Quick Method for the Analysis of Residues of Highly Polar Pesticides in Foods of Plant Origin involving simultaneous Extraction with Methanol and LC-MS/MS determination

- Version 4 (May 2010), Document History (p. 19) -

1. Scope and Short Description

A method is described for the analysis of polar, non-QuEChERS-amenable pesticides (log Kow < -2) in foods of plant origin such as fruits (including dried fruits), vegetables, cereals and processed products thereof.

Residues are extracted from the test portion following the addition of acidified methanol. The mixture is centrifuged, filtered and directly analyzed by LC-MS/MS. Quantification is performed with the help of isotopically labeled internal standards (analogues of the target analytes), which are added directly to the test portion at the beginning of the procedure.

2. Apparatus and Consumables

- Powerful sample processing equipment, for example Stephan UM 5
- 50 ml centrifuge tubes with screw caps, for example: a) 50 ml Teflon® centrifuge tubes with screw caps (e.g. Nalgene/Rochester, USA; Oak-ridge, article-no. 3114-0050) or b) disposable 50 ml centrifuge tubes (e.g. Sarstedt/Nümbrecht, Germany, 114x28 mm, PP, article-no. 62.548.004)
- Automatic pipettes, suitable for handling volumes of 10 to 100 µl, 200 to 1000 µl and 1 to 10 ml.
- 10 ml solvent-dispenser for methanol
- Centrifuge, suitable for the centrifuge tubes employed in the procedure and capable of achieving at least 2000 g
- Syringes, e.g. 2 or 5 mL disposable syringes
- Syringe filters, 0.45 µm pore size
- Injection vials, suitable for LC auto-sampler
- LC-columns (see in appropriate chapters)

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.

- Methanol, HPLC quality
- Acetonitrile, HPLC quality
- Formic acid (concentrated; > 95%)
- Citric acid monohydrate (p.a.)
- Dimethylamine (e.g. 40%, Fluka article-no. 38940)
- Ammonium formate (p.a.)
- Water (deionized)
- Pesticides or pesticide stock solutions
- Isotopically labeled analogues of the pesticides (see Annex)

4. Disclaimer

This method refers to several trade name 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.

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 and improve homogeneity.
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 (if possible by adding dry ice). 13.5 g of this homogenate will correspond to 5 g sample.

5.2. Extraction

- Weigh 10 g* of the comminuted sample into a 50 mL centrifuge tube.

  Notes:
  - *Use 13.5 g of the rehydrated dried-fruit homogenate as described under 5.1*
  - *When analyzing dry samples such as grains, raisins or flour, the sample amount should be reduced to 5 g followed by the addition of 10 g water.*
  - *Smaller test samples may have to be used for extract-rich commodities or commodities with high water absorbing capacity not allowing proper extraction.*

- Adjust the total water content in the sample to reach ca. 10 mL in total

  Note:
  - *The amount of water to be added to the different commodities is shown in Table 11 in the Annex.*
  - *Where ISTDs are employed water-volume adjustment is less critical and may be skipped for commodities containing more than 80% of water*

- Add 100 µL of internal standard solution containing one or more isotopically labeled analogues of the analytes (see Table 1);

Table 1 : Exemplary concentrations of ISTDs*:

Analyte	ISTD	Suggested Concentration of ISTD solution to be used for spiking in µg/mL	Expected concentration of ISTD in final extract in µg/mL **
Chlormequat	Chlormequat chloride D4	10-20	0.05-0.1
Mepiquat	Mepiquat iodide D3	10-20	0.05-0.1
Ethephon	Ethephon D4	10-20	0.05-0.1
Fosetyl-Al	Fosetyl-Al D15	10-20	0.05-0.1
Maleic acid hydrazide	Maleic acid hydrazide D2	20-200	0.1-1
Daminozide	Daminozide D6	10-20	0.05-0.1
Glyphosate	Glyphosate $^{13}C_2$ ^{15}N	10-20	0.05-0.1
AMPA	AMPA ^{13}C ^{15}N	10-20	0.05-0.1
HEPA	HEPA ethylene D4	10-20	0.05-0.1
Diquat	Diquat D4	10-20	0.05-0.1
Paraquat	Paraquat D6	10-20	0.05-0.1
Perchlorate	Perchlorate $^{18}O_4$	10-20	0.05-0.1
Streptomycin	Dehydrostreptomycin***	10-20	0.05-0.1

* Keep in mind that isotopically labeled ISTDs often contain small amounts of the non-labelled analogues. The amount of ISTD added to the samples should not be higher than necessary to avoid false positives. This cross-contamination effect is typically negligible, however in the case of maleic hydrazide, where the ITSD is added at much higher concentrations to the samples (due to the limited sensitivity) special care is necessary (see also under Method Fosetyl-MH).
** Having approximately the same concentration of ISTD in sample extract and calibration solutions is recommended to reduce errors originating from non-linear response.
*** not isotopically labeled but still suitable for compensation of matrix effects, adjust LC conditions in such a way to ensure coelution with Streptomycin

- Add 10 mL acidified methanol (methanol containing 1% concentrated formic acid).
- Close the tube and shake vigorously for 1 to 2 min.
- Centrifuge (e.g. 5 min at >2500 g)
- Transfer an aliquot (e.g. 3 mL) of the extract into a syringe and filter it into an autosampler vial. Employ for LC-MS/MS analysis

Note: *The resulting extract volume, taking into account the natural water content of the sample) is ca.* ***20 mL*** *(corresponding to ca.* ***0.5 g sample per mL extract****).*

5.3. Recovery experiments

Extraction of spiked samples: Using suitable blank commodities (not containing any detectable residues of the analyte), proceed sample preparation exactly as described in 5.2**.** Spiking (use small volumes of pesticide working solutions, e.g. 100 µL!) is to be preferably done directly to the matrix, prior to water addition.

Preparation of blank extract for matrix matched calibrations: Using suitable blank commodities (not containing any detectable residues of the analyte), proceed sample preparation exactly as described in 5.2 but **SKIP THE ADDITION OF ISTDs.** Transfer suitable aliquots of the blank extract to HPLC auto-sampler vials and proceed according to 5.4.

Note: *Where isotopically labeled ISTDs are used matrix-matched calibration is not essential as the ISTD compensates for any matrix-related signal suppressions / enhancements. Solvent-based calibrations can be used here. Where isotopically labeled ISTDs are <u>not</u> used matrix matching is, however, essential.*

5.4. Preparation of Calibration Standards

An exemplary pipetting scheme for the preparation of a calibration solution is shown in Table 2.

Table 2 Pipetting scheme for the preparation of a calibration solution (exemplary)

	Matrix-matched		Solvent based
	using ISTD	**without ISTD**	
Blank extract or methanol w. 1% formic acid	800 µL	900 µL	800 µL
ISTD solution (diluted): 1:20 dilution of the solution added to the test sample in step 5.2	100 µL*	-	100 µL*
Pesticide standard solution e.g. 0.5 µg/mL**	100 µL	100 µL	100 µL
Total volume	**1000 µL**	**1000 µL**	**1000 µL**

** Same volume as added in 5.2*

*** In this case the end-concentration of the pesticide in the calibration standard equals 0.05 µg/mL, which is equivalent to 0.1 mg/kg (when using 10 g test portions) or 0.2 mg/kg (when using 5 g test portions). It is of high importance to prepare the calibration solutions in a way that the ISTD-concentration approximately equals that of the final extracts of the test samples.*

5.5. LC-MS/MS Measurement Conditions

Any suitable LC and MS/MS conditions may be used. Below you will find some exemplary instrument measurement conditions.

Method: ***Glyphosate & Co.***

Table 3 : Proposed LC-MS/MS conditions for Ethephon, HEPA* (metabolite of Ethephon), Glyphosat, AMPA* (metabolite of Glyphosate), Glufosinate, MPPA* (metabolite of Glufosinate)

Instrument parameters	Conditions
Ionization mode	ESI neg
Column (see also notes)	Dionex IonPac AS 11 2 x 250 mm (P/N 44077)
Pre-column	Dionex IonPac AG11 2 x 50 mm (P/N 44079)
Pre-filters	e.g. Supelco column saver 2.0 µm Filter
Eluent A	Water
Eluent B Make sure your solvent filters of the eluent can handle alkaline solvents.	1 mM citric acid in water adjusted to pH 11 with dimethylamine (DMA)* * You will need ca. 1 mL DMA solution for 500 mL 1 mM citric acid in water
Gradient	Flow: 0.3 mL/min Gradient: 100% A in 8 min to 50 % B hold for ca. 3 min (Note: the column is equilibrated for 10 min with 100% A before every run)
Injection volume	20 µL (Note: in case of analyzing only ethephon 5 µL may be enough -depending on the instrument)
Calibration solutions and levels	e.g. 0.05 or 0.1 µg/ISTD + one level near the LOD is recommended
Aquired mass transitions (m/z)	AMPA: 110/63 (target ion), 110/79, 110/81 Ethephon: 143/107 (target ion), 143/79, 145/107 HEPA: 125/79 (target ion), 125/95, 125/63 Glufosinate: 180/63 (target ion), 180/136, 180/85, 180/95 Glyphosate: 168/63 (target ion), 168/124, 168/150, 168/81 MPPA: 151/63 (target ion), 151/107, 151/133 AMPA 13C15N (ISTD): 112/63 Ethephon D4 (ISTD): 147/111 Glyphosate 13C215N (ISTD): 171/63 HEPA D4 (ISTD): 129/79

AMPA: Aminomethylphosphonic acid; MPPA: 3-Methylphosphinicopropionic acid

Notes on LC-operation:

- For reconditioning, the column should be flushed from time to time (e.g. every 50 injections) with 30 mM NaOH. ***The NaOH solution has to go directly into a waste bottle and SHOULDN'T reach the MS ion source!***

- *To extent the lifespan of the column, the use of pre-filters is recommended. These should be exchanged regularly (e.g. every 100 injections) or when the pressure noticeably increases. As soon as a deterioration of the separation performance is noticed the guard column (pre-column) should be exchanged.*

- *Especially for AMPA, MPPA and ` iquat` t` te a peak shape worsening and larger matrix-dependent retention time shifts are observed as column performance deteriorates.*
- *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 glas bottles for eluent B; rotor-seals from alkali-persistent materials such as PEEK(polyetherketone) or Tefzel rather than Vespel.*

Figure 1: Typical chromatograms of Glyphosate, AMPA, Glufosinate, MPPA and Ethephon

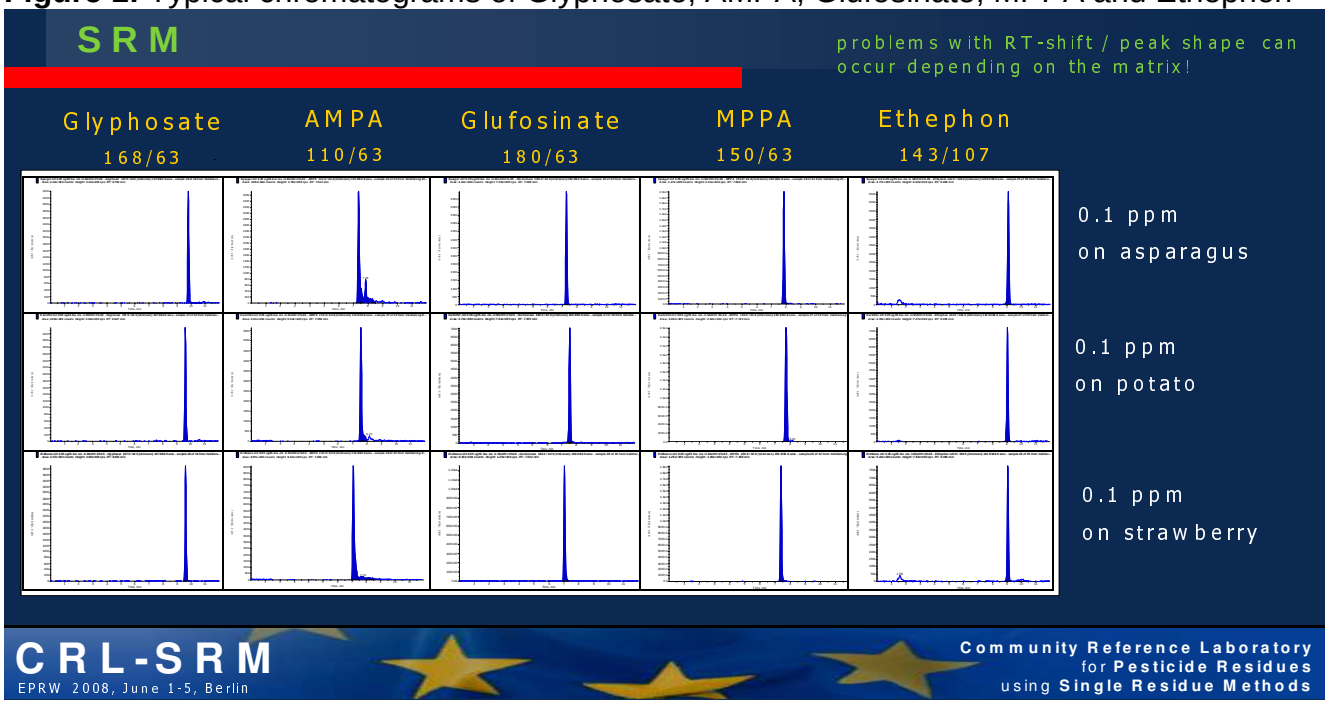
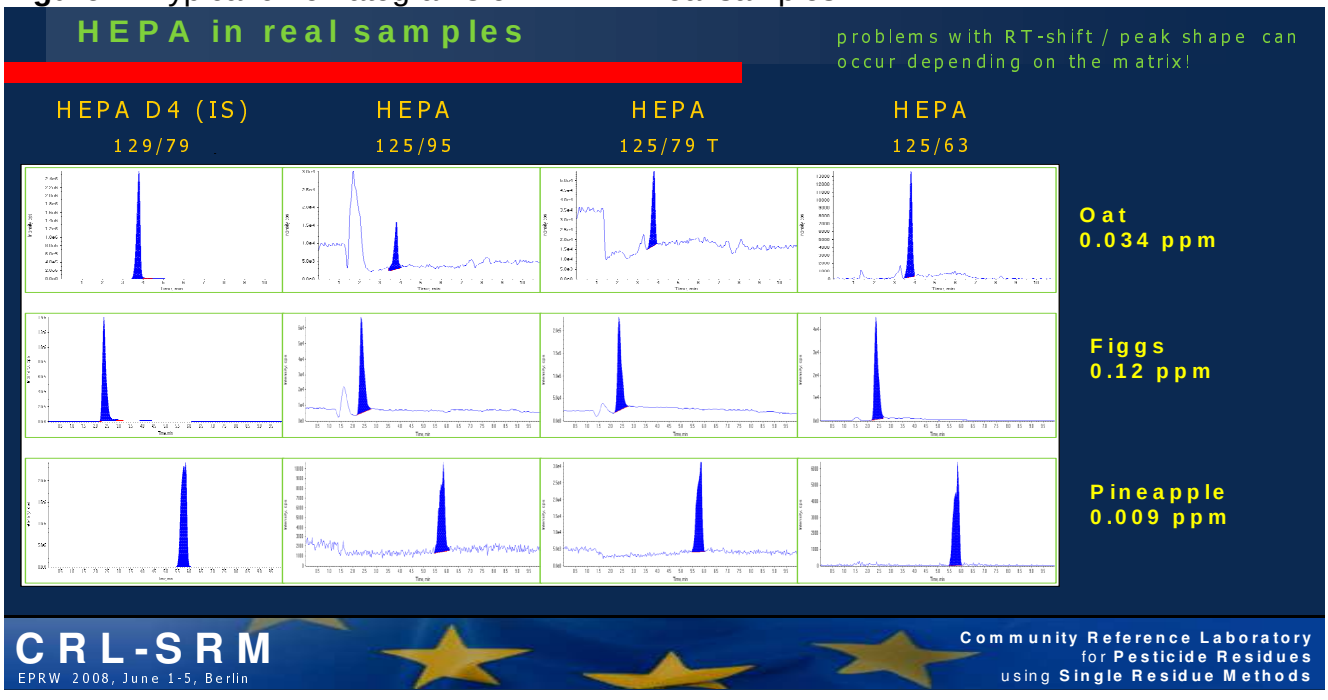


Figure 2: Typical chromatograms of HEPA in real samples



Method: ***Fosetyl-MH***

Table 4 Proposed LC-MS/MS conditions for Fosetyl-Al, Maleic Hydrazide and Perchlorate (screening)

Instrument parameters	
Ionization mode	ESI neg
Column	Obelisc R 2.1 x 150mm 5µm 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 solutions and levels	e.g. 0.05 or 0.1 µg/ml + one level near the LOD is recommended For maleic hydrazide (MH) a level of 1 µg/ml may be useful as well, due to high residue levels; consider that MH is typically only relevant for potatoes and Alliaceae
Acquired mass transitions	Fosetyl-Al (detected as ` iquat`): 109/81 (target ion), 109/63 Maleic hydrazide: 111/82 (target ion), 111/42, 111/55, 111/83 Perchlorate: 99/83, 101/85 Fosetyl-Al D15 (ISTD): 114/82 Maleic hydrazide D2 (ISTD): 113/42 Perchlorate $^{18}O_4$ (ISTD): 107/89

Note: It should be kept in mind that isotopically labeled maleic hydrazide (MH) may contain small amounts of unlabelled MH (in the example given in Figure 4, less than 0.2%). The influence of this impurity on quantitation is typically low (in this case corresponding to less than 0.005 mg MH/kg sample following addition of 20 µg ISTD to 10 g sample) compared to the typically high MH-levels encountered in treated samples and as the ISTD is also given to the calibration solutions which shifts the calibration curve and largely corrects quantification. However, in case of samples with low MRLs (e.g. baby food) or organic samples, care should be taken in quantification. Analysis of the sample without ISTD added will help to avoid false positives. The smaller the concentration of added ISTD the smaller these effects are.

Figure 3: Typical chromatograms of Fosetyl-Al

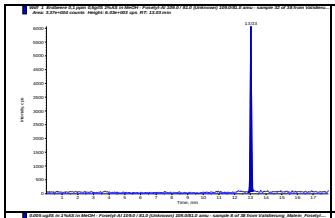
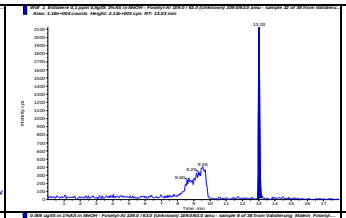
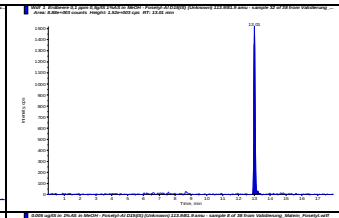
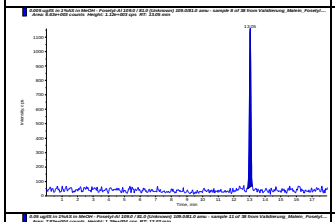
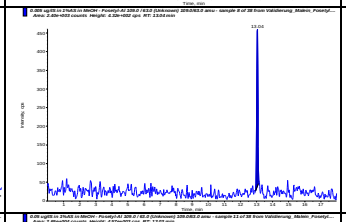
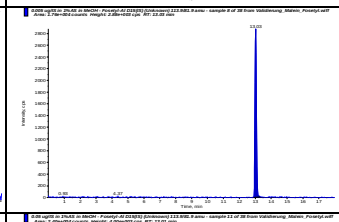
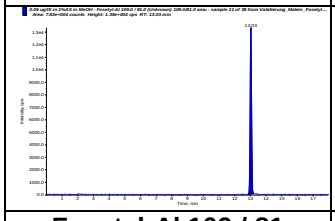
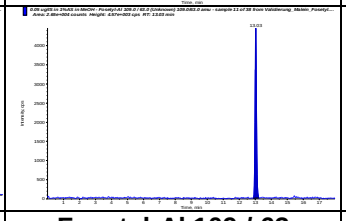
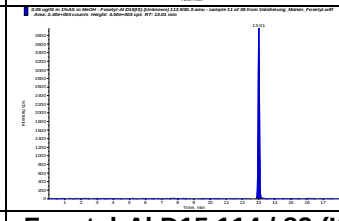
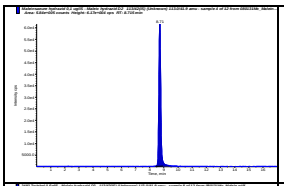
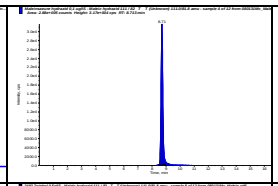
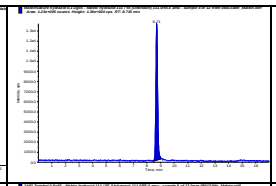
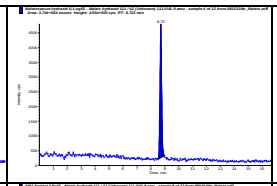
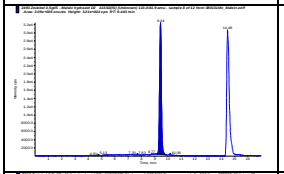
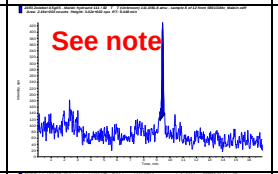
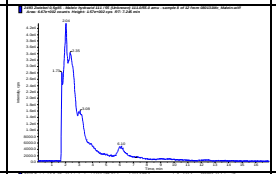
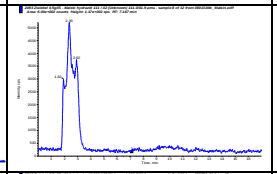
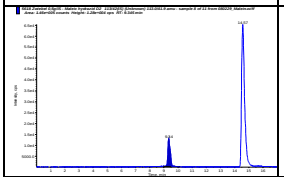
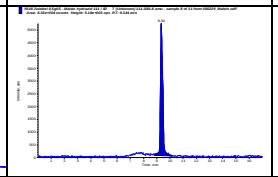
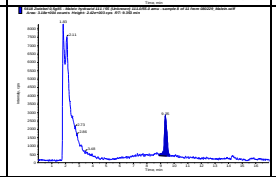
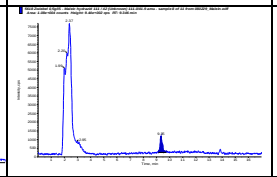
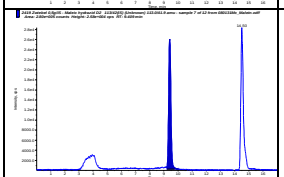
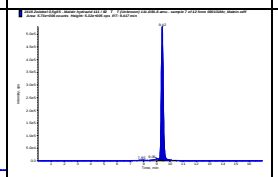
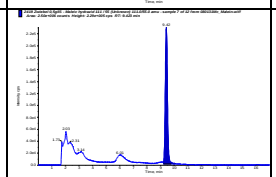
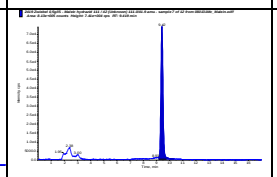
			**Recovery test on strawberry** **0.1 mg/kg** **= 0.05 µg/mL**
			**Fosetyl-Al solvent calib.** **0.005 µg/mL** **= 0.01 mg/kg**
			**Fosetyl-Al solvent calib.** **0.05 µg/mL** **= 0.1 mg/kg**
Fosetyl-Al 109 / 81	**Fosetyl-Al 109 / 63**	**Fosetyl-Al D15 114 / 82 (ISTD)**	

Figure 4: Typical chromatograms of Maleic Hydrazide

				**Maleic hydrazide solvent calib.** **= 0.05 µg/ml**
				**onion sample 0.5 g/ml** **n.d.**
				**onion sample 0.5 g/ml** **~ 0.1 mg/kg Maleic hydrazide**
				**onion sample 0.5 g/IS** **~ 4 mg/kg Maleic hydrazide**
Maleic hydrazide D2 113/42 (ISTD)	**Maleic hydrazide 111 / 82 (target ion)**	**Maleic hydrazide 111 / 55**	**Maleic hydrazide 111 / 42**	

Method: ***Quats & Co***

Table 5 : Proposed LC-MS/MS conditions Diquat, Paraquat (screening), Chlormequat, Mepiquat, Daminozide, and Trimethylsulfonium-Cation (counterion of Glyphosate)

Instrument parameters	Conditions
Ionisation mode	ESI pos
Column	Obelisc R 2.1 x 150mm 5µm 100 Å (SIELC; OR-21.150.0510); 40℃
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	20 mmol NH_4-formate in water (adjust to pH 3 w. formic Acid)*; Use brown glass bottles * you will need to add 1.8 mL formic acid (>96%) to 500 mL 20 mmol NH_4-formate in water
Eluent B	Acetonitrile
Gradient	Flow:: 0.4 mL/min Gradient: 20% A in 4 min to 80 % A
Injection volume	10 µL
Calibration solutions and levels	e.g. 0.05 or 0.1 µg/IS + one level near the LOD is recommended
Acquired mass transitions	Diquat: 184/128 (target ion), 183/157, 184/156 Paraquat: 186/171 (target ion), 171/77, 171/155 Chlormequat: 122/58 (target ion), 122/63, 124/58 Mepiquat: 114/98 (target ion), 114/58 Trimethylsulfonium-cation: 77/62 (target ion), 77/47 Daminozide: 161/143 (target ion), 161/101, 161/61, 161/115 N,N-dimethylhydrazine: 61/44, 61/45 Diquat D4 (ISTD): 188/160 Paraquat D6 (ISTD): 192/174 Chlormequat D4 (ISTD): 126/58 Mepiquat D3 (ISTD): 117/101 Daminozide D6 (ISTD): 167/149

Method: *Amitrol & Co*

Table 6 : Proposed LC-MS/MS conditions Amitrol, Chlormequat, Mepiquat and Daminozide

Instrument parameters	Conditions
Ionisation mode	ESI pos
Column	Obelisc R 2.1 x 150mm 5µm 100 Å (SIELC; OR-21.150.0510); 40°C
Pre-column	Obelisc R 2.1 x 10mm 5 µm (SIELC; OR-21.G.0510)
Pre-filters	e.g. Supelco column saver 2.0 µm Filter
Eluent A	50 mmol NH_4-formate in water Use brown glass bottles
Eluent B	Acetonitrile
Gradient	%A / Flow [mL/min] / Time [min]: 3 / 0.4 / 0 10 / 0.4 / 5 70 / 0.4 / 13 70 / 0.4 / 18 3 / 0.4 / 18.1 3 / 0.4 / 25.0
Injection volume	5 µL
Calibration solutions and levels	e.g. 0.05 or 0.1 µg/IS + one level near the LOD is recommended
Acquired mass transitions	Amitrol: 85/57 (target ion), 85/43 Chlormequat: 122/58 (target ion), 122/63, 124/58 Mepiquat: 114/98 (target ion), 114/58 Daminozide: 161/143 (target ion), 161/101, 161/61, 161/115 Chlormequat D4 (ISTD): 126/58 Mepiquat D3 (ISTD): 117/101 Daminozide D6 (ISTD): 167/149

Table 7 : Proposed alternative LC-MS/MS conditions for Chlormequat and Mepiquat

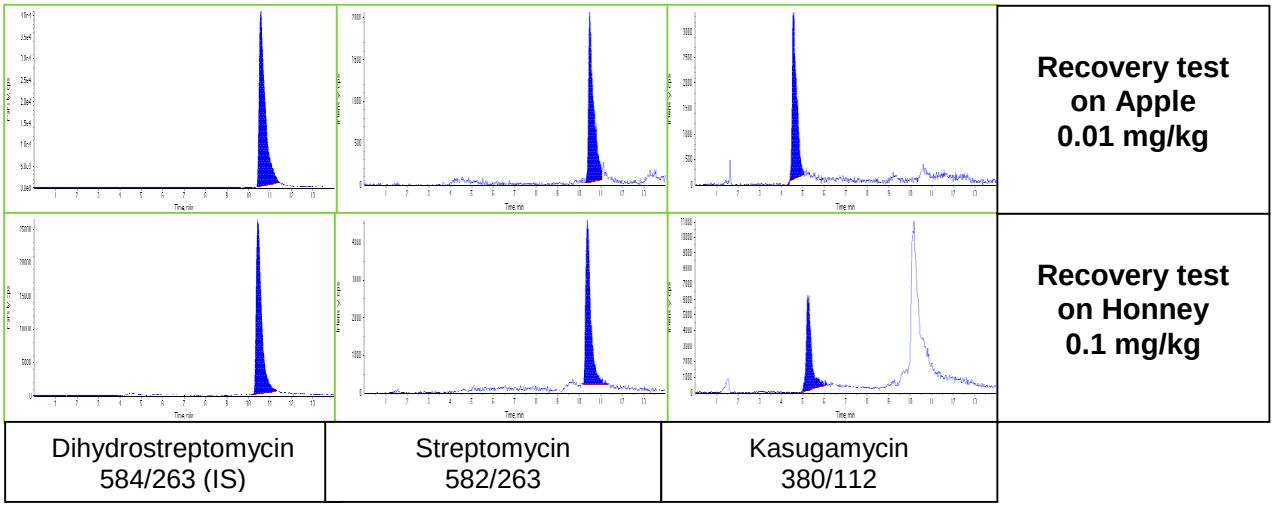
Instrument parameters	Conditions
Column:	MonoChrom MS 100x2 mm; 5 µm (Varian); at 40°C
Eluent A:	5 mmol/L NH_4-acetate + 0.1% acetic acid
Eluent B:	Acetonitrile
Gradient:	5% A in 2 min to 95% A
Flow rate:	0.4 mL/min
Injection volume:	5 µL

Method: ***Strepto & Kasuga***

Table 8 : Proposed LC-MS/MS conditions Streptomycin and Kasugamycin

Instrument parameters	**Conditions**
Ionisation mode	ESI pos
Column	Obelisc R 2.1 x 150mm 5µm 100 Å (SIELC; OR-21.150.0510); 40℃
Pre-column	Obelisc R 2.1 x 10mm 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 / 8 20 / 0.3 / 0 20 / 0.3 / 5 80 / 0.5 / 10 80 / 0.5 / 14
Injection volume	50 µL
Calibration solutions and levels	• µg/IS + one level near the LOD is recommended
Acquired mass transitions	**Streptomycin:** 582/263 (target ion), 582/246, 582/ 221 **Kasugamycin:** 380/112 (target ion), 380/200 **Dihydrostreptomycin (ISTD):** 584/263

Figure 5: Typical chromatograms of Streptomycin and Kasugamycin



Performance

Results of 5-fold analyses using matrix matched calibrations (for more information see method validation database at **www.crl-pesticides-datapool**).

Table 9 : Overview of Validation Data

Pesticide	Mean Recovery [%]	Variation-coefficient [%]	Spike Level [mg/kg]	Matrix	ISTD
Chlormequat-Cation (MRM 122/58)	97	4.6	0.1	Wheat Flour	Chlormequat D4
Mepiquat-Cation (MRM 114/98)	102	4.9	0.1	Wheat Flour	Mepiquat D3
Trimethylsulfonium-Cation (MRM 77/62)	104	1.9	0.1	Wheat Flour	-
Glyphosate (MRM 168/63)	124	7.6	0.1	Wheat Flour	Glyphosate $^{13}C_2{}^{15}N$
AMPA (MRM 110/63)	119	5.7	0.1	Wheat Flour	AMPA $^{13}C^{15}N$
Glufosinate (MRM 180/63)	85	4.6	0.1	Wheat Flour	-
MPPA (MRM 151/63)	85	1.3	0.1	Wheat Flour	-
Ethephon (MRM 143/107)	85	5.9	0.1	Wheat Flour	-
Fosetyl-Al (MRM 109/81)	95	4.1	0.1	Strawberry	Fosetyl-Al D15
Maleic Hydrazide (MRM 111/82)	106	3.7	0.1	Onion	Maleic Hydrazide D2
Daminozide (MRM 161/143)	101	2.8	0.1	Wheat Flour	Daminozide D6
HEPA (MRM 125/79)	96	3.5	0.1	Pear	HEPA D4
Diquat (MRM 184/128)	103	6.2	0.1	Wheat Flour	Diquat D4
Paraquat (MRM 186/171)	114	6.5	0.1	Wheat Flour	Paraquat D6
Amitrol (MRM 85/57)	107	4.6	0.01	Cucumber	-
Streptomycin (MRM 582/263)	115	5.8	0.01	Apple	Dihydrostreptomycin
Kasugamycin (MRM 380/112)	89	1.2	0.01	Apple	-

6. 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

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 10 : Isotopically labeled internal standards:

Name	Source (exem-plary)	Article-No.	Conc.	Amount	Price*	Price for 2 µg**	Price for 0.1 µg***
AMPA (^{13}C ^{15}N)	1	XA10205100WA	100 ng/µL	1.1 mL	280	560 c	28 c
Chlormequat chloride (1,1,2,2-D4)	1	X11340100DO	100 ng/µL	10 mL	310	6 c	0.3 c
	1	XA11340100DO	100 ng/µL	1.1 mL	73	146 c	7 c
Chlormequat-chloride D9	2	673151	5 mg		345	14 c	0.7 c
Daminozide D6	1	XA11960100AL	100 ng/µL	1.1 mL	89	180 c	9 c
Dihydrostreptomycin sesquisulfate hydrate	1	C12635300		100 mg	24	0.05 c	0.0025 c
Diquat dibromide D4 monohydrate	1	XA12960010DO	100 ng/µL	1.1 mL	84	8 c	0.4 c
Ethephon D4 (2-Chloroethyl-1,1,2,2-D4)	1	XA13230100AC	100 ng/µL	1.1 mL	128	13 c	0.65 c
Ethylene thiourea D4	1	C13330100		50 mg	310	1.2 c	0.06 c
		XA13330100AC	100 ng/µL		128	260 c	13 c
Fosetyl-aluminium D15	1	CA13940010		10 mg	380	8 c	0.4 c
Glyphosate (1,2-$^{13}C_2$ ^{15}N)	1	XA14050100WA	100 ng/µL	1.1 mL	330	660 c	33 c
HEPA D4	3	2004BRP042-247			donation		
Maleic hydrazide D2	1	C14730100		10 mg	230	12 c (5µg)	0.6 c 0.25 µg)
	2	673799		10 mg	199	10 c	0.5 c
Mepiquat iodide D3 (methyl D3)	1	X14880100DO	100 ng/µL	10 mL	410	140 c	7 c
	1	XA14880100DO	100 ng/µL	1.1 mL	68	80 c	4 c
Paraquat diiodide D6	1	C15870200		50 mg	146	150 c	7.5 c
Perchlorate $^{18}O_4$	4	OLM-7310-S	100 ng/µL	1.2 mL	389	780 c	39 c

Sources of compounds:
1: Dr. Ehrenstorfer
2: Bayer Crop Science
3: HPC (High Purity Compounds
4: Cambridge Isotope Lab. Inc

* market prices may be subject to changes
** 2 µg are typically employ if the ISTDs are added to the sample at the beginning of the procedure

*** 0.1 µg are typically added to a 1 mL sample portion of the extract

NOTES: *If detections of a compound are rather seldom the ISTD can be added in a second analysis in case the first one was positive. If however the retention time of a compound tends to shift, the addition of an ISTD is highly recommended, at least to the 1 mL aliquot. The Retention time of the ISTD will improve the certainty of identification.*

Table 11 : Water content of selected foods and amount of water, which has to be added to test sample prior to extraction (where ISTD is used)

Commodity group	Commodity	Typical water content g/100 g	Amount of water added to 10 g test portion g	Amount of water added to 5 g test portion g	Remarks
Fruits					
Citrus fruits	citrus juices	90			
	grapefruit	90			
	lemon/lime	85			
	orange	85			
	tangerine	90			
Pome fruit	apple	85			
	apple, dried	30		8.5 (see 5.1)	Weigh 13.5 g rehydratized homogenate
	apple sauce	80			
	apple juice	90			
	pear	85			
	quince	85			
Stone fruit	apricot	85			
	Apricot, dried	30		8.5 (see 5.1)	Weigh 13.5 g rehydratized homogenate
	apricot nectar	85			
	cherry	85			
	mirabelle	80			
	nectarine	85			
	peach	90			
	Peach, dried	20		8.5 (see 5.1)	Weigh 13.5 g rehydratized homogenate
	plum	85			
	plum. Dried	20		8.5 (see 5.1)	Weigh 13.5 g rehydratized homogenate
Soft and small fruits	blackberry	85			
	blueberry	85			
	currant	85			
	elderberry	80			

Commodity group	Commodity	Typical water content g/100 g	Amount of water added to 10 g test portion g	Amount of water added to 5 g test portion g	Remarks
	gooseberry	90			
	grapes	80			
	raspberry	85			
	raisin	20		8.5 (see 5.1)	Weigh 13.5 g rehydratized homogenate
	strawberry	90			
	pineapple	85			
Other fruits	banana	75	2.5		
	fig, dired	20		8.5 (see 5.1)	Weigh 13.5 g rehydratized homogenate
	kiwi	85			
	mango	80			
	papaya	90			
	papaya	90			
Vegetables					
Root and tuber vegetables	beetroot	90			
	carrot	90			
	celeriac	90			
	horseradish	75	2.5		
	parsley root	90			
	radish	95			
	black salsify	80			
	potato	80			
	garlic	60		7.0	
Leek plants	onion	90			
	leek	85			
	shallot	80			
	chive	85			
	aubergine	90			
Fruiting vegetables	cucumber	95			
	melon	90			
	pepper. Sweet	90			

Commodity group	Commodity	Typical water content g/100 g	Amount of water added to 10 g test portion g	Amount of water added to 5 g test portion g	Remarks
	pumpkin	95			
	tomato	95			
	zucchini (courgette)	95			
	broccoli	90			
Cabbage	` iquat` t sprouts	85			
	cauliflower	90			
	` iquat` cab-bage	95			
	kale	90			
	kohlrabi	90			
	red cabbage	90			
	savoy cabbage	90			
	white cabbage	90			
	lettuce varieties	95			
Leafy vegeta-bles and herbs	endive	95			
	cress	90			
	lamb's lettuce	85			
	parsley	80			
	rucola	85			
	spinach	90			
	asparagus	95			
Stem vegeta-bles	celery	95			
	leek	85			
	rhubarb	95			
	artichokes	85			
	beans, peas, lentils (dried)	<10		10	
Legumes	beans (fresh)	75	2.5		
	beans (fresh)	75	2.5		

Commodity group	Commodity	Typical water content g/100 g	Amount of water added to 10 g test portion g	Amount of water added to 5 g test portion g	Remarks
Miscellaneous					
Cereals	cereals (grain. Flour. Etc.)	10		10	
High Extract commodities	coffee beans	<10		10 (use 2 g test portion if extract-rich)	
	tea	<10			
	dry herbs and spices	<10			
Other	mushrooms	90			
	wine	90			

Table 12 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)	Jun. 2008	-
Drafting of the present document	Nov.-Dec. 2008	V 1
Placing of the present document in CRL-Website	Jan. 2009	V 1
Correction of Table 1, column “Expected concentration …” Expected concentrations of ISTDs were calculated with a wrong dilution factor in previous version. Arithmetical errors were corrected.	Aug. 2009	V 2
Introduction of measurement conditions for HEPA within the “Glyphosate & Co.” method		
Introduction of measurement conditions for the screening of ` iquat` t and ` iquat within the “Quats & Co. method”	Nov 2009	V3
Introduction of measurement conditions for Amitrol, chlormequat, mepiquat and daminozide “Amitrol & Co.” method	Nov 2009	V3
Extensive text revisions	Nov 2009	V3
Introduction of measurement conditions for Streptomycin Kasugamycin	May 2010	V4
Introduction of measurement conditions for the screening of Perchlorate ion	May 2010	V4
Extensive text revisions	May 2010	V4