European Commission

Combined Draft (Renewal) Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

GIBBERELLINS (GA4, GA7)

Volume 3 – B.5 (AS)

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Introduction

This document has been prepared to evaluate the European Gibberellins Task Force (Valent Biosciences Corporation (Sumitomo Chemical Agro Europe), Fine Agrochemicals Ltd, Globachem NV) application for EU renewal of the Annex I inclusion of active substance gibberellins (GA4, GA7). The document supplements and updates the corresponding Annex B section of the Draft Assessment Report produced during the first review of gibberellins (2005 - 2011).
Gibberelin has been identified as a presumed low-risk active substance in the Commission working document on the AIR-IV renewal programme (SANTE-2016-10616-rev 8). The EU Gibberellin Task Force (EGTF) proposes that Gibberelin is a low risk active substance according to Regulation (EC) 1107/2009 as amended by Commission Regulation 2017/1432.

In this report studies submitted for the first inclusion of gibberellins in Annex I to Directive 91/414/EEC and for the renewal of the approval of gibberellins have been evaluated.

Previous EU assessment

The dossier to support the first inclusion of gibberellins in Annex I to Directive 91/414/EEC was submitted to Hungary as the Rapporteur Member State in June 2005. The Draft Assessment Report is dated July 2006. Final Addendum to Draft Assessment Report, containing all individually submitted addenda on gibberellins, was compiled by EFSA in October 2011.

Structure of this document

Summaries of available data and overall assessments of each sub-section, as well as the exposure assessments, generally are not included in this document. Instead these parts of the assessment are included in Vol. 1, Level 2. The reason behind this structure is to avoid repetition and facilitate revisions of the assessment. As a result, this Annex B only contains the presentation and evaluation of individual study reports on the active substance.

In each section of this document, the following headings (a)-b)) occur:

a) Previous evaluation (2005-2011)

Under this heading study reports submitted for the first inclusion of gibberellins in Annex I to Directive 91/414/EEC are summarised. These studies have been re-evaluated for the purpose of the renewal in the light of current scientific and technical knowledge. The endpoints from the studies were also re-assessed and if considered relevant, re-calculated. However, full details from each study have not been repeated in this DRAR - therefore this DRAR is not a "stand-alone document" and for full reference sometimes the reader needs to consult the DAR (2005-2011).

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

Under this heading studies submitted prior to Annex I inclusion, but no evaluation of such material was presented in the form of Addenda to the DAR and studies that were submitted to support the application for renewal of Annex I inclusion are evaluated, i.e. new studies.

B.5. METHODS OF ANALYSIS

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

B.5.1.1. Methods for the analysis of the active substance as manufactured

B.5.1.1.1. Determination of the pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009

Validated analytical methods for the determination of the pure active substance in the active substance as manufactured are provided below in accordance with Commission Regulation (EU) No 283/2013. These methods were not previously evaluated at Annex I inclusion.

Previous evaluation:	None
Data point addressed:	KCA 4.1.1/01
Author(s) (year):	Parsons, A.H. (2006)
Title:	Validation of G C Laboratories Ltd. Analytical Method M564 "HPLC Determination of Gibberellins GA4 and GA7 in Technical Material and Formulations" for Gibberellin GA4 in GA4 Technical Material and the 'Novagib' Formulation
Laboratory report/project number:	J15061
Testing facility:	G C Laboratories Ltd.
Published:	No
Test guideline used:	PSD Guidelines for the Validation of Analytical Methods for Pesticides (PRD 2400) Commission Directive 96/46/EC SANCO/3030/99 rev. 4
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: M564
Test material: GA_4 Technical, 54GA490
Lot/Batch No.: 20040110
Purity: Not reported
Stability of test compound: Not reported

Principle of the method

Samples of GA4/7 technical material (ca. 0.05 g) were weighed into 100 mL volumetric flasks. An aliquot (20 mL) of 1 g/L valerophenone in methanol internal standard solution and methanol (50 mL) were added. The solution was sonicated, made to volume with 0.2% perchloric acid in water and mixed by inversion. Samples were filtered (only if solution was not clear) and analysed for GA4 content by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 210 nm. Quantification was performed using valerophenone internal standard and GA4 analytical standard.

Linearity

The linearity of the detector was demonstrated using five calibration solutions of GA4 reference standard in internal standard solution and methanol prepared over the nominal concentration range of ± 50% (actual range 0.03 to 0.065 µg). The coefficient of correlation was 0.9998 and the calibration curve equation is y = 0.0765x – 0.0005. Representative calibration curve plot is provided.

Specificity

No interferences from impurities or the internal standard were observed at the retention times of GA4 in the chromatograms of the blank formulation (1,2-propanediol) and internal standard, demonstrating specificity of the method. Furthermore, no interferences were observed at the retention time of the internal standard in the chromatogram of the technical material. Analyte (GA4) identity was confirmed by UV spectra (using a diode array detector over 190 to 400 nm) and retention time matching with analytical standards. Representative chromtograms are provided.

Accuracy

The determination of accuracy for the active substance in the technical material, in terms of recovery data, is not required in accordance with SANCO/3030/99 rev. 4. Nevertheless, data has been provided. Known amounts of GA4 were added to 1,2-propanediol, equivalent to 75, 100 and 125% of the nominal concentration and analysed by HPLC-UV in accordance with the method. A summary of the recovery results are presented in the table below.

Table B.5.1.1.1-01: Recovery results of GA4 from fortified solutions of 1,2-propanediol

Analyte	Fortification Level (% of nominal)	Individual Recoveries* (%)	Number of Analysis (n)	Mean Recovery* (%)	RSD** (%)	Recovery Range (%)
GA4	75	102	3	101	1.1	100-102
	100	100				
	125	100				

**Rounded to integer values; **Values calculated based on rounded figures and rounded to 1 d.p.*

The mean recovery was within the guideline range of 98 to 102% for an active substance content of >10% (SANCO/3030/99 rev.4), so is accepted. Further assessment of the accuracy of the method can be made by analysis of interference (specificity) and precision (repeatability).

Repeatability

Precision data were generated from five replicate determinations of GA4/7 technical material prepared at concentrations equivalent to 75, 87.5, 100, 112.5 and 125% nominal. The relative standard deviation (%RSD) obtained was 0.75%, which is below that calculated by the Horwitz equation (1.36%) for a mean content of 90.8% w/w, and so is accepted.

Conclusion

The method for the determination of GA4 in the technical active substance as manufactured was successfully validated in terms of specificity, linearity, precision and accuracy, in accordance with the requirements of SANCO/3030/99 rev.4.

RMS comments and conclusion:

The method is fully validated for determination of the GA4 active substance in the active substance as manufactured. No further data required.

Previous evaluation:	None
Data point addressed:	KCA 4.1.1/02
Author(s) (year):	Knowles, R.J. (2009)
Title:	Validation of G C Laboratories Ltd. Analytical Method M564 "HPLC Determination of Gibberellins GA4 and GA7 in Technical Materials and Formulations" Validation for GA7 in GA4/GA7 Technical Gibberellin
Laboratory report/project number:	J17176
Testing facility:	G C Laboratories Ltd.
Published:	No
Test guideline used:	PSD Guidelines for the Validation of Analytical Methods for Pesticides (PRD 2400) Commission Directive 96/46/EC SANCO/3030/99 rev. 4
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: M564
Test material: Technical $GA_{4/7}$
Lot/Batch No.: C321/08/0179, C321/08/1226 and C321/08/1229
Purity: Determined in the report, 0.582 % w/w (GA7) for Lot No. C321/08/0179
Stability of test compound: Not reported

Principle of the method

Samples of GA4/7 technical material (ca. 0.05 g) were weighed into 100 mL volumetric flasks. An aliquot (20 mL) of 1 g/L valerophenone in methanol internal standard solution and methanol (50 mL) were added. The solution was sonicated, made to volume with 0.2% perchloric acid in water and mixed by inversion. Samples were filtered (only if solution was not clear) and analysed for GA7 content by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 210 nm. Quantification was performed using valerophenone internal standard and GA7 analytical standard.

Linearity

The linearity of the detector was demonstrated using duplicate injection of five calibration solutions of GA7 analytical standard in internal standard solution and methanol prepared across the nominal concentration range equivalent to ca. 5 to 40% in technical GA4/7. A calibration plot was provided with a coefficient of determination (r^2) of 0.9997 and the equation $y = 18.8290x + 0.0032$.

Specificity

No interferences were observed at the retention times of GA7 in the chromatogram of the internal standard. Furthermore, no interferences were observed at the retention times of the internal standard in the chromatograms of technical material or GA4 and GA7 analytical standards, demonstrating specificity of the method. Analyte (GA7) identity was confirmed by UV spectra (scanning from 190 to 320 nm) and retention time matching with analytical standards. Representative chromtograms are provided.

Accuracy

The determination of accuracy for the active substance in the technical material, in terms of recovery data, is not required in accordance with SANCO/3030/99 rev. 4. Nevertheless, data has been provided. First, technical GA4/7 was analysed in accordance with the method to determine exact levels of GA7. Samples of the same technical GA4/7 samples were then fortified with GA7 at five levels corresponding to nominal concentrations of 5, 10, 20, 30 and 40% and analysed in duplicate in accordance with the method. A summary of the recovery results are presented in the table below.

Table B.5.1.1.1-02: Recovery results of GA7 from fortified solutions of technical GA4/7

Analyte	Fortification Level (% of nominal)	Individual Recoveries* (%)	Number of Analysis (n)	Mean Recovery* (%)	RSD** (%)	Recovery Range (%)
GA7	5	102	5	100	1.3	99-102
	10	101				
	20	100				
	30	99				
	40	99				

**Rounded to integer values; **Values calculated based on rounded figures and rounded to 1 d.p.*

The mean recovery was within the guideline range of 98 to 102% for an active substance content of >10% (SANCO/3030/99 rev.4), so is accepted. Further assessment of the accuracy of the method can be made by analysis of interference (specificity) and precision (repeatability).

Repeatability

Precision data were generated from duplicate injection of five replicate determinations of GA4/7 technical material (with a concentration of GA7 between 20 and 25%). The relative standard deviation (%RSD) obtained was 0.73%, which is below that calculated by the Horwitz equation (1.70%) for a mean content of 21.0% w/w, and therefore considered acceptable.

Conclusion

The method for determination of GA7 in the technical active substance was successfully validated in terms of specificity, linearity, precision and accuracy, in accordance with the requirements of SANCO/3030/99 rev.4.

RMS comments and conclusion:

The method is fully validated for determination of the GA7 active substance in the active substance as manufactured. No further data required.

Previous evaluation:	None
Data point addressed:	KCA 4.1.1/03 (N.B. This study has been added to the confidential Doc K)
Author(s) (year):	Comb, T (2018)
Title:	GA4/7: Method Validation
Laboratory report/project number:	ENV-17-057
Testing facility:	AgroChemex Environmental Ltd.
Published:	No
Test guideline used:	Regulation (EC) 1107/2009 OCSPP 830.1700 SANCO/3030/99 rev.4
Deviations:	None*
GLP:	Yes
EU agreed endpoint:	No

*Deviations were noted in the study, but they did not apply to the method validation of GA4/7.

Materials and methods
Method: Methods used to support five batch analyses, described in report ENV-17-058
Test material: $GA_{4/7}$
Lot/Batch No.: S016 0303 and S016 0604 (only two of the five batches were used for validation)
Purity: Determined in the report, ca. 92.1 % w/w (GA4) and ca. 1.8 % w/w (GA7)
Stability of test compound: Not reported

Principle of the method

Samples of GA4/7 technical material (ca. 200 mg) were weighed into 50 mL volumetric flasks and methanol added to dissolve the samples. Samples were made to volume with methanol. Aliquots (5 mL) of the solution were further diluted to 100 mL with mobile phase (methanol:pH 4.8 phosphate buffer*, 50:50 v/v) before analysis by high performance liquid chromatography with UV detection (HPLC-UV) at 204 nm.

*Buffer solution was typically prepared by dissolving potassium dihydrogen phosphate (25 g) in water (5 L) and adjusting the pH to ca. 4.8 with 1 M aqueous potassium hydroxide solution.

Specificity

Analyte identities were confirmed by retention time and spectra (UV and mass) matching with analytical standards. UV spectra were recorded with a diode array detector, mass spectra were recorded using liquid-chromatography mass spectroscopy (LC-MS). Representative chromatograms and spectra of the test item and analytical standards were provided in the report along with a chromatogram of the solvent blank.

Linearity

The linearity of the detector was demonstrated using five calibration solutions of GA4 or GA7 analytical standards in mobile phase prepared over the concentration ranges of 50 to 270 mg/L for GA4 or 2 to 11 mg/L for GA7. The coefficients of correlation were 0.9999 (GA4) and 1.0000 (GA7). Representative calibration curve plots are provided and the equations are as follows.

GA4: $y = 21.06x - 34.46$

GA7: $y = 28.75x - 2.203$

Accuracy

In accordance with SANCO/3030/99 rev. 4, the determination of accuracy for the active substance in the technical material, in terms of recovery data, is not required. Instead, assessment of accuracy can be made by analysis of interference (specificity) and precision (repeatability).

Repeatability

Precision data were generated from two batches of technical GA4/7, each assayed ten times over two separate days (five per day). The relative standard deviations (%RSD) obtained were within the acceptable criteria. Results are presented in table B.5.1.1.1-03 below.

Table B.5.1.1.1-03: Precision data

Analyte	Batch	Number of Samples	Mean Content (%)	RSD (%)	Acceptable RSD* (%)
GA4	S016 0303	10	90.9	0.65	1.36
	S016 0604	10	94.3	0.62	1.35
GA7	S016 0303	10	1.76	0.48	2.46
	S016 0604	10	1.76	0.52	2.46

**Modified Horwitz criteria, taken from SANCO/3030/99 rev. 4*

Conclusion

The method for determination of GA4 and GA7 in the technical active substance GA4/7 as manufactured was successfully validated in terms of specificity, linearity, precision and accuracy, in accordance with the requirements of SANCO/3030/99 rev.4.

RMS/comments and conclusion:

The method is fully validated for determination of the GA4 and GA7 active substances in the technical active substance as manufactured. No further data required.

B.5.1.1.2 Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

Please see the confidential section Vol.4 for analytical methods relating to the determination of significant impurities. There are no additives or relevant impurities in technical GA4/7 as manufactured.

B.5.1.2. Methods for risk assessment

B.5.1.2.1. Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Soil

A pre-registration method that has been used in support of an environmental fate study is presented below in accordance with Commission Regulation (EU) No 283/2013. The method was not considered previously at Annex I inclusion.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/01
Author(s) (year):	Traub M. (2014)
Title:	Gibberellins GA4, GA7: Aerobic Degradation in Four European Soils
Laboratory report / project number:	S14-01454
Testing facility:	Eurofins Agroscience Services
Published:	No
Test guideline used:	OECD 307 SANCO/3029/99 rev.4
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: Verification of active substance concentration, described in report S14-01454
Test material: GA4
Lot/Batch No.: 18603
Purity: 99 %

Test material: GA7
Lot/Batch No.: 18664
Purity: 100 %
Stability of test compound: Not reported

Principle of the method

Samples of German standard soil (LUFA 2.1, 2.2, 2.3 and 6S) were fortified with solutions of GA4 and GA7 in acetonitrile at concentrations equivalent to 0.0018 mg/kg (LOQ) and 0.0416 mg/kg for GA4 and 0.0009 mg/kg (LOQ) and 0.0196 mg/kg for GA7. Each soil sample was subject to triple ambient extraction by shaking with an acetonitrile:water mixture (80:20, v/v), followed by a further single extraction with acetonitrile:water (80:20, v/v) at 55 °C for 20 minutes using a microwave. Concentrations of GA4/7 in each sample were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Linearity

The linearity of the detector was confirmed using duplicate determinations of seven calibration solutions of GA4/7 in acetonitrile:water mixture (40:60, v/v) over the range 0.03 to 20 ng/mL for GA4 and 0.03 to 10 ng/mL for GA7. Representative calibration curves were provided in the report with values given for intercepts (GA4: 4000, GA7: 2760) and slopes (GA4: 148000, GA7: 522000) equations of the lines given. The coefficients of determinations were 0.9984 for GA7 and 0.9985 for GA4.

Specificity

Example chromatograms of control samples, standards at the lowest calibrated level and samples fortified at the LOQ were provided. Retention times of GA4/7 in sample extracts matched those of standard solutions and no peak interferences were observed at the retention times of the analytes. Concentrations of GA4/7 in blank samples were <30% the assigned LOQs. LC-MS/MS is considered to be a highly specific detection technique. The method included two quantification MS/MS transitions (GA4: 331.2 → 257.2 m/*z*; GA7: 329.2 → 223.0 m/*z*) and two confirmation MS/MS transitions (GA4: 331.2 → 243.1 m/*z*; GA7: 329.2 → 211.1 m/*z*). Mass spectra were given, justifying ion transition choices.

Accuracy

Samples of German standard soil (LUFA 2.1, 2.2, 2.3 and 6S) were fortified with GA4 or GA7 (in acetonitrile) at both the LOQ (5% of application level) and 110% of application level. For each fortification level per soil matrix, five test samples were prepared and analysed. The %RSD for GA4/7 at each fortification level for each matrix was <20% and therefore considered acceptable.

Recovery findings

A summary of the recovery results for GA4/7 are presented in the table below.

Table B.5.1.2.1-01: Recovery results for GA4/7 in German standard soil

Matrix	Analyte	Fortification Level (mg/kg)	Individual Recoveries (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
LUFA 2.1	GA4	0.0018	99, 102, 83, 90, 93	5	93	9.4	83-102
		0.0416	102, 103, 100, 103, 103	5	102	1.6	100-103
	GA7	0.0009	84, 82, 82, 88, 78	5	83	4.2	78-88
		0.0196	92, 91, 91, 96, 90	5	92	2.4	90-96
LUFA 2.2	GA4	0.0018	306*, 108, 102, 101, 102	4	103	3.2	101-108
		0.0416	99, 99, 96, 99, 97	5	98	1.5	96-99
	GA7	0.0009	86, 87, 83, 84, 86	5	85	1.8	83-87
		0.0196	90, 95, 91, 91, 91	5	91	2.1	90-95
LUFA 2.3	GA4	0.0018	87, 102, 91, 93, 95	5	94	5.8	87-102
		0.0416	101, 99, 98, 100, 100	5	100	1.4	98-101
	GA7	0.0009	87, 86, 85, 83, 85	5	85	1.9	83-87
		0.0196	93, 90, 92, 91, 91	5	92	1.2	90-93
LUFA 6S	GA4	0.0018	84, 99, 84, 81, 88	5	87	8.1	81-99

		0.0416	93, 97, 96, 97, 96	5	96	1.9	93-97
	GA7	0.0009	95, 86, 90, 82, 93	5	87	5.0	82-95
		0.0196	90, 91, 77, 93, 81	5	87	8.4	77-93

**Outlier, not used for evaluation*

Repeatability

Mean recoveries of GA4/7 at each fortification level per matrix were within the range 70 to 120% and therefore considered acceptable.

Limit of quantification and limit of detection

The limit of quantification (LOQ) of the method was 0.0018 mg/kg for GA4 and 0.0009 mg/kg for GA7. The limit of detection (LOD) was calculated as 1% of the applied dose, which is equivalent to 0.0004 mg/kg for GA4 and 0.0002 mg/kg for GA7 (approximately 20% the LOQ).

Conclusion

The method was successfully validated for the determination of GA4/7 in German standard soil LUFA 2.1, 2.2, 2.3 and 6S in accordance with SANCO/3029/99 rev. 4.

RMS comments:

The method for determination of GA4/7 in soil was not previously evaluated. It was successfully validated for linearity, accuracy, repeatability, specificity and LOQ according to SANCO/3029/99 rev. 4 and therefore could be accepted. No further data required.

Water and sediment

No new studies have been submitted in support of environmental fate studies as part of the Annex I renewal of GA4/7

Air

No new studies have been submitted in support of environmental fate studies as part of the Annex I renewal of GA4/7

B.5.1.2.2. Methods in soil, water and any additional matrices used in support of efficacy studies

No new efficacy studies have been submitted as part of the Annex I renewal of GA4/7.

B.5.1.2.3. Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

No new toxicological studies have been submitted as part of the Annex I renewal of GA4/7.

B.5.1.2.4. Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

No new exposure studies have been submitted as part of the Annex I renewal of GA4/7.

B.5.1.2.5. Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

Two residue trials, on apples and pears, have been presented in support of the Annex I renewal of GA4/7 and are summarised in Residues Section. These residue trials were previously evaluated as part of the original EU review for GA4/7. Full details of the studies are found in Annex II of the EU DAR (IIA 6.3.1/01). A summary of the method and validation data are presented below.

Previous evaluation:	This study was evaluated in the DAR (IIA 6.3.1/01) and has been considered by EFSA.
Data point addressed:	KCA 4.1.2/02
Author(s) (year):	Harrison C. (2005a)
Title:	VBC 30011: Residue Levels in Apples and Pears from Trials Carried Out in Northern France, Southern France, Italy and Northern Spain During 2002
Laboratory report / project number:	AF/6256/VB
Testing facility:	Agrisearch UK, Ltd
Published:	No
Test guideline used:	EU Working Document 1607/VI/97 rev. 2 Internal method VBC 30011/CROP/K/03/1
Deviations:	None
GLP:	Yes
EU agreed endpoint:	Yes

Materials and methods
Method: VBC30011/CROPS/KB/03/1 Test material: GA4/7 Lot/Batch No.: 21-973-CD Purity: 60.4% (GA4), 30.2% (GA7) Stability of test compound: Not reported

Principle of the method

See KCA 4.1.2/03 below.

Validation data

See KCA 4.1.2/03 below.

Conclusion

See KCA 4.1.2/03 below.

RMS comments: Calibration curves plots and equations were presented as well as representative chromatograms. Accuracy/recovery determinations were performed at three different levels with only single trials and results for repeatability are missing. Other validation criteria are successfully presented. Based on the lack of accuracy, and repeatability results this method for analysing GA4/7 residues in apples and pears, could not be considered validated and acceptable according to SANCO/3029/99. However due to the successful additional validation in the study KCA 4.1.2/04 below it could be taken as fit-for-purpose.

Previous evaluation:	This study was evaluated in the DAR (IIA 6.3.1/02) and has been considered by EFSA.
Data point addressed:	KCA 4.1.2/03
Author(s) (year):	Harrison C. (2005b)
Title:	VBC 30011: Residue Levels in Apples and Pears from Trials Carried Out in Northern France, Southern France, Italy and Northern Spain During 2003
Laboratory report / project number:	AF/6989/VB
Testing facility:	Agrisearch UK, Ltd
Published:	EU Working Document 1607/VI/97 rev. 2
Test guideline used:	None
Deviations:	Yes
GLP:	Yes
EU agreed endpoint	Yes

Materials and methods
Method: VBC30011/CROPS/KB/03/1
Test material: Gibberellin A4/A7
Lot/Batch No.: 21-973-CD
Purity: 90.6 % w/w: 60.4 % w/w (GA4), 30.2 % w/w (GA7)
Stability of test compound: Not reported

Principle of the method:

Samples of apple and pear were extracted by macerating in the presence of methanol, followed by centrifugation. An aliquot of the supernatant was filtered (syringe filter, PTFE, 0.45 μm) prior to dilution with 0.1% formic acid:water. The mixture was purified by reverse phase C18 solid phase extraction (SPE) cartridge clean-up (pre-conditioned with 0.1% formic acid:water) and eluted with acetonitrile. The extracts were concentrated to <0.25 mL before dilution with acetonitrile:water:formic acid (30:69.9:0.1, v/v/v). Levels of GA4/7 were quantified by LC-

MS/MS. Ions transitions (m/z) monitored were: 331.3→225.1, 331.3→243.2 and 331.3→287.4 for GA4 and 329.4→223.2 for GA7.

Validation Data

A summary of the method validation data for the determination of gibberellins (GA4/7) in apples and pears is given below.

Table B.5.1.2.5-01: Validation data for GA4/7 in apples and pears

Analyte	Matrix	Fortification Level (mg/kg)	LOQ (mg/kg)	No. of samples	Mean Recovery (%)	% RSD	Recovery Range (%)	Linearity	Study reference
GA4/7	Apple	0.05	0.05	1	112	n.a.	n.a.	Single matrix-matched analyses at 5 levels Range: 0.025 to 0.5 µg/mL r = 0.9983 slope :109 850 intercept : 1831.1	Harrison C. (2005a) Method ref: VBC 3001/CROPS/KB/03/1
	Pear	0.05		1	103	n.a.	n.a.		
	Overall	-		2	108	n.a.	103-112		
	Apple	0.01	0.05	1	94	n.a.	n.a.	Single analyses at 5 levels Range: 0.025 to 0.5 µg/mL r = 0.9998 slope :10^6 intercept : 37973	Harrison C. (2005b) Method ref: VBC 3001/CROPS/KB/03/1
		0.1		1	63	n.a.	n.a.		
	Pear	0.01		1	76	n.a.	n.a.		
		0.1		1	82	n.a.	n.a.		
	Overall	-		4	79	16.4	63-94		

Conclusion

Method VBC 30011/CROPS/KB/03/1 used in the two residue trial studies (AF/6256/VB and AF/6989/VB) was found to be acceptable to address the requirements for pre-registration methods under SANCO/3029/99 rev.4 as part of the original EU review for GA4/7 in the determination of GA4/7 in apples and pears. It is assumed, therefore, that this method is still considered acceptable here for renewal also.

RMS comments: Calibration curves plots and equations were presented as well as representative chromatograms. Accuracy/recovery determinations were performed at three different levels with only single trials and results for

repeatability are missing. Other validation criteria are successfully presented. Based on the lack of accuracy, and repeatability results this method for analysing GA4/7 residues in apples and pears, could not be considered validated and acceptable according to SANCO/3029/99. However due to the successful additional validation in the study KCA 4.1.2/04 below it could be taken as fit-for-purpose.

A freezer storage stability study, which has not been previously evaluated at EU level as part of the original review for GA4/7, is now included in this dossier. The study uses the method described above (VBC 3001/CROPS/KB/03/1) for the determination of GA4/7 in apples.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/04
Author(s) (year):	Harrison C. (2010)
Title:	To Determine the Stability of Gibberellin A4 (GA4) and Gibberellin A7 (GA7) in Pome Fruit Apple Specimens Following Storage at ca -18°C for 0, 1, 3, 12, 18 and 30 Months
Laboratory report / project number:	AD/6258/VB
Testing facility:	EUROFINS Agroscience services, UK
Published:	No
Test Guideline Used:	EU Working Document 7032/VI/95 rev 5. (Appendix H)
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: VBC30011/CROPS/KB/03/1 (based on a method validated in report V99.1181, KCA 4.2/02)
Test material: GA4/A7
Lot/Batch No.: 21-973-CD
Purity: 90.6 % w/w
Stability of test compound: Not reported

Principle of the method

See KCA 4.1.2/03 above.

Linearity

The linearity of the detector was confirmed using single analyses of matrix-matched standards in acetonitrile prepared at five concentration levels over the range 0.01 to 0.5 µg/mL. A representative calibration curve was provided in the report. The coefficient of determination was 0.9977 and the equation of the calibration curve is y = 458127x - 2756.6.

Specificity

Example chromatograms of control (untreated) samples, calibration standards, samples fortified at 0.5 g/kg were provided. Concentrations of GA4/7 in control samples were <30% of the assigned LOQ. LC-MS/MS is considered to be a highly specific detection technique, therefore no separate confirmatory method is required.

Accuracy

Six samples of apple were fortified with GA4/7 at the LOQ (0.5 mg/kg) and analysed according to the method. The %RSD was <20% and therefore considered acceptable.

Recovery findings

A summary of the recovery results for GA4/7 in apples is presented in the table below.

Table B.5.1.2.5-02: Procedural recovery results for GA4/7 in apples

Analyte	Matrix	Fortification Level (mg/kg)	Individual Recoveries (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
GA4/7	Apple	0.5	101, 79, 125, 109, 124, 85	6	104	18.6*	79-124

**Re-calculated based on rounded figures*

Repeatability

The mean recovery of GA4/7 was within the guideline range 70 to 110% and therefore considered acceptable.

Limit of quantification

The LOQ of the method was 0.02 mg/kg (see KCA 4.2/02), equivalent to the lowest validated level.

Conclusion

As the analytical method (VBC 3001/CROPS/KB/03/1) has been previously evaluated and accepted at EU level (during the original EU review for GA4/7) to address the requirements for pre-registration methods under SANCO/3029/99 rev.4; the method is considered acceptable here for renewal also.

RMS comments: Method was successfully validated regarding linearity, accuracy, precision, LOQ and selectivity and is therefore accepted for determination of GA4/7 in apples according to SANCO/3029/99. No further data required.

A series of published articles from literature have also been reviewed as part of the Annex I renewal of GA4/7 and have been summarised in Volume 3 Residues Section. Details of the associated analytical methods are presented below.

Data point addressed:	KCA 4.1.2/05
Author(s) (year):	Stephan M., Bangerth F., Schneider G. (1999)
Title:	Quantification of Endogenous Gibberellins in Exudates from Fruit from *Malus Domestica*. *J. Plant Growth Regul.*, 1999, **28**: 55.
Published:	Yes – This reference was briefly reported in the EU DAR (B7.1) but not in detail.

Principle of the method

Samples of fruits from four different varieties of apples were excised, placed pedicel end down in agar gel and incubated in the dark (20°C, 20 hours, almost 100% relative humidity). Plates were lyophilised, powdered by pestle and mortar and then extracted three times at 4°C in 80% methanol containing 1mM butyl hydroxytoluene (BHT). Deuterium labelled standards of relevant gibberellins (17-$^2H_2$-GA4 and 17-$^2H_2$-GA7) were used as internal standards for the procedure. The extracts were purified by anion exchange HPLC, concentrated and re-dissolved in methanol before determination of GA4/7 content by liquid chromatography coupled with mass spectrometry (LC-MS), where two ion transitions were monitored (17-$^2H_2$-GA4: 331→333 m/z and 17-$^2H_2$-GA7: 329→331 m/z).

No method validation data was provided in the report other than the determination of calibration parameters from plots of the logarithms of the peak area ratio of the ions from the unlabelled and radiolabelled standards against the logarithms of the molar ratio using the five different calibration mixtures: 10:1, 3:1, 1:1, 1:3 and 1:10. For GA4, the slope of the line was 1.22400 with an intercept of 0.07814. For GA7, the slope of the line was 1.29130 with an intercept of −0.05015.

RMS comments:

No method validation data except of some linearity data was presented in the paper therefore method performance cannot be assessed.

Data point addressed:	KCA 4.1.2/06
Author(s) (year):	Zhang C., Tateishi N., Tananbe K. (2010)
Title:	Pollen Density on the Stigma Affects Endogenous Gibberellin Metabolism, Seed and Fruit Set, and Fruit Quality in *Pyrus Pyrifolia.* *J. Exp. Bot.*, 2010, **61**, 15, 4291.
Published:	Yes

Principle of the method

The method of extraction, purification and determination of gibberellin content (GA1, GA3 and GA4) followed the procedure outlined by Zhang and Tanabe *et al.* (*Science*, **132**, 452). Samples of fruitlets from the Gold Nijisseiki variety of pears or growth medium containing pollen were homogenised and extracted overnight in 80% aqueous methanol containing BHT. Deuterium labelled standards of the relevant gibberellins (i.e. 17-$^2H_2$-GA) were used as internal standards for the procedure. The extracts were filtered, concentrated, partitioned against hexane and then acidified before partitioning against ethyl acetate. Further clean-up of extracts was conducted using C18 SPE cartridges, followed by fractionation by HPLC into solutions containing the individual gibberellins. Dried samples were methylated with ethereal diazomethane followed by trimethylsilylation. Final gibberellin

content was determined by GC-MS, where two ion transitions were monitored for GA4 (418→420 and 284→286 m/z).

RMS comments:

No method validation data was presented in the paper therefore method performance cannot be assessed.

A pre-registration method that is being used to support a further freezer storage stability study has been validated and is presented below in accordance with Commission Regulation (EU) No 283/2013. The method has not been considered previously at Annex I inclusion.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/07
Author(s) (year):	Jean-Baptiste C. (2011)
Title:	Frozen Storage Stability of Residues of Gibberellic Acid, Gibberellin A4 and Gibberellin A7 in Pears
Laboratory report / project number:	R A9206
Testing facility:	ANADIAG
Published:	No
Test guideline used:	SANCO/825/00 rev.7 SANCO/3029/99 rev.4
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

The analytical method was validated in KCA 4.1.2/08 below.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/08
Author(s) (year):	Jean-Baptiste C. (2009)
Title:	Validation of the Analytical Methods for the Determination of Gibberellic Acid, Gibberellin A4 and Gibberellin A7 Residues in Pears
Laboratory report / project number:	R A9006
Testing facility:	ANADIAG
Published:	No
Test guideline used:	SANCO/825/00 rev.7 SANCO/3029/99 rev.4 SANCO/2007/3131
Deviations:	None

GLP:	Yes
EU agreed endpoint:	No

Materials and methods

Method: R A9206 (see KCA 4.1.2/07), ANADIAG references: SOP MP 325, SOP MA 489 and SOP MA 647
Test material: Gibberellin A_4
Lot/Batch No.: 090505-CR-GIB-II
Purity: 95.83 % w/w
Stability of test compound: Not reported

Test material: Gibberellin A_7
Lot/Batch No.: 090505-CR-GIB-I
Purity: 97.13 % w/w
Stability of test compound: Not reported

Principle of the method

Pre-homogenised and frozen samples of pears were fortified with GA4/7 at concentrations equivalent to 0.02 and 0.2 mg/kg. Samples were mixed with Milli-Q water and the pH of the solution adjusted to pH 2. Samples were extracted with ethyl acetate, centrifuged and evaporated to dryness. Concentrations of GA4/7 were determined by dissolution of the residues in an accurate volume of methanol, sonication, filtration and analysis by LC-MS/MS.

Linearity

The linearity of the detector was demonstrated by single determinations of eight calibration solutions of GA4 and GA7 in pear sample matrix and ethyl acetate over the range 25 to 1200 ng/mL. Representative calibration plots were provided for GA4 and GA7. The coefficients of determination were 0.99646 for GA4 and 0.99257 for GA7. Calibration curve equations were:

GA: 0.082812x – 13.13

GA7: 0.40463x – 12.41

Specificity

Example chromatograms of control samples, standards at the lowest calibrated level and samples fortified at the LOQ were provided. No peak interferences occurred at retention times of the analytes with concentrations of GA4/7 in blank samples were found to be <30% the LOQ. LC-MS/MS as a detection technique can be considered to be highly specific. Two ion transitions were monitored for each analyte (GA4: 331.1 → 269.1 and 331.1 → 287.1 m/*z*; GA7: 331.1 → 269.1 and 331.1 → 295.1 m/*z*). Example mass spectra were provided, justifying ion choices. The relative abundances of the qualifier ions (% relative to the confirmation transition) in the spiked extracts were compared with those of the calibration standards. The differences of the relative abundances between sample extracts and standard were lower than maximum tolerances outlined in SANCO/2007/3131 (difference in relative abundance between extract and standards, ±20%), confirming sample extract identity and specificity of the method.

Accuracy

Samples of pears were fortified at concentrations equivalent to 0.02 and 0.2 mg/kg of GA4/7. For each fortification level, five test samples were analysed alongside two unfortified control samples. The %RSD for GA4/7 at each fortification level and overall was <20% and are therefore considered acceptable.

Recovery findings

A summary of the recovery results for GA4/7 in pears is presented in the table below.

Table B.5.1.2.5-03: Recovery results of GA4/7 in pears

Analyte	Fortification Level (mg/kg)	Individual Recoveries (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
GA4	0.02	71, 84, 79, 77, 82	5	79	6.6	71-84
	0.20	97, 103, 103, 104, 106	5	102	3.3	97-106
GA7	0.02	78, 90, 80, 76, 90	5	83	8.1	76-90
	0.20	94, 106, 110, 109, 108	5	105	5.9	94-110
Overall	-	-	20	92	14.2	71-110

Repeatability

Five recoveries were determined for each analyte at the LOQ (0.02 mg/kg) and at 10 times the LOQ (0.2 mg/kg). Mean recoveries at each fortification level and overall for GA4/7 in pears were within the range 70 to 120% and therefore considered acceptable.

Limit of quantification and limit of detection

The LOQ of the method for GA4 and GA7 was 0.02 mg/kg. LOD values were estimated to be three times the background noise under the analytical conditions used. The LOD for GA4 was 0.004 mg/kg and for GA7 was 0.003 mg/kg.

Conclusion

The method was successfully validated for the determination of GA4/7 in pears in accordance with SANCO/3029/99 rev. 4.

RMS comments: Method was successfully validated regarding linearity, accuracy, precision, LOQ and selectivity and is therefore accepted for determination of GA4/7 in pears according to SANCO/3029/99. No further data required.

B.5.1.2.6. Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Pre-registration methods that have been used in support of ecotoxicological studies are presented below in accordance with Commission Regulation (EU) No 283/2013. These methods have not been considered previously at Annex I inclusion.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/09
Author(s) (year):	Juckeland W. (2014)
Title:	Toxicity of Gibberellins (GA4/7) Technical to *daphnia Magna* in a 21-Day Semi-Static Reproduction Test
Laboratory report / project number:	14 10 48 073 W
Testing facility:	BioChem agrar
Published:	No
Test guideline used:	SANCO/3029/99 rev. 4 SANCO/12495/2011
Deviations:	Yes – minor
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: Verification of active substance concentration, described in report 14 10 048 078 W
Test material: Gibberellins (GA4/7) technical
Lot/Batch No.: 20130204
Purity: 90.6 % w/w
Stability of test compound: Stable under normal conditions

Principle of the method

Elendt M4 test medium was fortified with GA4/7 (in methanol and water) at concentrations of 0.22 and 0.9 mg /L. Total amounts of GA4/7 in each sample were quantified by LC-MS/MS. The target LOQ was 0.022 mg/L.

Linearity

The detector response was demonstrated to be quadratic using single determinations of five calibration solutions of GA4/7 test item in methanol and water in the range 0.02 to 10.8 mg/mL. Quadratic calibration curves were provided in the report for low (0.02 to 10.8 mg/L) and high (0.11 to 1.0 mg/L) validations. A quadratic fit and 1/c weighting was used. Equations of the line were:

Low range: $y = -0.003346x^2 + 19.112059x + 83.801043$, $R^2 = 0.999923$

High range: $y = -0.00059x^2 + 14.626495x + 781.910986$, $R^2 = 0.997479$

Specificity

No interferences were observed ≥30% of the LOQ in any of the blank samples analysed as part of the method validation. Example chromatograms of control samples, standards at the lowest calibration level and samples

fortified at the LOQ were provided. The method included two MS/MS transitions (348.2 → 295.2 m/z for GA7 and 315.2 → 241.2 for GA4 m/z). A mass spectrum was given, justifying ion transition choices.

Accuracy

Samples of Elendt M4 test medium were fortified with GA4/7 at concentrations equivalent to 0.22 and 9 mg/L. For each fortification level, five test samples were prepared and analysed. The %RSD for GA4/7 at each concentration and overall was <20% and are therefore considered acceptable.

Recovery findings

A summary of the recovery results for GA4/7 in the test medium is presented in the table below.

Table B.5.1.2.6-01: Recovery results for GA4/7 in Elendt M4

Analyte	Fortification Level (mg/L)	Individual Recoveries (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
GA4/7	0.22	N/R	5	102	5.9	N/R
	9	N/R	5	93	0.5	N/R
	Overall	-	10	98	6.5	N/A

N/R – Not reported; N/A – Not applicable

Repeatability

Mean recoveries at each fortification level for GA4/7 in Elendt M4 test medium were within the range 70 to 110% and therefore considered acceptable. The overall mean recovery was within the range 80 to 100%.

Limit of quantification

The LOQ for GA4/7 in this study was 0.022 mg/L, equivalent to the lowest validated fortification level.

Conclusion

The method was successfully validated for the determination of GA4/7 in Elendt M4 medium in accordance with SANCO/3029/99 rev. 4.

RMS comments: Method was successfully validated regarding linearity, accuracy, precision, LOQ and selectivity and is therefore accepted for determination of GA4/7 in Elendt M4 medium according to SANCO/3029/99. No further data required.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/10
Author(s) (year):	[redacted] (2016)

Title:	Gibberellic Acid (GA3): An Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales Promelas*)
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 210 OPPTS 850.1400
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: Verification of active substance concentration, described in report 529A-130, developed by Wildlife International
Test material: Gibberellic Acid (GA3) Technical Powder
Lot/Batch No.: 237-979-S4
Purity: 91.8 % w/w
Stability of test compound: Not reported

This study concerns the determination of gibberellic acid (GA3). Data from this study has been used as read-across to address endpoints within this supplementary dossier for gibberellins (GA4/7).

Principle of the method

The method was validated by fortifying samples of freshwater with stock solutions of GA3 (in methanol solution) at concentrations equivalent to 0.55, 2.5 and 11.0 mg/L. The concentration of GA3 in each sample was quantified by dilution with a methanol/freshwater/formic acid mixture prior to detection by liquid chromatography-tandem mass spectroscopy (LC-MS/MS). The target limit of quantification (LOQ) was 0.55 mg/L.

Recovery findings

Summaries of the recovery results for GA3 in freshwater are presented in Table B.5.1.2.6-01.

Table B.5.1.2.6-01: Recovery results of GA3 in freshwater samples during method validation

Fortification Level (mg/L)	Individual Recoveries (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
0.550	99, 104, 101, 96, 103, 97	6	100	3.2	96-104
2.50	97, 105, 111, 100, 100, 98	6	102	5.2	97-111
11.0	98, 107, 112, 98, 101, 100	6	103	5.5	98-112
Overall	-	18	102	4.6	96-112

Linearity

The linearity of the detector was demonstrated using multiple determinations of five concentrations of GA3 in methanol:freshwater:formic acid prepared over the range 0.05 to 0.5 mg/L, equivalent to 0.1 to 1.0 mg/L of freshwater. A representative calibration curve is provided in the report with values given for the intercept (10847.6) and slope (1744300) and a coefficient of determination of 0.9980.

Specificity

No interferences were observed at or above the LOQ in any of the blank samples analysed as part of the method validation. Example chromatograms for control samples, standards at the lowest calibrated level and samples fortified at 5.5 × the LOQ have been presented in the study report.

Accuracy

Samples of freshwater were fortified with stock solutions of GA3 in methanol at concentrations equivalent to 0.55, 2.5 and 11.0 mg/L. For each fortification level, six test samples were prepared and analysed. The %RSD for GA3 at each fortification level and overall was <20% and therefore considered acceptable.

Repeatability

Mean recoveries at each fortification level and overall for GA3 in freshwater samples were within the range 70-120% and therefore considered acceptable.

Limit of quantification and limit of detection

The LOQ for GA3 in freshwater was reported as 0.1 mg/L, equivalent to the lowest calibration concentration, however the lowest validated fortification level was equivalent to 0.55 mg/L. The LOQ was therefore 0.55 mg/L whilst the LOD was 0.1 mg/L.

Conclusion

The method was successfully validated for the determination of GA3 in freshwater in accordance with SANCO/3029/99 rev. 4.

RMS comment: The analytical method for the determination of GA3 in freshwater is considered valid and acceptable according to SANCO/3029/99 rev.4. No further data required.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/11
Author(s) (year):	Taylor K. (2017)
Title:	Gibberellins A4A7: Honey Bee (*Apis Mellifera*) Larval Toxicity Test, Single Exposure
Laboratory report / project number:	CR15QN

Testing facility:	Envigo CRS Limited
Published:	No
Test guideline used:	OECD 237
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: DFA/M100/16
Test material: Gibberellins A_4A_7 Technical
Lot/Batch No.: 1000048922
Purity: 91.6 % w/w
Stability of test compound: Not reported

Principle of the method

Diet C formulation was fortified with technical GA4/7 at concentrations of 0.208 and 3.333 mg/mL. Amounts of GA4/7 in each sample were then determined by ultra-performance liquid chromatography (UPLC) with UV detection at 205 nm.

Linearity

The linearity of the detector was demonstrated using single determinations of eight calibration solutions of GA4/7 in Diet C formulation prepared over the range 0.3 to 10 µg/mL. A representative calibration curve is provided in the report with values given for the intercept (147) and slope (10300). The coefficient of determination was >0.999.

Specificity

No interferences were observed at the retention times for gibberellins GA4/7 at or above the LOQ in any of the control samples analysed as part of the method validation. Example chromatograms for control samples, standards at the highest calibration level, samples fortified at the LOQ and 16 × the LOQ were presented in the study report.

Accuracy

Ten samples of GA4/7 technical in Diet C formulation were prepared, five at a concentration of 0.208 mg/mL and five at 3.333 mg/mL. Mean recoveries for GA4/7 in the diluent at each fortification level were within the range 70-120% and the overall recovery was within the range 80-100%, therefore are acceptable. The %RSD values were <20% and also considered acceptable.

Recovery findings

A summary of the recovery results for gibberellins (GA4/7) in Diet C formulation is presented in table B.5.1.2.6-02 below.

Table B.5.1.2.6-02: Recovery results of GA4/7 in Diet C formulation

Analyte	Fortification Level (mg/mL)	Individual Recoveries* (%)	Number of Analysis (n)	Mean Recovery** (%)	RSD** (%)	Recovery Range* (%)
GA4/7	0.208	94, 94, 95, 94, 93	5	94	0.8	93-95
	3.333	101, 100, 98, 101, 99	5	100	1.3	98-101
	Overall	-	10	97	3.3	93-101

**Values have been rounded; **Values have been re-calculated based on rounded figures*

Repeatability

Repeatability was assessed by analysing six replicate injections of the lowest and highest calibration standards (0.3 and 10 µg/mL, respectively). The %RSD values were <20% at each concentration level and therefore considered acceptable.

Limit of quantification and limit of detection

The LOQ for GA4/7 in Diet C formulation was reported as 0.0003 mg/mL, equivalent to ten times the baseline noise in the chromatogram of a control sample. However, the lowest validated fortification level was 0.208 mg/mL. The LOQ is therefore 0.208 mg/mL. The LOD was 0.0932 µg/mL, equivalent to three times the baseline noise in the chromatogram of a control sample.

Conclusion

The method was successfully validated for the determination of GA4/7 in Diet C formulation in accordance with SANCO/3029/99 rev. 4.

RMS comment: The analytical method for the determination of GA4/7 in Diet C formulation is considered valid and acceptable according to SANCO/3029/99 rev.4. No further data required.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/12
Author(s) (year):	Gray J. (2017)
Title:	Gibberellins A4A7: Honey Bees (*Apis mellifera L.*) Chronic Oral Toxicity Test 10 Day Feeding in the Laboratory
Laboratory report / project number:	FR30QH
Testing facility:	Envigo CRS Limited
Published:	No
Test guideline used:	OECD proposal for a new guideline for the testing of chemicals; Honey bee chronic oral toxicity test 10 day feeding test in the laboratory, February 2016.

Deviations:	None*
GLP:	Yes
EU agreed endpoint:	No

*Deviations were noted in the study, but they did not apply to the method validation.

Materials and methods
Method: DFA/M100/6
Test material: Gibberellins A4A7 Technical
Lot/Batch No.: 1000048922
Purity: 91.6 % w/w
Stability of test compound: Not reported

Principle of the method

50% (w/v) aqueous sugar solution was fortified with GA4/7 technical at concentrations of 9 µg/g and 150 µg/g. Amounts of GA4/7 in each sample were determined by ultra-performance liquid chromatography (UPLC) with UV detection at 205 nm.

Linearity

The linearity of the detector was demonstrated using single determinations of eight concentrations of GA4/7 in 50% (w/v) aqueous sugar solution prepared over the range 0.3 to 10 µg/mL. A representative calibration curve was provided in the report with values given for the intercept (612) and slope (11600) and a coefficient of determination of 0.999896.

Specificity

No interferences were observed at the retention times for GA4/7 at or above the LOQ in any of the control samples analysed as part of the method validation. Example chromatograms for control samples, standards at the highest calibration level, samples fortified at the LOQ and 16 × the LOQ have been presented in the study report.

Accuracy

Ten samples of GA4/7 in 50% (w/v) aqueous sugar solution were prepared, five at a concentration of 9 µg/g and five at 150 µg/g. Mean recoveries at each fortification level were within the range 70-110% and the overall recovery was within the range 80-100%, so are accepted. The %RSD values were <20% and so are also considered acceptable.

Recovery findings

A summary of the recovery results for GA4/7 in 50% (w/v) aqueous sugar solution is presented in Table B.5.1.2.6-03 below.

Table B.5.1.2.6-03: Recovery results of GA4/7 in 50% (w/v) aqueous sugar solution

Analyte	Fortification Level (µg/g)	Individual Recoveries* (%)	Number of Analysis (n)	Mean Recovery** (%)	RSD** (%)	Recovery Range* (%)
GA4/7	9	96, 96, 96, 98, 96	5	96	0.9	96-98
	150	99, 99, 98, 99, 98	5	99	0.6	98-99
	Overall	-	10	98	1.4	96-99

**Values have been rounded; **Values have been re-calculated based on rounded figures*

Repeatability

Repeatability was assessed by analysing six replicate injections of the lowest and highest calibration standards (0.3 and 10 µg/mL, respectively). The %RSD values were <20% at each fortification level and therefore considered acceptable.

Limit of quantification and limit of detection

The LOQ for GA4/7 in 50% (w/v) aqueous sugar solution was reported 0.111 µg/mL, equivalent to ten times the baseline noise in the chromatogram of a control sample, however the lowest validated fortification level was 9 µg/g. The LOQ is therefore 9 µg/g. The LOD was 0.0332 µg/mL, equivalent to three times the baseline noise in the chromatogram of a control sample.

Conclusion

The method was successfully validated for the determination of GA4/7 in 50% (w/v) aqueous sugar solution in accordance with SANCO/3029/99 rev. 4.

RMS comment: The analytical method for the determination of GA4/7 in aqueous sugar solution is considered valid and acceptable according to SANCO/3029/99 rev.4. No further data required.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/13
Author(s) (year):	Mantilacci, S. (2017)
Title:	Toxicity Evaluation of Test item Gibberellins GA4/7 Technical on *Navicula Pelliculosa* in a Growth Inhibition Limit Test and Validation of the Analytical Method
Laboratory report / project number:	BT264/17
Testing facility:	BioTecnologie B.T. Srl
Published:	No
Test guideline used:	SANCO/3029/99 rev. 4

Deviations:	No
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: Verification of GA4 concentration, described in report BT264/17
Test material: Gibberellins GA4/7 Technical
Lot/Batch No.: F0170901
Purity: 91.35 % w/w: 90.3 % w/w (GA4), 1.05 % w/w (GA7)
Stability of test compound: Not reported

Principle of the method

Acidified EPA medium was fortified with GA4/7 at two concentration levels. Samples were analysed for GA4 and GA7 contents by LC-MS/MS and quantified using external standards. Ion transitions monitored were: 331→243.2 m/z (quantification) and 331→257 m/z (confirmation) for GA4 and 329→223 m/z (quantification) and 329→211 m/z (confirmation) for GA7.

Linearity

The linearity of the detector was confirmed for each transition by duplicate determinations of five calibration solutions of the analytical standard (GA4 or GA7) in methanol and diluent (EPA medium acidified with 0.1% formic acid). The linear range was 102 to 1020 µg/L for GA4 and 1.2 to 12 µg/L for GA7. Representative calibration plots were provided in the report for each analyte and ion transition with values given the slopes and intercepts. Results are given in the table below.

Table B.5.1.2.6-04: Linearity data

Analyte	**Ion Transition (m/z)**	**r^2**
GA4	331→243.2	0.9934
	331→257	0.9935
GA7	329→223	0.9964
	329→211	0.9934

Specificity

No interferences were observed at the retention times of GA47 at or above 30% of the LOQ in any of the blank samples analysed as part of the method validation. Representative chromatograms of blank samples (i.e. EPA medium), analytical standards at the highest and lowest calibration levels, samples fortified at the LOQ and five times the LOQ were provided in the report for both GA4 and GA7. The method included two MS/MS transitions for GA4 and two for GA7 (a quantification and confirmation transitions), all of which have been validated.

Accuracy

Five samples of the test item in methanol and diluent (EPA medium acidified with 0.1% formic acid) were prepared at the LOQ (153 µg/L for GA4 and 1.8 µg/L for GA7) and five were prepared at five times the LOQ (765 µg/L for GA4 and 8.9 µg/L for GA7). Fortified samples were analysed alongside two control (EPA medium) samples. The %RSD values were <20% when using both the quantification and confirmation techniques and so are considered acceptable.

Recovery findings

A summary of the recovery results for GA4/7 in EPA medium is presented in the table below.

Table B.5.1.2.6-05: Recovery results for GA4/7 in EPA medium

Analyte	Ion Transition (m/z)	Fortification Level (µg/L)	Individual Recoveries* (%)	Number of Analysis (n)	Mean Recovery (%)	RSD** (%)	Recovery Range (%)
GA4	331→243.2	153.060	104, 100, 100, 101, 100	5	101	1.7	100-104
		765.300	99, 102, 103, 102, 99	5	101	1.9	99-103
		Overall	-	10	101	1.7	99-104
	331→257	153.060	101, 100, 101, 100, 101	5	101	0.5	100-101
GA7	329→223	1.780	108, 106, 105, 104, 109	5	106	1.9	104-109
		8.899	100, 99, 101, 99, 98	5	99	1.1	98-101
		Overall	-	10	103	3.9	98-109
	329→211	1.780	102, 128, 98, 112, 92	5	106	13.3	82-128

**Rounded to 2 d.p.; **Recalculated based on rounded figures*

Repeatability

Mean recoveries for GA4 and GA7 in EPA medium were within the range 70 to 110% and therefore considered acceptable.

Limit of quantification

The LOQs for GA4 and GA7 in EPA medium were 153 µg/L and 1.8 µ/L (respectively), corresponding to the lowest validated fortification level.

Conclusion

The method was successfully validated for the determination of GA4/7 in EPA medium in accordance with SANCO/3029/99 rev. 4.

RMS comment: The analytical method for the determination of GA4/7 in EPA medium is considered valid and acceptable according to SANCO/3029/99 rev.4. No further data required.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/14
Author(s) (year):	Stead, A. (2018)
Title:	Gibberellins A4A7: GLP Seedling Emergence and Seedling Growth Test Terrestrial Non-Target Plants (based on OECD Guideline 208) - 2017
Laboratory report / project number:	STC/17/E1126
Testing facility:	Stockbridge Technology Centre Ltd.
Published:	No
Test guideline used:	OECD Guideline 208
Deviations:	No
GLP:	Yes
EU agreed endpoint:	No

The analytical method was validated in KCA 4.1.2/15 below.

Previous evaluation:	None
Data point addressed:	KCA 4.1.2/15
Author(s) (year):	Turner, B. (2018)
Title:	Analysis of Gibberellins A4A7 Spray Solution
Laboratory report / project number:	TB20CD
Testing facility:	Stockbridge Technology Centre Ltd.
Published:	No
Test guideline used:	SANCO/3029/99 rev.4
Deviations:	No
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: Verification of active substance concentration, used in report STC/17/E1126 (see KCA 4.1.2/14)
Test material: Gibberellin A4A7

Lot/Batch No.: 21-973-CD Purity: 89.6 % w/w Stability of test compound: Not reported

Principle of the method

Spray solution containing GA4/7 test item was sonicated and stirred prior to sampling. Three 10 mL aliquots of the spray solution were added to separate volumetric flasks (100 mL) and diluted to volume with mobile phase (methanol:0.1% orthophosphoric acid, 60:40 v/v). Samples were analysed for GA4 and GA7 contents by HPLC-UV at 204 nm and quantified using external bracketing standard solutions.

Linearity

The linearity of the detector was confirmed by single determinations of five calibration solutions of GA4/7 analytical standard in methanol and 0.1% orthophosphoric acid. Solutions were prepared over the concentration range of ca. 30 to 150 mg/L. A representative calibration plot was provided in the report. The coefficient of correlation (r) was 0.9999.

Specificity

Representative chromatograms of GA4/7 standard solutions and sample solutions were provided. There were no interferences noted.

Accuracy and repeatability

Three samples of spray solution (containing GA4/7 test item at ca. 822.4 mg/L) were analysed in accordance with the method. The mean recovery of GA4/7 from the spray solution was within the guideline range of 80-100% (SANCO/3029/99 rev. 4), so is accepted.

The accuracy of the method was further confirmed by analysis of five laboratory prepared aqueous solutions of the test item prepared at concentrations from 850.4 to 915.9 mg/L (taking purity of the test item into account). Samples of the GA4/7 test item (ca. 0.11 g) were added to volumetric flasks (100 mL), isopropyl alcohol added (0.8 mL) and samples sonicated. Samples were made to volume with purified water and sonicated. Aliquots (10 mL) of the sample were transferred to separate volumetric flasks (100 mL) and diluted to volume with mobile phase (methanol:0.1% orthophosphoric acid, 60:40 v/v). Samples were analysed for GA4 and GA7 contents by HPLC-UV at 204 nm and quantified using external bracketing standard solutions. The mean recovery was within the guideline range 80-100% and the %RSD was ≤20% (SANCO/3029/99 rev. 4), so the method is considered accurate and precise.

Recovery findings

A summary of the recovery results for GA4/7 in the spray solution is presented in the table below.

Table B.5.1.2.6-06: Recovery results for GA4/7 in the spray solution

Analyte	Concentration of GA4/7 in Spray (mg/L)	Individual Recoveries (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
GA4/7	832.6	N/R	3	82	N/A	N/A
	835.9					
	798.7					

N/R = Not reported; N/A = Not applicable

Recovery data were also obtained from laboratory prepared solutions of the test item GA4/7 in isopropyl alcohol, water and mobile phase. Results are presented in the table below.

Table B.5.1.2.6-07: Recovery results for GA4/7 from solvent (isopropyl alcohol, water and mobile phase)

Analyte	Concentration of GA4/7 in Sample (mg/L)	Equivalent Concentration of GA4/7 in Spray (mg/L)*	Individual Recoveries** (%)	Number of Analysis (n)	Mean Recovery** (%)	RSD (%)	Recovery Range (%)
GA4/7	85.04	850.4	85	5	89	2.9	85-92
	89.00	890.0	89				
	89.48	894.8	90				
	89.81	898.1	89				
	91.59	915.9	92				

**Concentration of GA4/7 in sample × Dilution Factor (10); **Rounded to integer values*

Limit of quantification

The LOQ for GA4/7 in the spray solution was 822.4 mg/L.

Conclusion

The method was successfully validated for the determination of GA4/7 in the spray solution in accordance with SANCO/3029/99 rev. 4.

RMS comment: The analytical method for the determination of GA4/7 in the spray solution is considered valid and acceptable according to SANCO/3029/99 rev.4. No further data required.

B.5.1.2.7. Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physichal and chemical properties tests

No new physico-chemical studies have been submitted as part of the Annex I renewal of GA4/7.

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES

B.5.2.1. Methods for the determination of all components included in the monitoring residue definition as submitted in accordance with the provision of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin

Residues in or on food and feed of plant origin

During peer review under Directive 91/414/EEC, a residue analytical method was evaluated for the determination of GA4/7 in apples and pears. A summary of the method and validation data are provided in the Draft Assessment Report (Annex B: Section B.5, July 2006) and also below.

Previous evaluation:	This study was evaluated in the DAR (B.5.2) and has been considered by EFSA.
Data point addressed:	KCA 4.2/01
Author(s) (year):	Gian Carlo G. (1995)
Title:	Determination of Gibberellins A4-A7 Residues on Apple and Pear
Laboratory report / project number:	NEOT/GLP/LN 52A-95
Testing facility:	Neotron S.r.l.
Published:	No
Test guideline used:	None stated, but meets the requirements of SANCO/825/00 rev.7
Deviations:	None
GLP:	Yes
EU agreed endpoint:	Yes

Materials and methods
Method: NEOT/GLP/LN 52A-95
Test material: Gibberellins A4-A7
Lot/Batch No.: 21-973-CD
Purity: 90.6% (as sum of the two isomers), 60.4% (GA4), 30.2% (GA7)
Stability of test compound: Not reported

Principle of the method

Please see KCA 4.2/02 below.

Validation data

Please see KCA 4.2/02 below.

Conclusion

Please see KCA 4.2/02 below.

An independent laboratory validation was required to validate the method and is summarised below.

Previous evaluation:	This study was evaluated in the DAR (B.5.2) and has been considered by EFSA.
Data point addressed:	KCA 4.2/02
Author(s) (year):	Mol J.G.J. (2001)
Title:	Site Validation of the GA4/GA7 Residue Method in Apple and Pear
Laboratory report / project number:	V99.1181
Testing facility:	TNO Nutrition and Food Research
Published:	No
Test guideline used:	None stated, but meets the requirements of SANCO/825/00 rev.7
Deviations:	None
GLP:	Yes
EU agreed endpoint:	Yes

Materials and methods
Method: NEOT/GLP/LN 52A-95
Test material: Gibberellins GA4/GA7
Lot/Batch No.: 93-041-CD
Purity: 60.4 % w/w (GA4), 30.2 % w/w (GA7)
Stability of test compound: Not reported

Principle of the method

Gibberellins GA4A7 were extracted from crops by blending with acetone and buffer solution at pH 7. The extract was purified by liquid/liquid partitions with ethyl acetate followed by normal phase HPLC clean-up. The analysis was carried out by HPLC with UV detection at a wavelength of 206 nm. The limit of quantification of the method (LOQ) was 0.05 mg/kg. An independent laboratory validation (ILV) had been conducted using tandem mass spectrometric detection. (DAR, Annex B: Section B.5.2.1, July 2006).

Validation data

A summary of the method validation data for the determination of gibberellins (GA4/7) in apple and pear (report no: NEOT/GLP/LN 52A-95) and an independent laboratory validation of the method (report no.: V99.1181) are given below.

Table B.5.2.1-01: Validation data for determination of GA4/7 in apple and pear (adapted from DAR, Annex B: Section B.5.2.1, July 2006).

Analyte	**Matrix**	**Fortification Level (mg/kg)**	**LOQ (mg/kg)**	**No. of Samples**	**Mean Recovery (%)**	**RSD**	**Recovery Range (%)**	**Linearity**	**Study reference**

						(%)			
GA4/7	Apple	0.02	0.05*	-	92	-	-	Triplicate analyses at 4 levels Range: 0.2 to 5.0 µg/mL r^2 = 0.9998	Gian Carlo G. (1995) Method ref: NEOT/GLP/LN 52A-95
		0.20		-	89	-	-		
		2.0		-	87	-	-		
		Overall		3	89	2.8	87-92		
	Pear	0.02	0.05*	-	94	-	-		
		0.20		-	95	-	-		
		2.0		-	97	-	-		
		Overall		3	95	1.6	94-97		
	Apple	0.05	0.05	6	89	16	64-99	Duplicate analyses at 6 levels Range: 0.1 to 50 mg/mL $r^2 \geq 0.997$	Mol J.G.J. (2001) Method ref: V99.1181
		0.50		6	97	10	79-105		
		Overall		12	93	13	64-105		
	Pear	0.05	0.05	4**	103**	7.3	93-111		
		0.50		5	103	4.4	98-108		
		Overall		9	103	5.4	93-111		

**Limit of quantification is 0.05 mg/kg (lowest validated fortification level for ILV); **One low recovery value of 27% was excluded from the calculations and it was identified as an outlier using the Dixon test.*

Conclusion

The DAR states:

"A monitoring method for gibberellins GA_4/GA_7 in apple and pear was successfully evaluated and meets the EU criteria with respect to specificity, accuracy and precision according to the requirements of EU Commission Directive 96/46/EC and guidance document SANCO/825/00. The method requires equipment and instrumentation which are commonly available in most well-equipped laboratories. The procedures are consistent with standard multi-residue methods (i.e. solvent extraction, purification using a solid phase extraction cartridge and determination by LC/MS/MS). Therefore, a suitable method is available for generation of pre-registration data for risk assessment purposes and for post-registration monitoring and enforcement."

The method is considered acceptable in support of active substance renewal for GA4/7. The method has been validated by an independent laboratory using LC-MS/MS.

RMS comment:
The results from the method validation have in detail fulfilled the guideline SANCO/825/00 rev. 8.1. and the method is suitable for monitoring of GA4/7 in pears and apples.

Residues in or on food and feed of animal origin

Referring to Residues Section, gibberellins are a family of naturally occurring plant hormones which are widespread in plants and fungi. There is no significant difference between naturally occurring levels and levels arising from the use of GA4/7 as a plant protection product. No MRLs are proposed nor a residue definition for monitoring and enforcement in animals (EFSA Conclusion, 2012). As such, a monitoring method for residues in or on food and feed of animal origin is not required.

B.5.2.2. Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of point 7.4.2

Residues in soil

Residue analytical methods in soil were considered during the 2008 EU review. The DAR states:

"Gibberellins GA4 and GA7 are naturally occurring non-toxic compounds that are present in a wide range of plant species. Consequently, a continuous but variable background level will exist in the environment and monitoring of gibberellins GA4 and GA7 residues in soil are not considered relevant."

However, the 2012 EFSA conclusion identified methods of analysis for soil as a data gap. As such, a new method has been developed since first approval and is presented below.

Previous evaluation:	None
Data point addressed:	KCA 4.2/03
Author(s) (year):	Brewin S. (2017)
Title:	GA4A7: Validation of Methodology for the Determination of Residues of in Soil
Laboratory report / project number:	YR93VB
Testing facility:	Envigo CRS Limited
Published:	No
Test guideline used:	SANCO/825/00 rev. 8.1
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: Verification of active substance concentrations, annex to report YR93VB
Test material: Gibberellin A_4 (GA4)
Lot/Batch No.: 91-932-BD
Purity: 100.0%
Stability of test compound: Not reported

Test material: Gibberellin A_7 (GA7)
Lot/Batch No.: 65753-145
Purity: 92.1% w/w
Stability of test compound: Not reported

Principle of the method

Sub-samples of soil fortified with GA4/7 in solutions of water:methanol:formic acid were extracted with water:methanol:formic acid mixture by mechanical shaking followed by centrifugation. Samples were made to volume with water:methanol:formic acid mixture to achieve a matrix concentration of 0.1 g soil/mL. The analytes GA4 and GA7 were stable in the final extract for up to 7 days when stored at -20 °C in the dark, prior to analysis.

Quantification was achieved by liquid chromatography-tandem mass spectroscopy (LC-MS/MS). Four ion transitions were monitored; 331→243 and 329→223 m/z for quantification; 331→225 and 329→241 m/z for confirmation. The method was validated in sandy soil with low organic carbon content and clay soil with high organic carbon content.

Linearity

Matrix effects were assessed and not considered significant (<20%) for GA4 and GA7 in the final sample extracts. The linearity of the detector was confirmed using single determinations of eight calibration solutions of GA4/7 in methanol prepared over the range 0.25 to 20 ng/ml (equivalent to 0.0025 to 0.2 mg/kg in soil). Typical calibration curves were provided in the report for both analytes and ion transitions. Results are given in table B.5.2.2-01 below.

Table B.5.2.2.-01: Linearity data

Analyte	Ion Transition (m/z)	r^2	slope	intercept
GA4	331→243	0.9999	6534	-184.737
	331→225	0.9996	3876.21	-257.244
GA7	329→223	0.9997	32947	-1326.44
	329→241	0.9992	1280.69	-59.6584

Specificity

Labelled chromatograms of control samples, standards at the lowest calibrated level and samples fortified at the LOQ were provided. Control (untreated) samples of soil were analysed. There were no interferences (i.e. response ≥30% of the LOQ) from the matrix under the quantification or confirmation conditions. LC-MS/MS as a detection technique is considered to be highly specific. The method included four MS/MS transitions (two quantification and two confirmation), each of which were validated. A mass spectrum was given, justifying ion transition choices. Typical mass spectra were provided in the report.

Accuracy

Sandy and clay soil samples were fortified at the LOQ (0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg) with GA4/7. Five samples were prepared for each fortification level and run alongside two control samples. The relative standard deviations (%RSD) for GA4/7 recoveries were <20% for all transitions in both soil types and are therefore considered acceptable.

Recovery findings

A summary of the recovery results for GA4/7 in the soil matrices are presented in the table below.

Table B.5.2.2-02: Recovery results for GA4/7 in sandy and clay soils

Analyte	Matrix	Fortification	Individual	Number	Mean	RSD	Recovery

		Level (mg/kg)	**Recoveries (%)**	**of Analysis (n)**	**Recovery (%)**	**(%)**	**Range (%)**
GA4	Sandy soil m/z 331→243	0.01	92, 89, 87, 90, 94	5	91	3.0	87-94
		0.1	85, 88, 85, 83, 83	5	85	2.5	83-88
		Overall	-	10	88	4.4	83-94
	Sandy soil m/z 331→225	0.01	99, 92, 92, 87, 90	5	92	4.5	87-99
		0.1	85, 86, 89, 87, 84	5	86	2.3	84-89
		Overall	-	10	89	4.9	84-99
	Clay soil m/z 331→243	0.01	83, 85, 81, 86, 83	5	84	2.5	81-86
		0.1	78, 76, 82, 79, 81	5	79	3.0	76-82
		Overall	-	10	81	3.9	76-86
	Clay soil m/z 331→225	0.01	79, 84, 76, 81, 84	5	81	4.3	76-84
		0.1	78, 79, 79, 85, 81	5	80	3.4	78-85
		Overall	-	10	81	3.7	76-85
GA7	Sandy type soil m/z 329→223	0.01	84, 82, 86, 88, 90	5	86	4.0	82-90
		0.1	83, 83, 81, 81, 82	5	82	1.3	81-83
		Overall	-	10	84	3.8	81-90
	Sandy soil m/z 329→241	0.01	87, 84, 96, 97, 91	5	91	6.2	84-97
		0.1	75, 81, 83, 80, 84	5	81	4.3	75-84
		Overall	-	10	86	8.2	75-97
	Clay soil m/z 329→223	0.01	77, 76, 80, 79, 82	5	79	2.8	76-82
		0.1	77, 77, 77, 79, 75	5	77	1.8	75-79
		Overall	-	10	78	2.4	75-82
	Clay soil m/z 329→241	0.01	88, 92, 98, 94, 102	5	95	5.7	88-102
		0.1	74, 73, 77, 79, 75	5	75	3.2	73-79
		Overall	-	10	85	12.8	73-102

Soil sample characteristics

Sandy soil sample

parameter	**Found value**
Soil type	Sand
pH	5.8
Dry matter (%)	90.4
Total nitrogen (% w/w)	0.21
Cation exchange capacity (meq/100g)	6.3
Organic carbon content (% w/w)	2.6

Clay soil sample

parameter	Found value
Soil type	Clay
pH	6.0
Dry matter (%)	75.0
Total nitrogen (% w/w)	0.64
Cation exchange capacity (meq/100g)	35.7
Organic carbon content (% w/w)	5.6

Repeatability

At each fortification level and overall, the mean recoveries for GA4/7 were in the range 70 to 120% for each ion transition and are therefore considered acceptable.

Limit of quantification

The LOQ for GA4/7 was 0.01 mg/kg in the soil types tested, equivalent to the lowest fortification level validated.

Conclusion

The method was successfully validated for the determination of GA4/7 in soil matrices in accordance with SANCO/825/00 rev.8.1.

RMS comment:
The results from the method validation have in detail fulfilled all requirements in according to SANCO/825/00 rev. 8.1. and the method is suitable for monitoring of GA4/7 in soil. No further data required.

Residues in water

During peer review under Directive 91/414/EEC, analytical methods for the determination of GA4/7 in drinking and surface water were evaluated. Summaries of the methods and validation data are provided in the Draft Assessment Report (Annex B: Section B.5, July 2006) and also below.

Previous evaluation:	This study was evaluated in the DAR (B.5.3.2).
Data point addressed:	KCA 4.2/04
Author(s) (year):	de Wolf J.M. (2001)
Title:	Validation of the Determination of Gibberellin A4 and A7 in Drinking Water Using LC-MS
Laboratory report / project number:	V3044
Testing facility:	TNO Nutrition and Food Research Institute
Published:	No
Test guideline used:	None stated, but meets requirements of SANCO/825/00 rev. 8.1
Deviations:	None

GLP:	Yes
EU agreed endpoint:	Yes

Materials and methods
Method: Verification of active substance concentration, contained in report V3044
Test material: Gibberellin A4/A7
Lot/Batch No.: 93-041-CD
Purity: 68.9 % w/w (GA4), 21.3 % w/w (GA7)
Stability of test compound: Not reported

Principle of the method

Water samples were extracted using a C18 polar solid phase extraction cartridge. Gibberellins GA_4A_7 determination was by LC/MS/MS. (DAR, Annex B: Section B.5.3.2.1, July 2006).

Linearity

Good linearity was observed in the range of 0.05 to 4.3 µ/L for gibberellins GA_4A_7 (r^2 = 0.9997). (DAR, Annex B: Section B.5.2.1, July 2006). Representative calibration curve plots were provided.

Specificity

Analysis of control water showed no significant interference at the retention time of gibberellins GA_4A_7. The baseline interference was less than 30 % of the LOQ (0.11 µg/L). The LC/MS system used was sufficiently selective and sensitive to be considered inherently self-confirmatory. Therefore an additional confirmation method is not required. (DAR, Annex B: Section B.5.3.2.1, July 2006).

Recovery findings

A summary of the recovery results for GA4/7 in drinking water is presented in the table below.

Table B.5.2.2-03: Recovery results of GA4/7 in drinking water (adapted from DAR, Annex B: Section B.5.2.1, July 2006).

Analyte	**Fortification Level (µg/L)**	**Number of Analysis (n)**	**Mean Recovery (%)**	**RSD (%)**	**Recovery Range (%)**
GA4/7	0.11	5	89	4.3	83-93
	1.12	5	78	4.9	74-82
	Overall	10	83	7.8	74-93

Limit of quantification

The limit of quantification, defined as the lowest concentration at which acceptable recovery is obtained, is 0.11 µg/L for drinking water. (DAR, Annex B: Section B.5.3.2.1, July 2006).

Repeatability

The relative standard deviations measured with respect to recoveries following fortification in the range 0.11 µg/L to 1.12 µg/L were 4.3 and 4.9 %. The values obtained are indicative of the method having satisfactory repeatability (RSD <20 %). (DAR, Annex B: Section B.5.3.2.1, July 2006).

Conclusion

Please see KCA 4.2/05 below.

Previous evaluation:	This study was evaluated in the DAR (B.5.3.2) and has been considered by EFSA.
Data point addressed:	KCA 4.2/05
Author(s) (year):	Kruplak J.F. (2004)
Title:	Validation of a Method for the Determination of Gibberellin A4 and A7 (GA4/GA7) in Surface Water
Laboratory report / project number:	ADC 1880-1
Testing facility:	Analytical Development Corporation (ADC)
Published:	No
Test guideline used:	EPA Residue Chemistry Test Guidelines OPPTS 960.1340 SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
EU agreed endpoint:	Yes

Materials and methods
Method: Based on TNO Report V 3044, but used LC/MS/MS for quantitation of residues
Test material: Gibberellin A4/A7
Lot/Batch No.: 21-973-CD
Purity: 90.6 % w/w (GA4/7): 60.4 % w/w (GA4), 30.0 % w/w (GA7)
Stability of test compound: Dilutions of GA4/7 in methanol were stable for at least four months when stored refrigerated.

Principle of the method

Gibberellins GA4A7 in surface water is concentrated by C18 solid-phase extraction, eluted with methanol and analysed by LC/MS/MS. (DAR, Annex B: Section B.5.3.2.1, July 2006).

Recovery findings

A summary of the recovery results for GA4/7 in surface water is presented in the table below.

Table B.5.2.2-04: Recovery results of GA4/7 in surface water (adapted from DAR, Annex B: Section B.5.2.1, July 2006).

Analyte	Fortification Level (µg/L)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
GA4/7	10	5	84	6.4	78-90
	100	5	99	6.9	89-106
	Overall	10	92	11	78-106

Linearity

Good linearity was observed in the range of 10 to 100 µg/L for gibberellins GA4 and GA7 (r^2= 0.9986 and 0.9995, respectively). (DAR, Annex B: Section B.5.3.2.1, July 2006). Representative calibration curve plots, equations and coefficients were provided.

Specificity

Analysis of control water showed no significant interference at the retention time of gibberellins GA_4A_7. The baseline interference was less than 30 % of the LOQ (10 µg/L). The LC/MS/MS system used was sufficiently selective and sensitive to be considered inherently self- confirmatory. Therefore, an additional confirmation method is not required. (DAR, Annex B: Section B.5.3.2.1, July 2006). Representative mass spectra were provided.

Limit of quantification

The limit of quantification, defined as the lowest concentration at which an acceptable recovery is obtained, is 10 µg/L for surface water. (DAR, Annex B: Section B.5.3.2.1, July 2006).

Repeatability

*The relative standard deviations measured with respect to recoveries following fortification in the range 10 µg/L to 100 µg/L were 6.4 and 6.9 %. The values obtained are indicative of the method having satisfactory repeatability (RSD <20 %). (*DAR, Annex B: Section B.5.3.2.1, July 2006).

Conclusion

The DAR states:

"Monitoring methods for gibberellins GA4A7 in drinking and surface water were successfully evaluated and meet the EU criteria with respect to specificity, accuracy and precision according to the requirements of EU Commission Directive 96/46/EC and guidance document SANCO/825/00."

The methods are therefore considered acceptable in support of active substance renewal for GA4/7.

RMS comment:
The results from the method validation have in detail fulfilled all requirements in according to SANCO/825/00 rev. 8.1. and the method is suitable for monitoring of GA4/7 in drinking and surface water. No further data required.

An independent laboratory validation is required to validate the method in drinking water, in accordance with the requirements of Commission Regulation (EU) No 283/2013, and is summarised below.

Previous evaluation:	None
Data point addressed:	KCA 4.2/06
Author(s) (year):	Warnick J. (2017)
Title:	Independent Laboratory Validation (ILV) of the Determination of Gibberellin A4 and A7 (GA4GA7) in Drinking Water using Liquid Chromatography-Tandem Mass Spectrometry
Laboratory report / project number:	605G1561
Testing facility:	EPL Bio Analytical Services (EPL)
Published:	No
Test guideline used:	OCSPP 850.6100 SANCO/825/00 rev. 8.1
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: Based on TNO Report V 3044, but used LC/MS/MS for quantitation of residues
Test material: Gibberellin A4 + A7
Lot/Batch No.: 21-973-CD
Purity: 59.80 % w/w (GA4), 29.85 % w/w (GA7)
Stability of test compound: Not reported

Principle of the method

Samples of untreated drinking water were fortified with GA4/7 in acetonitrile:0.04% phosphoric acid (35:65, v/v) at 0.10 and 0.91 µg/L. Formic acid (0.1%, w/v) was added. Samples were then extracted using a SPE cartridge. Fortified solutions were applied to the cartridge with HPLC water. The cartridge was dried under vacuum and gibberellins (GA4 and GA7) eluted with acetonitrile. The eluent was evaporated to approx. 0.25 mL (by 40 °C heat and nitrogen stream) and made to 1 mL volume with acetonitrile:0.04% phosphoric acid (35:65, v/v) mixture. Solutions were analysed by LC-MS/MS. Ion transitions monitored were 330.7→287.1 m/*z* (quantification) and 330.7→243.2 m/*z* (confirmation) for GA4 and 328.8→223.0 m/*z* (quantification) and 328.8→211.0 m/*z* (confirmation) for GA7.

The following deviations to the method were noted:

- The column and SPE cartridge of analytical method were changed (originals are no longer manufactured);
- The mobile phase was changed (from acetonitrile:Milli Q water:formic acid, 30:69.9:0.1 v/v/v to 0.1%formic acid in HPLC water:0.1%formic acid in acetonitrile 95:5 to 5:95 v/v);
- A gradient solvent system was used (vs. isocratic) to better resolve analytes from co-eluting peaks
- A confirmatory transition for GA7 was added (328.8→211.0 m/z);

- The calibration curve lowest standard was decreased (from 26 to 2 µg/L) to meet the requirements of the detector response.

The changes were necessary and/or result in improvement of the method improvement, therefore the impact to the data is considered positive.

Linearity

Matrix effects were assessed by comparing injections of matrix-matched standards with injections of a solvent standard, both fortified at the LOQ. Effects were not considered significant (<20%) for GA4 and GA7 and so calibration solutions were prepared using solvent.

The linearity of the detector was confirmed using single determinations of nine calibration solutions of GA4 and GA7 in methanol:acetonitrile:0.04% phosphoric acid mixtures prepared over the concentration range 2.0 to 4048 µg/L. This range is equivalent to 0.004 to 8.1 µg/L in drinking water or, alternatively, 1.2 to 2421 µg/L for GA4 and 0.6 to 1208 µg/L for GA7 (correcting for test substance purities). Typical calibration curves were provided in the report for both analytes and ion transitions. Results are given in table B.2.2-05 below.

Table B.2.2-05: Linearity data

Analyte	Ion Transition (m/z)	r^2	slope	intercept
GA4	330.7→287.1	0.99862	4089.96492	4363.44077
	330.7→243.2	0.99898	6354.94771	2925.49414
GA7	328.8→223.0	0.99908	48471	3242.74934
	328.8→211.0	0.99966	4408.91386	459.69962

Specificity

Representative chromatograms of unfortified control samples, reagent blanks, calibration standards and samples fortified at the LOQ and ca. 10 × LOQ were provided for GA4 and GA7 analysis. Retention times of chromatographic peaks observed in fortified extracts matched those present in calibration standards, confirming analyte identities. No interfering peaks were observed in reagent blanks at the retention times of GA4 or GA7 however an interfering peak corresponding to the GA7 confirmatory transition was observed in the unfortified control samples. The peak was however minor (30% of the LOQ) and so the method was still considered specific. LC-MS/MS as a detection technique is considered to be highly specific. The method included four MS/MS transitions (two quantification and two confirmation), each of which were validated.

Accuracy

Samples of drinking water were fortified with GA4/7 at 0.1 µg/L and 0.91 µg/L. This is equivalent to 0.06 µg/L (GA4) and 0.03 µg/L (GA7), 0.545 µg/L (GA4) and 0.272 µg/L (GA7) respectively, after correcting for GA4 and GA7 purities in the test substance (GA4 – 59.80%, GA7 – 29.85%). Five samples were prepared for each fortification level and run alongside two control samples. The relative standard deviations (%RSD) for GA4/7 recoveries were <20% for each fortification level therefore are considered acceptable.

Recovery findings

A summary of the recovery results for GA4/7 in drinking water are presented in the table below.

Table B.2.2-06: Recovery results for determination of GA4/7 in drinking water

Analyte	Transition (m/z)	Fortification Level of Analyte[a] (µg/L)	Individual Recoveries[b] (%)	Number of Analysis (n)	Mean Recovery[c] (%)	RSD[c] (%)	Recovery Range (%)
GA4	330.7→287.1 (quantification)	0.06	110, 107, 107, 105, 113	5	108	2.9	105-113
		0.545	88, 95, 98, 96, 95	5	94	4.0	88-98
	330.7→243.2 (confirmation)	0.06	111, 108, 106, 108, 116	5	110	3.6	106-116
		0.545	87, 96, 100, 96, 92	5	94	5.2	87-100
GA7	328.8→223.0 (quantification)	0.03	100, 97, 99, 101, 102	5	100	1.9	97-102
		0.272	85, 93, 95, 89, 89	5	90	4.3	85-95
	328.8→211.0 (confirmation)	0.03	104, 104, 103, 104, 119	5	107	6.4	103-119
		0.272	88, 94, 99, 91, 90	5	92	4.6	88-99
GA4+GA7[d]	Quantification	0.09	108, 104, 105, 104, 110	5	106	2.5	104-110
		0.817	87, 94, 97, 94, 93	5	93	4.0	87-97
	Confirmation	0.09	110, 107, 105, 108, 118	5	110	4.6	105-118
		0.817	88, 95, 100, 94, 92	5	94	4.7	88-100

[a]Values re-calculated based on fortification solutions of 0.1 and 0.91 µg test substance/L and purities of GA4 and GA7 in the test substance (59.80 and 29.85%, respectively); [b]Values have been rounded to integers; [c]Values re-calculated based on rounded figures; [d]Individual recoveries calculation: $\frac{Amount\ found\ (GA4+GA7)\ in\ \mu g/L}{Fortified\ level\ (GA4+GA7)\ in\ \mu g/L} \times 100$

Repeatability

At each fortification level, the mean recoveries for GA4/7 were in the range 70 to 120% for each ion transition and are therefore considered acceptable.

Limit of quantification

The LOQ in the study for determination of GA4/7 in drinking water was 0.1 µg/L, equivalent to the lowest validated fortification level. This is equivalent to 0.06 µg/L for GA4 and 0.03 µg/L for GA7 after correcting for purities in the test substance.

Conclusion

The analytical method for the determination of GA4/7 in drinking water has been successfully validated by an independent laboratory (with modifications) in accordance with SANCO/825/00 rev.8.1.

RMS comment:
The results from the independent laboratory method validation have in detail fulfilled all requirements in according to SANCO/825/00 rev. 8.1. and confirmed that method the method de Wolf J.M. (2001) is suitable for monitoring of GA4/7 in drinking water. No further data required.

B.5.2.3. Methods for the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

The DAR indicates that GA4/7 has a vapour pressure of *ca.* 1 x 10^{-5} Pa m^3 mol^{-1} at 25 °C and as such can be regarded as a non-volatile substance. GA4/7 is also presented as a low-risk active substance that is a naturally occurring substance, with detected levels found to be close to background levels, as well as not being classified according to GHS. Monitoring methods in air are not generally required for naturally occurring, non-toxic substances and substances not classified as T, T+, Xi or Xn (SANCO/825/00 rev 8.1). Therefore a method for the determination of GA4/7 in air is not required.

B.5.2.4. Methods for the analysis in body fluids and tissues for active substances and relevant metabolites

A monitoring method for the analysis of gibberellin (GA4/7) in body fluids and tissues is not required as the substance is non-toxic (EFSA Journal 2012; 10(1); 2502). In further support of this, GA4/7 is presented as a low-risk active substance that is a naturally occurring substance with detected levels found to be close to background.

Regulation (EC) No. 1107/2009 concerning the placing of plant protection products on the market describes the criteria for approval of active substances as low-risk (Section 5, Annex II). This criterion has been amended in the guidance document SANTE/11953/2015 rev.5 (in draft, 22 March 2016). In accordance with the criteria outlined in SANTE/11953/2015 rev.5, GA4/7 is considered low risk as the representative uses do not require specific risk mitigation measures (i.e. measures deduced as a result of a risk assessment that must be applied to ensure safe use and are not a generalised precautionary approach). GA4/7 is not explosive nor classified as acutely toxic (Cat. 1, 2 or 3), a skin corrosive (Cat. 1A, 1B or 1C), a skin sensitiser (Cat. 1), mutagenic (Cat. 1A, 1B or 2), carcinogenic (Cat. 1A, 1B or 2), toxic to reproduction (Cat. 1A, 1B or 2), a Specific Target Organ Toxicant (Cat. 1 or 2) or causes serious damage to the eyes (Cat. 1). Furthermore, GA4/7 is not expected to display neurotoxic or immunotoxic effects and there is no evidence for GA4/7 as an endocrine disrupter. GA4/7 is neither persistent in soil/water-sediment nor expected to be bio-accumulative or leach to groundwater through soil. GA4/7 is also not classified as toxic to aquatic life (Cat. 1) and risk mitigation measures were not required to protect the environment based on the outcome of the ecotoxicological risk assessment.

B.5.3. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 4.1.1/01	Parsons, A.H.	2006	Validation of G C Laboratories Ltd. Analytical Method M564 "HPLC Determination of Gibberellins GA4 and GA7 in Technical Material and Formulations" for Gibberellin GA4 in GA4 Technical Material and the 'Novagib' Formulation G C Laboratories Ltd., 6 Fen End, Astwick Road, Stotfold, Hitchin, SG5 4BA GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd.	-
KCA 4.1.1/02	Knowles, R.J.	2009	Validation of G C Laboratories Ltd. Analytical Method M564 "HPLC Determination of Gibberellins GA4 and GA7 in Technical Materials and Formulations" Validation for GA7 in GA4/GA7 Technical Gibberellin G C Laboratories Ltd., 6 Fen End, Astwick Road, Stotfold, Hitchin, SG5	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd.	-

			4BA, UK GLP Unpublished					
KCA 4.1.1/03	Comb, T.	2018	GA4/7: Method Validation AgroChemex Environmental Ltd., Dead Lane, Essex, CO11 2NF, UK GLP Unpublished N.B. This study has been added to the confidential Doc K	N	Y	New study submitted for the purpose of renewal	Valent BioSciences	-
KCA 4.1.2/01	Traub, M.	2014	Gibberellins GA4, GA7: Aerobic Degradation in Four European Soils Report No. S14-01454 Eurofins Agroscience Services, Germany GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Globachem	-
KCA 4.1.2/02	Harrison, C.	2005a	VBC 30011: Residue Levels in Apples and Pears from Trials Carried Out in Northern France, Southern France, Italy and Northern Spain During 2002 Report No. AF/6256/VB Agrisearch UK, Ltd GLP Unpublished	N	Y	-	Valent BioSciences	In DAR: IIA 6.3.1/01
KCA 4.1.2/03	Harrison, C.	2005b	VBC 30011: Residue Levels in Apples and Pears from Trials Carried Out in Northern	N	Y	-	Valent BioSciences	In DAR: IIA 6.3.1/02

			France, Southern France, Italy and Northern Spain During 2003 Report No. AF/6989/VB Agrisearch UK, Ltd GLP Unpublished					
KCA 4.1.2/04	Harrison, C.	2010	To Determine the Stability of gibberellin A4 (GA4) and Gibberellin A7 (GA7) in Pome Fruit Apple Specimens Following Storage at ca -18°C for 0, 1, 3, 12, 18 and 30 Months Report No. AD/6258/VB EUROFINS Agroscience services, UK GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Valent BioSciences	-
KCA 4.1.2/05	Stephan, M., Bangerth, F. and Schneider, G.	1999	Quantification of Endogenous Gibberellins in Exudates from Fruit From *Malus Domestica* *J. Plant Growth Regul.*, 1999, **28**: 55. Non GLP Published	N	N	-	Published paper	In DAR: IIA 6.2.1/02
KCA 4.1.2/06	Zhang, C., Tateishi, N. and Tananbe, K.	2010	Pollen Density on the Stigma Affects Endogenous Gibberellin Metabolism, Seed and Fruit Set, and Fruit Quality in *Pyrus Pyrifolia.* *J. Exp. Bot.*,	N	N	-	Published paper	-

			2010, **61**, 15, 4291. Non GLP Published					
KCA 4.1.2/07	Jean-Baptiste, C.	2011	Frozen Storage Stability of Residues of Gibberellic Acid, Gibberellin A4 and Gibberellin A7 in Pears Study No. A9206 ANADIAG, 16, rue Ampére, 67500 Haguenau, France GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Globachem	-
KCA 4.1.2/08	Jean-Baptiste, C.	2009	Validation of the Analytical Methods for the Determination of Gibberellic Acid, Gibberellin A4 and Gibberellin A7 Residues in Pears Study No. R A9006 ANADIAG, 16, rue Ampére, 67500 Haguenau, France GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Globachem	-
KCA 4.1.2/09	Juckeland, D.	2014	Toxicity of Gibberellins (GA4/7) Technical to *Daphnia Magna* in a 21-Day Semi-Static Reproduction Test Report No. 14 10 48 073 W BioChem agrar, Gerichshain, Germany	N	Y	New study submitted for the purpose of renewal	Globachem	-

			GLP Unpublished					
KCA 4.1.2/10	[redacted]	2016	Gibberellic Acid (GA3): An Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales Promelas*) Report No. [redacted] GLP Unpublished	Y	Y	New study submitted for the purpose of renewal	Valent BioSciences	-
KCA 4.1.2/11	Taylor, K.	2017	Gibberellins A4A7: Honey Bee (Apis Mellifera) Larval Toxicity Test, Single Exposure Report No. CR15QN Envigo CRS limited, Huntingdon, Cambridgeshire, UK, PE28 4HS GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Valent BioSciences	-
KCA 4.1.2/12	Gray, J.	2017	Gibberellins A4A7: Honey Bees (Apis Mellifera L.) Chronic Oral Toxicity Test 10 Day Feeding in the Laboratory Report No. FR30QH Envigo CRS limited, Huntingdon, Cambridgeshire, UK, PE28 4HS GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Valent BioSciences	-

KCA 4.1.2/13	Mantilacci, S.	2017	Toxicity Evaluation of Test Item Gibberellins GA4/7 Technical on Navicula Pelliculosa in a Growth Inhibition Limit Test and Validation of the Analytical Method Report No. BT264/17 BioTecnologie B.T. Srl, 06059 Todi (PG), Italy GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd.	-
KCA 4.1.2/14	Stead, A.	2018	Gibberellins A4A7: GLP Seedling Emergence and Seedling Growth Test Terrestrial Non-Target Plants (Based on OECD Guideline 208) - 2017 Report No. STC/17/E1126 Stockbridge Technology Centre Ltd, Norht Yorkshire, UK GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Valent BioSciences	-
KCA 4.1.2/15	Turner, B.	2018	Analysis of Gibberellins A4A7 Spray Solution Report No. STC/17/E1126 Stockbridge Technology Centre Ltd, Norht Yorkshire, UK GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Valent BioSciences	-

KCA 4.2/01	Gian Carlo, G.	1995	Determination of Gibberellins A4-A7 Residues on Apple and Pear Report No. NEOT/GLP/LN52A-95 Neotron S.r.l., Via Puccini 185, 41058 Vignola GLP Unpublished	N	Y	-	Valent BioSciences	In DAR: IIA 4.3.1/01
KCA 4.2/02	Mol, J.G.J.	2001	Site Validation of the GA4/GA7 Residue Method in Apple and Pear Report No. V99.1181 TNO Nutrition and Food Research, The Netherlands GLP Unpublished	N	Y	-	Valent BioSciences	In DAR: IIA 4.3.1/02
KCA 4.2/03	Brewin, S.	2017	GA4A7: Validation of Methodology for the Determination of Residues of in Soil Study No. YR93VB Envigo CRS Limited, Eye, Suffolk, IP23 7PX, UK GLP Unpublished	N	Y	-	Valent BioSciences	-
KCA 4.2/04	de Wolf, J.M.	2001	Validation of the Determination of Gibberellin A4 and A7 (GA4/GA7) in Drinking Water Using LC-MS Report No. V3044 TNO Nutrition and Food	N	Y	-	Valent BioSciences	In DAR: IIA 4.5/01

			Research, Utrechtseweg 48, The Netherlands GLP Unpublished					
KCA 4.2/05	Kruplak, J.F.	2004	Validation of a Method for the Determination of Gibberellin A4 and A7 (GA4/GA7) in Surface Water Report No. ADC 1880-1 Analytical Development Corporation (ADC), Colorado Springs, CO 80907 GLP Unpublished	N	Y	-	Valent BioSciences	In DAR: IIA 4.5/02
KCA 4.2/06	Warnick, J.	2017	Independent Laboratory Validation (ILV) of the Determination of Gibberellin A4 and A7 (GA4GA7) in Drinking Water Using Liquid Chromatography-Tandem Mass Spectrometry Report No. 605G1561 EPL Bio Analytical Services (EPL), 9095 W. Harristown Blvd., IL 62551, USA GLP Unpublished	N	Y	New study for the purposes of renewal	GA4/7 Task Force	-