REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: ADM.09050.H.1.A

Product name(s): **STEMPER**

Chemical active substance:

Trinexapac-ethyl, 175 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: **ADAMA**

Submission date: May 2022

Evaluation date: March 2023

Version history

When	What
~~January 2021~~	~~dRR version 1 submitted by applicant~~
May 2022	dRR version 1 submitted by applicant
March 2023	Initial RR

DATA PROTECTION CLAIM

Under Article 59, Regulation 1107/2009/EC, on behalf of the Sponsor Company the applicant claims data protection for these studies. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A

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5 Analytical methods

This document summarizes the analytical methods on the plant protection product ADM.09050.H.1.A (former code AG-T3-175 EC1), an emulsifiable concentrate [Code: EC] containing 175 g/L trinexapac-ethyl for use in cereals (spring cereals, winter cereals) and grassland in the Central Zone under article 33 of the Regulation 1107/2009.
This application follows the data requirements for the active substance laid down in Regulation (EC) No. 283/2013 for the active substance trinexapac-ethyl, and Regulation (EC) No. 284/2013 for the plant protection product ADM.09050.H.1.A.

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance and relevant impurities in the plant protection product.
Noticed data gaps are:

- None

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

It should be noted that the applicant's dRR was not rewritten by the ZRMS and the RR resulted from the evaluation was prepared by an insertion into the dRR by zRMS comments/corrections on the grey background.

The applicant, as the TaskForce member has an access to the adequate analytical methods.
The residue definition for monitoring in plant and animal matrices was defined as sum of trinexapac and its salts, expressed as trinexapac. The quick, easy, cheap, effective, rugged and safe (QuEChERS) multi-residue enforcement method and also single residue methods with liquid chromatography with tandem mass spectrometry (LC–MS/MS) can be used for the determination of residues of trinexapac in food and feed of plant and animal origin with a limit of quantification (LOQ) of 0.01 mg/kg in each commodity group and in each animal matrix (EFSA Journal 2018;16(4):5229).
Moreover in the context of the authorization request the applicant submitted several acceptable analytical methods for the determination of residues in support of ecotoxicological studies (see Appendix 2)
Noticed data gaps are: none

Commodity/crop	**Supported/ Not supported**
Cereals (spring cereals, winter cereals) Grassland	**Supported**

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

The plant protection product ADM.09050.H.1.A has not been reviewed at EU level as a consequence of the review of trinexapac-ethyl.
An overview on the acceptable methods for analysis of trinexapac-ethyl in plant protection product ADM.09050.H.1.A is provided as follows:

Comments of zRMS:	Accepted. This method meets the requirements and may be applied for analysing trinexapac in the PPP.

Reference:	KCP 5.1.1/01 (also filed under KCP 2.1/01)
Report	Determination of Storage Stability and Phys-Chem Properties in AG-T3-175 EC1 (Trinexapac-ethyl 175 EC) Stored at 54°C for 14 Days and at 0°C for 7 Days Edelson T., 2016 Report no. F16-02/4, Sponsor study number 90019616
Guideline(s):	Yes, European Commission, Residues: Guidance Document, SANCO/3029/99 rev. 4, July 11, 2000 - Working Document Deviations: None
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A method was validated for the determination of the active substance trinexapac-ethyl in the formulation trinexapac-ethyl 175 EC (ADM.09050.H.1.A, formerly AG-T3-175 EC1). A defined amount of trinexapac-ethyl 175 EC was dissolved in acetonitrile. The analysis was performed by HPLC with UV detection using an external standard technique.

Test Material
Trinexapac-ethyl 175 g/L (ADM.09050.H.1.A, formerly AG-T3-175 EC1), Batch: 1601240
Trinexapac-ethyl, analytical standard, Batch: 182-2797, Purity: 99.14%, Source: ADAMA Agan Ltd. Northern Industrial Zone, Ha'Ashlag St. Ashdod 7752009, Israel

Sample solutions
Duplicate preparations of sample solutions (two separate weighing) were made. About 300 mg Trinexapac-ethyl 175 g/l EC formulation (representing ~50 mg active substance) were weighed and dissolved in 100 ml acetonitrile by sonicating for about 4 minutes. Five ml of sample solution were diluted to 50 ml with acetonitrile.

HPLC-UV Conditions:

Column:	Zorbax RX C8, 5µm, 250 x 4.6 mm ID

Mobile phase:	Acetonitrile : water containing 0.1% phosphoric acid (45:55; v/v)
Injection Volume:	10 µL
Flow:	1.8 mL/min
Temperature:	Ambient
Detector:	280 nm
Retention time:	Trinexapac-ethyl at about 6.7 min.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance Trinexapac-ethyl in plant protection product ADM.09050.H.1.A

	Trinexapac-ethyl
Author(s), year	Edelson, T., 2016
Principle of method	HPLC with UV detection using an external standard technique
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Six calibration solutions in the range of 25 – 75 mg/L (equal to 7.94 – 23.88% or 79.16 – 238.08 g/L) spanning about 45-135% of the nominal concentration of trinexapac-ethyl in the formulation were prepared in acetonitrile. Y = 6.53571e-005x – 0.119942, r^2 = 0.99999 Calibration data are presented in the report.
Precision – Repeatability Mean n = 10 (%RSD)	Ten separate weightings of one lot of Trinexapac-ethyl 175 EC, injected in triplicate. Average content of 17.85% with a RSD of 0.23% was obtained. Based on the modified Horwitz equation (%RSD <2(1 – 0.5 log C) · 0.67; For C = 0.1785, the proposed acceptable % RSD is 1.74%, which is larger than the % RSD obtained in the precision measurements (0.23%). So, Horrat is 0.13.
Accuracy n = 3 (% Recovery)	Spiked placebos were prepared to represent approximately 80%, 100% and 120% of the levels of trinexapac-ethyl expected in the formulated product. Recovery values were in the range of 100.41-100.75% for trinexapac-ethyl. The mean percent recovery was 100.56%.
Interference/ Specificity	Specificity was established by HPLC-MS. The similarity of the mass spectra from the standard and sample solutions confirms the identity of the active substance and indicates that the peak of the active substance is free from coeluents. Furthermore, an excipient was analyzed using the same HPLC-UV conditions as for the standard and sample solutions. The resulting chromatograms demonstrate that none of the excipients interferes with the peaks of the active substance
Comment	Acceptable

Conclusion

The validation data demonstrate that the analytical method is suitable for the specific, accurate and precise determination of trinexapac-ethyl in trinexapac-ethyl 175 EC1 (ADM.09050.H.1.A).

5.2.1.2 Description of analytical methods for the determination of relevant impurities

(KCP 5.1.1)

A study for determination of relevant impurities is still ongoing and will be submitted.

RMS comment:
Since no new requirements for trinexapac are in force due to the renewal is still pending. No relevant impurities are specified accordingly to the reg (EU) 540/2011. Summarising, the method is not required yet. Nevertheless, the renewal process is still pending.

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

None of the formulants or their constituents of the plant protection product in trinexapac-ethyl 175 EC1 (ADM.09050.H.1.A) are considered by the applicant to represent compounds of particular toxicological, ecotoxicological or environmental concern. The submission of analytical methods for such is therefore not considered to be required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method is not available.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

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

Table 5.2-2: Validated methods for the generation of pre-authorization data for trinexapac-ethyl in plant and animal products

Studies indicated as **new data** are currently under EU evaluation (reviewed by Lithuania, 2018 and EFSA, 2018).

Component of residue definition for plant matrices (EFSA, 2018): **- Trinexapac, free and conjugated (cereal grain) (provisional)** **- Trinexapac, free and conjugated plus CGA300405 (cereal fodder items/grass) (expressed as trinexapac or separate, pending its toxicological relevance) (provisional)** **Processed products: open** **Component of residue definition for animal matrices (EFSA, 2018):** **- Poultry: trinexapac** **- Ruminant: trinexapac + metabolite CGA 113745, expressed as trinexapac (provisional)**				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat grain (1) wheat straw Wheat and barley grain (1), straw (Residues)	Primary method REM 137.01 Trinexapac-ethyl (CGA163935)	0.04 mg/kg 0.04 mg/kg	HPLC-UV	Forrer, 1989 Sack, 1994 *EU agreed (DAR Oct. 2003, Vol 3 B.5.2* *EFSA (2005), 57,1-70)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-UV)	
Wheat and barley grain (1) wheat and barley straw, rape seed (3) (Residues)	Primary method REM 137.02 Trinexapac-acid (CGA179500)	0.02 mg/kg	HPLC-UV	Forrer, 1991a Sack, 1999 *EU agreed (DAR Oct. 2003, Vol 3 B.5.2* *EFSA (2005), 57,1-70)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-UV)	
Meat, liver, kidney eggs, milk Milk (ILV) meat (ILV) (Residues)	Primary method REM 137.12 Trinexapac-acid (CGA179500)	0.02 mg/kg 0.01 mg/kg 0.01 mg/kg 0.02 mg/kg	HPLC-UV	REM 137.12 xxxxx, 1995a xxxxx, 2001 (ILV) *EU agreed (DAR October, 2003, Vol 3, B5.2*)
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC-UV)	
Wheat grain (1) wheat forage wheat straw (Residues)	Primary method GRM020.01A (modified 110-01) Trinexapac acid (CGA179500) free and conjugated forms	0.01 mg/kg	HPLC-MS/MS	Method and validation Lin, 2008 ILV: Thomas, 2010 **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*

Component of residue definition for plant matrices (EFSA, 2018): **- Trinexapac, free and conjugated (cereal grain) (provisional)** **- Trinexapac, free and conjugated plus CGA300405 (cereal fodder items/grass) (expressed as trinexapac or separate, pending its toxicological relevance) (provisional)** **Processed products: open** **Component of residue definition for animal matrices (EFSA, 2018):** **- Poultry: trinexapac** **- Ruminant: trinexapac + metabolite CGA 113745, expressed as trinexapac (provisional)**				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	
Barley grain (1), sunflower seed (3), barley hay and straw Wheat: whole plant, grain, straw & barley: pot barley, pearl barley, flour, bran, brewing malt, malt sprouts, brewers grain (dried), brewer's yeast and beer; wheat processed commodities (Residues)	Primary method GRM020.05A GRM020.05A supercedes REM 137.13 Trinexapac acid (CGA179500)	0.01 mg/kg	HPLC-MS/MS	Hargreaves, 2008 Validation: Mayer, 2008, (amended 2016) **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	
Cereal grain (1) cereal straw dry broad beans (1) oilseed rape, seed (3) cereal grain (1) cereal straw Barley: pot barley, pearl barley, flour, bran, brewing malt, malt sprouts, brewers grain (dried), brewer's yeast and beer; wheat processed commodities (Residues)	Primary method GRM020.009A (Finalised as Method GRM020.09A) Trinexapac acid (CGA179500) free and conjugated forms	0.01 mg/kg 0.05 mg/kg 0.01 mg/kg 0.05 mg/kg 0.02 mg/kg 0.05 mg/kg	HPLC-MS/MS	Validation of GRM020.009A: Tsui, 2015 Validation of GRM020.09A: Braid & Tsui, 2016 GRM020.09B[1] Braid & Tsui, 2016 GRM020.16A[2] Braid & Tsui, 2016 Validation of GRM020.09B and GRM020.16A Tsui, 2015 **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	

Component of residue definition for plant matrices (EFSA, 2018): **- Trinexapac, free and conjugated (cereal grain) (provisional)** **- Trinexapac, free and conjugated plus CGA300405 (cereal fodder items/grass) (expressed as trinexapac or separate, pending its toxicological relevance) (provisional)** **Processed products: open** **Component of residue definition for animal matrices (EFSA, 2018):** **- Poultry: trinexapac** **- Ruminant: trinexapac + metabolite CGA 113745, expressed as trinexapac (provisional)**				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Muscle, fat, kidney, liver (bovine) and eggs (chicken) milk (Residues)	Primary method AGR/MOA/Trin-06 (based on Ciba-method REM 137.08) Trinexapac acid (CGA179500	0.01 mg/kg 0.005 mg/kg	HPLC-MS/MS	xxxx 2008 CHE/TRIN/08003 **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	
Wheat and barley grain (1) (Residues)	Primary method REM 137.08 Trinexapac-acid (CGA179500)	-	HPLC-UV	Hauck, 1993 *EU agreed (DAR Oct. 2003, Vol 3 B.5.2* *EFSA (2005), 57,1-70)*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- UV)	
Brewing and baking matrices: grain (1) beer bread bran flour (Residues)	Primary method GRM020.15A Cyclopropane carboxylic acid (CPCA) (CGA224439	0.01 mg/kg	HPLC-UV	Method Watson, 2016 Validation Watson, 2016a **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- UV)	
Processed food - beer, bread, bran, wheat grain and flour (Residues)	Primary method GRM020.013A* CGA313458	0.01 mg/kg	HPLC-UV	Braid, Langridge, 2016 **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- UV)	

Component of residue definition for plant matrices (EFSA, 2018): **- Trinexapac, free and conjugated (cereal grain) (provisional)** **- Trinexapac, free and conjugated plus CGA300405 (cereal fodder items/grass) (expressed as trinexapac or separate, pending its toxicological relevance) (provisional)** **Processed products: open** **Component of residue definition for animal matrices (EFSA, 2018):** **- Poultry: trinexapac** **- Ruminant: trinexapac + metabolite CGA 113745, expressed as trinexapac (provisional)**				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Processed commodities - beer, bread (Residues)	Primary method GRM020.014A** CGA113745	0.01 mg/kg	HPLC-UV	Braid, Brookes, Langridge, 2016 Validation Langridge, 2016 **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- UV)	
Feed, body fluids (Toxicology)	No additional data.			
	Confirmatory (if required)	--	--	--

The numbers in brackets according to GD SANCO 825/00. Commodities and four matrix groups: 1) dry commodities (high protein/high starch content) and commodities with high water content (2); high oil content (3), high acid content (4).

* Method REM 137.13 developed for data generation purposes could be used as a confirmatory method for REM 137.02 in support of storage stability study on wheat.
** Method GRM020.09B indicated to be an update of GRM020.09A to include new validation data for dry broad beans and oilseed rape seeds. .

Table 5.2-3: Validated methods for the generation of pre-authorization data for trinexapac-ethyl in soil, water and air

Component of residue definition: **Soil: trinexapac-ethyl, CGA179500, CGA300405 and CGA275537** **Surface water and Sediment: trinexapac-ethyl, CGA179500, CGA300405, CGA275537, M2 and M3** **Ground water: trinexapac-ethyl, CGA179500, CGA300405 and CGA275537** **Air: trinexapac-ethyl**				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Local tap water (drinking water quality) Toxicity to *Rainbow Trout* (OECD 203) (Ecotoxicology)	Primary	4.64 mg AG-T3-175 EC/L, corresp. to 0.861 mg trinexapac-ethyl/L	HPLC-UV	KCP 5.1.2/01 filed under KCP10.2.1/01 xxxxxxxxxxxxxx 2008 Report no: B93071 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- UV)	
ISO Standard water 6341 *Daphnia magna* acute toxicity (OECD 202) (Ecotoxicology)	Primary	10.1 mg AG-T3-175 EC/L, corresp. to 1.87 mg trinexapac-ethyl/L	HPLC-UV	KCP 5.1.2/02 filed under KCP 10.2.1/02 Höger S., 2008 Report no: B93082 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- UV)	
OECD 201 test medium *Anabaena flos-aquae* - algal growth inhibition test) (OECD 201) (Ecotoxicology)	Primary	22.1 mg AG-T3-175 EC/L, corresp. to 4.11 mg trinexapac-ethyl/L	HPLC-UV	KCP 5.1.2/03 filed under KCP 10.2.1/03 Bätscher R., 2008 Report no: B93093 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- UV)	
20X AAP growth medium *Lemna gibba* - toxicity to higher aquatic plants) (OECD 221) (Ecotoxicology)	Primary	3.27 mg AG-T3-175 EC/L, corresp. to 0.606 mg trinexapac-ethyl/L	HPLC-UV	KCP 5.1.2/04 filed under KCP 10.2.1/04 Höger S., 2009 Report no: C45577 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- UV)	

Component of residue definition: **Soil: trinexapac-ethyl, CGA179500, CGA300405 and CGA275537** **Surface water and Sediment: trinexapac-ethyl, CGA179500, CGA300405, CGA275537, M2 and M3** **Ground water: trinexapac-ethyl, CGA179500, CGA300405 and CGA275537** **Air: trinexapac-ethyl**				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
- 50 % (w/v) aqueous sucrose solution (diet) *Honey Bee* - Chronic Toxicity Test (OECD 245) (Ecotoxicology)	Primary	36.1 mg/kg of test item equal to 6.25 mg/kg of trinexapac-ethyl	HPLC-MS/MS	KCP 5.1.2/05 filed under KCP 10.3.1.2/01 Oberrauch S., 2018a, Report no: S18-00067, Sponsor no:90020907 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	
- deionized water *Honey Bee* - Larvae chronic toxicity test (OECD 239) (Ecotoxicology)	Primary	200 mg/L of test item equal to 34.6 mg/L of trinexapac ethyl	HPLC-MS/MS	KCP 5.1.2/06 filed under KCP 10.3.1.3/01 Oberrauch S., 2018b, Report no: S18-00066, Sponsor no:90020906 **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	
Vegetative vigour (OECD 227) (Ecotoxicology)	Primary	64 µg/L trinexapac-ethyl	HPLC-UV	KCP 5.1.2/07 and KCP 5.1.2/08 filed under KCA 10.6.2/01 and KCA 10.6.2/02 Friedrich S., 2008a, b Report no´s: 08 10 48 030 S and 08 10 48 029 S **See Appendix 2**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- UV)	
Body fluids, air (Exposure)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water, sediment,... (Environmental fate)	No additional data.			
	Confirmatory (if required)	--	--	--
Soil, water,... (Efficacy)	No additional data.			

Component of residue definition:
Soil: trinexapac-ethyl, CGA179500, CGA300405 and CGA275537
Surface water and Sediment: trinexapac-ethyl, CGA179500, CGA300405, CGA275537, M2 and M3
Ground water: trinexapac-ethyl, CGA179500, CGA300405 and CGA275537
Air: trinexapac-ethyl

Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory (if required)	--	--	--
Water, buffer solutions,... (Properties)	No additional data.			
	Confirmatory (if required)	--	--	--

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

Please refer to the analytical methods for the determination of the active substances in the plant protection product as provided in chapter 5.2.1. A study for analysis of the relevant impurities is ongoing.

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

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

For this application, it is referred to the following EU concluded residue definitions:

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Trinexapac (sum of trinexapac (acid) and its salts, expressed as trinexapac)	0.01 mg/kg	Lowest MRL MRL Regulation (EU) 2017/1016
Plant, high acid content		0.01 mg/kg	Lowest MRL MRL Regulation (EU) 2017/1016
Plant, high protein/high starch content (dry commodities)		0.02 mg/kg	Lowest MRL MRL Regulation (EU) 2017/1016
Plant, high oil content		0.01 mg/kg	Lowest MRL MRL Regulation (EU) 2017/1016
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg5	Lowest MRL MRL Regulation (EU) 2017/1016
Muscle	Trinexapac (sum of trinexapac (acid) and its salts, expressed as trinexapac)	0.01 mg/kg	Lowest MRL MRL Regulation (EU) 2017/1016
Milk		0.01 mg/kg	Lowest MRL MRL Regulation (EU) 2017/1016
Eggs		0.01 mg/kg	Lowest MRL MRL Regulation (EU) 2017/1016
Fat		0.01 mg/kg	Lowest MRL MRL Regulation (EU) 2017/1016
Liver, kidney		0.01 mg/kg	Lowest MRL MRL Regulation (EU)

Matrix	**Residue definition**	**MRL / limit**	**Reference for MRL/level Remarks**
			2017/1016
Soil (Ecotoxicology)	Trinexapac-ethyl (CGA 163935)	0.01 mg/kg	EFSA Journal 2018;16(4):5229 common limit
Drinking / ground water (Human toxicology)	Trinexapac-ethyl (CGA 163935) Trinexapac acid (CGA179500)	0.05 µg/L	EFSA Journal 2018;16(4):5229
Surface water (Ecotoxicology)	Trinexapac-ethyl (CGA 163935) Trinexapac acid (CGA179500)	0.05 µg/L	EFSA Journal 2018;16(4):5229
Air	Trinexapac-ethyl (CGA 163935)	10 $\mu g/m^3$	AOEL sys: 0.34 mg/kg bw/d (EFSA Journal 2018;16(4):5229)
Tissue (meat or liver)	Trinexapac acid (CGA179500)	0.01 mg/kg	EFSA Journal 2018;16(4):5229
Body fluids		0.01 mg/kg	EFSA Journal 2018;16(4):5229

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

An overview of the acceptable methods and possible data gaps for analysis of trinexapac-ethyl in plant matrices is given in the following table. **New data** are already published in the dRAR of Trinexapac-ethyl, Volume 3 – B.5 Methods of analysis, December 2017. No detailed evaluation of the already published data are presented in Appendix 2 of this dossier.

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

Studies indicated as new data are currently under EU evaluation (reviewed by Lithuania, 2018 and EF-SA, 2018).

Component of residue definition: Trinexapac (sum of trinexapac (acid) and its salts, expressed as trinexapac)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content - *Lettuce*	Primary	0.01 mg/kg	HPLC-MS/MS (data generation or monitoring)	Campbell, A.J and Crook, S.J. 2004 Syngenta Report no. REM 137.13 Nichols, C. Kwiatkowski, A., 2004 Syngenta Report no. 03-3001 ILV: Benazeraf, L. 2004a Syngenta Report no. SYN/TRIN/04091 *EU agreed (DAR Addendum, 2005 and EFSA Scientific Report (2005)*
	ILV	0.01 mg/kg		
High oil content - *Sunflower seed*	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
High protein/ high starch content (dry) - *Barley grain*	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
Difficult (if required, depends on intended use) - *Barley hay and straw*	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	
High protein/ high starch content (dry) - *Barley grain*	Primary	0.01 mg/kg	HPLC-MS/MS	GRM020.05A Hargreaves, 2008 **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
High water content - *Lettuce*				
High oil content - *Sunflower seed*				
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	
Dry matrix - *Barley hay and straw*	Data generation or monitoring	0.01 mg/kg	HPLC-MS/MS	Supercedes REM 137.13 Validation: Mayer L, 2008, (amended 2016) **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*

Component of residue definition: Trinexapac (sum of trinexapac (acid) and its salts, expressed as trinexapac)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	
High oil content - - *Sunflower seed*	Primary	0.01 mg/kg	QuEChERS (LC-MS/MS) (monitoring)	Richter, 2015a Report: P 3685 G and amend-ment to report (Syngenta File No. CGA179500_10993) Brown, 2015a (ILV) Report RES-00008; TK0255738 (Syngenta File No. CGA179500_11005) **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
	ILV	0.01 mg/kg		
High oil content - *Sunflower seed*	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
High protein/high starch content (dry) - *Wheat, grain* - *Broad bean, dried*	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
High acid content - *Orange*	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	
Difficult to analyse None	Primary	-	-	-
	ILV	-		

‘Two analytical methods (REM 137.13 and REM 137.14) have been previously assessed in the framework of Directive 91/414/EEC. In order to cover all types of matrices, further validation of REM 137.13 has been conducted; the method has been renamed GRM020.05A.’
No new data are presented in Appendix 2.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	The extractability of trinexapac acid (CGA179500) residues with methanol/water and acetonitrile/water mixtures has been demonstrated in radiolabelled metabolism studies (Study reports No 4/91 (CGA163935/0209, CGA163935/0308), No 20/90 (CGA163935/0086), No 6/93 (CGA163935/0303), No 11/96 (CGA163935/0482) and No 623-00 (CGA163935/0862). The polarity of the extraction solvent mixture (30/56/14 v/v/v MeOH/UPW/phosphate buffer) used in analytical method GRM020.05A is comparable to those used in the metabolism studies and efficient extractability is therefore demonstrated. No further study is required.

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

An overview of the acceptable methods and possible data gaps for analysis of trinexapac-ethyl in animal matrices is given in the following table. **New data** are already published in the dRAR of Trinexapac-ethyl, Volume 3 – B.5 Methods of analysis, December 2017. No detailed evaluation of the already published data are presented in Appendix 2 of this dossier.

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

Studies indicated as **new data** are currently under EU evaluation (reviewed by Lithuania, 2018 and EF-SA, 2018).

Component of residue definition: Trinexapac (sum of trinexapac (acid) and its salts, expressed as trinexapac)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Muscle	Primary	0.01 mg/kg	HPLC-MS/MS (data generation or monitoring)	xxxxx 2004 Method no. REM 137.14. Syngenta file no. CGA 179500/0039. and xxxxxxxxxxxxxxx., 2004 Syngenta Report no. RJ3570B ILV: xxxxxxxxxxxxxxxx Syngenta Report no. SYN/TRIN/04092 *EU agreed (DAR Addendum, 2005 and EFSA Scientific Report (2005)*
	ILV	0.01 mg/kg		
Fat	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
Kidney /liver	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
Eggs	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
Milk	Primary	0.005 mg/kg		
	ILV	0.01 mg/kg		
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	
Muscle	Primary	0.01 mg/kg	QuEChERS (LC-MS/MS) (monitoring)	xxxxxx 2015b Report: P 3686 G and amendment to report (Syngenta File No. CGA179500_10995) xxxxxx 2015b (ILV) Report RES-00009; TK0255742 (Syngenta File No. CGA179500_11006) **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
	ILV	0.01 mg/kg		
Liver	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
Egg	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
Fat	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
Milk	Primary	0.01 mg/kg		
	ILV	0.01 mg/kg		
	Confirmatory (if required)	--	Not required, highly specific detection system was used (HPLC- MS/MS)	

No new data are presented in Appendix 2.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	The extraction procedures using 80:20 acetonitrile:water used in the QuEChERS method for animals is similar to the one used in the metabolism studies (Reports 624-00 (CGA163935/0944), 5/93 (CGA163935/0305), 6/93(CGA163935/0306)). The QuEChERS method for the determination of trinexapac acid in fat samples is performed so that the fat is melted and partitioned between 80/20 v/v acetonitrile/water to extract trinexapac acid residues into the acetonitrile/water mixture which is immiscible with the melted fat. The sample is frozen to solidify the fat and leave trinexapac acid in the 80/20 v/v acetonitrile/water. This procedure is based on similar principles and uses similar polarity solvents to those used in ^{14}C metabolism studies (5/93 (poultry) and 6/93 (goat)) where the fat was dissolved in 80/20 chloroform/methanol which is then partitioned with aqueous buffer to extract the trinexapac acid residues. No new study is necessary.

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

An overview of the acceptable methods and possible data gaps for analysis of trinexapac-ethyl in soil is given in the following table. New data are already published in the dRAR of Trinexapac-ethyl, Volume 3 – B.5 Methods of analysis, December 2017. No detailed evaluation of the already published data are presented in Appendix 2 of this dossier.

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

Studies indicated as **new data** are currently under EU evaluation (reviewed by Lithuania, 2018 and EF-SA, 2018).

Component of residue definition: trinexapac ethyl (CGA163935), trinexapac acid (CGA179500), CGA300405				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Soil (not specified) Soil (sandy loam)	Primary and Confirmatory Trinexapac acid (CGA179500)	0.04 mg/kg	HPLC-UV	Methods REM 137.03, Forrer, 1991c REM 137.04, Forrer, 1991d REM 137.10, xxxxx 1995a *EU agreed (DAR Oct. 2003, Vol 3, B.5.3.1)*
Soil: (loamy sand) (silty clay loam)	Primary and Confirmatory Trinexapac ethyl (CGA163935)	0.01 mg/kg	HPLC-MS/MS	RAM 436.01 Hargreaves, 2004a (*REM 137.01 modified*) Validation: Nagra, 2004a, (study no. 04-S609) RAM 437.01 Hargreaves, 2004b

Component of residue definition: trinexapac ethyl (CGA163935), trinexapac acid (CGA179500), CGA300405				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Trinexapac acid (CGA179500)	0.01 mg/kg		(*REM 137.10 modified*) Validation: Nagra, 2004b, (study no. 04-S609) *EU agreed (DAR Addendum January 2005, Vol 3 B5.2.2, EFSA Scientific report (2005) 57, 1-70)*
Soil *-loamy silt* *-sandy loam*	Primary and Confirmatory Trinexapac ethyl (CGA163935)	0.01 mg/kg	HPLC-MS/MS	Method GRM020.03A, Hargreaves, 2008a Validation: Solé, 2008a **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
Soil *-loamy silt* *-sandy loam*	Primary and Confirmatory Trinexapac acid (CGA179500)	0.01 mg/kg	HPLC-MS/MS	Method GRM020.04A, Hargreaves, 2008b Validation: Solé, 2008a **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
Soil *-Loamy sand, LUFA 2.2* *-Sandy loam, LUFA 5M*	Primary and Confirmatory CGA300405	0.01 mg/kg	HPLC-MS/MS	Method GRM020.10A, Braid, 2015 Validation: Heinz, 2015 **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*

No new data are presented in Appendix 2.

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

An overview of the acceptable methods and possible data gaps for analysis of trinexapac-ethyl, trinexapac acid and CGA300405 in surface and drinking water is given in the following table. New data are already published in the dRAR of Trinexapac-ethyl, Volume 3 – B.5 Methods of analysis, December 2017. No detailed evaluation of the already published data are presented in Appendix 2 of this dossier.

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

Studies indicated as **new data** are currently under EU evaluation (reviewed by Lithuania, 2018 and EF-SA, 2018).

Component of residue definition: Trinexapac-acid (CGA179500), trinexapac-ethyl (CGA163935) and CGA300405				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Unspecified water tap water	Primary and Confirmatory Trinexapac-acid (CGA179500)	0.1 µg/L	HPLC-UV	REM 137.06 Hauck, 1991 *EU agreed (DAR Oct. 2003, Vol 3, B.5.3.2)*
Potable water tap water soil capillary water		0.05 µg/L		REM 137.09 Sack, 1995 *EU agreed (DAR Oct. 2003, Vol 3, B.5.3.2)*
Potable water Surface water		0.05 µg/L 0.1 µg/L		REM 137.09 modified validation Bourry, 2000 *EU agreed (DAR October 2003, Vol 3, B.5.3.2)*
Well water	Primary and Confirmatory Trinexapac-ethyl (CGA163935)	0.1 µg/L	HPLC-UV	AG 558A Ward, 1990 *EU agreed (DAR October 2003, Vol 3, B.5.3.2)*
Water: (river, ground and drinking water)	Primary and Confirmatory Trinexapac-ethyl (CGA 163935) Trinexapac acid (CGA179500)	0.1 µg/L 0.1 µg/L	HPLC-MS/MS	RAM 438/01 Hargreaves, 2004c (REM 137.09 modified) RAM 438.01, Validation Nagra, 2004 (Study no. RJ3531B) *EU agreed (DAR Addendum, 2005)*
Water (ground, drinking and surface water)	Primary and Confirmatory Trinexapac-ethyl (CGA 163935) Trinexapac acid (CGA179500)	0.05 µg/L 0.05 µg/L	HPLC-MS/MS	Method GRM020.02A Hargreaves, 2008c Validation: Solé, 2007 **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
Drinking water	ILV: Trinexapac-ethyl (CGA 163935) Trinexapac acid	0.05 µg/L 0.05 µg/L	HPLC-MS/MS	ILV Foster and Mumford, 2016 **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*

Component of residue definition: Trinexapac-acid (CGA179500), trinexapac-ethyl (CGA163935) and CGA300405				
Matrix type	**Method type and analyte**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
	(CGA179500)			
Surface water Drinking water	Primary and Confirmatory CGA300405	 0.05 µg/L	HPLC-MS/MS	GRM020.11A Crook, 2015 Validation: Heinz, 2015a **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
Surface water Drinking water	ILV: CGA300405	 0.05 µg/L	HPLC-MS/MS	ILV Hamberger, 2015 **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*

No new data are presented in Appendix 2.

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

An overview of the acceptable methods and possible data gaps for analysis of trinexapac-ethyl in air is given in the following table. **New data** are already published in the dRAR of Trinexapac-ethyl, Volume 3 – B.5 Methods of analysis, December 2017. No detailed evaluation of the already published data are presented in Appendix 2 of this dossier.

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

Studies indicated as **new data** are currently under EU evaluation (reviewed by Lithuania, 2018 and EF-SA, 2018).

Component of residue definition: trinexapac ethyl (CGA163935)				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Air	Primary Confirmatory	20 µg/m^3 10 µg/m^3	HPLC-UV	REM 137/07 Tribolet R, 1993 Validation Tribolet R, 1996 *EU agreed (DAR Oct. 2003, Vol 3, B.5.3.3)*
Air	Primary and confirmatory	10 µg/m^3	HPLC-MS/MS	Method GRM020.12A Wiltshire K, 2015a

Component of residue definition: trinexapac ethyl (CGA163935)				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				Validation Wiltshire K, 2015b **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*

No new data are presented in Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of trinexapac acid in body fluids and tissues is given in the following table.
New data for the validation in bovine meat and liver are already published in the dRAR of Trinexapac-ethyl, Volume 3 – B.5 Methods of analysis, Rev. 0, March 2017.

Table 5.3-9: Methods for body fluids and tissues (if appropriate)

Studies indicated as new data are currently under EU evaluation (reviewed by Lithuania, 2018 and EF-SA, 2018).

Component of residue definition: Trinexapac acid (CGA179500)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Bovine muscle, bovine liver	Primary and confirmatory	0.01 mg/kg	QuEChERS (LC-MS/MS)	xxxxxxx, 2015b Report: P 3686 G and amend-ment to report **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*
Bovine blood	Primary and confirmatory	0.01 mg/kg	QuEChERS (LC-MS/MS)	xxxxxxxxxxx, 2017 Report: P 4381 G **New data** *EU agreed (RAR 2017 Volume 3 – B.5 (AS))*

No new data are presented in Appendix 2.

5.3.2.8 Other studies/ information

No other studies and information are submitted in the framework of this application.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 5.1.1/01	Edelson, T.	2016	Determination of Storage Stability and Phys-Chem Properties in AG-T3-175 EC1 (Trinexapac-ethyl 175 EC) Stored at 54°C for 14 Days and at 0°C for 7 Days ADAMA Agan Ltd., Israel, report no. F16-02/4 and sponsor report no. 90019616 GLP Unpublished Also filed under KCP 2.1/01	N	ADAMA Agan Ltd.
KCP 5.1.2/01	xxxxxxxxxxxxxx	2008	AG-T3-175 EC: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-Hour Static Test xxxxxxxxxxxxxxxxxxxxxx GLP Unpublished Also filed under KCP 10.2.1/01	Y	ADAMA Agan Ltd.
KCP 5.1.2/02	Höger, S.	2008	AG-T3-175 EC: Acute Toxicity to *Daphnia magna* in a 48-Hour Immobilization Test RCC Ltd., Switzerland, report no. B93082 Celsius Property B.V., report no 90018031_000081126 GLP Unpublished Also filed under KCP 10.2.1/02	N	ADAMA Agan Ltd.
KCP 5.1.2/03	Bätscher, R.	2008	AG-T3-175 EC: Toxicity to *Anabaena flos-aquae* in a 72-Hour Algal Growth Inhibition Test RCC Ltd., Switzerland, report no. B93093 Celsius Property B.V., report no 90018032_000081127 GLP Unpublished	N	ADAMA Agan Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			Also filed under KCP 10.2.1/03		
KCP 5.1.2/04	Höger, S.	2009	AG-T3-175 EC: Toxicity of AG-T3-175 EC to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test RCC Ltd., Switzerland, report no. C45577 Celsius Property B.V., report no 90011801_000066083 GLP Unpublished Also filed under KCP 10.2.1/04	N	ADAMA Agan Ltd.
KCP 5.1.2/05	Oberrauch, S.	2018a	Trinexapac-ethyl 175 EC: Honey Bee (Apis mellifera L.) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory Eurofins Agroscience Services Ecotox GmbH, Germany, report no. S18-00067 ADAMA Agan Ltd., report no. 90020907 GLP Unpublished Also filed under KCP 10.3.1.2/01	N	ADAMA Agan Ltd.
KCP 5.1.2/06	Oberrauch, S.	2018b	Trinexapac-ethyl 175 EC - Honey Bee (Apis mellifera L.) 22 Day Larval Toxicity Test (Repeated Exposure) Eurofins Agroscience Services Ecotox GmbH, Germany, report no. S18-00066 ADAMA Agan Ltd., report no. 90020906 GLP Unpublished Also filed under KCP 10.3.1.3/01	N	ADAMA Agan Ltd.
KCP 5.1.2/07	Friedrich, S.	2008a	Terrestrial (non-target) plant test with Trinexapac-ethyl 175 EC: Vegetative vigour test of non-target terrestrial plants BioChem agrar, Germany, report no. 08 10 48 030 S Celsius Property B.V., report no 90018044_000081140	N	ADAMA Agan Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			GLP Unpublished Also filed under KCP 10.6.2/01		
KCP 5.1.2/08	Friedrich, S.	2008b	Terrestrial (non-target) plant test with Trinexapac-ethyl 175 EC: Seedling emergence and seedling growth test of non-target terrestrial plants BioChem agrar, Germany, report no. 08 10 48 029 S Celsius Property B.V., report no 90018045_000081141 GLP Unpublished Also filed under KCP 10.6.2/02	N	ADAMA Agan Ltd.

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evalu-ation (DAR) or New Data (RAR, 2018)
CP 5.1.2	Forrer K.	1989	CGA 163935 Liquid chromatographic determination of residues of parent, Plant material and soil Study Report No. REM 137.01 Ciba-Geigy Ltd., Basel, Switzerland GLP, inpublished	N	Trinexapac Task Force	CA 4.1.2 (B5.1.2.1/001) DAR October 2003
CP 5.1.2	Forrer K.	1991a	CGA 163935 - Determination of residues of the metabolite CGA 179500 by liquid chro-matography, Plant material Study Report No. REM 137.02 Ciba-Geigy Ltd., Basel, Switzerland GLP, unpublished	N	Trinexapac Task Force	CA 4.1.2 (B5.1.2.1/002) DAR October 2003
CP 5.1.2	Sack St.	1999	Validation (ILV) of method REM 137.02 (validation by analysis of fortified specimens and determination of recoveries), Plant material Study Report No. 304/99 Novartis Crop Protection AG, Basel, Switzerland GLP, unpublished	N	Trinexapac Task Force	CA 4.1.2 (B5.1.2.1/003) DAR October 2003
CP 5.1.2	xxxxxx	1995a	Determination of the metabolite CGA 179500 by liquid chromatography, Animal produce (tissue, milk, eggs) Study Report No. REM 137.12 xxxxxxxxx GLP, unpublished	N	Trinexapac Task Force	CA.4.1.2 (B5.1.2.2/001 DAR October 2003
CP 5.1.2	xxxxxx	2001	Independent Laboratory Validation (ILV) of REM 137.12 Study Report No.312/01 xxxxxxxxxxxxx GLP, unpublished	N	Trinexapac Task Force	CA.4.1.2 (B5.1.2.2/001) DAR October 2003

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evalu-ation (DAR) or New Data (RAR, 2018)
CP 5.1.2	Lin K.	2008	Validation of Analytical Method GRM020.01A for the Determination of Residues of Trinexapac-ethyl as CGA179500 in Crops by LC-MS/MS GRM020.01A Syngenta Crop Protection, Inc., Greensboro, USA GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/08
CP 5.1.2	Thomas C.	2010	Independent Laboratory Validation (ILV) of Syngenta Analytical Method GRM020.01A – Analytical Method for the Determination of Residues of Trinexapac-Ethyl as CGA179500 in Crops by LC-MS/MS 110.036 Syngenta Crop Protection, Inc., Greensboro, USA North Coast Laboratories, Arcata CA, USA, GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/09
CP 5.1.2	Hargreaves S.	2008	Trinexapac ethyl – Analytical Method for the Determination of Residues of the Metab-olite CGA179500 in Crops. Final Determination by LC-MS/MS GRM020.05A, T009081-06 Syngenta – Jealott's Hill, Bracknell, United Kingdom, Not GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/01 (B.5.1.2.1)
CP 5.1.2	Mayer L.	2008 2016	Trinexapac-Ethyl – Validation of Analytical Method GRM020.05 for the Determination of Residues of Trinexapac-Ethyl Metabolite CGA179500 in Crops by LC-MS/MS GRM020.05, T001300-08 Report has been amended 12 August 2016 Syngenta Crop Protection, Inc., Greensboro, USA GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/02 (B.5.1.2.1)
CP 5.1.2	Braid S., Tsui G.	2015 2016	Trinexapac Ethyl –Analytical Method GRM020.09A for the Determination of Residues of CGA179500 in Cereal Grain and Straw by LC-MS/MS GRM020.009A, Report was amended: 22 February 2016 and 2 August 2016	N	Trinexapac Task Force	KCA 4.1.2/03 (B.5.1.2.1)

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evalu-ation (DAR) or New Data (RAR, 2018)
			Syngenta - Jealott's Hill,Bracknell, United Kingdom Not GLP, unpublished			
CP 5.1.2	Tsui G.	2015	Trinexapac Ethyl - Validation of Analytical Method GRM020.00A for the Determination of Residues of CGA179500 in Cereal Grain and Straw by LC-MS/MS TK0252289, Report has been amended 19 November 2015 Battelle UK Ltd, Chelmsford, Essex, UK GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/04 (B.5.1.2.1)
CP 5.1.2	Braid S., Tsui G.	2016a	Trinexapac Ethyl - Analytical Method GRM020.09B for the Determination of Residues of CGA179500 in Various Crop Matrices by LC-MS/MS Syngenta - Jealott's Hill, Bracknell, United Kingdom GRM020.09B, Report was amended 2 August 2016 Not GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/05
CP 5.1.2	Tsui G.	2016	Trinexapac Ethyl - Validation of Analytical Methods GRM020.09B and GRM020.16A for the Determination of Residues of CGA179500 in Various Crop Matrices by LC-MS/MS Battelle UK Ltd, Chelmsford, Essex, UK TK0252289 GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/06
CP 5.1.2	Braid S., Tsui G.	2016b	Trinexapac Ethyl - Analytical Method GRM020.16A for the Determination of Residues of CGA179500 in Various Crop Matrices by LC-MS/MS Syngenta - Jealott's Hill, Bracknell, United Kingdom GRM020.16A TK0252289 Not GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/07
CP 5.1.2	xxxxxxx	2008	Validation of Residue Method AGR/MOA/TRIN-06 for the Determination of Trinexapac in Animal Matrices xxxxxxxxxxxx	N	Trinexapac Task Force	KCA 4.1.2 / 17 & KCA 4.2 / 01

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evalu-ation (DAR) or New Data (RAR, 2018)
			CHE/TRIN/08003 GLP, unpublished			
CP 5.1.2	Hauck M.	1993	Determination of residues of the metabolite CGA 179500 by liquid chromatography, Plant material Study Report No. REM 137.08 Ciba-Geigy Ltd., Basel, Switzerland GLP, unpublished	N	Trinexapac Task Force	CA 4.1.2 (B5.1.2.1/004) DAR Oactober 2003
CP 5.1.2	Watson G.	2016	Trinexapac-ethyl - Analytical Method GRM020.15A for the Determination of CGA224439 (Cyclopropanecarboxylic Acid) in Brewing and Baking Matrices GRM020.15A ResChem Analytical Limited, Derby, UK Not GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/15
CP 5.1.2	Watson G.	2016a	Trinexapac Ethyl - Validation of a method for the determination of residues of CPCA in processed commodity matrices by LC-MS/MS RES-00026 ResChem Analytical Limited, Derby, UK GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/14
CP 5.1.2	Braid S., Langridge G	2016	Trinexapac Ethyl - Analytical Method GRM020.013A for the Determination of the Metabolite CGA313458 in Brewing and Baking Commodities GRM020.013A Syngenta - Jealott's Hill, Bracknell, United Kingdom, Not GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/12
CP 5.1.2	Braid S., Brookes S., Langridge G.	2016	Trinexapac Ethyl - Analytical Method GRM020.014A for the Determination of the Metabolite CGA113745 in Brewing and Baking Commodities GRM020.014A Syngenta - Jealott's Hill, Bracknell, United Kingdom	N	Trinexapac Task Force	KCA 4.1.2/13

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evalu-ation (DAR) or New Data (RAR, 2018)
			Not GLP, unpublished			
CP 5.2	Campbell A.J., Crook S.J.	2004	Residue analytical method for the determination of residues of trinexapac acid (CGA 179500) in crop samples. Final determination by LC/MS/MS. Syngenta Ltd, Bracknell, United Kingdom. report no. REM 137.13 Not GPL, Unpublished.	N	Trinexapac Task Force	CA 4.1.2 (B5.1.2.1/005)
CP 5.2	Nichols C., Kwiatkowski A.	2004	Residue Study with Trinexapac-Ethyl (CGA 163935) in or on Broad Beans in France (South). Syngenta Ltd, Bracknell, United Kingdom. report no. 03-3001 GLP, unpublished.	N	Trinexapac Task Force	CA 4.1.2 (B5.1.2.1/005)
CP 5.2	Benazeraf L.	2004a	Independent laboratory validation of residue method REM 137.13 for the determination of trinexapac acid (CGA 179500) in oilseed rape, potato, apple and cereal grain. Syngenta Ltd, Bracknell, United Kingdom. report no. SYN/TRIN/04091 GLP, unpublished.	N	Trinexapac Task Force	CA 4.1.2 (B5.1.2.1/005)
CP 5.2	Hargreaves S.	2008	Trinexapac ethyl Analytical Method for the Determination of Residues of the Metabolite CGA17950 in Crops. Final Determination by LC-MS/MS Syngenta - Jealott's Hill Bracknell, United Kingdom, report no. GRM020.05A, T009081-06 Not GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/01 (B.5.1.2.1)
CP 5.2	Mayer L.	2008	Trinexapac-Ethyl - Validation of Analytical Method GRM020.05 for the Determination of Residues of Trinexapac-Ethyl Metabolite CGA179500 in Crops by LC-MS/MS Syngenta Crop Protection, Inc., Greensboro, USA, report no. GRM020.05, T001300-08, amended 2016 GLP, unpublished	N	Trinexapac Task Force	KCA 4.1.2/02 (B.5.1.2.1)
CP 5.2	Richter S.	2015a	Trinexapac– Validation of the QuEChERS Method for the Determination of Trinexapac (Acid) in Crop Matrices by LCMS/MS. Method Validation Report Amendment 1 PTRL Europe GmbH, Germany.	N	Trinexapac Task Force	KCA 4.2 / 02

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evalu-ation (DAR) or New Data (RAR, 2018)
			Report PTRL Europe ID P 3685 G, Task number TK0255737 GLP, unpublished			
CP 5.2	Brown D.	2015a	Trinexapac (acid) – Independent Laboratory Validation of the QuEChERS Analytical Method for the Determination of Trinexapac (acid) Residues in Crops. ResChem Analytical Limited, United Kingdom Report RES-00008; Task number TK0255738 GLP, unpublished	N	Trinexapac Task Force	KCA 4.2 / 03
CP 5.2	xxxxxxx	2004	Residue analytical method for the determination of residues of trinexapac acid (CGA 179500) in animal matrices. Final determination by LC/MS/MS. xxxxxxxxxxxxxxxxxxxxxx GLP, unpublished. Syngenta file no. CGA	N	Trinexapac Task Force	IIA 4.2.1
CP 5.2	xxxxxxx	2004	Trinexapac Acid (CGA179500): Validation of a Residue Analytical Method REM 137.14 for the Determination of Residues in Animal Products (Milk, Eggs, Muscle, Kidney, Fat and Liver). xxxxxxxxxxxxxxxxxxxxxx GLP, unpublished	N	Trinexapac Task Force	IIA 4.2.1
CP 5.2	xxxxxxxxx	2004b	Independent laboratory validation of residue method REM 137.14 for the determination of trinexapac acid (CGA 179500) in bovine muscle and bovine Milk. xxxxxxxxxxxxxxxxxxxxxx GLP, unpublished	N	Trinexapac Task Force	IIA 4.2.1
CP 5.2	xxxxxxxxx	2015b	Trinexapac: Validation of the QuEChERS Method for the Determination of Trinexapac (Acid) in Animal Matrices by LC-MS/MS. Method Validation Report Amendment 1 PTRL Europe GmbH, Germany. xxxxxxxxxxxxxxxxx GLP, unpublished	N	Trinexapac Task Force	KCA 4.2 / 04
CP 5.2	xxxxxxxx	2015b	Trinexapac (acid) –Independent Laboratory Validation of the QuEChERS Analytical Method for the Determination of Trinexapac (acid) Residues in animal matrices. xxxxxxxxxxxxxxxxxxx	N	Trinexapac Task Force	KCA 4.2 / 05

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evalu-ation (DAR) or New Data (RAR, 2018)
			Report RES-00009, TK0255742 GLP, unpublished			
CP 5.2	Forrer K.	1991c	CGA 163935 Determination of residues of the metabolite CGA 179500 by liquid chroma-tography, Soil Ciba-Geigy Ltd., Basel, Switzerland, report No. REM 137.03 GLP, unpublished	N	Trinexapac Task Force	CA.4.2.2/01 = IIIA.5.2.2 (DAR Vol3 B5)
CP 5.2	Forrer K.	1991d	CGA 163935, Liquid chromatographic determination of residues of parent compound, Soil Ciba-Geigy Ltd., Basel, Switzerland, study report no. REM 137.04 GLP, unpublished	N	Trinexapac Task Force	CA 4.2.2/02 = IIIA.5.2.2 (DAR Vol3 B5)
CP 5.2	Sack St.	1995a	Determination of the metabolite CGA 179500 by liquid chromatography, Soil Ciba-Geigy Ltd., Basel, Switzerland, study report no. REM 137.10 GLP, unpublished	N	Trinexapac Task Force	CA.4.2.2/03 = IIIA.5.2.2 (DAR Vol3 B5)
CP 5.2	Hargreaves S.L.	2004a	Residue Analytical Method for the Determination of Residues of Trinexapac-Ethyl in Soil. Syngenta Ltd., Bracknell, UK, method no. RAM 436/01 Not GLP, unpublished	N	Trinexapac Task Force	IIA 4.2.2
CP 5.2	Nagra B.S.	2004a	Trinexapac-ethyl: Validation of an Analytical Method for the Determination of Residues of Trinexapac-ethyl (CGA163935) in Soil. Syngenta Ltd., Bracknell, UK, report no. RJ3526B, GLP, unpublished	N	Trinexapac Task Force	IIA 4.2.2
CP 5.2	Hargreaves S.L.	2004b	Residue Analytical Method for the Determination of Residues of CGA 179500 in Soil. Syngenta Ltd., Bracknell, UK, method number RAM 437/01 Not GLP, unpublished	N	Trinexapac Task Force	IIA 4.2.2
CP 5.2	Nagra B.S.	2004b	Validation of an Analytical Method for the Determination of Residues of CGA 179500 in Soil. Syngenta Ltd., Bracknell, UK, report no. RJ3527B GLP, unpublished	N	Trinexapac Task Force	IIA 4.2.2
CP 5.2	Hargreaves S.L.	2008a	GRM020.03A – Trinexapac-ethyl – Residue Method for the Determination of trinexapac - ethyl in soil	N	Trinexapac Task Force	KCA 4.2/06

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evalu-ation (DAR) or New Data (RAR, 2018)
			Syngenta Ltd., Jealott´s Hill International Research Centre, UK SYN/TRN/07001, Report No. T010139-04 not GLP, unpublished			
CP 5.2	Solé C.	2008a	Validation of residue methods GRM020.03A and GRM020.04A for the determination of trinexapac-ethyl (CGA163935) and its metabolite (CGA179500) in soil. Eurofins ADME Bioanalysis, France Report No. T010139-04 GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/07 and KCA 4.2/09
CP 5.2	Hargreaves S.L.	2008b	GRM020.04A - Trinexapac ethyl - Residue Method for the Determination of Metabolite CGA179500 in Soil - Analytical Method. Syngenta Ltd., Jealott´s Hill International Research Centre, UK Report No. T010139-04, SYN/TRIN/07001 not GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/08
CP 5.2	Braid S.	2015	Trinexapac-ethyl – Analytical Method for the Determination of Metabolite CGA300405 in soil Syngenta Ltd., Jealott´s Hill International Research Centre, UK Report No. GRM020.10A, Task No. TK0253683 not GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/10
CP 5.2	Heinz N.	2015a	Validation of an Analytical Method for the Determination of Trinexapac-ethyl Metabolite CGA300405 in Soil Method/Validation, Report Amendment No. 1 PTRL Europe GmbH, Ulm, Germany. Report No. PTRL Europe ID P 3616G. GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/11
CP 5.2	Hauck M.	1991	CGA 163935, Determination of residues of the metabolite CGA 179500 by liquid chroma-tography (LC), Water. Ciba-Geigy Ltd., Basel, Switzerland, study report no. REM137.06 GLP, unpublished	N	Trinexapac Task Force	CA.4.2.3.1/01 = IIIA.5.2.3/01 (DAR Vol3 B5)
CP 5.2	Sack St.	1995b	Trinexapac-Ethyl (CGA 163935), Determination of the metabolite CGA 179500 by liquid	N	Trinexapac	CA.4.2.3.1/02 =

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evalu-ation (DAR) or New Data (RAR, 2018)
			chromatography, Water, Residue method validated Ciba-Geigy Ltd., Basel, Switzerland, study report No. REM 137.09, GLP, unpublished		Task Force	IIIA.5.2.3.1/ 02
CP 5.2	Bourry R.	2000	Validation of Method REM 137.09 Novartis Crop Protection, AG, Basel, Switzerland, study report No. 308/00, GLP, unpublished	N	Trinexapac Task Force	CA.4.2.3.1/03 = IIIA.5.2.3.1/03
CP 5.2	Ward M.K.	1990	Analytical method for the determination of CGA 163935 in water by HPLC with column switching Ciba-Geigy Corp., Greensboro, United States, study report no. AG558A, GLP, unpublished	N	Trinexapac Task Force	CA.4.2.3.1/04 = IIIA.5.2.3.1/04
CP 5.2	Hargreaves S.L.	2004c	Residue Analytical Method for the Determination of Residues of Trinexapac-Ethyl (CGA 163935) and its Metabolite CGA 179500 In Water Syngenta Ltd, Bracknell, UK, Method No. RAM 438/01 not GLP, unpublished	N	Trinexapac Task Force	IIA 4.2.3
CP 5.2	Nagra B.S.	2004c	Trinexapac-ethyl - Validation of an Analytical Method for the Determination of Residues of trinexapac-ethyl (CGA163935) and CGA179500 in Water. Syngenta Ltd., Bracknell, UK, report no. RJ3531B GLP, unpublished	N	Trinexapac Task Force	IIA 4.2.3
CP 5.2	Hargreaves S.L.	2008c	GRM020.02A Trinexapac ethyl – Residue Method for the Determination of Trinexapac ethyl and its metabolite CGA179500 in water – Analytical Method Syngenta Ltd., Jealott´s Hill International Research Centre, UK Report No. T012479-04 not GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/12
CP 5.2	Solé C.	2007	Validation of residue method GRM020.02A for the determination of trinexapac-ethyl (CGA163935) and its metabolite CGA179500 in water Eurofins ADME Bioanalysis, France Report No. T012479-04, SYN/TRIN/07002 GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/13
CP 5.2	Foster B. and	2016	Trinexapac-ethyl - Independent Laboratory Validation of Residue Method GRM020.02A	N	Trinexapac	KCA 4.2/14

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evaluation (DAR) or New Data (RAR, 2018)
	Mumford J.		for the Determination of Trinexapac ethyl and its Metabolite CGA179500 in Water Method Validation; Report Amendment No. 1 Smithers Viscient (ESG) Ltd., UK Report Number: 3201221, Task Number: TK0281334 GLP, unpublished		Task Force	
CP 5.2	Crook S.	2015	Trinexapac-ethyl – Analytical Method GRM020.11A for the Determination of the Metabolite CGA300405 in Water. Syngenta, Jealotts Hill International Research Centre, UK. Report No. GRM020.113A, Task Number TK0253682 not GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/15
CP 5.2	Heinz N.	2015	Heinz N. (2015a). - Trinexapac Ethyl – Validation of an Analytical Method for the Determination of Trinexapac Ethyl Metabolite CGA300405 in Water Method Validation Report Amendment No. 1 PTRL Europe GmbH, Germany Report no. P 3617 G, TK0253685 GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/16
CP 5.2	Hamberger R.	2015	Trinexapac ethyl – Independent Laboratory Validation of Analytical Method for the Determination of Metabolite CGA300405 in water. CIP Chemisches Institut Pforzheim GmbH, Germany Report No. SYNCGA300405DW, Study no. 15S08142-02-VMWA, TK0253686 GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/17
CP 5.2	Tribolet R.	1993	Sampling of air and determination of residues of parent compound by high performance liquid chromatography Ciba-Geigy Ltd., Basel, Switzerland, study report no. REM 137.07 GLP, unpublished	N	Trinexapac Task Force	IIIA 5.2.4.1/01
CP 5.2	Tribolet R.	1996	Validation of Method REM 137.07 in Air; Validation by analysis of fortified specimens and evaluation of recoveries Ciba-Geigy Ltd., Basel, Switzerland, study report no. 151/96 GLP, unpublished	N	Trinexapac Task Force	IIIA 5.2.4.1/02

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner	Previous evalu-ation (DAR) or New Data (RAR, 2018)
CP 5.2	Wiltshire K.	2015a	Trinexapac-ethyl – Residue Method GRM020.12A for the Determination of trinexapac – ethyl in Air by LC-MS/MS. CEM Analytical Services Ltd (CEMAS), UK Report No. GRM020.12A, TK0253684 not-GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/18
CP 5.2	Wiltshire K.	2015b	Trinexapac-ethyl – Validation of Draft Residue Method GRM020.12A for the Determination of trinexapac –ethyl in Air by LC-MS/MS. CEM Analytical Services Ltd (CEMAS), UK Report No. CEMR-7011-REG, TK0253684 GLP unpublished	N	Trinexapac Task Force	KCA 4.2/19
CP 5.2	xxxxxxxxxx	2017	Trinexapac - Validation of the QuEChERS Method for the Determination of Residues of Trinexapac in blood by LC-MS/MS, Final Report – Report Amendment 2 xxxxxxxxxxxxx Report Number: P 4381 G, TK0325240 GLP, unpublished	N	Trinexapac Task Force	KCA 4.2/20

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Trinexapac-ethyl analysis.

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1.2)

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

A 2.1.1.6.1 Analytical method

A 2.1.1.6.1.1 Method validation - Acute toxicity to fish

Comments of zRMS:	The validation has been accepted. The quantification of the test item was based on the analyte trinexapac-ethyl and was performed by HPLC analysis with UV/VIS-detection. 7 point calibration was made. The average recoveries were found to be 87% and 92% of the spiked values, with an overall mean of 89% (n = 4). No correction for the recovery rate was made. The method is suitable for the purpose.

Reference:	KCP 5.1.2/01 (also filed under KCP 10.2.1/01)
Report	xxxxxxxxxxx 2008: AG-T3-175 EC - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-Hour Static Test Study no: B93071
Guideline(s):	Not mentioned, in line with SANCO/3029/99 rev. 4
Deviations:	Yes. Repeatability and accuracy was assessed for 2 instead of 5 replicates at 2 fortification levels. This deviation is not relevant, as the samples were analysed without sample work-up procedure.
GLP:	Yes
Acceptability:	Yes. Validation meets guideline criteria (SANCO/3029/99 rev. 4, 11/07/2000) with minor deviations.

Materials and methods

The analytical method for determination of trinexapac-ethyl in tap water was validated. The limit of quantification (LOQ) of the method was 4.64 mg/L. The quantitative measurements of trinexapac-ethyl were performed using HPLC-UV (280 nm).

Test substances:

Test substance	AG-T3-175 EC
Batch No:	D-I0703
Active ingredient content	Trinexapac-ethyl, 180 g/L

Reference substance:	Trinexapac-Ethyl purified
Batch No.	D-TR-38
Purity:	98.2% (w/w)

Sample preparation for trinexapac-ethyl determination

Treatment samples and control samples were thawed at 25°C (water bath) for 1.5 hours and shaken manually to obtain homogeneous sample solutions. Aliquots of the final solutions were analyzed by HPLC with UV/VIS-detection.

Equipment for trinexapac-ethyl determination:

HPLC system	Auto sampler: Merck-Hitachi L-7200, Pump: Merck-Hitachi L-7100 Detector: Merck-Hitachi L-7400, Column oven: Merck-Hitachi L-7300

Column:	Phenomenex Luna C18 (2); 50 mm x 4.6 mm; 3 µm Pre-Column: Phenomenex C18 (Octadecyl ODS); 4 x 3 mm
Column Temperature:	Room temperature
Injection Volume:	100 µL
Mobile phases:	0.4% phosphoric acid in water/acetonitrile (v/v;7/3)
Flow rate:	1 mL/min
Detection wavelength:	280 nm
Retention time:	Trinexapac-ethyl: Approximately 11.2 minutes

Results and discussions

Table A 1: Recovery results from method validation of trinexapac-ethyl in tap water

Matrix	Nominal concentration of test item (mg a.s./L)	Concentration determined in the spiked sample (mg/L)	Recovery (%)	Mean recovery (%)	Overall mean (%)
Tap water	Control	<0.135 mg analyte/L (smallest calibration level)	n.a.	n.a.	
	4.64	4.00, 4.04	86, 87	87	89
	46.4	42.7, 42.3	92, 91	92	

n.a. = not applicable

Table A 2: Characteristics for the analytical method used for validation of trinexapac-ethyl residues in aqueous solution

	Trinexapac-ethyl
Specificity	Two dilution water blank samples were used to show specificity and blank values being <30 % of the LOQ.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in acetonitrile/water (v/v; 3/7). The linearity was determined with eleven standard solutions ranging from 135 mg/L to 0.135 mg/L. The calibrations were found linear with coefficients of determination $R \geq 0.999$ y = 1109595x + 10452, R2 = 0.9998, weighting 1/y. Calibration data and the graph is presented in the report.
Matrix effects	Not tested
Limit of determination / quantification	Limit of quantification is 4.64 mg a.s./L in tap water.

Conclusion

The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of trinexapac-ethyl in tap water.
Exception: only 2 recovery samples were prepared instead of 5 recovery samples. This deviation is not relevant, as the samples were analysed without sample work-up procedure.

A 2.1.1.6.2 Analytical method 2

A 2.1.1.6.2.1 Method validation - Acute toxicity to aquatic invertebrates

Comments of zRMS:	The validation has been accepted. The quantification of the test item was based on the analyte trinexapac-ethyl and was performed by HPLC analysis with UV/VIS-detection. The linearity: R2 fit of the calibration curve used was 0.9998, reflects the linearity of the HPLC-system within the calibration range of 0.135 - 135 mg analyte /L. The average recoveries were found to be 87% and 92% of the spiked values, with an overall mean of 89% (n = 4). No correction for the recovery rate was made. The average recoveries of AG-T3-175 EC on the basis of trinexapac-ethyl found in the treatment samples ranged from 96% to 113% (day 0) of the nominal concentrations, was 97% on day 1 and ranged from 95% to 108% on day 4. The method is suitable for the purpose.

Reference:	KCP 5.1.2/02 (also filed under KCP 10.2.1/02)
Report	Höger, S., 2008, AG-T3-175 EC: Acute Toxicity to *Daphnia magna* in a 48-Hour Immobilization Test, Study no: B93082, Sponsor reference number: 90018031_000081126
Guideline(s):	Not mentioned, in line with SANCO/3029/99 rev. 4
Deviations:	Yes. Repeatability and accuracy was assessed for 2 instead of 5 replicates at 2 fortification levels. This deviation is not relevant, as the samples were analysed without sample work-up procedure.
GLP:	Yes
Acceptability:	Yes. Validation meets guideline criteria (SANCO/3029/99 rev. 4, 11/07/2000) with minor deviations.

Materials and methods

The analytical method for determination of trinexapac-ethyl in ISO Standard water 6341 was validated. The limit of quantification (LOQ) of the method was 1.64 mg/L. The quantitative measurements of trinexapac-ethyl were performed using HPLC-UV (280 nm).

Test substances:

Test substance	AG-T3-175 EC
Batch No:	D-I0703
Active ingredient content	Trinexapac-ethyl, 180 g/L
Reference substance:	Trinexapac-Ethyl purified
Batch No.	D-TR-38
Purity:	98.2% (w/w)

Sample preparation for trinexapac-ethyl determination

Treatment samples and control samples were thawed at 25°C (water bath) for 1.5 hours and shaken manually to obtain homogeneous sample solutions. Aliquots of the final solutions were analyzed by HPLC with UV/VIS-detection.

Equipment for trinexapac-ethyl determination:

HPLC system	Auto sampler: Merck-Hitachi L-7200, Pump: Merck-Hitachi L-7100 Detector: Merck-Hitachi L-7400, Column oven: Merck-Hitachi L-7300
Column:	Phenomenex Luna C18 (2); 50 mm x 4.6 mm; 3 µm Pre-Column: Phenomenex C18 (Octadecyl ODS); 4 x 3 mm
Column Temperature:	Room temperature
Injection Volume:	100 µL
Mobile phases:	0.4% phosphoric acid in water/acetonitrile (v/v;7/3)
Flow rate:	1 mL/min
Detection wave length:	280 nm
Retention time:	Trinexapac-ethyl: Approximately 11.3 minutes

Results and discussions

Table A 3: Recovery results from method validation of trinexapac-ethyl in ISO Standard water 6341

Matrix	Nominal concentration of test item (mg as./L)	Concentration determined in the spiked sample (mg/L)	Recovery (%)	Mean recovery (%)	Overall mean (%)
ISO Standard water 6341	Control	<0.135 mg analyte/L (smallest calibration level)	n.a.	n.a.	
	10.1	8.85, 8.84	88, 88	88	90
	101	94.1, 93.0	93, 92	93	

n.a. = not applicable

Table A 4: Characteristics for the analytical method used for validation of trinexapac-ethyl residues in aqueous solution

	Trinexapac-ethyl
Specificity	Two dilution water blank samples were used to show specificity and blank values being <30 % of the LOQ.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in acetonitrile/water (v/v; 3/7). The linearity was determined with eleven standard solutions ranging from 135 mg/L to 0.135 mg/L. The calibrations were found linear with coefficients of determination $R \geq 0.999$ $y = 1109595x + 10452$, $R^2 = 0.9998$, weighting 1/y. Calibration data and the graph is presented in the report.
Matrix effects	Not tested
Limit of determination / quantification	Limit of quantification is 10.1 mg a.s./L in ISO Standard water 6341

Conclusion

The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of trinexapac-ethyl in ISO Standard water 6341.

Exception: only 2 recovery samples were prepared instead of 5 recovery samples. This deviation is not relevant, as the samples were analysed without sample work-up procedure.

A 2.1.1.6.3 Analytical method 3

A 2.1.1.6.3.1 Method validation - Effects on aquatic algae

Comments of zRMS:	The validation has been accepted. The quantification of the test item was based on the analyte trinexapac-ethyl and was performed by HPLC analysis with UV/VIS-detection. 7 point calibration was made. The average recoveries of the unfiltered samples were found to be 92% and 93% of the spiked values, with an overall mean of 92% (n = 4). The average recoveries of the filtered samples were found to be 92% (n = 4). No correction for the recovery rate was made. The method is suitable for the purpose.

Reference:	KCP 5.1.2/03 (also filed under KCP 10.2.1/03)
Report	Bätscher R., 2008, AG-T3-175 EC: Toxicity to *Anabaena flos-aquae* in a 72-Hour Algal Growth Inhibition Test Report no: B93093, Sponsor reference no: 90018032_000081127
Guideline(s):	Not mentioned, in line with SANCO/3029/99 rev. 4
Deviations:	Yes. Repeatability and accuracy was assessed for 2 instead of 5 replicates at 2 fortification levels. This deviation is not relevant, as the samples were analysed without sample work-up procedure.
GLP:	Yes
Acceptability:	Yes. Validation meets guideline criteria (SANCO/3029/99 rev. 4, 11/07/2000) with minor deviations.

Materials and methods

The analytical method for determination of trinexapac-ethyl in OECD TG 201 medium was validated. The limit of quantification (LOQ) of the method was 22.1 mg/L. The quantitative measurements of trinexapac-ethyl were performed using HPLC-UV (280 nm).

Test substances:

Test substance	AG-T3-175 EC
Batch No:	D-I0703
Active ingredient content	Trinexapac-ethyl, 180 g/L
Reference substance:	Trinexapac-Ethyl purified
Batch No.	D-TR-38
Purity:	98.2% (w/w)

Sample preparation for trinexapac-ethyl determination

Treatment samples and control samples were thawed at 25°C (water bath) for 30 minutes and shaken manually to obtain homogeneous sample solutions. The treatment and control samples from day 3 were filtered (PTFE, 0.45 µm) due to algal growth. Aliquots of the final solutions were analyzed by HPLC with UV/VIS-detection.

Equipment for trinexapac-ethyl determination:

HPLC system	Auto sampler: Merck-Hitachi L-7200, Pump: Merck-Hitachi L-7100 Detector: Merck-Hitachi L-7400, Column oven: Merck-Hitachi L-7300
Column:	Phenomenex Luna C18 (2); 50 mm x 4.6 mm; 3 µm Pre-Column: Phenomenex C18 (Octadecyl ODS); 4 x 3 mm

Column Temperature:	Room temperature
Injection Volume:	100 µL
Mobile phases:	0.4% phosphoric acid in water/acetonitrile (v/v;7/3)
Flow rate:	1 mL/min
Detection wavelength:	280 nm
Retention time:	Trinexapac-ethyl: Approximately 11.2 minutes

Results and discussions

Table A 5: Recovery results from method validation of trinexapac-ethyl in OECD 201 test medium

Matrix	Nominal concentration of test item (mg a.s./L)	Concentration determined in the spiked sample (mg/L)	Recovery (%)	Mean recovery (%)	Overall mean (%)
Unfiltered Sample					
OECD 201 test medium	Control	<0.135 mg analyte/L (smallest calibration level)	n.a.	n.a.	
	22.1	20.3, 20.2	92, 91	92	92
	100	91.9, 93.4	92, 93	93	
Filtered Sample					
OECD 201 test medium	Control	<0.135 mg analyte/L (smallest calibration level)	n.a.	n.a.	
	22.1	20.5, 20.3	93, 92	92	92
	100	91.5, 93.0	91, 93	92	

n.a. = not applicable

Table A 6: Characteristics for the analytical method used for validation of trinexapac-ethyl residues in aqueous solution

	Trinexapac-ethyl
Specificity	Two dilution water blank samples were used to show specificity and blank values being <30 % of the LOQ.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in acetonitrile/water (v/v; 3/7). The linearity was determined with eleven standard solutions ranging from 135 mg/L to 0.135 mg/L. The calibrations were found linear with coefficients of determination R ≥ 0.999 y = 1115460x + 2039.1, R2 = 0.9999, weighting 1/y. Calibration data and the graph is presented in the report.
Matrix effects	Not tested
Limit of determination / quantification	Limit of quantification is 22.1 mg a.s./L in OECD 201 test medium

Conclusion

The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of trinexapac-ethyl in OECD 201 test medium.

Exception: only 2 recovery samples were prepared instead of 5 recovery samples. This deviation is not relevant, as the samples were analysed without sample work-up procedure.

A 2.1.1.6.4 Analytical method 4

A 2.1.1.6.4.1 Method validation - Effects on aquatic macrophytes

Comments of zRMS:	The validation has been accepted. The quantification of the test item was based on the analyte trinexapac-ethyl and was performed by HPLC analysis with UV/VIS-detection. 7 point calibration was made. The average recoveries were found to be 93% of the spiked values (n = 4). No correction for the recovery rate was made. The method is suitable for the purpose.

Reference:	KCP 5.1.2/04 (also filed under KCP 10.2.1/04)
Report	Höger S., 2009, AG-T3-175 EC: Toxicity of AG-T3-175 EC to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test Report no: C45577, Sponsor reference no: 90011801_000066083
Guideline(s):	Not mentioned, in line with SANCO/3029/99 rev. 4
Deviations:	Yes. Repeatability and accuracy was assessed for 2 instead of 5 replicates at 2 fortification levels. This deviation is not relevant, as the samples were analysed without sample work-up procedure.
GLP:	Yes
Acceptability:	Yes. Validation meets guideline criteria (SANCO/3029/99 rev. 4, 11/07/2000) with minor deviations.

Materials and methods

The analytical method for determination of trinexapac-ethyl in 20X AAP growth medium was validated. The limit of quantification (LOQ) of the method was 22.1 mg/L. The quantitative measurements of trinexapac-ethyl were performed using HPLC-UV (280 nm).

Test substances:

Test substance	AG-T3-175 EC
Batch No:	D-I0703
Active ingredient content	Trinexapac-ethyl, 180 g/L
Reference substance:	Trinexapac-Ethyl purified
Batch No.	D-TR-38
Purity:	98.2% (w/w)

Sample preparation for trinexapac-ethyl determination

Treatment samples and control samples were thawed at 25 °C for 1.5 hours and shaken manually to obtain homogeneous sample solutions. Aliquots of the samples were analyzed by HPLC with UV/VIS detection.

Equipment for trinexapac-ethyl determination:

HPLC system	Auto sampler: Merck-Hitachi L-7200, Pump: Merck-Hitachi L-7100 Detector: Merck-Hitachi L-7400, Column oven: Merck-Hitachi L-7300
Column:	Phenomenex Luna C18 (2); 50 mm x 4.6 mm; 3 µm Pre-Column: Phenomenex C18 (Octadecyl ODS); 4 x 3 mm

Column Temperature:	Room temperature
Injection Volume:	100 µL
Mobile phases:	0.4% phosphoric acid in water/acetonitrile (v/v;7/3)
Flow rate:	1 mL/min
Detection wave length:	280 nm
Retention time:	Trinexapac-ethyl: Approximately 10.9 minutes

Results and discussions

Table A 7: Recovery results from method validation of trinexapac-ethyl in 20X AAP growth medium

Matrix	Nominal concentration of test item (mg a.s./L)	Concentration determined in the spiked sample (mg/L)	Recovery (%)	Mean recovery (%)	Overall mean (%)
20X AAP growth medium	Control	<0.135 mg analyte/L (smallest calibration level)	n.a.		
	3.27	3.05, 3.05	93, 93	93	93
	109	101, 102	93, 93	93	

n.a. = not applicable

Table A 8: Characteristics for the analytical method used for validation of trinexapac-ethyl residues in aqueous solution

	Trinexapac-ethyl
Specificity	Two dilution water blank samples were used to show specificity and blank values being <30 % of the LOQ.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in acetonitrile/water (v/v; 3/7). The linearity was determined with nine standard solutions ranging from 0.103 to 51.6 mg/L. The calibrations were found linear with coefficients of determination $R \geq 0.999$ y = 1146050x + 3928.6, weighting 1/y, R2 = 1.0000 Calibration data and the graph is presented in the report.
Matrix effects	Not tested
Limit of determination / quantification	Limit of quantification is 3.27 mg a.s./L in 20X AAP growth medium

Conclusion

The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of trinexapac-ethyl in 20X AAP growth medium.

Exception: only 2 recovery samples were prepared instead of 5 recovery samples. This deviation is not relevant, as the samples were analysed without sample work-up procedure.

A 2.1.1.6.5 Analytical method 5

A 2.1.1.6.5.1 Method validation - Chronic toxicity to the honeybee

Comments of zRMS:	The validation has been accepted. An analytical method for the determination of trinexapac-ethyl in 50 % (w/v) aqueous sucrose solution was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. Specimen analysis was performed by direct injection of 50 % (w/v) aqueous sucrose solution samples and quantification by HPLC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 36.1 mg/kg of test item (6.25 mg/kg of trinexapac-ethyl). The analyte was not detectable in the 50 % (w/v) aqueous sucrose solution used for recovery samples. The limit of detection (LOD) was defined as 30 % of the limit of quantification (1.88 mg/kg of trinexapac-ethyl). The calibration function was linear within the range from 1 ng/mL to 10 ng/mL with R2 0.999 covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a diluted sample. The recovery was determined by fortification of untreated 50 % (w/v) aqueous sucrose solution with the test item. All mean recovery values at fortification levels of 36.1 mg/kg of test item and 7960 mg/kg of the test item comply with the standard acceptance criteria of the guidance document SANCO/3029/99 rev. 4 11/07/2000, with evaluation of two mass transitions. The mean recoveries at each fortification level were in the range between 70 % and 110 % with relative standard deviations below 20 %.

Reference:	KCP 5.1.2/05 (also filed under KCP 10.2.1/03)
Report	Oberrauch, S., 2018a, Trinexapac-ethyl 175 EC: Honey Bee (*Apis mellifera L.*) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory, Report no: S18-00067, Sponsor reference no: 90020907
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	none
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for determination of trinexapac-ethyl in 50 % (w/v) aqueous sucrose solution was validated. Specimen analysis was performed by direct injection of 50 % (w/v) aqueous sucrose solution samples and quantification by HPLC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 36.1 mg/kg of test item (6.25 mg/kg of trinexapac-ethyl).

Test substances:

Test substance	Trinexapac-ethyl 175 EC (= AG-T3-175 EC1)
Batch No:	8227
Active ingredient content	Trinexapac-ethyl, 172.9 g/L
Analytical standard:	Trinexapac-Ethyl
Batch No.	BCBT3285

Purity: 98.7 area%

Sample preparation for trinexapac-ethyl determination

50 % (w/v) aqueous sucrose solution samples (5 mL + 50 µL formic acid in 20 mL glass bottle) were stored deep-frozen (- 18 °C) until analysis. In the analytical laboratory, the samples were thawed, shaken well followed by a two-step dilution. For first step the samples were diluted with 15 mL acetonitrile/water (1:1, v/v) + 0.1 % formic acid. For second step 1 mL of each sample was diluted with 9 mL acetonitrile/water (1:1, v/v) + 0.1 % formic acid in 15 mL plastic tubes.
Prior to analysis by HPLC-MS/MS the samples were further diluted with matrix blank extract.

Recovery samples were prepared by fortifying untreated 50 % (w/v) aqueous sucrose solution with the test item. 2 mL of recovery samples were mixed with 20 µL formic acid and shaken well and diluted in two steps. For first step the samples were diluted with 6 mL acetonitrile/water (1:1, v/v) + 0.1 % formic acid, ultrasonicated and shaken. For second step see above.
Prior to analysis by HPLC-MS/MS the samples were further diluted with matrix blank extract.
For matrix blank extract two untreated solution samples (solution blanks) were prepared. The first step of the preparation was performed as described above for recovery samples. For second step 8 mL of each sample was diluted with 72 mL acetonitrile/water (1:1, v/v) + 0.1 % formic acid and combined in a 250 mL glass bottle.

Equipment for trinexapac-ethyl determination:

HPLC system	Thermo Accela 1250 HPLC pump with Thermo Accela Open autosampler
Column:	Phenomenex Luna 3u C18 (2) 100A, 50 mm x 3 mm i.d., 3 µm mean particle size with 4 mm guard column
Column Temperature:	40°C
Injection Volume:	10 µL
Mobile phases:	Eluent A: Water Eluent B: Acetonitrile Eluent C: Methanol Eluent D: 1 % formic acid in water
Gradient:	

Time (min)	%Eluent A	% Eluent B	% Eluent C	% Eluent D	Flow (µL/min)
0.0	78.0	0	20.0	2.0	400
3.0	3.0	0	95.0	2.0	400
6.0	3.0	0	95.0	2.0	400
6.01	78.0	0	20.0	2.0	400
8.0	78.0	0	20.0	2.0	400

Retention time:	Approx. 4.1 min

Mass spectrometric conditions

MS System	Thermo TSQ Vantage triple quadrupole system
Ionisation type	Electrospray ionization (ESI)
Polarity	Positive ion mode
Scan type	MS/MS
Analyte monitored **Trinexapac-ethyl**	

Ion mass transition monitored [m/z]	Collision energy [V]	Quadrupole 1 width [amu]	Quadrupole 3 width [amu]
253 → 207*	11	0.7	0.7
253 → 69	24	0.7	0.7

*used as quantifier

Results and discussions

Table A 9: Recovery results from method validation of trinexapac-ethyl in 50 % (w/v) aqueous sucrose solution

Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).

Matrix	Test Item Fortification level [mg/kg]	Trinexapac-ethyl Nominal [mg/kg]	Recovery (%)	Mean recovery (%)	Rel. Std. Dev. [%]	Replicates
Quantifier: 253 → 207						
50 % (w/v) aqueous sucrose solution	36.1	6.25	103 107 104 106 102	104	2	5
	7960*	1380 1320 1340 1410 1430	96 98 96 98 92	96	3	5
Qualifier: 253 → 69						
50 % (w/v) aqueous sucrose solution	36.1	6.25	101 104 103 102 99	102	2	5
	7960*	1380 1320 1340 1410 1430	96 98 96 99 99	98	2	5

* mean value

Table A 10: Characteristics for the analytical method used for validation of trinexapac-ethyl residues in aqueous solution

	Trinexapac-ethyl
Specificity	The analyte was determined in the final specimen dilutions by use of LC-MS/MS detection. For the analyte, one MS/MS mass transition was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of specimens. Untreated 50 % (w/v) aqueous sucrose solution samples were analysed according to the method to investigate the presence of residue and/or background interference at the retention time of trinexapac-ethyl. The samples showed no significant interference (above 30 % of LOQ) at the retention time of the analyte in any investigated 50 % (w/v) aqueous sucrose solution, therefore showing that the method is highly specific.
Calibration (type, number of data points) **Calibration range**	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 1 ng/mL to 10 ng/mL This range covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detected in any (diluted) sample extract.

	Trinexapac-ethyl
	The calibration curve was linear with coefficients of determination (R^2) 0.999. Linear regression was performed with 1/x-weighting. $y = 861.312 + 9725.76x.1$, $R^2 = 0.9991$, weighting 1/x. Calibration data and the graph is presented in the report.
Matrix effects	Not tested, but matric matched calibration standard used.
Limit of determination / quantification	The LOQ of the method of 36.1 mg/kg of the test item (6.25 mg/kg of trinexapac-ethyl) was confirmed for trinexapac-ethyl in 50 % (w/v) aqueous sucrose solution. The LOD was set at 30 % of the LOQ which is 1.88 mg/kg of trinexapac-ethyl.

Conclusion

The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of trinexapac-ethyl in 50 % (w/v) aqueous sucrose solution with a LOQ of 36.1 mg/kg of the test item (6.25 mg/kg of trinexapac-ethyl).

A 2.1.1.6.6 Analytical method 6

A 2.1.1.6.6.1 Method validation - Toxicity to honeybee larvae

Comments of zRMS:	The validation has been accepted. An analytical method for the determination of trinexapac ethyl in deionized water was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. Specimen analysis was performed by direct injection of deionized water samples and quantification by HPLC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 200 mg/L of test item (34.6 mg/L of trinexapac ethyl). The analyte was not detectable in the deionized water used for recovery samples. The limit of detection (LOD) was defined as 30 % of the limit of quantification (10.4 mg/L of trinexapac ethyl). The calibration function was linear within the range from 1 ng/mL to 10 ng/mL with R2 0.998 covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a diluted sample. The recovery was determined by fortification of untreated deionized water with the test item. All mean recovery values at fortification levels of 200 mg/L of test item and 41500 mg/L of the test item comply with the standard acceptance criteria of the guidance document SANCO/3029/99 rev. 4 11/07/2000, with evaluation of two mass transitions. The mean recoveries at each fortification level were in the range between 70 % and 110 % with relative standard deviations below 20 %. The reason for amendment was typing error.

Reference:	KCP 5.1.2/06 (also filed under KCP 10.3.1.3/01)
Report	Oberrauch, S., 2018b, Trinexapac-ethyl 175 EC - Honey Bee (*Apis mellifera L.*) 22 Day Larval Toxicity Test (Repeated Exposure)- Report Amendment 1 Report no: S18-00066, Sponsor reference no: 90020906
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	none

GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for determination of trinexapac-ethyl in deionized water was validated. Specimen analysis was performed by direct injection of deionized water samples and quantification by HPLC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 200 mg/L of test item (34.6 mg/L of trinexapac ethyl).

Test substances:

Test substance	Trinexapac-ethyl 175 EC (= AG-T3-175 EC1)
Batch No:	8227
Active ingredient content	Trinexapac-ethyl, 172.9 g/L
Analytical standard:	Trinexapac-Ethyl
Batch No.	BCBT3285
Purity:	98.7 area%

Sample preparation for trinexapac-ethyl determination
After receipt in the analytical laboratory, deionized water samples (2 mL + 20 µL formic acid) in 20 mL glass bottle) were stored deep-frozen (- 18 °C) until analysis. In the analytical laboratory, the samples were thawed to ambient temperature, shaken well. 1 mL of the sample was mixed with 9 mL acetonitrile/water (1:1, v/v). Then 1 mL of diluted sample was further diluted with 9 mL acetonitrile/water (1:1, v/v). Prior to analysis by HPLCMS/MS the samples were further diluted with matrix blank extract.

Recovery samples (2mL) at LOQ fortification level were prepared by fortifying untreated deionized water with the test item. Each sample was stabilised with 20 µL formic acid and analysed as described above.
For recovery samples at 41500 mg test item/L the test item was weighed into 20 mL glass bottles. The samples were filled with 2 mL deionized water. Additionally, a volume of deionized water was added adjusted to the weight. Each sample was stabilized with 20 µL formic acid and analysed as described above.
For matrix blank extract 2 mL of deionized water were diluted with 198 mL acetonitrile/water (1:1, v/v). The mix was used for dilution of the samples and for calibration.

Equipment for trinexapac-ethyl determination:

HPLC system	Thermo Accela 1250 HPLC pump with Thermo Accela Open autosampler
Column:	Phenomenex Luna 3u C18 (2) 100A, 50 mm x 3 mm i.d., 3 µm mean particle size with 4 mm guard column
Column Temperature:	40°C
Injection Volume:	10 µL
Mobile phases:	Eluent A: Water Eluent B: Acetonitrile Eluent C: Methanol Eluent D: 1 % formic acid in water
Gradient:	

Time (min)	%Eluent A	% Eluent B	% Eluent C	% Eluent D	Flow (µL/min)
0.0	78.0	0	20.0	2.0	400
3.0	3.0	0	95.0	2.0	400
6.0	3.0	0	95.0	2.0	400
6.01	78.0	0	20.0	2.0	400
8.0	78.0	0	20.0	2.0	400

Retention time:	Approx. 4.1 min

Mass spectrometric conditions

MS System	Thermo TSQ Vantage triple quadrupole system			
Ionisation type	Electrospray ionization (ESI)			
Polarity	Positive ion mode			
Scan type	MS/MS			
Analyte monitored	Ion mass transition monitored [m/z]	Collision energy [V]	Quadrupole 1 width [amu]	Quadrupole 3 width [amu]
Trinexapac-ethyl	253 → 207*	11	0.7	0.7
	253 → 69	24	0.7	0.7

*used as quantifier

Results and discussions

Table A 11: Recovery results from method validation of trinexapac-ethyl in deionized water

Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).

Matrix	Test Item Fortification level [mg/L]	Trinexapac-ethyl Nominal [mg/L]	Recovery (%)	Mean recovery (%)	Rel. Std. Dev. [%]	Replicates
Quantifier: 253 → 207						
Deionized water	200	34.6	102, 103, 100, 102, 108	103	3	5
	41500	7180	96, 94, 101, 96, 101	98	3	5
Qualifier: 253 → 69						
Deionized water	200	34.6	100, 97, 95, 95, 104	98	4	5
	41500	7180	97, 93, 99, 99, 97	97	3	5

* mean value

Table A 12: Characteristics for the analytical method used for validation of trinexapac-ethyl residues in aqueous solution

	Trinexapac-ethyl
Specificity and Selectivity	The analyte was determined in the final specimen extracts by use of LC-MS/MS detection. For the analyte, one MS/MS mass transition was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of specimens. Untreated deionized water samples were analysed according to the method to investigate the presence of residue and/or background interference at the retention time of trinexapac ethyl. The samples showed no significant interference (above 30 % of LOQ) at the retention time of the analyte in any investigated deionized water, therefore showing that the method is highly specific.
Calibration (type, number of data points) **Calibration range**	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 1 ng/mL to 10 ng/mL This range covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detected in any (diluted) sample. The calibration curve was linear with coefficients of determination (R^2) ≥ 0.998. Linear regression was performed with 1/x-weighting

	Trinexapac-ethyl
	$y = 2342.44 + 12830.2x$, $R^2 = 0.9995$, weighting 1/x. Calibration data and the graph is presented in the report
Matrix effects	Not tested, but matric matched calibration standard used.
Limit of determination / quantification	The limit of quantification (LOQ) of the analytical method was 200 mg/L of test item (34.6 mg/L of trinexapac ethyl). The LOD was set at 30 % of the LOQ which is 10.4 mg/L of trinexapac ethyl.
Storage Stability	The results show that trinexapac ethyl was stable under deep-frozen conditions (- 18°C with minor fluctuations -16 °C for 0.5 h until sample preparation) in deionized water for at least 66 days.

Conclusion

The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of trinexapac-ethyl in deionized water with a LOQ of 200 mg/L of the test item (34.6 mg/L of trinexapac-ethyl).

A 2.1.1.6.7 Analytical method 7

A 2.1.1.6.7.1 Method validation – Analytic to vegetative vigour and seedling emergence

Comments of zRMS:	The method validation has been accepted. The recovery determined was within 70-110%. RSD was < 20%. SANCO/3029/99 rev. 4 criteria are fulfilled. The method is suitable for the intended purpose.

Reference:	KCP 5.1.2/07 (also filed under KCP 10.6.2/01)
Report	Friedrich, S., 2008a: Terrestrial (non-target) plant test with Trinexapac-ethyl 175 EC: Vegetative vigour test of non-target terrestrial plants Report no: 08 10 48 030 S, Sponsor reference no: 90018044_000081140
and	KCP 5.1.2/08 (filed under KCP 10.6.2/02) Friedrich, S., 2008b: Terrestrial (non-target) plant test with Trinexapac-ethyl 175 EC: Seedling emergence and seedling growth test of non-target terres-trial plants Report no: 08 10 48 029 S, Sponsor reference no: 90018045_000081141
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	none
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method for determination of trinexapac-ethyl in the test solutions of the vegetative vigour test was validated. Specimen analysis was performed after dilution by HPLC-UV detection.

<u>Test substances:</u>

Test substance	Trinexapac-ethyl 175 EC
Batch No:	D-I0703

Active ingredient content	Trinexapac-ethyl, 172.9 g/L (nominal), 180 g/L analysed
Analytical standard:	Trinexapac-ethyl, Dr Ehrenstorfer
Batch No.	60105
Purity:	99.0 ± 0.5%

Sample preparation for trinexapac-ethyl determination
The samples were analysed direct after the preparation of the test and control solution. The specimen were diluted during the first dilution step with water followed by a second dilution step 1:10 with fluid phase (500 mL acetonitrile mixed with 500 mL water + 1 mL phosphoric acid).

Fortification solution
The trinexapac-ethyl concentration of the validation low solutions and validation high solutions were approximately 64 and 2050 µg/l respectively. Five replicates of each concentration level were weighted diluted and measured. The samples were diluted during the first dilution step with water followed by a second dilution step 1:10 with fluid phase (500 mL acetonitrile mixed with 500 mL water + 1 mL phosphoric acid). Two unfortified control samples (water) and two fluid phase samples were used as fortification blank samples.

Equipment for trinexapac-ethyl determination:

HPLC system	Jasco Plus series
Column:	Phenomenex Aqua 5 µm C18 125A, 150 x 2 mm with 4 mm Aqua 5 µm C18 guard column
Column Temperature:	25°C
Injection Volume:	10 µL
Mobile phases:	Acetonitrile / water, (50:50; v/v) + 0.1% phosphoric acid
Flow rate:	0.3 mL/min
Gradient:	isocratic
Retention time:	Approx. 5 min for trinexapac-ethyl
Wavelength:	280 nm

Results and discussions

Table A 13: Recovery results from method validation of trinexapac-ethyl in deionized water

The determination of the recovery was performed at two concentration levels; at the lower working range and at the upper working range of the biological test.
Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70 - 110 % mean recovery, 20 % RSD).

Matrix	Nominal concentration of trinexapac-ethyl [mg/L]	Dilution factor	Nominal concentration of trinexapac-ethyl [µg/L]	Concentration of trinexapac-ethyl [µg/L]	Recovery ± Rel. Std. Dev. (%)	Mean Recovery ± Rel. Std. Dev. (%)	Replicates
Fluid phase	0.00	0	0.00	0.00	-	-	2
Blank	0.00	0	0.00	0.00	-	-	2
Deionized water	64.20 63.95 93.90 64.20 64.15	100	642.0 639.5 639.0 642.0 641.5	605.76 591.21 607.21 619.80 614.06	94.4 92.5 95.5 96.6 95.7	94.8 ± 1.6	5
Deionized wate	2055.0 2047.0 2045.4 2055.1 2053.5	1000	2055.0 2047.0 2045.4 2055.1 2053.5	1943.91 1938.62 1911.78 1991.30 1945.96	94.8 94.7 93.5 96.6 94.8	94.9 ± 1.3	5

Table A 14: Characteristics for the analytical method used for validation of trinexapac-ethyl residues in aqueous solution

	Trinexapac-ethyl
Specificity	The method is specific for to analyte in the test solution since no interfering result of the analyte > 30 % of the LOQ in the validation blank solution could be observed.
Calibration (type, number of data points) **Calibration range**	The calibration of the analytical method was performed in the calibration range of 312 - 3122 µg/L. The calibration line was measured with 6 calibration points in duplicate. The plot of a linear calibration function is resented in the report.
Matrix effects	Not relevant
Limit of determination / quantification	The LOQ of the analytical method in the report was defined as the UV signal height ration of 1:10 to be 30 µg/L. But in line with SANCO/83029/99 rev 4 requirements the LOQ was defined as to the lowest acceptably validated fortification level (see tables above) to be 64 µg/L.

Conclusion

The analytical method fulfils the requirements of SANCO/3029/99 rev. 4, suitable for the determination of trinexapac-ethyl in deionized water.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.1.2.1 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted