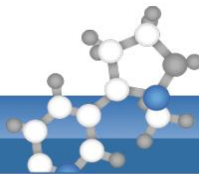

Quick Method for the Analysis of Numerous Highly Polar Pesticides in Food Involving Extraction with Acidified Methanol and LC-MS/MS Measurement

I. Food of Plant Origin (QuPPe-PO-Method)

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Note: Changes from V9.3 to V10 are highlighted in yellow, changes from V10 to V10.1 are highlighted in green

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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, vegetables, cereals, dry pulses, oily seeds and nuts as well as in honey.
Residues are extracted from the test portion following water adjustment and addition of acidified methanol. In the case of pulses, nuts and oily seeds, EDTA is added for the complexation of metal ions, such as calcium and magnesium, which can affect the analysis of certain compounds (e.g. glyphosate and AMPA). The mixture is centrifuged, filtered and directly analyzed by LC-MS/MS. Various LC-MS/MS methods allowing simultaneous analysis of different combinations of pesticides are provided. Quantification is in most cases performed employing isotope labeled analogues of the target analytes 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. Due to the simplicity of the procedure matrix-effects can be very pronounced in many cases due to the simplicity

2. Apparatus and Consumables

2.1. Powerful sample processing equipment,

for milling samples. For fruits and vegetables, e.g. Stephan UM 5 or Retsch200 by Retsch Grindomix GM 300 or Vorwerk-Thermomix TM31-1. For dry commodities such as cereals, e.g. ZM 200 by Retsch equipped with a 0.5 mm sieve.

2.1. LC-Plastic tub,

for filling-in liquid nitrogen to immerse the samples prior to milling. e.g. 20 to 40 L polypropylene or polyethylene tub with handles. Styrofoam boxes are also suitable. Take the necessary precautions when working with liquid nitrogen.

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; Oak-ridge, 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. 10 mL centrifuge tubes with screw caps,

for the d-SPE step (**5.2.5**), e.g.: disposable 10 mL PP-tubes by Simport/Beloeil (Canada), article-no. T550-10AT.

2.4. Automatic pipettes,

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

2.5. 10 mL solvent-dispenser,

for the acidified methanol (**3.6**).

2.6. Centrifuge,

suitable for the centrifuge tubes employed in the procedure (**2.2**) and capable of achieving > 3,000 g. E.g. Rotanta 460 by Hettich, Tuttlingen/Germany. Centrifuges capable of achieving higher centrifugal forces and of refrigerating the sample during centrifugation (e.g. Avanti JXN-26 by Beckman Coulter, Brea/USA) are to be preferred.

Notes: Higher relative centrifugal forces (e.g. RCFs > 10,000 g) and cooling during centrifugation (e.g. to -10°C) are beneficial by causing increased precipitation of matrix components. Check centrifuge tubes for suitability for higher velocities.

2.7. Disposable syringes,

suitable to the filters used; e.g. 2 or 5 mL disposable polypropylene syringes with luer tip by Macherey-Nagel, Düren / Germany (Ref. 729100 and 729101 respectively). These are suitable for the syringe filters listed below (**2.8**).

2.8. Disposable syringe filters,

e.g. . Ø 25 mm CHROMAFIL® filters and 0.2 µm pore size filters of the following materials: Hydrophilized polytetrafluoroethylene (H-PTFE) or Cellulose Mixed Ester or Polyester (Ref. No. 729245, 729006 and 729021 respectively) all by Macherey-Nagel, Düren / Germany. 0.45 µm pore size filters of the above types may be attached in front of the 0.2 µm filters if the latter get clogged when used directly. -

Note: Check filters for any contamination with analytes of interest. Significant levels of Perchlorate and Chlorate were detected in the above mentioned polyester filters. For testing suitability consider the worst-case scenario, where filters are clogged quickly (e.g. elute only 200 µL through each filter). Such severe clogging was for example observed with industrially milled cereals, pears and pineapples.

2.9. Ultrafiltration filters,

5 or 10 kDa molecular weight cutoff filters suitable for centrifuges, e.g. Vivaspin® 6 mL 5 kDa entailing Polyethersulfone membranes OR Amicon® Ultra-15 10K entailing Ultracel® low binding regenerated cellulose.

2.10. Autosampler vials,

suitable for LC auto-samplers, e.g. Vials Screw top 2 mL Cat No. 9502S-PP-CLEAR, 12x32 mm MicroSolv Technology Corporation (MTC), USA; Lids for plastic vials: Lid G9-L/Sil-CS Art.-No. 2.301398-Blau WE13989, Ziemer GmbH, Langerwehe / Germany

Notes: The use of plastic vials is highly recommended as several of the compounds covered by this method (e.g. Phosphonate, Nicotine, Paraquat, Diquat, Streptomycin and Glyphosate)[1] tend to interact with glass-surfaces. 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 reduces such interactions. Percent losses due to such interactions are typically higher at low concentrations

2.11. Volumetric flask with stoppers,

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

Notes: The use of plastic flasks and stoppers is highly recommended as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.10**).

2.12. LC-MS/MS instrumentation,

equipped with ESI source and appropriate columns, see details in chapters **5.6.1** till **5.6.14**.

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 (LC-MS quality)

3.3. Acetonitrile (LC-MS quality)

3.4. Formic acid (concentrated; > 98%)

[1] The list of compounds requiring plastic vessels is not comprehensive (this remark applies to the entire document).

3.5. Acetic Acid (concentrated; >98%)

3.6. Acidified methanol,

for the extraction of the majority of samples, prepared by pipetting 10 mL formic acid (**3.4**) into a 1000 mL volumetric flask and filling up to volume with methanol (**3.2**).

3.7. C-18 sorbent (ODS-sorbent),

e.g. Polygoprep 30-300 µm Macherey-Nagel GmbH & Co KG/Düren (Germany), article-no. 711720.100).

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

3.9. Dimethylamine,

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

3.10. Ammonium formate (p.a.)

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

3.12. Sodium hydroxide (p.a.)

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

3.14. Ethylenediaminetetraacetic acid tetrasodium

e.g. tetrasodium dihydrate salt (CAS Number 10378-23-1): E6511 Sigma Aldrich (MW=416.20)
OR tetrasodium tetrahydrate salt (CAS No.: 13235-36-4): 34103-M EMD Millipore/Merck (MW=452.23)

3.15. 10% aqueous EDTA solution,

prepared by weighing 15.85 EDTA tetrasodium tetrahydrate (OR 14.59 g EDTA tetrasodium dihydrate) into a 100 mL volumetric flask with stopper, dissolving it in 80 mL water and filling up to 100 mL with water. This solution contains 10 % (w/v) EDTA tetra-anion.

3.16. Dry ice,

technical grade can be used; periodically check that it does not contain compounds of interest at relevant levels.

3.17. Pesticide Standards,

of known purity.

3.18. Pesticide stock solutions,

e.g. 1 mg/mL solutions of pesticide standards (**3.17**) in a water miscible solvent. Suggestions of solvents suitable for the preparation of stock solutions can be found in **Table 28**.

Notes: Keep in mind that some standards are sold as salts or hydrates. Some exemplary **conversion factors** to be applied between typical standards and the analytes are shown in **Table 27.** Keep solutions in **plastic vessels** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.10**).

3.19. Pesticide working solutions / mixtures,

prepared at appropriate concentrations by diluting pesticide stock solutions (**3.18**) 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**). Suggestions of solvents for preparing stock solutions can be found in **Table 28**.

Notes: Keep solutions in **plastic vessels** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.10**).

3.20. Internal Standards (ISs),

Exemplary sources are shown in

Table 29. Check whether the ISs contain native compounds at levels, which would lead to false positives or quantification errors.

3.21. IS-stock solutions,

e.g. 1 mg/mL solutions of ISs (**3.20**) in a water miscible solvent (e.g. methanol, acetonitrile, water or mixtures thereof). For solvent-suggestions see **Table 28** in the ANNEX.

Notes: Keep solutions in **plastic vessels** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.10**).

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 relevantly disturbed by co-eluting matrix components. An ILIS standard with a relatively low purity may be still acceptable as long as the content of native analytes (irrespective whether they were initially present - as impurity - or whether they were formed during storage of working solutions) is low enough to exclude false positive results and to ensure that any influence on quantification of positive results is negligible. Some examples where care is needed to avoid formation of native analytes from ILISs are N-Acetyl-Glyphosate (acetyl D_3) that may de-acetylate into native Glyphosate, Fosetyl-D_5 that tends to hydrolyse to native Phosphonic acid (see **5.6.2** under Hints on Method 1.2) and Maleic Hydrazide D_2 the standard of which typically contains a small, but relevant, fraction of the native compound as impurity (see **5.6.8**).

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.

3.22. IS-working solution I (IS-WSln-1) for spiking samples prior to extraction,

prepared at appropriate concentrations by diluting IS-stock solutions (**3.21**) of one or more ISs with water-miscible solvents. Suggestions for solvents are shown in **Table 28** and suggestions for the concentrations in **Table 30**.

Notes: Keep solutions in **plastic vessels** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.10**).

In presence of water and especially at high pH levels, Phosphonic acid $^{18}O_3$ will gradually convert to $^{18}O_2{}^{16}O_1$, $^{18}O_1{}^{16}O_2$ and eventually of $^{16}O_3$ (native) Phosphonic acid. The **$^{18}O_3$ Phosphonic acid standard solution provided by the EURLs should be preferably diluted in acetonitrile**, where it was shown to be stable for long periods.

3.23. IS-working solution II (IS-WSln-2) for preparation of calibration standards,

prepared at appropriate concentrations by diluting IS-WSln-1 (**3.22**) with water-miscible solvents. Suggestions for solvents are shown in **Table 28** and for concentrations in **Table 30**.

Notes: Keep solutions in **plastic vessels** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.10**).

For short term usage (e.g. up to one month) the ILIS of Phosphonic acid can be diluted in acidified methanol (**3.6**).

3.24. LC-MS/MS mobile phases,

see details in chapters **5.6.16** to **5.6.16.**

4. Disclaimer

This method refers to several trade names 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 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 valid regulations and guidelines.

Fruits and vegetables are preferably milled cryogenically (e.g. using dry ice). This is done to reduce analyte degradation and particle sizes, with the latter resulting in improved homogeneity and residue accessibility. One possibility for cryogenic milling is to cut large units coarsely to ca 3x3 cm pieces, freeze them and then mill them for ca. 1-2 minutes with a powerful mill. Then add dry ice (ca. 150-200 g per 500 g sample) and continue milling until barely any carbon dioxide fumes are observed. Alternatively fill a plastic or polystyrene container with a ca 5-10 cm thick layer of liquid nitrogen and immerse the sample pieces into liquid nitrogen. When completely frozen transfer the material into a powerful knife mill and grind at high speed until it gets a free flowing snow-like consistency. If necessary crush large units with a hammer before milling. If the material starts defrosting during milling, add some more liquid nitrogen or dry ice and continue milling as described above.

Dry commodities (e.g. cereals, pulses) are intensively milled to reduce particle size and improve the accessibility of residues enclosed in the interior of the materials. Particle sizes (e.g. <500 µm) are preferable. The larger the particles are the longer the extraction times required to achieve quantitative extraction of systemically distributed compounds. Ultra centrifugal mills with 500 µm sieves were found to be suitable for this purpose. Addition of dry ice during milling (e.g. at a sample: dry ice ratio of 2:1) reduces heat. The use of knife mills is also possible but prolonged milling times are needed to reduce the size of particles. Add some dry ice periodically to reduce heat formation. Alternatively a two stage milling can be helpful. For this a representative portion of the first milling step is transferred to a second smaller mill and homogenized further.

Dry and oily commodities (e.g. oily seeds and nuts) tend to form a thick paste that prevents proper milling and is difficult to handle, when using knife mills at room temperature. Milling with ultra centrifugal mills typically leads to a clogging of the filters. For such materials cryogenic grinding with a powerful knife mill is recommended. Precool the mill with dry ice and then mill the material at a sample to dry ice ratio of ca 2:1 until a fine powder is obtained. Keep temperature low to avoid that the material becomes clumpy and thus more difficult to handle. Alternatively immerse the sample in a plastic or polystyrene container containning liquid nitrogen. When completely frozen transfer it into a powerful knife mill and grind until a fine powder is obtained. Do not mill too long as the material with thaw and become clumpy and thus more difficult to handle.

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. Alternatively immerse the sample material in a plastic or polystyrol container containning liquid nitrogen. When completely frozen transfer it into a powerful knife mill and grind until a fine powder is obtained. Do not mill too long and quickly transfer the frozen powder into a storage container and place it into the freezer to avoid that it becomes clumpy and more difficult to handle.

5.2. Extraction / Freeze-Out / Centrifugation / Cleanup / Filtration

A flow chart of the **analytical procedure** is shown in Figure 1 (for most commodities) and in Figure 2 (for pulses, nuts and oily seeds)

5.2.1. **Weighing of analytical portions**

Weigh a representative analytical portion (ma) of the sample homogenate (**5.1**) into a 50 mL centrifuge tube (**2.2**). In case of fresh fruits and vegetables and juices weigh 10 g ± 0.1 g of the homogenized sample. In case of cereals, dried pulses, oily seeds, nuts, dried fruits, dried vegetables, dried mushrooms and honey weigh 5 g ± 0.05 g of the homogenates. In case of dry fruits rehydrated according to **5.1**, weigh 13.5 g ± 0.1 g of the re-hydrated and homogenized material (corresponding to 5 g sample). Smaller analytical 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

For **commodities with ≥ 80% of natural moisture**, water adjustment to 10 mL is not essential and may be skipped when appropriate ISs are employed before any aliquotation. If no IS is used, **add water (3.1)**, as indicated in **Table 31** to minimize volumetric errors. Continue with step **5.2.3.**
For **commodities with < 80% of natural moisture** (except chia seeds and lineseeds, see notes)**, add water (3.1)** to the analytical portion (**5.2.1**) to reach a total water content of approx. 10 g according to the indications in **Table 31**. No further water adjustment is needed where re-hydrated commodities (see **5.1**) are employed. Continue with step **5.2.3.**

Notes:
Keep in mind that the water volume adjustments in **Table 31** are approximate.

For **oily seeds, nuts and pulses** the water contained in the aqueous EDTA solution (added during the extraction step (**5.2.3**) is also considered in the overall water content. Therefore 9 mL of water + 1 mL of aqueous EDTA solution are added in total. See also **Table 31**.

In the case of **chia seeds and lineseeds (flaxseeds)** adding water directly to the samples leads to a soaking of the formation of a gellike layer, which hinders the accessibility of residues. To suppress this phenomenon, change the order of solvent addition as follows. First add 10 mL acidified methanol (**3.6**) and 100 µL formic acid (**3.4**), shake shortly, and then add the 9 mL water and the 1 mL EDTA solution and continue with 15 min shaking as described under **5.2.3.** Then continue with step **5.2.4-(2)** or **(3)** and further with step **5.2.5-(2)**.

5.2.3.Extraction

A) General procedure

(1) **All commodities of plant origin except pulses, nuts and oily seeds:** Add 10 mL acidified methanol (**3.6**) and 100 µL (or another appropriate small volume) of the IS-WSln-1 (**3.22**) containing isotopically labeled analogues of the analytes of interest (added IS mass = m_{IS}^{sample}). Close the tube and shake vigorously for 1 to 15 min by hand or a mechanical shaker.

(2) **Pulses, nuts and oily seeds:** Add 100 µL (or another appropriate small volume) of the IS-WSln-1 (**3.22**) containing isotopically labeled analogues of the analytes of interest (added IS mass = m_{IS}^{sample}) and agitate shortly to distribute the ISs. Add an extra amount of 100 µL formic acid (**3.4**). Close the tube and shake for a few seconds to distribute the acid and allow proteins to coagulate. Add 1 mL 10% aqueous EDTA solution (**3.15**) and shake for 15 min by an automatic shaker. For chia seeds and lineseeds please refer to the notes under **5.2.2**. Where no automatic shaker is available, dry products may be shaken for 1 min by hand followed by a soaking period of 15 min and a subsequent second 1 min vigorous shaking by hand.

Notes: Where no ISs are used the aim should be to reach a total volume of the liquid phase as close as possible to 20 mL. This volume will mainly consist of the water naturally contained in the sample, the water added during the procedure (including that of the EDTA solution), the extraction solvent added, the IS solution added as well as the extra formic acid added. A volume contraction is also taking place and is partly matched by the IS and the formic acid. Further alternatives to avoid errors due to volumetric deviations are calibrations that compensate for recovery, such as standard additions to sample portions and procedural calibrations using a suitable blank matrix. The 20 mL volume of the extractant corresponds to 0.5 g / 0.25 g sample per mL extract if 10 g / 5 g sample are used. Where the raw extract is diluted with acetonitrile for cleanup purposes (see **5.2.5-(2)** concerning pulses, oily seeds and nuts) the final concentration in the extract is reduced to 0.125 g/mL.
For screening purposes the IS can be alternatively added to a sample extract aliquot (e.g. the 1 mL aliquot transferred to the autosampler vial, see below), assuming that 1 mL extract entails 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 but not for recovery and volume deviations. The quantitative result should therefore be considered tentative. For more accuracy samples should be re-extracted with the IS being added to the analytical portion before aliquotation.

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 or the extraction will need to involve addition additional breakup of the sample particles, e.g. by the use of a high speed dispenser (e.g. Ultra Turrax).

B) Procedure for Paraquat and Diquat

For the analysis of Paraquat and Diquat add 10 mL of a 1:1 mixture of methanol + aqueous HCl 0.1M to the water-adjusted analytical portion (from **5.2.2**), close the vessel and shake initially for 1 minute. Then place the extraction vessel in shaking water bath at 80 °C for 15 minutes. Then shake again for 1 minute and wait for the sample to cool down to room temperature or lower before centrifuging.

Notes: The above mentioned extraction conditions for paraquat and diquat are needed for the quantitative extraction of incurred residues[2]. Extractions with the normal QuPPe solvent (methanol containing 1% formic acid) at room temperature lead to poorer extraction yields but are still well suitable for screening. Extractions with the normal QuPPe solvent involving thermal treatment (15 minute 80 °C for 15 minutes) were shown to provide quantitative extraction yields of incurred Diquat and Paraquat residues in wheat and potatoes but not in lentils.

5.2.4. Freeze-Out and Centrifugation

Depending on the available centrifugation equipment there is various options, e.g.:

(1) **Ambient centrifugation:** Centrifuge the extracts from **5.2.3** for 5 min at ≥3,000 g (the higher the centrifugation force the better). This procedure is **NOT** recommended for extracts of commodities that pose difficulties in filtration (e.g. finely milled cereals, pineapples and pears). For such commodities better use the following options (2) or (3).

(2) **Ambient centrifugation following freeze-out:** Place the extracts from **5.2.3** into a freezer (e.g. at ca, -80 °C for 30 min or for > 120 min at ca. -20 °C) and centrifuge while still cold for 5 min at ≥3,000 g. Higher centrifugation forces (e.g. ≥10,000 g) are preferable. This procedure is suitable for the extracts of all samples and especially recommended for those posing difficulties in filtration.

(3) **Refrigerated high-speed centrifugation:** Centrifuge the extracts from **5.2.3** for >20 min at high centrifugation speed (e.g. >10,000 g) and low temperatures (e.g. lower than -5 °C). Centrifugation time may be reduced to 5 min if the extract is pre-frozen. This procedure is suitable for extracts of all samples and especially recommended for those posing difficulties in filtration.

Notes: Solid metal racks suitable for falcon tubes (e.g. VWR® Modular Blocks for Conical-Bottom 50 mL Centrifuge Tubes) may be used to speed up freeze-out.

Low temperatures reduce the solubility of interfering matrix components resulting in increased precipitation, which considerably facilitates the filtration step as well as the subsequent LC-MS/MS analysis by reducing matrix effects and increasing the lifespan of columns. In cases **(2)** and **(3)** it is recommended to proceed immediately with the next steps to avoid redissolvation of matrix components. Otherwise transfer an aliquot of the cold supernatant into a sealable container for later use.

5.2.5. Removal of proteins and lipids

(1) **Pulses:** transfer 2 mL of the supernatant into a 10 mL centrifuge vial containing 2 mL of acetonitrile (**3.3**) and shake for 1 min. Then centrifuge for 5 minutes at >3000 g (see **2.6**).

(2) **Nuts and oily seeds:** transfer 2 mL of the supernatant into a 10 mL centrifuge vial containing 2 mL of acetonitrile (**3.3**) and 100 mg of C-18 sorbent and shake for 1 min. Then centrifuge for 5 minutes at >3000 g (see **2.6**).

(3) **Oily fruits (e.g. avocado):** transfer 4 mL of the supernatant (from **5.2.4)** into a 10 mL centrifuge vial containing 200 mg of C-18 sorbent and shake for 1 min. Centrifuge for 5 min at >3000 g (see **2.6**). This step may be skipped if the sample was centrifuged frozen (**5.2.4-(2)** and **5.2.4-(3)**, with the supernatant being removed while still very cold.

5.2.6. Filtration

Withdraw an aliquot (e.g. 2-3 mL) of the supernatant from **5.2.4** or **5.2.5** using a syringe (**2.7**) and **filter** it through a syringe filter (**2.8**) either directly into an auto-sampler vial (**2.10**) or into a sealable storage vessel.

Notes: Where centrifugation with the available means results in extracts that are difficult to filter a 2-step filtration may be performed by connecting a 0.45 µm syringe filter on top of a 0.2 µm one (**2.7**).

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[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.

Where a high lipid and low protein content commodity (e.g. avocado) was centrifuged frozen (under **5.2.4),** and step **5.2.5** was skipped, filter the supernatant quickly to avoid that lipids redissolve.

Pulses, nuts and oily seeds: transfer a 3 mL aliquot of the supernatant from 5.2.5 into an ultrafiltration unit (**2.9**) and centrifuge at ca. 3,000 g until enough filtrate is accumulated in the reservoir (5 min are typically enough). Transfer an aliquot of the filtrate into an autosampler vial for measurement.

5.3. Generation of blank extracts

Using suitable blank commodities (not containing any relevant 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 (**3.19** and **Table 28**). Spike directly to the matrix, prior to any water or solvent addition. Use small volumes of pesticide working solutions (e.g. 50-300 µL), to avoid too strong dilution. Conduct sample preparation as described in **5.2**.

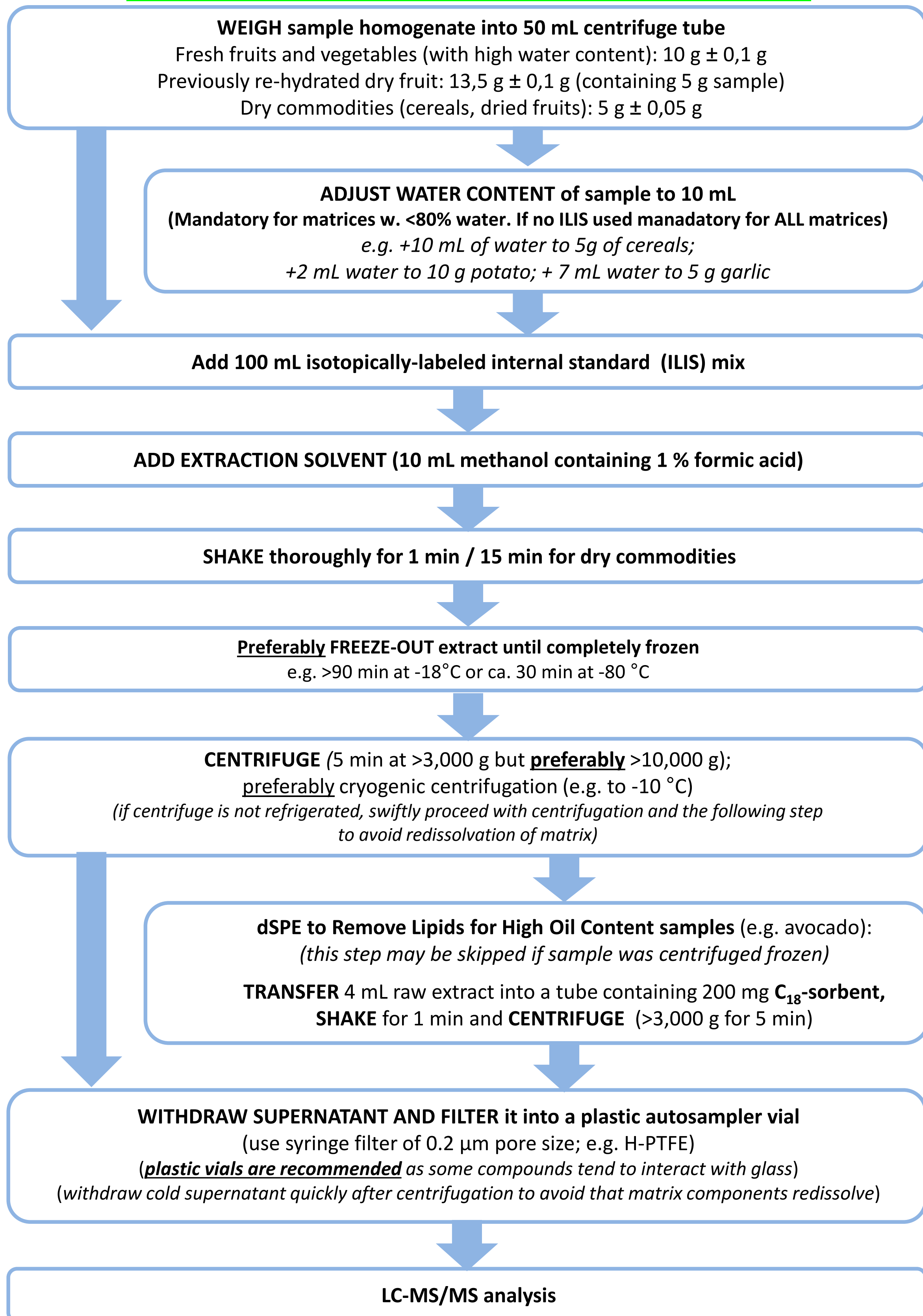


Figure 1: QuPPe-PO-Method at a glance (general procedure for most commodities, not considering paraquat and diquat)

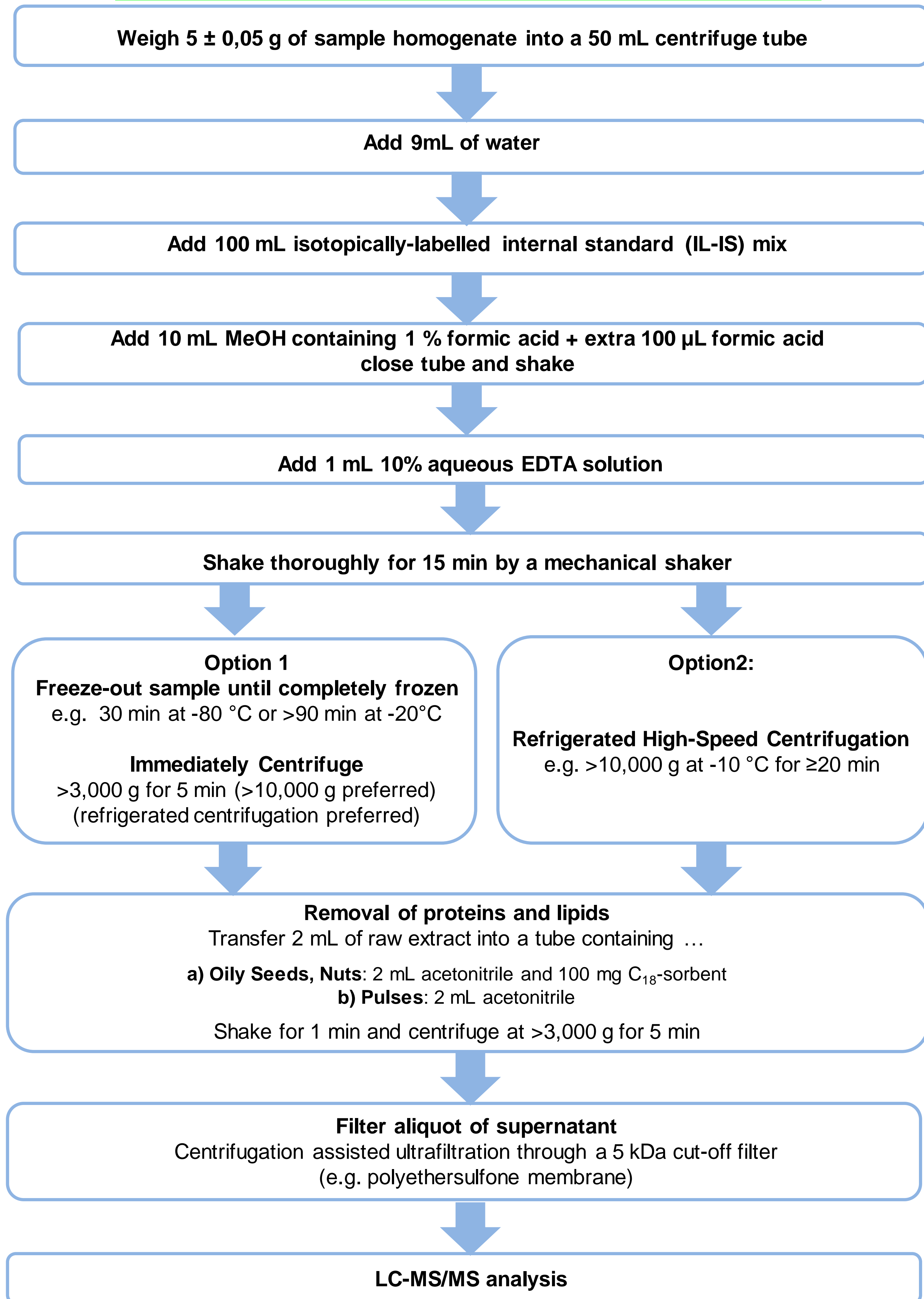


Figure 2: QuPPe-PO-Method at a glance; procedure for pulses, oily seeds and nuts

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 W_R of the pesticide in the sample, when IS is used, is shown in **5.7.1**. 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.

Notes: Though matrix-matched calibration is considered the best option, solvent-based calibrations can also produce accurate results as IL-ISs can compensate for errors irrespective on whether the calibration is solvent-based, matrix-based or matrix-matched. Nevertheless, in some cases the use of matrix-based calibrations are to be preferred over solvent-based calibrations as the matrix present can decrease unwanted interactions with surfaces (e.g. in the injector area) thus leading to peak shapes and retention times that are closer to those observed from sample extracts.

5.5.2. Matrix-based and matrix-matched calibration standards

Transfer suitable aliquots of a blank extract (**5.3**) to auto-sampler vials and proceed as shown in **Table 1**.
The calculation of the mass-fraction W_R of the pesticide in the sample using matrix-matched calibration standards, with and without the use of ILIS, is shown in **5.7.1** and **5.7.2** respectively.

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

		Calibration standards								
		Solvent based (5.5.1)			Matrix-matched (5.5.2)					
		using IS[4]			without IS[5]			using IS[4]		
Calibr. levels in µg pesticide /mL OR in µg pesticide/ "IS-portion"[1]		0.05[6]	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 (3.19) [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-WSln-2 (3.23)[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-WSln-2 (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 highly recommended to prepare the IS-WSln-2 **(3.23)** by diluting IS-WSln-1 **(3.22)** 20-fold. The same volume and pipette as in **5.2.3** can be used for preparing 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).
[4] Where ILISs are not available/employed, matrix-matched standards **Table 1**) or the standard additions approach (**5.5.3**) 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. Where the raw extract is diluted further and when using 5 g test portions (e.g. pulses, nuts and oily seeds) 0.05 µg/mL calibration level corresponds to 0.4 mg pesticide /kg sample.

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 ($w_{R(approx)}$). 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 (**3.19**) 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 x $w_{R(approx)}$, vial 3) 1 x $w_{R(approx)}$, and vial 4) 1.5 x $w_{R(approx)}$,
Example B: Vial 1) no addition; vial 2) 1 x $w_{R(approx)}$, vial 3) 2 x $w_{R(approx)}$, and vial 4) 3 x $w_{R(approx)}$.
Adjust the volume within all vials by adding the corresponding solvent amounts.
An exemplary pipetting scheme according to Example B in shown in **Table 2**. The calculation of the mass fraction of the pesticide in the sample w_R is shown in **5.7.2**.

Table 2: *Exemplary pipetting scheme of a standard additions to extract aliquots approach (for a sample extract containing 0.5 g sample equivalents per mL and an estimated residue level ($w_{R(approx)}$) of 0.5 mg/kg = 0.25 µg/1000 µl*

Additions	Vial 1	Vial 2	Vial 3	Vial 4
Volume of sample extract	1000 µL (= 0.5 g sample)	1000 µL (= 0.5 g sample)	1000 µL (= 0.5 g sample)	1000 µL (= 0.5 g sample)
Internal Standard (IS)	none	none	none	none
Added volume of pesticide working solution containing 5 µg/mL (3.19)	-	50 µL	100 µL	150 µL
Mass of pesticide added to each vial ($m_{pest}^{std\ add}$ **)**	-	0.25 µg	0.5 µg	0.75 µg
Volume of solvent (for volume equalization)	150 µL	100 µL	50 µL	-
Final volume	1150 µL	1150 µL	1150 µL	1150 µL

5.5.4. **Procedural calibration standards**

Procedural calibration is most useful where numerous samples of the same commodity type are analyzed within the same badge and can help to largely compensate for recovery losses and matrix effects. An ideal precondition is the availability of a blank matrix of exactly the same type as the samples to be analyzed. For this prepare 4 analytical portions of a suitable blank sample and spike three of them with increasing amounts of the pesticides of interest (as done in recovery experiments, see also **5.4**). The aim should be to cover the concentration range of the analytes expected in the samples. These spiked samples are extracted as described above and the obtained extracts are used in the same way as any other matrix-matched standards.

5.6. LC-MS/MS Analysis

Any suitable LC-MS/MS conditions that generate peaks that can be well integradet may be used. The use of IL-ISs typically ensures a good method accuracy and robustness even when matrix components have a strong influence on signals or retention times. Some exemplary instrument measurement conditions are given below. An overview of LC-MS/MS conditions proposed within this document is given in **Table 3** and **Table 4**

.

Table 3: *Overview and scope of QuPPe-LC-Methods of analytes presented within this document (see legent under* ***Table 4****)*

QuPPe method code	M 1.1 (5.6.1)	M 1.2 (5.6.2)	M 1.3 (5.6.3)	M 1.4 (5.6.4)	M 1.5 (5.6.5)	M 1.6 (5.6.6)	M 1.7 (5.6.7)	M 2 (5.6.8)	M 3 (5.6.9)	M 4.1 (5.6.10)	M 4.2 (5.6.11)	M 5 (5.6.12)	M 6 (5.6.13)	M 7 (5.6.14)	M8 (5.6.15)	M 9 (5.6.16)
Separation principle	Anion Ex.	Anion Ex.	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon	HILIC
Column type	AS 11	AS 11-HC	Hypercarb	Hypercarb	Trinity Q1	Torus DEA	Torus DEA	Obelisc-R	Obelisc-R	Obelisc-R	BEH-Amide	PFP	Obelisc-R	Trinity P1	Hypercarb	Trinity P1
ANALYTES COVERED BY LC-MS/MS IN THE ESI-POSITIVE MODE																
Amitrole	NT	NT	-	NT	NT	NT	NT	NT	✓	-	✓	NT	NT	NT	NT	NT
ETU	NT	NT	✓	NT	NT	NT	NT	NT	✓	-	✓	✓	NT	NT	NT	NT
PTU	NT	NT	✓	NT	NT	NT	NT	NT	✓	-	✓	✓	NT	NT	NT	NT
Cyromazine	NT	NT	NT	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT	NT
Trimesium	NT	NT	NT	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT	NT
Daminozide	NT	NT	NT	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT	NT
Chlormequat	NT	NT	✓	NT	NT	NT	NT	NT	✓	✓	✓	✓	NT	NT	NT	NT
Mepiquat	NT	NT	✓	NT	NT	NT	NT	NT	✓	✓	✓	✓	NT	NT	NT	NT
Difenzoquat	NT	NT	-	NT	NT	NT	NT	NT	✓	✓	✓	✓	NT	NT	NT	NT
Propamocarb	NT	NT	NT	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT	NT
Melamine	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	✓	NT	NT	NT	NT	NT
Diquat	NT	NT	-	NT	NT	NT	NT	NT	NT	✓	-	NT	NT	NT	NT	NT
Paraquat	NT	NT	-	NT	NT	NT	NT	NT	NT	✓	-	NT	NT	NT	NT	NT
N,N-Dimethylhydrazine	NT	NT	-	NT	NT	NT	NT	NT	NT	✓	-	NT	NT	NT	NT	NT
Nereistoxin	NT	NT	✓	NT	NT	NT	NT	NT	NT	✓	✓	NT	NT	NT	NT	NT
Streptomycin	NT	NT	NT	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	✓	NT	NT	NT
Morpholine	NT	NT	NT	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT	✓
Diethanolamine	NT	NT	NT	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT	NT
Triethanolamine	NT	NT	NT	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT	NT
1,2,4-Triazole	NT	NT	NT	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓	NT
Triazole-alanine	NT	NT	NT	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓	NT
Triazole-acetic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓	NT
Triazole-lactic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓	NT
Aminocyclopyrachlor	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Chloridazon-desphenyl	NT	NT	NT	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	NT	NT	NT
Propamocarb-N-desmethyl	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Propamocarb-N-oxide	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Maleic Hydrazide	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT

Table 4: *Overview and scope of the methods proposed within this document for the QuPPe method*

QuPPe method code	M 1.1 (5.6.1)	M 1.2 (5.6.2)	M 1.3 (5.6.3)	M 1.4 (5.6.4)	M 1.5 (5.6.5)	M 1.6 (5.6.6)	M 1.7 (5.6.7)	M 2 (5.6.8)	M 3 (5.6.9)	M 4.1 (5.6.10)	M 4.2 (5.6.11)	M 5 (5.6.12)	M 6 (5.6.13)	M 7 (5.6.14)	M8 (5.6.15)	M 9 (5.6.16)
Separation principle	Anion Ex.	Anion Ex.	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon	HILIC
Column type	AS 11	AS 11-HC	Hypercarb	Hypercarb	Trinity Q1	Torus DEA	Torus DEA	Obelisc-R	Obelisc-R	Obelisc-R	BEH-Amide	PFP	Obelisc-R	Trinity P1	Hypercarb	Trinity P1
ANALYTES COVERED BY LC-MS/MS IN THE ESI-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	-	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	NT
Bromate	NT	NT	(✓)	✓	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT
N-Acetyl-Glyphosate	NT	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	NT	NT	NT	✓
Trifluoroacetic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓

✓ = satisfactory chomatography 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 limitations in the detection of qualifiers compromising 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)
** Different LC-conditions required to improve peaks (see M1.7)

Table 5 : *Methods mainly used by CVUA Stuttgart*

Method	Special remarks on Substances	LC-MS/MS	Comments
M3: Glyphosate & Co. Hyper-carb (see 5.6.3)	Glyphosate AMPA N-Acetyl-AMPA N-Acetyl-Glyphosate Ethephon HEPA Glufosinate N-Acetyl-Glufosinate MPPA Fosetyl-Al Phosphonic acid (screening) Maleic Hydrazide Perchlorate (screening)) Chlorate (screening) Cyanuric acid Bialaphos	Agilent 1200 Sciex QTRAP 5500	Evaluation via solvent calibration and ILISs except for Bialaphos and N-Acetyl-AMPA (ILIS not yet available) M 1.5 and M 1.6 are currently being tested for their suitability to replace M 1.3
M1.4: Method 1.4 (M 1.4): "PerChloPhos" **(see 5.6.4)**	Perchlorate (quantitative) Chlorate (quantitative) Phosphonic acid (quantitative) Bromide (Screening, quantitative) Bromate (quantitative)	Agilent 1200 Sciex QTRAP 5500	Mostly employed directly (option: screening by M 1.3, if postive -> M 1.4) Dilution 5-fold Evaluation via solvent calibration and ILISs
M 4.1: "Quats & Co Obelisc R (see 5.6.10)	Paraquat (for specific commodities) Diquat (for specific commodities)	Waters Acquity UPLC I-Class Sciex QTRAP 5500	Analysis of specific relevant commodities. Evaluation via matrix-based calibration and ILISs
M4.2: "Quats & Co BEH Am-ide" (see 5.6.11)	Amitrole ETU Chlormequat Mepiquat Daminozide PTU Cyromazine Trimethylsulfonium Nereistoxin Difenzoquat Melamine Propamocarb Morpholine (1st screening) Diethanolamine (1st screening) Triethanolamine (1st 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, Aminocyclopy-rachlor, Mepiquat-4-hydroxy, Propamocarb-N-desmethyl, Propamocarb-N-oxide)
M6: "Streptomycin and Ka-sugamycin" (see 5.6.13)	Streptomycin Kasugamycin	Agilent 1200 Sciex QTRAP 5500	Seasonal analyses of selected commodities Evaluation via solvent calibration (using Dihydrostreptomycin as IS for Streptomycin)
M7: "Morpholine, Diethano-lamine and Triethanolamine" (see 5.6.14)	Morpholine (quantitative) Diethanolamine (quantitative) Triethanolamine (quantitative)	Waters Acquity UPLC I-Class Sciex QTRAP 5500	Employed if screening by M 4.2 was positive and in matrices where DEA tends to give false negative results in M 4.2 (e.g. in cereals, dried mushrooms). Quantification via sol-vent-based calibration and ILISs
Method 8 (M 8): "Triazole derivative metabolites (TDMs)" **(see 5.6.15)**	1,2,4-Triazole Triazol-alanine Triazole-acetic acid Triazole-lactic acid	Waters Acquity UPLC I-Class Sciex SelexION Q-Trap® 5500	Method used to be employed to collect data on residue situation. Now dormant. Quantifi-cation via solvent-based calibration and ILISs.
Method 9 (M 9): "Difluoroace-tic acid and Trifluoroacetic acid" **"Difluoroacetic acid and Trifluoroacetic acid" (see 5.6.16)**	Difluoroacetic acid Trfluoroacetic acid	Waters Acquity UPLC I-Class Sciex SelexION Q-Trap® 5500	Method used to be employed to collect data on residue situation. Now dormant. Quantifi-cation via matrix-based calibration and ILISs

5.6.1. **Method 1.1 (M 1.1): "Glyphosate & Co. AS 11"**

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), 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	**%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)	**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-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 "Hints on Method 1.1" concerning the potential interference of AMPA signals by Fosetyl.

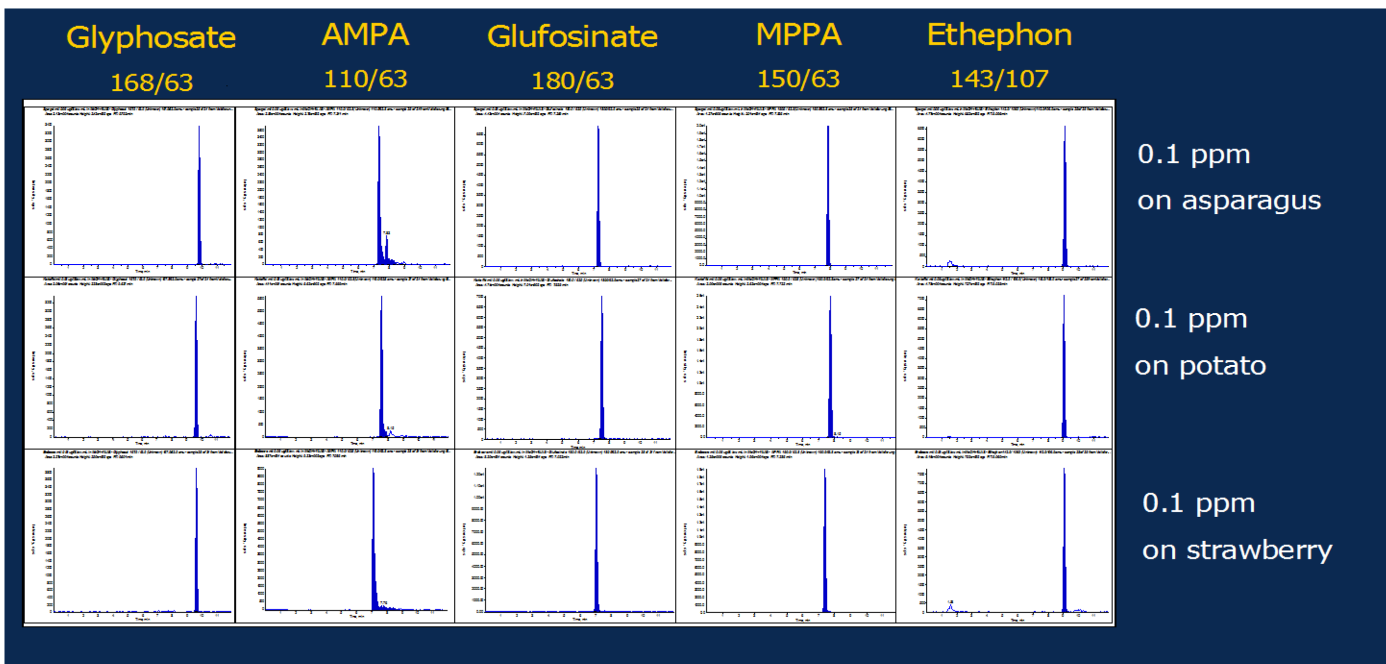


Figure 3: *Typical chromatograms of Glyphosate, AMPA, Glufosinate, MPPA and Ethephon spiked on blank-QuPPe extracts of various commodities*

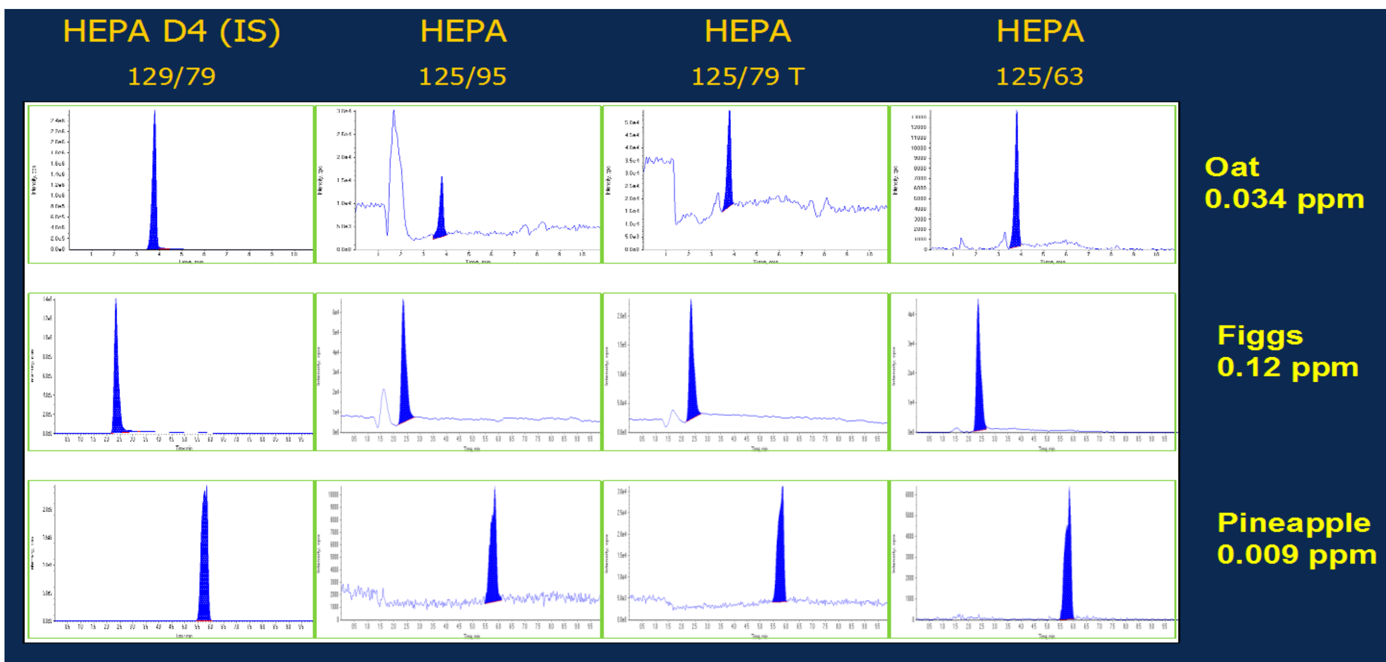


Figure 4: *Typical chromatograms of HEPA in real samples*

Hints on Method 1.1

1. **Mass spectrometric interference:** AMPA and Fosetyl share the mass-transition 110/81. Chromatographic separation is thus needed.
2. **pH-related precautions:** 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.
3. **Priming and reconditioning of column:** before first use, after long storage (e.g. >2 weeks), after injection of 50-100 sample extracts):
   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**
   b. Flush for 1 hour with 30 mM NaOH (240 mg NaOH in 200 mL water) at 0.3 mL/min
   c. Flush column for 30 minutes with **Eluent A** (water) at 0.3 mL/minRun 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!.
4. **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 **3.a - 3.c**.
5. Pre-filters: If pre-filters are used exchange them as soon as backpressure increases significantly. For practical and convenience reasons it is highly recommended to exchange pre-filters when performing other maintenance operations such as reconditioning or pre-column exchange. Losses of glyphosate, that could be clearly linked to interactions with a dirty pre-filter, have been once observed.
6. 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 M 1.1. needs to be exchanged more often than that of M 1.2 and M 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, see: http://www.dionex.com/en-us/webdocs/113497-Man-065463-03-IonPac-AS11-HC-4um-Nov12.pdf

5.6.2. **Method 1.2 (M 1,2): “Glyphosate & Co. AS 11-HC”**

Table 7: *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	Dionex IonPac AS 11-HC 2 x 250 mm (P/N 052961); 40°C **(see also notes below)**		
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: **Glyphosate-$^{13}C_2$,^{15}N (ILIS):**	168/63, 168/124, 168/150, 168/81 171/63	
	AMPA: **AMPA-^{13}C,^{15}N (ILIS):**	110/63, 110/79, 110/81** 112/63	
	N-Acetyl-AMPA:	152/63, 152/79, 152/110	
	Ethephon: **Ethephon-D_4 (ILIS):**	143/107, 143/79, 145/107 147/111	
	HEPA: **HEPA-D_4 (ILIS):**	125/79, 125/95, 125/63 129/79	
	Glufosinate: **Glufosinate-D_3 (ILIS):**	180/63, 180/136, 180/85, 180/95 183/63	
	N-Acetyl-Glufosinate: **N-Acetyl-Glufosinate-D_3 (ILIS):**	222/63, 222/59, 222/136 225/63	
	MPPA: **MPPA-D_3 (ILIS):**	151/63, 151/107, 151/133 154/63	
	Fosetyl-Al: **Fosetyl-Al-D_{15} (ILIS):**	109/81, 109/63 (Fosetyl) 114/82 (Fosetyl-D_5)	
	Phosphonic acid*:** **Phosphonic acid-$^{18}O_3$ (ILIS):**	81/79, 81/63 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 **2** under “Hints on Method 1.2” concerning the potential interference of AMPA signals by Fosetyl.
*** See comment **3** and comment **4** on Phosphonic acid under “Hints on Method 1.2”

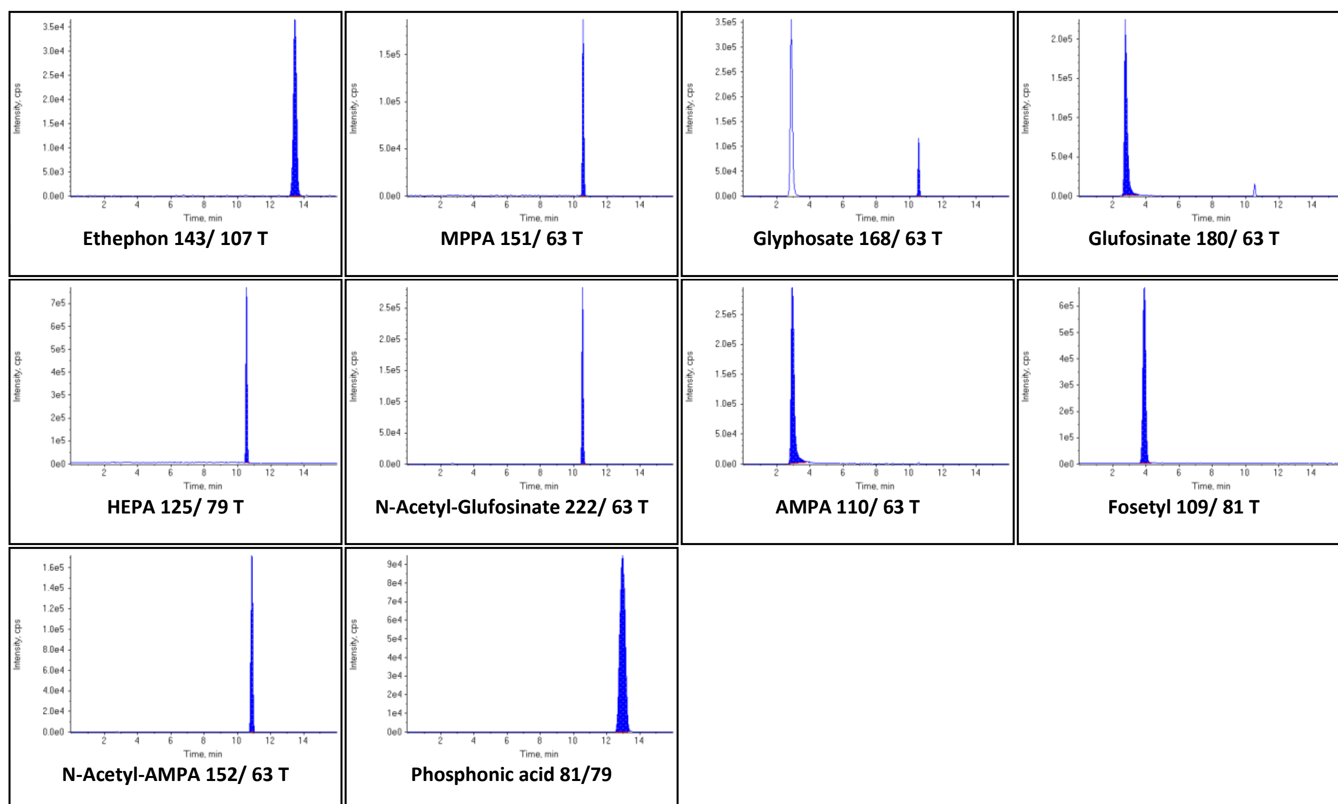


Figure 5: *Typical chromatograms of Ethephon, HEPA, Glyphosat, AMPA, Glufosinate, MPPA, N-Acetyl-AMPA, N-Acetyl-Glufosinate, Fosetyl-Al and Phosphonic acid at 0.1 mg/L in methanol with 1% formic acid.*

Hints on Method 1.2

1. **Peak splitting:** Using this M 1.2 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
2. **Mass spectrometric interference:** **AMPA and Fosetyl** share the mass-transition (110/81). Chromatographic separation is thus needed (typically this is the case).
3. **Intereference of Phosphonic acid by Fosetyl:** Fosetyl and Fosetyl-D_5 tend to degrade to Phosphonic acid both in solutions and in the LC-MS/MS via in-sorce fragmentation. A good chromatographic separation between the two is thus necessary (typically this is the case).
4. **Intereference of Phosphonic acid by Phosphoric acid:** 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.

5. **Priming and reconditioning of column:** before first use, after long storage (e.g. >2 weeks), after injection of 100-200 extracts
   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!.

6. **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) re-condition the column as described under **5.a to 5.c**.
7. **Pre-filters:** If pre-filters are used exchange them as soon as backpressure increases significantly. For practical and convenience reasons it is highly recommended to exchange pre-filters when performing other maintenance operations such as reconditioning or pre-column exchange. Losses of glyphosate, that could be clearly linked to interactions with a dirty pre-filter, have been once observed.
8. **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 M 1.2. needs to be exchanged less often than that of M 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.

5.6.3. Method 1.3 (M 1.3): “Glyphosate & Co. Hypercarb”

***Table 8:** Proposed LC-MS/MS conditions for Ethephon, HEPA (Ethephon metabolite), Glyphosat, AMPA (Glyphosate metabolite), N-Acetyl-Glyphosate (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		
Dilution	Not regularly; in case of strong matrix interferences 5-10-fold (see also Hints 8.)		
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, 171/126	
	AMPA:**	110/63, 110/79, **110/81****	
	AMPA-^{13}C,^{15}N (ILIS):	112/63, 112/81	
	N-Acetyl-AMPA:	152/63, 152/79, 152/110	
	N-Acetyl-Glyphosate	210/63, 210/150, 210/79, 210/148	
	N-Acetyl-Glyphosate-D_3 (ILIS)	213/63, 213/153	
	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, 129/97	
	Glufosinate:	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (ILIS):	183/63, 183/98	
	N-Acetyl-Glufosinate:	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-D_3 (ILIS):	225/63, 225/137	
	MPPA:	151/63, 151/107, 151/133	
	MPPA-D_3 (ILIS):	154/63, 154/136	
	Fosetyl-Al:	109/81, 109/63 (detected as Fosetyl)	
	Fosetyl-Al-D_{15} (ILIS):	114/82, 114/63 (detected as Fosetyl-D_5)	
	Maleic Hydrazide:	111/82, 111/42, 111/55, 111/83	
	Maleic Hydrazide-D_2 (ILIS):	113/42, 113/85	
	Cyanuric acid:	128/42, 128/85	
	Cyanuric acid-$^{13}C_3$:	131/43, 131/87	
	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 5** under “Hints on Method 1.3" concerning the potential interference of AMPA signals by Fosetyl.
*** See **comment 6** on Phosphonic acid under “Hints on Method 1.3"

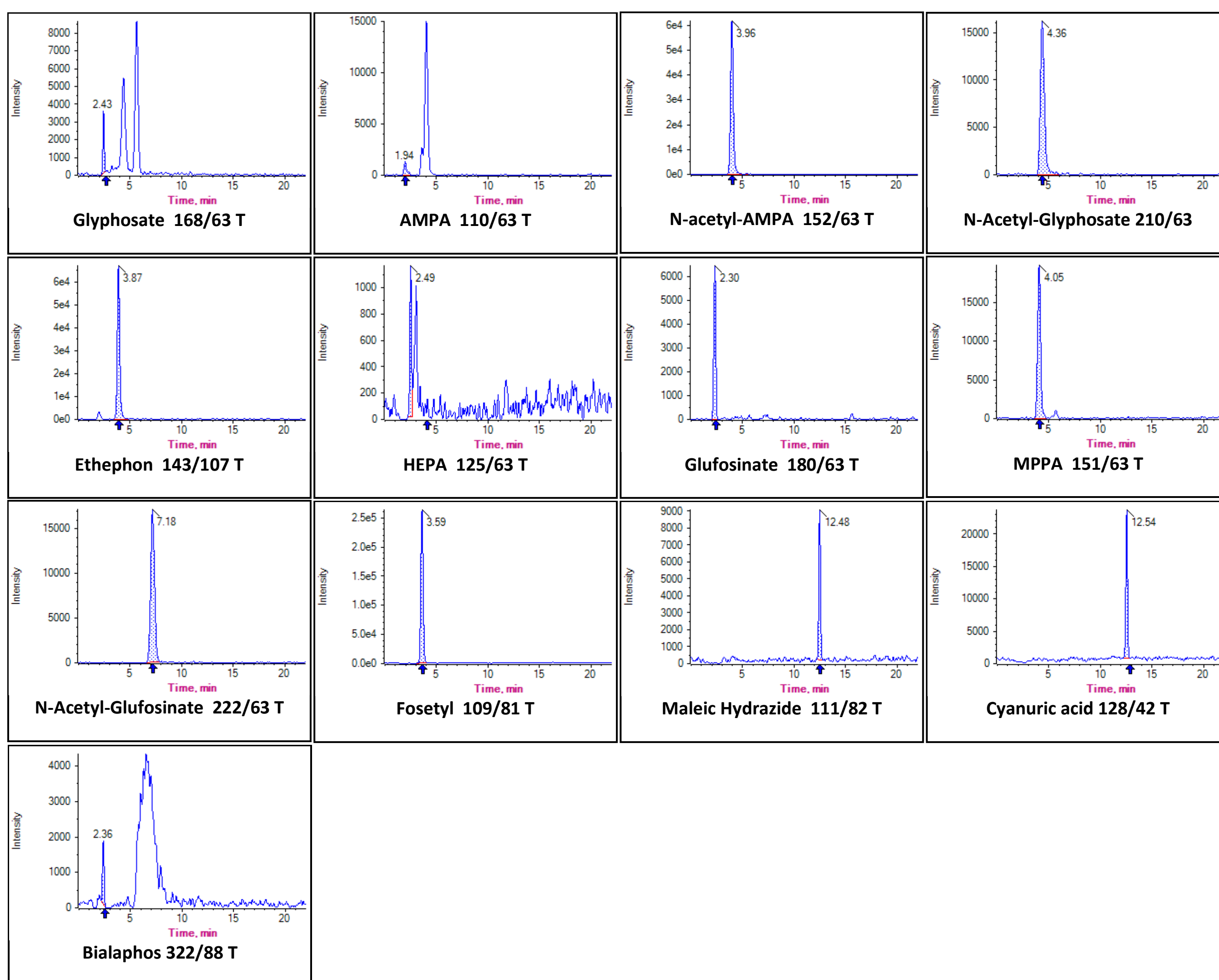


Figure 6: *Chromatograms of Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Fosetyl, Maleic Hydrazide, Cyanuric acid and Bialaphos at 0.02 mg/kg on apple extract.*

Hints on Method 1.3

1. **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 anthocyans 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-H_2O 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 **Figure 7**, **Figure 8** and **Figure 9**.

Table 9: 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.		

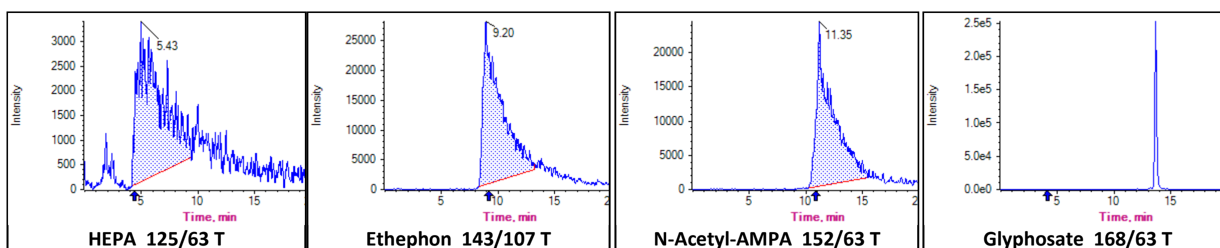


Figure 7: *Chromatograms obtained using a new Hypercarb column, poor chromatographic behavior due to strong interactions of analytes with active sites. Same behavior is observed when the pre-column is new.*

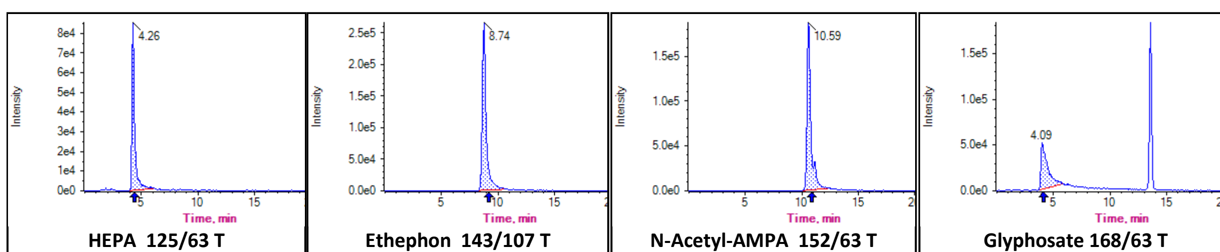


Figure 8: *Chromatograms following priming with 25 injections QuPPe extracts of spinach. Injection volume 50 µL per injection*

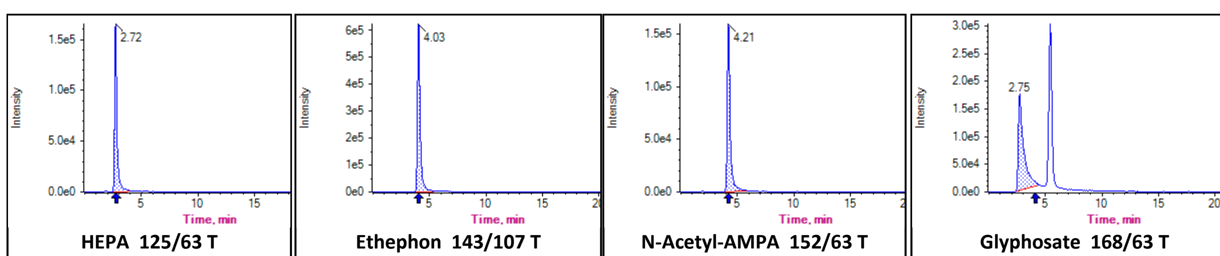


Figure 9: *Chromatograms after additional injection of approximately 100 QuPPe-extracts of various fruit and vegetables during normal routine use.*

2. **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 M 1.3 needs to be clearly less often exchanged compared to the pre-columns of M 1.1 and M 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.
3. **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 stand-

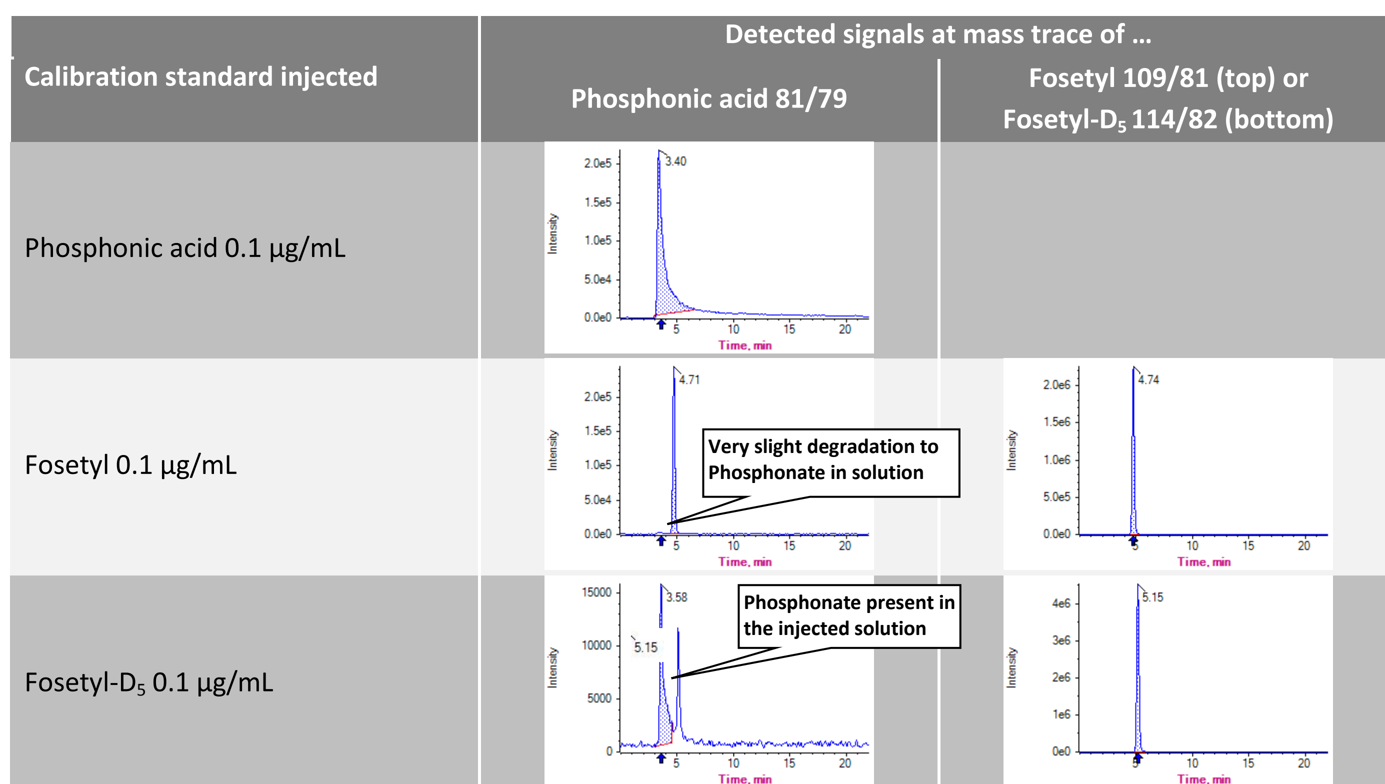
ards in matrix) before starting the sequence. If to be stored for longer periods (e.g. >2 months) it is highly recommended to recondition the column as described above.

4. **Pre-filters**: If pre-filters are used exchange them as soon as backpressure increases significantly. For practical and convenience reasons it is highly 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.

5. **Mass spectrometric interference: AMPA and Fosetyl** share the mass-transition (110/81). Chromatographic separation is thus needed (typically this is the case).

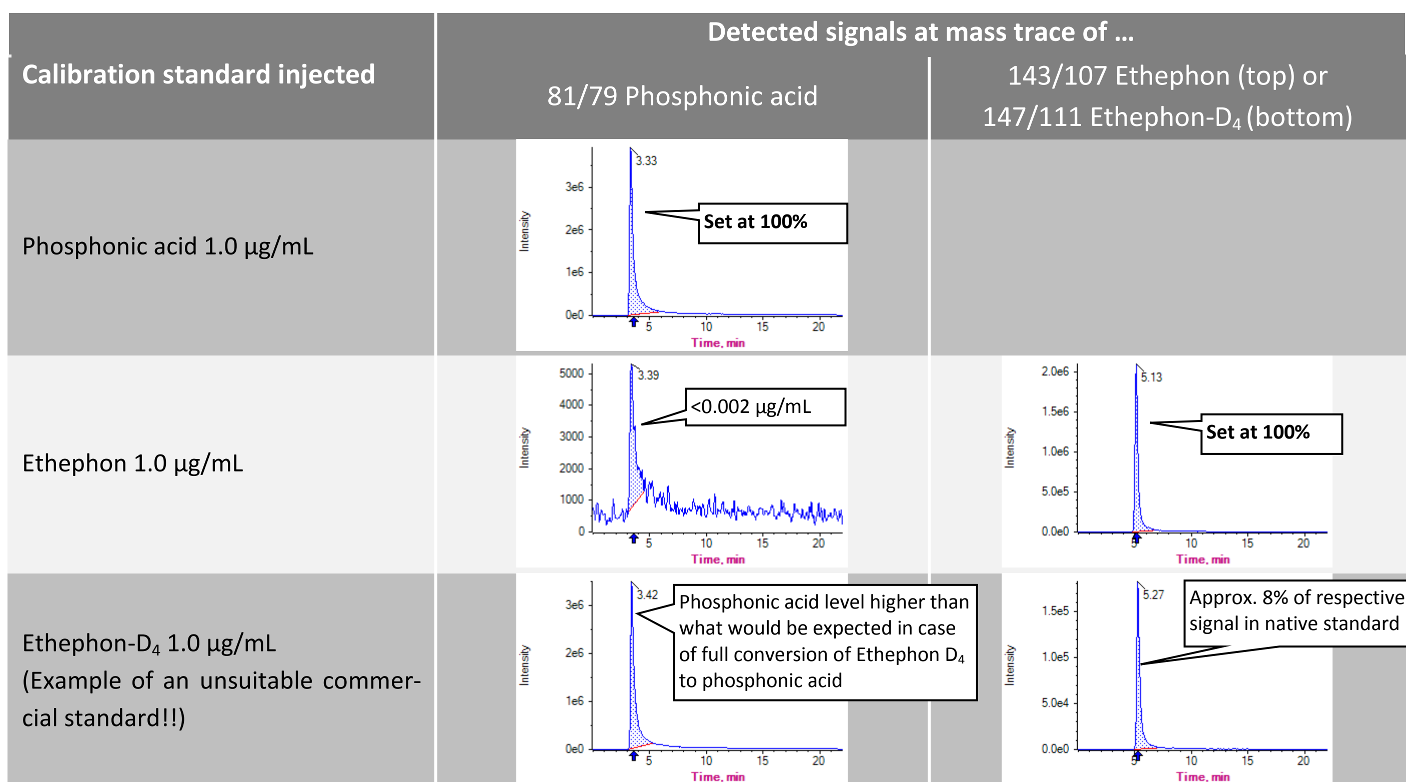
6. **Intereference of Phosphonic acid by Fosetyl:** Fosetyl and its D_5-analogon tend to degrade to Phosphonic acid both in solutions and via in-source fragmentation in LC-MS/MS. A good chromatographic separation between Fosetyl and Phosphonic acid is thus necessary (and is typically the case). **Figure 10** 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-D_5 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.



Calibration standard injected	Detected signals at mass trace of …	
	Phosphonic acid 81/79	Fosetyl 109/81 (top) or Fosetyl-D_5 114/82 (bottom)
Phosphonic acid 0.1 µg/mL		
Fosetyl 0.1 µg/mL		
Fosetyl-D_5 0.1 µg/mL		

Figure 10: *Chromatograms of Phosphonic acid, Fosetyl and Fosetyl-D_5 (each at 0.1 µg/mL). In addition to the proper mass-traces of Fosetyl and Fosetyl-D_5 the mass trace of Phosphonic acid is also shown to demonstrate the occurrence of in-source fragmentation of Fosetyl and Fosetyl-D_5 towards Phosphonic acid as well as the presence of Phosphonic acid as an impurity of the Fosetyl-D_5 standard solution.*

7. **Degradation of Ethephon to Phosphonic acid:** A degradation of Ethephon to Phosphonic acid in solution is also observed. **Figure 11** 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 11** also shows chromatograms of an unsuitable Ethephon-D_4 standard containing only ca. 0.08 µg/mL instead of the expected 1 µg/mL Ethephon-D_4 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.



Calibration standard injected	Detected signals at mass trace of …	
	81/79 Phosphonic acid	143/107 Ethephon (top) or 147/111 Ethephon-D_4 (bottom)
Phosphonic acid 1.0 µg/mL		
Ethephon 1.0 µg/mL		
Ethephon-D_4 1.0 µg/mL (Example of an unsuitable commercial standard!!)		

Figure 11: *Chromatograms of Phosphonic acid, Ethephon and an unsuitable Ethephon-D_4 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-D_4 standard is unacceptably high. That is caused by the Phosphonic acid having already been present at high amounts in the purchased standard.*

8. **Dilution:** For certain matrixes it can be beneficial to dilute the sample extract 5-10-fold before injection or to inject smaller volumes (1-2 µL). Dilution of the sample extract is highly recommended for matrixes that contain high amounts of Protein (e.g. oily seeds and pulses) and samples of animal origin in general. In routine analysis it is possible to perform a screening in the undiluted extract and to repeat the measurement procedure in diluted extraxts.
9. **Reference**: 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).

5.6.4. Method 1.4 (M 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, 91/73	
	Perchlorate:	99/83, 101/85	
	Perchlorate-$^{18}O_4$ (ILIS):	107/89, 109/91	
	Phosphonic acid:	81/79, 81/63	
	Phosphonic acid-$^{18}O_3$ (ILIS):	87/85, 87/67	

* The 1:5 dilution is used for Bromide screening. For quantification purposes where Bromide exceeds approx. 1 mg/kg, the sample extracts should be diluted e.g. 1:250 (1:50 manually and 1:5 by the HPLC).

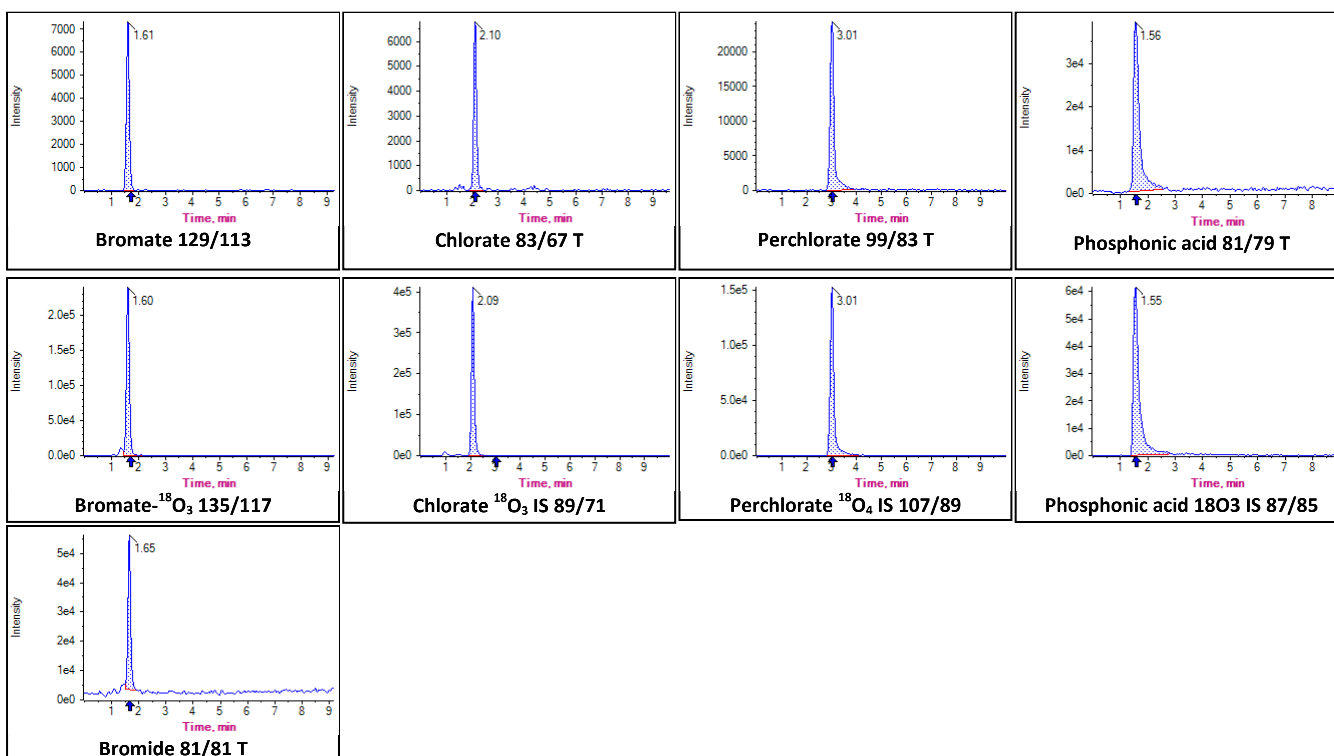


***Figure 12:** Chromatograms of Bromate (0.02 mg/kg), Bromide (1 mg/kg), Phosphonic acid (0.05 mg/kg), Perchlorate (0.01 mg/kg) and Chlorate (0.01 mg/kg); all in currant extract.*

Hints on Method 1.4

1. The hypercarb column and its pre-column should be thoroughly primed before usage, see hint on Method 1.3.
2. Check the filters for any cross-contamination of Perchlorate and Chlorate. Cellulose mixed ester filters were found to be suitable for this application (see comments under **2.7**)
3. Fosetyl and Ethephon as well as their respective ILIS's degrade to Phosphonic acid. To be on the safe side Fosetyl, Ethephon 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.
4. **Intereference of Phosphonic acid by Phosphoric acid:** 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 13**). 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 13** 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.

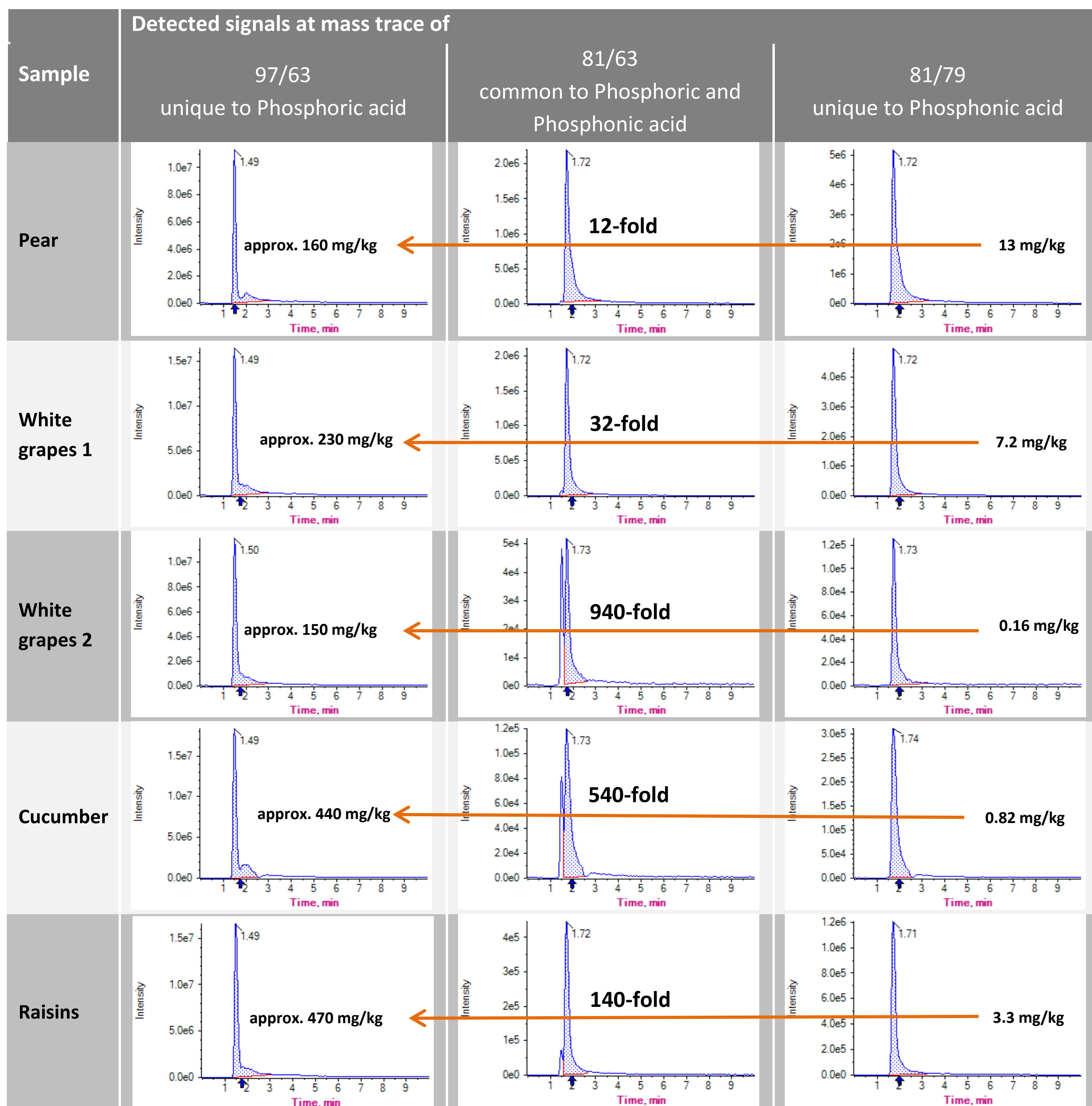


Figure 13: *Chromatographic and mass-spectrometric separation of Phosphoric and Phosphonic acid.*

5. 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 ($^{79}Br^-$ and $^{81}Br^-$). 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 highly 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 $[H_2PO_3]$-=81) whereas m/z 79/79 is highly affected by Phosphoric acid due to in-source fragmentation (
   Figure 14, 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 14, 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.

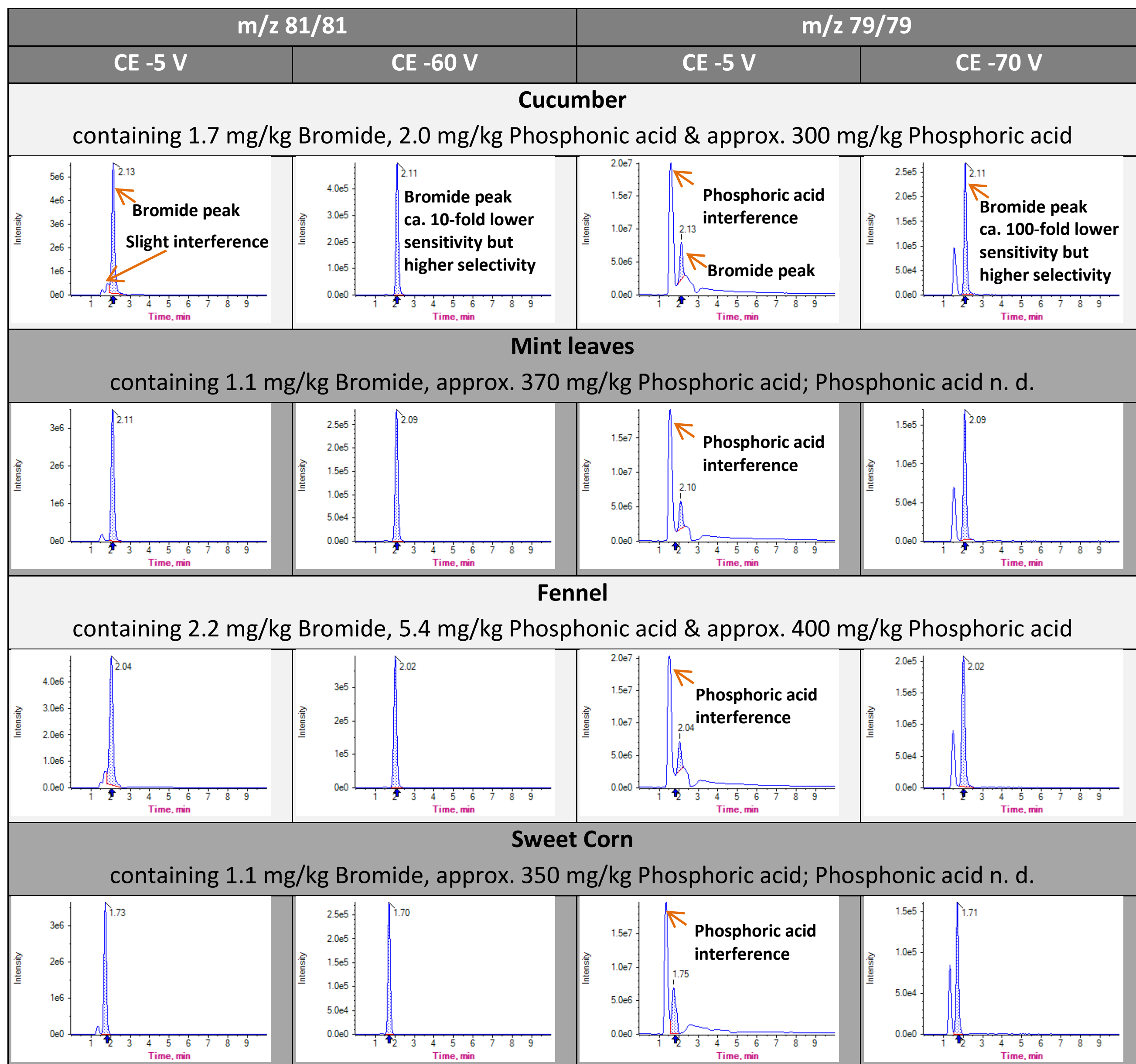


Figure 14: *Chromatograms of Bromide using non-optimized collision energies (CE -5 V) showing the interference by Phosphoric acid and Phosphonic acid as well as optimized collision energies (CE -60 V and -70 V, the) showing reduced interferences.*

6. 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 **Figure 15**). 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.

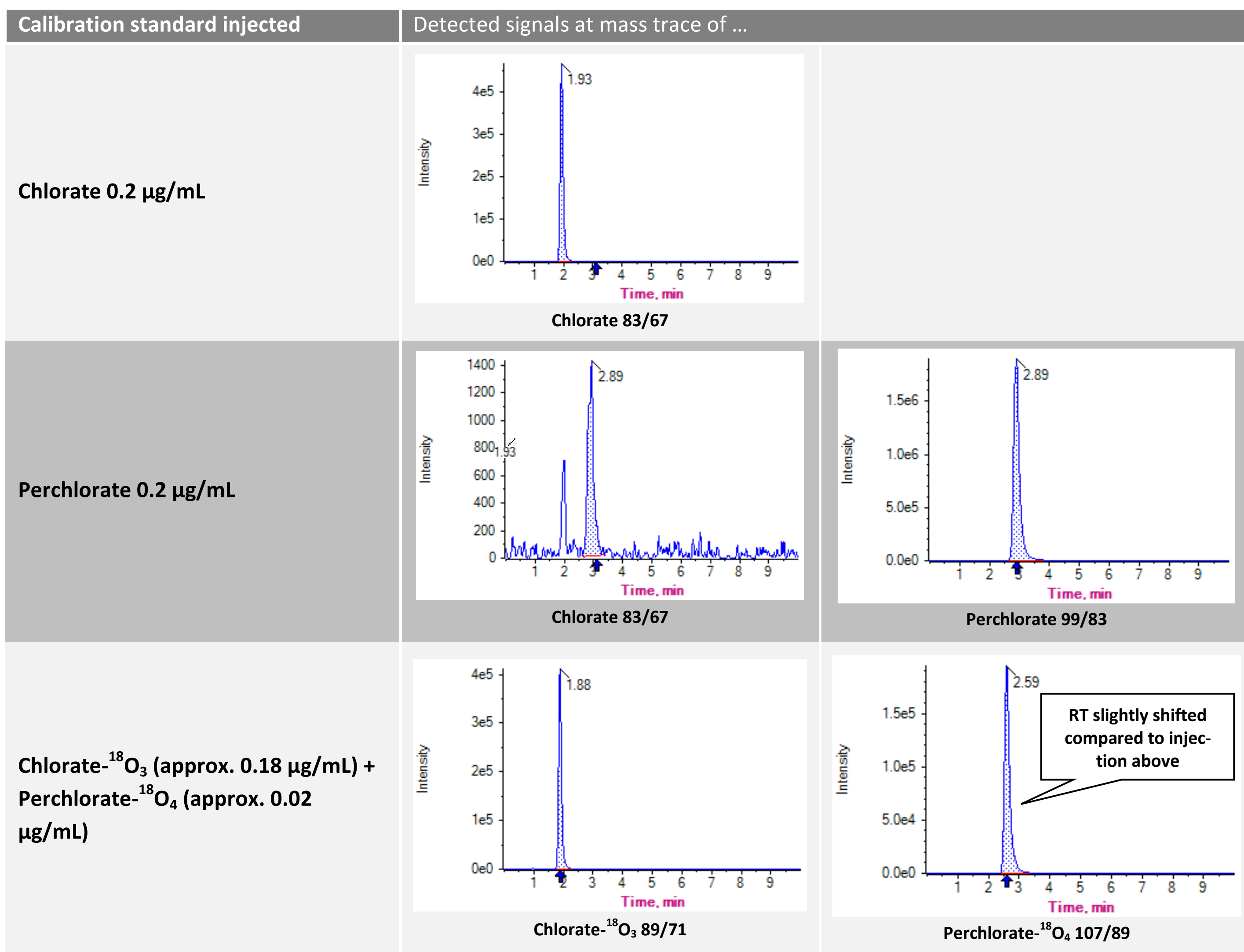


__Figure 15:__ Chromatograms of Chlorate and Perchlorate at 0.2 µg/mL and of a mixture of Chlorate-$^{18}O_3$ and Perchlorate-$^{18}O_4$, containing approx. 0.2 µg/mL Chlorate $^{18}O_3$ and approx. 0.02 µg/mL Perchlorate-$^{18}O_4$.

5.6.5. Method 1.5 (M 1.5): "Glyphosate & Co on Trinity Q1

Table 11: *Proposed LC-MS/MS conditions for Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-glyphosate, Ethephon, HEPA, Glufosinate, N-Acetyl-Glufosinate, MPPA and Fosetyl-Al, Maleic Hydrazide, Cyanuric acid, Bialaphos, Bromide, Chlorate, Perchlorate, Phosphonic acid*

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Acclaim Trinity Q1 100x2.1 mm; 3 µm (P/N 079717; Thermo Fisher Scientific); 30 °C		
Pre-column	Acclaim Trinity Q1 Guard Cartridge 2.1x10 mm, 5 µm (P/N 083244; Thermo Fisher Scientific)		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter (optional)		
Eluent A	50 mM Ammonium formate (pH 2.9) in water+acetonitrile 6+4 5 mL 5 M Ammoniumformate and 4.5 mL Formic acid ad 300 mL water, add 200 mL Acetonitrile		
Eluent B	Acetonitrile		
Gradient	**Time [min]**	**Flow [mL/min]**	**%A**
	0	0.5	100
	10	0.5	100
	10.1	0.5	18.2 (≙ 90 % acetonitrile)
	13	0.5	18.2 (≙ 90 % acetonitrile)
	13.1	0.5	100
	18	0.5	100
Injection volume	10 µL		
Dilution	Not regularly; in case of many matrix interferences 5-10-fold		
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, 171/126	
	AMPA:**	110/63, 110/79, 110/81**	
	AMPA-^{13}C,^{15}N (ILIS):	112/63, 112/81	
	N-Acetyl-AMPA:	152/63, 152/79, 152/110	
	N-Acetyl-Glyphosate	210/63, 210/150, 210/79, 210/148	
	N-Acetyl-Glyphosate-D_3 (ILIS)	213/63, 213/153	
	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, 129/97	
	Glufosinate:	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (ILIS):	183/63, 183/98	
	N-Acetyl-Glufosinate:	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-D_3 (ILIS):	225/63, 225/137	
	MPPA:	151/63, 151/107, 151/133	
	MPPA-D_3 (ILIS):	154/63, 154/136	
	Fosetyl-Al:	109/81, 109/63 (each detected as Fosetyl)	
	Fosetyl-Al-D_{15} (ILIS):	114/82, 114/63 (each detected as Fosetyl- D_5)	
	Maleic Hydrazide:	111/82, 111/42, 111/55, 111/83	
	Maleic Hydrazide-D_2 (ILIS):	113/42, 113/85	
	Cyanuric acid:	128/42, 128/85	
	Cyanuric acid-$^{13}C_3$:	131/43, 131/87	
	Bialaphos:	322/88, 322/94, 322/134	
	Bromide*:	81/81, 79/79	
	Chlorate:	83/67, 85/69	
	Chlorate-$^{18}O_3$ (ILIS):	89/71, 91/73	
	Perchlorate:	99/83, 101/85	
	Perchlorate-18O4 (ILIS):	107/89, 109/91	
	Phosphonic acid:	81/79, 81/63	
	Phosphonic acid $^{18}O_3$ (ILIS):	87/85, 87/67	

* We recommend to use an optimized collision energy for Bromide as described in **5** under "Hints on Method 1.4".

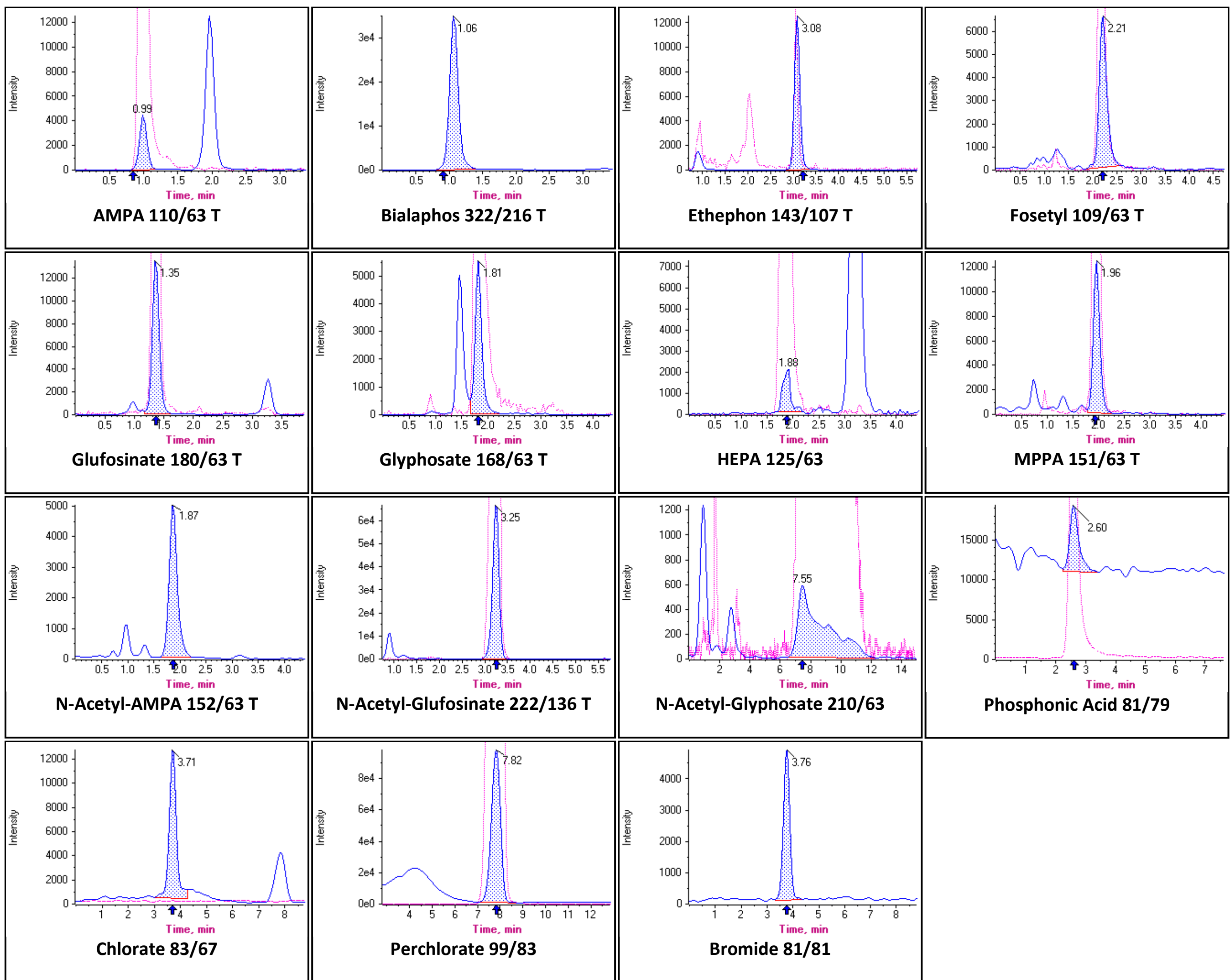


Figure 16: *Chromatograms of Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Fosetyl, Maleic Hydrazide, Cyanuric acid, Bialaphos and Bromide at 0.02 mg/kg each, Phosphonic acid at 0.05 mg/kg, as well as Chlorate and Perchlorate at 0.005 mg/kg each, all in black currant extract.*

5.6.6. **Method 1.6 (M 1.6): Glyphosate & Co. on Torus DEA**

Table 12: *Proposed LC-MS/MS conditions for Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-glyphosate, Ethephon, HEPA, Glufosinate, N-Acetyl-Glufosinate, MPPA and Fosetyl-Al*

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Waters Torus™DEA 2.1 mm x 100 mm; 1.7 µm; 50 °C		
Pre-column	Waters Torus™DEA VanGuard™ 2.1 mm x 5 mm; 1.7 µm		
Pre-filters	Waters ACQUITY UPLC Column In-Line Filter Kit [205000343]		
Eluent A	1.2% formic acid in water		
Eluent B	0.5 % formic acid in Acetonitrile		
Gradient	**%A**	**Flow [mL/min]**	**Time [min]**
	10	0.5	0
	10	0.5	0.5
	80	0.5	1.5
	90	0.5	4.5
	90	0.5	17.5
	10	0.5	17.6
	10	0.5	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, 171/126	
	AMPA:**	110/63, 110/79, 110/81**	
	AMPA-^{13}C,^{15}N (ILIS):	112/63, 112/81	
	N-Acetyl-AMPA:	152/63, 152/79, 152/110	
	N-Acetyl-Glyphosate:	210/63, 210/150, 210/79, 210/148	
	N-Acetyl-Glyphosate-D_3 (ILIS):	213/63, 213/153	
	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, 129/97	
	Glufosinate:	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (ILIS):	183/63, 183/98	
	N-Acetyl-Glufosinate:	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-D_3 (ILIS):	225/63, 225/137	
	MPPA:	151/63, 151/107, 151/133	
	MPPA-D_3 (ILIS):	154/63, 154/136	
	Fosetyl-Al:	109/81, 109/63 (each detected as Fosetyl)	
	Fosetyl-Al-D_{15} (ILIS):	114/82, 114/63 (each detected as Fosetyl- D_5)	

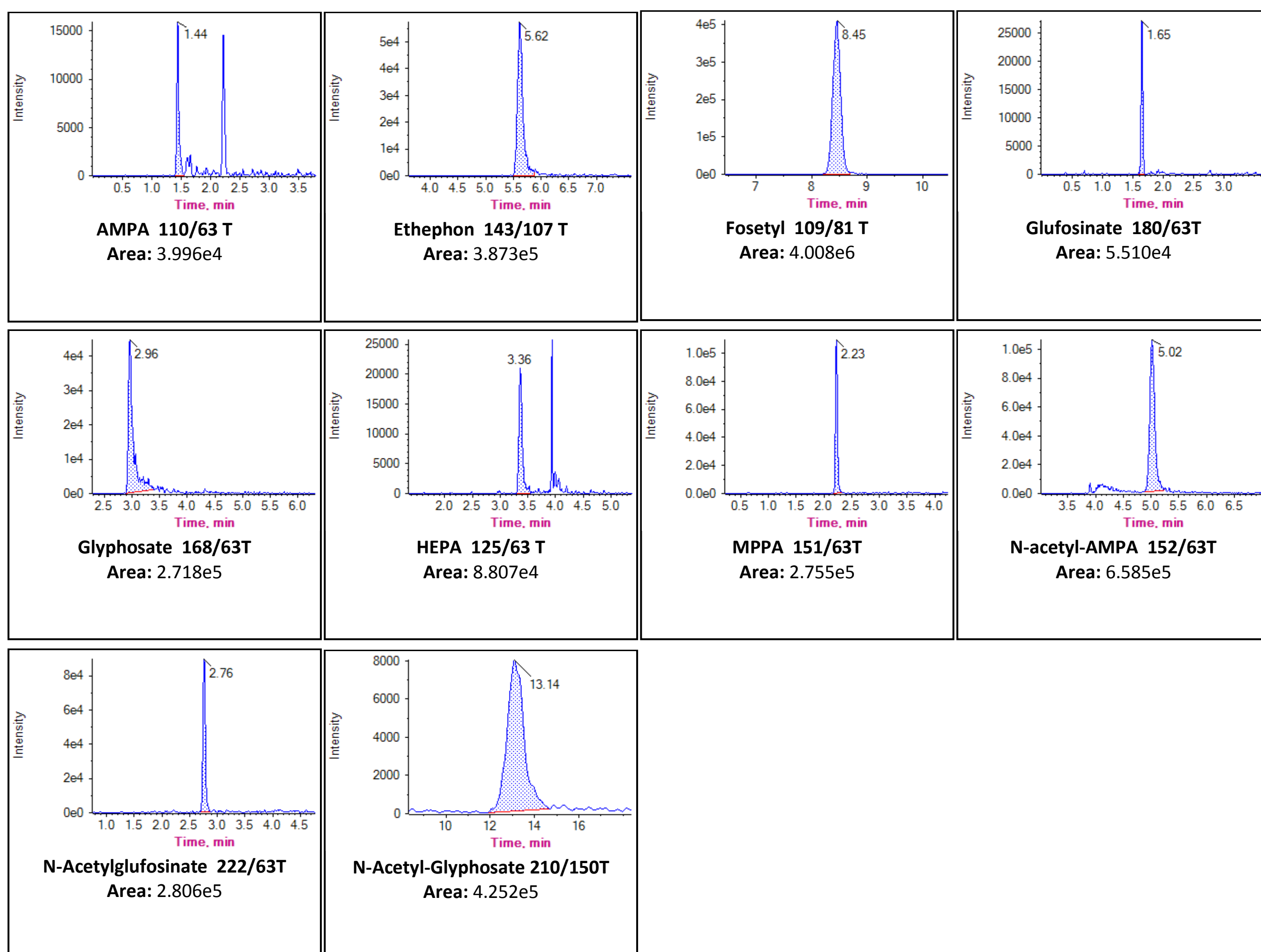


Figure 17: *Chromatograms of Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Fosetyl at 0.04 mg/kg on cucumber extract.*

Hints on Method 1.6:

1. The Torus DEA column should be conditioned before use following the manufacturer's **Start-up Guide**, which foresees flushing the column with a 5 mmol/L solution of Na_2EDTA. Afterwards it is important to prime thoroughly.
2. **Maleic hydrazide and Cyanuric acid on Torus DEA:** The intention was to cover all analytes of M1.3 with M1.6. During method development, however, it became clear that Maleic hydrazide and Cyanuric acid showed a very poor retention behaviour on this column, with retention times close to the dead-time, heavy interefernces of matrix components on detection signals, peak shapes as well as the peak intensities (signal suppression). **Figure 18** shows exemplarily chromatograms obtained upon injection of standards in solvent and in extracts of plum, broccoli, soy and onion at 0.1 µg/mL. Proper evaluation of the peaks is often not possible. Fortunately Maleic Hydrazide can also be covered by M 4.2 (5.6.11). Cyanuric acid is not regulated.

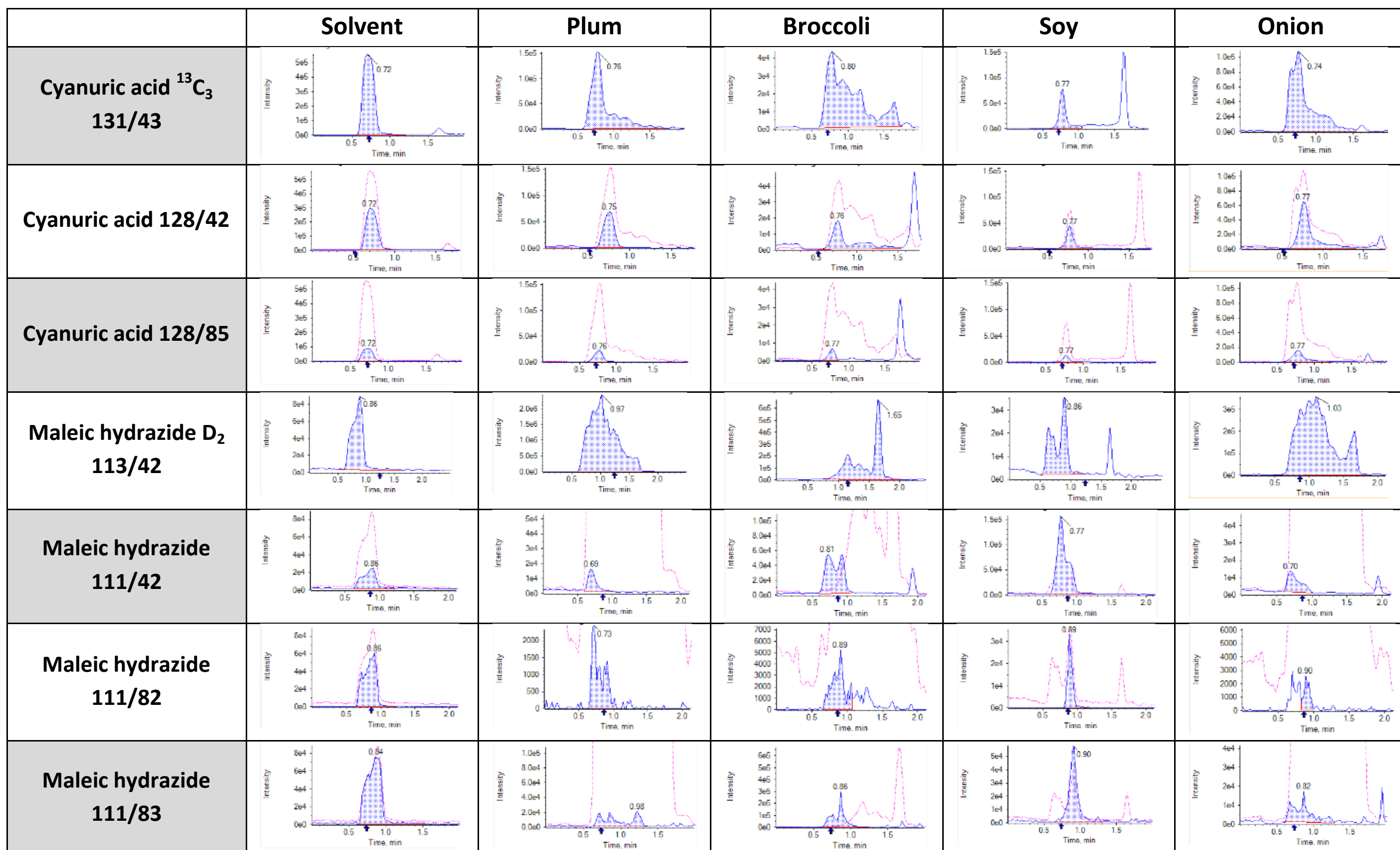


Figure 18: Exemplary peak shapes of Cyanuric acid and Maleic hydrazide in solvent-based standards and in standards of plum, broccoli, soy and onion extracts at 0.1 µg/mL.

5.6.7. Method 1.7 (M 1.7): PerChloPhos on Torus DEA

Table 13: *Proposed LC-MS/MS conditions for PerChlorate, Chlorate, Phosphonic acid and Bormide*

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Waters Torus™DEA 2.1 mm x 100 mm; 1.7 µm; 50 °C		
Pre-column	Waters Torus™DEA VanGuard™ 2.1 mm x 5 mm; 1.7 µm		
Pre-filters	Waters ACQUITY UPLC Column In-Line Filter Kit [205000343]		
Eluent A	1.2% formic acid + 10 mmol ammonium formate in water		
Eluent B	0.5 % formic acid in Acetonitrile		
Gradient	**%A**	**Flow [mL/min]**	**Time [min]**
	10	0.5	0
	10	0.5	0.5
	80	0.5	1.5
	90	0.5	4.5
	90	0.5	17.5
	10	0.5	17.6
	10	0.5	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)**	
	Bromide:	81/81, 79/79	
	Chlorate:	83/67, 85/69	
	Chlorate-$^{18}O_3$ (ILIS):	89/71, 91/73	
	Perchlorate:	99/83, 101/85	
	Perchlorate-$^{18}O_4$ (ILIS):	107/89, 109/91	
	Phosphonic acid:	81/79, 81/63	
	Phosphonic acid-$^{18}O_3$ (ILIS):	87/85, 87/67	

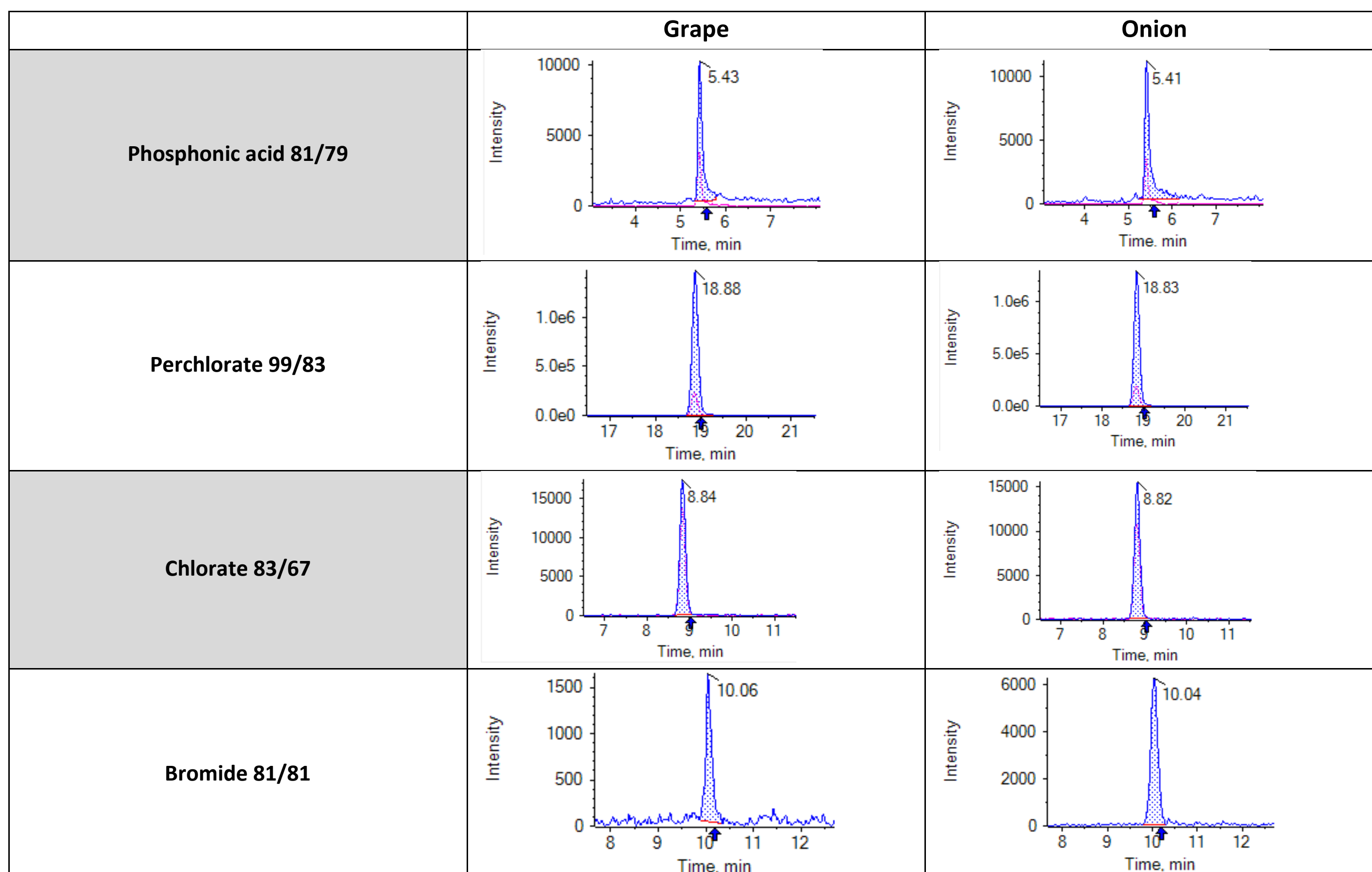


Figure 19: *Exemplary chromatograms of Phosphonic acid, Perchlorate, Chlorate and Bromide at 0.01 mg/kg on Garpe and Onion*

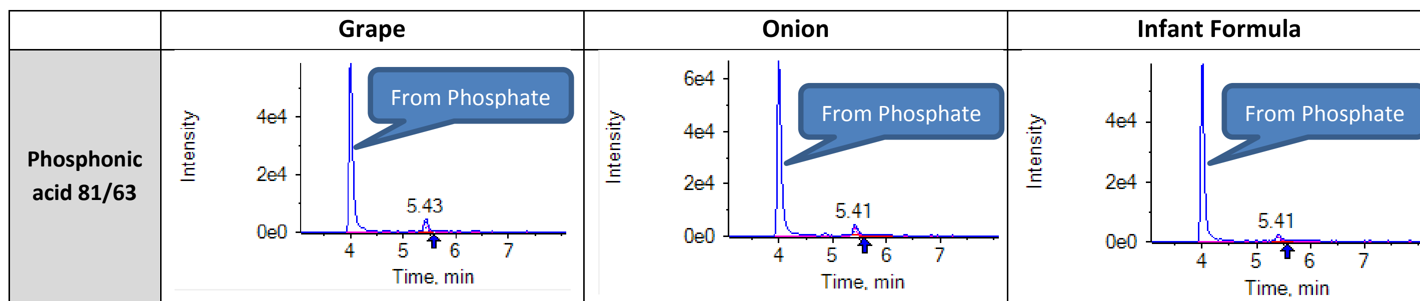


Figure 20: Chromatograms of Phosphonate transition 81/63 which is also common to Phosphate at 0.01 µg/mL in grape, onion and infant formula. Both substances are separated sufficiently

Hints on Method 1.7:

1. The Torus DEA column should be conditioned before use following the manufacturer's **Start-up Guide**, which foresees flushing the column with a 5 mmol/L solution of Na_2EDTA. Afterwards it is important to prime thoroughly.
2. **Intereference of Phosphonic acid by Phosphoric acid:** As it is described in the Hints on M 1.4 the chromatographic separation of Phosphonic acid (Phosphonate) and phosphoric acid (phosphate) is of high importance because the most intensive mass-transition of phosphonic acid (m/z 81/63) also occurs as a minor mass-transition of Phosphoric acid (following in-source fragmentation). As phosphate is naturally present in most samples at much higher levels compared Phosphonate the interference on this mass transition can be very relevant if these two compounds are not separated. As shown in **Figure 20** a sufficient chromatographic separation of phosphate and Phosphonate is obtained by M 1.7 without the need of a major dilution as in the case of M1.4.

5.6.8. Method 2 (M 2): “Fosetyl and Maleic Hydrazide”

Table 14: *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 NH_4-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: **Fosetyl-Al-D_{15} (ILIS):**	109/81, 109/63 (detected as fosetyl) 114/82 (detected as fosetyl-D_5)	
	Maleic Hydrazide: **Maleic Hydrazide-D_2 (ILIS):**	111/82, 111/42, 111/55, 111/83 113/42	
	Perchlorate: **Perchlorate-$^{18}O_4$ (ILIS):**	99/83, 101/85 107/89	

* 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 D_2-MH at 0.25 % the resulting concentration of native MH following the addition of 20 µg D_2-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 !

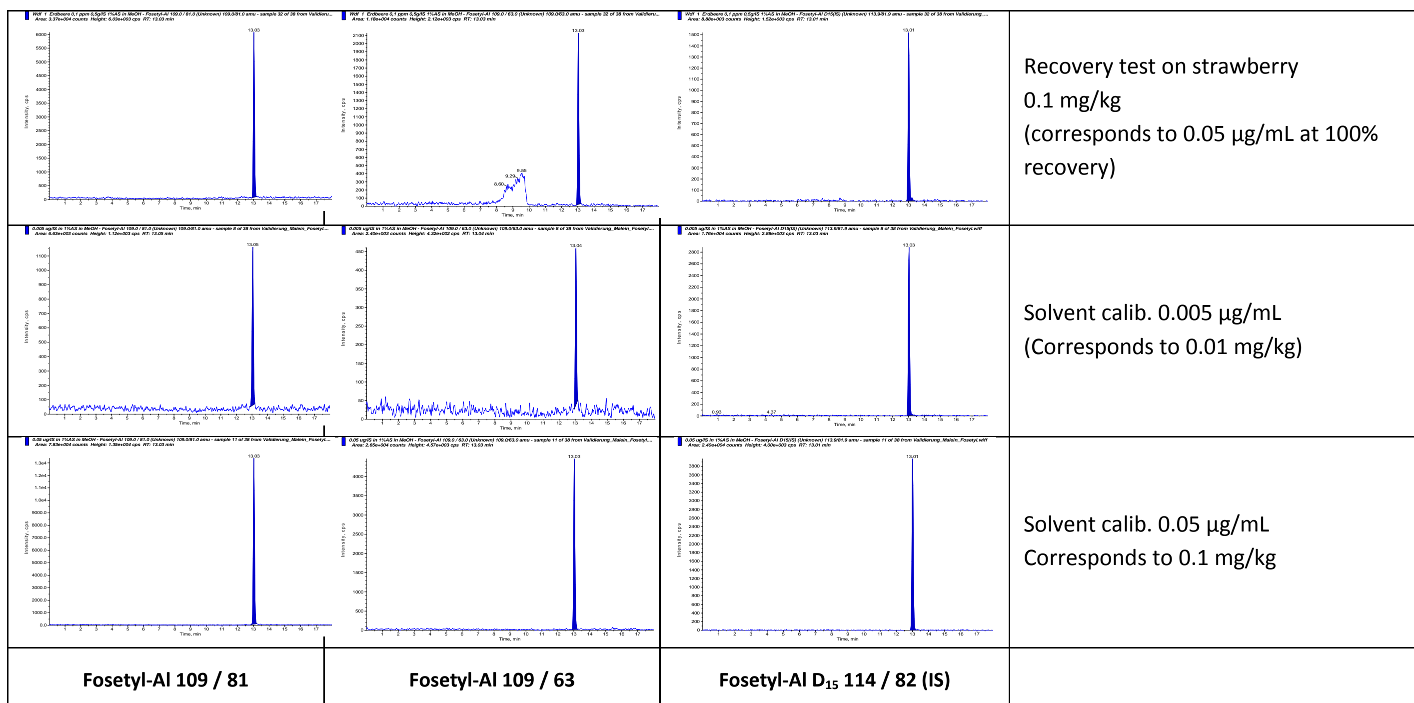


Figure 21: *Typical chromatograms of Fosetyl-Al in strawberry extract and in solvent-based standards*

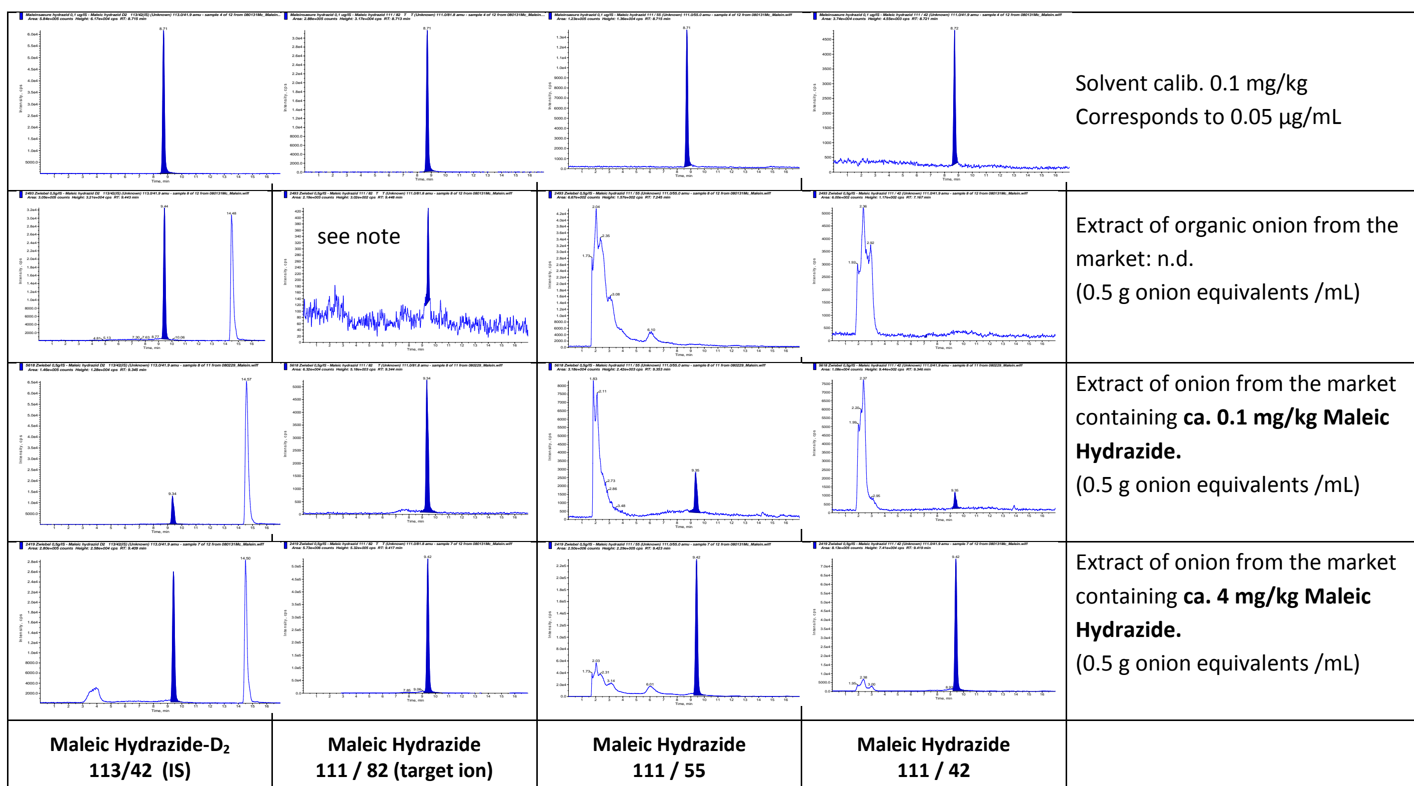


Figure 22: *Typical chromatograms of Maleic Hydrazide in onion extracts and in solvent-based standards*

5.6.9. **Method 3 (M 3): "Amitrole & Co"**

Table 15: *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: **Amitrole-^{15}N (ILIS):** **Amitrole-$^{15}N_2$,$^{13}C_2$ (ILIS):**	85/43, 85/57, 85/58 86/43 89/44	
	Chlormequat: **Chlormequat-D_4 (ILIS):**	122/58, 122/63, 124/58 126/58	
	Mepiquat: **Mepiquat-D_3 (ILIS):**	114/98, 114/58 117/101	
	Daminozide: **Daminozide-$^{13}C_4$ (ILIS):** **Daminozide-D_6 (ILIS):**	161/143, 161/61, 161/101 , 161/115, 161/44 165/147 167/149	
	Cyromazine: **Cyromazine-D_4 (ILIS):**	167/68, 167/125, 167/85, 167/108, 171/86	
	ETU (Ethylenethiourea): **ETU-D_4 (ILIS):**	103/44, 103/60, 103/86 107/48	
	PTU - N,N'-(1,2-Propylene)thiourea**)**:** **PTU-D6** - N,N'-(1,2-Propylene)thiourea –D6****:** *PTU-D6 - N,N'-(1,3-Propylene)thiourea -D6)***	117/100, 117/58, 117/60, 117/72 123/64 *(123/64)*	
	Trimethylsulfonium: **Trimethylsulfonium-D_9 (ILIS):**	77/62, 77/47 86/68	
	Difenzoquat: **No ILIS currently available**	249/77, 249/130, 249/193 -	

* 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'-(1,2-Propylene)thiourea = N,N'-iso-propylenethiourea (CAS No. 2055-46-1). The same accronym is, however, also used for N,N'-propylenethiourea = N,N'-(1,3-Propylene)thiourea = N,N'-Trimethylenethiourea (CAS No.: 2122-19-2).

Note: For Paraquat, Diquat, Trimethylsulfonium and N,N-Dimethylhydrazine better run Method 4 (5.6.10)

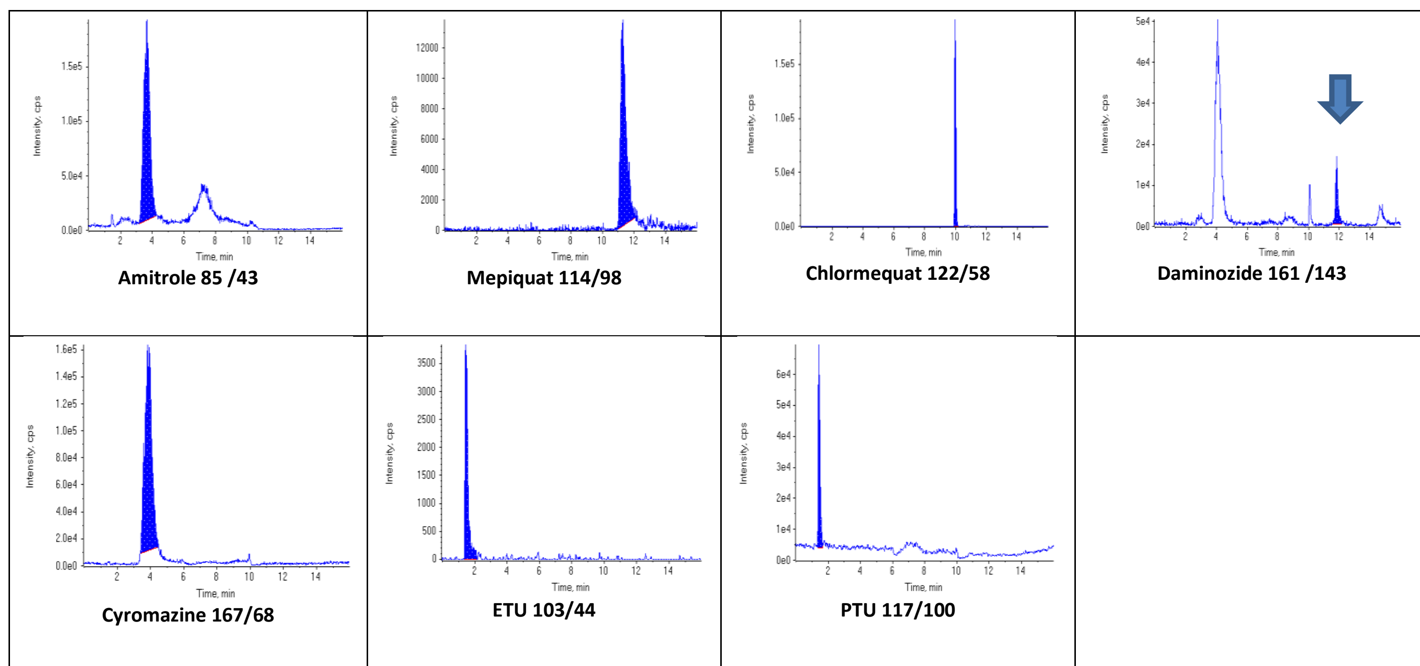


Figure 23: *Typical chromatograms of Amitrole, Chlormequat, Mepiquat, Daminozide, ETU, PTU and Cyromazine in apple extract at 0.01 mg/kg*

5.6.10. **Method 4.1 (M 4.1): “Quats & Co Obelisc R”**

Table 16: *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
	20	0.4	20
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 **5.2.3-B)**

Note: For Morpholin, Diethanolamine (DEA) and Triethanolamine (TEA) better run Method 7 (5.6.14). As DEA converts to Morpholine in the ion source, chromatographic separation of these two is paramount. With Method 4.1 (**5.6.10**) these two peaks do not sufficiently separate.

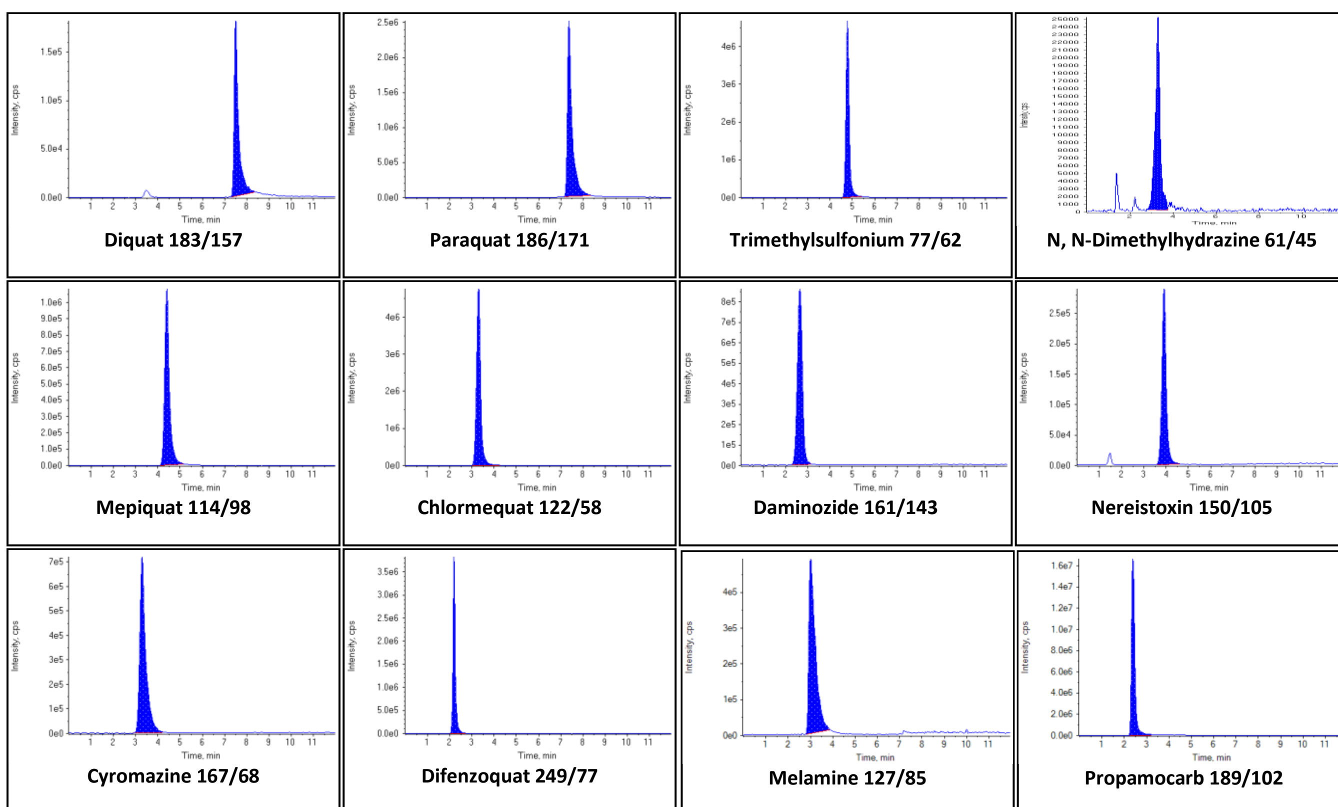


Figure 24: *Typical chromatograms of Diquat, Paraquat, Chlormequat, Mepiquat, Daminozide, N,N-Dimethylhydrazine, Trimethyl-sulfonium, Cyromazine, Nereistoxin, Difenzoquat, Melamine and Propamocarb in apple extract at 0.1 mg/kg*

5.6.11. **Method 4.2 (M 4.2): "Quats & Co BEH Amide"**

Table 17: *Proposed LC-MS/MS conditions for Aminocyclopyrachlor, Amitrole, Chlormequat, Chloridazon-desphenyl, Cyromazine, Daminozide, Diethanolamine, Difenzoquat, ETU, Melamine, Mepiquat, Mepiquat-4-hydroxy, Morpholine, Nereistoxin, Nicotine, 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
	3	0.5	10
Injection volume	2 µL		
Calibration standards and levels	e.g. one level at the reporting limit plus 0.05 or 0.1 µg/IS portion* +		
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, 161/44	
	Daminozide-$^{13}C_4$ (ILIS);	165/147 ;	
	Daminozide-D_6 (ILIS):	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	
	Maleic Hydrazide	113/67, 113/40	
	Maleic Hydrazide D2	115/69	
	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	
	Nicotine	163/130, 163/132, 163/84	
	Nicotine D4	167/84	
	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 - N,N'-(1,2-Propylene)thiourea)**:	117/100, 117/58, 117/60, 117/72	
	PTU-D6 - N,N'-(1,2-Propylene)thiourea –D6**:	123/64	
	Triethanolamine*** (TEA):	150/132, 150/70, 150/88	
	Triethanolamine-D_{12} (ILIS):	162/144	
	Trimethylsulfonium:	77/62, 77/47	
	Trimethylsulfonium-D_9 (ILIS):	86/68	

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

**See comments on PTU under M 3 (5.6.9).

***For Morpholin, DEA and TEA better run Method 7 (**5.6.9**) as these compounds are often strongly suppressed by matrix using these LC-conditions. For DEA even false negative results are observed in some cases.This effect is reduced if the extract is diluted e.g. 5/10 fold.

****The **screening option for diquat was removed** as the diquat peak is very broad. Deprotonated diquat (which is formed, e.g. in methanolic standards) gives an earlier eluting sharp peak, but this peak does not appear in fresh extracts of real samples and is thus unsuited for screening

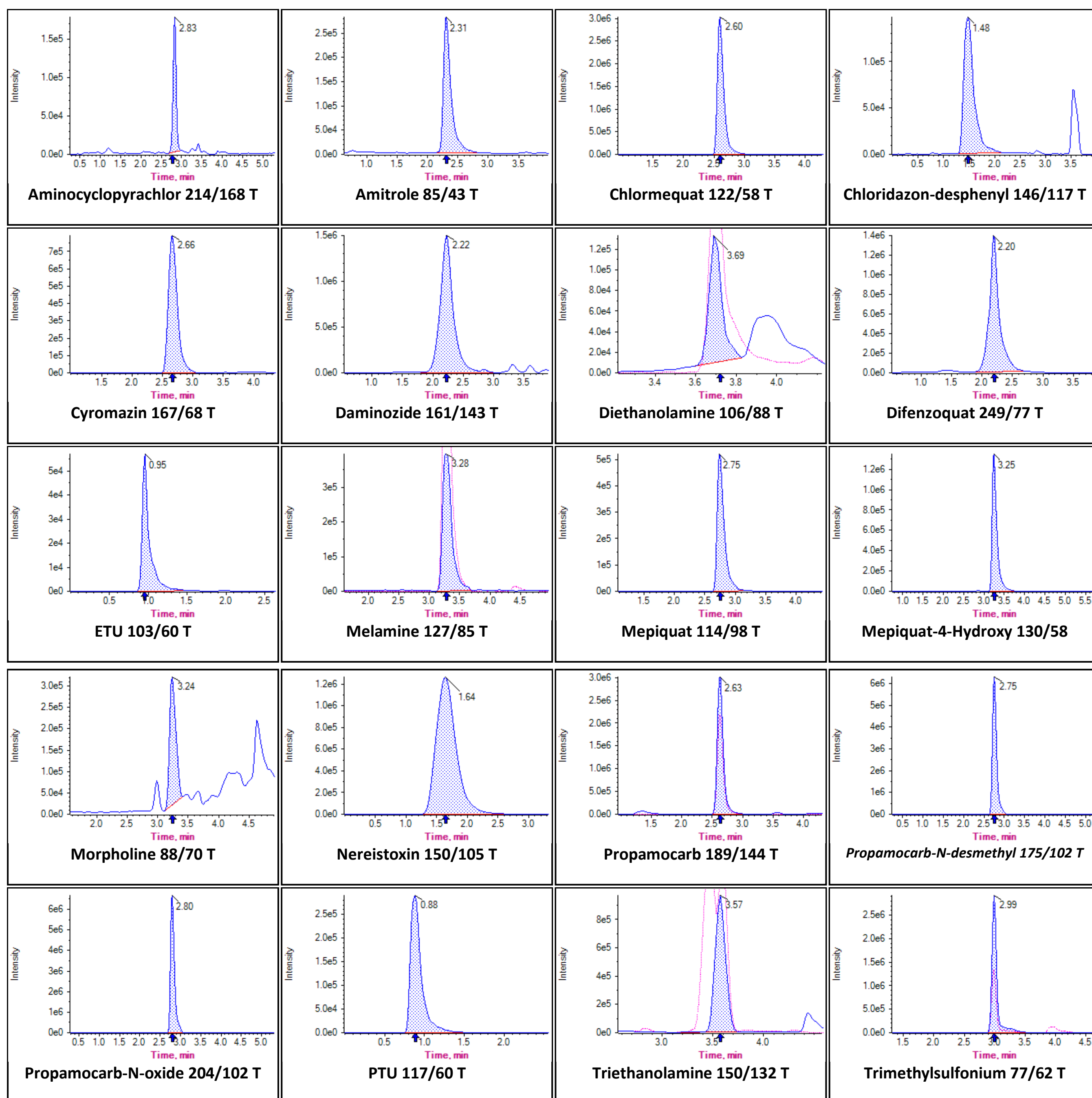


Figure 25: *Typical chromatograms of Aminocyclopyrachlor, Amitrole, Chlormequat, Chloridazon-desphenyl, Cyromazine, Daminozide, Diethanolamine, Difenzoquat, ETU, Melamine, Mepiquat, Mepiquat-4-hydroxy, Morpholine, Nereistoxin, Propamocarb, Propamocarb-N-desmethyl, Propamocarb-N-oxide, PTU, Triethanolamine, Trimesium (Trimethylsulfonium) in tomato extracts spiked at 0.05 mg/kg.*

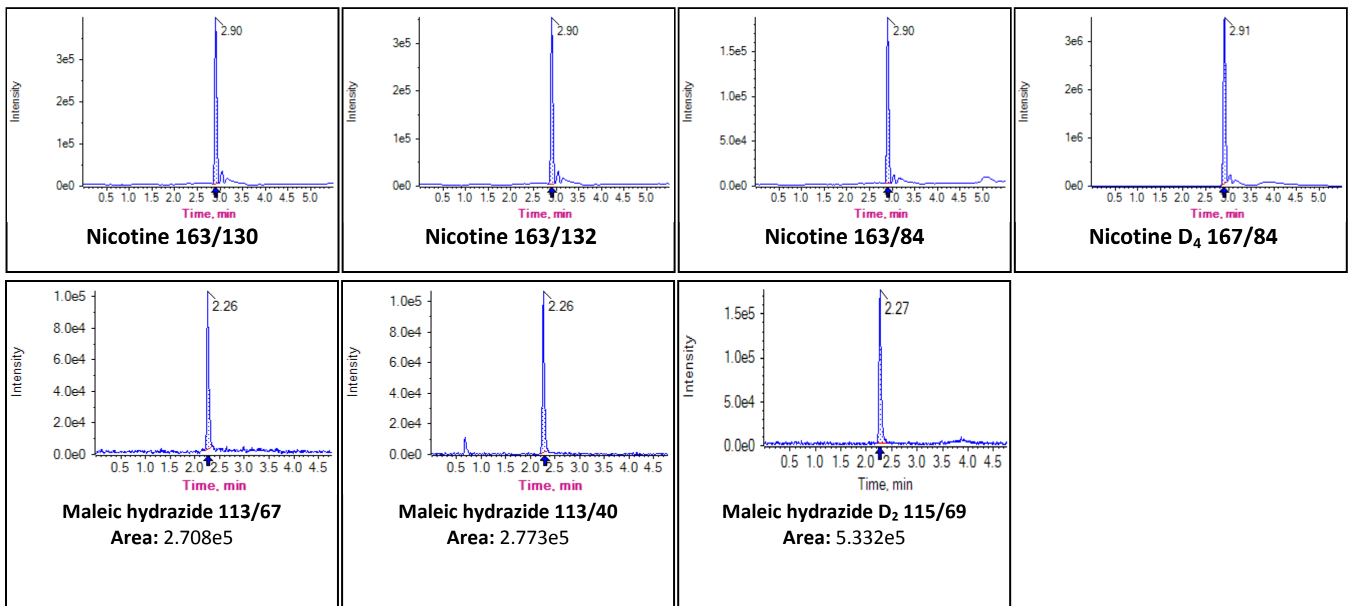


Figure 26: *Exemplary chromatograms of nicotine in flour (spelt, whole-grain) Maleic hydrazide in solvent. Nicotine at 0.01 mg/kg (0.005 µg/mL); nicotine D_4 at 0.1 mg/kg (0.05 µg/mL); Maleic hydrazide and Maleic hydrazide D_2 at 0.2 mg/kg (0.1 µg/mL).*

5.6.12. **Method 5 (M 5): “Quats & Co. MonoChrom MS”**

Table 18: *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 NH$_4$-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 - N,N’-(1,2-Propylene)thiourea**)**:**	117/100, 117/58, 117/60, 117/72	
	PTU-D6 - N,N’-(1,2-Propylene)thiourea –D6****:**	123/64	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also **Table 1**).
** See comments on PTU under M 3 (**5.6.9**).

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_CrlSrm.pdf

5.6.13. **Method 6 (M 6): "Streptomycin and Kasugamycin"**

Table 19: *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	8
	20	0.3	13
	80	0.5	18
	80	0.5	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**).
** Dihydrostreptomycin is a veterinary drug itself. It may be used as IS for the quantification of strepromycin if shown to be absent from the sample (and vice versa)

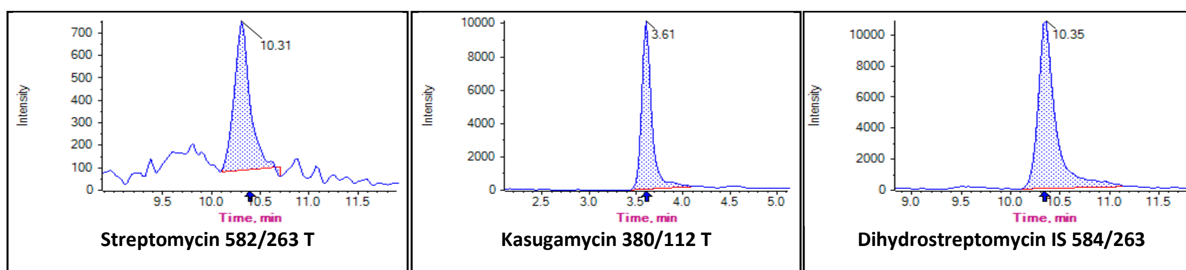


Figure 27: *Typical chromatograms of Streptomycin and Kasugamycin in apple extracts spiked at 0.01 mg/kg.*

5.6.14. **Method 7 (M 7): “Morpholine, Diethanolamine and Triethanolamine”**

Table 20: *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 (**5.5.3**)

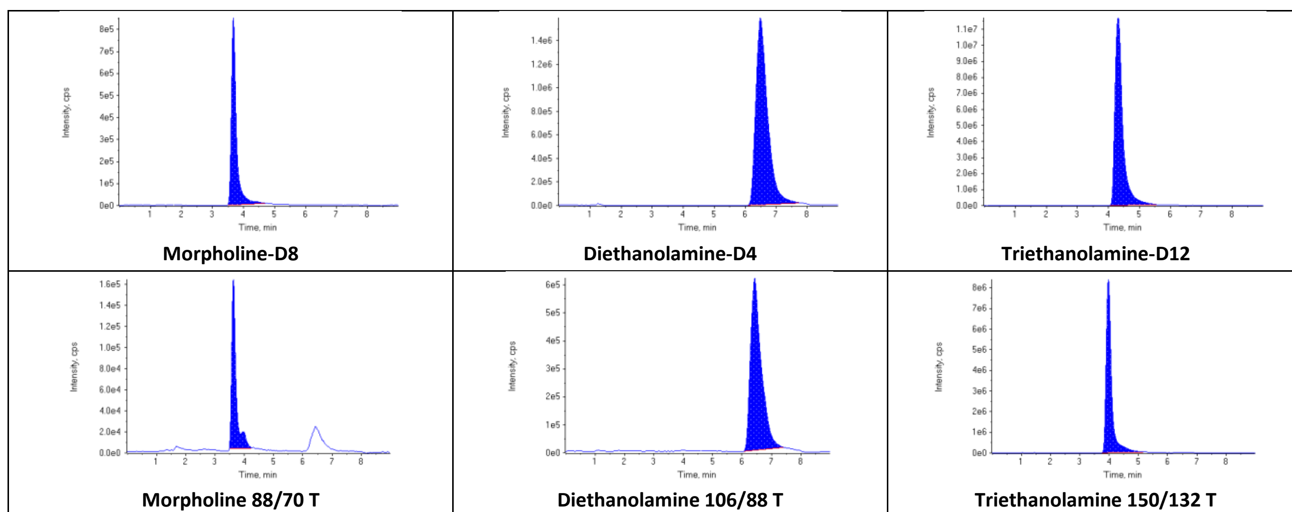


Figure 28: *Typical chromatograms of Morpholine, Diethanolamine and Triethanolamine in apple extracts at 0.05 mg/kg (extract were diluted 10-fold before injection)*

5.6.15. Method 8 (M 8): "Triazole derivative metabolites (TDMs)"

Table 21: *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	
	100	0.6	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			
Acquired mass transitions	**Compound****	**Mass Transitions (m/z)**	**DMS-Settings (SelexIon Q-Trap® 5500) *****	
			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, 157/42	-2.0	3000
	Triazole-alanine-$^{13}C_2$,$^{15}N_3$ (IS):	162/75	-1.75	3100
	Triazole-acetic acid:	128/70, 128/43, 128/73	-6.0	3100
	Triazole-acetic acid-$^{13}C_2$,$^{15}N_3$ (IS):	133/75	-6.0	3500
	Triazole-lactic acid:	158/70, 158/43, 158/112	-3.0	3300
	Triazole-lactic acid-$^{13}C_2$,$^{15}N_3$ (IS):	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: DMS temp.: low; CUR 20, GS1 60, GS2 70, DMO -3.0; DMS condition differ to some extent from instrument to instrument 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

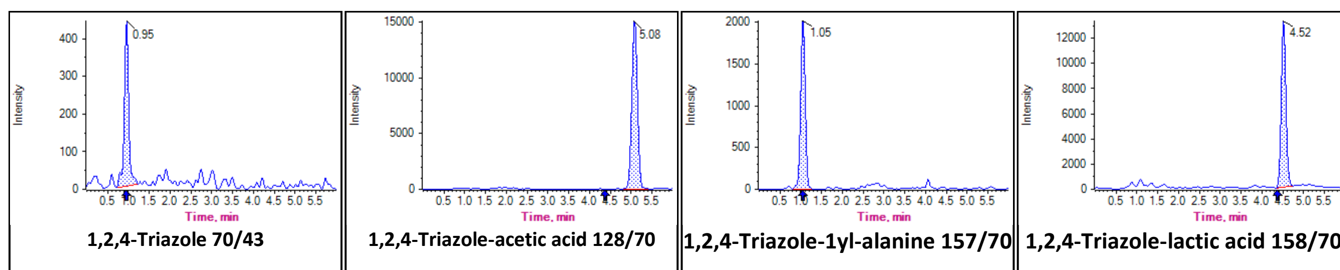


Figure 29: *Typical chromatograms of TDMs in avocado extracts spiked at 0.01 mg/kg.*

5.6.16. **Method 9 (M 9): “Difluoroacetic acid and Trifluoroacetic acid”**

***Table 22**: 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 Cartrige 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 NH4-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)**	**DMS-Settings** (SelexIon Q-Trap® 5500) ****	
			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
**** Further parameters: DMS temp.: medium; CUR 20, GS1 60, GS2 70, DMO -3.0; DMS condition differ to some extent from instrument to instrument

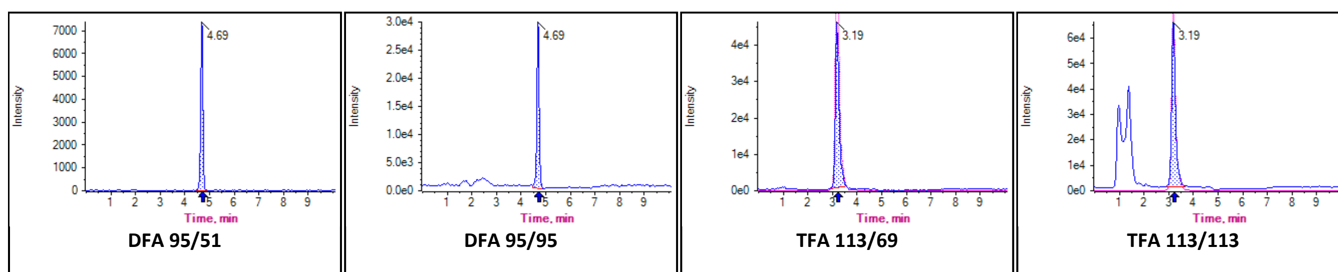


***Figure 30:** Typical chromatograms of DFA and TFA in tomato extracts spiked at 0.05 mg/kg*

5.7. Calibration and Calculations

5.7.1. **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) (**0**) (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:

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

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).

$$w_R = \frac{(PR^{Sample} - b_{cal})}{a_{cal}} \times \frac{1}{m_a} \times \frac{m_{ISTD}^{Sample}}{m_{ISTD}^{cal\,mix}} \left(\frac{\text{mg}}{\text{kg}}\right) \qquad (2)$$

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 21,** 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 $m_{IS\text{-}sample}$ being the IS mass that would have been added to the entire sample portion according to **5.2.2** and **Table 21**.

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 ($m_{aliquot} = m_a \times V_{aliquot}/20$).

$$w_R = \frac{(PR^{sample} - b_{cal})}{a_{cal}} \times \frac{1}{m_{aliquot}} \times \frac{m_{ISTD}^{aliquot}}{m_{ISTD}^{cal\,mix}} \left(\frac{\text{mg}}{\text{kg}}\right) \qquad (3)$$

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 (0 and 5.5.3) 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)

5.7.2. **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 (**5.5.3**) 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).

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

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

All other variables are listed in **5.7.1.**

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 31**. The Y-intercept of the calibration graph will indicate the pesticide mass contained in the non-fortified aliquot of the sample extract.*

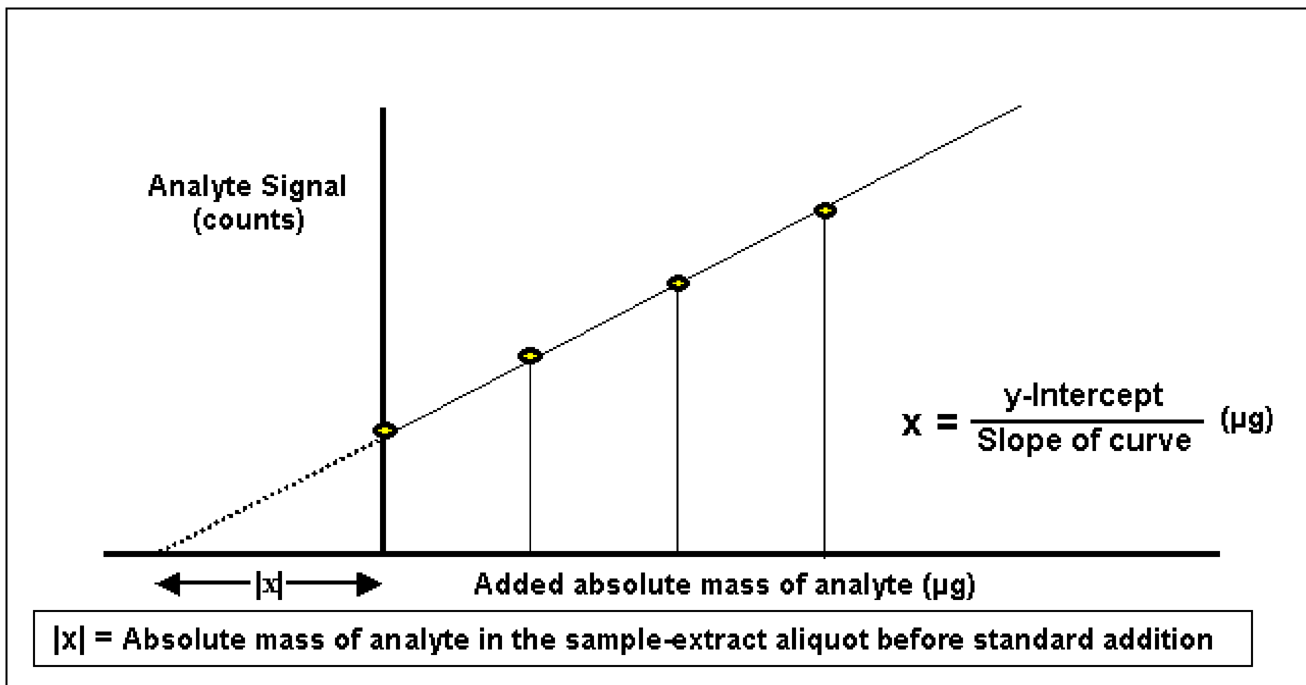


Figure 31: *Internal calibration using the procedure of standard additions, schematically*

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)

With x = $\frac{y - \text{int}\,ercept\ (b)}{slope\ of\ the\ curve\,(a)}$ (µg)

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/µg);

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. Analyte stability

A general overview regarding the stability of Glyphosate & Co. compounds in stock solutions is given in **Table 23**. For the compounds of this method (Maleic Hydrazide and Cyanuric acid excluded) the use of water with 10 % acetonitrile was shown to be a suitable solvent, see also **Table 28**.

In case of Ethephon (native compound or ILIS), which is sensitive towards neutral and alkaline pH, acidifying the stock solution with hydrochloric acid is recommended. 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 the need of 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 for some compounds, such as glyphosate.

To some extent degradation also takes place in QuPPe extracts (consisting of Water/Methanol+1 % Formic acid (1/1, v/v)) with AMPA and N-Acetyl-Glyphosate being most affected. In general degradation is negligible if extracts stored at room temperature are analyzed within 14 days . In any case such losses can be effectively corrected by the respective ILISs (if added at any stage prior to extract storage). The stability of compounds of the "Glyphosate & Co. group" in water containin g 10% acetonitrile, over a period of 7 months in the refrigerator, is demonstrated in **Figure 32**. The stability in stock solutions is generally better that in working solutions.

Table 23: *Overview of experiments on long-term stability "Glyphosate & Co." compounds dissolved in differently composed solvents. Concentration of analytes in stored mixtures 10 µg/mL ; storage duration: 6 months; storage temperature: 6°C*

	Composition of storage solvent											
	Pure Water				Water / MeOH (MeOH 25 and 50 %)*			Pure MeOH		Water / ACN (ACN 25 and 50 %)*		
Native Compound	w/o acid	1% FA	1% AA	0.1% HCl**	w/o acid	1% FA	1% AA	w/o acid	1% FA	w/o acid	1% FA	1% AA
AMPA	NT	NT	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
Ethephon	✗	✓	✓	✓	NT	NT	NT	NT	✓	NT	NT	NT
Fosetyl-Al	✓	✗	✓	✗	✓	NT	NT	NT	NT	NT	NT	NT
Glufosinate	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Glyphosate	✓	✗	✗	NT	✗	✗	✗	NT	NT	✗	✗	✗
HEPA	NT	NT	NT	NT	NT	NT	NT	✓	✓	NT	NT	NT
Maleic Hydrazide	NT	NT	NT	NT	NT	NT	NT	✓	✓	NT	NT	NT
MPPA	✓	✓	✓	NT	✗	✗	✗	NT	NT	✓	✓	✓
N-Acetyl-AMPA	✓	✓	✓	NT	✗	✗	✗	NT	NT	✓	✓	✓
N-Acetyl-Glufosinate	NT	NT	NT	NT	✓	**NT**	**NT**	NT	NT	NT	NT	NT
N-Acetyl-Glyphosate	✓	✓	✓	NT	✗	✗	✗	NT	NT	✓	✓	✓

✓= sufficiently stable (deviating less than ±10 % from a freshly prepared standard of the same composition) ; ✗=not stable
MeOH = Methanol; ACN = Acetonitrile
* Solutions of both 25 % and 50 % of organic solvent have been tested.
** 0.1% HCl-conc. (37%) in water (v/v)

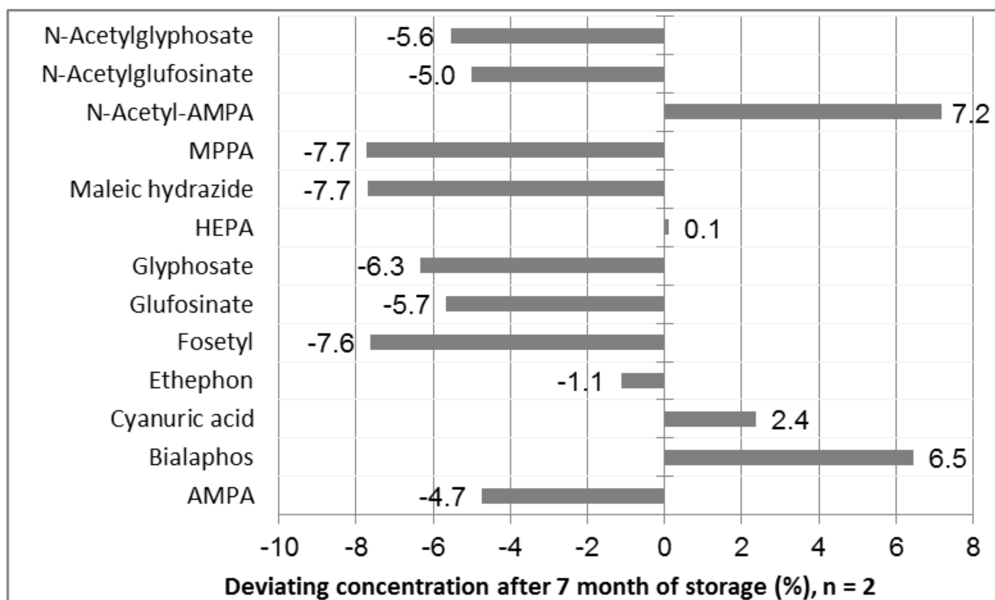


Figure 32: *Deviations of the concentration of Glyphosate & Co. compounds in a working solution of 10 µg/mL water containing 10 % acetonitrile and 1‰ HCl conc. (v/v), following 7 months of storage at 6 °C. Compared against a freshly prepared standard of same composition*

7. 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 24.**

Table 24: *Overview of lowest successfully validated levels per matrix*

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
M 1.3	AMPA	High water content + acidic	Grapes	0.02	12	110	9
	AMPA	Dry (cereals)	Barley	0.02	5	101	14
	AMPA	Dry (pulses)*	Lentil	0.1	10	95	17
	AMPA	Dry (cereals)	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)	Barley	0.02	5	110	2
	Ethephon	Dry (cereals)	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
	Ethephon	High water content + acidic	Grapes	0.01	5	104	4
	Fosetyl	High water content + acidic	Strawberry	0.1	6	94	4
	Fosetyl	Dry (cereals)	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
	Fosetyl	High water content + acidic	Grapes	0.01	5	105	2
	Glufosinate	High water content + acidic	Grapes	0.05	5	96	10
	Glufosinate	Dry (cereals)	Barley	0.02	5	101	13
	Glufosinate	Dry (cereals)	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 water content + acidic	Grapes	0.02	12	112	8
	Glyphosate	High water content + acidic	Grapes	0.02	5	102	6
	Glyphosate	Dry (cereals)	Barley	0.02	5	105	8
	Glyphosate	Dry (pulses)*	Lentil	0.1	11	107	18
	Glyphosate	High oil content, dry (oily 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)	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)	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
	Maleic Hydrazide	High water content + acidic	Grapes	0.01	5	110	11
	MPPA	Dry (cereals)	Barley	0.02	5	106	10
	MPPA	Dry (cereals)	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
	MPPA	High water content + acidic	Grapes	0.02	5	102	3
	N-Acetyl AMPA	Dry (cereals)	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)	Barley	0.02	5	103	5
	N-Acetyl Glufosinate	High water content	Apple	0.02	7	112	9

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	N-Acetyl Glufosinate	High water content	Cucumber	0.02	3	101	3
	N-Acetyl Glufosinate	High water content + acidic	Grapes	0.01	5	97	4
	N-Acetyl Glyphosate	High water content + acidic	Grapes	0.01	10	109	8
	N-Acetyl Glyphosate	Dry (cereals)	Corn flour	0.02	10	104	10
	N-Acetyl Glyphosate	Dry (pulses)*	Lentil	0.05	10	104	8
	N-Acetyl Glyphosate	High oil content, dry (oily 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 water content + acidic	Currant	1	5	98	4
	Bromide (inorg.)	High water content	Cauliflower	1	5	94	12
	Chlorate	High water content + acidic	Currant	0.01	5	102	7
	Chlorate	Dry (cereals)	Rice	0.02	5	108	2
	Chlorate	High water content	Cauliflower	0.01	5	100	5
	Perchlorate	High water content + acidic	Currant	0.01	5	100	4
	Perchlorate	Dry (cereals)	Barley	0.01	5	106	2
	Perchlorate	Dry (cereals)	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 water content + acidic	Currant	0.1	5	102	3
	Phosphonic Acid	High water content + acidic	Mandarine	0.1	5	99	10
	Phosphonic Acid	Dry (cereals)	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 1.5	AMPA	High water content	Apple	0.02	5	102	8
	AMPA	High water content + acidic	Grape	0.02	5	112	8
	AMPA	Dry (oily seeds)*	Soy flour	0.1	5	92	7
	AMPA	Dry (pulses)*	Lentils	0.1	5	103	6
	Ethephon	High water content	Apple	0.025	5	97	0
	Ethephon	High water content + acidic	Grape	0.025	5	80	8
	Ethephon	Dry (oily seeds)*	Soy flour	0.05	5	92	7
	Ethephon	Dry (pulses)*	Lentils	0.05	5	97	4
	Fosetyl	High water content	Apple	0.01	5	99	9
	Fosetyl	High water content + acidic	Grape	0.01	5	100	3
	Fosetyl	Dry (oily seeds)*	Soy flour	0.1	5	98	3
	Fosetyl	Dry (pulses)*	Lentils	0.1	5	99	1
	Glufosinate	High water content	Apple	0.02	5	94	2
	Glufosinate	High water content + acidic	Grape	0.02	5	106	4
	Glufosinate	Dry (oily seeds)*	Soy flour	0.1	5	98	4
	Glufosinate	Dry (pulses)*	Lentils	0.1	5	101	5
	Glyphosate	High water content	Apple	0.02	5	98	8
	Glyphosate	High water content + acidic	Grape	0.02	5	106	5
	Glyphosate	Dry (oily seeds)*	Soy flour	0.1	5	102	3
	Glyphosate	Dry (pulses)*	Lentils	0.1	5	102	4
	HEPA	High water content	Apple	0.02	5	102	8
	HEPA	High water content + acidic	Grape	0.02	5	100	6
	HEPA	Dry (oily seeds)*	Soy flour	0.1	5	96	4
	HEPA	Dry (pulses)*	Lentils	0.1	5	98	2
	MPPA	High water content	Apple	0.02	5	97	4
	MPPA	High water content + acidic	Grape	0.02	5	106	3
	MPPA	Dry (oily seeds)*	Soy flour	0.1	5	103	1
	MPPA	Dry (pulses)*	Lentils	0.1	5	103	2
	N-Acetyl-Glufosinate	High water content	Apple	0.01	5	100	3
	N-Acetyl-Glufosinate	High water content + acidic	Grape	0.01	5	103	3

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	N-Acetyl-Glufosinate	Dry (oily seeds)*	Soy flour	0.05	5	99	3
	N-Acetyl-Glufosinate	Dry (pulses)*	Lentils	0.05	5	102	1
	N-Acetyl-Glyphosate	Dry (oily seeds)*	Soy flour	0.05	5	105	10
	N-Acetyl-Glyphosate	Dry (pulses)*	Lentils	0.05	5	99	12
M 1.6	AMPA	High water content	Cucumber	0.02	5	99	7
	Ethephon	High water content	Cucumber	0.02	5	95	5
	Fosetyl	High water content	Cucumber	0.02	5	98	0
	Glufosinat	High water content	Cucumber	0.02	5	95	4
	Glyphosat	High water content	Cucumber	0.02	5	107	3
	HEPA	High water content	Cucumber	0.02	5	108	8
	MPPA	High water content	Cucumber	0.02	5	103	3
	N-Acetyl-AMPA	High water content	Cucumber	0.02	5	104	1
	N-Acetyl-Glufosinat	High water content	Cucumber	0.02	5	98	2
	N-Acetyl-Glyphosat	High water content	Cucumber	0.02	5	98	2
M 4.1	Amitrole	High water content + acidic	Orange	0.01	6	107	5
	Amitrole	Dry (cereals)	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 water content + acidic	Currant	0.02	5	99	4
	Chloridazon, Desphenyl-	Other	Swine meat	0.02	5	94	4
	Chloridazon, Desphenyl-	High water content	Lettuce varieties	0.02	5	97	3
	Chlormequat	High water content + acidic	Grapes	0.01	6	93	10
	Chlormequat	High water content + acidic	Grapes	0.2	5	102	1
	Chlormequat	Dry (cereals)	Barley	0.01	5	97	5
	Chlormequat	Dry (cereals)	Wheat flour	0.1	5	97	5
	Chlormequat	High oil content, 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 water content + acidic	Grapes	0.01	6	101	4
	Cyromazine	Dry (cereals)	Barley	0.01	5	109	6
	Cyromazine	High oil content, 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 water content + acidic	Orange	0.01	3	113	1
	Daminozide	Dry (cereals)	Barley	0.01	5	113	6
	Daminozide	High oil content, 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
	Diethanolamine	High water content + acidic	Mandarine	0.1	5	103	1
	Diethanolamine	High water content	Apple	0.1	5	103	3
	Diethanolamine	High water content	Mango	0.1	6	101	14
	Difenzoquat	Dry (cereals)	Barley	0.01	5	99	8
	Difenzoquat	High water content	Apple	0.01	6	99	11
	Diquat	Dry (cereals)	Barley	0.01	10	103	7
	Diquat	High water content	Apple	0.01	5	107	4
	ETU	Dry (cereals)	Barley	0.01	5	96	10
	ETU	High water content	Apple	0.01	7	102	9
	Melamine	High water content + acidic	Grapes	0.01	6	87	13
	Melamine	High oil content, dry (oily seeds, nuts)*	Bean, Soya	0.02	3	109	5
	Melamine	High oil content, wet (oily fruits)	Avocado	0.01	7	108	6
	Mepiquat	High water content + acidic	Grapes	0.01	6	95	5
	Mepiquat	High water content + acidic	Orange	0.01	6	101	9
	Mepiquat	Dry (cereals)	Barley	0.01	5	108	3

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	Mepiquat	Dry (cereals)	Wheat flour	0.1	5	102	5
	Mepiquat	High oil content, 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 water content + acidic	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 water content + acidic	Grapes	0.01	6	93	9
	Nereistoxin	Dry (cereals)	Barley	0.01	5	104	13
	Nereistoxin	High oil content, 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	Dry (cereals)	Barley	0.01	10	106	15
	Paraquat	High oil content, 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)	Barley	0.01	5	113	3
	Triethanolamine	High water content + acidic	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 water content + acidic	Grapes	0.01	6	93	7
	Trimesium	Dry (cereals)	Barley	0.01	5	118	3
	Trimesium	Dry (cereals)	Wheat flour	0.1	5	105	2
	Trimesium	High oil content, wet (oily fruits)	Avocado	0.01	7	93	14
	Trimesium	High water content	Potato	0.01	6	84	5
M 4.2	Aminocyclopyrachlor	High water content	Apple	0.01	5	110	5
	Aminocyclopyrachlor	Dry (cereals)	Oat	0.02	5	106	7
	Aminocyclopyrachlor	High water content	Cucumber	0.01	5	101	6
	Aminocyclopyrachlor	High water content + acidic	Lemon	0.01	5	112	9
	Aminocyclopyrachlor	High water content	Mint	0.01	5	108	7
	Amitole	High water content	Apple	0.01	5	99	6
	Amitole	Dry (cereals)	Oat	0.02	5	117	4
	Amitole	High water content + acidic	Raspberry	0.01	5	120	5
	Amitole	High water content	Cucumber	0.01	5	104	6
	Amitole	High water content + acidic	Lemon	0.01	5	96	4
	Chlormequat	High water content	Cucumber	0.01	5	106	3
	Chlormequat	High water content + acidic	Lemon	0.01	5	103	2
	Chlormequat	High water content	Mint	0.01	5	102	1
	Chlormequat	High water content	Apple	0.01	5	101	2
	Chlormequat	Dry (cereals)	Oat	0.02	5	119	2
	Chloridazon-desphenyl	High water content	Cucumber	0.01	5	104	3
	Chloridazon-desphenyl	High water content + acidic	Lemon	0.01	5	108	8
	Chloridazon-desphenyl	High water content	Mint	0.01	5	108	10
	Chloridazon-desphenyl	High water content	Apple	0.01	5	97	5
	Chloridazon-desphenyl	Dry (cereals)	Oat	0.02	5	113	9
	Cyromazine	High water content	Cucumber	0.01	5	101	5
	Cyromazine	High water content + acidic	Lemon	0.01	5	95	3
	Cyromazine	High water content	Mint	0.01	5	100	5
	Cyromazine	High water content	Apple	0.01	5	98	3
	Cyromazine	Dry (cereals)	Oat	0.02	5	114	4
	Daminozide	High water content	Apple	0.01	5	101	2
	Daminozide	Dry (cereals)	Oat	0.02	5	116	2

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	Daminozide	High water content + acidic	Raspberry	0.01	5	119	3
	Daminozide	High water content	Cucumber	0.01	5	103	6
	Daminozide	High water content + acidic	Lemon	0.01	5	102	1
	Daminozide	High water content	Mint	0.01	5	104	3
	Diethanolamin	Dry (cereals)	Oat	0.02	5	106	14
	Difenzoquat	High water content	Cucumber	0.01	5	105	1
	Difenzoquat	High water content + acidic	Lemon	0.01	5	105	3
	Difenzoquat	High water content	Apple	0.01	5	105	4
	Difenzoquat	Dry (cereals)	Oat	0.02	5	97	6
	ETU	High water content	Cucumber	0.01	5	87	10
	ETU	High water content + acidic	Lemon	0.01	5	104	11
	ETU	Dry (cereals)	Oat	0.02	5	103	14
	ETU	High water content + acidic	Raspberry	0.01	5	109	5
	Melamine	High water content	Cucumber	0.01	5	90	13
	Melamine	High water content + acidic	Lemon	0.01	5	91	11
	Melamine	High water content	Mint	0.01	5	93	11
	Melamine	High water content	Apple	0.01	5	97	8
	Melamine	Dry (cereals)	Oat	0.02	5	117	8
	Mepiquat	High water content	Cucumber	0.01	5	102	3
	Mepiquat	High water content + acidic	Lemon	0.01	5	104	4
	Mepiquat	High water content	Mint	0.01	5	96	3
	Mepiquat	High water content	Apple	0.01	5	104	3
	Mepiquat	Dry (cereals)	Oat	0.02	5	114	5
	Mepiquat, 4-Hydroxy	High water content	Cucumber	0.01	5	108	2
	Mepiquat, 4-Hydroxy	High water content + acidic	Lemon	0.01	5	107	4
	Mepiquat, 4-Hydroxy	High water content	Mint	0.01	5	105	2
	Mepiquat, 4-Hydroxy	High water content	Apple	0.01	5	110	2
	Mepiquat, 4-Hydroxy	Dry (cereals)	Oat	0.02	5	112	3
	Morpholine	High water content	Cucumber	0.01	5	97	10
	Morpholine	High water content + acidic	Lemon	0.01	5	92	9
	Morpholine	High water content	Apple	0.01	5	84	15
	Morpholine	High water content + acidic	Raspberry	0.01	5	84	18
	Nereistoxin	High water content	Cucumber	0.01	5	94	8
	Nereistoxin	High water content + acidic	Lemon	0.01	5	99	2
	Nereistoxin	High water content	Mint	0.01	5	90	3
	Nereistoxin	High water content	Apple	0.01	5	101	3
	Nereistoxin	Dry (cereals)	Oat	0.02	5	113	2
	Nereistoxin	High water content + acidic	Raspberry	0.01	5	114	2
	Nicotine	High water content	Apple	0.01	5	90	3
	Nicotine	High water content	Lamb's lettuce	0.01	5	95	8
	Nicotine	High water content + acidic	Orange	0.01	5	104	4
	Nicotine	High water content + acidic	Grape	0.01	5	99	2
	Nicotine	Dry (cereals)	Whole flour (spelt)	0.01	5	101	4
	Propamocarb	High water content	Cucumber	0.01	5	99	2
	Propamocarb	High water content + acidic	Lemon	0.01	5	84	6
	Propamocarb	High water content	Mint	0.01	5	102	2
	Propamocarb	High water content	Apple	0.01	5	102	2
	Propamocarb	Dry (cereals)	Oat	0.02	5	113	3
	Propamocarb-N-Desmethyl	High water content	Apple	0.01	5	113	3
	Propamocarb-N-Desmethyl	Dry (cereals)	Oat	0.02	5	94	3
	Propamocarb-N-Desmethyl	High water content	Cucumber	0.01	5	106	2
	Propamocarb-N-Oxide	High water content	Cucumber	0.01	5	102	2
	Propamocarb-N-Oxide	High water content + acidic	Lemon	0.01	5	109	4

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	Propamocarb-N-Oxide	High water content	Mint	0.01	5	111	3
	Propamocarb-N-Oxide	High water content	Apple	0.01	5	110	4
	PTU	High water content	Cucumber	0.01	5	97	4
	PTU	High water content + acidic	Lemon	0.01	5	100	5
	PTU	Dry (cereals)	Oat	0.02	5	113	6
	PTU	High water content + acidic	Raspberry	0.01	5	115	6
	Triethanolamine	High water content	Apple	0.01	5	73	15
	Triethanolamine	High water content + acidic	Raspberry	0.01	5	106	4
	Trimethylsulfonium	High water content	Cucumber	0.01	5	119	3
	Trimethylsulfonium	High water content + acidic	Lemon	0.01	5	110	2
	Trimethylsulfonium	High water content	Mint	0.01	5	116	3
	Trimethylsulfonium	High water content	Apple	0.01	5	119	2
M 5	See un der http://www.crl-pesticides.eu/library/docs/srm/meth_ChlormequatMepiquat_CrlSrm.pdf						
M 6	Kasugamycin	High water content	Apple	0.01	5	98	4
	Streptomycin	High water content	Apple	0.01	10	106	9
M 7	Morpholine	High water content	Apple	0.1	5	94	3
	Morpholine	High water content	Mango	0.1	5	95	2
	Morpholine	High water content + acidic	Mandarin	0.1	5	95	7
	Diethanolamine	High water content	Apple	0.1	5	103	3
	Diethanolamine	High water content	Mango	0.1	5	107	1
	Diethanolamine	High water content + acidic	Mandarin	0.1	5	103	1
	Triethanolamine	High water content	Apple	0.1	5	108	6
	Triethanolamine	High water content	Mango	0.1	5	118	3
	Triethanolamine	High water content + acidic	Mandarin	0.1	5	112	4
M 8	1,2,4-Triazole	High water content	Cucumber	0.1	5	85	12
	1,2,4-Triazole	High water content	Potatoes	0.01	5	100	8
	1,2,4-Triazole	High acid content	Orange	0.1	5	94	20
	1,2,4-Triazole	High acid content	Grapes	0.01	5	90	10
	1,2,4-Triazole	Dry (cereals)	Rice	0.2	5	86	3
	1,2,4-Triazole	Dry (cereals)	Barley	0.1	5	104	6
	1,2,4-Triazole	Fatty, wet	Avocado	0.01	5	94	10
	Triazole-acetic acid	High water content	Cucumber	0.01	5	100	2
	Triazole-acetic acid	High water content	Potatoes	0.01	5	96	6
	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)	Rice	0.02	5	74	5
	Triazole-acetic acid	Dry (cereals)	Barley	0.01	5	109	5
	Triazole-acetic acid	Fatty, wet	Avocado	0.01	5	97	2
	Triazole-alanine	High water content	Cucumber	0.01	5	100	19
	Triazole-alanine	High water content	Potatoes	0.01	5	102	18
	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)	Rice	0.02	5	88	4
	Triazole-alanine	Dry (cereals)	Barley	0.02	5	119	9
	Triazole-alanine	Fatty, wet	Avocado	0.01	5	91	13
	Triazole-lactic acid	High water content	Cucumber	0.01	5	107	3
	Triazole-lactic acid	High water content	Potatoes	0.01	5	102	6
	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)	Rice	0.02	5	71	4
	Triazole-lactic acid	Dry (cereals)	Barley	0.02	5	99	4
	Triazole-lactic acid	Fatty, wet	Avocado	0.01	5	97	4
	See also: **http://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EurlSrm_meth_TriazoleDerivativeMetabolites.pdf**						

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
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)	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)	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)	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
	Trifluoroacetic acid	High acid content	Orange	0.01	5	107	3
	Trifluoroacetic acid	Dry (cereals)	Rice	0.04	5	72	4
	Trifluoroacetic acid	High water content	Tomato	0.02	5	76	15

*The extract of pulses was prepared according to the former Version of QuPPe for dry commodities, oily seeds and pulses (V9) without use of EDTA solution.

Table 25: *Data of Recovery experiments on Lentils with M 1.6. The extract was prepared using the current version of QuPPe (V10-1) with use of EDTA solution and dilution.*

Method	Analyte	Commodity/Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
M 1.6	AMPA	Dry (pulses)	Lentil	0.1	5	107	10
	Ethephon	Dry (pulses)	Lentil	0.1	5	89	12
	Fosetyl	Dry (pulses)	Lentil	0.1	5	104	3
	Glufosinat	Dry (pulses)	Lentil	0.1	5	82	8
	Glyphosat	Dry (pulses)	Lentil	0.1	5	115	9
	HEPA	Dry (pulses)	Lentil	0.1	5	100	5
	MPPA	Dry (pulses)	Lentil	0.1	5	108	5
	N-Acetyl-Glufosinat	Dry (pulses)	Lentil	0.1	5	113	4
	N-Acetyl-Glyphosat	Dry (pulses)	Lentil	0.1	5	101	8

Table 26: *Validation data deriving from two QuPPe interlaboratory validation studies organized by the EURL-SRM.*

Matrix	Level (mg/kg)	Solvent + ILIS Calibration			Matrix Matched Calibration			Matrix + ILIS Calibration		
		Mean Recovery (%)	RSD (± %)	No. Labs	Mean Recovery (%)	RSD (± %)	No. Labs	Mean Recovery (%)	RSD (± %)	No. Labs
Cyromazine										
Potatoes	0.01	102	4	7	89	5	9	100	5	9
	0.05	115	4	8	91	4	9	102	3	9
	0.2	100	3	9	92	2	9	102	3	9
Grapes	0.01	101	4	8	96	6	10	103	4	10
	0.05	99	3	8	96	3	9	103	3	10
	0.2	97	2	8	96	3	10	102	2	10
Rye flour	0.01	119	7	6	85	13	7	102	9	7
	0.05	104	6	8	85	5	9	100	7	9
	0.2	97	4	8	86	4	9	98	3	9
Avocados	0.01	100	4	6	92	4	8	104	4	7
	0.05	102	2	9	93	3	10	102	3	9
	0.2	99	2	8	86	4	11	102	2	10
Daminozide										
Potatoes	0.01	107	2	3	100	6	8	89	4	8
	0.05	99	3	7	97	4	10	97	4	9
	0.2	93	4	7	99	3	10	94	4	10
Grapes	0.01	102	6	4	97	5	10	97	7	8
	0.05	97	2	8	97	3	11	97	4	11
	0.2	97	2	8	97	3	11	97	4	11
Rye flour	0.01	107	11	5	105	6	5	97	5	5
	0.05	90	5	7	113	5	7	106	4	8
	0.2	89	4	7	109	3	7	101	4	8
Avocados	0.01	110	2	5	100	7	7	95	6	6
	0.05	99	4	8	104	4	10	99	3	9
	0.2	95	2	8	99	3	10	99	2	9
Chlormequat										
Potatoes	0.01	99	3	9	98	4	10	101	3	10
	0.05	99	3	9	98	3	10	102	3	10
	0.2	105	4	10	101	3	10	102	4	10
Grapes	0.01	97	3	10	99	4	10	104	3	10
	0.05	99	3	10	95	2	10	101	2	11
	0.2	101	2	10	97	2	11	103	2	11
Rye flour	0.01	109	5	9	105	7	10	102	4	10
	0.05	103	5	9	101	4	10	106	5	10
	0.2	102	3	9	103	3	10	104	3	10
Avocados	0.01	97	3	10	97	4	10	103	3	10
	0.05	99	3	9	96	3	10	102	3	10
	0.2	101	2	10	91	3	11	103	2	10
Trimesium										
Potatoes	0.01	87	5	9	98	4	10	104	4	10
	0.05	92	3	7	97	4	10	104	4	10
	0.2	93	2	8	97	3	10	101	3	10
Grapes	0.01	93	3	10	95	3	11	101	2	11
	0.05	96	2	9	95	2	10	100	2	11
	0.2	97	2	9	95	3	11	102	2	11
Rye flour	0.01	126	6	9	102	7	10	107	5	10
	0.05	122	5	9	100	4	10	106	4	10
	0.2	120	4	9	101	3	10	101	4	10
Avocados	0.01	93	4	9	89	4	10	98	4	10
	0.05	92	3	9	91	4	10	101	3	10
	0.2	93	3	10	88	4	10	99	3	9

Matrix	Level (mg/kg)	Solvent + ILIS Calibration Mean Recovery (%)	RSD (± %)	No. Labs	Matrix Matched Calibration Mean Recovery (%)	RSD (± %)	No. Labs	Matrix + ILIS Calibration Mean Recovery (%)	RSD (± %)	No. Labs
Nereistoxin										
Potatoes	0.01	128	6	5	91	8	6	105	9	6
	0.05	110	7	5	91	5	8	98	5	7
	0.2	111	3	8	94	3	9	100	3	9
Grapes	0.01	109	7	4	94	7	9	97	6	9
	0.05	107	5	8	96	5	10	100	6	11
	0.2	115	3	9	94	4	11	100	4	11
Rye flour	0.01	184	8	6	93	9	7	99	7	7
	0.05	137	6	8	89	7	9	104	6	9
	0.2	131	4	8	90	4	9	102	5	9
Avocados	0.01	100	6	3	84	8	5	102	7	5
	0.05	108	2	7	84	4	7	102	3	8
	0.2	108	3	7	80	4	9	106	5	9
Melamine										
Potatoes	0.01	121	4	4	78	6	7	103	7	7
	0.05	105	4	6	73	5	9	101	5	9
	0.2	97	3	7	81	4	9	104	5	9
Grapes	0.01	102	6	5	91	7	7	102	7	8
	0.05	95	5	8	94	3	9	101	4	10
	0.2	99	3	9	96	4	10	102	2	10
Rye flour	0.01	196	5	5	71	7	6	148	13	7
	0.05	109	5	7	63	14	8	115	8	8
	0.2	106	5	8	60	7	9	105	5	9
Avocados	0.01	120	3	4	88	6	5	109	4	4
	0.05	102	6	7	90	4	8	101	3	9
	0.2	96	5	9	82	4	10	102	4	9
Perchlorate										
Carrot	0.01	131	22	7	101	41	7	98	9	7
	0.02	116	17	7	96	34	7	98	14	7
	0.2	93	16	6	79	38	6	94	11	6
Lemon	0.01	123	11	7	107	23	7	105	8	7
	0.02	117	12	7	103	12	7	104	8	7
	0.2	131	50	8	103	12	8	102	5	8
Rye flour	0.01	107	14	7	57	46	7	100	7	7
	0.02	101	11	8	62	36	8	101	9	8
	0.2	99	11	9	59	35	9	101	11	9
Avocado	0.01	104	17	5	97	27	5	103	15	5
	0.02	104	8	6	94	18	6	101	10	6
	0.2	94	26	5	92	10	5	99	6	5
Chlorate										
Carrot	0.01	102	12	8	103	31	8	101	10	8
	0.02	104	9	7	95	37	7	101	9	7
	0.2	99	6	6	84	37	6	99	5	6
Lemon	0.01	103	10	7	109	20	7	100	11	7
	0.02	119	31	7	108	15	7	111	15	7
	0.2	129	39	8	102	11	8	100	5	8
Rye flour	0.01	100	29	8	80	34	8	109	14	8
	0.02	103	11	8	72	39	8	105	11	8
	0.2	98	9	9	72	33	9	105	13	9
Avocado	0.01	130	30	6	103	22	6	111	21	6
	0.02	101	8	6	107	22	6	107	12	6
	0.2	100	8	5	98	6	5	104	8	5

Matrix	Level (mg/kg)	Solvent + ILIS Calibration Mean Recovery (%)	RSD (± %)	No. Labs	Matrix Matched Calibration Mean Recovery (%)	RSD (± %)	No. Labs	Matrix + ILIS Calibration Mean Recovery (%)	RSD (± %)	No. Labs
Phosphonic acid										
Carrot	0.01	102	21	3	94	5	3	93	6	3
	0.02	107	8	4	111	14	4	104	3	4
	0.2	97	5	3	106	3	3	102	2	3
Lemon	0.01	87	32	3	94	12	3	91	18	3
	0.02	87	26	3	100	6	3	99	13	3
	0.2	117	43	7	118	33	7	98	8	7
Rye flour	0.01	91	37	3	82	7	3	91	18	3
	0.02	111	30	5	83	33	5	100	21	5
	0.2	103	13	7	78	28	7	104	15	7
Avocado	0.01	125	19	4	96	8	4	94	9	4
	0.02	114	27	4	99	4	4	99	6	4
	0.2	119	37	5	97	17	5	101	7	5
Bromide										
	No ILIS used as not available for bromide									
Carrot	0.01	*110*	*34*	*5*	82	12	5			
	0.02	*128*	*52*	*6*	80	11	6			
	0.2	*97*	*12*	*5*	78	13	5			
Lemon	0.01	*88*	*62*	*7*	102	13	7			
	0.02	*92*	*32*	*7*	107	9	7			
	0.2	*88*	*25*	*6*	118	19	6			
Rye flour	0.01	*97*	*29*	*6*	97	9	6			
	0.02	*90*	*26*	*7*	90	19	7			
	0.2	*84*	*25*	*6*	99	8	6			
Avocado	0.01	*125*	*39*	*6*	96	14	6			
	0.02	*124*	*28*	*6*	99	10	6			
	0.2	*114*	*14*	*6*	104	7	6			

8. References

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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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9. ANNEX

Table 27: *Conversion factors between typical purchased standards and target analytes (**3.18**).*

Compound	MW [g/mol]	Compound as sold	MW [g/mol]	Conv. Factor (CF)	Inverse CF
Bialaphos	323.3	Bialaphos-sodium	345.3	0.94	1.07
Bromate (anion)	127.9	Potassium bromate	167.0	0.77	1.31
Bromide (anion)	79.9	Potassium bromide	119.0	0.67	1.49
Chlorate (anion)	83.5	Chlorate-sodium	106.4	0.78	1.27
Chlormequat (cation)*	122.6	Chlormequat-chloride*	158.1	0.78	1.29
Chlormequat-D_4 (cation)	126.6	Chlormequat-D_4-chloride	162.1	0.78	1.28
Difenzoquat (cation)	249.3	Difenzoquat-methylsulfate	360.4	0.69	1.45
Difluoroacetic acid-$^{13}C_2$	96.0	Sodium difluoroacetate-$^{13}C_2$	120.0	0.80	1.25
Dihydrostreptomycin	583.6	Dihydrostreptomycin-sesquisulfate	730.7	0.80	1.25
Diquat (dication)	184.2	Diquat-dibromide-monohydrate	362.1	0.51	1.97
Diquat-D_4 (dication)	188.2	Diquat-D_4-dibromide-monohydrate	366.1	0.51	1.95
Fosetyl	110.0	Fosetyl-Al	118.0	0.93	1.07
Fosetyl-D_5	115.0	Fosetyl-D_5-1/3 aluminium	123.0	0.93	1.07
		Fosetyl-D_5-sodium	137.0	0.84	1.19
Glufosinate	181.1	Glufosinate-ammonium	198.2	0.91	1.09
Glufosinate-D_3	184.1	Glufosinate-D_3-hydrochloride	220.6	0.83	1.20
Kasugamycin	379.4	Kasugamycin-hydrochloride-monohydrate	433.8	0.87	1.14
Mepiquat (cation)*	114.2	Mepiquat-chloride*	149.7	0.76	1.31
Mepiquat-D_3 (cation)	117.2	Mepiquat-D_3-iodide	244.1	0.48	2.08
Mepiquat-4-hydroxy	130.2	Mepiquat-4-hydroxy-chloride	165.7	0.79	1.27
N,N'-Dimethylhydrazine-D_6	66.1	Dimethylhydrazine-D_6–hydrochloride	102.6	0.64	1.55
N-Acetyl-Glufosinate	223.2	N-Acetyl-Glufosinate-disodium	267.2	0.84	1.20
N-Acetyl-Glufosinate-D_3	226.2	N-Acetyl-Glufosinate-D_3-disodium	270.2	0.84	1.19
Nereistoxin	149.3	Nereistoxin-oxalate	239.3	0.62	1.60
Nereistoxin-D_6	155.3	Nereistoxin-D_6-oxalate	245.3	0.63	1.58
Nicotine	162.2	Nicotine hemisulfate	422.5**	0.77	1.30
Paraquat (dication)	186.3	Paraquat-dichloride	257.2	0.72	1.38
Paraquat-D_6 (dication)	192.3	Paraquat-D_6-diiodide	446.1	0.43	2.32
Propamocarb-N-oxide	204.3	Propamocarb-N-oxide hydrochloride	240.7	0.85	1.17
Streptomycin	581.6	Streptomycin-sesquisulfate	728.7	0.80	1.25
Trifluoroacetic acid -$^{13}C_2$	114.0	Sodium trifluoroacetate-$^{13}C_2$	138.0	0.83	1.21
Trimethylsulfonium (cation)	77.2	Trimethylsulfonium-iodide	204.1	0.38	2.64
Trimethylsulfonium-D_9 (cation)	86.2	Trimethylsulfonium-D_9-iodide	213.1	0.40	2.47

* Attention: The EU – Maximum Residue Levels are now expressed as the respective chloride salts. **Thus no conversion of the chloride to the cation is needed.**

** MW refers to the following formula $C_{10}H_{14}N_2)_2 \cdot H_2SO_4$ which entails two nicotine molecules

Table 28: *Exemplary concentrations of pesticide stock and working solutions (**3.18** and **3.19**), solvent proposals also apply to ILISs (see **3.21**, **3.22** and **3.23**). Prefereably **use plastic vials** (e.g. PP) as many of the compounds listed below 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	MeOH	1	MeOH	10 / 1 / 0.1
Amitrole	MeOH	1	MeOH	10 / 1 / 0.1
AMPA	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
Bromate	Water/MeOH (50:50)	1	MeOH	10 / 1 / 0.1 / 0.01
Bromide	MeOH	1	MeOH	10 / 1 / 0.1 / 0.01
Chlorate	MeOH	1	MeOH	10 / 1 / 0.1 / 0.01
Chloridazon-desphenyl	MeOH	1	MeOH	10 / 1 / 0.1
Chlormequat	MeOH	1	MeOH	10 / 1 / 0.1
Cyanuric acid	MeOH	1	10 % ACN in water	10 / 1 / 0.1
Cyromazine	MeOH	1	MeOH	10 / 1 / 0.1
Daminozide	MeOH	1	MeOH	10 / 1 / 0.1
Diethanolamine	CAN	1	MeOH	10 / 1 / 0.1
Difenzoquat	ACN	1	MeOH	10 / 1 / 0.1
Difluoroacetic acid	ACN with 5% water	1	ACN with 5% water	10 / 1/ 0.1
Diquat**	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
Ethephon	10 % ACN in water + 0,1 % HCl	1	10 % ACN in water + 0,1 % HCl	10 / 1 / 0.1
ETU	MeOH	1	MeOH	10 / 1 / 0.1
Fosetyl	10 % ACN in water	0.1	10 % ACN in water	10 / 1 / 0.1
Glufosinate	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
Glyphosate*	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
HEPA	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
Kasugamycin	MeOH	1	MeOH	10 / 1 / 0.1
Maleic Hydrazide	MeOH	1	10 % ACN in water	10 / 1 / 0.1
Mepiquat	MeOH	1	MeOH	10 / 1 / 0.1
Mepiquat-4-hydroxy	MeOH	1	MeOH	10 / 1 / 0.1
Morpholine	MeOH	1	MeOH	10 / 1 / 0.1
MPPA	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
N,N-Dimethylhydrazine	MeOH	1	MeOH	10 / 1 / 0.1
N-Acetyl- AMPA	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
N-Acetyl-Glufosinate	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
N-Acetyl-Glyphosate	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
Nereistoxin	MeOH / water (3:1)	1	MeOH	10 / 1 / 0.1
Nicotine*	ACN	1	ACN	1 / 0.1
Paraquat**	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
Perchlorate	MeOH	1	MeOH	10 / 1 / 0.1 / 0.01
Phosphonic acid*	Water (^{18}O-H_2O for the ILIS)	1	ACN***	10 / 1 / 0.1 / 0.01
Propamocarb	ACN	1	MeOH	10 / 1 / 0.1
Propamocarb-N-desmethyl	ACN:Acetone (1 mL acetone to initially dissolve)	1	MeOH	10 / 1 / 0.1
Propamocarb-N-oxide	MeOH	1	MeOH	10 / 1 / 0.1
PTU	MeOH	1	MeOH	10 / 1 / 0.1
Streptomycin*	Water / MeOH (1:1)	0,5	MeOH	10 / 1 / 0.1
Triethanolamine	MeOH	1	MeOH	10 / 1 / 0.1
Trifluoroacetic acid	ACN with 5% water	1	ACN with 5% water	10 / 1/ 0.1
Trimethylsulfonium	MeOH	1	MeOH	10 / 1 / 0.1

* Use plastic vessels and stoppers for compounds that tend to interact with glass surfaces
** Use plastic vials and protect solutions from light exposure
*** Pure water (^{18}O-H_2O for the ILIS) is also suitable for the working solution. 10% ACN will reduce growth of microorganisms
MeOH: Methanol; ACN: Acetonitrile; FA: Formic acid

Table 29:** Exemplary providers of isotopically labelled internal standards* ***3.20.

Isotope labelled compound		Source	Article-No.	Conc. (µg/mL)	Amount per unit	Prices in €-cent (see diclaimer)		
						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
	$^{15}N2$ $^{13}C_2$	7	A633382		10 mg	1.500 €	30 c	1.5 c
	$^{15}N4$ / $^{13}C_2$	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	1.690 €	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	No indication	1 mL	4.300 €		
		12***	-	200	5 mL	250 €	50 c	2.5 c
Chloridazon-desphenyl-$^{15}N_2$		13	8399.4-10MG		10 mg	1.380 €	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
		9	00291		5 mg	485 €	19 c	1.0 c
	D_9	3	673151		5 mg	320 €	13 c	0.6 c
Cyanuric acid	**$^{13}C_3$**	7	C987717		5 mg	164 €	6.6 c	0.3 c
		9	32679		10 mg	470 €	9.4 c	0.5 c
	$^{18}O3$	3	673141		10 mg	299 €	6.0 c	0.3 c
Cyromazine-D_4		1	DRE-C11920010		10 mg	366 €	7.3 c	0.4 c
		1	XA11920010EA	100	1.1 mL	118 €	215 c	11 c
		7	C989302		10 mg	1255 €	25.1 c	1.3 c
		9	93101		5 mg	164 €	6.6 c	0.3 c
Daminozide-D_6		1	XA11960100AL	100	1.1 mL	87 €	158 c	7.9 c
		7	D416717		25 mg	647 €	5.2 c	0.3 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 -$^{13}C_2$ (Sodium salt)		2	friendly donation					
Dihydrostreptomycin	**sesquisulfate-hydrate**	1	C 12635300		100 mg	29 €	0.1 c	0.003 c
	sulfate	1	EPD1954000		25 mg	120 €	1.0 c	0.048
Diquat-D_4-dibromide (ethylene-D_4)		1	DRE-CA12960010		50 mg	315 €	1.3 c	0.06 c
		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	1.100 €	22 c	1.1 c
		11	sc-218246		5 mg	234 €	9.4 c	0.5 c
Ethephon	**D_4**	1	XA13230100AC	100	1.1 mL	127 €	231 c	12 c
			DRE-C13230100		10 mg	1.200 €	24 c	1.2 c
		6	D8328		5 mg	1.400 €	56 c	2.8 c
		7	C366177		10 mg	1.120 €	22 c	1.1 c
	$^{13}C_2$	7	C366178		0,25 mg	210 €	170 c	8 c
Ethylenethiourea-D_4 (ETU-D_4)		1	C 13330100		50 mg	316 €	1.3 c	0.06 c
			XA13330100AC	100	1.1 mL	127 €	231 c	12 c
		6	D1965		100 mg	733 €	1.5 c	0.07 c
		7	I367002		10 mg	98 €	2.0 c	0.1 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

Isotope labelled compound		Source	Article-No.	Conc. (µg/mL)	Amount per unit	Prices in €-cent (see diclaimer)		
						1 unit	2 µg*	0,1 µg**
Glufosinate	D$_3$	2	Friendly donation					
		3	680888	100	1 mL	380 €	760 c	38 c
		14	LBS2J3L1604	1000	1.1 mL	385 €	70 c	3.5 c
	D$_3$-Chloride	3	681220	100	1 mL	380 €	760 c	38 c
		7	G596952		10 mg	1.900 €	38 c	1.9 c
Glyphosate	^{13}C2,^{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	1.170 €	196 c	9.8 c
		1	CIL-CNLM-4666-1.2	100	1.2 mL	344 €	573 c	29 c
		6	CN10570		5 mg	1.990€	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
	^{13}C,^{15}N	9	90479		5 mg	536 €	21 c	1.1 c
	^{13}C	7	G765001		5 mg	210 €	8.4 c	0.4 c
		9	606502		10 mg	785 €	16 c	0.9 c
HEPA (Hydroxy-Ethephon)-D$_4$		1	CA13230200		10 mg	256 €	5.1 c	0.3 c
		7	H939652		25 mg	1.125 €	9.0 c	0.5 c
		2	Friendly donation					
		3	676639	100	1 mL	99 €	200 c	10 c
Maleic Hydrazide-D$_2$		1	C 14730100		10 mg	235 €	4.7 c	0.2 c
		3	673799		10 mg	199 €	20c (10µg)	1 c (0.5 µg)
		7	M124502		5 mg	141 €	5.6 c	0.3 c
Melamine-^{13}C$_3$, ^{15}N$_3$		1	CIL-CNLM-8150-10X-1.2	1000	1.2 mL	1300 €	260 c	13 c
Melamine-^{15}N$_3$		9	80038		10 mg	647 €	13	0.7 c
		3	673055		10 mg	289 €	5.8 c	0.3 c
Melamine-^{13}C$_3$		3	679703		10 mg	480 €	9.6 c	0.5 c
		1	B-MYC8020-1.2	100	1.2 mL	528	8.8 €	440 c c
Mepiquat-	D$_{16}$ –chloride-	6	D14539		50 mg	1.350 €	5.4 c	0.3 c
		9	52485		5 mg	214 €	8.6 c	0.4 c
	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
		9	78278		10 mg	379 €	7.6 c	0.4 c
		3	677008		10 mg	320 €	6.4 c	0.3 c
Morpholine	D$_8$	4	D-1895/0.5		500 mg	468 €	0.94 c (10µg)	0.05c (0.5µg)
		7	M723728		25 mg	131 €	1.1 c	0.05 c
	^{13}C$_4$	7	M723727		1 mg	131 €	26 c	1.3 c
N-Acetyl-Glufosinate	D$_3$ (methyl-D$_3$)	2	Friendly donation					
	D$_3$ (Acetylamino-D$_3$)	7	A178237		5 mg	141 €	5.6 c	0.3 c
		9	05567		5 mg	97.50 €	3.9 c	0.2 c
		3	680264	100	1 mL	280 €	560 c	28 c
		14	LBS9AZ3L1606	1000	1.1 mL	180 €	70 c	3.5 c
N-Acetyl-glyphosate	D$_3$ (methyl-D$_3$)	7	A178248		25 mg	1.153 €	9.2 c	0.5 c
	^{13}C$_2$,^{15}N	7	A178247		10 mg	1.326 €	26,5 c	1,3 c
Nereistoxin-oxalate-D6		1	C 15502010		10 mg	245 €	5 c	0.3 c
Nicotine-D4		4	D-5098		100 mg	400 €	0.8 c	0.04 c
MPPA-D3		2	Friendly donation					
		7	M326162		10 mg	1.921 €	38 c	1.9 c
		3	680891	100	1mL	380 €	760 c	38 c
Paraquat	D$_6$-diiodide	1	C 15870200		50 mg	256 €	1.0 c	0.05 c
	D$_6$-dichloride (dimethyl D$_6$)	1	DRE-C15870050		50 mg	390 €	1.6 c	0.08 c
	D$_8$-dichloride	1	DRE-CA15870100		50 mg	390 €	1.6 c	0.08 c
		7	P191902		25 mg	920 €	7.3 c	0.4 c
Perchlorate-^{18}O$_4$		5	OLM-7310-1.2	100	1.2 mL	326 €	272 c	14 c

Isotope labelled compound		Source	Article-No.	Conc. (µg/mL)	Amount per unit	Prices in €-cent (see diclaimer)		
						1 unit	2 µg*	0,1 µg**
		12***		40	5 mL	250 €	125 c	6.3 c
		9	631981		10 mg	4.500 €	90 c	4.5 c
Phosphonic acid-$^{18}O_3$		12		2000	1 mL	125	6.3 c	0.3 c
Propamocarb	**D_6**	7	P758462		10 mg	1050 €	21 c	1.1 c
	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
		7	P836802****		10 mg	1.100 €	22 c	1.1 c
	D_3	9	07359		5 mg	205 €	8.2 c	0.4 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})**	1	CIL-DLM-7663		1 mg	153 €	31 c	1.5 c
	D_{12}	7	T775582		10 mg	141 €	2.8 c	0.15 c
Trifluoroacetic acid -$^{13}C_2$ (Sodium acetate)		7	S673752		10 mg	2.670 €	53 c	2.7 c
Trimethylsulfonium-D_9 (Iodide)	**D_9**	6	D2677		100 mg	730 €	0.7 c	0.04 c
		6	D2677		10 mg	270 €	2.6 c	0.13 c
		4	D-6093		500mg	430 €	0.2 c	0.009 c
	D_3	3	684243		10 mg	100 €	2 c	0.1 c

Providers of compounds::

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-Supelco (Merck)

10. Cerilliant (by Sigma Aldrich)

11. Santa Cruz biotechnology. Inc.

12. EURL-SRM (hosted at CVUA Stuttgart)

13. Campro Scientific **/** Chiron AS

14. Lab Instruments

(**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.

* 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 $^{18}O_3$-Chlorate is accompanied by ca 20% $^{18}O_4$-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.

**** The PTU-D_6 offered by (7) used to be the non-branched 1,3 propylene variant. This product did not exactly co-elute with the target analyte and thus not compensating mtrix effects. It now seems to be the right product N,N'-(1,2-Propylene)thiourea-D_6

***Table 30**: Exemplary concentrations of Internal Standard Working Solutions (IS-WS) (**3.22**)*

Internal Standard (IS)*	**IS –Addition to samples (5.2.3)**		**IS-Addition to calibration standard(s) (5.5)**		**Expected approx.. IS-concentration in sample extracts (~20 mL) and calibration standards (~1 mL)**
	Suggested concentration of IS-WSIn1 (3.22)	Absolute mass of IS spiked to sample (100 µL IS-WSIn1) (m_{IS}^{sample})	Suggested concentration of IS-WSIn2 (3.23) **	Absolute mass of IS spiked to calibration standard (100 µL IS-WSIn2) ($m_{IS}^{cal\ mix}$)	
	µg/mL	µg	µg/mL	µg	µg/mL
Amitrole-(^{15}N)/ ($^{15}N_2$, $^{13}C_2$)	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-$^{15}N_3$	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
Nicotine-D_4	10	1	0.5	0.05	0.05
Paraquat-D_6	40	4	2	0.2	0.2
Perchlorate-$^{18\circ}{}_4$	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 extend 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 **5.6.2**).

** 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 **Table 29**). 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 31: *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	Sample weight	Typical natural water content g/100 g	Water to be added	Water addition may be skipped if suitable IS is used before aliquotation	Remarks
Fruits						
Citrus fruit	**Citrus juices**	10 g	90	1	Yes	
	Grapefruit	10 g	90	1	Yes	
	Lemon/lime	10 g	85	1.5	Yes	
	Orange	10 g	85	1.5	Yes	
	Tangerine	10 g	90	1	Yes	
Pome fruit	**Apple**	10 g	85	1.5	Yes	
	Apple (dried)	5 g (13.5 g rehydra-tized homogenate)	30	8.5 (see 5.2.2)	**No**	Weigh 13.5 g rehy-dratized homogenate
	Apple sauce	10 g	80	2	Yes	
	Apple juice	10 g	90	1	Yes	
	Pear	10 g	85	1.5	Yes	
	Quince	10 g	85	1.5	Yes	
Stone fruit	**Apricot**	10 g	85	1.5	Yes	
	Apricot (dried)	5 g (13.5 g rehydra-tized homogenate	30	8.5 (see 5.2.2)	**No**	Weigh 13.5 g rehy-dratized homogenate
	Apricot nectar	10 g	85	1.5	Yes	
	Cherry	10 g	85	1.5	Yes	
	Mirabelle	10 g	80	2	Yes	
	Nectarine	10 g	85	1.5	Yes	
	Peach	10 g	90	1	Yes	
	Peach (dried)	5 g (13.5 g rehydra-tized homogenate	20	8.5 (see 5.2.2)	**No**	Weigh 13.5 g rehy-dratized homogenate
	Plum	10 g	85	1.5	Yes	
	Plum (dried)	5 g (13.5 g rehydra-tized homogenate	20	8.5 (see 5.2.2)	**No**	Weigh 13.5 g rehy-dratized homogenate
Soft and small fruit	**Blackberry**	10 g	85	1.5	Yes	
	Blueberry	10 g	85	1.5	Yes	
	Currant	10 g	85	1.5	Yes	
	Elderberry	10 g	80	2	Yes	
	Gooseberry	10 g	90	1	Yes	
	Grapes	10 g	80	2	Yes	
	Raspberry	10 g	85	1.5	Yes	
	Raisins	5 g (13.5 g rehydra-tized homogenate	20	8.5 (see 5.2.2)	**No**	Weigh 13.5 g rehy-dratized homogenate
	Strawberry	10 g	90	1	Yes	
	Pineapple	10 g	85	1.5	Yes	
Other fruits	**Banana**	10 g	75	2.5	**No**	
	Fig	10 g	80	2	Yes	
	Fig (dried)	5 g (13.5 g rehydra-tized homogenate	20	8.5 (see 5.2.2)	**No**	Weigh 13.5 g rehy-dratized homogenate
	Kiwi	10 g	85	1.5	Yes	
	Mango	10 g	80	2	Yes	
	Papaya	10 g	90	1	Yes	
Vegetables						
Root and tuber vegetables	**Beetroot**	10 g	90	1	Yes	
	Carrot	10 g	90	1	Yes	
	Celeriac	10 g	90	1	Yes	
	Horseradish	10 g	75	2.5	**No**	
	Parsley root	10 g	90	1	Yes	
	Radish	10 g	95	0.5	Yes	
	Black salsify	10 g	80	2	Yes	

Commodity group	Commodity	Sample weight	Typical natural water content g/100 g	Water to be added	Water addition may be skipped if suitable IS is used before aliquotation	Remarks
	Potato	10 g	80	2	Yes	
	Garlic	**5 g**	60	7	**No**	
Leek plants	**Onion**	10 g	90	1	Yes	
	Leek	10 g	85	1.5	Yes	
	Shallot	10 g	80	2	Yes	
	Chives	10 g	85	1.5	Yes	
Fruiting vegetables	**Aubergine**	10 g	90	1	Yes	
	Cucumber	10 g	95	0.5	Yes	
	Melon	10 g	90	1	Yes	
	Pepper. Sweet	10 g	90	1	Yes	
	Pumpkin	10 g	95	0.5	Yes	
	Tomato	10 g	95	0.5	Yes	
	Zucchini	10 g	95	0.5	Yes	
	Broccoli	10 g	90	1	Yes	
Cabbage	**Brussel sprouts**	10 g	85	1.5	Yes	
	Cauliflower	10 g	90	1	Yes	
	Chinese cabbage	10 g	95	0.5	Yes	
	Kale	10 g	90	1	Yes	
	Kohlrabi	10 g	90	1	Yes	
	Red cabbage	10 g	90	1	Yes	
	Savoy cabbage	10 g	90	1	Yes	
	White cabbage	10 g	90	1	Yes	
	Lettuce varieties	10 g	95	0.5	Yes	
	Endive	10 g	95	0.5	Yes	
Leafy vegetables and herbs	**Cress**	10 g	90	1	Yes	
	Lamb's lettuce	10 g	85	1.5	Yes	
	Parsley	10 g	80	2	Yes	
	Rucola	10 g	85	1.5	Yes	
	Spinach	10 g	90	1	Yes	
Stem vegetables	**Asparagus**	10 g	95	0.5	Yes	
	Celery	10 g	95	0.5	Yes	
	Leek	10 g	85	1.5	Yes	
	Rhubarb	10 g	95	0.5	Yes	
	Artichokes	10 g	85	1.5	Yes	
Legumes / Pulses	**Pulses (dried Beans. Peas. Lentils)**	**5 g**	<10	9 mL water and 1 mL EDTA solution	**No**	Sample amount may need to be reduced if material strongly absorbs water
	Fresh Peas	10 g	75	2.5	No	
	Green Beans.	10 g	90	1	Yes	
Cereals	**Grain. Flour etc.**	**5 g**	10	10	No	Sample amount may need to be reduced if material strongly absorbs water
Oily seeds	**Peanuts, Poppy seeds, Pumpkin seeds, Sesame seeds, Soyabeans, Sunflower seeds**	**5 g**	<10	9 mL water and 1 mL EDTA solution	**No**	
	Linseeds, Chiaseeds	**5 g**	<10	9 mL water and 1 mL EDTA solution	**No**	To reduce slime formation, which hinders residue accesibility, **change sequence!** First add acidified methanol and then EDTA/water

Commodity group	Commodity	Sample weight	Typical natural water content g/100 g	Water to be added	Water addition may be skipped if suitable IS is used before aliquotation	Remarks
Nuts	**Almonds, Cashew nuts, Dried coconuts, Hazel-nuts, Macadamias, Pecans, Pistachios, Walnuts**	**5 g**	<10	9 mL water and 1 mL EDTA solution	**No**	
Miscellaneous						
Extract-rich (“diffi-cult”) commodities	**Coffee beans**	2 g	<10	10	No	Different sample amounts may be used depending on extract-richness
	Tea	2 g	<10	10	No	
	Dry herbs and spices	2 g	<10	10	**No**	
Miscellaneous Other	**Mushrooms**	10 g	90	1	Yes	
	Wine	10 g	90	1	Yes	
	Honey	**5 g**	20	9	**No**	
	Avocado	10 g	70	3	**No**	

Table 32: *Exemplary LC-MS/MS parameters for Sciex Qtrap 5500*

Parameters	Methods 1.1 / 1.2	Method 1.3	Method 1.4	Method 1.5	Method 1.6	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	negative	negative	positive	positive	positive	positive	pos. / neg. SelexIon™
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)	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	-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)	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)	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)
Temperature of Gas 2	600°C	550°C	550°C	600°C	600°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	20	10	50	20	10	50	20	20 / 40

*FWHM = full width at half maximum

Table 33: *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 Iss 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 D6 in M4		
New method (M7) for the analysis of Morpholin/Morpholin D_8; Diethanonamine/diethanolanmine 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 Iss		
Some additions in “Apparatus and Consumables” chapter	Dec. 2012	V7
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.”)		
New LC-method (Method 1.2) for “Glyphosate & Co.” using a Dionex ionPac AS11-HC column and an Eluent with near to neutral pH; additionallycovering Fosetyl		
New LC-method (Method 1.3) for “Glyphosate & Co.” using a Hypercarb column and an acidic 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	Nov. 2013	V7.1
Update of practical considerations for methods 1.1-1.3 (Column C)		
Update of table with performance data		

Action	When?	Version
Update of table with LOQs		
Introduction of Trimethylsulfonium-D9 and N.N-Dimethylhydrazine-D6 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 acidic 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 corresponsing ILISs		
Update of Table 17: Providers of isotopically labeled internal standards		
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 **4**: Overview and scope of the methods proposed within this document for the QuPPe method		
Update of **Table 5**: 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. Chloridazon-desphenyl. 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 (M 8): "Triazole derivative metabolites (TDMs)" new DMS parameters		
Update of Table 24: *Overview of approximate limits of quantification (LOQs)**		
Update of Table 27: Conversion factors between typical purchased standards and target analytes (3.18):		
Update of Table 28: Exemplary concentrations of pesticide stock and working solutions		
Update of **Table 29**: *Providers of isotopically labeled internal standards*		
Update of Table 30: *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-Acetyl-Glyphosate in Table 3: Overview and scope of the methods proposed within this document for the QuPPe method:	October 2016	V9.2
Inclusion of N-Acetyl-Glyphosate in Table 4: Practical Information: Mainly used methods used at CVUA		

Action	When?	Version
Stuttgart		
Addition of a further Ethephon-ILIS mass trace and inclusion of N-Acetyl-Glyphosate in Table 7: Proposed LC-MS/MS conditions for Ethephon. HEPA (Ethephon metabolite). Glyphosat. AMPA (Glyphosate metabolite). N-Acetyl-Glyphosate (Glyphosate metabolite). N-Acetyl-AMPA (Glyphosate 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-Acetyl-Glyphosate at 0.1 mg/kg on almond extract.		
Inclusion of N-Acetyl-Glyphosate 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-Acetyl-Glyphosate 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 5.6.16	April 2017	V9.3
Proposed volume of IS-WS II changed to match with volume of IS-WS I (see Table 1)		
Update of Table **4**: data on M 9 were included		
Update of Table **4**: data on M 9 were included		
Hints on stability of standard solutions added in 0, including Table 23		
Overview of lowest successfully validated levels (Table 24)		
Update of Table 27 : DFA and TFA added		
Update of *Table 28*: DFA and TFA added; solvents for Ethephon, Fosetyl and Maleic Hydrazide changed		
Table 29 updated		
Update of Table 30: DFA and TFA added		
Update of Table 32: data on M 9 were included		
Extensive general revision of text, tables and figures	Dec 2018	V10
Addition of nuts and oily seeds to the scope of the method		
Update of centrifuge information under 2.5		
Update of syringe filters information under 2.6		
Revision of sample preparation conditions section (5.1) to include milling of oily seeds and nuts and more details on how to accomplish cryogenic milling using carbon dioxide and liquid nitrogen		
Revision of the chapter concerning centrifugation (5.2). Inclusion of pre-centrifugation freeze-out and cryogenic centrifugation as an option to improve the subsequent filtration behaviour		
Revision of Figure 1 QuPPe-PO-Method at a glance		
Splitting of Table 3 (Overview and scope of methods) and spitting into Table 3 and 4		
New method M 1.5 (Glyphosate&Co. using Trinity Q1)		
New Method M 1.6 (Glyphosate&Co. using DEA Torus)		
Inclusion of Nicotine under Method 4.2		
Introduction of Chapter 6 on Analyte Stability		
Extention of Table 22 (Overview of lowest successfully validated levels per matrix)		
Addition of Table 23 (Validation data deriving from Interlab validation studies)		
Update of Table 24 (Conversion factors between typical purchased standards and target analytes)		
Update of Table 25 (Exemplary concentrations of pesticide stock and working solutions)		
Update of Table 26 (Exemplary providers of isotopically labeled internal standards)		
Change of the wording of the document title	April 2019	V10.1
Update of method for pulses, oily seeds and nuts. Method now involves addition of EDTA during the		

Action	When?	Version
extraction step for complexation metals that may interfere with analysis of certain analytes		
Update of cleanup procedure for the removal of lipids and proteins		
Addition of Method 1.7 for phosphonate, bromide, chlorate and perchlorate		
Maleic hydrazide added to Methode 4.2		