

Analytical method for [Propiconazole, and its degradates: CGA-217495, CGA-91305, CGA-136735, CGA-118244, CGA-118245, and CGA-71019 in soil and water.

Reports: ECM: Analytical Method for the Determination of Propiconazole (CGA64250) and Its Degradates CGA217495, CGA91305, CGA118244, CGA118245, CGA136735, and CGA71019 in Soil and Water by High Performance Liquid Chromatography with Mass Spectrometric Detection Including Method Validation Data, Final Report.


ILV: Independent Laboratory Validation: Syngenta Residue Analytical Method No. AG-677 and Modified Method AG-677 for Water, with a 0.02 ppb Limit of Quantitation: "Analytical Method for the Determination of Propiconazole (CGA-64250) and its Degradates CGA-217495, CGA-91305, CGA-1 18244, CGA-1 18245, CGA-136735, and CGA-71019 in Soil and Water by High Performance Liquid Chromatography with Mass Spectrometric Detection Including Method Validation Data". May 4, 2004.

Document No.: MRIDs 48697002 & 48713801
Guideline: 850.7100


Statements: The studies were conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice Standards (40 CFR Part 160). A signed and dated Data Confidentiality, GLP Compliance, Quality Assurance, and Authenticity Certification statements were provided for the method and ILV reports.

Classification: This analytical method is classified as *acceptable for* Propiconazole (CGA64250) and Its Degradates CGA217495, CGA91305, CGA118244, CGA118245, CGA136735, and CGA71019 in Soil and Water.

PC Code: 122101
Reviewer: Ibrahim Abdel-Saheb
Environmental Scientist
ERB6/Environmental Fate and
Effects Division (7507P)

Signature: 
Date: 9-18-13

Secondary Reviewer: Andrew Shelby
Environmental Scientist
ERB6/Environmental Fate and
Effects Division (7507P)

Signature: 
Date: 9-18-13

Analytical method for [Propiconazole, and its degradates: CGA-217495, CGA-91305, CGA-136735, CGA-118244, CGA-118245, and CGA-71019 in soil and water.

Executive Summary

This analytical method, [EPA OPPTS 860. 1340], is designed for the quantitative determination of Propiconazole, and its degradates: CGA-217495, CGA-91305, CGA-136735, CGA-118244, CGA-118245, and CGA-71019 in soil and water using High Performance Liquid Chromatography with Mass Spectrometric Detection. The method is quantitative for the analytes or a subset thereof at the stated LOQ(s) of 5 ppb for all analytes in soil and 0.1 ppb for all analytes in water.

The LOQ is less than the lowest toxicological level of concern in Nonvascular Plants.

For nearly all of the summary results the average recoveries were between 70 and 120 percent of theoretical with relative standard deviations of less than 20 percent as recommended in the EPA data reporting guidelines (EPA 712-C-96-348). One exception was for CGA-136735 in well water, using the modified AG-677 method, where the average recovery was 125 percent at the 0.02 ppb LOQ. In addition, RSDs of 22.0% and 22.7% were obtained for the CGA-71019 analyses at the 0.1 ppb LOQ for well water and surface water, respectively, using the original AG-677 method.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Propiconazole, and its degradates: CGA-217495, CGA-91305, CGA-136735, CGA-118244, CGA-118245, and CGA-71019	48697002	48713801	Acceptable	Water/ Soil	10/30/1997	Syngenta Crop Protection, LLC	HPLC/MS	5 ppb (soil) 0.1 ppb (water)

I. Principle of the Method

SOIL

Weigh and record 20 ± 0.1 g of soil sample into a 250-mL round bottom flask. Add 100 mL of 70% methanol in water. Briefly swirl the contents. Attach to a reflux condenser, chilled with cold water, and heat under reflux for one hour using an electrical heating mantle. Remove the heating mantle from the round bottom flask and permit the sample to cool before disconnecting from the reflux condenser. Transfer the sample to a centrifugable polyallomer bottle. Centrifuge the sample at approximately 9,000 RPM for 10 minutes, or at an alternate speed and time if the results are considered satisfactory. Decant the

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sample extract through filter paper into a 50-mL graduated cylinder. Collect 50 mL for subsequent cleanup. Precondition a SAX SPE column with approximately 5 mL of methanol, followed by 5 mL of the SAX SPE basic rinse solvent. Discard the eluate. Add 100 µL of concentrated ammonium hydroxide to each sample. Place a stopper on the cylinder and mix the contents well. Pass the sample at a fast drip rate through the SAX SPE column via the reservoir, collecting the eluate in a 100-mL round bottom flask, or alternatively in a beaker and then transferring the eluate to a 100-mL round bottom flask. Rinse the graduated cylinder with approximately 5 mL of the SAX SPE basic rinse solvent, making sure to rinse the walls of the reservoir. Pass through the SPE column. Collect this eluate in the 100-ml round bottom flask. Remove the methanol solvent from the samples via rotary evaporation using a water bath temperature of approximately 40 °C. Add 100 µL of concentrated acetic acid to the sample. Mix. Add purified water, if necessary, to the sample so that the sample volume is approximately 15 mL. Pass the sample through a Varian ENV SPE column attached piggy-back style to a Varian SCX SPE. Discard the eluate. (The SPE columns are preconditioned by passing 5 mL of methanol and then 5 mL of 0.2% acetic acid through each column. Add 2-3 mL of 0.2% acetic acid to the lower SCX column just prior to adding the sample to the top ENV column. Do not permit the SCX column to go dry while the top ENV column still contains sample. Add 0.2% acetic acid to lower SCX column, as needed, to prevent it from going dry. Add approximately 5.0 mL of 0.2% acetic acid to the round bottom flask from Step 2.11 and swirl to dissolve any residues still on the glass. Pass through both SPE columns. Discard the eluate. Rinse the SPE columns (columns are still attached) with approximately 5 mL of purified water. Disconnect the two SPE columns. Rinse the SCX column with approximately 5 mL of 70% methanol/water. Discard the eluate.

ENV Analytes

Place a precalibrated 50-mL concentration tube containing approximately 2 mL of purified water beneath the ENV SPE column. Elute the analytes (CGA-64250, CGA-91305, CGA-217495, CGA-118244, CGA-118245, and CGA-136735) with 15 mL of acetonitrile. Remove the organic solvent via rotary evaporation with a water bath temperature of approximately 40°C until less than 3 mL of aqueous remains. Add 1.5 mL of acetonitrile and dilute to the 5.0 mL calibration mark using purified water. The sample may be further diluted using 30% acetonitrile/water, if needed. Store the samples in a refrigerator (<5°C) until the time of analysis. Analyze the ENV analytes (CGA-64250, CGA-91305, CGA-217495, CGA-118244, CGA-118245, and CGA-136735) by LC/MS with the reversed phase HPLC system.

SCX Analyte (CGA-71019)

Place a precalibrated 50-mL concentration tube beneath the SCX SPE column. Elute CGA-71019 with 10 mL of 2.5% ammonium hydroxide in 70% methanol/water. Remove the organic solvent via rotary evaporation with a water bath temperature of approximately 40°C until less than 3 mL of aqueous remains. Add approximately 1.25 mL of methanol and dilute to the 5.0 mL calibration mark using purified water. The sample may be further diluted using 25% methanol/water, if needed. Store the samples in a refrigerator (<5°C) until the time of analysis. Analyze the CGA-71019 by LC/MS with the cation exchange (SCX) HPLC system.

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WATER

Measure 100 mL of a water sample, using a 100-mL graduated cylinder. Sample fortification, if required for this particular sample, is to be done at this time. If suspended solids are present in the sample, centrifuge the sample in a centrifugable polyallomer bottle at approximately 9,000 RPM for 10 minutes, or at an alternate speed and time if the results are considered satisfactory. Pass the sample through filter paper and collect in a 100-mL graduated cylinder. Record the volume of the sample. Add 200 µL of concentrated acetic acid. Mix with the sample. The remainder of the cleanup procedure follows Steps 2.12 through 2.21 of the soil procedure (the SAX SPE cleanup step to remove soil matrix is not used). The final volume of the water samples may need to be reduced to approximately 2.0 mL, depending on instrument sensitivity. The volume of organic solvent added to the final sample should be adjusted to maintain the same ratio of organic/aqueous as in the soil samples.

CGA-118244, CGA-118245, and CGA-136735 contain up to four stereoisomers for each analyte. The stereoisomers of the three hydroxy degradates cannot all be chromatographically resolved from one another. Therefore, the peak area for all of these isomers is summed for quantitative calculations.

II. Recovery Findings

Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) as shown in Tables 2-6, below.

Analytical method for [Propiconazole, and its degradates: CGA-217495, CGA-91305, CGA-136735, CGA-118244, CGA-118245, and CGA-71019 in soil and water.

Table 2. Initial Validation Method Recoveries for Analytes in Soil

Analyte	Fortification Level (units)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Propiconazole	[LOQ] 5 ppb	5	91-110	100	7.3	7.3
	[10x LOQ] 50 ppb	5	86-92	88	2.6	2.9
CGA-91305	[LOQ] 5 ppb	5	84-120	103	16.0	15.5
	[10x LOQ] 50 ppb	5	94-100	97	2.4	2.5
Hydroxy Isomers (CGA-118244, CGA-118245, CGA-136735)	[LOQ] 5 ppb	5	95-120	105	11.5	11
	[10x LOQ] 50 ppb	5	95-97	96	0.84	0.87
CGA-217495	[LOQ] 5 ppb	5	84-105	94	8.8	9.4
	[10x LOQ] 50 ppb	5	88-94	91	4.6	5.1
CGA-71019	[LOQ] 5 ppb	5	73-104	90	13.8	15.3
	[10x LOQ] 50 ppb	5	75-98	89	9.9	11.2

Analytical method for [Propiconazole, and its degradates: CGA-217495, CGA-91305, CGA-136735, CGA-118244, CGA-118245, and CGA-71019 in soil and water.

Table 3. Initial Validation Method Recoveries for Analytes in **Water**

Analyte	Fortification Level (units)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Propiconazole	[LOQ] 0.1 ppb	5	81-89	85	4.1	4.8
	[10x LOQ] 1 ppb	5	83-104	93	7.6	12.2
CGA-91305	[LOQ] 0.1 ppb	5	85-97	91	4.5	4.9
	[10x LOQ] 1 ppb	5	82-99	93	6.5	7.0
Hydroxy Isomers (CGA-118244, CGA-118245, CGA-136735)	[LOQ] 0.1 ppb	5	83-97	90	5.3	5.9
	[10x LOQ] 1 ppb	5	87-100	96	5.2	5.5
CGA-217495	[LOQ] 0.1 ppb	5	82-96	89	5.4	6.0
	[10x LOQ] 1 ppb	5	83-98	93	6.0	6.5
CGA-71019	[LOQ] 0.1 ppb	5	90-103	99	5.3	5.4
	[10x LOQ] 1 ppb	5	87-94	90	2.7	3.0

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Table 4. Independent Validation Method Recoveries for Analytes in well water

Analyte	Fortification Level (units)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Propiconazole	[LOQ] 0.1 ppb	5	86-105	96	10.3	10.7
	[10x LOQ] 1 ppb	5	78-106	91	10.8	11.8
CGA-91305	[LOQ] 0.1 ppb	5	85-105	94	8.7	9.3
	[10x LOQ] 1 ppb	5	81-104	92	10.0	10.8
Hydroxy Isomers (CGA-118244, CGA-118245, CGA-136735)	[LOQ] 0.1 ppb	5	87-105	95	8.8	9.3
	[10x LOQ] 1 ppb	5	78-101	92	8.8	9.6
CGA-217495	[LOQ] 0.1 ppb	5	81-103	91	9.7	10.7
	[10x LOQ] 1 ppb	5	77-96	86	6.7	7.8
CGA-71019	[LOQ] 0.1 ppb	5	57-90	72	15.8	9.3
	[10x LOQ] 1 ppb	5	61-93	78	13.3	9.6

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Table 5. Independent Validation Method Recoveries for Analytes in surface water

Analyte	Fortification Level (units)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Propiconazole	[LOQ] 0.1 ppb	5	90-111	98	9.9	10.1
	[10x LOQ] 1 ppb	5	104-114	109	4.5	4.1
CGA-91305	[LOQ] 0.1 ppb	5	87-107	97	8.9	9.2
	[10x LOQ] 1 ppb	5	105-112	109	3.0	2.8
Hydroxy Isomers (CGA-118244, CGA-118245, CGA-136735)	[LOQ] 0.1 ppb	5	90-112	100	9.5	9.5
	[10x LOQ] 1 ppb	5	107-114	110	3.2	3.0
CGA-217495	[LOQ] 0.1 ppb	5	92-107	98	5.5	5.6
	[10x LOQ] 1 ppb	5	107-132	117	10.8	9.3
CGA-71019	[LOQ] 0.1 ppb	5	79-136	99	22.4	22.7
	[10x LOQ] 1 ppb	5	74-95	83	10.2	12.2

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Table 6. Independent Validation Method Recoveries for Analytes in soil

Analyte	Fortification Level (units)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Propiconazole	[LOQ] 5.0 ppb	5	91-98	95	2.8	3.0
	[10x LOQ] 50 ppb	5	87-105	92	7.2	7.7
CGA-91305	[LOQ] 5.0 ppb	5	88-91	89	1.3	1.5
	[10x LOQ] 50 ppb	5	84-102	91	6.9	7.6
Hydroxy Isomers (CGA-118244, CGA-118245, CGA-136735)	[LOQ] 5.0 ppb	5	88-93	90	2.2	2.4
	[10x LOQ] 50 ppb	5	87-108	94	8.2	8.8
CGA-217495	[LOQ] 5.0 ppb	5	83-91	86	3.7	4.3
	[10x LOQ] 50 ppb	5	85-115	95	11.5	12.2
CGA-71019	[LOQ] 5.0 ppb	5	88-104	100	11.2	11.2
	[10x LOQ] 50 ppb	5	81-114	96	12.0	12.5

III. Method Characteristics

The limit of determination (the lowest fortification specified by the method which gives adequate recovery. The limit of detection by LC/MS (smallest standard amount injected during the chromatographic run).

Analytical method for [Propiconazole, and its degradates: CGA-217495, CGA-91305, CGA-136735, CGA-118244, CGA-118245, and CGA-71019 in soil and water.

Table 7. Method Characteristics

	Analyte- Soil	Analyte- Water
Limit of Quantitation (LOQ)	5 µg/L- All analytes	0.1 µg/L- All analytes
Limit of Detection (LOD)	0.25 ng/ µl - for propiconazole, CGA-91305, CGA-217495,CGA-118244, CGA-118245,and CGA-136735. 0.375 ng/µl for CGA-71019.	
Linearity (calibration curve r ² and concentration range)	r ² 0.999 –For all analysis sets 0.0025-0.05 ng/µl	
Repeatable	Yes	Yes
Reproducible	Yes	Yes
Specific	No interferences were observed in either water or soil control samples other than trace, nonquantifiable residues that were due to contamination.	

IV. Method Deficiencies and Reviewer's Comments

Method reports provide clearly written procedures for sample preparation, extraction, cleanup, and analysis. The analytical procedures include information on the sample preparation technique, type of instrument and analytical column(s) used, instrument setup and operating parameters, standard and reagent preparation and calibration procedures. Method performance is demonstrated by acceptable recovery data (*i.e.*, method repeatability). Chromatograms for one standard, one matrix blank, and matrix spike at the LOQ and 10 x LOQ spiking levels with response values are included, as well as a regression analysis that defines the slope, intercept, and standard error of the calibration curve. Required equipment and glassware are generally available. An ILV has been performed on the same version of the analytical method and produced acceptable performance data.

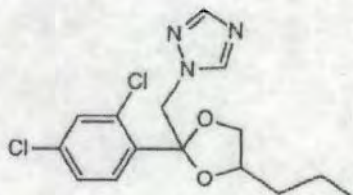
V. References

- 1- Vargo, J. D., "Validation of "Draft" Analytical Method AG-677 for the Determination of Propiconazole (CGA-64250) and its Degradates CGA-217495, CGA-91305, CGA-118244, CGA-118245, CGA-136735, and CGA-71019 in Soil and Water by High Performance Liquid Chromatography with Mass Spectrometric Detection," including Protocol Amendment 1.
- 2- Vargo, J. D., Novartis Residue Test Report RI-MV-013-97, Report Number 1.

**Analytical method for [Propiconazole, and its degradates: CGA-217495,
CGA-91305, CGA-136735, CGA-118244, CGA-118245, and CGA-71019
in soil and water.**

Attachment A: CHEMICAL NAMES AND STRUCTURES

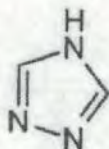
Analytical method for [Propiconazole, and its degradates: CGA-217495, CGA-91305, CGA-136735, CGA-118244, CGA-118245, and CGA-71019 in soil and water.



Propiconazole (CGA-64250)

CAS Number: 60207-90-1

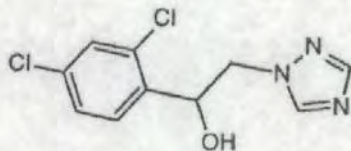
1H-1,2,4-Triazole, 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-



CGA-71019

CAS Number: 288-88-0

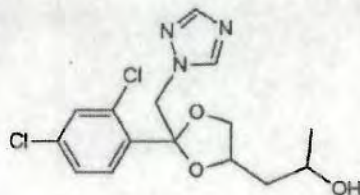
1H-1,2,4-Triazole



CGA-91305

CAS Number: 58905-18-3

1H-1,2,4-Triazole-1-ethanol, .alpha.-(2,4-dichlorophenyl)-

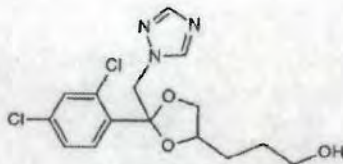


CGA-118244

CAS Number: 104390-57-0

1,3-Dioxolane-4-ethanol, 2-(2,4-dichlorophenyl)-.alpha.-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-

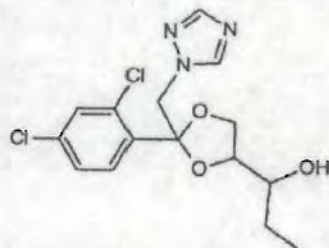
Analytical method for [Propiconazole, and its degradates: CGA-217495, CGA-91305, CGA-136735, CGA-118244, CGA-118245, and CGA-71019 in soil and water.



CGA-118245

CAS Number: 104390-58-1

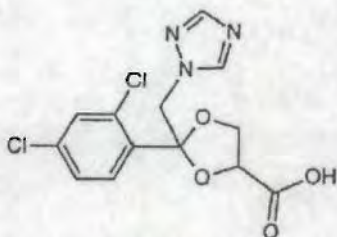
1,3-Dioxolane-4-propanol, 2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-



CGA-136735

CAS Number: 119725-85-8

1,3-Dioxolane-4-methanol, 2-(2,4-dichlorophenyl)-.alpha.-ethyl-2-(1H-1,2,4-triazol-1-ylmethyl)-



CGA-217495

CAS Number: 119725-91-6

1,3-Dioxolane-4-carboxylic acid, 2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-
