

Test Material: Trinexapac-ethyl

MRID: 49282805

Title: Trinexapac-ethyl - Residue Method (GRM020.08B) for the Determination of CGA300405 and CGA313458 Metabolites of Trinexapac-ethyl in Surface Water

MRID: 49282806

Title: Trinexapac-ethyl - Independent Laboratory Validation of "Trinexapac-ethyl - Residue Method (GRM020.08B) for the Determination of CGA300405 and CGA313458 Metabolites of Trinexapac-ethyl in Surface Water"

EPA PC Code: 112602

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lynne Binari

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Date: 8/11/14

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Date: 8/11/14

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Date: 8/11/14

Analytical method for trinexapac-ethyl transformation products CGA300405 and CGA313458 in water

Reports: ECM: EPA MRID No.: 49282805. Mayer, L. 2013. Trinexapac-ethyl - Residue Method (GRM020.08B) for the Determination of CGA300405 and CGA313458 Metabolites of Trinexapac-ethyl in Surface Water. Report No.: GRM020.08B. Task No.: TK0101067. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 53 pages. Final report issued January 17, 2013.
ILV: EPA MRID No.: 49282806. Schierhoff, R. 2012. Trinexapac-ethyl - Independent Laboratory Validation of "Trinexapac-ethyl - Residue Method (GRM020.08B) for the Determination of CGA300405 and CGA313458 Metabolites of Trinexapac-ethyl in Surface Water". Report No.: ABC Study No. 68826. Task No.: TK0060917. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 144 pages. Final report issued December 11, 2012.

Document No.: MRIDs 49282805 & 49282806

Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with either USEPA FIFRA or OECD Good Laboratory Practice (GLP) standards (p. 3 of MRID 49282805). Signed and dated No Data Confidentiality and GLP non-compliance statements were provided (pp. 2-3). Quality Assurance and Authenticity Certification statements were not provided.
ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 49282806). Signed and dated No Data Confidentiality, GLP, Quality Assurance and [authenticity] Certification statements were provided (pp. 2-4, 6).

Classification: This analytical method is classified as **Supplemental**. No originating ECM performance data were reported. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures.

PC Code: 122602

Reviewer: Christopher M. Koper, M.S., Chemist **Signature:**
Date: September 30, 2014

Executive Summary

This analytical method, Syngenta Residue Method GRM020.08B, is designed for the quantitative determination of trinexapac-ethyl transformation products CGA300405 and CGA313458 in water using LC/MS/MS. The method is quantitative for both analytes at the stated LOQ of 10 µg/L. The LOQ is less than the lowest toxicological level of concern in water. The method specifies the correction of procedural recoveries for residues in the controls; however, the reported recovery results were not corrected. No major issues were discovered by the independent laboratory.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
CGA300405	49282805	49282806		Water	17/01/2013	Syngenta Crop Protection	LC/MS/MS	10 µg/L
CGA313458								10 µg/L

I. Principle of the Method

Water (1.0 mL) was diluted to 10 mL with ultra pure water (UPW):acetonitrile (70:30, v:v) and vortexed (p. 14; Appendix 4, p. 53 of MRID 49282805). The method LOQ (10 µg/L, ppb) can be diluted 1 to 10 while maintaining acceptable signal/noise ratio at 1 µg/L (p. 14 of MRID 49282805). An aliquot (1.0 mL) was transferred to an autosampler vial and analyzed by LC/MS/MS (p. 14; Appendix 1, p. 50; Appendix 4, p. 53 of MRID 49282805).

Samples were analyzed for trinexapac-ethyl products CGA300405 and CGA313458 by HPLC (ACE 3 C18, 3.0 mm x 50 mm column) using a mobile phase of (A) methanol:water (50:50, v:v) in 10mM ammonium acetate and (B) acetonitrile:water (90:10, v:v) in 10mM ammonium acetate [percent A:B at 0.0-0.5 min. 0:100, 1.0-2.0 min. 100:0, 3.0 min. 0:100) with MS/MS (TurboIonSpray, negative) detection and multiple reaction monitoring (MRM; pp. 15-16 of MRID 49282805). Injection volume was 50 µL. Analytes were identified using two ion transitions; one for quantitation (Q) and one for confirmation (C; p. 17 of MRID 49282805). Ion transitions monitored were as follows: m/z 203→69 (Q) and m/z 203→157 (C) for CGA300405 and m/z 241→83 (Q) and m/z 241→69 (C) for CGA313458.

In the ILV, the 1.0 mL water sample was diluted to 14 mL with water:acetonitrile (70:30, v:v; p. 19 of MRID 49282806). HPLC injection volume was 20 µL (p. 20). In addition, a minimum of 50 equilibration injections were required to achieve constant instrumental response prior to acquiring data for the sample set (p. 19). None of these modifications were significant deviations from the original ECM.

LOQs and LODs for both analytes were the same in the ECM and ILV at 10 µg/L and 0.1 µg/L, respectively (p. 11 of MRID 49282805; p. 13; Figures 1-2, p. 36; Figures 15-16, p. 43; Appendix 2, pp. 97, 102 of MRID 49282806).

II. Recovery Findings

ECM (MRID 49282805): Originating ECM performance data were not reported. All reported results (Tables 3-6, pp. 26-29; Figures 3-36, pp. 32-48) were generated in the ILV (p. 23; Tables 7-9, pp. 32-34; Figures 1-34, pp. 36-53 of MRID 49282806).

ILV (MRID 49282806): Mean recoveries and RSDs were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of trinexapac-ethyl products CGA300405 and CGA313458 in surface water (p. 23). The method was validated with the first trial (p. 22). The water matrix was well characterized (Table 1, p. 27).

Table 2. Initial Validation Method Recoveries for Analytes in Water

Analyte	Fortification Level ($\mu\text{g/L}$)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ¹	Relative Standard Deviation (%)
CGA300405	LOQ					
	10x LOQ		No originating ECM performance data were reported. ¹			
CGA313458	LOQ					
	10x LOQ					

¹ Results reported in the ECM (Tables 1-6, pp. 25-29; Figures 3-36, pp. 32-48 of MRID 49282805) are from the ILV (p. 23; Tables 1-2, p. 27; Tables 7-9, pp. 32-34; Figures 1-34, pp. 36-53 of MRID 49282806).

Table 3. Independent Validation Method Recoveries for Analytes in Surface Water

Analyte	Fortification Level ($\mu\text{g/L}$)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
CGA300405	Quantitation (Primary) ion					
	10 (LOQ)	5	100-111	106	4.7	4.4
	100	5	103-114	109	3.9	3.6
	Confirmation ion					
	10 (LOQ)	5	79-115	102	14.3	13.9
	100	5	104-115	109	5.0	4.6
CGA313458	Quantitation (Primary) ion					
	10 (LOQ)	5	68-82	76	6.1	8.0
	100	5	101-116	107	7.2	6.7
	Confirmation ion					
	10 (LOQ)	5	75-84	79	3.8	4.9
	100	5	99-117	106	7.9	7.4

Data were obtained from Tables 3-6, pp. 28-31 of MRID 49282806.

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD values for CGA300405 and CGA313458 in water were 10 $\mu\text{g/L}$ and 0.1 $\mu\text{g/L}$, respectively (p. 21 of MRID 49282805; p. 24; Figures 1-2, p. 36; Figures 15-16, p. 43; Appendix 2, pp. 97, 102 of MRID 49282806). The ECM defined the LOQ as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-120% with a RSD of \leq 20% has been obtained (p. 21 of MRID 49282805). And, in general, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in a matrix control sample at the corresponding retention

times of the analytes. The LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in a matrix control sample at the corresponding retention times of the analytes; an estimate of the LOD can be taken as three times background noise.

Table 4. Method Characteristics

	CGA300405	CGA313458
Limit of Quantitation (LOQ)	10 µg/L	10 µg/L
Limit of Detection (LOD)	0.1 µg/L (0.093 µg/L)	0.1 µg/L (0.093 µg/L)
	Quantitation (Primary) ion	
Linearity (calibration curve r^2 and concentration range) ¹	$r^2 = 0.9962$ (0.19-9.3 ng/mL)	$r^2 = 0.9910$ (0.093-9.3 ng/mL)
	Confirmation ion	
	$r^2 = 0.9884$ (0.093-9.3 ng/mL)	$r^2 = 0.9922$ (0.19-9.3 ng/mL)
Repeatable	Yes	Yes
Reproducible ²	Yes	Yes
Specific	Yes	Yes
	There were significant matrix effects from the water used in the ILV; however, matrix matched external calibration standards were used to compensate, and the methodology is included in the ECM (pp. 11, 13-14, 20 of MRID 49282805; p. 24; Table 9, p. 34 of MRID 49282806).	

Data were obtained from p. 24; Figures 1-2, p. 36; Figures 15-16, p. 43 of MRID 49282806.

¹ Reviewer-calculated r^2 values from reported r values of 0.9981 (Q) and 0.9942 (C) for CGA300405, and 0.9955 (Q) and 0.9961 (C) for CGA313458 (Figures 29-32, pp. 50-51; Appendix 5, pp. 122-125 of MRID 49282806).

Reviewer-calculated calibration curves verified linearity for the ILV ($r^2 = 0.9959-0.9982$; see DER Attachment 2).

² The ILV validated the method at the LOQ; performance data establishing the LOQ at 10 µg/L were not provided with the ECM.

IV. Method Deficiencies and Reviewer's Comments

1. This method, Residue Method GRM020.08B, supersedes Residue Method GRM020.08A; version B has the addition of transformation product CGA313458 and improved chromatography (p. 11 of MRID 49282805).
2. No originating ECM performance data were reported. All results reported in the ECM (Tables 1-6, pp. 25-29; Figures 3-36, pp. 32-48 of MRID 49282805) are from the ILV (p. 23; Tables 1-2, p. 27; Tables 7-9, pp. 32-34; Figures 1-34, pp. 36-53 of MRID 49282806). A Draft ECM was appended to the ILV which contained chromatograms of standards, matrix control samples, and samples fortified at LOQ with CGA300405 and CGA313458 apparently generated during method development; recovery results were not included (Appendix 2, pp. 66-113).
3. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. The LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-120% with a RSD of $\leq 20\%$ has been obtained (p. 21 of MRID 49282805). And, in general, the response for

an analyte peak should be no lower than four times the mean amplitude of the background noise in a matrix control sample at the corresponding retention times of the analytes. The LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in a matrix control sample at the corresponding retention times of the analytes; an estimate of the LOD can be taken as three times background noise. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

4. There were significant matrix effects from the water used in the ILV; however, matrix matched external calibration standards were used to compensate, and the methodology is included in the ECM (pp. 11, 13-14, 20 of MRID 49282805; p. 24; Table 9, p. 34 of MRID 49282806).
5. Interferences in the reagent blank and matrix control samples were $\leq 20\%$ of the LOQ and were considered negligible (p. 12; Figures 7-12, pp. 39-41; Figures 21-26, pp. 46-48; Appendix 5, pp. 120-125 of MRID 49282806).
6. Although the method calculations specify the correction of sample recoveries for any residues found in the matrix control samples (p. 18 of MRID 49282805; Appendix 4, pp. 118-119 of MRID 49282806), the ILV spreadsheets indicate that the reported sample recoveries were not corrected (Appendix 5, pp. 122-125).
7. It was reported for the ILV that a single analyst completed a sample set consisting of 12 samples in an approximate 4 hour working period with LC/MS/MS analysis performed overnight (p. 23 of MRID 49282806).
8. CGA300405 and CGA313458, at 10 and 100 $\mu\text{g/L}$, were found to be stable in the sample final solution (water:acetonitrile, 70:30, v:v) after 9 days of storage at 4°C (p. 24; Tables 7-8, pp. 32-33 of MRID 49282806).

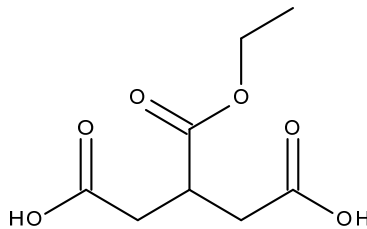
V. References

- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

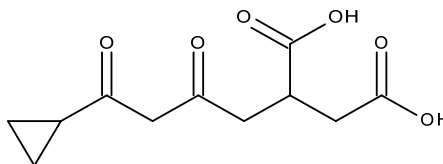
CGA300405 (CSAA382277, Tricarballic acid, ethyl ester; Figure 1, p. 31 of MRID 49282805; Appendix 3, p. 114 of MRID 49282806)

IUPAC Name: 3-Ethoxycarbonyl-pentanedioic acid
CAS Name: 1,2,3-Propanetricarboxylic acid, 2-ethyl ester
CAS Number: Not reported.
SMILES String: CCOC(=O)C(CC(=O)O)CC(=O)O



CGA313458 (CSAA394888)

IUPAC Name: 2-(4-Cyclopropyl-2,4-dioxo-butyl)-succinic acid
CAS Name: Butanedioic acid, (4-cyclopropyl-2,4-dioxobutyl)-
CAS Number: Not reported.
SMILES String: C1CC1C(=O)CC(=O)CC(CC(=O)O)C(=O)O

**Attachment 2: Calculations**

Calculations