

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 1100 D

Product name(s): CANDELA

Chemical active substance:

Glyphosate, 540 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

Submission date: February 2018

Update date: August 2020

Finalisation date: 18/10/2022

Version history

When	What
10/2018	Dossier sent for evaluation to Merit Mark (PL)
August 2020	Applicant update
10/2021	zRMS finalised evaluation
10/2022	Final version prepared by zRMS after Commenting period

Table of Contents

5	Analytical methods.....	4
5.1	Conclusion and summary of assessment.....	4
5.2	Methods used for the generation of pre-authorization data (KCP 5.1).....	4
5.2.1	Analysis of the plant protection product (KCP 5.1.1)	4
5.2.1.1	Determination of active substance and/or variant in the plant protection product (KCP 5.1.1).....	4
5.2.1.2	Description of analytical methods for the determination of relevant impurities (KCP 5.1.1).....	7
5.2.1.3	Description of analytical methods for the determination of formulants (KCP 5.1.1)	12
5.2.1.4	Applicability of existing CIPAC methods (KCP 5.1.1).....	12
5.2.2	Methods for the determination of residues (KCP 5.1.2).....	12
5.3	Methods for post-authorization control and monitoring purposes (KCP 5.2)	13
5.3.1	Analysis of the plant protection product (KCP 5.2)	13
5.3.2	Description of analytical methods for the determination of residues of Glyphosate (KCP 5.2).....	13
5.3.2.1	Overview of residue definitions and levels for which compliance is required	13
5.3.2.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2).....	14
5.3.2.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2).....	15
5.3.2.4	Description of methods for the analysis of soil (KCP 5.2).....	17
5.3.2.5	Description of methods for the analysis of water (KCP 5.2).....	18
5.3.2.6	Description of methods for the analysis of air (KCP 5.2).....	18
5.3.2.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2)	19
5.3.2.8	Other studies/ information	19
Appendix 1	Lists of data considered in support of the evaluation.....	20
Appendix 2	Detailed evaluation of submitted analytical methods	24
A 2.1	Analytical methods for Glyphosate	24
A 2.1.1	Methods used for the generation of pre-authorization data (KCP 5.1).....	24
A 2.1.2	Methods for post-authorization control and monitoring purposes (KCP 5.2)	24

Evaluator comments:

The text highlighted in grey was provided by the evaluator.

5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.

zRMS comment on residue analytical methods:

No new data are submitted. Sufficiently sensitive and selective analytical residue methods are available for the active substance. All data are discussed and accepted during glyphosate renewal.

Noticed data gaps are:

- none

Commodity/crop	Supported/ Not supported
Cereals (wheat, barley, rye, oats, triticale)	Supported
Maize	Supported
Oilseed rape	Supported
Sunflower	Supported
Pome fruits (apple, pear)	Supported
Grapevine	Supported
Stone fruit (peach, apricot, plums, cherry)	Supported

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of Glyphosate in plant protection product is provided as follows:

Comments of zRMS:	The method is accepted and may be applied for analysing active substance in the PPP.
-------------------	--

Reference: KCP 5.1.1-01

Report Validation of analytical method for determination of active ingredient content of Glyphosate 54% SL, Hetal K. Desai, 2017, report No. 228-2-12-15993

Guideline(s): Yes, SANCO/3030/99 rev. 4, OCSPP 830.1800 and ABNT NBR: 14029

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Principle of method:

The active ingredient content of Glyphosate 54% SL is determined by high performance liquid chromatography on a reversed phase column [C 8 (Inertsil)] using 0.1% ortho phosphoric acid in milli-Q water (100%) as mobile phase and UV detection at 195 nm.

Equipment:

Instruments	Model	Make / Supplier
Balance	MYA 5/2 Y	Radwag
	GR 202	Adair dutt
HPLC	LC 2030 C	Shimadzu
Sonicator	UCH-500W	Laboratory Instruments

Reagents:

Name	Grade	Source
Water	Milli-Q	Milli pore milli-Q water purification system
Orthophosphoric acid	HPLC	Fisher Scientific

Reference material:

Glyphosate, analytical standard.

Preparation of the Glyphosate reference standard solutions:

Weight (mg) of reference standard	Final volume (mL)	Volume made using	Purity (%)	Obtained concentration (mg/L)	Identification of reference standard stock solution
2.54	50	Mobile phase*	99.5	50.55	L1
5.12	50			101.89	L2
7.56	50			150.44	L3
5.10	25			202.98	L4
6.37	25			253.53	L5
7.76	25			308.85	L6
10.23	25			407.15	L7

*Mobile phase: 0.1% ortho phosphoric acid in milli-Q water

The reference standard solution (L4), concentration 202.98 mg/L was used for specificity.

Preparation of the blank formulation stock solution:

Weight (mg) of blank formulation	Final volume (mL)	Solution sonicated	Volume made up with
12.20	25	For 5 minutes	Mobile phase

Preparation of the sample solution:

Weight (mg) of blank formulation	Final volume (mL)	Solution sonicated	Volume made up with
12.01	25	For 5 minutes	Mobile phase

Instrumental parameters:

Instrument : HPLC [Shimadzu LC2010 AHT with LC Solution Software]
Column : C8 [Inertsil] [250mm x 4.6mm (i.d.), 5µm particle size]
Wave length : 195 nm
Flow rate : 0.5 mL/minute
Injection volume : 20 µL
Mobile phase : 0.1% ortho phosphoric acid in milli-Q water (100%)
Retention time (approx.) : 6.9 minutes

Validation - Results and discussions

Validation of HPLC Analytical Method

The analytical method for determination of active ingredient content of glyphosate 54% SL was validated. The validation covered the aspects namely: specificity, linearity, precision (%RSD), intermediate precision, accuracy, limit of detection, limit of quantitation and sensitivity.

Specificity

The specificity of the method was studied by injecting mobile phase, blank formulation, reference standard solution and sample solution. Since there was no interference between the peaks of active ingredient in reference standard solution, sample solution, blank formulation as well as mobile phase, the analytical method was considered to be specific for the analyte.

Linearity

The linearity of the method was established by injecting seven different concentrations (50.55 – 407.15 mg/L) of glyphosate reference standard solutions into HPLC in singlet and plotting the peak area against concentration (mg/L). The correlation coefficient (r) was 0.999.

Limit of Detection (LOD)

The LOD of the method was evaluated by injecting the lowest concentration linearity standard solution. The signal to noise ratio (S/N) was recorded in the chromatogram. The LOD was defined as the analyte concentration for which the signal to noise ratio would be 3. The calculated LOD of the method was 0.04 % w/w.

Limit of Quantitation (LOQ)

The LOQ of the method was evaluated by injecting the lowest concentration linearity standard solution. The signal to noise ratio (S/N) was recorded in the chromatogram. The LOQ was defined as the analyte concentration for which the signal to noise ratio would be 10. The calculated LOQ of the method was 0.14 % w/w.

Precision (% RSD)

Repeatability of the analytical method was determined by analysing 7 replicate preparations by analyst I of test item solutions and assayed for active ingredient content of test item in each replicate. According to Grubbs test, there were no outliers in the set of 7 results as the G_{MAX} and G_{MIN} were lower than the critical value of 2.02 for $n=7$.

Sample	Mean Content		
	(% w/w)	(g/L)	Precision (%RSD)
Glyphosate	44.06	543.84	0.09
Glyphosate IPA Salt	59.46	733.91	0.10

Accuracy (% Recovery)

Accuracy of the analytical method was determined by the method of standard addition to the blank formulation of the same test item. The blank formulation sample used for specificity fortified with reference substance in three fortification levels. The seven replicates determination was analysed for each level. The mean accuracy (%recovery) was 100.37 % for level I, 99.65% for level II and 100.65% for level III. The overall mean accuracy (% recovery) was 100.22%.

Sensitivity

Sensitivity of the method was expressed by gradient of linear regression curve and calculated using following formula:

$$\text{Sensitivity} = \frac{(\text{Peak area of Highest Concentration Calibration Standard} - \text{Peak area of Lowest Concentration Calibration Standard})}{(\text{Calculated concentration of Highest Concentration Calibration Standard} - \text{Calculated Concentration of Lowest Concentration Calibration Standard})}$$

The Sensitivity of analytical method was 1385.79 for glyphosate.

Table 5.2-1: Methods suitable for the determination of Glyphosate in plant protection product Glyphosate 54% SL

	Glyphosate
Author(s), year	Hetal K. Desai, 2017
Principle of method	high performance liquid chromatography on a reversed phase column [C 8 (Inertsil)] using 0.1% ortho phosphoric acid in milli-Q water (100%) as mobile phase and UV detection at 195 nm
Linearity (n=7) (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Concentration range: 50.55 to 407.15 Intercept (a): 3437.74 Slope (b): 1385.76 Correlation coefficient: 0.999
Precision – Repeatability Mean n = 7 (%RSD)	Mean Glyphosae content: 44.06 ± 0.04 % w/w (543.84 ± 0.51 g/L) Mean Glyphosate IPA salt: 59.46 ± 0.06 % w/w (733.91 ± 0.70 g/L) % RSD: 0.09 Acceptable % RSD (Horwitz): 1.52
Accuracy n = 7 (% Recovery)	Lower level (154.90) % recovery: 100.37 Nominal level (203.86) % recovery: 99.65 Upper level (250.78) % recovery: 100.65 Mean % recovery: 100.22 Acceptable limit (SANCO): 98 – 102
Interference/ Specificity	No interference
Comment	-

Conclusion

From the results of the analytical method validation, it is concluded that the analytical method is specific, sensitive, precise, and accurate for the analysis of Glyphosate 54% SL. The results of validation criteria are within the specified limits of SANCO/3030/99 rev.4 (2000), OCSPP 830.1800 and ABNT NBR 14029 guidelines.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

~~Study on-going.~~

An overview on the acceptable methods and possible data gaps for analysis of Glyphosate in plant protection product is provided as follows:

Comments of zRMS:	The method is accepted for analysing N-Nitroso-glyphosate (NNG) in the PPP.
-------------------	---

Reference: KCP 5.1.2-01

Report Glyphosate 54% w/v SL: Validation of the Analytical Method for the Determination of the N-Nitroso-glyphosate as Relevant Impurity Content, Mercedes Pardo Martinez, 2020, report No. CH – 0482/2020

Guideline(s): Yes, SANCO/3030/99 rev. 5

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Materials and methods

Principle of method:

This method is applicable to the quantitative determination of the N-Nitroso-glyphosate impurity in Glyphosate 54% w/v SL samples. The method has been validated by the analysis of N-Nitroso-glyphosate impurity standard and test item solutions. The determination of the N-Nitroso-glyphosate impurity is performed by HPLC, using an external standard and UV detector.

Equipment:

Equipment

- High performance liquid chromatograph equipped with UV/Vis or DAD detector, quaternary pump, auto sampler and software for instrument management and data reprocessing
- Analytical balance, 0.1 mg precision
- Technical balance, 0.1 g precision
- Refrigerator
- pHmeter
- Freezer
- Volumetric glassware: pipettes, flasks, measuring cylinders
- Usual laboratory glassware.

Reagents:

- Water, HPLC grade
- Dipotassium hydrogen phosphate (K_2HPO_4)
- Phosphoric acid 85% (H_3PO_4), reagent grade

Reference material:

- Impurity N-Nitroso-glyphosate (N-NO-glyphosate), analytical standard

Preparation of the test item solution

Using the analytical balance, weigh about 2500 mg of the test item into a 10.00 mL volumetric flask and make to volume with water.

If an impurity result is greater than 0.80 $\mu\text{g/g}$, the final solution must be suitably diluted using volumetric glassware.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of N-Nitroso-glyphosate impurity content in Glyphosate 54% w/v SL samples

	N-Nitroso-glyphosate (max. limit 0.44 $\mu\text{g/g}$ (0.44 mg/kg))
Author(s), year	Mercedes Pardo Martinez, 2020
Principle of method	HPLC/UV Confirmatory: HPLC/DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Five Working Standard Solutions. Nominal injected range from 20 ng/mL to 200 ng/mL, corresponding to a nominal content in the test item from 0.08 $\mu\text{g/g}$ to 0.80 $\mu\text{g/g}$. Correlation coefficient $r > 0.99$
Precision – Repeatability Mean n = 5	1.89

	N-Nitroso-glyphosate (max. limit 0.44 µg/g (0.44 mg/kg))
(%RSD)	
Accuracy (% Recovery)	Low level: 96 % High level: 100.4 %
Interference/ Specificity	The N-Nitroso-glyphosate (N-NO Glyphosate) impurity content in the Glyphosate 54% w/v SL test item was quantified by use of liquid chromatography HPLC/UV. The quantity of the impurity in sample solutions was determined by external standard method. The analytical method was shown to be specific for N-Nitroso-glyphosate impurity in Glyphosate 54% w/v SL sample.
LOQ	0.10 µg/g
Comment	

Conclusion

The method has been validated by the analysis of N-Nitroso-glyphosate impurity standard and test item solutions.

An overview on the acceptable methods and possible data gaps for analysis of Glyphosate in plant protection product is provided as follows:

Comments of zRMS:	Comment on study; acceptable or not; deficiencies, corrections, according to recent guidelines or not, used in evaluation or only as additional information
-------------------	---

Reference: KCP 5.1.2 01

Report Glyphosate 54% w/v SL: Validation of the Analytical Method for the Determination of the N Nitroso-glyphosate as Relevant Impurity Content, Mercedes Pardo Martinez, 2020, report No. CH 0482/2020

Guideline(s): Yes, SANCO/3030/99 rev. 5

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Materials and methods

Principle of method:

This method is applicable to the quantitative determination of the N Nitroso glyphosate impurity in Glyphosate 54% w/v SL samples. The method has been validated by the analysis of N Nitroso-glyphosate impurity standard and test item solutions. The determination of the N Nitroso-glyphosate impurity is performed by HPLC, using an external standard and UV detector.

Equipment:

Equipment

- High performance liquid chromatograph equipped with UV/Vis or DAD detector, quaternary pump, auto sampler and software for instrument management and data reprocessing
- Analytical balance, 0.1 mg precision
- Technical balance, 0.1 g precision
- Refrigerator

pHmeter
Freezer
Volumetric glassware: pipettes, flasks, measuring cylinders
Usual laboratory glassware.

Reagents:
Water, HPLC grade
Dipotassium hydrogen phosphate (K₂HPO₄)
Phosphoric acid 85% (H₃PO₄), reagent grade

Reference material:
Impurity N Nitroso glyphosate (N NO glyphosate), analytical standard

Preparation of the test item solution
Using the analytical balance, weigh about 2500 mg of the test item into a 10.00 mL volumetric flask and make to volume with water.
If an impurity result is greater than 0.80 µg/g, the final solution must be suitably diluted using volumetric glassware.

Validation – Results and discussions

Table 5.2-3: Methods suitable for the determination of N-Nitroso-glyphosate impurity content in Glyphosate 54% w/v SL samples

	N-Nitroso-glyphosate (max. limit 0.44 µg/g (0.44 mg/kg))
Author(s), year	Mercedes Pardo Martinez, 2020
Principle of method	HPLC/UV Confirmatory: HPLC/DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Five Working Standard Solutions. Nominal injected range from 20 ng/mL to 200 ng/mL, corresponding to a nominal content in the test item from 0.08 µg/g to 0.80 µg/g. Correlation coefficient $r > 0.99$
Precision – Repeatability Mean $n = 5$ (%RSD)	1.89
Accuracy (% Recovery)	Low level: 96 % High level: 100.4 %
Interference/ Specificity	The N Nitroso glyphosate (N NO Glyphosate) impurity content in the Glyphosate 54% w/v SL test item was quantified by use of liquid chromatography HPLC/UV. The quantity of the impurity in sample solutions was determined by external standard method. The analytical method was shown to be specific for N Nitroso-glyphosate impurity in Glyphosate 54% w/v SL sample.
LOQ	0.10 µg/g
Comment	

Conclusion

The method has been validated by the analysis of N-Nitroso-glyphosate impurity standard and test item solutions.

Comments of zRMS:	The method can be used for analysing Formaldehyde in the PPP.
-------------------	---

Reference: KCP 5.1.2-02

Report Glyphosate 54% w/v SL: Validation of the Analytical Method for the Determination of the Formaldehyde as Relevant Impurity Content, Mercedes Pardo Martinez, 2020, report No. CH – 0483/2020

Guideline(s): Yes, SANCO/3030/99 rev. 5

Deviations: No

GLP: Yes

Acceptability: Yes/No/Supplementary

Materials and methods

Principle of method:

This method is applicable to the quantitative determination of the Formaldehyde impurity in Glyphosate 54% w/v SL samples. The method has been validated by the analysis of Formaldehyde impurity standard and test item solutions. All solutions (test item and standard solutions) were dissolved in water and derivatized with an acid solution of 2,4-dinitrophenylhydrazine in order to obtain the derivatized product Formaldehyde- dinitrophenylhydrazine (Formaldehyde-DNPH) which is UV detected.

The determination of the Formaldehyde impurity is performed by HPLC, using an external standard and UV detector.

Equipment:

Equipment

- High performance liquid chromatograph equipped with UV/Vis or DAD detector, quaternary pump, auto sampler and software for instrument management and data reprocessing
- Analytical balance, 0.1 mg precision
- Technical balance, 0.1 g precision
- Ultrasonic bath
- Magnetic stirrer
- Mechanical shaker
- Refrigerator
- Volumetric glassware for titration: pipettes, flasks, measuring cylinders
- Usual laboratory glassware.

Reagents:

- Water, HPLC grade
- Acetonitrile, HPLC grade
- 2,4-dinitrophenylhydrazine (2,4-DNPH), reagent grade
- Hydrochloric acid 37 %, reagent grade
- Iodine, analytical grade
- Potassium iodide, analytical grade
- Starch paste 1 % water solution use free
- Sodium hydroxide 32 % solution
- Sulphuric acid 95-97 %, reagent grade.

Reference material:

Sodium thiosulphate 0.1 N standard solution

Formaldehyde, analytical standard.

Preparation of the test item solution

Using the analytical balance, an aliquot of about 1000 mg of the test item were weighed into five 25.00 mL volumetric flask and made up to volume with water.

Using volumetric pipettes, 1.00 mL of the test item and 2.00 mL of derivatization solution were transferred into a conical flask and let react for 15 minutes before analysis.

If an impurity result is greater than 600 µg/g, the final solution must be suitably diluted using volumetric glassware.

Validation - Results and discussions

Table 5.2-4: Methods suitable for the determination of formaldehyde impurity content in Glyphosate 54% w/v SL samples

	Formaldehyde (max. limit 0.44 µg/g (0.44 mg/kg))
Author(s), year	Mercedes Pardo Martinez, 2020
Principle of method	HPLC/UV Confirmatory: HPLC/MS/DAD.
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Five Working Standard Solutions. Nominal injected range from 80 ng/mL to 8000 ng/mL, corresponding to a nominal content in the test item from 6.00 µg/g to 600.00 µg/g. Each correlation coefficient $r > 0.99$
Precision – Repeatability Mean n = 5 (%RSD)	the Formaldehyde impurity content was not quantifiable in repeatability test, the precision was determined via the accuracy test with the lowest fortification level. 3.01%
Accuracy (% Recovery)	Low level: 79.7 % High level: 93.3 %
Interference/ Specificity	The analytical method results to be specific for formaldehyde impurity in Glyphosate 54% w/v SLsamples.
LOQ	16 µg/g
Comment	

Conclusion

The method has been validated by the analysis of formaldehyde impurity standard and test item solutions.

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

Not relevant.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method No. 284 is available for Glyphosate.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Please refer to post-registration methods.

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

5.3.2 Description of analytical methods for the determination of residues of Glyphosate (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	For sweet corn, oilseed rape, soya beans and maize: Glyphosate and N-acetyl-glyphosate Other commodities: Glyphosate	0.1 mg/kg	Reg. (EU) No 293/2013
Plant, high acid content		0.1 mg/kg	Reg. (EU) No 293/2013
Plant, high protein/high starch content (dry commodities)		0.1 mg/kg	Reg. (EU) No 293/2013
Plant, high oil content		0.1 mg/kg	Reg. (EU) No 293/2013
Plant, difficult matrices (hops, spices, tea)		0.1 mg/kg	Reg. (EU) No 293/2013
Muscle	Sum of Glyphosate and N-acetyl-glyphosate expressed as Glyphosate	0.05 mg/kg	Reg. (EU) No 293/2013
Milk		0.05 mg/kg	Reg. (EU) No 293/2013
Eggs		0.05 mg/kg	Reg. (EU) No 293/2013
Fat		0.05 mg/kg	Reg. (EU) No 293/2013
Liver, kidney		0.05 mg/kg	Reg. (EU) No 293/2013
Soil (Ecotoxicology)	Glyphosate and AMPA	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Glyphosate and AMPA	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Glyphosate and AMPA	38000 µg/L (Glyphosate) 12000 µg/L (AMPA)	Lowest LC ₅₀ from aquatic toxicity study on <i>Oncorhynchus mykiss</i> for Glyphosate Lowest NOEC from aquatic toxicity study on <i>Pimephales promelas</i> for AMPA
Air	Glyphosate	30 µg/m ³	AOEL sys: 0.1 mg/kg bw/d
Tissue (meat or liver)	-	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Glyphosate in plant matrices is given in the following tables.

Table 5.3-2: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: glyphosate and N-acetyl-glyphosate, expressed as Glyphosate (for sweet corn, oilseed rape, soya beans and maize), Glyphosate (for other plant commodities)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content	Primary	0.05 mg/kg	HPLC-MS/MS of underivatised analytes with phenyl-hexyl column	EU agreed (Pentz, A.M. and Bramble, F.Q. 2007)
	ILV	0.05 mg/kg	HPLC-MS/MS of underivatised analytes with phenyl-hexyl column	EU agreed
	Confirmatory (if required)	0.05 mg/kg	HPLC-MS/MS MRM with post-column derivatization or GC-MS after derivatization with trifluoroacetic acid and heptafluorobutanol (for Glyphosate)	EU agreed
High acid content	Primary	0.05 mg/kg	HPLC-MS/MS of underivatised analytes with phenyl-hexyl column	EU agreed
	ILV	0.05 mg/kg	HPLC-MS/MS of underivatised analytes with phenyl-hexyl column	EU agreed
	Confirmatory (if required)	0.05 mg/kg	HPLC with post-column derivatization and fluorescence detection or GC-MS after derivatization with trifluoroacetic acid and heptafluorobutanol (for Glyphosate, not required for N-acetyl-glyphosate)	EU agreed
High oil content	Primary	0.05 mg/kg	HPLC-MS/MS of underivatised analytes with phenyl-hexyl column	EU agreed
	ILV	0.05 mg/kg	HPLC-MS/MS of underivatised analytes with phenyl-hexyl column	EU agreed
	Confirmatory (if required)	0.05 mg/kg	HPLC with post-column derivatization and fluorescence detection or GC-MS after derivatization with trifluoroacetic acid and heptafluorobutanol (for Glyphosate)	EU agreed
High protein/high starch content (dry)	Primary	0.05 mg/kg	HPLC-MS/MS of underivatised analytes with phenyl-hexyl column	EU agreed
	ILV	0.05 mg/kg	HPLC-MS/MS of underivatised analytes with phenyl-hexyl column	EU agreed
	Confirmatory (if required)	0.05 mg/kg	HPLC with post-column derivatization and fluorescence detection or GC-MS after derivatization with trifluoroacetic acid and heptafluorobutanol (for Glyphosate)	EU agreed

Component of residue definition: glyphosate and N-acetyl-glyphosate, expressed as Glyphosate (for sweet corn, oilseed rape, soya beans and maize), Glyphosate (for other plant commodities)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: N-acetyl-glyphosate				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / EU agreed
High water content	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen P.K., 2016b
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber H. and Zetsch A., 2016a
High acid content	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen P.K., 2016b
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber H. and Zetsch A., 2016a
High oil content	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen P.K., 2016b
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber H. and Zetsch A., 2016a
High protein/high starch content (dry)	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen P.K., 2016b
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber H. and Zetsch A., 2016a

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Glyphosate RAR, October 2015
Not required, because:	-

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Glyphosate in animal matrices is given in the following tables.

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.025 mg/kg	HPLC-MS/MS of underivatized analytes with phenyl-hexyl column	EU agreed
	ILV	0.025 mg/kg	HPLC-MS/MS of underivatized analytes with phenyl-hexyl column	EU agreed
	Confirmatory (if required)	0.025 mg/kg	GC-MS based on derivatization with a mixture of trifluoroacetic anhydride and trifluoroethanol (for Glyphosate)	EU agreed
Eggs	Primary	0.025 mg/kg	HPLC-MS/MS of underivatized analytes with phenyl-hexyl column	EU agreed

Component of residue definition: sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	ILV	0.025 mg/kg	HPLC-MS/MS of underivatized analytes with phenyl-hexyl column	EU agreed
	Confirmatory (if required)	0.025 mg/kg	GC-MS based on derivatization with a mixture of trifluoroacetic anhydride and trifluoroethanol (for Glyphosate)	EU agreed
Muscle	Primary	0.025 mg/kg	HPLC-MS/MS of underivatized analytes with phenyl-hexyl column	EU agreed
	ILV	0.025 mg/kg	HPLC-MS/MS of underivatized analytes with phenyl-hexyl column	EU agreed
	Confirmatory (if required)	0.025 mg/kg	GC-MS based on derivatization with a mixture of trifluoroacetic anhydride and trifluoroethanol (for Glyphosate)	EU agreed
Fat	Primary	0.05 mg/kg	HPLC-MS/MS of underivatized analytes with phenyl-hexyl column	EU agreed
	ILV	0.05 mg/kg	Not required	EU agreed
Kidney, liver	Primary	0.05 mg/kg	HPLC-MS/MS of underivatized analytes with phenyl-hexyl column	EU agreed
	ILV	0.05 mg/kg	HPLC-MS/MS of underivatized analytes with phenyl-hexyl column	EU agreed

Component of residue definition: glyphosate				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / EU agreed
Milk	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016c
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Eggs	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016c
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Muscle	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016c
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Fat	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016c
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Kidney, liver	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016c
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b

Component of residue definition: AMPA				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / EU agreed
Milk	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016c
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Eggs	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016c
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Muscle	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016c
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Fat	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016c
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Kidney, liver	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016c
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b

Component of residue definition: N-acetyl-glyphosate				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / EU agreed
Milk	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016d
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Eggs	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016d
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Muscle	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016d
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Fat	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016d
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b
Kidney, liver	Primary	0.025 mg/kg	HPLC-MS/MS	Jensen K.P., 2016d
	ILV	0.025 mg/kg	HPLC-MS/MS	Weber J. and Zetzsch A., 2016b

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Glyphosate RAR, October 2015
Not required, because:	-

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Glyphosate in soil is given in the following tables.

Table 5.3-6: Validated methods for soil (if appropriate)

Component of residue definition: Glyphosate and AMPA			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.05 mg/kg	GC-MS after derivatization in a mixture of trifluoroacetic anhydride and trifluoroethanol	EU agreed Schneider V. 2001 – report PR01/006, MET2005-371
Confirmatory	0.05 mg/kg	GC-MS after derivatization in a mixture of trifluoroacetic anhydride and trifluoroethanol	EU agreed Scuter S.L. 1996 – report RR 96-059B, MET2000-699

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Glyphosate in surface and drinking water is given in the following tables.

Table 5.3-7: Validated methods for water (if appropriate)

Component of residue definition: Glyphosate and AMPA				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.03 µg/L	LC-MS/MS after derivatization with 9-Fluorenylmethylchlorformate (FMOc)	EU agreed Knoch, E. 2010 – report IF-10/01618859, ASB2012-12445
	ILV	0.03 µg/L	LC-MS/MS after derivatization with 9-Fluorenylmethylchlorformate (FMOc)	EU agreed Geschke, S. 2011 – report S10-02882, ASB2012-12426
	Confirmatory	0.03 µg/L	LC-MS/MS transition	EU agreed Knoch, 2010 ASB2012-12445 RAR, 2015
Surface water	Primary	0.03 µg/L	LC-MS/MS after derivatization with 9-Fluorenylmethylchlorformate (FMOc)	EU agreed Knoch, E. 2010 – report IF-10/01618859, ASB2012-12445
	Confirmatory	0.03 µg/L	LC-MS/MS transition	EU agreed RAR, 2015 Knoch, E. 2010 – report IF-10/01618859, ASB2012-12445

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Glyphosate in air is given in the following tables.

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: Glyphosate			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	5 µg/m ³	GC-MS after derivatization in a mixture of trifluoroacetic anhydride and trifluoroethanol	EU agreed Schenider V. 2001 – report PR01/007, MET2005-368
Confirmatory	-	Not required	EU agreed RAR, 2015

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

The active substance is not classified as toxic according to Regulation (EC) No 1272/2008 (CLP Regulation), therefore a method of analysis is not required for body fluids and tissues.

5.3.2.8 Other studies/ information

No new or additional studies have been submitted

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1-01	Hetal K. Desai	2017	Validation of analytical method for determination of active ingredient content of Glyphosate 54% SL, Hetal K. Desai, 2017, report No. 228-2-12-15993 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.1.2-01	Mercedes Pardo Martinez	2020	Glyphosate 54% w/v SL: Validation of the Analytical Method for the Determination of the N-Nitroso-glyphosate as Relevant Impurity Content Report No. CH – 0482/2020 GLP Unpublished	N	Sharda Cropchem Limited
KCP 5.1.2-02	Mercedes Pardo Martinez	2020	Glyphosate 54% w/v SL: Validation of the Analytical Method for the Determination of the Formaldehyde as Relevant Impurity Content Report No. CH – 0483/2020 GLP Unpublished	N	Sharda Cropchem Limited

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.1	Pentz, A.M., Bramble, F.Q.	2007	Analytical method for the determination of glyphosate and degradate residues in various crop matrices using LC/MS/MS DuPont-15444 revision-1 BVL-1748765, ASB2008-2635	N	DPB

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.1	Seal, S., Dillom, R.	2007	Independent laboratory validation of DuPont-15444, "Analytical method for the determination of glyphosate and degradate residues in various crop matrices using LC/MS/MS" Dupont-21313, Pyxant Labs Project no. 1763 BVL-1748767, ASB2008-2637	N	DPB
KCP 5.2.1	Klimmek, S.	2007	Validation of the analytical method DFG Method 405 for determination of residues of glyphosate and its metabolite AMPA in various plant materials 0FC00014427 ! FCS-0703V BVL-2309041, ASB2008-5606	N	EGT
KCP 5.2.1	Klimmek, S. Webber, H.	2008	First amendment to final report - Validation of the analytical method DFG Method 405 for determination of residues of glyphosate and its metabolite AMPA in various plant materials FCS-0703V BVL-2309043, ASB2008-5607	N	EGT
KCP 5.2.1	Webber H.	2012	Validation of an analytical method for the determination of glyphosate and AMPA in raw agricultural commodities using LC/MS/MS S11-03331 BVL-2309045, ASB2012-12489	N	EGT
KCP 5.2.2	Pentz, A.M., Bramble, F.Q.	2007	Analytical method for the determination of N-acetylglyphosate and other analytes in various animal matrices using LC/MS/MS DuPont-20009 BVL-1748766, ASB2008-2636	N	DPB
KCP 5.2.2	Karnik, S., Dillom, R.	2007	Independent laboratory validation of DuPont-2009, "Analytical method for the determination of N-acetylglyphosate and other analytes in various animal matrices using LC/MS/MS" DuPont-21372, Pyxant Labs Inc. ID: 1806 BVL-1748764, ASB2008-2634	N	DPB
KCP 5.2.2	Schneider, E.	2001	Validation of an analytical method for the determination of glyphosate in foodstuff of animal origin (meat, eggs, milk) (monitoring method) PR-1/005 BVL-2309057, MET2005-367	N	EGT

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2.3	Schneider, E.	2001	Validation of an analytical method for the determination of glyphosate in soil PR01/006 BVL-2309063, MET2005-371	N	EGT
KCP 5.2.4	Knoch, E.	2010	Validation of an analytical method: determination of glyphosate and AMPA in water matrices using FMOC derivatization, manual SPE cleanup and LC-MS/MS quantitation IF-10/01618859 BVL-2309065, ABS2012-12445	N	EGT
KCP 5.2.4	Geschke, S.	2011	Independent laboratory validation of an analytical method for the determination of residues of glyphosate and AMPA in drinking water S10-02882 BVL-2309067, ABS2012-12426	N	EGT
KCP 5.2.5	Schneider, E.	2001	Validation of analytical method for the determination of glyphosate in air PR01/007 BVL-2309069, MET2005-368	N	EGT
KCP 5.2.1/03	Jensen, P.K.	2016b	Analytical method for the determination of N-Acetyl glyphosate in matrices of plant origin MSL0027300 Monsanto Company GLP Unpublished	N	EGT
KCP 5.2.1/04	Weber, H., Zetzsch A.	2016a	Independent Laboratory Validation of an Analytical Method for the Determination of N-Acetyl glyphosate in Matrices of Plant Origin S15-04467/MOS-1503V Eurofins Agroscience Services GLP Unpublished	N	EGT
KCP 5.2.1/05	Jensen, P.K.	2016c	Analytical Method for the Determination of Glyphosate and AMPA in Matrices of Animal Origin MSL0027299 Monsanto Company GLP	N	EGT

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 5.2.1/06	Jensen, P.K.	2016d	Analytical Method for the Determination of N-Acetyl Glyphosate in Matrices of Animal Origin MSL0027301 Monsanto Company GLP Unpublished	N	EGT
KCP 5.2.1/07	Weber, H., Zetzsch A.	2016b	Independent Laboratory Validation of Analytical Methods for the determination of Glyphosate and its Metabolites N-Acetyl Glyphosate and AMPA in matrices of animal origin S15-04468/MOS-1504V Eurofins Agroscience Services GLP Unpublished	N	EGT

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Glyphosate

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

No new or additional studies have been submitted

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.1.2.1.1 ME-2000-01

A 2.1.2.1.1.1 Method validation

Reference: KCP 5.2.1/03

Report: Analytical method for the determination of N-Acetyl glyphosate in matrices of plant origin, Jensen P.K., 2016b, Unpublished study No. MSL0027300, Authority registration No.: N/A

Guideline(s): Guidance document SANCO/825/00 rev.8.1 of November 16, 2010, European Commission

US EPA OCSPP 860.1340 Residue Analytical Method

OECD Series on Testing and Assessment No. 72, Series on Pesticides No. 39 Guidance Document on Pesticide Residue Analytical Methods, 2007

Deviations: No

GLP: No

Acceptability: Yes

Materials and methods

N-acetyl glyphosate is isolated from crop matrices by extraction using high speed shaking with 0.1 % formic acid in water and methylene chloride containing stable isotope labelled internal standards. Following centrifugation, an aliquot of the aqueous phase extract is filtered prior to analysis.

N-acetyl glyphosate residue is determined by liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ion transitions (quantifier: 210→63, qualifier: 210→124 or 210→150) and quantitated using internal standards.

The limit of quantification (LOQ) is 0.025 mg/kg for all crop matrices.

Method performance and recovery data in crop matrices representing high water content (corn forage), high oil content (soybean and canola seed), dry (corn grain) and fruits with high acid content (oranges) are presented in this summary.

Results and discussions

Recoveries were obtained for the analysis of residues of N-acetyl-glyphosate for a high water (corn forage), high oil (soybean and canola seed), a high acid containing (oranges) and a dry crop (corn grain).

Results obtained are within guideline SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1 requirements (Mean recovery 70-110%; RSD ≤ 20%). The recovery results for N-acetyl-glyphosate are presented in

Tables below

Table A 1: Recovery results from method validation of N-acetyl glyphosate using the analytical method ME-2000-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
N-acetyl glyphosate, MRM <i>m/z</i> 210 → 63 (quantification)						
Corn grain	0.025	90-94	92	1.9	6	
	0.25	94-100	97	2.3	6	
	0.5	89-92	91	1.3	6	
	Overall	89-100	93	3.1	18	
Corn forage	0.025	92-99	96	2.6	6	
	0.25	98-101	99	1.1	6	
	0.5	91-94	93	1.4	6	
	Overall	91-101	96	3.2	18	
Soybean	0.025	84-99	92	6.6	6	
	0.25	89-94	92	2.2	6	
	0.5	88-98	92	4.4	6	
	Overall	84-99	92	4.5	18	
Canola	0.025	91-100	94	3.7	6	
	0.25	88-96	92	3.0	6	
	0.5	84-90	88	3.2	6	
	Overall	84-100	91	4.4	18	
Oranges	0.025	96-100	98	1.6	6	
	0.25	88-92	90	1.8	6	
	0.5	83-93	87	3.5	6	
	Overall	83-100	92	5.3	18	
N-acetyl glyphosate, MRM <i>m/z</i> 210 → 124 (confirmation) <i>m/z</i> 210 → 150 (confirmation) [#]						
Corn grain	0.025	92-100	96	3.5	6	
	0.25	92-97	93	2.0	6	
	0.5	91-94	92	0.9	6	
	Overall	91-100	94	2.7	18	
Corn forage	0.025	94-100	97	2.3	6	
	0.25	92-94	93	1.0	6	
	0.5	89-93	91	1.9	6	
	Overall	89-100	94	3.4	18	
Soybean	0.025	89-115	97	9.2	6	
	0.25	88-96	90	3.5	6	
	0.5	84-94	90	4.6	6	

Table A 1: Recovery results from method validation of N-acetyl glyphosate using the analytical method ME-2000-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
	Overall	84-115	92	7.3	18	
Canola	0.025	87-93	90	2.9	6	
	0.25	89-94	91	1.9	6	
	0.5	83-92	89	3.8	6	
	Overall	83-94	90	2.9	18	
Oranges	0.025	91-97	95	2.6	6	
	0.25	89-94	91	2.2	6	
	0.5	87-91	88	2.1	6	
	Overall	87-97	92	4.0	18	

For canola seed and soybean seed, an m/z 210 \rightarrow 150 was used for confirmation

Table A 2: Characteristics for the analytical method used for validation of N-acetyl glyphosate residues in crop matrices

Parameter	N-Acetyl glyphosate
Specificity	Analysis of control specimens of the different plant matrices with HPLC-MS/MS using two mass transitions yielded no residues of N-acetyl glyphosate above 30 % of the LOQ indicating that no significant interferences were present. Two mass transitions were monitored: m/z 210 \rightarrow 63 (quantification) m/z 210 \rightarrow 124 (confirmation) or m/z 210 \rightarrow 150 (confirmation)
Calibration (type, number of data points)	The linearity of the detector response was confirmed by making single determinations of 10 concentrations covering the ranges 1.5 to 1200 ng/mL (equivalent to 0.0075 to 6.0 mg/kg) for N-acetyl glyphosate in all plant matrices investigated. The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations.
Assessment of matrix effects is presented	Since matrix effects on detection are generally corrected by the use of the response ratio of analyte to isotopically enriched internal standard, no matrix effects were determined within this study.
Limit of determination (LOD)/quantification (LOQ)	The limit of quantification (LOQ) was established at 0.025 mg/kg for each matrix. The limit of detection (LOD) was determined separately in each matrix, but all LOD values were $\leq 30\%$ of the LOQ.

Conclusion

The applicability of method ME-2000-01 for analysis of residues of N-acetyl glyphosate in different crop matrices was tested, i.e. corn forage (high water), soybean and canola seed (high oil), oranges (high acid content matrix and corn grain (dry matrix).

The specimen extracts were analysed using liquid chromatography with mass selective detection (HPLC-MS/MS) and validated successfully according to SANCO/825/00 rev.8.1 (2010).

The method was proven to be specific, accurate and precise and good repeatability and recovery was found in all matrices. Therefore, this method can be used for monitoring of N-acetyl glyphosate in all tested matrix groups.

A 2.1.2.1.1.2 Independent laboratory validation

Reference:	KCP 5.2.1/04
Report	Independent Laboratory Validation of an Analytical Method for the Determination of N-Acetyl glyphosate in Matrices of Plant Origin, Weber H., Zetzsch A., 2016a, report No MSL0027695, document No S15-04467/MOS-1503V, Authority registration No: N/A
Guideline(s):	Guidance document SANCO/825/00 rev. 8.1 of 16-Nov-2010, European Commission
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

No addition or modification to the original method other than optimization of instrumental parameters was made.

Results and discussions

Recoveries were obtained for the analysis of residues of N-acetyl-glyphosate in tomato (fruit), orange (whole fruit), wheat (grain) and oilseed rape (seed). The validation test included analysis of five replicates fortified at the LOQ, and 10x LOQ for each matrix. All mean recovery values at fortification levels of 0.025 mg/kg and 0.25 mg/kg for N-Acetyl glyphosate for both ion mass transitions comply with the standard acceptance criteria of the guidance document SANCO/825/00 rev. 8.1., where mean recovery at each fortification level should be in the range of 70 - 120% with a relative standard deviation of $\leq 20\%$. The validation data are shown in the tables below.

Table A 3: Recovery results from independent laboratory validation of N-acetyl glyphosate for tomato, orange, wheat and oilseed rape using the analytical method ME-2000

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
N-acetyl glyphosate, MRM <i>m/z</i> 210 → 63 (quantification)						
Tomato fruit	0.025	101-105	104	1.5	5	
	0.25	104-107	105	1.3	5	
	Overall	101-107	104	1.4	10	
Orange whole fruit	0.025	98-102	101	1.8	5	
	0.25	100-105	102	2.2	5	
	Overall	100-105	102	2.1	10	

Table A 3: Recovery results from independent laboratory validation of N-acetyl glyphosate for tomato, orange, wheat and oilseed rape using the analytical method ME-2000

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
Wheat grain	0.025	90-98	99	1.1	5	
	0.25	94-99	100	3.2	5	
	Overall	90-99	95	3.5	10	
Oilseed rape seed	0.025	102-107	93	3.2	5	
	0.25	102-104	98	2.2	5	
	Overall	102-107	104	1.5	10	
N-acetyl glyphosate, MRM <i>m/z</i> 210 → 124 (confirmation)						
Tomato fruit	0.025	98-105	102	2.6	5	
	0.25	101-107	104	2.1	5	
	Overall	98-107	103	2.3	10	
Orange whole fruit	0.025	98-101	99	1.1	5	
	0.25	95-103	100	3.2	5	
	Overall	95-103	100	2.3	10	
Wheat grain	0.025	92-98	94	2.5	5	
	0.25	95-100	98	1.9	5	
	Overall	92-100	96	3.0	10	
Oilseed rape seed	0.025	103-111	106	2.9	5	
	0.25	105-109	106	1.5	5	
	Overall	103-111	106	2.2	10	

Table A 4: Characteristics for the analytical method used for independent laboratory validation of N-acetyl glyphosate residues in tomato, orange, wheat and oilseed rape

Parameter	N-Acetyl glyphosate
Specificity	Analysis of control specimens of the different plant matrices with HPLC-MS/MS using two mass transitions yielded no residues of N-acetyl glyphosate above 30 % of the LOQ indicating that no significant interferences were present. Two mass transitions were monitored: m/z 210 \rightarrow 63 (quantification) m/z 210 \rightarrow 124 (confirmation)
Calibration (type, number of data points)	The linearity of the detector response was confirmed by making single determinations of 8 concentrations covering the ranges 1.0 to 200 ng/mL (equivalent to 0.005 to 1.0 mg/kg) for N-acetyl glyphosate in all plant matrices investigated. The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations.
Assessment of matrix effects is presented	Since matrix effects on detection are generally corrected by the use of the

Table A 4: Characteristics for the analytical method used for independent laboratory validation of N-acetyl glyphosate residues in tomato, orange, wheat and oilseed rape

Parameter	N-Acetyl glyphosate
	response ratio of analyte to isotopically enriched internal standard, no matrix effects were determined within this study.
Limit of determination (LOD)/quantification (LOQ)	The limit of quantification (LOQ) was established at 0.025 mg/kg for each matrix. The limit of detection (LOD) was set at 0.0075 mg/kg, which is 30 % of the LOQ.

Conclusion

The analytical method ME-2000 validated for the determination of N-acetyl glyphosate residues in crops was successfully and independently validated for tomato (fruit), orange (whole fruit), wheat (grain) and oilseed rape (seed) at concentrations levels of LOQ at 0.025 mg/kg and 10x LOQ at 0.25 mg/kg.

A 2.1.2.1.1.3 Confirmatory method (if required)

Since two characteristic mass transitions were used to quantify N-acetyl glyphosate, the method achieves a high level of specificity and **no confirmatory method is required** to demonstrate the selectivity of the primary method.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.1.2.2.1 Analytical method 1

A 2.1.2.2.1.1 Method validation

Reference: KCP 5.2.1/05

Report: Analytical Method for the Determination of Glyphosate and AMPA in Matrices of Animal Origin, Jensen, P.K., 2016c, report No MSL0027299, document No ME-1951, Authority registration No: N/A

Guideline(s): In agreement with Guidance document SANCO/825/00 rev.8.1 of November 16, 2010, European Commission
US EPA OCSPP 860.1340 Residue Analytical Method
OECD Series on Testing and Assessment No. 72, Series on Pesticides No. 39 Guidance Document on Pesticide Residue Analytical Methods, 2007

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Glyphosate and AMPA are isolated from animal matrices by extraction using high speed shaking with 0.1 % formic acid in water and methylene chloride containing stable isotope labelled internal standards. Following centrifugation, an aliquot of the aqueous phase extract is purified using solid phase extraction and filtration.

Glyphosate and AMPA residues are determined by liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ion transitions (glyphosate: quantifier: 168→63, qualifier: 168→79; AMPA: quantifier: 110→63, qualifier: 110→79). The limit of quantification (LOQ) is 0.025 mg/kg for both analytes for all animal matrices.

Method performance and recovery data in animal matrices of cow milk, chicken egg, beef muscle, beef liver, and beef fat are presented in this summary.

Results and discussions

Recoveries were obtained for the analysis of residues of glyphosate and AMPA in beef muscle, liver, fat, chicken egg and cow milk. The validation test included analysis of six replicates fortified at the LOQ, 10x LOQ and 200x LOQ for each matrix.

All mean recovery values at fortification levels of 0.025 mg/kg, 0.25 mg/kg and 5.0 mg/kg for glyphosate and AMPA and for both ion mass transitions comply with the standard acceptance criteria of the guidance document SANCO/825/00 rev. 8.1., where mean recovery at each fortification level should be in the range of 70 - 120% with a relative standard deviation of ≤ 20 %. The validation data are shown in Tables below.

Table A 5: Recovery results from method validation of glyphosate for animal matrices using the analytical method ME-1951-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
Glyphosate, MRM <i>m/z</i> 168 → 63 (quantification)						
Meat	0.025	93-98	96	2.0	6	
	0.25	95-97	96	1.1	6	
	5.0	93-98	96	2.1	6	
	Overall	93-98	96	1.7	18	
Liver	0.025	100-106	103	2.4	6	
	0.25	96-98	97	1.1	6	
	5.0	94-98	96	1.6	6	
	Overall	94-106	98	3.6	18	
Fat	0.025	94-97	96	1.1	6	
	0.25	94-98	95	1.9	5	
	5.0	95-96	96	1.3	6	
	Overall	94-98	96	1.4	17	
Egg	0.025	94-101	99	2.7	6	
	0.25	97-99	98	0.8	6	
	5.0	95-101	98	2.0	6	

Table A 5: Recovery results from method validation of glyphosate for animal matrices using the analytical method ME-1951-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
	Overall	94-101	98	1.9	18	
Milk	0.025	97-102	99	1.6	6	
	0.25	95-99	96	1.4	6	
	5.0	92-96	94	1.5	6	
	Overall	92-102	97	2.5	18	
Glyphosate, MRM m/z 168 → 79 (confirmation) m/z 168 → 150 (alternate confirmation)[#]						
Meat	0.025	94-105	99	4.0	6	
	0.25	90-95	92	2.0	6	
	5.0	90-94	92	2.0	6	
	Overall	90-105	94	4.5	18	
Liver	0.025	92-101	96	3.9	6	
	0.25	87-94	91	2.7	6	
	5.0	87-102	91	7.2	6	
	Overall	87-102	93	5.2	18	
	0.025	100-111	103	4.6	6	m/z 168 → 150
	0.25	94-97	96	1.1	6	
	5.0	92-95	94	1.5	6	
	Overall	92-111	97	5.0	18	
Fat	0.025	99-106	103	2.7	6	
	0.25	92-98	95	2.7	5	
	5.0	95-100	97	2.3	6	
	Overall	92-106	98	4.1	17	
Egg	0.025	93-102	98	4.4	6	
	0.25	95-101	98	2.2	6	
	5.0	96-103	98	2.9	6	
	Overall	93-102	98	3.1	18	
Milk	0.025	91-98	94	2.8	6	
	0.25	91-99	94	3.3	6	
	5.0	90-94	92	1.9	6	
	Overall	90-99	93	2.8	18	

[#] For liver, an alternate ion transition m/z 168 → 150 was used for confirmation, due to elevated background levels in liver

Table A 6: Recovery results from method validation of AMPA for animal matrices using the analytical method ME-1951-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
AMPA, MRM m/z 110 \rightarrow 63 (quantification)						
Meat	0.025	100-104	102	1.4	6	
	0.25	99-102	100	1.0	6	
	5.0	97-101	99	1.1	6	
	Overall	97-104	101	1.8	18	
Liver	0.025	96-104	100	2.8	6	
	0.25	99-102	101	1.5	6	
	5.0	98-102	100	1.5	6	
	Overall	96-104	100	1.9	18	
Fat	0.025	96-102	100	2.7	6	
	0.25	97-103	100	2.3	5	
	5.0	98-102	99	1.5	6	
	Overall	96-103	100	2.1	17	
Egg	0.025	99-105	101	3.0	6	
	0.25	98-104	101	2.3	6	
	5.0	98-102	100	1.4	6	
	Overall	98-105	101	2.3	18	
Milk	0.025	92-104	97	4.1	6	
	0.25	99-103	101	1.1	6	
	5.0	98-101	100	1.4	6	
	Overall	92-104	100	2.9	18	
AMPA, MRM m/z 110 \rightarrow 79 (confirmation)						
Meat	0.025	99-104	101	1.6	6	
	0.25	98-102	100	1.8	6	
	5.0	97-102	99	2.0	6	
	Overall	97-104	100	1.9	18	
Liver	0.025	94-100	97	2.5	6	
	0.25	92-100	96	3.2	6	
	5.0	98-99	98	0.7	6	
	Overall	92-100	97	2.4	18	
Fat	0.025	100-107	103	2.4	6	
	0.25	98-102	100	1.6	5	
	5.0	96-99	98	1.5	6	
	Overall	96-107	100	3.1	17	
Egg	0.025	96-105	102	3.5	6	

Table A 6: Recovery results from method validation of AMPA for animal matrices using the analytical method ME-1951-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
	0.25	99-103	101	1.5	6	
	5.0	102-103	102	0.4	6	
	Overall	96-105	102	2.1	18	
Milk	0.025	100-103	102	1.0	6	
	0.25	99-104	101	2.2	6	
	5.0	98-102	101	1.6	6	
	Overall	98-104	101	1.6	18	

Table A 7: Characteristics for the analytical method used for validation of glyphosate and AMPA residues in animal matrices

	Glyphosate	AMPA
Specificity and selectivity	Analysis of control specimens of the different plant matrices with HPLC-MS/MS using two mass transitions yielded no residues of glyphosate above 30% of the LOQ indicating that no significant interferences were present. Two mass transitions were monitored: m/z 168 \rightarrow 63 (quantification) m/z 168 \rightarrow 79 (confirmation)	Analysis of control specimens of the different plant matrices with HPLC-MS/MS using two mass transitions yielded no residues of AMPA above 30% of the LOQ indicating that no significant interferences were present. Two mass transitions were monitored: m/z 110 \rightarrow 63 (quantification) m/z 110 \rightarrow 79 (confirmation)
Calibration (type, number of data points)	The linearity of the detector response was confirmed by making single determinations of 10 concentrations covering the ranges 0.0075 to 6.0 mg/kg for glyphosate and its metabolite AMPA in all animal matrices investigated. The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations.	
Assessment of matrix effects is presented	Since matrix effects on detection are generally corrected by the use of the response ratio of analyte to isotopically enriched internal standard, no matrix effects were determined within this study.	
Limit of determination (LOD)/quantification (LOQ)	The limit of quantification (LOQ) was established at 0.025 mg/kg for glyphosate and its metabolite AMPA in meat, liver, egg, milk and fat. The limit of detection (LOD) for each analyte in each matrix was determined separately, but all LOD values are $<30\%$ of the LOQ.	

Conclusion

The applicability of method ME-1951-01 was tested for analysis of residues of glyphosate and AMPA in beef muscle, liver, fat, chicken egg and cow milk.

The specimen extracts were analysed using liquid chromatography with mass selective detection (HPLC-MS/MS) and validated successfully according to SANCO/825/00 rev.8.1 (2010).

The method was proven to be specific, accurate and precise and good repeatability and recovery was found in all matrices. Therefore, this method can be used for monitoring of glyphosate and AMPA in all

tested animal matrices.

A 2.1.2.2.2 ME-1999-01

A 2.1.2.2.2.1 Method validation

Reference: KCP 5.2.1/06

Report Analytical Method for the Determination of N-Acetyl Glyphosate in Matrices of Animal Origin, Jensen, P.K., 2016d, report No MSL0027301, document No: N/A, Authority registration No: N/A

Guideline(s): In agreement with Guidance document SANCO/825/00 rev.8.1 of November 16, 2010, European Commission

US EPA OCSPP 860.1340 Residue Analytical Method

OECD Series on Testing and Assessment No. 72, Series on Pesticides No. 39 Guidance Document on Pesticide Residue Analytical Methods, 2007

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

N-acetyl glyphosate is isolated from animal matrices by extraction using high speed shaking with 0.1 % formic acid in water and methylene chloride containing stable isotope labelled internal standards. Following centrifugation, an aliquot of the aqueous phase extract is filtered prior to analysis.

N-acetyl glyphosate residue is determined by liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ion transitions (quantifier: 210→63, qualifier: 210→124 or 210→150). The limit of quantification (LOQ) is 0.025 mg/kg for all animal matrices.

Method performance and recovery data in animal matrices of cow milk, chicken egg, beef muscle, beef liver, and beef fat are presented in this summary.

Results and discussions

Recoveries were obtained for the analysis of residues of N-acetyl glyphosate in beef muscle, liver, fat, chicken egg and cow milk. The validation test included analysis of six replicates fortified at the LOQ, 10x LOQ and 200x LOQ for each matrix.

All mean recovery values at fortification levels of 0.025 mg/kg, 0.25 mg/kg and 5.0 mg/kg for N-acetyl glyphosate and for both ion mass transitions comply with the standard acceptance criteria of the guidance document SANCO/825/00 rev. 8.1., where mean recovery at each fortification level should be in the range of 70 - 120% with a relative standard deviation of ≤ 20 %. The validation data are shown in the tables below.

Table A 8: Recovery results from method validation of N-acetyl glyphosate for animal matrices using the analytical method ME-1999-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
N-acetyl glyphosate, MRM <i>m/z</i> 210 → 63 (quantification)						
Meat	0.025	93-102	98	4.2	6	
	0.25	91-100	95	4.6	6	
	5.0	86-92	89	2.7	6	
	Overall	86-92	94	5.4	18	
Liver	0.025	93-97	94	2.1	6	
	0.25	94-96	95	0.7	6	
	5.0	88-93	91	2.0	6	
	Overall	88-97	94	5.4	18	
Fat	0.025	88-99	95	4.6	6	
	0.25	90-96	93	2.7	6	
	5.0	88-93	89	2.5	6	
	Overall	88-99	92	4.1	18	
Egg	0.025	92-96	94	1.7	6	
	0.25	93-97	95	1.5	6	
	5.0	90-94	92	1.6	6	
	Overall	90-97	94	2.1	18	
Milk	0.025	93-96	94	99	6	
	0.25	92-93	93	0.4	6	
	5.0	88-90	89	1.1	6	
	Overall	92-96	92	2.8	18	
N-acetyl glyphosate, MRM <i>m/z</i> 210 → 124 (confirmation) <i>m/z</i> 210 → 150 (confirmation) [#]						
Meat	0.025	91-103	96	5.5	6	
	0.25	93-102	98	3.8	6	
	5.0	84-94	90	4.0	6	
	Overall	84-102	95	5.7	18	
Liver	0.025	81-97	88	6.9	6	
	0.25	90-95	93	2.1	6	
	5.0	90-96	93	2.4	6	
	Overall	81-96	91	4.7	18	
Fat	0.025	96-103	99	4.2	6	
	0.25	84-91	87	3.2	6	
	5.0	84-91	87	3.2	6	
	Overall	84-103	91	6.9	18	

Table A 8: Recovery results from method validation of N-acetyl glyphosate for animal matrices using the analytical method ME-1999-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
Egg	0.025	92-98	95	2.8	6	
	0.25	92-94	93	0.9	6	
	5.0	89-91	90	1.1	6	
	Overall	89-98	93	3.0	18	
Milk	0.025	92-97	95	2.1	6	
	0.25	91-93	92	0.7	6	
	5.0	87-90	89	1.2	6	
	Overall	87-97	92	3.1	18	

For liver, a ion transition m/z 210 \rightarrow 150 was used for confirmation

Table A 9: Characteristics for the analytical method used for validation of N-acetyl glyphosate residues in animal matrices

Parameter	N-Acetyl glyphosate
Specificity	Analysis of control specimens of the different plant matrices with HPLC-MS/MS using two mass transitions yielded no residues of N-acetyl glyphosate above 30 % of the LOQ indicating that no significant interferences were present. Two mass transitions were monitored: m/z 210 \rightarrow 63 (quantification) m/z 210 \rightarrow 124 (confirmation) or m/z 210 \rightarrow 150 (confirmation)
Calibration (type, number of data points)	The linearity of the detector response was confirmed by making single determinations of 10 concentrations covering the ranges 0.0075 to 6.0 mg/kg for N-acetyl glyphosate in all animal matrices investigated. The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations.
Assessment of matrix effects is presented	Since matrix effects on detection are generally corrected by the use of the response ratio of analyte to isotopically enriched internal standard, no matrix effects were determined within this study.
Limit of determination (LOD)/quantification (LOQ)	The limit of quantification (LOQ) was established at 0.025 mg/kg for each matrix. The limit of detection (LOD) for N-acetyl glyphosate in each matrix was determined separately, but all LOD values are <30% of the LOQ.

Conclusion

The applicability of method ME-1999-01 was tested for analysis of N-acetyl glyphosate residues in beef muscle, liver, fat, chicken egg and cow milk.

The specimen extracts were analysed using liquid chromatography with mass selective detection (HPLC-MS/MS) and validated successfully according to SANCO/825/00 rev.8.1 (2010).

The method was proven to be specific, accurate and precise and good repeatability and recovery was found in all matrices. Therefore, this method can be used for monitoring of N-acetyl glyphosate in all tested animal matrices.

A 2.1.2.2.2 Independent laboratory validation

Reference:	KCP 5.2.1/07
Report	Independent Laboratory Validation of Analytical Methods for the determination of Glyphosate and its Metabolites N-Acetyl Glyphosate and AMPA in matrices of animal origin, Weber H., Zetzsch A., 2016b, report No MSL0027696, document No S15-04468/MOS-1504V, Authority registration No: N/A
Guideline(s):	Guidance document SANCO/825/00 rev. 8.1 of 16-Nov-2010, European Commission
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

No addition or modification to the original method other than optimization of instrumental parameters was made.

Results and discussions

Recoveries were obtained for the analysis of residues of glyphosate, AMPA and N-acetyl-glyphosate in meat, liver, egg, milk and fat. The validation test included analysis of five replicates fortified at the LOQ, and 10x LOQ for each matrix.

All mean recovery values at fortification levels of 0.025 mg/kg and 0.25 mg/kg for glyphosate, AMPA and N-acetyl glyphosate and for both ion mass transitions comply with the standard acceptance criteria of the guidance document SANCO/825/00 rev. 8.1., where mean recovery at each fortification level should be in the range of 70 - 120% with a relative standard deviation of ≤ 20 %. The validation data are shown in Tables below.

Table A 10: Recovery results from independent laboratory validation of glyphosate for animal matrices using the analytical method ME-1951-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
Glyphosate, MRM <i>m/z</i> 168 → 63 (quantification)						
Meat	0.025	95-102	99	2.6	5	
	0.25	102-104	103	0.8	5	
	Overall	95-104	101	2.8	10	
Liver	0.025	94-99	96	1.9	5	
	0.25	93-103	98	4.1	5	
	Overall	93-103	97	3.2	10	
Milk	0.025	101-102	101	0.5	5	
	0.25	102-104	103	1.0	5	
	Overall	101-104	102	1.1	10	
Egg	0.025	86-91	88	2.2	5	
	0.25	101-106	103	1.9	5	

Table A 10: Recovery results from independent laboratory validation of glyphosate for animal matrices using the analytical method ME-1951-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
	Overall	86-91	96	8.4	10	
Fat	0.025	98-104	101	2.4	5	
	0.25	104-107	105	1.0	5	
	Overall	98-107	103	2.8	10	
Glyphosate, MRM <i>m/z</i> 168 → 79 (confirmation)						
Meat	0.025	88-98	93	4.6	5	
	0.25	104-109	107	1.9	5	
	Overall	88-109	100	8.4	10	
Liver	0.025	77-85	82	4.1	5	
	0.25	88-98	92	4.4	5	
	Overall	77-98	87	7.5	10	
Milk	0.025	99-109	104	4.4	5	
	0.25	107-110	109	1.0	5	
	Overall	99-110	106	3.8	10	
Egg	0.025	95-102	98	3.2	5	
	0.25	91-110	102	8.1	5	
	Overall	95-102	100	6.3	10	
Fat	0.025	92-105	98	5.4	5	
	0.25	104-109	106	2.0	5	
	Overall	92-109	102	5.7	10	

Table A 11: Recovery results from independent laboratory validation of AMPA for animal matrices using the analytical method ME-1951-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
AMPA, MRM <i>m/z</i> 110→ 63 (quantification)						
Meat	0.025	93-102	98	4.2	5	
	0.25	102-106	104	1.6	5	
	Overall	93-106	101	4.1	10	
Liver	0.025	104-109	108	1.9	5	
	0.25	108-110	109	0.6	5	
	Overall	104-110	108	1.5	10	
Milk	0.025	101-104	102	1.3	5	
	0.25	102-104	103	0.8	5	
	Overall	101-104	103	1.1	10	

Table A 11: Recovery results from independent laboratory validation of AMPA for animal matrices using the analytical method ME-1951-01

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
Egg	0.025	100-104	102	1.8	5	
	0.25	97-109	102	5.3	5	
	Overall	97-109	102	3.8	10	
Fat	0.025	98-99	98	0.5	5	
	0.25	104-105	104	0.4	5	
	Overall	98-105	102	3.5	10	
AMPA, MRM <i>m/z</i> 110 → 79 (confirmation)						
Meat	0.025	93-102	98	4.7	5	
	0.25	102-105	104	1.1	5	
	Overall	93-105	101	4.1	10	
Liver	0.025	103-114	107	3.9	5	
	0.25	108-110	109	0.8	5	
	Overall	103-114	108	2.8	10	
Milk	0.025	98-106	101	3.1	5	
	0.25	101-104	103	1.1	5	
	Overall	98-106	102	2.5	10	
Egg	0.025	95-101	97	2.4	5	
	0.25	100-109	104	3.4	5	
	Overall	95-109	101	4.4	10	
Fat	0.025	98-102	100	1.7	5	
	0.25	106-108	106	0.8	5	
	Overall	98-108	103	3.3	10	

Table A 12: Recovery results from independent laboratory validation of N-acetyl glyphosate for animal matrices using the analytical method ME-1999

Table A 13: Recovery results from independent laboratory validation of N-acetyl glyphosate for animal matrices using the analytical method ME-1999

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
N-acetyl glyphosate, MRM <i>m/z</i> 210 → 63 (quantification)						
Meat	0.025	88-92	89	1.9	5	
	0.25	91-101	95	4.4	5	
	Overall	88-101	92	4.7	10	

Table A 13: Recovery results from independent laboratory validation of N-acetyl glyphosate for animal matrices using the analytical method ME-1999

Matrix	Fortification level (mg/kg)	Recovery				Comments
		Range (%)	Mean (%)	RSD (%)	No. of analyses	
Liver	0.025	93-96	94	1.4	5	
	0.25	91-99	94	3.3	5	
	Overall	91-99	94	2.4	10	
Milk	0.025	85-89	86	1.9	5	
	0.25	91-94	93	1.2	5	
	Overall	85-94	90	4.2	10	
Egg	0.025	90-96	94	2.7	5	
	0.25	93-96	94	1.6	5	
	Overall	90-96	94	2.1	10	
Fat	0.025	95-100	97	2.2	5	
	0.25	96-100	98	1.7	5	
	Overall	95-100	97	1.9	10	
N-acetyl glyphosate, MRM <i>m/z</i> 210 → 124 (confirmation)						
Meat	0.025	80-92	86	5.1	5	
	0.25	85-91	89	2.6	5	
	Overall	80-91	87	4.3	10	
Liver	0.025	89-104	98	5.7	5	
	0.25	92-97	95	2.5	5	
	Overall	89-104	97	4.4	10	
Milk	0.025	92-99	96	2.9	5	
	0.25	90-98	94	3.1	5	
	Overall	90-99	95	2.9	10	
Egg	0.025	92-99	94	2.9	5	
	0.25	92-97	94	2.3	5	
	Overall	92-99	94	2.4	10	
Fat	0.025	94-99	97	2.2	5	
	0.25	94-97	95	1.4	5	
	Overall	94-99	96	2.0	10	

Table A 14: Characteristics for the analytical method used for independent laboratory validation of glyphosate, AMPA and N-acetyl glyphosate residues in animal matrices

	Glyphosate	AMPA	N-Acetyl glyphosate
Specificity	Analysis of control specimens of the different plant matrices with HPLC-MS/MS using two mass transitions yielded no residues of glyphosate above 30 % of the LOQ indicating that no significant interferences were present. Two mass transitions were monitored: m/z 168 → 63 (quantification) m/z 168 → 79 (confirmation)	Analysis of control specimens of the different plant matrices with HPLC-MS/MS using two mass transitions yielded no residues of AMPA above 30 % of the LOQ indicating that no significant interferences were present. Two mass transitions were monitored: m/z 110 → 63 (quantification) m/z 110 → 79 (confirmation)	Analysis of control specimens of the different plant matrices with HPLC-MS/MS using two mass transitions yielded no residues of N-acetyl glyphosate above 30 % of the LOQ indicating that no significant interferences were present. Two mass transitions were monitored: m/z 210 → 63 (quantification) m/z 210 → 124 (confirmation)
Calibration (type, number of data points)	The linearity of the detector response was confirmed by making single determinations of 8 concentrations covering the ranges 1.0 to 200 ng/mL (equivalent to 0.005 to 1.0 mg/kg) for glyphosate and its metabolite AMPA as well as for N-acetyl glyphosate in all plant matrices investigated. The coefficient of determination (R^2) was ≥ 0.99 for all analytical determinations.		
Assessment of matrix effects is presented	Since matrix effects on detection are generally corrected by the use of the response ratio of analyte to isotopically enriched internal standard, no matrix effects were determined within this study.		
Limit of determination (LOD) / quantification (LOQ)	The limit of quantification (LOQ) was established at 0.025 mg/kg for glyphosate and its metabolite AMPA as well as for N-acetyl glyphosate in meat, liver, egg, milk and fat. The limit of detection (LOD) was set at 0.0075 mg/kg, which is 30 % of the LOQ for all analytes in each matrix.		

Conclusion

Two analytical methods, ME-1951-01 and ME-1999 were successfully and independently validated for the determination of residues of glyphosate and its metabolite AMPA, and of N-acetyl glyphosate residues, respectively in meat, liver, egg, milk and fat at the LOQ of 0.025 mg/kg and 10x LOQ of 0.25 mg/kg.

The methods have been independently validated according to the EU guidelines SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1 (2010) and all validation requirements for the determination of glyphosate, AMPA and N-acetyl glyphosate residues in animal matrices at the LOQ (0.025 mg/kg) and $10 \times$ LOQ (0.25 mg/kg) were met.

A 2.1.2.2.2.3 Confirmatory method (if required)

Since two characteristic mass transitions were used to quantify glyphosate and its metabolites AMPA and

N-acetyl glyphosate, the method achieves a high level of specificity and **no confirmatory method is required** to demonstrate the selectivity of the primary method.

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted