DRAFT REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: A20607B

Product name(s): Vibrance SB

Chemical active substance(s):

Fludioxonil, 22.5 g/L
Metalaxyl-M, 14.4 g/L
Sedaxane, 15 g/L

~~United Kingdom~~

Great Britain (GB)

NATIONAL ASSESSMENT

~~(Renewal of authorisation)~~

Submitted to support Article 7 amendment of approval of Metalaxyl-M in GB

Applicant: Syngenta

Submission date: 21/10/2021

Finalisation date: 31/01/2024

Version history

When	What
October 2021	Applicant submission to support amendment of approval under Article 7 of retained Regulation (EC) No 1107/2009
December 2023	HSE (GB) assessment added in green boxes

This is an application from Syngenta for the renewal of VIBRANCE SB (A20607B) under Article 43 of Regulation (EC) No. 1107/2009 following the renewal of EU approval of the active substance Metalaxyl-M.

No equivalence assessment is required.

This application follows the data requirements for the active substance laid down in Regulation (EU) No. 544/2011 and the data requirements for the plant protection product laid down in Regulation (EU) No. 545/2011, also called 'old' data requirements. Metalaxyl-M is an 'AIR-2' substance which approval has been renewed in accordance with Regulation (EU) No 1141/2010, therefore Regulations (EU) No 283/2013 and (EU) No 284/2013 are not applicable to the renewal of authorizations for Metalaxyl-M-containing plant protection products (derogation by Commission Regulation (EU) No 2015/1475; further details in the guidance document SANTE/11509/2013 rev. 5.2).

Following the renewal of EU approval of the active substance Metalaxyl-M, the submission for the product renewal of VIBRANCE SB (A20607B) was made by 01 September 2020, in accordance with Article 43 of Regulation (EC) No 1107/2009.
All data relied on are provided with this application. The reference lists at Appendix 1 of dRR Part B Sections 1-10 define the data owner and data access. Data protection is a national concern and is addressed in Part A, Appendix 4.

The guidance on Renewal of Authorization according to Art 43 (SANCO/2010/13170 rev 14) requests that within the dRR 'changes to the risk assessment are highlighted'. This is the first submission of VIBRANCE SB (A20607B) in the dRR format of April 2015, consequently all of the summary text is previously unreviewed and should be considered as 'changed'. To facilitate the review, Syngenta has highlighted the summaries of reports not previously reviewed by the zRMS in yellow.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	The applicant, Syngenta Crop Protection AG, submitted this application to amend the conditions of approval of metalaxyl-M in accordance to Article 7 of Regulation 1107/2009 in Great Britain (GB). On the 5 May 2020 the Commission Implementing Regulation (EU) 2020/617 renewing the approval of the active substance metalaxyl-M, and restricting the use of seed treated with a plant protection product containing it to be sown only in greenhouses, was published[1]. The renewal of metalaxyl-M applies since 1 June 2020. Since this was before UK

[1] Commission Implementing Regulation (EU) 2020/617 of 5 May 2020 renewing the approval of the active substance metalaxyl-M, and restricting the use of seeds treated with plant protection products containing it, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of

	withdrawal from the EU, the Commission Implementing Regulation for the renewal of metalaxyl-M applies direct in GB. Two representative formulations were considered in the renewal of approval for metalaxyl-M, 'Apron XL' (A9642C) and 'Ridomil Gold Mz'/68 WG Fubol Gold' (A9651D). For this Article 7 amendment application in GB, two different formulations have been considered. The formulation 'Vibrance SB' (A20607B) containing 14.4 g/L metalaxyl-M, 22.5 g/L fludioxonil and 15.0 g/L sedaxane to support the field seed treatment use on sugar and fodder beet, and the formulation 'Wakil XL' (A9873C) containing 169.6 g/Kg metalaxyl-M, 100 g/Kg cymoxanil and 50 g/Kg fludioxonil) to support the field seed treatment use on peas (vining) are the basis of this Article 7 application for metalaxyl-M to GB. The applicant has re-submitted the draft registration reports prepared for the product renewals of 'Vibrance SB' and 'Wakil XL' under Article 43 of Regulation No 1107/2009 following the renewal of approval of the active substance metalaxyl-M. The information and data submitted within these draft registration reports have been considered previously by HSE for the applications for authorisation of a new product under Article 33 of Regulation No 1107/2009. Where relevant, re-evaluation of data or information has not occurred where studies have been performed in accordance with the current requirements and the results have been deemed acceptable. This draft registration report has been provided by the applicant, where required, comments have been inserted in green boxes by HSE or the text amended by the HSE in green (applicant's text has been struck through in green where necessary). HSE notes that the product authorisations for 'Vibrance SB' and 'Wakil XL' were withdrawn in GB by the applicant. This was based on the approval restriction provided for in Commission Implementing Regulation (EU) 2020/617 that only the treatment of seeds intended to be sown in greenhouses may be authorised. Since all authorised GB uses of 'Vibrance SB' and 'Wakil XL' products are on seeds which are direct drilled in the field, these products do not comply with the restriction and therefore could not be renewed under Article 43 of Regulation No 1107/2009. HSE notes that no authorisation for 'Vibrance SB' or 'Wakil XL' is sought within this Article 7 amendment application. Therefore, HSE has only considered the information presented in the draft registration reports that relate to metalaxyl-M. For a future GB authorisation of these products a separate application would be required with a full evaluation of the data and information for all active substances present in the formulation. Note that as of 1st January 2024, The Retained EU Law (Revocation and Reform) Act 2023 has taken effect and retained EU law are now known as assimilated law. As this assessment has been prepared prior to the Retained EU Law Act taking effect, assessment may still refer to "retained" regulation as opposed to "assimilated".

plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011

Table of Contents

5 Analytical methods

5.1 Conclusion and summary of assessment

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	'Vibrance SB' was not the representative product for the approval of metalaxyl-M. 'Vibrance SB' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Vibrance SB' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and sedaxane have not been considered further. 'Vibrance SB' is a FS formulation containing 14.4 g/L metalaxyl-M, 15 g/L sedaxane and 22.5 g/L fludioxonil. The applicant has access to the data considered in the DAR/RAR for metalaxyl-M as they are the data owner. This evaluation has been carried out in accordance with the Uniform Principles (as defined in Article 29 of Regulation (EC) No. 1107/2009) for active substance and product evaluation concerning the placing of plant protection products on the market. The renewal of 'metalaxyl-M' was assessed in accordance with the data requirements outlined in Regulation (No) 544/2011. Therefore, as methods of analysis data is considered active substance data, in accordance with the guidance document SANTE/11509 /2013– rev. 5.2 this methods assessment has been conducted in accordance with the same data requirements applied to the active. The information presented below in the RR has been written by the applicant, where required, comments have been inserted in green boxes by the HSE or the text amended by the HSE in green (applicant's text has been struck through in green where necessary). Sufficiently validated analytical methods are available for: • the active substance, metalaxyl-M in the plant protection product • the relevant impurities: CGA72649, CGA363736 in the plant protection product; methods are not available for CGA226048, however the current application seeks to remove CGA226048 as a relevant impurity in the GB approval, therefore this has not been considered further, depending on the outcome of the Art 7 application, methods may be required for CGA226048 for a future authorisation New data generation methods in support of support of efficacy, environmental fate, residues in plants, residues in animal products, and toxicology studies were not submitted and are not required. 2 additional methods were evaluated to support ecotoxicology studies; however, this was for dose verification only, see Appendix 2.2. Sufficiently validated analytical methods are available to allow monitoring of residues of metalaxyl-M in

	• plants in all crop groups (further data was submitted but not evaluated or required) • animal matrices (further data was submitted but not evaluated or required) • soil, water, and air • body fluids and tissues **Conclusion**: Sufficiently sensitive and selective analytical methods are available to support the plant protection product for the proposed uses.

State whether submitted data are sufficient for evaluation. Data gaps and conditions for authorization should be listed, if appropriate.

Sufficiently sensitive and selective analytical methods are not available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gaps are:

- data gap 1
- data gap 2
- data gap 3

Sufficiently sensitive and selective analytical methods are not available for all analytes included in the residue definitions.

Noticed data gaps are:

- data gap 1
- data gap 2
- data gap 3

Commodity/crop	Supported/ Not supported
Commodity/crop 1	
Commodity/crop 2	
Commodity/crop 3	

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)

The plant protection product A20607B has not been reviewed at EU level as a consequence of the review of Sedaxane, Fludioxonil and Metalaxyl-M.

An overview on the acceptable methods and possible data gaps for analysis of Sedaxane, Fludioxonil and

Metalaxyl-M (sum of R and S enantiomers), in plant protection product A20607B is provided as follows:

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	The applicant's summary as provided is an accurate representation of the study and validation data. The data below was previously relied upon for the original authorisation of the product in GB. This was based on a following zonal application, and the data was not specifically assessed by HSE; nevertheless, as the product will not be authorised based on this application, no further consideration has been made.

Reference:	KCP 5.1.1
Report	[redacted] (2014), ST-35/1 – Sedaxane, Fludioxonil and Metalxyl-M in Formulation FS (15/22.5/15) by HPLC. Syngenta Crop Protection, Münchwilen, Switzerland. Unpublished Report No. 300021938. Syngenta File No. VV-128321
Guideline(s):	No (method technical procedure)
Deviations:	N/A
GLP:	No (method technical procedure)
Acceptability:	Yes

Reference:	KCP 5.1.1
Report	[redacted] (2014), A20607B - Validation of Analytical Method ST-35/1. Syngenta Crop Protection, Münchwilen, Switzerland. Unpublished Report No. CHMU140402 Syngenta File No. VV-412231
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	N/A
GLP:	Yes
Acceptability:	Yes

An analytical method has been developed for the determination of the active substances Sedaxane, Fludioxonil and Metalaxyl-M (sum of R- and S-enantiomers) in formulation A20607B. In a first step, the active substances are analysed by non-chiral HPLC. For Metalaxyl-M, the analysis determines the sum of the active substance CGA329351 and its S-enantiomer CGA351920 (see analytical method STA-35/1). Then in a second step, the enantiomers are separated on a chiral column (see, analytical method STA-35/2).

Non Chiral Method ST-35/1 for the determination of Sedaxane, Fludioxonil and Metalaxyl-M (sum of R- and S-enantiomers) in A20607B

Materials and methods

The method provides for the simultaneous determination of Sedaxane (in the form of the stereoisomers SYN508210 and SYN508211), Fludioxonil and Metalaxyl-M in A20607B.

The active substances Sedaxane (stereoisomers SYN508210 and SYN508211), Fludioxonil and Metalaxyl-M (including the S-enantiomer) are determined in A20607B simultaneously by HPLC using a Agilent Zorbax XDB-C18 column and UV detection. Elution is done by a water/acetonitrile gradient. Detection was spectrophotometrically by a DAD UV detector operating at 220nm. Quantification was obtained by comparing peak areas of test samples with the areas from calibrated analytical standard solutions.

Validation - Results and discussions

Full validation of the method ST-35/1 has been conducted for A20607B. The method has been shown to be specific for the determination of Sedaxane (in the form of the stereoisomers SYN508210 and SYN508211), Fludioxonil and Metalaxyl-M (including its S-enantiomer) in the product A20607B and no significant interference was observed. Based on the results for repeatability, recovery, linearity and specificity, precision and accuracy of the method are established.

Table 5.2-1: Method suitable for the determination of active substances Sedaxane, Fludioxonil and Metalaxyl-M (including S-enantiomer) in product A20607B

	Sedaxane *trans*-isomers (SYN508210)	**Sedaxane *cis*-isomers (SYN508211)**	**Sedaxane**	**Fludioxonil**	**Metalaxyl-M (inc. S-enantiomer)**
Author(s), year	[redacted] (2014)				
Principle of method	HPLC-UV				
Linearity N=5 Tested between 50% - 150% of declared content	r = 0.99999 y = 1.010*X-0.176	r = 0.99981 y = 1.028*X-0.160	r = 0.99981 y = 1.011*X-0.358	r = 0.99999 y = 1.001*X+0.327	r = 0.99999 y = 1.000*X+0.505
Precision – Repeatability Mean value n = 5 (%RSD)	%RSD = 0.31 mean = 1.29% w/w	%RSD = 2.00 mean = 0.20% w/w	%RSD = 0.34 mean = 1.49% w/w	%RSD = 0.36 mean = 2.21% w/w	%RSD = 0.33 mean = 1.50% w/w
Accuracy n = 4 (% Recovery)	Mean recovery: 100.8%	Mean recovery: 100.9%	Mean recovery: 100.8	Mean recovery: 100.3%	Mean recovery: 100.5%
Interference/ Specificity	Specificity was established, no significant interference was observed. The analytical method is able to separate the active substances from the formulation blank and internal standard.				
Comment	The analytical method has been adequtely validated.				

Conclusion

Analytical method ST-35/1 is suitable for the specific, accurate and precise determination of Sedaxane (stereoisomers SYN508210 and SYN508211), Fludioxonil and Metalaxyl-M (including the S-enantiomer) in product Vibrance SB (A20607B).

Chiral Method STA-35/2 for the determination of Metalaxyl-M (CGA329351) and S-enatiomer (CGA351920) in A20607B

An analytical method has been developed for the determination of the enantiomers of Metalaxyl-M in A20607B. Metalaxyl-M consists of CGA329351 (R-enantiomer) and its manufacturing impurity CGA351920 (S-enantiomer).

Reference:	KCP 5.1.1
Report	[redacted] (2015), A20607B - Determination of CGA329351 and CGA351920 in Sedaxane/Fludioxonil/Metalaxyl-M FS (015/022.5/015) by chiral HPLC. Syngenta Crop Protection, Münchwilen, Switzerland. Unpublished Report No. 300036892 Syngenta File No. VV-128322
Guideline(s):	No (method technical procedure)
Deviations:	N/A
GLP:	No (method technical procedure)
Acceptability:	Yes

Reference:	KCP 5.1.1
Report	[redacted] (2015), A20607B - Validation of Analytical Method STA-35/2. Syngenta Crop Protection, Münchwilen, Switzerland. Unpublished Report No. 109713 Syngenta File No. VV-413006
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	N/A
GLP:	Yes
Acceptability:	Yes

Materials and methods

An analytical method has been developed for the determination of the enantiomers of Metalaxyl-M in A20607B. Metalaxyl-M consists of CGA329351 (R-enantiomer) and its manufacturing impurity CGA351920 (S-enantiomer).
The following analytical method for the determination of CGA329351 and CGA351920 in product A20607B has not previously been reviewed and is provided in support of this assessment.

Validation - Results and discussions

Full validation of the method STA-35/2 has been conducted for A20607B. The method has been shown to be specific for the determination of Metalaxyl-M (CGA329351) and its S-enantiomer (CGA351920) in the product performed on A20607B and no significant interference was observed. Based on the results for

repeatability, recovery, linearity and specificity, precision and accuracy of the method are established.

Table 5.2-2: Method suitable for the determination of active substances Metalaxyl-M (CGA329351) and its S-enantiomer (CGA351920) in A20607B

	Metalaxyl-M CGA329351 (R-enantiomer)	CGA351920 (S-enantiomer)	Metalaxyl-M (inc. its S-enantiomer)
Author(s), year	[redacted] (2015)		
Principle of method	HPLC-UV		
Linearity N=6 Tested between 50% - 150% of declared content	r = 0.99999 Y´= 0.989*X+0.015	r = 0.99885 Y´= 1.065*X-0.058	r = 0.99999 Y´= 0.991*X-0.074
Precision – Repeatability Mean value n = 5 (%RSD)	%RSD = 0.69 mean = 1.44% w/w	%RSD = 0.00 mean = 0.05% w/w	%RSD = 0.68 mean = 1.48% w/w
Accuracy n = 4 (% Recovery)	mean recovery: 98.9%	mean recovery: 102.6%	mean recovery: 98.9%
Interference/ Specificity	The specificity is confirmed, no interference was observed. The method is able to separate the active substances from the formulation blank and the internal standard.		
Comment	The analytical method has been adequtely validated.		

Conclusion

Analytical method STA-35/2 is suitable for the specific, accurate and precise determination of Metalaxyl-M (CGA329351) and S-enantiomer (CGA351920) in product Vibrance SB (A20607B).

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

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	The levels of the relevant impurities was not determined in the storage stability studies, neither pre- or post-storage. Nevertheless, the following methods have been described below. The Commission Implementing Regulation (EU) 2020/617 and GB approvals register state a maximum content of 0.5 g/kg (500 ppm) CGA72649 (2,6-dimethylphenylamine), 1.0 g/kg (1000 ppm) CGA363736 (4-methoxy-5-methyl-5H-[1,2]oxathiole 2,2-dioxide) and 0.18 g/kg CGA226048 (2-[(2,6-dimethyl-phenyl)-(2-methoxyacetyl)-amino]-propionic acid 1-methoxycarbonyl-ethyl ester in the technical material for metalaxyl-M. Considering a technical content of 14.4 g metalaxyl-M/L in 'A20607B', the theoretical maximum level of CGA72649, CGA363736 and CGA226048 are 0.007 g/L, 0.0145 g/L and 0.0025 g/L re-

spectively (equivalent to ~ 0.007 g/kg, 0.0145 g/kg and 0.0025 g/kg, based on the product density of 1032 g/L).

A method (AG-1837/2) for the determination of relevant impurity CGA72649 was presented in the RAR. However linearity and repeatability were determined using two other formulations (A9407A and A9408B). Therefore the RMS concluded that the method is not fully validated for linearity and repeatability. In addition, a method for the relevant impurity CGA363736 was not provided. Therefore at renewal there was a data gap for a method(s) to determine relevant impurities CGA72649 and CGA363736 in 'Apron XL'. The method SD-1751/1 for the determination of CGA72649 and CGA363736 in 'Apron XL' has been submitted under this application to address this data gap.

A new method for the determination of CGA72649 and CGA363736 in 'A9651D', a water dispersible granule formulation (WG) fungicide containing metalaxyl-M and mancozeb, has been submitted in the framework of the current application, but was also submitted for the previous evaluation of 'Apron XL'.

Three study reports have been submitted: Study report numbers 'VV-411110' and 'VV-128413' have been evaluated for a previous product evaluation. Study number 'VV-854722' is a statement which includes data, which describes the methods applicability to the current formulation ('A20607B').

Studies 'VV-411110' and 'VV-128413' were evaluated previously for 'Apron XL', using formulation 'A9651D', a WG formulation containing 3.88 w/w % metalaxyl-M and 64 w/w % mancozeb. The different nominal concentration of metalaxyl-M in the formulation is not considered to be an issue, the sample weighting is adjusted accordingly, to give a consistent concentration of metalaxyl-M, and the corresponding levels of impurities.

Method SD-1751/1 uses the standard addition procedure, calibration solutions are prepared by adding known amounts of CGA72649 and CGA363736 directly to formulation samples and diluting all samples to the same final volume. The resulting spiked solutions contain different known levels of CGA72649, ranging from between approx. 100 ppm to 700 ppm relative to metalaxyl-M, and of CGA363736, ranging from between approx. 200 ppm to 1400 ppm relative to metalaxyl-M, and by plotting the amounts of CGA72649 and CGA363736 added against their respective instrument responses (area of CGA72649 and CGA363736), the calibration curve is generated. One of the samples is prepared without the addition of CGA72649 and CGA363736, as it is from this sample that the actual content of CGA72649 and CGA363736 can be calculated using the calibration curve generated. Due to the fact that the analytes of interest, in this case CGA72649 and CGA363736, are directly added to the sample, all sample matrix effects with a potential influence on specificity, linearity, recovery, repeatability or the limit of quantification, can be accounted for.

The nominal for which the concentrations used in method validation are compared against for determination of the acceptability of the method are the maximum theoretical content of the impurities. For CGA72649 this is 500 ppm and for CGA363736 this is 1000 ppm (based on implementing reg).

The pre-spiking levels of the impurities were not given, for a future product authorisation, this information should be provided.

GC Conditions:

Chromatograph:	Thermo Trace GC ultra
Detector (mass spectrometer):	Thermo TSQ Quantum XLS For CGA72649: the selective reaction monitoring transition is m/z 121 → 106 using 15 V collision energy, segment start time 7 min, segment end time 9 min For CGA363736: the selective reaction monitoring transition is m/z 164 → 121 using 12 V collision energy, segment start time 9 min, segment end time 13 min
Column:	type: fused silica length: 15 m inside diameter: 0.32 mm stationary phase: DB-1701 film thickness: 1 µm
Column temperature:	75 °C,1 minute isothermal 10 °C/minute to 140 °C 25 °C/minute to 305 °C, 10 minute isothermal
Transfer line temperature:	295 °C
Injector temperature:	250 °C, split injector equipped with a split liner (5 mm straight without wool)
Carrier gas:	helium, flow rate 2.5 ml/minute, constant flow
Size of sample:	1 µl of test solution / spiked test solution
Split ratio:	20:1
Duration of chromatography:	approx. 24 minutes
Retention times:	CGA72649: approx. 7.8 min CGA363736: approx. 11.1 min

Summary of method validation data

Matrix	Analyte	LOQ (ppm)	Recovery fortification level (ppm)	% Recovery	Linearity	Specificity
A20607B	CGA72649	100 (equiva-lent to 0.1 g/kg)	98 (equivalent to 0.098 g/kg)	79.5	98 - 685 ppm (0.098 – 0.685 g/kg CGA72649 in TGAI in formulation, equivalent to 19.6 - 137% theoretical maximum CGA72649 in test solutions) y = 1074.93 x – 20626.36	Using MS/MS and standard addition mode, the specificity is established and no significant interference was observed.
			198 (equivalent to 0.198 g/kg)	95.0		
			290 (equivalent to 0.290 g/kg)	97.5		

			490 (equivalent to 0.490 g/kg)	106.6	r = 0.9967	
			685 (equivalent to 0.685 g/kg)	97.9		
A20607B	CGA363736	200 (equiva-lent to 0.2 g/kg)	184 (equivalent to 0.184 g/kg)	94.4	184 -1286 ppm (0.184 – 1.286 g/kg CGA363736 in TGAI in formulation, equivalent to 18 – 129 % theoretical maximum CGA363736 in test solutions) y = 40.53 x + 439.26 r = 0.9956	Using MS/MS and standard addition mode, the specificity is established and no significant interference was observed.
			371 (equivalent to 0.371 g/kg)	97.9		
			545 (equivalent to 0.545 g/kg)	100.6		
			920 (equivalent to 0.920 g/kg)	108.9		
			1286 (equivalent to 1.286 g/kg)	95.7		

Specificity:
Using a specific detection technique (MS/MS) and standard addition mode, the specificity was established and no significant interference was observed. Chromatograms of 'A20607B', batch SMU8B001 blank and spiked with approx. 300 ppm CGA72649 and 600 ppm CGA363736, relative to the amount of metalaxyl-M present in formulation, were presented. There was no interference of peaks. Therefore the method has been shown to be specific for the determination of CGA72649 and CGA363736 in formulation A20607B.

Linearity:
Linearity was demonstrated by the analysis of five standards of increasing concentration in

duplicate. The range of standard concentrations used was 98 - 660 ppm for CGA72649, equivalent to 0.098 – 0.660 g/kg in the product and 188 - 1264 ppm for CGA363736, equivalent to 0.188 – 1.264 g/kg in the product. The response was linear with a correlation coefficient (r) > 0.999.

Accuracy:
Recovery samples were prepared by spiking blank formulation with active substance standard at concentrations of 98 - 684 ppm for CGA72649 and 184 - 1286 ppm for CGA363736 and analysing them by the method described. Two samples were prepared at each fortification level. The spike concentrations were equivalent to 19.6 - 137% of the nominal concentration of 500 ppm for CGA72649 and 18 - 129% of the nominal concentration of 1000 ppm for CGA363736. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Mean recovery levels were within the range 79.5 to 106.6 for CGA72649 and 94.4 to 108.9 % for CGA363736.

Precision:
Repeatability was only originally tested for formulation 'A9651D'; additional repeatability data was submitted for another product authorisation ('Apron XL') using the formulation 'A9642C', an ES formulation. The repeatability data was as follows:
'The relative standard deviation obtained for CGA72649 was within the guideline requirements of a HORRAT (Hr) of ≤ 1. The RSD obtained for CGA363736 was >1 but <2; as such justification is typically required to support the high RSD. However, as the applicants case has been accepted with regards to the potential for formation of CGA363736 in the formulation, no justification has been requested at this time.'

This conclusion on repeatability is also accepted in this case, the validation data submitted for all other parameters for 'A20607B' is acceptable. Repeatability data is acceptable for two formulation types, including an ES formulation. Additionally, as stated, the case for the inability of the impurities to form on storage is accepted; and as the manufacturing sites for the active comply with the conditions of approval, the available information is acceptable in this case.

Conclusion:
The method for the determination of CGA72649 and CGA363736 in 'A20607B' is satisfactory validated in accordance with SANCO/3030/99 rev.5.

The LOQ is 100 ppm (0.1 g/kg) CGA72649 and 200 ppm (0.2 g/kg) for CGA363736. This is sufficient to cover the respective limits of 0.5 g/kg (500 ppm) CGA72649 (2,6-dimethylphenylamine) and 1.0 g/kg (1000 ppm) in the technical material as given in the approval conditions for metalaxyl-M (Commission Implementing Regulation (EU) 2020/617 and GB approvals register).

The pre-spiking levels of the impurities were not given, for a future product authorisation, this information should be provided.

The active substance Metalaxyl-M present in the formulated product A20607B contains the following relevant impurities:

- CGA72649 (2,6-dimethylaniline)
- CGA363736 (4-methoxy-5-methyl-5H-[1,2]oxathiole-2,2-dioxide)

These impurities may be formed in trace amounts during the chemical synthesis of Metalaxyl-M technical material however, it cannot be formed from Metalaxyl-M or from other formulation components of A20607B; storage stability data for CGA72649 and CGA363736 in formulated product Vibrance SB (A20607B) is therefore not required.

The following analytical methods for the determination of the relevant impurities CGA72649 and CGA363736 in the product A20607B have not previously been reviewed and are provided in support of this assessment.

Method SD-1751/1 for determination of relevant impurities CGA72649 and CGA363736 in A20607B

Reference:	KCP 5.1.1
Report	[redacted], [redacted], [redacted] (2014), Analytical method SD-1751/1: Determination of Metalaxyl-M Relevant Impurities CGA72649 and CGA363736 in formulation by GC/MS/MS. Syngenta Crop Protection Münchwilen AG Switzerland. Unpublished Report No. 300021240 Syngenta File No. VV-128413 (A9651D_10487)
Guideline(s):	No (method technical procedure)
Deviations:	N/A
GLP:	No (method technical procedure)
Acceptability:	Yes

Reference:	KCP 5.1.1
Report	[redacted] (2014), A9651D - Validation of analytical method SD-1751/1. Syngenta Crop Protection, Münchwilen, Switzerland. Unpublished Report No. CHMU140410 Syngenta File No. VV-411110 (A9651D_10488)
Guideline(s):	No
Deviations:	N/A
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.1.1
Report	[redacted] (2020), Statement on Validation of the Analytical Method SD-1751/1 for the determination of CGA72649 and CGA363736 in A20607B Sedaxane/Fludioxonil/Metalaxyl-M FS /015/022.5/015). Syngenta Crop Protection, Münchwilen, Switzerland. Unpublished Report no. 300162446 Syngenta File No. VV-854722

Guideline(s):	No (statement)
Deviations:	N/A
GLP:	No
Acceptability:	Yes

Materials and methods

The relevant impurities CGA72649 and CGA363736 are determined in formulation by gas chromatography on a 15 m fused silica DB-1701 column using helium as a carrier gas. Column temperature: 75°C up to 305°C. Detection is done by MS, monitoring for CGA72649 the transition m/z 121-> 106 and for CGA363736 the transition m/z 164-121. Quantification is achieved by standard addition method (internal standard).

Validation - Results and discussions

Full validation of the method SD-1751/1 has been performed for the Metalaxyl-M containing formulated product A9651D. Additionally, the method has been shown to be specific for the determination of CGA72649 and CGA363736 in product A20607B and no significant interference was observed. Based on the results for recovery, linearity and specificity the method is suitable for the specific, accurate and precise determination of CGA72649 and CGA363736 in product A20607B.

Table 5.2-3: Method suitable for the determination of relevant impurity CGA72649 and CGA363736 in A20607B

	CGA72649	**CGA363736**
Author(s), year	[redacted] (2020)	
Principle of method	GC/MS/MS	GC/MS/MS
Linearity **N=5**	r = 0.9967 y = 1074.9 x – 20626.36 (range of 98 ppm to 685 ppm relative to the amount of Metalaxyl-M)	r = 0.9956 y = 40.53 x – 439.26 (range of 184 ppm to 1286 ppm relative to the amount of Metalaxyl-M)
Precision – Repeatability **Mean value** **n = 6** **(%RSD)**	%RSD = 4.22 mean =291.39 ppm	%RSD = 2.94 mean =554.11 ppm
Accuracy **n = 5** **(% Recovery)**	Mean recovery = 95.3%	Mean recovery = 99.5
Interference/ Specificity	Using a specific detection technique (MS/MS) and standard addition mode, the specificity is established and no significant interference was observed	
Limit of quantification	The validation data prove that the limit of quantification for CGA72649 is below the 100 ppm level and for CGA363736 below the 200 ppm level (relative to the aount of Metalaxyl-M)	
Comment	The analytical method has been adequtely validated.	

Conclusion

Analytical method SD-1751/1 is suitable for the specific, accurate and precise determination of relevant impurities CGA72649 and CGA363736 in Vibrance SB (A20607B).

CGA226048 in Metalaxyl-M

Please note: Regarding impurity CGA226048 (2-[(2,6-dimethyl-phenyl)-(2-methoxyacetyl)-amino]-propionic acid 1-methoxycarbonyl-ethyl ester) as stated in Annex 1 of the Metalaxyl-M Implementing Regulation (EU) 2020/617 of 5 May 2020, an on-going EU evaluation is currently being finalised by the active substance RMS Belgium under Article 7 (submission of documentation 25th July 2019). Impurity CGA226048 is shown to be non-genotoxic and non-relevant. Hence, there is no need to provide an analytical method for the determination of CGA226048 within this product.

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

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	There are no relevant co-formulants in 'A20607B', therefore methods are not required

There are no formulants or constituents of formulants within the preparation or formed during storage, that are of toxicological, ecotoxicological or environmental relevance.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	'A20607B' contains more than one active substance. CIPAC methods for products that contain more than one active substance are not available

There are no CIPAC methods for the determination of Sedaxane, Fludioxonil and Metalaxyl-M.

There are no CIPAC methods for the determination of Sedaxane, Fludioxonil and Metalaxyl-M in FS formulations.

5.2.2 Methods for the determination of residues of Fludioxonil (KCP 5.1.2).

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	'Vibrance SB' was not the representative product for the approval of metalaxyl-M. 'Vibrance SB' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Vibrance SB' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and sedaxane have not been considered further.

An overview on the acceptable methods and possible data gaps for analysis of residues of Fludioxonil for the generation of pre-authorization data is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.2-4: Validated methods for the generation of pre-authorization data for Fludioxonil in soil, water, air (KCP 5.1.2.1 in support of environmental fate studies)

Component of residue definition: Fludioxonil				
Method type	**Matrix**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
REM 133.04	Soil	0.02 mg/kg	HPLC-UV	Method: [redacted], 1993 Report No.: REM 133.04 EU agreed (Denmark, 2005) Validation: [redacted], 2001 Report No.: 210/01 EU agreed (Denmark, 2005)

No analytical methods were used to support the efficacy data generated on this product.

No specific analytical methods were used to support the toxicological data generated on this product.

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-5: Validated methods for the generation of pre-authorization data for Fludioxonil in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Component of residue definition for plant and animal products: Fludioxonil				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
REM 133.01	High acid content *grape*	0.02 mg/kg	HPLC-UV	***Method:*** [redacted] 1989 Report No.:: REM 133.01 ***Validation:*** [redacted] 1989 Report No.: REM 133.01
	No group *wine*	0.005 mg/kg		

Component of residue definition for plant and animal products: Fludioxonil				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				EU agreed (Denmark 2005)
REM 133.04	High water content *tomato* *aubergine* *apple*	0.02 mg/kg	HPLC-UV	***Method:*** [redacted], 1993 Report No.: REM 133.04 ***Validation:*** [redacted], 1993 Report No.: REM 133.04 [redacted], 2001 Report No.: 210/01 EU agreed (Denmark 2005)
	High acid content *grape* *strawberry*	0.02 mg/kg		
	High starch content *Wheat grain*	0.02 mg/kg		
	No group *wine*	0.005 mg/kg		
REM 133.06	High water content *lettuce* *cauliflower* *pea seed/haulm/peas with pods* *apple* *cherry* *peach* *bulb onion* *carrot* *tomato* *melon* *asparagus* *celery* *witloof chicory*	0.01 mg/kg	LC-MS/MS	***Method:*** [redacted], 2006 Report No.: REM 133.06 ***Validation:*** [redacted] & [redacted], 2006 Report No.: RJ3773B [redacted], 2012 Report No.: R B0074 [redacted], 2014 Report No.: R B3113 [redacted], 2018 Report No.: R B7376 **New data**
	High protein/high starch content (dry) *wheat grain* *pea seed* *dried beans*	0.01 mg/kg		
	High oil *sunflower*	0.01 mg/kg		
	High acid content *orange* *kiwi* *strawberry* *blackcurrant*	0.01 mg/kg		
	No group *grape wine* *wheat straw* *pea haulm*	0.01 mg/kg		
AG-597	High water content: *corn forage* *sorghum fodder* *rice stalks*	0.01 mg/kg	HPLC-UV	***Method:*** [redacted], 1993 Report No.: AG-597 ***Validation:***
	High starch content	0.01 mg/kg		

Component of residue definition for plant and animal products: Fludioxonil				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	corn grain *sorghum grain* *rice grain* *potato tuber*	(0.05 mg/kg sorghum grain)		[redacted], 1993 Report No.: AG-597 EU agreed (Denmark 2005)
	No group *sorghum hay*	0.01 mg/kg		
AG-631A	High water content: *cherry* *apple* *pear* *peach* *plum* *forage/fodder*	0.02 mg/kg	HPLC-UV GP-NPD	***Method:*** [redacted] & van [redacted]., 1996 Report No.: AG-631A ***Validation:*** [redacted] & van [redacted]., 1996 Report No.: AG-631A EU agreed (Denmark 2005)
	High starch content *Cereal grain*	0.02 mg/kg		
	High acid content *grape*	0.02 mg/kg		
	No group *prune* *straw* *wine*	0.02 mg/kg 0.05 mg/kg 0.01 mg/kg		
-	Animal products, food of animal origin, (Residues)	Pre-authorisation methods are not required for animal products for the supported uses of A20607B as a seed treatment.		

Details of new studies not previously reviewed at EU level are given in appendix 2.

Methods and relationship to studies presented in the Part B Section 7 document.

The below table indicates which method has been used for each of the studies included within the Part B Section 7 document as well as the respective data point and report reference.

Method Identifier	**Study (Part B Section 7)**	
	Data Point	**Report Reference**
REM 133.01	KCA1 6.1	621/7-1012
REM 133.04		131/93, 221/98, 222/98, 210/00
AG-597		115-93
REM 133.04	KCA1 6.3/01	04-0315
	KCA1 6.3/02	04-0313
REM 133.06	KCA1 6.3/03	S18-02806
	KCA1 6.3/04	S18-02806
AG-631A	KCA1 6.6.2	174-97

No specific analytical methods were used to support the ecotoxicology data generated on this product.

No specific analytical methods were used to support the physical and chemical properties generated on this product.

5.2.3 Methods for the determination of residues of Metalayl-M (KCP 5.1.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	No new methods have been evaluated in the context of this evaluation, the sections below describe the available methods for various matrices. A number of ecotox methods have been evaluated in support of the current evaluation, these are detailed in Appendix 2.2.1.

An overview on the acceptable methods and possible data gaps for analysis of residues of Metalaxyl-M for the generation of pre-authorization data is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

No specific analytical methods for the generation of pre-authorization data for Metalaxyl-M in soil, water, air (in support of environmental fate studies) were used. Please see post-authorization methods for relevant soil, water and air methods.

No analytical methods were used to support the efficacy data generated on this product.

No analytical methods were used to support the toxicology data generated on this product.

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-6: Validated methods for the generation of pre-authorization data for Metalaxyl-M in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Component of residue definition: Metalaxyl-M				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
REM 181.01	High water content *Tomato*	0.02 mg/kg	GC-NPD (original method) GC-MSD (confirmation) LC-MS/MS (updated method)	**Method and Validation[(a)]:** [redacted], 1995 Report No.: REM 181.01 EU agreed (Belgium, 2014)
	High starch content *Potato*	0.02 mg/kg		
	High acid content *Grape*	0.02 mg/kg		
	High acid content *Citrus*	0.02 mg/kg		Validation: [redacted], 1999 Report No.: 517/99 EU agreed (Belgium, 2014)
	High acid content *Citrus peel, citrus pulp*	0.04 mg/kg		
	High oil content *Cotton*	0.02 mg/kg		Validation: [redacted], 1999

Component of residue definition: Metalaxyl-M				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	No group *Cotton hulls*	0.04mg/kg		Report No.: 518/99 EU agreed (Belgium, 2014)
	High oil content *Sunflower*	0.02 mg/kg		Validation: [redacted], 1999 Report No.: 519/99 EU agreed (Belgium, 2014)
	High water content *Witloof chicory leaves*	0.01 mg/kg		Validation: [redacted], 2005 Report No.: T004798-04 EU agreed (Belgium, 2014)
	High water content *Pome fruit, stone fruit pulp, carrot, onion, tomato, pepper, cucumber, melon, melon peel, melon pulp, flowering brassica, cabbage, lettuce, spinach, witloof chicory sprouts, bean pods, bean seeds, globe artichoke, leek, potato*	0.02 mg/kg		
	High water content *Tobacco green leaves*	0.1 mg/kg		
	High protein content *Dry bean*	0.02 mg/kg		
	High starch content *witloof chicory roots*	0.02 mg/kg		
	High acid content *Citrus, citrus peel, citrus pulp, berries, strawberry, kiwi peel, kiwi pulp*	0.02 mg/kg		
	High acid content *Kiwi peel*	0.04 mg/kg		
	No group *Wine, cocoa*	0.02 mg/kg		

Component of residue definition: Metalaxyl-M				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	No group *Tobacco dried leaves*	0.2 mg/kg		
-	Animal products, food of animal origin, (Residues)	Pre-authorisation methods are not required for animal products for the supported uses of A20607B as a seed treatment.		

(a) Metalaxyl-M was indicated as analyte because recovery was determined with samples spiked with Metalaxyl-M but it has to be noted that the method is not enantioselective. Therefore, Metalaxyl (R+S) is detected.

Component of residue definition: Metalaxyl-M				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
REM 181.13/A	High water content *Peach, tomato*	0.01 mg/kg	LC-MS/MS	Method[(a)]: [redacted] 2005 Reports: REM 181.13 Validation: [redacted] 2005 Report: RJ3585B 04-S624 [redacted] 2005 Reports: REM 181.13A[(b)] EU agreed (Belgium, 2014)
	High oil content *Oilseed rape*	0.01 mg/kg		
	High starch content *Carrot*	0.01 mg/kg		
	High acid content *Orange*	0.01 mg/kg		
	No group *Hops*	0.01 mg/kg		

(a) Metalaxyl-M was indicated as analyte because recovery was determined with samples spiked with Metalaxyl-M but it has to be noted that the method is not enantioselective. Therefore, Metalaxyl (R+S) is detected.
(b) This method is a minor modification of REM 181.13, due to the addition of text to the method. No further validation was performed.

Methods and relationship to studies presented in the Part B Section 7 document.

The below table indicates which method has been used for each of the studies included within the Part B Section 7 document as well as the respective data point and report reference.

Method	**Study (Part B Section 7)**	
Identifier	**Data Point**	**Report Reference**
REM181.06	KCA2 6.1	201/01 (plant)
REM181.13A	KCA2 6.3.1/01	S18-02612
REM181.13A	KCA2 6.3.1/02	S18-02613
REM181.01	KCA2 6.6.2	208/98
REM181.01		209/98
REM181.01		210/98
REM 181.13A		S11-00510
REM 181.13A		S11-00511

No specific analytical methods were used to support the ecotoxicology data generated on this product.

No specific analytical methods were used to support the physical and chemical properties generated on this product.

5.2.4 Methods for the determination of residues of Sedaxane (KCP 5.1.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	'Vibrance SB' was not the representative product for the approval of metalaxyl-M. 'Vibrance SB' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Vibrance SB' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and sedaxane have not been considered further.

An overview on the acceptable methods and possible data gaps for analysis of residues of Sedaxane for the generation of pre-authorization data is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

No specific analytical methods for the generation of pre-authorization data for Sedaxane in soil, water, air (in support of environmental fate studies) were used. Please see post-authorization methods for relevant soil, water and air methods.

No specific analytical methods were used to support the efficacy data generated on this product.

No analytical methods were used to support the toxicology data generated on this product.

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-7: Validated methods for the generation of pre-authorization data for Sedaxane in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Component of residue definition for plant and animal products: Sedaxane (sum of SYN508210 and SYN508211)				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
GRM023.01A GRM023.01B	High protein/high starch content (dry) *Wheat grain, lentil, potato, carrot*	0.01 mg/kg [(a)]	LC-MS/MS	Method: [redacted] & [redacted], 2008 & 2009 Report: GRM023.01A & GRM023.01B Validation: [redacted], 2008 Report: SYN-0705V EU agreed (France, 2012)
	High water content *Spinach, tomato*	0.01 mg/kg [(a)]		
	High acid content *Orange*	0.01 mg/kg [(a)]		
	High oil content *Oilseed rape*	0.01 mg/kg [(a)]		

Component of residue definition for plant and animal products: Sedaxane (sum of SYN508210 and SYN508211)				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	No group *Wheat straw, whole plant*	0.01 mg/kg [(a)]		Validation (additional potato data): North, 2015 Report: S14-01326 **New data**
GRM023.03A	High protein/high starch content (dry) *Wheat grain, maize kernel, lentil, potato, carrot*	0.01 mg/kg [(a)]	LC-MS/MS	Method: [redacted], 2010 Report: GRM023.03A Validation: [redacted] & [redacted], 2009 Report SYN-0843V [redacted], 2008 CEMR-3642(c) EU agreed (France, 2012)
	High water content *Spinach, tomato*	0.01 mg/kg [(a)]		
	High acid content *Orange*	0.01 mg/kg [(a)]		
	High oil content *Oilseed rape, soybean*	0.01 mg/kg [(a)]		
	No group *Wheat straw, whole plant*	0.01 mg/kg [(a)]		
GRM023.10A	Milk, eggs and tissues	0.01 mg/kg [(a)]	LC-MS/MS	Method: [redacted], 2009 Report: GRM023.10A Validation: [redacted], 2009 Report : T014679-05 EU agreed (France, 2012) See also 5.3.2.
GRM023.11A	High protein/high starch content (dry) *Wheat grain, carrot*	0.01 mg/kg [(a)]	LC-MS/MS	Method: [redacted], 2010 Report: GRM023.11A Validation: [redacted] & [redacted], 2010 Report: SYN-0951V EU agreed (France, 2012)
	High water content *Spinach*	0.01 mg/kg [(a)]		
	High oil content *Oilseed rape*	0.01 mg/kg [(a)]		
	No group *Wheat straw, whole plant*	0.01 mg/kg [(a)]		

(a): 0.005 mg/kg for each isomer (SYN508210 and SYN508211)

Methods and relationship to studies presented in the Part B Section 7 document.

The below table indicates which method has been used for each of the studies included within the Part B Section 7 document as well as the respective data point and report reference.

Method Identifier	Study (Part B Section 7)	
	Data Point	Report Reference
GRM023.11A	KCA3 7.4.1	T012299-05-REG
GRM023.03A	KCA3 7.4.1	KP-2009-02
GRM023.01A/B†	KCA3 A 2.3.3	S13-01026
		S13-01027
GRM023.010A	KCA3 7.4.4	Report 30634

†Minor differences between revision A and B – essentially same method.

No specific analytical methods were used to support the ecotoxicology data generated on this product.

No specific analytical methods were used to support the physical and chemical properties generated on this product.

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 Fludioxonil (KCP 5.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	'Vibrance SB' was not the representative product for the approval of metalaxyl-M. 'Vibrance SB' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Vibrance SB' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and sedaxane have not been considered further.

5.3.2.1 Overview of residue definitions and levels of Fludioxonil 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	Fludioxonil	0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2019/1791
Plant, high acid content		0.01 mg/kg	
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	
Plant, high oil content		0.01 mg/kg	
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	
Muscle	Sum of Fludioxonil and its metabolites oxidized to metabolite 2,2-difluoro-benzo[1,3]dioxole- 4 carboxylic acid (CGA 192155), expressed as Fludioxonil	0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2019/1791
Milk		0.01 mg/kg	
Eggs		0.05 mg/kg	
Fat		0.05 mg/kg	
Liver, kidney		0.05 mg/kg	
Soil (Ecotoxicology)	Fludioxonil	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Fludioxonil	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Fludioxonil	5 µg/L	NOEC Daphnia magna EFSA Scientific Report (2007) 110, 1-85. ASB2012- 3640
Air	Fludioxonil	177 µg/m^3	AOEL sys: 0.59 mg/kg bw/d (EFSA 2007)
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 Fludioxonil in plant matrices (KCP 5.2.1)

An overview on the acceptable methods and possible data gaps for analysis of Fludioxonil in plant matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: fludioxonil				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	DFG S19	0.02 mg/kg	LC-MS/MS (multi-residue)	**DFG S19** Method: [redacted], 2001 Validation (tomato, orange, oilseed rape, wheat grain): [redacted], 2001 Report: SYN-0103V Validation (citrus, kiwi, wheat grain): [redacted], 2005 Report: SYN-0503V ILV (tomato): [redacted], 2001 Report: SYN-0104V ILV (kiwi, oilseed rape, avocado): [redacted], 2006 Report: IF-05/00362984 EU agreed (Denmark, 2005) --- **QuEChERS** Validation (lettuce, orange, dried bean, oilseed rape seed, wheat straw): [redacted] & [redacted], 2014 Report: P-3446 G ILV (lettuce, dried bean, oilseed rape seed, wheat straw): [redacted] & [redacted], 2014 Report: 20140189 **New data**
	ILV (DFG S19)	0.02 mg/kg		
	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (QuEChERS)	0.01 mg/kg		
High acid content	DFG S19	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (DFG S19)	0.01 mg/kg		
	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (QuEChERS)	--		
High oil content	DFG S19	0.02 mg/kg	LC-MS/MS (multi-residue)	
	ILV (DFG S19)	0.01 mg/kg		
	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (QuEChERS)	0.01 mg/kg		
High protein/high starch content (dry)	DFG S19	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (DFG S19)	--		
	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (QuEChERS)	0.01 mg/kg		

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	**DFG S19:** The polarity of the acetone/water extraction solution used in DFG S19 is similar to that of the extraction procedures used in metabolism studies previously reviewed (see section 7.3 of the dRR). Therefore, DFG S19 method for crops extraction efficiency has been adequately demonstrated.

	Method for products of plant origin
Not required, because:	**QuEChERS** (EN 15662:2009-02) is a standard multi-residue method Data according to SANTE 2017/10632 Rev. 3 is not required for this active substance in the context of the product renewal of Metalaxyl-M. Data will be provided during the product renewal of fludioxonil.

5.3.2.3 Description of analytical methods for the determination of residues Fludioxonil in animal matrices (KCP 5.2.2)

The use of A20607B is expected to result in residues of Fludioxonil below the LOQ in relevant animal feed items. Therefore, the use of A20607B will not result in residues of Fludioxonil in animal feed items, and so the possible transfer of residues in animal commodities from the proposed uses does not need to be considered. Methods of analysis for residues in animal matrices are not required; however an overview on the acceptable methods for monitoring and possible data gaps for analysis of residues of Fludioxonil in animal matrices is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-4: Validated methods for food and feed of animal origin

Component of residue definition: Sum of Fludioxonil and its metabolites oxidized to metabolite 2,2-difluoro-benzo[1,3]dioxole- 4 carboxylic acid (CGA 192155), expressed as Fludioxonil				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Milk	AG-616B	0.01 mg/kg	HPLC-UV	**AG-616B** *Method:* [redacted], 1996 *Validation (milk, eggs, muscle, fat liver, kidney):* [redacted] 1996 Report: AG-616B *ILV (milk, eggs, liver):* [redacted], 1996 Report: 96-0010 EU agreed (Denmark, 2005) --- **GRM025.03A** *Method:* [redacted], 2008 Report: GRM025.03A (*not submitted*) [redacted], 2009 Report: GRM025.03A version 2 *Validation (milk, eggs, muscle, fat, liver, kidney, whole blood):* Sole C, 2009 Report: T001341-08-REG
	ILV (AG-616B)	0.01 mg/kg		
	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	--		
Eggs	AG-616B	0.05 mg/kg	HPLC-UV	
	ILV (AG-616B)	0.05 mg/kg		
	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	0.01 mg/kg		
Muscle	AG-616B	0.05 mg/kg	HPLC-UV	
	ILV (AG-616B)	--		
	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	0.01 mg/kg		
Fat	AG-616B	0.05 mg/kg	HPLC-UV	
	ILV (AG-616B)	--		
	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	0.01 mg/kg		
Kidney, liver	AG-616B	0.05 mg/kg	HPLC-UV	
	ILV (AG-616B)	0.05 mg/kg		

Component of residue definition: Sum of Fludioxonil and its metabolites oxidized to metabolite 2,2-difluoro-benzo[1,3]dioxole- 4 carboxylic acid (CGA 192155), expressed as Fludioxonil				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	GRM025.03A	0.01 mg/kg	LC-MS/MS	*ILV (Eggs, muscle, fat, liver):* [redacted], 2009 Report: 1983/108-D2149 (T001339-08) **New data**
	ILV (GRM025.03A)	0.01 mg/kg		
Blood (whole)	GRM025.03A	0.01 mg/kg	LC-MS/MS	
	ILV (GRM025.03A)	--		

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to Appendix 2.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	**AG-616B:** Radio validation of analytical method AG-616 was reported for the EU review (Denmark, 2005). Radiovalidation of analytical method AG-616 has been carried out and reported (ref. "Validation of "Draft" Analytical Method AG-616 for the Determination of Total Resiudes of CGA-173506 and Metabolites as CGA-192155 in Animal Tissues, Milk and Eggs", [redacted] 1993, ABR-95063). Fludioxonil is shown to be effectively extracted from animal matrices. **GRM025.03A** The extraction procedures used in analytical methods AG-616B and GRM025.03A are the very similar, so extractability efficiency of analytical method GRM025.03A has been adequately demonstrated. Data according to SANTE 2017/10632 Rev. 3 is not required for this active substance in the context of the product renewal of Metalaxyl-M. Data will be provided during the product renewal of fludioxonil.

5.3.2.4 Description of methods for the analysis of Fludioxonil body fluids and tissues (KCP 5.2.3)

Fludioxonil is not classified as toxic or highly toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required. However, should a method be required for monitoring of Fludioxonil in body fluids method GRM025.03A has been successfully validated in whole blood.

5.3.2.5 Description of methods for the analysis of Fludioxonil in soil (KCP 5.2.4)

An overview on the acceptable methods and possible data gaps for analysis of active substance in soil is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-6: Validated methods for soil

Component of residue definition: Fludioxonil			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	LC-MS/MS with 2 mass transitions	RAM 423/01 Method: [redacted] & [redacted], 2004 Report: RAM 423/01 Validation: [redacted], 2004 Report: RJ3493B EU agreed (Denmark, 2007)
Confirmatory	-	-	Not required: 2 mass transitions validated in primary method

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

5.3.2.6 Description of methods for the analysis of Fludioxonil in water (KCP 5.2.5)

An overview on the acceptable methods and possible data gaps for analysis of active substance in surface and drinking water is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-7: Validated methods for water

Component of residue definition: Fludioxonil and metabolites CGA192155 and CGA339833 *				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	LC-MS/MS with 2 mass transitions	**GRM025.01A** Method: [redacted], 2007 Report : GRM025.01A Validation: [redacted], 2007 Report: T003490-06-REG EU agreed (Denmark, 2007)
	ILV	0.05 µg/L	LC-MS/MS with 2 mass transitions	ILV: [redacted], 2016 Report: CGA173506DW **New data**
	Confirmatory	-	-	Not required: 2 ion transitions validated in primary method
Surface water	Primary	0.05 µg/L	LC-MS/MS with 2 mass transitions	**GRM025.01A** Method: [redacted], 2007

Component of residue definition: Fludioxonil and metabolites CGA192155 and CGA339833 *				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				Report : GRM025.01A Validation: [redacted], 2007 Report: T003490-06-REG EU agreed (Denmark, 2007)
	Confirmatory	-	-	Not required: 2 ion transitions validated in primary method

* Metabolites CGA192155 and CGA339833 are not part of the residue definition for monitoring, but included in the method and fully validated.

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

5.3.2.7 Description of methods for the analysis of Fludioxonil in air (KCP 5.2.6)

An overview on the acceptable methods and possible data gaps for analysis of active substance in air is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-8: Validated methods for air

Component of residue definition: Fludioxonil			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	2 µg/m^3	Normal phase HPLC-UV	REM 133.3 Method: [redacted], 1992 Report: REM 133.3 Validation (normal phase): [redacted], 1996 Report: 103/96 Validation (reverse phase): [redacted], 2001 Report: 133.03 29/11/2001 EU agreed (Denmark, 2005)
Confirmatory	2 µg/m^3	Reverse phase HPLC-UV	

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

5.3.2.8 Other studies/ information

5.3.3 Description of analytical methods for the determination of residues of

Metalaxyl-M (KCP 5.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	The table below gives a mostly accurate representation of the residue definitions for monitoring methods for metalaxyl-M; where necessary the text has been amended by HSE in green (applicant's text has been struck through in green where necessary).

5.3.3.1 Overview of residue definitions and levels of Metalaxyl-M 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. In the most recent EU assessment a residue definition for animal products was not proposed, however as part of the MRL review conducted in 2015[2] EFSA proposed a residue definition for monitoring of the sum of Metalaxyl (sum of isomers) and its metabolites containing the 2,6- dimethylaniline moiety, expressed as Metalaxyl. The current EU MRL legislation (Regulation (EU) No 2017/1164) states the residue definition for products of animal origin as: Metalaxyl and Metalaxyl-M (Metalaxyl including other mixtures of constituent isomers including Metalaxyl-M (sum of isomers)). Although the uses of A9873C do not give rise to residues in animal products (See dRR Part B Section 7) methods of analysis to determine the "EFSA MRL" residue definition of sum of metalaxyl (sum of isomers) and its metabolites containing the 2,6- dimethylaniline moiety, expressed as metalaxyl are available.

Table 5.3-9: 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	Metalaxyl and Metalaxyl-M (Metalaxyl including other mixtures of constituents isomers including Metalaxyl-M (sum of isomers))	0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Plant, high acid content		0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Plant, high oil content		0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Muscle	~~sum of metalaxyl (sum of isomers) and its metabolites containing the 2,6-dimethylaniline moiety, expressed as metalaxyl~~ Not required for the	0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Milk		0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Eggs		0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164

[2] Combined review of the existing maximum residue levels (MRLs) for the active substances Metalaxyl and Metalaxyl-M, EFSA Journal 2015; 13(4):4076

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Fat	representative uses EFSA, 2015a	0.01 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Liver, kidney		0.05 mg/kg	LOQ MRL according to Reg. (EU) No 2017/1164
Soil (Ecotoxicology)	Metalaxyl including other mixtures of constituents isomers including Metalaxyl-M (sum of isomers)	0.05 mg/kg	General limit
Drinking water (Human toxicology)	Metalaxyl including other mixtures of constituents isomers including Metalaxyl-M (sum of isomers)	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Metalaxyl including other mixtures of constituents isomers including Metalaxyl-M (sum of isomers)	1.2 mg/L	NOEC (Daphnia) (EFSA, 2015a)
Air	Metalaxyl including other mixtures of constituents isomers including Metalaxyl-M (sum of isomers)	24 µg/m^3	AOEL sys: 0.08 mg/kg bw/d (EFSA, 2015a)
Body fluids	~~sum of metalaxyl (sum of isomers) and its metabolites containing the 2,6-dimethylaniline moiety, expressed as metalaxyl~~ The active substance is not classified as a Health Hazard under CLP and therefore a method of analysis is not required for body fluids and tissues. EFSA, 2015a	0.01 mg/L	Default LOQ

5.3.3.2 Description of analytical methods for the determination of residues of Metalaxyl-M in plant matrices (KCP 5.2.1)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	Monitoring methods for the determination of residues of metalaxyl-M in plant matrices were evaluated for the active approval (EU RAR, 2014) and are considered fully validated. The applicant is the data owner. The available methods are validated for the crops in the high water, high acid, high oil, high protein, high starch crop groups. These are considered sufficient to cover the proposed uses of 'A20607B' on sugar beet. The EFSA conclusion states '*The compounds in the residue definition for plants can be*

	determined with a multi-residue method (QuEChERs) however a data gap was identified for extraction efficiency.' Data to address the extraction efficiency will be addressed the next renewal of the active substance. The applicant has provided a justification for not requiring extraction efficiency data. The proposed uses are expected to give to residues <LOQ therefore extraction efficiency of the method are not critical in the framework of this assessment. Hence, this case is accepted. The applicant has provided new data however this is not required in the context of this assessment so has not been evaluated. No further consideration is required.

An overview on the acceptable methods and possible data gaps for analysis of Metalaxyl-M in plant matrices is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-10: Validated methods for food and feed of plant origin

Component of residue definition: metalaxyl				
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	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Validation (tomato, potato, orange oilseed rape seed, dried bean): [redacted] & [redacted], 2011 Report: S11-01731 (two mass transitions validated) EU agreed (Belgium, 2014) ILV (tomatoes and oilseed rape) : [redacted], 2012 Report: S11-03712 **New data** New data is not required in the context of this assessment so has not been evaluated.
High acid content	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
High oil content	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
High protein/high starch content (dry)	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
Difficult (if required, depends on intended use)	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Validation (hop, cocoa bean): [redacted], 2016 Report: RES-00055 (two mass transitions validated) **New data** Not required in the context of this assessment; however this method was evaluated for a GB import tolerance application. ILV (hop, cocoa bean):
	ILV (QuEChERS)	0.01 mg/kg		

Component of residue definition: metalaxyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				[redacted], 2016 Report: YB27DB **New data** Not required in the context of this assessment; however this method was evaluated for a GB import tolerance application.

Table 5.3-11: Validated methods for food and feed of plant origin

Component of residue definition: Metalaxyl-M (enantiomer specific)				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	REM 181.06	0.02 mg/kg	GC-MSD (single residue)	<u>REM 181.06</u> Method: [redacted], 2001 Report: REM 181.06 Validation: [redacted], 2001 Report: 212/00 ILV: [redacted], 2001 Report: NOV/MET00111 EU agreed (Belgium, 2014) --- <u>DFG S19</u> Validation: [redacted] & [redacted], 2012 Report: S11-03698 EU agreed (Belgium, 2014)
	ILV (REM 181.06)	0.02 mg/kg		
	DFG S19	0.01 mg/kg	LC-MS/MS (multi-residue)	
High acid content	REM 181.06	0.02 mg/kg	GC-MSD (single residue)	
	ILV (REM 181.06)	0.02 mg/kg		
High oil content	REM 181.06	0.02 mg/kg	GC-MSD (single residue)	
	ILV (REM 181.06)	0.02 mg/kg		
High protein/high starch content (dry)	REM 181.06	0.02 mg/kg	GC-MSD (single residue)	
	ILV (REM 181.06)	0.02 mg/kg	GC-MSD (single residue)	

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	**Extraction Efficiency (SANTE 2017/10632 Rev. 3)** Based on SANTE 2017/10632, for renewal of product authorisations for which no change of the MRL is needed, the data requirements used for the latest renewal should be considered. The current MRL for crops associated with this submission is set at

	Method for products of plant origin
	0.01* (default). In the case of Metalaxyl-M as an AIR2 compound this application follows the data requirements for the active substance laid down in Regulation (EU) No. 544/2011 and the data requirements for the plant protection product laid down in Regulation (EU) No. 545/2011. Therefore, when considering these data requirements, no additional proof of extraction efficiency is required in the context of this product submission as in SANTE 2017/10632 Rev. 3 guidance. (page 19) In addition, the uses under consideration as part of this product submission result in <LOQ residues in all cases. On the basis of <LOQ exposure, extraction efficiency is not needed as per the decision tree for post registration methods (figure 1) and the decision tree for pre-registration methods (figure 2) outlined in the guidance.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	See green box above.

5.3.3.3 Description of analytical methods for the determination of residues of Metalaxyl- M in animal matrices (KCP 5.2.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	Monitoring methods for the determination of residues of metalaxyl-M in products of animal origin were presented in the RAR (EU RAR, 2014) and summarised below. It is noted that the EFSA conclusion states: '*Analytical methods for food of animal origin are not required in this regulatory context as there is no significant intake by livestock, when solely considering the supported representative uses.'* Based on the proposed uses of 'A20607B' residues in animal products will not be significant; therefore methods are not required. The applicant has provided new data for milk, eggs, muscle, fat, liver, kidney and blood. However this is not required in the context of this assessment so has not been evaluated. Data will be considered at the next renew of the active. No further consideration is required.

The use of A20607B is expected to result in residues of Metalaxyl-M below the LOQ in animal feed items. Therefore, the use of A20607B will not result in residues of Metalaxyl-M in animal feed items,

and so the possible transfer of residues in animal commodities from the proposed uses does not need to be considered. Methods of analysis for residues in animal matrices are not required; however an overview on the acceptable methods and possible data gaps for analysis of residues of Metalaxyl-M in animal matrices is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-13: Validated methods for food and feed of animal origin

Component of residue definition: Metalaxyl-M				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Milk	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	QuEChERS Validation (milk, eggs, muscle, fat, liver, kidney and blood): [redacted], 2011 Report: S11-01732 **New data** New data is not required in the context of this assessment so has not been evaluated. ILV (milk, eggs, muscle, liver, and fat) : [redacted], 2018 Report: MM87YQ **New data** New data is not required in the context of this assessment so has not been evaluated.
	ILV (QuEChERS)	0.01 mg/kg		
Eggs	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	
	ILV (QuEChERS)	0.01 mg/kg		
Muscle/meat	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	
	ILV (QuEChERS)	--		
Fat	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	
	ILV (QuEChERS)	0.01 mg/kg		
Liver	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	
	ILV (QuEChERS)	0.01 mg/kg		
Kidney	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	
	ILV (QuEChERS)	0.01 mg/kg		

Table 5.3-14: Validated methods for food and feed of animal origin

Component of residue definition: 2,6-dimethylaniline				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Milk	GRM031.06A	0.01 mg/kg	LC-MS/MS two mass transitions validated	GRM031.06A Method: [redacted] & [redacted], 2012 Report: GRM031.06A Validation: [redacted] & [redacted], 2012 Report: S11-03382 ILV (milk, eggs, liver, kidney) : [redacted], 2012
	ILV (GRM031.06A)	0.01 mg/kg		
Eggs	GRM031.06A	0.01 mg/kg	LC-MS/MS two mass transitions validated	
	ILV (GRM031.06A)	0.01 mg/kg		
Muscle/meat	GRM031.06A	0.01 mg/kg	LC-MS/MS two mass transitions validated	
	ILV	--		

Component of residue definition: 2,6-dimethylaniline				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	(GRM031.06A)			Report: S12-03412 EU agreed (Belgium, 2014) ILV (fat): [redacted], 2016 Report: TK0261461 **New data** New data is not required in the context of this assessment so has not been evaluated.
Fat	GRM031.06A	0.01 mg/kg	LC-MS/MS two mass transitions validated	
	ILV (GRM031.06A)	0.01 mg/kg		
Liver, Kidney	GRM031.06A	0.01 mg/kg	LC-MS/MS two mass transitions validated	
	ILV (GRM031.06A)	0.01 mg/kg		

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to Appendix 2.

Table 5.3-15: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	**<u>Extraction Efficiency (SANTE 2017/10632 Rev. 3)</u>** Based on SANTE 2017/10632, for renewal of product authorisations for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. In the case of Metalaxyl-M as an AIR2 compound this application follows the data requirements for the active substance laid down in Regulation (EU) No. 544/2011 and the data requirements for the plant protection product laid down in Regulation (EU) No. 545/2011. Therefore, when considering these data requirements, no additional proof of extraction efficiency is required in the context of this product submission as in SANTE 2017/10632 Rev. 3 guidance. (page 19) Also, according to SANTE 2017/10632, it is "not expected that new animal metabolism studies or new animal feeding studies should be set up only in order to evaluate aspects of analytical methods and extraction efficiency", as these would have to be carried out with vertebrate animals.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	The extraction efficiency of the monitoring method for the determination of residues of metalaxyl-M in animal matrices was <u>not</u> considered during the active substance renewal (EU RAR, 2014). Data to address the extraction efficiency will be addressed at the next renewal of the active substance.

	In addition, significant residues in products of animal origin are not expected based on the proposed uses therefore the extraction efficiency is not a critical concern. No further consideration is required.

5.3.3.4 Description of methods for the analysis of Metalaxyl-M in body fluids and tissues (KCP 5.2.3)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	Monitoring methods for the determination of residues of Metalaxyl-M in body fluids and tissues were not evaluated for the active approval. Metalaxyl-M was assessed using the data requirements under Reg (EU) 544/2011, and is not classified as toxic or very toxic, therefore it was not a requirement to provide methods of analysis. The EFSA Conclusion states *'The active substance is not classified as a Health Hazard under CLP and therefore a method of analysis is not required for body fluids and tissues.*' The applicant has provided a study; however, it is not necessary to consider these data until renewal of the active substance and the study has not been evaluated in this RR. No further consideration is required.

Metalaxyl-M is not classified as toxic or highly toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required. However the following method can be used to determine residues of Metalaxyl-M in body fluids and tissues.

Table 5.3-16: Methods for body fluids and tissues

Component of residue definition: Metalaxyl-M				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Blood	QuEChERS	0.01 mg/kg	LC-MS/MS (multi residue) two mass transitions validated	QuEChERS Validation (milk, eggs, muscle, fat, liver, kidney and **blood**): [redacted], 2011 Report: S11-01732 **New data** New data is not required in the context of this assessment so has not been evaluated.

Table 5.3-17: Methods for body fluids and tissues

Components of residue method: Metalaxyl-M and 2,6-dimethylaniline				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**

Components of residue method: Metalaxyl-M and 2,6-dimethylaniline				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Blood	Primary & confirmatory	0.01 mg/kg 0.01 mg/L	LC-MS/MS two mass transitions validated	Method: [redacted] & [redacted], 2012 Report: GRM031.06A Validation: [redacted] & [redacted], 2012 Report: S11-03382 ILV: [redacted], 2012 Report: S12-03412 EU agreed (Belgium, 2014)

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

5.3.3.5 Description of methods for the analysis of of Metalaxyl-M in soil (KCP 5.2.4)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	The table below gives an accurate representation of the available monitoring methods for residues of metalaxyl-M in soil. Monitoring methods for the determination of residues of metalaxyl-M in soil were evaluated for the active renewal (EU RAR, 2014) and are considered fully validated. The applicant is the data owner. No further consideration is required.

An overview on the acceptable methods and possible data gaps for analysis of active substance in soil is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-18: Validated methods for soil

Component of residue definition: Metalaxyl-M (sum of isomers) and NOA409045*			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.03A Method and validation: [redacted], 2008a Report: GRM031.03A EU agreed (Belgium, 2014)
Confirmatory	-	-	Not required: two mass transi-

Component of residue definition: Metalaxyl-M (sum of isomers) and NOA409045*			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
			tions validated in primary method

* Metabolite NOA409045 is not part of the residue definition for monitoring, but included in the method and fully validated. Metalaxyl-M and NOA409045 each contain 1 chiral centre. NOA409045 is the R-enantiomer of this metalaxyl metabolite, the RS-enantiomer of which is CGA62826. GRM031.03A is a non-enantiospecific method and will detect and quantify both R- and RS-enantiomers of each analyte as single chromatographic peaks.

5.3.3.6 Description of methods for the analysis of Metalaxyl-M in water (KCP 5.2.5)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	Monitoring methods for the determination of residues of metalaxyl-M in water were evaluated for the active renewal (EU RAR, 2014) and are considered fully validated. The applicant is the data owner. The applicant has provided new studies for drinking water, surface water and ground water. However, it is not necessary to consider these data until renewal of the active substance and therefore studies have not been evaluated in this RR. No further consideration is required.

An overview on the acceptable methods and possible data gaps for analysis of active substance in surface and drinking water is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-19: Validated methods for water

Component of residue definition: Metalaxyl-M (sum of isomers) and metabolites NOA409045 and CGA108906*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.02A Validation: [redacted], 2008b Report: GRM031.02A EU agreed (Belgium, 2014)
	ILV	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	ILV: [redacted], 2016 Report: IF-15/03469803-TK **New data** New data is not required in the context of this assessment so has not been evaluated.

Component of residue definition: Metalaxyl-M (sum of isomers) and metabolites NOA409045 and CGA108906*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method
Surface water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.02A Validation: [redacted], 2008b Report: GRM031.02A EU agreed (Belgium, 2014)
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method
Ground water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.02A Validation: [redacted], 2008b Report: GRM031.02A EU agreed (Belgium, 2014)
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method

* Metabolites NOA409045 and CGA108906 are not part of the residue definition for monitoring, but included in the method and fully validated. Metalaxyl-M, NOA409045 and CGA108906 each contain 1 chiral centre. NOA409045 is the R-enantiomer of this metalaxyl metabolite, the RS-enantiomer of which is CGA62826. CGA108906 is the racemic form (RS-enantiomer) of metalaxyl diacid metabolite, the R-enantiomer of which is SYN546520. GRM031.02A is a non-enantiospecific method and will detect and quantify both R- and RS-enantiomers of each analyte as single chromatographic peaks.

Table 5.3-20: Validated methods for water

Component: Metalaxyl-M (sum of isomers) and metabolites NOA409045, CGA108906 and CGA67868*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.08A Method: [redacted] & [redacted], 2015 Report: GRM031.08A **New data** New data is not required in the context of this assessment so has not been evaluated. Validation: [redacted], 2015 Report: TK0222545 **New data** New data is not required in the context of this assessment so has not

Component: Metalaxyl-M (sum of isomers) and metabolites NOA409045, CGA108906 and CGA67868*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				been evaluated.
	ILV	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	ILV: [redacted], 2016 Report: IF-15/03469803-TK **New data** New data is not required in the context of this assessment so has not been evaluated.
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method
Surface water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.08A Method: [redacted] & [redacted], 2015 Report: GRM031.08A **New data** New data is not required in the context of this assessment so has not been evaluated. Validation: [redacted], 2015 Report: TK0222545 **New data** New data is not required in the context of this assessment so has not been evaluated.
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method
Ground water	Primary	0.05 µg/L	LC-MS/MS two mass transitions validated (*non-enantiospecific*)	GRM031.08A Method: [redacted] & [redacted], 2015 Report: GRM031.08A **New data** New data is not required in the context of this assessment so has not been evaluated. Validation: [redacted], 2015 Report: TK0222545 **New data**

Component: Metalaxyl-M (sum of isomers) and metabolites NOA409045, CGA108906 and CGA67868*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				New data is not required in the context of this assessment so has not been evaluated.
	Confirmatory	-	-	Not required: 2 mass transitions validated in primary method

* Metabolites NOA409045, CGA108906 and CGA67868 are not part of the residue definition for monitoring, but included in the method and fully validated. Metalaxyl-M, NOA409045 and CGA108906 each contain 1 chiral centre. NOA409045 is the R-enantiomer of this metalaxyl metabolite, the RS-enantiomer of which is CGA62826. CGA108906 is the racemic form (RS-enantiomer) of metalaxyl diacid metabolite, the R-enantiomer of which is SYN546520. GRM031.02A is a non-enantiospecific method and will detect and quantify both R- and RS-enantiomers of each analyte as single chromatographic peaks. CGA67868 is not a chiral molecule.

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

5.3.3.7 Description of methods for the analysis of Metalaxyl-M in air (KCP 5.2.6)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD)**
Reviewer's comments	The table below gives an accurate representation of the available monitoring methods for residues of metalaxyl-M in air. Monitoring methods for the determination of residues of metalaxyl-M in air were evaluated for the active renewal (EU RAR, 2014) and are considered fully validated. The applicant is the data owner. No further consideration is required.

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

Table 5.3-21: Validated methods for air

Component of residue definition: Metalaxyl-M (sum of isomers)			
Method	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	10 µg/m^3	LC-MS/MS with two mass transitions validated *(non-enantiospecific)*	GRM011.01A [redacted], 2006 Report: T003619-05-REG EU agreed (Belgium, 2014)
Confirmatory	-	-	Not required: 2 ion transitions validated in primary method

* Metalaxyl-M contains 1 chiral centre. GRM011.01A is a non-enantiospecific method and will detect and quantify both R- and RS-enantiomers of metalaxyl as single chromatographic peaks.

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

5.3.3.8 Other studies/ information

No new or additional studies have been submitted.

5.3.4 Description of analytical methods for the determination of residues of Sedaxane (KCP 5.2)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	'Vibrance SB' was not the representative product for the approval of metalaxyl-M. 'Vibrance SB' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Vibrance SB' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and sedaxane have not been considered further.

5.3.4.1 Overview of residue definitions and levels of Sedaxane 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 5.3-22: 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	Sedaxane (sum of isomers)	0.01 mg/kg	LOQ MRL according to Reg. SANTE/10304/2019
Plant, high acid content		0.01 mg/kg	LOQ MRL according to Reg. SANTE/10304/2019
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	LOQ MRL according to Reg. SANTE/10304/2019
Plant, high oil content		0.01 mg/kg	LOQ MRL according to Reg. SANTE/10304/2019
Muscle	Sedaxane (sum of isomers)	0.01 mg/kg	LOQ MRL according to Reg. SANTE/10304/2019

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Milk		0.01 mg/kg	LOQ MRL according to Reg. SANTE/10304/2019
Eggs		0.01 mg/kg	LOQ MRL according to Reg. SANTE/10304/2019
Fat		0.01 mg/kg	LOQ MRL according to Reg. SANTE/10304/2019
Liver, kidney		0.01 mg/kg	LOQ MRL according to Reg. SANTE/10304/2019
Soil (Ecotoxicology)	Sedaxane (sum of isomers)	0.05 mg/kg	Default LOQ
Drinking water (Human toxicology)	Sedaxane (sum of isomers) CSAA798670 CSCD465008	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Sedaxane (sum of isomers)	62 µg/L	Toxic endpoint fish with SF of 10 for not tested metabolites (EFSA, 2013)
Air	Sedaxane (sum of isomers)	84 µg/m^3	AOEL sys: 0.28 mg/kg bw/d (EFSA, 2013)
Tissue (meat or liver)	Sedaxane (sum of isomers)	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

5.3.4.2 Description of analytical methods for the determination of residues of Sedaxane in plant matrices (KCP 5.2.1)

An overview on the acceptable methods and possible data gaps for analysis of Sedaxane in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-23: Validated methods for food and feed of plant origin

Component of residue definition: Sedaxane (sum of isomers)				
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 *Cereal green forage*	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	**QuEChERS** Method: [redacted], 2007 Report: P-14.141.04 Validation *: [redacted] & [redacted], 2009 Report SYN-0953V ILV *: [redacted], 2010
	ILV	0.01 mg/kg		
High acid content *Orange*	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
High oil content *Oilseed rape seed*	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV	0.01 mg/kg	LC-MS/MS	

Component of residue definition: Sedaxane (sum of isomers)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
			(multi-residue)	Report: TK0009697-REG EU agreed (France, 2012) ILV *: [redacted], 2019 Report: TK0395483 **New data**
High protein/high starch content (dry) *Wheat grain*	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV	0.01 mg/kg		
No group *Wheat straw*	ILV	0.01 mg/kg	LC-MS/MS (multi-residue)	

* Two mass transitions were validated. Therefore, no confirmatory method required.

Table 5.3-24: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	The efficiency of the extraction procedures using 80:20 acetonitrile:water for parent residues of SYN524464 used in the QuEChERS method for crops was demonstrated as part of a radiovalidation study performed for analytical methods GRM023.03A and GRM023.12A (parent + metabolites). [[redacted] & [redacted] (2010), Report No. 8210736-D2149. EU agreed (France, 2012) Data according to SANTE 2017/10632 Rev. 3 is not required for this active substance in the context of the product renewal of Metalaxyl-M. Data will be provided during the EU AIR review or product renewal of sedaxane.

5.3.4.3 Description of analytical methods for the determination of residues of Sedaxane in animal matrices (KCP 5.2.2)

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

Table 5.3-25: Validated methods for food and feed of animal origin

Component of residue definition: Sedaxane (sum of isomers) and metabolites CSCD658906 and CSCD659087 *				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Milk	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Validation **: [redacted] & [redacted], 2009 Report: SYN-0952V ILV **:
	ILV (QuEChERS)	0.01 mg/kg		
	GRM023.10A	0.01 mg/kg	LC-MS/MS (single residue)	
	ILV (GRM023.10A)	0.01 mg/kg		

Component of residue definition: Sedaxane (sum of isomers) and metabolites CSCD658906 and CSCD659087 *				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Eggs	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	Class & Sensiuc, 2010 Report: B 1844 G EU agreed (France, 2012) --- GRM023.10A * Method: [redacted], 2009 Report: GRM023.10A Validation **: [redacted], 2009 Report : T014679-05 ILV **: [redacted], 2009 Report : 30549 EU agreed (France, 2012)
	ILV (QuEChERS)	0.01 mg/kg		
	GRM023.10A	0.01 mg/kg	LC-MS/MS (single residue)	
Muscle/meat	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	GRM023.10A	0.01 mg/kg	LC-MS/MS (single residue)	
	ILV (GRM023.10A)	0.01 mg/kg		
Fat	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	GRM023.10A	0.01 mg/kg	LC-MS/MS (single residue)	
Liver	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	ILV (QuEChERS)	0.01 mg/kg		
	GRM023.10A	0.01 mg/kg	LC-MS/MS (single residue)	
	ILV (GRM023.10A)	0.01 mg/kg		
Kidney	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	GRM023.10A	0.01 mg/kg	LC-MS/MS (single residue)	
Blood	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	
	GRM023.10A	0.01 mg/kg	LC-MS/MS (single residue)	

* GRM023.10A: Metabolites CSCD658906 and CSCD659087are not part of the residue definition for monitoring, but included in the method and fully validated.
** Two mass transitions were validated. Therefore, no confirmatory method required.

Table 5.3-26: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	The efficiency of the extraction procedure using acetonitrile water (80:20 v/v) used in the QuEChERS method and GRM023.10A for animal matrices was demonstrated as part of a radiovalidation which validated this as a suitable procedure for use in residue methods for the measurement of SYN524464 and its metabolites in livestock tissues and milk samples (goat metabolism study, report 30258). Enzyme hydrolysis using β-glucuronisade with an incubation time of 6 hr was validated as a suitable procedure for the deconjugation of SYN524464 animal metabolites method. EU agreed (France, 2012)

	Method for products of animal origin
	Data according to SANTE 2017/10632 Rev. 3 is not required for this active substance in the context of the product renewal of Metalaxyl-M. Data will be provided during the EU AIR review or product renewal of sedaxane.

5.3.4.4 Description of methods of Sedaxane for the analysis in body fluids and tissues (KCP 5.2.3)

Sedaxane is not classified as toxic or highly toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required.

Nevertheless, both QuEChERS and GRM023.10A were validated in blood and animal tissues (please refer to 5.3.2.3 above).

5.3.4.5 Description of methods of Sedaxane for the analysis in soil (KCP 5.2.4)

An overview on the acceptable methods and possible data gaps for analysis of active substance in soil is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-27: Validated methods for soil

Component of residue definition: sedaxane (sum of isomers) and metabolites CSAA798670 and CSCD465008 *			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary & confirmatory (Sedaxane)	0.0001 mg/kg	LC-MS/MS two mass transitions validated	Method: [redacted], 2008 Report: GRM023.02A Validation: [redacted], 2008 Report : 20071456/01-RVS EU agreed (France, 2012)
Primary & confirmatory (CSAA78670 and CSCD465008)	0.0005 mg/kg	LC-MS/MS two mass transitions validated	Method: [redacted], 2009 Report: GRM023.05A Validation: [redacted], 2008 Report: S09-00917 EU agreed (France, 2012)

* GRM023.10A: Metabolites CSCD658906 and CSCD659087are not part of the residue definition for monitoring, but included in the method and fully validated.

5.3.4.6 Description of methods of Sedaxane for the analysis in water (KCP 5.2.5)

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

Table 5.3-28: Validated methods for water

Component of residue definition: sedaxane (sum of isomers), CSCC210616, CSAA798670 and CSCD465008 *				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water and Surface water	Primary & confirmatory	0.05 µg/L for each analyte	LC-MS/MS two mass transitions validated	Method & validation: [redacted], 2009; [redacted] & [redacted], 2010 Report: GRM023.06A EU agreed (France, 2012)
	ILV	0.05 µg/L for each analyte	LC-MS/MS two mass transitions validated	ILV: [redacted], 2019 Report: SYN508210_10298 **New data**

* Metabolite CSCC210616 is not part of the residue definition for monitoring, but included in the method and fully validated.

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

5.3.4.7 Description of methods of Sedaxane for the analysis of air (KCP 5.2)

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

Table 5.3-29: Validated methods for air

Component of residue definition: sedaxane (sum of isomers)			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary & confirmatory	0.375[(c)] µg/m^3	LC-MS/MS two mass transitions validated	Method & validation: [redacted] & [redacted], 2010; [redacted], 2009 Report: GRM023.09A EU agreed (France, 2012)

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

5.3.4.8 Other studies/information

No new or additional studies have been submitted.

5.4 References

Fludioxonil

Denmark, 2005. Draft assessment report on the active substance fludioxonil prepared by the rapporteur Member State Denmark in the framework of Council Directive 91/414/EEC, June 2005.

Denmark, 2007. Final addendum to the draft assessment report on the active substance fludioxonil prepared by the rapporteur Member State Denmark in the framework of Council Directive 91/414/EEC, compiled by EFSA, June 2007.

EC (European Commission), 2007. Review report for the active substance fludioxonil. Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 9 October 2007 in view of the inclusion of fludioxonil in Annex I of Council Directive 91/414/EEC. SANCO/2818/07 – rev. 2, 10 September 2007.

EFSA (European Food Safety Authority), 2007. Conclusion regarding the peer review of the pesticide risk assessment of the active substance fludioxonil. EFSA Scientific Report (2007) 110, 1-85.

Metalaxyl-M

Belgium, 1999. Draft assessment report on the active substance metalaxyl-M prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, July 1999.

Belgium, 2001. Addendum to the draft assessment report on the active substance metalaxyl-M prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, September 2001.

Belgium, 2014. Renewal assessment report on the active substance metalaxyl-M prepared by the rapporteur Member State Belgium under Regulation (EC) No 1107/2009, December 2013.

EFSA (European Food Safety Authority), 2015a. Conclusion on the peer review of the pesticide risk assessment of the active substance metalaxyl-M. EFSA Journal 2015; 13(3):3999, [105 pp.] doi:10.2903/j.efsa.2015.3999.

EFSA (European Food Safety Authority), 2015b. Combined review of the existing maximum residue levels (MRLs) for the active substances metalaxyl and metalaxyl-M, EFSA Journal 2015; 13(4):4076, [56 pp.] doi:10.2903/j.efsa.2015.4076.

Sedaxane

France, 2011. Draft assessment report on the active substance Sedaxane prepared by the rapporteur Member State France in the framework of Council Directive 91/414/EEC, May 2011.

France, 2012. Final addendum to the draft assessment report on the active substance Sedaxane prepared by the rapporteur Member State France in the framework of Council Directive 91/414/EEC, compiled by EFSA, June 2012

EFSA, 2013. Conclusion on the peer review of the pesticide risk assessment of the active substance sedaxane, EFSA Journal 2013; 11(1):3057, [76 pp.]; doi:10.2903/j.efsa.2013.3057.

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
A20607B					
~~KCP 5.1.1~~	[redacted]	~~26/06/2014~~	~~A20607B - Determination of Sedaxane, Fludioxonil and Metalaxyl-M in Formulation FS (015/022.5/015)~~ ~~Report No. 300021938~~ ~~Document No. VV-128321 , A20607B_10177~~ ~~Test Facility Syngenta Crop Protection~~ ~~Not GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.1~~	[redacted]	~~17/09/2014~~	~~A20607B - Validation of Analytical Method ST-35/1~~ ~~Report No. CHMU140402~~ ~~Document No. VV-412231 , A20607B_10187~~ ~~Test Facility Syngenta Crop Protection~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.1~~	[redacted]	~~30/03/2015~~	~~A20607B - Determination of CGA329351 and CGA351920 in sedaxane/fludioxonil/metalaxyl-M FS (015/022.5/015) by chiral HPLC~~ ~~Report No. 300036892~~ ~~Document No. VV-128322 , A20607B_10181~~ ~~Test Facility Syngenta Crop Protection~~ ~~Not GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.1~~	[redacted]	~~09/07/2015~~	~~A20607B - Validation of Analytical Method STA-35/2~~ ~~Report No. CHMU150540~~ ~~Document No. VV-413006 , A20607B_10208~~ ~~Test Facility Syngenta Crop Protection~~	~~N~~	~~SYN~~

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**
			~~GLP~~ ~~Unpublished~~		
KCP 5.1.1	[redacted]	11/12/2014	A9651D - Analytical Method SD-1751/1 Report No. 300021240 Document No. VV-128413 , A9651D_10487 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	SYN
KCP 5.1.1	[redacted]	25/11/2014	A9651D - Validation Analytical Method SD-1751/1 Report No. CHMU140410 Document No. VV-411110 , A9651D_10488 Test Facility Syngenta Crop Protection GLP Unpublished	N	SYN
KCP 5.1.1	[redacted]	04/05/2020	Statement on Validation of the Analytical Method SD-1751/1 for the determination of CGA72649 and CGA363736 in A20607B sedaxane/fludioxonil/metalaxyl-M FS (015/022.5/015) Report No. N/A Document No. VV-854722 Test Facility Syngenta Crop Protection Munchwilen AG GLP Unpublished	N	SYN
Fludioxonil					
~~KCP 5.1.2~~	[redacted]	~~30/06/2006~~	~~Analytical Method for the determination of Residues of Fludioxonil (CGA173506) in Crop Matrices. Final Determination by LC-MS/MS~~ ~~Report No. REM 133.06~~ ~~Document No. VV-124731 , CGA173506/6932~~ ~~Test Facility Syngenta - Jealott's Hill International~~ ~~Not GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~

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 5.1.2~~	[redacted]	~~29/06/2006~~	~~Fludioxonil (CGA173506): Validation of Residue Analytical Method REM 133.06 for the determination of Residues in Crops. Final Determination by LC-MS/MS~~ ~~Report No. RJ3773B - 05-S604~~ ~~Document No. VV-337212 , CGA173506/6933~~ ~~Test Facility Syngenta - Jealott's Hill International~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.2~~	[redacted]	~~23/01/2012~~	~~Cyprodinil and Fludioxonil - Residue study on Cauliflower in Northern France, Poland and United Kingdom in 2010~~ ~~Report No. R-B0074~~ ~~Document No. VV-401158 , A9219B_11593~~ ~~Test Facility Anadiag SA~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.2~~	[redacted]	~~19/05/2014~~	~~Fludioxonil - Validation of the Analytical Method for the Determination of Fludioxonil residues in Peas (seeds and haulm)~~ ~~Report No. B3113~~ ~~Document No. VV-407927 , CGA173506_11705~~ ~~Test Facility Anadiag SA~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.1.2~~	[redacted]	~~16/01/2018~~	~~Fludioxonil (CGA173506) - Validation of Analytical Method REM133.06 for the Determination of Residues of Fludioxonil in multiple crops~~ ~~Report No. R-B7376~~ ~~Document No. VV-469007 , CGA173506_12273~~ ~~Test Facility Anadiag SA~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.1~~	[redacted]	~~15/10/2014~~	~~Fludioxonil - Validation of the QuEChERS Method for the Determination of Fludioxonil Residues in Crop Matrices by LC-MS/MS~~	~~N~~	~~SYN~~

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
			~~Report No. P 3446 G~~ ~~Document No. VV 410631 , CGA173506_11710~~ ~~Test Facility PTRL Europe~~ ~~GLP~~ ~~Unpublished~~		
~~KCP 5.2.1~~	[redacted]	~~05/12/2014~~	~~Fludioxonil - Independent Laboratory Validation of the QuEChERS Method for the Determination of Fludioxonil Residues in Crop Matrices by LC-MS/MS~~ ~~Report No. 20140189~~ ~~Document No. VV 410968 , CGA173506_11723~~ ~~Test Facility Innovative Environmental Services~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.2~~	[redacted]	~~26/02/2009~~	~~Fludioxonil - Analytical Method for the Determination of Residues of Total Fludioxonil (CGA173506) and Metabolites as CGA192155 in Animal Matrices (milk, eggs, muscle, fat, liver, kidney and whole blood). Final Determination by LC-MS/MS~~ ~~Report No. GRM025.03A~~ ~~Document No. VV 127758 , CGA173506_11402~~ ~~Test Facility ADME - Bioanalyses~~ ~~Not GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.2~~	[redacted]	~~24/02/2009~~	~~Validation of residue method GRM025.03A for total fludioxonil (CGA173506) and metabolites as CGA192155 in animal matrices (milk, eggs, muscle, fat, liver, kidney and whole blood)~~ ~~Report No. T001341-08-REG~~ ~~Document No. VV 382790 , CGA173506_11403~~ ~~Test Facility ADME - Bioanalyses~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.2~~	[redacted]	~~02/04/2009~~	~~Fludioxonil - Magnitude of residues in animal tissues following repeated oral administration to the laying hen~~ ~~Report No. T001339-08|1983/108-D2149~~	~~Y~~	~~SYN~~

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
			~~Document No. VV-383645 , CGA173506_11440~~ ~~Test Facility Covance Laboratories Ltd.~~ ~~GLP~~ ~~Unpublished~~		
~~KCP 5.2.5~~	[redacted]	~~04/04/2016~~	~~Fludioxonil – Independent Laboratory Validation (ILV) of Analytical Method GRM025.01A for the Determination of Residues of Fludioxonil (CGA173506) and its Metabolites CGA192155 and CGA339833 in Water~~ ~~Report No. CGA173506DW~~ ~~Document No. VV-462757 , CGA173506_11942~~ ~~Test Facility CIP Chemisches Institut Pforzheim GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
Metalaxyl-M					
KCP 5.2.1	[redacted]	07/01/2014	Metalaxyl-M – Independent Laboratory Validation (ILV) of an Analytical Method for Determination of Residues of Metalaxyl-M in Crops Report No. S11-03712 Document No. VV-407367 , CGA329351_11643 Test Facility Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	SYN
KCP 5.2.1	[redacted]	15/06/2016	Metalaxyl-M – Validation of the QuEChERS Multiple Residue Method in Hops and Cocoa Beans Report No. RES-00055 Document No. VV-465427 , CGA329351_11743 Test Facility ResChem Analytical Limited GLP Unpublished	N	SYN
~~KCP 5.2.1~~	[redacted]	~~16/08/2016~~	~~Metalaxyl-M: Independent Laboratory Validation of the QuEChERS Multiple Residue Method in Hops and Cocoa Beans~~ ~~Report No. YB27DB~~ ~~Document No. VV-465743 , CGA329351_11745~~	~~N~~	~~SYN~~

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
			~~Test Facility Envigo CRS Limited~~ ~~GLP~~ ~~Unpublished~~		
~~KCP 5.2.2~~	[redacted]	~~10/10/2011~~	~~Metalaxyl-M – Validation of the Multiple Residue Method QuEChERS for the Determination in Animal Matrices~~ ~~Report No. S11-01732~~ ~~Document No. VV-400487 , CGA329351_11472~~ ~~Test Facility Eurofins Agroscience Services Chem GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.2~~	[redacted]	~~19/11/2018~~	~~Metalaxyl-M – Independent Laboratory Validation of Analytical Method QuEChERS for the Determination of Residues of Metalaxyl-M in Animal Matrices by LC-MS/MS~~ ~~Report No. MM87YQ~~ ~~Document No. VV-470901 , CGA329351_11851~~ ~~Test Facility Envigo CRS Limited~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.2~~	[redacted]	~~30/03/2016~~	~~Metalaxyl-M – Independent Laboratory Validation of Analytical Method GRM031.06A for the Determination of Metalaxyl-M and Structurally Related Metabolites as the Common Moiety 2,6-Dimethylaniline (CGA72649) in Animal Fat~~ ~~Report No. S16-00573~~ ~~Document No. VV-463097 , CGA329351_11737~~ ~~Test Facility Eurofins Agroscience Services EcoChem GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.5~~	[redacted]	~~01/10/2015~~	~~Metalaxyl-M – Residue Method GRM031.08A for the Determination of Metalaxyl-M (CGA329351) and Metabolites NOA409045, CGA108906 and CGA67868 in water. Non-enantiospecific method. Final determination by LC-MS/MS~~ ~~Report No. GRM031.08A~~ ~~Document No. VV-132583 , CGA329351_11693~~	~~N~~	~~SYN~~

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
			~~Test Facility Syngenta – Jealott's Hill~~ ~~Not GLP~~ ~~Unpublished~~		
~~KCP 5.2.5~~	[redacted]	~~01/07/2015~~	~~Metalaxyl-M – Validation of Analytical Method for the Determination of Metalaxyl-M Metabolite CGA67868 in Water~~ ~~Report No. S14-05740~~ ~~Document No. VV-412805 , CGA092370_10006~~ ~~Test Facility Eurofins Agroscience Services Chem SAS~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.5~~	[redacted]	~~12/02/2016~~	~~Metalaxyl-M – Independent Laboratory Validation of Analytical Method GRM031.08A for the Determination of Metalaxyl-M (CGA329351) and its Metabolites NOA409045, CGA108906 and CGA67868 in Drinking Water~~ ~~Report No. IF-15/03469803-TK~~ ~~Document No. VV-415481 , CGA329351_11732~~ ~~Test Facility SGS Germany GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
Sedaxane					
~~KCP 5.1.2~~	[redacted]	~~01/10/2015~~	~~Sedaxane – Residue Study following seed treatment with A20110E, on Potato in Southern France and Spain in 2014~~ ~~Report No. S14-01326~~ ~~Document No. VV-413257 , A20110E_10061~~ ~~Test Facility Eurofins Agroscience Services Ltd~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~
~~KCP 5.2.1~~	[redacted]	~~19/09/2019~~	~~SYN524464 – Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of SYN508210 and SYN508211 in Crop Matrices by LC-MS/MS~~ ~~Report No. 20190112~~ ~~Document No. VV-619363 , SYN508210_10296~~	~~N~~	~~SYN~~

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
			~~Test Facility Innovative Environmental Services~~ ~~GLP~~ ~~Unpublished~~		
~~KCP 5.2.5~~	[redacted]	~~23/09/2019~~	~~SYN524464 - Independent Laboratory Validation of Analytical Method GRM023.06A for the Determination of Residues of SYN508210 and SYN508211 and the Metabolites CSCC210616, CSCD465008 and CSAA798670 in Water~~ ~~Report No. S18-05320~~ ~~Document No. VV-619368 , SYN508210_10298~~ ~~Test Facility Eurofins Agroscience Services Chem GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~SYN~~

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 XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

List of data submitted by the applicant and not 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 5.2.1	[redacted]	16/08/2016	Metalaxyl-M: Independent Laboratory Validation of the QuEChERS Multiple Residue Method in Hops and Cocoa Beans Report No. YB27DB Document No. VV-465743 , CGA329351_11745 Test Facility Envigo CRS Limited GLP Unpublished	N	SYN
KCP 5.2.2	[redacted]	10/10/2011	Metalaxyl-M – Validation of the Multiple Residue Method QuEChERS for the Determination in Animal Matrices Report No. S11-01732 Document No. VV-400487 , CGA329351_11472 Test Facility Eurofins Agroscience Services Chem GmbH	N	SYN

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
			GLP Unpublished		
KCP 5.2.2	[redacted]	19/11/2018	Metalaxyl-M - Independent Laboratory Validation of Analytical Method QuEChERS for the Determination of Residues of Metalaxyl-M in Animal Matrices by LC-MS/MS Report No. MM87YQ Document No. VV-470901 , CGA329351_11851 Test Facility Envigo CRS Limited GLP Unpublished	N	SYN
KCP 5.2.2	[redacted]	30/03/2016	Metalaxyl-M – Independent Laboratory Validation of Analytical Method GRM031.06A for the Determination of Metalaxyl-M and Structurally Related Metabolites as the Common Moiety 2,6-Dimethylaniline (CGA72649) in Animal Fat Report No. S16-00573 Document No. VV-463097 , CGA329351_11737 Test Facility Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	SYN
KCP 5.2.5	[redacted]	01/10/2015	Metalaxyl-M - Residue Method GRM031.08A for the Determination of Metalaxyl-M (CGA329351) and Metabolites NOA409045, CGA108906 and CGA67868 in water. Non-enantiospecific method. Final determination by LC-MS/MS Report No. GRM031.08A Document No. VV-132583 , CGA329351_11693 Test Facility Syngenta - Jealott's Hill Not GLP Unpublished	N	SYN
KCP 5.2.5	[redacted]	01/07/2015	Metalaxyl-M – Validation of Analytical Method for the Determination of Metalaxyl-M Metabolite CGA67868 in Water Report No. S14-05740 Document No. VV-412805 , CGA092370_10006 Test Facility Eurofins Agroscience Services Chem SAS	N	SYN

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
			GLP Unpublished		
KCP 5.2.5	[redacted]	12/02/2016	Metalaxyl-M – Independent Laboratory Validation of Analytical Method GRM031.08A for the Determination of Metalaxyl-M (CGA329351) and its Metabolites NOA409045, CGA108906 and CGA67868 in Drinking Water Report No. IF-15/03469803-TK Document No. VV-415481 , CGA329351_11732 Test Facility SGS Germany GmbH GLP Unpublished	N	SYN

List of data relied on not submitted by the applicant but necessary for evaluation

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 XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Fludioxonil

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	'Vibrance SB' was not the representative product for the approval of metalaxyl-M. 'Vibrance SB' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Vibrance SB' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and sedaxane have not been considered further.

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

A 2.1.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted.

A 2.1.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted.

A 2.1.1.3 Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2.3)

No new or additional studies have been submitted.

A 2.1.1.4 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

No new or additional studies have been submitted.

A 2.1.1.5 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

A 2.1.1.5.1 REM 133.06

Reference:	KCP 5.1.2
Report	Analytical Method for the Determination of Residues of Fludioxonil (CGA173506) in Crop Matrices. Final Determination by LC-MS/MS.
Guideline(s):	[redacted], 2006.
Deviations:	Report No. REM 133.06. Syngenta document No. CGA173506/6932.
GLP:	No – study is method description only.
Acceptability:	Yes.

Principle of the method

Crop samples are extracted by homogenisation with methanol. Extracts are centrifuged and aliquots diluted with acetonitrile: water (30:70 v/v). Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

A 2.1.1.5.1.1 Method validation

Reference:	KCP 5.1.2
Report	[redacted] & [redacted] (2006) Validation of Residue Analytical Method REM 133.06 for the Determination of Residues in Crops. Final Determination by LC-MS/MS. Report No. RJ3773B. Syngenta document No. CGA173506/6933.
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev.7). OPPTS 860.1340.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Materials and methods

Crop samples are extracted by homogenisation with methanol. Extracts are centrifuged and aliquots diluted with acetonitrile: water (30:70 v/v). Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

Results and discussions

Control samples were fortified at the limit of quantification (0.01 mg/kg) for all matrices and at 10X LOQ for wheat grain, wheat straw, wine and sunflower seed and at 10 mg/kg for orange fruit, 20 mg/kg for kiwi fruit, 5.0 mg/kg for lettuce and 3.0 mg/kg for grapes (the expected range of residues for each ma-

trix). Mean recoveries of all matrices were 70% to 110% with an RSD <20% for all six matrices. The recoveries obtained using non-matrix matched standards are summarised below.

Table A 1: Recovery results from method validation of Fludioxonil using the analytical method REM 133.06

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Range
Orange fruit	Fludioxonil (m/z 247.0→179.9)	0.01*	109	5	102-115
		10.0	99	2	96-100
		Overall	*104*	*6*	96-115
	Fludioxonil (*m/z* 247.0→126.1)	0.01*	105	4	100-110
		10.0	98	2	96-100
		Overall	101	5	96-110
Kiwi fruit	Fludioxonil (m/z 247.0→179.9)	0.01*	87	8	81-98
		20.0	87	2	84-88
		Overall	*87*	*6*	81-98
	Fludioxonil (*m/z* 247.0→126.1)	0.01*	83	9	76-95
		20.0	87	2	84-89
		Overall	87	6	76-95
Lettuce	Fludioxonil (m/z 247.0→179.9)	0.01*	102	5	97-110
		5.0	90	2	88-92
		Overall	*96*	*7*	88-110
	Fludioxonil (*m/z* 247.0→126.1)	0.01*	101	7	94-111
		5.0	91	1	90-93
		Overall	96	7	90-111
Wheat grain	Fludioxonil (m/z 247.0→179.9)	0.01*	103	4	98-109
		0.1	99	8	81-98
		Overall	*97*	*9*	81-109
	Fludioxonil (*m/z* 247.0→126.1)	0.01*	101	13	88-121
		0.1	97	5	90-102
		Overall	99	10	88-121
Wheat straw	Fludioxonil (m/z 247.0→179.9)	0.01*	97	8	88-106
		0.1	98	4	94-105
		Overall	*97*	*6*	88-106
	Fludioxonil (*m/z* 247.0→126.1)	0.01*	92	9	81-103
		0.1	93	5	86-97
		Overall	93	7	81-103
Grape	Fludioxonil (m/z 247.0→179.9)	0.01*	100	12	86-114
		3.0	102	4	96-107
		Overall	*101*	*8*	86-114

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Range
	Fludioxonil (*m/z* 247.0→126.1)	0.01*	109	12	89-123
		3.0	99	3	93-102
		Overall	104	10	89-123
Wine	Fludioxonil (m/z 247.0→179.9)	0.01*	104	10	91-119
		0.1	101	3	93-114
		Overall	*102*	*9*	91-119
	Fludioxonil (*m/z* 247.0→126.1)	0.01*	98	9	86-109
		0.1	101	3	96-109
		Overall	100	7	86-109
Sunflower seed	Fludioxonil (m/z 247.0→179.9)	0.01*	90	7	81-97
		0.1	82	1	80-83
		Overall	*86*	*7*	80-97
	Fludioxonil (*m/z* 247.0→126.1)	0.01*	85	11	76-99
		0.1	79	4	76-84
		Overall	82	9	76-99

* Denotes LOQ

Table A 2: Characteristics for the analytical method used for validation of Fludioxonil residues in plants

	Fludioxonil
Specificity	LC-MS/MS with two transitions is considered to be highly selective and the method is therefore specific. Residues of Fludioxonil measured in control samples were <30% of the LOQ during method validation.
Calibration (type, number of data points)	Linear, R^2 = 1.00.
Calibration range	0.00005 to 0.012 µg/mL (equivalent to 1.0 to 240 pg injected using a 20 µL injection volume). Residues from 0.01 mg/kg to 1.0 mg/kg may be analysed directly (without additional dilution) while remaining within at least ± 20% of the linear range of the instrument.
Assessment of matrix effects is presented	Yes-Some suppression or enhancement of LC-MS/MS response to Fludioxonil in the presence of matrix was demonstrated but this was less than 10% for most matrices so that samples may be quantified using non-matrix matched standards.
Limit of determination/quantification	0.01 mg/kg.

Conclusion

Fludioxonil residues may be reliably and accurately determined in crop matrices by Method 133.06. The limit of quantification is 0.01 mg/kg Fludioxonil in crops (when using either the primary or confirmatory transition for quantification).

Reference:	KCP 5.1.2
Report:	[redacted], (2012) Cyprodinil and Fludioxonil – Residue Study on Cauliflower in Northern France in 2010 Syngenta Report No. R B0074, Syngenta File No. A9219B_11593
Guideline(s):	FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials (Rome, 1990). Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Annex I of Directive 91/414/EEC (Article 5.3 and 8.2), 1996.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Crop samples are extracted by homogenisation with methanol. Extracts are centrifuged and aliquots re-diluted with acetonitrile/water (30/70, v/v). Final determination is high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS; m/z 247→179.9 for quantification and m/z 247→126.1 for confirmation).

Results and discussions

Control samples were fortified at the limit of quantification (0.01 mg/kg) for cauliflower inflorescence and at 100X LOQ. Mean recoveries were between 70% to 110% with an RSD <20%. The recoveries obtained using non-marix matched standards are summarised below.

Table A 3: Recovery results from method validation on cauliflower using the analytical method REM 133.06

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Range (%)
Cauliflower inflorescence	Fludioxonil (m/z 247.2→179.9)	0.01*	96	3.7	92-100
		1.0	98	2.1	95-101
		Overall	*97*	*2.9*	*92-101*
	Fludioxonil (m/z 247.2→125.9)	0.01*	98	3.8	93-102
		1.0	94	3.7	92-100
		Overall	*96*	*3.8*	*92-102*

* Denotes LOQ

Table A 4: Characteristics for the analytical method used for validation of Fludioxonil residues in plants

Fludioxonil	
Specificity	LC-MS/MS with two transitions is considered to be highly selective and the method is therefore specific. Residues of Fludioxonil measured in control samples were <30% of the LOQ during method validation.
Calibration (type, number of data points)	Linear, 8 standards, r = 0.99979
Calibration range	From 0.03 to 1.2 ng/L. Residues from 0.01 mg/kg to 1.0 mg/kg may be analysed directly (without additional dilution) while remaining within at least ± 20% of the linear range of the instrument.
Assessment of matrix effects is presented	Yes-Some suppression or enhancement of LC-MS/MS response to Fludioxonil in the presence of matrix was demonstrated but this was less than 10% for most matrices so that samples may be quantified using non-matrix matched standards.
Limit of determination/quantification	0.01 mg/kg.

Conclusion

Analytical method REM 133.06 has been successfully validated for the analysis of Fludioxonil in cauliflower. Results obtained were within the guideline requirements (mean recovery 70-110%; RSD <20%) for both mass transitions. The method is valid for the determination of Fludioxonil in crops.

Reference: KCP 5.1.2

Report [redacted], (2014)

Fludioxonil – Validation of the Analytical Method for the Determination of Fludioxonil residues in Peas (Seeds and Haulm)

Syngenta Report No. R B3113. Syngenta File No. CGA173506_11705

Guideline(s): Regulation (EC) No. 1107/2009; Concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

Regulation (EU) No.545/2011

SANCO/825/00 rev.8.1; 16 November 2010

SANCO/3029/99 rev.4, 11 July 2000

OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO (2007)17 (Unclassified, 13 Aug 2007).

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Crop samples are extracted by homogenisation with methanol. Extracts are centrifuged and aliquots re-diluted with acetonitrile/water (30/70, v/v). Final determination is high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS; m/z 247→179.9 for quantification and m/z 247→126.1 for confirmation).

Results and discussions

Control samples were fortified at the limit of quantification (0.01 mg/kg) for all pea matricies and at 10X LOQ for pea seeds and 100XLOQ for pea haulm. Mean recoveries were between 70% to 110% with an RSD <20%. The recoveries obtained using non-marix matched standards are summarised below.

Table A 5: Recovery results from method validation on peas using the analytical method REM 133.06.

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Range (%)
Pea seeds	Fludioxonil (m/z 247.2→179.9)	0.01*	102	9	92-114
		0.1	94	2	91-96
		Overall (n = 10)	*98*	*5.5*	*91-114*
	Fludioxonil (m/z 247.2→125.9)	0.01*	111	4	106-116
Pea haulm	Fludioxonil (m/z 247.2→179.9)	0.01*	98	12	80-114
		10	94	6	84-114
		Overall (n = 10)	*96*	*8*	*80-114*
	Fludioxonil (m/z 247.2→125.9)	0.01*	87	3	83-90

* Denotes LOQ

Table A 6: Characteristics for the analytical method used for validation of Fludioxonil residues in plants

Fludioxonil	
Specificity	LC-MS/MS with two transitions is considered to be highly selective and the method is therefore specific. Residues of Fludioxonil measured in control samples were <30% of the LOQ during method validation.
Calibration (type, number of data points)	Linear, 7 standards, r = 0.99947.
Calibration range	0.03 to 1.2 mg/mL (equivalent to 0.003 to 0.12 mg/kg) for all matrices.
Assessment of matrix effects is presented	Yes-Some suppression or enhancement of LC-MS/MS response to Fludioxonil in the presence of matrix was demonstrated but this was less than 10% for most matrices so that

	samples may be quantified using non-matrix matched standards.
Limit of determination/quantification	0.01 mg/kg.

Stability of residues in extracts

The stability results proved that Fludioxonil standards were stable in milli-Q water/acetonitrile (70/30, v/v) when stored at a target temperature of -18°C for a period of 16 days.

Conclusion

The purpose of this study was to demonstrate the suitability and perform the validation of the method REM 133.06 to determine residues of the Fludioxonil in peas (seeds and haulm). The analytical method has been successfully validated for the determination of Fludioxonil in peas (seeds and haulm). The repeatability and specificity of the method have been demonstrated, and the method REM 133.06 is therefore considered valid for the determination of residues of Fludioxonil in peas (seeds and haulm) at the LOQ of 0.01 mg/kg over concentration ranges typical of those for which the method will be used. The method has been validated according to the EU guideline SANCO/3029/99 Rev.4 and SANCO/825/00 Rev. 8.1.

Reference:	KCP 5.1.2
Report:	[redacted], (2018) Fludioxonil (CGA173506) - Validation of Analytical Method REM133.06 for the Determination of Residues of Fludioxonil in multiple crops Syngenta Report No. R B7376. Syngenta File No. CGA173506_12273
Guideline(s):	Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000). OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007). EPA Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174 (Aug 1996).
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Crop samples were extracted by homogenisation with methanol. Extracts were centrifuged and aliquots (0.1 mL) were diluted with 0.9 mL of acetonitrile:water (30:70 v/v). Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 247.0-179.9) and the confirmatory transition (m/z 247.0-

125.9).

Analytical method REM 133.06 was validated in a wide range of crops, apple, cherry, peach, fresh peas (with pods), dried beans, bulb onion, carrot, tomato, melon, strawberry, blackcurrant, asparagus, celery and witloof chicory (chicon).

Results and discussion

Control samples were fortified at the limit of quantification (0.01 mg/kg) for all matrics and at a fortification level given below.

- 500 x LOQ (5 mg/Kg) for apple
- 500 x LOQ (5 mg/Kg) for cherry
- 1000 x LOQ (10 mg/Kg) for peach
- 100 x LOQ (1 mg/Kg) for fresh peas (with pods)
- 50 x LOQ (0.5 mg/Kg) for dried beans
- 50 x LOQ (0.5 mg/Kg) for bulb onion
- 100 x LOQ (1 mg/Kg) for carrot
- 300 x LOQ (3 mg/Kg) for tomato
- 30 x LOQ (0.3 mg/Kg) for melon
- 500 x LOQ (5 mg/Kg) for strawberry
- 300 x LOQ (3 mg/Kg) for blackcurrant
- 10 x LOQ (0.1 mg/Kg) for asparagus
- 150 x LOQ (1.5 mg/Kg) for celery
- 10 x LOQ (0.1 mg/Kg) for witloof chicory (chicon).

Mean recoveries were between 70% to 110% with an RSD <20%. The recoveries obtained using non-marix matched standards are summarised below.

Table A 7: Recovery results from validation of REM 133.06 for Fludioxonil in various crops

Matrix	**Analyte**	**Fortification level (mg/kg) (*n* =5)**	**Mean recovery (%)**	**RSD (%)**	**Range (%)**
Apple	Fludioxonil (m/z 247.2→179.9)	0.01*	81	3.5	77 - 84
		5	98	3.7	94 - 104
		Overall (n = 10)	*90*	*10.8*	*77 - 104*
	Fludioxonil (m/z 247.2→125.9)	0.01*	80	3.6	78 - 85
		5	99	4.0	95 - 105
		Overall (n = 10)	*89*	*12.1*	*78 - 105*
Cherry	Fludioxonil (m/z 247.2→179.9)	0.01*	86	3.0	83 - 89
		5	91	1.9	89 - 93
		Overall (n = 10)	*88*	*4.0*	*83 - 93*
	Fludioxonil (m/z 247.2→125.9)	0.01*	85	4.8	78 - 89
		5	91	1.1	90 - 92
		Overall (n = 10)	*88*	*5.0*	*78 - 92*
Peach	Fludioxonil (m/z 247.2→179.9)	0.01*	82	2.0	79 - 83
		10	101	9.0	91 - 116
		Overall (n = 10)	*92*	*13.2*	*79 - 116*
	Fludioxonil (m/z 247.2→125.9)	0.01*	82	4.7	78 - 86
		10	102	9.5	92 - 118
		Overall (n = 10)	*92*	*13.9*	*78 - 118*
Fresh pea (with pod)	Fludioxonil (m/z 247.2→179.9)	0.01*	74	4.3	69 - 78
		1	98	4.3	93 - 101
		Overall (n = 10)	*86*	*14.7*	*69 - 101*
	Fludioxonil (m/z 247.2→125.9)	0.01*	76	4.9	72 - 81
		1	99	3.6	95 - 102
		Overall (n = 10)	*87*	*14.4*	*72 - 102*
Dried bean	Fludioxonil (m/z 247.2→179.9)	0.01*	83	3.5	78 - 85
		0.5	95	1.9	93 - 97
		Overall (n = 10)	*89*	*7.7*	*78 - 97*
	Fludioxonil (m/z 247.2→125.9)	0.01*	83	2.4	81 - 86
		0.5	96	1.9	93 - 98
		Overall (n = 10)	*89*	*8.0*	*81 - 98*
Bulb onion	Fludioxonil (m/z 247.2→179.9)	0.01*	78	2.7	75 - 80
		0.5	92	2.3	89 - 95
		Overall (n = 10)	*85*	*8.8*	*75 - 95*
	Fludioxonil (m/z 247.2→125.9)	0.01*	79	2.0	77 - 81
		0.5	91	2.3	88 - 93
		Overall (n = 10)	*85*	*7.8*	*77 - 93*

Matrix	**Analyte**	**Fortification level (mg/kg) (*n* =5)**	**Mean recovery (%)**	**RSD (%)**	**Range (%)**
Carrot	Fludioxonil (m/z 247.2→179.9)	0.01*	84	2.4	82 - 86
		1	95	3.4	91 - 98
		Overall (n = 10)	*89*	*6.9*	*82 - 98*
	Fludioxonil (m/z 247.2→125.9)	0.01*	78	7.7	69 - 83
		1	94	3.5	89 - 97
		Overall (n = 10)	*86*	*10.9*	*69 - 97*
Tomato	Fludioxonil (m/z 247.2→179.9)	0.01*	90	5.3	87 - 99
		3	99	3.1	96 - 104
		Overall (n = 10)	*95*	*6.4*	*87 - 104*
	Fludioxonil (m/z 247.2→125.9)	0.01*	94	6.7	87 - 103
		3	98	2.2	95 - 100
		Overall (n = 10)	*96*	*5.1*	*87 - 103*
Melon	Fludioxonil (m/z 247.2→179.9)	0.01*	86	5.1	81 - 90
		0.3	98	3.9	94 - 102
		Overall (n = 10)	*92*	*8.3*	*81 - 102*
	Fludioxonil (m/z 247.2→125.9)	0.01*	94	6.7	87 - 103
		0.3	98	2.2	95 - 100
		Overall (n = 10)	*96*	*5.1*	*87 - 103*
Strawberry	Fludioxonil (m/z 247.2→179.9)	0.01*	90	2.2	87 - 92
		5	100	3.3	98 - 105
		Overall (n = 10)	*95*	*6.4*	*87 - 105*
	Fludioxonil (m/z 247.2→125.9)	0.01*	90	4.9	85 - 96
		5	101	3.0	98 - 105
		Overall (n = 10)	*95*	*7.1*	*85 - 105*
Blackcurrant	Fludioxonil (m/z 247.2→179.9)	0.01*	*77*	4.2	73 - 80
		3	88	2.3	84 - 89
		Overall (n = 10)	*82*	*7.7*	*73 - 89*
	Fludioxonil (m/z 247.2→125.9)	0.01*	76	6.4	71 - 82
		3	87	2.5	83 - 89
		Overall (n = 10)	*81*	*8.3*	*71 - 89*
Asparagus	Fludioxonil (m/z 247.2→179.9)	0.01*	89	0.9	88 - 90
		0.1	98	1.0	96 - 99
		Overall (n = 10)	*94*	*4.9*	*88 - 99*
	Fludioxonil (m/z 247.2→125.9)	0.01*	87	2.0	86 - 89
		0.1	98	1.1	97 - 99
		Overall (n = 10)	*93*	*6.5*	*86 - 99*

Matrix	Analyte	Fortification level (mg/kg) (*n* =5)	Mean recovery (%)	RSD (%)	Range (%)
Celery	Fludioxonil (m/z 247.2→179.9)	0.01*	79	2.7	77 - 82
		1.5	98	2.5	96 - 100
		Overall (n = 10)	*88*	*11.4*	*77 - 100*
	Fludioxonil (m/z 247.2→125.9)	0.01*	78	4.6	74 - 83
		1.5	99	2.1	97 - 101
		Overall (n = 10)	*89*	*12.9*	*74 - 101*
Witloof chicory (chicon)	Fludioxonil (m/z 247.2→179.9)	0.01*	91	3.4	85 - 93
		1.5	95	1.7	93 - 96
		Overall (n = 10)	*93*	*3.4*	*85 - 96*
	Fludioxonil (m/z 247.2→125.9)	0.01*	88	3.5	85 - 93
		1.5	96	1.3	94 - 97
		Overall (n = 10)	*92*	*5.0*	*85 - 97*

0.01 mg/Kg = limit of quantification, defined by the lowest validated fortification level.

Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 8: Characteristics for the analytical method used for validation of Fludioxonil residues in plants

Fludioxonil	
Specificity	LC-MS/MS with two transitions is considered to be highly selective and the method is therefore specific. Residues of Fludioxonil measured in control samples were <30% of the LOQ during method validation. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest.
Calibration (type, number of data points)	Linear, 7 standards, r > 0.99
Calibration range	From 0.03 to 1.21 ng/mL. Residues from 0.01 mg/kg to 1.0 mg/kg may be analysed directly (without additional dilution) while remaining within at least ± 20% of the linear range of the instrument.
Assessment of matrix effects is presented	No significant matrix effects were observed in the crop matrices tested during method validation, therefore non-matrix matched linearity standards were used for quantification.
Limit of determination/quantification	0.01 mg/kg.

Stability of Final Extracts

The stability of sample extracts fortified with Fludioxonil at the LOQ level (0.01 mg/Kg) was assessed up to 14 days for carrot, tomato and melon, 15 days for apple, peach, fresh peas (with pods), dried beans, strawberry, asparagus, celery and witloof chicory (chicon) and 17 days for cherry, bulb onion and blackcurrant. The results demonstrated that the Fludioxonil residues in the stored fortified samples were stable over these time periods. The mean recovery values at the LOQ level were between 70 and 110%, with a RSD of ≤ 20% and the difference from the original analysis was ≤ 20% when re-analysed.

Stability of Standard Solutions

The stability of the stored working standard solutions of Fludioxonil at 1.01 ng/mL were assessed after a storage period of 17 days in a refrigerator between 1 - 7°C against freshly prepared calibration standards. The results demonstrated that Fludioxonil residues in the stored working standard solutions were stable. The mean response factors from three replicate measurements for each of two solutions (old and new) did not differ by more than 10%.

Stability of Spiking Solutions

The stability of the stored working spiking solutions of Fludioxonil at 1007 ng/mL was assessed after a storage period of 21 days in a refrigerator between 1 - 7°C against freshly prepared spiking solution. The results demonstrated that Fludioxonil residues in the stored working spiking solutions were stable. The mean response factors from three replicate measurements for each of two solutions (old and new) did not differ by more than 10%.

Conclusion

Analytical method REM 133.06 has been demonstrated to be a reliable and accurate procedure for the determination of Fludioxonil in crops to a limit of quantification of 0.01 mg/Kg, using commercially available laboratory equipment and reagents.

A 2.1.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

No new or additional studies have been submitted.

A 2.1.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

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 5.2.1)

A 2.1.2.1.1 QuEChERS (EN 15662:2009-02)

A 2.1.2.1.1.1 Method validation

Reference:	KCP 5.2.1
Report	[redacted] and [redacted] (2014). Fludioxonil – Validation of the QuEChERS Method for the Determination of Fludioxonil Residues in Crop Matrices by LC-MS/MS. Report No. P-3446 G. Syngenta document No. CGA173506/11710.
Guideline(s):	Commission of the European Communities. Guidance Document on Resi-

due Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).

OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO (2007)17.

Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

The analytical method was derived from the QuEChERS (EN 15662:2009-02) multi-residue method. It is based on extraction and clean-up procedures, and subsequent LC-MS/MS determination.

Sample material (lettuce, oilseed rape seed, dried broad bean, wheat straw and whole orange) was extracted by shaking with acetonitrile, after the addition of a suitable volume of water if necessary (i.e. taking into account the natural water content of the samples). After the addition of a mixture of magnesium sulphate, sodium chloride, and buffering citrate salts (available pre-mixed commercially: dispersive SPE citrate extraction tube, Supelco 55227-U) the extracts were shaken and then centrifuged. In the case of oilseed rape seed and dried broad bean the fat was frozen out, and then an aliquot of each extract (for all matrices) was cleaned up using a pre-mixed, commercially available dispersive SPE clean up tube (Supelco 55228-U). For oilseed rape seed and dried broad bean extracts, a portion of C18 was added prior to shaking. After centrifugation, extracts were acidified with a small amount of 5% formic acid solution and diluted to within the calibration range with acetonitrile/water (20/80, v/v, containing 0.1% formic acid) and blank matrix (if necessary). Final determination was by high-performance liquid chromatography with triple quadrupole mass-spectrometric detection (LC MS/MS), monitoring for a primary (m/z 247→ 169) and confirmatory (m/z 247→ 126) transition.

The QuEChERS method was validated for a wide range of crop types:

Lettuce (high water content), oilseed rape seed (high oil content), dried broad bean (high protein content), wheat straw (dry commodity) and whole orange (high acid content).

Results and discussions

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at a higher level: 0.10 mg/kg (oilseed rape seed), 0.50 mg/kg (dried broad bean and wheat straw), 10 mg/kg (whole orange) and 15 mg/kg (lettuce). Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (SANCO 825/00 rev.8.1 16/11/10) demonstrate the method has satisfactory accuracy.

The relative standard deviations (RSDs) of the recoveries at each fortification level and overall for each crop tested during method validation were <20% and therefore according to the EU guidance (SANCO 825/00 rev.8.1 16/11/10) demonstrate the method has satisfactory repeatability.

Table A 9: Recovery results from method validation of Fludioxonil using the QuEChERS analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)

Matrix	**Analyte**	**Fortification level (mg/kg) (*n* = x)**	**Mean recovery (%)**	**RSD (%)**	**Recovery Range (%)**
Lettuce	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	110	1	109-112
		15 (n = 5)	107	3	102-109
		Overall	*108*	*3*	*102-112*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	110	2	108-113
		15 (n = 5)	107	2	103-110
		Overall	*109*	*2*	*103-113*
Whole orange	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	110	2	106-113
		10 (n = 5)	99	5	93-103
		Overall	*105*	*7*	*93-113*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	110	4	104-116
		10 (n = 5)	97	4	91-101
		Overall	*104*	*8*	*91-116*
Wheat straw	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	91	3	87-95
		0.5 (n = 5)	96	5	88-98
		Overall	*93*	*5*	*87-98*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	91	4	86-95
		0.5 (n = 5)	96	4	88-98
		Overall	*93*	*5*	*86-98*
Dried broad bean	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	85	4	81-89
		0.5 (n = 5)	83	5	81-90
		Overall	*84*	*4*	*81-90*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	85	3	82-87
		0.5 (n = 5)	83	4	81-89
		Overall	*84*	*3*	*81-89*
Oil seed rape seed	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	100	2	98-102
		0.1 (n = 5)	98	1	97-100
		Overall	*99*	*2*	*97-102*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	100	1	98-101
		0.1 (n = 5)	98	2	95-101
		Overall	*99*	*2*	*95-101*

Table A 10: Characteristics for the analytical method used for validation of Fludioxonil residues in plants

	Fludioxonil
Specificity	No significant interferences arising from the crop matrices, the lab ware, reagents or solvents have been observed at the retention times of interest.
Calibration (type, number of data points)	The linearity was tested using standard solutions in solvent

	Fludioxonil
Specificity	No significant interferences arising from the crop matrices, the lab ware, reagents or solvents have been observed at the retention times of interest.
	(for lettuce and dried broad bean) or matrix-matched standard solutions (for whole orange, oilseed rape seed, wheat straw), over a concentration range of 0.05 ng/mL to 5.0 ng/mL. Linearity was tested for both the primary and confirmatory MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9993 to 1.000 were obtained for Fludioxonil.
Calibration range	0.05 ng/mL to 5.0 ng/mL.
Assessment of matrix effects is presented	Yes: Insignificant matrix effects (suppression or enhancement, ≤ ± 20%) were observed for lettuce and dried broad bean matrices when matrix-matched standards and standards in solvent (0.1% formic acid in acetonitrile/water (20/80, v/v)) were compared. Significant matrix effects on LC-MS/MS response were observed for whole orange, oilseed rape seed and wheat straw matrices; thus, whole orange, oilseed rape seed and wheat straw extracts were evaluated using matrix-matched standards
Limit of determination/quantification	The limit of quantification was established at 0.01 mg/kg. The limit of detection (LOD) was demonstrated to be ≤ 0.002 mg/kg for both the primary and confirmatory transitions, for all matrices tested.

Conclusion

The QuEChERS method has been demonstrated to be a reliable and accurate procedure for the determination of Fludioxonil in crops to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.1.2.1.1.2 Independent laboratory validation

Reference: KCP 5.2.1

Report [redacted] and [redacted] (2014).

Fludioxonil – Independent Laboratory Validation of the QuEChERS Method for the Determination of Fludioxonil Residues in Crop Matrices by LC-MS/MS.

Report No. 20140189. Syngenta document No. CGA173506/11723.

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).

OECD Guidance Document on Pesticide Residue Analytical Methods

ENV/JM/MONO (2007)17.

Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

The QuEChERS method was independently validated in lettuce (high water content), oilseed rape seed (high oil content), dried broad bean (high protein content) and wheat straw (high starch content).

Results and discussions

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and 10 x LOQ (0.10 mg/kg) for oilseed rape seed matrix; at the LOQ (0.01 mg/kg) and 50 x LOQ (0.50 mg/kg) for dried broad bean and wheat straw matrices; and at the LOQ (0.01 mg/kg) and 1500 x LOQ (15 mg/kg) for lettuce matrix. Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010) demonstrate the method has satisfactory accuracy.

The relative standard deviations (RSDs) of the recoveries at each fortification level and overall for each crop tested during method validation were ≤20% and therefore according to the EU guidance (SANCO/825/00 rev.8.1, 16/11/2010) demonstrate the method has satisfactory repeatability.

Table A 11: Recovery results from independent laboratory validation of Fludioxonil using the QuEChERS analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Lettuce	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	109	4.1	101-112
		15 (n = 5)	100	2.5	97-103
		Overall	*105*	*5.3*	*97-112*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	104	6.8	92-109
		15 (n = 5)	102	1.4	100-104
		Overall	*103*	*4.7*	*92-109*
Wheat straw	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	94	10.5	82-107
		0.5 (n = 5)	94	15.5	76-112
		Overall	*94*	*12.5*	*76-112*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	84	8.8	78-97
		0.5 (n = 5)	93	15.0	78-112
		Overall	*89*	*12.9*	*78-112*
Oilseed rape seed	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	77	18.1	59-90
		0.1 (n = 5)	107	2.7	103-109
		Overall	*92*	*19.9*	*59-109*

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	83	3.8	78-87
		0.1 (n = 5)	110	2.1	107-112
		Overall	*96*	*15.2*	*78-112*
Dried broad beans	Fludioxonil *m/z* 247→ 169 (quantification)	0.01 (n = 5)	74	15.5	60-86
		0.5 (n = 5)	91	7.4	82-98
		Overall	*82*	*15.4*	*60-98*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.01 (n = 5)	81	14.2	61-89
		0.5 (n = 5)	90	6.5	81-95
		Overall	*85*	*11.5*	*61-95*

Table A 12: Characteristics for the analytical method used for independent laboratory validation of Fludioxonil residues in plants

	Fludioxonil
Specificity	No significant interferences arising from the crop matrices, the lab ware, reagents or solvents have been observed at the retention times of interest.
Calibration (type, number of data points)	The linearity was tested using standard solutions in solvent or matrix-matched standard solutions, over a concentration range of 0.05 ng/mL to 10 ng/mL. Linearity was tested for both the primary and confirmatory MS/MS transitions At least six different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9987 to 1.000 were obtained for Fludioxonil.
Calibration range	0.05 ng/mL to 10 ng/mL.
Assessment of matrix effects is presented	Yes: Insignificant matrix effects (suppression or enhancement, < ± 20%) were observed for oilseed rape seed matrix when matrix-matched standards and standards in solvent (acetonitrile: water (20:80, v/v, containing 0.1% formic acid)) were compared. Significant matrix effects (suppression) on LC-MS/MS response were observed for wheat straw, lettuce and dried broad bean matrices. Matrix matched linearity standards were used for quantification for all crop matrices.
Limit of determination/quantification	The limit of quantification was established at 0.01 mg/kg. The limit of detection (LOD) was demonstrated to be ≤ 0.002 mg/kg for both the primary and confirmatory transitions, for all matrices tested.

Conclusion

The repeatability and specificity of the method have been independently demonstrated, and QuEChERS method EN 15662:2009-2 is therefore considered valid for the determination of residues of Fludioxonil in crop matrices to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

A 2.1.2.2.1 GRM025.03A

A 2.1.2.2.1.1 Method validation

Reference:	KCP 5.2.2
Report	Fludioxonil – Analytical Method for the Determination of Residues of Total Fludioxonil (CGA173506) and Metabolites as CGA192155 in Animal Matrices (Milk, Eggs, Muscle, Fat, Liver, Kidney and Whole Blood). Final Determination by LC-MS/MS [redacted], 2009 Report No. GRM025.03A – version 2. Syngenta document No. CGA173506_11402, VV-127758
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev.7). OPPTS 860.1340.
Deviations:	No.
GLP:	No
Acceptability:	Yes.

Reference:	KCP 5.2.2
Report	Fludioxonil: Validation of Residue Method GRM025.03A for Total Fludioxonil (CGA173506) and Metabolites as CGA192155 in Animal Matrices (Milk, Eggs, Muscle, Fat, Liver, Kidney and Whole Blood). [redacted], 2009 Report No. T-001341-08-REG. Syngenta document No. VV-382790
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev.7). OPPTS 860.1340.
Deviations:	No.
GLP:	Yes.

Acceptability: Yes.

Materials and methods

Method GRM025.03A determines fludioxonil and its metabolites oxidisable to CGA192155 as CGA192155 (expressed as fludioxonil equivalents).

Samples are homogenised and then extracted by refluxing with ammonium hydroxide/acetonitrile (80/20, v/v). After filtration the aqueous phase is acidified and partitioned with toluene following addition of sodium chloride. Conversion of CGA173506 and its metabolites to CGA192155 is carried out by heating in the presence of potassium permanganate and aqueous sodium hydroxide; the oxidation is then quenched with sodium metabisulfite, the extracts are filtered, acidified, and partitioned into dichloro-methane/ ethyl acetate (80/20, v/v). After evaporation, the residues are dissolved in acetonitrile/water (50/50, v/v) and determined as total fludioxonil by LC-MS/MS.

The analytical procedure converts fludioxonil and structurally-related metabolites to the common moiety CGA192155. A molecular weight correction factor of 1.23 is applied when calculating procedural recovery values and quantifying residues of CGA192155.

Results and discussions

A reagent blank sample was analysed, control samples were analysed in duplicate and samples fortified with fludioxonil were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) for each matrix tested.

Acceptable mean recoveries of between 70% and 110% with relative standard deviations (RSD) of <20% were found for both transitions for all analytes in all matrices.

Table A 13: Recovery results from method validation of total fludioxonil residues using the analytical method GRM025.03A

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Recovery range (%)
Eggs	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	109	2	107-113
		0.1 (n=5)	97	2	95-99
		Overall	*103*	*6*	*95-113*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	103	2	99-105
		0.1 (n=5)	97	2	95-100
		Overall	*100*	*3*	*95-105*
Milk	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	87	11	74-98
		0.1 (n=5)	78	2	76-80
		Overall	*83*	*9*	*74-98*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	84	6	80-92
		0.1 (n=5)	80	2	77-81
		Overall	*82*	*5*	*77-92*
Muscle	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	77	6	70-82
		0.1 (n=5)	78	3	75-80
		Overall	*78*	*4*	*70-82*

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Recovery range (%)
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	79	4	74-82
		0.1 (n=5)	79	2	76-80
		Overall	*79*	*3*	*74-82*
Liver	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	86	2	85-89
		0.1 (n=5)	87	1	87-88
		Overall	*87*	*1*	*85-89*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	88	1	86-89
		0.1 (n=5)	87	1	86-87
		Overall	*87*	*1*	*86-89*
Kidney	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	79	4	74-82
		0.1 (n=5)	82	3	80-84
		Overall	*80*	*4*	*74-84*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	79	6	73-83
		0.1 (n=5)	81	2	80-83
		Overall	*80*	*4*	*73-83*
Fat	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	78	2	76-81
		0.1 (n=5)	79	2	77-81
		Overall	*78*	*2*	*76-81*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	72	5	65-75
		0.1 (n=5)	79	2	77-81
		Overall	*75*	*6*	*65-81*
Blood	CGA192155 *m/z* 201→ 157 (quantification)	0.01 (n=5)	80	2	77-82
		0.1 (n=5)	83	1	82-84
		Overall	*81*	*2*	*77-84*
	CGA192155 *m/z* 201→ 91 (confirmation)	0.01 (n=5)	79	3	77-82
		0.1 (n=5)	83	1	82-84
		Overall	*81*	*3*	*77-84*

Table A 14: Characteristics for the analytical method used for validation of fludioxonil residues in animal matrices

	Total fludioxonil
Specificity	Residues of fludioxonil as CGA192155 measured in control samples were <30% of the limit of quantification (LOQ) in any of the control or reagent blank samples.
Calibration (type, number of data points)	Standard solutions containing CGA192155 at concentrations ranging from 0.0005 to 0.05 µg/mL were analysed. The response of the LC-MS/MS system was shown to be linear for CGA192155 primary transition and the confirmatory transition over the concentration range tested. Correlation coefficients ranged from 0.9994 to 0.9999.

	Total fludioxonil
Calibration range	0.0005 to 0.05 µg/mL.
Assessment of matrix effects is presented	The effect of matrix on the LC-MS/MS response was assessed by preparing standards with and without matrix and comparing the peak areas of CGA192155 at equivalent concentrations. Matrix effects (enhancement or suppression) were greater than 10% for several matrices, and the use of matrix-matched standards is recommended.
Limit of determination/quantification	The validated limit of quantification for fludioxonil and metabolites as CGA192155 in animal tissues was 0.01 mg/kg for fludioxonil (= 0.0081 mg/kg for CGA192155) when measured as CGA192155 for all animal matrices tested.

Conclusion

Method GRM025.03 has been demonstrated to be a reliable and accurate procedure for the determination of fludioxonil as CGA192155 in animal matrices using commercially available laboratory equipment and reagents. Bovine milk, muscle, liver, fat, kidney, hen's eggs and bovine blood have been used as representative matrices. The limit of quantification is 0.01 mg/kg for all matrices tested using either the primary or confirmatory transition. The method complies with the requirements of SANCO 3029/99 rev 4 11/07/00 and SANCO 825/00 rev.8.1 16/11/10.

A 2.1.2.2.1.2 Independent laboratory validation

Reference: KCP 5.2.2

Report: Fludioxonil: Magnitude of Residues in Animal Tissues Following Repeated Oral Administration to the Laying Hen.

[redacted] (2009).

Report No. 1983/108-D2149. Syngenta document No CGA 173506_11440

Guideline(s): European Union Council Directive 91/414/EEC of 15 July 1991, as amended by Commission Directive 96/68/EC of 21 October 1996.

SANCO/7031/VI/95 (Livestock Feeding Studies - Appendix G).

OECD guideline 50.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

Analytical method GRM025.03A was used to determine the content of CGA192155 in egg and tissue samples. The method involved extraction of homogenised samples by refluxing with ammonium hydroxide/acetonitrile (80/20, v/v). Following filtration the aqueous phase was acidified and partitioned with the addition of salt and toluene. Fludioxonil and its metabolites were converted to CGA192155 by heating in the presence of potassium permanganate and aqueous sodium hydroxide. The oxidation was then

quenched by the addition of sodium metabisulphite, with the extracts filtered and acidified prior to partition into dichloromethane/ethyl acetate (80/20, v/v). Following evaporation residues were dissolved in acetonitrile/water (50/50, v/v) with final quantification by Liquid Chromatography with tandem Mass Spectrometry detection (LC-MS/MS) in the multiple reaction monitoring (MRM) mode.

All results reported were quantified using the transition, m/z 200.9→91.0 with m/z 200.9→156.9 employed for confirmation.

The analytical procedure converts Fludioxonil and structurally-related metabolites to the common moiety CGA192155. A molecular weight correction factor of 1.23 is applied when calculating procedural recovery values and quantifying residues of CGA192155.

The extraction and clean-up procedure was identical to the primary method GRM025.03A.

Results and discussions

Recovery of CGA192155 from each matrix fortified at the LOQ and expected residues levels was determined in quintuplicate.

Acceptable mean recoveries of between 70% and 110% with relative standard deviations (RSD) of <20% were found for both transitions in all matrices.

Table A 15: Recovery results from independent laboratory validation of total Fludioxonil using the analytical method GRM025.03A

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Recovery range
Eggs	CGA192155 *m/z* 201→ 91 (quantification)	0.01 (n=5)	70	5	66-74
		0.6 (n=5)	77	3	73-79
		Overall	*74*	*6*	*66-79*
	CGA192155 *m/z* 201→ 157 (confirmation)	0.01 (n=5)	70	7	64-76
		0.6 (n=5)	77	3	74-80
		Overall	*74*	*7*	*64-80*
Poultry muscle	CGA192155 *m/z* 201→ 91 (quantification)	0.01 (n=5)	90	4	84-94
		0.1 (n=5)	73	2	72-75
		Overall	*82*	*11*	*72-94*
	CGA192155 *m/z* 201→ 157 (confirmation)	0.01 (n=5)	95	5	88-99
		0.1 (n=5)	73	2	71-74
		Overall	*84*	*14*	*71-99*
Poultry fat	CGA192155 *m/z* 201→ 91 (quantification)	0.01 (n=5)	83	7	76-90
		0.1 (n=5)	76	4	73-80
		Overall	*80*	*7*	*73-90*
	CGA192155 *m/z* 201→ 157 (confirmation)	0.01 (n=5)	91	6	86-99
		0.1 (n=5)	77	4	74-81
		Overall	*84*	*10*	*74-99*
Poultry liver	CGA192155 *m/z* 201→ 91 (quantification)	0.01 (n=5)	*94*	*11*	79-108
		0.1 (n=5)	*84*	*5*	77-88
		Overall	*89*	*10*	*77-108*

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Recovery range
	CGA192155 *m/z* 201→ 157 (confirmation)	0.01 (n=5)	102	10	88-114
		0.1 (n=5)	84	5	77-88
		Overall	*93*	*13*	*77-114*

Table A 16: Characteristics for the analytical method used for independent laboratory validation of Fludioxonil residues in animal matrices

	Total Fludioxonil
Specificity	Residues of Fludioxonil as CGA192155 measured in control samples were <30% of the limit of quantification (LOQ) in any of the control or reagent blank samples.
Calibration (type, number of data points)	Standard solutions containing CGA192155 at concentrations ranging from 0.0005 to 0.05 µg/mL were analysed. The response of the LC-MS/MS system was shown to be linear for CGA192155 primary transition and the confirmatory transition over the concentration range tested. Correlation coefficients were > 0.99.
Calibration range	0.0005 to 0.05 µg/mL.
Assessment of matrix effects is presented	No significant interferences arising from animal matrices were observed and there was no significant enhancement or suppression of detector response.
Limit of determination/quantification	The limit of quantification for Fludioxonil residues was established at 0.01 mg/kg.

Conclusion

The study is suitable as an independent laboratory validation study.

A 2.1.2.2.1.3 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required.

A 2.1.2.2.1.4 Extraction efficiency

The extraction procedures used in analytical methods GRM025.03 and AG-616B are very similar, so extractability efficiency of analytical method GRM02.03 can be demonstrated by reference to AG-616. Radio validation of analytical method AG-616 has been carried out and reported (Denmark, 2005).

A 2.1.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

No new or additional studies have been submitted.

A 2.1.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

No new or additional studies have been submitted

A 2.1.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

No new or additional studies have been submitted

A 2.1.2.5.1 GRM025.01A

A 2.1.2.5.1.1 Independent laboratory validation

Reference:	KCP 5.2.5
Report	Fludioxonil- Independent Laboratory Validation (ILV) of Analytical Method GRM025.01A for the Determination of Residues of Fludioxonil (CGA173506) and its Metabolites CGA192155 and CGA339833 in Water. [redacted], 2016. Report Number CGA173506DW. Syngenta File No. CGA173506_11942.
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). Residue Chemistry Test Guidelines OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation, EPA 712-C-001, January 2012.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Materials and methods

Analytical method GRM025.01A was independently validated in drinking water samples for Fludioxonil and its metabolites CGA192155 and CGA339833, at the limit of quantification (LOQ) of the method (0.05 µg/L) and at 10 x LOQ (0.5 µg/L).

By following the method and washing the SPE column with 1 mL of water, the method was successfully validated for Fludioxonil and CGA192155. However, CGA339833 failed the validation. The method procedure was slightly modified and the SPE column was washed with 2 mL of water. Using this modified procedure, the method was successfully validated for Fludioxonil and CGA339833 but not for CGA192155. Therefore, two SPE columns should be prepared and post application of the water specimens, one SPE columns should be washed with 1 mL of water to enable Fludioxonil and CGA192155 analysis and the other one should be washed with 2 mL of water to enable Fludioxonil and CGA339833 analysis.

Results and discussions

Recoveries at the LOQ and at ten times the LOQ were determined in quintuplicate.

Acceptable mean recoveries of between 70% and 110% with relative standard deviations (RSD) of <20% were found for Fludioxonil, CGA129155 and CGA339833 for both transitions.

Table A 17: Recovery results from independent laboratory validation of Fludioxonil using the analytical method – original procedure

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery range
Drinking water	Fludioxonil *m/z* 247→ 180 (quantification)	0.05 (n=5)	78	5	74-84
		0.5 (n=5)	78	4	75-83
		Overall	*78*	*4*	*74-84*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.05 (n=5)	78	5	73-82
		0.5 (n=5)	79	4	76-83
		Overall	*79*	*4*	*73-83*
	CGA192155 *m/z* 201→ 91 (quantification)	0.05 (n=5)	80	5	77-86
		0.5 (n=5)	76	5	70-79
		Overall	*78*	*6*	*70-86*
	CGA192155 *m/z* 201→ 157 (confirmation)	0.05 (n=5)	81	6	76-88
		0.5 (n=5)	75	6	70-80
		Overall	*78*	*6*	*70-88*

Table A 18: Recovery results from independent laboratory validation of Fludioxonil using the analytical method – modified procedure

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Recovery range
Drinking water	Fludioxonil *m/z* 247→ 180 (quantification)	0.05 (n=5)	76	6	70-81
		0.5 (n=5)	77	5	71-80
		Overall	*77*	*5*	*70-81*
	Fludioxonil *m/z* 247→ 126 (confirmation)	0.05 (n=5)	80	9	69-87
		0.5 (n=5)	78	5	73-81
		Overall	*79*	*7*	*69-87*
	CGA339833 *m/z* 311→ 267 (quantification)	0.05 (n=5)	78	3	75-81
		0.5 (n=5)	84	5	77-87
		Overall	*81*	*5*	*75-87*
	CGA339833 *m/z* 311→ 66 (confirmation)	0.05 (n=5)	78	5	72-82
		0.5 (n=5)	83	6	75-88
		Overall	*81*	*6*	*72-88*

Table A 19: Characteristics for the analytical method used for independent laboratory validation of Fludioxonil residues in drinking water

	Fludioxonil	**CGA129155**	**CGA339833**
Specificity	Residues in control samples and reagent blanks were less than 30% of the LOQ.		
Calibration (type, number of data points)	The detector response for Fludioxonil, CGA192155 and CGA339833 was shown to be linear over the range 1 pg to 500 pg injected (n=9). Straight lines with correlation coefficients ranging from 0.9986 to 0.9999 were obtained.		
Calibration range	0.1 µg/L to 50 µg/L when using a 10 µL injection volume.		
Assessment of matrix effects is presented	No significant interferences, above 30% of the LOQ, arising from the drinking water matrix, the lab ware, reagents or solvents have been observed at the retention times of interest.		
Limit of determination/quantification	The limit of quantification for Fludioxonil, CGA192155 and CGA339833 was established at 0.05 µg/L.		

Conclusion

Analytical method GRM025.01A has been independently validated for the determination of Fludioxonil, CGA192155 and CGA339833 in drinking water with a limit of quantification of 0.05 µg/L

A 2.1.2.5.1.2 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required.

A 2.1.2.5.1.3 Extraction efficiency

Not required for an ILV study.

A 2.1.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

No new or additional studies have been submitted

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for the Metalaxyl-M

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.2.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted.

A 2.2.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted.

A 2.2.1.3 Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2.3)

No new or additional studies have been submitted.

A 2.2.1.4 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

No new or additional studies have been submitted.

A 2.2.1.5 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

No new or additional studies have been submitted.

A 2.2.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY
Name of authority: HSE Chemicals Regulation Division (CRD) **Comments:**
Two ecotox studies have been submitted which are supported by method validation data, these are: *Sedaxane/Metalaxyl-M/Fludioxonil FS (A20607B) – Toxicity to the Water Flea Daphnia magna Straus under Laboratory Conditions (Acute Immobilisation Test – Static);* [redacted] *2015. S14-04365.* *Sedaxane/Metalaxyl-M/Fludioxonil FS (A20607B) - Toxicity to the Rainbow Trout Oncorhynchus mykiss under*

Laboratory Conditions (Acute Toxicity Test – Static); [redacted] *2014. S14-04366.*

The concentration of the test item was analysed in the medium (water) to ensure adequate dosing of the ecotox studies. The concentration of the test item (A20607B) was measured by determination of fludioxonil in the test water.

The method was used to determine the content of fludioxonil in samples of treated aquarium water from ecotoxicological studies on rainbow trout and *Daphnia magna.* The method was used in both of the above studies. Acceptable procedural recoveries were presented for each study, as detailed below.

Principle of the method

Samples of aquarium water (0.5 mL) were collected and diluted with acetonitrile (0.5 mL). Samples were further diluted, if necessary, to ensure they were within the linear range. An aliquot of the sample was analysed by HPLC-MS/MS by the conditions below:

Chormatographic system:	Thermo Surveyor MS pump with Thermo Surveyor autosampler Thermo TSQ Quantum triple quadrupole system		
Analytical column:	Phenomenex Synergi Fusion-RP 80 A, 50 mm x 2.1 mm i.d., 4 µm mean particle size (No. 00B-4424-B0) with 4 mm guard column		
Target column temperature:	40 °C		
Injection volume:	10 µL		
Mobile phase A: Mobile phase B:	Water Methanol		
Flow rate:	500 µL/min		
Gradient	Time (min) / Phase A (%) / Phase B (%) 0 / 80 / 20 4.00 / 10 / 90 5.00 / 10 / 90 5.01 / 80 / 20 7.00 / 80 / 20		
Analyte:	Transitions	Polarity	Expected Retention Time
Fludioxonil	247 → 180 [1] 247 → 126	negative	approx. 3.65 min

1 – primary transition

Specificity/ Confirmation of analyte identity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample. Additionally, the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is necessary.

Matrix effects

Matrix-matched standards were not used. No interference was observed at the retention time of interest in any of the blank samples; therefore, matrix matched standards are not considered necessary, this is acceptable.

Linearity

Linearity was demonstrated by the analysis of eight standards of increasing concentration. Only single measurements were made. The range of standard concentrations used was 0.01 – 10 µg/mL. This is equivalent to 0.02 – 20 mg/L BAS 550 I in the samples. The response was linear, with a correlation coefficient (r^2) of 0.9997. The equations of the calibration curve is presented in the table below. Samples were diluted according to their concentrations, so as to remain within the calibration curve. This is acceptable.

Precision (repeatability)

Precision was determined from the accuracy recovery data. A sufficient number of samples were prepared at each fortification level. The % RSD at each fortification level was < 20%.

Accuracy (recovery)

Recovery samples were prepared by spiking blank aquarium water samples with fludioxonil and analysing them by the method described. The spike concentrations were in the range 0.05 to 120 mg/L. A sufficient number of samples were prepared at each fortification level. Mean recovery levels were within the range 101 – 110 % and are acceptable.

Summary of validation data – Aquarium water – fludioxonil

Analyte	Matrix	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Equation of calibration curve
Fludioxonil	*Daphnia magna* test water	0.4	0.4	100 – 125 (110)	10 (5)	0.1 – 50 ng/mL* n=9	y = -661.7 x + 7476 r^2=0.9986
			120	100 – 110 (105)	4 (5)		
	Oncorhynchus mykiss (rainbow trout) test water	0.05	0.05	88 – 113 (103)	10 (5)	0.1 – 50 ng/mL* n=12	y = -24.99x^2 + 8703.5x – 978.8 † r^2=0.9990
			100	97 – 104 (101)	3 (5)		

* No equivalent range of conc. in sample, sample diluted as required
† Justification is typically required when a non-linear calibration curve is used; some justification was given: *Second order calibration resulted in much lower deviations from all nominal concentrations than linear calibration and is therefore more accurate*. As the method is only used for dose verification purposes, further data is not required at this time.

Limit of Quantification

The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.05 mg/L for fludioxonil in aquarium water.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of fludioxonil aquarium water samples.

No new or additional studies have been submitted.

A 2.2.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

No new or additional studies have been submitted.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)

A 2.2.2.1.1 QuEChERS (BS EN 15662:2008)

A 2.2.2.1.1.1 Independent Laboratory Validation (tomatoes and oilseed rape)

Reference:	KCP2 5.2.1
Report	Metalaxyl-M – Independent Laboratory Validation (ILV) of an Analytical Method for Determination of Residues of Metalaxyl-M in Crops. [redacted] (2012). Report No S11-03712. Syngenta File No. CGA329351_11643 (Syngenta Task No. TK0055473)
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17. Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Principle of the method

The specimens were analysed for residues of Metalaxyl-M using QuEChERS Multiple Residue Method and detected by means of liquid chromatography with mass selective detection (module LC-MS/MS). The limit of quantitation (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was 0.003 mg/kg.

Recovery Findings
Summaries of the results for Metalaxyl-M are presented in the Tables below

Table A 20: Recovery results from method validation of Metalaxyl- M in crops: primary transition m/z 280 → 192

Matrix	Fortification Level (mg/kg)	Recovery (%)	Number of Analysis (n)	Mean Recovery(%)	RSD (%)	Recovery Range (%)
Tomato	0.01	97, 106, 104, 93, 97	5	99	5.0	93-106
	0.10	102, 103, 104, 105, 98	5	102	3.0	98-105
	Overall	-	10	101	4.0	93-106
Oilseed Rape	0.01	91, 97, 94, 88, 90	5	93	4.0	88-97
	0.10	93, 92, 93, 91, 88	5	91	2.0	88-93
	Overall	-	10	92	3.0	88-97

Table A 21: Recovery results from validation for Metalaxyl-M in crops: confirmatory transition m/z 280 → 160

Matrix	Fortification Level (mg/kg)	Recovery (%)	Number of Analysis (n)	Mean Recovery(%)	RSD (%)	Recovery Range (%)
Tomato	0.01	97, 106, 104, 93, 97	5	99	5.0	93-106
	0.10	102, 103, 104, 105, 98	5	102	3.0	98-105
	Overall	-	10	101	4.0	93-106
Oilseed Rape	0.01	91, 97, 94, 88, 90	5	93	4.0	88-97
	0.10	93, 92, 93, 91, 88	5	91	2.0	88-93
	Overall	-	10	92	3.0	88-97

RSD: relative standard deviation

Table A 22: Characteristics for the analytical method used for independent laboratory validation of metalaxtyl-M residues in tomatoes and oilseed rape

	Metalaxy-M
Specificity / Interferences	LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required (SANCO/825/00 rev.8.1, 16/11/2010). No significant interferences arising from the matrices, the labware, reagents or solvents have been observed at the retention times of interest
Linearity / Calibration	The linearity of the LC-MS/MS detector was tested using matrix matched standard solutions (0.25 ng/mL to 50 ng/mL). Standards at seven different concentrations were injected and the response plotted against standard concentration for both primary and confirmatory transitions. Straight lines with coefficients of determination $R^2 \geq 0.99$ were obtained for Metalaxyl-M..
Accuracy / Recovery	Fortified samples were analysed in quintuplet at the limit of

	Metalaxy-M
	quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 87% and 112% were found for both transitions on all matrices tested and therefore according to EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory accuracy.
Repeatability	The relative standard deviations (RSDs) of Metalaxyl-M recoveries at each fortification level and overall for each matrix tested during method validation were < 20% and therefore accord-ing to the EU guideline (SANCO 3029/99 rev. 4 11/7/00) demonstrate the method was satisfac-tory repeatability.
Limit of quantification	The limit of quantitation was 0.01 mg/kg for tomato and oilseed rape. No interfering peaks around the retention time of Metalaxyl-M were found in any of the control samples at levels above 30% of the limit of quantification.
Limit of detection	The limit of quantitation was calculated to be 0.003 mg/kg for the primary and confirmatory transition for the matrices tomato and oilseed rape.
Matrix effects	Significant matrix effects (suppression) were found in the crop matrices tested during method validation, therefore matrix matched linearity standards were used for quantification..

A 2.2.2.1.1.2 Method validation – Difficult commodities (hops and cocoa beans)

Comments:	Study previously evaluated.

Reference: KCP2 5.2.1

Report: Metalaxyl-M – Validation of the QuEChERS multiple residue method in hops and cocoa beans by LC-MS/MS.

[redacted] (2016).

Report No RES-00055. Syngenta File No. CGA329351_11743 (Syngenta Task No. TK0308525)

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.

Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

Metalaxyl-M was extracted from hops and cocoa beans by hydration of the matrix using water followed by mixing with acetonitrile. After addition of QuEChERS salts, samples were vortex mixed and centrifuged. Extracts were frozen overnight to freeze-out co-extracted fats and oils. Aliquots were then further purified by addition of QuEChERS dispersive SPE reagents followed by vortex mixing and centrifugation of the extracts. Supernatants were diluted with water. Extracts were analysed for Metalaxyl-M residues by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS), monitoring for the primary transition (*m/z* 280→192) and the confirmatory transition (*m/z* 280→160).

Results and discussions

Summaries of the results for Metalaxyl-M are presented below.

Table A 23: Recovery results from method validation of Metalaxyl-M using the QuEChERS analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Hops	Metalaxyl-M *m/z* 280→192 (primary)	0.01***** (n=5)	94, 94, 91, 88, 92	92	2.9	88 – 94
		0.1 (n=5)	83, 83, 85, 82, 84	83	1.5	82 – 85
		Overall	-	*88*	*5.5*	*82 – 94*
	Metalaxyl-M *m/z* 280→160 (confirmatory)	0.01***** (n=5)	101, 99, 94, 90, 98	96	4.5	90 – 101
		0.1 (n=5)	83, 81, 85, 82, 84	83	2.0	81 – 85
		Overall	-	*90*	*8.5*	*81 – 101*
Cocoa beans	Metalaxyl-M *m/z* 280→192 (primary)	0.01***** (n=5)	95, 99, 96, 92, 95	95	2.7	92 – 99
		0.1 (n=5)	97, 92, 87, 87, 86	90	4.9	86 – 97
		Overall	-	92	4.8	86 – 99
	Metalaxyl-M *m/z* 280→160 (confirmatory)	0.01***** (n=5)	94, 97, 93, 90, 97	94	3.2	90 – 97
		0.1 (n=5)	94, 92, 86, 89, 87	90	3.7	86 – 94
		Overall	-	92	4.0	86 – 97

Table A 24: Characteristics for the analytical method used for independent laboratory validation of metalaxtyl-M residues in hops and cocoa

	Metalaxyl-M
Specificity	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010), no further confirmatory technique is required. The method includes two MS/MS transitions for Metalaxyl-M, both of which have been validated. No significant interferences arising from the crop matrix, the lab ware, reagents or solvents have been observed at the retention time of interest.

Calibration (type, number of data points)	The linearity was tested using matrix matched standard solutions for all MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9991 to 0.9997 were obtained.
Calibration range	0.06 - 10 ng/ml
Assessment of matrix effects is presented	Significant matrix effects (i.e. suppression ≥ 20%) were observed for hops during method validation, therefore matrix matched linearity standards were used for quantification. Insignificant matrix effects (i.e. suppression ≤ 20%) were observed for cocoa beans during method validation, however matrix matched linearity standards were used for quantification.
Limit of determination/quantification	The limit of quantification for Metalaxyl-M residues in the matrix tested using the QuEChERS method was established at 0.01 mg/kg. No interfering peaks around the retention time of Metalaxyl-M were found in any of the control samples at levels above 30% of the limit of quantification.

Stability of Final Extracts

The stability of final sample extracts fortified with Metalaxyl-M at the LOQ level (0.01 mg/kg) was checked after a storage period of 12 days in a refrigerator at 4-8ºC against freshly prepared calibration standards. The results proved that Metalaxyl-M residues in the stored fortified samples were stable. The mean recovery values for hops at the LOQ level were 95% with a RSD of ≤ 20% when re-analysed, and were found to be within 20% of the original result when re-analysed. The mean recovery values for cocoa beans at the LOQ level were 96% with a RSD of ≤ 20% when re-analysed, and were found to be within 20% of the original result when re-analysed.

Stability of Standard Solutions

The stability of stored working standard solutions of Metalaxyl-M at 0.0002 µg/mL was assessed after a storage period of 15 days in a refrigerator at 4-8ºC against freshly prepared calibration standards. The mean peak area of the stored standard solution was found to be within ± 10% of the mean peak area of the freshly prepared standard solution for Metalaxyl-M, demonstrating that the standard solutions were stable for the storage period assessed when stored under the described conditions.

Conclusion

The QuEChERS analytical method has been demonstrated to be a reliable and accurate procedure for the determination of Metalaxyl-M in hops and cocoa beans to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.2.1.1.3 Independent Laboratory validation

Comments:	Study previously evaluated.

Reference: KCP2 5.2.1

Report Metalaxyl-M - Independent laboratory validation of the QuEChERS multi-

ple residue method in hops and cocoa beans.

[redacted] (2016).

Report No YB27DB. Syngenta File No. CGA329351_11745.

Guideline(s): European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000).

European Commission Guidance Document on Residue Analytical Method, SANCO/825/00 revision 8.1 (16 Nov 2010).

OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007).

OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

1 g sub-samples were extracted by the multi-residue QuEChERS method with extraction by homogenisation.

Samples were extracted by homogenisation with acetonitrile in the presence of buffering salts and cleaned-up by dispersive solid phase extraction. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) monitoring for the primary transition (m/z 280 → 192) and the confirmatory transition (m/z 280 → 160). The limit of quantification of the method was 0.01 mg/kg (0.01 ppm, 10 ppb).

The QuEChERS analytical method was independently validated in two crop types; dried hops and cocoa beans.

Results and discussions

Summaries of the results for Metalaxyl-M are presented below.

Table A 25: Recovery results from confirmatory method validation of Metalaxyl-M using the confirmatory analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Hops	Metalaxyl-M *m/z* 280→192 (primary)	0.01***** (n=5)	102, 98, 90, 97, 93	96	4.8	90 – 102
		0.1 (n=5)	102, 95, 99, 98, 95	98	3.0	95 - 102
		Overall	-	97	3.9	90 - 102
	Metalaxyl-M *m/z* 280→160 (confirmatory)	0.01***** (n=5)	105, 99, 83, 86, 89	92	10.0	83 - 105
		0.1 (n=5)	104, 100, 101, 102, 96	101	2.9	96 - 104
		Overall	-	97	8.1	83 - 105
Cocoa beans	Metalaxyl-M	0.01***** (n=5)	91, 94, 99, 98, 94	95	3.4	91 - 99

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
	m/z 280→192 (primary)	0.1 (n=5)	104, 102, 98, 106, 102	102	2.9	98 - 106
		Overall	-	99	4.9	91 - 106
	Metalaxyl-M *m/z* 280→160 (confirmatory)	0.01***** (n=5)	90, 96, 99, 95, 99	96	3.9	90 – 99
		0.1 (n=5)	105, 102, 98, 105, 102	102	2.8	98 - 105
		Overall	-	99	4.7	90 - 105

Table A 26: Characteristics for the analytical method used for independent laboratory validation of Metalaxyl-M residues in hops and cocoa

	Metalaxyl-M
Specificity	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010), no further confirmatory technique is required. The method includes two MS/MS transitions for Metalaxyl-M, both of which have been validated. No significant interferences arising from the crop matrix, the lab ware, reagents or solvents have been observed at the retention time of interest.
Calibration (type, number of data points)	The linearity was tested using matrix matched standard solutions for all MS/MS transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9973 to 0.9996 were obtained.
Calibration range	0.05 - 5 ng/ml
Assessment of matrix effects is presented	No significant matrix effects were observed in the crop matrices tested during method validation, however matrix matched linearity standards were used for quantification.
Limit of determination/quantification	The limit of quantification for Metalaxyl-M residues in the matrix tested using the QuEChERS method was established at 0.01 mg/kg. No interfering peaks around the retention time of Metalaxyl-M were found in any of the control samples at levels above 25% of the limit of quantification

Conclusion

The QuEChERS analytical method has been demonstrated to be a reliable and accurate procedure for the determination of Metalaxyl-M in crops to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.2.1.1.4 Confirmatory method

No confirmatory method is required. LC-MS/MS with two transitions is considered to be a highly specific detection technique. The method includes two MS/MS transitions for Metalaxyl-M, both of which have been validated.

A 2.2.2.1.1.5 Extraction efficiency

No extraction efficiency required for an ILV study.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

A 2.2.2.2.1 QuEChERS – Validation (milk, egg, fat, liver, kidney and blood)

Comments:	Study not required

Reference:	KCP2 5.2.2
Report	Metalaxyl-M - Validation of the Multiple Residue Method QuEChERS for the Determination in Animal Matrices . [redacted] 2011. Report No. S11-01732. Syngenta document No. CGA329351_11472.
Guideline(s):	OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996. EU Directive 91/414/EC (as amended by 96/46/EC 4.2) Guidance document SANCO/825/00 rev. 8.1 of 16/11/2010 of the European Commission, BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes.

Materials and methods

Principle of the method

5 g homogenised sub-samples were extracted by the multi-residue QuEChERS method with extraction by shaking.

Samples were extracted by homogenisation with acetonitrile and water, followed by a buffer salt mixture and cleaned-up by dispersive solid phase extraction. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) monitoring for the primary transition (m/z 280 → 160) and the confirmatory transition (m/z 280 → 192). The limit of quantification of the method was 0.01 mg/kg (0.01 ppm, 10 ppb).

The QuEChERS analytical method was validated in seven animal matrices (milk, eggs, meat, fat, liver, kidney and blood).

Recovery Findings

Summaries of the results for Metalaxyl-M are presented below.

Table A 27: Recovery results from independent laboratory validation of the QuEChERS method for Metalaxyl-M in animal commodities: primary transition m/z 280 160

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Milk	0.01	97, 107, 108, 108, 106	5	105	4.4	97 – 108
	0.10	91, 107, 107, 110, 108	5	105	7.4	91 - 110
	Overall	-	10	105	5.7	91 - 110
Eggs	0.01	103, 104, 96, 105, 108	5	103	4.3	96 -108
	0.10	91, 107, 101, 105, 105	5	102	6.3	91 – 107
	Overall	-	10	103	5.1	91 – 108
Meat	0.01	88, 105, 104, 101, 110	5	102	8.1	88 -110
	0.10	92, 107, 106, 105, 104	5	103	6.0	92 – 107
	Overall	-	10	102	6.7	88 - 110
Fat	0.01	94, 109, 108, 106, 110	5	105	6.2	94 – 110
	0.10	92, 107, 108, 104, 104	5	103	6.2	92 – 108
	Overall	-	10	104	6.0	92 – 110
Liver	0.01	93, 103, 108, 105, 107	5	103	5.8	93 – 108
	0.10	93, 106, 103, 105, 108	5	103	5.7	93 – 108
	Overall	-	10	103	5.4	93 - 108
Kidney	0.01	101, 102, 105, 103, 105	5	103	1.7	101 - 105
	0.10	110, 104, 103, 103, 100	5	104	3.5	100 - 110
	Overall	-	10	104	2.7	100 - 110
Blood	0.01	96, 105, 103, 104, 10	5	103	3.7	96 - 105
	0.10	92, 109, 105, 107, 108	5	104	6.7	92 - 109
	Overall		10	103	5.2	92 - 109

Table A 28: Recovery results from independent laboratory validation of the QuEChERS method for Metalaxyl-M in animal commodities: confirmatory transition m/z 280-192

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean Recovery(%)	RSD (%)	Recovery Range (%)
Milk	0.01	95, 103, 105, 106, 105	5	103	4.4	95 -106
	0.10	87, 108, 107, 108, 106	5	103	8.8	87 – 108
	Overall	-	10	96	8.7	80 - 104
Eggs	0.01	97, 106, 103, 107, 112	5	105	5.3	97 - 112
	0.10	93, 107, 104, 103, 105,	5	102	5.3	93 – 107
	Overall	-	10	104	5.2	93 – 112
Meat	0.01	96, 110, 102, 107, 104	5	104	5.1	96 – 107
	0.10	93, 107, 103, 108, 107	5	104	6.0	93 – 108
	Overall	-	10	104	5.3	93 - 110
Fat	0.01	91, 105, 100,105, 108	5	102	6.6	91 - 108
	0.10	94, 106, 105, 104, 100	5	102	4.8	94 – 106
	Overall	-	10	102	5.4	91 – 108
Liver	0.01	98, 94, 107, 107, 100	5	101	5.7	94 – 107
	0.10	95, 101, 102, 101, 105	5	101	3.6	95 – 105
	Overall	-	10	95	12.6	78 - 114
Kidney	0.01	99, 100, 105, 107, 105	5	103	3.4	99 - 107
	0.10	106, 104, 102, 104, 101	5	103	1.9	101 - 106
	Overall	-	10	103	2.6	99 - 107
Blood	0.01	95, 104, 106, 106, 108	5	104	4.9	95 - 108
	0.10	94, 109, 108, 109, 106	5	105	6.1	94 - 109
	Overall	-	10	105	5.3	94 - 109

Table A 29: Characteristics for the analytical method used for validation of Metalaxyl-M residues in animal commodities

	Metalaxy-M
Specificity / Interferences	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010), no further confirmatory technique is required. The method includes two MS/MS transitions for Metalaxyl-M, both of which have been validated. No significant interferences arising from the crop matrix, the lab ware, reagents or solvents have been observed at the retention time of interest.
Linearity / Calibration	The linearity was tested using matrix matched standard solutions for all MS/MS transitions. Standards at nine different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients were found to be ≥ 0.9962 to 0.9988 were obtained for Metalaxyl-M.
Accuracy / Recovery	Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 87% and 112% were found for both transitions on all matrices tested and therefore according to EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory accuracy.

	Metalaxy-M
Repeatability	The relative standard deviations (RSDs) of Metalaxyl-M recoveries at each fortification level and overall for each animal commodity tested during method validation were <20% and there-fore according to the EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory repeatability.
Limit of quantification	The limit of quantification for Metalaxyl-M residues in animal commodities using The QuEChERS analytical method was established at 0.01 mg/kg. No interfering peaks around the retention time of Metalaxyl-M were found in any of the control samples at levels above 30% of the limit of quantification
Limit of detection	Limit of Detection The limit of detection (LOD) was defined in this study as the lowest prepared instrument cali-bration solution that gave rise to a measureable chromatographic response. The LOD for this study is 0.003 mg/kg.
Matrix effects	No significant matrix effects were observed in the animal commodities tested during method validation, therefore non-matrix matched linearity standards were used for quantification.

Conclusion

The QuEChERS analytical method has been demonstrated to be a reliable and accurate procedure for the determination of Metalaxyl-M in animal commodities to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.2.2.2 QuEChERS – Independent laboratory validation (milk, egg, fat, liver and kidney)

Reference: KCP2 5.2.2

Report: Metalaxyl-M - Independent Laboratory Validation of Analytical Method QuEChERS for the Determination of Residues of Metalaxyl-M in Animal Matrices by LC-MS/MS.

[redacted]. 2018.

Report No. MM87YQ. Syngenta document No. CGA329351_11851.

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.

Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Principle of the method
5 g homogenised sub-samples were extracted by the multi-residue QuEChERS method with extraction by shaking.

Samples were extracted by homogenisation with acetonitrile and water, followed by a buffer salt mixture and cleaned-up by dispersive solid phase extraction. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) monitoring for the primary transition (m/z 280 → 160) and the confirmatory transition (m/z 280 → 192). The limit of quantification of the method was 0.01 mg/kg (0.01 ppm, 10 ppb).

The QuEChERS analytical method was independently validated in five animal matrices (milk, eggs, meat, fat, liver).

Recovery Findings
Summaries of the results for Metalaxyl-M are presented below.

Table A 30: Recovery results from independent laboratory validation of the QuEChERS method for Metalaxyl-M in animal commodities: primary transition m/z 280 160

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Milk	0.01	99, 96, 97, 103, 102	5	99	3.1	96 – 103
	0.10	93, 85, 98, 88, 79	5	89	8.2	79 - 98
	Overall	-	10	94	8.3	79 - 103
Eggs	0.01	90, 88, 110, 103, 100	5	98	9.3	88 – 110
	0.10	100, 102, 109, 105, 90	5	101	7.0	90 – 109
	Overall	-	10	100	7.9	88 – 110
Meat	0.01	91, 82, 88, 87, 94	5	88	5.1	82 – 94
	0.10	106, 96, 116, 116, 102	5	107	8.2	96 – 116
	Overall	-	10	98	12.2	82 - 116
Fat	0.01	105, 93, 110, 100, 106	5	103	6.4	93 – 110
	0.10	96, 95, 105, 111, 103	5	102	6.5	95 – 111
	Overall	-	10	102	6.1	93 – 111
Liver	0.01	96, 83, 90, 76, 90	5	87	8.8	76 – 96
	0.10	73, 107, 100, 107, 97	5	97	14.5	73 – 107
	Overall	-	10	92	12.9	73 - 107

Table A 31: Recovery results from independent laboratory validation of the QuEChERS method for Metalaxyl-M in animal commodities: confirmatory transition m/z 280-192

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean Recovery(%)	RSD (%)	Recovery Range (%)
Milk	0.01	103, 96, 104, 104, 99	5	101	3.5	96 – 104
	0.10	94, 86, 101, 89, 80	5	90	8.9	80 – 101
	Overall	-	10	96	8.7	80 - 104
Eggs	0.01	85, 82, 106, 90, 81	5	89	11.5	82 – 106
	0.10	98, 100, 101, 105, 91	5	99	5.2	91 – 105
	Overall	-	10	94	9.9	82 – 106
Meat	0.01	110, 80, 77, 98, 106	5	94	15.9	77 – 110
	0.10	108, 97, 118, 118, 104	5	109	8.4	97 – 118
	Overall	-	10	102	13.8	77 - 118
Fat	0.01	93, 96, 94, 82, 87	5	90	6.4	82 – 96
	0.10	97, 98, 108, 110, 104	5	103	5.6	97 – 110
	Overall	-	10	97	9.0	82 – 110
Liver	0.01	87, 83, 95, 88, 91	5	89	5.1	83 – 95
	0.10	78, 114, 105, 109, 104	5	102	13.7	78 – 114
	Overall	-	10	95	12.6	78 - 114

Table A 32: Characteristics for the analytical method used for independent laboratory validation of Metalaxyl-M residues in animal commodities

	Metalaxy-M
Specificity / Interferences	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010) no further confirma-tory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the animal matrices, the labware, rea-gents or solvents have been observed at the re-tention times of interest.
Linearity / Calibration	The linearity of the LC-MS/MS detector was tested using solvent standard solutions (0.25 ng/ml to 100 ng/ml). Linearity was tested for both MS/MS transitions. Standards at nine different concentrations were injected and the response plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9962 to 0.9988 were obtained for Metalaxyl-M.
Accuracy / Recovery	Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory accuracy.
Repeatability	The relative standard deviations (RSDs) of Metalaxyl-M recoveries at each fortification level and overall for each animal commodity tested during method validation were <20% and there-fore according to the EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory repeatability.

	Metalaxy-M
Limit of quantification	The limit of quantification for Metalaxyl-M residues in animal commodities using The QuEChERS analytical method was established at 0.01 mg/kg. No interfering peaks around the retention time of Metalaxyl-M were found in any of the control samples at levels above 30% of the limit of quantification
Limit of detection	The limit of detection (LOD) was defined in this study as the lowest prepared instrument cali-bration solution that gave rise to a measureable chromatographic response. For this study, it was shown to be 0.25 ng/mL (equivalent to 0.0025 mg/kg in sample matrix).
Matrix effects	No significant matrix effects were observed in the animal commodities tested during method validation, therefore non-matrix matched linearity standards were used for quantification.

Conclusion

The QuEChERS analytical method has been demonstrated to be a reliable and accurate proce-dure for the determination of Metalaxyl-M in animal commodities to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.2.2.2.1 Confirmatory method

Confirmatory study not required. LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required.

A 2.2.2.2.2.2 Extraction efficiency

Not required for an ILV study.

A 2.2.2.2.3 Analytical method GRM031.06A

A 2.2.2.2.3.1 Independent laboratory validation

Reference: KCP2 5.2.2

Report: Metalaxyl-M – Independent laboratory validation of analytical method GRM031.06A for the determination of Metalaxyl-M and structurally related metabolites as the common moiety 2,6-dimethylaniline (CGA72649) in animal fat.

[redacted] 2016.

Report No. TK0261461. Syngenta document No. CGA329351_11737.

Guideline(s): Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

Commission of the European Communities. Guidance Document on Resi-

due Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).

OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17.

Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

Analytical method GRM031.06A was independently validated in animal fat.

Residues of Metalaxyl-M were extracted from animal fat by adding ethyl acetate/cyclohexane (1:1, v/v) and dissolving the fat at 40°C in a water bath. Acetonitrile was added and the samples were stored for 1 h at -20°C. The precipitating fat was separated from the extract by filtration. Water was added to the extract and the solution was evaporated to near dryness. The remainder was heated under reflux in methane sulfonic acid for 20 minutes. The extract was diluted with water, a solution of sodium hydroxide and methanol. Final determination of Metalaxyl-M (analysed as 2,6-dimethylaniline) was done by LC-MS/MS, monitoring for the primary transition (*m/z* 122-105) and the confirmatory transition (*m/z* 122-103). The limit of quantification of the method was 0.01 mg/kg.

Results and discussions

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore demonstrate the method has satisfactory accuracy.

The relative standard deviations (RSDs) of Metalaxyl-M recoveries at each fortification level and overall for each crop tested during method validation were <20% and therefore demonstrate the method has satisfactory repeatability.

Table A 33: Recovery results from the independent laboratory validation of Metalaxyl-M (as 2,6-dimethylaniline) using the analytical method GRM031.06A

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Animal fat	2,6-dimethylaniline *m/z* 122→105 (primary)	0.01 (n=5)	117, 73, 91, 117, 100	100	19	73 – 117
		0.1 (n=5)	91, 84, 87, 88, 86	87	3	84 – 91
		Overall	-	*93*	*15*	*73 – 117*
	2,6-dimethylaniline *m/z* 122→103 (confirmatory)	0.01 (n=5)	113, 72, 88, 115, 100	98	18	72 – 115
		0.1 (n=5)	91, 84, 84, 88, 86	87	3	84 – 91
		Overall	-	*92*	*15*	*72 – 115*

Table A 34: Characteristics for the analytical method used for the independent laboratory validation of Metalaxyl-M (as 2,6-dimethylaniline) residues in animal fat

	2,6-dimethylaniline
Specificity	No significant interferences arising from the crop matrices, the lab ware, reagents or solvents have been observed at the retention time of interest. No interfering peaks around the retention time of Metalaxyl-M (analysed as 2,6-dimethylaniline) were found in any of the control samples at levels above 30% of the limit of quantification.
Calibration (type, number of data points)	The linearity of the LC-MS/MS detector was tested using standard solutions and matrix matched standard solutions. Linearity was tested in both solvent mixtures used and for both MS/MS transitions. Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients 0.9999 were obtained for Metalaxyl-M (analysed as 2,6-dimethylaniline).
Calibration range	0.025 µg/ml to 10 µg/ml.
Assessment of matrix effects is presented	No significant matrix effects were observed in the matrices tested during method validation.
Limit of determination/quantification	The limit of quantification for Metalaxyl-M residues in animal matrices using method GRM031.06A was established at 0.01 mg/kg.

Conclusion

The repeatability and specificity of the method have been independently demonstrated, and GRM031.06A is therefore considered valid for the determination of residues of Metalaxyl-M in animal fat at the LOQ of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.2.2.2.3.2 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required.

A 2.2.2.2.3.3 Extraction efficiency

Not applicable for an ILV study.

A 2.2.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

No new or additional studies have been submitted

A 2.2.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

No new or additional studies have been submitted

A 2.2.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

A 2.2.2.5.1 Analytical method GRM031.08A

A 2.2.2.5.1.1 Method validation

Reference:	KCP 5.2.5
Report	Metalaxyl-M - Residue Method GRM031.08A for the Determination of Metalaxyl-M (CGA329351) and Metabolites NOA409045, CGA108906 and CGA67868 in water. Non-enantiospecific Method. Final Determination by LC-MS/MS. [redacted] and [redacted] (2015). Report No. TK0222544. Syngenta document No. CGA329351_11693.
Guideline(s):	None (method description only).
Deviations:	No.
GLP:	No (method description only).
Acceptability:	Yes.

Reference:	KCP 5.2.5
Report	Metalaxyl-M - Validation of an Analytical Method for the Determination of the Metalaxyl-M Metabolite CGA67868 in Water. [redacted] (2015). Report No. TK0222545. Syngenta document No. CGA092370_10006.
Guideline(s):	Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712C-96-174, August 1996. EPA Field Test Data Reporting Guideline, Environmental Chemistry Methods and Associated Independent Laboratory Validation, OCSPP 850.6100. Commission of the European Communities. Guidance Document on Resi-

due Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).

Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).

Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market.

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

Water samples were acidified and passed through Phenomenex Strata-X solid phase extraction cartridges. The columns were dried under vacuum and eluted from the columns with methanol. The column eluates were evaporated to dryness and the residual material re-dissolved in acetonitrile/ultra-pure water (10/90, v/v) solution. The samples were analysed by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS), monitoring for the primary transition *m/z* 194.1-134.2 and the confirmatory transition *m/z* 194.1-91.1 for CGA67868.

The analytical method GRM031.08A was validated for the determination of CGA67868 in surface water and groundwater matrices. GRM031.08A was based on GRM031.02A with the inclusion of CGA67868.

Results and discussions

Fortified samples were analysed in quintuplet at the limit of quantification (0.05 µg/L) and at ten times the LOQ (0.5 µg/L) for surface water and ground water matrices. Acceptable mean recoveries of between 70% and 110% were found for both transitions. The relative standard deviations (RSDs) at each fortification level for both transitions and overall for each water matrix tested were <20%. The method has satisfactory accuracy and repeatability.

Table A 35: Recovery results from the method validation of CGA67868 using the analytical method GRM031.08A

Matrix	Analyte	Fortification level (µg/L) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Surface water	CGA67868 *m/z* 194→134 (primary)	0.05 (n=5)	94, 95, 96, 92, 108	97	7	92-108
		0.5 (n=5)	105, 95, 88, 98, 99	97	6	88-105
		Overall	-	*97*	*6*	*88-108*
	CGA67868 *m/z* 194→91 (confirmatory)	0.05 (n=5)	99, 88, 94, 90, 99	94	5	88-99
		0.5 (n=5)	99, 90, 83, 91, 93	91	6	83-99
		Overall	-	*93*	*5*	*83-99*
Ground water	CGA67868 *m/z* 194→134 (primary)	0.05 (n=5)	102, 98, 92, 107, 106	101	6	92-107
		0.5 (n=5)	101, 94, 93, 106, 75	94	13	75-106
		Overall	-	*97*	*10*	*75-107*
	CGA67868 *m/z* 194→91	0.05 (n=5)	96, 90, 95, 111, 110	100	9	90-111
		0.5 (n=5)	98, 91, 89, 102, 74	91	12	74-102

Matrix	Analyte	Fortification level (µg/L) (n = x)	Recovery (%)	Mean recovery (%)	RSD (%)	Recovery Range (%)
	(confirmatory)	*Overall*	-	*96*	*11*	*74-111*

Table A 36: Characteristics for the analytical method used for validation of CGA67868 residues in surface and ground water

	CGA67868
Specificity	No interfering peaks around the retention time of CGA67868 were found in any of the control samples at levels above 30% of the limit of quantification.
Calibration (type, number of data points)	Linearity was assessed using matrix matched standard solutions for both MS/MS transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9960 to 0.9994 were obtained.
Calibration range	0.075 to 10 µg/L.
Assessment of matrix effects is presented	No significant matrix effects (i.e. suppression or enhancement of the detector response ≤ ± 20%) were observed in the ground water matrix tested for the primary and confirmatory transitions. Significant matrix effects (i.e. suppression or enhancement of the detector response ≥ ± 20%) were observed in the surface water matrix tested. Matrix matched linearity standards were used for the quantification of CGA67868 during this study.
Limit of determination/quantification	The limit of quantification for CGA67868 residues in water matrices was 0.05 µg/L. The limits of detection (LODs) were calculated in each Matrix and ranged from 0.0003 to 0.0005 mg/kg for the primary transition and 0.0002 to 0.0143 mg/kg for the confirmatory transition.

Conclusion

Analytical method GRM031.08A has been demonstrated to be a reliable and accurate procedure for the determination of CGA67868 in surface water and ground water to a limit of quantification of 0.05 µg/L using commercially available laboratory equipment and reagents.

A 2.2.2.5.1.2 Independent laboratory validation

Reference: KCP 5.2.5

Report: Metalaxyl-M - Independent Laboratory Validation of Analytical Method GRM031.08A for the Determination of Metalaxyl-M (CGA329351) and its Metabolites NOA409045, CGA108906 and CGA67868 in Drinking Water.

[redacted] (2016).

Report No. IF-15/03469803-TK. Syngenta document No. CGA329351_11732.

Guideline(s): EPA OCSPP 850.6100 (2012).

SANCO/3029/99 Rev. 4 (2000).

SANCO/825/00 Rev. 8.1 (2010).

Deviations: No.

GLP: Yes.

Acceptability: Yes.

Materials and methods

In summary, acidified drinking water samples are concentrated using solid phase extraction (SPE). After elution with methanol, samples are evaporated to dryness and dissolved in acetonitrile/ultra-pure water and analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 0.05 µg/L for all analytes.

Results and discussions

The mean metalaxyl-M, CGA62826 (NOA409045), CGA108906 and CGA67868 recoveries for both primary and confirmatory ion transitions at each fortification level and overall were in the range 70% to 102%. The relative standard deviations (RSDs) of recoveries for all analytes for both primary and confirmatory ion transitions at each fortification level and overall were in the range 1 to 12%. These results demonstrate the method has satisfactory accuracy and repeatability.

Table A 37: Recovery results from the independent laboratory validation of metalaxyl-M residues using the analytical method GRM031.08A

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)
Drinking water	Metalaxyl-M *m/z* 280→220 (primary)	0.05 (n=5)	74	8	69-84
		0.5 (n=5)	82	1	80-82
		Overall	*78*	*7*	*69-84*
	Metalaxyl-M *m/z* 280→192 (confirmatory)	0.05 (n=5)	80	11	74-94
		0.5 (n=5)	82	3	80-86
		Overall	*81*	*7*	*74-94*
Drinking water	CGA62826 (NOA409045) *m/z* 266→192 (primary)	0.05 (n=5)	97	10	89-113
		0.5 (n=5)	96	2	94-98
		Overall	*97*	*7*	*89-113*
	CGA62826 (NOA409045) *m/z* 266→160 (confirmatory)	0.05 (n=5)	102	8	90-110
		0.5 (n=5)	96	3	93-99
		Overall	*99*	*6*	*90-110*
Drinking water	CGA108906 *m/z* 296→160 (primary)	0.05 (n=5)	93	10	87-110
		0.5 (n=5)	96	2	93-97
		Overall	*95*	*7*	*87-110*
	CGA108906 *m/z* 296→178	0.05 (n=5)	89	8	82-101
		0.5 (n=5)	96	2	94-98

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Recovery Range (%)
	(confirmatory)	*Overall*	*92*	*7*	*82-101*
Drinking water	CGA67868 *m/z* 194→134 (primary)	0.05 (n=5)	72	9	64-80
		0.5 (n=5)	71	1	70-72
		Overall	*71*	*6*	*64-80*
	CGA67868 *m/z* 194→91 (confirmatory)	0.05 (n=5)	74	12	66-88
		0.5 (n=5)	70	3	68-73
		Overall	*72*	*9*	*66-88*

Table A 38: Characteristics for the analytical method used for independent laboratory validation of metalaxyl-M residues in water

	Metalaxyl-M	CGA62826 (NOA409045)	CGA108906	CGA67868
Specificity	Residues of all analytes measured in the control samples were always below 30% of the LOQ during method validation.			
Calibration (type, number of data points)	A minimum of 5 standard solutions were injected, the lowest concentration injected was at 30% of the LOQ of the method and the upper margin was higher by at least 20% above the highest concentrations in the final extracts. The LC-MS/MS detector response for metalaxyl-M, NOA409045, CGA108906 and CGA67868 was found to be linear.			
Calibration range	0.07 to 4.3 ng/mL			
Assessment of matrix effects is presented	Matrix effects (either enhancement or suppression) were not considered to be significant for metalaxyl-M, CGA62826 (NOA409045), CGA108906 and CGA67868 and as such non-matrix calibration standards could be used if necessary.			
Limit of determination/quantification	The LOQ for metalaxyl-M, CGA62826 (NOA409045), CGA108906 and CGA67868 residues was confirmed at 0.05 µg/L in drinking water.			

Conclusion

Method GRM031.08A was successfully validated by an independent laboratory for the analysis of residues of metalaxyl-M, CGA62826 (NOA409045), CGA108906 and CGA67868 in drinking water with an LOQ of 0.05 µg/L for each analyte.

A 2.2.2.5.1.3 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore no further confirmatory technique is required.

A 2.2.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

No new or additional studies have been submitted

A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted

A 2.3 Analytical methods for the Sedaxane

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	**HSE Chemicals Regulation Division (CRD), UK**
Reviewer's comments	'Vibrance SB' was not the representative product for the approval of metalaxyl-M. 'Vibrance SB' has been assessed in the current evaluation as a representative product for the Article 7 amendment to the GB approval for metalaxyl-M. As this Article 7 amendment only concerns metalaxyl-M, and as the product 'Vibrance SB' is not to be approved for use – the product has only been evaluated with respect to metalaxyl-M. Fludioxonil and sedaxane have not been considered further.

A 2.3.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.3.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted.

A 2.3.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted

A 2.3.1.3 Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2.3)

No new or additional studies have been submitted

A 2.3.1.4 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

No new or additional studies have been submitted

A 2.3.1.5 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

A 2.3.1.5.1 Analytical method GRM023.01B

A 2.3.1.5.1.1 Method validation

Reference:	KCP 5.1.2
Report	[redacted], 2015 Sedaxane - Residue Study following seed treatment with A20110E, on Potato in Southern France and Spain in 2014, Syngenta Report No S14-01326. Syngenta File No. A20110E_10061.
Guideline(s):	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). OECD Guidance Document on Crop Field Trials, Series on Pesticides No. 66 and Series on Testing and Assessment No. 164, ENV/JM/MONO(2011)50. OECD Guidance Document on Overview of Residue Chemistry Studies (as revised 2009), Series on Testing and Assessment (No. 64) and Series on Pesticides (No. 32), ENV/JM/MONO(2009)31. Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009. European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000). European Commission Guidance Document on Residue Analytical Method, SANCO/825/00 revision 8.1 (16 Nov 2010). OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007).
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The potato samples were analysed for residues of sedaxane using analytical method GRM023.01B detected by liquid chromatography (LC) with MS/MS detection.
Full details of the methodology and the chromatographic conditions used in this study are given in the method itself, which was already peer-reviewed (France, 2012).

Results and discussions

Summaries of the results for sedaxane are presented in the tables below. Three fortifications of untreated

control samples at the level 0.2 mg/kg (per isomer) were performed, representing a reduced validation data set, for this fortification level. Validation results are acceptable. No confirmatory method is needed.

Table A 39: Recovery results from reduced method validation of sedaxane using the analytical method GRM023.01B

Matrix	m/z transition	Fortification level (mg/kg) (*n* = x)	Recovery range (%)	Mean recovery (%)	RSD (%)	n	Comments
SYN508211							
Potato	332 → 159	0.2	98 - 100	99	1.0	3	Reduced validation
	332 → 292	0.2	100 - 1002	100	1.1	3	
SYN508210							
Potato	332 → 159	0.2	98 – 100	99	1.2	3	Reduced validation
	332 → 292	0.2	97 - 99	98	1.2	3	

Table A 40: Characteristics for the analytical method used for reduced validation of Sedaxane residues in potato

	Sedexane
Specificity / Interference	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated.
Linearity / Calibration	The linearity of the LC-MS/MS detector was assessed using 6 solvent calibration standards over a concentration range of 0.1 ng/mL to 10 ng/mL. The lower margin of the linearity test was at least 30% of the LOQ and the upper margin was at least 20% above the highest fortification concentrations in the final extracts. The response of the analytes was shown to be linear with a correlation coefficients (R2) ≥ 0.99 for both transitions. Calibration curve parameters were generated either with '1/x weighting' or 'no weighting' using an appropriate regression package.
Acuracy / Recovery	Fortified samples were analysed in three replicates at 0.2 mg/kg (reduced validation). Acceptable mean recoveries of between 70% and 110% were found for both transitions and therefore according to EU guidance (SANCO 3029/99 rev.4 11/7/00) demonstrate the method has satisfactory accuracy.
Repeatability	The relative standard deviations (RSDs) of sedaxane recoveries for the matrix tested during reduced method validation were < 20% and therefore, according to the EU guidance (SANCO 3029/99 rev.4 11/7/00), demonstrate the method has satisfactory repeatability.
Limit of quantification	The limit of quantification for sedaxane residues in potato using method GRM023.01B was established at 0.01 mg/kg (0.005 mg/kg per isomer) during full method validation ([redacted], 2008. Syngenta Report No. SYN-0705V).
Matrix effects	Matrix effects (enhancement or suppression) were deemed to be insignificant for potato matrix since procedural recoveries were within the range of 70-110%.

Conclusion

The peer-reviewed analytical method GRM023.01B was successfully validated for sedaxane in potato according to the EU guidelines SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1. The validation also complies with OECD guidance document ENV/JM/MONO (2007) 17. It is suitable for data generation purposes.

A 2.3.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

No new or additional studies have been submitted

A 2.3.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

No new or additional studies have been submitted

A 2.3.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.3.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2.1)

A 2.3.2.1.1 QuEChERS method

A 2.3.2.1.1.1 Independent laboratory validation

Reference:	KCP 5.2.1
Report	[redacted], 2019 SYN524464 - Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of SYN508210 and SYN508211 in Crop Matrices by LC-MS/MS, Syngenta Report No 20190112 (TK0395483). Syngenta File No. SYN508210_10296
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17. Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and

91/414/EEC.

Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Samples were extracted with acetonitrile plus a suitable volume of water, taking into account the natural water content of the specimens. After addition of a mixture of magnesium sulphate, sodium chloride, trisodium citrate dihydrate and disodium hydrogen citrate, the extract was shaken. After centrifugation an aliquot of the extract was cleaned by PSA and C18 sorbents. The extracts were then analysed for residues of SYN508210 and SYN508211 by gh-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS) monitoring the primary transition (m/z 332 → 159) and the confirmatory transition (m/z 332 → 292).
Analytical multi-residue QuEChERS method EN 15662:2009 was independently validated in wheat grain and oilseed rape seeds matrices.

Results and discussions

Summaries of the recovery results for SYN508210 and SYN508211 are presented in Tables A 15 to A 18.

Table A 41: Recovery results from validation of QuEChERS method for SYN508210 in wheat grain and oilseed rape seed: primary transition m/z 332 → 159

Matrix	Fortification Level (mg/kg)	Recovery** (%)					Number of Analyses (n)	Mean Recovery (%)	SD (±)	RSD (%)	Recovery Range (%)
Wheat Grain	0.005*	98	97	97	97	97	5	97	0.4	0.4	97 - 98
	0.05	98	99	95	98	98	5	98	1.5	1.5	95 - 99
						Overall:	10	97	1.0	1.1	95 - 99
Oilseed Rape Seed	0.005*	82	83	82	81	80	5	82	1.4	1.7	80 - 83
	0.05	77	80	75	74	73	5	76	2.9	3.8	73 - 80
						Overall:	10	79	3.6	4.6	73 - 83

Table A 42: Recovery results from validation of QuEChERS method for SYN508210 in wheat grain and oilseed rape seed: confirmatory transition m/z 332 → 292

Matrix	Fortification Level (mg/kg)	Recovery** (%)					Number of Analyses (n)	Mean Recovery (%)	SD (±)	RSD (%)	Recovery Range (%)
Wheat Grain	0.005*	96	98	95	97	98	5	97	1.4	1.4	95 - 98
	0.05	97	98	94	94	95	5	96	1.7	1.8	94 - 98
						Overall:	10	96	1.6	1.6	94 - 98
Oilseed Rape Seed	0.005*	82	82	80	78	80	5	81	1.8	2.3	78 - 82
	0.05	77	79	73	72	71	5	74	3.4	4.6	71 - 79
						Overall:	10	77	4.2	5.5	71 - 82

Table A 43: Recovery results from validation of QuEChERS method for SYN508211 in wheat grain and oilseed rape seed: primary transition m/z 332 → 159

Matrix	Fortification Level (mg/kg)	Recovery** (%)					Number of Analyses (n)	Mean Recovery (%)	SD (±)	RSD (%)	Recovery Range (%)
Wheat Grain	0.005*	100	96	95	93	96	5	96	2.8	2.9	93 - 100
	0.05	97	97	96	96	97	5	97	0.5	0.5	96 - 97
						Overall:	10	96	1.9	2.0	93 - 100
Oilseed Rape Seed	0.005*	83	83	80	82	80	5	82	1.1	1.4	80 - 83
	0.05	79	80	75	74	74	5	76	3.0	3.9	74 - 80
						Overall:	10	79	3.5	4.4	74 - 83

Table A 44: Recovery results from validation of QuEChERS method for SYN508211 in wheat grain and oilseed rape seed: confirmatory transition m/z 332 → 292

Matrix	Fortification Level (mg/kg)	Recovery** (%)					Number of Analyses (n)	Mean Recovery (%)	SD (±)	RSD (%)	Recovery Range (%)
Wheat Grain	0.005*	99	96	94	95	92	5	95	2.5	2.6	92 - 99
	0.05	100	96	98	95	97	5	97	1.8	1.9	95 - 100
						Overall:	10	96	2.3	2.3	92 - 100
Oilseed Rape Seed	0.005*	81	86	77	83	85	5	83	3.4	4.1	77 - 86
	0.05	80	82	76	75	74	5	77	3.3	4.3	74 - 82
						Overall:	10	80	4.2	5.2	74 - 86

Table A 45: Characteristics for the analytical method used for independent laboratory validation of Sedaxane residues in plant matrices

	Sedaxane
Specificity / Interferences	LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (***SANCO/825/00 rev.8.1, 16/11/2010***) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crops matrices, the labware, reagents or solvents have been observed at the retention times of interest.
Linearity / Calibration	The linearity of the LC-MS/MS detector was tested using standard solutions (0.125 ng/ml to 25 ng/ml) and also matrix matched standard solutions for both MS/MS transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging (r^2) ≥ 0.99 were obtained for SYN508210 and SYN50821.
Accuracy / Recovery	Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.005 mg/kg) and at ten times the LOQ (0.05 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions on the matrix tested (wheat grain and oilseed rape seed) and therefore according to EU guidance (***SANCO 3029/99 rev.4 11/7/00***) demonstrate the method has satisfactory accuracy.
Repeatability	The relative standard deviations (RSDs) of SYN508210 and SYN508211 recoveries at each fortification level and overall

	Sedaxane
	for wheat grain and oilseed rape seed test systems during independent laboratory validation were <20% and therefore according to the EU guidance (***SANCO 3029/99 rev.4 11/7/00***) demonstrate the method has satisfactory repeatability.
Limit of quantification	The limit of quantification for SYN508210 and SYN508211 residues in wheat grain and oilseed rape seed matrices using QuEChERS method was established at 0.005 mg/kg. No interfering peaks around the retention time of SYN508210 and SYN508211 were found in any of the control samples at levels above 30% of the limit of quantification.
Limit of detection	The limit of detection (LOD) was calculated to be 0.0006 mg/kg and 0.0006 mg/kg for SYN508210 and SYN508211 primary transitions in wheat grain and 0.0002 mg/kg and 0.0002 mg/kg for SYN508210 and SYN508211 primary transitions in oilseed rape seeds, respectively and was calculated to be 0.0006 mg/kg and 0.0008 mg/kg for SYN508210 and SYN508211 confirmatory transitions in wheat grain and 0.0002 mg/kg and 0.0003 mg/kg for SYN508210 and SYN508211 confirmatory transitions in oilseed rape seeds, respectively.
Matrix effects	No significant matrix effects (suppression or enhancement, ≤ ± 20%) were observed in wheat grain and oilseed rape seed matrices tested during method validation, therefore non-matrix matched linearity standards were used for quantification.
Stability of extracts	Following storage of final extracts in vials in refrigerated conditions between 2 and 8°C for 7-8 days, the mean recovery from matrix fortified at the LOQ using the primary transition was found to be in the range 70 – 110% and the relative standard deviation was found to be less than 20%. This demonstrates that the stability of final extract in vials stored in refrigerated conditions is acceptable.

Conclusion

The repeatability and specificity of the method have been independently demonstrated, and QuEChERS method is therefore considered valid for the determination of residues of SYN508210 and SYN508211 in crop matrices at the LOQ of 0.005 mg/kg, using commercially available laboratory equipment and reagents.

A 2.3.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

No new or additional studies have been submitted

A 2.3.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

No new or additional studies have been submitted

A 2.3.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

No new or additional studies have been submitted

A 2.3.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

A 2.3.2.5.1.1 Independent laboratory validation

Reference:	KCP 5.2.5
Report	[redacted] (2019): SYN524464 - Independent Laboratory Validation of Analytical Method GRM023.06A for the Determination of Residues of SYN508210 and SYN508211 and the Metabolites CSCC210616, CSCD465008 and CSAA798670 in Water. Syngenta Report No. S18-05320. Syngenta File No. VV-619368
Guideline(s):	Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. European Commission Guidance Document on Pesticide Residue Analytical Methods, SANCO/825/00 revision 8.1 (16 Nov 2010). Ecological Effects Test Guidelines OCSPP 850.6100 (2012), EPA 712-C-001, January 2012.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Principle of the method

In summary, using analytical method GRM023.06A, sub-samples of drinking water and surface water were analysed as per the following methods:

a) Direct Analysis of Water Samples for SYN508210 and SYN508211

A sub-sample (20 mL) of water is diluted with an equal volume of methanol (20 mL) in a polypropylene centrifuge tube (50 mL size).

The final sample solution is analyzed for SYN508210 and SYN508211 by high-performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) with negative ESI. Two mass transitions were monitored for each analyte:
SYN508210: Primary transition 330→131 *m/z* and confirmatory transition 330→91 *m/z*
SYN508211: Primary transition 330→131 *m/z* and confirmatory transition 330→91 *m/z*.

b) Direct Analysis of Water Samples for CSCC201616

A sub-sample (50 mL) of water is transferred into a polypropylene centrifuge tube (50 mL size).

The sample solution is analyzed for CSCC210616 by high-performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) with positive ESI. Two mass transitions were monitored:
Primary transition 176→136 *m/z* and confirmatory transition 176→156 *m/z*.

c) Solid Phase Extraction Procedure for SYN508210, SYN508211 and CSCC210616

A sub-sample (50 mL) of water is cleaned up by SPE using a Waters Oasis™ HLB cartridge (size 60 mg, 3 mL). The analytes are eluted with acetonitrile (5 mL).

Aliquots are diluted 1.20 with 50/50 v/v methanol/ultra pure water for SYN508210 and SYN508211 analysis, and 1:10 with ultra pure water for CSCC210616 analysis. Final sample solutions are analyzed for SYN508210, SYN508211 and CSCC210616 by high-performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). Two mass transitions were monitored for each analyte:
SYN508210: Primary transition 330→131 *m/z* and confirmatory transition 330→91 *m/z*
SYN508211: Primary transition 330→131 *m/z* and confirmatory transition 330→91 *m/z*.
CSCC210616: Primary transition 176→136 *m/z* and confirmatory transition 176→156 *m/z*.

d) Solid Phase Extraction Procedure for CSAA465008 and CSAA798670

A sub-sample (50 mL) of water is cleaned up by SPE using a Waters Oasis™ HLB cartridge (size 60 mg, 3 mL). The analytes are eluted with 50/50 v/v/acetonitrile/ultra-pure water (2 mL). The acetonitrile is evaporated under a stream of air or nitrogen in a sample concentrator, and the volume is made up to 1 mL with ultra-pure water.

The final sample solution is analyzed for CSCD465008 and CSAA798670 by high-performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) with negative ESI. Two mass transitions were monitored for each analyte:
CSCD465008: Primary transition 161→141 *m/z* and confirmatory transition 161→66 *m/z*
CSAA798670: Primary transition 175→91 *m/z* and confirmatory transition 175→111 *m/z*.

Recovery Findings

Summaries of the results for SYN508210 and SYN508211 as well as for the metabolites CSCC210616, CSCD465008 and CSAA798670 in drinking water and surface water are presented in the tables below.

Table A 46: Recovery Results from the Validation (ILV) of GRM023.06A for SYN508210 in Drinking Water and Surface Water (Direct Analysis): Primary Transition 330□131 m/z

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	86, 88, 103, 98, 92	5	93	7.6	86-103	87-100
	0.50	101, 98, 98, 98, 97	5	98	1.5	97-101	97-100
	Overall	-	10	96	5.7	86-103	92-99
Surface water	0.05 *	95, 93, 97, 101, 94	5	96	3.3	93-101	93-99
	0.50	97, 96, 94, 96, 97	5	96	1.3	94-97	95-97
	Overall	-	10	96	2.4	93-101	95-97

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 47: Recovery Results from the Validation (ILV) of GRM023.06A for SYN508210 in Drinking Water and Surface Water (Direct Analysis): Confirmatory Transition 330□91 m/z

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	86, 87, 104, 101, 104	5	96	9.5	86-104	88-104
	0.50	105, 103, 101, 101, 100	5	102	2.0	100-105	100-104
	Overall	-	10	99	6.9	86-105	95-103
Surface water	0.05 *	98, 100, 98, 102, 95	5	99	2.6	95-102	96-101
	0.50	100, 98, 96, 97, 95	5	97	2.0	95-100	96-99
	Overall	-	10	98	2.3	95-102	96-99

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 48: Recovery Results from the Validation (ILV) of GRM023.06A for SYN508211 in Drinking Water and Surface Water (Direct Analysis): Primary Transition 330□131 m/z

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	86, 83, 100, 97, 101	5	93	8.9	83-101	86-101
	0.50	100, 97, 96, 96, 97	5	97	1.7	96-100	96-99
	Overall	-	10	95	6.3	83-101	92-99
Surface water	0.05 *	95, 101, 97, 94, 94	5	96	3.1	94-101	94-99
	0.50	95, 95, 92, 94, 94	5	94	1.3	92-95	93-95
	Overall	-	10	95	2.5	92-101	94-97

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 49: Recovery Results from the Validation (ILV) of GRM023.06A for SYN508211 in Drinking Water and Surface Water (Direct Analysis): Confirmatory Transition 330□91 m/z

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	85, 87, 103, 100, 102	5	95	9.1	85-103	88-103
	0.50	100, 98, 97, 99, 97	5	98	1.3	97-100	97-99
	Overall	-	10	97	6.2	85-103	93-101
Surface water	0.05 *	93, 99, 96, 97, 95	5	96	2.3	93-99	94-98
	0.50	94, 92, 94, 93, 94	5	93	1.0	92-94	93-94
	Overall	-	10	95	2.2	92-99	93-96

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.

Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 50: Recovery Results from the Validation (ILV) of GRM023.06A for CSCC210616 in Drinking Water and Surface Water (Direct Analysis): Primary Transition 176→136 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	96, 104, 104, 108, 102	5	103	4.3	96-108	99-107
	0.50	104, 105, 107, 109, 109	5	107	2.1	104-109	105-109
	Overall	-	10	105	3.7	96-109	102-107
Surface water	0.05 *	100, 106, 108, 101, 106	5	104	3.4	100-108	101-107
	0.50	107, 108, 110, 110, 111	5	109	1.5	107-111	108-111
	Overall	-	10	107	3.5	100-111	104-109

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 51: Recovery Results from the Validation (ILV) of GRM023.06A for CSCC210616 in Drinking Water and Surface Water (Direct Analysis): Confirmatory Transition 176→156 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	100, 102, 105, 103, 103	5	103	1.8	100-105	101-104
	0.50	106, 106, 109, 110, 109	5	108	1.7	106-110	106-110
	Overall	-	10	105	3.2	100-110	103-107
Surface water	0.05 *	96, 101, 105, 103, 103	5	102	3.4	96-105	99-105
	0.50	107, 108, 109, 109, 109	5	108	0.8	107-109	108-109
	Overall	-	10	105	4.1	96-109	102-108

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 52: Recovery Results from the Validation (ILV) of GRM023.06A for SYN508210 in Drinking Water and Surface Water (SPE Method): Primary Transition 330□131 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	77, 101, 100, 101, 99	5	96	11	77-101	86-105
	0.50	99, 98, 99, 96, 98	5	98	1.2	96-99	97-99
	Overall	-	10	97	7.4	77-101	92-101
Surface water	0.05 *	99, 104, 102, 102, 93	5	100	4.3	93-104	96-104
	0.50	100, 102, 101, 98, 101	5	100	1.5	98-102	99-102
	Overall	-	10	100	3.0	93-104	98-102

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 53: Recovery Results from the Validation (ILV) of GRM023.06A for SYN508210 in Drinking Water and Surface Water (SPE Method): Primary Transition 330□91 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	82, 104, 100, 103, 98	5	97	9.2	82-104	90-105
	0.50	100, 104, 101, 99, 101	5	101	1.9	99-104	99-103
	Overall	-	10	99	6.4	82-104	95-103
Surface water	0.05 *	99, 110, 112, 104, 101	5	105	5.4	99-112	100-110
	0.50	103, 101, 101, 102, 104	5	102	1.3	101-104	101-103
	Overall	-	10	104	4.0	99-112	101-106

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 54: Recovery Results from the Validation (ILV) of GRM023.06A for SYN508211 in Drinking Water and Surface Water (SPE Method): Primary Transition 330□131 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	83, 99, 100, 99, 96	5	95	7.4	83-100	89-102
	0.50	97, 95, 95, 94, 95	5	95	1.2	94-97	94-96
	Overall	-	10	95	5.0	83-100	92-98
Surface water	0.05 *	99, 98, 96, 91, 93	5	95	3.5	91-99	92-98
	0.50	95, 96, 95, 94, 95	5	95	0.7	94-95	94-96
	Overall	-	10	95	2.4	91-99	94-97

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.

Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 55: Recovery Results from the Validation (ILV) of GRM023.06A for SYN508211 in Drinking Water and Surface Water (SPE Method): Primary Transition 330□91 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	79, 98, 103, 100, 97	5	95	9.9	79-103	87-104
	0.50	95, 98, 95, 95, 95	5	96	1.4	95-98	94-97
	Overall	-	10	96	6.7	79-103	92-99
Surface water	0.05 *	95, 97, 93, 96, 98	5	96	2.0	93-98	94-97
	0.50	97, 98, 97, 97, 99	5	98	0.9	97-99	97-98
	Overall	-	10	97	1.8	93-99	96-98

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 56: Recovery Results from the Validation (ILV) of GRM023.06A for CSCC210616 in Drinking Water and Surface Water (SPE Method): Primary Transition 176□136 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	95, 99, 95, 98, 94	5	96	2.3	94-99	94-98
	0.50	95, 91, 101, 110, 98	5	99	7.2	91-110	93-105
	Overall	-	10	98	5.3	91-110	94-101
Surface water	0.05 *	98, 97, 103, 90, 92	5	96	5.4	90-103	91-101
	0.50	103, 101, 101, 100, 108	5	103	3.1	100-108	100-105
	Overall	-	10	99	5.4	90-108	96-103

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 57: Recovery Results from the Validation (ILV) of GRM023.06A for CSCC210616 in Drinking Water and Surface Water (SPE Method): Confirmatory Transition 176→156 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	97, 98, 99, 100, 99	5	99	1.2	97-100	98-100
	0.50	95, 91, 100, 110, 96	5	98	7.3	91-110	92-105
	Overall	-	10	99	5.0	91-110	95-102
Surface water	0.05 *	95, 93, 101, 97, 94	5	96	3.3	93-101	93-99
	0.50	103, 100, 102, 100, 108	5	103	3.2	100-108	100-105
	Overall	-	10	99	4.7	93-108	96-102

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 58: Recovery Results from the Validation (ILV) of GRM023.06A for CSCD465008 in Drinking and Surface Water: Primary Transition 161→141 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	80, 76, 72, 70, 76	5	75	5.2	70-80	71-78
	0.50	73, 71, 71, 67, 68	5	70	3.5	67-73	68-72
	Overall	-	10	72	5.5	67-80	70-75
Surface water	0.05 *	74, 74, 76, 80, 78	5	76	3.4	74-80	74-79
	0.50	72, 71, 74, 72, 73	5	72	1.6	71-74	71-73
	Overall	-	10	74	3.8	71-80	73-76

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 59: Recovery Results from the Validation (ILV) of GRM023.06A for CSCD465008 in Drinking and Surface Water: Confirmatory Transition 161→66 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	81, 77, 75, 79, 76	5	78	3.1	75-81	75-80
	0.50	76, 74, 71, 69, 72	5	72	3.7	69-76	70-75
	Overall	-	10	75	4.9	69-81	73-77
Surface water	0.05 *	80, 74, 72, 80, 81	5	77	5.3	72-81	74-81
	0.50	70, 75, 71, 74, 73	5	73	2.9	70-75	71-74
	Overall	-	10	75	5.3	70-81	73-77

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 60: Recovery Results from the Validation (ILV) of GRM023.06A for CSAA798670 in Drinking and Surface Water: Primary Transition 175□91 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	76, 77, 76, 76, 76	5	76	0.6	76-77	76-77
	0.50	79, 78, 79, 77, 78	5	78	1.1	77-79	77-79
	Overall	-	10	77	1.6	76-79	76-78
Surface water	0.05 *	74, 77, 74, 73, 75	5	75	2.0	73-77	73-76
	0.50	79, 82, 79, 80, 80	5	80	1.5	79-82	79-81
	Overall	-	10	77	4.0	73-82	75-79

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Table A 61: Recovery Results from the Validation (ILV) of GRM023.06A for CSAA798670 in Drinking and Surface Water: Confirmatory Transition 175□111 *m/z*

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analyses (n)	Mean Recovery (%)	RSD (%)	Range (%)	95% conf. interval (%)
Drinking water	0.05 *	79, 81, 79, 77, 77	5	79	2.1	77-81	77-80
	0.50	79, 78, 79, 78, 79	5	79	0.7	78-79	78-79
	Overall	-	10	79	1.5	77-81	78-79
Surface water	0.05 *	80, 73, 79, 77, 82	5	78	4.4	73-82	75-81
	0.50	81, 80, 84, 82, 82	5	82	1.8	80-84	80-83
	Overall	-	10	80	3.9	73-84	78-82

* 0.05 µg/L = limit of quantification, defined by the lowest validated fortification level.
Residues in duplicate control samples and single reagent blanks were less than 30 % of the LOQ.

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore, according to EU guidance (*SANCO/825/00 rev.8.1, 16/11/2010*), no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been independently validated. No significant interferences arising from the matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the detector response was confirmed by injecting seven solvent or matrix-matched standard solutions covering the working range of 0.0075 – 0.50 ng/mL for SYN508210 and SYN508211, of 0.015 – 1.0 ng/mL for CSCC210616, and of 0.75 – 50 ng/mL for CSCD465008 and CSAA798670. The lower margin of the linearity tests was 30 % of the LOQ, and the upper margin was at least 20 % above the 10x LOQ concentration in the final extracts. These margins cover the range as demanded in SANCO/825/00 rev. 8.1 (16/11/2010). Straight lines with coefficients of determination (R^2) ≥ 0.995 were obtained for all analytes and all mass transitions.

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.05 µg/L) and in quintuplet at a higher level (0.50 µg/L). Acceptable mean recoveries between 70% and 120% were found for

both mass transitions in drinking water and surface water, and therefore, according to EU guidance (*SANCO/825/00 rev.8.1, 16/11/2010*), demonstrate the method has satisfactory accuracy.

Repeatability

The relative standard deviations (RSDs) of recoveries of SYN508210 and SYN508211 and the metabolites CSCC210616, CSCD465008 and CSAA798670 at each fortification level and overall for both water types tested during the ILV were ≤ 20% for the fortification levels 0.05 µg/L and 0.50 µg/L, and therefore, according to the EU guidance (*SANCO/825/00 rev.8.1, 16/11/2010*), demonstrate the method has satisfactory repeatability.

Limit of Quantification

The limit of quantification (LOQ) for residues of of SYN508210 and SYN508211 and the metabolites CSCC210616, CSCD465008 and CSAA798670 in drinking water and surface water using method GRM023.06A was 0.05 µg/L as in the method validation. No interfering peaks around the retention times of of SYN508210 and SYN508211 and the metabolites CSCC210616, CSCD465008 and CSAA798670 were found in any of the control samples at levels above 30% of the limit of quantification.

Limit of Detection

The limit of detection (LOD) was estimated to be below 0.0075 µg/L (15 % of the LOQ) for both primary and confirmatory transitions for all analytes.

Matrix Effects

A comparison of the response obtained from matrix-matched standards against the response obtained from solvent standards was performed (enhancement (+) or suppression (-) on the instrument response).

Matrix effects on the detector response caused by drinking water and surface water for SYN508210, SYN508211 and CSCC210616 were considered to be insignificant (< ± 20%); therefore solvent standards were used for quantification, except for the quantification of CSCC210616 in drinking water after direct injection where matrix-matched standards were used. For CSCD465008 and CSAA798670, matrix effects were deemed to be significant (> 20%) for surface water, but insignificant (≤ 20%) for drinking water. Nevertheless, matrix-matched standards were used for quantification of CSCD465008 and CSAA798670 in both water types.

Conclusion

The data presented demonstrate that the analytical method GRM023.06A permits the determination of residues of SYN508210 and SYN508211 and the metabolites CSCC210616, CSCD465008 and CSAA798670 in the validated water types (drinking water and surface water) with satisfactory accuracy, precision and repeatability using LC-MS/MS detection.

The method is therefore considered valid for the determination of residues of SYN508210 and SYN508211 and the metabolites CSCC210616, CSCD465008 and CSAA798670 in drinking water and surface water at the LOQ of 0.05 µg/L over concentration ranges typical of those for which the method will be used.

The method GRM023.06A was successfully independently validated according to the EU guideline SANCO/825/00 rev. 8.1. The validation also complies with US EPA guideline OCSPP 850.6100.

A 2.3.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

No new or additional studies have been submitted

A 2.3.2.7 Other Studies/ Information

No new or additional studies have been submitted