used for the propineb degradant 4-Methyl-2-imidazolidinethione (N,N'-iso-propylenethiourea), is also used for N,N'-propylenethiourea (= N,N'-Trimethylenethiourea). The IS tested corresponds to N,N'-propylenethiourea D₆.

Note: For Paraquat, Diquat, Trimethylsulfonium and N,N-Dimethylhydrazine better run Method 4 (5.7.7)

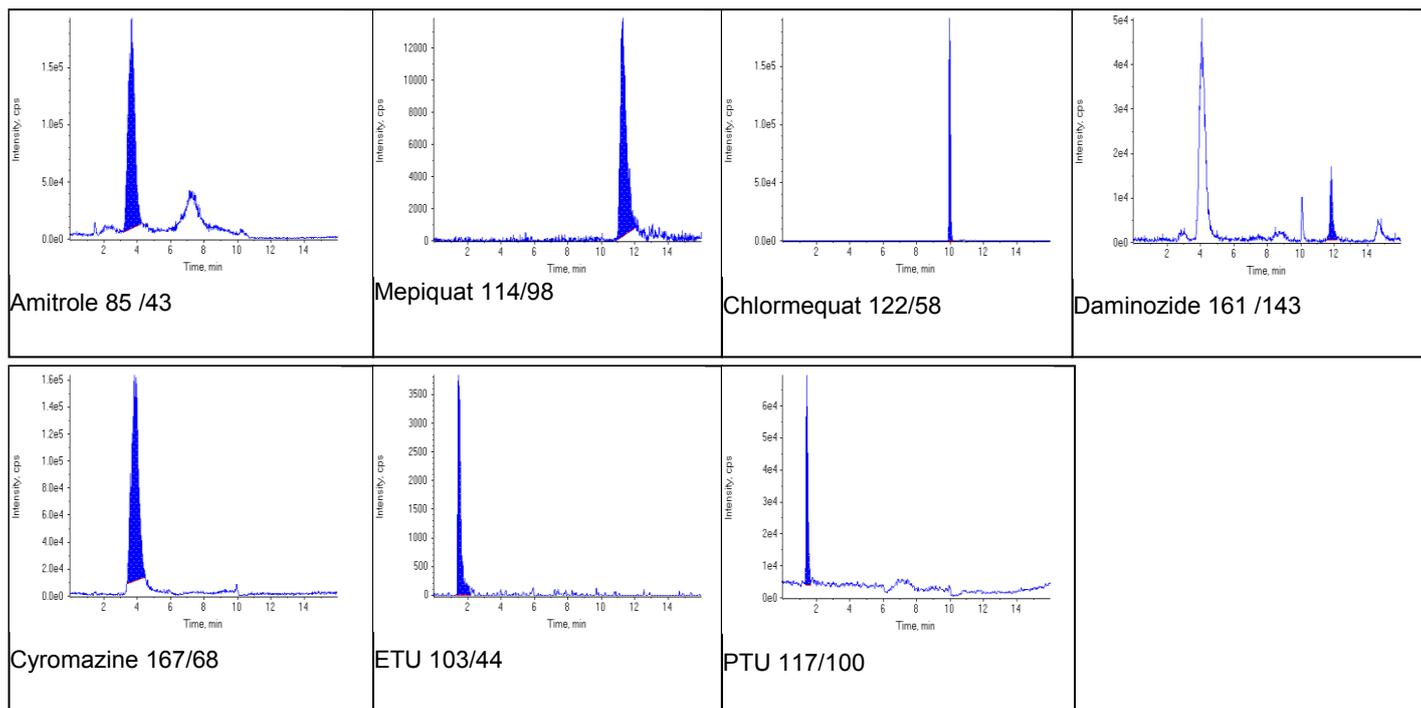


Figure 15: Typical chromatograms of Amitrole, Chlormequat, Mepiquat, Daminozide, ETU, PTU and Cyromazine in apple extract at 0.01 mg/kg

5.7.7.Method 4.1 “Quats & Co Obelisc R”

Table 13: Proposed LC-MS/MS conditions Diquat, Paraquat, Chlormequat, Mepiquat, Daminozide N,N-Dimethylhydrazine, Cyromazine, Trimethylsulfonium, Nereistoxin, Difenzoquat, Melamine and Propamocarb.

Instrument parameters	Conditions		
Ionisation mode	ESI pos		
Column/temperature	Obelisc R 2.1 x 150 mm 5 µm 100 Å (SIELC; OR-21.150.0510); 40°C		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter		
Pre-column	Obelisc R 2.1 x 10 mm 5 µm (SIELC; OR-21.G.0510)		
Eluent A	20 mmol NH ₄ -formate in water (adjust to pH 3 with formic acid), for this mix 1.8 mL formic acid (3.4) with 500 mL 20 mmol NH ₄ -formate in water Use brown glass bottles!		
Eluent B	Acetonitrile		
Gradient	%A	Flow [mL/min]	Time [min]
	20	0.4	0
	80	0.4	4
	80	0.4	12
	20	0.4	12.1
Injection volume	10 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion* + one level at the reporting limit (use plastic vials if Paraquat and Diquat are within your scope!)		
Acquired mass transitions	Compound	Mass Transitions (m/z)	
	Diquat**:	184/128, 183/157, 184/156	
	Diquat-D ₄ (ILIS):	188/160	
	Paraquat**:	186/171, 171/77, 171/155	
	Paraquat-D ₆ (ILIS):	192/174	
	Chlormequat:	122/58, 122/63, 124/58	
	Chlormequat-D ₄ (ILIS):	126/58	
	Mepiquat:	114/98, 114/58	
	Mepiquat-D ₃ (ILIS):	117/101	
	Daminozide:	161/143, 161/61, 161/101, 161/115, 161/44	
	Daminozide- ¹³ C ₄ (ILIS):	165/147	
	Daminozide-D ₆ (ILIS):	167/149	
	N,N-Dimethylhydrazine:	61/44, 61/45	
	N,N-Dimethylhydrazine-D ₆ (ILIS):	67/49	
	Cyromazine:	167/68, 167/125, 167/85, 167/108,	
	Cyromazine-D ₄ (ILIS):	171/86	
	Trimethylsulfonium:	77/62, 77/47	
	Trimethylsulfonium-D ₉ (ILIS):	86/68	
Nereistoxin:	150/105, 150/61, 150/71		
Nereistoxin-D ₆ (ILIS):	156/105		
Difenzoquat:	249/77, 249/130, 249/193		
No ILIS currently available	-		
Melamine:	127/85, 127/68, (127/60)		
Melamine- ¹⁵ N ₃ (ILIS):	130/87		
Propamocarb:	189/144, 189/102, 189/74		
Propamocarb-D ₇ (ILIS):	196/103		

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

** Diquat and Paraquat require special extraction conditions (see 0)

Note: For Morpholin, Diethanolamine (DEA) and Triethanolamine (TEA) better run Method 7 (5.6.9). As DEA converts to Morpholine in the ion source, chromatographic separation of these two is paramount. With Method 4.1 these two peaks do not sufficiently separate.

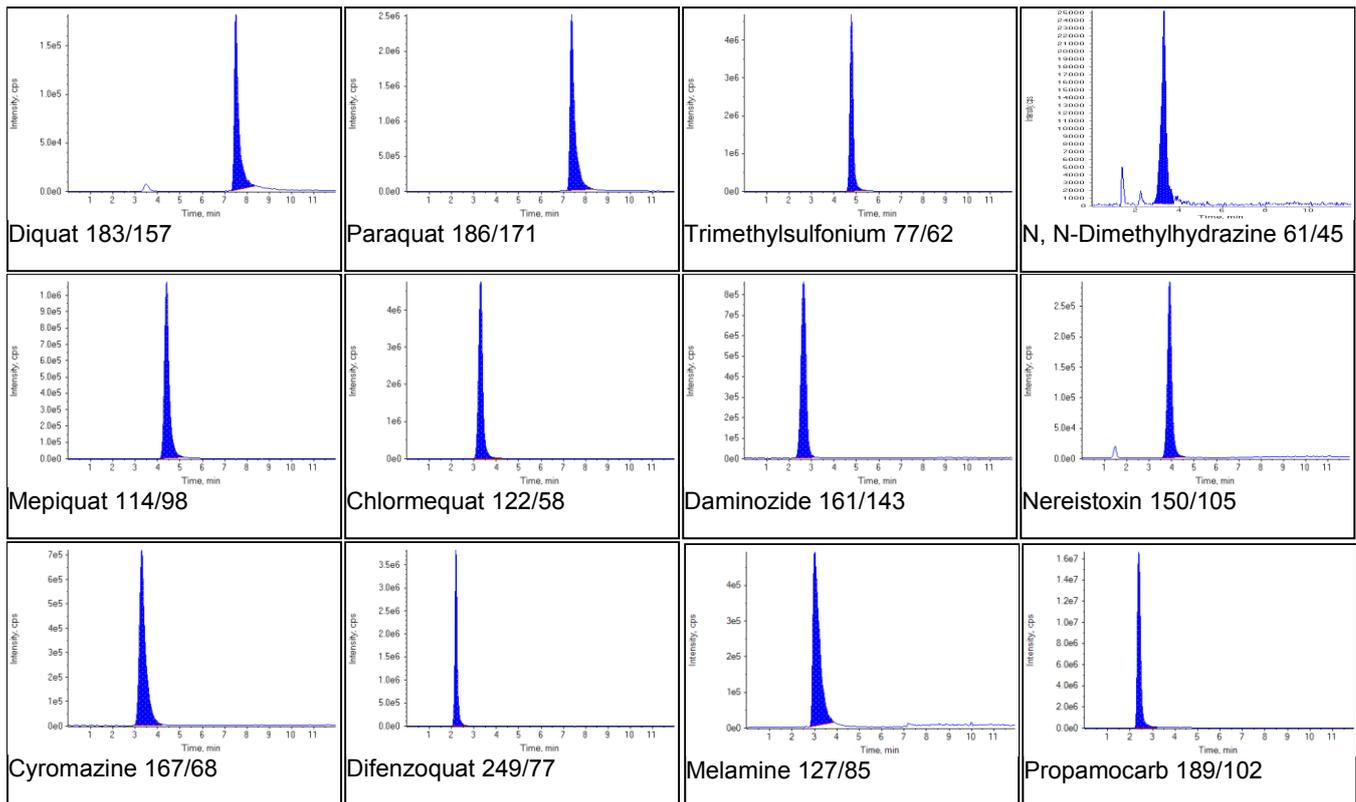


Figure 16: Typical chromatograms of Diquat, Paraquat, Chlormequat, Mepiquat, Daminozide, N,N-Dimethylhydrazine, Trimethylsulfonium, Cyromazine, Nereistoxin, Difenzoquat, Melamine and Propamocarb in apple extract at 0.1 mg/kg

5.7.8. Method 4.2 “Quats & Co BEH Amide”

Table 14: Proposed LC-MS/MS conditions for Aminocyclopyrachlor, Amitrole, Chlormequat, Chloridazon-desphenyl, Cyromazine, Daminozide, Diethanolamine, Difenzoquat, ETU, Melamine, Mepiquat, Mepiquat-4-hydroxy, Morpholine, Nereistoxin, Propamocarb, Propamocarb-N-desmethyl, Propamocarb-N-oxide, PTU, Triethanolamine, Trimesium (Trimethylsulfonium).

Instrument parameters	Conditions		
Ionisation mode	ESI pos.		
Column/temperature	BEH Amide 2.1 x 100mm 1.7 µm (P/N: 186004801); 40°C		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter		
Pre-column	BEH Amide 1.7 µm (P/N: 186004799)		
Eluent A	50 mmol NH ₄ -formate in water (adjust to pH 3 with formic acid) Use brown glass !		
Eluent B	Acetonitrile		
Gradient	%A	Flow [mL/min]	Time [min]
	3	0.5	0
	3	0.5	0.5
	30	0.5	4.0
	60	0.5	5.0
	60	0.5	6.0
	3	0.5	6.1
Injection volume	2 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion* + one level at the reporting limit		
Acquired mass transitions	Compound	Mass Transitions (m/z)	
	Aminocyclopyrachlor:	214/170, 214/168, 214/101	
	Amitrole:	85/43, 85/57, 85/58	
	Amitrole- ¹⁵ N (ILIS):	86/43	
	Amitrole- ¹⁵ N ₂ ¹³ C ₂ (ILIS):	89/44	
	Chlormequat:	122/58, 124/58, 122/63	
	Chlormequat-D ₄ (ILIS):	126/58	
	Chloridazon-desphenyl:	146/117, 146/101, 146/66	
	Chloridazon-desphenyl- ¹⁵ N ₂ (ILIS):	148/117	
	Cyromazine:	167/68, 167/125, 167/108, 167/85	
	Cyromazine-D ₄ (ILIS):	171/86	
	Daminozide:	161/143, 161/61, 161/101, 161/115,	
	Daminozide- ¹³ C ₄ (ILIS):	161/44	
	Daminozide-D ₆ (ILIS):	165/147 ; 167/149	
	Diethanolamine*** (DEA):	106/88, 106/70, 106/45	
	Diethanolamine-D ₄ (ILIS):	110/92	
	Difenzoquat:	249/130, 249/77, 249/193,	
	No ILIS currently available	-	
	ETU (Ethylenethiourea):	103/60, 103/44, 103/86	
	ETU-D ₄ (IS):	107/48	
Melamine:	127/85, 127/68, (127/60)		
Melamine- ¹⁵ N ₃ (ILIS):	130/87		
Mepiquat:	114/98, 114/58		
Mepiquat-D ₃ (ILIS):	117/101		
Mepiquat-4-hydroxy:	130/58, 130/96, 130/114		
Morpholine***:	88/70, 88/45, 88/44		
Morpholine-D ₈ (ILIS):	96/78		
Nereistoxin:	150/105, 150/61, 150/71		
Nereistoxin-D ₆ (ILIS):	156/105		
Propamocarb:	189/144, 189/74, 189/102		
Propamocarb-D ₇ (ILIS):	196/103		
Propamocarb-N-desmethyl:	175/102, 175/144, 175/74		
Propamocarb-N-oxide:	205/102, 205/144, 205/74		

PTU (4-Methyl-2-imidazolidinethione)**: PTU-D ₆ (N,N'-Propylenethiourea-D ₆):	117/100, 117/58, 117/60, 117/72 123/64
Triethanolamine*** (TEA): Triethanolamine-D ₁₂ (ILIS):	150/132, 150/70, 150/88 162/144
Trimethylsulfonium: Trimethylsulfonium-D ₉ (ILIS):	77/62, 77/47 86/68

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).
 **The acronym PTU, commonly used for the propineb degradant 4-Methyl-2-imidazolidinethione (N,N'-iso-propylenethiourea), is also used for N,N'-propylenethiourea (= N,N'-Trimethylenethiourea). The IS tested corresponds to N,N'-propylenethiourea-D₆.
 ***For Morpholin, Diethanolamine and Triethanolamine better run Method 7 (5.6.9) as these compounds seem to be strongly suppressed by matrix using these LC-conditions. For Diethanolamine even false negative results are observed in some cases. This effect is reduced if the extract is diluted e.g. 5/10 fold.

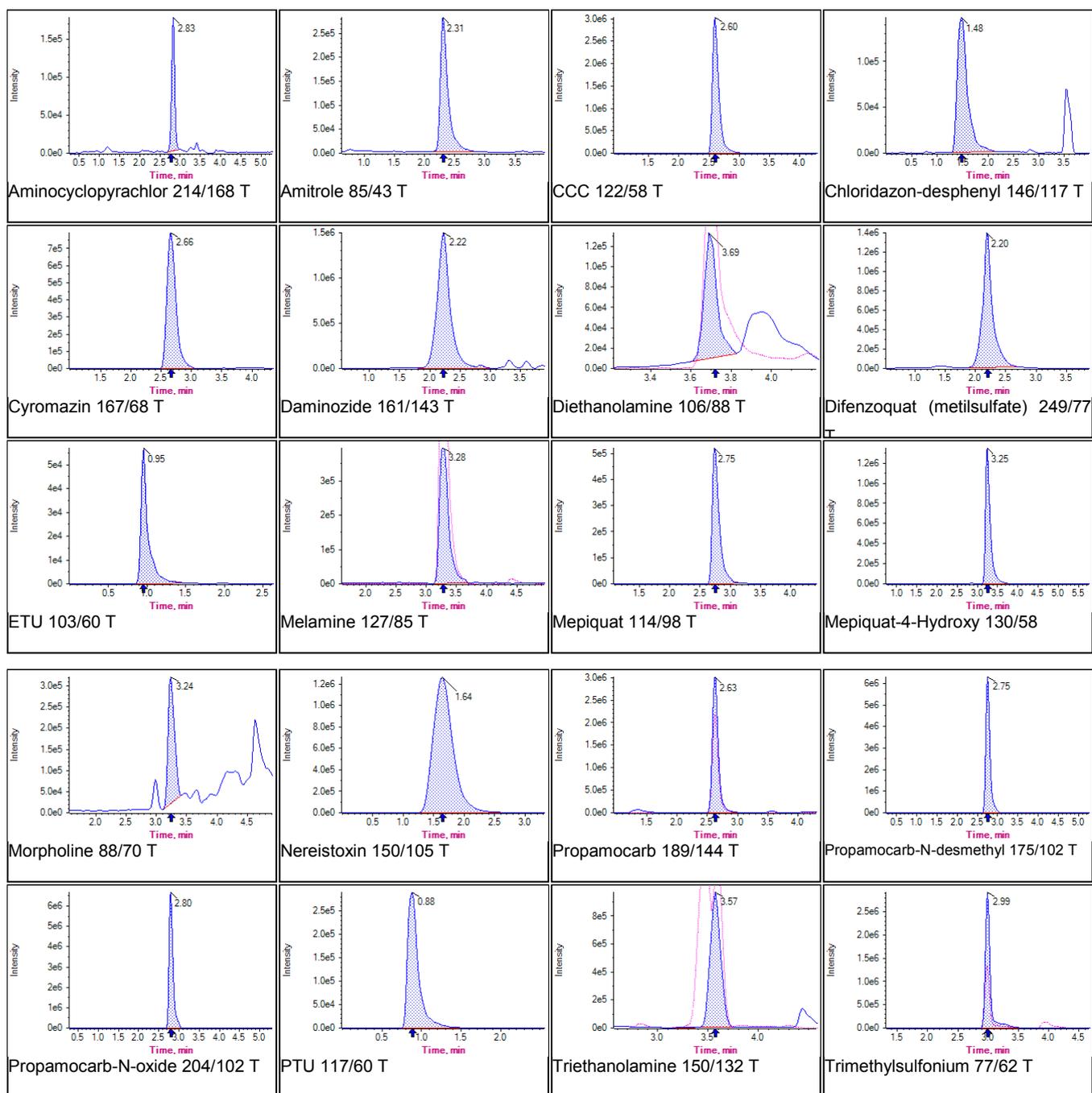


Figure 17: Typical chromatograms of Aminocyclopyrachlor, Amitrole, Chlormequat, Chloridazon-desphenyl, Cyromazine, Daminozide, Diethanolamine, Difenzoquat, ETU, Melamine, Mepiquat, Mepiquat-

4-hydroxy, Morpholine, Nereistoxin, Propamocarb, Propamocarb-N-desmethyl, Propamocarb-N-oxide, PTU, Triethanolamine, Trimesium (Trimethylsulfonium) in tomato extracts spiked at 0.05 mg/kg.

5.7.9. Method 5 “Quats & Co. MonoChrom MS”

Table 15: Proposed alternative LC-MS/MS conditions for Chlormequat and Mepiquat

Instrument parameters	Conditions		
Ionisation mode	ESI pos		
Column/temperature	MonoChrom MS 100x2 mm; 5 µm (Varian); at 40°C		
Eluent A	5 mmol/L NH4-acetate + 0.1% acetic acid in water		
Eluent B	Acetonitrile		
Gradient	%A	Flow [mL/min]	Time [min]
	5	0.4	0
	95	0.4	2
	95	0.4	5
	5	0.4	5.1
	5	0.4	15
Injection volume	5 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion*+ one level at the reporting limit		
Acquired mass transitions	Compound	Mass Transitions (m/z)	
	Chlormequat:	122/58, 122/63, 124/58	
	Chlormequat-D ₄ (ILIS):	126/58	
	Mepiquat:	114/98, 114/58	
	Mepiquat-D ₃ (ILIS):	117/101	
	Difenzoquat:	249/77, 249/130, 249/193	
	No IS currently available	-	
	ETU (Ethylenethiourea):	103/44, 103/60, 103/86	
	ETU-D ₄ (ILIS):	107/48	
PTU (4-Methyl-2-imidazolidinethione)**:	117/100, 117/58, 117/60, 117/72		
PTU-D ₆ (N,N'-Propylenethiourea-D ₆):	123/64		

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

**The acronym PTU, commonly used for the propineb degradant 4-Methyl-2-imidazolidinethione (N,N'-isopropylenethiourea), is also used for N,N'-propylenethiourea (= N,N'-Trimethylenethiourea). The IS tested corresponds to N,N'-propylenethiourea-D₆.

For more information on method 5 please refer to the following document within the EURL homepage:

http://www.crl-pesticides.eu/library/docs/srm/meth_ChlormequatMepiquat_CrISrm.pdf

5.7.10. Method 6 “Streptomycin and Kasugamycin”

Table 16: Proposed LC-MS/MS conditions Streptomycin and Kasugamycin

Instrument parameters	Conditions		
Ionisation mode	ESI pos		
Column	Obelisc R 2.1 x 150 mm 5µm 100 Å (SIELC; OR-21.150.0510); 40°C		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter		
Pre-column	Obelisc R 2.1 x 10 mm 5 µm (SIELC; OR-21.G.0510)		
Eluent A	0.1% formic acid in water		
Eluent B	0.1% formic acid in acetonitrile		
Gradient	%A	Flow [mL/min]	Time [min]
	20	0.3	0
	20	0.3	5
	80	0.5	10
	80	0.5	15
	20	0.3	15.1
20	0.3	23	
Injection volume	20 µL; dwell time increased to 200 ms		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion* one level at the reporting limit (use plastic vials if Streptomycin is within your scope)		
Acquired mass transitions	Compound	Mass Transitions (m/z)	
	Streptomycin:	582/263, 582/246, 582/ 221	
	Dihydrostreptomycin (IS):	584/263	
	Kasugamycin:	380/112, 380/200	
	No IS currently available	-	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

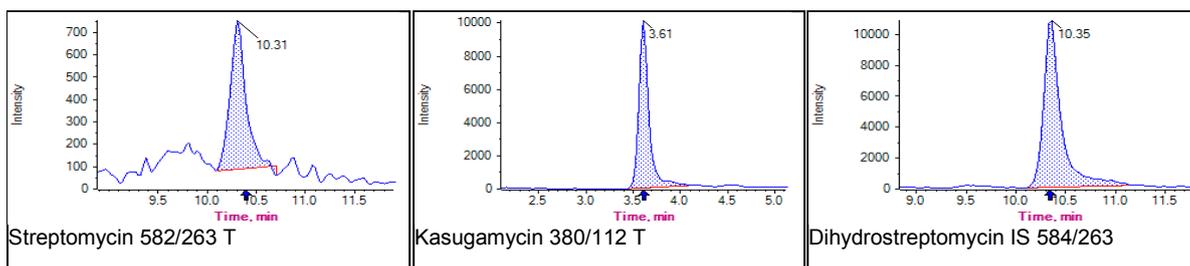


Figure 18: Typical chromatograms of Streptomycin and Kasugamycin in apple extracts spiked at 0.01 mg/kg.

5.7.11. Method 7 “Morpholine, Diethanolamine and Triethanolamine”

Table 17: Proposed LC-MS/MS conditions Morpholine, Diethanolamine and Triethanolamine

Instrument parameters	Conditions		
Ionisation mode	ESI pos		
Column	Dionex Acclaim Trinity P1 2.1 x 100 mm (3 µm) (P/N 071389); 40°C		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter		
Pre-column	Dionex Acclaim Trinity P1 2.1 x 10 mm (3 µm) (P/N 071391)		
Eluent A	50 mmol NH ₄ -formate in water (adjust to pH 3 with formic acid) Use brown glass bottles!		
Eluent B	Acetonitrile		
Gradient	%A	Flow [mL/min]	Time [min]
	10	0.4	0
	10	0.4	10
Injection volume	5 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion+ one level at the reporting limit		
Acquired mass transitions	Compound	Mass Transitions (m/z)	
	Morpholine:	88/70, 88/45, 88/44	
	Morpholine-D ₈ (IS):	96/78	
	Diethanolamine (DEA):	106/88, 106/70, 106/45	
	Diethanolamine-D ₄ (IS):	110/92	
Triethanolamine (TEA):	150/132, 150/70, 150/88		
Triethanolamine-D ₁₂ (IS):	162/144		

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

Morpholin, DEA and TEA are not pesticides, they are additive of waxes used to coat crops (citrus, apples and mangoes etc). They are included in this method for the sake of convenience and synergy. As these three compounds can be analyzed very sensitively 5-10-fold dilution of the extracts before injection is recommendable where possible, especially in absence of an IS requiring standard additions approach (0)

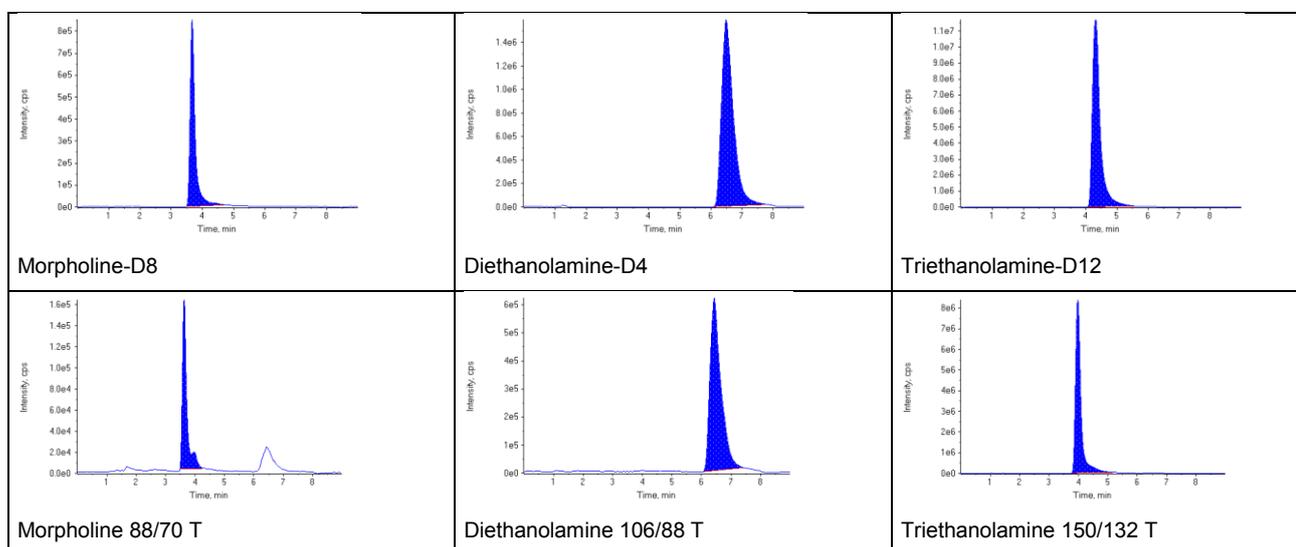


Figure 19: Typical chromatograms of Morpholine, Diethanolamine and Triethanolamine in apple extracts at 0.05 mg/kg (extract were diluted 10-fold before injection)

5.7.12. Method 8 “Triazole derivative metabolites (TDMs)”

Table 18: Proposed LC-MS/MS conditions 1,2,4-Triazole, Triazole-alanine, Triazole-acetic acid, Triazole-lactic acid and 1,2,3- Triazole

Instrument parameters	Conditions			
Ionisation mode	ESI pos			
Column	Hypercarb 2.1 x 100 mm 5 µm (P/N 35005-102130); 40°C			
Pre-column	Hypercarb Guard 2.1 x 10 mm 5 µm (P/N 35005-102101)			
Pre-filter	e.g. Supelco column saver 2.0 µm Filter (optional)			
Eluent A	1% acetic acid in water + 5% methanol			
Eluent B	1% acetic acid in methanol			
Gradient	%A	Flow [mL/min]	Time [min]	
	100	0.6	0	
	10	0.6	5	
	10	0.6	6	
	100	0.6	6.1	
Injection volume	2 µL			
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion* one level at the reporting limit			
Acquired mass transitions	Compound**	Mass Transitions (m/z)	Selexion Q-Trap® 550 DMS-Conditions (DMS temperature: low)***	
			COV (V)	SV (V)
	1,2,4-Triazole [#] :	70/43, 70/70	-10	2600
	1,2,4-Triazole- ¹³ C ₂ , ¹⁵ N ₃ (IS):	75/46	-13.75	3000
	Triazole-alanine:	157/70, 157/88,	-2.0	3000
	Triazole-alanine- ¹³ C ₂ , ¹⁵ N ₃ (IS):	157/42, 162/75	-1.75	3100
	Triazole-acetic acid:	128/70, 128/43,	-6.0	3100
	Triazole-acetic acid- ¹³ C ₂ , ¹⁵ N ₃ (IS):	128/73, 133/75	-6.0	3500
Triazole-lactic acid:	158/70, 158/43,	-3.0	3300	
Triazole-lactic acid- ¹³ C ₂ , ¹⁵ N ₃ (IS):	158/112, 163/75	-2.25	3500	
1,2,3-Triazole [#] :	70/43	-12	3000	
No IS currently available	-	-	-	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

** All ILISs were a friendly donation and are at the time not commercially available.

*** Further parameters: CUR 20, GS1 60, GS2 70, DMO -3.0. DMS condition differ to some extent from instrument to instrument.

1,2,4-Triazole and 1,2,3-Triazole are used as nitrification inhibitors in fertilizers

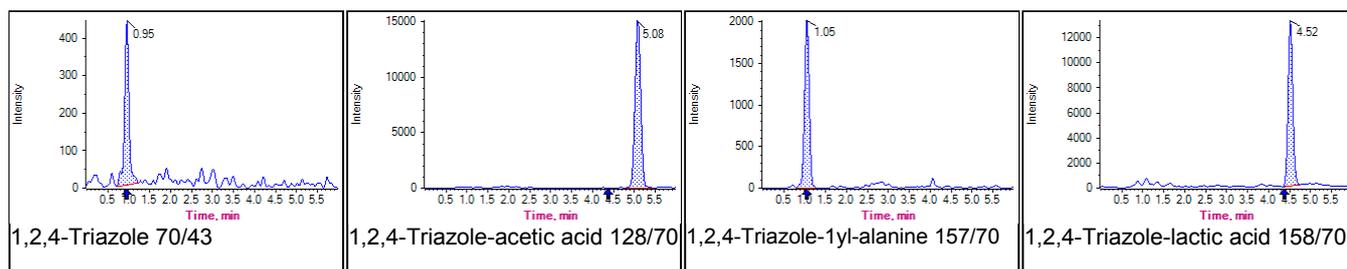


Figure 20: Typical chromatograms of TDMs in avocado extracts spiked at 0.01 mg/kg.

5.7.13.Method 9 “Difluoroacetic acid and Trifluoroacetic acid”

Table 19: Proposed LC-MS/MS and Selexion conditions Difluoroacetic and Trifluoroacetic acid

Instrument parameters	Conditions			
Ionisation mode	ESI neg			
Column	Dionex/Thermo, Acclaim Trinity P1, 2.1 x 100 mm, (3 µm) (P/N 071389); 40°C			
Pre-column	Thermo Guard Cartridge Acclaim Trinity P1, 2.1 x 10 mm, (3 µm) (P/N 071391)			
Pre-filter	e.g. Supelco column saver 2.0 µm Filter (optional)			
Eluent A	50 mmol NH ₄ -formate, adjusted to pH 3 with formic acid			
Eluent B	acetonitrile			
Gradient	%A	Flow [mL/min]	Time [min]	
	10	0.45	0	
	10	0.45	3.5	
	50	0.45	4	
	50	0.45	6	
	10	0.45	6.1	
10	0.45	10		
Injection volume	2 µL			
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion*, one level at the reporting limit. Always use matrix based calibrations (e.g. blank tomato extract) instead of solvent based.			
Acquired mass transitions	Compound	Mass Transitions (m/z)	Selexion Q-Trap® 5500 DMS-Conditions (DMS temp.: medium)****	
			COV (V)	SV (V)
	Difluoroacetic acid (DFA):	95/51, 95/95***	-9.5	2500
	Difluoroacetic acid - ¹³ C ₂ (ILIS)**:	75/46	-12	3000
	Trifluoroacetic acid (TFA)	113/69, 113/113***	-5.6	2200
Trifluoroacetic acid - ¹³ C ₂ (ILIS):	115/70	-5.5	2300	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also Table 1).

** ILIS was a friendly donation and is at the time not commercially available.

*** Despite not having a mass transition the DMS provides good selectivity

**** DMS condition differ to some extent from instrument to instrument (further parameters: CUR 20, GS1 60, GS2 70, DMO -3.0)

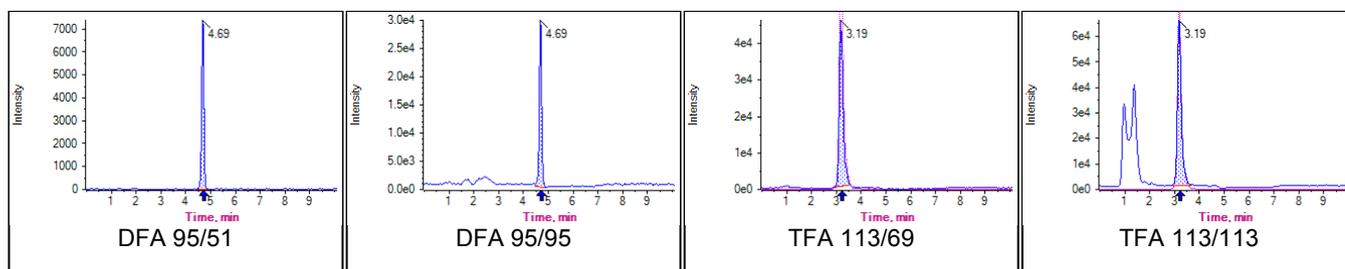


Figure 21: Typical chromatograms of DFA and TFA in tomato extracts spiked at 0.05 mg/kg.

5.8. Calibration and Calculations

5.8.1. Using IS

Where IS is added to the sample before any aliquotation:

The following calculation approach requires that the ratio of the IS masses added to the test portions (5.2.3) and to the calibration standard(s) (5.5) ($m_{IS}^{sample} / m_{IS}^{cal\ mix}$) is known and constant. By keeping the IS constant throughout the calibration levels the peak ratio $PR^{cal\ mix}$ ($A_{pest}^{cal\ mix} / A_{IS}^{cal\ mix}$) of each calibration level can be plotted against the absolute mass of the pesticide $m_{pest\ cal\ mix}$ rather than the ratio $m_{pest\ cal\ mix} / m_{IS}^{cal\ mix}$ (the $m_{IS}^{cal\ mix}$ is set as 1).

The calibration graph (to be plotted for each pesticide separately) is described by the following formula:

$$PR^{cal\ mix} = a_{cal} \times m_{pest}^{cal\ mix} + b_{cal} \quad (1)$$

The mass fraction (w_R) of a given pesticide in a given sample can be calculated as follows using the respective peak ratio of pesticide and internal standard obtained from the sample extract ($PR^{sample} = A_{pest}^{sample} / A_{IS}^{sample}$), the correction factor ($m_{IS}^{sample} / m_{IS}^{cal\ mix}$) as well as the weight of the test portion (m_a).

$$w_R = \frac{(PR^{sample} - b_{cal})}{a_{cal}} \times \frac{1}{m_a} \times \frac{m_{ISTD}^{sample}}{m_{ISTD}^{cal\ mix}} \begin{pmatrix} \text{mg} \\ \text{kg} \end{pmatrix} \quad (2)$$

Reasonably (but not necessarily) the calibration standards should be prepared in such a way that the ratio $m_{IS}^{sample} / m_{IS}^{cal\ mix}$ equals 20 (the assumed volume ratio of sample extract versus calibration standard). The absolute masses of the IS-WS I and II do not need to be necessarily known (see also the notes of Table 1).

Where IS is added to an aliquot of the extract

When adding the IS to an aliquot of the extract (e.g. 1 mL) the knowledge of the exact total volume of the sample extract becomes important. Water adjustment is thus essential and if it is done as described in 5.2.2 and **Table 18**, the total volume can be assumed to be exactly 20 mL. In this case 1 mL sample extract will correspond to 1/20th of the test portion (m_a). The mass of the IS to be added to an aliquot ($m_{IS}^{aliquot}$) should be scaled according to the aliquot volume used ($V_{aliquot}$) with the IS mass ratio ($m_{IS}^{aliquot} / m_{IS}^{cal\ mix}$) being important for the calculation. Reasonably (but not necessarily) $m_{IS}^{aliquot}$ should be derived using the following formula $m_{IS}^{aliquot} = m_{IS}^{sample} \times V_{aliquot}/20$, with m_{IS}^{sample} being the IS mass that would have been added to the entire sample portion according to 5.2.2 and **Table 18**.

Following the above, the mass fraction (w_R) of a given pesticide in a given sample can be calculated as follows using the respective peak ratio of pesticide and internal standard obtained from the sample extract ($PR^{sample} = A_{pest}^{sample} / A_{IS}^{sample}$), the correction factor ($m_{IS}^{aliquot} / m_{IS}^{cal\ mix}$) as well as the weight of the sample equivalents in the aliquot ($m_{aliquot} = m_a \times V_{aliquot}/20$).

$$w_R = \frac{(PR^{sample} - b_{cal})}{a_{cal}} \times \frac{1}{m_{aliquot}} \times \frac{m_{ISTD}^{aliquot}}{m_{ISTD}^{cal\ mix}} \begin{pmatrix} \text{mg} \\ \text{kg} \end{pmatrix} \quad (3)$$

Variables used

Mass of pesticide in calibration mixture	$m_{pest}^{cal\ mix}$	µg
Mass of pesticide in final extract	m_{pest}^{sample}	µg
Mass of internal standard in calibration mixture	$m_{ISTD}^{cal\ mix}$	µg
Mass of internal standard added to test portion (sample)	m_{ISTD}^{sample}	µg
Mass of internal standard added to aliquot of sample extract	$m_{ISTD}^{aliquot}$	µg
Volume of sample extract aliquot used (5.8.1 and 0) to spike the IS or for standard additions	$V_{aliquot}$	mL
Mass of test portion	m_a	g
Mass of test portion represented in an aliquot	$m_{aliquot}$	g
Mass fraction of pesticide in the sample	w_R	mg/kg
Peak area of pesticide obtained from calibration standard (mixture)	$A_{pest}^{cal\ mix}$	(counts)
Peak area of IS obtained from calibration standard (mixture)	$A_{ISTD}^{cal\ mix}$	(counts)
Peak area of pesticide obtained from the injected extract	A_{pest}^{sample}	(counts)
Peak area of IS obtained from the injected extract	A_{ISTD}^{sample}	(counts)
Peak ratio of pesticide vs. IS obtained from calibration mixture	$PR^{cal\ mix}$	(dimensionless)
Peak ratio of pesticide vs. IS obtained from injected extract	PR^{sample}	(dimensionless)
Slope of calibration graph	a_{cal}	(dimensionless)
Bias of calibration graph (intercept)	b_{cal}	(dimensionless)

5.8.2. Not using IS

If no appropriate ISs are used it is of high importance to properly compensate for matrix effects. For the compensation of matrix effects matrix-matched calibrations (5.5.2) and the standard additions approach (0) are recommended. In both cases the assumption is made that the total volume of the sample extract is exactly 20 mL. Adjustment of the water content (and extract volume) in the sample is thus paramount.

Calculations when employing matrix-matched calibration without IS

The calibration graph (to be plotted for each pesticide separately) is described by the following formula:

$$A_{pest}^{cal\ mix} = a_{cal} \times C_{pest}^{cal\ mix} + b_{cal} \quad (1)$$

The mass fraction (w_R) of a given pesticide in a given sample can be calculated as follows using the respective peak area of the pesticide obtained from the sample extract (A_{pest}^{sample}) and a correction factor (V) as well as the weight of the test portion (m_a).

$$w_R = \frac{(A_{pest}^{sample} - b_{cal})}{a_{cal}} \times \frac{1}{m_a} \times V_{end} \left(\frac{mg}{kg} \right) \quad (2)$$

where V_{end} is the total volume of the sample extract (20 mL).

All other variables are listed in 5.8.1.

Calculations when employing the standard additions approach

The standard additions approach is the method of choice where no appropriate IL-IS is available. This approach typically compensates matrix effect better than the matrix-matched calibrations (5.5.2). The mass fraction of the pesticide in the sample (w_R) is calculated via linear regression using a graphical presentation as shown in Figure 1. The Y-intercept of the calibration graph will indicate the pesticide mass contained in the non-fortified aliquot of the sample extract.

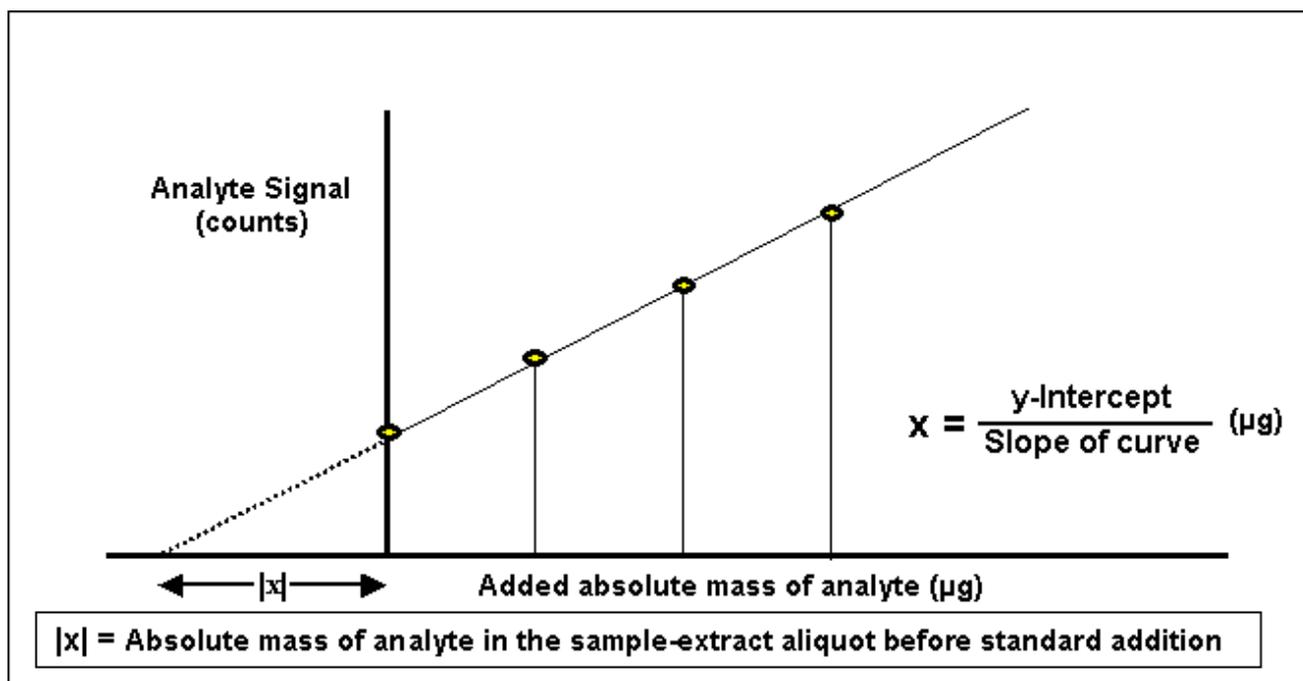


Figure 21: Internal calibration using the procedure of standard additions, schematically

Key:

Y Peak area of analyte

X Added absolute mass of analyte $m_{pest}^{std\ add}$ in μg

|x| absolute amount of analyte in the sample extract (in μg) before standard addition ($y = 0$)

$$\text{With } x = \frac{\text{y-intercept } (b)}{\text{slope of the curve } (a)} \quad (\mu\text{g})$$

The calculation is performed as follows using the regression graph shown in

$$w_R = \frac{b}{a} \times \frac{V_{end}}{V_{al} \times m_a} \left(\frac{\text{mg}}{\text{kg}} \right)$$

where:

b Y-intercept of the calibration graph of the analyte in question;

a Slope of the calibration graph of the analyte in question ($1/\mu\text{g}$);

V_{end} Volume of sample extract (mL) (should be 20 mL)

V_{al} Volume of aliquots used for the standard additions approach (mL)

m_a Weight of initial sample portion (g)

6. Performance Data

Validation data of the presented methods according to SANTE/11945/2015 guidance document are shown at the EURL validation database at www.eurl-pesticides-datapool.eu. Exemplary LOQs of the presented methods are listed in Table 19.

Table 19: Overview of lowest successful validated levels per matrix

Method	Analyte	Commodity Group	Matrix	Spiking Level (ppm)	n	Rec.	RSD
M 1.3	AMPA	High acid content	Grapes	0.02	12	110	9
	AMPA	Dry (cereals, dry pulses)	Barley	0.02	5	101	14
	AMPA	Dry (cereals, dry pulses)	Lentil	0.1	10	95	17
	AMPA	Dry (cereals, dry pulses)	Wheat flour	0.1	5	119	6
	AMPA	High water content	Apple	0.02	17	100	12
	Cyanuric Acid	High water content	Cucumber	0.02	3	106	13
	Ethephon	Dry (cereals, dry pulses)	Barley	0.02	5	110	2
	Ethephon	Dry (cereals, dry pulses)	Wheat flour	0.1	5	85	6
	Ethephon	High water content	Apple	0.02	7	105	11
	Ethephon	High water content	Cucumber	0.02	3	101	11
	Fosetyl	High acid content	Strawberry	0.1	6	94	4
	Fosetyl	Dry (cereals, dry pulses)	Barley	0.02	5	106	7
	Fosetyl	High water content	Apple	0.02	7	104	5
	Fosetyl	High water content	Cucumber	0.02	3	103	5
	Glufosinate	Dry (cereals, dry pulses)	Barley	0.02	5	101	13
	Glufosinate	Dry (cereals, dry pulses)	Wheat flour	0.1	5	85	5
	Glufosinate	High water content	Apple	0.02	7	106	8
	Glufosinate	High water content	Cucumber	0.02	3	115	4
	Glyphosate	High acid content	Grapes	0.02	12	112	8
	Glyphosate	Dry (cereals, dry pulses)	Barley	0.02	5	105	8
	Glyphosate	Dry (cereals, dry pulses)	Lentil	0.1	11	107	18
	Glyphosate	Fatty, dry (oil seeds, nuts)	Bean, Soya	0.1	10	95	10
	Glyphosate	High water content	Apple	0.02	16	93	12
	Glyphosate	High water content	Cucumber	0.02	3	94	3
	HEPA	Dry (cereals, dry pulses)	Barley	0.02	5	106	17
	HEPA	High water content	Apple	0.02	7	109	14
	HEPA	High water content	Cucumber	0.02	3	104	6
	Maleic hydrazide	Dry (cereals, dry pulses)	Barley	0.02	5	100	9
	Maleic hydrazide	High water content	Apple	0.02	7	110	9
	Maleic hydrazide	High water content	Cucumber	0.02	3	103	13
	Maleic hydrazide	High water content, extract rich	Onion	0.1	5	106	4
	MPPA	Dry (cereals, dry pulses)	Barley	0.02	5	106	10
	MPPA	Dry (cereals, dry pulses)	Wheat flour	0.1	5	85	1
	MPPA	High water content	Apple	0.02	7	88	11
	MPPA	High water content	Cucumber	0.02	3	107	14
	N-Acetyl AMPA	Dry (cereals, dry pulses)	Barley	0.02	5	108	3
	N-Acetyl AMPA	High water content	Apple	0.02	7	120	11
	N-Acetyl AMPA	High water content	Cucumber	0.02	3	89	7
	N-Acetyl Glufosinate	Dry (cereals, dry pulses)	Barley	0.02	5	103	5
	N-Acetyl Glufosinate	High water content	Apple	0.02	7	112	9
N-Acetyl Glufosinate	High water content	Cucumber	0.02	3	101	3	

Method	Analyte	Commodity Group	Matrix	Spiking Level (ppm)	n	Rec.	RSD
	N-Acetyl Glyphosate	High acid content	Grapes	0.01	10	109	8
	N-Acetyl Glyphosate	Dry (cereals, dry pulses)	Corn flour	0.02	10	104	10
	N-Acetyl Glyphosate	Dry (cereals, dry pulses)	Lentil	0.05	10	104	8
	N-Acetyl Glyphosate	Fatty, dry (oil seeds, nuts)	Bean, Soya	0.05	10	102	7
	N-Acetyl Glyphosate	High water content	Apple	0.01	10	109	8
M 1.4	Bromate	High water content	Lettuce varieties	0.02	5	103	6
	Bromide (inorg.)	High acid content	Currant	1	5	98	4
	Bromide (inorg.)	High water content	Cauliflower	1	5	94	12
	Chlorate	Acidic	Currant	0.01	5	102	7
	Chlorate	Dry (cereals, dry pulses)	Rice	0.02	5	108	2
	Chlorate	High water content	Cauliflower	0.01	5	100	5
	Perchlorate	High acid content	Currant	0.01	5	100	4
	Perchlorate	Dry (cereals, dry pulses)	Barley	0.01	5	106	2
	Perchlorate	Dry (cereals, dry pulses)	Rice	0.02	5	100	7
	Perchlorate	High water content	Apple	0.01	5	108	3
	Perchlorate	High water content	Cauliflower	0.01	5	97	3
	Phosphonic Acid	High acid content	Currant	0.1	5	102	3
	Phosphonic Acid	High acid content	Mandarine	0.1	5	99	10
	Phosphonic Acid	Dry (cereals, dry pulses)	Rice	0.2	5	97	4
	Phosphonic Acid	High water content	Apple	0.1	6	102	9
	Phosphonic Acid	High water content	Cauliflower	0.1	5	87	2
	Phosphonic Acid	High water content	Mango	0.1	5	99	9
M 4.1	Amitrole	High acid content	Orange	0.01	6	107	5
	Amitrole	Dry (cereals, dry pulses)	Barley	0.01	5	111	2
	Amitrole	High water content	Apple	0.01	7	93	11
	Amitrole	High water content	Cucumber	0.01	6	92	4
	Chloridazon, Desphenyl-	High acid content	Currant	0.02	5	99	4
	Chloridazon, Desphenyl-	High water content	Lettuce varieties	0.02	5	97	3
	Chlormequat	High acid content	Grapes	0.01	6	93	10
	Chlormequat	High acid content	Grapes	0.2	5	102	1
	Chlormequat	Dry (cereals, dry pulses)	Barley	0.01	5	97	5
	Chlormequat	Dry (cereals, dry pulses)	Wheat flour	0.1	5	97	5
	Chlormequat	Fatty, wet (oily fruits)	Avocado	0.01	7	103	8
	Chlormequat	High water content	Apple	0.01	6	102	6
	Chlormequat	High water content	Cucumber	0.01	6	103	4
	Chlormequat	High water content	Potato	0.01	6	99	4
	Cyromazine	High acid content	Grapes	0.01	6	101	4
	Cyromazine	Dry (cereals, dry pulses)	Barley	0.01	5	109	6
	Cyromazine	Fatty, wet (oily fruits)	Avocado	0.01	7	107	2
	Cyromazine	High water content	Apple	0.01	6	102	8
	Cyromazine	High water content	Potato	0.01	6	103	8
	Daminozide	High acid content	Grapes	0.01	6	213	24
	Daminozide	High acid content	Orange	0.01	3	113	1
	Daminozide	Dry (cereals, dry pulses)	Barley	0.01	5	113	6
	Daminozide	Fatty, wet (oily fruits)	Avocado	0.01	6	112	10
	Daminozide	High water content	Apple	0.01	6	100	9
	Daminozide	High water content	Cucumber	0.01	6	93	12
	Daminozide	High water content	Potato	0.01	6	158	15
	Diethanolamine	High acid content	Mandarine	0.1	5	103	1
	Diethanolamine	High water content	Apple	0.1	5	103	3

Method	Analyte	Commodity Group	Matrix	Spiking Level (ppm)	n	Rec.	RSD
	Diethanolamine	High water content	Mango	0.1	6	101	14
	Difenzoquat	Dry (cereals, dry pulses)	Barley	0.01	5	99	8
	Difenzoquat	High water content	Apple	0.01	6	99	11
	Diquat	High acid content	Grapes	0.01	5	44	126
	Diquat	Dry (cereals, dry pulses)	Barley	0.01	10	103	7
	Diquat	Fatty, wet (oily fruits)	Avocado	0.05	5	186	6
	Diquat	High water content	Apple	0.01	5	107	4
	Diquat	High water content	Potato	0.01	10	75	50
	ETU	Dry (cereals, dry pulses)	Barley	0.01	5	96	10
	ETU	High water content	Apple	0.01	7	102	9
	Melamine	High acid content	Grapes	0.01	6	87	13
	Melamine	Fatty, dry (oil seeds, nuts)	Bean, Soya	0.02	3	109	5
	Melamine	Fatty, wet (oily fruits)	Avocado	0.01	7	108	6
	Melamine	High water content	Potato	0.01	5	141	6
	Mepiquat	High acid content	Grapes	0.01	6	95	5
	Mepiquat	High acid content	Orange	0.01	6	101	9
	Mepiquat	Dry (cereals, dry pulses)	Barley	0.01	5	108	3
	Mepiquat	Dry (cereals, dry pulses)	Wheat flour	0.1	5	102	5
	Mepiquat	Fatty, wet (oily fruits)	Avocado	0.01	6	104	5
	Mepiquat	High water content	Apple	0.01	6	98	7
	Mepiquat	High water content	Cucumber	0.01	6	107	6
	Mepiquat	High water content	Potato	0.01	6	99	3
	Morpholine	High acid content	Mandarine	0.1	5	95	7
	Morpholine	High water content	Apple	0.1	5	94	3
	Morpholine	High water content	Mango	0.1	5	95	2
	Nereistoxin	High acid content	Grapes	0.01	6	93	9
	Nereistoxin	Dry (cereals, dry pulses)	Barley	0.01	5	104	13
	Nereistoxin	Fatty, wet (oily fruits)	Avocado	0.01	5	103	6
	Nereistoxin	High water content	Apple	0.01	6	118	2
	Nereistoxin	High water content	Potato	0.01	6	113	9
	Paraquat	High acid content	Grapes	0.01	5	128	7
	Paraquat	Dry (cereals, dry pulses)	Barley	0.01	10	106	15
	Paraquat	Fatty, wet (oily fruits)	Avocado	0.05	5	83	10
	Paraquat	High water content	Apple	0.01	5	106	5
	Paraquat	High water content	Potato	0.01	10	103	13
	PTU	Dry (cereals, dry pulses)	Barley	0.01	5	113	3
	Triethanolamine	High acid content	Mandarine	0.1	5	112	4
	Triethanolamine	High water content	Apple	0.1	5	108	6
	Triethanolamine	High water content	Mango	0.1	5	120	5
	Triethanolamine	High water content	Pear	0.1	3	107	11
	Trimesium	High acid content	Grapes	0.01	6	93	7
	Trimesium	Dry (cereals, dry pulses)	Barley	0.01	5	118	3
	Trimesium	Dry (cereals, dry pulses)	Wheat flour	0.1	5	105	2
	Trimesium	Fatty, wet (oily fruits)	Avocado	0.01	7	93	14
	Trimesium	High water content	Apple	0.01	5	147	5
	Trimesium	High water content	Potato	0.01	6	84	5
M 4.2	Aminocyclopyrachlor	High acid content	Raspberry	0.01	5	149	7
	Aminocyclopyrachlor	Dry (cereals, dry pulses)	Oat	0.02	5	106	7
	Aminocyclopyrachlor	High water content	Apple	0.01	5	110	6
	Aminocyclopyrachlor	High water content	Cucumber	0.01	5	101	6

Method	Analyte	Commodity Group	Matrix	Spiking Level (ppm)	n	Rec.	RSD
	Aminocyclopyrachlor	High acid content	Lemon	0.01	5	112	10
	Aminocyclopyrachlor	High water content, extract rich	Mint	0.01	5	108	8
	Amitrol	Dry (cereals, dry pulses)	Oat	0.02	5	110	4
	Amitrol	High water content	Apple	0.01	5	99	6
	Amitrol	High water content	Cucumber	0.01	5	104	6
	Amitrol	High acid content	Lemon	0.01	5	96	4
	Amitrol	High water content, extract rich	Mint	0.02	5	111	6
	Amitrol	High acid content	Raspberry	0.01	5	120	5
	Chloridazon-desphenyl	High acid content	Raspberry	0.01	5	156	10
	Chloridazon-desphenyl	Dry (cereals, dry pulses)	Oat	0.02	5	113	10
	Chloridazon-desphenyl	High water content	Apple	0.01	5	97	4
	Chloridazon-desphenyl	High water content	Cucumber	0.01	5	104	3
	Chloridazon-desphenyl	High acid content	Lemon	0.01	5	108	9
	Chloridazon-desphenyl	High water content, extract rich	Mint	0.01	5	108	10
	Chlormequat	High acid content	Raspberry	0.01	5	124	6
	Chlormequat	Dry (cereals, dry pulses)	Oat	0.02	5	105	3
	Chlormequat	Dry (cereals, dry pulses)	Oat	0.1	5	111	2
	Chlormequat	High water content	Apple	0.01	5	101	2
	Chlormequat	High water content	Apple	0.05	5	103	2
	Chlormequat	High water content	Cucumber	0.01	5	106	3
	Chlormequat	High water content	Cucumber	0.02	5	103	3
	Chlormequat	High acid content	Lemon	0.01	5	103	2
	Chlormequat	High acid content	Lemon	0.02	5	107	4
	Chlormequat	High water content, extract rich	Mint	0.02	5	101	2
	Chlormequat	High water content, extract rich	Mint	0.01	5	102	1
	Cyromazine	High acid content	Raspberry	0.01	5	124	6
	Cyromazine	Dry (cereals, dry pulses)	Oat	0.02	5	106	4
	Cyromazine	High water content	Apple	0.01	5	98	3
	Cyromazine	High water content	Cucumber	0.01	5	101	5
	Cyromazine	High acid content	Lemon	0.01	5	95	3
	Cyromazine	High water content, extract rich	Mint	0.02	5	106	5
	Daminozide	High acid content	Raspberry	0.01	5	119	3
	Daminozide	Dry (cereals, dry pulses)	Oat	0.02	5	125	3
	Daminozide	High water content	Apple	0.01	5	101	2
	Daminozide	High water content	Cucumber	0.01	5	103	6
	Daminozide	High acid content	Lemon	0.01	5	102	1
	Daminozide	High water content, extract rich	Mint	0.01	5	104	3
	Diethanolamine	High acid content	Raspberry	0.05	5	109	4
	Diethanolamine	Dry (cereals, dry pulses)	Oat	0.02	5	105	14
	Diethanolamine	High water content	Apple	0.05	5	120	11
	Difenzoquat	High acid content	Raspberry	0.01	5	155	7
	Difenzoquat	Dry (cereals, dry pulses)	Oat	0.02	5	97	6
	Difenzoquat	High water content	Apple	0.01	5	105	4
	ETU	High acid content	Raspberry	0.01	5	109	6
	ETU	Dry (cereals, dry pulses)	Oat	0.02	5	98	12
	ETU	High water content	Apple	0.01	5	90	25
	ETU	High water content	Cucumber	0.01	5	87	9

Method	Analyte	Commodity Group	Matrix	Spiking Level (ppm)	n	Rec.	RSD
	ETU	High acid content	Lemon	0.01	5	104	11
	ETU	High water content, extract rich	Mint	0.02	5	107	15
	Melamine	High acid content	Raspberry	0.01	5	133	13
	Melamine	Dry (cereals, dry pulses)	Oat	0.02	5	94	20
	Melamine	High water content	Apple	0.01	5	97	8
	Melamine	High water content	Cucumber	0.01	5	90	12
	Melamine	High acid content	Lemon	0.01	5	91	10
	Melamine	High water content, extract rich	Mint	0.01	5	93	11
	Mepiquat	High acid content	Raspberry	0.01	5	122	4
	Mepiquat	Dry (cereals, dry pulses)	Oat	0.02	5	104	6
	Mepiquat	High water content	Apple	0.01	5	104	3
	Mepiquat	High water content	Cucumber	0.01	5	102	3
	Mepiquat	High acid content	Lemon	0.01	5	104	4
	Mepiquat	High water content, extract rich	Mint	0.01	5	96	3
	Mepiquat, 4-hydroxy	High acid content	Raspberry	0.01	5	133	3
	Mepiquat, 4-hydroxy	Dry (cereals, dry pulses)	Oat	0.02	5	112	4
	Mepiquat, 4-hydroxy	High water content	Apple	0.01	5	110	2
	Mepiquat, 4-hydroxy	High water content	Cucumber	0.01	5	108	2
	Mepiquat, 4-hydroxy	High acid content	Lemon	0.01	5	107	4
	Mepiquat, 4-hydroxy	High water content, extract rich	Mint	0.01	5	105	3
	Morpholine	High acid content	Raspberry	0.01	5	84	15
	Morpholine	Dry (cereals, dry pulses)	Oat	0.02	5	119	30
	Morpholine	High water content	Apple	0.01	5	84	13
	Morpholine	High water content	Cucumber	0.01	5	97	10
	Morpholine	High acid content	Lemon	0.01	5	92	8
	Morpholine	High water content, extract rich	Mint	0.02	5	87	38
	Nereistoxin	High acid content	Raspberry	0.01	5	114	3
	Nereistoxin	Dry (cereals, dry pulses)	Oat	0.02	5	97	2
	Nereistoxin	High water content	Apple	0.01	5	101	3
	Nereistoxin	High water content	Cucumber	0.01	5	94	7
	Nereistoxin	High acid content	Lemon	0.01	5	99	2
	Nereistoxin	High water content, extract rich	Mint	0.01	5	90	3
	Propamocarb	High acid content	Raspberry	0.01	5	121	4
	Propamocarb	Dry (cereals, dry pulses)	Oat	0.02	5	122	4
	Propamocarb	High water content	Apple	0.01	5	102	2
	Propamocarb	High water content	Cucumber	0.01	5	99	2
	Propamocarb	High acid content	Lemon	0.01	5	84	5
	Propamocarb	High water content, extract rich	Mint	0.01	5	102	2
	Propamocarb-N-desmethyl	High acid content	Raspberry	0.01	5	135	3
	Propamocarb-N-desmethyl	Dry (cereals, dry pulses)	Oat	0.02	5	113	5
	Propamocarb-N-desmethyl	High water content	Apple	0.01	5	94	3
	Propamocarb-N-desmethyl	High water content	Cucumber	0.01	5	113	2
	Propamocarb-N-desmethyl	High acid content	Lemon	0.01	5	128	4
	Propamocarb-N-desmethyl	High water content, extract rich	Mint	0.01	5	121	3
	Propamocarb-N-oxide	High acid content	Raspberry	0.01	5	154	3
	Propamocarb-N-oxide	Dry (cereals, dry pulses)	Oat	0.02	5	137	3

Method	Analyte	Commodity Group	Matrix	Spiking Level (ppm)	n	Rec.	RSD	
	Propamocarb-N-oxide	High water content	Apple	0.01	5	110	4	
	Propamocarb-N-oxide	High water content	Cucumber	0.01	5	102	2	
	Propamocarb-N-oxide	High acid content	Lemon	0.01	5	109	4	
	Propamocarb-N-oxide	High water content, extract rich	Mint	0.01	5	111	4	
	PTU	High acid content	Raspberry	0.01	5	115	7	
	PTU	Dry (cereals, dry pulses)	Oat	0.02	5	110	6	
	PTU	High water content	Apple	0.05	5	71	5	
	PTU	High water content	Cucumber	0.01	5	97	4	
	PTU	High acid content	Lemon	0.01	5	100	5	
	Triethanolamine	High acid content	Raspberry	0.01	5	106	4	
	Triethanolamine	Dry (cereals, dry pulses)	Oat	0.02	5	129	36	
	Triethanolamine	High water content	Apple	0.01	5	73	11	
	Trimethylsulfonium	High acid content	Raspberry	0.01	5	140	5	
	Trimethylsulfonium	Dry (cereals, dry pulses)	Oat	0.02	5	109	2	
	Trimethylsulfonium	High water content	Apple	0.01	5	119	3	
	Trimethylsulfonium	High water content	Cucumber	0.01	5	119	3	
	Trimethylsulfonium	High acid content	Lemon	0.01	5	110	2	
	Trimethylsulfonium	High water content, extract rich	Mint	0.01	5	116	4	
M 6	Kasugamycin	High water content	Apple	0.01	5	98	4	
	Streptomycin	High water content	Apple	0.01	10	106	9	
M 8	1,2,4-Triazole	High water content	Cucumber	0.01	5	92	25	
	1,2,4-Triazole	High acid content	Orange	0.01	5	128	21	
	1,2,4-Triazole	High acid content	Grapes	0.01	5	115	21	
	1,2,4-Triazole	Dry (cereals, dry pulses)	Rice	0.2	5	86	3	
	Triazole-acetic acid	High water content	Cucumber	0.01	5	100	2	
	Triazole-acetic acid	High acid content	Orange	0.01	5	104	9	
	Triazole-acetic acid	High acid content	Grapes	0.01	5	95	4	
	Triazole-acetic acid	Dry (cereals, dry pulses)	Rice	0.02	5	74	5	
	Triazole-alanine	High water content	Cucumber	0.01	5	100	19	
	Triazole-alanine	High acid content	Orange	0.01	5	98	5	
	Triazole-alanine	High acid content	Grapes	0.01	5	95	11	
	Triazole-alanine	Dry (cereals, dry pulses)	Rice	0.02	5	88	4	
	Triazole-lactic acid	High water content	Cucumber	0.01	5	107	3	
	Triazole-lactic acid	High acid content	Orange	0.01	5	111	12	
	Triazole-lactic acid	High acid content	Grapes	0.01	5	100	5	
	Triazole-lactic acid	Dry (cereals, dry pulses)	Rice	0.02	5	71	4	
	M 9	Difluoroacetic acid	High water content	Apple	0.01	5	94	7
		Difluoroacetic acid	Fatty, wet (oily fruits)	Avocado	0.02	5	103	8
Difluoroacetic acid		High water content	Cucumber	0.01	5	70	2	
Difluoroacetic acid		Dry (cereals, dry pulses)	Flour	0.02	5	77	9	
Difluoroacetic acid		High acid content	Grapes	0.01	5	80	5	
Difluoroacetic acid		High acid content	Grapes	0.01	5	106	15	
Difluoroacetic acid		High acid content	Orange	0.01	5	109	11	
Difluoroacetic acid		Dry (cereals, dry pulses)	Rice	0.02	5	80	3	
Trifluoroacetic acid		High water content	Apple	0.01	5	93	6	
Trifluoroacetic acid		Fatty, wet (oily fruits)	Avocado	0.04	5	77	4	
Trifluoroacetic acid		Dry (cereals, dry pulses)	Flour	0.04	5	84	6	
Trifluoroacetic acid		High acid content	Gooseberry	0.02	5	128	11	
Trifluoroacetic acid		High acid content	Grapes	0.01	5	87	14	

Method	Analyte	Commodity Group	Matrix	Spiking Level (ppm)	n	Rec.	RSD
	Trifluoroacetic acid	High acid content	Orange	0.01	5	107	3
	Trifluoroacetic acid	Dry (cereals, dry pulses)	Rice	0.04	5	72	4
	Trifluoroacetic acid	High water content	Tomato	0.02	5	76	15

7. References

Anastassiades, M and Mack, D (2008); New Developments in the Analysis of Pesticides Typically not Covered by Multiresidue Methods; European Pesticide Residue Workshop, EPRW 2008, Berlin, oral presentation O1, Book of Abstracts

Kolberg DI, Mack D, Anastassiades M, Hetmanski MT, Fussell RJ, Meijer T, Mol HG. *Anal Bioanal Chem.* 404(8):2465-74 (2012); Development and independent laboratory validation of a simple method for the determination of paraquat and diquat in potato, cereals and pulses

Alder L. and Startin J. R. (2005); Determination of Chlormequat and Mepiquat in Foods by Liquid Chromatography/Mass Spectrometry or Liquid Chromatography/Tandem Mass Spectrometry: Interlaboratory Study; *Journal of AOAC International* Vol. 88, No. 6: 1762-1776

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8. ANNEX

Table 20: Conversion factors between typical purchased standards and target analytes (3.15):

Compound	MW [g/mol]	Compound as sold	MW [g/mol]	Conversion factor
Bialaphos	323.3	Bialaphos-sodium	345.3	1.07
Bromate (anion)	127.9	Potassium bromate	167.0	1.31
Bromide (anion)	79.9	Potassium bromide	119.0	1.49
Chlorate (anion)	83.5	Chlorate-sodium	106.4	1.27
Chlormequat (cation)	122.6	Chlormequat-chloride	158.1	1.29
Chlormequat-D ₄ (cation)	126.6	Chlormequat-D ₄ -chloride	162.1	1.28
Difenzoquat (cation)	249.3	Difenzoquat-methylsulfate	360.4	1.45
Difluoroacetic acid- ¹³ C ₂	96.0	Sodium difluoroacetate- ¹³ C ₂	120.0	1.25
Dihydrostreptomycin	583.6	Dihydrostreptomycin-sesquisulfate	730.7	1.25
Diquat (dication)	184.2	Diquat-dibromide-monohydrate	362.1	1.97
Diquat-D ₄ (dication)	188.2	Diquat-D ₄ -dibromide-monohydrate	366.1	1.95
Fosetyl	110.0	Fosetyl-Al	118.0	1.07
Fosetyl-D ₅	115.0	Fosetyl-D ₅ -1/3 aluminium	123.0	1.07
		Fosetyl-D ₅ -sodium	137.0	1.19
Glufosinate	181.1	Glufosinate-ammonium	198.2	1.09
Glufosinate-D ₃	184.1	Glufosinate-D ₃ -hydrochloride	220.6	1.20
Kasugamycin	379.4	Kasugamycin-hydrochloride-monohydrate	433.8	1.14
Mepiquat (cation)	114.2	Mepiquat-chloride	149.7	1.31
Mepiquat-D ₃ (cation)	117.2	Mepiquat-D ₃ -iodide	244.1	2.08
Mepiquat-4-hydroxy	130.2	Mepiquat-4-hydroxy-chloride	165.7	1.27
N, N-Dimethylhydrazine-D ₆	66.1	Dimethylhydrazine-D ₆ -hydrochloride	102.6	1.55
N-Acetyl-glufosinate	223.2	N-Acetyl-glufosinate-disodium	267.2	1.20
N-Acetyl-glufosinate-D ₃	226.2	N-Acetyl-glufosinate-D ₃ -disodium	270.2	1.19
Nereistoxin	149.3	Nereistoxin-oxalate	239.3	1.60
Nereistoxin-D ₆	155.3	Nereistoxin-D ₆ -oxalate	245.3	1.58
Paraquat (dication)	186.3	Paraquat-dichloride	257.2	1.38
Paraquat-D ₆ (dication)	192.3	Paraquat-D ₆ -diiodide	446.1	2.32
Propamocarb-N-oxide	204.3	Propamocarb-N-oxide hydrochloride	240.7	1.17
Streptomycin	581.6	Streptomycin-sesquisulfate	728.7	1.25
Trifluoroacetic acid - ¹³ C ₂	114.0	Sodium trifluoroacetate- ¹³ C ₂	138.0	1.21
Trimethylsulfonium (cation)	77.2	Trimethylsulfonium-iodide	204.1	2.64
Trimethylsulfonium-D ₉ (cation)	86.2	Trimethylsulfonium-D ₉ -iodide	213.1	2.47

Table 21: Exemplary concentrations of pesticide stock and working solutions (3.15 and 0), solvent proposals also apply to ILISs (see 3.17, 3.18 and 3.19). * Use plastic vessels and stoppers for compounds that tend to interact with glass surfaces

Compound	Stock Solution (exemplary)		Working Solutions including mixtures (exemplary)	
	Solvent used to prepare	[mg/mL]	Solvent used to prepare	[µg/mL]
Aminocyclopyrachlor	Methanol	1	Methanol	10 / 1 / 0.1
Amitrole	Methanol	1	Methanol	10 / 1 / 0.1
AMPA	10% acetonitrile in water	1	10% acetonitrile in water	10 / 1 / 0.1
Bromate	Water/methanol (50:50)	1	Methanol	10 / 1 / 0.1 / 0.01
Bromide	Methanol	1	Methanol	10 / 1 / 0.1 / 0.01
Chlorate	Methanol	1	Methanol	10 / 1 / 0.1 / 0.01
Chloridazon-desphenyl	Methanol	1	Methanol	10 / 1 / 0.1
Chlormequat	Methanol	1	Methanol	10 / 1 / 0.1
Cyromazine	Methanol	1	Methanol	10 / 1 / 0.1
Daminozide	Methanol	1	Methanol	10 / 1 / 0.1
Diethanolamine	Acetonitrile	1	Methanol	10 / 1 / 0.1
Difenzoquat	Acetonitrile	1	Methanol	10 / 1 / 0.1
Difluoroacetic acid	Acetonitrile	1	Acetonitrile	10 / 1 / 0.1
Diquat*	Methanol + 1% formic acid	1	Methanol + 1% formic acid	10 / 1 / 0.1
Ethephon	10% acetonitrile in water + 0,1% Hydrochloric acid (37%)	1	10% acetonitrile in water + 0,1% Hydrochloric acid (37%)	10 / 1 / 0.1
ETU	Methanol	1	Methanol	10 / 1 / 0.1
Fosetyl	Water / methanol (3/1)	0.1	10% acetonitrile in water	10 / 1 / 0.1
Glufosinate	10% acetonitrile in water	1	10% acetonitrile in water	10 / 1 / 0.1
Glyphosate*	10% acetonitrile in water	0.2	10% acetonitrile in water	10 / 1 / 0.1
HEPA	10% acetonitrile in water	1	10% acetonitrile in water	10 / 1 / 0.1
Kasugamycin	Methanol	1	Methanol	10 / 1 / 0.1
Maleic hydrazide	Methanol	1	10% acetonitrile in water	10 / 1 / 0.1
Mepiquat	Methanol	1	Methanol	10 / 1 / 0.1
Mepiquat-4-hydroxy	Methanol	1	Methanol	10 / 1 / 0.1
Morpholine	Methanol	1	Methanol	10 / 1 / 0.1
MPPA	10% acetonitrile in water	1	10% acetonitrile in water	10 / 1 / 0.1
N,N-Dimethylhydrazine	Methanol	1	Methanol	10 / 1 / 0.1
N-Acetyl- AMPA	10% acetonitrile in water	1	10% acetonitrile in water	10 / 1 / 0.1
N-Acetyl-glufosinate	10% acetonitrile in water	1	10% acetonitrile in water	10 / 1 / 0.1
N-Acetylglyphosate	10% acetonitrile in water	1	10% acetonitrile in water	10 / 1 / 0.1
Nereistoxin	Methanol / water (3:1)	1	Methanol	10 / 1 / 0.1
Paraquat**	Methanol	1	Methanol	10 / 1 / 0.1
Perchlorate	Methanol	1	Methanol	10 / 1 / 0.1 / 0.01
Phosphonic acid	Water (¹⁸ O-H ₂ O for the ILIS)	1	Methanol	10 / 1 / 0.1 / 0.01
Propamocarb	Acetonitrile	1	Methanol	10 / 1 / 0.1
Propamocarb-N-desmethyl	Acetonitrile:Acetone (1 mL Acetone to initially dissolve)	1	Methanol	10 / 1 / 0.1
Propamocarb-N-oxide	Methanol	1	Methanol	10 / 1 / 0.1
PTU	Methanol	1	Methanol	10 / 1 / 0.1
Streptomycin*	Water / methanol (1:1)	0,5	Methanol	10 / 1 / 0.1
Triethanolamine	Methanol	1	Methanol	10 / 1 / 0.1

Compound	Stock Solution (exemplary)		Working Solutions including mixtures (exemplary)	
	Solvent used to prepare	[mg/mL]	Solvent used to prepare	[µg/mL]
Trifluoroacetic acid	Acetonitrile	1	Acetonitrile	10 / 1 / 0.1
Trimethylsulfonium (trimesium)	Methanol	1	Methanol	10 / 1 / 0.1

Table 22: Exemplary providers of isotopically labeled internal standards 3.17.

Isotope labelled compound	Source	Article-No.	Conc. (µg/mL)	Amount per unit	Prices in €-cent (see disclaimer)			
					1 unit	2 µg*	0,1 µg**	
Amitrole	¹⁵ N	1	XA10240100ME	100	1.1 mL	165 €	300 c	15 c
	¹⁵ N ¹³ C	1	XA10240110AL	100	1.1 mL	332 €	604 c	30 c
	¹⁵ N ₂ ¹³ C ₂	7	A633382		10 mg	1.500 €	30 c	1.5 c
	¹⁵ N ₄ / ¹³ C ₂	8	C4313		10 mg			
AMPA	¹³ C, ¹⁵ N, D ₂	1	CIL-CDNLM-6786-1.2	100	1.2 mL	464 €	773 c	39 c
		5	CDNLM-6786-1.2	100	1.2 mL	464 €	773 c	39 c
		10	CDNLM-6786-1.2	100	1.2 mL	465 €	775 c	39 c
	¹³ C, ¹⁵ N	7	A617342		10 mg	1.687 €	34 c	1.7 c
		1	XA10205100WA	100	1.1 mL	332 €	604 c	30 c
Bromate	¹⁸ O ₃	1	CIL-OLM-8283-18O-1.2	100	1.2 mL	406 €	677 c	34 c
Chlorate	¹⁸ O ₃	7	C292762	-	1 mL	4.291 €		
		12***	-	200	5 mL	250 €	50 c	2.5 c
Chloridazon-desphenyl	¹⁵ N ₂	13	8399.4-10MG		10 mg	1382	28 c	1.4 c
Chlormequat-chloride	1.1.2.2-D ₄	1	X 11340100DO	100	10 mL	286 €	57 c	2.9 c
		1	XA11340100DO	100	1.1 mL	73 €	133 c	6.6 c
		6	D3386		10 mg	756 €	15 c	0.8 c
		1	CA11340100		5 mg	389 €	16 c	0.8 c
	D ₉	3	673151		5 mg	310 €	12 c	0.6 c
Cyanuric acid	¹³ C ₃	7	C987717		5 mg	164.03 €	6.6 c	0.3 c
	¹⁸ O ₃	9	32679		10 mg	459.1	9.2 c	0.5 c
		3	673141		10 mg	299 €	6.0 c	0.3 c
Cyromazine	D ₄	1	XA11920010EA	100	1.1 mL	118 €	215 c	11 c
		7	C989302		10 mg	1.047 €	21 c	1.1 c
		9	93101		5 mg	160 €	6.4 c	0.3 c
Daminozide	D ₆	1	XA11960100AL	100	1.1 mL	87 €	158 c	7.9 c
Diethanolamine	D ₄	4	D-5307		100 mg	432 €	0.9 c	0.04 c
	D ₈	7	D441902		100 mg	1.100 €	2.2 c	0.1 c
Difluoroacetic acid-(Sodium salt)	¹³ C ₂	2	-	friendly donation				
Dihydrostreptomycin	sesquisulfate-hydrate sulfate	1	C 12635300		100 mg	29 €	0.1 c	0.003 c
		1	EPD1954000		25 mg	120 €	1.0 c	0.048
Diquat-D ₄ -dibromide (ethylene-D ₄)		1	XA12960010DO	100	1.1 mL	82 €	149 c	7.5 c
		4	D-3932		10 mg	144 €	2.9 c	0.1 c
		6	D17071		50 mg	840 €	3.4 c	0.2 c
		7	D492902		5 mg	117 €	4.7 c	0.2 c
		9	3627		5 mg	152 €	6.1 c	0.3 c
		10	B130022-10		10 mg	1.109 €	22 c	1.1 c
Ethephon	D ₄	1	XA13230100AC	100	1.1 mL	127 €	231 c	12 c
		6	DRE-C13230100		10 mg	1.197 €	24 c	1.2 c
		6	D8328		5 mg	1.387 €	56 c	2.8 c
		7	C366177		10 mg	1.122 €	22 c	1.1 c
	¹³ C ₂	7	C366178		0,25 mg	210.90 €	170 c	8 c
Ethylenethiourea-D ₄ (ETU-D ₄)		1	C 13330100	100	50 mg	316 €	1.3 c	0.06 c
		6	XA13330100AC	100	1.1 mL	127 €	231 c	12 c
		7	D1965		100 mg	733 €	1.5 c	0.07 c
Fosetyl	D ₁₅ (Aluminium salt)	1	CA13940010		10 mg	380 €	7.6 c	0.4 c
	D ₅ (Sodium salt)	8	C5607		10 mg	825 €	17 c	0.8 c
Glufosinate	D ₃	2	-	Friendly donation				
		7	G596952		10 mg	1.870 €	37 c	1.9 c
Glyphosate	¹³ C ₂ , ¹⁵ N	1	XA14050100WA	100	1.1 mL	304 €	553 c	28 c
		5	CNLM-4666-1.2	100	1.2 mL	361 €	602 c	30 c
			CNLM-4666-10X-1.2	1000	1.2 mL	1.173 €	196 c	9.8 c
		1	CIL-CNLM-4666-1.2	100	1.2 mL	344 €	573 c	29 c

Isotope labelled compound	Source	Article-No.	Conc. (µg/mL)	Amount per unit	Prices in €-cent (see disclaimer)				
					1 unit	2 µg*	0,1 µg**		
	¹³ C, ¹⁵ N	6	CN10570		5 mg	1.991 €	80 c	4.0 c	
		7	G765002		10 mg	1.048 €	21 c	1.0 c	
		11	sc-280758		1 mg	262 €	52 c	2.6 c	
		9	90479		5 mg	524 €	21 c	1 c	
		7	G765001		5 mg	210.90 €	8.4 c	0.4 c	
	¹³ C	7	606502		10 mg	785 €	16 c	0.9 c	
		1	CA13230200		10 mg	256 €	5.1 c	0.3 c	
		7	H939652		25 mg	1.125 €	9.0 c	0.5 c	
HEPA (Hydroxy-Ethephon)	D ₄	2	-	Friendly donation 100	1 mL	99 €	200 c	10 c	
		3	676639						
		1	C 14730100			10 mg	235 €	4.7 c	0.2 c
		3	673799			10 mg	199 €	20 c	1 c
Maleic hydrazide	D ₂	7	M124502		5 mg	141 €	5.6 c	0.3 c	
		3	673055		10 mg	289 €	5.8 c	0.3 c	
Melamine	¹³ C ₃ , ¹⁵ N ₃	1	CIL-CNLM-8150-10X-1.2	1000	1.2 mL	1.145 €	229 c	12 c	
		6	D14539		50 mg	1.350 €	5.4 c	0.3 c	
Mepiquat-	D ₁₆ -chloride-D ₃ (methyl-D ₃) - iodide	1	X 14880100DO	100	10 mL	378 €	76 c	3.8 c	
		1	XA14880100DO	100	1.1 mL	68 €	124 c	6.2 c	
		4	D-1895/0.5		500 mg	468 €	0.94 c	0.05 c	
Morpholine	D ₈	7	M723728		25 mg	131.23 €	1.1 c	0.05 c	
		7	M723727		1 mg	131.23 €	26 c	1.3 c	
		2	-	Friendly donation					
7	A178237		5 mg		141 €	5.6 c	0.3 c		
N-Acetylglufosinate	D ₃ (methyl-D ₃)	9	5567		5 mg	95 €	3.8 c	0.2 c	
		7	A178248		25 mg	1.075 €	8,6 c	0,4 c	
N-Acetyl-glyphosate	D ₃ (methyl-D ₃) ¹³ C ₂ , ¹⁵ N	7	A178247		10 mg	1.326 €	26,5 c	1,3 c	
		1	C 15502010		10 mg	245 €	5 c	0.3 c	
Nereistoxin-oxalate	D ₆	2	-	Friendly donation					
		7	M326162			10 mg	1.825 €	37 c	1.8 c
MPPA	D ₃	1	C 15870200		50 mg	256 €	1.0 c	0.05 c	
		7	P191902		25 mg	918.59 €	7.3 c	0.4 c	
Paraquat	D ₆ -diiodide D ₈ -dichloride	5	OLM-7310-1.2	100	1.2 mL	326 €	272 c	14 c	
		12***		40	5 mL	250 €	125 c	6.3 c	
		9	631981		10 mg	4.455 €	89 c	4.5 c	
Phosphonic acid	¹⁸ O ₃ D ₆	12		2000	1 mL	125	6.3 c	0.3 c	
		7	P758462		10 mg	1050 €	21 c	1.1 c	
Propamocarb	D ₇	4	DER-XA16390100AC	100	1.1 mL	82 €	149 c	7.5 c	
		9	80757		5 mg	230 €	9.2 c	0.5 c	
PTU	D ₆	6	D535 (not available)		100 mg	756 €	1.5 c	0.1 c	
PTU	D ₆ (not exactly co-eluting with target analyte)	7	P836802		10 mg	1.100 €	22 c	1.1 c	
1, 2, 4-Triazole	¹³ C ₂ , ¹⁵ N ₃	2	-	Friendly donation					
1, 2, 4-Triazole-acetic acid	¹³ C ₂ , ¹⁵ N ₃	2	-	Friendly donation					
1, 2, 4-Triazole-alanine	¹³ C ₂ , ¹⁵ N ₃	2	-	Friendly donation					
1, 2, 4-Triazole-lactic acid	¹³ C ₂ , ¹⁵ N ₃	2	-	Friendly donation					
Triethanolamine	"D ₁₅ " (in reality D ₁₂) D ₁₂	1	CIL-DLM-7663		1 mg	153 €	31 c	1.5 c	
		7	T775582		10 mg	141 €	2.8 c	0.15 c	
Trifluoroacetic acid (Sodium acetate)	¹³ C ₂	7	S673752		10 mg	2.666 €	53 c	2.7 c	
Trimethylsulfonium-	D ₉	6	D2677		100 mg	733 €	0.7 c	0.04 c	

Isotope labelled compound	Source	Article-No.	Conc. (µg/mL)	Amount per unit	Prices in €-cent (see disclaimer)		
					1 unit	2 µg*	0,1 µg**
(Iodide)							
<p>Providers of compounds::</p> <ol style="list-style-type: none"> 1: LGC Standards 2: Bayer Crop Science 3: HPC (High Purity Compounds) 4: CDN Isotopes (via Dr. Ehrenstorfer) 5: Cambridge Isotope Lab. Inc. 6: Medical isotopes 7: Toronto Research Chemicals 8: ALSACHIM 9: Sigma-Aldrich 10. Cerilliant (by Sigma Aldrich) 11. Santa Cruz biotechnology. inc. 12. EURL-SRM (hosted at CVUA Stuttgart) 13. Campro Scientific / Chiron AS 							
<p>(Disclaimer: The use of trade names is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the EURL of any product to the exclusion of others. Market prices and currency exchange rates may be subject to changes. Shipping costs are not included in the pricing.</p>							

* 2 µg IS are typically employed to samples (typically 10 g) at the beginning of the procedure

** 0.1 µg are typically added to 1 mL aliquots of sample extracts (typically corresponding to 0.5 g sample), in this case only matrix-effects are compensated

*** Due to manufacturing process the stock solution of ¹⁸O₃-Chlorate is accompanied by ca 20% ¹⁸O₄-Perchlorate.. As perchlorate typically exhibits a ca. 5-fold higher LC-MS/MS-sensitivity compared to chlorate the signal intensities of the two are end up within the same range.

Table 23: Exemplary concentrations of Internal Standard Working Solutions (IS-WS) (3.18)

Internal Standard (IS)*	IS -Addition to samples (5.2.3)		IS-Addition to calibration standard(s) (0)		Expected approx. IS-concentration in sample extracts (~20 mL) and calibration standards (~1 mL)
	Suggested concentration of IS-WS I (3.18)	Absolute mass of IS spiked to sample (100 µL IS-WS I) (mISsample)	Suggested concentration of IS- WS II (3.19) **	Absolute mass of IS spiked to calibration standard (100 µL IS-WS II) (mIScal mix)	
	µg/mL	µg	µg/mL	µg	
Amitrole-(15N)/ (¹⁵ N ₂ , ¹³ C ₂)	20	2	1	0.1	0.1
AMPA- ¹³ C, ¹⁵ N	20	2	1	0.1	0.1
Bromate- ¹⁸ O ₃	200	20	10	1	1
Chlorate- ¹⁸ O ₃	20	2	1	0.1	0.1
Chloridazon-desphenyl- ¹⁵ N ₂ (ILIS)	40	2	2	0.2	0.2
Chlormequat-D ₄	10	1	0.5	0.05	0.05
Cyromazine-D ₄	20	2	1	0.1	0.1
Daminozid-D ₆	10	1	0.5	0.05	0.05
Diethanolamine-D ₆	20	2	1	0.1	0.1
Difluoroacetic acid - ¹³ C ₂	10	1	1	0.05	0.05
Dihydrostreptomycin****	20	2	1	0.1	0.1
Diquat-D ₄	40	4	2	0.2	0.2
Ethephon-D ₄	20	2	1	0.1	0.1
ETU-D ₄	20	2	1	0.1	0.1
Fosetyl-D ₅ (from fosetyl-aluminium-D ₁₅)	20	2	1	0.1	0.1
Glufosinate-D ₃	20	2	1	0.1	0.1
Glyphosate- ¹³ C ₂ , ¹⁵ N	20	2	1	0.1	0.1
HEPA-D ₄	20	2	1	0.1	0.1
Maleic hydrazide-D ₂	20	2	1	0.1	0.1
Melamine-N ₃	20	2	1	0.1	0.1
Mepiquat-D ₃	10	1	0.5	0.05	0.05
Morpholine-D ₈	20	2	1	0.1	0.1
MPPA-D ₃	20	2	1	0.1	0.1
N-Acetyl-glufosinate-D ₃	20	2	1	0.1	0.1
N-Acetyl-glyphosate- ¹³ C ₂ , ¹⁵ N	20	2	1	0.1	0.1
Nereistoxin-D ₄	10	1	0.5	0.05	0.05
Paraquat-D ₆	40	4	2	0.2	0.2
Perchlorate- ¹⁸ O ₄	20	2	1	0.1	0.1
Phosphonic acid- ¹⁸ O ₃	20	2	1	0.1	0.1
Propamocarb-D ₇	2	0.2	0.1	0.01	0.01
PTU-D ₆	10	1	0.5	0.05	0.05
Triethanolamine-D ₁₂	10	1	0.5	0.05	0.05
Trifluoroacetic acid - ¹³ C ₂	10	1	1	0.05	0.05
Trimethylsulfonium-D ₁₀	10	1	0.5	0.05	0.05

* The concentration of the IS should be high enough to ensure good detection with little influence of signal noise (S/N>20 is typically fine). It should be kept in mind, however, that isotopically labeled ISs (IL-ISs) sometimes contain small amounts of the non-labeled analogues. To minimize the risk of false positives the amount of IL-IS added to the samples should thus not be higher than necessary. Quantification of the parent is typically not affected to a great extent as the cross-contamination is typically at low levels and as similar concentrations of the native pesticide originating from the IL-IS will also be present in the calibration standards and thus subtracted via the intercept. In the case of Maleic hydrazide, where the IL-IS is added at higher concentrations to the samples special attention is necessary (see also comments under 5.7.2).

** a 20-fold dilution of the IS working solution used to spike samples in step 5.2.3 .

*** Dihydrostreptomycin is not isotopically labeled but still suitable for compensation of matrix effects on Streptomycin, if LC conditions are adjusted to ensure exact co-elution and thus equivalent matrix-effects.

NOTE: If detections of a compound are rather seldom and the IS expensive it is advisable to add the IS to the 1 mL aliquot transferred to the auto-sampler vial (see 5.2.6). Alternatively, it can be even skipped entirely in the first screening analysis and only added in a second analysis in case the first one was positive. The first approach is to be preferred especially where the retention times of a compound tends to shift. By comparing the retention time between the IS and the suspected peak as well as the peak shape the certainty of identification significantly improves.

Table 24: Water content of selected foods and water amount to be added to test portions prior to extraction (5.2.2) depending on the analytical approach

Commodity group	Commodity	Typical water content g/100 g	mL of water to be added to 10 g test portions [g] (where water-addition refers to different sample weights this is specified)		Remarks
			When quantifying with IS that was added at the beginning of the procedure (5.8.1)	When no IS is used or when IS is added after aliquotation (5.8.2)	
Fruits					
Citrus fruit	Citrus juices	90	-	1	
	Grapefruit	90	-	1	
	Lemon/lime	85	-	1.5	
	Orange	85	-	1.5	
	Tangerine	90	-	1	
Pome fruit	Apple	85	-	1.5	
	Apple (dried)	30	8.5 to 5 g sample (see 5.2.2)	8.5 to 5 g sample (see 5.2.2)	Weigh 13.5 g rehydrated homogenate
	Apple sauce	80	-	2	
	Apple juice	90	-	1	
	Pear	85	-	1.5	
	Quince	85	-	1.5	
Stone fruit	Apricot	85	-	1.5	
	Apricot (dried)	30	8.5 to 5 g sample (see 5.2.2)	8.5 to 5 g sample (see 5.2.2)	Weigh 13.5 g rehydrated homogenate
	Apricot nectar	85	-	1.5	
	Cherry	85	-	1.5	
	Mirabelle	80	-	2	
	Nectarine	85	-	1.5	
	Peach	90	-	1	
	Peach (dried)	20	8.5 to 5 g sample (see 5.2.2)	8.5 to 5 g sample (see 5.2.2)	Weigh 13.5 g rehydrated homogenate
	Plum	85	-	1.5	
	Plum (dried)	20	8.5 to 5 g sample (see 5.2.2)	8.5 to 5 g sample (see 5.2.2)	Weigh 13.5 g rehydrated homogenate
Soft and small fruit	Blackberry	85	-	1.5	
	Blueberry	85	-	1.5	
	Currant	85	-	1.5	
	Elderberry	80	-	2	
	Gooseberry	90	-	1	
	Grapes	80	-	2	
	Raspberry	85	-	1.5	
	Raisins	20	8.5 to 5 g sample (see 5.2.2)	8.5 to 5 g sample (see 5.2.2)	Weigh 13.5 g rehydrated homogenate
	Strawberry	90	-	1	
	Pineapple	85	-	1.5	
Other fruits	Banana	75	2.5	2.5	
	Fig	80	-	2	
	Fig (dried)	20	8.5 to 5 g sample (see 5.2.2)	8.5 to 5 g sample (see 5.2.2)	Weigh 13.5 g rehydrated homogenate
	Kiwi	85	-	1.5	
	Mango	80	-	2	
	Papaya	90	-	1	
Vegetables					
Root and tuber vegetables	Beetroot	90	-	1	
	Carrot	90	-	1	
	Celeriac	90	-	1	
	Horseradish	75	2.5	2.5	
	Parsley root	90	-	1	
	Radish	95	-	0.5	
	Black salsify	80	-	2	
	Potato	80	-	2	

Commodity group	Commodity	Typical water content g/100 g	mL of water to be added to 10 g test portions [g] (where water-addition refers to different sample weights this is specified)		Remarks
			When quantifying with IS that was added at the beginning of the procedure (5.8.1)	When no IS is used or when IS is added after aliquotation (5.8.2)	
	Garlic	60	7 to 5 g sample	7 to 5 g sample	
Leek plants	Onion	90	-	1	
	Leek	85	-	1.5	
	Shallot	80	-	2	
	Chives	85	-	1.5	
Fruiting vegetables	Aubergine	90	-	1	
	Cucumber	95	-	0.5	
	Melon	90	-	1	
	Pepper, sweet	90	-	1	
	Pumpkin	95	-	0.5	
	Tomato	95	-	0.5	
	Zucchini	95	-	0.5	
	Broccoli	90	-	1	
Cabbage	Brussel sprouts	85	-	1.5	
	Cauliflower	90	-	1	
	Chinese cabbage	95	-	0.5	
	Kale	90	-	1	
	Kohlrabi	90	-	1	
	Red cabbage	90	-	1	
	Savoy cabbage	90	-	1	
	White cabbage	90	-	1	
	Lettuce varieties	95	-	0.5	
	Endive	95	-	0.5	
Leafy vegetables and herbs	Cress	90	-	1	
	Lamb's lettuce	85	-	1.5	
	Parsley	80	-	2	
	Rucola	85	-	1.5	
	Spinach	90	-	1	
Stem vegetables	Asparagus	95	-	0.5	
	Celery	95	-	0.5	
	Leek	85	-	1.5	
	Rhubarb	95	-	0.5	
	Artichokes	85	-	1.5	
Legumes	Beans, peas, lentils (dried)	<10	10 to 5 g sample	10 to 5 g sample	
	Beans, peas	75	2.5	2.5	
Miscellaneous					
Cereals	Grain, flour etc.	10	10 to 5 g sample	10 to 5 g sample	Different sample amounts may be used depending on water-absorbing properties of material
Extract-rich ("difficult") commodities	Coffee beans	<10	10 to 2 g sample	10 to 2 g sample	Different sample amounts may be used depending on extract-richness
	Tea	<10	10 to 2 g sample	10 to 2 g sample	
	Dry herbs and spices	<10	10 to 2 g sample	10 to 2 g sample	
Other	Mushrooms	90	-	1	
	Wine	90	-	1	
	Honey	20	9 to 5 g sample	9 to 5 g sample	
	Avocado	70	3 to 10 g sample	3 to 10 g sample	

Table 25: Exemplary LC-MS/MS parameters for Sciex QTrap 5500

Parameters	Methods 1.1 / 1.2	Method 1.3	Method 1.4	Method 2	Method 3 + 4.1 + 5	Method 4.2	Method 6	Method 7	Method 8 / 9
Ion source (ESI. Turbo Ion Spray) Mode	negative	negative	negative	negative	positive	positive	positive	positive	pos. / neg. SelexI-onTM
Curtain gas (Nitrogen)	30 psi (2.07 bar)	40 psi (2.76 bar)	40 psi (2.76 bar)	30 psi (2.07 bar)	30 psi (2.07 bar)	30 psi (2.07 bar)	30 psi (2.07 bar)	40 psi (2.76 bar)	20 psi (1.38 bar)
Collision gas	medium								
Ion spray voltage	-4500	-4500	-4500	-4500	1500	5000	5500	1500	5500 / -5500
Gas 1 (Zero Grade Air or Nitrogen)	50 psi (3.45 bar)	60 psi (4.14 bar)	60 psi (4.14 bar)	50 psi (3.45 bar)	50 psi (3.45 bar)	60 psi (4.14 bar)	50 psi (3.45 bar)	60 psi (4.14 bar)	60 psi (4.14 bar)
Gas 2 (Zero Grade Air or Nitrogen)	60 psi (4.14 bar)	60 psi (4.14 bar)	70 psi (4.83 bar)	60 psi (4.14 bar)	60 psi (4.14 bar)	50 psi (3.45 bar)	60 psi (4.14 bar)	70 psi (4.83 bar)	70 psi (4.83 bar)
Temperatur Gas 2	600°C	550°C	550°C	500°C	500°C	500°C	550°C	500°C	550°C
Resolution MS 1	unit (approx. 0.7 amu FWHM*)								
Resolution MS 2	unit (approx. 0.7 amu FWHM)								
Dwell time	20	20	20	50	20	10	50	20	20 / 40

*FWHM = full width at half maximum

Table 26: Document History

Action	When?	Version
Development of Method by the CRL-SRM	2006-2008	
Presentation of method at the EPRW in Berlin (oral presentation plus poster)	June 2008	-
Drafting of V1	Nov.-Dec. 2008	V1
Placing of V1 in CRL-Website	Jan. 2009	
Update of Table 1. Expected concentrations of ISSs were calculated with a wrong dilution factor in previous version. Arithmetical errors were corrected.	Aug. 2009	V2
Introduction of measurement conditions for HEPA within the "Glyphosate & Co." method		
Introduction of measurement conditions for the screening of diquat and paraquat within the "Quats & Co. method"	Nov 2009	V3
Introduction of measurement conditions for Amitrole. chlormequat. mepiquat and daminozide "Amitrole & Co." method		
Extensive text revisions		
Introduction of measurement conditions for Streptomycin Kasugamycin	May 2010	V4
Introduction of measurement conditions for the screening of Perchlorate ion		
Extensive text revisions		
Extensive text revisions and restructuring of document	Nov 2010	V5
Introduction of measurement conditions for ETU. ETU D ₄ . PTU. PTU D ₆ . Cyromazine. Cyromazine D ₄ . N-Acetyl-Glufosinate. N-Acetyl-Glufosinate D ₃ . Glufosinate D ₃ . MPPA D ₃ . Morpholin. Morpholin D ₈		
Introduction of an acronym for the method (QuPPE)	July 2011	V6
Advice to use plastic vessels and stoppers for Glyphosate		
Minor modification and additional instructions in Method 1 (M1)		
Modification of mobile phase of M3 to improve analysis of ETU and PTU		
Introd. of measurement cond. for Amitrole ¹⁵ N ¹³ C and Amitrole ¹⁵ N in M3		
Introd. of measurement cond. for Nereistoxin and Nereistoxin D ₆ in M4		
New method (M7) for the analysis of Morpholin/Morpholin D ₈ ; Diethanolamine/diethanolamine D ₆ ; Triethanolamine/Triethanolamine D ₁₂ (M7)		
Removal of Morpholin from M4 as it does not separate from the interfering diethanolamine		
Introduction of ETU and PTU and their corresponding ILISs in Method 5		
Correction of dimension of stock solutions conc. in Table 12 (to mg/mL)		
Text and Table revisions		
Extensive revision of table concerning possible sources of purchase of ISSs		
Some additions in "Apparatus and Consumables" chapter		
Clarifications in chapter concerning standard additions		
Overview table concerning the scope of the methods 1.1. 1.2. 1.3 and 2		
Addition of Phosphonic acid in Method 1.1 ("Glyphosate & Co.")		

Action	When?	Version
New LC-method (Method 1.2) for "Glyphosate & Co." using a Dionex ionPac AS11-HC column and an Eluent with near to neutral pH; additionally covering Fosetyl		
New LC-method (Method 1.3) for "Glyphosate & Co." using a Hypercarb column and an High acid content Eluent covering all analytes covered by Method 1.1. Method 1.2 and Method 2 (including perchlorate).		
Update of practical considerations for methods 1.1-1.3		
Update of table with performance data		
Table with exemplary recovery data was deleted (recovery figures can be obtained in the EURL-DataPool)		
Update of table with LOQs		
Update of table with providers of ILISs		
Elimination of errors in text		
Addition of Chlorate in Method 1.3		
Update of practical considerations for methods 1.1-1.3 (Column C)	Nov. 2013	V7.1
Update of table with performance data		
Update of table with LOQs		
Introduction of Trimethylsulfonium-D ₉ and N.N-Dimethylhydrazine-D ₆ in Method 4		
Thorough revision of text and elimination of errors	Mar. 2015	V8
Practical advices on the choice of filter materials		
New Table 15: Conversion factors between standard materials and analytes		
Advices as regards the use of ILISs		
Update of Table 5.6: LC-MS/MS measurement conditions		
New chapters "Hints on Method 1.1 – 1.4" and replacement of the section "Practical care and use considerations concerning the columns of methods 1.1-1.3. This includes information on various potential sources of errors such as in-source fragmentations of Fosetyl and Ethephon to Phosphonic acid and of Perchlorate to Chlorate as well as degradation of compounds in solution.		
Introduction of Cyanuric acid and Bialaphos in M1.3		
Correction of a typing error concerning the mass-transitions of Phosphonic acid (81/79 instead of 81/81)		
Introduction of the ILIs of Phosphonic acid and chlorate in M1.3 and 1.4		
New LC Method (1.4) for "PerChloPhos" using a Hypercarb column and an High acid content Eluent optimized for chlorate. perchlorate. phosphonic acid compared to Method 1.3		
Change of name of former M4 to M4.1		
Introduction of Melamine and Propamocarb as well as the corresponding ILISs in M4.1		
New LC Method (M4.2) employing a Hilic-Type BEH Amide column allowing the simultaneous analysis of many polar pesticides		
Reduction of injection volume and increase of dwell-time in method M6		
New LC-method (M8) for the analysis of triazole derivative metabolite (TDMs) and their corresponding ILISs		
Update of Table 17: Providers of isotopically labeled internal standards		

Action	When?	Version
5.1 Sample preparation: note to importance of having small particle sizes	May 2015	V8.1
5.2.4 notes to extraction time for dry products and the influence of particle size		
5.6 information on the methods currently routinely used at CVUA Stuttgart		
Update Table 20: Exemplary LC-MS/MS parameters for Sciex QTRAP 5500		
Update of Chapter 5: Procedure including the extraction procedure at a glance	Mar. 2016	V9
Update of Table 3: Overview and scope of the methods proposed within this document for the QuPPE method		
Update of		
Table 4: Practical Information: Methods mainly used by CVUA Stuttgart		
Update of Chapter 5.7.3.1.: Hints on Method 1.3		
Update of Method 1.4: Introduction of measurement conditions for the measurement of Bromate and Bromide ion		
Update of Chapter 5.7.4.1.: Hints on Method 1.4		
Update of Method 4.2 : "Quats & Co BEH Amide" including Aminocyclopyrachlor. Chloridazonesphenyl. Mepiquat-4-hydroxy. Propamocarb-N-desmethyl. Propamocarb-N-oxide		
Update of Method 6 : "Streptomycin and Kasugamycin". change of gradient and new chromatograms		
Update of Method 8 "Triazole derivative metabolites (TDMs)" new DMS parameters		
Update of Table 19: Overview of approximate limits of quantification (LOQs)*		
Update of Table 20: Conversion factors between typical purchased standards and target analytes (3.15):		
Update of Table 21: Exemplary concentrations of pesticide stock and working solutions		
Update of Table 22: Providers of isotopically labeled internal standards		
Update of Table 23: Exemplary concentrations of IS working solutions		
Elimination of an error in method 1.4 (Change in dilution procedure)	May. 2016	V9.1
Inclusion of N-Acetylglyphosate in Table 3: Overview and scope of the methods proposed within this document for the QuPPE method:	October 2016	V9.2
Inclusion of N-Acetylglyphosate in Table 4: Practical Information: Mainly used methods used at CVUA Stuttgart		
Addition of a further Ethephon-ILIS mass trace and inclusion of N-Acetylglyphosate in Table 7: Proposed LC-MS/MS conditions for Ethephon. HEPA (Ethephon metabolite). Glyphosat. AMPA (Glyphosate metabolite). N-Acetylglyphosate (Glyphosate metabolite). N-Acetyl-AMPA (Glufosinate metabolite). Glufosinate. MPPA (Glufosinate metabolite). N-Acetyl-glufosinate (Glufosinate metabolite). Fosetyl-Al. Maleic hydrazide. Cyanuric acid and Bialaphos.		
Update of Figure 4: Chromatograms of Ethephon. HEPA. Glyphosat. AMPA. Glufosinate. MPPA. N-Acetyl-AMPA. N-Acetyl-Glufosinate. Fosetyl-Al. Maleic hydrazide. Cyanuric acid. Bialaphos and N-Acetylglyphosate at 0.1 ppm on almond extract.		
Inclusion of N-Acetylglyphosate in Table 18: Overview of approximate limits of quantification (LOQs)		
Update of Table 19: Conversion factors between typical purchased standards and target analytes (3.15)		
Update of Table 20: Exemplary concentrations of pesticide stock and working solutions (3.15 and 3.16). solvent proposals also apply to ILISs (see 3.18. 3.19 and 3.20).		
Inclusion of N-Acetylglyphosate in Table 21: Exemplary providers of isotopically labeled internal standards 3.17.		
Update of Table 22: Exemplary concentrations of IS working solutions (3.19)		
New Method: (Method 9 "Difluoroacetic acid and Trifluoroacetic acid"), see 5.7.13		

Action	When?	Version
Proposed volume of IS-WS II changed to match with volume of IS-WS I (see Table 1)		
Update of Method 6: Modification of the LC-Gradient		
Update of Table 3: data on M 9 were included		
Update of Table 3: data on M 9 were included		
Hints on stability of standard solutions added in 5.7.3 h), including Table 9		
Overview of lowest successfully validated levels (Table 19)		
Update of Table 20 : DFA and TFA added		
Update of Table 21: DFA and TFA added; solvents for Ethephon, Fosetyl and Maleic hydrazide changed		
Table 22 updated		
Update of Table 23: DFA and TFA added		
Update of Table 25: data on M 9 were included		