EU Reference Laboratories for Residues of Pesticides
Single Residue Methods

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

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

Version 12 (22.07.2021, Document History, see page **98**)

Check for latest version of this Method under www.quppe.eu ; older versions: obsolete versions

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Note: Changes from V11.1 to V12 are highlighted in yellow

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 cereals, 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- or IC-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 (IL-ISs). So far available, these IL-ISs 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 strong matrix-effects are frequently observed.

Shortcut-Links to useful information

- Flow Chart - QuPPe-PO-Method at a glance (procedure for most commodities)
- Flow Chart - QuPPe-PO-Method at a glance (procedure for cereals, pulses, nuts and oily seeds)
- Pipetting Scheme (exemplary) - Preparation of Calibration Standards
- Pipetting Scheme (exemplary) - Standard-Additions-Approach
- Scope Overview- LC-Methods covering ESI-pos. Analytes
- Scope Overview - LC-Methods covering ESI-neg Analytes
- General hints on analytes to avoid pitfalls
- Calibration and Calculations
- Analyte Stability
- Performance Data
- Conversion Factors (between purchased standards and target analytes)
- Analyte Stock and Working Solutions (exemplary)
- Exemplary Providers of IL-ISs (Isotopically Labelled Internal Standards)
- IL-IS-Working Solutions (exemplary)
- Water Addition Overview

How to Cite (proposal):

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URL: **https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN**

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.

2.2. 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.

2.3. 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).

2.4. 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.

2.5. Automatic pipettes,

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

2.6. 10 mL solvent-dispenser,

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

2.7. Mechanical shaker,

suitable for 50 mL-centrifuge tubes, e.g. Geno/Grinder® 2010; SPEX® SamplePrep.

2.8. Water Bath,

adjustable to at least 80°C and automatically shaking.

2.9. Centrifuge,

suitable for the centrifuge tubes employed in the procedure (**2.3**) 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.

2.10. 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 (**2.11**).

2.11. 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.

2.12. 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.

2.13. 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.

2.14. 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 **2.13**).

2.15. Screw-cap storage vessels,

for storage of sample extracts or storage of stock and working solutions, e.g. 20 mL.

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 2.13).

2.16. LC-MS/MS instrumentation,

equipped with ESI source and appropriate columns, see details in chapters 5.6.2 to 5.6.225.6.21.

2.17. IC-MS/MS instrumentation,

equipped with ESI source and appropriate columns, see details in chapters 5.6.22.

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),

for water additions to the samples

3.1. Water, ultrapure

e.g. prepared by a laboratory water purification system. Commercially available MS-quality water can be used for LC-MS/MS mobile phases and IC-quality water for IC-MS/MS mobile phases.

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

3.2. Methanol at least HPLC quality,

for the preparation of mobile phases preferably use MS-quality methanol.

3.3. Acetonitrile at least HPLC quality,

for the preparation of mobile phases preferably use MS-quality acetonitrile.

3.4. Formic acid (concentrated; > 98%),

for the preparation of mobile phases preferably use MS-quality formic acid.

3.5. Acetic Acid (concentrated; >98%),

for the preparation of mobile phases preferably use MS-quality acetic acid.

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 (**3.1**).

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 43**.

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 42.** Keep solutions in **plastic vessels** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.13**).

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 43**.

Notes: Keep solutions in **plastic vessels** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.13**).

3.20. Internal Standards (ISs),

Exemplary sources are shown in **Table 44**. 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 43** 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 **2.13**).

In general the absolute concentrations of the IL-IS-solutions are not important as long as the IL-IS-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 IL-IS 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 IL-ISs 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 **5.6.3** 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 **5.6.9**).

For quantification purposes it is of foremost importance that the ratio between the absolute IL-IS amount added to the sample prior to extraction (or to the isolated aliquot of the sample extract) and the absolute amount of IL-IS 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 43** and suggestions for the concentrations in **Table 45**.

Notes: Keep solutions in **plastic vessels 2.15** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.13**).

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 43** and for concentrations in **Table 45**.

Notes: Keep solutions in **plastic vessels** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.13**).

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

3.24. LC-MS/MS mobile phases and other consumables,

see details in chapters 5.6.2 to 5.6.21**.**

3.25. IC-MS/MS mobile phases and other consumables,

see details in chapter 5.6.22.

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** (~15 to ≤ 40% water content) the following procedure is proposed: Weigh 500 g of frozen dried fruits, add X g* of cold water (see **Table 1**) 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**). Weigh Y g* of homogenate (see **Table 1**; corresponding to 5 g sample).

Table 1: *Weight of dried fruits required for slurry homogenization and analztical portions of rehydratized homogenates to be emplozed for analzsis*

Moisture content of product	Water amount added (X g)	Weight of analytical portion (Y g; corresponding to 5 g of original dry sample)
~15 to <25 %	900 g	14 g
25 to <35 %	850 g	13.5 g
≥35 to 40 %	800 g	13 g

Alternatively, immerse the sample material in a plastic or polystyrol container containing 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.

For freeze-dried fruit and vegetables homogenize with a high speed knife mill preferably adding dry ice to keep the sample cool. Thereof 2 g sample may be employed for analysis (as in the case of spices, herbs and other extract-rich commodities).

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 (**2.3**). 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 Y g (e.g. 13.5 g ± 0.1 g, see **Table 1**) 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, herbs 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 46** 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 46**. 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 46** 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 46.**

In the case of **chia seeds and lineseeds (flaxseeds**) adding water directly to the samples leads to a soaking and 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) **<u>All commodities of plant origin except cereals, pulses, nuts and oily seeds</u>:** 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) **<u>Cereals, pulses, nuts and oily seeds</u>:** 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}) 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 IL-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 or 0.25 or 0.125 g sample equivalents, see above. 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 during extraction. 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, otherwise the extraction will need to involve additional breakup of the sample particles, e.g. by the use of a high speed dispenser (e.g. Ultra Turrax).
The addition of EDTA solution is highly recommended when targeting analytes showing poor recoveries in absence of EDTA. Affected are compounds with a tendency to form complexes with metals, such as Glyphosate and metabolites, Glufosinate and metabolites. **If affected analytes are not targeted, EDTA addition may be skipped and 10 mL of water are added.**

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 a shaking water bath (**2.8**) 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 (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 **<u>NOT</u>** recommended for extracts of commodities that pose diffi-

[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.

culties in filtration (e.g. finely milled cereals, pineapples, strawberry, asparagus, kaki, banana 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) and cold centrifugation are preferred. 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. To avoid redissolvation of the matrix components in the cases **(2)** and **(3)**, it is recommended to **transfer an aliquot of the cold supernatant into a sealable container** for later use, or to proceed immediately with the next steps, while the extract is still cold.

5.2.5. Removal of proteins and lipids

(1) **Cereals and 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 **2.7**).

(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 **2.7**).

(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 **2.7**). 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

(1) **All commodities of plant origin except cereals, pulses, nuts and oily seeds:** Withdraw an aliquot (e.g. 2-3 mL) of the supernatant from **5.2.4** or **5.2.5** using a syringe (**2.10**) and **filter** it through a syringe filter (**2.11**) either directly into an auto-sampler vial (**2.13**) 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 (**2.10**).
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.

(2) **Cereals, pulses, nuts and oily seeds:** Transfer a 3 mL aliquot of the supernatant from 5.2.5 into an ultrafiltration unit (**2.12**) 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.

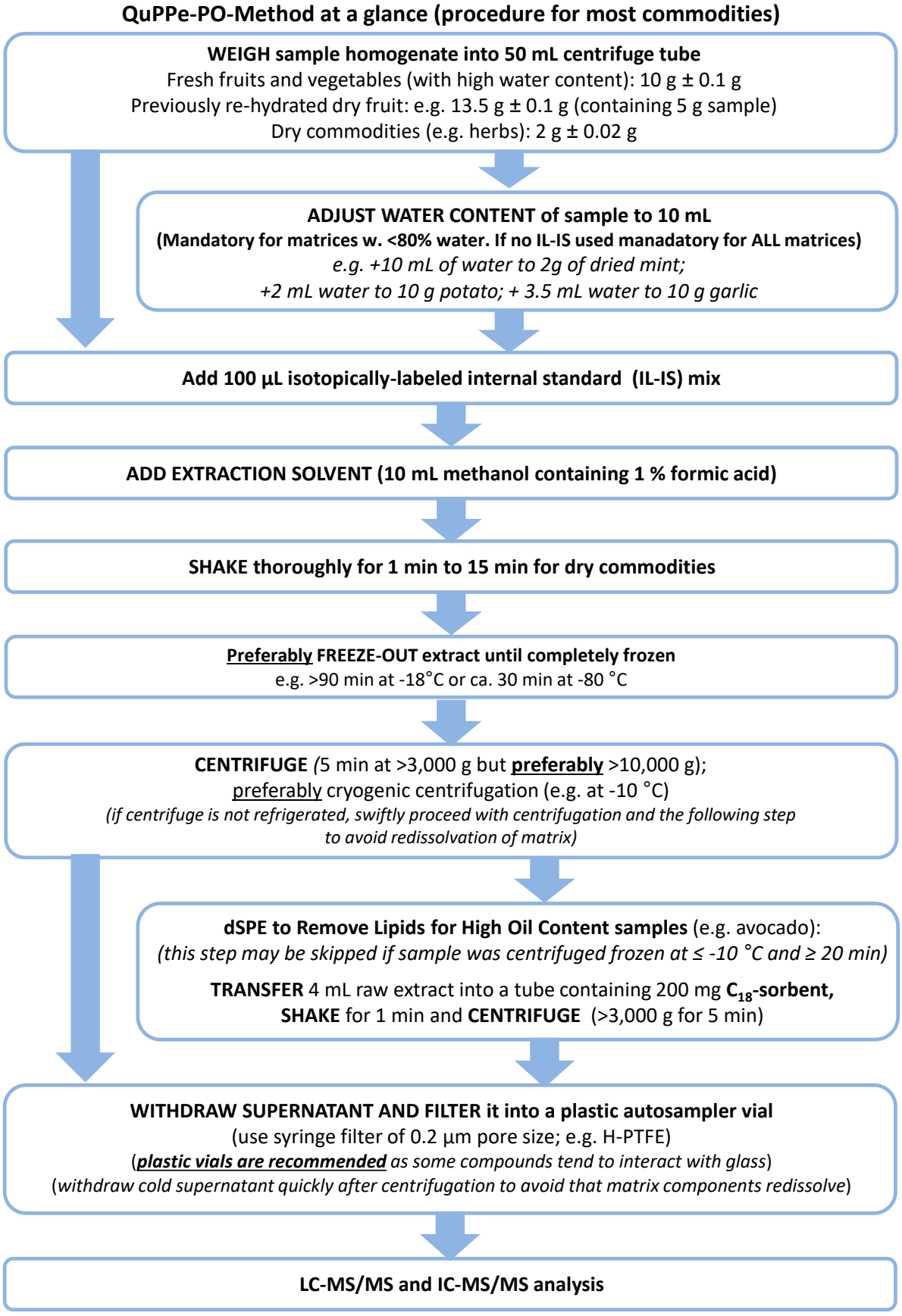


Figure 1: QuPPe-PO-Method at a glance (general procedure for most commodities, not considering paraquat and diquat)

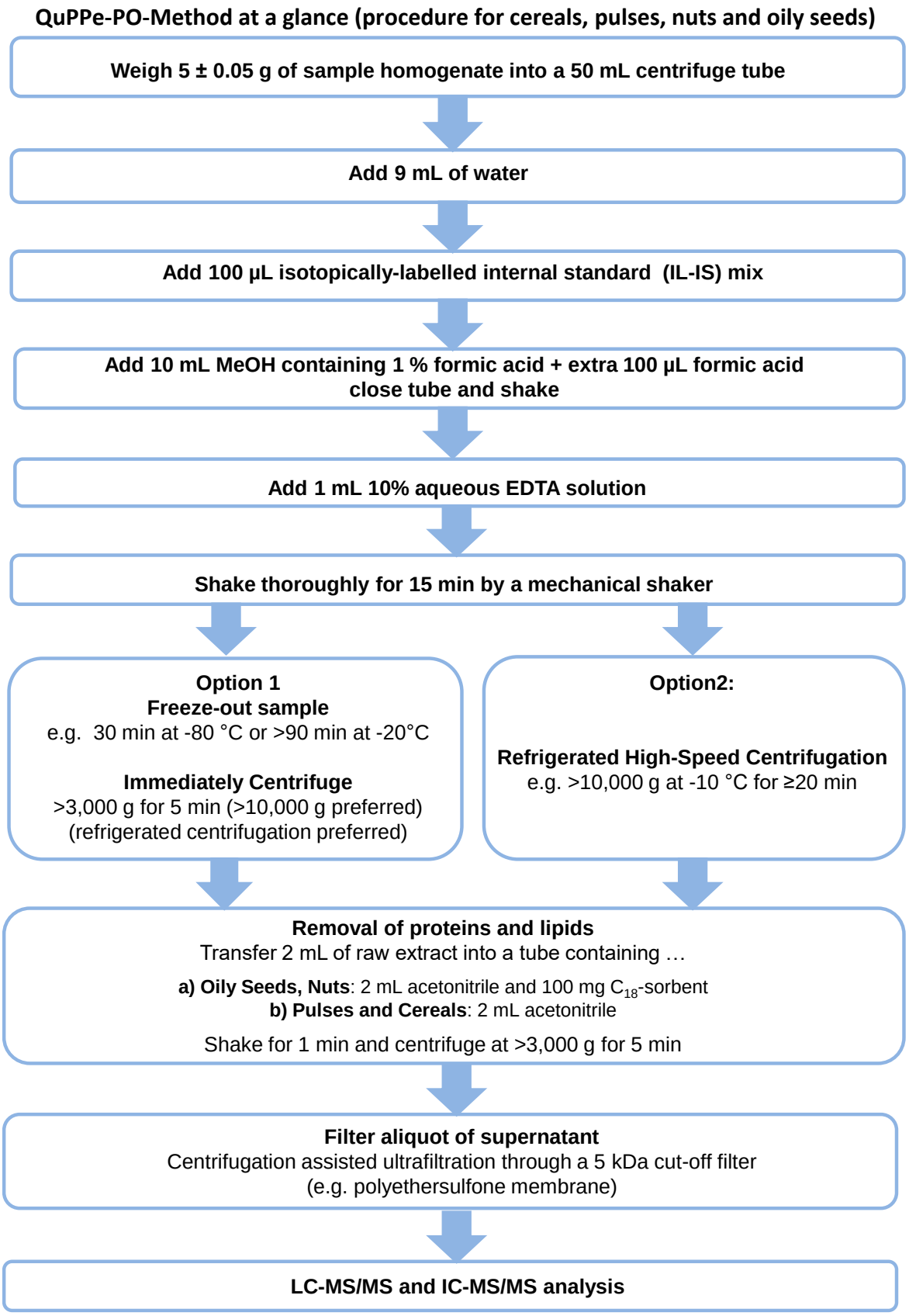


Figure 2: QuPPe-PO-Method at a glance; procedure for cereals, pulses, oily seeds and nuts

5.3. Preparation of blank extracts

Using homogenates of 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 (**2.3**) and spike it with a suitable pesticide working solution (**3.19** and **Table 43**). 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**.

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 2**.
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 IL-ISs 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 2**.
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 IL-IS, is shown in **5.7.1** and **5.7.2** respectively.

Table 2: *Exemplary pipetting scheme for the preparation of calibration standards*

		Calibration standards								
		Solvent based (5.5.1)			Matrix-matched (5.5.2)					
		using IL-IS[4]			Without IL-IS[5]			using IL-IS[4]		
Calibr. levels in µg pesticide /mL OR in µg pesticide/ "IL-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 IL-IS portion would correspond to the IL-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)** appropriately (e.g. 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, volume adjustments are of less importance as the IL-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 IL-IS in the respective calibration standards and b) the ratio between the IL-IS mass added to the sample (**5.2.3**) and the IL-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 IL-ISs are not available/employed, matrix-matched standards **Table 2**) 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 raw extracts is assumed to be exactly 20 mL. This translates into 0.5 g sample equivalents per mL when using 10 g test portions.

[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), the 0.05 µg/mL calibration level corresponds to 0.4 mg pesticide /kg sample.

5.5.3. **Standard-Additions approach**

Where no appropriate IL-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 3**. The calculation of the mass fraction of the pesticide in the sample w_R is shown in **5.7.2**.

Table 3: *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 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-and IC-MS/MS analysis

Any suitable LC- or IC-MS/MS conditions generating peaks that can be well integrated 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- and IC-MS/MS conditions proposed within this document is given in **Table 4** and following.

***Table 4**: Scope of QuPPe-LC-Methods of analytes analyzed in the ESI-pos. mode (see legent under **Table 7**) Part.I*

QuPPe method code	M 1.1 (5.6.2)	M 1.2 (5.6.3)	M 1.3 (5.6.4)	M 1.4 (5.6.5)	M 1.5 (5.6.6)	M 1.6a/b (5.6.7)	M 1.7 a/b (5.6.8)	M 1.10 (5.6.9)	M 1.11 (5.6.10)	M 1.12 (5.6.11)
Separation principle	Anion Ex.	Anion Ex.	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC
Column type	AS 11	AS 11-HC	Hypercarb	Hypercarb	Trinity Q1	**a:** Torus DEA/ **b:** APPC	**a:** Torus DEA/ **b:** APPC	APPC	Raptor PolarX	ObeliscN
	ANALYTES COVERED BY LC-MS/MS IN THE ESI-POSITIVE MODE									
Amitrole	NT	NT	-	NT	NT	NT	NT	NT	NT	NT
ETU	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
PTU	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Cyromazine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Trimesium	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Daminozide	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Chlormequat	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Mepiquat	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Difenzoquat	NT	NT	-	NT	NT	NT	NT	NT	NT	NT
Propamocarb	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Melamine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Diquat	NT	NT	-	NT	NT	NT	NT	NT	NT	NT
Paraquat	NT	NT	-	NT	NT	NT	NT	NT	NT	NT
N,N-Dimethylhydrazine	NT	NT	-	NT	NT	NT	NT	NT	NT	NT
Nereistoxin	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Streptomycin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Kasugamycin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Morpholine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Diethanolamine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Triethanolamine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
1,2,4-Triazole	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Triazole-alanine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Triazole-acetic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Triazole-lactic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Aminocyclopyrachlor	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Chloridazon-desphenyl	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Mepiquat-4-hydroxy	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Propamocarb-N-desmethyl	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Propamocarb-N-oxide	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Maleic Hydrazide	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nicotine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Matrine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Oxymatrine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Table 5: *Scope of QuPPe-LC- and IC-Methods of analytes analyzed in the ESI-pos. mode (see legent under* ***Table 7****) Part.II*

QuPPe method code	M 2 (5.6.12)	M 3 (5.6.13)	M 4.1 (5.6.14)	M 4.2 (5.6.15)	M 5 (5.6.16)	M 6 (5.6.17)	M 7 (5.6.18)	M8 (5.6.19)	M 9 (5.6.20)	M 10 (5.6.21)	M 11 (5.6.22)
Separation principle	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon	HILIC	HILIC	Ion Chroma-tography
Column type	Obelisc-R	Obelisc-R	Obelisc-R	BEH-Amide	PFP	Obelisc-R	Trinity P1	Hypercarb	Trinity P1	Torus DEA	AS19
ANALYTES COVERED BY LC-and IC-MS/MS IN THE ESI-POSITIVE MODE											
Amitrole	NT	✓	-	✓	NT	NT	NT	NT	NT	NT	NT
ETU	NT	✓	-	✓	✓	NT	NT	NT	NT	NT	NT
PTU	NT	✓	-	✓	✓	NT	NT	NT	NT	NT	NT
Cyromazine	NT	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT
Trimesium	NT	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT
Daminozide	NT	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT
Chlormequat	NT	✓	✓	✓	✓	NT	NT	NT	NT	NT	NT
Mepiquat	NT	✓	✓	✓	✓	NT	NT	NT	NT	NT	NT
Difenzoquat	NT	✓	✓	✓	✓	NT	NT	NT	NT	NT	NT
Propamocarb	NT	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT
Melamine	NT	NT	✓	✓	NT	NT	NT	NT	NT	NT	NT
Diquat	NT	NT	✓	(✓)****	NT	NT	NT	NT	NT	NT	NT
Paraquat	NT	NT	✓	(✓)****	NT	NT	NT	NT	NT	NT	NT
N,N-Dimethylhydrazine	NT	NT	✓	-	NT	NT	NT	NT	NT	NT	NT
Nereistoxin	NT	NT	✓	✓	NT	NT	NT	NT	NT	NT	NT
Streptomycin	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Kasugamycin	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Morpholine	NT	NT	(✓)	(✓)	NT	NT	✓	NT	✓	NT	NT
Diethanolamine	NT	NT	(✓)	(✓)	NT	NT	✓	NT	NT	NT	NT
Triethanolamine	NT	NT	(✓)	(✓)	NT	NT	✓	NT	NT	NT	NT
1,2,4-Triazole	NT	NT	(✓)	-	NT	NT	NT	✓	NT	(✓)	NT
Triazole-alanine	NT	NT	(✓)	-	NT	NT	NT	✓	NT	✓	NT
Triazole-acetic acid	NT	NT	(✓)	-	NT	NT	NT	✓	NT	✓	NT
Triazole-lactic acid	NT	NT	NT	-	NT	NT	NT	✓	NT	✓	NT
Aminocyclopyrachlor	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Chloridazon-desphenyl	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Mepiquat-4-hydroxy	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Propamocarb-N-desmethyl	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Propamocarb-N-oxide	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Maleic Hydrazide	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Nicotine	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Matrine	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Oxymatrine	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT

Table 6: *Scope of QuPPe-LC-Methods of analytes analyzed in the ESI-neg. mode Part.I*

QuPPe method code	M 1.1 (5.6.2)	M 1.2 (5.6.3)	M 1.3 (5.6.4)	M 1.4 (5.6.5)	M 1.5 (5.6.6)	M 1.6a/b (5.6.7)	M 1.7 a/b (5.6.8)	M 1.10 (5.6.9)	M 1.11 (5.6.10)	M 1.12 (5.6.11)
Separation principle	Anion Ex.	Anion Ex.	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC
Column type	AS 11	AS 11-HC	Hypercarb	Hypercarb	Trinity Q1	**a:** Torus DEA/ **b:** APPC	**a:** Torus DEA/ **b:** APPC	APPC	Raptor PolarX	ObeliscN
ANALYTES COVERED BY LC-MS/MS IN THE ESI-NEGATIVE MODE										
Ethephon	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
HEPA	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
Glufosinate	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
N-Acetyl-Glufosinate	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
MPPA	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
Glyphosate	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
AMPA	✓	✓	✓	NT	✓	✓	(✓)		✓	(✓)
Phosphonic acid	(✓)	(✓)	✓	✓	✓	✓	✓		✓	(✓)***
N-Acetyl-AMPA	NT	✓	✓	NT	✓	✓	(✓)		NT	NT
Fosetyl-Al	-	✓	✓	NT	✓	✓	(✓)		✓	(✓)***
Maleic Hydrazide	-	-	✓	NT	-	-	-	(✓)	(✓)	(✓)
Perchlorate	NT	-	✓	✓	✓	(✓)**	✓	✓	✓	✓
Chlorate	NT	-	✓	✓	✓	(✓)**	✓	✓	(✓)	✓
Bialaphos	NT	NT	✓	NT	✓	NT	NT		✓	NT
Cyanuric acid	NT	NT	✓	NT	-	-	-	✓	(✓)	(✓)
Bromide	NT	NT	-	✓	✓	(✓)**	✓		✓	✓
Bromate	NT	NT	(✓)	✓	NT	NT	✓		NT	NT
N-Acetyl-Glyphosate	NT	NT	✓	NT	(✓)**	✓	(✓)		-	✓
Difluoroacetic acid	NT	NT	NT	NT	NT	NT	NT		✓	NT
Trifluoroacetic acid	NT	NT	NT	NT	NT	NT	NT		✓	✓
Thiocyanate	NT	NT	(✓)**	✓	NT	NT	NT		✓	NT
Desmethyl-Dimethoate	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 or M1.9)
*** Compromised quantitation of Phosphonic acid due to co-elution of Phosphonic acid and Fosetyl, see also General Hints **5.6.1**
**** Quality of analysis may strongly depend on instrument type and condition

Table 7: *Scope of QuPPe-LC- and IC-Methods of analytes analyzed in the ESI-neg. mode Part.II*

QuPPe method code	M 2 (5.6.12)	M 3 (5.6.13)	M 4.1 (5.6.14)	M 4.2 (5.6.15)	M 5 (5.6.16)	M 6 (5.6.17)	M 7 (5.6.18)	M8 (5.6.19)	M 9 (5.6.20)	M 10 (5.6.21)	M 11 (5.6.22)
Separation principle	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon	HILIC	HILIC	Ion Chroma-tography
Column type	Obelisc-R	Obelisc-R	Obelisc-R	BEH-Amide	PFP	Obelisc-R	Trinity P1	Hypercarb	Trinity P1	Torus DEA	AS19
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	NT	✓
N-Acetyl-AMPA	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	-
Cyanuric acid	NT	NT	NT	NT	NT	NT	✓*	NT	NT	NT	✓
Bromide	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓
Bromate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
N-Acetyl-Glyphosate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓
Difluoroacetic acid	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	✓
Trifluoroacetic acid	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	✓
Thiocyanate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓
Desmethyl-Dimethoate	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 or M1.9)

Table 8 : *Methods mainly used by CVUA Stuttgart*

Method	Special remarks on Substances	LC-MS/MS	Comments
M 1.3: Glyphosate & Co. Hyper-carb (see 5.6.4)	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 Desmethyl-Dimethoate	1290 Agilent Infinity II and Sciex QTRAP 6500+	Evaluation via solvent calibration and IL-ISs except for Bialaphos and N-Acetyl-AMPA (IL-IS not yet available) M 1.5 and M 1.6 are currently being tested for their suitability to replace M 1.3
M 1.4: Method 1.4 (M1.4): "Per-ChloPhos" (see 5.6.5)	Perchlorate (quantitative) Chlorate (quantitative) Phosphonic acid (quantitative) Bromide (Screening, quantitative) Bromate (quantitative) Thiocyanate	1290 Agilent Infinity II and Sciex QTRAP 6500+	Mostly employed directly (option: screening by M 1.3, if postive -> M 1.4) Dilution 5-fold Evaluation via solvent calibration and IL-ISs
M 4.1: "Quats & Co Obelisc R (see 5.6.14)	Paraquat (for specific commodities) Diquat (for specific commodities)	1290 Agilent Infinity II and Sciex QTRAP 5500	Analysis of specific relevant commod-ities. Evaluation via matrix-based calibration and IL-ISs
M 4.2: "Quats & Co BEH Amide" (see 5.6.15)	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 Nicotine Matrine Oxamatrine	1290 Agilent Infinity II and Sciex QTRAP 5500	Evaluation via matrix-based calibra-tion and IL-ISs (except for Difen-zoquat, Aminocyclopyrachlor, Mepi-quat-4-hydroxy, Propamocarb-N-desmethyl, Propamocarb-N-oxide)
M 7: "Morpholine, Diethanola-mine and Triethanolamine" (see 5.6.18)	Morpholine (quantitative) Diethanolamine (quantitative) Triethanolamine (quantitative)	1290 Agilent Infinity II and 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 mush-rooms). Quantification via solvent-based calibration and IL-ISs

5.6.1. General hints on analytes to avoid pitfalls

1. **Mass spectrometric interferences:**

   a) **AMPA and Fosetyl** share the mass-transition 110/81. Chromatographic separation is thus needed.

   b) **Interference of Phosphonic acid by Phosphoric acid; take care when using M 1.4 (Hypercarb column)**:
   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 compounds elute in close vicinity (e.g. M 1.4 using Hypercarb column). Exemplary chormatograms demonstrating this effect are shown in **Table 10**.
   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. When using method M 1.4 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 individual mass-transition (97/63 and 81/79 respectively, shown in **Table 10**), 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 IL-IS 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. Different instrument settings may result in a different degree of interference.
   In an experiment using Differential Mobility Separation (DMS) a separation of the phosphonate generated in-source from Phosphate and the original Phosphonate (mass trace m/z 81/63) was achieved, possibly due to a different molecular structure of the $P(OH)_3$ species and the $HPO(OH)_2$ species.

   Tip: Using method M 1.7 (Torus DEA and APPC) and method M 1.8 (Raptor Polar X), sufficient chromatographic separation was achieved without dilution of the sample extract. Exemplary chromatograms for M1.7 are shown in **Table 9**

Table 9: *Chromatograms of Phosphonate transition 81/63 (also common to Phosphate) at 0.01 µg phosphonate/mL in grape, onion and infant formula using* ***M 1.7****. Both substances are separated sufficiently.*

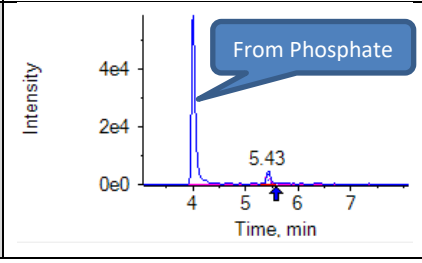
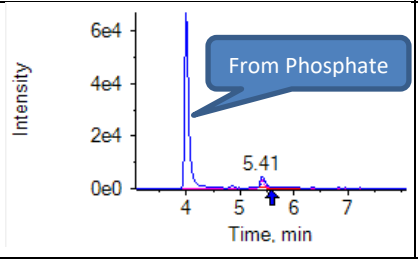
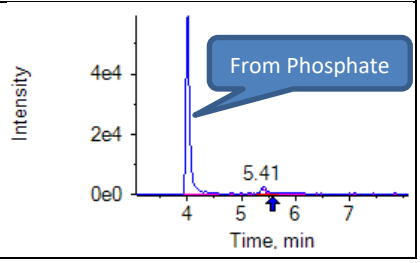
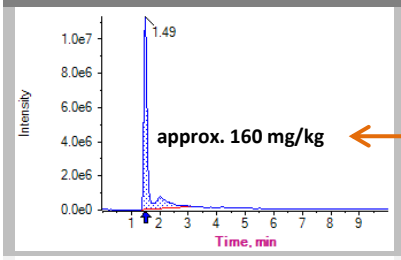
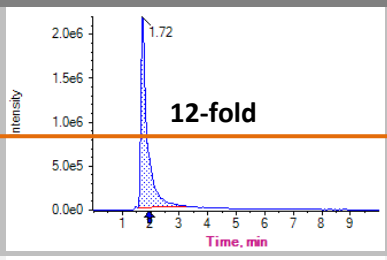
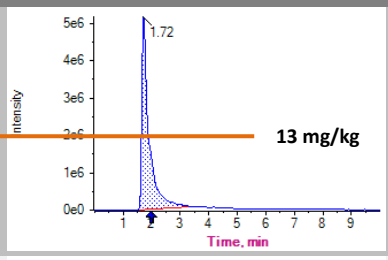
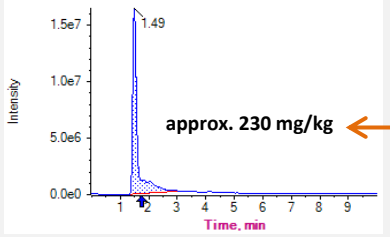
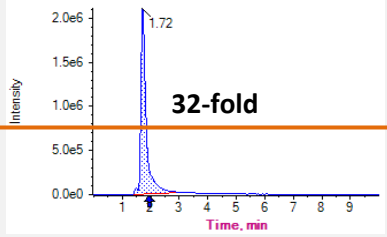
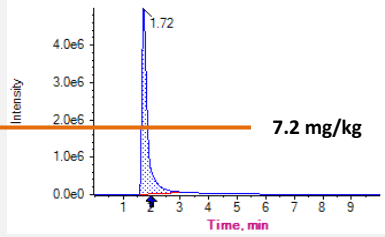
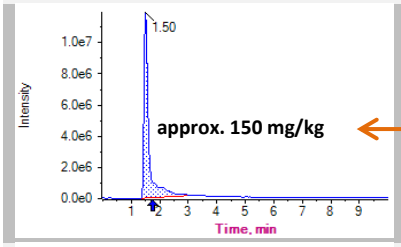
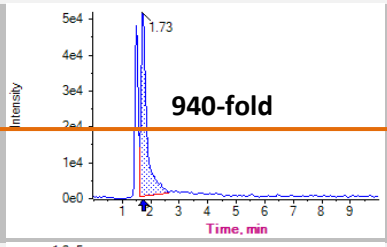
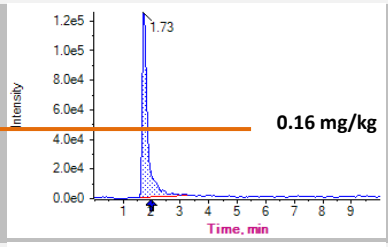
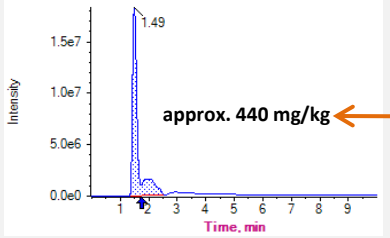
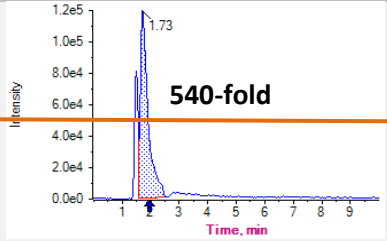
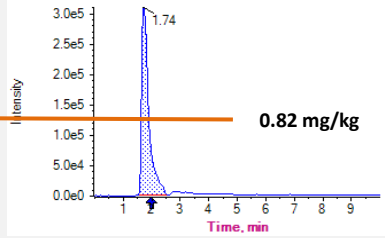
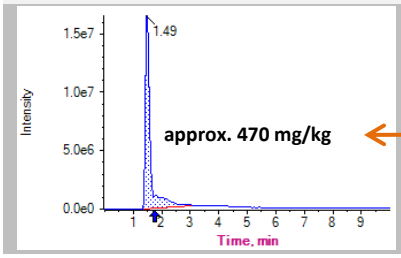
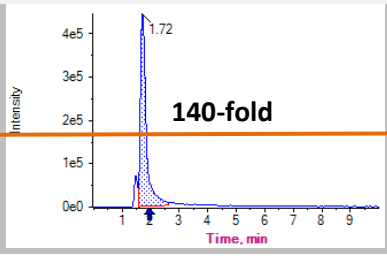
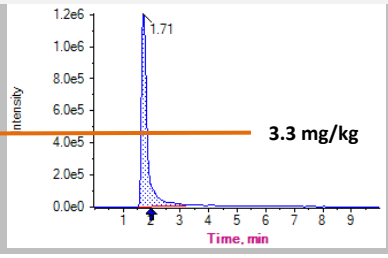
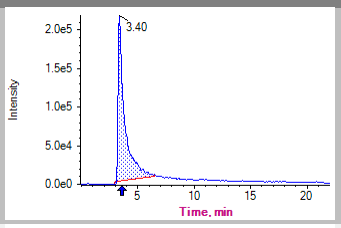
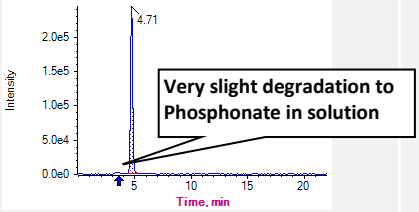
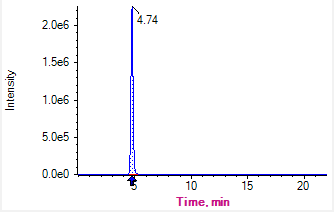
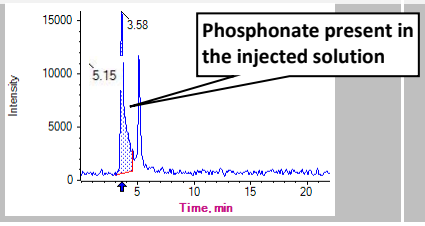
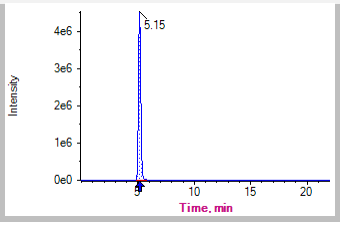
	Grape	Onion	Infant Formula
Phosphonic acid 81/63			

Table 10: *Chromatographic and mass-spectrometric separation of Phosphoric and Phosphonic acid using* ***M 1.4.***

Sample	Detected signals at mass trace of		
	97/63 unique to Phosphoric acid	81/63 common to Phosphoric and Phosphonic acid	81/79 unique to Phosphonic acid
Pear			
White grapes 1			
White grapes 2			
Cucumber			
Raisins			

c) **Intereference of Phosphonic acid by Fosetyl and Fosetyl-D_5:**
Fosetyl and its D_5-analogon tend to degrade to Phosphonic acid both in solutions and via in-source fragmentation in LC-MS/MS, see also **below** and **6**. **A good chromatographic separation between Fosetyl and Phosphonic acid is thus necessary**.
Table 11 shows an example of this in-source fragmentation using M 1.3 and M 1.4 (Hypercarb column). 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.
Tip: To be on the safe side, Fosetyl-IL-IS should not be added to calibration solutions, sample test portions or sample extracts intended to be used for the analysing native Phosphonic acid. Further, calibration solutions used for the analysis of Phosphonic acid should better not contain any native Fosetyl.

Table 11: *Chromatograms of Phosphonic acid, Fosetyl and Fosetyl-D_5 (each at 0.1 µg/mL) using Method M 1.3 and M 1.4.*

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		

Note: 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.

d) **Paraquat interfered by Diquat:**

Tranistions of Paraquat ($[M^{2+}- H^+]^+$ 185/#) are interfered by Diquat. Diquat and Paraquat produce several parent ions within the ion-source, each one fragmenting to various product ions, see 3.a). MRMs of singly charged protonated Paraquat ($[M^{2+}- H^+]^+$ 185/#) tend to be interfered by Diquat, e.g. $[M^{2+}- H^+]^+$ 185/170 and $[M^{2+}- H^+]^+$ 185/169. Same applies to the respective MRM of Paraquat D_8 ($[M^{2+} - H^+]^+$193/#) , which is interfered by Diquat D_8. Furthermore, those transitions are less sensitive.

e) **Bromide; take care when using M 1.4:**

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, especially when using M 1.4 (Hypercarb column).

Bromide is mainly composed of two naturally occurring stable isotopes, that are almost equally frequent ($^{79}Br^-$ and $^{81}Br^-$). For bromide (element-ion), 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.

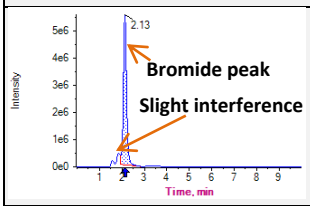
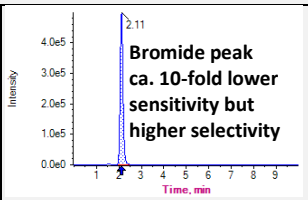
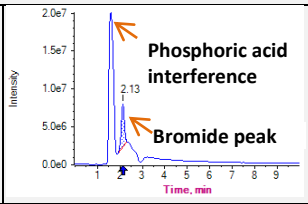
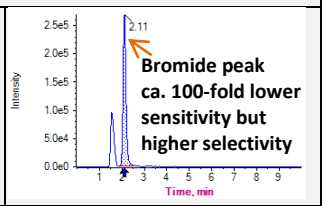
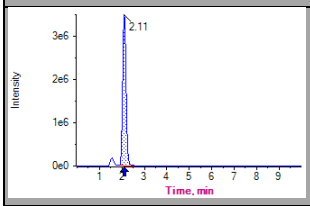
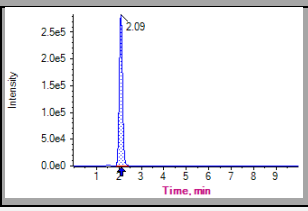
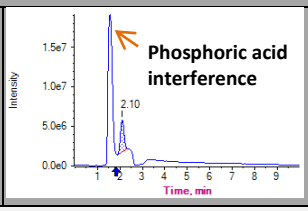
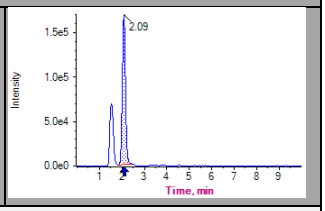
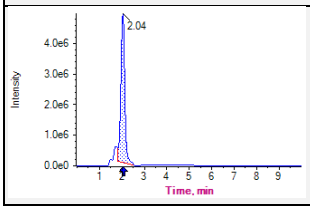
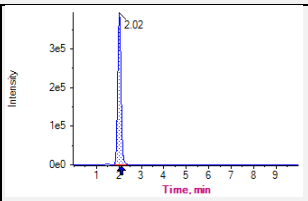
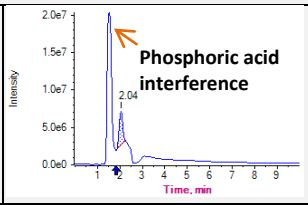
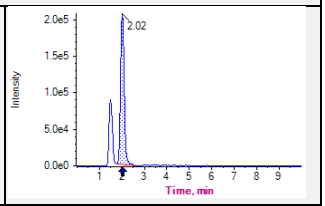
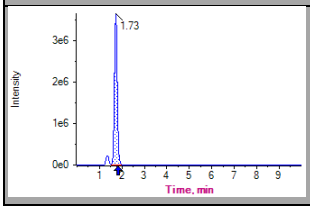
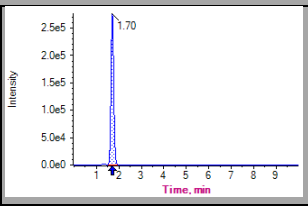
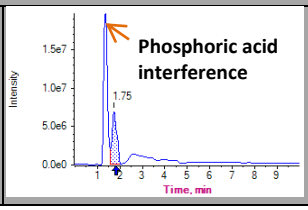
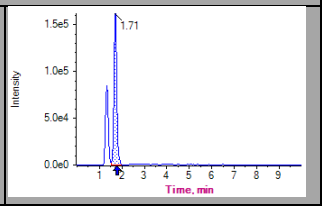
The mass trace m/z 81/81 is interfered by Phosphonic acid (m/z of $[H_2PO_3]^-$ = 81) whereas m/z 79/79 is highly affected by Phosphoric acid due to in-source fragmentation (**12**, the two columns declared as "CE -5 V"). In practice the interference by Phosphoric acid is more critical as it is naturally contained at high levels (e.g. 100-2000 mg/kg) in various samples.

Tip: 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 (when using M 1.4, Hypercarb).

To improve selectivity and increase quantification accuracy and identification certainty, the interfer-

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

12: *Chromatograms of Bromide using non-optimized collision energies (CE -5 V) showing the interference by Phosphoric and Phosphonic acid as well as optimized collision energies (CE -60 V and -70 V, the) showing reduced interferences using M 1.4.*

m/z 81/81		m/z 79/79	
CE -5 V	CE -60 V	CE -5 V	CE -70 V
Cucumber containing 1.7 mg/kg Bromide, 2.0 mg/kg Phosphonic acid & approx. 300 mg/kg Phosphoric acid			
			
Mint leaves containing 1.1 mg/kg Bromide, approx. 370 mg/kg Phosphoric acid; Phosphonic acid n. d.			
			
Fennel containing 2.2 mg/kg Bromide, 5.4 mg/kg Phosphonic acid & approx. 400 mg/kg Phosphoric acid			
			
Sweet Corn containing 1.1 mg/kg Bromide, approx. 350 mg/kg Phosphoric acid; Phosphonic acid n. d.			
			

2. Degradation and Contamination:

a) **Issues concerning the purity of N-Acetyl-Glufosinate D_3:**

There is two types of N-Acetyl-Glufosinate D_3 standards on the market. Both contain the three deuterium atoms on a methyl group, but the first one contains them on the methyl group of the acetyl moiety and the other one on the methyl group that is attached to the phosphorus atom. In theory, the acetyl group can be hydrolytically detached, so that native glufosinate may be formed in working solutions of N-Acetyl-Glufosinate (acetyl-D_3), leading to false positive results. Fortunately the degradation rate observed in the water:acetonitrile 9:1 mixture (see **Figure 36**) was negligible. More important is the content of native glufosinate in purchased N-Acetyl-Glufosinate (acetyl-D_3) standards.

Tip: Before first use, the standards should be checked for the presence of native glufosinate impurities and not used if the levels of native compound are considered critical. The levels of native glufosinate impurities depend on the manufacturer and the badge. Where e.g. 0.5 µg IL-IS is added to 1 g sample, the presence of 2% native glufosinate (a level once encountered) can lead to glufosinate levels of 0.01 mg/kg. See also chapter **6**.

b) **Possible contaminations by consumables:**

Check the filters and other consumables used for sample preparation for any contamination of Perchlorate and Chlorate. Cellulose mixed ester filters were found to be suitable for this application (see comments under 2.11).

c) **Stability of the Phosphonic acid IL-IS:**

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.

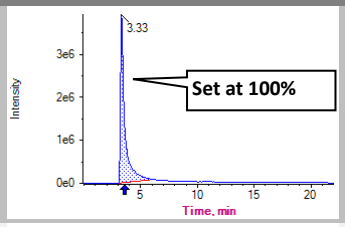
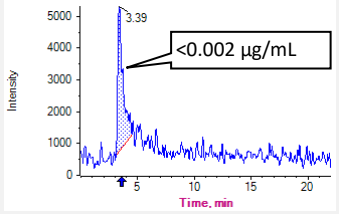
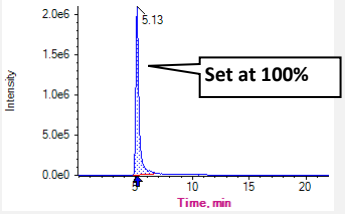
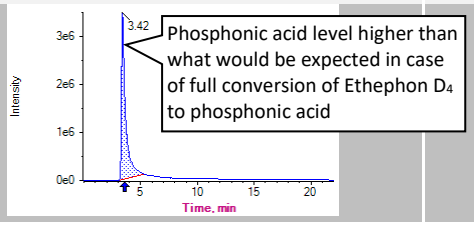
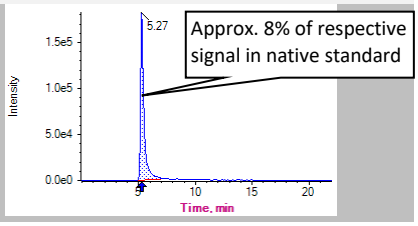
d) **Degradation of Ethephon and Fosetyl to Phosphonic acid:**

Fosetyl and Ethephon as well as their respective IL-IS's degrade to Phosphonic acid. **Table 13** 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 using M 1.4. This contamination is considered negligible. However **Table 13** 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 IL-IS would contaminate the sample with Phosphonic acid leading to false positive results.

Tip: To be on the safe side Fosetyl, Ethephon and their respective IL-IS'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 Ethephon or Fosetyl.

See also "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" and Chapter **6.**

Table 13: *Chromatograms of Phosphonic acid, Ethephon and an unsuitable Ethephon-D_4 standard (each at 1.0 µg/mL) using Method M 1.3 and M 1.4 (Hypercarb column).*

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!!)		

Note: 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.

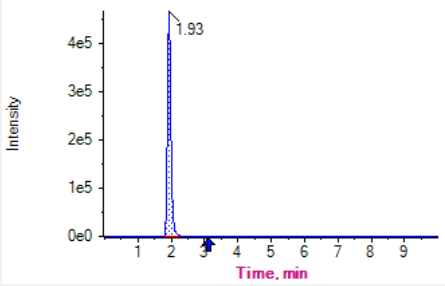
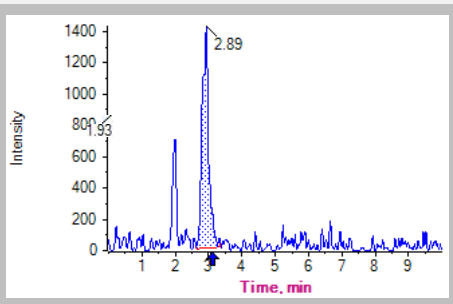
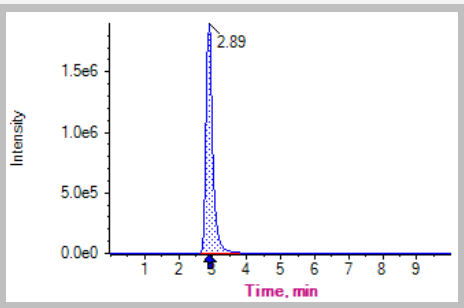
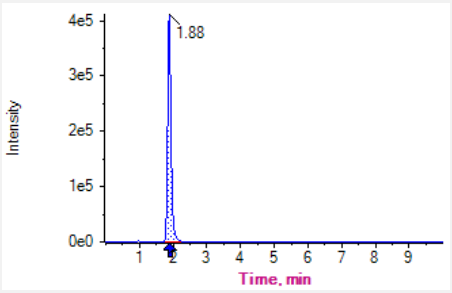
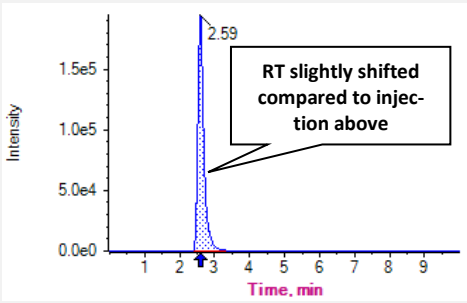
e) **Contamination of Maleic hydrazide D_2 with native Maleic hydrazide:**

In the case of Maleic Hydrazide (MH), the amount of IL-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 % (a typical level encountered) 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.

f) **Chlorate can be a minor contaminant of Perchlorate solutions**

Chlorate can be a minor contaminant of Perchlorate solutions and is also a minor in-source fragment of Perchlorate. In the example below, Perchlorate standard at 0.2 µg/mL was injected resulting in two peaks on the mass traces of Chlorate (see **Table 14**). The first one originating from Chlorate contained as impurity in the Perchlorate solution (at approx. 0.35%) and the second 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 IL-IS is co-injected misidentification is unlikely as the two compounds typically separate well chromatographically.

Table 14: *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-$^{18}O_4$.*

Calibration standard injected	Detected signals at mass trace of …	
Chlorate 0.2 µg/mL	 **Chlorate 83/67**	
Perchlorate 0.2 µg/mL	 **Chlorate 83/67**	 **Perchlorate 99/83**
Chlorate-$^{18}O_3$ (approx. 0.18 µg/mL) + Perchlorate-$^{18}O_4$ (approx. 0.02 µg/mL)	 **Chlorate-$^{18}O_3$ 89/71**	 **Perchlorate-$^{18}O_4$ 107/89**

3. Miscellaneous

a) **Diquat and Paraquat:**

Diquat and Paraquat produce several parent ions within the ion-source, each one fragmenting to various product ions. The most prominent parent ions observed are the doubly charged ones ($[M]^{2+}$), the singly charged protonated ones ($[M^{2+} - H^+]^+$) and the singly charged radical ones ($[M]^{+\bullet}$). The relative yields of the various parent ions were shown to greatly depend on the co-eluting matrix, which gives an additional dimension to the matrix-effects. Mass transitions originating from the same parent ion are simmilarly affected by co-eluting matrix (this also applies to the IL-IS), unlike those originating from different parent ions. For a proper equalization of matrix effects and correct quantitations, it is thus paramount to use equivalent parent ions (or even better, equivalent mass-transitions) of native analyte and the corresponsing IL-IS. Generally, measurements achieved when using transitions from doubly charged parent ions ($[M]^{2+}$) were more robust and validation results fluctuating less.

Table 15 gives an overview of mass transitions of Diquat while Table 16 shows matrix effects for various mass-transitions in infant formula powder.

Table 15: *Individual transitions and MS/MS settings (Sciex API 5500) for Diquat and Paraquat and their respective IL-ISs on Sciex 5500 QTrap ESI(+). Transitions are grouped by parent type.*

	Suitable IL-IS transition	Sensitivity Ranking*	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)	CXP (V)
Diquat $[M]^{2+}$ 92/84	**IL-IS Diquat D_8 $[M]^{2+}$ 96/88**	**1**	**92**	**84.4**	**61**	**21**	**4**
Diquat $[M]^{2+}$ 92/157		**3**	**92**	**157**	**61**	**19**	**12**
Diquat $[M]^{2+}$ 92/78		**6**	**92**	**78**	**61**	**31**	**12**
Diquat $[M]^{2+}$ 92/130		**5**	**92**	**130**	**61**	**25**	**8**
Diquat $[M^{2+} - H^+]^+$ 183/157	**IL-IS Diquat D_8 $[M^{2+}- H^+]^+$ 191/165**	**2**	**183**	**157**	**161**	**31**	**10**
Diquat $[M^{2+} - H^+]^+$ 183/130		**4**	**183**	**130**	**161**	**43**	**8**
Diquat $[M^{2+} - H^+]^+$ 183/168		**5**	**183**	**168**	**161**	**37**	**10**
Diquat $[M^{2+} - H^+]^+$ 183/78		**4**	**183**	**78**	**161**	**51**	**12**
Diquat $[M]^{+\bullet}$ 184/128***	IL-IS Diquat D_8 $[M]^{+\bullet}$ 192/134	4	184	128	60	55	8
Diquat $[M]^{+\bullet}$ 184/106		5	184	106	60	23	8
Diquat $[M^{+\bullet}$184/78		5	184	78	60	65	12
Diquat $[M]^{+\bullet}$ 184/156***		4	184	156	60	29	10
Diquat $[M]^{+\bullet}$ 184/169		5	184	169	60	27	12
Diquat $[M]^{+\bullet}$ 184/155		5	184	155	60	43	12
Diquat $[M]^{+\bullet}$ 184/168		5	184	168	60	45	12
IL-IS Diquat D_8 $[M]^{2+}$ 96/88	**-**		**96**	**88.4**	**61**	**21**	**4**
IL-IS Diquat D_8 $[M^{2+}- H^+]^+$ 191/165	**-**		**191**	**165**	**101**	**31**	**10**
IL-IS Diquat D_8 $[M]^{+\bullet}$ 192/134	-		192	134	156	55	8
Paraquat $[M]^{2+}$ 93/171	**IL-IS Paraquat D_8 $[M]^{2+}$ 97/179**	**2**	**93**	**171**	**46**	**15**	**12**
Paraquat $[M]^{2+}$ 93/77		**3**	**93**	**77**	**46**	**31**	**12**
Paraquat $[M]^{2+}$ 93/155		**4**	**93**	**155**	**46**	**25**	**10**
Paraquat $[M]^{2+}$ 93/144		**4**	**93**	**144**	**46**	**17**	**8**
Paraquat $[M]^{+\bullet}$ 186/171	**IL-IS Paraquat D_8 $[M]^{+\bullet}$ 194/179**	**1**	**186**	**171**	**41**	**25**	**12**
Paraquat $[M]^{+\bullet}$ 186/77		**3**	**186**	**77**	**41**	**57**	**4**
Paraquat $[M]^{+\bullet}$ 186/155		**4**	**186**	**155**	**41**	**55**	**8**
Paraquat $[M]^{+\bullet}$ 186/128		**5**	**186**	**128**	**41**	**57**	**12**
Paraquat $[M]^{+\bullet}$ 186/103		**4**	**186**	**103**	**41**	**49**	**12**
Paraquat $[M^{2+}- H^+]^+$ 185/170	*IL-IS Paraquat D_8 $[M^{2+}- H^+]^+$* 193/178	**	*185*	*170*	*61*	*23*	*8*
Paraquat $[M^{2+}- H^+]^+$ 185/169		**	*185*	*169*	*61*	*37*	*8*
Paraquat $[M^{2+}- H^+]^+$ 185/144		**	*185*	*144*	*61*	*29*	*8*
Paraquat $[M^{2+}- H^+]^+$ 185/115		**	*185*	*115*	*61*	*55*	*8*
IL-IS Paraquat D_8 $[M]^{2+}$ 97/179	**-**		**97**	**179**	**46**	**15**	**12**
IL-IS Paraquat D_8 $[M]^{+\bullet}$ 194/179	**-**		**194**	**179**	**71**	**27**	**10**
IL-IS Paraquat D_8 $[M^{2+}- H^+]^+$	-		193	178	86	29	12

* The ranking in this table only refers to the signal to noise ratio. Further experiments are planned to study signal repeatability of various mass transitions also in comparison with the transitions of the respective IL-IS.

** MRMs of singly charged protonated Paraquat ($[M^{2+}- H^+]^+$ 185/#) are typically less sensitive and tend to show more variable signals than the MRMs of the other two parent ions ($[M]^{2+}$ and $[M]^{+\bullet}$). Furthermore these transitions tend to be interfered by Diquat. Same applies to the respective MRM of Paraquat D_8 (*$[M^{2+}- H^+]^+$193/#) , which is interfered by Diquat D_8.*

*** Removed from this Table as the signals showed more variability than the ones newly included

Table 16: *Exemplary matrix effects of Diquat in infant formula powder considering mass transitions resulting from different parents (the conc. of Diquat and Paraquat in the final extract was 0.015 µg/mL each)*

Analyte	Type of parent ion	Matrix Effect of parent (%) (MRM)	Matrix Effect of corresponding IL-IS D_8 (%) (MRM)
Diquat	**$[M]^{2+}$**	**+57** ***(92/84)***	**+54** ***(96/88)***
	$[M^{2+} - H^+]^+$	**-92** ***(183/157)***	**-91** ***(191/165)***
	$[M]^{+\bullet}$	-91 *(184/128)*	-93 *(192/134)*
Paraquat	**$[M]^{2+}$**	**+111** ***(93/171)***	**+112** ***(97/179)***
	$[M^{2+} - H^+]^+$	-74 *(185/170)*	-78 *(193/178)*
	$[M]^{+\bullet}$	**-81** ***(186/171)***	**-78** ***(194/179)***

b) **Carry-over:**
Be aware that some analytes are prone to carry-over effects and regularly observe blank solvent or blank matrix extract injections for the occurrence of any carry-over. In some cases signals deriving from carry-over are more intensive when injecting blank matrix extracts compared to pure solvent.
Examples of analytes where carry-over has been observed: Diquat, Paraquat, Phosphonic acid, Chlorate, Glyphosate.

c) **Avoid glass containers for certain analytes:**
Keep solutions in plastic vessels **2.15** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.12**).

4. Handling of column, pre-column and pre-filters:

a) **AS11 and AS11HC:**
Priming and reconditioning of column: before first use, after long storage (e.g. >2 weeks), after injection of 50-100 sample extracts):

- 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
- 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!.

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 a sequence. If to be stored for longer periods (e.g. >2 months) recondition the column as described above.

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.

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**

b) **Hypercarb**

Priming and reconditioning of column: Before first use, 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. Priming may be performed by multiple injection of a QuPPe extract of spinach or of a grape skin extract solution (prepared by dissolving 100 mg grape skin extract in 20 mL methanol + 1% FA-H_2O 1:1). For a quick equilibration, the LC-conditions shown in **Table 17** may be used. 10-15 injections of spinach extracts are typically required for the pre-column and ca. 50 injections for the column and pre-column combined. If possible inject 50 µL each time. This masking of the active sites is temporary as 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 reobtain sufficient column masking. The impact of priming on the chromatographic properties of the column is exemplary shown in Figure 3, Figure 4 and Figure 5.

Table 17: *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.		

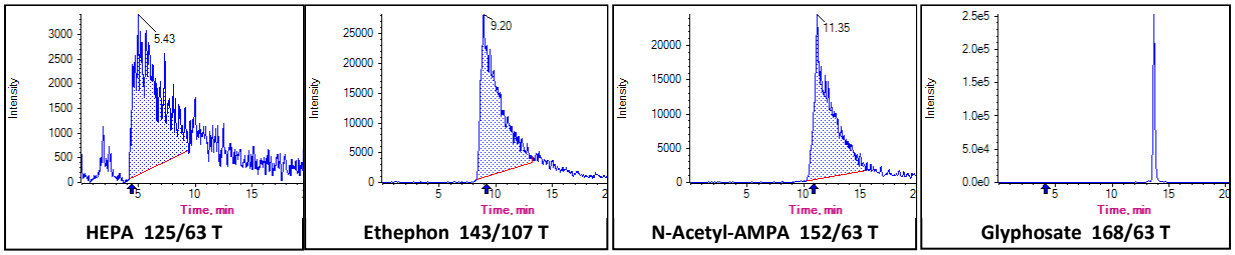


Figure 3: *Chromatograms obtained using a new Hypercarb column, poor chromatographic behavior due to strong interactions of analytes with active sites. Same behavior is observed when the pre-column is new.*

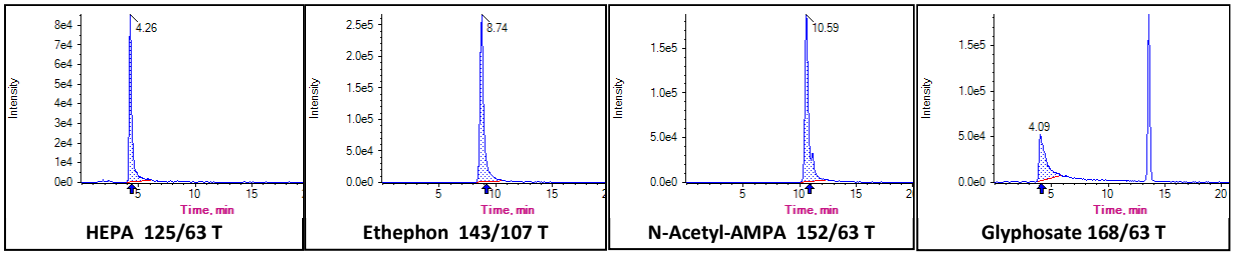


Figure 4: *Chromatograms following priming with 25 injections QuPPe extracts of spinach. Injection volume 50 µL per injection*

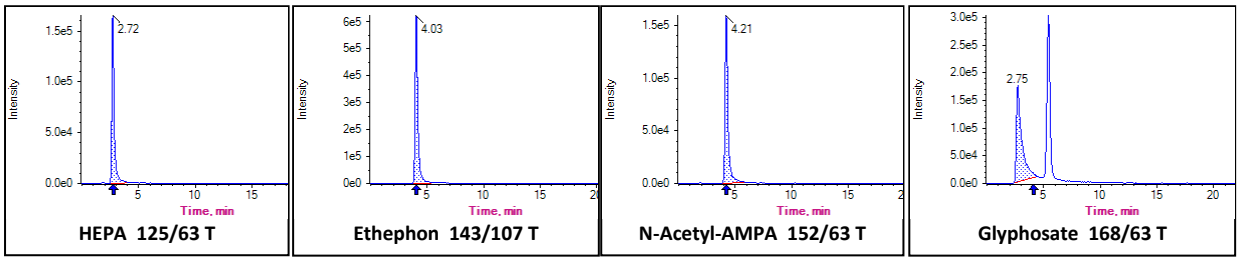


Figure 5: *Chromatograms after additional injection of approximately 100 QuPPe-extracts of various fruit and vegetables during normal routine use.*

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.

Storage of columns: Following normal operation the column can be stored directly after any normal sequence/run (full gradient). Run system 3-4 times with full gradient to reactivate the column (inject standards in matrix) before starting the sequence. If to be stored for longer periods (e.g. >2 months) it is highly recommended to recondition the column as described above.

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.

c) **Torus DEA**

Priming:

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.

d) **Raptor Polar X**

Priming:

The manufacturing company of this column recommends "passivating" the LC-system with a methanolic solution of Methylenediphosphonic Acid (Medronic Acid) (1984-15-2).

5.6.2. **Method 1.1 (M1.1): "Gly&Co. AS 11"**

Table 18: *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-$^{13}C_2$,$^{15}N_1$ (IL-IS)	171/63, 171/126	
	AMPA	110/63, 110/79, 110/81**	
	AMPA-$^{13}C_1$$^{15}N_1$ (IL-IS)	112/63, 112/81	
	Ethephon	143/107, 143/79, 145/107	
	Ethephon-D_4 (IL-IS)	147/111, 147/79 (optional, in case of interferences)	
	HEPA	125/79, 125/95, 125/63	
	HEPA-D_4 (IL-IS)	129/79, 129/97	
	Glufosinate	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (IL-IS)	183/63, 183/98	
	N-Acetyl-Glufosinate	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-[acetyl]D_3 (IL-IS)	225/63, 225/137	
	N-Acetyl-Glufosinate-[methyl]D_3 (IL-IS)	225/63	
	MPPA	151/63, 151/107, 151/133	
	MPPA-D_3 (IL-IS)	154/63, 154/136	

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 2).
** See also 5.6.1.

Hints on Method 1.1

1. **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.
2. **Handling of column, pre-column and pre-filters:** See 5.6.1. point **4.a)**
3. **For general hints on analytes:** See 5.6.1

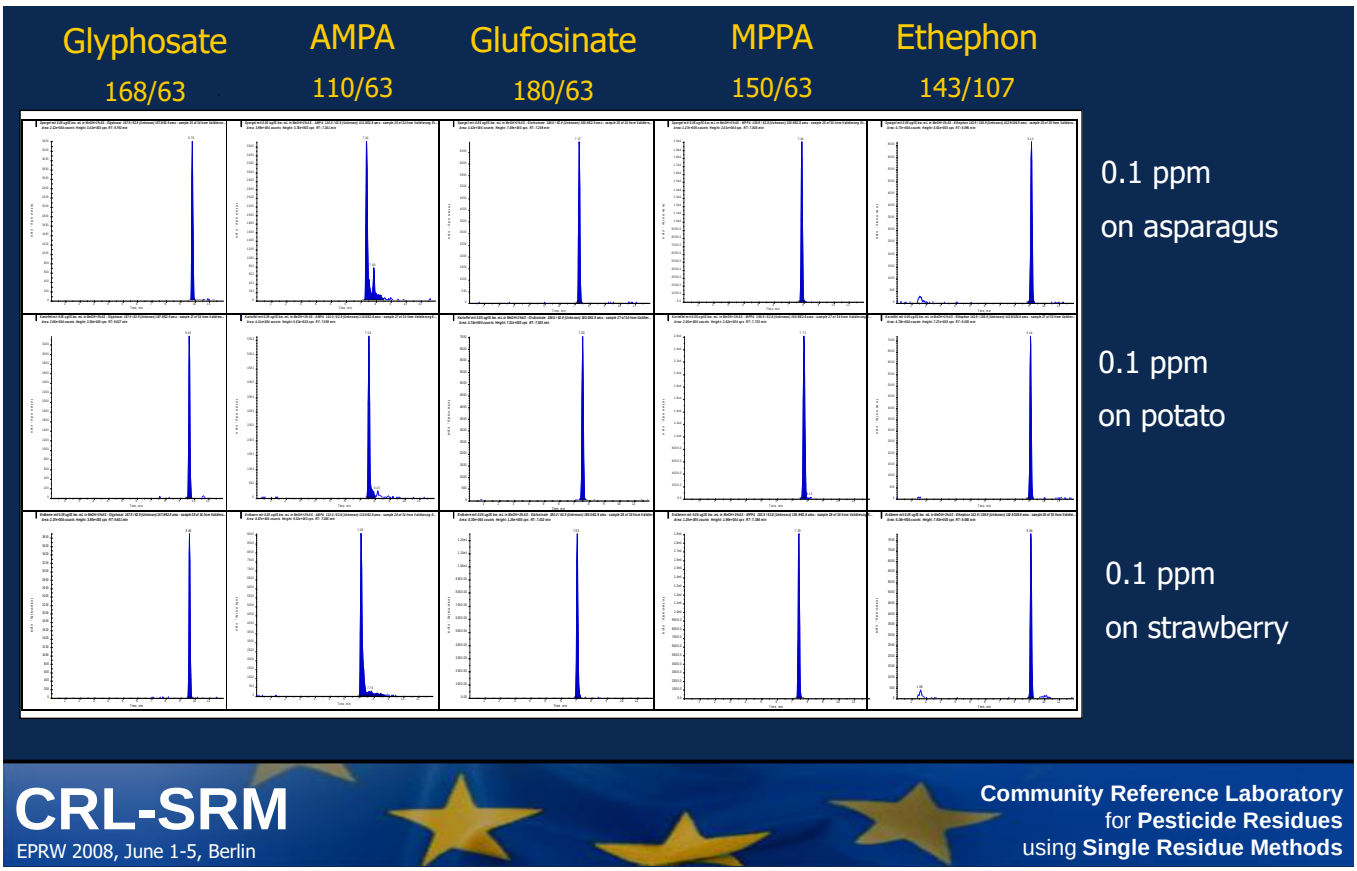


Figure 6: *Typical chromatograms of Glyphosate, AMPA, Glufosinate, MPPA and Ethephon spiked on blank-QuPPe extracts*

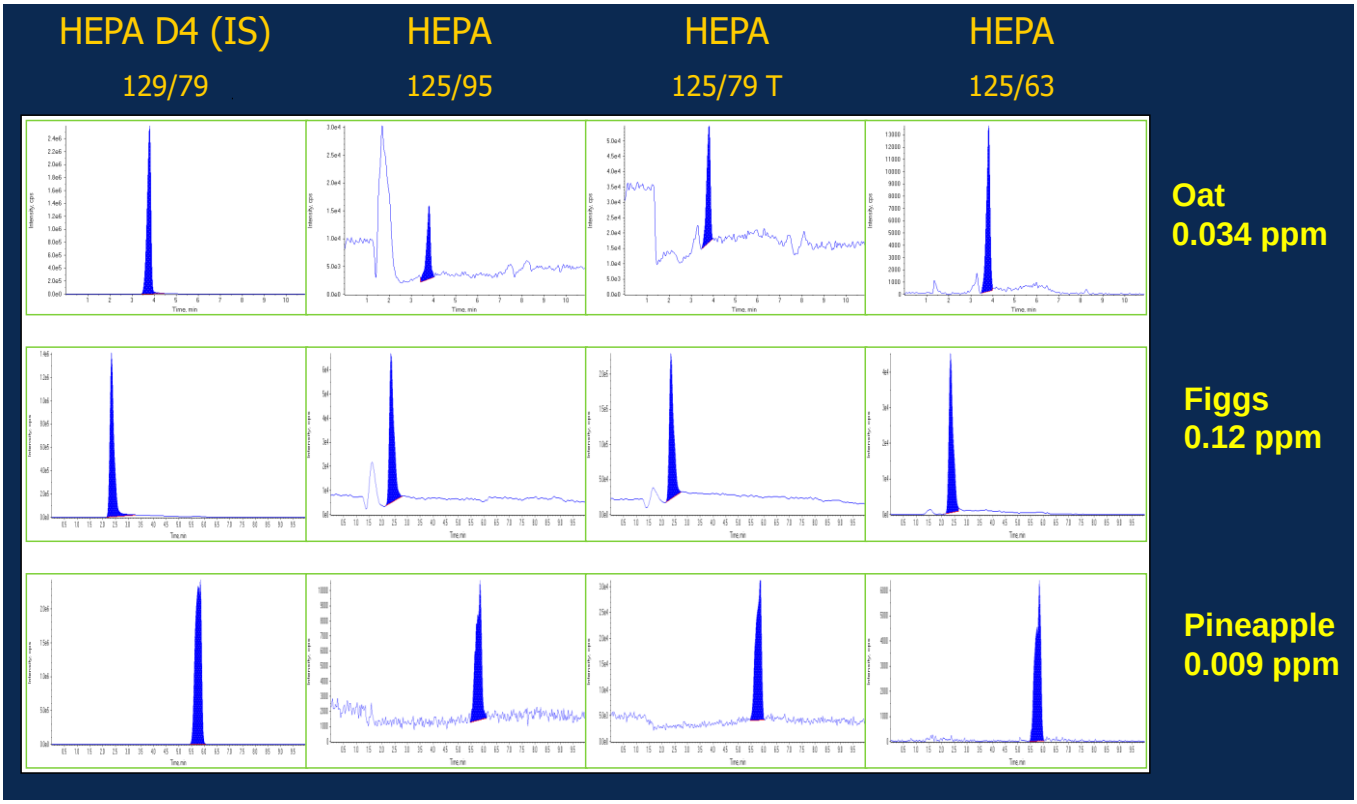


Figure 7: *Typical chromatograms of HEPA in real samples*

5.6.3. **Method 1.2 (M1.2): "Gly&Co. AS 11-HC"**

Table 19: *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	168/63, 168/124, 168/150, 168/81	
	Glyphosate-$^{13}C_2$,^{15}N (IL-IS)	171/63, 171/126	
	AMPA	110/63, 110/79, 110/81**	
	AMPA-^{13}C,^{15}N (IL-IS)	112/63, 112/81	
	N-Acetyl-AMPA	152/63, 152/79, 152/110	
	Ethephon	143/107, 143/79, 145/107	
	Ethephon-D_4 (IL-IS)	147/111, 147/79 (optional, in case of interferences)	
	HEPA	125/79, 125/95, 125/63	
	HEPA-D_4 (IL-IS)	129/79, 129/97	
	Glufosinate	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (IL-IS)	183/63, 183/98	
	N-Acetyl-Glufosinate	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-[acetyl]D_3 (IL-IS)	225/63, 225/137	
	N-Acetyl-Glufosinate-[methyl]D_3 (IL-IS)	225/63	
	MPPA	151/63, 151/107, 151/133	
	MPPA-D_3 (IL-IS)	154/63, 154/136	
	Fosetyl-Al:	109/81, 109/63 (Fosetyl)	
	Fosetyl-Al-D_{15} (IL-IS):	114/82, 114/63 (Fosetyl-D_5)	
	Phosphonic acid* **	81/79, 81/63	
	Phosphonic acid-$^{18}O_3$ (IL-IS)	87/85, 87/67	

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 2**).
** See also 5.6.1
*** See also 5.6.1

Hints on Method 1.2

1. **Handling of column, pre-column and pre-filters:** See 5.6.1. point **4.a)**
2. **Peak splitting:** Using this M 1.2 some compounds (e.g. Glyphosate) in some commodities tend to give two sharp peaks. The corresponding IL-IS typically behaves equally, so that quantification with any of the two peaks remains accurate
3. **Intereference of Phosphonic acid by Fosetyl:** See 5.6.1.
4. **Intereference of Phosphonic acid by Phosphoric acid:** See 5.6.1.
5. **IL-IS of N-Acetyl-Glufosinate D_3:** See 6
6. **For general hints on analytes:** See 5.6.1

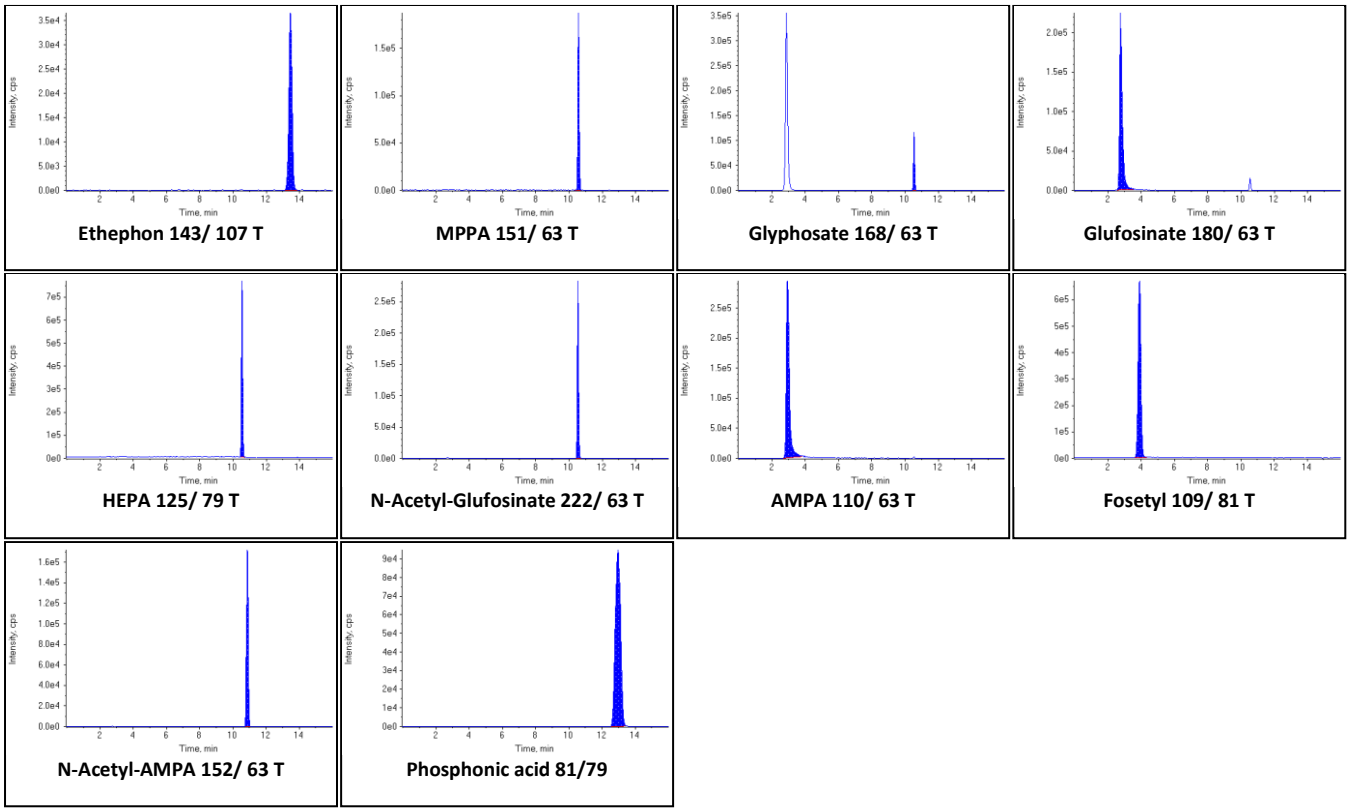


Figure 8: *Typical chromatograms of Ethephon, HEPA, Glyphosat, AMPA, Glufosinate, MPPA, N-Acetyl-AMPA, N-Acetyl-Glufosinate, Fosetyl-Al and Phosphonic acid at 0.1 mg/L in methanol with 1% formic acid.*

5.6.4.**Method 1.3 (M1.3): "Gly&Co. Hypercarb"**

Table 20: *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 (IL-IS)	171/63, 171/126	
	AMPA**	110/63, 110/79, **110/81****	
	AMPA-^{13}C,^{15}N (IL-IS)	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 (IL-IS)	213/63, 213/153	
	Ethephon	143/107, 143/79, 145/107	
	Ethephon-D_4 (IL-IS)	147/111, 147/79	
	HEPA	125/79, 125/95, 125/63	
	HEPA-D_4 (IL-IS)	129/79, 129/97	
	Glufosinate	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (IL-IS)	183/63, 183/98	
	N-Acetyl-Glufosinate:	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-[acetyl]D_3 (IL-IS)	225/63, 225/137	
	N-Acetyl-Glufosinate-[methyl]D_3 (IL-IS)	225/63	
	MPPA	151/63, 151/107, 151/133	
	MPPA-D_3 (IL-IS)	154/63, 154/136	
	Fosetyl-Al	109/81, 109/63 (detected as Fosetyl)	
	Fosetyl-Al-D_{15} (IL-IS)	114/82, 114/63 (detected as Fosetyl-D_5)	
	Maleic Hydrazide	111/82, 111/42, 111/55, 111/83	
	Maleic Hydrazide-D_2 (IL-IS)	113/42, 113/85	
	Maleic Hydrazide-$^{13}C_4$ (IL-IS)	115/87, 115/58	
	Cyanuric acid	128/42, 128/85	
	Cyanuric acid-$^{13}C_3$	131/43, 131/87	
	Bialaphos	322/88, 322/94, 322/134	
	Desmethyl-Dimethoate	214/104, 214/95, 214/136	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also **Table 2**).
** See **also 5.6.1**
*** See **also 5.6.1**

Hints on Method 1.3

1. **Handling of column, pre-column and pre-filters:** See **5.6.1** point **4.a)4.b)**
2. **Mass spectrometric interference of AMPA and Fosetyl: see 5.6.1**
3. **Intereference of Phosphonic acid by Fosetyl: see 5.6.1**
4. **Degradation of Ethephon to Phosphonic acid: see 5.6.1**
5. **Dilution:** For certain matrices 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 matrices containing high amounts of protein such as oily seeds, pulses and commodities of animal origin in general. In routine analysis there is an option run undiluted extracts for screening and in case of a positive result repeat measurement with a diluted extract (provided that there is no issues with false negatives, in non-diluted extracts are injected, **see also 5.6.1**).
6. **IL-IS of N-Acetyl-Glufosinate D_3** see 6
7. **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).
8. **For general hints on analytes**: See 5.6.1

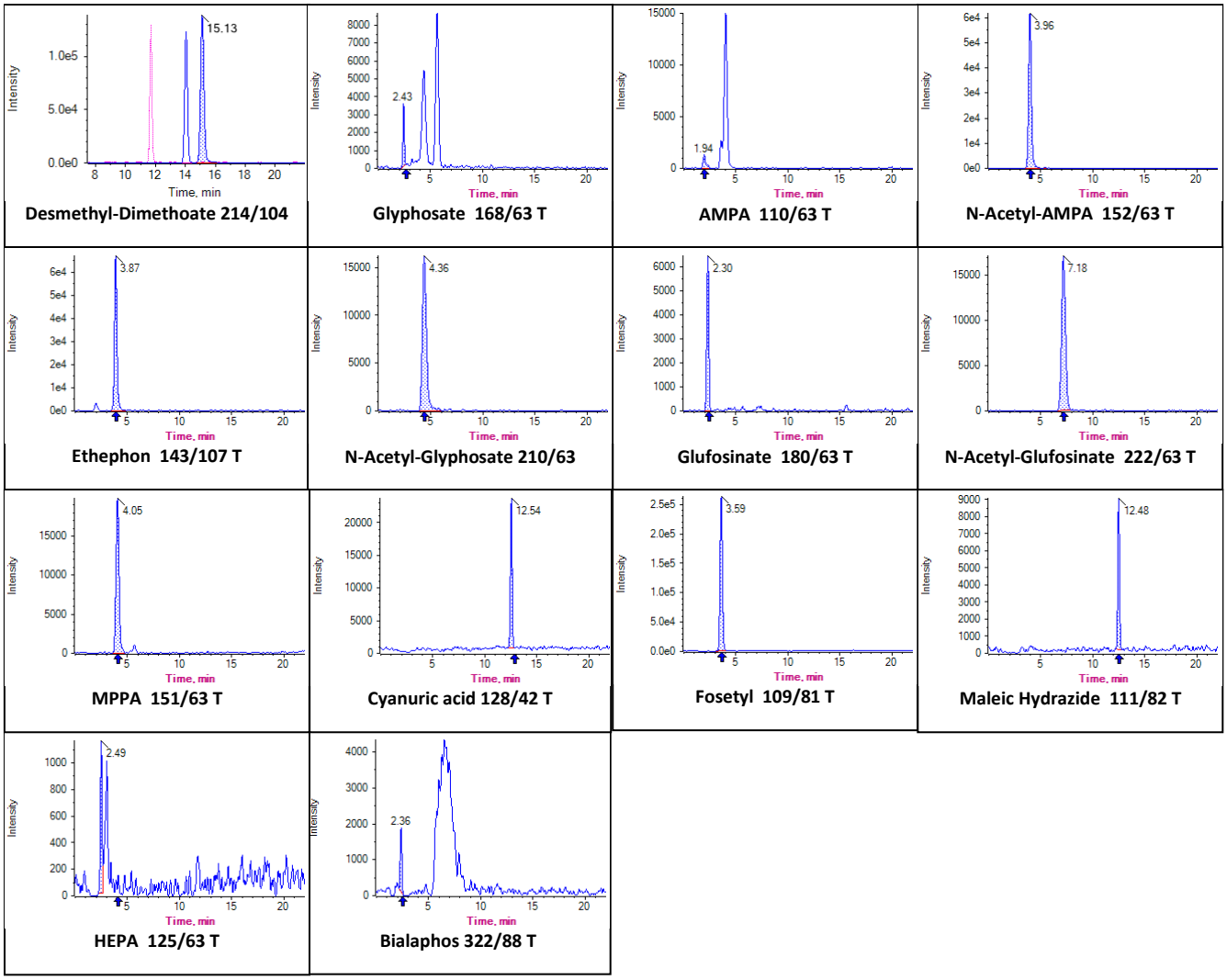


Figure 9: *Chromatograms of Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Fosetyl, Maleic Hydrazide, Cyanuric acid and Bialaphos at 0.02 mg/kg on apple extract and Desmethyl-Dimethoate at 0.03 mg/kg on cherry extract.*

5.6.5.**Method 1.4 (M1.4): "PerChloPhos"**

Table 21: *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	5-fold dilution with 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-$^{18}O_3$ (IL-IS)	135/117	
	Bromide*	81/81, 79/79	
	Chlorate	83/67, 85/69	
	Chlorate-$^{18}O_3$ (IL-IS)	89/71, 91/73	
	Perchlorate	99/83, 101/85	
	Perchlorate-$^{18}O_4$ (IL-IS)	107/89, 109/91	
	Phosphonic acid	81/79, 81/63	
	Phosphonic acid-$^{18}O_3$ (IL-IS)	87/85, 87/67	
	Thiocyanate	58/58	
	Thiocyanate ^{13}C ^{15}N	60/60	

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

Hints on Method 1.4

1. **Handling of column, pre-column and pre-filters:** see 5.6.1 point **4.a)4.b).**
2. **Cross-contamination and other issues on Perchlorate and Chlorate**: See 5.6.1
3. **Degradation of Ethephon and Fosetyl to Phosphonic acid**: See 5.6.1.
4. **Intereference of Phosphonic acid by Phosphoric acid and impact of dilution:** See 5.6.1.
5. **Improving selectivity of Bromide analysis:** See 5.6.1.
6. **For general hints on analytes:** See 5.6.1

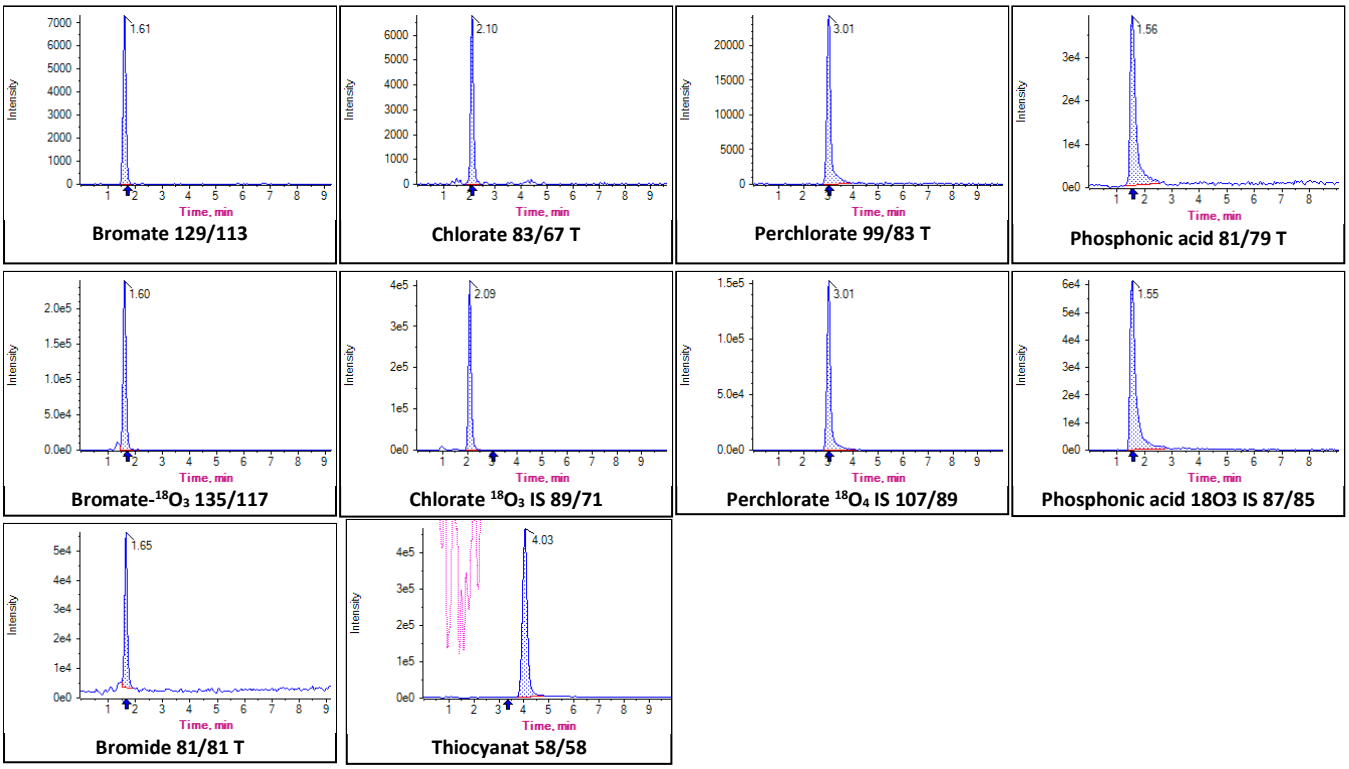


Figure 10: *Chromatograms of Bromate (0.02 mg/kg) in currant extract, Bromide (1 mg/kg) in currant extract, Phosphonic acid (0.05 mg/kg) in currant extract, Perchlorate (0.01 mg/kg) in currant extract, Chlorate (0.01 mg/kg) in currant extract and Thiocyanate (1 mg/kg) in porree extract.*

5.6.6. Method 1.5 (M1.5): "Gly&Co. on Trinity Q1"

Table 22: *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 ACN		
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 (IL-IS)	171/63, 171/126	
	AMPA**	110/63, 110/79, 110/81**	
	AMPA-^{13}C,^{15}N (IL-IS)	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 (IL-IS)	213/63, 213/153	
	Ethephon	143/107, 143/79, 145/107	
	Ethephon-D_4 (IL-IS)	147/111, 147/79 (optional, when interferences)	
	HEPA	125/79, 125/95, 125/63	
	HEPA-D_4 (IL-IS)	129/79, 129/97	
	Glufosinate:	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (IL-IS):	183/63, 183/98	
	N-Acetyl-Glufosinate	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-[acetyl]D_3 (IL-IS)	225/63, 225/137	
	N-Acetyl-Glufosinate-[methyl]D_3 (IL-IS)	225/63	
	MPPA:	151/63, 151/107, 151/133	
	MPPA-D_3 (IL-IS)	154/63, 154/136	
	Fosetyl-Al	109/81, 109/63 (each detected as Fosetyl)	
	Fosetyl-Al-D_{15} (IL-IS)	114/82, 114/63 (each detected as Fosetyl- D_5)	
	Maleic Hydrazide	111/82, 111/42, 111/55, 111/83	
	Maleic Hydrazide-D_2 (IL-IS)	113/42, 113/85	
	Maleic Hydrazide-$^{13}C_4$ (IL-IS)	115/87, 115/58	
	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-$^{18}O_3$ (IL-IS)	89/71, 91/73	
	Perchlorate	99/83, 101/85	
	Perchlorate-18O4 (IL-IS)	107/89, 109/91	
	Phosphonic acid	81/79, 81/63	
	Phosphonic acid $^{18}O_3$ (IL-IS)	87/85, 87/67	

* It is recommended to use an optimized collision energy for Bromide as described in 5.6.1.

Hints on Method 1.5

1. **For general hints on analytes see 5.6.1**

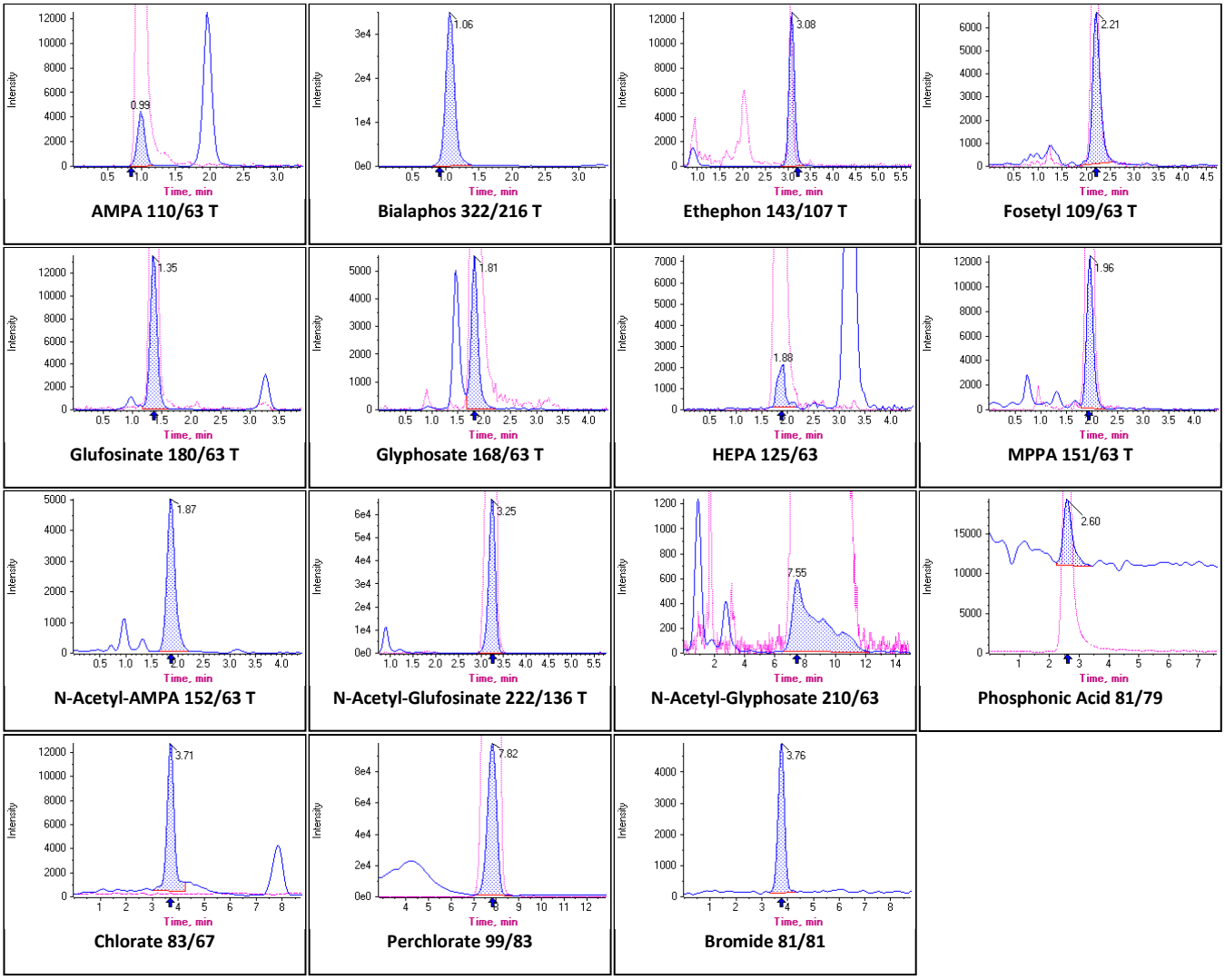


Figure 11: *Chromatograms of Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Fosetyl, Maleic Hydrazide, Cyanuric acid, Bialaphos and Bromide at 0.02 mg/kg each, Phosphonic acid at 0.05 mg/kg, as well as Chlorate and Perchlorate at 0.005 mg/kg each, all in black currant extract.*

5.6.7. Method 1.6 "Gly&Co. on Torus DEA; (M1.6a)" or "Gly&Co. on Anionic Polar Pesticide Column (APPC); (M1.6b)"

Table 23: *Proposed LC-MS/MS conditions for Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, N-Acetyl-Glufosinate, MPPA, Fosetyl-Al, Trifluoroacetic acid, Phosphonic acid and Bromide.*

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	M1.6a: Waters Torus™DEA 2.1 mm x 100 mm; 1.7 µm; 50 °C M1.6b: Waters Anionic Polar Pesticide Column (APPC), 130Å, 5 µm, 2.1 mm x 100 mm; 50 °C		
Pre-column	M1.6a: Waters Torus™DEA VanGuard™ 2.1 mm x 5 mm; 1.7 µm M1.6b: Waters Anionic Polar Pesticide VanGuard Cartridge, 130Å, 5µm, 2.1 mm X 5 mm		
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; Standard solutions of Fosetyl and Ethephon (and their IL-ISs) may be contaminated with native Phosphonic acid which may potentially lead to false positives or shifted calibration, see 5.6.1.		
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 (IL-IS)	171/63, 171/126	
	AMPA	110/63, 110/79, 110/81	
	AMPA-^{13}C,^{15}N (IL-IS)	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 (IL-IS)	213/63, 213/153	
	Ethephon	143/107, 143/79, 145/107	
	Ethephon-D_4 (IL-IS)	147/111, 147/79 (optional, when interferences)	
	HEPA	125/79, 125/95, 125/63	
	HEPA-D_4 (IL-IS)	129/79, 129/97	
	Glufosinate	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (IL-IS)	183/63, 183/98	
	N-Acetyl-Glufosinate	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-[acetyl]D_3 (IL-IS)	225/63, 225/137	
	N-Acetyl-Glufosinate-[methyl]D_3 (IL-IS)	225/63	
	MPPA	151/63, 151/107, 151/133	
	MPPA-D_3 (IL-IS)	154/63, 154/136	
	Fosetyl-Al	109/81, 109/63 (each detected as Fosetyl)	
	Fosetyl-Al-D_{15} (IL-IS)	114/82, 114/63 (each detected as Fosetyl- D_5)	
	Trifluoroacetic acid (TFA)	113/69, 113/113	
	Trifluoroacetic acid -$^{13}C_2$ (IL-IS)	115/70	
	Phosphonic acid	81/79, 81/63	
	Phosphonic acid-$^{18}O_3$ (IL-IS)	87/85, 87/67	
	Bromide	81/81, 79/79	

Hints on Method 1.6a and Method 1.6b

1. **Handling of column, pre-column and pre-filters:** see 5.6.1 point **4.a)4.c)**
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 on this column, with retention times close to the dead-time, heavy interefernces of matrix components on peak shapes and intensities (signal suppression). Figure **14** 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 at low concentrations is often not possible. Fortunately Maleic Hydrazide can also be covered by M 4.2 (5.6.15), whereas Cyanuric acid is not regulated.
3. **For general hints on analytes:** See 5.6.1

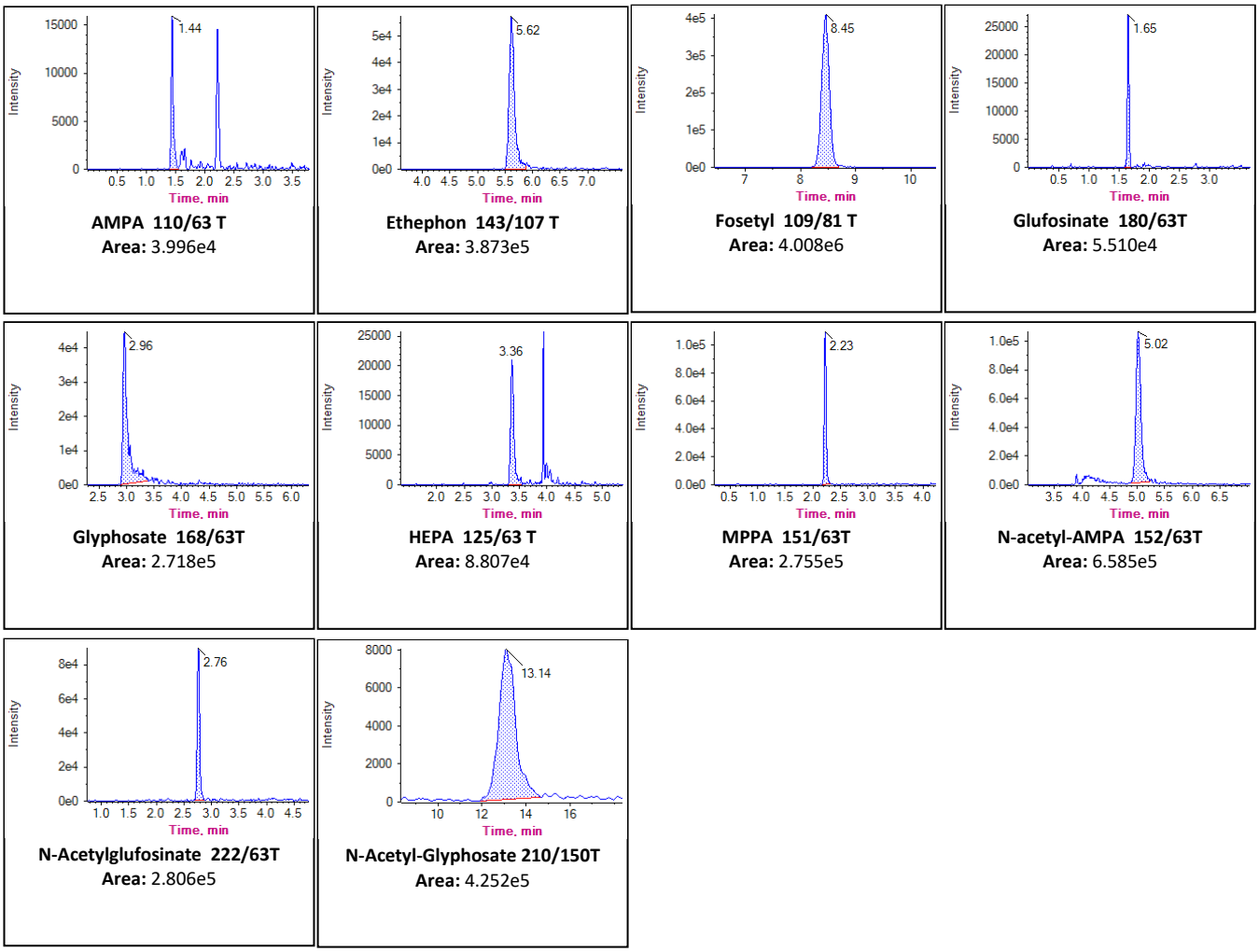


Figure 12: *Chromatograms of Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Fosetyl at 0.04 mg/kg on cucumber extract using* ***i:*** *Waters Torus™DEA 2.1 mm x 100 mm .*

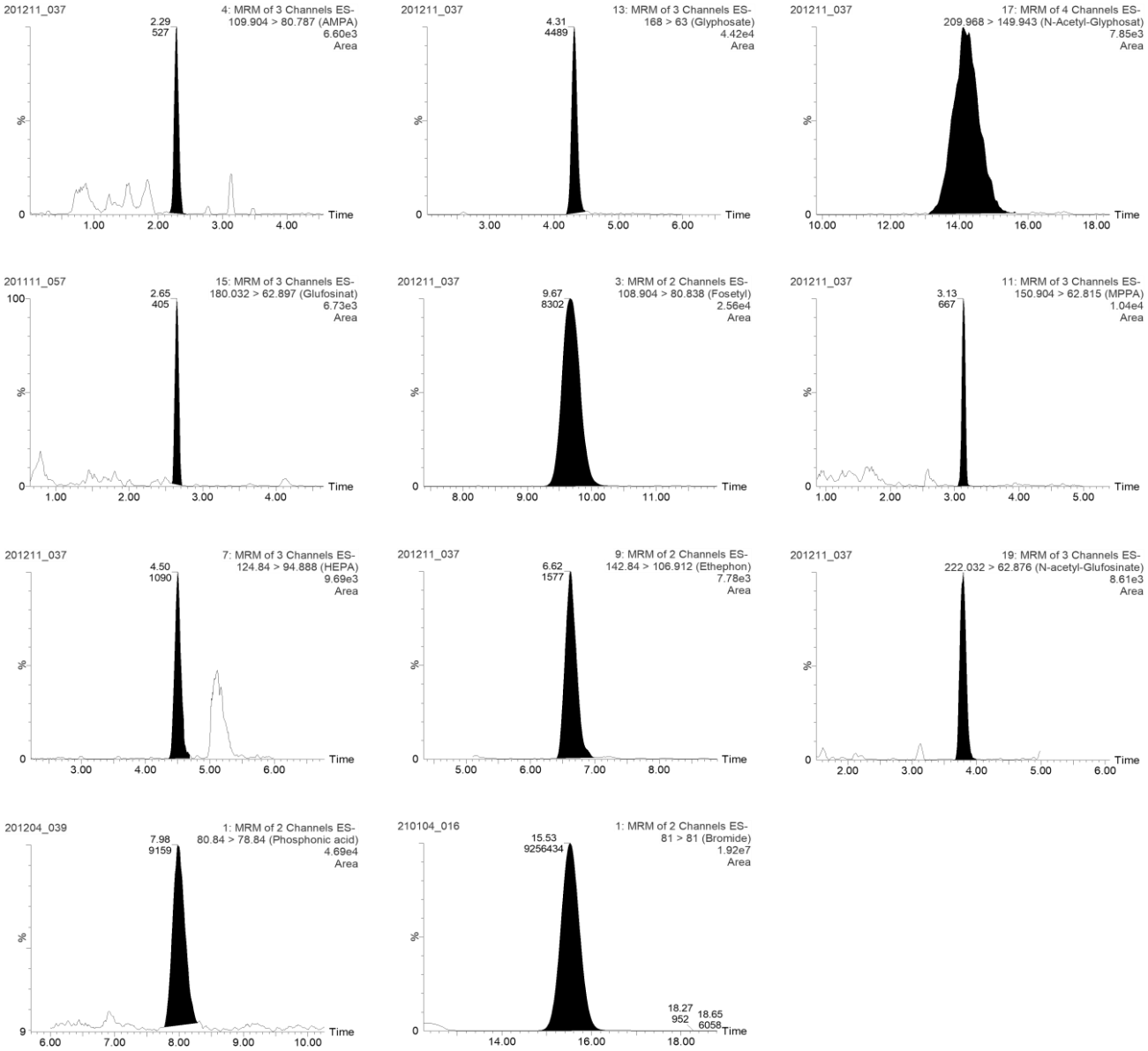


Figure 13: *Chromatograms of Glyphosate, AMPA, N-Acetyl-Glyphosate at 0.06 mg/kg, Glufosinate at 0.036 mg/kg, HEPA, MPPA, N-Acetyl-Glufosinate at 0.024 mg/kg, Ethephon, Fosetyl at 0.012 mg/kg, all in strawberry extract and Phosphonic acid at 0.06 mg/kg, Bromide at 6 mg/kg both in lemon extract using ii: Waters Anionic Polar Pesticide Column, 130Å, 5 µm, 2.1 mm x 100 mm.*

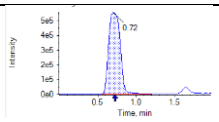
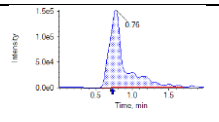
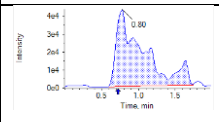
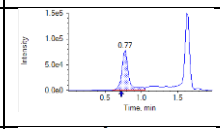
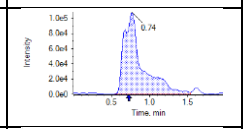
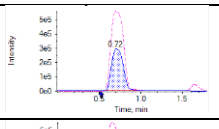
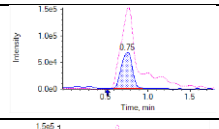
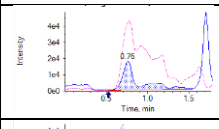
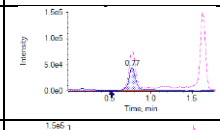
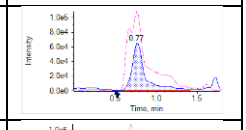
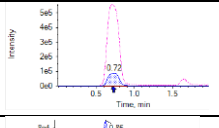
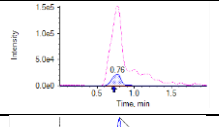
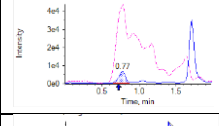
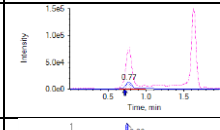
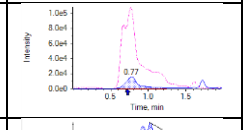
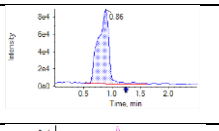
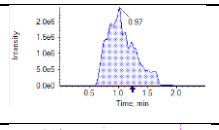
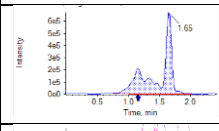
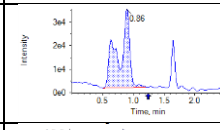
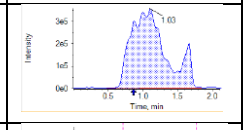
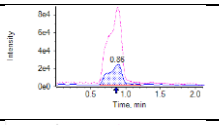
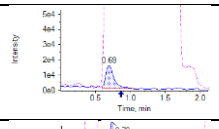
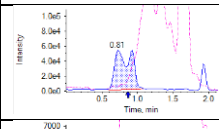
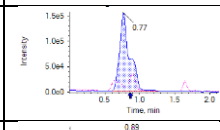
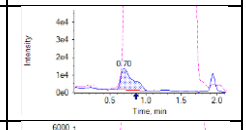
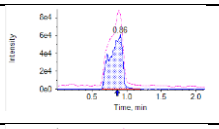
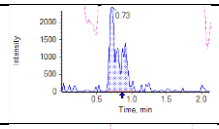
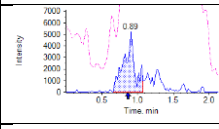
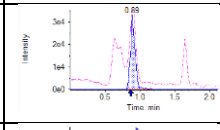
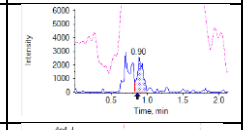
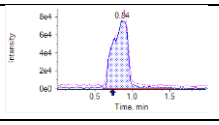
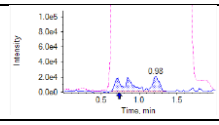
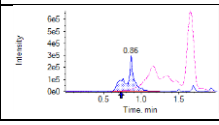
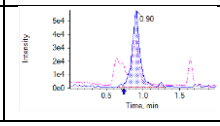
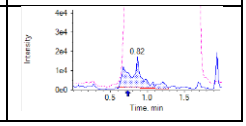
	Solvent	Plum	Broccoli	Soy	Onion
Cyanuric acid $^{13}C_3$ 131/43					
Cyanuric acid 128/42					
Cyanuric acid 128/85					
Maleic hydrazide D_2 113/42					
Maleic hydrazide 111/42					
Maleic hydrazide 111/82					
Maleic hydrazide 111/83					

Figure 14: 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 *(Please also read the note under "Hints on Method 1.6a and Method 1.6b" above)*

5.6.8. Method 1.7 "PerChloPhos on Torus DEA; (M1.7a)" or "PerChloPhos on Anionic Polar Pesticide Column (APPC); (M1.7b)"

Table 24: *Proposed LC-MS/MS conditions for PerChlorate, Chlorate, Phosphonic acid and Bormide*

Instrument parameters	Conditions					
Ionisation mode	ESI neg					
	M1.7a			**M1.7b**		
Column/temperature	Waters Torus™DEA 2.1 mm x 100 mm; 1.7 µm; 50 °C			Waters Anionic Polar Pesticide Column, 130Å, 5 µm, 2.1 mm x 100 mm; 50 °C		
Pre-column	Waters Torus™DEA VanGuard™ 2.1 mm x 5 mm; 1.7 µm			Waters Anionic Polar Pesticide VanGuard Cartridge, 130Å, 5µm, 2.1 mm X 5 mm		
Pre-filters	Waters ACQUITY UPLC Column In-Line Filter Kit [205000343]			Waters ACQUITY UPLC Column In-Line Filter Kit [205000343]		
Eluent A	1.2% formic acid + 10 mmol ammonium formate in water			1.2% formic acid + 15 mmol ammonium formate in water		
Eluent B	0.5 % formic acid in Acetonitrile			0.5 % formic acid in Acetonitrile		
Gradient	**%A**	**Flow [mL/min]**	**Time [min]**	**%A**	**Flow [mL/min]**	**Time [min]**
	10	0.5	0	10	0.5	0
	10	0.5	0.5	10	0.5	0.5
	80	0.5	1.5	80	0.5	1.5
	90	0.5	4.5	90	0.5	4.5
	90	0.5	17.5	90	0.5	13.5
	10	0.5	17.6	10	0.5	13.6
	10	0.5	23	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-$^{18}O_3$ (IL-IS)		89/71, 91/73			
	Perchlorate		99/83, 101/85			
	Perchlorate-$^{18}O_4$ (IL-IS)		107/89, 109/91			
	Phosphonic acid		81/79, 81/63			
	Phosphonic acid-$^{18}O_3$ (IL-IS)		87/85, 87/67			

Hints on Method 1.7

1. **Handling of column, pre-column and pre-filters:** See 5.6.1 point **4.a)4.c)**
2. **Intereference of Phosphonic acid by Phosphoric acid**: See 5.6.1
3. **For general hints on analytes:** See 5.6.1

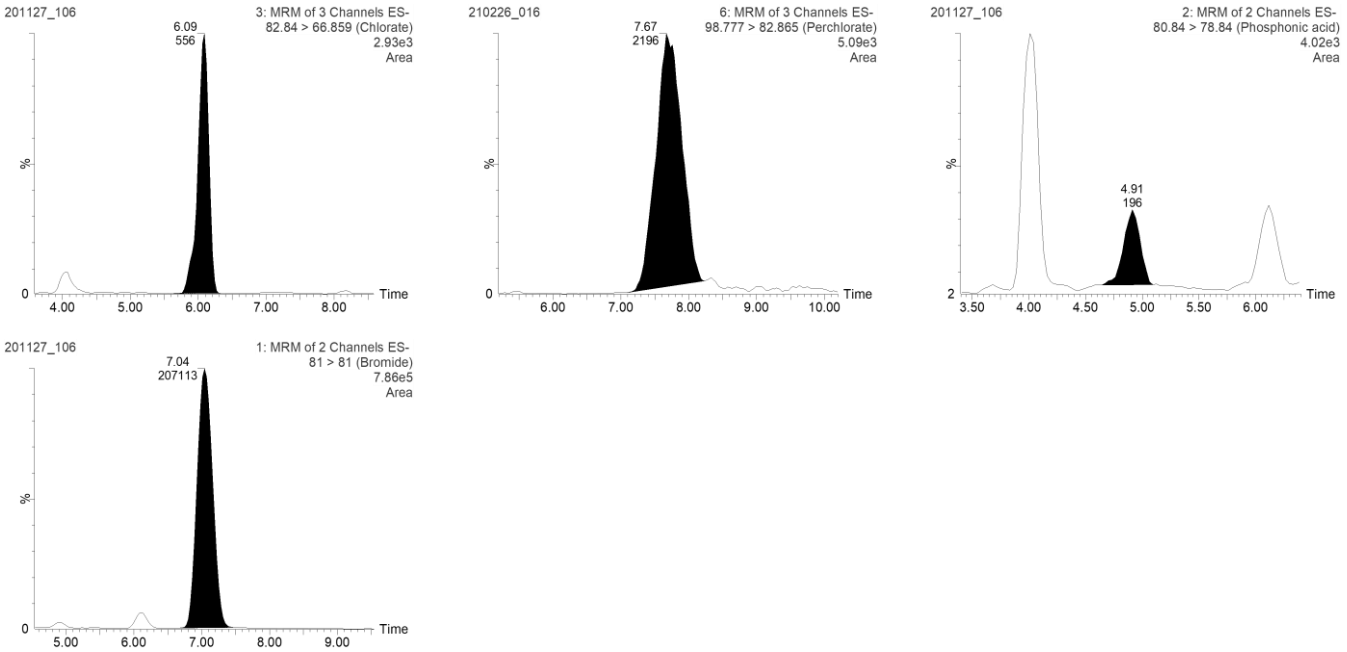


Figure 15: *Chromatograms of Chlorate at 0.03mg/kg, Perchlorate at 0.01 mg/kg, Phosphonic acid at 0.05 mg/kg and Bromide at 5.0 mg/kg, all in lemon extract using ii: Waters Anionic Polar Pesticide Column, 130Å, 5 µm, 2.1 mm x 100 mm; 50 °C.*

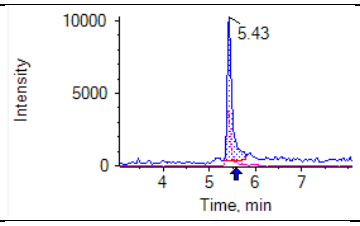
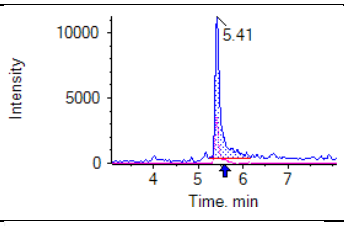
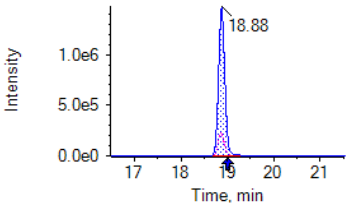
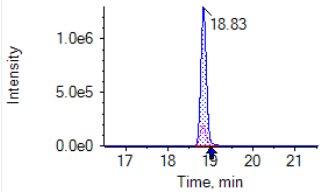
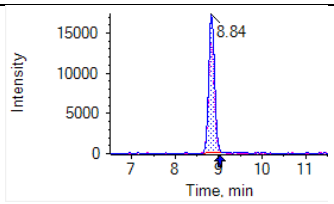
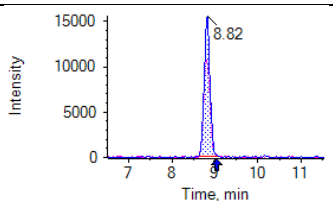
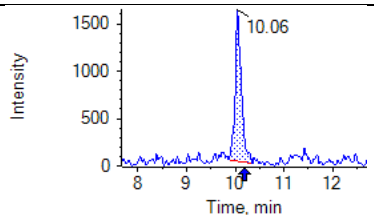
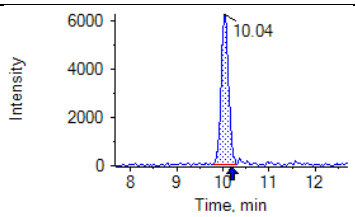
	Grape	Onion
Phosphonic acid 81/79		
Perchlorate 99/83		
Chlorate 83/67		
Bromide 81/81		

Figure 16: *Exemplary chromatograms of Phosphonic acid, Perchlorate, Chlorate and Bromide at 0.01 mg/kg on Garpe and Onion using i: Waters Torus™DEA 2.1 mm x 100 mm; 1.7 µm; 50 °C.*

5.6.9. Method 1.8 (M1.8): "PerChloCyanMalein on Anionic Polar Pesticide Column (APPC)"

Table 25: *Proposed LC-MS/MS conditions for Perchlorate, Chlorate, Cyanuric acid and Maleic hydrazide*

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Waters Anionic Polar Pesticide Column, 130Å, 5 µm, 2.1 mm x 100 mm; 50 °C		
Pre-column	Waters Anionic Polar Pesticide VanGuard Cartridge, 130Å, 5µm, 2.1 mm X 5 mm		
Pre-filters	Waters ACQUITY UPLC Column In-Line Filter Kit [205000343]		
Eluent A	1.2% formic acid and 50mM NH_4-formate in water		
Eluent B	85 % ACN : 10 % MeOH : 5 % water		
Gradient	**%A**	**Flow [mL/min]**	**Time [min]**
	0	0.5	0
	0	0.5	1.5
	70	0.5	4.5
	70	0.5	7.0
	0	0.5	7.1
	0	0.5	15
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)**	
	Cyanuric acid	128/42, 128/85	
	Cyanuric acid $^{13}C_3$ (IL-IS)	131/43	
	Chlorate	83/67, 85/69, 83/51	
	Chlorate-$^{18}O_3$ (IL-IS)	89/71, 91/73	
	Perchlorate	99/83, 101/85, 99/67	
	Perchlorate-$^{18}O_4$ (IL-IS)	107/89, 109/91	
	Maleic hydrazide	111/83, 111/55, 111/41	
	Maleic hydrazide D2 (IL-IS)	113/85	
	Maleic Hydrazide-$^{13}C_4$ (IL-IS)	115/87, 115/58	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also **Table 2**).
** Depending on matrix; better use M 1.3

Hints on Method 1.8

1. **For general hints on analytes:** See 5.6.1

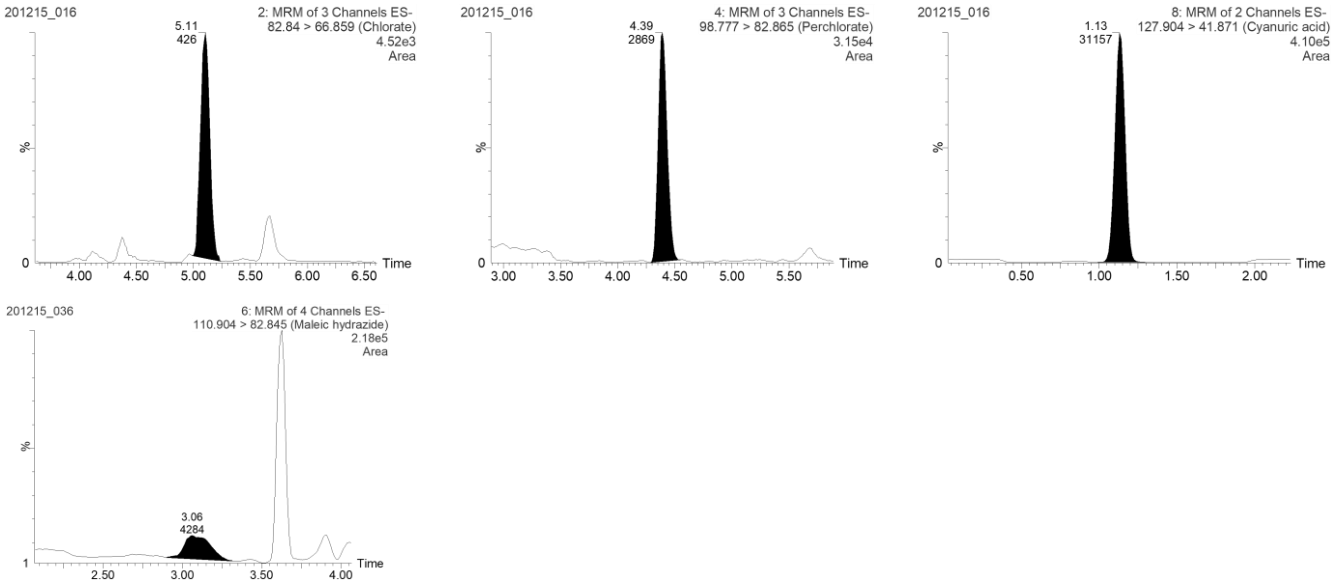


Figure 17: *Chromatograms of Chlorate at 0.03mg/kg, Perchlorate at 0.01 mg/kg, Cyanuric acid and Maleic hydrazide at 0.05 mg/kg, all in lemon extract*

5.6.10.Method 1.9 (M1.9): "Gly&Co. on Raptor Polar X"

Table 26: *Proposed LC-MS/MS conditions for Glyphosate, AMPA, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Fosetyl-Al, Bialaphos, Bromide, (Chlorate,) Perchlorate, Phosphonic acid*

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Restek Raptor Polar X LC column, 90Å, 2,7 µm, 2.1 mm x 30 mm; 50 °C		
Pre-column	Restek Raptor Polar X LC column, 90Å, 2,7 µm, 2.1 mm x 5 mm; 50 °C		
Eluent A	0.5% formic acid in water		
Eluent B	0.5% formic acid in acetonitrile		
Gradient	**%A**	**Flow [mL/min]**	**Time [min]**
	10	0.5	0.5
	60	0.5	1.5
	90	0.5	11.5
	90	0.5	14
	10	0.5	14.1
	10	0.5	17
Injection volume	10 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion + one level at the reporting limit; Standard solutions of Fosetyl and Ethephon (and their IL-ISs) may be contaminated with native Phosphonic acid which may potentially lead to false positives or shifted calibration, see 5.6.1.		
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 (IL-IS)	171/63, 171/126	
	AMPA	110/63, 110/79, 110/81	
	AMPA-^{13}C,^{15}N (IL-IS)	112/63, 112/81	
	Ethephon	143/107, 143/79, 145/107	
	Ethephon-D_4 (IL-IS)	147/111, 147/79 (optional, when interferences)	
	HEPA	125/79, 125/95, 125/63	
	HEPA-D_4 (IL-IS)	129/79, 129/97	
	Glufosinate	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (IL-IS)	183/63, 183/98	
	N-Acetyl-Glufosinate	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-[acetyl]D_3 (IL-IS)	225/63, 225/137	
	N-Acetyl-Glufosinate-[methyl]D_3 (IL-IS)	225/63	
	MPPA	151/63, 151/107, 151/133	
	MPPA-D_3 (IL-IS)	154/63, 154/136	
	Fosetyl-Al	109/81, 109/63 (each detected as Fosetyl)	
	Fosetyl-Al-D_{15} (IL-IS)	114/82, 114/63 (each detected as Fosetyl- D_5)	
	Trifluoroacetic acid (TFA)	113/69, 113/113	
	Trifluoroacetic acid -$^{13}C_2$ (IL-IS)	115/70	
	Bialaphos	322/88, 322/94, 322/134	
	Bromide	81/81, 79/79	
	Chlorate	83/67, 85/69	
	Chlorate-$^{18}O_3$ (IL-IS)	89/71, 91/73	
	Perchlorate	99/83, 101/85	
	Perchlorate-$^{18}O_4$ (IL-IS)	107/89, 109/91	
	Phosphonic acid	81/79, 81/63	
	Phosphonic acid-$^{18}O_3$ (IL-IS)	87/85, 87/67	

Hints on Method 1.9

1. **Handling of column, pre-column and pre-filters:** See 5.6.1 point **4.a)4.c)**
2. **For general hints on analytes:** See 5.6.1

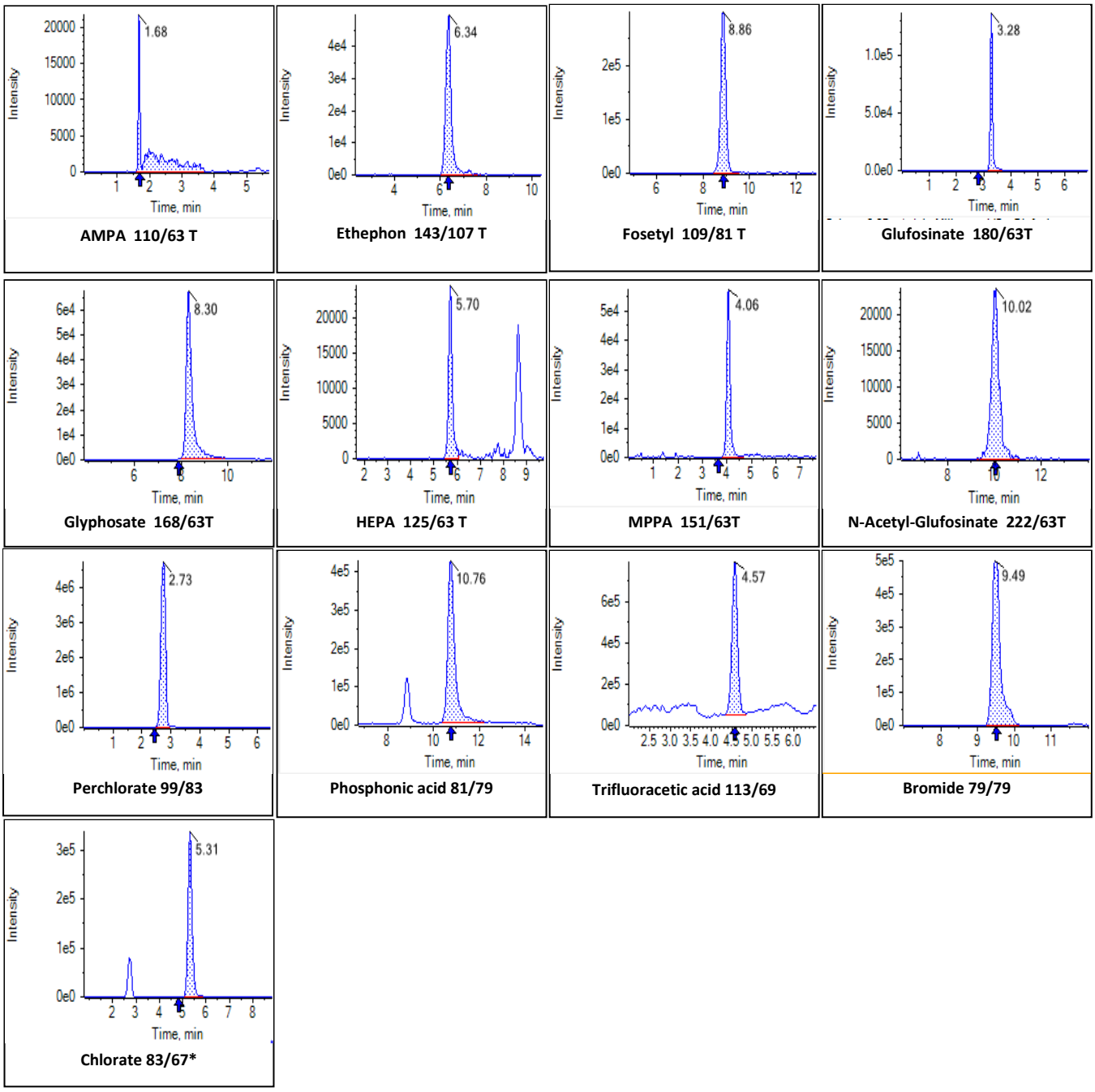


Figure 18: *Typical chromatograms in strawberry extracts spiked at 0.1 mg/kg.*
**for Chlorate matrix dependent retention time shifts were observed*

5.6.11. Method 1.10 (M1.10): "Gly&Co. on Obelisc N"

Table 27: *Proposed LC-MS/MS conditions for for Glyphosate, AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Bromide, Chlorate, Perchlorate, (Fosetyl-Al and Phosphonic acid)*

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Sielc Obelisc N, 100Å, 5 µm, 2.1 mm x 150 mm (SIELC; ON-21.150.0510), 50°C		
Pre-column	Sielc Obelisc N, 100Å, 5 µm, 2.1 mm x 10 mm (SIELC; ON-21.G.0510), 50°C		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter		
Eluent A	1% formic acid in water		
Eluent B	0.5 % formic acid in Acetonitrile		
Gradient	**%A**	**Flow [mL/min]**	**Time [min]**
	10	0.4	0.5
	80	0.4	2
	90	0.4	9
	10	0.4	9.1
	10	0.4	13
Injection volume	10 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion + one level at the reporting limit; Standard solutions of Fosetyl and Ethephon (and their IL-ISs) may be contaminated with native Phosphonic acid which may potentially lead to false positives or shifted calibration, see 5.6.1.		
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 (IL-IS)	171/63, 171/126	
	AMPA	110/63, 110/79, 110/81	
	AMPA-^{13}C,^{15}N (IL-IS)	112/63, 112/81	
	N-Acetyl-Glyphosate	210/63, 210/150, 210/79, 210/148	
	N-Acetyl-Glyphosate-D_3 (IL-IS)	213/63, 213/153	
	Ethephon	143/107, 143/79, 145/107	
	Ethephon-D_4 (IL-IS)	147/111, 147/79	
	HEPA	125/79, 125/95, 125/63	
	HEPA-D_4 (IL-IS)	129/79, 129/97	
	Glufosinate	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (IL-IS	183/63, 183/98	
	N-Acetyl-Glufosinate	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-[acetyl]D_3 (IL-IS)	225/63, 225/137	
	N-Acetyl-Glufosinate-[methyl]D_3 (IL-IS)	225/63	
	MPPA	151/63, 151/107, 151/133	
	MPPA-D_3 (IL-IS)	154/63, 154/136	
	Fosetyl-Al	109/81, 109/63 (each detected as Fosetyl)	
	Fosetyl-Al-D_{15} (IL-IS)	114/82, 114/63 (each detected as Fosetyl- D_5)	
	Trifluoroacetic acid (TFA)	113/69, 113/113	
	Trifluoroacetic acid -$^{13}C_2$ (IL-IS)	115/70	
	Maleic Hydrazide	111/82, 111/42, 111/55, 111/83	
	Maleic Hydrazide-D_2 (IL-IS)	113/42, 113/85	
	Maleic Hydrazide-$^{13}C_4$ (IL-IS)	115/87, 115/58	
	Cyanuric acid	128/42, 128/85	
	Cyanuric acid-$^{13}C_3$	131/43, 131/87	
	Bromide:	81/81, 79/79	
	Chlorate	83/67, 85/69	
	Chlorate-$^{18}O_3$ (IL-IS)	89/71, 91/73	
	Perchlorate	99/83, 101/85	
	Perchlorate-$^{18}O_4$ (IL-IS)	107/89, 109/91	
	Phosphonic acid	81/79, 81/63	
	Phosphonic acid-$^{18}O_3$ (IL-IS)	87/85, 87/67	

Hints on Method 1.9

1. **For general hints on analytes:** See 5.6.1

5.6.12.Method 2 (M2): "Fosetyl and Maleic Hydrazide"

Table 28: *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	109/81, 109/63 (detected as fosetyl)	
	Fosetyl-Al-D_{15} (IL-IS)	114/82, 114/63 (detected as fosetyl-D_5)	
	Maleic Hydrazide	111/82, 111/42, 111/55, 111/83	
	Maleic Hydrazide-D_2 (IL-IS)	113/42, 113/85	
	Maleic Hydrazide-$^{13}C_4$ (IL-IS)	115/87, 115/58	
	Perchlorate	99/83, 101/85	
	Perchlorate-$^{18}O_4$ (IL-IS)	107/89, 109/91	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also **Table 2**).

Hints on Method 2

1. **Contamination of Maleic hydrazide D_2 with native Maleic hydrazide:** See 5.6.1
2. **For Perchlorate better run Method 1.3 or 1.4** !
3. **For general hints on analytes:** See 5.6.1

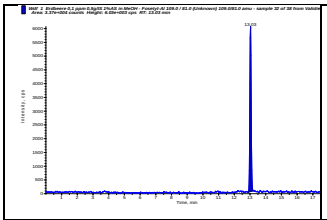
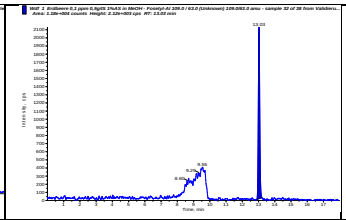
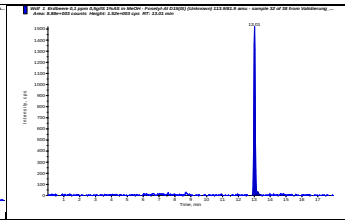
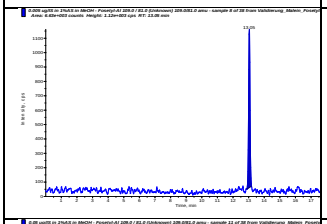
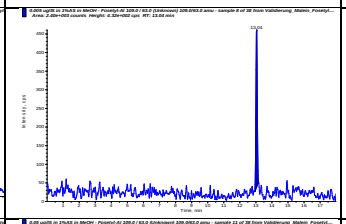
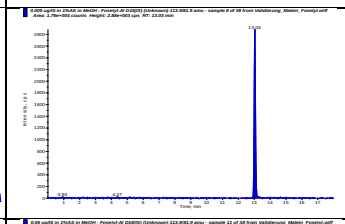
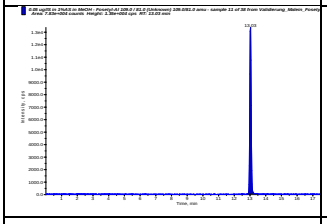
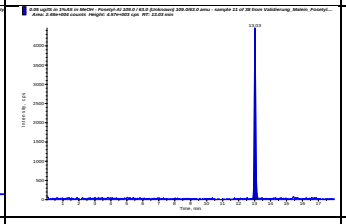
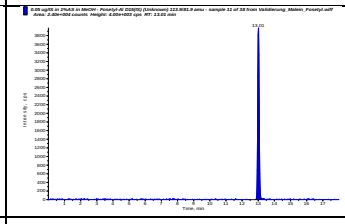
			Recovery test on strawberry 0.1 mg/kg (corresponds to 0.05 µg/mL at 100% recovery)
			Solvent calib. 0.005 µg/mL (Corresponds to 0.01 mg/kg)
			Solvent calib. 0.05 µg/mL Corresponds to 0.1 mg/kg
Fosetyl-Al 109 / 81	**Fosetyl-Al 109 / 63**	**Fosetyl-Al D_{15} 114 / 82 (IS)**	

Figure 19: *Typical chromatograms of Fosetyl-Al in strawberry extract and in solvent-based standards*

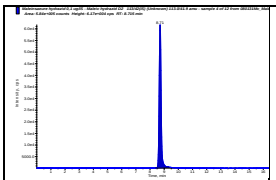
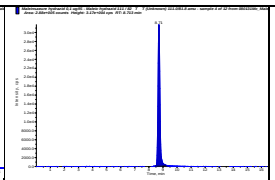
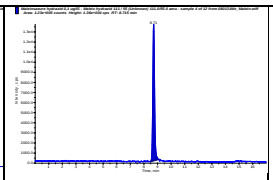
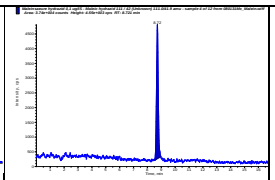
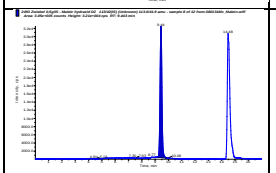
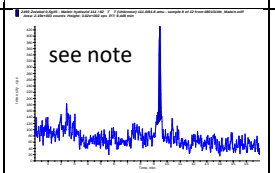
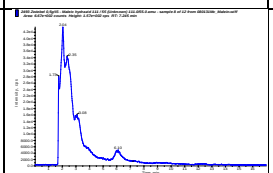
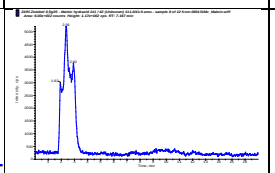
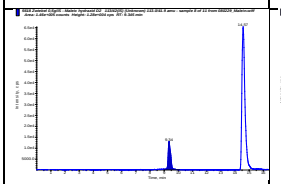
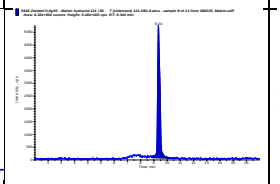
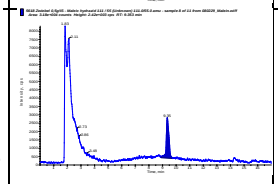
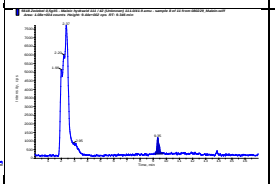
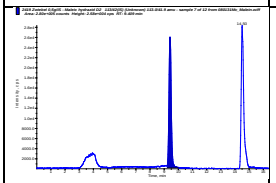
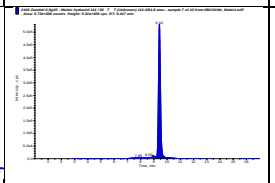
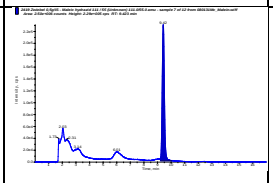
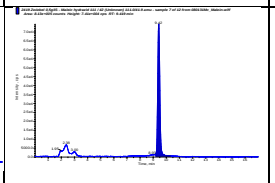
				Solvent calib. 0.1 mg/kg Corresponds to 0.05 µg/mL
				Extract of organic onion from the market: n.d. (0.5 g onion equivalents /mL)
				Extract of onion from the market containing **ca. 0.1 mg/kg Maleic Hydrazide.** (0.5 g onion equivalents /mL)
				Extract of onion from the market containing **ca. 4 mg/kg Maleic Hydrazide.** (0.5 g onion equivalents /mL)
Maleic Hydrazide-D_2 113/42 (IS)	**Maleic Hydrazide 111 / 82 (target ion)**	**Maleic Hydrazide 111 / 55**	**Maleic Hydrazide 111 / 42**	

Figure 20: *Typical chromatograms of Maleic Hydrazide in onion extracts and in solvent-based standards*

5.6.13. **Method 3 (M3): "Amitrole&Co."**

Table 29: *Proposed LC-MS/MS conditions for Amitrole, Chlormequat, Mepiquat, Daminozide, ETU, PTU, Trimesium, Difenzoquat and Cyromazine.*

Instrument parameters	Conditions		
Ionisation mode	ESI pos		
Column/temperature	Obelisc R 2.1 x 150 mm 5 µm 100 Å (SIELC; OR-21.150.0510); 40°C		
Pre-column	Obelisc R 2.1 x 10 mm 5 µm (SIELC; OR-21.G.0510)		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter		
Eluent A	5 mmol NH_4-formate in water Use brown glass bottles		
Eluent B	5 mmol NH_4-formate acetonitrile/water 95 :5 (v/v)		
Gradient	**%A**	**Flow [mL/min]**	**Time [min]**
	2	0.4	0
	2	0.4	2.5
	80	0.4	5
	80	0.4	11
	2	0.4	11.1
	2	0.4	18
Injection volume	5 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion* + one level at the reporting limit		
Acquired mass transitions	**Compound**	**Mass Transitions (m/z)**	
	Amitrole	85/43, 85/57, 85/58	
	Amitrole-^{15}N (IL-IS)	86/43	
	Amitrole-$^{15}N_2$,$^{13}C_2$ (IL-IS)	89/44	
	Chlormequat	122/58, 122/63, 124/58	
	Chlormequat-D_4 (IL-IS)	126/58	
	Mepiquat	114/98, 114/58	
	Mepiquat-D_3 (IL-IS)	117/101	
	Daminozide	161/143, 161/61, 161/101 , 161/115, 161/44	
	Daminozide-$^{13}C_4$ (IL-IS)	165/147, 165/44	
	Daminozide-D_6 (IL-IS)	167/149, 165/97	
	Cyromazine	167/68, 167/125, 167/85, 167/108,	
	Cyromazine-D_4 (IL-IS)	171/86, 171/68	
	ETU (Ethylenethiourea)	103/44, 103/60, 103/86	
	ETU-D_4 (IL-IS)	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, 126/74	
	*PTU-D6 - N,N'-(1,3-Propylene)thiourea -D6)***	*(123/64)*	
	Trimethylsulfonium	77/62, 77/47	
	Trimethylsulfonium-D_9 (IL-IS)	86/68, 86/50	
	Difenzoquat	249/77, 249/130, 249/193	
	No IL-IS currently available	-	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also **Table 2**).
** 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).

Hints on Method 3

1. **For Paraquat, Diquat, Trimethylsulfonium and N,N-Dimethylhydrazine better run Method 4 (5.6.14)**
2. **For general hints on analytes:** See 5.6.1

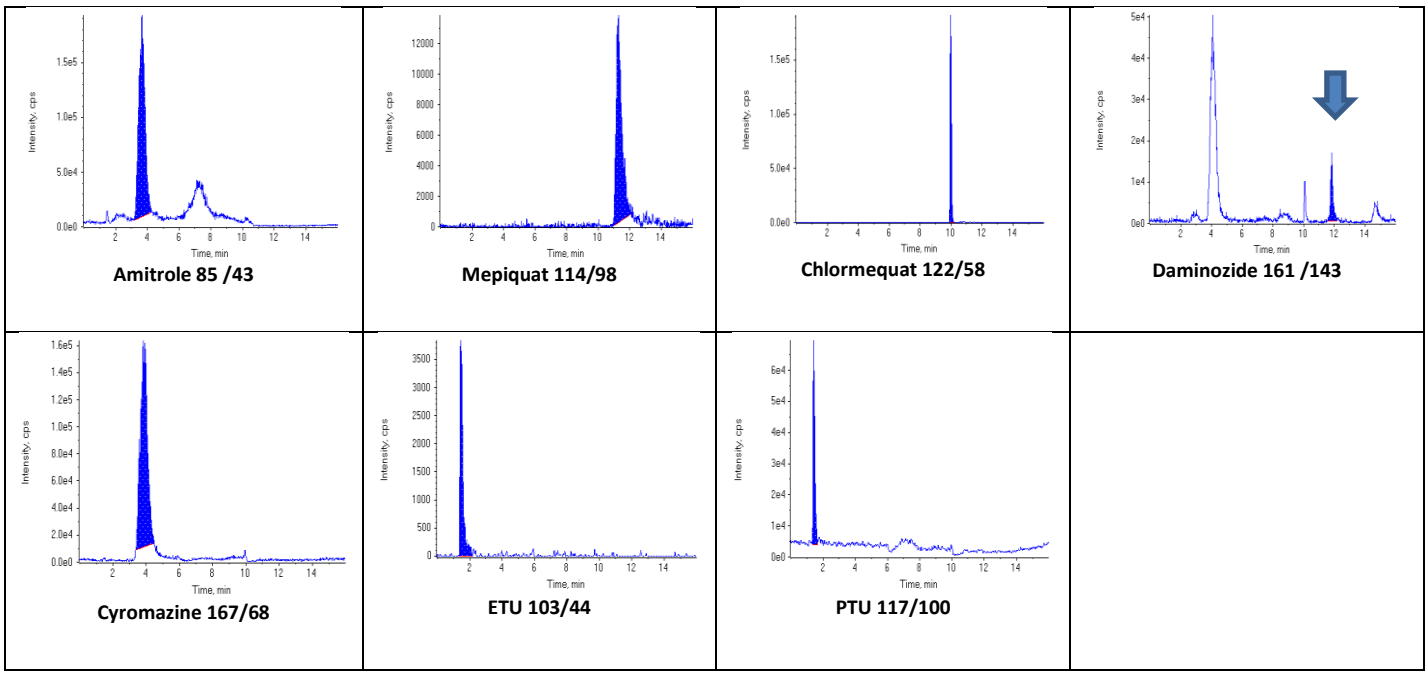


Figure 21: *Typical chromatograms of Amitrole, Chlormequat, Mepiquat, Daminozide, ETU, PTU and Cyromazine in apple extract at 0.01 mg/kg*

5.6.14.Method 4.1 (M4.1): "Quats&Co. Obelisc R"

Table 30: *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! Alternative eluent A: 50 mmol NH_4formate in water (adjust to pH 3 with formic acid). This eluent components is also used in M 4.2 "Quats & Co BEH Amide"		
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	92/84, 183/157, 92/157, ~~184/156, 184/128~~	
	Diquat-D_4 (IL-IS)	188/160 (this IL-IS showed problems with stability)	
	Diquat-D_8 (IL-IS)	96/88, 191/165	
	Paraquat	186/171, 93/171, 93/77, ~~171/77, 171/155~~	
	Paraquat-D_8 (IL-IS)	194/179, 97/179	
	Chlormequat	122/58, 122/63, 124/58	
	Chlormequat-D_4 (IL-IS)	126/58	
	Mepiquat	114/98, 114/58	
	Mepiquat-D_3 (IL-IS)	117/101	
	Daminozide	161/143, 161/61, 161/101 , 161/115, 161/44	
	Daminozide-$^{13}C_4$ (IL-IS)	165/147	
	Daminozide-D_6 (IL-IS)	167/149	
	N,N-Dimethylhydrazine	61/44, 61/45	
	N,N-Dimethylhydrazine-D_6 (IL-IS)	67/49	
	Cyromazine	167/68, 167/125, 167/85, 167/108,	
	Cyromazine-D_4 (IL-IS)	171/86	
	Trimethylsulfonium	77/62, 77/47	
	Trimethylsulfonium-D_9 (IL-IS)	86/68	
	Nereistoxin	150/105, 150/61, 150/71	
	Nereistoxin-D_6 (IL-IS)	156/105	
	Difenzoquat	249/77, 249/130, 249/193	
	No IL-IS currently available	-	
	Melamine	127/85, 127/68, (127/60)	
	Melamine-$^{15}N_3$ (IL-IS)	130/87	
	Propamocarb	189/144, 189/102, 189/74	
	Propamocarb-D_7 (IL-IS)	196/103	

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

Hints on Method 4.1

1. **For Morpholin, DEA and TEA better run Method 7 (5.6.18).** As DEA converts to Morpholine in the ion source, chromatogr. separation is paramount. With Method 4.1 **(5.6.14)** these two peaks do not sufficiently separate.
2. **Diquat and Paraquat require special extraction conditions** (see **5.2.3-B)**
3. **For general hints on analytes:** See 5.6.1

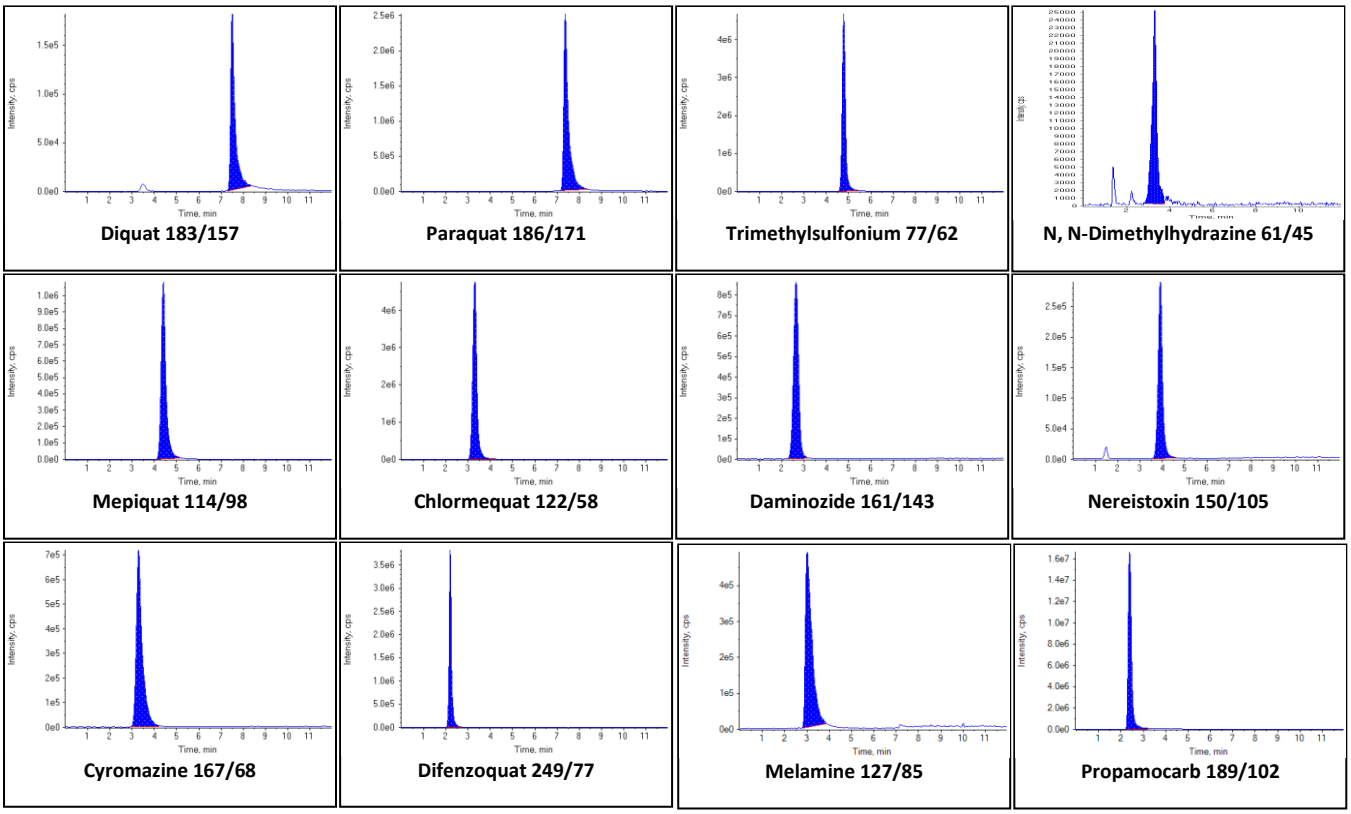


Figure 22: *Typical chromatograms of Diquat, Paraquat, Chlormequat, Mepiquat, Daminozide, N,N-Dimethylhydrazine, Trimethylsulfonium, Cyromazine, Nereistoxin, Difenzoquat, Melamine and Propamocarb in apple extract at 0.1 mg/kg.*

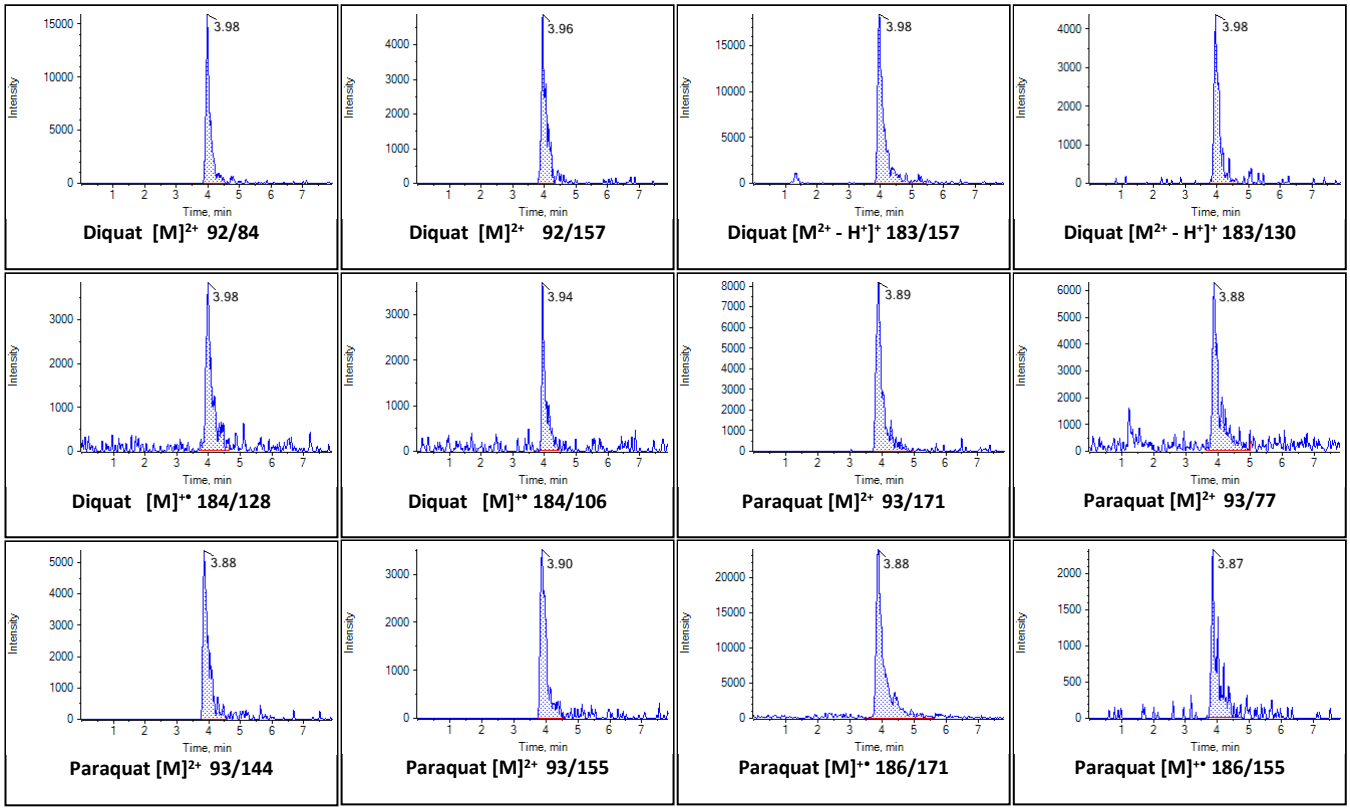


Figure 23: *Typical chromatograms of Diquat and Paraquat in rice 0.005 µg/mL final extract (corresponding to 0,04 mg/kg)*

5.6.15. **Method 4.2 (M4.2): "Quats&Co. BEH Amide"**

Table 31: *Proposed LC-MS/MS conditions for ESI-pos. compounds listed in the table below.*

Instrument parameters	Conditions		
Ionisation mode	ESI pos.		
Column/temperature	BEH Amide 2.1 x 100mm 1.7 µm (P/N: 186004801); 40°C		
Pre-column / Pre-filters	BEH Amide 1.7 µm (P/N: 186004799) / e.g. Supelco column saver 2.0 µm Filter		
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 (0.5 µL for Waters Xevo TQ-Sµ)		
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, 214/68	
	Amitrole	85/43, 85/57, 85/58	
	Amitrole-^{15}N (IL-IS)	86/43	
	Amitrole-$^{15}N_2$ $^{13}C_2$ (IL-IS)	89/44	
	Chlormequat	122/58, 124/58, 122/63, 122/59, 124/59	
	Chlormequat-D_4 (IL-IS)	126/58; 126/59	
	Chloridazon-desphenyl	146/117, 146/101, 146/66, 148/119	
	Chloridazon-desphenyl-$^{15}N_2$ (IL-IS)	148/117, 148/102	
	Cyromazine	167/68, 167/125, 167/108, 167/85, 167/60	
	Cyromazine-D_4 (IL-IS)	171/86, 171/68	
	Daminozide	161/143, 161/61, 161/101, 161/115, 161/44	
	Daminozide-$^{13}C_4$ (IL-IS)	165/147, 165/44	
	Daminozide-D_6 (IL-IS)	167/149, 165/97	
	Diethanolamine (DEA)	106/88, 106/70, 106/45	
	Diethanolamine-D_4 (IL-IS)	110/92	
	Difenzoquat	249/130, 249/77, 249/193,	
	Diquat:***	92/84, 92/157, 183/157	
	Diquat D_8 (IL-IS)	96/89, 191/165	
	ETU (Ethylenethiourea)	103/60, 103/44, 103/86	
	ETU-D_4 (IS)	107/48	
	Melamine	127/85, 127/68, (127/60)	
	Melamine-$^{15}N_3$ (IL-IS)	130/87, 130/44	
	Maleic Hydrazide	113/67, 113/40	
	Maleic Hydrazide D2	115/69, 115/87	
	Maleic Hydrazide-$^{13}C_4$ (IL-IS)	115/87, 115/58	
	Matrine	249/148, 249/150, 249/110, 249/55	
	Matrine D_3	252/148, 252/150, 252/96	
	Mepiquat	114/98, 114/58	
	Mepiquat-D_3 (IL-IS)	117/101	
	Mepiquat-4-hydroxy	130/58, 130/96, 130/114	
	Morpholine	88/70, 88/45, 88/44	
	Morpholine-D_8 (IL-IS)	96/78, 96/46	
	Nereistoxin:	150/105, 150/61, 150/71, 150/72	
	Nereistoxin-D_6 (IL-IS):	156/105, 156/61	
	Nicotine	163/130, 163/132, 163/84, 163/106	
	Nicotine D_4	167/84	
	Oxymatrine	265/247, 265/205, 265/148, 265/136	
	Oxymatrine D_3	268/250, 268/208	
	Paraquat***	93/171, 93/85, 185/170	
	Paraquat D_8 (IL-IS)	97/179, 193/178	
	Propamocarb:	189/144, 189/74, 189/102	
	Propamocarb-D_7 (IL-IS)	196/103, 196/75	
	Propamocarb-N-desmethyl	175/102, 175/144, 175/74, 175/116	
	Propamocarb-N-oxide:	205/102, 205/144, 205/74	
	PTU - N,N'-(1,2-Propylene)thiourea)**	117/100, 117/58, 117/60, 117/72, 117/41	
	PTU-D_6 - N,N'-(1,2-Propylene)thiourea –D_6**	123/64, 123/74	
	Triethanolamine (TEA)	150/132, 150/70, 150/88	
	Triethanolamine-D_{12} (IL-IS)	162/144	
	Trimethylsulfonium	77/62, 77/47	
	Trimethylsulfonium-D_9 (IL-IS)	86/68, 86/50	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also **Table 2**).
**See comments on PTU under M3 (5.6.13).
*** Diquat and paraquat were only measured on the Waters Xevo TQ-Sµ

Hints on Method 4.2

1. As the signals of **Morpholin, DEA and TEA better run Method 7 (5.6.18): 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.
2. **Diquat and Paraquat require special extraction conditions** (see **5.2.3-B).** 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
3. **For general hints on analytes:** See 5.6.1

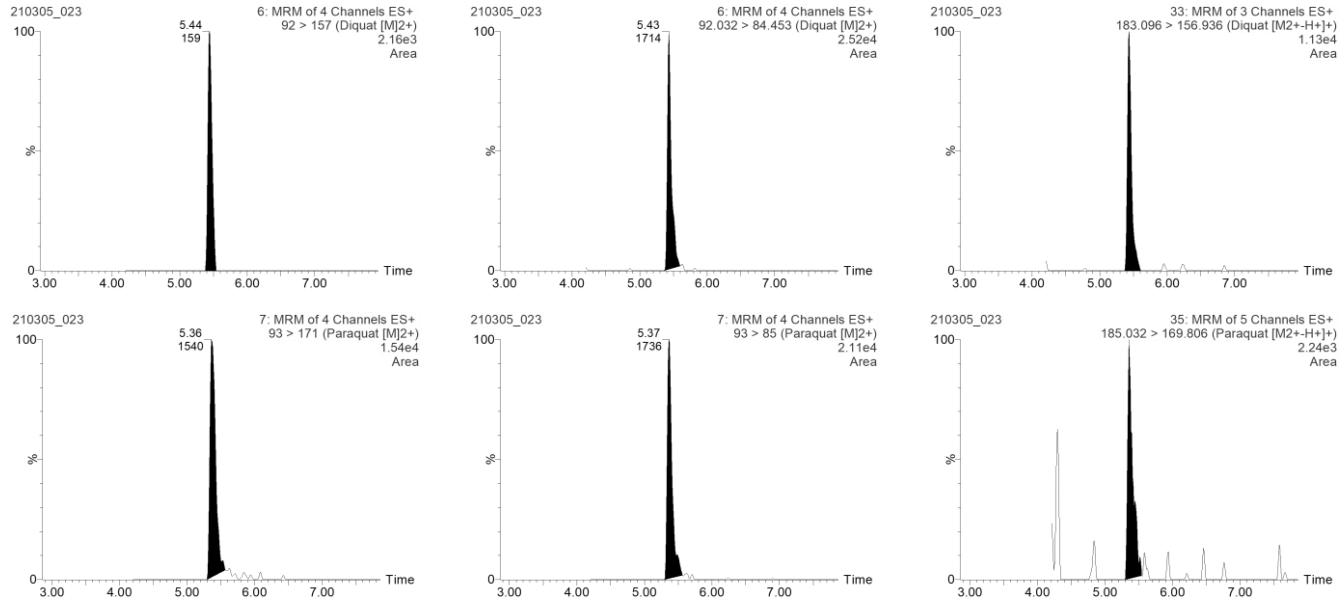


Figure 24: *Typical chromatograms of Diquat and Paraquat in sesame extracts spiked at 0.05 mg/kg using Waters Xevo TQ-Sμ.*

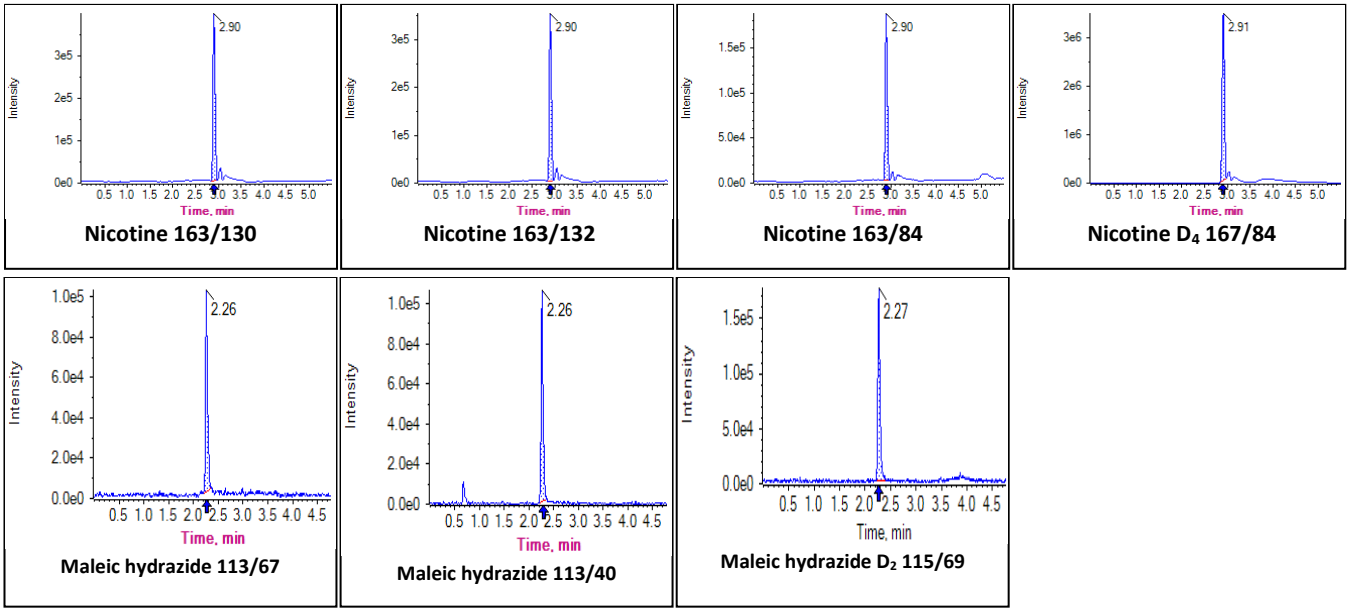


Figure 25: *Exemplary chromatograms of nicotine in flour (spelt, whole-grain) Maleic hydrazide in solvent. Nicotine at 0.01 mg/kg (0.005 µg/mL); nicotine D_4 at 0.1 mg/kg (0.05 µg/mL); Maleic hydrazide and Maleic hydrazide D_2 at 0.2 mg/kg (0.1 µg/mL).*

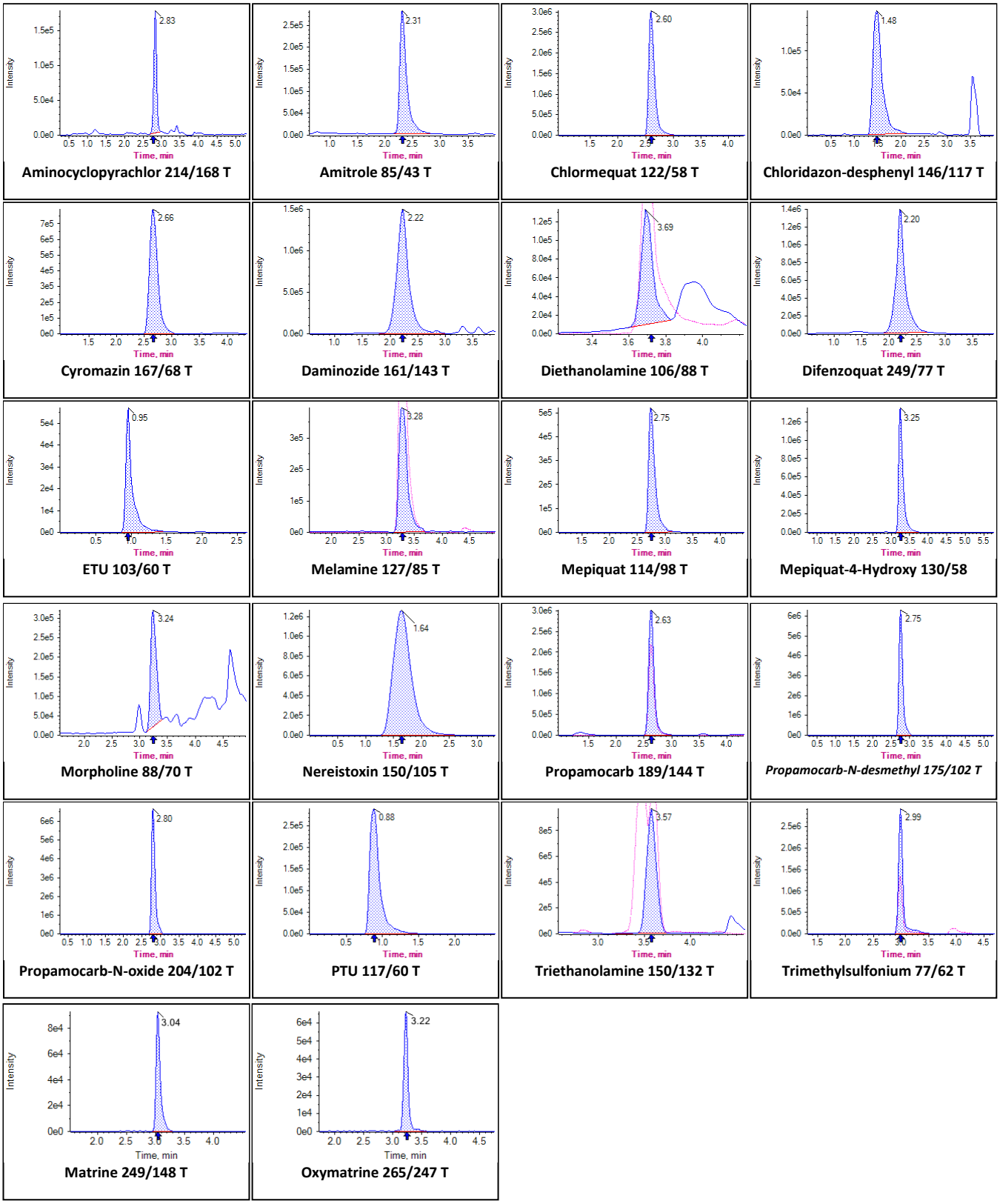


Figure 26: *Typical chromatograms of Aminocyclopyrachlor, Amitrole, Chlormequat, Chloridazon-desphenyl, Cyromazine, Daminozide, Diethanolamine, Difenzoquat, ETU, Melamine, Mepiquat, Mepiquat-4-hydroxy, Morpholine, Nereistoxin, Propamocarb, Propamocarb-N-desmethyl, Propamocarb-N-oxide, PTU, Triethanolamine, Trimesium (Trimethylsulfonium) in tomato extracts spiked at 0.05 mg/kg; additionally Matrine and Oxymatrine at 0.01mg/kg in grape extract.*

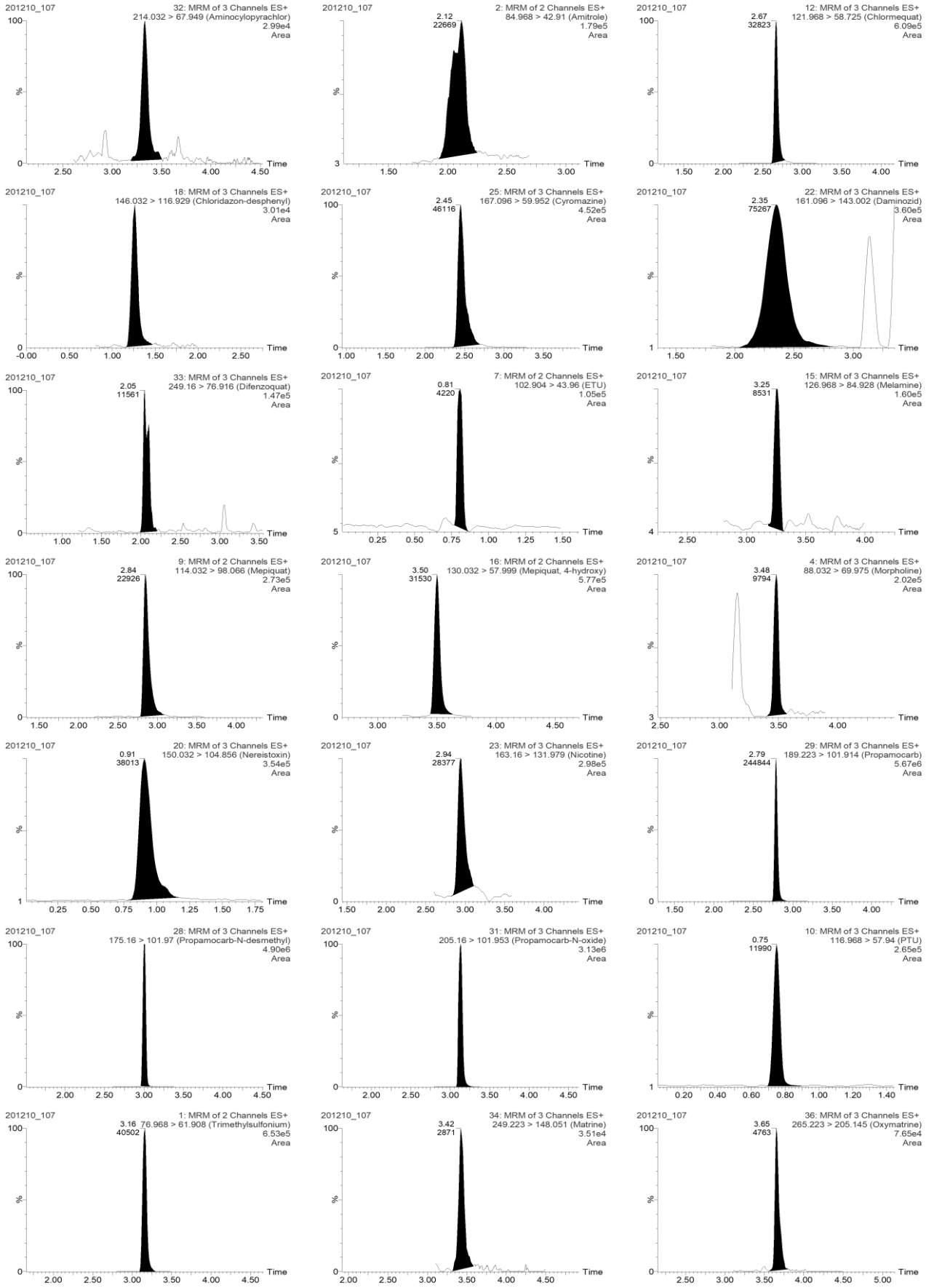


Figure 27: *Typical chromatograms of Aminocyclopyrachlor, Amitrole, Chlormequat, Chloridazon-desphenyl, Cyromazine, Daminozide, Diethanolamine, Difenzoquat, ETU, Melamine, Mepiquat, Mepiquat-4-hydroxy, Morpholine, Nereistoxin, Propamocarb, Propamocarb-N-desmethyl, Propamocarb-N-oxide, PTU, Triethanolamine, Trimesium (Trimethylsulfonium) in tomato extracts spiked at 0.06 mg/kg.*

5.6.16. **Method 5 (M5): "Quats&Co. MonoChrom MS"**

Table 32: *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 (IL-IS)	126/58	
	Mepiquat	114/98, 114/58	
	Mepiquat-D_3 (IL-IS)	117/101	
	Difenzoquat:	249/77, 249/130, 249/193	
	No IS currently available	-	
	ETU (Ethylenethiourea)	103/44, 103/60, 103/86	
	ETU-D_4 (IL-IS)	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, 123/74	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also **Table 2**).
** See comments on PTU under M 3 (**5.6.13**).

Hints on Method 5

1. **For general hints on analytes:** See 5.6.1
2. 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

5.6.17. **Method 6 (M6): "Streptomycin and Kasugamycin"**

Table 33: *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	
	No IS currently available	-	
	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 2**).

Hints on Method 6

1. **For general hints on analytes:** See 5.6.1
2. **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)

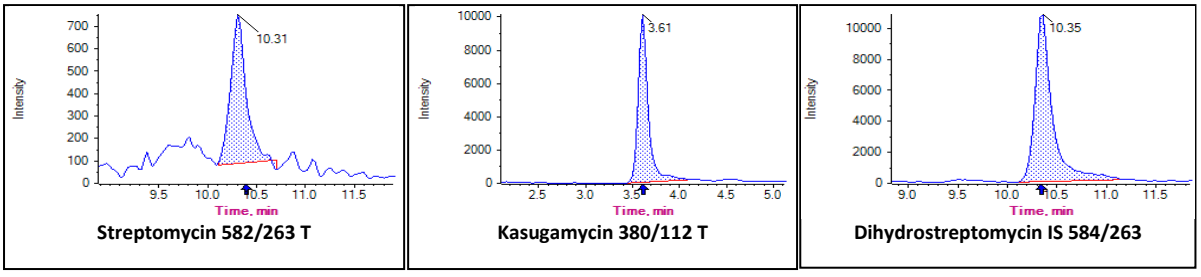


Figure 28: *Typical chromatograms of Streptomycin and Kasugamycin in apple extracts spiked at 0.01 mg/kg.*

5.6.18. **Method 7 (M7): "Morpholine, Diethanolamine and Triethanolamine"**

Table 34: *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, 96/46	
	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 2**).

Hints on Method 7

1. **For general hints on analytes:** See 5.6.1
2. **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**)

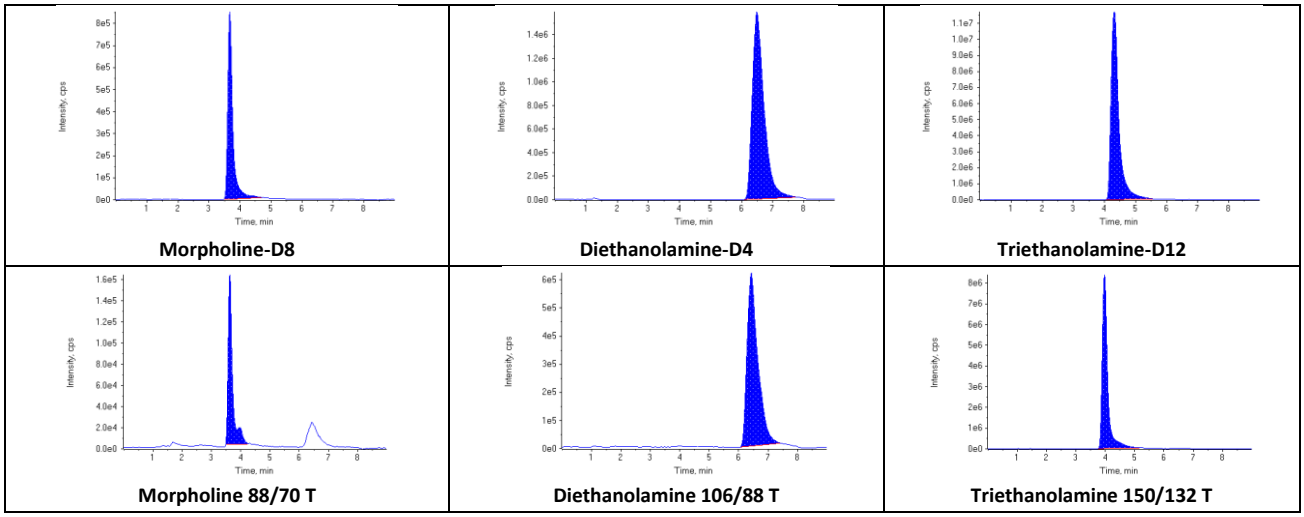


Figure 29: *Typical chromatograms of Morpholine, Diethanolamine and Triethanolamine in apple extracts at 0.05 mg/kg (extract were diluted 10-fold before injection)*

5.6.19.Method 8 (M8): "Triazole derivative metabolites (TDMs)"

Table 35: *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

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also **Table 2**).
** 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

Hints on Method 8

1. **For general hints on analytes:** See 5.6.1
2. **1,2,4-Triazole is used as nitrification inhibitors in fertilizers**
3. **The following commercially available isotopically labelled components were not tested with this method:** 1,2,4-Triazole-D_2, 1, 2, 4-Triazole-acetic acid-D_2, 1, 2, 4-Triazole-alanine-D_2, 1, 2, 4-Triazole-lactic acid-D_2

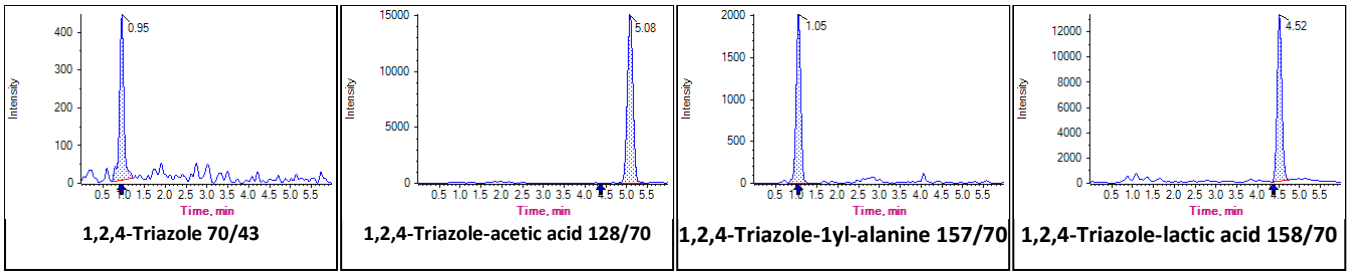


Figure 30: *Typical chromatograms of TDMs in avocado extracts spiked at 0.01 mg/kg.*

5.6.20. **Method 9 (M9): "Difluoroacetic acid and Trifluoroacetic acid"**

Table 36: *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 -$^{13}C_2$ (IL-IS)	75/46	-12	3000
	Trifluoroacetic acid (TFA)	113/69, 113/113**	-5.6	2200
	Trifluoroacetic acid -$^{13}C_2$ (IL-IS)	115/70	-5.5	2300

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also **Table 2**).
** 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

Hints on Method 9

1. **For general hints on analytes:** See 5.6.1

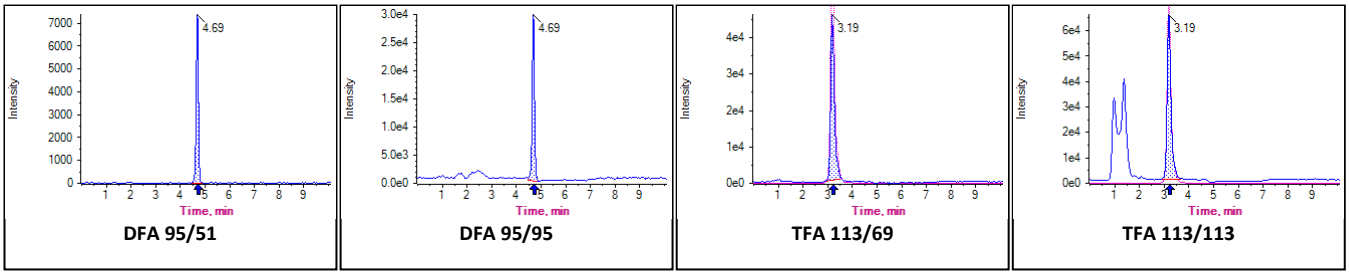


Figure 31: *Typical chromatograms of DFA and TFA in tomato extracts spiked at 0.05 mg/kg*

5.6.21. Method 10 (M10): "Triazole derivative metabolites (TDMs) on Torus DEA"

Table 37: *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	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-filter	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	5
	10	0.5	5.5
	10	0.5	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)**	
	Triazole-alanine:	157/88, 157/70, 157/42	
	Triazole-alanine-$^{13}C_2$,$^{15}N_3$ (IS):	162/75	
	Triazole-alanine D_2:	159/42	
	Triazole-acetic acid:	128/70, 128/43, 128/73	
	Triazole-acetic acid-$^{13}C_2$,$^{15}N_3$ (IS):	133/75	
	Triazole-acetic acid D_2:	130/72	
	Triazole-lactic acid:	158/70, 158/43, 158/112	
	Triazole-lactic acid-$^{13}C_2$,$^{15}N_3$ (IS):	163/75	
	Triazole-lactic acid D_2:	160/72	

* One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also **Table 2**).

Hints on Method 10

1. **For general hints on analytes:** See 5.6.1
2. **1,2,4-Triazole is measured by M8:** See 5.6.19

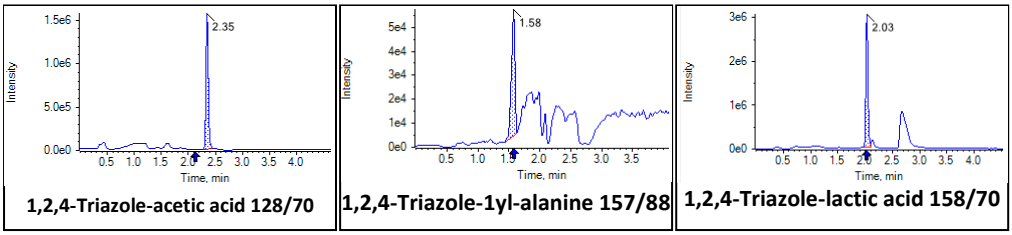


Figure 32: *Typical chromatograms of TDMs in strawberry extracts spiked at 0.05 mg/kg.*

5.6.22. Method 11 (M11): "Gly&Co. by IC on AS19"[3]

Table 38: *Proposed IC-MS/MS conditions for Glyphosate, AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, Fosetyl-Al, Cyanuric acid, Bromide, Chlorate, Perchlorate, Phosphonic acid and TFA*

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Thermo Scientific™ Dionex™ IonPac™ AS19,2x250mm; 32°C		
Pre-column	Thermo Scientific™ Dionex™ IonPac™ AG19,2x50mm		
Eluent	Thermo Scientific™ Dionex™ EGC 500™ KOH eluent generator cartridge		
Gradient	**c [KOH]**	**Flow [mL/min]**	**Time [min]**
	15	0.3	0
	15	0.3	8
	36	0.3	13
	36	0.3	21
	70	0.3	21.5
	70	0.3	25
	15	0.3	25.5
	15	0.3	30
Injection volume	5 µL of 5-fold diluted extracts in water (preferably ultrapure)		
Flow Make-up Solvent before ion source	0.15 mL/min acetonitrile		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion + one level at the reporting limit; Standard solutions of Fosetyl and Ethephon (and their IL-ISs) may be contaminated with native Phosphonic acid which may potentially lead to false positives or shifted calibration, see 5.6.1.		
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 (IL-IS):	171/63, 171/126	
	AMPA:	110/63, 110/79, 110/81	
	AMPA-^{13}C,^{15}N (IL-IS):	112/63, 112/81	
	N-Acetyl-Glyphosate:	210/63, 210/150, 210/79, 210/148	
	N-Acetyl-Glyphosate-D_3 (IL-IS):	213/63, 213/153	
	Ethephon:	143/107, 143/79, 145/107	
	Ethephon-D_4 (IL-IS):	147/111, 147/79	
	HEPA:	125/79, 125/95, 125/63	
	HEPA-D_4 (IL-IS):	129/79, 129/97	
	Glufosinate:	180/63, 180/136, 180/85, 180/95	
	Glufosinate-D_3 (IL-IS):	183/63, 183/98	
	N-Acetyl-Glufosinate:	222/63, 222/59, 222/136	
	N-Acetyl-Glufosinate-[acetyl]D_3 (IL-IS):	225/63, 225/137	
	N-Acetyl-Glufosinate-[methyl]D_3 (IL-IS):	225/63	
	MPPA:	151/63, 151/107, 151/133	
	MPPA-D_3 (IL-IS):	154/63, 154/136	
	Fosetyl-Al:	109/81, 109/63 (each detected as Fosetyl)	
	Fosetyl-Al-D_{15} (IL-IS):	114/82, 114/63 (each detected as Fosetyl- D_5)	
	Trifluoroacetic acid (TFA)	113/69, 113/113	
	Trifluoroacetic acid -$^{13}C_2$ (IL-IS):	115/70	
	Cyanuric acid:	128/42, 128/85	
	Cyanuric acid-$^{13}C_3$:	131/43, 131/87	
	Bromide:	81/81, 79/79	
	Chlorate:	83/67, 85/69	
	Chlorate-$^{18}O_3$ (IL-IS):	89/71, 91/73	
	Perchlorate:	99/83, 101/85	
	Perchlorate-$^{18}O_4$ (IL-IS):	107/89, 109/91	
	Phosphonic acid:	81/79, 81/63	
	Phosphonic acid-$^{18}O_3$ (IL-IS):	87/85, 87/67	

Hints on Method 11

1. **For general hints on analytes:** See 5.6.1

[3] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EPRW%202020%20-%20PD87.pdf

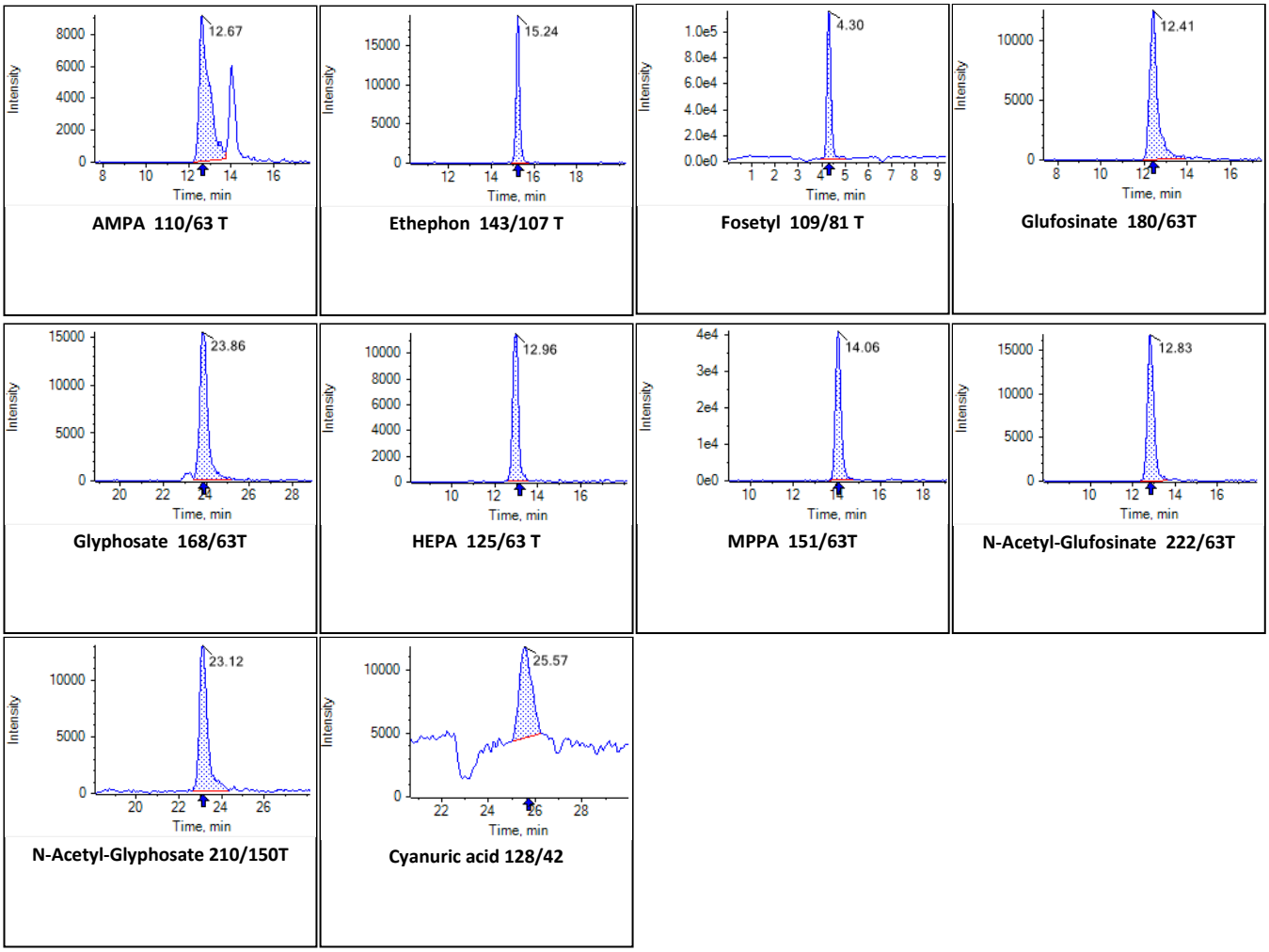


Figure 33: *Typical chromatograms in cucumber extracts spiked at 0.05 mg/kg.*

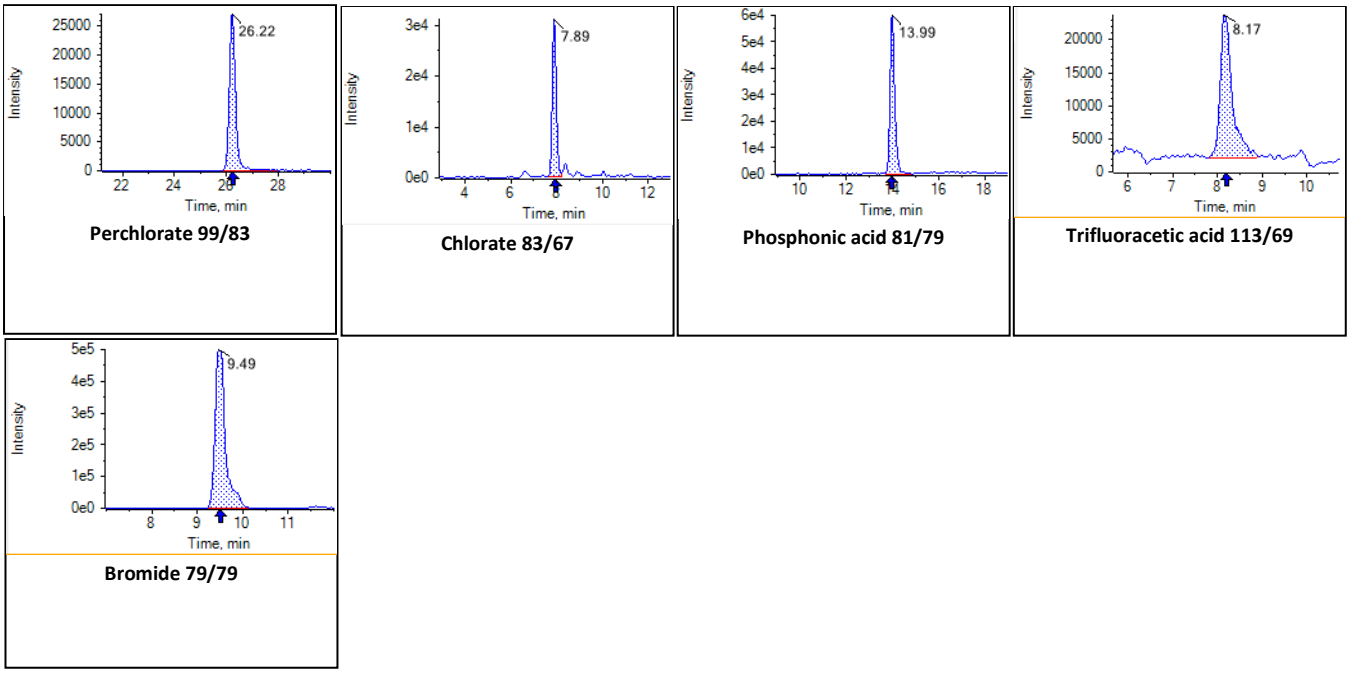


Figure 34: *Typical chromatograms in milk extracts spiked at 0.01 mg/kg (Perchlorate and Chlorate); at 0.05 mg/kg (Phosphonic acid); 0.025 mg/kg (Trifluoracetic acid) and 5 mg/kg (Bromide).*

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) (13**5.5**) (m_{IS}^{sample} / $m_{IS}^{cal\ mix}$) is known and constant. By keeping the IS constant throughout the calibration levels the peak ratio $PR^{cal\ mix}$ ($A_{pest}^{cal\ mix}$/ $A_{IS}^{cal\ mix}$) of each calibration level can be plotted against the absolute mass of the pesticide $m_{pest}^{cal\ mix}$ rather than the ratio $m_{pest}^{cal\ mix}$ / $m_{IS}^{cal\ mix}$(the $m_{IS}^{cal\ mix}$ is set as 1).

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

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

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

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

Reasonably (but not necessarily) the calibration standards should be prepared in such a way that the ratio m_{IS}^{sample} / $m_{IS}^{cal\ mix}$ equals the assumed volume ratio of sample extract versus calibration standard (20 for most samples and 40 for cereals, pulses, nuts and oily seeds). The absolute masses of the IS-WS I and II do not need to be necessarily known (see also the notes of **Table 2**).

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 35,** the total volume can be assumed to be exactly 20 mL. 1 mL sample extract will correspond to 1/20th of the test portion (m_a) in case of most samples (or to 1/40th. in case of cereals, pulses, nuts and oily seeds, where extracts are diluted 2-fold during cleanup). 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 $m_{IS}^{aliquot} = m_{IS}^{sample} \times V_{aliquot}/20$ (or $m_{IS}^{aliquot} = m_{IS}^{sample} \times V_{aliquot}/40$ in case of cereals, pulses, nuts and oily seeds), with m_{IS}^{sample} being the IS mass that would have been added to the entire sample portion according to **5.2.2** and **Table 35**.

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 ($m_{aliquot} = m_a \times V_{aliquot}/20$ or $m_{aliquot} = m_a \times V_{aliquot}/40$ in case of a 2-fold dilution during cleanup).

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

Variables used

Mass of pesticide in calibration mixture	$m_{pest}^{cal\ mix}$	µg
Mass of pesticide in final extract	m_{pest}^{sample}	µg
Mass of internal standard in calibration mixture	$m_{ISTD}^{cal\ mix}$	µg
Mass of internal standard added to test portion (sample)	m_{ISTD}^{sample}	µg

Mass of internal standard added to aliquot of sample extract	$m_{ISTD}^{aliquot}$	µg
Volume of sample extract aliquot used (5.7.1 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 or 40 mL in case of a 2-fold dilution during cleanup).

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

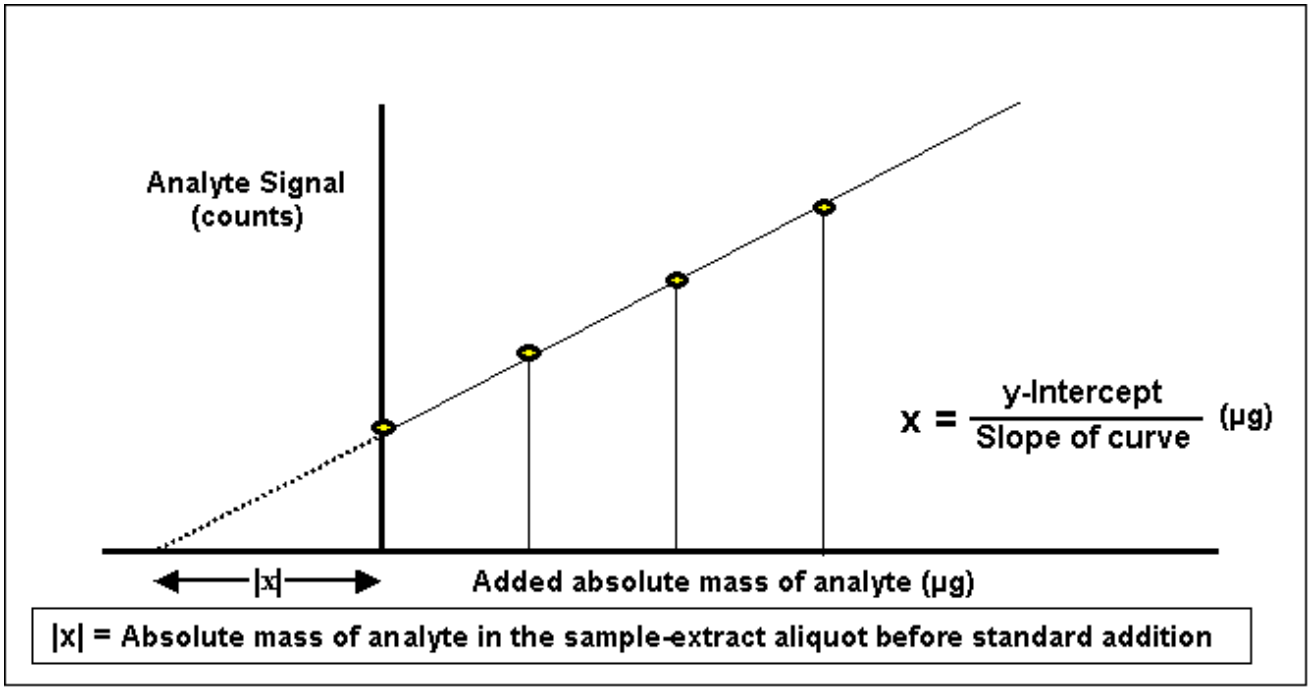


Figure 35: *Internal calibration using the procedure of standard additions, schematically*

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. Stability and purity of standards

A general overview regarding the stability of Glyphosate & Co. compounds in stock solutions is given in **Table 39**. 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 43**.

In case of Ethephon (native compound or IL-IS), 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 IL-ISs (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 36**. The stability in stock solutions is generally better that in working solutions.

Table 39: *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)

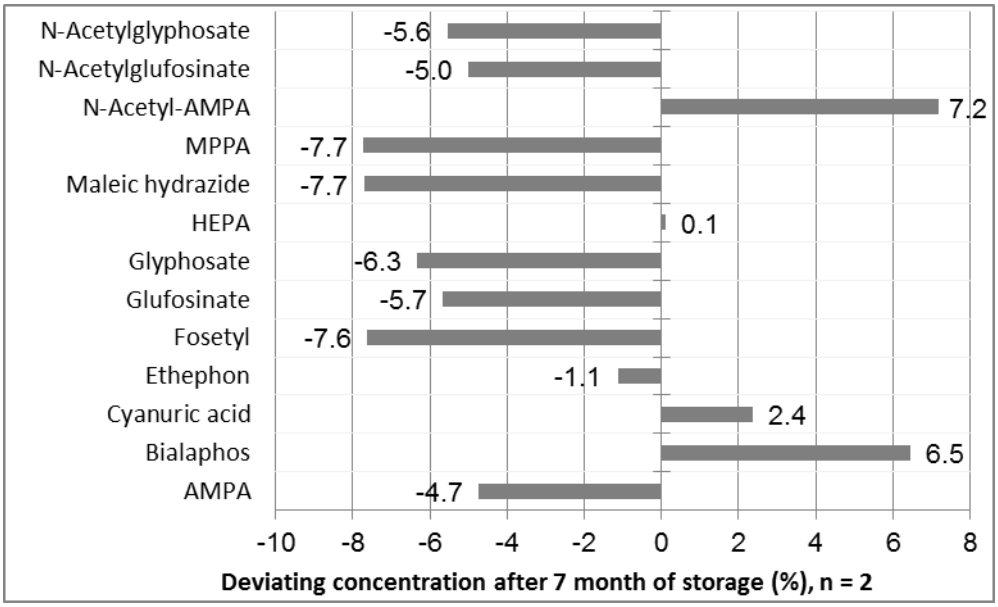


Figure 36: *Deviations of the concentration of Glyphosate & Co. compounds in a working solution of 10 µg/mL water containing 10 % acetonitrile and 1‰ HCl conc. (v/v), following 7 months of storage at 6 °C. Compared against a freshly prepared standard of same composition*

Issues concerning the purity of N-Acetyl-Glufosinate D_3: There is two types of N-Acetyl-Glufosinate D_3 standards on the market. Both contain the three deuterium atoms on a methyl group, but the first one contains them on the methyl group of the acetyl moiety and the other one on the methyl group that is attached to the phosphorus atom. In theory the acetyl group can be hydrolytically detached, native glufosinate may be formed in working solutions of N-Acetyl-Glufosinate (acetyl-D_3), leading to false positive results. Fortunately the degradation rate observed in the water:acetonitrile 9:1 mixture (see Figure 36) was negligible. More important is the content of native glufosinate in purchased N-Acetyl-Glufosinate (acetyl-D_3) standards. Before first use the standards should be checked for the presence of native glufosinate impurities and object the product if it does not meet the producer's specifications. The levels of native glufosinate impurities depend on the manufacturer and the badge. Where e.g. 0.5 µg IL-IS is added to 1 g sample, the presence of 2% native glufosinate (a typical level encountered) can lead to glufosinate levels of 0.01 mg/kg.

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 40.**

Table 40: *Overview of lowest successfully validated levels per matrix*

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
M1.3	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
	AMPA	Dry (cereals)*	Rice	0,1	5	99	6
	AMPA	Dry (pulses)*	Soybean	0,1	5	106	6
	Cyanuric Acid	High water content	Cucumber	0.02	3	106	13
	Cyanuric Acid	Dry (cereals)*	Rice	0,1	5	105	5
	Cyanuric Acid	Dry (pulses)*	Soybean	0,1	5	115	12
	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
	Ethephon	Dry (cereals)*	Rice	0,02	5	92	8
	Ethephon	Dry (pulses)*	Soybean	0,02	5	107	11
	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
	Fosetyl	Dry (cereals)*	Rice	0,02	5	91	3
	Fosetyl	Dry (pulses)*	Soybean	0,02	5	96	4
	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
	Glufosinate	Dry (cereals)*	Rice	0,06	5	96	5
	Glufosinate	Dry (pulses)*	Soybean	0,06	5	105	8
	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
	Glyphosate	Dry (cereals)*	Rice	0,1	5	91	6
	Glyphosate	Dry (pulses)*	Soybean	0,06	5	99	5
	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
	HEPA	Dry (cereals)*	Rice	0,04	5	98	1
	HEPA	Dry (pulses)*	Soybean	0,04	5	93	6
	Maleic Hydrazide	Dry (cereals)	Barley	0.02	5	100	9

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	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
	Maleic Hydrazide	Dry (cereals)*	Rice	0,08	5	96	8
	Maleic Hydrazide	Dry (pulses)*	Soybean	0,08	5	97	14
	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
	MPPA	Dry (cereals)*	Rice	0,04	5	97	3
	MPPA	Dry (pulses)*	Soybean	0,04	5	101	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
	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 Glufosinate	Dry (cereals)*	Rice	0,04	5	99	2
	N-Acetyl Glufosinate	Dry (pulses)*	Soybean	0,04	5	98	3
	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
	N-Acetyl Glyphosate	Dry (cereals)*	Rice	0,1	5	94	2
	N-Acetyl Glyphosate	Dry (pulses)*	Soybean	0,1	5	101	3
M1.4	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
M1.5	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

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	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
	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
M1.6a: Torus DEA	AMPA	High water content	Cucumber	0.02	5	99	7
	AMPA	Dry (pulses)*	Lentil	0.1	5	107	10
	Ethephon	High water content	Cucumber	0.02	5	95	5
	Ethephon	Dry (pulses)*	Lentil	0.1	5	89	12
	Fosetyl	High water content	Cucumber	0.02	5	98	3
	Fosetyl	Dry (pulses)*	Lentil	0.1	5	104	3
	Glufosinat	High water content	Cucumber	0.02	5	95	4
	Glufosinat	Dry (pulses)*	Lentil	0.1	5	82	8
	Glyphosat	High water content	Cucumber	0.02	5	107	3
	Glyphosat	Dry (pulses)*	Lentil	0.1	5	115	9
	HEPA	High water content	Cucumber	0.02	5	108	8
	HEPA	Dry (pulses)*	Lentil	0.1	5	100	5
	MPPA	High water content	Cucumber	0.02	5	103	3
	MPPA	Dry (pulses)*	Lentil	0.1	5	108	5
	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-Glufosinat	Dry (pulses)*	Lentil	0.1	5	113	4
	N-Acetyl-Glyphosat	High water content	Cucumber	0.02	5	98	2
	N-Acetyl-Glyphosat	Dry (pulses)*	Lentil	0.1	5	101	8
M1.6b: APPC	AMPA	High water content + acidic	Strawberry	0.05	5	106	6
	Bromide (inorg.)	High water content + acidic	Lemon	5	5	101	6
	Ethephon	High water content + acidic	Strawberry	0.01	5	105	2
	Ethephon	Dry (oily seeds)*	Soybean	0.02	5	110	10
	Fosetyl	High water content + acidic	Strawberry	0.01	5	99	1
	Fosetyl	Dry (oily seeds)*	Soybean	0.02	5	98	3
	Glufosinate	High water content + acidic	Strawberry	0.03	5	98	10
	Glufosinate	Dry (oily seeds)*	Soybean	0.06	5	96	6
	Glyphosate	High water content + acidic	Strawberry	0.05	5	104	8

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	Glyphosate	Dry (oily seeds)*	Soybean	0.1	5	101	5
	HEPA	High water content + acidic	Strawberry	0.02	5	107	8
	HEPA	Dry (oily seeds)*	Soybean	0.04	5	99	4
	MPPA	High water content + acidic	Strawberry	0.02	5	96	13
	MPPA	Dry (oily seeds)*	Soybean	0.04	5	92	13
	N-Acetyl-Glufosinate	High water content + acidic	Strawberry	0.02	5	100	6
	N-Acetyl-Glufosinate	Dry (oily seeds)*	Soybean	0.04	5	92	5
	N-Acetyl-Glyphosate	High water content + acidic	Strawberry	0.05	5	97	1
	N-Acetyl-Glyphosate	Dry (oily seeds)*	Soybean	0.1	5	95	5
	Phosphonic Acid	High water content + acidic	Lemon	0.05	5	99	3
	Phosphonic Acid	Dry (oily seeds)*	Sesame	0.2	5	99	7
M1.7a: Torus DEA	Bromide (inorg.)	High water content + acidic	Lemon	5	5	99	2.1
	Chlorate	High water content + acidic	Lemon	0.03	5	100	5.6
	Perchlorate	High water content + acidic	Lemon	0.01	5	112	2.6
	Phosphonic acid	High water content + acidic	Lemon	0.05	5	100	4.6
M1.7b: APPC	Bromide (inorg.)	High water content + acidic	Lemon	5	5	100	3
	Bromide (inorg.)	Dry (oily seeds)*	Sesame	20	5	81	1
	Chlorate	High water content + acidic	Lemon	0.03	5	115	6
	Chlorate	Dry (oily seeds)*	Sesame	0.12	5	91	6
	Perchlorate	High water content + acidic	Lemon	0.01	5	104	14
	Phosphonic acid	High water content + acidic	Lemon	0.05	5	100	8
	Phosphonic acid	Dry (oily seeds)*	Sesame	0.2	5	102	18
M1.8	Chlorate	High water content + acidic	Lemon	0.03	5	99	4
	Chlorate	Dry (oily seeds)*	Sesame	0.12	5	102	6
	Cyanuric Acid	High water content + acidic	Lemon	0.05	5	99	1
	Cyanuric Acid	Dry (oily seeds)*	Sesame	0.2	5	76	2
	Maleic Hydrazide	Dry (oily seeds)*	Sesame	0.2	5	107	16
	Perchlorate	High water content + acidic	Lemon	0.01	5	103	5
	Perchlorate	Dry (oily seeds)*	Sesame	0.04	5	101	6
M1.9	AMPA	Dry (oily seeds)*	Soybean	0.2	5	88	5.9
	Ethephon	Dry (oily seeds)*	Soybean	0.02	5	111	11.7
	Fosetyl	Dry (oily seeds)*	Soybean	0.02	5	108	3.9
	Glufosinat	Dry (oily seeds)*	Soybean	0.06	5	102	4.8
	Glyphosat	Dry (oily seeds)*	Soybean	0.1	5	103	2.0
	HEPA	Dry (oily seeds)*	Soybean	0.04	5	99	5.3
	MPPA	Dry (oily seeds)*	Soybean	0.08	5	94	6.9
	N-Acetyl-Glufosinat	Dry (oily seeds)*	Soybean	0.08	5	96	11.9
	Bromide (inorg.)	High water content + acidic	Lemon	5	5	98	4.1
	Chlorate	High water content + acidic	Lemon	0.03	5	100	1.3
	Perchlorate	High water content + acidic	Lemon	0.02	5	100	14.1
	Phosphonic acid	High water content + acidic	Lemon	0.05	5	95	2.2
M1.10	Fosetyl	High water content + acidic	Strawberry	0.01	5	104	3.2
	Glyphosat	High water content + acidic	Strawberry	0.1	5	101	4.3
	MPPA	High water content + acidic	Strawberry	0.04	5	97	2.7
	N-Acetyl-Glufosinat	High water content + acidic	Strawberry	0.04	5	103	4.6
	N-Acetyl-Glyphosat	High water content + acidic	Strawberry	0.05	5	106	1.8
	Chlorate	High water content + acidic	Lemon	0.03	5	98	8.1
	Perchlorate	High water content + acidic	Lemon	0.01	5	106	8.3
	Phosphonic acid***	High water content + acidic	Lemon	0.05	5	101	5.3
	Trifluoro-acetic acid	High water content + acidic	Lemon	0.025	5	102	9.3
M4.1	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

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	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
	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

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	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
M4.2	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
	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

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	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
	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
	Diquat**	Dry (oily seeds)**	Sesame	0.05	5	105	7
	Paraquat**	Dry (oily seeds)**	Sesame	0.02	5	100	10
M5	See un der http://www.crl-pesticides.eu/library/docs/srm/meth_ChlormequatMepiquat_CrlSrm.pdf						
M6	Kasugamycin	High water content	Apple	0.01	5	98	4
	Streptomycin	High water content	Apple	0.01	10	106	9
M7	Morpholine	High water content	Apple	0.1	5	94	3

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
	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
M8	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						
M9	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

Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
M10							
M11	AMPA	High water content	Cucumber	0.02	5	97	5.9
	Ethephon	High water content	Cucumber	0.02	5	90	5.2
	Fosetyl	High water content	Cucumber	0.02	5	101	3.1
	Glufosinat	High water content	Cucumber	0.02	5	99	4.6
	Glyphosat	High water content	Cucumber	0.02	5	96	2.3
	HEPA	High water content	Cucumber	0.02	5	101	14.5
	MPPA	High water content	Cucumber	0.02	5	103	4.0
	N-Acetyl-Glufosinat	High water content	Cucumber	0.02	5	103	3.0
	N-Acetyl-Glyphosat	High water content	Cucumber	0.02	5	104	2.7
	Bromide (inorg.)	High water content + acidic	Lemon	10	5	110	11.3
	Chlorate	High water content + acidic	Lemon	0.06	5	96	2.7
	Perchlorate	High water content + acidic	Lemon	0.02	5	97	2.0
	Phosphonic acid	High water content + acidic	Lemon	0.1	5	100	4.1
	TFA	High water content + acidic	Lemon	0.05	5	100	1.5

*The extract was prepared according to QuPPe PO V10 or newer versions, so EDTA solution was used during extraction (for oily seeds, nuts pulses and cereals). All other data was generated without using EDTA solution during extraction.

**analysed with Waters Xevo TQ-Sμ

*** spiked separately from co-eluting Fosetyl to avoid interference that affects quantification

Table 41: *Validation data deriving from two QuPPe interlaboratory validation studies organized by the EURL-SRM, Round 1.*

Matrix	Level (mg/kg)	Solvent + IL-IS Calibration			Matrix Matched Calibration			Matrix + IL-IS 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										

Matrix	Level (mg/kg)	Solvent + IL-IS Calibration			Matrix Matched Calibration			Matrix + IL-IS Calibration		
		Mean Recovery (%)	RSD (± %)	No. Labs	Mean Recovery (%)	RSD (± %)	No. Labs	Mean Recovery (%)	RSD (± %)	No. Labs
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
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
	0.2	*114*	*14*	*6*	104	7	6			
Perchlorate										
Carrot	0.01							99	9.3	11
	0.02							99	11.4	11

Matrix	Level (mg/kg)	Solvent + IL-IS Calibration			Matrix Matched Calibration			Matrix + IL-IS Calibration		
		Mean Recovery (%)	RSD (± %)	No. Labs	Mean Recovery (%)	RSD (± %)	No. Labs	Mean Recovery (%)	RSD (± %)	No. Labs
	0.2							96	10.5	10
Lemon	0.01							105	6.4	11
	0.02							104	7.4	11
	0.2							102	5.4	12
Rye flour	0.01							100	8.6	11
	0.02							102	8.7	12
	0.2							101	9.8	14
Avocado	0.01							104	11.5	10
	0.02							105	10.2	11
	0.2							102	8.1	11
Chlorate										
Carrot	0.01							105	11.6	12
	0.02							103	8.5	11
	0.2							100	4.9	10
Lemon	0.01							99	13.4	12
	0.02							104	18.1	11
	0.2							100	5.2	12
Rye flour	0.01							108	13.3	12
	0.02							105	15.9	14
	0.2							101	6.5	13
Avocado	0.01							99	4.1	8
	0.02							102	5.5	8
	0.2							106	8.7	11
Phosphonic acid										
Carrot	0.01							100	18.8	6
	0.02							106	7.2	7
	0.2							108	14.3	6
Lemon	0.01							104	8.0	5
	0.02							101	10.1	5
	0.2							98	8.7	10
Rye flour	0.01							104	20.3	5
	0.02							103	18.1	7
	0.2							105	14.1	11
Avocado	0.01							99	10.2	7
	0.02							105	12.1	8
	0.2							102	7.9	9
Bromide										
Carrot	0.01				103	11.2	7			
	0.02				106	8.7	8			
	0.2				115	17.0	7			
Lemon	0.01				99	10.5	10			
	0.02				94	18.9	10			
	0.2				100	10.6	10			
Rye flour	0.01				82	10.9	9			
	0.02				83	10.3	10			
	0.2				85	34.8	11			
Avocado	0.01				102	15.9	10			
	0.02				102	10.5	11			
	0.2				106	9.6	11			

8. References

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

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

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

Vahl, M. et al. (1998); Analysis of Chlormequat residues in grain using liquid chromatography-mass spectrometry (LC-MS/MS); Fresenius J Anal Chem 361:817-820

9. ANNEX

Table 42: *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 (neutral = protonated)	110.05x3 = 330.14**	Fosetyl-Al	354.10	0.932	1.07
Fosetyl-D_5 (neutral = protonated)	115.08x3=345.23**	Fosetyl-Al D_{15}	369.19	0.935	1.07
	115.08	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.**

** Taking into account that 1 mol fosetyl-Al (MW 354.10) contains 3 mols of fosetyl anion (MW 109.04x3=327,12) leading to 3 mols fosetyl acid (MW 110.05x3=330,14). For the ILIS the following numbers apply 1 mol fosetyl-Al D_{15} (MW 369.19) contains 3 mols of fosetyl D_5 anion (MW 114.07x3=342.21) leading to 3 mols fosetyl D_5 acid (MW 115.08*3=345.23)

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

Table 43: *Exemplary concentrations of pesticide stock and working solutions (3.18 and 3.19), (solvent proposals also apply to IL-IS, see 3.21, 3.22, 3.23). Prefereably use plastic vials (e.g. PP) as many of the compounds 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	10 % ACN in water	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	ACN	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
Matrine	ACN	1	ACN	10 / 1 / 0.1
Maleic Hydrazide	MeOH	1	10 % ACN in water	10 / 1 / 0.1
Melamine	MeOH:water (90:10)	1	MeOH	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
Oxymatrine	ACN	1	ACN	10 / 1 / 0.1
Paraquat**	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0.1
Perchlorate	10 % ACN in water	1	MeOH	10 / 1 / 0.1 / 0.01
Phosphonic acid*	10 % ACN in water	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	ACN	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
Triazole	MeOH	1	MeOH	10 / 1 / 0.1
Triazole-lactic acid	MeOH	1	MeOH	10 / 1 / 0.1
Triazole-acetic acid	MeOH	1	MeOH	10 / 1 / 0.1
Triazole-alanine	MeOH/Water (1:3)	1	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 IL-IS) is also suitable for the working solution. 10% ACN will reduce growth of microorganisms
MeOH: Methanol; ACN: Acetonitrile; FA: Formic acid

Table 44: *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
	$^{15}N_2$, $^{13}C_2$	7	A633382		10 mg	1.500 €	30 c	1.5 c
		14	LBS9AZ3L3293	1000	1.1 mL	1.380 €	251 c	12.5 c
	$^{15}N_4$, $^{13}C_2$	8	C4313		10 mg			
AMPA	^{13}C, ^{15}N, D_2	1	CIL-CDNLM-6786-1.2	100	1.2 mL	464 €	773 c	39 c
		5	CDNLM-6786-1.2	100	1.2 mL	464 €	773 c	39 c
		10	CDNLM-6786-1.2	100	1.2 mL	465 €	775 c	39 c
	^{13}C, ^{15}N	7	A617342		10 mg	1.690 €	34 c	1.7 c
		1	XA10205100WA	100	1.1 mL	332 €	604 c	30 c
		14	LBS9AZ3L1603	1000	1.1 mL	1.380 €	251 c	12.5 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
		14	LBS9G3L3294	1000	1.1 mL	1.380 €	251 c	12.5 c
		3	679027		5 mg	790 €	31.6 c	1.6 c
		11	sc-218161		1 mg	326 €	65.2 c	3.3 c
		18	20273		10 mg	810 €	16.2 c	0.8 c
Chloridazon-methyl-desphenyl-D3		18	20229		10 mg	695 €	13.9 c	0.7 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
		14	CRM9G3L1612	1000	1.1 mL	320 €	58 c	2.9 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
		14	LBS9G3L1609	1000	1.1 mL	200 €	36 c	1.8 c
	$^{18}O_3$	3	673141		10 mg	299 €	6.0 c	0.3 c
	$^{13}C_3$, $^{15}N_3$	15	S-O-C695-A-1.2ML	100	1.2 mL	378 €	630 c	31.4 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	1.255 €	25.1 c	1.3 c
		9	93101		5 mg	164 €	6.6 c	0.3 c
		14	LBS9G3L1613	1000	1.1 mL	170 €	31 c	1.5 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
	D_4	14	LBS9G3L2291	1000	1.1 mL	320 €	58 c	2.9 c
		6	D45297		50 mg	441 €	1.8 c	0.09 c
Diethanolamine	D_4	4	D-5307		100 mg	432 €	0.9 c	0.04 c
		14	LBS9B3L3152	1000	1.1 mL	180 €	33 c	1.6 c
	D_8	7	D441902		100 mg	1.100 €	2.2 c	0.1 c
		14	LBS9B3L3095	1000	1.1 mL	180 €	33 c	1.6 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_8 Dibromide (dipyridine-D_8)		4	D-7990		10 mg			
~~Diquat-D_4-dibromide (ethylene-D_4)*~~ *stability problems		~~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~~

Isotope labelled compound		Source	Article-No.	Conc. (µg/mL)	Amount per unit	Prices in €-cent (see diclaimer)		
						1 unit	2 µg*	0.1 µg**
		~~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~~
		~~14~~	~~LBS9AZ3L2482~~	~~1000~~	~~1.1 mL~~	~~240 €~~	~~44 c~~	~~2.2 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
		14	LBS9BK3L1600	1000	1.1 mL	250 €	45.5 c	2.3 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
		1	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
		14	LBS9G3L2293	1000	1.1 mL	150 €	27 c	1.4 c
Fosetyl	**D_{15} (Aluminium salt)**	1	CA13940010		10 mg	380 €	7.6 c	0.4 c
		14	LBS2AZ3L1607	100	1.1 mL	178 €	324 c	16.2 c
	D_5 (Sodium salt)	8	C5607		10 mg	825 €	17 c	0.8 c
Glufosinate	**D_3**	2	Friendly donation					
		3	680888	100	1 mL	380 €	760 c	38 c
		14	CRM9AZ3L1604	1000	1.1 mL	1.380 €	251 c	12.5 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	**$^{13}C_2$, ^{15}N**	1	XA14050100WA	100	1.1 mL	304 €	553 c	28 c
		5	CNLM-4666-1.2	100	1.2 mL	361 €	602 c	30 c
			CNLM-4666-10X-1.2	1000	1.2 mL	1.170 €	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
		14	CRM17AZ3L1602	200	1.1 mL	470 €	427 c	21.4 c
		15	S-FCN1104S-1.2ML	100	1.2 mL	393 €	655 c	32.7 c
		17	R009984		10 mg	2.165 €	43.4 c	2.2 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
		14	LBS9AZ3L1601	1000	1.1 mL	580 €	105.5 c	5.3 c
Matrine-D_3		7	M197872		10 mg	820 €	16 c	0.8 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
		14	LBS9G3L1608	1000	1.1 mL	270 €	49 c	2.5 c
	$^{13}C_4$	9	04311-10MG		10 mg	228 €	4.6 c	0.2 c
Melamine	**$^{13}C_3$, $^{15}N_3$**	1	CIL-CNLM-8150-10X-1.2	1000	1.2 mL	1.300 €	260 c	13 c
	$^{15}N_3$	9	80038		10 mg	647 €	13	0.7 c
		3	673055		10 mg	289 €	5.8 c	0.3 c
		14	LBS9G3L1616	1000	1.1 mL	270 €	49 c	2.5 c
	$^{13}C_3$	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

Isotope labelled compound		Source	Article-No.	Conc. (µg/mL)	Amount per unit	Prices in €-cent (see diclaimer)		
						1 unit	2 µg*	0.1 µg**
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
		14	CRM9G3L2292	1000	1.1 mL	300 €	54.5 c	2.7 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
		14	LBS9G3L1531	1000	1.1 mL	290 €	53 c	2.6 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
		14	LBS9G3L3094	1000	1.1 mL	200 €	36 c	1.8 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 €	33 c	1.6 c
		18	20053		20 mg	240 €	2.4 c	0.12 c
		11	sc-479498		5 mg	230 €	9.2 c	0.46 c
N-Acetyl-Glyphosate	D_3 (methyl-D_3)	7	A178248		25 mg	1.153 €	9.2 c	0.5 c
		14	LBS9AZ3L2868	1000	1.1 mL	580 €	105.5 c	5.3 c
	$^{13}C_2$, ^{15}N	7	A178247		10 mg	1.326 €	26.5 c	1.3 c
		17	R052712		10 mg	2.982 €	59.6 c	3.0 c
Nereistoxin-oxalate-D_6		1	C 15502010		10 mg	245 €	5 c	0.3 c
		14	LBS9AR3L1615	1000	1.1 mL	270 €	49 c	2.5 c
Nicotine-D_4		4	D-5098		100 mg	400 €	0.8 c	0.04 c
		14	LBS9B3L3297	1000	1.1 mL	420 €	76 c	3.8 c
Oxymatrine-D3		7	O876302		5 mg	600 €	24 c	1.2 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
		14	LBS9AZ3L1605	1000	1.1 mL	1.800 €	327 c	16.4 c
Paraquat	D_6-diiodide	1	C 15870200		50 mg	256 €	1.0 c	0.05 c
		14	CRM9AZ3L1611	1000	1.1 mL	180 €	33 c	1.6 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
		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
		14	LBS9G3L3296	1000	1.1 mL	320 €	58 c	2.9 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
		14	LBS9G3L3151	1000	1.1 mL	220 €	40 c	2.0 c
1, 2, 4-Triazole	$^{13}C_2$, $^{15}N_3$	2	Friendly donation					
		16	3201		5 mg	1.854 $	74.2 c	3.7 c
	D_2	19	RCG-401		10 mg	350.61 €	7 c	0,35 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**
1, 2, 4-Triazole-acetic acid	**$^{13}C_2$, $^{15}N_3$**	2	Friendly donation					
		16	15297		1 mg	420 $	84 c	4.2 c
	D_2	19	RCG-398		10 mg	350.61 €	7 c	0,35 c
1, 2, 4-Triazole-alanine	**$^{13}C_2$, $^{15}N_3$**	2	Friendly donation					
	D_2	19	RCG-399		10 mg	350.61 €	7 c	0,35 c
1, 2, 4-Triazole-lactic acid	**$^{13}C_2$, $^{15}N_3$**	2	Friendly donation					
		16	15295		1 mg	420 $	84 c	4.2 c
	D_2	19	RCG-400		10 mg	350.61 €	7 c	0,35 c
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
		14	LBS9G3L3096	1000	1.1 mL	180 €	33 c	1.6 c
Trifluoroacetic acid -$^{13}C_2$ (Sodium acetate)		7	S673752		10 mg	2.670 €	53 c	2.7 c
Trimethylsulfonium-(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
		14	LBS9G3L1614	1000	1.1 mL	605.71 €	110 c	5.5 c
	D_3	3	684243		10 mg	100 €	2 c	0.1 c

Providers of compounds:

1: LGC Stndards
2: Bayer Crop Science
3: HPC (High Purity Compounds)
4: CDN Isotopes (distributed in Germany by EQ Laboratories GmbH)
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
15: Chem Service Inc.
16: IsoSciences
17: MuseChem
18: ASCA GmbH
19: ReseaChem GmbH

(**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 45: *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)		
	Suggested concentration of IS-WSln1 (3.22) µg/mL	Absolute mass of IS spiked to sample (100 µL IS-WSln1) (m_{IS}^{sample}) µg	Suggested con-centration of IS-WSln2 (3.23) ** µg/mL	Absolute mass of IS spiked to calibration standard (100 µL IS-WSln2) ($m_{IS}^{cal\ mix}$) µg	**Expected approx.. IS-concentration in sample extracts (~20 mL) and cali-bration standards (~1 mL)** 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-$^{15}N_2$ (IL-IS)	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-$^{18}°_4$	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 IL-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 **5.6.3**).

** 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 IL-IS to the 1 mL aliquot transferred to the auto-sampler vial (see **Table 44**). 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 46: *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 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 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	
	Plum	10 g	85	1.5	Yes	
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	
	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	
	Kiwi	10 g	85	1.5	Yes	
	Mango	10 g	80	2	Yes	
	Papaya	10 g	90	1	Yes	
	Kaki/persimmon	10 g	90	1	Yes	
Dried fruit	Apple, dried	5 g	20	9	No	Weigh 14 g rehydratized homogenate (500 g +900 g water)
	Apricot, dried	5 g	20	9	No	
	Figs, dried	5 g	20	9	No	
	Prunes (dried plums)	5 g	20	9	No	
	Raisins	5 g	15	9	No	
	Apricot, dried soft	5 g	30-35	8,5	No	Weigh 13.5 g rehydratized homogenate (500 g +850 g water)
	Figs, dried soft	5 g	30-35	8,5	No	
	Prunes, soft	5 g	35-40	8	No	Weigh 13 g rehydratized homogenate (500 g +800 g water)
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	
	Potato	10 g	80	2	Yes	
	Garlic	10 g	65	3.5	**No**	

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
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	9 mL water and 1 mL EDTA solution*	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	9 mL water and 1 mL EDTA solution*	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 fresh**	10 g	90	1	Yes	
	Mushrooms dried	2g	<10	10	No	
	Wine	10 g	90	1	Yes	
	Honey	**5 g**	20	9	**No**	
	Avocado	10 g	70	3	**No**	
	Coconut copra	5 g	<10	0		Fat melting needed, see QuPPe-AO (ani-mal fat)
	Olives	10 g	70	3	**No**	

** The addition of EDTA solution is highly recommended when targeting analytes showing poor recoveries in absence of EDTA. Affected are compounds with a tendency to form complexes with metals, such as Glyphosate and metabolites, Glufosinate and metabolites. If affected analytes are not targeted, EDTA addition may be skipped and 10 mL of water are added.*

Table 47: *Exemplary LC-MS/MS parameters for Sciex Qtrap 5500*

Parameters	Methods 1.1/1.2/1.5/ 1.6/ 1.7/1.9/1.10	Method 1.3	Method 1.4	Method 2	Method 3/ 4.1/ 5	Method 4.2	Method 6	Method 7	Method 8 / 9	Method 10	Method 11
Ion source (ESI. Turbo Ion Spray) Mode	negative	negative	negative	negative	positive	positive	positive	positive	pos. / neg. Selexlon™	positive	negative
Curtain gas (N_2)	30 psi (2.07 bar)	40 psi (2.76 bar)	40 psi (2.76 bar)	30 psi (2.07 bar)	30 psi (2.07 bar)	30 psi (2.07 bar)	30 psi (2.07 bar)	40 psi (2.76 bar)	20 psi (1.38 bar)	20 psi (1.38 bar)	40 psi (2.76 bar)
Collision gas	medium										high
Ion spray voltage	-4500	-4500	-4500	-4500	1500	5000	5500	1500	5500 / -5500	5500	-4500
Gas 1 (Zero Grade Air or N_2)	50 psi (3.45 bar)	60 psi (4.14 bar)	60 psi (4.14 bar)	50 psi (3.45 bar)	50 psi (3.45 bar)	60 psi (4.14 bar)	50 psi (3.45 bar)	60 psi (4.14 bar)	60 psi (4.14 bar)	60 psi (4.14 bar)	60 psi (4.14 bar)
Gas 2 (Zero Grade Air or N_2)	60 psi (4.14 bar)	60 psi (4.14 bar)	70 psi (4.83 bar)	60 psi (4.14 bar)	60 psi (4.14 bar)	50 psi (3.45 bar)	60 psi (4.14 bar)	70 psi (4.83 bar)	70 psi (4.83 bar)	70 psi (4.83 bar)	60 psi (4.14 bar)
Tempera-ture of Gas 2	600°C	550°C	550°C	500°C	500°C	500°C	550°C	500°C	550°C	550°C	600°C
Resolution MS 1	unit (approx.. 0.7 amu FWHM*)										
Resolution MS 2	unit (approx.. 0.7 amu FWHM)										
Dwell time	20	20	20	50	20	10	50	20	20 / 40	20	20

*FWHM = full width at half maximum

Table 48: *Exemplary LC-MS/MS parameters for Waters Xevo TQ-Sμ*

Parameters	Method 1 M1.6b/M1.7b/M1.8	Method M4.2
Ion source (ESI)	negative	Positive
Source Temperature	150 °C	150 °C
Desolvation Temperature	600 °C	600 °C
Cone Gas Flow	50 L/h	150 L/h
Desolvation Gas Flow	1000 L/h	1000 L/h
Capillary	0.5 kV	0.5 kV
Resolution MS	unit	unit

Table 49: *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 IL-ISs 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 IL-ISs		
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 IL-ISs		
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 IL-IS 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 IL-ISs 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 IL-ISs		
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 8**: 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 (M8): "Triazole derivative** metabolites (TDMs)" new DMS parameters		
Update of Table 40: *Overview of approximate limits of quantification (LOQs)**		
Update of Table 42: Conversion factors between typical purchased standards and target analytes (3.18):		
Update of Table 43: Exemplary concentrations of pesticide stock and working solutions		
Update of **Table 44**: *Providers of isotopically labeled internal standards*		
Update of Table 45: *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

Action	When?	Version
Inclusion of N-Acetyl-Glyphosate in Table 4: Practical Information: Mainly used methods used at CVUA Stuttgart		
Addition of a further Ethephon-IL-IS 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 IL-ISs (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 5.6.20	April 2017	V9.3
Proposed volume of IS-WS II changed to match with volume of IS-WS I (see Table 2)		
Update of Table 4: data on M 9 were included		
Hints on stability of standard solutions added in 0, including Table 39		
Overview of lowest successfully validated levels (Table 40)		
Update of Table 42 : DFA and TFA added		
Update of *Table 43*: DFA and TFA added; solvents for Ethephon, Fosetyl and Maleic Hydrazide changed		
Update of Table 48		
Update of Table 45: DFA and TFA added		
Table 47 : 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 splitting 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 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		

Action	When?	Version
Maleic hydrazide added to Methode 4.2		
Introduction of a list with shortcut-links	Feb 2020	V11
Update of Table 4 (Overview of scope)		
Update of Table 5 (Overview of main methods)		
Update of method for cereals. It now involves addition of EDTA during the extraction step for complexation metals that may interfere with analysis of certain analytes		
Update of Method M 4.1 (Quats & Co. Obelisc R), new IL-IS and additional MRMs for Diquat		
Update of Table 31 (Exemplary providers of isotopically labeled internal standards)		
Update of Table 33 (Water contents of selected commodities)		
Update of Chapter 6 (information on purity of N-acetyl-glufosinate D_3 standards)		
Inclusion of Thiocyanate (M1.3 and M1.4) and Desmethyl-Dimethoate (M1.3) to the scope		
Inclusion of Matrine and Oxymatrine (M 4.2) to the scope	March 2021	V11.1
Restructuring of document to improve clarity (e.g. Hints and comments applying to more than one method are merged)	July 2021	V12
Introduction of a Chapter containing collected hints (5.6.1: Hints on analytes to avoid pitfalls)		
Thorough revision of text and elimination of errors		
Additional differentiation in solvent grades		
Extension of Apparatus list		
Revision of text for dried fruits		
Introduction of Method M1.6b, M1.7b, M 1.8, M 1.9, M 1.10, M 10, M 11		
Update of Table 40: Additional validation data		
Update of Table 45: data on Melamine, Matrine, Oxymatrine, Triazole, Triazole-lactic acid, Triazole-acetic acid, Triazole-alanine included		
Update of Table 46: dried fruits; olives, coconut copra, dried mushrooms, kaki included; garlic updated		
New ILISs: Maleic Hydrazide $^{13}C_4$, 1, 2, 4-Triazole-D_2, 1, 2, 4-Triazole-acetic acid-D_2, 1, 2, 4-Triazole-alanine-D_2, 1, 2, 4-Triazole-lactic acid-D_2		
Table 47 : data on M 1.10, M 10, M 11		
Introduction of Table 48: MS Parameters for Waters Xevo TQ-Sμ		