

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 13 (18.09.2026, Document History, see page 104)

Check for latest version of this Method under <https://www.quppe.eu>; older versions: [obsolete versions](#)

Authors: M. Anastassiades; A.-K. Schäfer; E. Eichhorn; D. I. Kolberg; H. Dias; A. Benkenstein; S. Zechmann; D. Mack; C. Wildgrube; A. Barth; B. Sauer; S. Fieberg; I. Sigalov; S. Goerlich; D. Dörk; G. Cerchia

EU Reference Laboratory for pesticides requiring Single Residue Methods (EURL-SRM)

Contact: CVUA Stuttgart, Schaflandstr. 3/2, DE-70736 Fellbach, Germany, www.eurl-pesticides.eu; EURL@cvas.bwl.de

Reference to EN 18032:2025 and its supplements.

The QuPpe procedure has become a CEN standard [EN 18032:2025](#). If you wish to establish the QuPpe method as an official standardized procedure in your laboratory, please refer to the official standard. Please note that supplementary information of the CEN standard is hosted within the QuPpe-Website under the following link: <https://www.quppe.eu/supplements.asp>

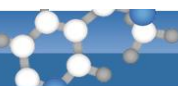
The supplementary information of the standard includes the following:

- method at a glance figures,
- decision trees for the use of quantifications modules,
- aggregated validation data of three interlaboratory validation studies and the respective protocols.

The methods described in the present document are largely equivalent to the CEN standard, but keep in mind that the present document does not legally correspond to [EN 18032:2025](#) and does not aim to replace it neither in full nor in part. The present document complements the standard and its official supplements with additional up-to date information and practical hints.

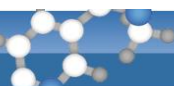
How to Cite (proposal):

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 13 (published on EURL-SRM website in August 2026); M. Anastassiades; A.-K. Schäfer; E. Eichhorn; D. I. Kolberg; H. Dias; A. Benkenstein; S. Zechmann; D. Mack; C. Wildgrube; A. Barth; B. Sauer; S. Fieberg; I. Sigalov; S. Goerlich; D. Dörk and G. Cerchia. URL: https://www.quppe.eu/quppe_doc.asp



Note: Substantial changes from V12.3 to V13 are highlighted in green

1.	Scope and Short Description	3
2.	Apparatus and Consumables	4
3.	Chemicals	6
4.	Disclaimer	9
5.	Procedure	9
5.1.	Sample preparation	9
5.2.	Extraction / Freeze-Out / Centrifugation / Cleanup / Filtration	10
5.3.	Preparation of blank extracts	20
5.4.	Recovery experiments	20
5.5.	Preparation of calibration standards	20
5.6.	LC-and IC-MS/MS analysis	22
5.6.1.	General recommendations and hints on analytes to avoid pitfalls	28
5.6.2.	Method 1.1 (M1.1): "Gly&Co. AS 11"	41
5.6.3.	Method 1.2 (M1.2): "Gly&Co. AS 11-HC"	43
5.6.4.	Method 1.3 (M1.3): "Gly&Co. Hypercarb"	45
5.6.5.	Method 1.4 (M1.4): "PerChloPhos"	47
5.6.6.	Method 1.5 (M1.5): "Gly&Co. on Trinity Q1"	49
5.6.7.	Method 1.6 "Gly&Co. on Torus DEA; (M1.6a)" or "Gly&Co. on Anionic Polar Pesticide Column (APPC); (M1.6b)"	51
5.6.8.	Method 1.7 "PerChloPhos on Torus DEA; (M1.7a)" or "PerChloPhos on Anionic Polar Pesticide Column (APPC); (M1.7b)"	55
5.6.9.	Method 1.8 (M1.8): "PerChloCyanMalein on Anionic Polar Pesticide Column (APPC)"	58
5.6.10.	Method 1.9 (M1.9): "Gly&Co. on Raptor Polar X"	59
5.6.11.	Method 1.10 (M1.10): "Gly&Co. on Obelisc N"	61
5.6.12.	Method 1.11 (M1.11): "Gly&Co. on Luna Polar Pesticides"	62
5.6.13.	Method 1.12 (M1.12): "PerChloPhos on Luna Polar Pesticides"	64
5.6.14.	Method 2 (M2): "Fosetyl and Maleic Hydrazide"	66
5.6.15.	Method 3 (M3): "Amitrole&Co."	68
5.6.16.	Method 4.1 (M4.1): "Quats&Co. Obelisc R"	70
5.6.17.	Method 4.2 (M4.2): "Quats&Co. BEH Amide"	72
5.6.18.	Method 4.3 (M4.3): "Quats&Co. Luna Polar Pesticides"	76
5.6.19.	Method 5 (M5): "Quats&Co. MonoChrom MS"	78
5.6.20.	Method 6 (M6): "Streptomycin and Kasugamycin"	79
5.6.21.	Method 7 (M7): "Morpholine, Diethanolamine and Triethanolamine"	80
5.6.22.	Method 8 (M8): "Triazole derivative metabolites (TDMs)"	81
5.6.23.	Method 9 (M9): "Difluoroacetic acid and Trifluoroacetic acid"	82
5.6.24.	Method 10 (M10): "Triazole derivative metabolites (TDMs) on Torus DEA"	83
5.6.25.	Method 11 (M11): "Gly&Co. by IC on AS19"	84
5.6.26.	Method 12.1 (M12.1): "Quats&Co by IC on CS17"	87
5.6.27.	Method 12.2 (M12.2): "Quats&Co by IC on CS21"	89
5.7.	Calibration and Calculations	91
6.	Stability and purity of standards	94
7.	Performance Data	95
8.	References	96
9.	ANNEX	97



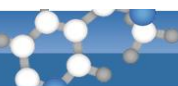
1. Scope and Short Description

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, oilseeds and nuts as well as in honey. A separate QuPPe document focusing on the analysis of highly polar pesticides in food of animal origin is available on the QuPPe website. Please check for the latest version.

The residues are extracted from the test portion following water adjustment and addition of acidified methanol. For most commodities (e.g. fruits vegetables, honey), the mixture is centrifuged, filtered and directly analyzed by LC-MS/MS. In the case of commodities with high protein content (i.e. cereals, pulses, nuts and oilseeds), 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). For the latter group of commodities residual protein is removed from the extracts through precipitation by diluting the extract with acetonitrile (1+1) prior to instrumental analysis. The protein removal step is accompanied by the removal of residual fat via dispersive SPE (d-SPE) with C₁₈ sorbent. **For glyphosate analysis in raw coffee, oxalate is used instead of EDTA for metal complexation.** In the case of plant oil, the extractant contains a higher methanol content to ensure that the lipids form the bottom layer. 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 largely 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 \(procedure for cereals, pulses, nuts and oilseeds\)](#)
- [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](#)



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 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 / 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 (3.7).

2.7. Mechanical shaker,

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

2.8. Mechanical grinding/shaking aids,

e.g. stainless steel grinding balls (Ø 7-10 mm).

2.9. Water Bath,

adjustable to at least 80°C and automatically shaking.

2.10. 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.

Note:

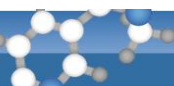
- Higher relative centrifugal forces (e.g. RCFs > 10,000 g) and cooling during centrifugation (e.g. to -10°C) are beneficial by causing increased precipitation of matrix components. Check centrifuge tubes for suitability for higher velocities.

2.11. 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 (2.12).

2.12. 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 (Ref. No. 729246 H-PTFE) may be attached in front of the 0.2 µm filters if the latter get clogged when used directly. In case of filter-clogging during honey extracts filtration, filters with 5.0 µm pore sizes (e.g. Rotilab® PTFE filters by ROTH Ref. No. SE4M075I99) may be used for pre-filtration (or even instead of the filters with smaller pore-sizes), as pollen grains are typically > 10 µm in diameter.

Note:

- Check filters for any contamination with analytes of interest. Variable and in some cases significant levels of Perchlorate, and Chlorate were detected in the above-mentioned polyester filters, while Phosphonic acid was detected in the H-PTFE filters. For testing the suitability of the filters consider a quasi worst-case scenario, where filters are clogged quickly so that only a small volume of the extract (e.g. 200 µL) is passed through each filter. Severe clogging was for example observed with industrially milled cereals and well as in extracts of pears and pineapples. Hints on how to reduced clogging can be found in chapters 5.2.4 and 5.2.6 .

2.13. 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.

2.14. 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

Note:

- 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.

2.15. Volumetric flask with stoppers,

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

Note:

- 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 2.14).

2.16. Screw-cap storage vessels,

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

Note:

- 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 2.14).

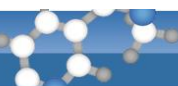
2.17. LC-MS/MS instrumentation,

equipped with ESI source and appropriate columns, see details in chapters 5.6.2 to 5.6.25.6.24.

2.18. IC-MS/MS instrumentation,

equipped with ESI source and appropriate columns, see details in chapters 5.6.25.

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



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

3.2. 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.

3.3. Methanol at least HPLC quality,

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

3.4. Acetonitrile at least HPLC quality,

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

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

for the preparation of mobile phases preferably use MS-quality formic acid. **Note: Check the purity of formic acid by injecting e.g. a 1% solution. Contaminations with e.g. chlorate or phosphonate were observed. These vary from provider to provider and from batch to batch. If no sufficiently clean formic acid can be obtained, distillation is an effective measure to reduce contamination.**

3.6. Acetic acid (concentrated; >98%),

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

3.7. Acidified methanol,

for the extraction of the majority of samples, prepared by pipetting 10 mL formic acid (3.5) into a 1000 mL volumetric flask and filling up to volume with methanol (3.3).

3.8. Acidified methanol with 30% water,

for the extraction of vegetable oil samples, prepared by pipetting 10 mL formic acid (3.5) into a 1000 mL volumetric flask, followed by 300 mL water (3.1) and filling up to volume with methanol (3.3).

3.9. Hydrochloric acid 1M,

aqueous, for the preparation of HCl-methanol mix (3.10).

3.10. HCl-methanol mix,

for the extraction of Diquat and Paraquat in most types of commodities, prepared by filling 500 mL methanol into a 1000 mL volumetric flask and filling up to volume with 1M hydrochloric acid (3.3).



3.11. C18 sorbent (ODS-sorbent),

e.g. Polyoprep 30-300 µm Macherey-Nagel GmbH & Co KG/Düren (Germany), article-no. 711720.100). **Note: Check the purity of the sorbent in regard to the measured analytes.** Contamination with e.g. Morpholine was observed. For checking, conduct d-SPE reasonably using a higher C18/solvent ratio compared to the method. Acetonitrile/Water 1:1 may be used as solvent.

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

3.13. Dimethylamine,

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

3.14. Ammonium formate (p.a.)

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

3.16. Sodium hydroxide (p.a.)

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

3.18. 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).

3.19. 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.

3.20. Disodium oxalate (p.a.)

e.g. di-sodium oxalate (CAS Number 62-76-0): 71800 Sigma Aldrich (MW=134).

3.21. Dry ice,

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

3.22. Pesticide Standards,

of known purity.

3.23. Pesticide stock solutions,

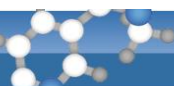
e.g. 1 mg/mL solutions of pesticide standards (3.22) in a water miscible solvent. Suggestions of solvents suitable for the preparation of stock solutions can be found in **Table 46**.

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 45**. Keep solutions in plastic vessels as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under 2.14).

3.24. Pesticide working solutions / mixtures,

prepared at appropriate concentrations by diluting pesticide stock solutions (3.23) 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 46**.

**Notes:**

- Keep solutions in **plastic vessels** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.14**).

3.25. Internal Standards (ISs),

Exemplary sources are **shown in the online list of suppliers of analytical standards (Excel)**. Check whether the ISs contain native compounds at levels, which would lead to false positives or quantification errors.

3.26. IS-stock solutions,

e.g. 1 mg/mL solutions of ISs (**3.25**) in a water miscible solvent (e.g. methanol, acetonitrile, water or mixtures thereof). For solvent-suggestions see **Table 46** 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 **2.14**).
- 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 analyte** (irrespective on whether it was initially present - as impurity - or formed in the working solutions during storage) 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 the native analyte from the IL-IS are N-Acetyl-Glyphosate (acetyl D₃), that may de-acetylate into native Glyphosate, Fosetyl-D₅, that tends to hydrolyse to native Phosphonic acid (see **5.6.1-1c** under Hints on Method 1.2). As far as the presence of original impurities is concerned, it was observed that Maleic Hydrazide D₂ standards typically contain a small, but relevant, share of the native compound (see **2.e** **5.6.9**), whereas fosetyl D₅ was found to contain relevant amounts of native Phosphonic acid (see **1.c**).
- 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.

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

prepared at appropriate concentrations by diluting IS-stock solutions (**3.26**) of one or more ISs with water-miscible solvents. Suggestions for solvents are shown in **Table 46** and suggestions for the concentrations in **Table 47**.

Notes:

- Keep solutions in **plastic vessels 2.16** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.14**).
- In presence of water and especially at high pH levels, Phosphonic acid ¹⁸O₃ will gradually convert to ¹⁸O₂¹⁶O₁, ¹⁸O₁¹⁶O₂ and eventually of ¹⁶O₃ (native) Phosphonic acid. The ¹⁸O₃ **Phosphonic acid standard solution provided by the EURLs should be preferably diluted in acetonitrile**, where it was shown to be stable for long periods.

3.28. IS-working solution II (IS-WSIn-2) for preparation of calibration standards,

prepared at appropriate concentrations by diluting IS-WSIn-1 (**3.27**) with water-miscible solvents. Suggestions for solvents are shown in **Table 46** and for concentrations in **Table 47**.

Notes:

- Keep solutions in **plastic vessels** as several of the compounds covered by this method tend to interact with glass-surfaces (see examples under **2.14**).
- For short term usage (e.g. up to one month) the IL-IS of Phosphonic acid can be diluted in acidified methanol (**3.7**).

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

see details in chapters 5.6.2 to 5.6.24.

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

see details in chapter 5.6.25.



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.

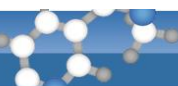
Fruits and vegetables are preferably milled cryogenically (e.g. using dry ice). This is done to reduce analyte degradation and particle sizes, with the latter resulting in improved homogeneity and residue accessibility. One possibility for cryogenic milling is to cut large units coarsely to ca 3x3 cm pieces, freeze them and then mill them for ca. 1-2 min 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. oilseeds 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 ring-sieve. 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 containing liquid nitrogen. When completely frozen transfer it into a powerful knife mill and grind until a fine powder is obtained. Do not mill for too long as the material will thaw and become clumpy and thus more difficult to handle. **Plant oil and fat can be used as is.**

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 (**3.21**). Weigh Y g* (see **Table 1**) of homogenate, corresponding to 5 g of the original dry sample.

Table 1: Sample-Water-ratios required for slurry homogenization of dried fruits and analytical portions employed for analysis



Initial moisture content of dried fruit product	Water amount (X g) added to 500 g of dried fruit	Analytical portion of rehydratized product (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, **liquid nitrogen (LN) assisted milling** may be conducted. For this, immerse the sample into a plastic or polystyrol container containing LN and when completely frozen, use a powerful knife mill to grind until a fine powder is obtained. Do not mill for too long and quickly transfer the frozen powder into a storage container which should be swiftly placed into a 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 adding 10 mL of water (as with spices, herbs and other extract-rich commodities).

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

Flow charts of the procedure are shown in Figure 1 (for most commodities) and in Figure 2 (for pulses, nuts and oilseeds)

5.2.1. Weighing of analytical portions

Weigh a representative analytical portion (m_a) of the sample homogenate (5.1) into a 50 mL centrifuge tube (2.3). In case of **fresh fruits and vegetables and juices** weigh $10 \text{ g} \pm 0.1 \text{ g}$ of the homogenized sample. In case of **cereals, dried pulses, oilseeds, nuts, oil, dried fruits, dried vegetables, dried mushrooms and honey** weigh $5 \text{ g} \pm 0.05 \text{ g}$ of the homogenates. In case of **dry fruits rehydrated** according to 5.1, weigh Y g (e.g. $13.5 \text{ g} \pm 0.1 \text{ g}$, 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.

Notes:

- Lowering the analytical portion size (e.g. from 5 g to 2 g) can help increase the recovery rates of compounds that tend to interact with matrix components, such as glyphosate, paraquat and diquat. This measure also reduces matrix induced effects in analyte ionization but it also affects sensitivity as the analyte concentration is reduced. See also comments under 5.6.1

5.2.2. Adjustment of water content

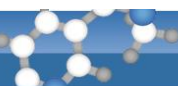
For **commodities containing ≥ 80% natural moisture**, water adjustment to 10 mL is not essential and may be skipped when appropriate ISs are employed before any aliquotation. If no IS is used, **add water (3.1)**, as indicated in **Table 48** to minimize volumetric errors. Continue with step 5.2.3.

For **commodities with < 80% of natural moisture** (except honey, 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 as indicated in **Table 48**. No water adjustment is needed where re-hydrated commodities (see 5.1) are employed. Continue with step 5.2.3.

For vegetable oils, no water is added at this stage.

Notes:

- Keep in mind that the water volume adjustments in **Table 48** are approximate.
- In the case of **honey**, it needs to be taken into account, that sugars completely dissolve in the methanol/water mixture and also contribute to the total volume. So, instead of adding 9 mL of water (to account for ~1 mL of water contained in honey with ~20% moisture content), the amount of water to be added to a 5 g portion of honey is 7.5 mL.
- For **oilseeds, 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 48**.



- In the case of **chia seeds, psyllium husk and linseeds (flaxseeds)** adding water directly to the samples leads to a soaking and the formation of a gel-like 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 (3.7) and 100 µL formic acid (3.5), 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). Similarly, for the extraction of **Diquat** and **Paraquat**, first add 10 mL HCl-methanol mix (3.10), shake shortly, and then add 10 mL water and continue with 15 min shaking as described under 5.2.3. Then continue with step 5.2.4-(2) or 5.2.4-(3) and further with step 5.2.5-(2).

5.2.3. Extraction

A) General procedure

- (1) **For all commodities of plant origin except cereals, pulses, nuts and oilseeds (Figure 1):** Add 10 mL acidified methanol (3.7) and 100 µL (or another appropriate small volume) of the IS-WSIn-1 (3.27) 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) **For cereals, pulses, nuts and oilseeds (Figure 2):** Add 10 mL acidified methanol (3.7) and 100 µL (or another appropriate small volume) of the IS-WSIn-1 (3.27) 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 (3.5). 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 (3.19) and shake for 15 min by an automatic shaker. For chia seeds and linseeds 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.
- (3) **Alternative procedure for raw coffee beans (recommended for glyphosate) (Figure 5):** Add 10 mL methanol (3.3) and 100 µL (or another appropriate small volume) of the IS-WSIn-1 (3.27) containing isotopically labeled analogues of the analytes of interest (added IS mass = m_{IS}^{sample}) and agitate shortly to distribute the ISs. Add 250 mg disodium oxalate (3.20) and agitate shortly to distribute it. Then close the tube and shake for one minute. Add 200 µL formic acid (3.5) and shake for 15 min by a mechanical shaker. This procedure may be also used for the other highly polar pesticides analytes listed in the present document.
- (4) **For vegetable oils (Figure 6):** Add 10 mL acidified methanol with 30% water (3.8) and 100 µL (or another appropriate small volume) of the IS-WSIn-1 (3.27) containing isotopically labeled analogues of the analytes of interest (added IS mass = m_{IS}^{sample}) and agitate shortly to distribute the ISs. In case of liquid vegetable oil, proceed with shaking by a mechanical shaker for 2-15 min. In case of solid/pasty fat (where compound partitioning between the phases is hindered), add mechanical aids, e.g. stainless steel grinding balls (Ø 7-10 mm) (2.8), close the tube and shake by a mechanical shaker for 5-20 min. Alternatively, heat up the tube until the fat is melted completely, e.g. by placing the tube into a water bath, and shake by a mechanical shaker for 2-15 min.

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 phase will mainly consist of the water naturally contained in the sample, the water supplemented 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, oilseeds and nuts) the final concentration in the extract is reduced to 0.125 g/mL.
- **Adding the IS to a sample extract aliquot** (e.g. the 1 mL aliquot transferred to the autosampler vial, see below), helps to drastically reduce the amount of IS used (e.g. 20-fold if added to 1 mL extract). This approach is mainly recommended for screening purposes. The IS added at this step will compensate for matrix effects including retention-time shifts but not for recovery and volume deviations during extraction. If this approach is used for quantification, it is of foremost



importance to make sure that the volume of the liquid phase is close to 20 mL (allowing to assume that 1 mL extract entails exactly 0.5 or 0.25 or 0.125 g sample equivalents, see above), otherwise the quantitative result should be considered tentative. For more accuracy, samples should be re-extracted with the IS being added to the analytical portion before aliquotation.

- **Particle size of dry products** (e.g. cereals, pulses) plays an important role in analyte extractability. If a considerable fraction of 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** 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, Glufosinate and their metabolites. **If affected analytes are not targeted, EDTA addition may be skipped adding 10 rather than 9 mL of water.**
- For **Diquat** and **Paraquat**, the addition of the IL-IS directly to a dry matrix may reduce the IL-IS recovery, thus leading to overestimated results.

B) Procedure for Paraquat and Diquat (Figure 3)

For the analysis of Paraquat and Diquat add 10 mL of HCl-methanol mix (3.10), to the water-adjusted analytical portion (from 5.2.2), add 100 µL (or another appropriate small volume) of the IS-WSIn-1 (3.27) containing isotope-labeled analogues of Paraquat and Diquat (added IS mass = m_{IS}^{sample}). Close the tube and shake vigorously for 15 min by a mechanical shaker.

Notes:

- **Extracting with the normal QuPpe solvent** (methanol containing 1% formic acid) at room temperature leads to poor extraction yields. Poor extraction yields are typically corrected for by the IL-IS, but this approach is associated with a high risk of false negatives, which limiting its suitability for screening purposes.
- Extractions with the previously published solvent (1:1 mixture of methanol + aqueous HCl 0.1M)², resulted in satisfactory recoveries of Paraquat and Diquat from various fruits and vegetables as well as cereals, but the recoveries from more challenging commodities, such as pulses and oily seeds remained poor.
- **Extracting with the HCl-methanol mix (3.10)**, ensures much higher absolute recoveries (yields) compared to the normal QuPpe as well as compared to the previous procedure for paraquat and diquat, which employed less HCl. The yields/recoveries, however, strongly vary depending on the matrix. In some cases, absolute recoveries may even fall below 50% with Paraquat tending to show lower absolute recoveries than diquat. The results of numerous experiments suggest that Diquat and Paraquat (as well as their respective IL-ISs), enter into a dynamic equilibrium with the matrix, with the recovery rates being defined by the affinity of the analytes towards the matrix under the selected extraction conditions. As the IL-ISs behave in an equivalent way as the native analytes (incurred or spiked), they can very well correct for analyte losses caused by them attaching to the matrix surface. A precondition is that the signals of both (native analyte and IL-IS) remain well measurable. Exemplary commodities for which poor recoveries have been observed include linseed, dried pulses (especially of dark colour), bananas, potatoes with soil-residues, as well as other products that may be contaminated with dust or soil, e.g. spices, root vegetables.
- **Highly retentive matrices** causing recovery drop or strong signal suppression will increase the **risk of false negatives**. Monitoring the IL-IS signal is therefore an important QC-measure to minimize the occurrence of false negative results.

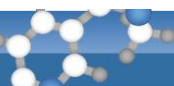
Where abs. recoveries fall below the acceptable level of 30% (SANTE/11312/2021), it is recommended to lower the sample weight to 2 g. An separate LOQ for this method variant should be established.

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 $\geq 3,000$ g (the higher the centrifugation force the better). This procedure is **NOT** recommended for extracts of commodities that pose difficulties in filtration (e.g. finely milled cereals, pineapples, 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 $\geq 3,000$ g. Higher centrifugation forces (e.g. $\geq 10,000$ 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.

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.



- (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. These racks can be also used for keeping the tubes cold after removing them from the centrifuge.
- Low temperatures reduce the solubility of interfering matrix components (e.g. proteins, lipids) 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 redissolution 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 (3.4), shake for 1 min and then centrifuge for 5 min at >3000 g (see 2.7).
- (2) **Nuts and oilseeds:** transfer 2 mL of the supernatant into a 10 mL centrifuge vial containing 2 mL of acetonitrile (3.4) and 100 mg of C18 sorbent, shake for 1 min and then centrifuge for 5 min 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 C18 sorbent, shake for 1 min and then 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.

- (4) **Vegetable oils (e.g. olive oil):** transfer 4 mL of the supernatant (from 5.2.4) into a 10 mL centrifuge vial containing 200 mg of C18 sorbent, shake for 1 min and then 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 oilseeds:** Withdraw an aliquot (e.g. 2-3 mL) of the supernatant from 5.2.4 or 5.2.5 using a syringe (2.11) and filter it through a syringe filter (2.12) either directly into an auto-sampler vial (2.14) 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 (2.11). In the case of **honey** extracts pollen and wax particles may be the cause of filter clogging. In this case use 5.0 µm pore size syringe filters (2.12) for pre-filtration (rather than filters with smaller pore-sizes), as pollen grains are typically > 10 µm in diameter. High-speed centrifugation (see 5.2.4 (3)) also helps to separate pollen.
- 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.
- **Optional Ultrafiltration:** for cereals, pulses, nuts and oilseeds transfer a 3 mL aliquot of the supernatant from 5.2.5 into an ultrafiltration unit (2.13) 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. Protein-removal through precipitation (assisted by low temperatures, high speed centrifugation and/or acetonitrile dilution) is preferable than ultrafiltration. Some UF-units tend to clog easily and the polycarbonate vessels in 2.13 tend to slowly dissolve in the extraction solvent with unknown consequences for the analytical column.



QuPPe-PO-Method at a glance

Procedure for most commodities of Plant Origin

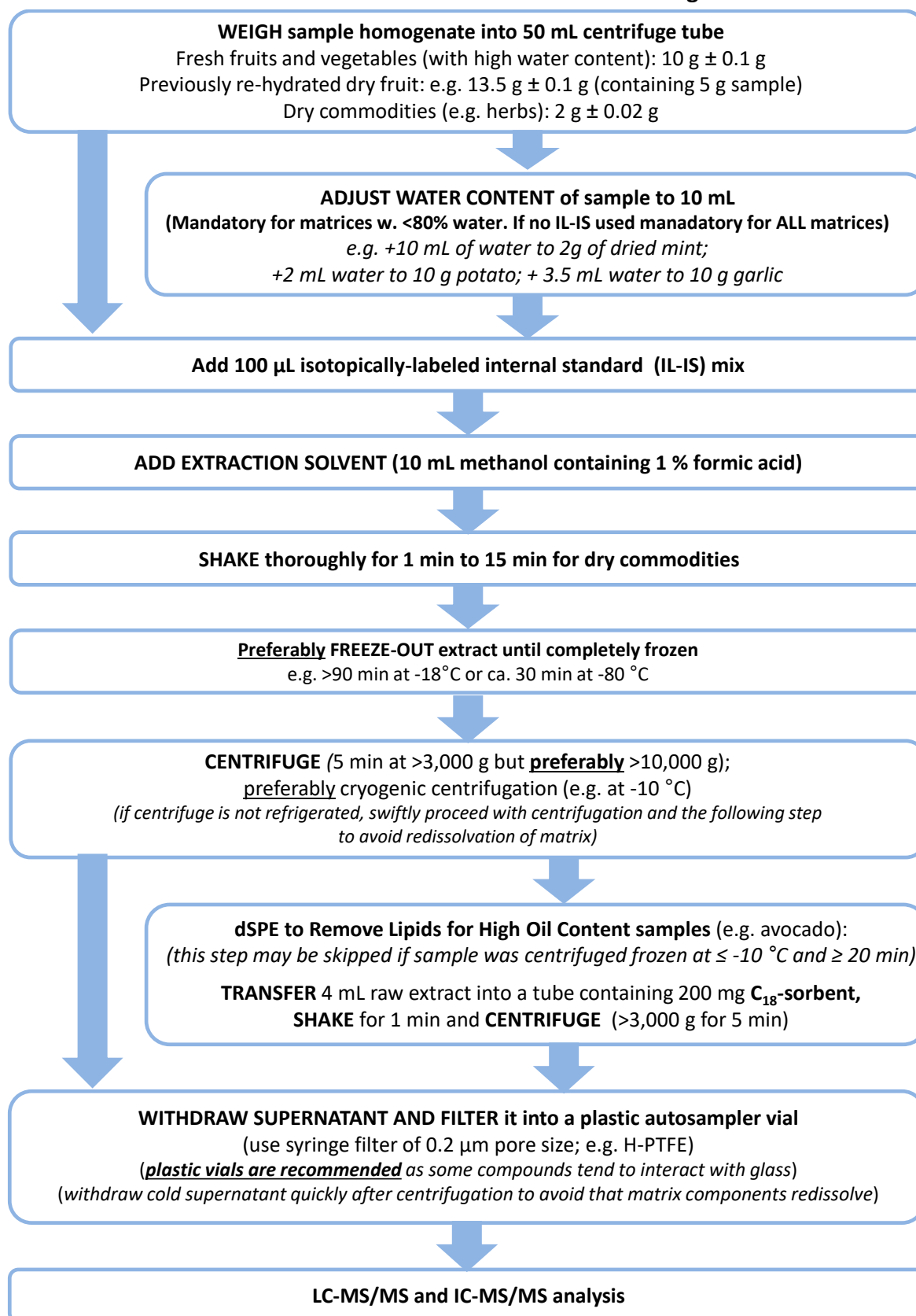


Figure 1: QuPPe-PO-Method at a glance (general procedure for most commodities, not considering paraquat and Diquat)

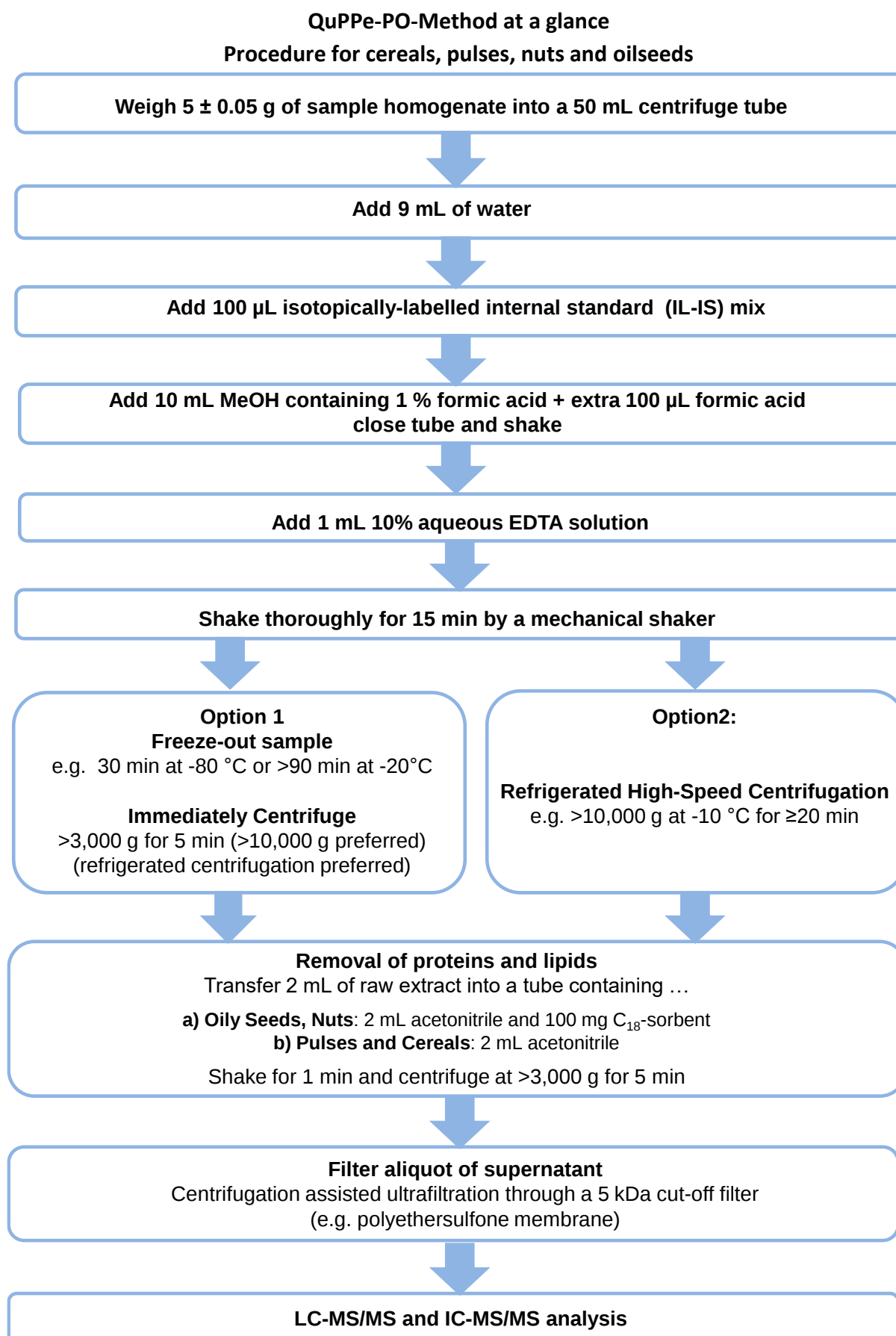


Figure 2: QuPpe-PO-Method at a glance; procedure for cereals, pulses, oilseeds and nuts

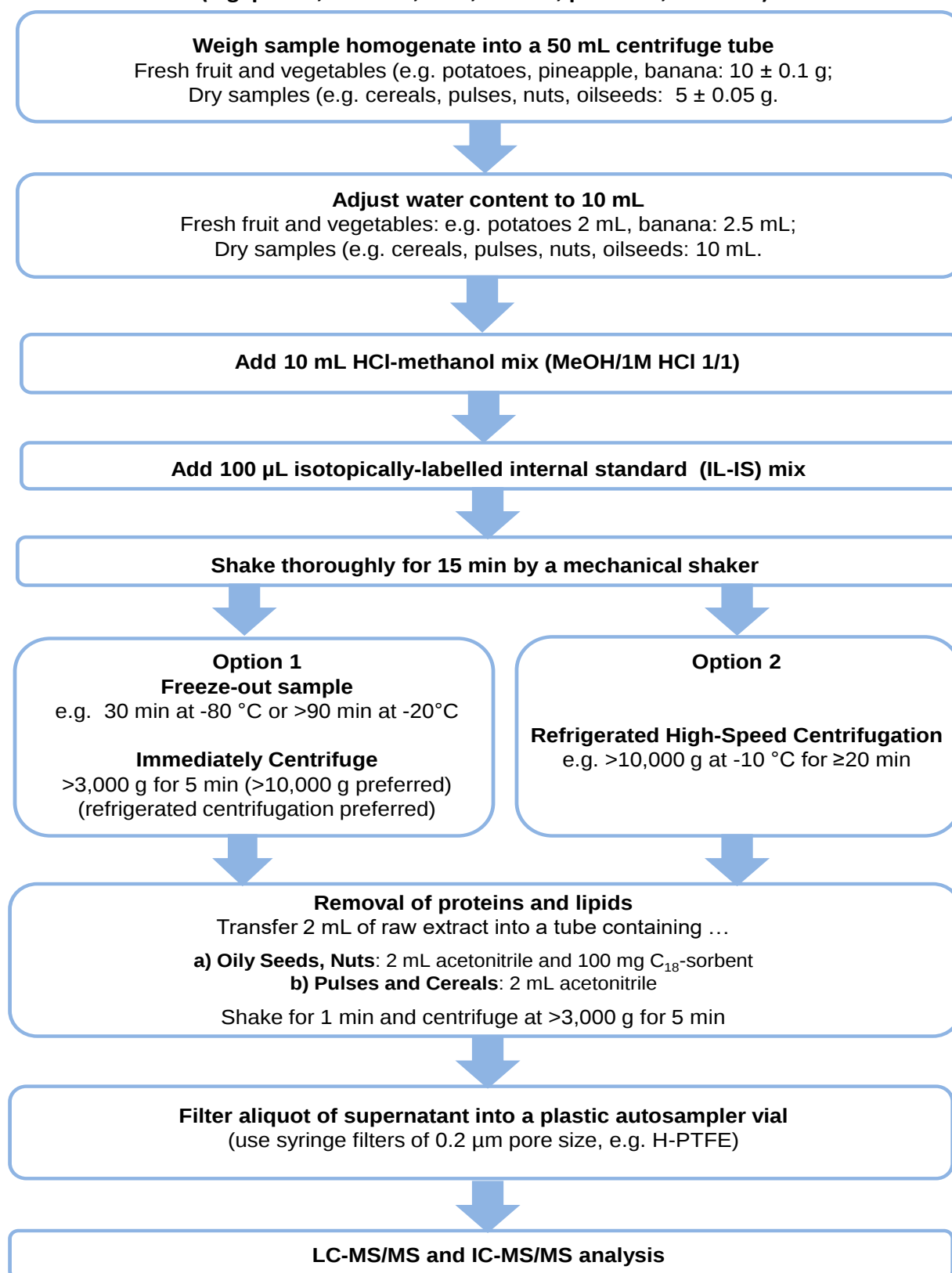
QuPpe-PO-Method at a glance**Procedure for Diquat and Paraquat in various commodities****(e.g. pulses, oilseeds, nuts, cereals, potatoes, bananas)**

Figure 3: QuPpe PO Method at a glance; procedure for the extraction of Diquat and Paraquat from various commodities, e.g. from potatoes, bananas, cereals, pulses, oilseeds and nuts

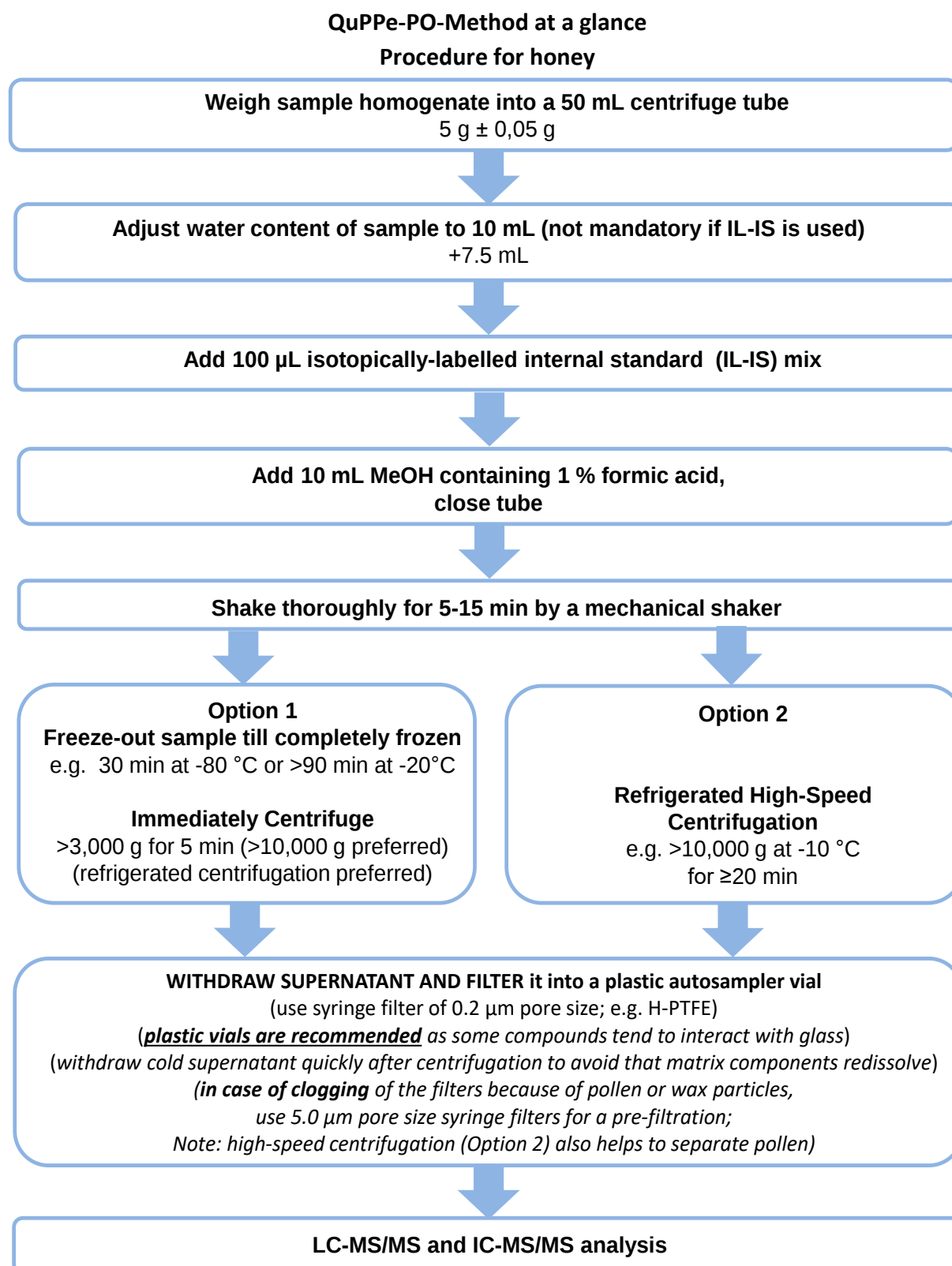


Figure 4: QuPPe-PO-Method at a glance; procedure for honey

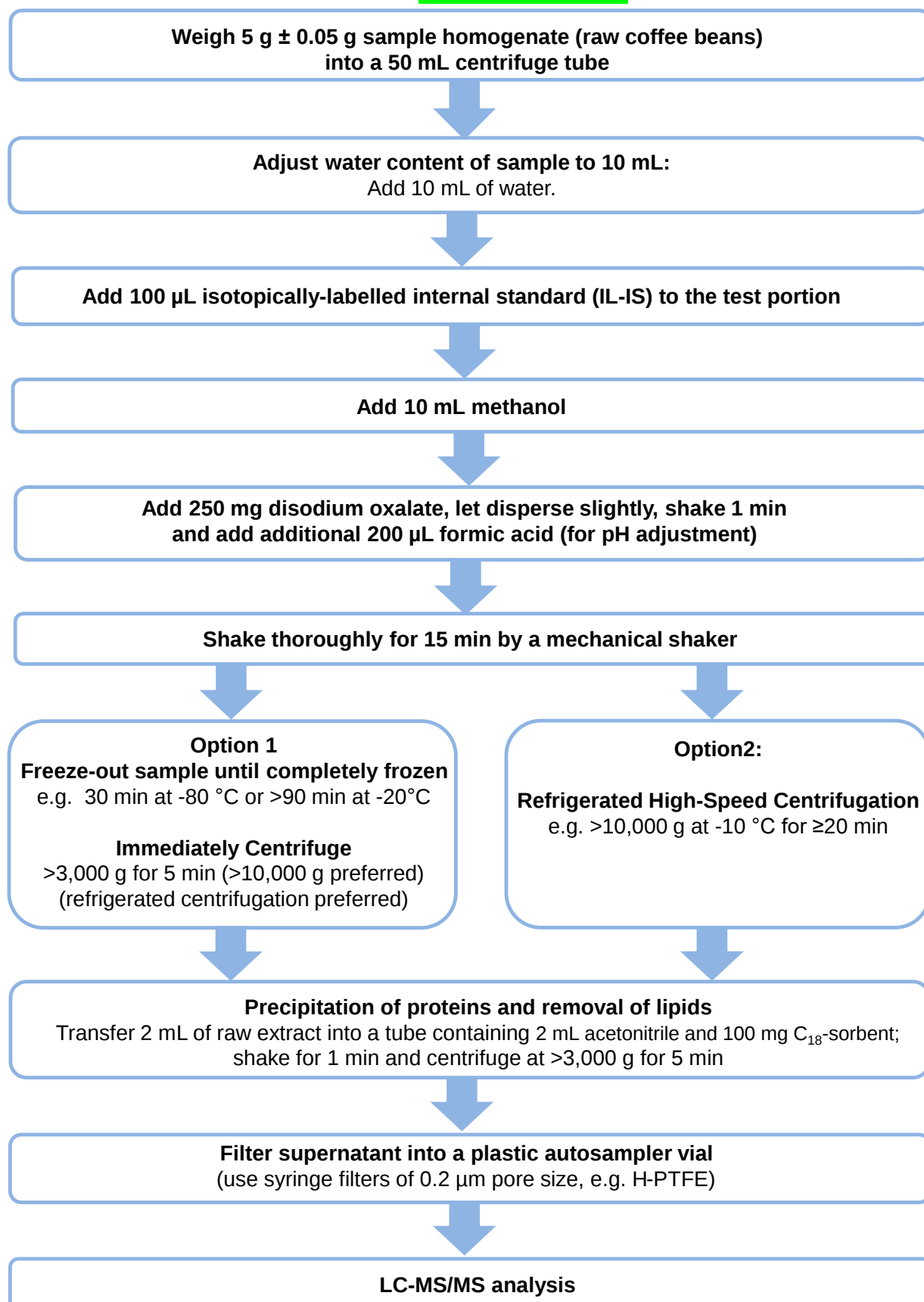
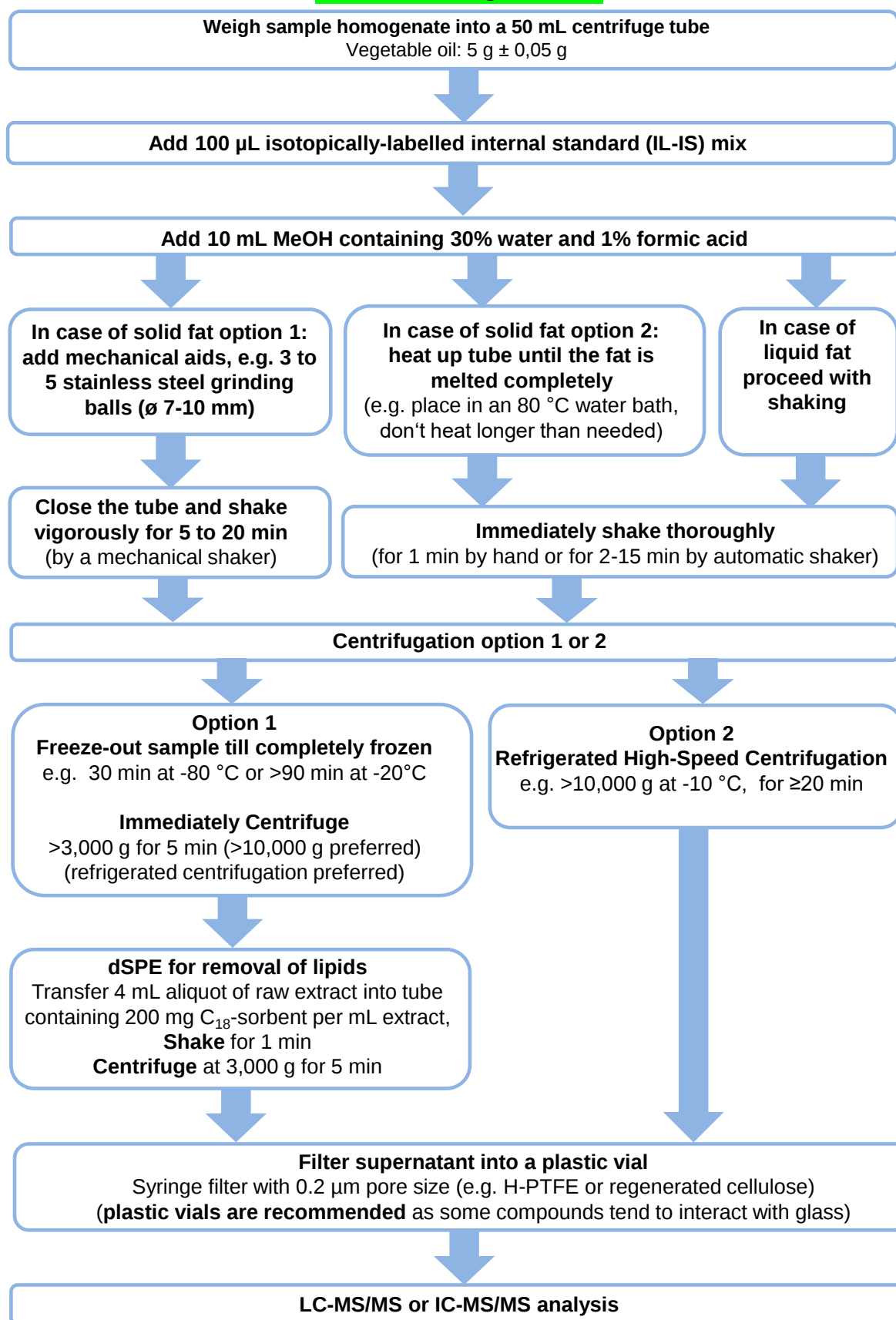
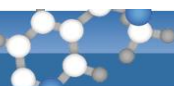
QuPpe-PO-Method at a glance**Procedure for Coffee**

Figure 5: QuPpe-PO-Method at a glance; alternative procedure for raw coffee (especially recommended for Glyphosate)

QuPPE-PO-Method at a glance**Procedure for vegetable oils****Figure 6:** QuPPE-PO-Method at a glance; procedure for vegetable oils



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 (3.24 and Table 46). 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.

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 (3.7)		850 µL	800 µL	850 µL	100 µL	50 µL	100 µL	50 µL	-	50 µL
Pesticide working solutions (3.24) 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-WSIn-2 (3.28) ^{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 the IS-WSIn-2 solution added to the calibration standards (in the particular example 100 µL are 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-WSIn-2 (3.28) by diluting IS-WSIn-1 (3.27) 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 and volume adjustments are of less importance as the IL-IS compensates for any matrix-related signal shifts. 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).



⁵ 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 oilseeds), the 0.05 µg/mL calibration level corresponds to 0.4 mg pesticide /kg sample.

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.

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

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

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

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

An exemplary pipetting scheme according to Example B is 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 (3.24)	-	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 legend 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 1.8 (5.6.9)	M 1.9 (5.6.10)	M 1.10 (5.6.11)	M 1.11 (5.6.12)	M 1.12 (5.6.13)
Separation principle	Anion Ex.	Anion Ex.	Carbon	Carbon	HILIC	HILIC	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	Obelisc N	Luna PP	Luna PP
ANALYTES COVERED BY LC-MS/MS IN THE ESI-POSITIVE MODE												
Amitrole	NT	NT	-	NT	NT	NT	NT	NT	NT	NT	NT	NT
ETU	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT
PTU	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT
Cyromazine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Trimesium	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Daminozide	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Chlormequat	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT
Mepiquat	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT
Difenzoquat	NT	NT	-	NT	NT	NT	NT	NT	NT	NT	NT	NT
Propamocarb	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Melamine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Diquat	NT	NT	-	NT	NT	NT	NT	NT	NT	NT	NT	NT
Paraquat	NT	NT	-	NT	NT	NT	NT	NT	NT	NT	NT	NT
N,N-Dimethylhydrazine	NT	NT	-	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nereistoxin	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT
Streptomycin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Kasugamycin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Morpholine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Diethanolamine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Triethanolamine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
1,2,4-Triazole	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Triazole-alanine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Triazole-acetic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Triazole-lactic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Aminocyclopyrachlor	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Chloridazon-desphenyl	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Mepiquat-4-hydroxy	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Propamocarb-N-desmethyl	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Propamocarb-N-oxide	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Pymetrozine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Maleic Hydrazide	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nicotine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Matrine	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Oxymatrine	NT	NT	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 legend under Table 7) Part II

QuPpe method code	M 2 (5.6.12)	M 3 (5.6.15)	M 4.1 (5.6.16)	M 4.2 (5.6.17)	M 4.3 (5.6.18)	M 5 (5.6.19)	M 6 (5.6.20)	M 7 (5.6.21)	M 8 (5.6.22)	M 9 (5.6.23)	M 10 (5.6.24)	M 11 (5.6.25)	M 12.1 (5.6.26)	M 12.2 (5.6.27)
Separation principle	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon	HILIC	HILIC	IC	IC	IC
Column type	Obelisc R	Obelisc R	Obelisc R	BEH-Amide	Luna PP	PFP	Obelisc R	Trinity P1	Hypercarb	Trinity P1	Torus DEA	AS19	CS17	CS21
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	NT	(✓)	✓
Paraquat	NT	NT	✓	(✓)****	NT	NT	NT	NT	NT	NT	NT	NT	(✓)	✓
N,N-Dimethylhydrazine	NT	NT	✓	-	NT	NT	NT	NT	NT	NT	NT	NT	-	-
Nereistoxin	NT	NT	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	✓	✓
Streptomycin	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Kasugamycin	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT
Morpholine	NT	NT	(✓)	(✓)	(✓)	NT	NT	✓	NT	✓	NT	NT	✓	✓
Diethanolamine	NT	NT	(✓)	(✓)	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT
Triethanolamine	NT	NT	(✓)	(✓)	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT
1,2,4-Triazole	NT	NT	(✓)	-	NT	NT	NT	NT	✓	NT	(✓)	NT	NT	NT
Triazole-alanine	NT	NT	(✓)	-	NT	NT	NT	NT	✓	NT	✓	NT	NT	NT
Triazole-acetic acid	NT	NT	(✓)	-	NT	NT	NT	NT	✓	NT	✓	NT	NT	NT
Triazole-lactic acid	NT	NT	NT	-	NT	NT	NT	NT	✓	NT	✓	NT	NT	NT
Aminocyclopyrachlor	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	NT	-	-
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	✓	-
Pymetrozine	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Maleic Hydrazide	NT	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
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 1.8 (5.6.9)	M 1.9 (5.6.10)	M 1.10 (5.6.11)	M 1.11 (5.6.12)	M 1.12 (5.6.13)
Separation principle	Anion Ex.	Anion Ex.	Carbon	Carbon	HILIC	HILIC	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	Luna PP	Luna PP
ANALYTES COVERED BY LC-MS/MS IN THE ESI-NEGATIVE MODE												
Ethephon	✓	✓	✓	NT	✓	✓	(✓)	NT	✓	✓	✓	-
HEPA	✓	✓	✓	NT	✓	✓	(✓)	NT	✓	✓	✓	-
Glufosinate	✓	✓	✓	NT	✓	✓	(✓)	NT	✓	✓	✓	-
N-Acetyl-Glufosinate	✓	✓	✓	NT	✓	✓	(✓)	NT	✓	✓	✓	-
MPPA	✓	✓	✓	NT	✓	✓	(✓)	NT	✓	✓	✓	-
Glyphosate	✓	✓	✓	NT	✓	✓	(✓)	NT	✓	✓	✓	-
AMPA	✓	✓	✓	NT	✓	✓	(✓)	NT	✓	(✓)	(✓)	-
Phosphonic acid	(✓)	(✓)	✓	✓	✓	✓	✓	NT	✓	(✓)***	(✓)	✓
N-Acetyl-AMPA	NT	✓	✓	NT	✓	✓	(✓)	NT	NT	NT	NT	NT
Fosetyl-Al	-	✓	✓	NT	✓	✓	(✓)	NT	✓	(✓)***	✓	-
Maleic Hydrazide	-	-	✓	NT	-	-	-	(✓)	(✓)	(✓)	-	-
Perchlorate	NT	-	✓	✓	✓	(✓)**	✓	✓	✓	✓	-	✓
Chlorate	NT	-	✓	✓	✓	(✓)**	✓	✓	(✓)	✓	-	(✓)
Bialaphos	NT	NT	✓	NT	✓	NT	NT	NT	✓	NT	NT	NT
Cyanuric acid	NT	NT	✓	NT	-	-	-	✓	(✓)	(✓)	-	-
Bromide	NT	NT	-	✓	✓	(✓)**	✓	NT	✓	✓	(✓)	✓
Bromate	NT	NT	(✓)	✓	NT	NT	✓	NT	NT	NT	NT	NT
N-Acetyl-Glyphosate	NT	NT	✓	NT	(✓)**	✓	(✓)	NT	-	✓	✓	-
Difluoroacetic acid	NT	NT	NT	NT	NT	✓	✓	NT	✓	NT	NT	NT
Trifluoroacetic acid	NT	NT	NT	NT	NT	✓	✓	NT	✓	✓	-	(✓)
Thiocyanate	NT	NT	(✓)**	✓	NT	NT	✓	NT	✓	NT	-	(✓)
Desmethyl-Dimethoate	NT	NT	✓	-	NT	NT	NT	NT	NT	NT	NT	NT
Diethyl thiophosphate	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Diethyl phosphate	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
O,O-dimethyl dithiophosphate	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
O,O-diemthyl thiophosphate	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Diethyl dithiophosphate	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT
Dimethyl phosphate	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT	NT

✓ = satisfactory chromatography and detection sensitivity achieved,

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

(✓) = possible but compromised due to matrix effects or lacking separation or limited sensitivity or 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 (5.6.15)	M 4.1 (5.6.16)	M 4.2 (5.6.17)	M 4.3 (5.6.18)	M 5 (5.6.19)	M 6 (5.6.20)	M 7 (5.6.21)	M 8 (5.6.22)	M 9 (5.6.23)	M 10 (5.6.24)	M 11 (5.6.25)	M 12.1 (5.6.26)	M 12.2 (5.6.27)
Separation principle	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon	HILIC	HILIC	IC	IC	IC
Column type	Obelisc R	Obelisc R	Obelisc R	BEH-Amide	Luna PP	PFP	Obelisc R	Trinity P1	Hypercarb	Trinity P1	Torus DEA	AS19	CS17	CS21
ANALYTES COVERED BY LC-MS/MS IN THE ESI-NEGATIVE MODE														
Ethephon	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓	NT	NT
HEPA	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓	NT	NT
Glufosinate	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓	NT	NT
N-Acetyl-Glufosinate	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓	NT	NT
MPPA	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓	NT	NT
Glyphosate	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	a: ✓ / b: (✓)	NT	NT
AMPA	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓	NT	NT
Phosphonic acid	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓	NT	NT
N-Acetyl-AMPA	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓	NT	NT
Fosetyl-Al	✓	NT	NT	NT	NT	NT	NT	✓*	NT	NT	NT	✓	NT	NT
Maleic Hydrazide	✓	NT	NT	NT	NT	NT	NT	✓*	NT	NT	NT	(✓)	NT	NT
Perchlorate	✓	NT	NT	NT	NT	NT	NT	✓*	NT	NT	NT	✓	NT	NT
Chlorate	NT	NT	NT	NT	NT	NT	NT	✓*	NT	NT	NT	✓	NT	NT
Bialaphos	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	-	NT	NT
Cyanuric acid	NT	NT	NT	NT	NT	NT	NT	✓*	NT	NT	NT	✓	NT	NT
Bromide	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT
Bromate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
N-Acetyl-Glyphosate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	a: ✓ / b: (✓)	NT	NT
Difluoroacetic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	✓	NT	NT
Trifluoroacetic acid	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	✓	NT	NT
Thiocyanate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT
Desmethyl-Dimethoate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	NT	NT
Diethyl thiophosphate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Diethyl phosphate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
O,O-dimethyl dithiophosphate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
O,O-diethylthiophosphate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Diethyl dithiophosphate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Dimethyl phosphate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

✓ = satisfactory chromatography and detection sensitivity achieved,

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

(✓) = possible but compromised due to matrix effects or lacking separation or limited sensitivity or 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. Hypercarb (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 7500+	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 11: Method 1.4 (M1.4): "Per-ChloPhos" (see 5.6.5)	Perchlorate (quantitative) Chlorate (quantitative) Phosphonic acid (quantitative) Bromide (Screening, quantitative) Bromate (quantitative) Thiocyanate (Screening, quantitative) TFA (quantitative) DFA (quantitative)	1290 Agilent Infinity II and Sciex QTRAP 7500+	Mostly employed directly (option: screening by M 1.3, if positive -> M 1.4 or M 11) Dilution 10-fold Evaluation via solvent calibration and IL-ISs
M 4.1: "Quats & Co Obelisc R (see 5.6.16)	Paraquat (for specific commodities) Diquat (for specific commodities)	1290 Agilent Infinity II and Sciex QTRAP 5500	Analysis of specific relevant commodities. Evaluation via matrix-based calibration and IL-ISs
M 4.2: "Quats & Co BEH Amide" (see 5.6.17)	Amitrole ETU Chlormequat Mepiquat Daminozide PTU Cyromazine Trimethylsulfonium Nereistoxin Difenzoquat Melamine Propamocarb Morpholine (1 st screening) Diethanolamine (1 st screening) Triethanolamine (1 st screening) Aminocyclopyrachlor Chloridazon-desphenyl Mepiquat-4-hydroxy Propamocarb-N-desmethyl Propamocarb-N-oxide Nicotine Matrine Oxamatriene	1290 Agilent Infinity II and Sciex QTRAP 5500	Evaluation via matrix-based calibration and IL-ISs (except for Difenzoquat, Aminocyclopyrachlor, Mepiquat-4-hydroxy, Propamocarb-N-desmethyl, Propamocarb-N-oxide)
M 7: "Morpholine, Diethanolamine and Triethanolamine" (see 5.6.21)	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 mushrooms). Quantification via solvent-based calibration and IL-ISs

5.6.1. General recommendations and 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 chromatograms 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.

Hint: 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.

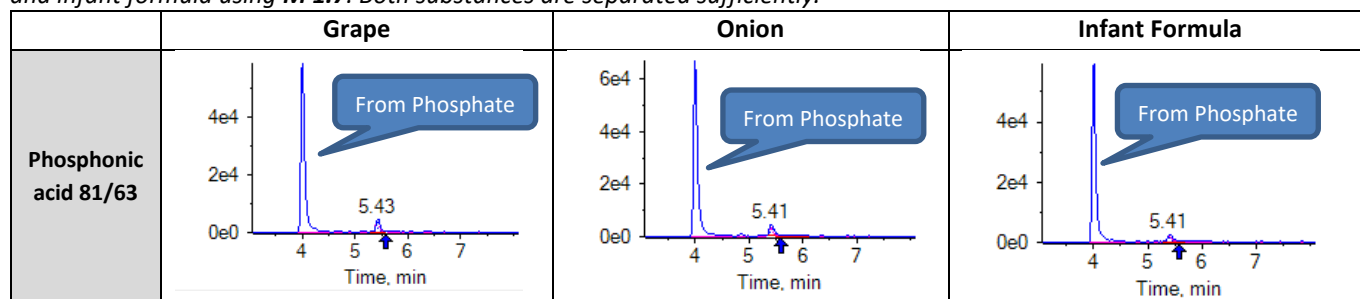
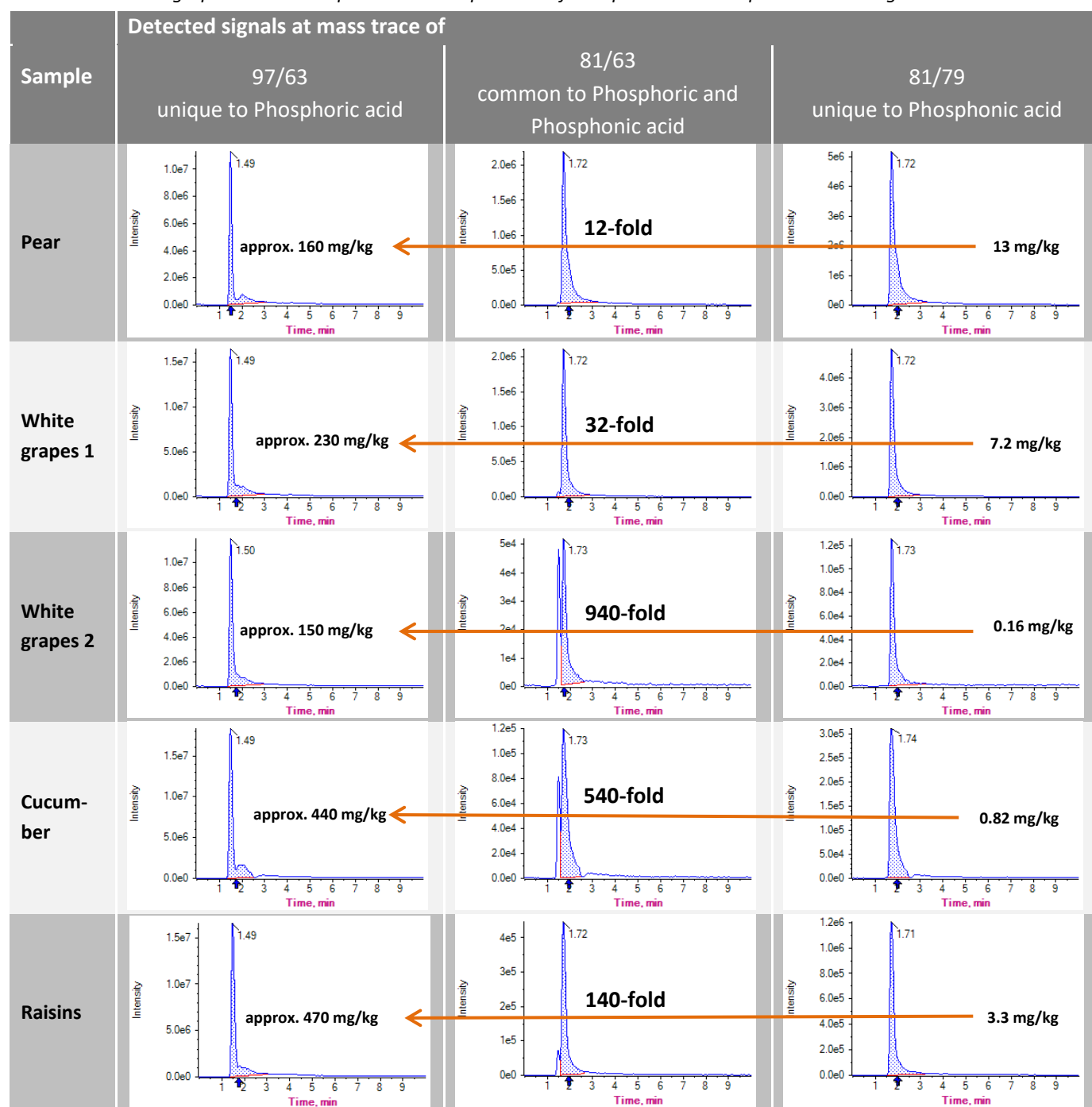


Table 10: Chromatographic and mass-spectrometric separation of Phosphoric and Phosphonic acid using M 1.4.


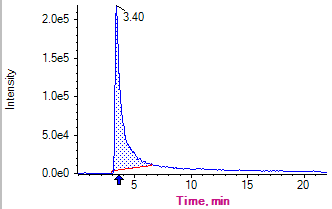
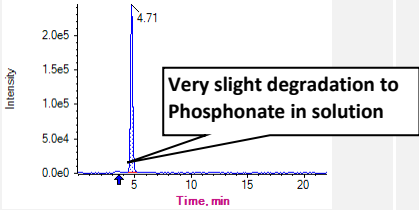
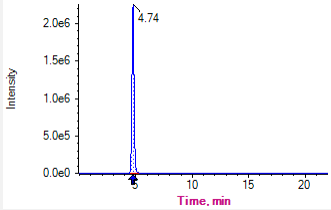
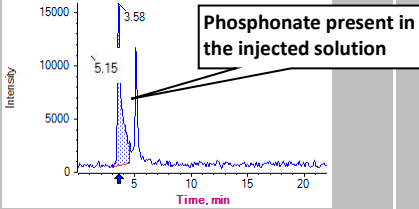
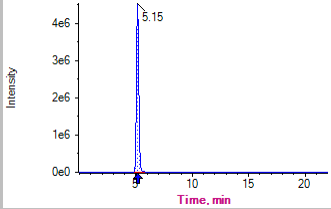
c) **Interference of Phosphonic acid by Fosetyl and Fosetyl-D₅:**

Fosetyl and its D₅-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.**

- d) **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-D₅ at 0.1 µg/kg the in-source fragmentation was less abundant (corresponding to approx. 0.001 µg/mL Phosphonic acid) but Phosphonic acid as impurity showed up at its proper retention time at a concentration corresponding to approx. 0.007 µg/mL.

Hint: 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-D₅ (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-D₅ the mass trace of Phosphonic acid is also shown to demonstrate the occurrence of in-source fragmentation of Fosetyl and Fosetyl-D₅ towards Phosphonic acid as well as the presence of Phosphonic acid as an impurity of the Fosetyl-D₅ standard solution.

e) Paraquat interfered by Diquat:

Transitions 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 6.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 D₈ ($[M^{2+} - H^+]^+ 193/\#$), which is interfered by Diquat D₈. Furthermore, those transitions are less sensitive.

f) 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).

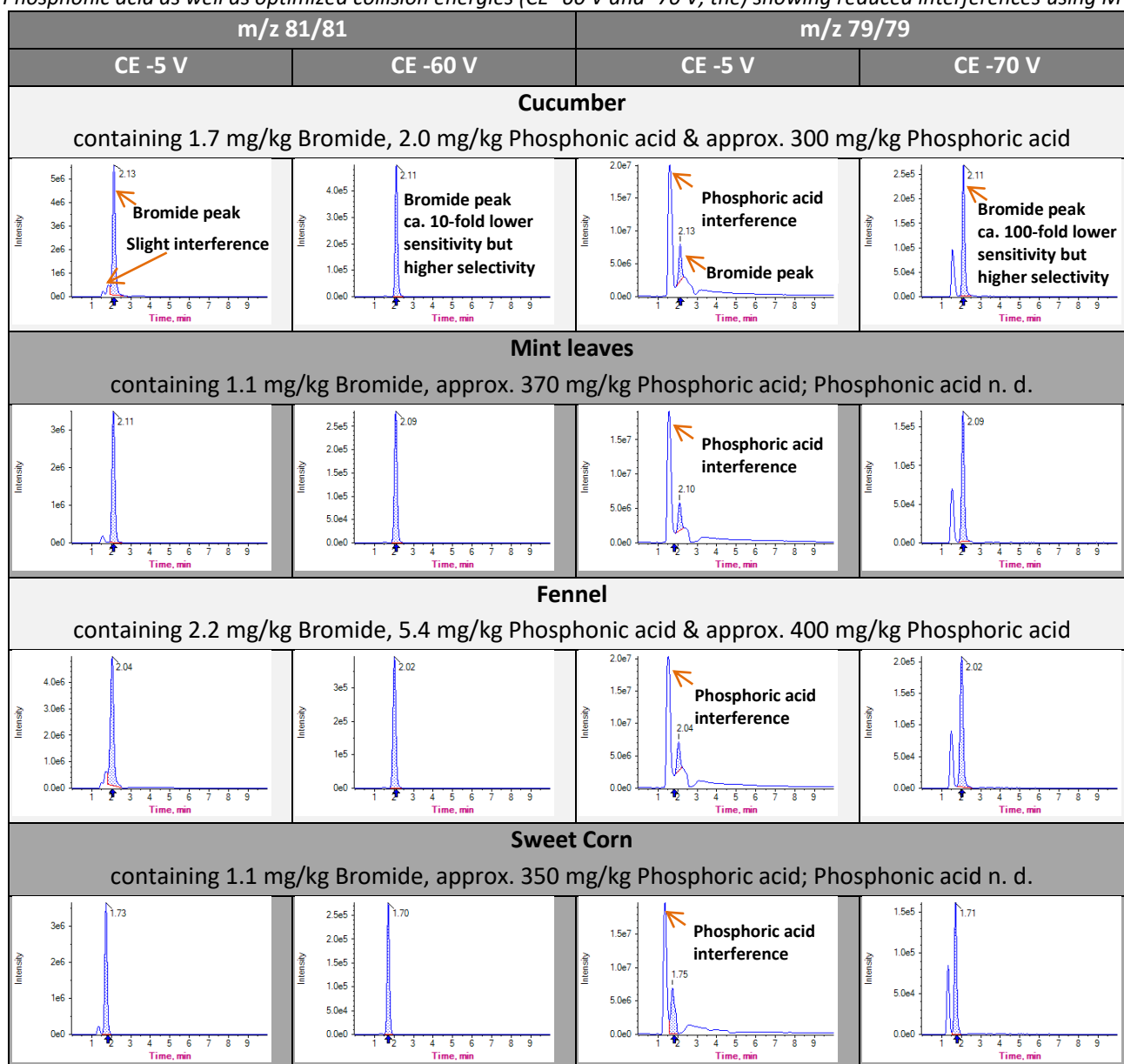
Bromide is mainly composed of two naturally occurring stable isotopes, that are almost equally frequent ($^{79}\text{Br}^-$ and $^{81}\text{Br}^-$). For bromide (element-ion), no MS/MS fragmentation is possible so that MS/MS analysis has to rely on "parent/parent" analysis. The mass trace $m/z 81/81$ 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 $[\text{H}_2\text{PO}_3]^- = 81$) whereas $m/z 79/79$ is highly affected by Phosphoric acid due to in-source fragmentation (**Table 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.

Hint: 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 interferences caused by Phosphoric and Phosphonic acid can be further reduced by increasing the Collision Energy (CE) for the m/z 81 and 79 (Table 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.

Table 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.



2. Degradation and Contamination:

a) Possible contaminations by consumables:

Check filters, sorbents, chemicals and other consumables used in sample preparation for any contamination. Contamination originating from filters was observed, e.g. in the case of Perchlorate, Chlorate and Phosphonic acid (see comments under 2.12). Contamination originating from the C18 sorbent was observed with Morpholine. Contamination originating from the formic acid used in extraction was observed in the case of Perchlorate, Phosphonic acid and Trifluoroacetic acid. Reagent blanks, as QC measure are recommended at least as soon as one of the relevant consumables is changed (e.g. different batch). **Contamination originating from EDTA (p.a.) was observed in the case of Chlorate.**

Note

- Contaminations occurring during or after a dilution step have a more severe influence on the results and are thus more critical.
- For contaminations originating from IL-IS or other analytical standards check among others points: b), d), e), f)

b) Issues concerning the purity of N-Acetyl-Glufosinate D₃:

There are two types of N-Acetyl-Glufosinate D₃ 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-D₃), leading to false positive results. Fortunately the degradation rate observed in the water:acetonitrile 9:1 mixture (see **Figure 50**) was negligible. More important is the content of native glufosinate in purchased N-Acetyl-Glufosinate (acetyl-D₃) standards.

Hint: 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**.

c) Stability of the Phosphonic acid IL-IS:

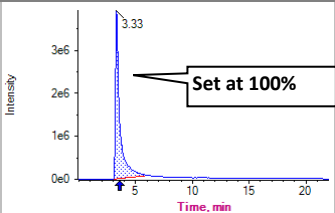
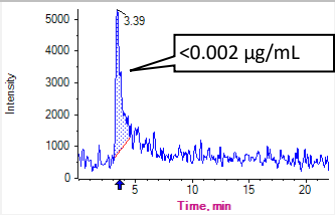
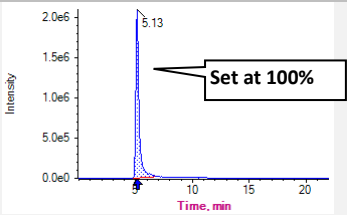
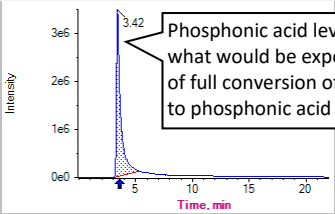
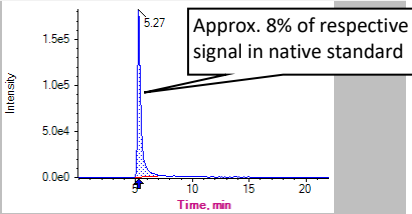
In presence of water and especially at high pH levels, Phosphonic acid ¹⁸O₃ will gradually convert to ¹⁸O₂¹⁶O₁, ¹⁸O₁¹⁶O₂ and eventually of ¹⁶O₃ (native) Phosphonic acid. The ¹⁸O₃ **Phosphonic acid standard solution provided by the EURLs should be preferably diluted in acetonitrile**, where it was shown to be stable for long periods.

d) Degradation of Ethephon, Fosetyl and their respective IL-ISs to Phosphonic acid:

Fosetyl and Ethephon as well as their respective IL-ISs 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-D₄ standard containing only ca. 0.08 µg/mL instead of the expected 1 µg/mL Ethephon-D₄ and ca. 0.8 µg/mL Phosphonic acid. The use of such an IL-IS would contaminate the sample with Phosphonic acid leading to false positive results.

Hint: To be on the safe side Fosetyl, Ethephon and their respective IL-ISs 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 **“Interference of Phosphonic acid by Fosetyl:** Fosetyl and its D₅-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-D₄ 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-D₄ 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 D₂ 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 D₂-MH at 0.25 % (a typical level encountered) the resulting concentration of native MH following the addition of 20 µg D₂-MH to 10 g sample will be at 0.005 mg /kg sample. This aspect is to be considered when setting the Reporting Limits of MH as well as when judging residue levels in samples having low MRLs (e.g. baby food) or organic food. Alternatively,

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-¹⁸O₃ and Perchlorate-¹⁸O₄, containing approx. 0.2 µg/mL Chlorate-¹⁸O₃ and approx. 0.02 µg/mL Perchlorate-¹⁸O₄.

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-¹⁸O₃ 89/71	Perchlorate-¹⁸O₄ 107/89

g) Degradation of Diquat and Paraquat:

Both compounds tend to undergo redox reactions via radicals and are therefore sensitive to light, as well as to certain chemicals. It was for example observed that Diquat-D₄ shows the tendency for D/H replacement, especially in methanol. Additionally, a degradation of both native Diquat and Diquat-D₄ was observed in methanol, as well as in acidified methanol (3.7). This degradation was accelerated by sun light. A good stability of stock solutions (>2 years) was observed in 10% acetonitrile in water.

3. Using Collision Energy dependent signal shifts to increase identification certainty:

In its 2026 version, the **SANTE quality control procedures** included the use of “different instrumental settings (e.g. different collision energies)” as a measure to verify identification where the requirements for identification of Table 3 (Chapter D8) cannot be met. This is particularly interesting for analytes having only one sufficiently sensitive mass transition with the sensitivity of the second mass-transition essentially dictating the concentration at which the SANTE ion-ratio criteria for a successful identification are met. By conducting parallel measurements on the most intensive mass transition at collision energies deviating from the optimized value the signal intensities are expected to shift at a different rate than those of interfering matrix compounds. The ratios of the signal intensities obtained for the selected mass transition using the different collision energies can serve as a complementary criterion for analyte identification (by comparing them with those of a reference standard in the same way as traditional “ion ratios” are used). A thorough description how this can be utilized is given in the following document Analytical Observation on TFA and DFA (SRM-54).

4. Dealing with high background and unavailability of residue-free sample material for validation experiments

A practical guidance on how to deal with background levels in blanks was implemented in the **SANTE quality control procedures** in Appendix A - 1. Initial full validation. It is differentiated between backgrounds of ubiquitous compounds, which may arise from contamination in the laboratory, such as from glassware, solvents (e.g. DDAC, BAC, TFA, chlorate, phosphonic acid, perchlorate, chlormequat, morpholine), and compounds that are absent in procedural blanks but present in known non-treated or organic products (e.g. bromide, copper, TFA, phosphonic acid, chlorate, cyanuric acid and nicotine). In such cases, the guidelines foresee replicate analysis of a sample with the lowest level. If the criteria for identification and repeatability are met, this particular level may be established as the LOQ. Recovery rates are determined by spiking at a level of at least three times the previously determined background.

5. Dealing with low absolute recovery rates

For some analytes (e.g. glyphosate, paraquat, diquat), recovery rates depend both on the type of matrix as well as the amount of matrix employed for extraction. Where the equilibria are dynamic and there is no issue of analyte extractability/accessibility, IL-ISs efficiently correct for reduced recovery. According to the **SANTE quality control procedures** the absolute recovery of analyte needs to range between 30 and 140%, otherwise the procedure needs to be adjusted. In cases of recoveries <30%, it is typically helpful to reduce the sample weight, see **Figure 7** and **Figure 8**. As this measure also affects the LOQ this modification needs to be validated to determine the LOQ.

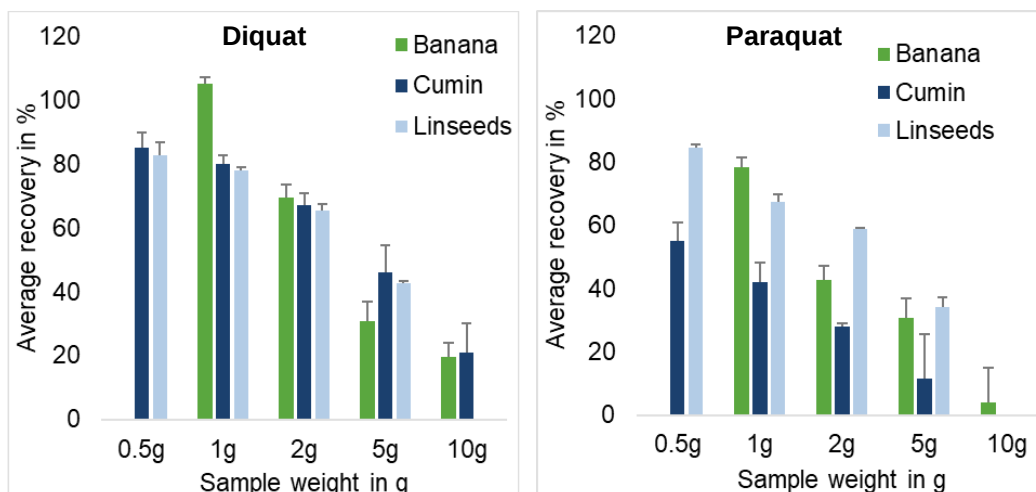


Figure 7: Average recovery rates (n=3) of diquat and paraquat in spiked banana, linseeds and cumin at different sample weights: 0.5 g (linseeds, cumin), 1 g, 2g, 5g (banana, linseeds, cumin), and 10 g (banana, cumin, note paraquat was not detected in 10 g cumin). During spiking, 1 µg diquat or paraquat (absolute quantity) was spiked on each sample portion resulting in a concentration of 0.05 µg/mL in the measurement solutions, i.e. 2 mg/kg on 0.5 g sample weight, 1 mg/kg on 1 g sample weight, 0.5 mg/kg on 2 g sample weight, 0.2 mg/kg on 5 g sample weight, 0.1 mg/kg on 10 g sample weight.

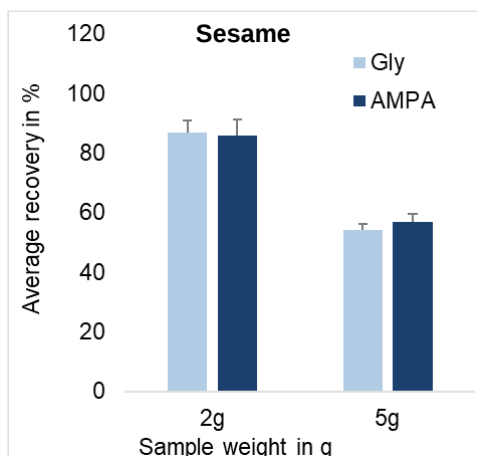


Figure 8: Average recovery rates (n=3) of AMPA and Glyphosate (Gly) spiked at 0.2 mg/kg each in sesame with using different sample weights: 2 g and 5 g.

6. 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 ($[M]^{+•}$). 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 similarly 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 corresponding 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

b)

c) **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 $[M]^{+•}$ 184/128***	IL-IS Diquat D_8 $[M]^{+•}$ 192/134	4	184	128	60	55	8
Diquat $[M]^{+•}$ 184/106		5	184	106	60	23	8
Diquat $[M^{+•}]$ 184/78		5	184	78	60	65	12
Diquat $[M]^{+•}$ 184/156***		4	184	156	60	29	10
Diquat $[M]^{+•}$ 184/169		5	184	169	60	27	12
Diquat $[M]^{+•}$ 184/155		5	184	155	60	43	12
Diquat $[M]^{+•}$ 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] ⁺⁺ 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] ⁺⁺ 186/171	IL-IS Paraquat D ₈ [M] ⁺⁺ 194/179	1	186	171	41	25	12
Paraquat [M] ⁺⁺ 186/77		3	186	77	41	57	4
Paraquat [M] ⁺⁺ 186/155		4	186	155	41	55	8
Paraquat [M] ⁺⁺ 186/128		5	186	128	41	57	12
Paraquat [M] ⁺⁺ 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] ⁺⁺ 194/179	-		194	179	71	27	10
IL-IS Paraquat D ₈ [M ²⁺ - H ⁺] ⁺ 193/178	-		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 ([M²⁺- 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]⁺⁺). Furthermore these transitions tend to be interfered by Diquat. Same applies to the respective MRM of Paraquat D₈ ([M²⁺- H⁺]⁺193/#), which is interfered by Diquat D₈.

*** 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)

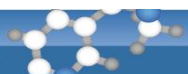
Analyte	Type of parent ion	Matrix Effect of parent (%) (MRM)	Matrix Effect of corresponding IL-IS D ₈ (%) (MRM)
Diquat	[M] ²⁺	+57 (92/84)	+54 (96/88)
	[M ²⁺ - H ⁺] ⁺	-92 (183/157)	-91 (191/165)
	[M] ⁺⁺	-91 (184/128)	-93 (192/134)
Paraquat	[M] ²⁺	+111 (93/171)	+112 (97/179)
	[M ²⁺ - H ⁺] ⁺	-74 (185/170)	-78 (193/178)
	[M] ⁺⁺	-81 (186/171)	-78 (194/179)

d) Unwanted retention in the tubing leading to peak tailing and/or carry-over effects:

Be aware, that some analytes tend to interact with surfaces within the tubing of the LC-system. This effect contributes to **peak tailing** and increases the risk of significant **carry-over** phenomena. Examples of such analytes are Diquat, Paraquat, Phosphonic acid, Glyphosate.

Carry over: The extend of carry-over effects depends on the condition of the tubing (active sites, degree of surface contamination) as well as the composition of the injected extract, the eluent and the washing solutions. It is not uncommon, that carry-over is more pronounced when injecting blank matrix extracts rather than pure solvent, as the matrix-components contained in the former may displace the analytes from the active sites. For the same reason a standard in pure solvent will typically cause a greater carry-over to the subsequent injection than an equally concentrated standard in blank matrix extract. It is of high importance to **regularly check for carry-over effects** by injecting both blank solvent as well as blank matrix extracts. Where significant carry-over effects occur be aware to run blanks after injecting calibration solutions or extract with high levels of the analyte. Samples suspected of having been affected by carry over may need to be re-injected.

Peak tailing: The effect of analytes interacting with surfaces within the tubing will also contribute to peak tailing. Long-term observations suggest the presence of active sites of different affinity strength within the



LC- system. For some analytes this might lead to a complete loss of the analyte signals and/or severe tailing (e.g. Glyphosate and Paraquat). Conditioning/priming with the affected compound via multiple injections (e.g. >10) of a standard solution at a relatively high concentration (e.g. 0.5 µg/mL) can help to saturate the system and reduce such effects.

e) **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**).

f) **Analysis of bromate:**

Although bromate is not listed as an active substance for plant protection, it can be a by-product of the oxidative treatment of water that is used for preparing or washing food. Issues in the stability of native standards, a contamination of the bromate IL-IS with bromide and an interference in the mass transition 135/117 of bromate IL-IS were observed, which lead to poor performance.

g) **Nereistoxin-D₆ (IL-IS):**

The retention time of Nereistoxin-D₆ (IL-IS) is slightly shifted from the retention time of the native substance (~ 0.2 min). Unfortunately, no other IL-IS is currently available.

7. **Handling of column, pre-column, pre-filters and maintenance:**

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 min 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 min with **Eluent A** (water) at 0.3 mL/min Run system 3-4 times with full gradient (inject standards in matrix)

Note: When flushing NaOH or Borax solution through the column make sure that it will go directly into waste and not to the MS ion source!.

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/web-docs/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 anthocyanins 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-H₂O 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 9, Figure 10 and Figure 11.

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.		

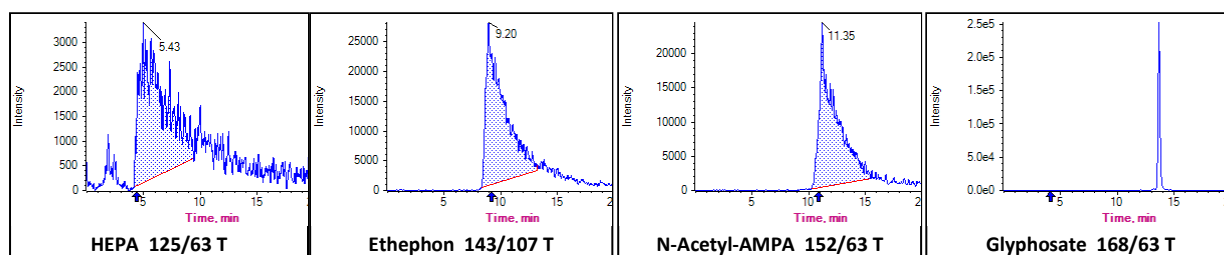


Figure 9: Chromatograms obtained using a new Hypercarb column, poor chromatographic behavior due to strong interactions of analytes with active sites. Same behavior is observed when the pre-column is new.

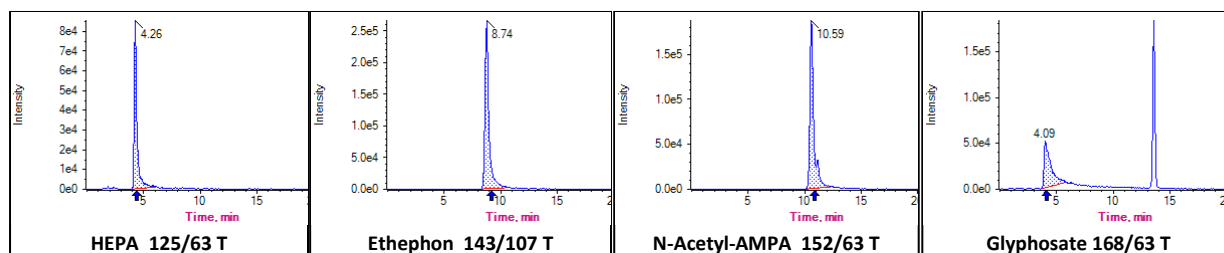


Figure 10: Chromatograms following priming with 25 injections QuPPE extracts of spinach. Injection volume 50 µL per injection

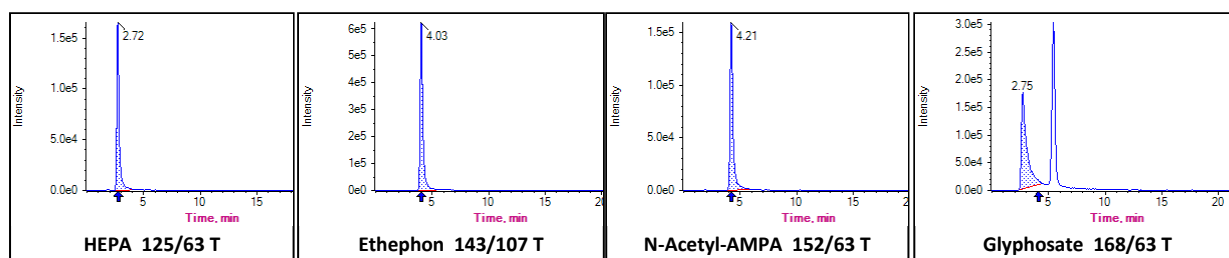


Figure 11: Chromatograms after additional injection of approximately 100 QuPPE-extracts of various fruit and vegetables during normal routine use.

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 Na₂EDTA. 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 Methylene diphosphonic Acid (Medronic Acid) (1984-15-2).

e) IC-MS/MS analysis

Using IC-MS/MS regular maintenance of the autosampler can help to obtain a stable performance of the system regarding sensitivity and reproducibility, see also hints on M11.

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;

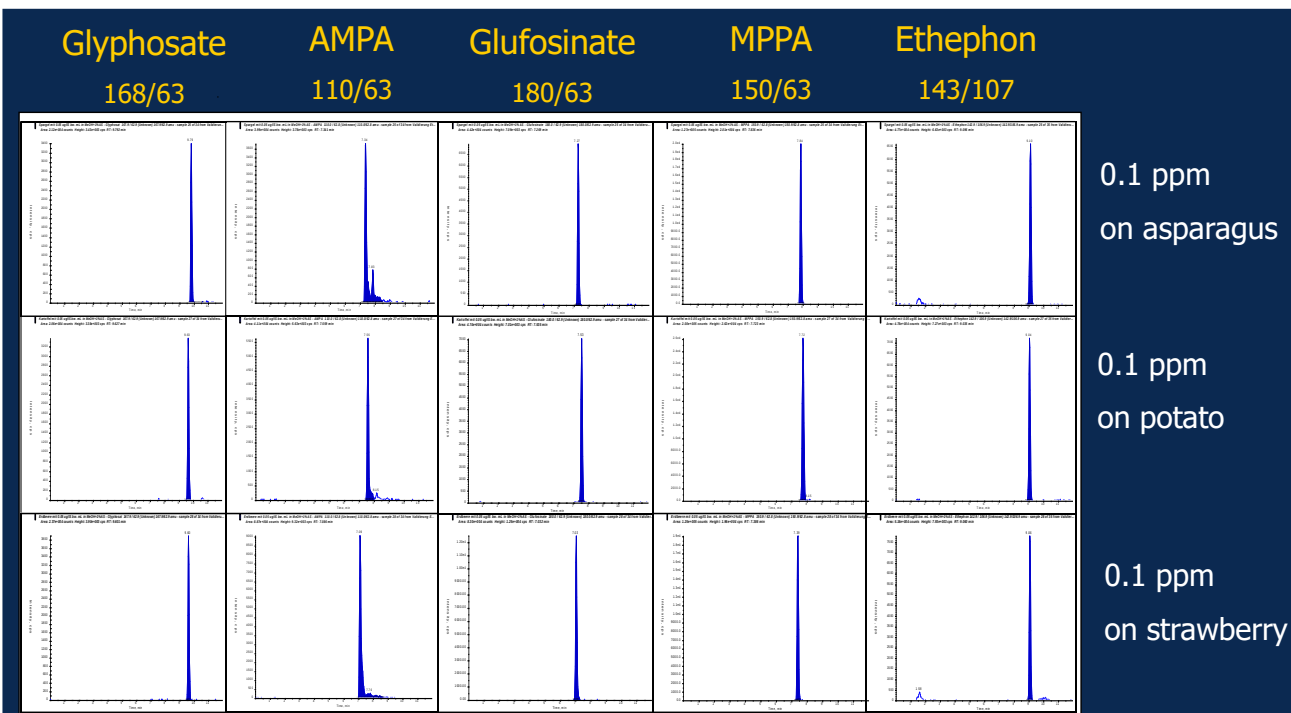
HEPA: 2-Hydroxyethylphosphonic acid (= hydroxy-ethephon),

* 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

- 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.
- Handling of column, pre-column and pre-filters:** See 5.6.1. point **7.a)**
- For general hints on analytes:** See 5.6.1



CRL-SRM

EPRW 2008, June 1-5, Berlin

Community Reference Laboratory
for Pesticide Residues
using Single Residue Methods

Figure 12: Typical chromatograms of Glyphosate, AMPA, Glufosinate, MPPA and Ethephon spiked on blank-QuPPE extracts

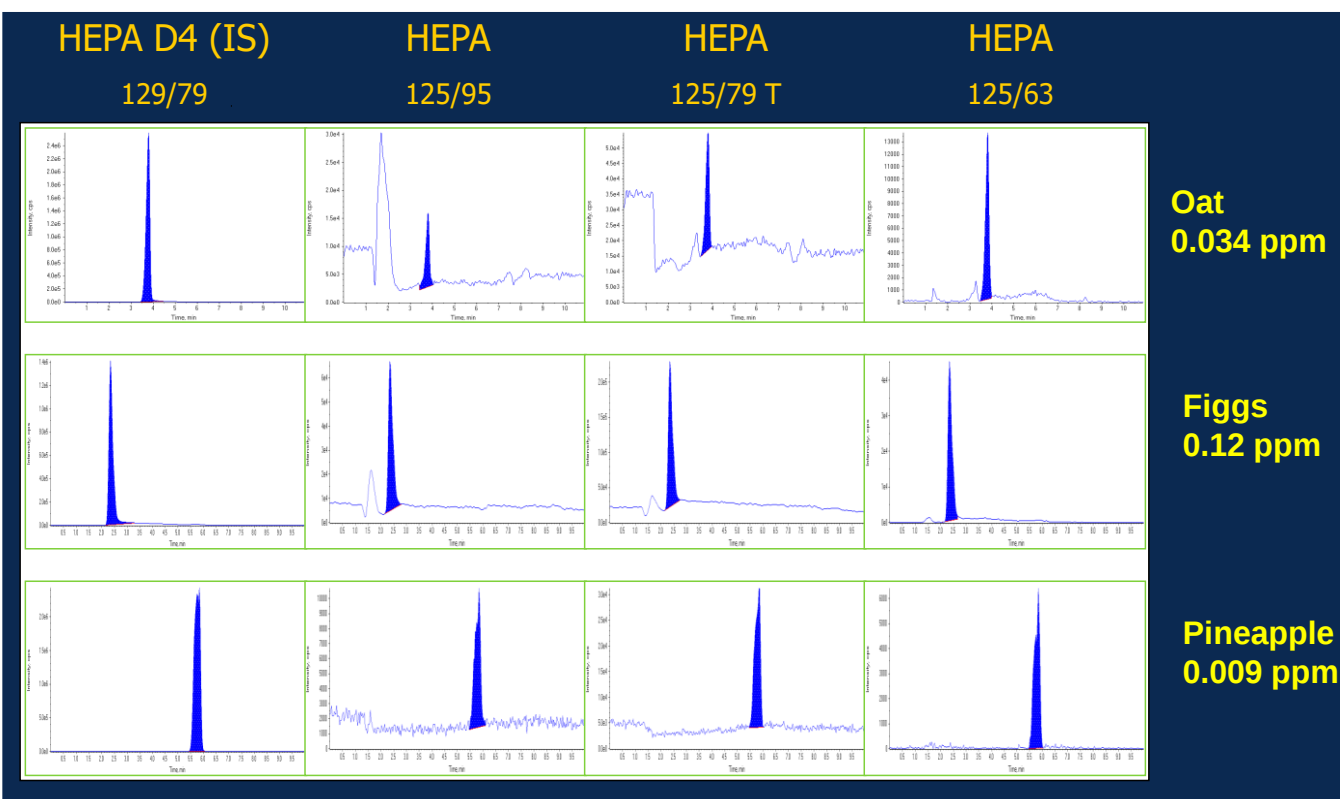


Figure 13: Typical chromatograms of HEPA in real samples

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 7.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. **Interference of Phosphonic acid by Fosetyl:** See 5.6.1.
4. **Interference of Phosphonic acid by Phosphoric acid:** See 5.6.1.
5. **IL-IS of N-Acetyl-Glufosinate D₃:** See 6
6. **For general hints on analytes:** See 5.6.1

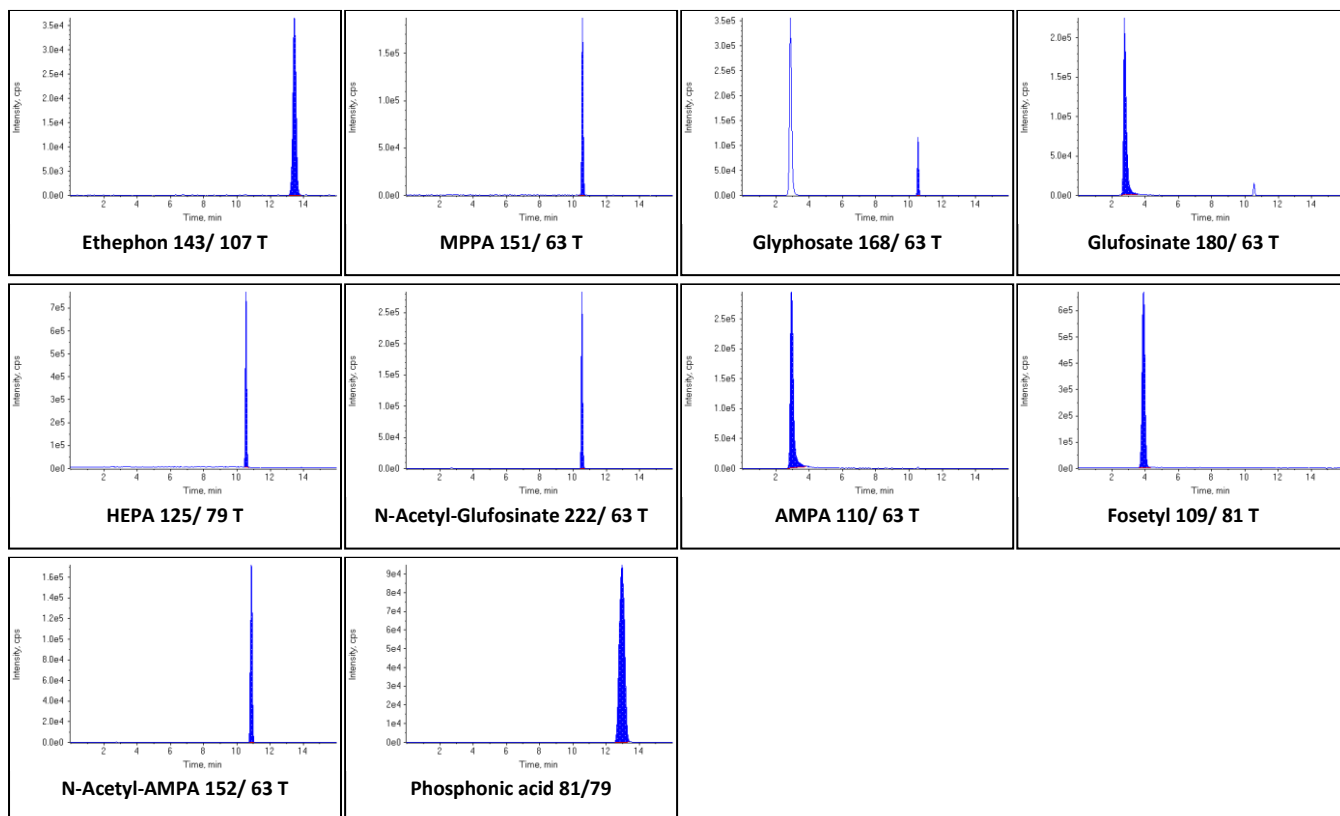


Figure 14: Typical chromatograms of Ethephon, HEPA, Glyphosat, AMPA, Glufosinate, MPPA, N-Acetyl-AMPA, N-Acetyl-Glufosinate, Fosetyl-Al and Phosphonic acid at 0.1 mg/L in methanol with 1% formic acid.

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 7.a) and 7.b)
2. **Mass spectrometric interference of AMPA and Fosetyl:** See 5.6.1
3. **Interference 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 oilseeds, 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 D₃** 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

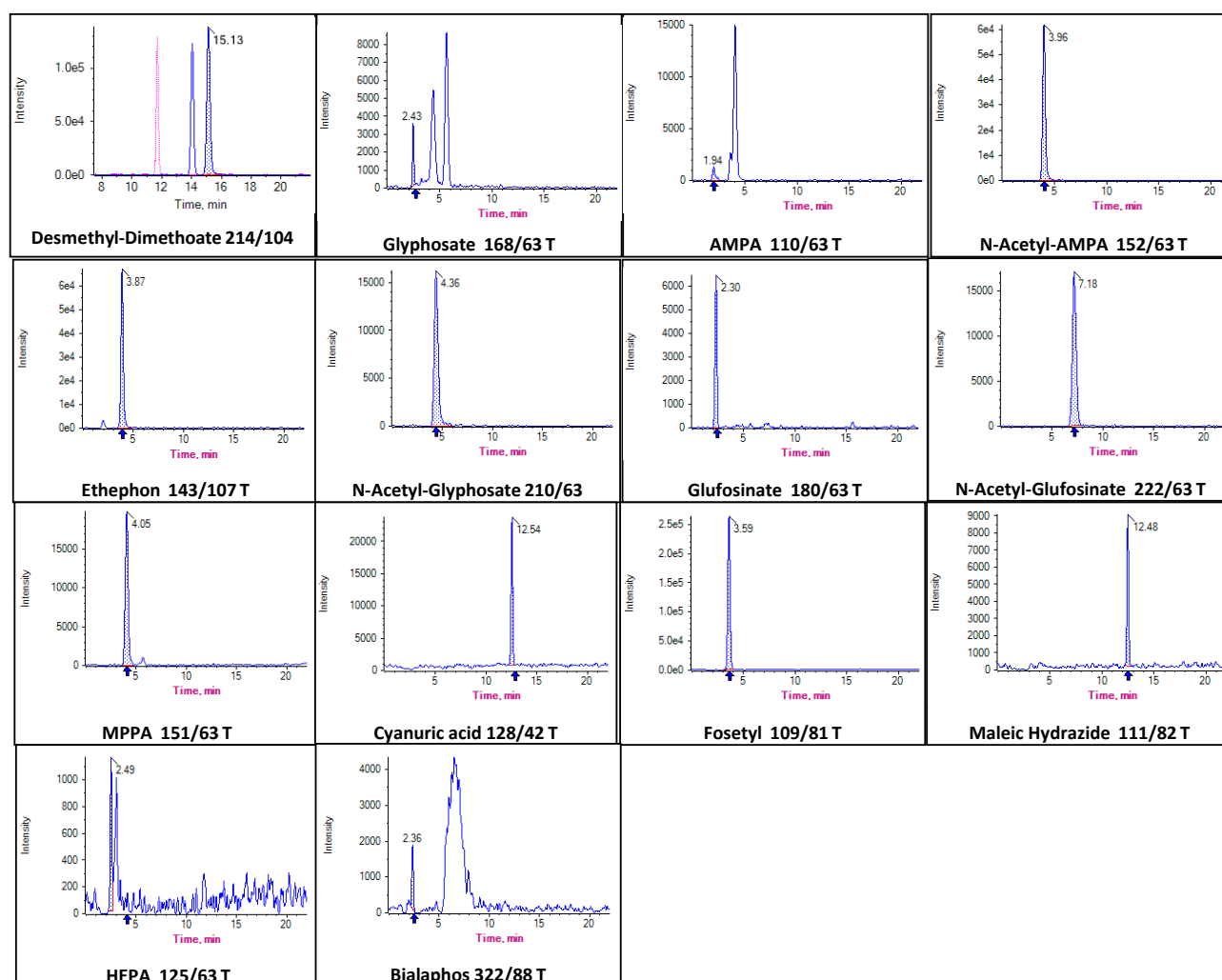


Figure 15: Chromatograms of Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Fosetyl, Maleic Hydrazide, Cyanuric acid and Bialaphos at 0.02 mg/kg on apple extract and Desmethyl-Dimethoate at 0.03 mg/kg on cherry extract.

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-¹⁸O₃ (IL-IS)	135/117	
	Bromide*	81/81, 79/79	
	Chlorate	83/67, 85/69	
	Chlorate-¹⁸O₃ (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-¹⁸O₃ (IL-IS)	87/85, 87/67	
	Thiocyanate	58/58	
	Thiocyanate ¹³C ¹⁵N	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 **7.a)** and **7.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. **Interference 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

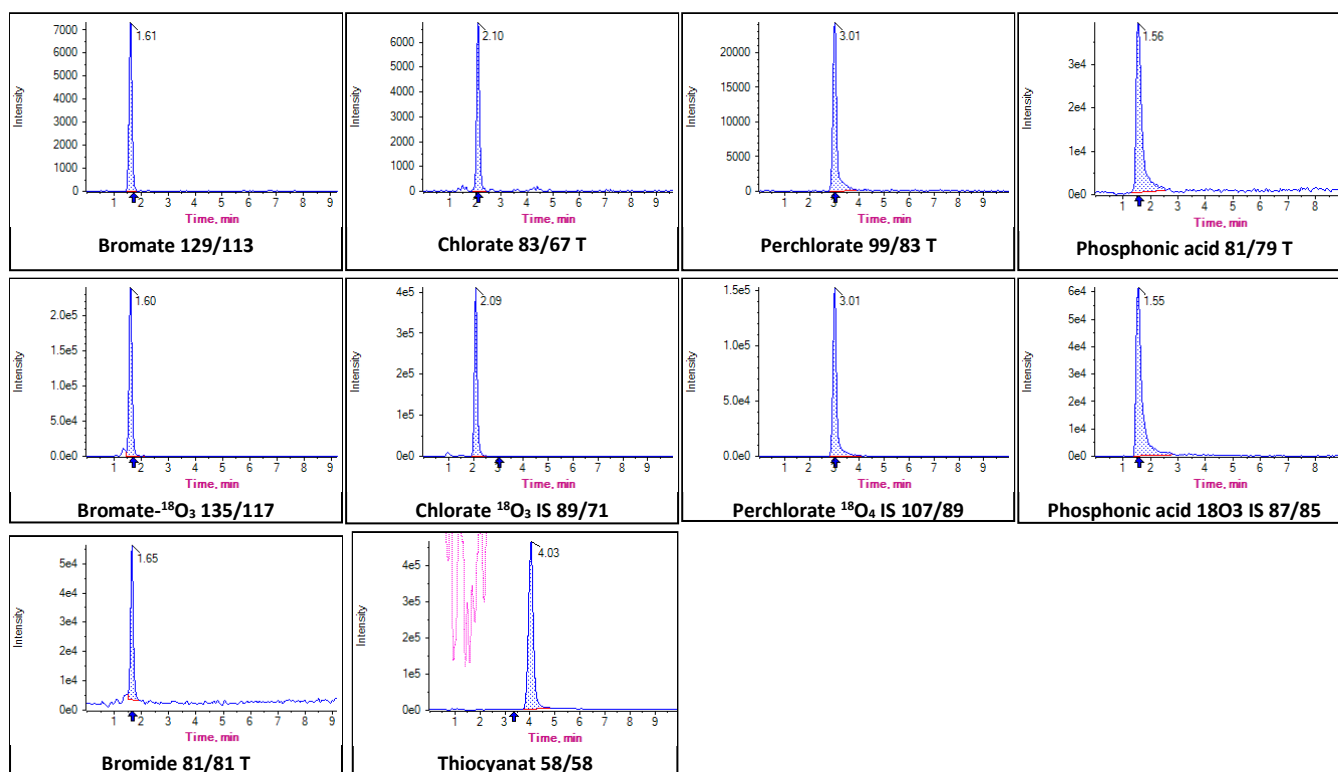


Figure 16: Chromatograms of Bromate (0.02 mg/kg) in currant extract, Bromide (1 mg/kg) in currant extract, Phosphonic acid (0.05 mg/kg) in currant extract, Perchlorate (0.01 mg/kg) in currant extract, Chlorate (0.01 mg/kg) in currant extract and Thiocyanate (1 mg/kg) in porree extract.

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- 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	

* 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

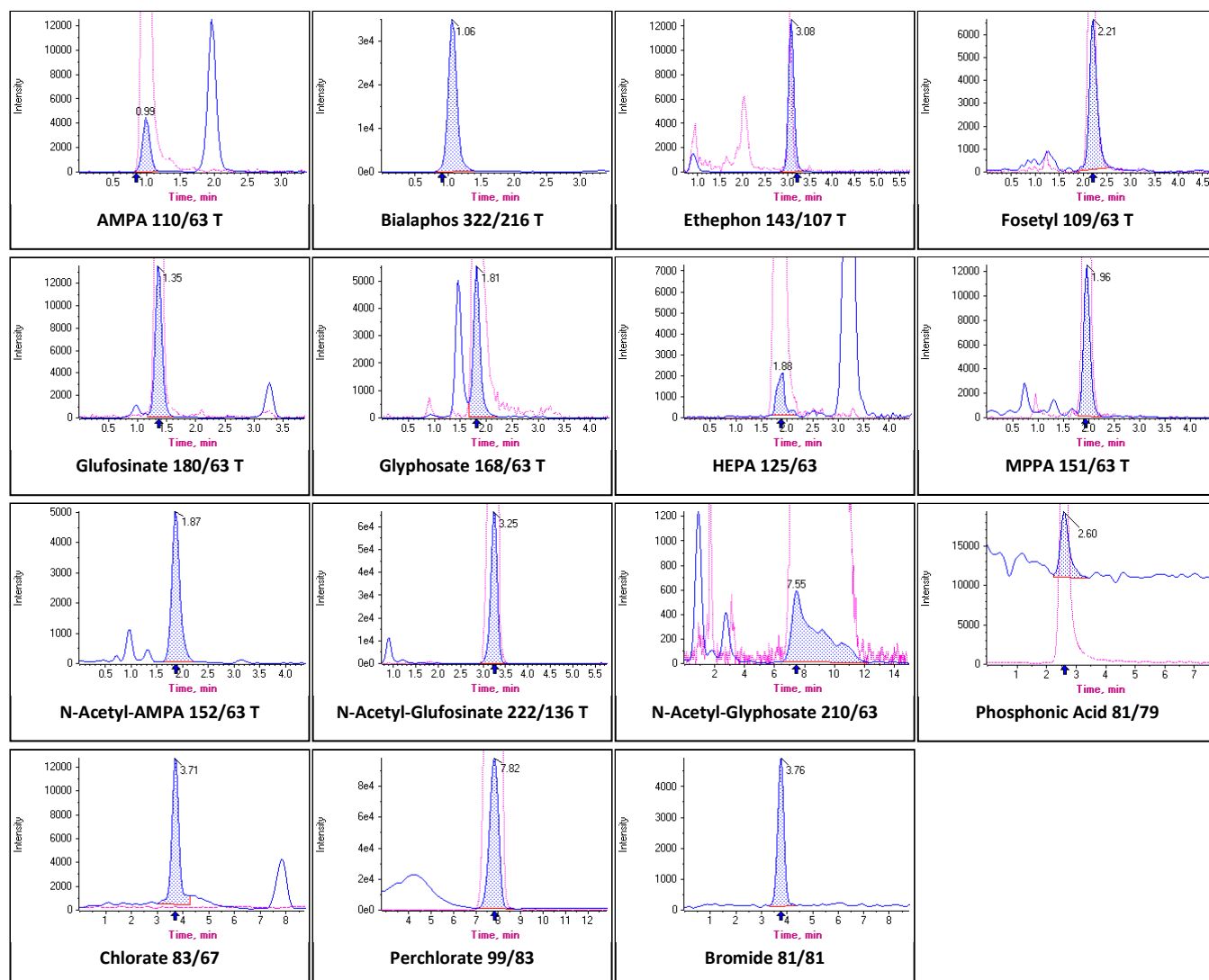


Figure 17: Chromatograms of Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Fosetyl, Maleic Hydrazide, Cyanuric acid, Bialaphos and Bromide at 0.02 mg/kg each, Phosphonic acid at 0.05 mg/kg, as well as Chlorate and Perchlorate at 0.005 mg/kg 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, 69/19	
	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	
	Difluoroacetic acid (DFA)	95/51, 95/95	
	Difluoroacetic acid - ¹³ C (IL-IS)	97/52	

Hints on Method 1.6a and Method 1.6b

1. **Handling of column, pre-column and pre-filters:** see 5.6.1 point 7.a) and 7.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 interferences of matrix components on peak shapes and intensities (signal suppression). **Figure 20** 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 (5.6.17), whereas Cyanuric acid is not regulated.
3. **For general hints on analytes:** See 5.6.1

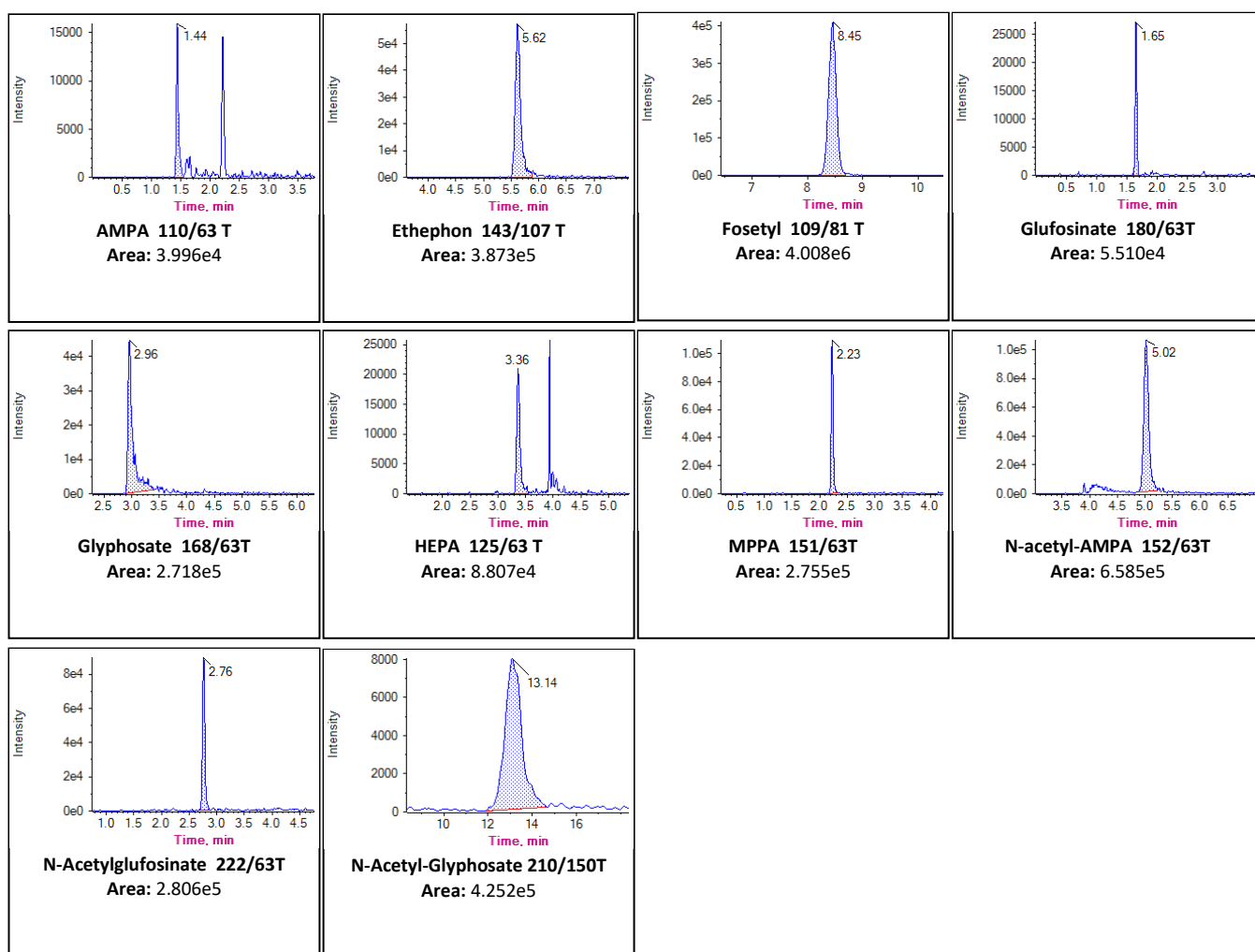


Figure 18: Chromatograms of Glyphosate, AMPA, N-Acetyl-AMPA, N-Acetyl-Glyphosate, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate, Fosetyl at 0.04 mg/kg on cucumber extract using a: Waters Torus™DEA 2.1 mm x 100 mm.

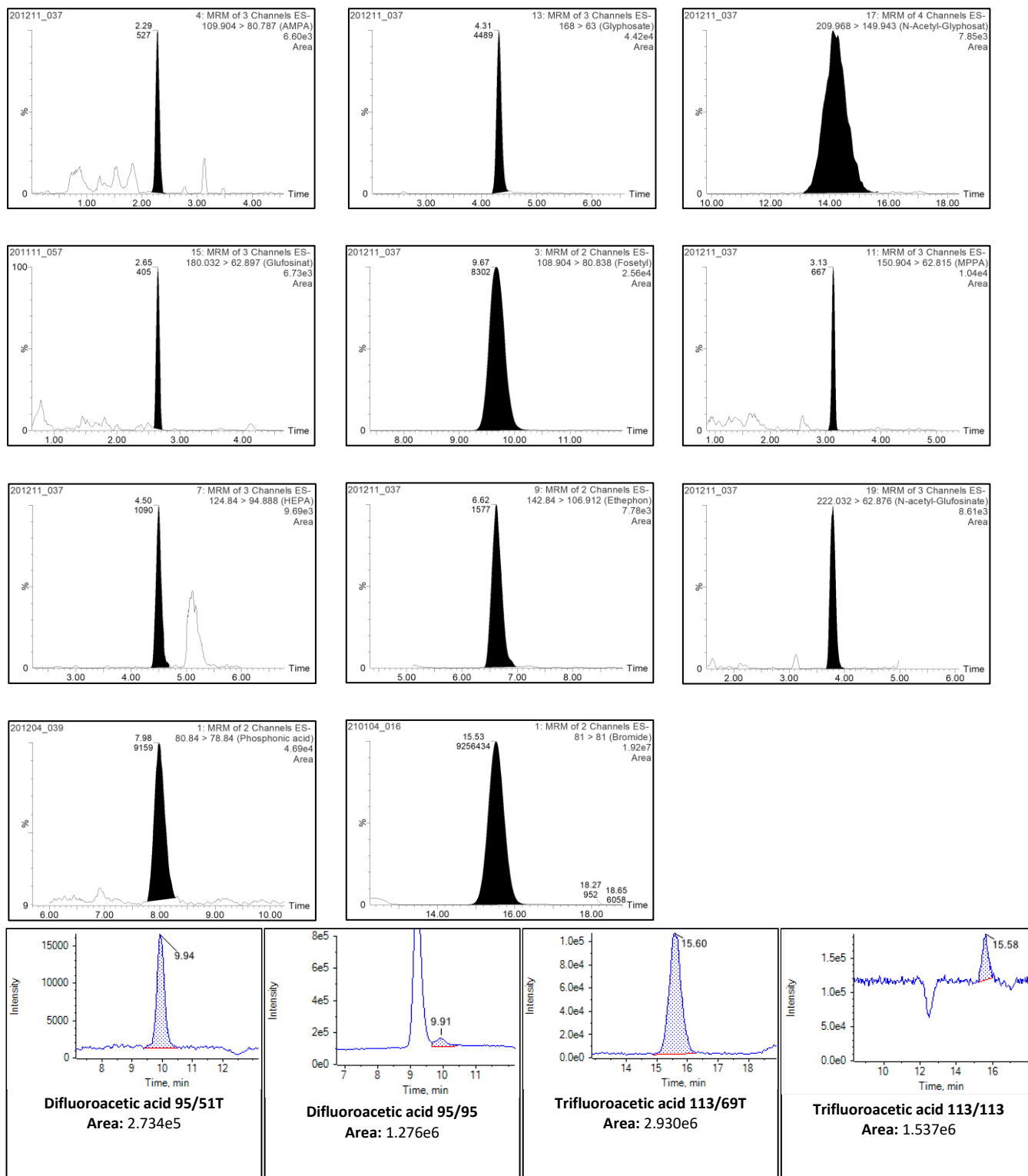


Figure 19: Chromatograms of Glyphosate, AMPA, N-Acetyl-Glyphosate at 0.06 mg/kg, Glufosinate at 0.036 mg/kg, HEPA, MPPA, N-Acetyl-Glufosinate at 0.024 mg/kg, Ethephon, Fosetyl at 0.012 mg/kg, all in strawberry extract and Phosphonic acid at 0.06 mg/kg, Bromide at 6 mg/kg both in lemon extract; difluoroacetic acid at 0.02 mg/kg, trifluoroacetic acid at 0.05 mg/kg in grape extract using *b*: Waters Anionic Polar Pesticide Column, 130Å, 5 µm, 2.1 mm x 100 mm.

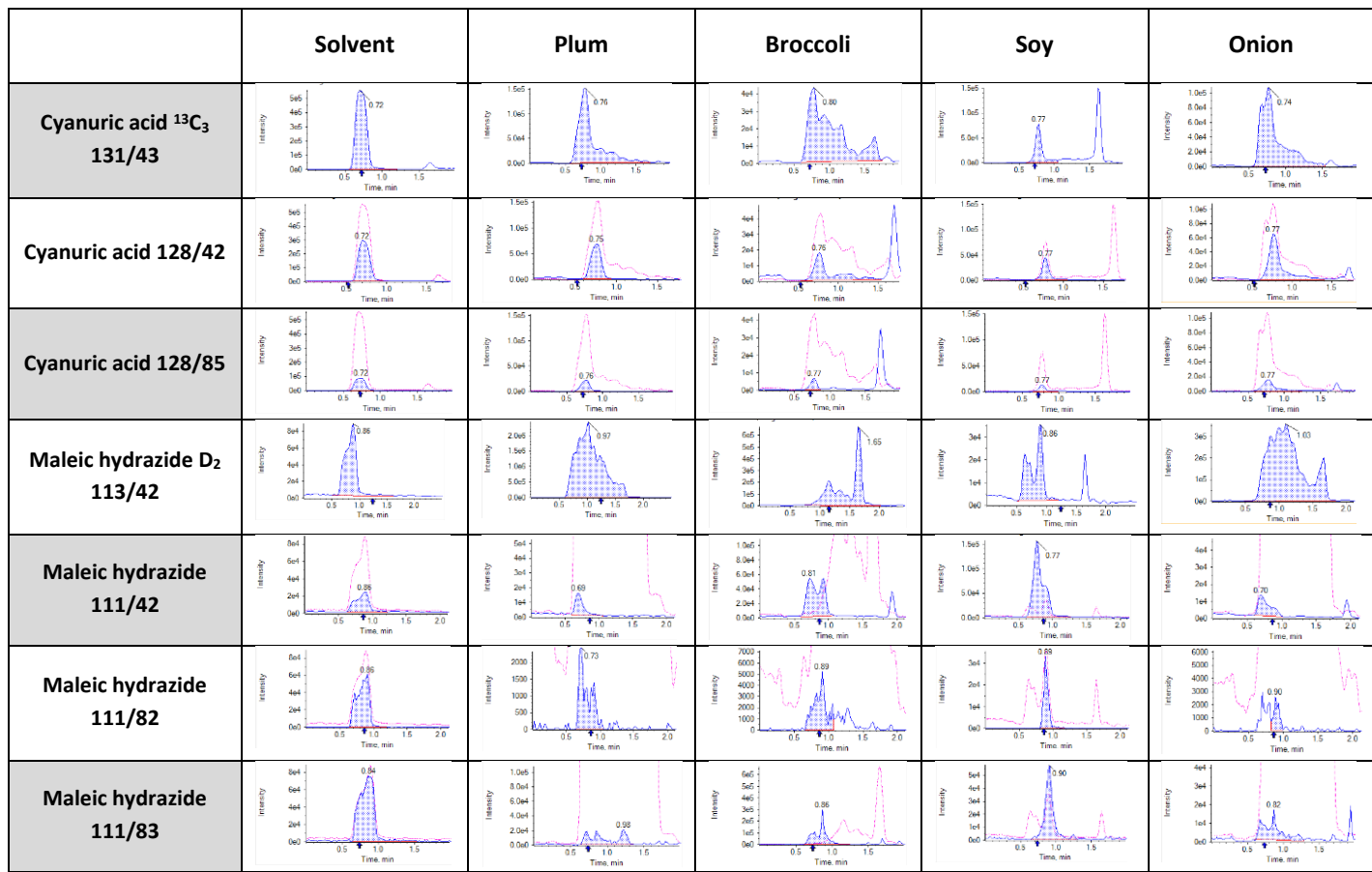


Figure 20: Exemplary peak shapes of Cyanuric acid and Maleic hydrazide in solvent-based standards and in standards of plum, broccoli, soy and onion extracts at 0.1 µg/mL (Please also read the note under “Hints on Method 1.6a and Method 1.6b” above)

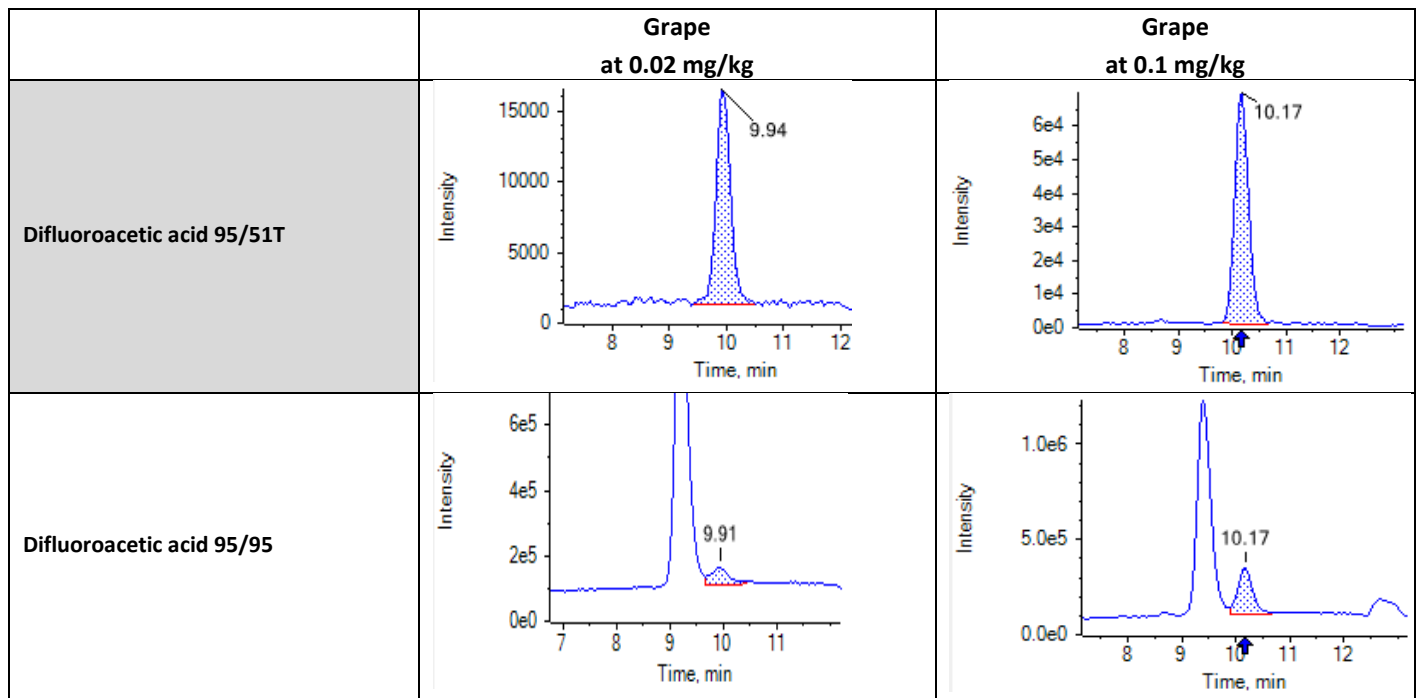


Figure 21: Chromatograms of difluoroacetic acid at 0.02 mg/kg and 0.1 in grape extract using b: Waters Anionic Polar Pesticide Column, 130Å, 5 µm, 2.1 mm x 100 mm. Sensitivity of the parent/parent trace is compromised at 0.02 mg/kg due to interferences, but satisfying at 0.1 mg/kg.

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			
	Trifluoroacetic acid (TFA)		113/69, 113/113, 69/19			
	Trifluoroacetic acid - ¹³ C ₂ (IL-IS)		115/70			
	Difluoroacetic acid (DFA)		95/51, 95/95			
	Difluoroacetic acid - ¹³ C (IL-IS)		97/52			
	Thiocyanate		58/58			
	Thiocyanate ¹³ C ¹⁵ N (IL-IS)		60/60			
	Dimethyl phosphate (DMP)		125/63, 125/110, 125/62			
	Diethyl phosphate (DEP)		153/125, 153/79, 153/63			
	O,O-Dimethyl thiophosphate (DMTP)		141/79, 141/47, 141/63			
	O,O-Diethyl thiophosphate (DETP)		169/95, 169/141, 169/63			
	O,O-Dimethyl dithiophosphate (DMDTP)		157/142, 157/112, 157/111			
	O,O-Diethyl dithiophosphate (DEDTP)		185/111, 185/79, 185/157			

Hints on Method 1.7

1. Handling of column, pre-column and pre-filters: See 5.6.1 point 7.a) and 7.c)
2. Interference of Phosphonic acid by Phosphoric acid: See 5.6.1
3. For general hints on analytes: See 5.6.1

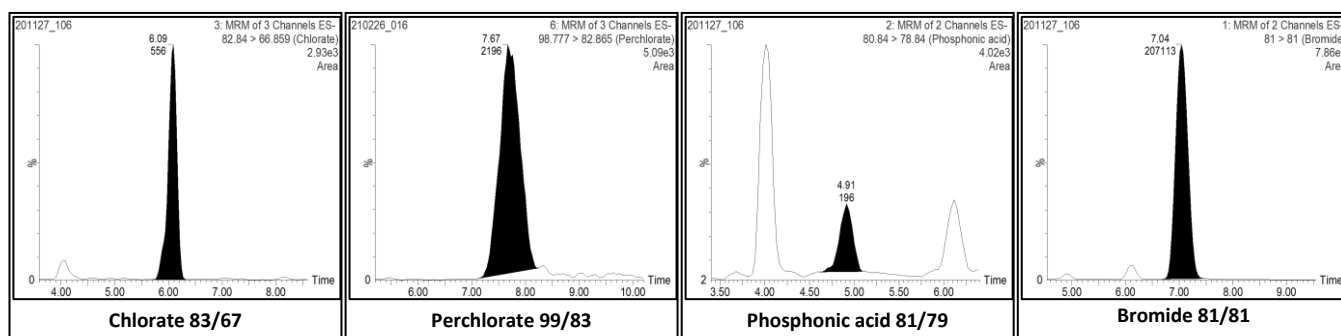


Figure 22: Chromatograms of Chlorate at 0.03 mg/kg, Perchlorate at 0.01 mg/kg, Phosphonic acid at 0.05 mg/kg and Bromide at 5.0 mg/kg, all in lemon extract; using b: Waters Anionic Polar Pesticide Column, 130Å, 5 µm, 2.1 mm x 100 mm; 50 °C.

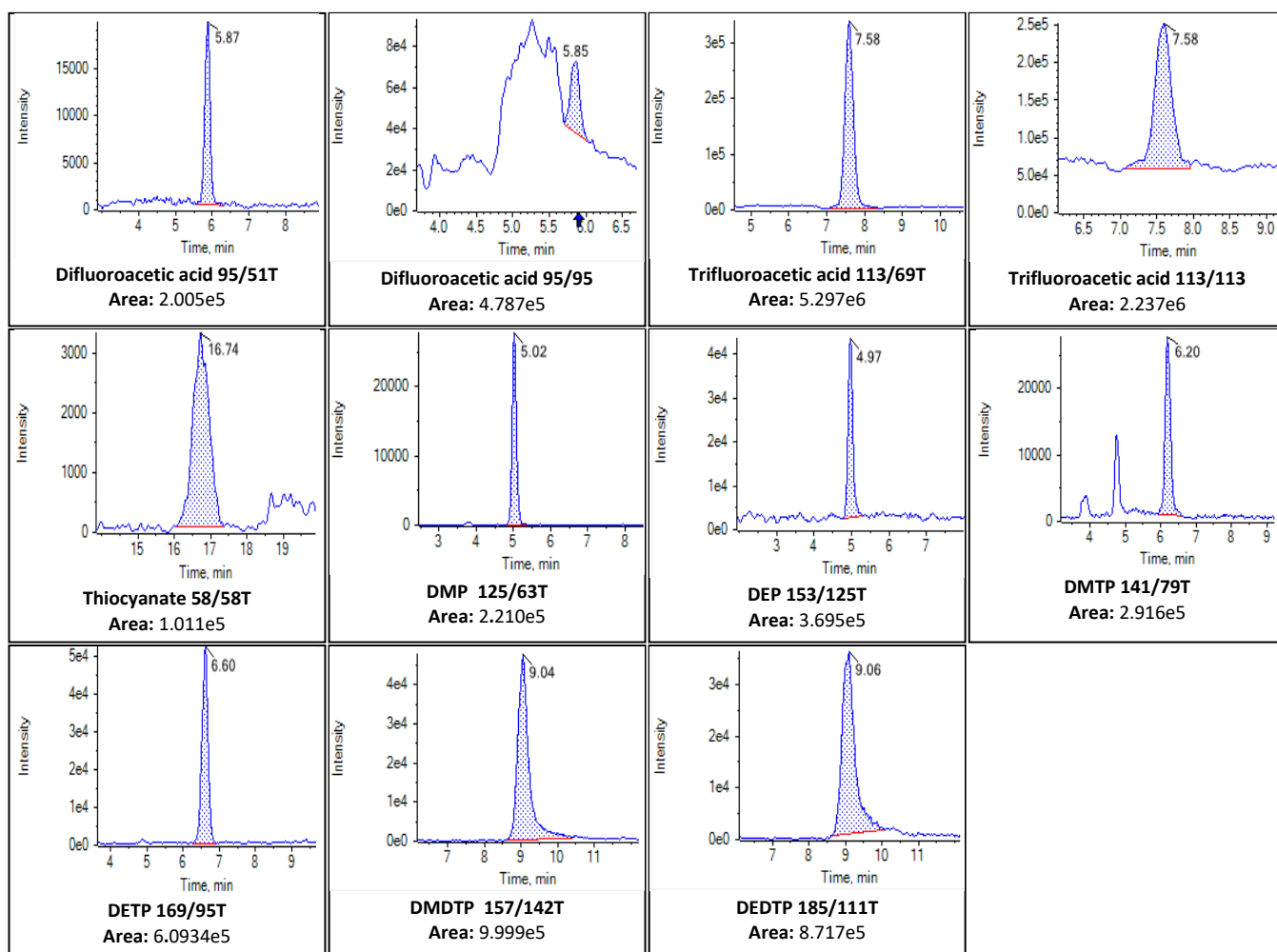


Figure 23: Chromatograms of Difluoroacetic acid at 0.02 mg/kg, Trifluoroacetic acid at 0.05 mg/kg, Thiocyanate at 0.02 mg/kg, Diethylphosphate at 0.025 mg/kg and Diethyl Thiophosphate, O,O-Dimethyl Dithiophosphate, O,O-Dimethyl Thiophosphate, O,O-Diethyl Dithiophosphate, Dimethyl Phosphate at 0.01 mg/kg in grape extract using b: Waters Anionic Polar Pesticide Column, 130Å, 5 µm, 2.1 mm x 100 mm; 50 °C.

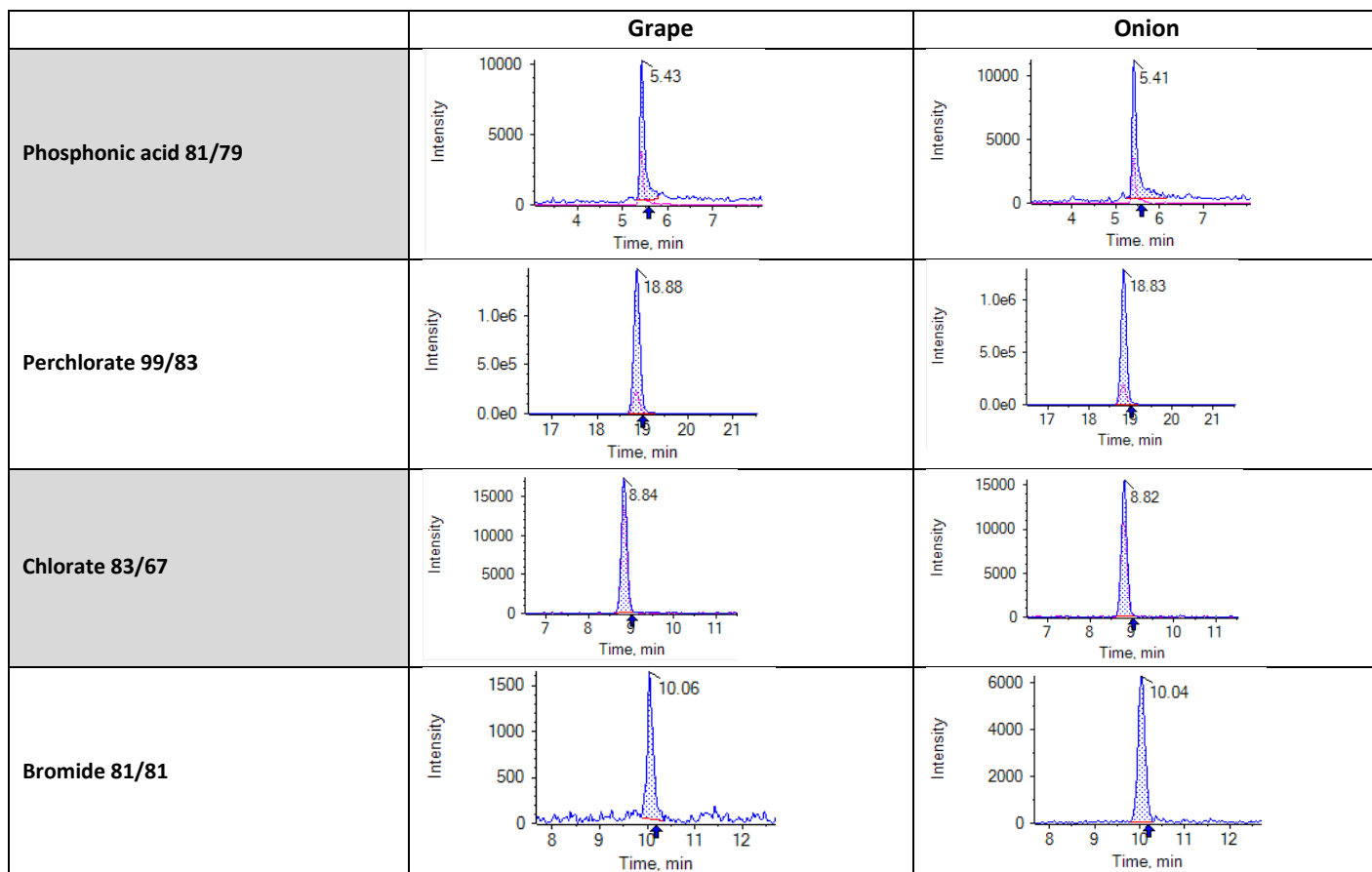


Figure 24: Exemplary chromatograms of Phosphonic acid, Perchlorate, Chlorate and Bromide at 0.01 mg/kg on Grape and Onion using *a*: Waters Torus™DEA 2.1 mm x 100 mm; 1.7 µm; 50 °C.

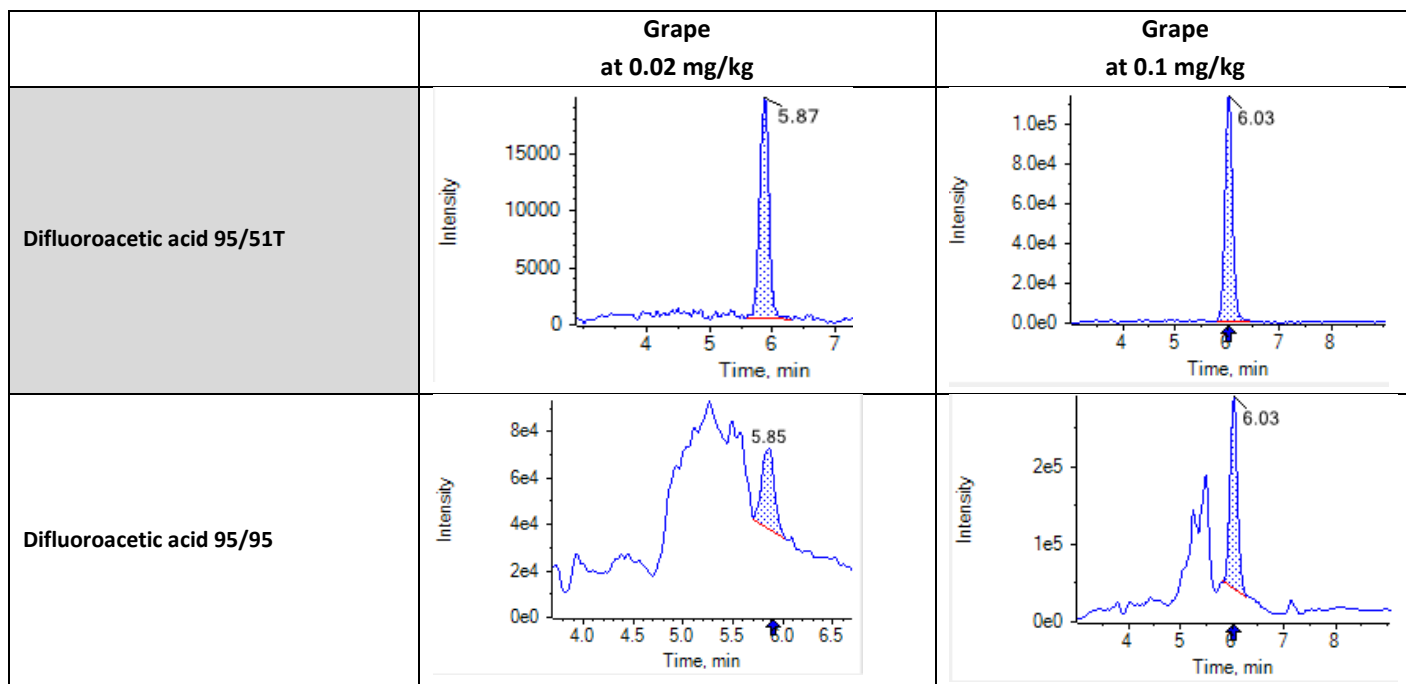


Figure 25: Chromatograms of difluoroacetic acid at 0.1 mg/kg in grape extract using *b*: Waters Anionic Polar Pesticide Column, 130Å, 5 µm, 2.1 mm x 100 mm. Sensitivity of the parent/parent trace is compromised at 0.02 mg/kg due to interferences, but satisfying at 0.1 mg/kg.

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

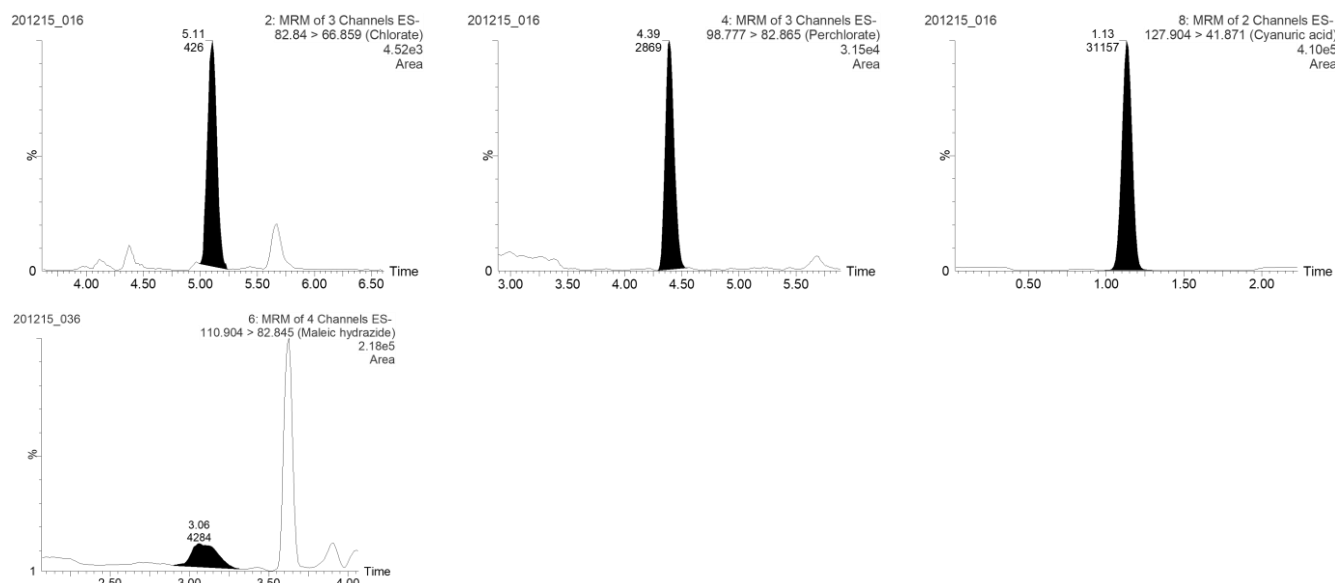


Figure 26: Chromatograms of Chlorate at 0.03mg/kg, Perchlorate at 0.01 mg/kg, Cyanuric acid and Maleic hydrazide at 0.05 mg/kg, all in lemon extract

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, 69/19	
	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 7.a) and 7.c)
2. For general hints on analytes: See 5.6.1

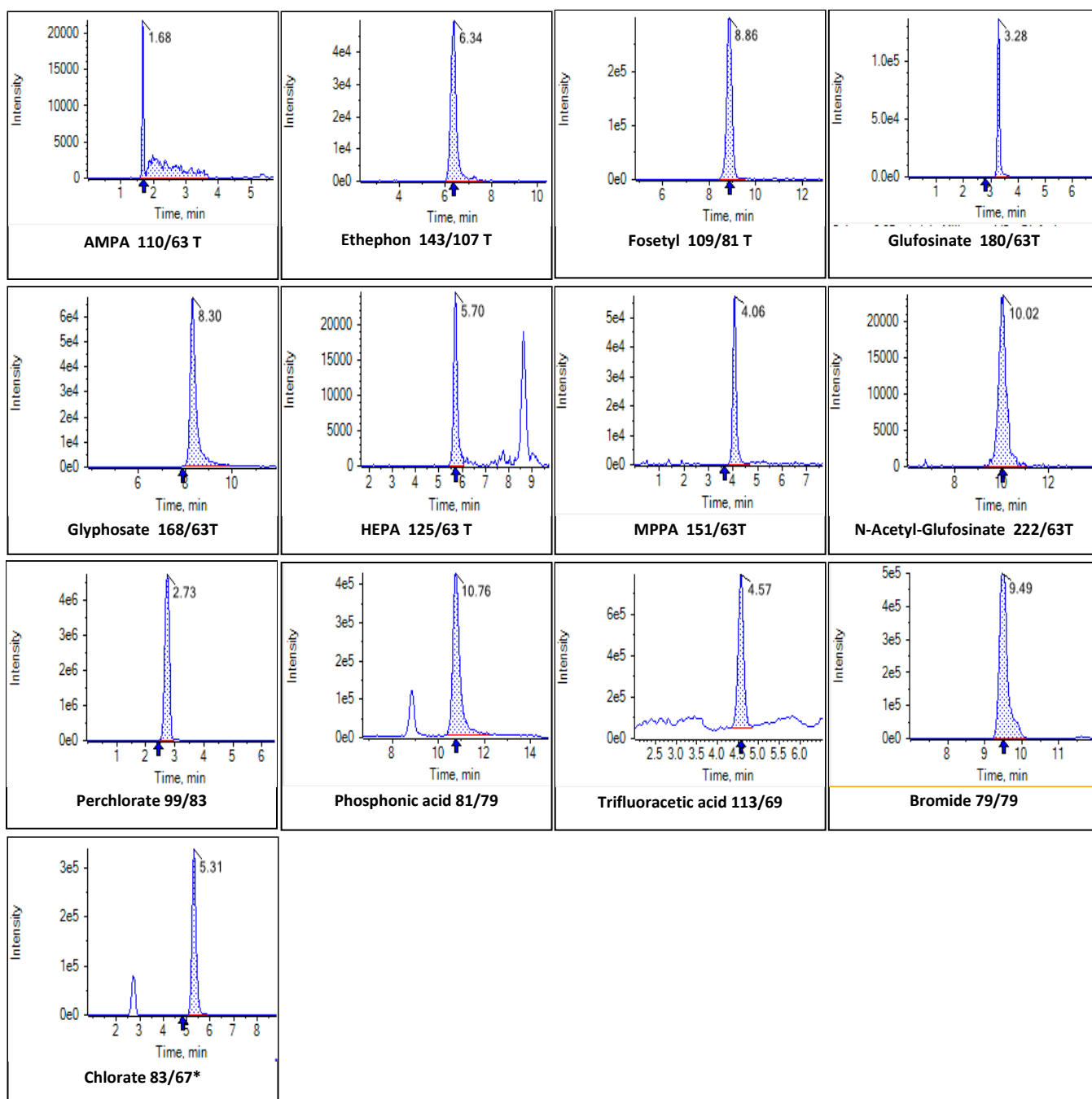


Figure 27: Typical chromatograms in strawberry extracts spiked at 0.1 mg/kg.

*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 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, 69/19	
	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 ion	81/81, 79/79 (pseudotransitions, consider notes)	
	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 1.10

For general hints on analytes: See 5.6.1

5.6.12. Method 1.11 (M1.11): “Gly&Co. on Luna Polar Pesticides”

Table 28: Proposed LC-MS/MS conditions for Glyphosate, AMPA, N-Acetyl-Glyphosate, Fosetyl, Ethephon, HEPA, Glufosinate, MPPA, N-Acetyl-Glufosinate

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Luna Polar Pesticides, 3 µm, 2.1 mm x 100 mm, 40°C		
Pre-column	Phenomenex SecurityGuard ULTRA Cartridges, For Luna Polar Pesticides Columns (AJ0-8789 3/pk), 40°C		
Eluent A	0.3% formic acid in water		
Eluent B	0.5 % formic acid in Acetonitrile		
Gradient	%A	Flow [mL/min]	Time [min]
	5	0.4	0
	5	0.4	1
	65	0.4	5
	90	0.4	5.1
	90	0.4	11
	5	0.4	11.1
	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; 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)	

Hints on Method 1.11

For general hints on analytes: See 5.6.1

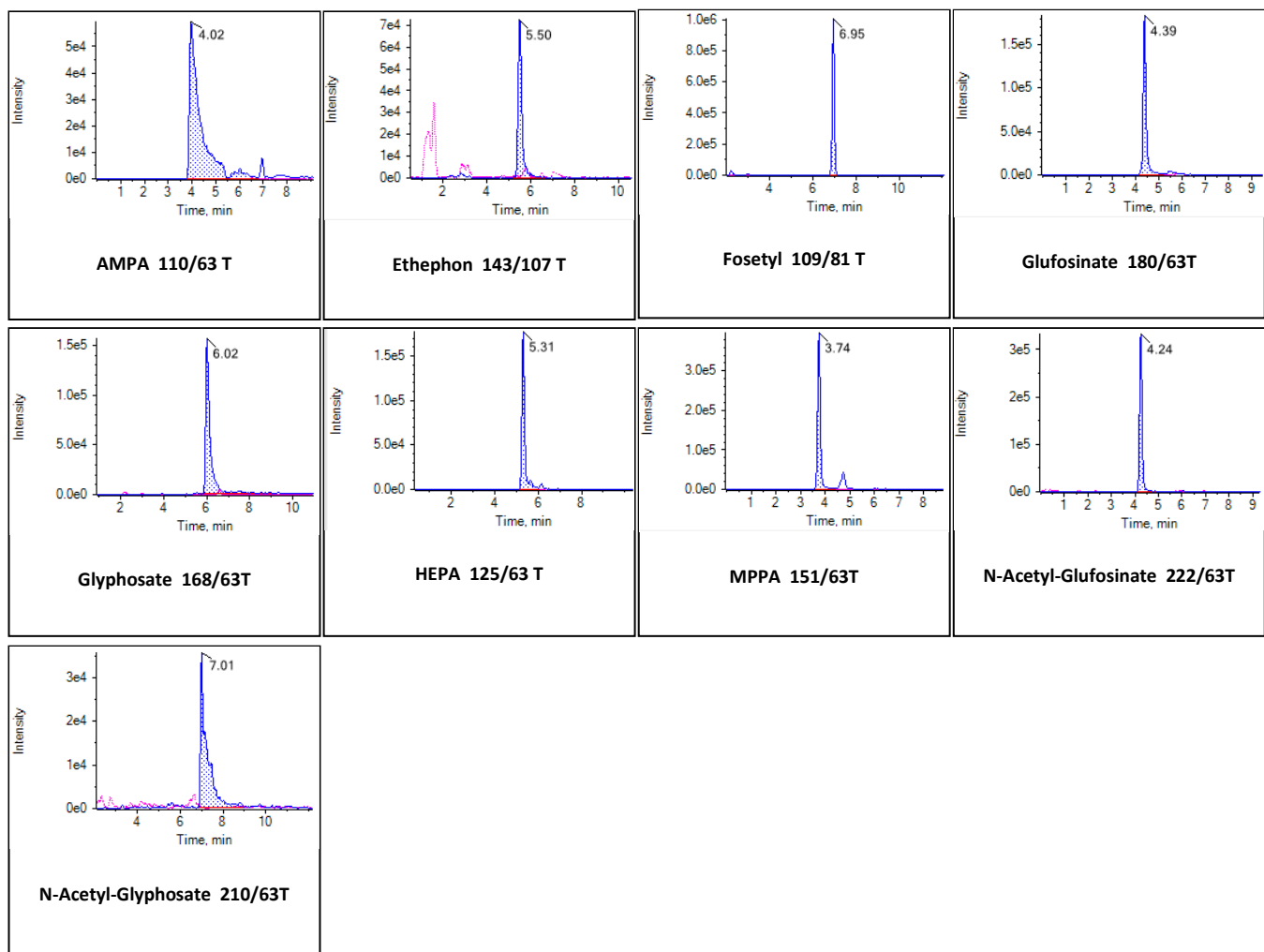


Figure 28: Typical chromatograms in tomato extracts spiked at 0.1 mg/kg.

5.6.13. Method 1.12 (M1.12): “PerChloPhos on Luna Polar Pesticides”

Table 29: Proposed LC-MS/MS conditions for Bromide, Chlorate, Perchlorate, Phosphonic acid and Thiocyanate

Instrument parameters	Conditions		
Ionisation mode	ESI neg		
Column/temperature	Luna Polar Pesticides		
Pre-column	Luna Polar Pesticides, 3 µm, 2.1 mm x 100 mm, 40°C		
Pre-filters	Phenomenex SecurityGuard UHPL HILIC 2.1 mm x 10 mm, 40°C		
Eluent A	0.3% formic acid in water		
Eluent B	0.5 % formic acid in Acetonitrile		
Gradient	%A	Flow [mL/min]	Time [min]
	95	0.4	0
	95	0.4	1.5
	50	0.4	9
	50	0.4	10
	95	0.4	10.1
Injection volume	95	0.4	13
	2 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)	
	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	
	Trifluoroacetic acid (TFA)	113/69, 113/113, 69/19	
	Trifluoroacetic acid - ¹³ C ₂ (IL-IS)	115/70	
	Difluoroacetic acid (DFA)	95/51, 95/95	
	Difluoroacetic acid - ¹³ C (IL-IS)	97/52	

Hints on Method 1.11

For general hints on analytes: See 5.6.1

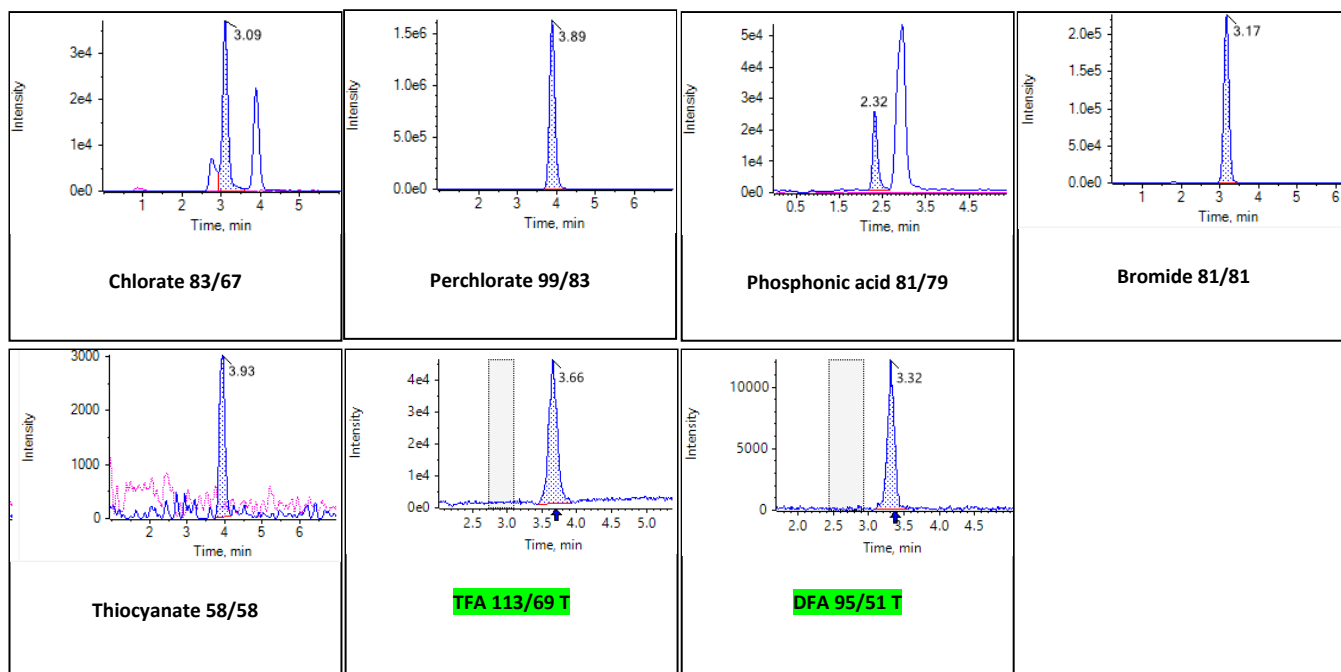


Figure 29: Typical chromatograms of Chlorate, Perchlorate, Phosphonic Acid, Bromide and Thiocyanate in tomato extracts spiked at 0.1 mg/kg. And TFA and DFA in tomato extracts spiked at 0.05 mg/kg.

5.6.14. Method 2 (M2): “Fosetyl and Maleic Hydrazide”

Table 30: 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 D₂ 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

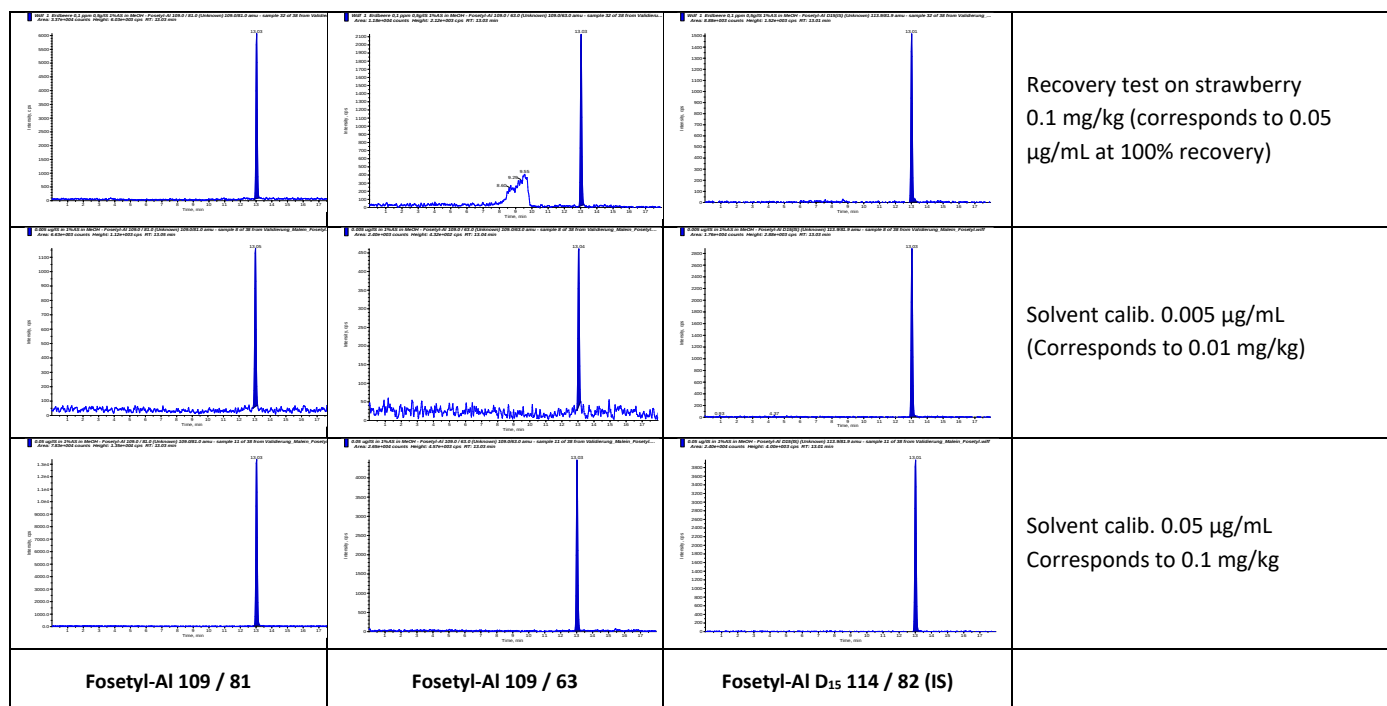


Figure 30: Typical chromatograms of Fosetyl-Al in strawberry extract and in solvent-based standards

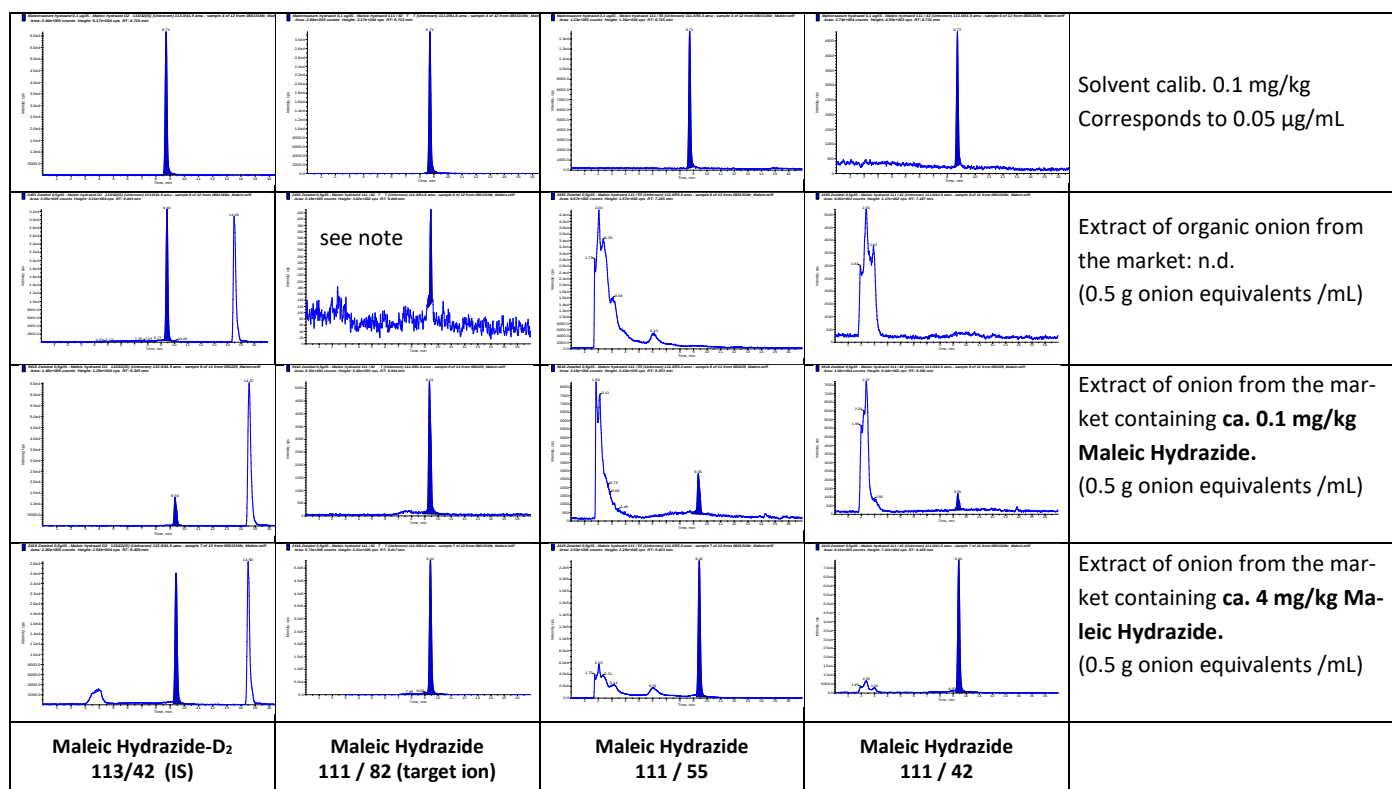


Figure 31: Typical chromatograms of Maleic Hydrazide in onion extracts and in solvent-based standards

5.6.15. Method 3 (M3): “Amitrole&Co.”

Table 31: 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	
	Daminozide-D ₄ (IL-IS)	165/147	
	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 acronym is, however, also used for N,N'-propylenethiourea = N,N'-(1,3-Propylene)thiourea = N,N'-Trimethylenethiourea (CAS No.: 2122-19-2).

***not available any more to our knowledge

Hints on Method 3

1. For Paraquat, Diquat, Trimethylsulfonium and N,N-Dimethylhydrazine better run Method 4 (5.6.16)
2. For general hints on analytes: See 5.6.1

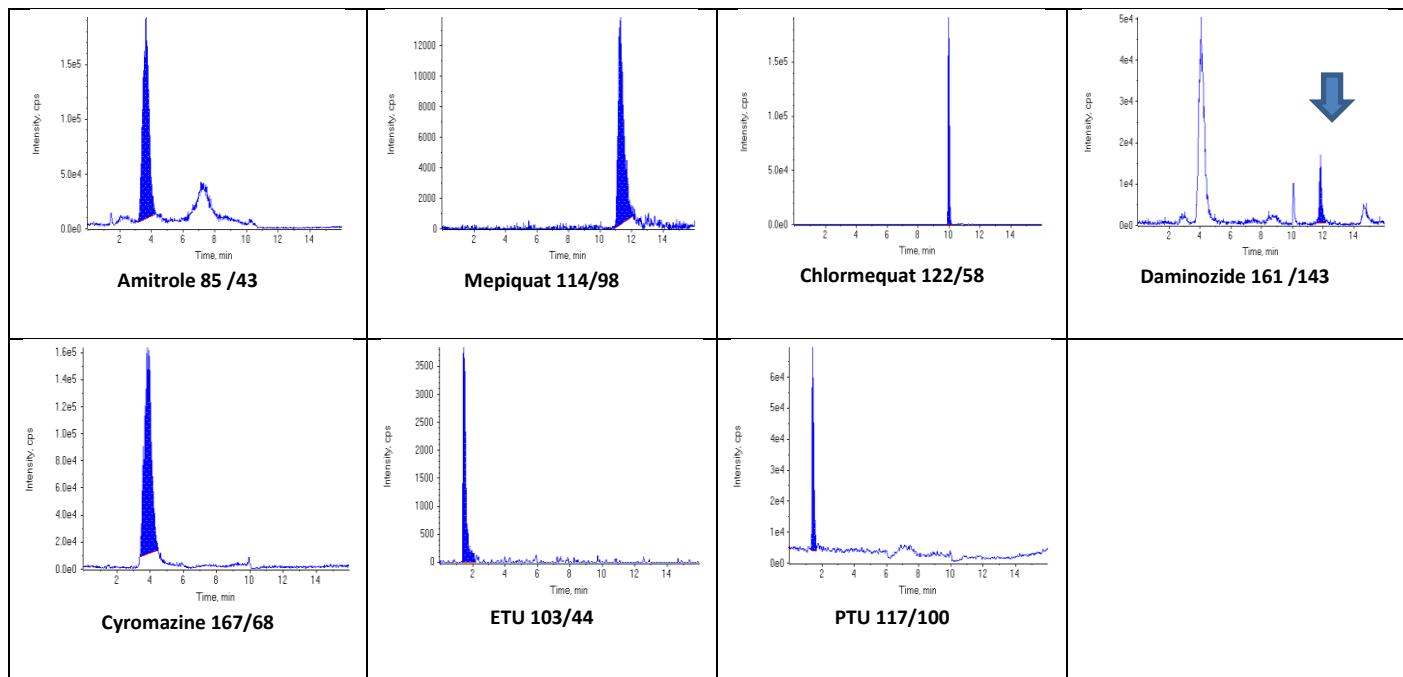


Figure 32: Typical chromatograms of Amitrole, Chlormequat, Mepiquat, Daminozide, ETU, PTU and Cyromazine in apple extract at 0.01 mg/kg

5.6.16.Method 4.1 (M4.1): “Quats&Co. Obelisc R”

Table 32: 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 (3.5) with 500 mL 20 mmol NH ₄ -formate in water Use brown glass bottles! Alternative eluent A: 50 mmol NH ₄ formate 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-D₄ (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, 165/44	
	Daminozide-D ₆ (IL-IS)	167/149, 165/97	
	Daminozide-D ₄ (IL-IS)	165/147	
	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).

**not available any more to our knowledge

Hints on Method 4.1

1. For Morpholin, DEA and TEA better run Method 7 (5.6.21). In the hot ESI-ion source, DEA seems to convert to Morpholine through condensation (water loss). Chromatographic separation is therefore paramount. With Method 4.1 (5.6.16) these two peaks do not sufficiently separate.

2. Diquat and Paraquat require special extraction conditions (see 5.2.3-B)
3. Variations in the performance of this column have been observed from batch to batch.
4. For general hints on analytes: See 5.6.1

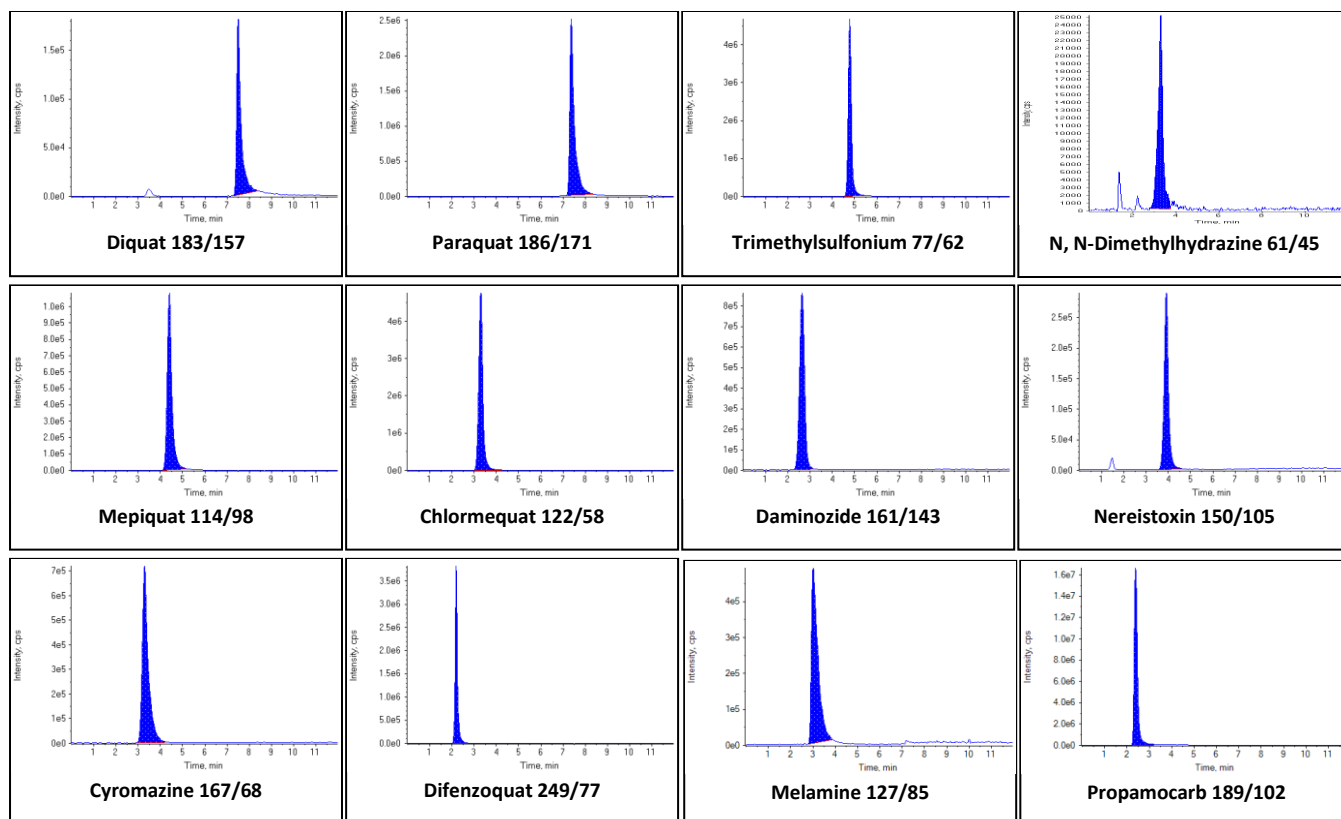


Figure 33: Typical chromatograms of Diquat, Paraquat, Chlormequat, Mepiquat, Daminozide, N,N-Dimethylhydrazine, Trimethylsulfonium, Cyromazine, Nereistoxin, Difenzoquat, Melamine and Propamocarb in apple extract at 0.1 mg/kg.

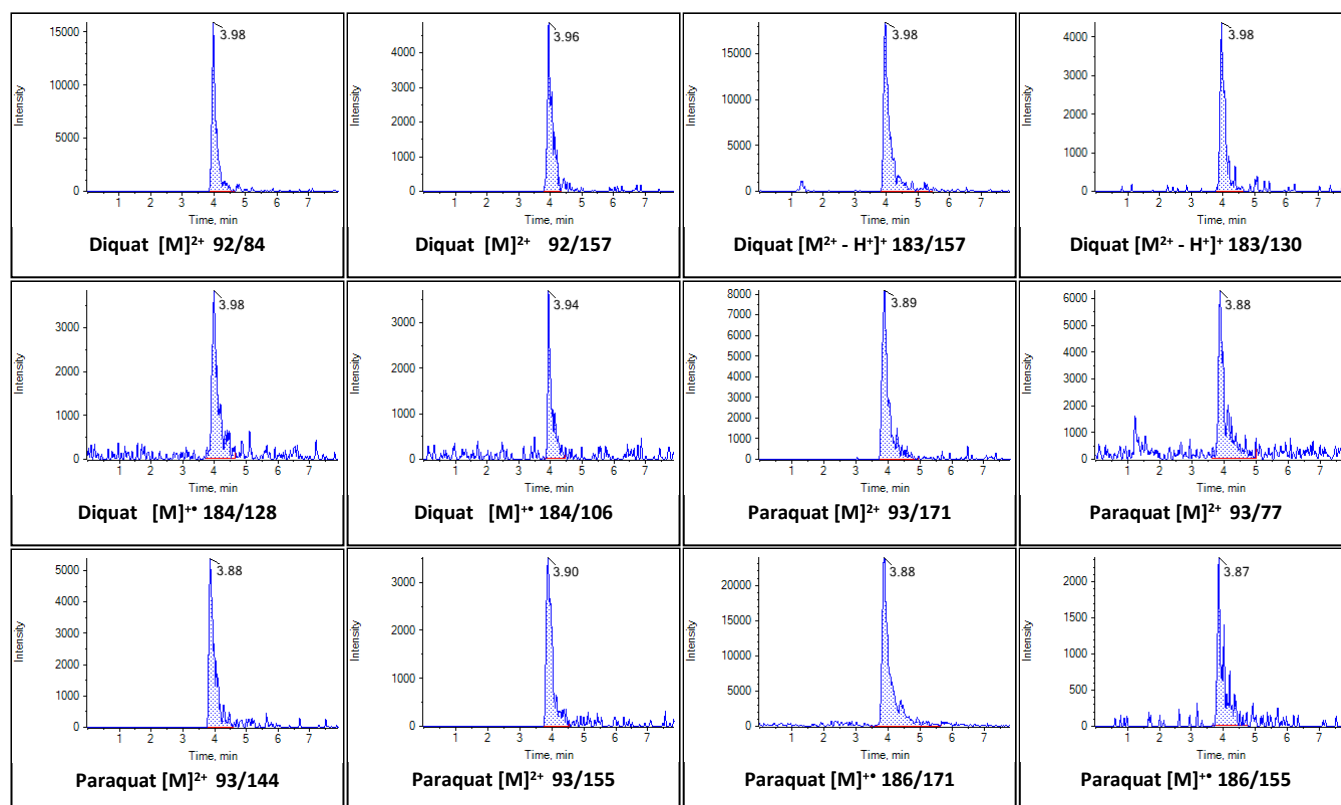


Figure 34: Typical chromatograms of Diquat and Paraquat in rice 0.005 µg/mL final extract (corresponding to 0.04 mg/kg)

5.6.17. Method 4.2 (M4.2): “Quats&Co. BEH Amide”

Table 33: 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
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-D ₆ (IL-IS)	167/149, 165/97	
	Daminozide-D ₄ (IL-IS)	165/147	
	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	
	Pymetrozine	218/105, 218/78, 218/79	
	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 (5.6.15).

*** 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 (5.6.21)**: 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

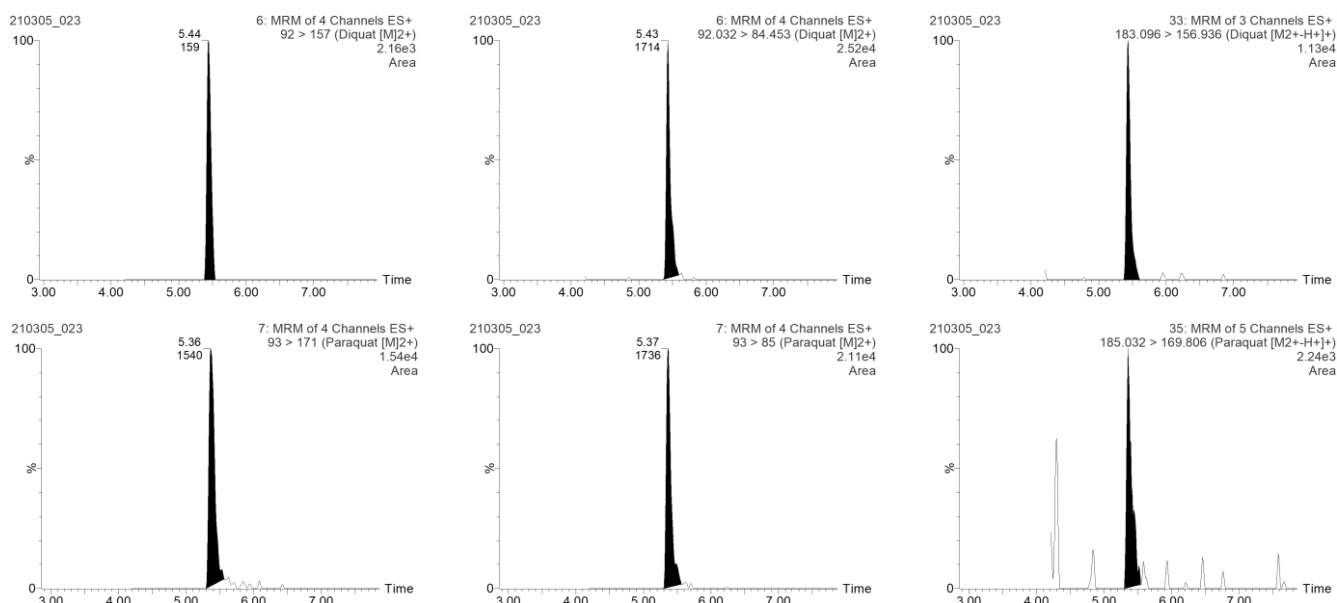


Figure 35: Typical chromatograms of Diquat and Paraquat in sesame extracts spiked at 0.05 mg/kg using Waters Xevo TQ-Sμ.

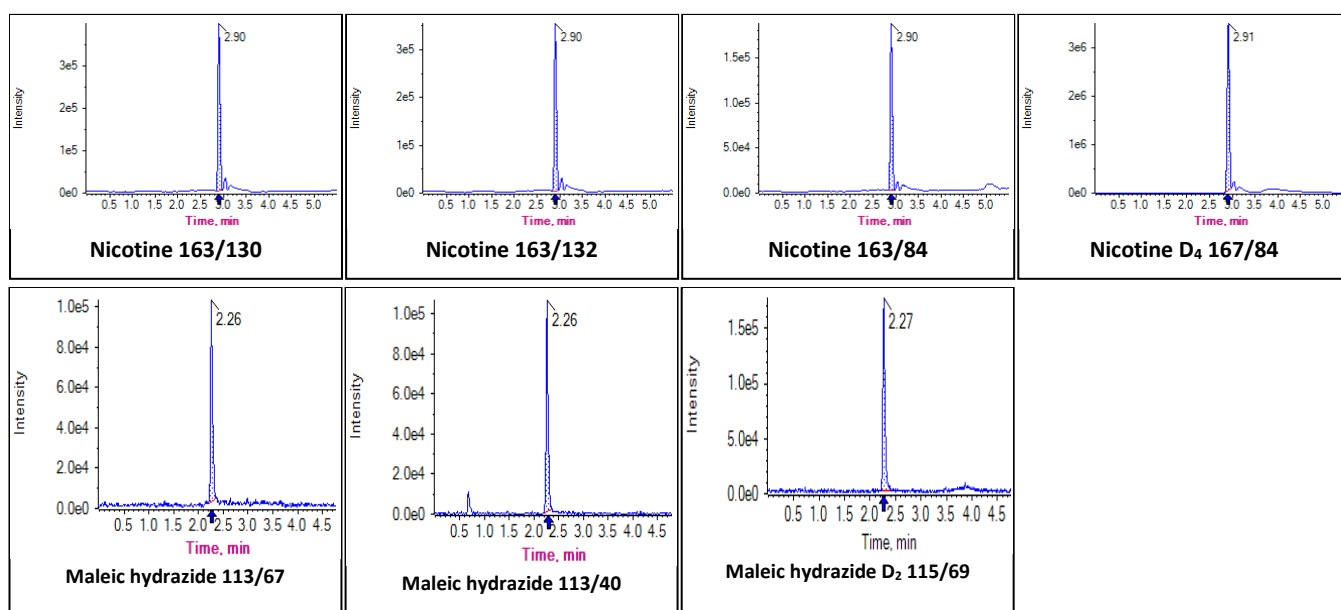


Figure 36: Exemplary chromatograms of Nicotine in flour (spelt, whole-grain) Maleic Hydrazide in solvent. Nicotine at 0.01 mg/kg (0.005 μg/mL); Nicotine D₄ at 0.1 mg/kg (0.05 μg/mL); Maleic hydrazide and Maleic Hydrazide D₂ at 0.2 mg/kg (0.1 μg/mL).

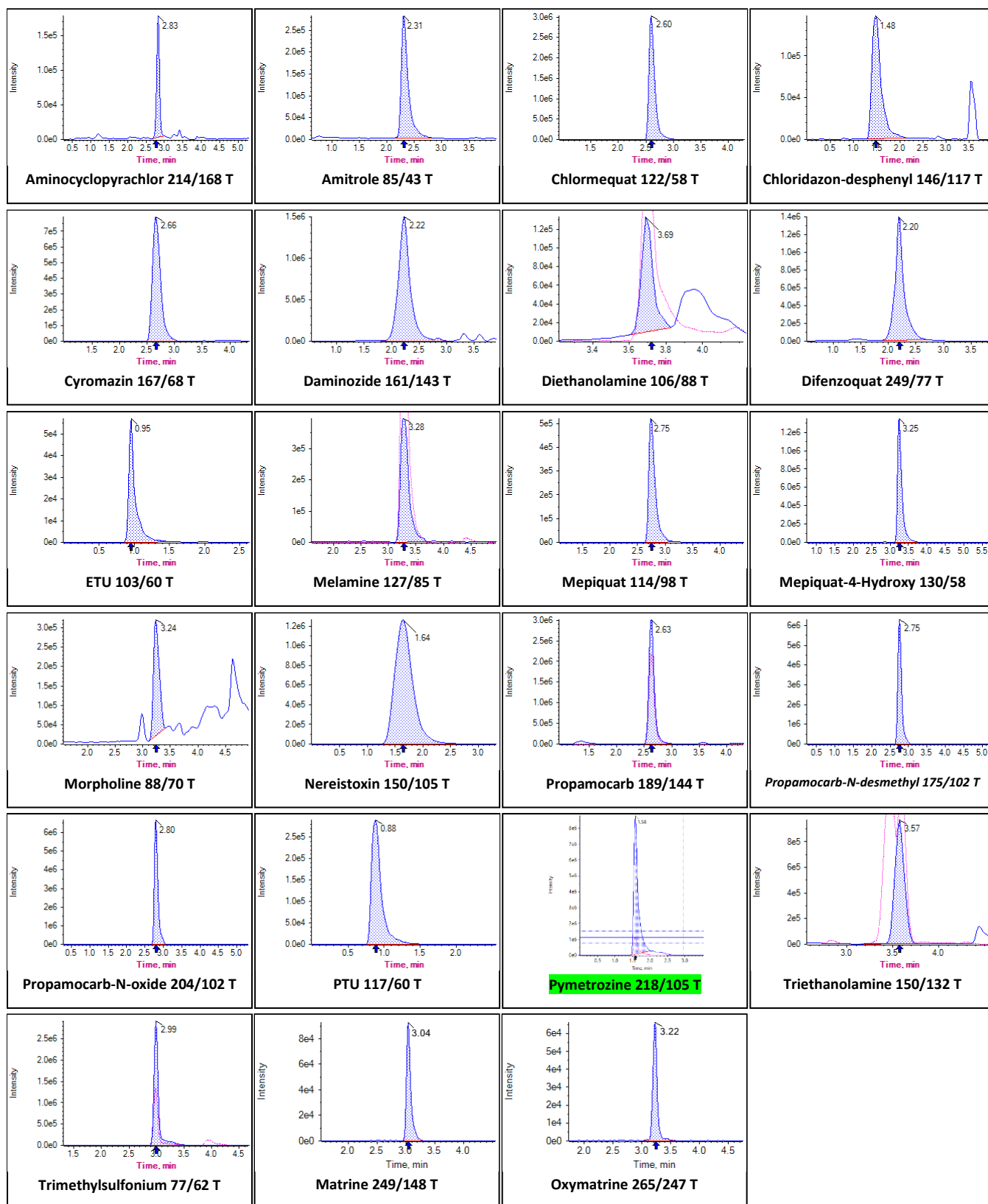


Figure 37: Typical chromatograms of Aminocyclopyrachlor, Amitrole, Chlormequat, Chloridazon-desphenyl, Cyromazine, Daminozide, Diethanolamine, Difenzoquat, ETU, Melamine, Mepiquat, Mepiquat-4-hydroxy, Morpholine, Nereistoxin, Propamocarb, Propamocarb-N-desmethyl, Propamocarb-N-oxide, PTU, Pymetrozine, Triethanolamine, Trimesium (Trimethylsulfonium) in tomato extracts spiked at 0.05 mg/kg; additionally Matrine and Oxymatrine at 0.01mg/kg in grape extract.

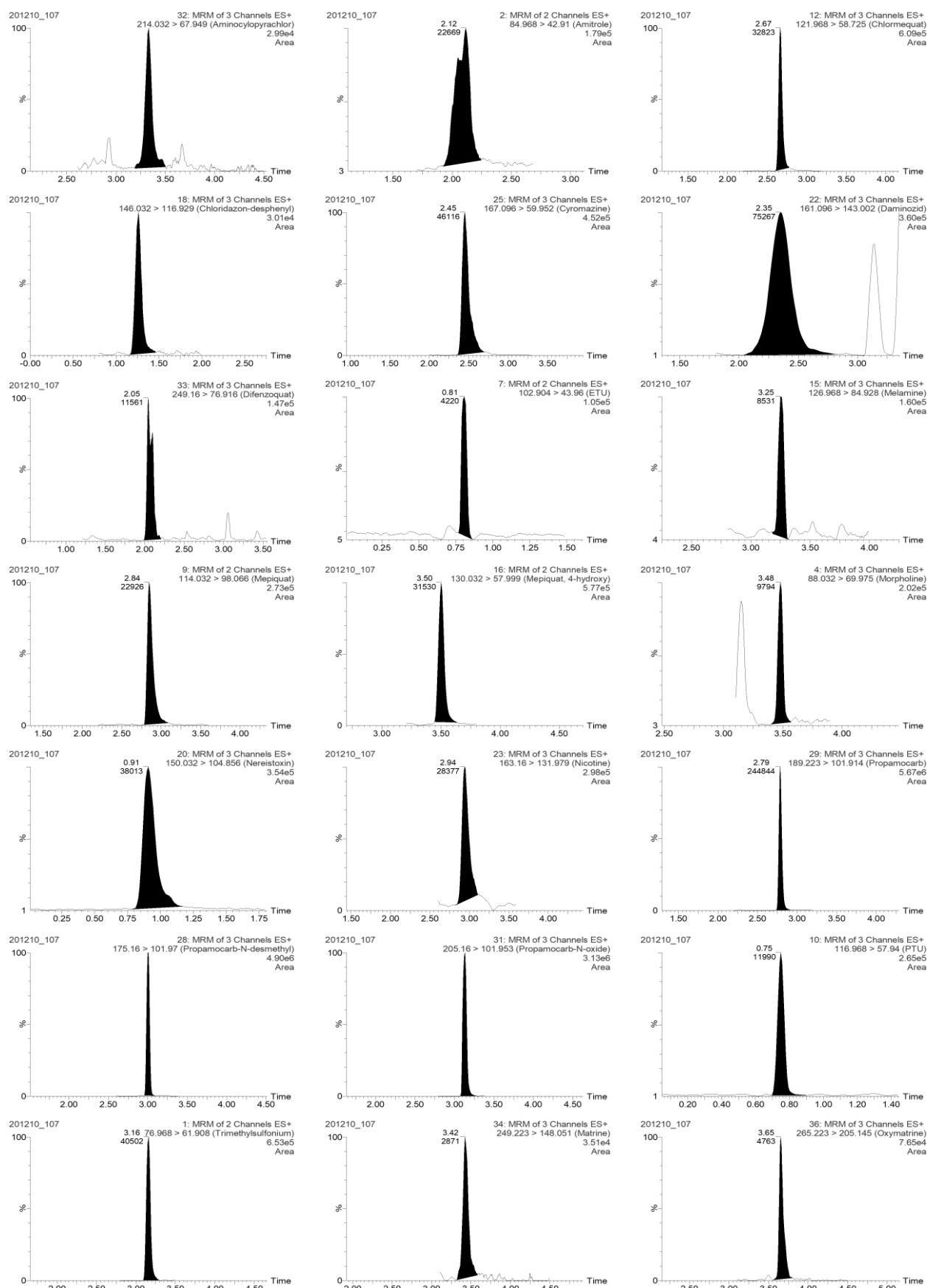


Figure 38: Typical chromatograms of Aminocyclopyrachlor, Amitrole, Chlormequat, Chloridazon-desphenyl, Cyromazine, Daminozide, Diethanolamine, Difenzoquat, ETU, Melamine, Mepiquat, Mepiquat-4-hydroxy, Morpholine, Nereistoxin, Propamocarb, Propamocarb-N-desmethyl, Propamocarb-N-oxide, PTU, Triethanolamine, Trimesium (Trimethylsulfonium) in tomato extracts spiked at 0.06 mg/kg.

5.6.18.Method 4.3 (M4.3): “Quats&Co. Luna Polar Pesticides”

Table 34: Proposed LC-MS/MS conditions for ESI-pos. compounds listed in the table below.

Instrument parameters	Conditions		
Ionisation mode	ESI pos.		
Column/temperature	Luna Polar Pesticides, 3 µm, 2.1 mm x 100 mm, 40°C		
Pre-column / Pre-filters	Phenomenex SecurityGuard ULTRA Cartridges, For Luna Polar Pesticides Columns (AJ0-8789 3/pk), 40°C		
Eluent A	50 mmol NH ₄ -formate in water (adjust to pH 3 with formic acid) Use brown glass !		
Eluent B	Acetonitrile		
Gradient	%A	Flow [mL/min]	Time [min]
	3	0.5	0
	3	0.5	0.5
	30	0.5	4.0
	60	0.5	5.0
	60	0.5	6.0
	3	0.5	6.1
	3	0.5	10
Injection volume	2 µL		
Calibration standards and levels	e.g. one level at the reporting limit plus 0.05 or 0.1 µg/IS portion		
Acquired mass transitions	Compound	Mass Transitions (m/z)	
	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	
	Daminozide-D ₄ (IL-IS)	165/147	
	Difenzoquat	249/130, 249/77, 249/193,	
	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	
	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	
	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	
	Trimethylsulfonium	77/62, 77/47	
	Trimethylsulfonium-D ₉ (IL-IS)	86/68, 86/50	

*not available any more to our knowledge

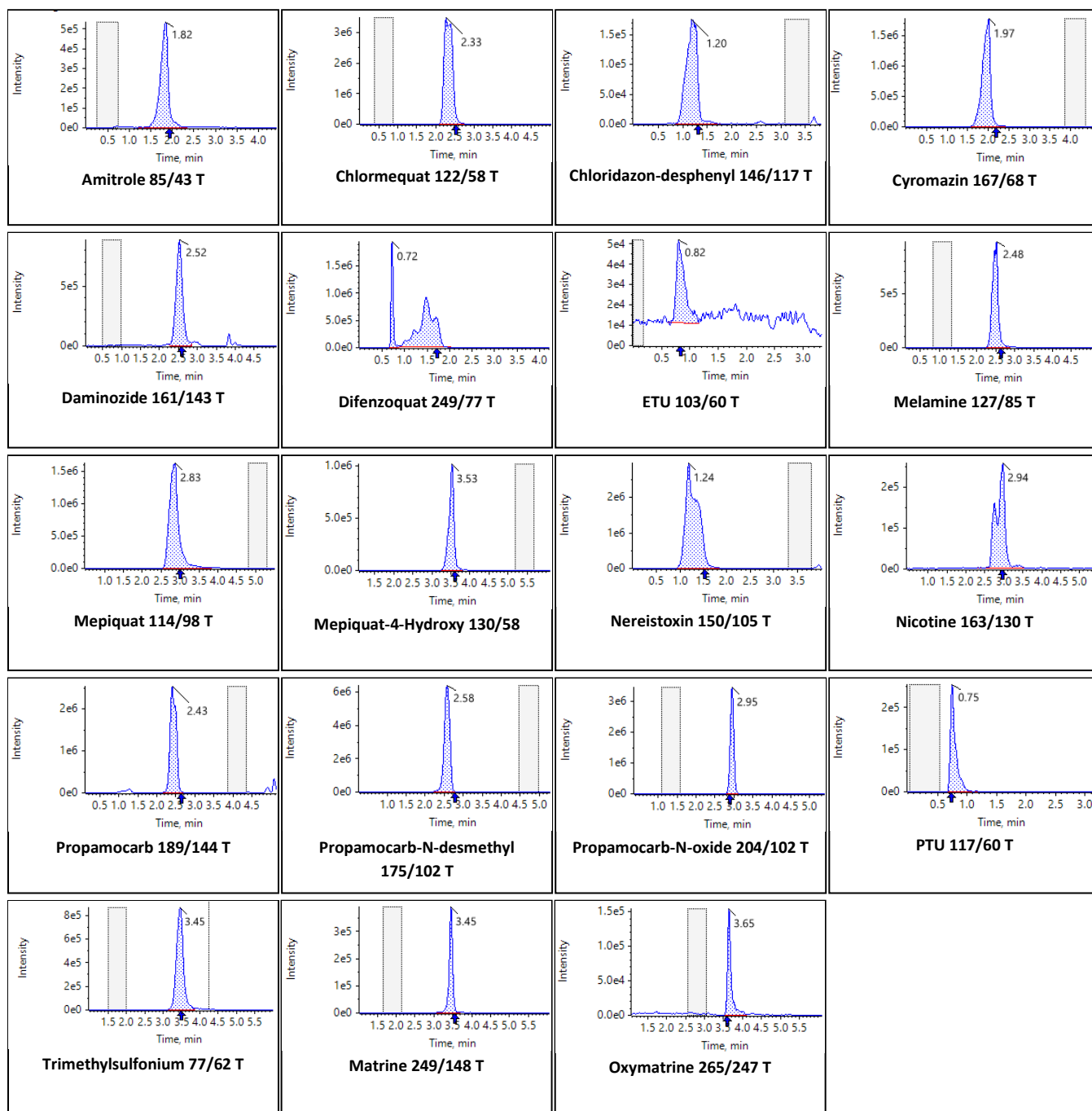


Figure 39: Typical chromatograms of Amitrole, Chlormequat, Chloridazon-desphenyl, Cyromazine, Daminozide, Difenzoquat, ETU, Melamine, Mepiquat, Mepiquat-4-hydroxy, Nereistoxin, Nicotine, Propamocarb, Propamocarb-N-desmethyl, Propamocarb-N-oxide, PTU, Trimesium (Trimethylsulfonium), Matrine and Oxymatrine in tomato extracts at 0.1 µg/mL.

5.6.19. Method 5 (M5): “Quats&Co. MonoChrom MS”

Table 35: 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-D ₆ - N,N'-(1,2-Propylene)thiourea –D ₆ **	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 (**5.6.15**).

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_CrISrm.pdf

5.6.20. Method 6 (M6): “Streptomycin and Kasugamycin”

Table 36: 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	584/263, 584/246	
	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 streptomycin if shown to be absent from the sample (and vice versa)

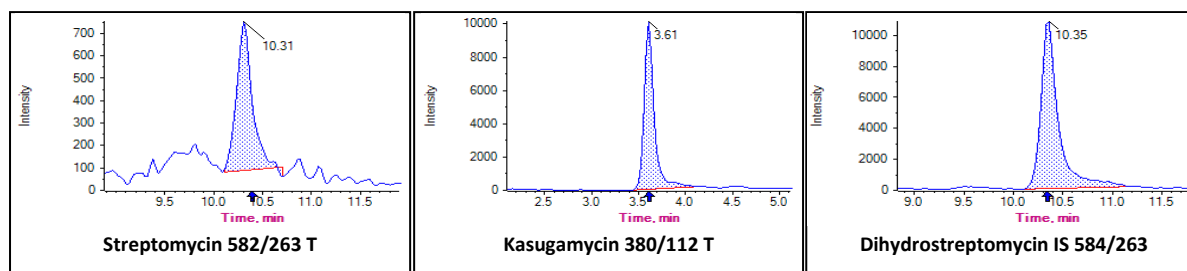


Figure 40: Typical chromatograms of Streptomycin and Kasugamycin in apple extracts spiked at 0.01 mg/kg.

5.6.21. Method 7 (M7): “Morpholine, Diethanolamine and Triethanolamine”

Table 37: 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

- For general hints on analytes: See 5.6.1
- Morpholin, DEA and TEA are not pesticides, they are additive of waxes used to coat crops (citrus, apples and mangoes etc). They are included in this method for the sake of convenience and synergy. As these three compounds can be analyzed very sensitively 5-10-fold dilution of the extracts before injection is recommendable where possible, especially in absence of an IS requiring standard additions approach (5.5.3)

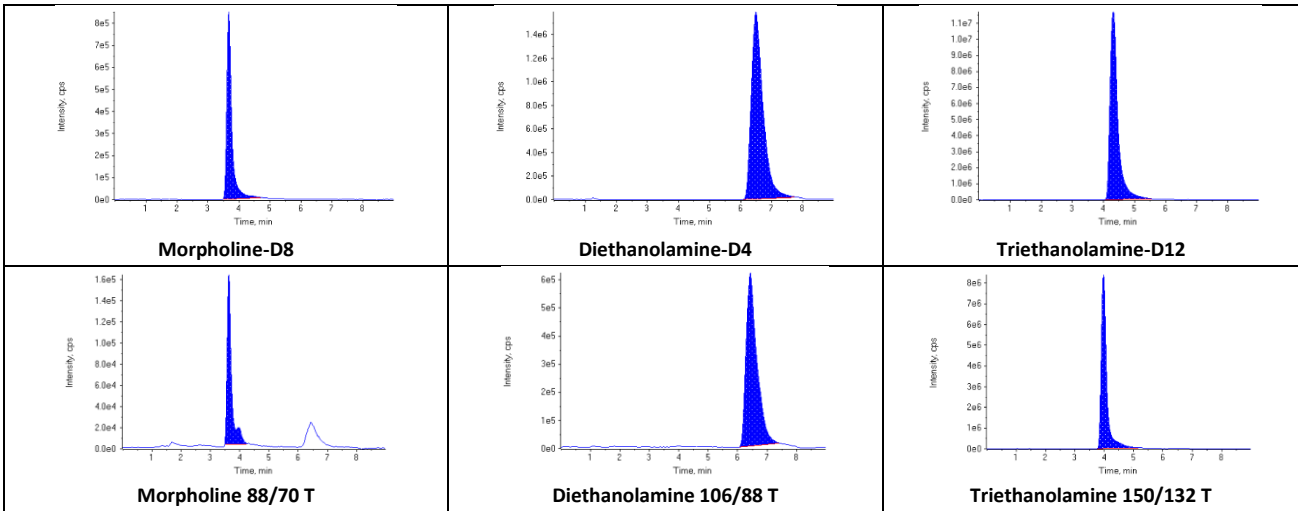


Figure 41: Typical chromatograms of Morpholine, Diethanolamine and Triethanolamine in apple extracts at 0.05 mg/kg (extract were diluted 10-fold before injection)

5.6.22.Method 8 (M8): “Triazole derivative metabolites (TDMs)”

Table 38: 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

Hints on Method 8

- For general hints on analytes: See 5.6.1
- 1,2,4-Triazole is used as nitrification inhibitors in fertilizers
- The following commercially available isotopically labelled components were not tested with this method: 1,2,4-Triazole-D₂, 1, 2, 4-Triazole-acetic acid-D₂, 1, 2, 4-Triazole-alanine-D₂, 1, 2, 4-Triazole-lactic acid-D₂

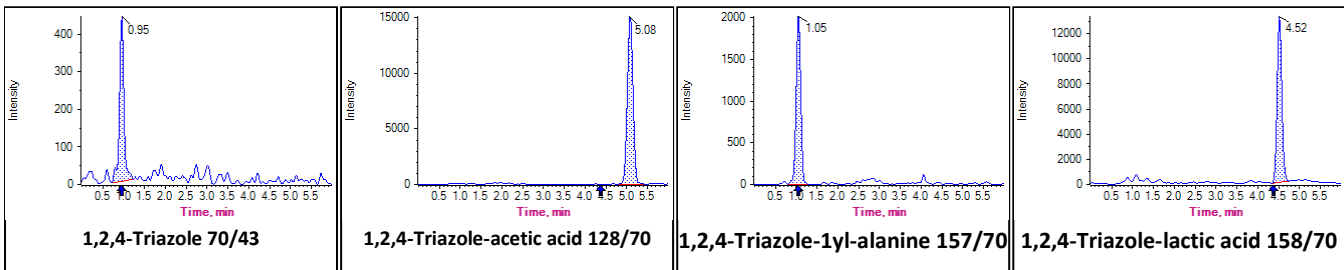


Figure 42: Typical chromatograms of TDMs in avocado extracts spiked at 0.01 mg/kg.

5.6.23. Method 9 (M9): “Difluoroacetic acid and Trifluoroacetic acid”

Table 39: Proposed LC-MS/MS and Selexion conditions Difluoroacetic acid and Trifluoroacetic acid

Instrument parameters	Conditions			
Ionisation mode	ESI neg			
Column	Dionex/Thermo, Acclaim Trinity P1, 2.1 x 100 mm, (3 µm) (P/N 071389); 40°C			
Pre-column	Thermo Guard Cartridge Acclaim Trinity P1, 2.1 x 10 mm, (3 µm) (P/N 071391)			
Pre-filter	e.g. Supelco column saver 2.0 µm Filter (optional)			
Eluent A	50 mmol 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

- For general hints on analytes: See 5.6.1

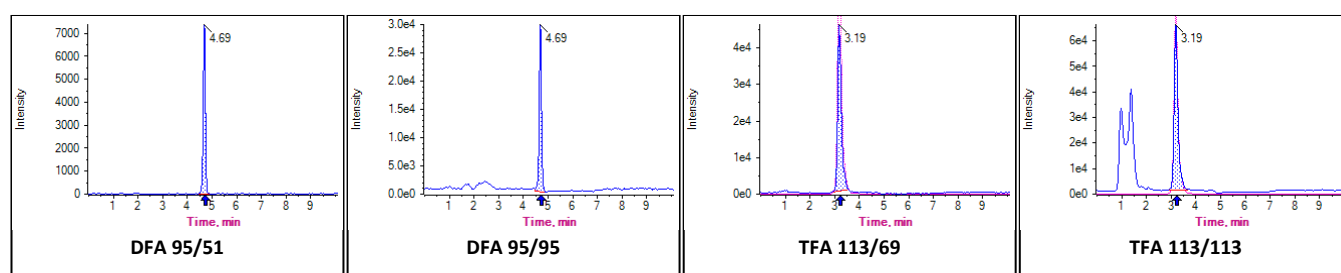


Figure 43: Typical chromatograms of DFA and TFA in tomato extracts spiked at 0.05 mg/kg

5.6.24. Method 10 (M10): “Triazole derivative metabolites (TDMs) on Torus DEA”

Table 40: 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

- For general hints on analytes: See 5.6.1
- 1,2,4-Triazole is measured by M8: See 5.6.22

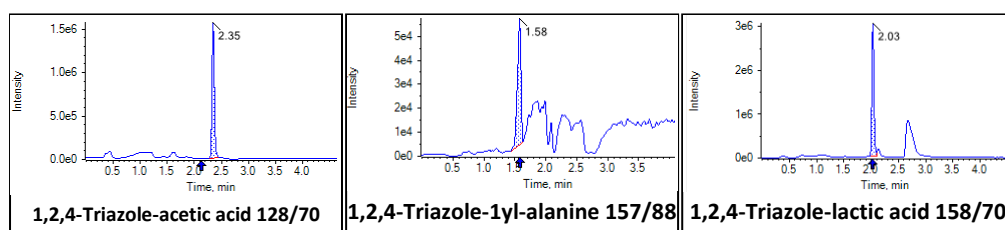


Figure 44: Typical chromatograms of TDMs in strawberry extracts spiked at 0.05 mg/kg.

5.6.25. Method 11 (M11): “Gly&Co. by IC on AS19”³

Table 41: 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 a: longer gradient with focus on the separation of Glyphosate from N-Acetyl-Glyphosate		
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
	Gradient b: shorter gradient with focus on the analysis of Chlorate, Perchlorate, Phosphonic acid, Bromide, TFA and DFA		
	30	0.3	0
	30	0.3	6
	40	0.3	7
	40	0.3	9
	90	0.3	10
	90	0.3	17.5
	30	0.3	17.6
	30	0.3	22
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, 69/19	

³ <https://doi.org/10.1007/s00216-024-05389-4>

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
Difluoroacetic acid (DFA)	95/51, 95/95
Difluoroacetic acid - ¹³ C (IL-IS)	97/52

Hints on Method 11

- For general hints on analytes: See 5.6.1

Sensitivity and Reproducibility: Where issues with fluctuating signals or reduced sensitivity of e.g. phosphonic acid or bromide are observed, maintenance of the autosampler can be helpful and is recommended. "Injection port" and "wash port" should be cleaned (e.g. with methanol), and the buffer loop and if needed also the seals in the injection valve should be replaced (according to the maintenance procedures of the manufacturer). Issues likely arise from microbiological activity in the injection port and tubing of the autosampler due to the inability to wash those with organic solvents. Periodic injections of acidified methanol (containing 1% formic acid) to the system (e.g. twice at the beginning and in the end of a sequence) can help in reducing these effects.

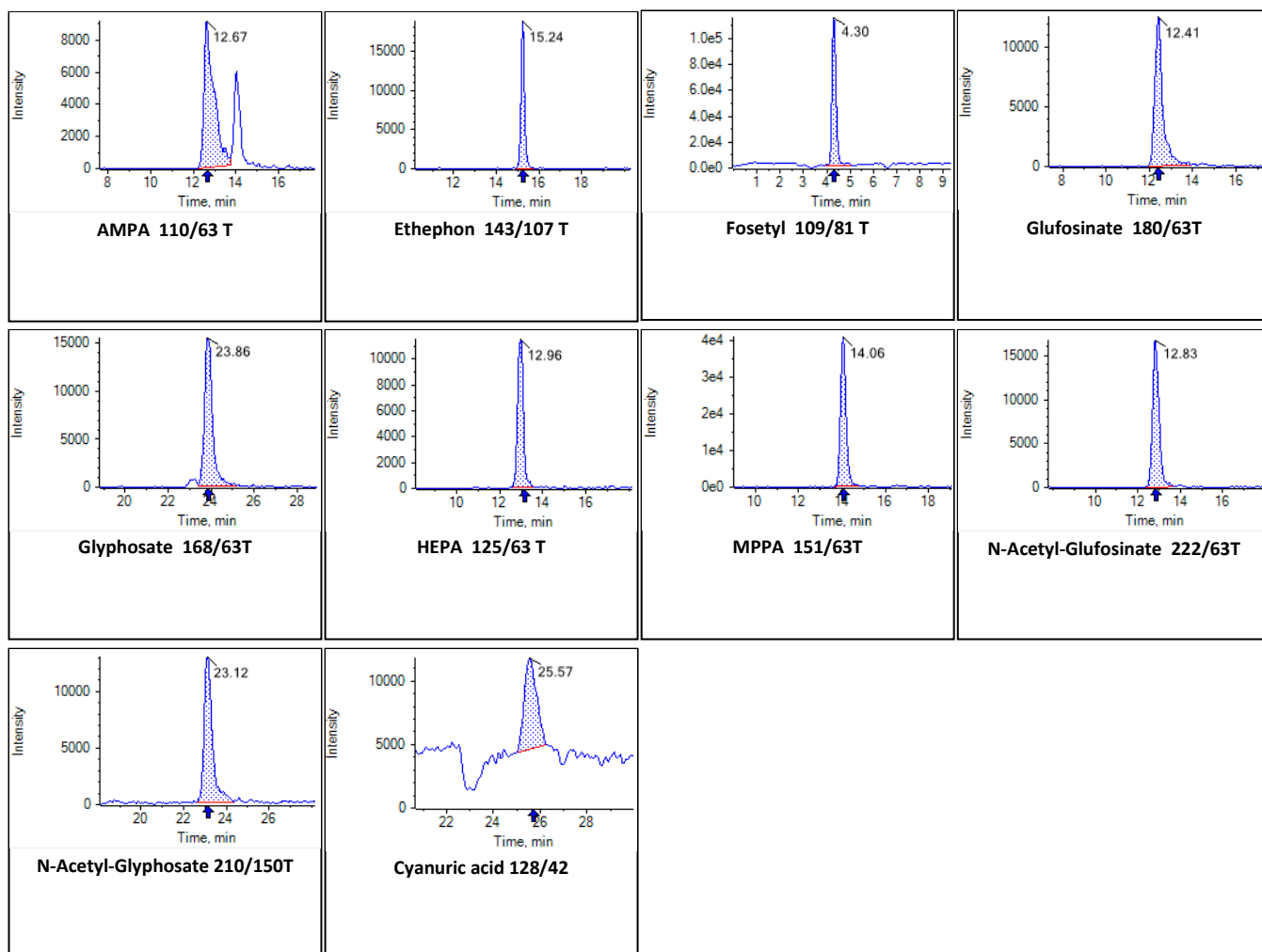


Figure 45: Typical chromatograms in cucumber extracts spiked at 0.05 mg/kg.

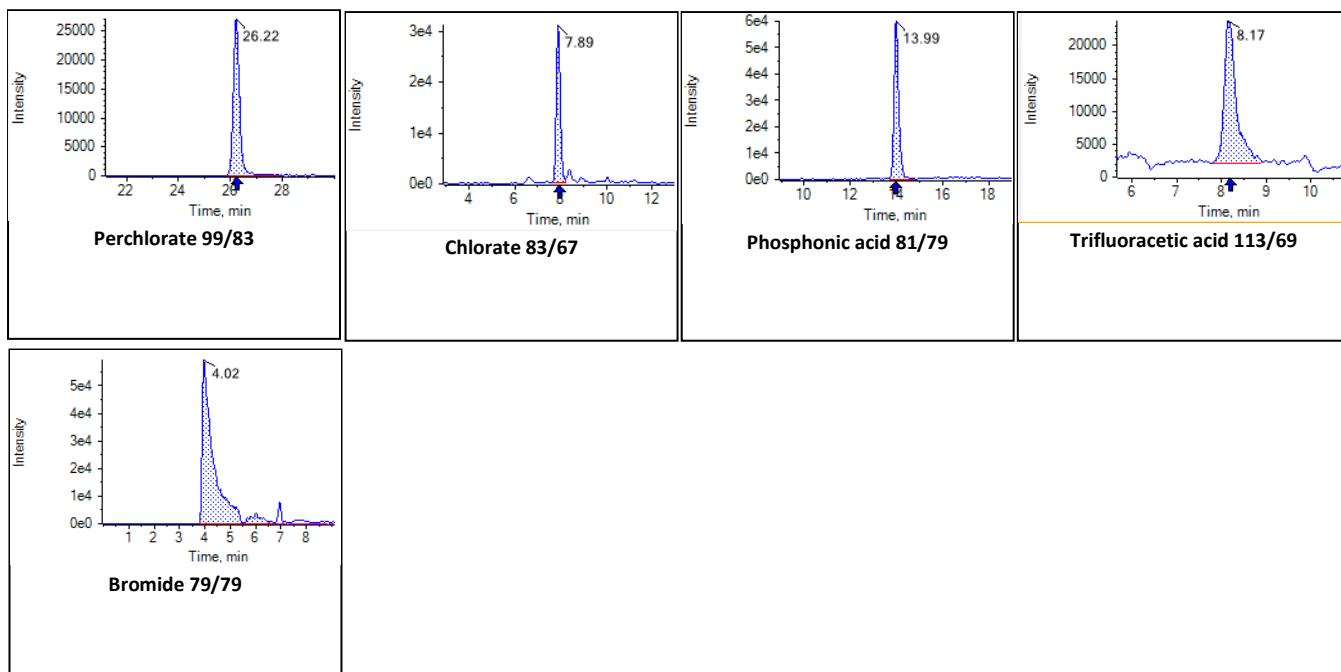


Figure 46: Typical chromatograms in milk extracts spiked at 0.01 mg/kg (Perchlorate and Chlorate); at 0.05 mg/kg (Phosphonic acid); 0.025 mg/kg (Trifluoroacetic acid) and 5 mg/kg (Bromide).

5.6.26. Method 12.1 (M12.1): “Quats&Co by IC on CS17”⁴

Table 42: Proposed IC-MS/MS conditions for cationic compounds on the CS17 (chlormequat, cyromazine, melamine, matrine, mepiquat, mepiquat-4-hydroxy, morpholine, nereistoxin, nicotine, oxymatrine, propamocarb, propamocarb-N-desmethyl, propamocarb-N-oxide, trimethylsulfonium).

Instrument parameters	Conditions		
Ionisation mode	ESI pos		
Column/temperature	Thermo Scientific™ Dionex™ IonPac™ CS17, 2x250mm; 55°C		
Pre-column	Thermo Scientific™ Dionex™ IonPac™ CG17, 2x50mm		
Eluent	Thermo Scientific™ Dionex™ EGC 500™ MSA eluent generator cartridge		
Gradient	c [MSA]	Flow [mL/min]	Time [min]
	15	0.4	0
	15	0.4	5
	60	0.4	6
	60	0.4	12.5
	15	0.4	12.5
	15	0.4	19
Injection volume	5 µL of 5-fold diluted extracts in water (preferably ultrapure)		
Flow Make-up Solvent before ion source	0.2 mL/min acetonitrile		
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)	
	Chlormequat	122/58, 124/58, 122/63, 122/59, 124/59	
	Chlormequat-D ₄ (IL-IS)	126/58; 126/59	
	Cyromazine	167/68, 167/125, 167/108, 167/85, 167/60	
	Cyromazine-D ₄ (IL-IS)	171/86, 171/68	
	Diquat:*	92/84, 92/157, 183/157	
	Diquat D ₈ (IL-IS)	96/89, 191/165	
	Melamine	127/85, 127/68, (127/60)	
	Melamine- ¹⁵ N ₃ (IL-IS)	130/87, 130/44	
	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	
	Trimethylsulfonium	77/62, 77/47	
	Trimethylsulfonium-D ₉ (IL-IS)	86/68, 86/50	

* Diquat and paraquat are not separated on the CS17.

Hints on Method 12.1

- For general hints on analytes: See 5.6.1

⁴ <https://doi.org/10.1016/j.chroma.2025.466430>

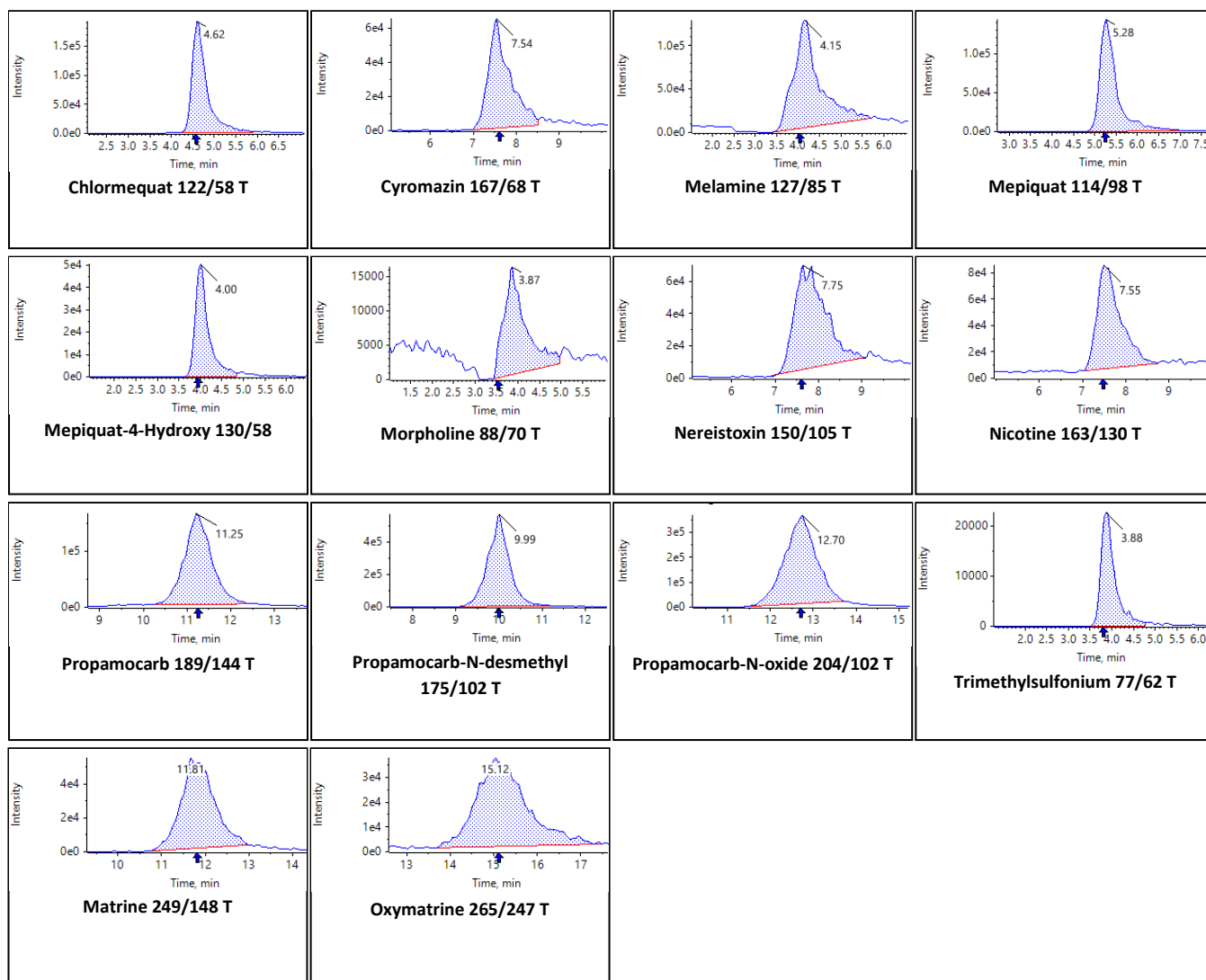


Figure 47: Typical chromatograms of Chlormequat, Cyromazine, Melamine, Mepiquat, Mepiquat-4-hydroxy, Morpholine, Nereistoxin, Nicotine in tomato extracts spiked 0.01 mg/kg. And Propamocarb, Propamocarb-N-desmethyl, Propamocarb-N-oxide, Trimethylsulfonium (Trimethylsulfonium), Matrine and Oxymatrine in tomato extract at 0.01 µg/mL.

5.6.27. Method 12.2 (M12.2): “Quats&Co by IC on CS21”⁵

Table 43: Proposed IC-MS/MS conditions for cationic compounds on the CS21 (chlormequat, cyromazine, diquat, melamine, matrine, mepiquat, mepiquat-4-hydroxy, morpholine, nereistoxin, nicotine, oxymatrine, propamocarb, propamocarb-N-desmethyl, propamocarb-N-oxide, trimethylsulfonium).

Instrument parameters	Conditions		
Ionisation mode	ESI pos		
Column/temperature	Thermo Scientific™ Dionex™ IonPac™ CS21, 2x150mm; 50°C		
Pre-column	Thermo Scientific™ Dionex™ IonPac™ CG21, 2x30mm		
Eluent	Thermo Scientific™ Dionex™ EGC 500™ MSA eluent generator cartridge		
Gradient	c [MSA]	Flow [mL/min]	Time [min]
	4	0.3	0
	4	0.3	2
	20	0.3	12
	40	0.3	13
	40	0.3	17
	4	0.3	17
	4	0.3	22
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		
Acquired mass transitions (m/z)	Compound	Mass Transitions (m/z)	
	Chlormequat	122/58, 124/58, 122/63, 122/59, 124/59	
	Chlormequat-D ₄ (IL-IS)	126/58; 126/59	
	Cyromazine	167/68, 167/125, 167/108, 167/85, 167/60	
	Cyromazine-D ₄ (IL-IS)	171/86, 171/68	
	Diquat:	92/84, 92/157, 183/157	
	Diquat D ₈ (IL-IS)	96/89, 191/165	
	Melamine	127/85, 127/68, (127/60)	
	Melamine- ¹⁵ N ₃ (IL-IS)	130/87, 130/44	
	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	
	Paraquat	93/171, 93/85, 185/170	
	Paraquat D ₈ (IL-IS)	97/179, 193/178	
	Trimethylsulfonium	77/62, 77/47	
	Trimethylsulfonium-D ₉ (IL-IS)	86/68, 86/50	

Hints on Method 12.2

1. For general hints on analytes: See 5.6.1

⁵ <https://doi.org/10.1016/j.chroma.2025.466430>

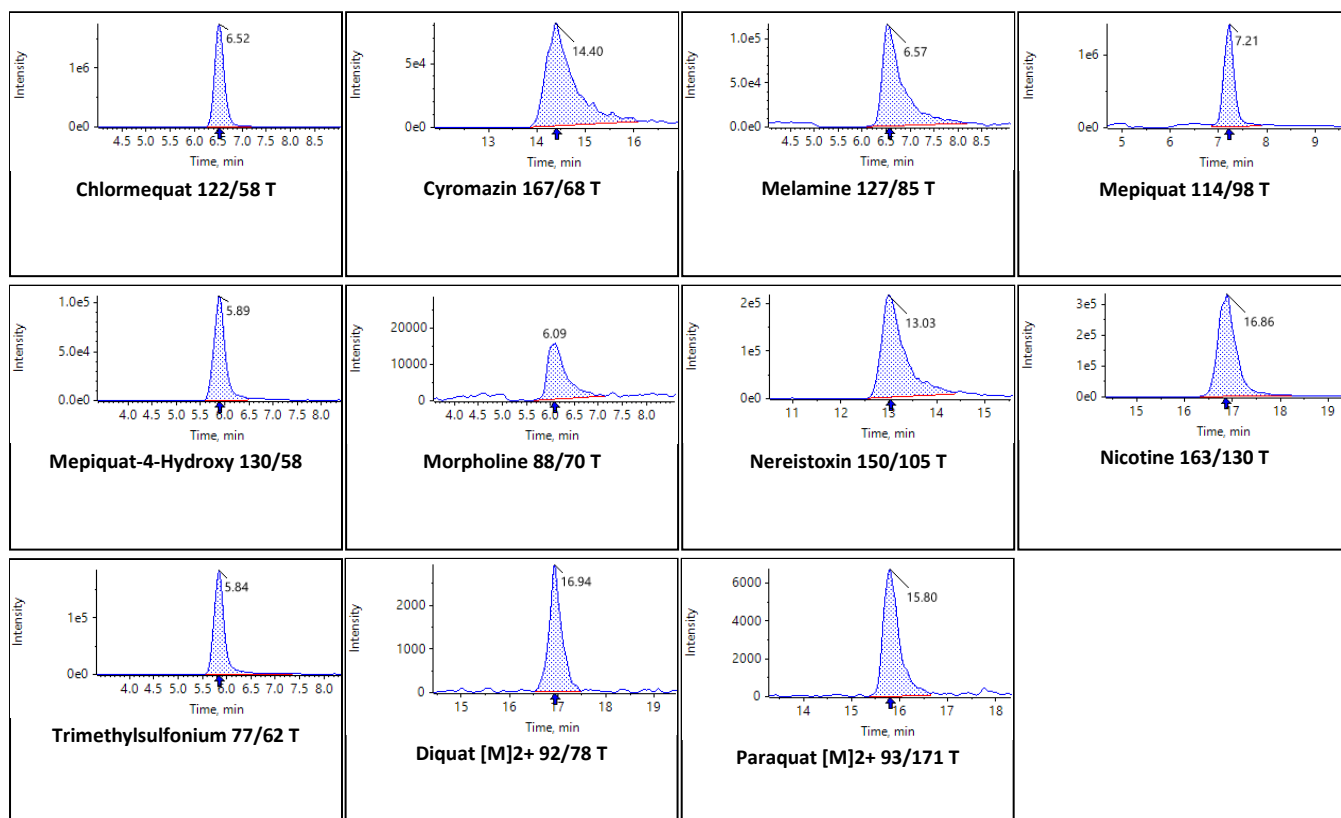


Figure 48: Typical chromatograms of Chlormequat, Cyromazine, Melamine, Mepiquat, Mepiquat-4-hydroxy, Morpholine, Nereistoxin, Nicotine and Trimesium (Trimethylsulfonium) in tomato extract at 0.01 µg/mL. And Paraquat and Diquat in rice spiked at 0.02 mg/kg.

5.7. Calibration and Calculations

5.7.1. Using IS

Where IS is added to the sample before any aliquotation:

The following calculation approach requires that the ratio of the IS masses added to the test portions (5.2.3) and to the calibration standard(s) (205.5) ($m_{IS}^{sample} / m_{IS}^{cal mix}$) 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).

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

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 oilseeds). 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 38, 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 oilseeds, 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 oilseeds), 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 38.

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).

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

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).

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

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

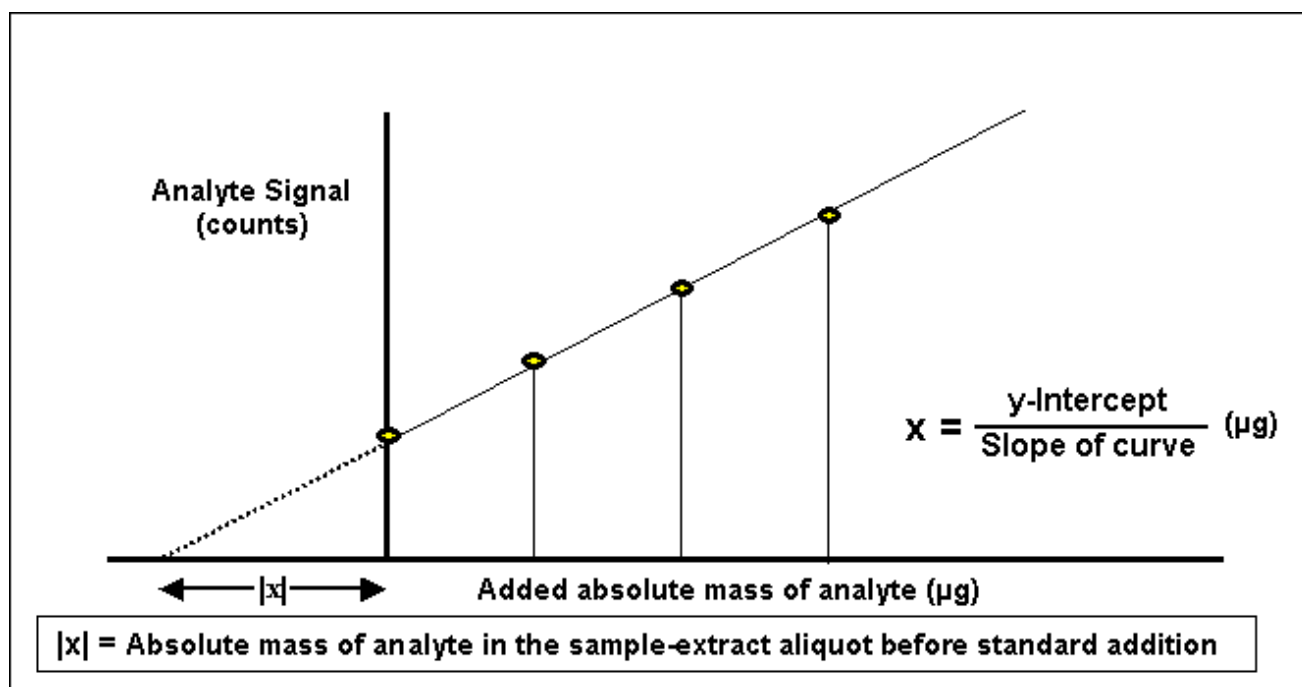


Figure 49: Internal calibration using the procedure of standard additions, schematically

Key:

Y Peak area of analyte

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

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

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

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

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

where:

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

a Slope of the calibration graph of the analyte in question (1/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 44**. 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 46**.

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 containing 10% acetonitrile, over a period of 7 months in the refrigerator, is demonstrated in **Figure 50**. The stability in stock solutions is generally better than in working solutions.

Table 44: 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

Native Compound	Composition of storage solvent											
	Pure Water				Water / MeOH (MeOH 25 and 50 %)*			Pure MeOH		Water / ACN (ACN 25 and 50 %)*		
	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)

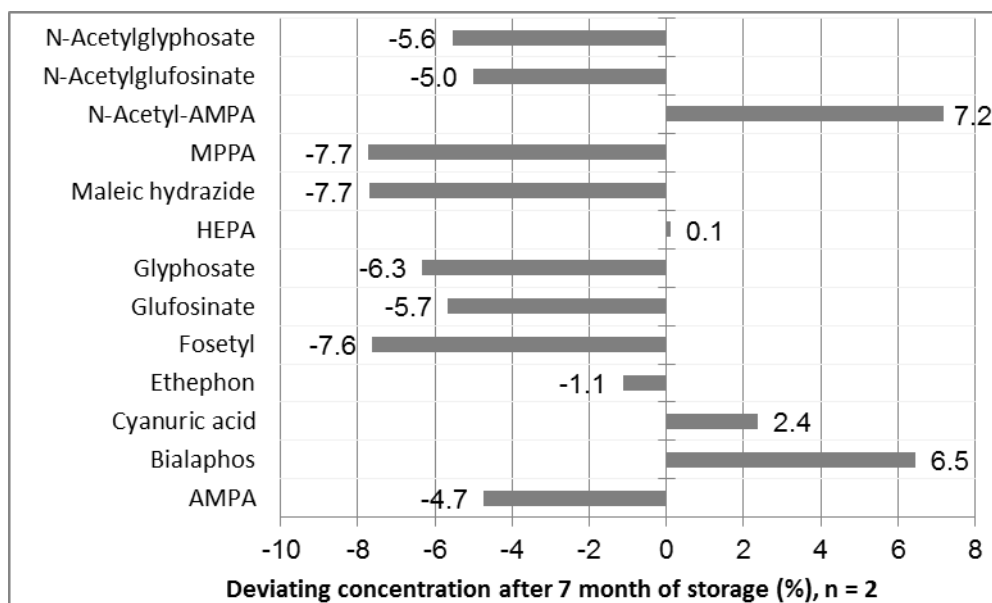


Figure 50: Deviations of the concentration of Glyphosate & Co. compounds in a working solution of 10 µg/mL water containing 10 % acetonitrile and 1% HCl conc. (v/v), following 7 months of storage at 6 °C. Compared against a freshly prepared standard of same composition

Issues concerning the purity of N-Acetyl-Glufosinate D₃: There is two types of N-Acetyl-Glufosinate D₃ 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-D₃), leading to false positive results. Fortunately the degradation rate observed in the water:acetonitrile 9:1 mixture (see Figure 50) was negligible. More important is the content of native glufosinate in purchased N-Acetyl-Glufosinate (acetyl-D₃) 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/11312/2021](#) guidance document are shown at the EURL validation database at www.eurl-pesticides-datapool.eu.

A compilation of comprehensive validation data that was generated during standardization of EN 18032:2025 is available as Supplement of EN 18032:2025 under <https://www.quppe.eu/supplements.asp>.

Exemplary validation data of the presented methods are listed in an external Table, which was formerly Table 45 in this document and which was removed as from Version V13.

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 45: Conversion factors between typical purchased standards and target analytes (3.23). *See also Supplementary Information on EN 18032.*

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	94.0	Sodium difluoroacetate	118.0	0.8	1.26
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
Diquat-D ₈	192.2	Diquat-D ₈ -dibromide	352.1	0.54	1.87
Fosetyl	110.05x3 = 330.14**	Fosetyl-Al	354.10	0.932	1.07
Fosetyl-D ₅	115.08x3=345.23**	Fosetyl-Al D ₁₅	369.19	0.935	1.07
Fosetyl-D ₅	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
Glufosinate-D ₃	184.1	Glufosinate-D ₃ -ammonium hydrate	243.2	0.76	1.32
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	60.1	Dimethylhydrazine hydrochloride	96.6	0.59	1.71
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
Paraquat-D ₈	194.3	Paraquat-D ₈ -dichloride	265.2	0.73	1.16
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	113.0	Sodium trifluoroacetate	136.0	0.83	1.20
Trifluoroacetic acid - ¹³ C ₂	115.0	Sodium trifluoroacetate- ¹³ C ₂	138.0	0.83	1.20
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 free fosetyl acid (MW 110.05x3=330,14). For the IL-IS the following numbers apply 1 mol fosetyl-Al D₁₅ (MW 369.19) contains 3 mols of fosetyl D₅ anion (MW 114.07x3=342.21) leading to 3 mols fosetyl D₅ acid (MW 115.08*3=345.23)

*** MW refers to the following formula (C₁₀H₁₄N₂)₂ · H₂SO₄ which entails two nicotine molecules

****to be avoided due to stability problems

Table 46: Exemplary concentrations of pesticide stock and working solutions (3.23 and 3.24), (solvent proposals also apply to IL-IS, see 3.26, 3.27, 3.28). Preferably use plastic vials (e.g. PP) as many of the compounds tend to interact with glass surfaces. [See also Supplementary Information on EN 18032.](#)

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 (¹⁸O-H₂O 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

Exemplary providers of isotopically labelled internal standards are available in the online available list of suppliers of analytical standards ([Excel](#)). The former **Table 49** was removed as from **Version V13** and was replaced by the online Excel-list.

Table 47: Exemplary concentrations of Internal Standard Working Solutions (IS-WS) (3.27)

Internal Standard (IS)*	IS –Addition to samples (5.2.3)		IS-Addition to calibration standard(s) (5.5)		Expected approx. IS-concentration in sample extracts (~20 mL) and calibration standards (~1 mL)
	Suggested concentration of IS-WSIn1 (3.27) µg/mL	Absolute mass of IS spiked to sample (100 µL IS-WSIn1) (m _{IS sample}) µg	Suggested concentration of IS-WSIn2 (3.28) ** µg/mL	Absolute mass of IS spiked to calibration standard (100 µL IS-WSIn2) (m _{IS cal mix}) µg	
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	20	2	1	0.1	0.1
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
Diquat D ₈	20	2	1	0.1	0.1
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
Paraquat-D ₈	20	2	1	0.1	0.1
Perchlorate- ¹⁸ O ₄	20	2	1	0.1	0.1
Phosphonic acid- ¹⁸ O ₃	20	2	1	0.1	0.1
Propamocarb-D ₇	2	0.2	0.1	0.01	0.01
PTU-D ₆	10	1	0.5	0.05	0.05
Triethanolamine-D ₁₂	10	1	0.5	0.05	0.05
Trifluoroacetic acid - ¹³ C ₂	10	1	1	0.05	0.05
Trimethylsulfonium-D ₁₀	10	1	0.5	0.05	0.05

* The concentration of the 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 extent as the cross-contamination is typically at low levels and as similar concentrations of the native pesticide originating from the IL-IS will also be present in the calibration standards and thus subtracted via the intercept. In the case of Maleic Hydrazide. Where the IL-IS is added at higher concentrations to the samples special attention is necessary (see also comments under **5.6.3**).

** 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.

**** to be avoided due to stability problems

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 Fehler! Verweisquelle konnte nicht gefunden werden.). 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 48: 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 fresh 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)

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
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	
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	
Brassica crops	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	
Leafy vegetables and herbs	Lettuce varieties	10 g	95	0.5	Yes	
	Endive	10 g	95	0.5	Yes	
	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	
Fresh legumes	Fresh Peas	10 g	75	2.5	No	
	Green Beans	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	
Pulses / Cereals Oilseeds						
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
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

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
Oilseeds	Peanuts, Poppy seeds, Pumpkin seeds, Sesame seeds, Soyabeans, Sunflower seeds	5 g	<10	9 mL water and 1 mL EDTA solution*	No	
Oilseeds developing a mucilage/slime on their surface upon wetting	Linseeds, Chiseeds	5 g	<10	9 mL water and 1 mL EDTA solution*	No	To reduce slime formation, which hinders residue accessibility, first add acidified methanol and then EDTA/water
Nuts	Almonds, Cashew nuts, Dried coconuts, Hazelnuts, Macadamias, Pecans, Pistachios, Walnuts	5 g	<10	9 mL water and 1 mL EDTA solution*	No	
Miscellaneous						
Extract-rich ("difficult") 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
	Coffee beans (alternative procedure especially recommended for glyphosate)	5 g	<10	10 mL water (+ 250 mg disodium oxalate)	No	
	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	None*	Yes	The wine will mix with the extractant, thus fully contributing to the final extract volume.
	Honey	5 g	20	7.5	No	
	Avocado	10 g	70	3	No	
	Coconut copra	5 g	<10	0		Fat-melting or assistance by grinding balls is needed, see QuPPE-AO (animal 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. For the extraction of **Diquat** and **Paraquat**, from this commodity, 10 mL water are added instead of 9 mL water and 1 mL EDTA solution.

Table 49: 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. SelexIon™	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 50: 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 51: 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 D ₆ in M4		
New method (M7) for the analysis of Morpholin/Morpholin D ₈ ; Diethanonamine/diethanolamine D ₆ ; Triethanolamine/Triethanolamine D ₁₂ (M7)		
Removal of Morpholin from M4 as it does not separate from the interfering diethanolamine		
Introduction of ETU and PTU and their corresponding 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; additionally covering 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		
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	Mar. 2015	V8
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 corresponding IL-ISs		
Update of Table 17: Providers of isotopically labeled internal standards		
5.1 Sample preparation: note to importance of having small particle sizes		
5.2.4 notes to extraction time for dry products and the influence of particle size	May 2015	V8.1
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		
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.: <i>Hints on Method 1.3</i>		
Update of Method 1.4: Introduction of measurement conditions for the measurement of Bromate and Bromide ion		
Update of Chapter 5.7.4.1.: <i>Hints on Method 1.4</i>		
Update of Method 4.2 : "Quats & Co BEH Amide" including Aminocyclopyrachlor. Chloridazon-desphenyl. Mepiquat-4-hydroxy. Propamocarb-N-desmethyl. Propamocarb-N-oxide	Mar. 2016	V9
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 former Table 42: Overview of approximate LOQs		
Update of Table 44: Conversion factors		
Update of Table 45: Exemplary concentrations of pesticide stock and working solutions		
Update of former Table 49 on exemplary providers of isotopically labelled internal standards		
Update of Table 47: 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:		
Inclusion of N-Acetyl-Glyphosate in Table 4: Practical Information: Mainly used methods used at CVUA Stuttgart	October 2016	V9.2

Action	When?	Version
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 5.6.23	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 44		
Overview of lowest successfully validated levels (former Table 45)		
Update of former Table 45 : DFA and TFA added		
Update of Table 46: DFA and TFA added; solvents for Ethephon, Fosetyl and Maleic Hydrazide changed		
Update of Table 48		
Update of Table 47: DFA and TFA added	Dec 2018	V10
Former Table 49: data on M 9 were included		
Extensive general revision of text, tables and figures		
Addition of nuts and oilseeds 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 oilseeds 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		
Extension 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)	April 2019	V10.1
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		
Update of method for pulses, oilseeds 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	Feb 2020	V11
Addition of Method 1.7 for phosphonate, bromide, chlorate and perchlorate		
Maleic hydrazide added to Methode 4.2		
Introduction of a list with shortcut-links		

Action	When?	Version
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 former Table 45: Validation data		
Update of Table 47: data on Melamine, Matrine, Oxymatrine, Triazole, Triazole-lactic acid, Triazole-acetic acid, Triazole-alanine included		
Update of Table 48: dried fruits; olives, coconut copra, dried mushrooms, kaki included; garlic updated		
New IL-ISs: 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	March 2023	V12.1
Former Table 49: data on M 1.10, M 10, M 11		
Introduction of Table 50: MS Parameters for Waters Xevo TQ-Sμ		
Revision of procedure for honey including water adjustment for honey, hints on how to use syringe / particle filters. New figure showing the procedure at a glance for honey, and validation data	December 2023	V12.2
Introduction of new measurement methods: M1.11 (on Phenomenex Luna Polar Pesticides) and M1.12 (Phenomenex Luna Polar Pesticides)		
Addition of difluoroacetic acid (DFA) to the scope of method M1.6b (LC-MS/MS on Waters APPC)		
Addition of difluoroacetic acid (DFA), trifluoroacetic acid (TFA), thiocyanate as well as of several metabolites of organophosphorous pesticides (diethyl phosphate, diethyl thiophosphate, O,O-dimethyl dithiophosphate, O,O-dimethyl thiophosphate, diethyl dithiophosphate, dimethyl phosphate) to the scope of method M1.7b (LC-MS/MS on Waters APPC)		
Addition of difluoroacetic acid (DFA) to the scope of M10 (IC-MS/MS)		
Addition of further IL-ISs		
Addition of further validation data		
Introduction of new procedure for the extraction of Diquat and Paraquat in various matrices	December 2024	V12.3
Introduction of a flow chart: "QuPPe PO Method at a glance - Procedure for Diquat and Paraquat in various commodities (e.g. pulses, oilseeds, nuts, cereals, potatoes, bananas)"		
Addition of a mass transition for the determination of TFA		
Update of "How to cite"		
Update of list of chapter 3 Chemicals		
Update of chapter 5.6.1 as regards Contamination Sources and the Stability of Diquat and Paraquat		
Update of former Table 45, Table 47, Table 48	September 2026	V13
Correction of typos and syntax errors and revision of text passages		
Introduction of a note highlighting that formic acid may be a source of contamination and the need to check this by running procedural blanks. Hint for distilling formic acid as a measure to reduce contamination.		

Action	When?	Version
Introduction of a note highlighting that in special cases of analytes showing low absolute recoveries due to dynamic interactions with the samples (e.g. Glyphosate, Paraquat, Diquat), reducing the weight of the analytical portion can help to exceed the required 30% threshold for the absolute method recovery. Examples are given in Chapter 5.6.1.		
Introduction of a procedure for plant oils		
Introduction of an alternative procedure for raw coffee based on the use of oxalalate for chelation, especially recommended for the analysis of glyphosate in this commodity.		
Introduction of recommendations on how to support analyte identification by using ion ratios between signals of the same mass transition obtained at different collision energies, which is particularly of interest for analytes with only one useful mass transition (e.g. TFA). (Chapter 5.6.1)		
Introduction of recommendations on how to validate compounds showing a high frequency of background which prevent validation at low levels when following the original rule requiring that the background should not exceed 30% of the spiked concentration in validation experiments. (Chapter 5.6.1)		
Thorough revision of the text on “Unwanted retention in the tubing leading to peak tailing and/or carry-over effects”		
Introduction of a note on the analysis of bromate		
Introduction of a note on the use of nereistoxin IL-IS		
Introduction of a note on the maintenance of IC autosamplers		
Addition of TFA and DFA into M1.12. And addition of equilibration time into the gradient of M1.12		
Addition of Pymetrozine into M4.2.		
Introduction of a new HILIC-based measurement method for ESI-pos. analytes: M4.3 (on Phenomenex Luna Polar Pesticides)		
Introduction of an additional shorter gradient for M11		
Introduction of new measurement methods based on ion chromatography for for ESI-pos. analytes: M12.1 (CS 17) and M12.2 CS21)		
Correction of typos and syntax errors and revision of text passages		
Removal of Table 45 which was showing exemplary validation data of the presented methods to an external table and linking to the external table		
Removal of Table 49 showing exemplary providers of isotopically labelled internal standards, which was replaced by the online available list in Excel format		