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

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1. Scope and Short Description

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

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

2. Apparatus and Consumables

2.1. Powerful sample processing equipment,

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

2.2. 50 mL centrifuge tubes with screw caps,

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

2.3. Automatic pipettes,

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

2.4. 10 mL solvent-dispenser,

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

2.5. Centrifuge,

suitable for the centrifuge tubes employed in the procedure (**2.2**) and capable of achieving > 2500 rpm.

2.6. Syringe filters,

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

Note:

- ***Significant levels of Perchlorate and Chlorate were detected in the above mentioned polyester filters.*** *Cellulose mixed esters filters were found to be appropriate for these two compounds. For this suitability test take the worst case scenario into account where the filters are clogged by the extracts, not allowing large volumes (e.g. 200 µL) to pass. Thus elute only small volumes through the filters (e.g. 200 µL). Such clogging was observed using commodities such as industrially milled cereals, pears and pineapples. Furthermore,* ***special attention is required if filters are used to filter diluted extracts as any detected levels in the extracts will have to be multiplied accordingly when calculating the levels in the sample.***

2.7. Syringes

e.g. 2 or 5 mL disposable polypropylene syringes suitable for the above mentioned filters **2.6.**

2.8. Autosampler vials,

suitable for LC auto-samplers,

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

2.9. Volumetric flask with stoppers,

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

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

2.10. LC-MS/MS instrumentation,

equipped with ESI source and appropriate columns, see details in chapters **5.6.1** till **5.6.11**.

[1]The list of compounds requiring plastic vessels might not be comprehensive (this remark applies to the entire document). Such interactions with glass surfaces are typically more pronounced when solutions have low water content and low acidity.

3. Chemicals

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

3.1. Water (deionized)

3.2. Methanol (HPLC quality)

3.3. Acetonitrile (HPLC quality)

3.4. Formic acid (concentrated; > 95%)

3.5. Acetic Acid (concentrated; >98%)

3.6. Acidified methanol,

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

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

3.8. Dimethylamine,

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

3.9. Ammonium formate (p.a.)

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

3.11. Sodium hydroxide (p.a.)

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

3.13. Dry ice,

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

3.14. Pesticide Standards,

of known purity.

3.15. Pesticide stock solutions,

e.g. 1 mg/mL solutions of pesticide standards (**3.14**) in a water miscible solvent (e.g. water (**3.1**), methanol (**3.2**), acidified methanol (**3.6**), acetonitrile (**3.3**) or mixtures thereof). See **Table 15** for the conversion factors to be applied between typical purchased standards and analytes and **Table 16** for suggested solvents for the preparation of the stock solutions.

Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. Paraquat, Diquat, Streptomycin and Glyphosate). Keep in mind that some standards are sold as salts or hydrates. Some exemplary conversion factors are shown in **Table 15.**

3.16. Pesticide working solutions / mixtures,

prepared at appropriate concentrations by diluting pesticide stock solutions (**3.15**) of one or more pesticides with water-miscible solvents as required for the spiking of samples in recovery experiments (**5.4**) or for the preparation of calibration standards (**5.5**). See suggestions in **Table 16** in the Annex.
Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. Paraquat, Diquat, Streptomycin and Glyphosate).

3.17. Internal Standards (ISs),

Exemplary sources are shown in **Table 17**.

3.18. IS Stock solutions,

e.g. 1 mg/mL solutions of ISs (**3.17**) in a water miscible solvent (e.g. methanol, acetonitrile, water or mixtures thereof). For solvent-suggestions see **Table 16** in the Annex.
Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. ILISs of Paraquat, Diquat and Glyphosate as well as Dihydrostreptomycin). Keep in mind that some standards are sold as salts or hydrates. Some exemplary conversion factors are shown in **Table 15.**

Notes:

- *In general the absolute concentrations of the ILIS-solutions are not important as long as the ILIS-concentration in the final extract is high enough to produce a well measurable signal that is not disturbed by co-eluting matrix components. Important is furthermore that any content of the native analyte within the ILIS-standard (irrespective whether it was present as an impurity of the purchased standard or whether it was generated in the lab during storage of the ILIS-solution or during sample preparation) is low enough to exclude false positive results or significant influence on quantification. For quantification purposes it is of foremost importance that the ratio between the absolute ILIS amount added to the sample prior to extraction (or to the isolated aliquot of the sample extract) and the absolute amount of ILIS added to the calibration standard solutions is known as it is used in calculations.*

3.19. IS-working solution I (IS-WS I) for spiking samples prior to extraction,

prepared at appropriate concentrations by diluting IS stock solutions (**3.18**) of one or more ISs with water-miscible solvents. Suggestions for solvents are shown in **Table 16** and suggestions for the concentrations in Table 18.

Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. ILIS of Paraquat, Diquat and Glyphosate as well as Dihydrostreptomycin). In presence of water and especially at high pH levels, Phosphonic acid $^{18}O_3$ will gradually convert to $^{18}O_2{}^{16}O_1$, $^{18}O_1{}^{16}O_2$ and eventually of $^{16}O_3$ (native) phosphonic acid. The $^{16}O_3$ phosophonic acid standard solution provided by the EURLs should be preferably diluted in acetonitrile, where it was shown to be stable for long periods.

3.20. IS-working solution II (IS-WS II) for preparation of calibration standards,

prepared at appropriate concentrations by diluting IS-WS-I (**3.19**) with water-miscible solvents. Suggestions for solvents are shown in **Table 16** and for concentrations in **Table 18**.

Use plastic flasks and stoppers if pesticides that tend to interact with glass-surfaces are present (e.g. ILIS of Paraquat, Diquat and Glyphosate as well as Dihydrostreptomycin). See also sub-note 3 in **Table 1**. For short term usage (e.g. up to one month) the ILIS of Phosphonic acid can be diluted in acidified methanol (3.6).

3.21. LC-MS/MS mobile phases,

see details in chapters **5.6.1** till **5.6.12.**

4. Disclaimer

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

5. Procedure

5.1. Sample preparation

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

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

5.2. Extraction / Centrifugation / Filtration

5.2.1. Weigh a representative portion (m_a) of the sample homogenate (**5.1**) into a 50 mL centrifuge tube (**2.2**). In case of fresh fruits and vegetables as well as juices take 10 g ± 0.1 g of the homogenized sample. In case of dried fruits, dried vegetables, dried mushrooms take 5 g ± 0.05 g or 13.5 g ± 0.1 g of the re-hydrated and homogenized material according to **5.1.1** (corresponding to 5 g sample). In case of cereals, dried pulses and honey also take 5 g ± 0.05 g of the homogenate.

Notes:

- *Smaller sample portions may have to be used for extract-rich commodities, such as spices or fermented products, or commodities with very high water absorbing capacity not allowing proper extraction.*

5.2.2. Add water (**3.1**) to a total content of ca. 10 g according to the indications in **Table 19.**

Notes:

- *No further water adjustment is needed where re-hydrated commodities (**5.1.1**) are employed.*
- *Where no ISs are used or where they are added after extract aliquotation, water adjustment to 10g is essential. Where the appropriate ISs are employed before any aliquotation has taken place water adjustment is less critical and may be skipped for commodities containing ≥80% water (see **Table 19**)*

5.2.3. Add 10 mL acidified methanol (**3.6**) and 50 µL of the IS-WS I (**3.19**) containing isotopically labeled analogues of one or more of the analytes of interest (added IS mass = m_{IS}^{sample}).

Notes:

- *The resulting extract volume, taking into account the natural water content of the sample and the water added in **5.2.2** sum up ca. 20 mL (corresponds to ca. 0.5 g sample per mL extract if 10 g sample is employed for extraction). Where no ISs are used the aim should be to reach a total volume of the liquid phase that is as close as possible to 20 mL. Keep in mind that the water volume adjustments in **Table 19** are approximate and that there is a ca. 2.5% volume contraction occurring when methanol is mixed with water. In any case water adjustment will help to reduce the bias related to the volume deviation from 20 mL to an acceptable level.*

- *For screening purposes the IS can be alternatively added to a sample extract aliquot (e.g. 1 mL, see **5.2.8**), assuming that 1 mL extract corresponds to exactly 0.5 g sample equivalents. This way the added amount of IS per sample can be drastically reduced (e.g. 20-fold if added to 1 mL extract). The IS added at this step will compensate for matrix effects including retention-time shifts. The quantitative result should however be considered as tentative. For more accuracy samples should be re-analyzed with the IS being added in step **5.2.3**.*

5.2.4. Close the tube and shake vigorously for 1 min by hand or for 5-20 minutes by a mechanical shaker.

Notes:

- *In case of dry products the 1 minute shaking is to be followed by a soaking period of 10 minutes and a subsequent second 1 minute vigorous shaking. Where mechanical shaking is employed no soaking period for dry commodities is necessary.*

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

Notes:

- *1 minute extractions at room temperature with methanol containing 1% formic acid are well suitable Paraquat and Diquat screening. 15-minute extractions at 80 °C using the same extraction solvent were shown to provide quantitative extraction yields of incurred Diquat and Paraquat residues in wheat and potatoes. In an experiment on **Lentils** containing incurred Diquat residues a stronger extraction solvent was necessary (MeOH/aqueous HCl 0,1M (1:1)) using the same volume, extraction temperature and extraction time as described above[2].*

5.2.6. Centrifuge (e.g. for 5 min at 4000 rpm).

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

Note:

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

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

Note for 5.2.7 and 5.2.8:

- *Use plastic storage vessels/vials if pesticides that tend to interact with glass-surfaces are or expected to be present (e.g. ILISs of Paraquat, Diquat and Glyphosate as well as Dihydrostreptomycin).*

5.3. Blank extracts

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

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

5.4. Recovery experiments

Weigh an appropriate portion (see **5.2.1)** of a blank commodity homogenate into a 50 mL centrifuge tube (**2.2**) and spike it with a suitable pesticide working solution (**3.16** and **Table 16**).

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 exactly 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 1**.

The calculation of the mass-fraction W_R of the pesticide in the sample, when IS is used, is shown in **5.7.1**.

> ***Note:*** *Where solvent-based calibrations are used the use of ILISs for quantification is essential as the IS compensates for any matrix-related signal suppressions / enhancements.*

5.5.2. **Matrix matched calibration standards**

Transfer suitable aliquots of the blank extract **(5.3)** to auto-sampler vials and proceed as shown in **Table 1**.

The calculation of the mass-fraction W_R of the pesticide in the sample using matrix-matched calibration standards, with and without the use of ILIS, is shown in **5.7.1.1** and **5.7.2.1** respectively.

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

		Calibration standards								
		Solvent based (5.5.1)			**Matrix-matched (5.5.2)**					
		using IS[4]			**without IS[5]**			**using IS[4]**		
Calibration levels in µg pesticide /mL OR in µg pesticide/ "IS-portion"[1]		**0.05[6]**	**0.1**	**0.25**	**0.05**	**0.1**	**0.25**	**0.05**	**0.1**	**0.25**
Blank extract (5.3)		-	-	-	900 µL	900 µL	900 µL	850 µL	850 µL	850 µL
1:1 (v/v) mix of water (3.1) and acidified MeOH (3.6)		900 µL	850 µL	900 µL	50 µL	-	50 µL	50 µL	-	50 µL
Pesticide working solutions (3.16) [2]	**1 µg/mL**	50 µL	100 µL	-	50 µL	100 µL	-	50 µL	100 µL	-
	5 µg/mL	-	-	50 µL	-	-	50 µL	-	-	50 µL
IS-WS II (3.20)[1,3]		50 µL	50 µL	50 µL	-	-	-	50 µL	50 µL	50 µL
Total volume		**1000 µL**	**1000 µL**	**1000 µL**	**1000 µL**	**1000 µL**	**1000 µL**	**1000 µL**	**1000 µL**	**1000 µL**

[1] One IS portion would correspond to the IS mass contained in 50 µL IS-WS II (which in the particular example is added to each calibration standard).

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

*[3]For calibration standards of 1 mL it is recommended to prepare the IS-WS II **(3.20)** by diluting 20-fold the IS-WS I **(3.19)**. The same volume and pipette as in **5.2.3** can then be used for the preparation of the calibration standards.*

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

*[4] Where ILISs are <u>not</u> available/employed, matrix-matching via matrix-matched standards (**Table 1**) or via the standard additions approach (**5.5.3**) are particularly important to compensate for matrix effects in measurement. In both cases the total volume of the sample extracts is assumed to be exactly 20 mL, which translates into 0.5 g sample equivalents per mL.*

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

5.5.3.**Standard-Additions-Approach**

Where no appropriate ISs are available the method of standard additions is a very effective approach for compensating matrix-induced enhancement or suppression phenomena. As this procedure involves a linear extrapolation it is mandatory that pesticide concentrations and detection signals show a linear relationship throughout the relevant concentration range. The procedure furthermore requires knowledge of the approximate (estimated) residue level in the sample ($w_{R(approx)}$). 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 (**3.16**) of the analyte at a concentration level where 50 or 100 µL of the solution contain the lowest amount of analyte to be added.

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

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

Table 2 shows an example according to Example B. The calculation of the mass fraction of the pesticide in the sample w_R is shown in **5.7.2.2**.

Table 2 : Exemplary pipetting scheme of a standard additions approach (for a sample extract containing 0.5 g sample equivalents per mL and an estimated residue level ($w_{R(approx)}$) 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)
IS	none	none	none	none
Added volume of pesticide working solution containing 5 µg/mL (**3.16**)	-	50 µL	100 µL	150 µL
Resulting mass ($m_{pest}^{std\ add}$) of pesticide added to each vial		0.25 µg	0.5 µg	0.75 µg
Volume of solvent	150 µL	100 µL	50 µL	-
Final volume	**1150 µL**	**1150 µL**	**1150 µL**	**1150 µL**

5.6. LC-MS/MS Measurement

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

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

	M 1.1	M 1.2	M 1.3	M 1.4	M 2	M 3	M 4.1	M 4.2	M 5	M 6	M 7	M8
ESI-mode	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
Separation principle	Anion Exchange	Anion Exchange	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon
Column type	AS-11	AS11-HC	Hyper-carb	Hyper-carb	Obelisc-R	Obelisc-R	Obelisc-R	BEH-Amide	PFP	Obelisc-R	Trinity P1	Hyper-carb
NEGATIVE MODE												
Ethephon	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
HEPA	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
Glufosinate	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
N-Acetyl-glufosinate	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
MPPA	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
Glyphosate	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
AMPA	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
Phosphonic acid	(✓)	(✓)	✓	✓	NT	NT	NT	NT	NT	NT	-	NT
N-Acetyl-AMPA	NT	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
Fosetyl-Al	-	✓	✓	NT	✓	NT	NT	NT	NT	NT	✓*	NT
Maleic hydrazide	-	-	✓	NT	✓	NT	NT	NT	NT	NT	✓*	NT
Perchlorate	NT	-	✓	✓	✓	NT	NT	NT	NT	NT	✓*	NT
Chlorate	NT	-	✓	✓	NT	NT	NT	NT	NT	NT	✓*	NT
Bialaphos	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
Cyanuric acid	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	✓*	NT
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	NT
Trimesium	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT
Daminozide	NT	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	NT
Melamine	NT	NT	NT	NT	NT	NT	✓	✓	NT	NT	NT	NT
Diquat	NT	NT	-	NT	NT	NT	✓		NT	NT	NT	NT
Paraquat	NT	NT	-	NT	NT	NT	✓		NT	NT	NT	NT
N,N-Dimethylhydrazine	NT	NT	-	NT	NT	NT	✓		NT	NT	NT	NT
Nereistoxin	NT	NT	✓	NT	NT	NT	✓	✓	NT	NT	NT	NT
Streptomycin	NT	NT	NT	NT	NT	NT	NT		NT	✓	NT	NT
Kasugamycin	NT	NT	NT	NT	NT	NT	NT		NT	✓	NT	NT
Morpholine	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT
Diethanolamine	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT
Triethanolamine	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT
1,2,4-Triazole	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓
Triazole-alanine	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓
Triazole-acetic acid	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓
Triazole-lactic acid	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓

NT= Not tested under the conditions shown in the respective sections , **(✓) =** possible but compromised due to matrix effects or lacking separation or limited sensitivity or limited information for proper identification, "-" analysis was tested and found to be poor under the described conditions
* Using a gradient (98% B -> 60% B in 5 min, hold 2 min)

5.6.1. **Method 1.1 (for "Glyphosate & Co.")**

Table 4: 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 AG11 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 ca. **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 Glyphosate-$^{13}C_2$,$^{15}N_1$ (ILIS)	168/63, 168/124, 168/150, 168/81 171/63
	AMPA AMPA-$^{13}C_1$$^{15}N_1$ (ILIS)	110/63, 110/79, **110/81**** 112/63
	Ethephon Ethephon-D_4 (ILIS)	143/107, 143/79, 145/107 147/111
	HEPA HEPA-D_4 (ILIS)	125/79, 125/95, 125/63 129/79
	Glufosinate Glufosinate-D_3 (ILIS)	180/63, 180/136, 180/85, 180/95 183/63
	N-Acetyl-glufosinate N-Acetyl-glufosinate-D_3 (ILIS)	222/63, 222/59, 222/136 225/63
	MPPA MPPA-D_3 (ILIS)	151/63, 151/107, 151/133 154/63

AMPA: Aminomethylphosphonic acid; MPPA: 3-Methylphosphinicopropionic acid; HEPA: 2-Hydroxyethylphosphonic acid (= hydroxy-ethephon),
** One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also* **Table 1***).*
*** See comment 1 under* ***5.6.1.1*** *concerning potential interference of AMPA by Fosetyl.*

Figure 1: Typical chromatograms of Glyphosate, AMPA, Glufosinate, MPPA and Ethephon spiked on blank-QuPPe extracts of various commodities

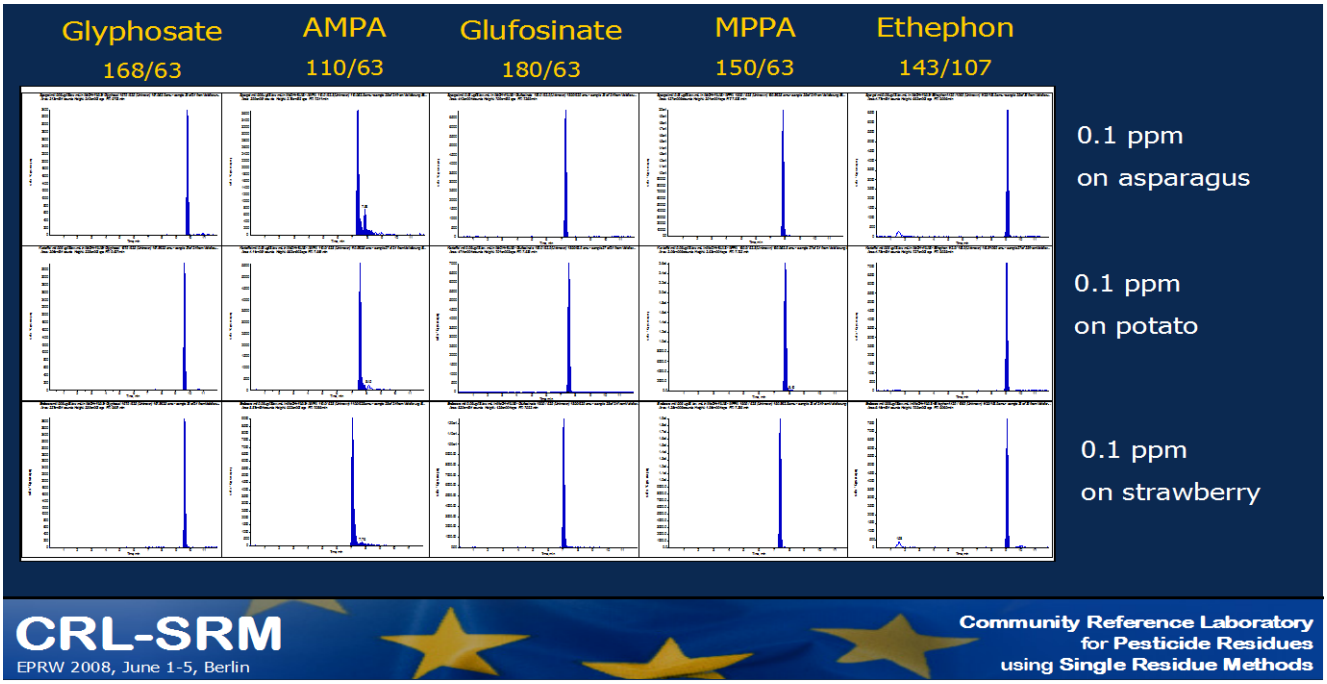
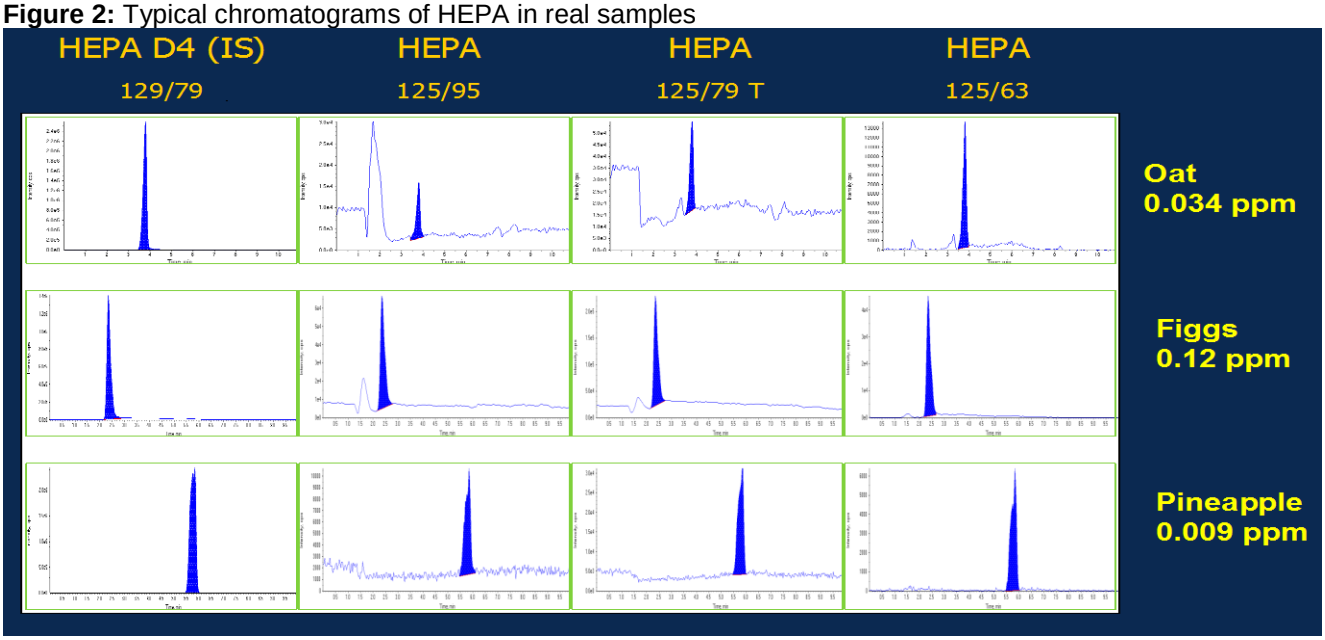


Figure 2: Typical chromatograms of HEPA in real samples



5.6.1.1. Hints on Method 1.1

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

2) *As the pH of the mobile phase is quite high, it is recommendable to **use alkali-compatible components**, e.g. metal frits instead of silica frits in the Eluent B reservoir; borosilicate 3.3 bottles instead of glass bottles for eluent B; rotor-seals from alkali-persistent materials, such as PEEK (polyetherketone) or Tefzel,* rather than Vespel.

3) ***Priming and reconditioning of column:*** *before first use, after long storage (e.g. >2 weeks), after injection of 50-100 sample extracts for column A or 100-200 extracts for column B):*
   a. *Flush column for 30 minutes with **100 mmol aqueous Borax solution** (7,62 g di-sodium tetraborate decahydrate in 200 mL water) at 0.3 mL/min **OR** Flush for 1 hour with 30 mM NaOH (240 mg NaOH in 200 mL water) at 0.3 mL/min*
   b. *Flush column for 30 minutes with **Eluent A** (water) at 0.3 mL/min*
   c. *Run system 3-4 times with full gradient (inject standards in matrix)*

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

4) ***Storage of column:*** *If to be stored for short periods (<2 weeks), columns can be put aside after any normal sequence/run (full gradient). Run system 3-4 times with full gradient to reactivate the column (inject standards in matrix) before starting the sequence. If to be stored for longer periods (e.g. >2 months) recondition the column as described under I.1.a-c*

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

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

6) ***Pre-columns (guard columns)****:*
   a. *The pre-column should be exchanged as soon as a clear deterioration of the separation performance (worsening of peak-shape) is noticed. The pre-column of method 1.1. needs to be exchanged more often than that of 1.2 and 1.3. If after pre-filter exchange (see above) the pressure does not come back to normal levels, the frit of the pre-column should be exchanged.*

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

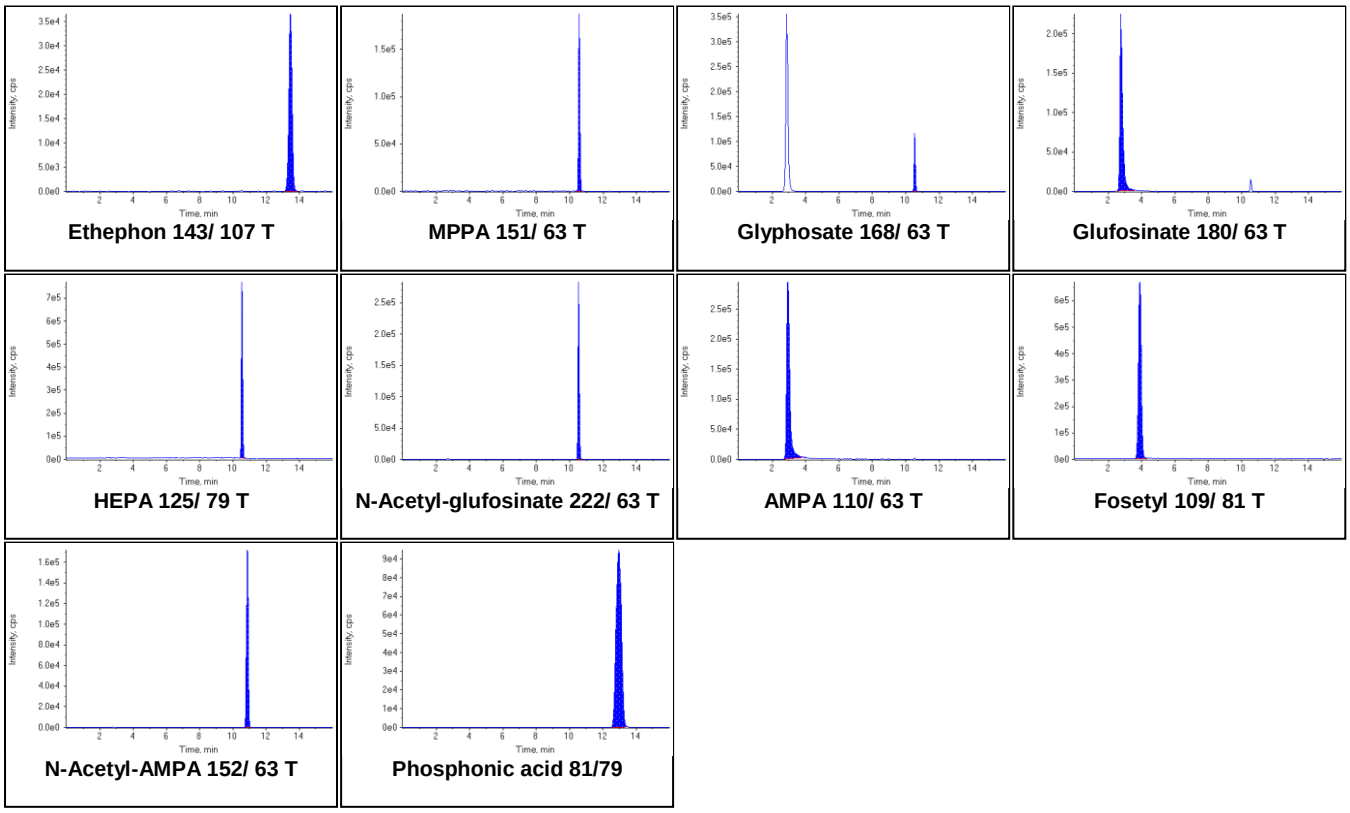
5.6.2. **Method 1.2 (for "Glyphosate & Co.")**

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

Instrument parameters	**Conditions**	
Ionization mode	ESI neg	
Column/temperature **(see also notes below)**	Dionex IonPac AS 11-**HC** 2 x 250 mm (P/N 052961); 40°C	
Pre-column	Dionex IonPac AG11-**HC** 2 x 50 mm (P/N 052963)	
Pre-filters	e.g. Supelco column saver 2.0 µm Filter (optional)	
Eluent A	Water (**3.1**)	
Eluent B	1 mM tribasic ammonium citrate in water	
Gradient	**%A** / **Flow [mL/min]** / **Time [min]**: 100 / 0.3 / 0; 0 / 0.3 / 8; 0 / 0.3 / 16; 100 / 0.3 / 16.1; 100 / 0.3 / 23	
Injection volume	10 µL	
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS-portion* + one level at the reporting limit	
Acquired mass transitions (m/z)	**Compound**	**Mass Transitions (m/z)**
	Glyphosate Glyphosate-$^{13}C_2$,^{15}N (ILIS)	168/63, 168/124, 168/150, 168/81 171/63
	AMPA AMPA-^{13}C,^{15}N (ILIS)	110/63, 110/79, **110/81**** 112/63
	N-Acetyl-AMPA	152/63, 152/79, 152/110
	Ethephon Ethephon-D_4 (ILIS)	143/107, 143/79, 145/107 147/111
	HEPA HEPA-D_4 (ILIS)	125/79, 125/95, 125/63 129/79
	Glufosinate Glufosinate-D_3 (ILIS)	180/63, 180/136, 180/85, 180/95 183/63
	N-Acetyl-glufosinate N-Acetyl-glufosinate-D_3 (ILIS)	222/63, 222/59, 222/136 225/63
	MPPA MPPA-D_3 (ILIS)	151/63, 151/107, 151/133 154/63
	Fosetyl-Al Fosetyl-Al-D_{15} (ILIS)	109/81, 109/63 (Fosetyl) 114/82 (Fosetyl-D_5)
	Phosphonic acid*** Phosphonic acid-$^{18}O_3$ (ILIS)	81/79, 81/63 87/85

AMPA: Aminomethylphosphonic acid; MPPA: 3-Methylphosphinicopropionic acid; HEPA: 2-Hydroxyethylphosphonic acid (=hydroxy-ethephon)
** One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also* **Table 1***).*
*** See comment 1 under* ***5.6.1.1*** *concerning potential interference of AMPA by Fosetyl.*
*** See comment 3 on Phosphonic acid under* ***5.6.2.1***

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



5.6.2.1. Hints on Method 1.2

1) Using this M1.2 some compounds (e.g. Glyphosate) in some commodities tend to give two sharp peaks. The corresponding ILIS typically behaves equally, so that quantification with any of the two peaks remains accurate

2) AMPA and Fosetyl share the mass-transition (110/81). Chromatographic separation is thus needed (typically the case).

3) Fosetyl and Fosetyl-D_5 tend to degrade to Phosphonic acid both in solutions and in the LC-MS/MS via in-sorce fragmentation. A good chromatographic separation between the two is thus necessary (typically the case).

4) *When extracts containing high levels of Phosphoric acid (which is naturally contained at high concentrations in many samples) are injected the chromatographic separation of Phosphoric and Phosphonic acid is compromised. This often results in a suppression of the Phosphonic acid signal and in some cases even leads to false negative results.* ***The most important qualifier mass-transition of Phosphonic acid (81/63) also occurs as a minor transition of Phosphoric acid,*** *but as the latter is often present at much higher levels than Phosphonic acid its interference on this mass transition can still be significant, especially if these two elute in close vicinity. The chromatographic separation of Phosphoric and Phosphonic acid considerably improves following dilution of the extracts typically allowing proper detection, identification and quantification of Phosphonic acid next to high levels of phosphoric acid. It is thus beneficial to inject smaller volumes of sample extract (e.g. 1-2 µL) or to* ***dilute QuPPe extracts 5-10-fold before injection****. Fortunately both,* ***Phosphoric and Phosphonic acid have at least 1 proper mass-transition (97/63 and 81/79 respectively), which in the case of Phosphonic acid can be used for quantitation and to improve identification certainty****. The elution time and peak shape of the Phosphonic acid ILIS can also be used to distinguish it from Phosphoric acid and to avoid false positives. Using signals on the 81/63 mass trace it was calculated that 20 mg/kg Phosphoric acid would simulate 0.1 mg/kg Phosphonic acid if this mass transition was used for quantification.*

5) ***Priming and reconditioning of column:*** *before first use, after long storage (e.g. >2 weeks), after injection of 50-100 sample extracts for column A or 100-200 extracts for column B):*

   a. *Flush column for 30 minutes with* ***100 mmol aqueous Borax solution*** *(7,62 g di-sodium tetraborate decahydrate in 200 mL water) at 0.3 mL/min* ***OR*** *Flush for 1 hour with 30 mM NaOH (240 mg NaOH in 200 mL water) at 0.3 mL/min*
   b. *Flush column for 30 minutes with* ***Eluent A*** *(water) at 0.3 mL/min*
   c. *Run system 3-4 times with full gradient (inject standards in matrix)*

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

6) ***Storage of column:*** *If to be stored for short periods (<2 weeks), columns can be put aside after any normal sequence/run (full gradient). Run system 3-4 times with full gradient to reactivate the column (inject standards in matrix) before starting the sequence. If to be stored for longer periods (e.g. >2 months) recondition the column as described under I.1.a-c*

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

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

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

5.6.3. **Method 1.3 (for "Glyphosate & Co.")**

Table 6: Proposed LC-MS/MS conditions for Ethephon, HEPA (Ethephon metabolite), Glyphosat, AMPA (Glyphosate metabolite), , N-Acetyl-AMPA (Glyphosate metabolite), Glufosinate, MPPA (Glufosinate metabolite), N-Acetyl-glufosinate (Glufosinate metabolite), Fosetyl-Al, Phosphonic acid (Fosetyl metabolite), Maleic hydrazide, Perchlorate, Chlorate, 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% MeOH
Eluent B	1% Acetic acid in MeOH

Gradient

%A	Flow [mL/min]	Time [min]
100	0.2	0
70	0.2	10
70	0.4	11
70	0.4	18
10	0.4	19
10	0.4	22
100	0.2	22.1
100	0.2	30

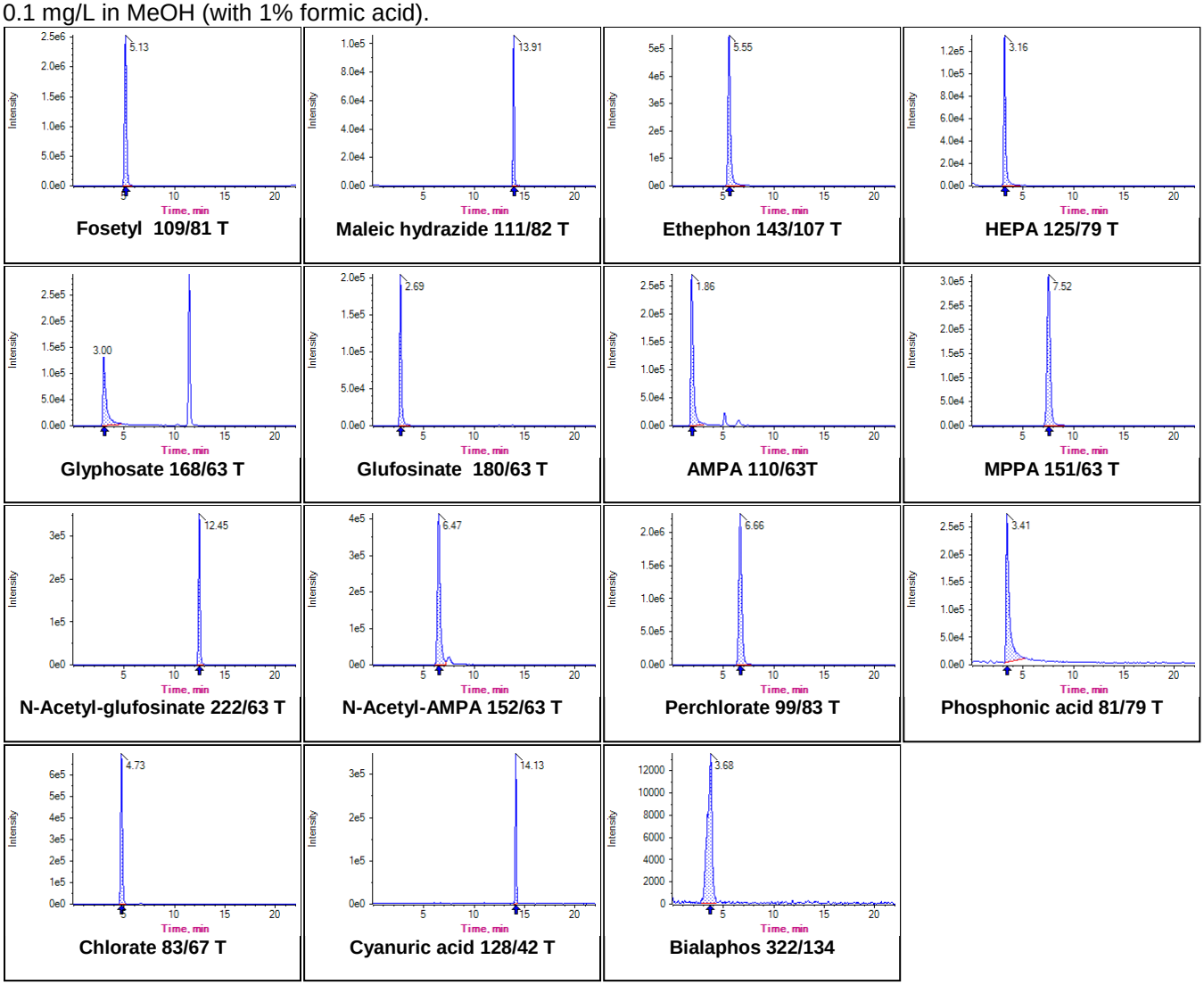
Injection volume	5 µL
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS-portion* + one level at the reporting limit

Acquired mass transitions (m/z)

Compound	**Mass Transitions (m/z)**
Glyphosate Glyphosate-$^{13}C_2$,^{15}N (ILIS)	168/63, 168/124, 168/150, 168/81 171/63
AMPA** AMPA-^{13}C,^{15}N (ILIS)	110/63, 110/79, **110/81**** 112/63
N-Acetyl-AMPA	152/63, 152/79, 152/110
Ethephon Ethephon-D_4 (ILIS)	143/107, 143/79, 145/107 147/111
HEPA HEPA-D_4 (ILIS)	125/79, 125/95, 125/63 129/79
Glufosinate Glufosinate-D_3 (ILIS)	180/63, 180/136, 180/85, 180/95 183/63
N-Acetyl-glufosinate N-Acetyl-glufosinate-D_3 (ILIS)	222/63, 222/59, 222/136 225/63
MPPA MPPA-D_3 (ILIS)	151/63, 151/107, 151/133 154/63
Fosetyl-Al Fosetyl-Al-D_{15} (ILIS)	109/81, 109/63 (detected as Fosetyl) 114/82 (detected as Fosetyl- D_5)
Phosphonic acid*** Phosphonic acid-$^{18}O_3$ (ILIS)	81/79, 81/63 (detected as Phosphonate anion) 87/85
Maleic hydrazide Maleic hydrazide-D_2 (ILIS)	111/82, 111/42, 111/55, 111/83 113/42
Perchlorate Perchlorate-$^{18}O_4$ (ILIS)	99/83, 101/85 107/89
Chlorate Chlorate-$^{18}O_3$ (ILIS)	83/67, 85/69 89/71
Cyanuric acid Cyanuric acid-$^{13}C_3$	128/42, 128/85 131/43
Bialaphos	322/88, 322/94, 322/134

** One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also* **Table 1***).*
*** See comment 1 under 5.6.3.1concerning potential interference of AMPA by Fosetyl.*
**** See comment 3 on Phosphonic acid under* ***5.6.3.1***

Figure 4: Chromatograms of Ethephon, HEPA, Glyphosat, AMPA, Glufosinate, MPPA, N-Acetyl-AMPA, N-Acetyl-Glufosinate, Fosetyl-Al, Maleic hydrazide, Phosphonic acid, Perchlorate, Chlorate, Cyanuric acid and Bialaphos at 0.1 mg/L in MeOH (with 1% formic acid).



5.6.3.1. Hints on Method 1.3 and 1.4

1) *AMPA and Fosetyl share the mass-transition 110/81. Chromatographic separation is thus needed (typically the case).*

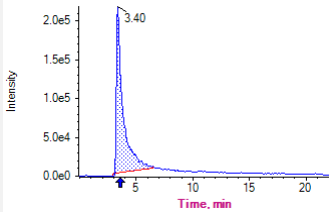
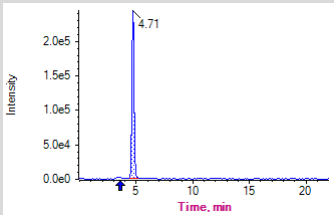
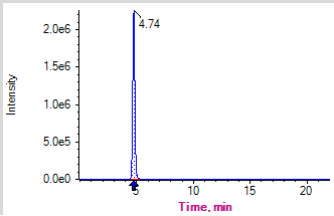
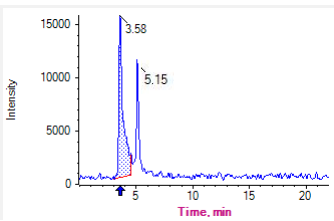
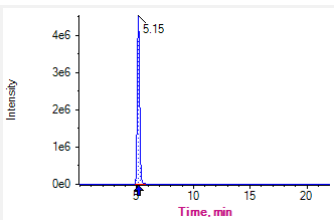
2) *In case of the determination of Fosetyl and Phosphonic acid on the Hypercarb-column, we refer to the patent of D. Rosati and C. Venet from Bayer CropScience (Patent-No. WO 2006079566 A1).*

3) *For the analysis of Perchlorate, Chlorate and Phosphonic acid it is recommended to dilute the QuPPe extracts 5 or 10-fold (see also comment 7)*

4) *Check the filters for any cross-contamination of Perchlorate and Chlorate. See comments under **2.6.** Cellulose mixed ester filters were found to be suitable for this application!*

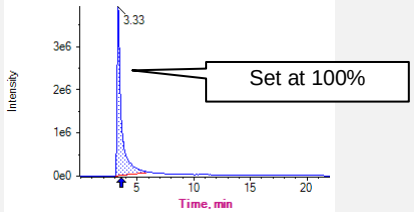
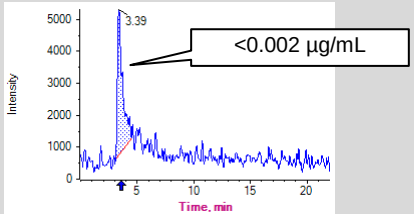
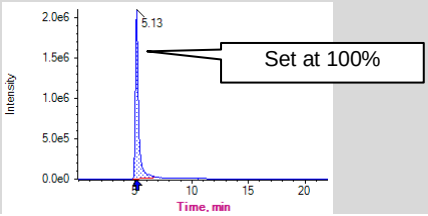
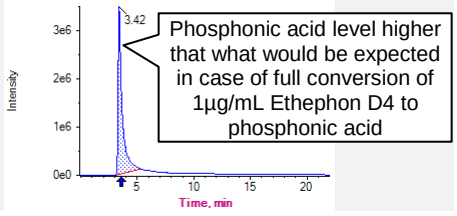
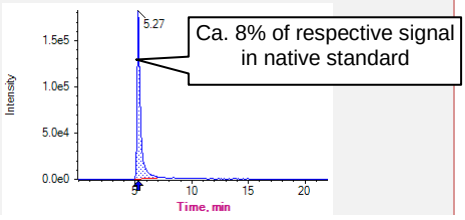
5) *Fosetyl and its D_5-analogon tend to degrade to Phosphonic acid both in solutions and via in-source fragmentation in LC-MS/MS. A good chromatographic separation between Fosetyl and Phosphonic acid is thus necessary (and is typically the case). Figure 5 shows an example of this in-source fragmentation. Upon injection of 0.1µg/mL Fosetyl a peak showed up on the mass traces of Phosphonic acid at the retention time of Fosetyl. The signal intensity of this peak corresponded to 0,04 µg/mL Phosphonic acid. When injecting Fosetyl-D_5 at 0.1 µg/kg the in-source fragmentation was less abundant (corresponding to ca. 0.001 µg/mL Phosphonic acid) but Phosphonic acid as impurity showed up at it proper retention time at a concentration corresponding to ca. 0.007 µg/mL. **To be on the safe side Fosetyl-ILIS should thus not be added to calibration solutions or samples or sample extracts intended to be used for the analysis of native phosphonic acid. Furthermore calibration solutions used for the analysis of phosphonic acid should better not contain any native Fosetyl**. (see also comment 6).*

Figure 5: Chromatograms of Phosphonic acid, Fosetyl and Fosetyl-D_5 (each at 1,0 µg/mL). In addition to the proper mass-traces of Fosetyl and Fosetyl-D_5 the mass trace of Phosphonic acid is also shown to demonstrate the occurrence of in-source fragmentation of Fosetyl and Fosetyl-D_5 towards Phosphonic acid as well as the presence of Phosphonic acid as an impurity of the Fosetyl-D_5 standard solution.

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		

6) *A degradation of Ethephon to Phosphonic acid in solution is also observed. Figure 6 shows a small peak of Phosphonic acid (corresponding to 0,002 µg/mL) showing up when Ethephon standard at 1 µg/ mL was injected. This contamination is considered negligible. However Figure 6 also shows chromatograms of an extensively degraded and thus* ***unsuitable Ethephon-D_4 standard*** *containing only ca. 0.08 µg/mL Ethephon-D_4 and ca. 0.8 µg/mL Phosphonic acid instead of the expected 1 µg/mL Phosphonic acid. The use of such an ILIS would contaminate the sample with Phosphonic acid leading to false positive results.* ***To be on the safe side Ethephon-ILIS should thus not be added to calibration solutions, samples or sample extracts intended for the analysis of native phosphonic acid. Furthermore calibration solutions used to analyse phosphonic acid should better not contain any native Ethephon****. (see also comment 5).*

Figure 6: Chromatograms of Phosphonic acid, Ethephon and an unsuitable Ethephon-D_4 standard (each at 1,0 µg/mL). Whereas Phosphonic acid is only contained at very low concentrations in the Ethephon standard the content of Phosphonic acid in the Ethephon-D4 standard is unacceptably high with the Phosphonic acid having been already been present at high contents in the purchased standard.

Calibration standard injected	Detected signals at mass trace of …	
	81/79 Phosphonic acid	143/107 Ethephon (top) or 114/82 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 !!)**		

7) *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 the in-source fragment of Phosphoric acid,*** *but as the latter is often present at much higher levels than Phosphonic acid its interference on this mass transition can still be significant, especially if these two elute in close vicinity (exemplarily shown at the chromatograms of blueberry extract in* ***Figure 7a****). The chromatographic separation of Phosphoric and Phosphonic acid considerably improves following dilution of the extracts typically allowing proper detection, identification and quantification of Phosphonic acid next to high levels of phosphoric acid. It is thus beneficial to inject smaller volumes of sample extract (e.g. 1-2 µL) or to* ***dilute QuPPe extracts 5-10-fold before injection****. Fortunately both, Phosphoric and Phosphonic acid have at least 1 proper mass-transition (97/63 and 81/79 respectively, exemplarily shown for the particular spiked blueberry extracts in* ***Figure 7a****), which in the case of Phosphonic acid can be used for quantitation and to improve identification certainty. The elution time and peak shape of the Phosphonic acid ILIS can also be used to distinguish it from Phosphoric acid and to avoid false positives. Using signals on the 81/63 mass trace it was calculated that 20 mg/kg Phosphoric acid would simulate 0.1 mg/kg Phosphonic acid if this mass transition was used for quantification. In an experiment using Differential Mobility Separation (DMS) technique (see* ***Figures 8a and 8b****)*

Figure 7: Chromatographic and mass-spectrometric separation of Phosphoric and Phosphonic acid.

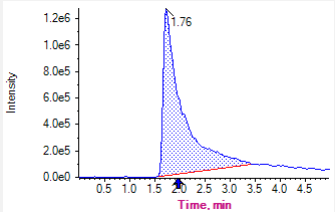
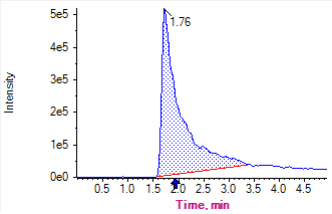
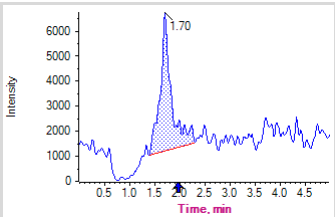
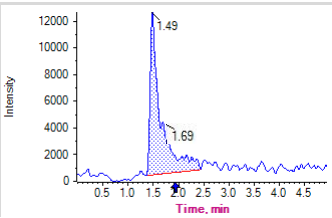
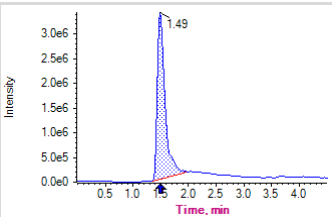
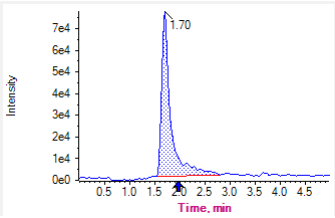
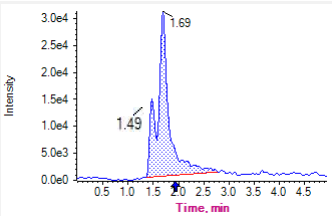
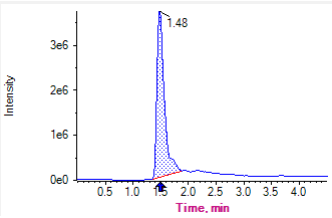
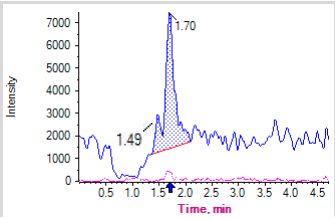
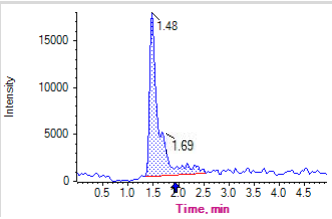
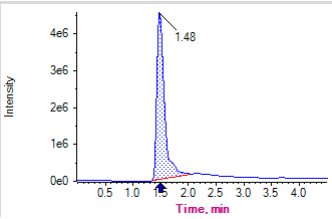
Injected sample	Detected signals at mass trace of …		
	81/79 ("unique" to Phosphonic acid)	81/63 (common to Phosphonic acid and Phosphoric acid)	97/63 ("unique to Phosphoric acid)
Phosphonic acid (2,5 µg/mL)			
Blueberry extract (containing ca. 20 mg/kg phosphoric acid)			
Blueberry extract spiked with Phosphonic acid (0,05 µg/mL)			
Blueberry extract spiked with Phosphoric acid (5 µg/mL)			

Figure 8a: Chromatographic and mass-spectrometric of Phosphoric and Phosphonic acid and indications that Phosphonic acid differs from the isobaric (both m/z 81) in-source fragment of Phosphoric acid (81/81 versus 81/63 ratio different and 81/79 transition is missing)

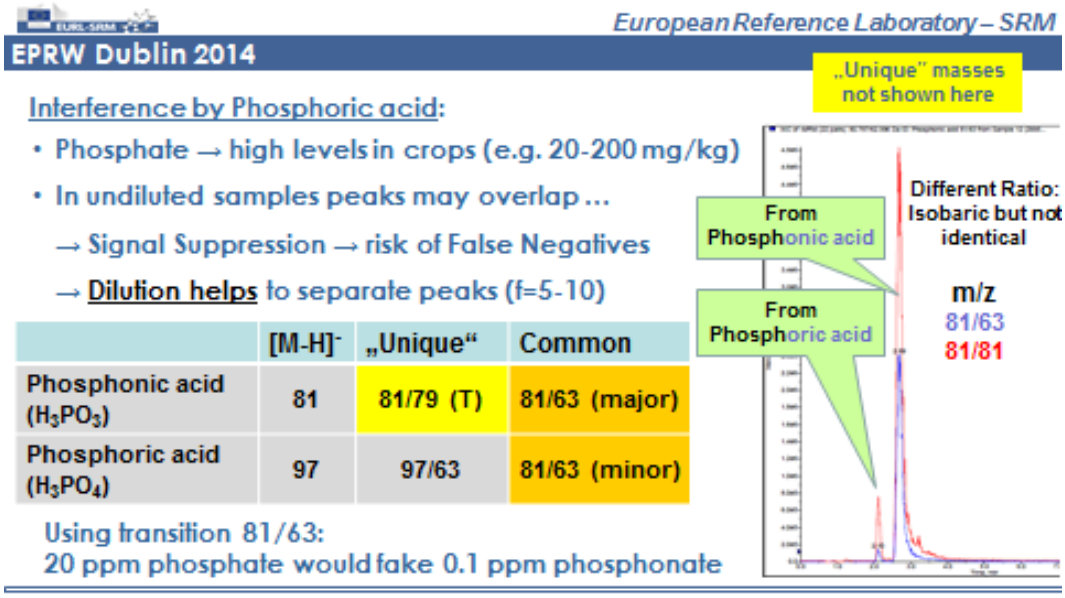
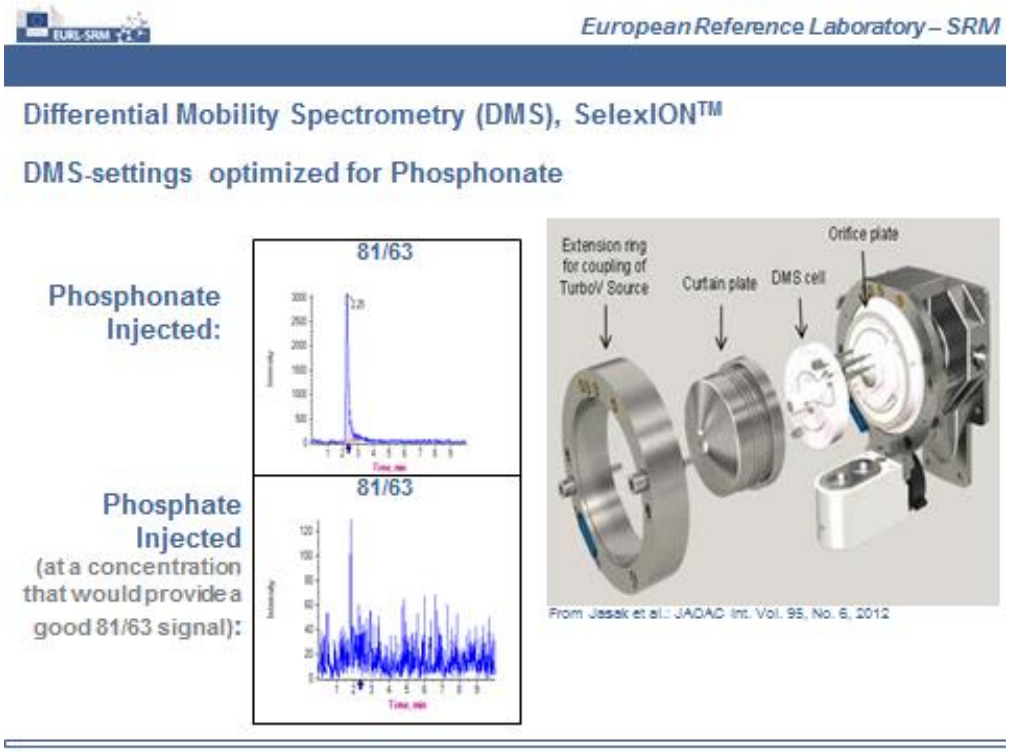
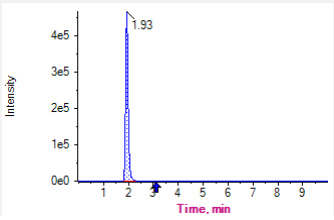
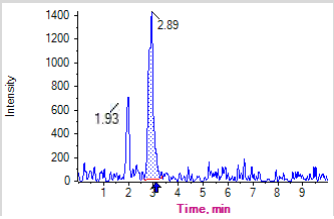
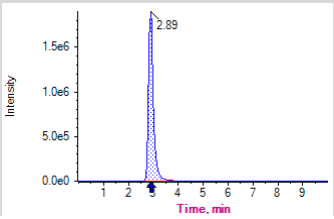
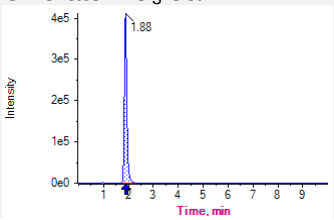
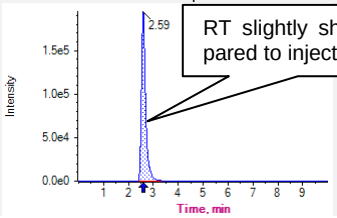


Figure 8b: DMI separation between Phosphonic acid (dihydrophosphonate, m/z=81) and the isobaric in-source fragment of Phosphoric acid (both m/z 81)



8) *Chlorate can be a minor contaminant of Perchlorate solutions and is also a minor in-source fragment of Perchlorate. In one experiment Perchlorate standard at 0.2 µg/mL was injected resulting in two peaks on the mass traces of Chlorate (see **Figure. 9**). One originating from Chlorate contained as impurity in the Perchlorate solution (at ca. 0.35%) and one originating from in-source fragmentation at the retention time of Perchlorate, corresponding to a Chlorate amount of 0,001 µg/mL.*

Figure 9: Chromatograms of Chlorate and Perchlorate at 0.2 µg/mL and of a mixture of Chlorate-$^{18}O_3$ and Perchlorate-$^{18}O_4$, containing ca. 0.2 µg/mL Chlorate $^{18}O_3$ and ca. 0.02 µg/mL Perchlorate-$^{18}O_4$.

Calibration standard injected	Detected signals at mass trace of …	
Chlorate 0.2 µg/mL	Chlorate 83/67 	
Perchlorate 0.2 µg/mL	Chlorate 83/67 	Perchlorate 99/83 
Chlorate-$^{18}O_3$ (ca. 0.18 µg/mL + Perchlorate-$^{18}O_4$ (ca. 0.02 µg/mL)	Chlorate-$^{18}O_3$ 89/71 	Perchlorate-$^{18}O_4$ 107/89  RT slightly shifted compared to injection above

9) ***Priming and reconditioning of column:*** *before first use the Hypercarb columns and pre-columns have to be thoroughly primed to cover certain active sites on the surface. Priming with solutions containing planar molecules such as chlorophyll and anthocyans accelerates the priming period. A recommendable procedure for priming is the injection of QuPPe extract of spinach (15-20 times, if possible inject 20 µL) or the injection grape skin extract[3] solution, prepared by dissolving 100 mg grape skin extract in 20 mL MeOH + 1% FA-H_2O 1:1. This masking of the active sites is temporary and the activity of the column gradually increases with the injection of solvent or diluted extracts. Following a sequence of injections with low or no matrix load will typically raise the need for intermediate conditioning with extracts to restore the column.* The impact of priming on the chromatographic properties of the column is exemplary shown *in **Figures 10, 11 and 12***.

[3] *available as Dietary Supplement*

Figure 10: 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.

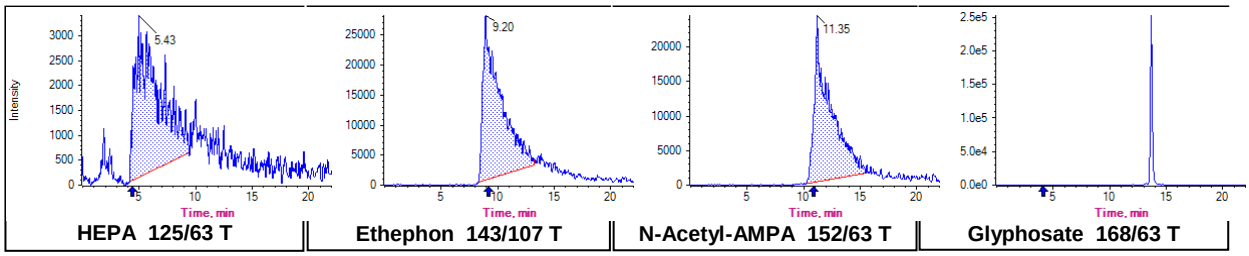


Figure 11: Chromatograms following priming with 10 injections (20µL) of Spinach QuPPe extracts.

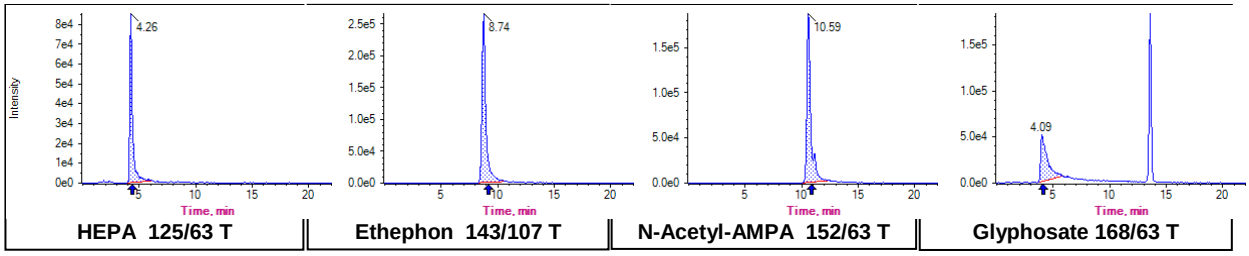
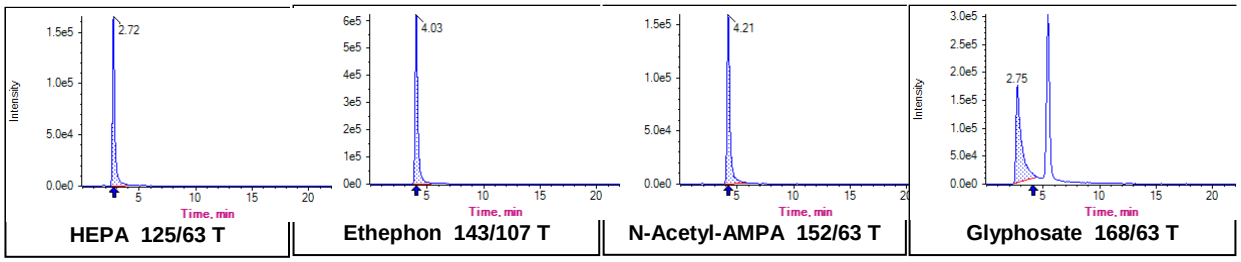


Figure 12: Chromatograms after additional injection of ca. 100 extracts of various fruit and vegetable QuPPe-extracts during normal routine use.



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

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

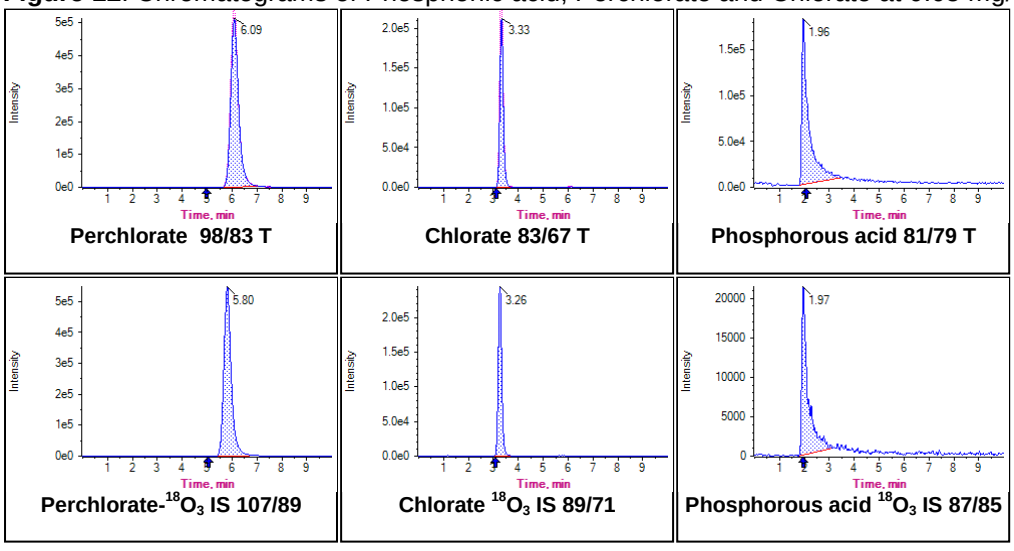
12) ***Pre-columns (guard columns)****: The pre-column should be exchanged as soon as a clear deterioration of the separation performance (worsening of peak-shape) is noticed. The pre-column of method 1.3 needs to be clearly less often exchanged compared to the pre-columns of methods 1.1 and 1.2. Any exchange of the pre-column requires priming as described above. For this the pre-column does not have to be attached to the column. Connecting several pre-columns in a row and priming them simultaneously is also an option.*

5.6.4.**Method 1.4 (for PerChloPhos)**

Table 7: Proposed LC-MS/MS conditions for Phosphonic acid (Fosetyl metabolite), Perchlorate and Chlorate.

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% MeOH		
Eluent B	1% Acetic acid in MeOH		
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		
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)**	
	Perchlorate Perchlorate-$^{18}O_4$ (ILIS)	99/83, 101/85 107/89	
	Chlorate Chlorate-$^{18}O_3$ (ILIS)	83/67, 85/69 89/71	
	Phosphonic acid Phosphonic acid $^{18}O_3$ (ILIS)	81/79, 81/63 87/85	

Figure 12: Chromatograms of Phosphonic acid, Perchlorate and Chlorate at 0.05 mg/L in MeOH.



5.6.5. **Method 2 (for Fosetyl and Maleic Hydrazide):**

Table 7: Proposed LC-MS/MS conditions for Fosetyl-Al, Maleic hydrazide and Perchlorate

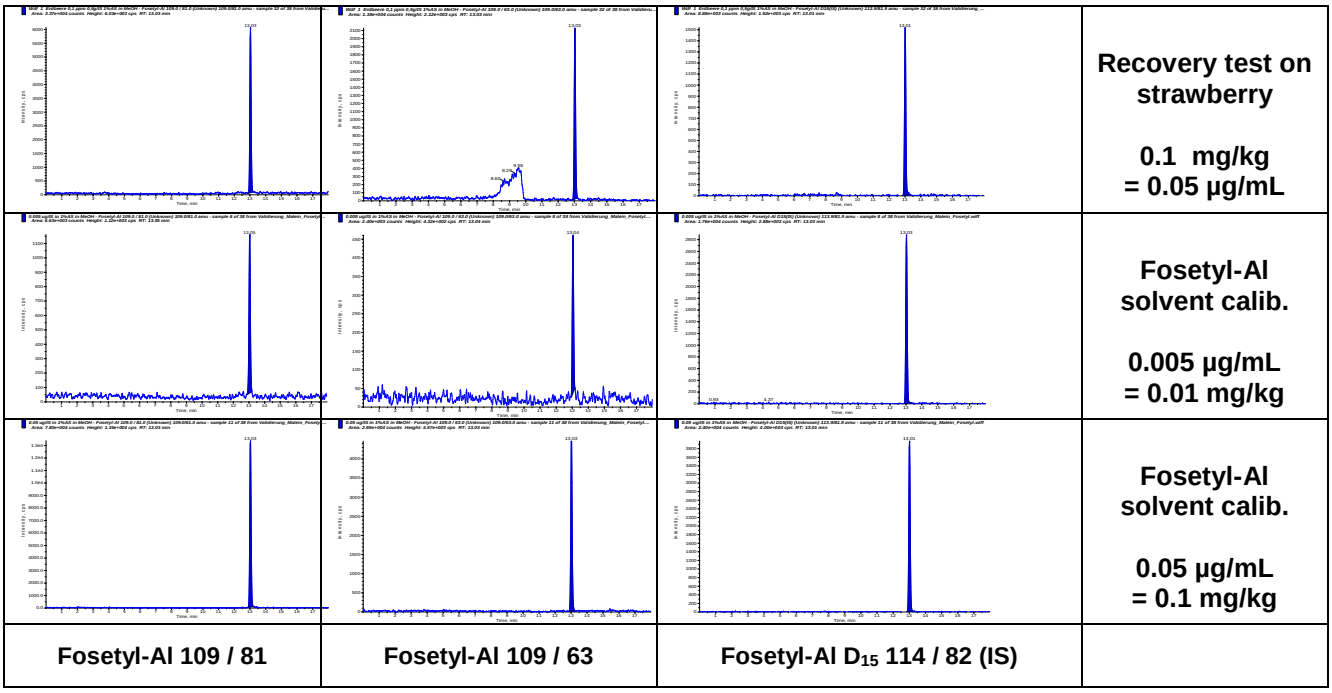
Instrument parameters	
Ionization mode	ESI neg
Column/temperature	Obelisc R 2.1 x 150 mm 5 µm 100 Å; (SIELC; OR-21.150.0510)
Pre-filters	e.g. Supelco column saver 2.0 µm Filter
Pre-column	Obelisc R 2.1 x 10mm 5 µm (SIELC; OR-21.G.0510)
Eluent A	50 mmol NH_4-formate in water + 0.1 % formic acid use brown glass bottles
Eluent B	Acetonitrile
Gradient	**%A** / **Flow [mL/min]** / **Time [min]** 3 / 0.3 / 0 10 / 0.3 / 6 70 / 0.5 / 15 70 / 0.5 / 18 3 / 0.5 / 18.1 3 / 0.5 / 28
Injection volume	5 µL
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion*, + one level at the reporting limit For Maleic hydrazide (MH) an additional level at 1 or 2 µg/mL may be useful as well, due to high residue levels; consider that MH is typically only relevant for potatoes and crops of the leek family (onions etc.)
Acquired mass transitions	**Compound** / **Mass Transitions (m/z)**
	Fosetyl-Al Fosetyl-Al-D_{15} (ILIS) / 109/81, 109/63 (detected as fosetyl) 114/82 (detected as fosetyl-D_5)
	Maleic hydrazide Maleic hydrazide-D_2 (ILIS) / 111/82, 111/42, 111/55, 111/83 113/42
	Perchlorate Perchlorate-$^{18}O_4$ (ILIS) / 99/83, 101/85 107/89

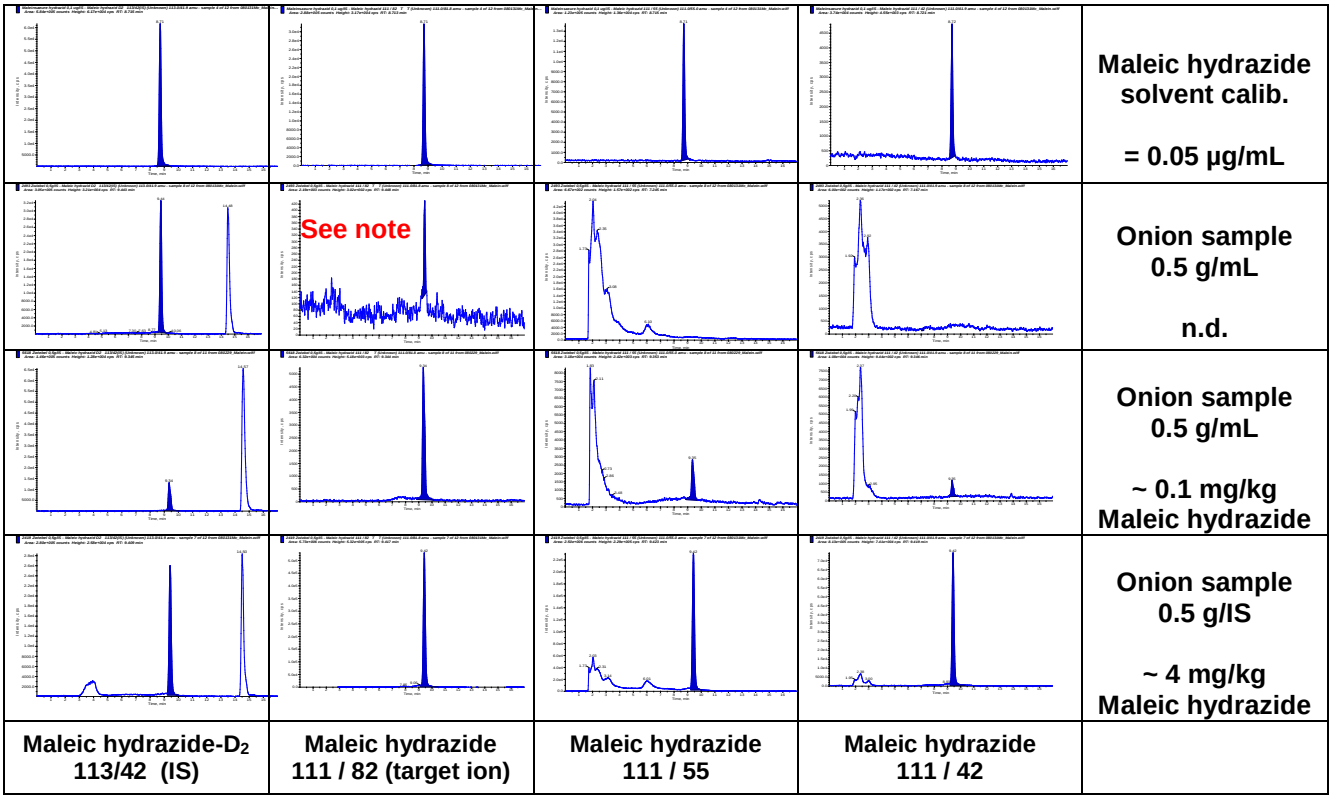
** One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also* **Table 1***).*

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

For Perchlorate better run Method 1.3 or 1.4

Figure 13: Typical chromatograms of Fosetyl-Al and Maleic hydrazide in various types of extracts and in pure solvent





5.6.6. **Method 3 (for Amitrole & Co)**

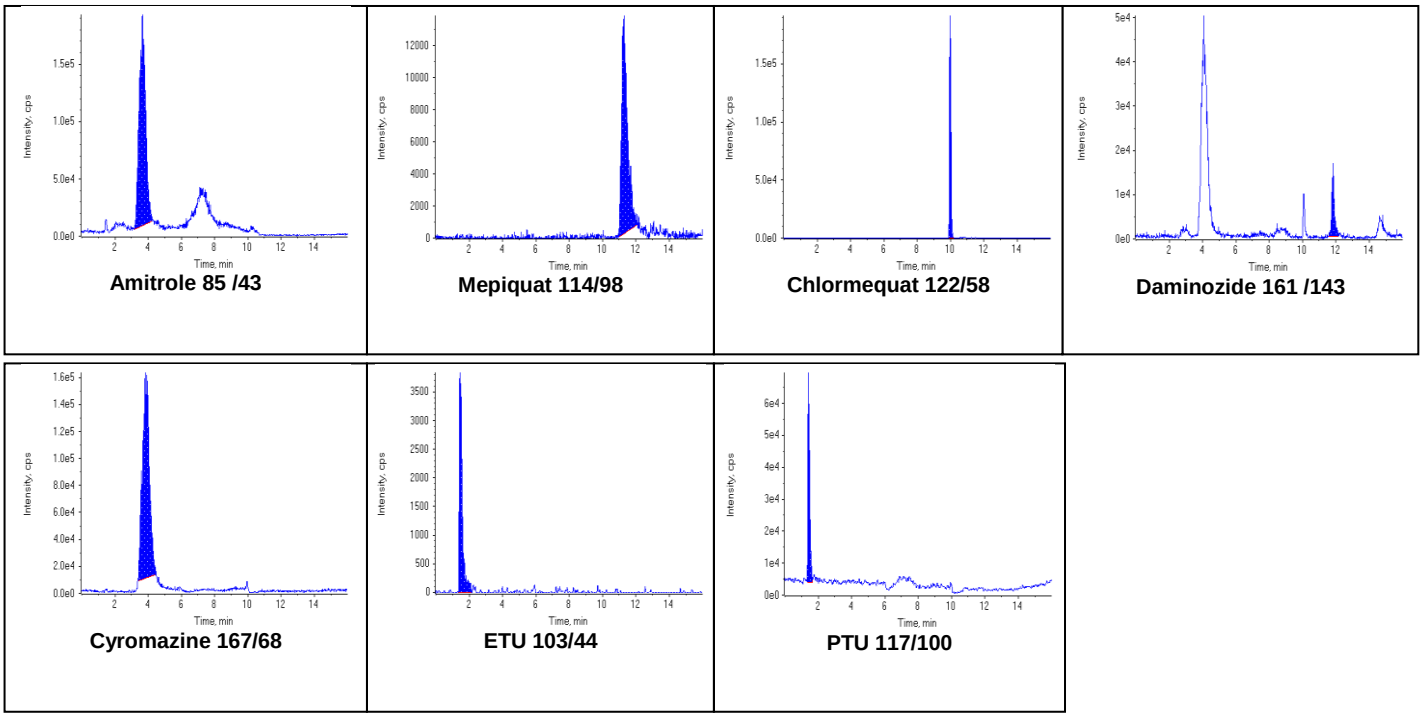
Table 8: Proposed LC-MS/MS conditions for Amitrole, Chlormequat, Mepiquat, Daminozide, ETU, PTU, Trimesium, Difenzoquat and Cyromazine.

Instrument parameters	**Conditions**		
Ionisation mode	ESI pos		
Column/temperature	Obelisc R 2.1 x 150 mm 5 µm 100 Å (SIELC; OR-21.150.0510); 40°C		
Pre-column	Obelisc R 2.1 x 10 mm 5 µm (SIELC; OR-21.G.0510)		
Pre-filters	e.g. Supelco column saver 2.0 µm Filter		
Eluent A	5 mmol NH_4-formate in water Use brown glass bottles		
Eluent B	5 mmol NH_4-formate Acetonitrile/Water 95 :5 (v/v)		
Gradient	**%A**	**Flow [mL/min]**	**Time [min]**
	2	0.4	0
	2	0.4	2.5
	80	0.4	5
	80	0.4	11
	2	0.4	11.1
	2	0.4	18
Injection volume	5 µL		
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion* + one level at the reporting limit		
Acquired mass transitions	**Compound**	**Mass Transitions (m/z)**	
	Amitrole: Amitrole-^{15}N (ILIS): Amitrole-$^{15}N_2$,$^{13}C_2$ (ILIS):	85/43, 85/57, 85/58 86/43 89/44	
	Chlormequat: Chlormequat-D_4 (ILIS):	122/58, 122/63, 124/58 126/58	
	Mepiquat: Mepiquat-D_3 (ILIS):	114/98, 114/58 117/101	
	Daminozide: Daminozide-$^{13}C_4$ (ILIS): Daminozide-D_6 (ILIS):	161/143, 161/61, 161/101 , 161/115, 161/44 165/147 167/149	
	Cyromazine: Cyromazine-D_4 (ILIS):	167/68, 167/125, 167/85, 167/108, 171/86	
	ETU (Ethylenethiourea): ETU-D_4 (ILIS):	103/44, 103/60, 103/86 107/48	
	PTU (4-Methyl-2-imidazolidinethione)**: PTU-D6 (N,N′-Propylenethiourea-D_6):	117/100, 117/58, 117/60, 117/72 *(123/64)*	
	Trimethylsulfonium: Trimethylsulfonium-D_9 (ILIS):	77/62, 77/47 86/68	
	Difenzoquat: No ILIS currently available:	249/77, 249/130, 249/193 -	

** One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also* **Table 1***).*
****** The acronym PTU, commonly used for the propineb degradant 4-Methyl-2-imidazolidinethione (N,N'-iso-propylenethiourea), is also used for N,N'-propylenethiourea (= N,N'-Trimethylenethiourea). The IS tested corresponds to N,N'-propylenethiourea D6.*

Note: *For Paraquat, Diquat,* Trimethylsulfonium *and N,N-Dimethylhydrazine better run Method 4 (**5.6.7**)*

Figure 14: Typical chromatograms of Amitrole, Chlormequat, Mepiquat, Daminozide, ETU, PTU and Cyromazine in apple extract at 0.01 mg/kg



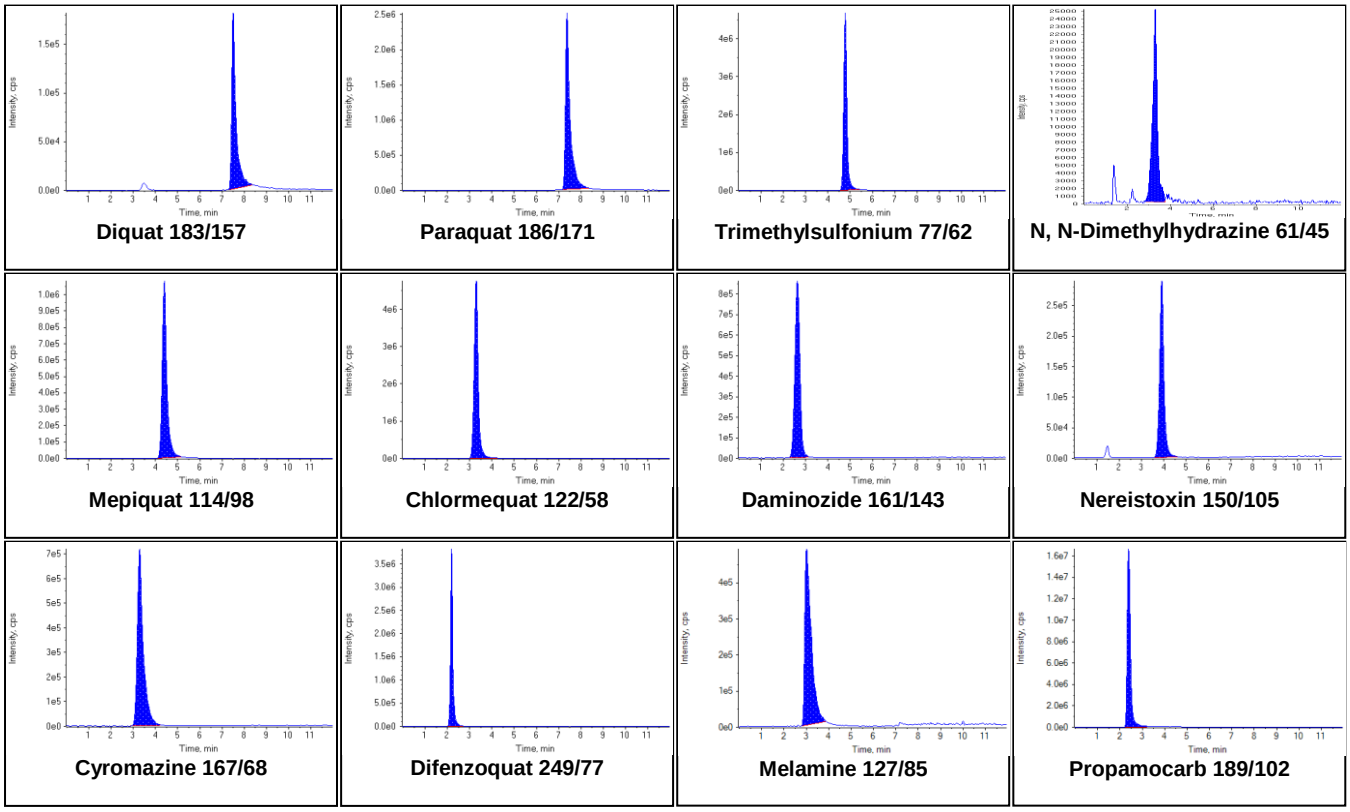
5.6.7. **Method 4.1 (for "Quats & Co")**

Table 9: Proposed LC-MS/MS conditions Diquat, Paraquat, Chlormequat, Mepiquat, Daminozide N,N-Dimethylhydrazine, Cyromazine, Trimethylsulfonium, Nereistoxin, Difenzoquat, Melamine and Propamocarb.

Instrument parameters	**Conditions**	
Ionisation mode	ESI pos	
Column/temperature	Obelisc R 2.1 x 150 mm 5 µm 100 Å (SIELC; OR-21.150.0510); 40°C	
Pre-filters	e.g. Supelco column saver 2.0 µm Filter	
Pre-column	Obelisc R 2.1 x 10 mm 5 µm (SIELC; OR-21.G.0510)	
Eluent A	20 mmol NH_4-formate in water (adjust to pH 3 with formic acid), for this mix 1.8 mL formic acid (**3.4**) with 500 mL 20 mmol NH_4-formate in water **Use brown glass bottles!**	
Eluent B	Acetonitrile	
Gradient	**%A** / **Flow [mL/min]** / **Time [min]** 20 / 0.4 / 0 80 / 0.4 / 4 80 / 0.4 / 12 20 / 0.4 / 12.1 20 / 0.4 / 20	
Injection volume	10 µL	
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion* + one level at the reporting limit (**use plastic vials if Paraquat and Diquat are within your scope**!)	
Acquired mass transitions	**Compound**	**Mass Transitions (m/z)**
	Diquat** : Diquat-D_4 (ILIS) :	184/128, 183/157, 184/156 188/160
	Paraquat** : Paraquat-D_6 (ILIS) :	186/171, 171/77, 171/155 192/174
	Chlormequat: Chlormequat-D_4 (ILIS):	122/58, 122/63, 124/58 126/58
	Mepiquat: Mepiquat-D_3 (ILIS):	114/98, 114/58 117/101
	Daminozide: Daminozide-$^{13}C_4$ (ILIS): Daminozide-D_6 (ILIS):	161/143, 161/61, 161/101 , 161/115, 161/44 165/147 167/149
	N,N-Dimethylhydrazine: N,N-Dimethylhydrazine-D_6 (ILIS):	61/44, 61/45 67/49
	Cyromazine: Cyromazine-D_4 (ILIS):	167/68, 167/125, 167/85, 167/108, 171/86
	Trimethylsulfonium: Trimethylsulfonium-D_9 (ILIS):	77/62, 77/47 86/68
	Nereistoxin: Nereistoxin-D_6 (ILIS):	150/105, 150/61, 150/71 156/105
	Difenzoquat: No ILIS currently available:	249/77, 249/130, 249/193 -
	Melamine: Melamine-$^{15}N_3$ (ILIS):	127/85, 127/68, (127/60) 130/87
	Propamocarb: Propamocarb-D_7 (ILIS):	189/144, 189/102, 189/74 196/103

** One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also* **Table 1***).*
*** **Diquat and Paraquat require special extraction conditions** (see **5.2.5**)*
***Note:** For Morpholin, Diethanolamine (DEA) and Triethanolamine (TEA) better run Method 7 (5.6.9). As DEA converts to Morpholine in the ion source, chromatographic separation of these two is paramount. With Method 4.1 these two peaks do not sufficiently separate.*

Figure 15: Typical chromatograms of Diquat, Paraquat, Chlormequat, Mepiquat, Daminozide, N,N-Dimethylhydrazine, Trimethylsulfonium, Cyromazine, Nereistoxin, Difenzoquat, Melamine and Propamocarb in apple extract at 0.1 mg/kg



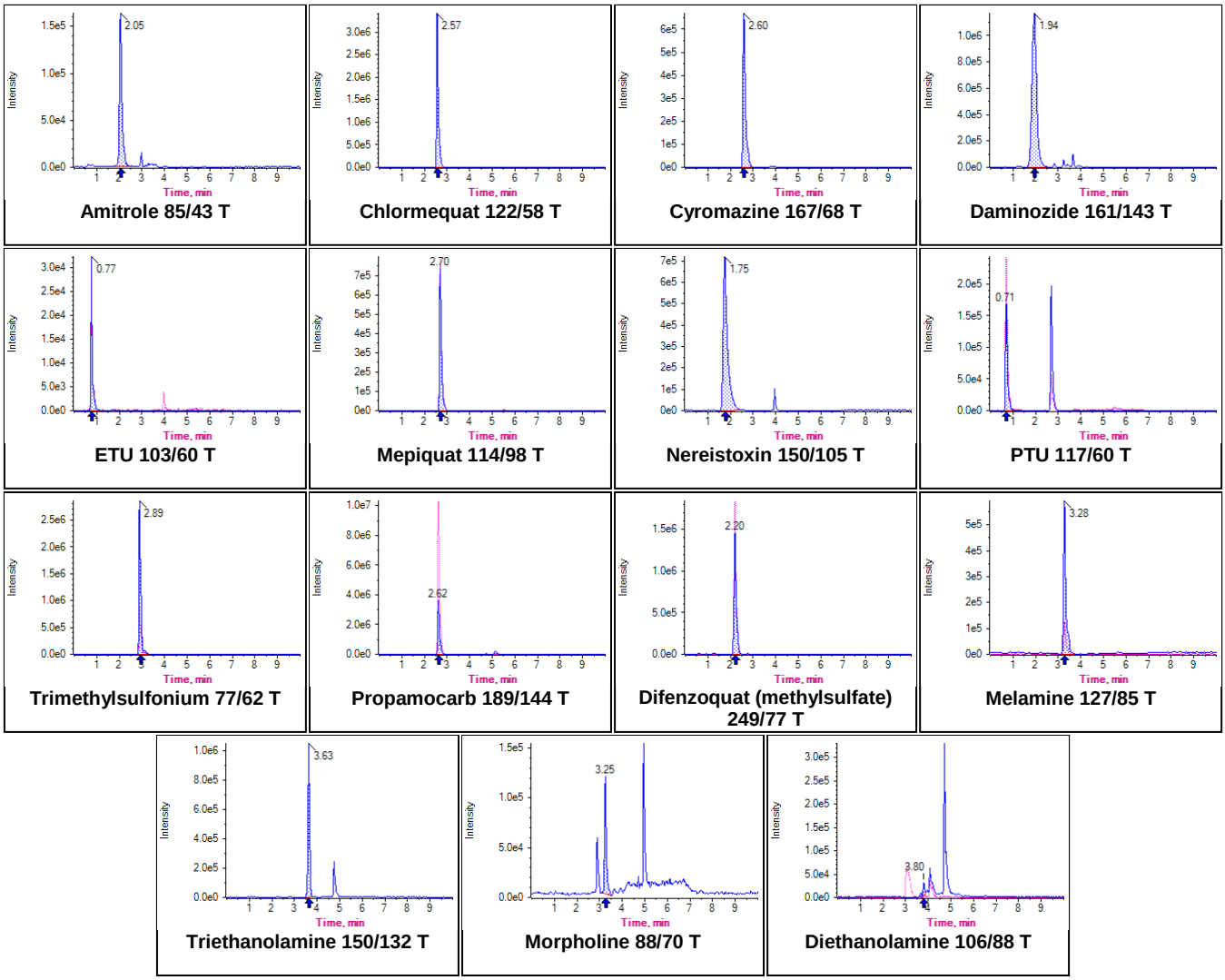
5.6.8. Method 4.2 (for Amide)

Table 10: Proposed LC-MS/MS conditions for Amitrole, ETU, Chlormequat, Mepiquat, Daminozide, PTU, Cyromazine, Trimesium, Nereistoxin, Difenzoquat, Melamine, Propamocarb, Morpholine, Diethanolamine, Triethanolamine.

Instrument parameters	**Conditions**	
Ionisation mode	ESI pos.	
Column/temperature	BEH Amide 2.1 x 100mm 1.7 µm (P/N: 186004801); 40°C	
Pre-filters	e.g. Supelco column saver 2.0 µm Filter	
Pre-column	BEH Amide 1.7 µm (P/N: 186004799)	
Eluent A	50 mmol NH_4-formate in water (adjust to pH 3 with formic acid) **Use brown glass !**	
Eluent B	Acetonitrile	
Gradient	**%A** / **Flow [mL/min]** / **Time [min]**: 3 / 0.5 / 0; 3 / 0.5 / 0.5; 30 / 0.5 / 4.0; 60 / 0.5 / 5.0; 60 / 0.5 / 6.0; 3 / 0.5 / 6.1; 3 / 0.5 / 10	
Injection volume	2 µL	
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion* + one level at the reporting limit	
Acquired mass transitions	**Compound**	**Mass Transitions (m/z)**
	Amitrole : Amitrole-^{15}N (ILIS): Amitrole-$^{15}N_2$ $^{13}C_2$ (ILIS):	85/43, 85/57, 85/58 86/43 89/44
	ETU (Ethylenethiourea): ETU-D_4 (IS):	103/60, 103/44, 103/86 107/48
	Chlormequat: Chlormequat-D_4 (ILIS):	122/58, 124/58, 122/63 126/58
	Mepiquat: Mepiquat-D_3 (ILIS):	114/98, 114/58 117/101
	Daminozide: Daminozide-$^{13}C_4$ (ILIS) ; Daminozide-D_6 (ILIS):	161/143, 161/61, 161/101 , 161/115, 161/44 165/147 ; 167/149
	PTU (4-Methyl-2-imidazolidinethione)** : PTU-D6 (N,N'-Propylenethiourea-D_6)	117/100, 117/58, 117/60, 117/72 *(123/64)*
	Cyromazine: Cyromazine-D_4 (ILIS):	167/68, 167/125, 167/85, 167/108, 171/86
	Trimethylsulfonium: Trimethylsulfonium-D_9 (ILIS):	77/62, 77/47 86/68
	Nereistoxin: Nereistoxin-D_6 (ILIS):	150/105, 150/61, 150/71 156/105
	Difenzoquat: No ILIS currently available:	249/130, 249/77, 249/193, -
	Melamine: Melamine-$^{15}N_3$ (ILIS):	127/85, 127/68, (127/60) 130/87
	Propamocarb: Propamocarb-D_7 (ILIS):	189/144, 189/74, 189/102 196/103
	Morpholine***: Morpholine-D_8 (ILIS):	88/70, 88/45, 88/44 96/78
	Diethanolamine*** (DEA): Diethanolamine-D_4 (ILIS):	106/88, 106/70, 106/45 110/92
	Triethanolamine*** (TEA): Triethanolamine-D_{12} (ILIS):	150/132, 150/70, 150/88 162/144

** One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also* **Table 1***).*
***The acronym PTU, commonly used for the propineb degradant* 4-Methyl-2-imidazolidinethione *(N,N'-iso-propylenethiourea), is also used for N,N'-propylenethiourea (= N,N'-Trimethylenethiourea). The IS tested corresponds to N,N'-propylenethiourea D_6.*
****For Morpholin, Diethanolamine and Triethanolamine better run Method 7 (5.6.9) as these compounds seem to be strongly suppressed by matrix using these LC-conditions.*

Figure 16: Typical chromatograms of Amitrole, ETU, Chlormequat, Mepiquat, Daminozide, PTU, Cyromazine, Trimethylsulfonium, Nereistoxin, Difenzoquat, Melamine, Propamocarb, Triethanolamine, Morpholine and Diethanolamine in tomato extracts spiked at 0.05 mg/kg.



5.6.9. **Method 5 (alternative method for Chlormequat and Mepiquat)**

Table 11: 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: Chlormequat-D_4 (ILIS):		122/58, 122/63, 124/58 126/58
	Mepiquat : Mepiquat-D_3 (ILIS):		114/98, 114/58 117/101
	Difenzoquat: No IS currently available		249/77, 249/130, 249/193 -
	ETU (Ethylenethiourea) : ETU-D_4 (ILIS):		103/44, 103/60, 103/86 107/48
	PTU (4-Methyl-2-imidazolidinethione)** : PTU-D6 (N,N′-Propylenethiourea-D_6)		117/100, 117/58, 117/60, 117/72 *(123/64)*

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

***The acronym PTU, commonly used for the propineb degradant 4-Methyl-2-imidazolidinethione (N,N'-iso-propylenethiourea), is also used for N,N'-propylenethiourea (= N,N'-Trimethylenethiourea). The IS tested corresponds to N,N'-propylenethiourea D_6.*

For more information on method 5 please refer to the following document within the EURL homepage: http://www.crl-pesticides.eu/library/docs/srm/meth_ChlormequatMepiquat_CrlSrm.pdf

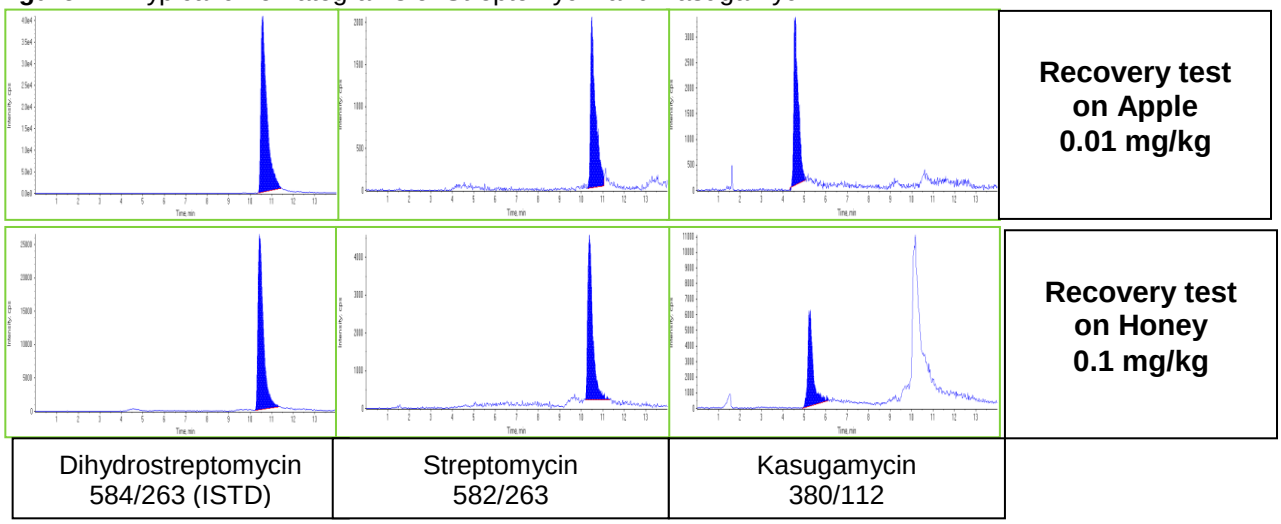
5.6.10. **Method 6 (for Streptomycin and Kasugamycin)**

Table 12: Proposed LC-MS/MS conditions Streptomycin and Kasugamycin

Instrument parameters	**Conditions**
Ionisation mode	ESI pos
Column	Obelisc R 2.1 x 150 mm 5µm 100 Å (SIELC; OR-21.150.0510); 40°C
Pre-filters	e.g. Supelco column saver 2.0 µm Filter
Pre-column	Obelisc R 2.1 x 10 mm 5 µm (SIELC; OR-21.G.0510)
Eluent A	0.1% formic acid in water
Eluent B	0.1% formic acid in acetonitrile
Gradient	**%A** / **Flow [mL/min]** / **Time [min]** 20 / 0.3 / 0 20 / 0.3 / 5 80 / 0.5 / 10 80 / 0.5 / 14 20 / 0.3 / 14.1 20 / 0.3 / 22
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 Dihydrostreptomycin (IS) / 582/263, 582/246, 582/ 221 584/263 Kasugamycin No IS currently available / 380/112, 380/200 -

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

Figure 17: Typical chromatograms of Streptomycin and Kasugamycin



5.6.11. **Method 7 (for Morpholine, Diethanolamine and Triethanolamine)**

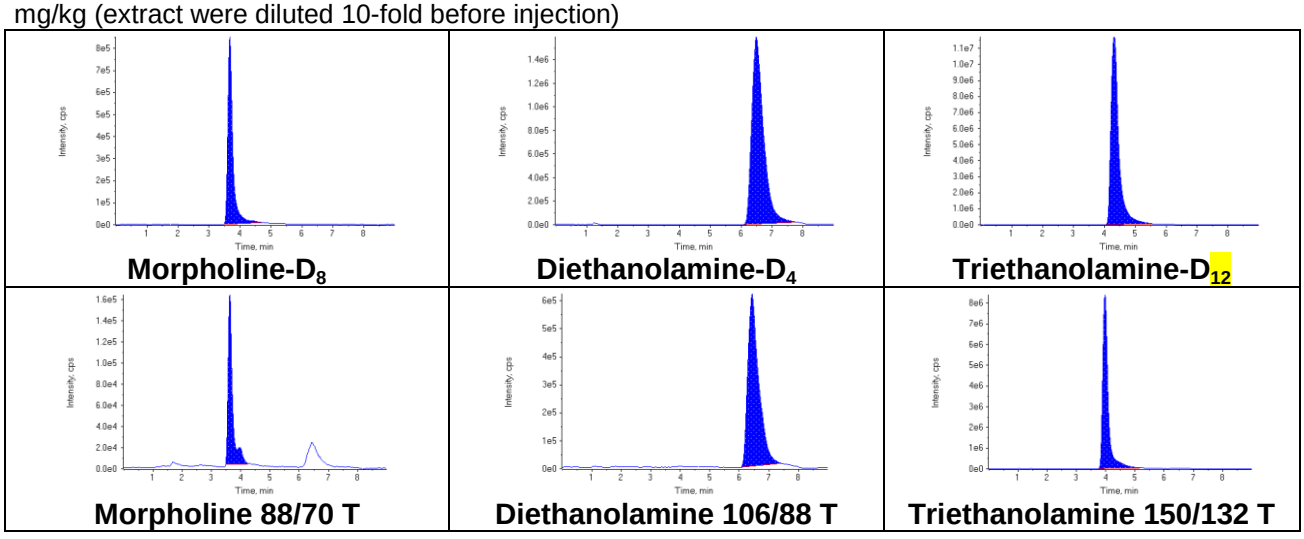
Table 13: Proposed LC-MS/MS conditions Morpholine, Diethanolamine and Triethanolamine

Instrument parameters	**Conditions**
Ionisation mode	ESI pos
Column	Dionex Acclaim Trinity P1 2.1 x 100 mm (3 µm) (P/N 071389); 40°C
Pre-filters	e.g. Supelco column saver 2.0 µm Filter
Pre-column	Dionex Acclaim Trinity P1 2.1 x 10 mm (3 µm) (P/N 071391)
Eluent A	50 mmol NH_4-formate in water (adjust to pH 3 with formic acid) **Use brown glass bottles!**
Eluent B	Acetonitrile
Gradient	**%A** / **Flow [mL/min]** / **Time [min]** 10 / 0.4 / 0 10 / 0.4 / 10
Injection volume	5 µL
Calibration standards and levels	e.g. 0.05 or 0.1 µg/IS portion+ one level at the reporting limit
Acquired mass transitions	**Compound** / **Mass Transitions (m/z)** Morpholine / 88/70, 88/45, 88/44 Morpholine-D_8 (IS) / 96/78 Diethanolamine (DEA) / 106/88, 106/70, 106/45 Diethanolamine-D_4 (IS) / 110/92 Triethanolamine (TEA) / 150/132, 150/70, 150/88 Triethanolamine-D_{12} (IS) / 162/144

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

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

Figure 18: Typical chromatograms of Morpholine, Diethanolamine and Triethanolamine in apple extracts at 0.05 mg/kg (extract were diluted 10-fold before injection)



5.6.12. Method 8 (for Triazole derivative metabolites (TDMs))

Table 13: 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% MeOH
Eluent B	1% Acetic acid in MeOH

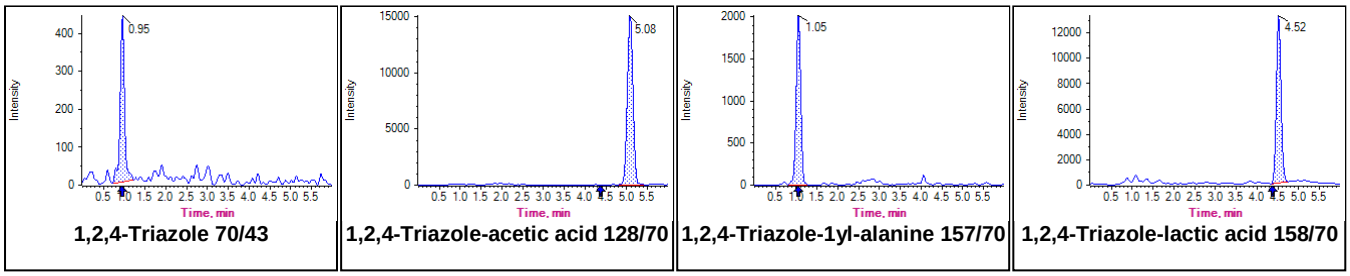
Gradient

%A	**Flow [mL/min]**	**Time [min]**
100	0.6	0
10	0.6	5
100	0.6	5.1
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-Conditions*** using a SelexION Q-Trap® 5500 DMS temperature: high**
			COV (V) / **SV (V)**
	1,2,4-Triazole*** 1,2,4-Triazole (IS)	70/43 75/46	-13 /-13.5 -5 /-13 / 3000 /3000 2000 /3000
	Triazole-alanine Triazole-alanine-$^{13}C_2$,$^{15}N_3$ (IS)	157/70 162/75	2 /0.5 0 /3.0 / 3500 /3000 2500 /3000
	Triazole-acetic acid Triazole-acetic acid-$^{13}C_2$,$^{15}N_3$ (IS)	128/70 133/75	-1.5 /-3.0 -1.5 /-3.0 / 2000 /3000 2000 /3000
	Triazole-lactic acid Triazole-lactic acid-$^{13}C_2$,$^{15}N_3$ (IS)	158/70 163/75	-1 /-0.5 -0.5 /-0.5 / 2500 /3000 2500 /3000
	1,2,3-Triazole** No IS currently available	70/43 -	-12 /-12.5 - / 3000 /3000 -

** One IS portion is the absolute IS-mass contained in the prepared calibration standard solution (see also* **Table 1***).*
*** DMS condition differ to some extent from instrument to instrument*
**** 1,2,4-Triazole and 1,2,3-Triazole are used as nitrification inhibitors in fertilizers*

Figure 18: Typical chromatograms of TDMs in avocado extracts spiked at 0.01 mg/kg.



5.7. Calibration and Calculations

5.7.1. **Using IS**

5.7.1.1. Where IS is added to the sample before any aliquotation:

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

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

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

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

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

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

5.7.1.2. 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 **Table18,** the total volume can be assumed to be exactly 20 mL. In this case 1 mL sample extract will correspond to 1/20th of the test portion (m_a). The mass of the IS to be added to an aliquot ($m_{IS}^{aliquot}$) should be scaled according to the aliquot volume used ($V_{aliquot}$) with the IS mass ratio ($m_{IS}^{aliquot}$ / $m_{IS}^{cal\ mix}$) being important for the calculation. Reasonably (but not necessarily) $m_{IS}^{aliquot}$ should be derived using the following formula $m_{IS}^{aliquot}$ = m_{IS}^{sample} x $V_{aliquot}$/20 , with m_{IS}^{sample} being the IS mass that would have been added to the entire sample portion according to **5.2.2** and **Table 18**.

Following the above, the mass fraction (w_R) of a given pesticide in a given sample can be calculated as follows using the respective peak ratio of pesticide and internal standard obtained from the sample extract

($PR^{sample} = A_{pest}^{sample} / A_{IS}^{sample}$), the correction factor ($m_{IS}^{aliquot} / m_{IS}^{cal\ mix}$) as well as the weight of the sample equivalents in the aliquot ($m_{aliquot} = m_a \times V_{aliquot}/20$).

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

Variables used

Mass of pesticide in calibration mixture	$m_{pest}^{cal\ mix}$	µg
Mass of pesticide in final extract	m_{pest}^{sample}	µg
Mass of internal standard in calibration mixture	$m_{ISTD}^{cal\ mix}$	µg
Mass of internal standard added to test portion (sample)	m_{ISTD}^{sample}	µg
Mass of internal standard added to aliquot of sample extract	$m_{ISTD}^{aliquot}$	µg
Volume of sample extract aliquot used (**5.7.1.2** 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.

5.7.2.1. Calculations when employing matrix-matched calibration without IS

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

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

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

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

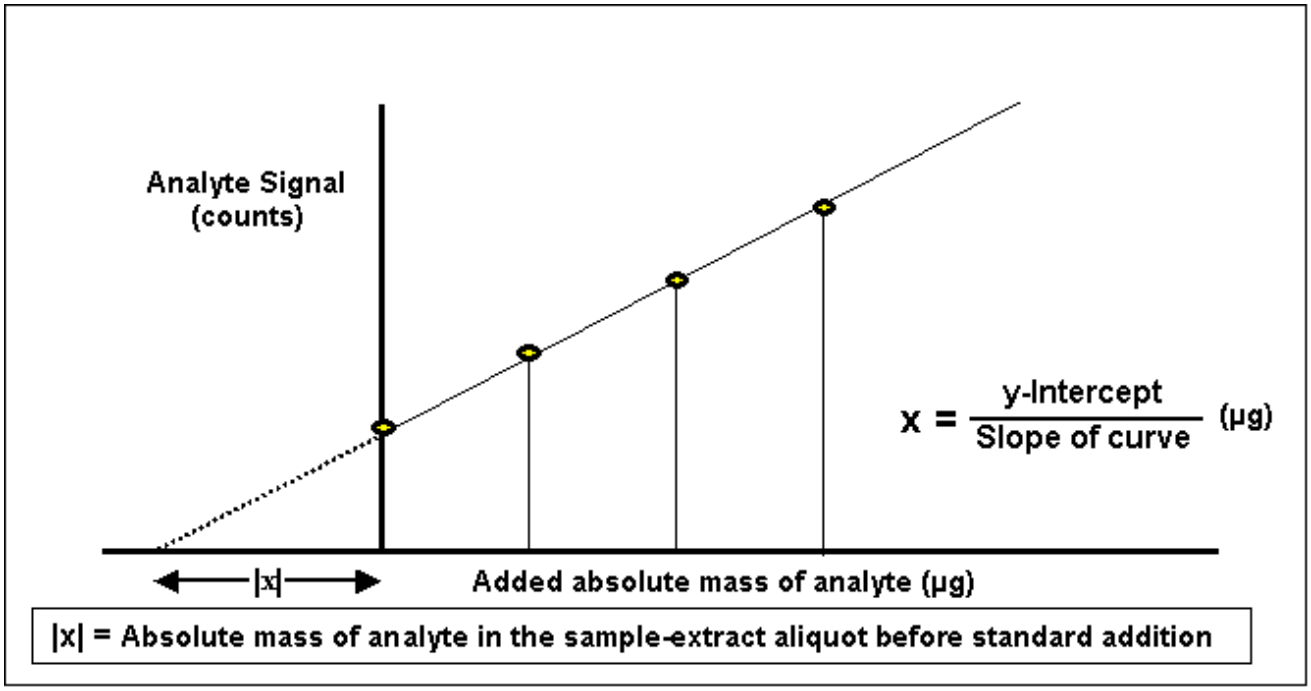
where V_{end} is the total volume of the sample extract (20 mL).

All other variables are listed in **5.7.1.2.**

5.7.2.2. Calculations when employing the standard additions approach

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

Figure 20: Internal calibration using the procedure of standard additions, schematically



Key:

Y Peak area of analyte

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

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

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

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

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

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. Performance Data

Exemplary results of recovery experiments (n=5) using matrix matched calibrations (for more information see method validation database at **www.crl-pesticides-datapool**).

Table 14: Overview of approximate limits of quantification (LOQs)*

Method	Pesticide	Most fruits and Vegetables (tested on Tomato, Cucumber, Apples) [mg/kg]	Citrus (tested on Orange) [mg/kg]	Cereals (tested on Barley) [mg/kg]
M1.1/M1.2/M1.3	Ethephon	0.01/0.01/0.01	0.01/0.01/0.01	0.02/0.02/0.02
M1.1/M1.2/M1.3	HEPA	0.01/0.01/0.02	0.01/0.01/0.02	0.02/0.02/0.02
M1.1/M1.2/M1.3	Glyphosate	0.01/0.01/0.02	0.02/0.01/0.02	0.02/0.02/0.02
M1.1/M1.2/M1.3	AMPA	0.01/0.01/0.02	0.02/0.01/0.02	0.02/0.02/0.02
M1.1/M1.2/M1.3	Glufosinate	0.01/0.01/0.02	0.02/0.02/0.02	0.02/0.02/0.02
M1.1/M1.2/M1.3	MPPA	0.01/0.01/0.01	0.02/0.02/0.01	0.02/0.02/0.02
M1.1/M1.2/M1.3	N-Acetyl-glufosinate	0.02/0.02/0.01	0.02/0.02/0.01	0.02/0.02/0.02
M1.2/M1.3	N-Acetyl-AMPA	0.01/0.01	0.01/0.01	0.02/0.02
M1.3 / M2	Perchlorate	0.01/0.01	0.01/0.01	0.01/0.01
M1.2 / M1.3	Phosphonic acid**	0.1/0.1	0.1/0.1	0.1/0.1
M1.3	Chlorate**	0.01	n.a.	n.a.
M1.1/ M1.3/M2	Fosetyl	0.1 / 0.005/0.005	n.a. / 0.005/0.005	n.a. / 0.005/0.005
M2 / M1.3	Maleic hydrazide	0.01	0.01	0.02
M3	Amitrole	0.01	0.01	0.02
M3 / M5	ETU	0.01 / 0.01	0.02 / n.a.	0.02 / n.a.
M3 / M5	PTU	0.01 / 0.01	0.02 / n.a.	0.02 / n.a.
M3 / M4 / M5	Chlormequat	0.005 / 0.005	0.005 / 0.005	0.01 / 0.01
M3 / M4 / M5	Mepiquat	0.005 / 0.01	0.005 / 0.01	0.001 / 0.02
M3 / M4	Cyromazine	0.01 / 0.01	0.01 / 0.01	0.02 / 0.02
M3 / M4	Daminozide	0.01 / 0.02	0.01 / 0.02	0.02 / 0.04
M3 / M4	Trimethylsulfonium-Cation	0.01 / 0.005	0.01 / 0.005	0.02 / 0.01
M3 / M4	Nereistoxin	0.01 / 0.01	n.a. / n.a.	n.a. / n.a.
M4	N,N-Dimethylhydrazine	0.005	0.005	0.01
M4	Diquat	0.005	0.005	0.005

M4	Paraquat	0.005	0.005	0.005
M6	Streptomycin	0.01	n.a.	n.a.
M6	Kasugamycin	0.01	n.a.	n.a.
M7	Morpholine**	0.01**	0.01**	n.a.
M7	Diethanolamine**	0.01**	0.01**	n.a.
M7	Triethanolamine**	0.01**	0.01**	n.a.

* using Q-Trap 5500 instrument;
** value derived from 10-fold diluted extract (0,05 g sample equivalents/mL)

7. References

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

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

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

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

ANNEX

Table 15: Conversion factors between typical purchased standards and target analytes **(3.15):**

Compound	MW [g/mol]	Compound as sold	MW [g/mol]	Conversion factor
Bialaphos	323,3	Bialaphos-sodium	345,3	1,07
Chlorate (anion)	83,6	Chlorate-sodium	106,1	1,27
Chlormequat (cation)	117,6	Chlormequat-chloride	158,1	1,34
Chlormequat-D_4 (cation)	121,6	Chlormequat-D_4-chloride	162,1	1,33
Difenzoquat (cation)	249,3	Difenzoquat-methylsulfate	360,4	1,45
Diquat (dication)	184,2	Diquat-dibromide-monohydrate	362,1	1,97
Diquat-D_4 (dication)	188,2	Diquat-D_4-dibromide-monohydrate	366,1	1,95
Fosetyl	110,0	Fosetyl-Al	118,0	1,07
Fosetyl-D_5	115,0	Fosetyl-D_5-1/3aluminium	123,0	1,07
		Fosetyl-D_5-sodium	137,0	1,19
Glufosinate	182,2	Glufosinate-ammonium	198,2	1,09
Glufosinate-D_3	185,1	Glufosinate-D_3-hydrochloride	220,6	1,19
Kasugamycin	379,4	Kasugamycin-hydrochloride-monohydrate	433,8	1,14
Mepiquat (cation)	114,2	Mepiquat-chloride	149,7	1,31
Mepiquat-D_3 (cation)	117,2	Mepiquat-D_3-iodide	244,1	2,08
N, N-Dimethylhydrazine-D_6	66,1	Dimethylhydrazine-D_6--hydrochloride	102,6	1,55
N-Acetyl-glufosinate	223,2	N-Acetyl-glufosinate-disodium	267,2	1,20
N-Acetyl-glufosinate-D_3	226,2	N-Acetyl-glufosinate-D_3-disodium	270,2	1,19
Nereistoxin	149,3	Nereistoxin-oxalate	239,3	1,60
Nereistoxin-D_6	155,3	Nereistoxin-D_6-oxalate	245,3	1,58
Paraquat (dication)	186,3	Paraquat-dichloride	257,2	1,38
Paraquat-D_6 (dication)	192,3	Paraquat-D_6-diiodide	446,0	2,32
Streptomycin	581,6	Streptomycin-sesquisulfate	728,7	1,25
Trimethylsulfonium	77,2	Trimethylsulfonium-iodide	204,1	2,64
Trimethylsulfonium-D_9	86,2	Trimethylsulfonium-D_9-iodide	213,1	2,47

Table 16: Exemplary concentrations of pesticide stock and working solutions (**3.15** and **3.16**), solvent proposals also apply to ILISs (see **3.18, 3.19, 3.20**):

Compound	Method	Stock Solution (exemplary)		Working Solutions including mixtures (exemplary)	
		Solvent used to prepare	[mg/mL]	Solvent used to prepare	[µg/mL]
Ethephon	M1.(1.2.3)	Methanol + 1% formic acid	1	Methanol + 1% formic acid	5 / 1 / 0.1
HEPA	M1.(1.2.3)	Methanol	1	Methanol + 1% formic acid	5 / 1 / 0.1
Glyphosate	M1.(1.2.3)	Water / methanol (3:1)	0.2	Methanol + 1% formic acid	5 / 1 / 0.1
AMPA*	M1.(1.2.3)	Water *	0.01	Methanol + 1% formic acid	5 / 1 / 0.1
Glufosinate	M1.(1.2.3)	Water / methanol (2:1)	1	Methanol + 1% formic acid	5 / 1 / 0.1
MPPA*	M1.(1.2.3)	Acetonitrile*	0.01	Methanol + 1% formic acid	5 / 1 / 0.1
N-Acetyl-glufosinate	M1.(1.2.3)	Methanol	1	Methanol + 1% formic acid	5 / 1 / 0.1
N-Acetyl- AMPA*	M1.(2.3)	Water*	0.1	Methanol + 1% formic acid	5 / 1 / 0.1
Phosphonic acid	M1.(2.3)	Water (Acetonitrile for the ILIS)	1	Methanol + 1% formic acid***	5 / 1 / 0.1
Fosetyl	M1.(2.3)/M2	Water / methanol (3:1)	0.1	Methanol + 1% formic acid	5 / 1 / 0.1
Maleic hydrazide	M1.3/M2	Methanol	1	Methanol + 1% formic acid	5 / 1 / 0.1
Perchlorate	M1.3/M2	Methanol	1	Methanol + 1% formic acid	5 / 1 / 0.1
Chlorate	M1.3	Methanol	1	Methanol + 1% formic acid	5 / 1 / 0.1
Amitrole	M3	Methanol	1	Methanol	5 / 1 / 0.1
ETU	M3	Methanol	1	Methanol	5 / 1 / 0.1
PTU	M3	Methanol	1	Methanol	5 / 1 / 0.1
Trimethylsulfonium (trimesium)	M3,4	Methanol	1	Methanol	5 / 1 / 0.1
Cyromazine	M3,4	Methanol	1	Methanol	5 / 1 / 0.1
Daminozide	M3,4	Methanol	1	Methanol	5 / 1 / 0.1
Chlormequat	M3,4,5	Methanol	1	Methanol	5 / 1 / 0.1
Mepiquat	M3,4,5	Methanol	1	Methanol	5 / 1 / 0.1
Diquat**	M4	Methanol + 1% formic acid	1	Methanol + 1% formic acid	5 / 1 / 0.1
Paraquat**	M4	Methanol + 1% formic acid	1	Methanol + 1% formic acid	5 / 1 / 0.1
Nereistoxin	M4	Methanol / water (3:1)	1	Methanol	5 / 1 / 0.1
Difenzoquat	M4	Acetonitrile	1	Methanol	5 / 1 / 0.1
N,N-Dimethylhydrazine	M4	Acetonitrile	1	Methanol	5 / 1 / 0.1
Streptomycin**	M6	Water / methanol (1:1)	1	Methanol	5 / 1 / 0.1
Kasugamycin	M6	Methanol	1	Methanol	5 / 1 / 0.1
Morpholine	M7	Methanol	1	Methanol	5 / 1 / 0.1
Diethanolamine	M7	Methanol	1	Methanol	5 / 1 / 0.1
Triethanolamine	M7	Methanol	1	Methanol	5 / 1 / 0.1

* Solutions as purchased by the provider

** Use plastic vessels and stoppers for compounds that tend to interact with glass surfaces
*** in methanol + 1% formic acid solution Phosphonic acid ILIS was shown to be sufficiently stable over 1 month (insignificant formation of native Phosphonate).

Table 17: Providers of isotopically labeled internal standards **3.17**, (exemplary)

Name		Source	Article-No.	Conc. [µg/mL]	Amount per unit	Prices in €-cent		
						1 unit	2 µg*	0.1 µg**
Amitrole	^{15}N	1	XA10240100ME	100	1.1 mL	165 €	300 c	15 c
	^{15}N ^{13}C	1	XA10240110AL	100	1.1 mL	332 €	604 c	30 c
	$^{15}N_2$ $^{13}C_2$	7	A633382		10 mg	1496€	30 c	1,5 c
	$^{15}N_4$ / $^{13}C_2$	8	C4313		10 mg			
AMPA	$^{13}C_2$, $^{15}N_2$	1	CIL-CDNLM-6786-1.2	100	1.2 mL	464 €	773 c	39 c
		5	CDNLM-6786-1.2	100	1.2 mL	464 €	773 c	39 c
	^{13}C, ^{15}N	7	A617342		10 mg	1687 €	34 c	1,7 c
		1	XA10205100WA	100	1.1 mL	332 €	604 c	30 c
	^{13}C, ^{15}N,D_2	10	CDNLM-6786-1.2	100	1.2 mL	465 €	775 c	39 c
Chlorate-$^{18}O_3$***		12		200	5 mL	250 €	50 c	2,5 c
Chlormequat-chloride	1,1,2,2-D_4	1	X 11340100DO	100	10 mL	286 €	57 c	2,9 c
		1	XA11340100DO	100	1.1 mL	73 €	133 c	6,6 c
		6	D3386		10 mg	756 €	15 c	0,8 c
		1	CA11340100		5 mg	389 €	16 c	0,8 c
	D_9	3	673151		5 mg	310 €	12 c	0.6 c
Cyanuric acid	$^{13}C_3$	9	32679		10 mg	408	8,2 c	0,4 c
	$^{18}O_3$	3	673141		10 mg	299 €	6,0 c	0,3 c
Cyromazine-D_4		1	XA11920010EA	100	1.1 mL	118 €	215 c	11 c
		7	C989302		10 mg	1047 €	21 c	1,1 c
Daminozide-D_6		1	XA11960100AL	100	1.1 mL	87 €	158 c	7,9 c
Diethanolamine	D_4	4	D-5307		100 mg	432 €	0.9 c	0.04 c
	D_8	7	D441902		100 mg	1100 €	2,2 c	0,1 c
Dihydrostreptomycin	sesquisulfate-hydrate	1	C 12635300		100 mg	29 €	0,1 c	0,003 c
	sulfate	1	EPD1954000		25 mg	120 €	1,0 c	0.048
Diquat-D_4-dibromide (ethylene-D_4) (mostly as monohydrate !)		1	XA12960010DO	100	1.1 mL	82 €	149 c	7,5 c
		4	D-3932		10 mg	144 €	2,9 c	0,1 c
		6	D17071		50 mg	840 €	3,4 c	0,2 c
		7	D492902		5 mg	117 €	4,7 c	0,2 c
		10	B130022-10		10 mg	1109 €	22 c	1,1 c
		11	sc-218246		5 mg	234 €	9,4 c	0,5 c
Ethephon	D_4	1	XA13230100AC	100	1.1 mL	127 €	231 c	12 c
			DRE-C13230100		10 mg	1197 €	24 c	1,2 c
		6	D8328		5 mg	1387 €	56 c	2.8 c
		7	C366177		10 mg	1122 €	22 c	1,1 c
	$^{13}C_2$	7	C366178		2,5 mg	1650 €	132 c	6,6 c
Ethylenethiourea-D_4 (ETU-D_4)		1	C 13330100		50 mg	316 €	1.3 c	0.06 c
			XA13330100AC	100	1.1 mL	127 €	231 c	12 c
		6	D1965		100 mg	733 €	1,5 c	0,07 c
		7	I367002		10 mg	98 €	2,0 c	0,1 c
Fosetyl	D_{15} (Aluminium)	1	CA13940010		10 mg	380 €	7,6 c	0.4 c

Name		Source	Article-No.	Conc. [µg/mL]	Amount per unit	Prices in €-cent		
						1 unit	2 µg*	0.1 µg**
	D_5 (Sodium)	8	C5607		10 mg	825 €	17 c	0,8 c
Glufosinate-D_3		2	-	friendly donation				
		7	G596952		10 mg	1870 €	37 c	1,9 c
Glyphosate-1,2-$^{13}C_2$,^{15}N		1	XA14050100WA	100	1.1 mL	304 €	553 c	28 c
		5	CNLM-4666-1.2	100	1.2 mL	361 €	602 c	30 c
			CNLM-4666-10X-1.2	1000	1.2 mL	1173 €	196 c	9,8 c
		1	CIL-CNLM-4666-1.2	100	1.2 mL	344 €	573 c	29 c
		6	CN10570		5 mg	1991 €	80 c	4,0 c
		7	G765002		10 mg	1048 €	21 c	1,0 c
		9	608629-SPEC		10 mg	247 €	4,9 c	0.25 c
		11	sc-280758		1 mg	262 €	52 c	2,6 c
HEPA (Hydroxy-Ethephon)-D_4		1	CA13230200		10 mg	256 €	5,1 c	0,3 c
		7	H939652		25 mg	1125 €	9,0 c	0,5 c
		2	-			friendly donation		
Maleic hydrazide-D_2 (MH-D_2)		1	C 14730100		10 mg	235 €	4,7 c	0,2 c
		3	673799		10 mg	199 €	20 c (10µg)	1 c (0.5 µg)
Melamine-$^{13}C_3$, $^{15}N_3$		3	673055		10 mg	289 €	5,8 c	0,3 c
		1	CIL-CNLM-8150-10X-1.2	1000	1,2 mL	1145 €	229 c	12 c
Mepiquat-	D_{16} -chloride-	6	D14539		50 mg	1350 €	5.4 c	0.3 c
	D_3 (methyl-D_3) -iodide	1	X 14880100DO	100	10 mL	378 €	76 c	3,8 c
		1	XA14880100DO	100	1.1 mL	68 €	124 c	6,2 c
Morpholine-D8		4	D-1895/0.5		500 mg	468 €	0.94 c (10µg)	0.05 c (0.5µg)
N-Acetyl-glufosinate	D_3 (methyl-D_3)	2	-	friendly donation				
	D_3, (Acetyl amino-D_3, -disodium salt	7	A178237		5 mg	141 €	5,6 c	0,3 c
Nereistoxin-oxalate-D_6		1	C 15502010		10 mg	245 €	5 c	0.3 c
MPPA-D_3		2	-	friendly donation				
		7	M326162		10 mg	1825 €	37 c	1.8 c
Paraquat-	D_6-diiodide	1	C 15870200		50 mg	256 €	1,0 c	0.05 c
	D_8-dichloride	7	P191902		25 mg	1125 €	9,0 c	0,5 c
Perchlorate-$^{18}O_4$		5	OLM-7310-1.2	100	1.2 mL	326 €	272 c	14 c
		12***		40	5 mL	250 €	125 c	6,3 c
Phosphonic acid-$^{18}O_3$		12		2000	1 mL	125	6,3 c	0,3 c
Propamocarb-D_7		4	DER-XA16390100AC	100	1,1 mL	82 €	149 c	7,5 c
PTU-D_6 = N,N´-(1,2-Propylene)thiourea-D_6 ; = (4-Methyl-2-imidazolidinethione-D_6)		6	D535 (not available)		100 mg	756 €	1,5 c	0.1 c
PTU-D_6 (1,3-Propylene-d6 Thiourea) (not exactly co-eluting with target analyte)		7	P836802		10 mg	1100 €	22 c	1,1 c
1, 2, 4-Triazole-$^{13}C_2$,$^{15}N_3$		2	-	friendly donation				
1, 2, 4-Triazole-acetic acid-$^{13}C_2$,$^{15}N_3$		2	-	friendly donation				
1, 2, 4-Triazole-alanine-$^{13}C_2$,$^{15}N_3$		2	-	friendly donation				
1, 2, 4-Triazole-lactic acid-$^{13}C_2$,$^{15}N_3$		2	-	friendly donation				
Triethanolamine	"D_{15}" (in reality D_{12})	1	CIL-DLM-7663		1 mg	153 €	31 c	1,5 c
	D_{12}	7	T775582		10mg	141 €	2,8 c	0,15 c

Name	Source	Article-No.	Conc. [µg/mL]	Amount per unit	Prices in €-cent		
					1 unit	2 µg*	0.1 µg**
Trimethylsulfonium-D_9 (Iodide)	6	D2677		100 mg	733 €	0,7 c	0,04 c

Providers of compounds:

1: LGC Sta dards

2: Bayer Crop Science

3: HPC (High Purity Compounds)

4: CDN Isotopes (via Dr. Ehrenstorfer)

5: Cambridge Isotope Lab. Inc.

6: Medical isotopes

7: Toronto Research Chemicals

8: ALSACHIM

9: Sigma-Aldrich

10. Cerilliant (by Sigma Aldrich)

11. Santa Cruz biotechnology, inc.

12. EURL-SRM (hosted at CVUA Stuttgart)

(**Disclaimer**: The use of trade names is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the EURL of any product to the exclusion of others. Market prices may be subject to changes, shipping costs are not included in the pricing):

* 2 µg IS are typically employed to samples (typically 10 g) at the beginning of the procedure
** 0.1 µg are typically added to 1 mL aliquots of sample extracts (typically corresponding to 0.5 g sample), in this case only matrix-effects are compensated
*** Due to manufacturing process the stock solution of $^{18}O_3$-Chlorate is accompanied by $^{18}O_4$-Perchlorate (ca. 40 µg/mL). As perchlorate has typically a 5-fold higher sensitivity compared to chlorate the signal intensities of the two are typically within the same range.

Table 18: Exemplary concentrations of IS working solutions (**3.19**)

Method	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-WS I (3.19)	Absolute mass of IS spiked to sample (50 µL IS-WS I) (m_{IS}^{sample})	Suggested concentration of IS- WS II (3.20) **	Absolute mass of IS spiked to calibration standard (50 µL IS-WS II) ($m_{IS}^{cal\ mix}$)	
		µg/mL	µg	µg/mL	µg	µg/mL
M1.(1.2.3)	Ethephon-D_4	40	2	2	0,1	0,1
M1.(1.2.3)	HEPA-D_4	40	2	2	0,1	0,1
M1.(1.2.3)	Glyphosat-$^{13}C_2$,^{15}N	40	2	2	0,1	0,1
M1.(1.2.3)	AMPA-^{13}C,^{15}N	40	2	2	0,1	0,1
M1.(2.3)	N-Acetyl-AMPA*	40	2	2	0,1	0,1
M1.(1.2.3)	Glufosinat-D_3	40	2	2	0,1	0,1
M1.(1.2.3)	MPPA-D_3	40	2	2	0,1	0,1
M1.(1.2.3)	N-Acetyl-glufosinate-D_3	40	2	2	0,1	0,1
M1.(2.3),2	Fosetyl-D_5 (from fosetyl-aluminium-D_{15})	40	2	2	0,1	0,1
M1.3,2	Maleic hydrazide-D_2	200	10	10	0,5	0,5
M1.3,2	Perchlorate-$^{18}O_4$	40	2	2	0,1	0,1
M3	Amitrole-(^{15}N)/ ($^{15}N_2$,$^{13}C_2$)	40	2	2	0,1	0,1
M3,5	ETU-D_4	40	2	2	0,1	0,1
M3,5	PTU-D_6	40	2	2	0,1	0,1
M3,4	Cyromazine-D_4	40	2	2	0,1	0,1
M3,4	Daminozid-D_6	40	2	2	0,1	0,1
M3,4	Nereistoxin-D_4	40	2	2	0,1	0,1
M3,4,5	Chlormequat-D_4	40	2	2	0,1	0,1
M3,4,5	Mepiquat-D_3	40	2	2	0,1	0,1
M4	Diquat-D_4	40	2	2	0,1	0,1
M4	Paraquat-D_6	40	2	2	0,1	0,1
M6	Dihydrostreptomycin****	40	2	2	0,1	0,1
M7	Morpholine-D_8	40	2	2	0,1	0,1
M7	Diethanolamine-D_6	40	2	2	0,1	0,1
M7	Triethanolamine-D_{12}	40	2	2	0,1	0,1

* The concentration of the IS should be high enough to ensure good detection with little influence of the noise on the signal (e.g. S/N>20 is fine). It should be kept in mind, however, that isotopically labeled ISs (IL-ISs) typically contain small amounts of the non-labeled analogues. To minimize the risk of false positives the amount of IL-IS added to the samples should thus not be higher than necessary. Quantification of the parent is typically not affected to a great extend as the cross-contamination is typically at low levels and as similar concentrations of the native pesticide originating from the IL-IS will also be present in the calibration standards and thus subtracted via the intercept. In the case of Maleic hydrazide, where the IL-IS is added at higher concentrations to the samples special attention is necessary (see also comments under **5.6.2**).

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

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

NOTE: *If detections of a compound are rather seldom and the IS expensive it is advisable to add the IS to the 1 mL aliquot transferred to the autosampler vial (see **5.2.7**). 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 19: Water content of selected foods and water amount to be added to test portions prior to extraction (**5.2.2**) depending on the analytical approach

Commodity group	Commodity	Typical water content g/100 g	mL of water to be added to 10 g test portions [g] (where water-addition refers to different sample weights this is specified)		Remarks
			When quantifying with IS that was added at the beginning of the procedure (5.2.3)	When no IS is used or when IS is added after aliquotation (5.7.1.2)	
Fruits					
Citrus fruit	Citrus juices	90	-	1	
	Grapefruit	90	-	1	
	Lemon/lime	85	-	1.5	
	Orange	85	-	1.5	
	Tangerine	90	-	1	
Pome fruit	Apple	85	-	1.5	
	Apple (dried)	30	8.5 to 5 g sample (see **5.1.1**)	8.5 to 5 g sample (see **5.1.1**)	Weigh 13.5 g rehydratized homogenate
	Apple sauce	80	-	2	
	Apple juice	90	-	1	
	Pear	85	-	1.5	
	Quince	85	-	1.5	
Stone fruit	Apricot	85	-	1.5	
	Apricot (dried)	30	8.5 to 5 g sample (see **5.1.1**)	8.5 to 5 g sample (see **5.1.1**)	Weigh 13.5 g rehydratized homogenate
	Apricot nectar	85	-	1.5	
	Cherry	85	-	1.5	
	Mirabelle	80	-	2	
	Nectarine	85	-	1.5	
	Peach	90	-	1	
	Peach (dried)	20	8.5 to 5 g sample (see **5.1.1**)	8.5 to 5 g sample (see **5.1.1**)	Weigh 13.5 g rehydratized homogenate
	Plum	85	-	1.5	
	Plum (dried)	20	8.5 to 5 g sample (see **5.1.1**)	8.5 to 5 g sample (see **5.1.1**)	Weigh 13.5 g rehydratized homogenate
Soft and small fruit	Blackberry	85	-	1.5	
	Blueberry	85	-	1.5	

Commodity group	Commodity	Typical water content g/100 g	mL of water to be added to 10 g test portions [g] (where water-addition refers to different sample weights this is specified)		Remarks
			When quantifying with IS that was added at the beginning of the procedure (5.2.3)	When no IS is used or when IS is added after aliquotation (5.7.1.2)	
	Currant	85	-	1.5	
	Elderberry	80	-	2	
	Gooseberry	90	-	1	
	Grapes	80	-	2	
	Raspberry	85	-	1.5	
	Raisins	20	8.5 to 5 g sample (see **5.1.1**)	8.5 to 5 g sample (see **5.1.1**)	Weigh 13.5 g rehydratized homogenate
	Strawberry	90	-	1	
	Pineapple	85	-	1.5	
Other fruits	Banana	75	2.5	2.5	
	Fig	80	-	2	
	Fig (dired)	20	8.5 to 5 g sample (see **5.1.1**)	8.5 to 5 g sample (see **5.1.1**)	Weigh 13.5 g rehydratized homogenate
	Kiwi	85	-	1.5	
	Mango	80	-	2	
	Papaya	90	-	1	
Vegetables					
Root and tuber vegetables	Beetroot	90	-	1	
	Carrot	90	-	1	
	Celeriac	90	-	1	
	Horseradish	75	2.5	2.5	
	Parsley root	90	-	1	
	Radish	95	-	0.5	
	Black salsify	80	-	2	
	Potato	80	-	2	
	Garlic	60	7 to 5 g sample	7 to 5 g sample	
Leek plants	Onion	90	-	1	
	Leek	85	-	1.5	
	Shallot	80	-	2	

Commodity group	Commodity	Typical water content g/100 g	mL of water to be added to 10 g test portions [g] (where water-addition refers to different sample weights this is specified)		Remarks
			When quantifying with IS that was added at the beginning of the procedure (5.2.3)	When no IS is used or when IS is added after aliquotation (5.7.1.2)	
	Chive	85	-	1.5	
Fruiting vegetables	Aubergine	90	-	1	
	Cucumber	95	-	0.5	
	Melon	90	-	1	
	Pepper, sweet	90	-	1	
	Pumpkin	95	-	0.5	
	Tomato	95	-	0.5	
	Zucchini	95	-	0.5	
	Broccoli	90	-	1	
Cabbage	Brussel sprouts	85	-	1.5	
	Cauliflower	90	-	1	
	Chinese cabbage	95	-	0.5	
	Kale	90	-	1	
	Kohlrabi	90	-	1	
	Red cabbage	90	-	1	
	Savoy cabbage	90	-	1	
	White cabbage	90	-	1	
	Lettuce varieties	95	-	0.5	
	Endive	95	-	0.5	
Leafy vegetables and herbs	Cress	90	-	1	
	Lamb's lettuce	85	-	1.5	
	Parsley	80	-	2	
	Rucola	85	-	1.5	
	Spinach	90	-	1	
Stem vegetables	Asparagus	95	-	0.5	
	Celery	95	-	0.5	
	Leek	85	-	1.5	
	Rhubarb	95	-	0.5	

Commodity group	Commodity	Typical water content g/100 g	mL of water to be added to 10 g test portions [g] (where water-addition refers to different sample weights this is specified)		Remarks
			When quantifying with IS that was added at the beginning of the procedure (5.2.3)	When no IS is used or when IS is added after aliquotation (5.7.1.2)	
	Artichokes	85	-	1.5	
Legumes	Beans, peas, lentils (dried)	<10	10 to 5 g sample	10 to 5 g sample	
	Beans, peas	75	2.5	2.5	
Miscellaneous					
Cereals	Grain, flour etc.	10	10 to 5 g sample	10 to 5 g sample	Different sample amounts may be used depending on water-absorbing properties of material
Extract-rich ("difficult") commodities	Coffee beans	<10	10 to 2 g sample	10 to 2 g sample	Different sample amounts may be used depending on extract-richness
	Tea	<10	10 to 2 g sample	10 to 2 g sample	
	Dry herbs and spices	<10	10 to 2 g sample	10 to 2 g sample	
Other	Mushrooms	90	-	1	
	Wine	90	-	1	
	Honey	20	9 to 5 g sample	9 to 5 g sample	

Table 20: Exemplary LC-MS/MS parameters for ABI 5500

	Methods 1.1 / 1.2	Method 1.3	Method 2	Method 3+4+5	Method 6
Ion source/Mode	Turbo Ion Spray (ESI)/negative	Turbo Ion Spray (ESI)/negative	Turbo Ion Spray (ESI)/negative	Turbo Ion Spray (ESI)/positive	Turbo Ion Spray (ESI)/positive
Curtain gas	Nitrogen 30 psi (2,07 bar)	Nitrogen 30 psi (2,07 bar)	Nitrogen 30 psi (2,07 bar)	Nitrogen 40 psi (2,76 bar)	Nitrogen 40 psi (2,76 bar)
Collision gas	med	med	med	med	med
Ion spray voltage	-4500	-4500	-4500	1500	5500
Gas 1	Nitrogen 50 psi (3,45 bar)				
Gas 2	Nitrogen 50 psi (4,14 bar)				
Temperatur Gas 2	600°C	420°C	500°C	500°C	550°C
Resolution MS 1	unit (ca. 0.7 amu FWHM*)				
Resolution MS 2	unit (ca. 0.7 amu FWHM)				
Dwell time	20	20	50	20	50

*FWHM = full width at half maximum

Table 21: Document History

Action	When?	Version
Development of Method by the CRL-SRM	2006-2008	-
Presentation of method at the EPRW in Berlin (oral presentation plus poster)	June 2008	
Drafting of V1	Nov.-Dec. 2008	V1
Placing of V1 in CRL-Website	Jan. 2009	
Update of Table 1, Expected concentrations of ISs were calculated with a wrong dilution factor in previous version. Arithmetical errors were corrected.	Aug. 2009	V2
Introduction of measurement conditions for HEPA within the “Glyphosate & Co.” method		
Introduction of measurement conditions for the screening of diquat and paraquat within the “Quats & Co. method”	Nov 2009	V3
Introduction of measurement conditions for Amitrole, chlormequat, mepiquat and daminozide “Amitrole & Co.” method		
Extensive text revisions		
Introduction of measurement conditions for Streptomycin Kasugamycin	May 2010	V4
Introduction of measurement conditions for the screening of Perchlorate ion		
Extensive text revisions		
Extensive text revisions and restructuring of document	Nov 2010	V5
Introduction of measurement conditions for ETU, ETU D_4, PTU, PTU D_6, Cyromazine, Cyromazine D_4, N-Acetyl-Glufosinate, N-Acetyl-Glufosinate D_3, Glufosinate D_3, MPPA D_3, Morpholin, Morpholin D_8		
Introduction of an acronym for the method (QuPPe)	July 2011	V6
Advice to use plastic vessels and stoppers for Glyphosate		
Minor modification and additional instructions in Method 1 (M1)		
Modification of mobile phase of M3 to improve analysis of ETU and PTU		
Introd. of measurement cond. for Amitrole $^{15}N^{13}C$ and Amitrole ^{15}N in M3		
Introd. of measurement cond. for Nereistoxin and Nereistoxin D6 in M4		
New method (M7) for the analysis of Morpholin/Morpholin D_8; Diethanonamine/diethanolanmine D_6; Triethanolamine/Triethanolamine D_{12} (M7)		
Removal of Morpholin from M4 as it does not separate from the interfering diethanolamine		
Introduction of ETU and PTU and their corresponding ILISs in Method 5		
Correction of dimension of stock solutions conc. in Table 12 (to mg/mL)		
Text and Table revisions		

Action	When?	Version
Extensive revision of table concerning possible sources of purchase of ISs		
Some additions in “Apparatus and Consumables” chapter	Dec. 2012	V7
Clarifications in chapter concerning standard additions		
Overview table concerning the scope of the methods 1.1, 1.2, 1.3 and 2		
Addition of Phosphonic acid in Method 1.1 ("Glyphosate & Co.")		
New LC-method (Method 1.2) for "Glyphosate & Co." using a Dionex ionPac AS11-HC column and an Eluent with near to neutral pH; additionallycovering Fosetyl		
New LC-method (Method 1.3) for "Glyphosate & Co." using a Hypercarb column and an acidic Eluent covering all analytes covered by Method 1.1, Method 1.2 and Method 2 (including perchlorate).		
Update of practical considerations for methods 1.1-1.3		
Update of table with performance data		
Table with exemplary recovery data was deleted (recovery figures can be obtained in the EURL-DataPool		
Update of table with LOQs		
Update of table with providers of ILISs		
Elimination of errors in text		
Addition of Chlorate in Method 1.3	Nov. 2013	V7.1
Update of practical considerations for methods 1.1-1.3 (Column C)		
Update of table with performance data		
Update of table with LOQs		
Introduction of Trimethylsulfonium-D9 and N,N-Dimethylhydrazine-D6 in Method 4		
Thorough revision of text and elimination of errors	Mar. 2015	V8
Practical advices on the choice of filter materials		
New Table 15: Conversion factors between standard materials and analytes		
Advices as regards the use of ILISs		
Update of Table 5.6 LC-MS/MS measurement conditions		
New chapters “Hints on Method 1.1 – 1.4” and replacement of the section “Practical care and use considerations concerning the columns of methods 1.1-1.3. This includes information on various potential sources of errors such as in-source fragmentations of Fosetyl and Ethephon to Phosphonic acid and of Perchlorate to Chlorate as well as degradation of compounds in solution.		
Introduction of Cyanuric acid and Bialaphos in M1.3		

Action	When?	Version
Correction of a typing error concerning the mass-transitions of Phosphonic acid (81/79 instead of 81/81)		
Introduction of the ILIs of Phosphonic acid and chlorate in M1.3 and 1.4		
New LC Method (1.4) for "PerChloPhos" using a Hypercarb column and an acidic Eluent optimized for chlorate, perchlorate, phosphonic acid compared to Method 1.3		
Change of name of former M4 to M4.1		
Introduction of Melamine and Propamocarb as well as the corresponding ILISs in M4.1		
New LC Method (M4.2) employing a Hilic-Type BEH Amide column allowing the simultaneous analysis of many polar pesticides		
Reduction of injection volume and increase of dwell-time in method M6		
New LC-method (M8) for the analysis of triazole derivative metabolite (TDMs) and their corresponsing ILISs		
Update of Table 17: Providers of isotopically labeled internal standards		