syngenta

Pinoxaden

Pinoxaden - Independent Laboratory Validation of Residue Method (GRM017.07A) for the Determination of Pinoxaden and its Metabolites NOA407854 and NOA447204 in Water by LC-MS/MS Analysis

Final Report

DATA REQUIREMENT(S): EPA 850.6100 (2012)
EC SANCO/825/00 Rev.8.1 (2010)
EC SANCO/3029/99 Rev.4 (2000)

2.0 INTRODUCTION

The purpose of this study was to conduct an independent laboratory validation of Syngenta Analytical Method GRM017.07A entitled "Pinoxaden - Residue Method GRM017.07A for the Determination of Pinoxaden and Its Metabolites NOA407854 and NOA447204 in Water by LC-MS/MS Analysis", as written.

This study was designed to satisfy guideline requirements described in US EPA OCSPP 850.6100[1], EC SANCO/3029/99 rev. 4 (2000)[2], and EC SANCO/825/00 rev. 8.1 (2010)[3] guidelines. This study was conducted in compliance with US EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160, which are compatible with the OECD Principles of Good Laboratory Practice (as revised in 2007).

The residue analytical method is suitable for the determination of Pinoxaden and its Metabolites NOA407854 and NOA447204 in water. Surface and ground water were selected for evaluation in this validation study to meet all requirements.

The method was successfully validated for Pinoxaden and its Metabolites NOA407854 and NOA447204 at 0.05 µg/L (LOQ) and 0.50 µg/L (10X LOQ) in ground water (drinking water) and surface water using external solvent calibration.

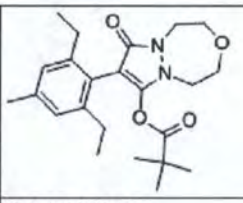
To summarize the method, 20 mL of water samples were acidified with formic acid. Samples were processed through an Oasis HLB SPE column and eluted with ultra-pure water/acetonitrile (50:50 v/v). An aliquot was transferred into suitable autosampler vials, and submitted for LC-MS/MS analysis. The limit of quantitation (LOQ) is 0.05 µg/L (ppb) for Pinoxaden and its Metabolites NOA407854 and NOA447204.

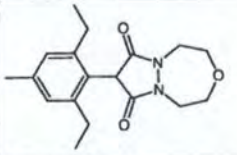
The analytical procedure was performed as written with the following exceptions. Surface water was filtered through Whatman Qualitative Circles, Grade 1, 110 mm filter paper after collection. An additional 300 µL of formic acid was needed to acidify the samples to a pH $\leq$ 2. Autosampler injection volumes were increased to overcome detector sensitivity limits.

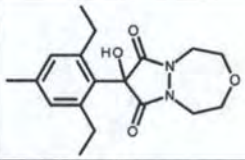
3.0 MATERIALS AND METHODS

3.1 Test Substance/Reference Substance

The test substance, Pinoxaden, was received on February 10, 2017 from Syngenta Crop Protection, Greensboro, North Carolina. The test substances, NOA407854, and NOA447204 were received on March 1, 2017 from Syngenta Crop Protection, Greensboro, North Carolina. The following information was provided:

Compound Structure	
Syngenta Code:	NOA407855
Common Name:	Pinoxaden
Chemical Name:	Propanoic acid, 2,2-dimethyl-, 8-(2,6-diethyl-4-methylphenyl)-1,2,4,5-tetrahydro-7-oxo-7H-pyrazolo[1,2-d][1,4,5]oxadiazepin-9-yl ester
CAS Number:	243973-20-8
IUPAC Name:	[8-(2,6-diethyl-4-methylphenyl)-7-oxo-1,2,4,5-tetrahydropyrazolo[1,2-d][1,4,5]oxadiazepin-9-yl] 2,2-dimethylpropanoate
Batch ID:	731946
Molecular Mass:	400.5
Structural Formula	$C_{23}H_{32}N_2O_4$
Storage Conditions:	< -10°C (long term storage); < 30°C in desiccator (short term storage, routine dispensed, to be disposed of after 6 months)
Purity:	99.4% ± 0.3% (wt/wt)
Recertification Date:	End of March 2019

Compound Structure	
Syngenta Code:	NOA407854
Common Name:	NOA407854
Chemical Name:	7H-Pyrazolo[1,2-d][1,4,5]oxadiazepine-7,9(8H)-dione,8-(2,6-diethyl-4-methylphenyl)tetrahydro-
CAS Number:	314020-44-5
IUPAC Name:	8-(2,6-diethyl-4-methylphenyl)-1,2,4,5-tetrahydropyrazolo[1,2-d][1,4,5]oxadiazepine-7,9-dione
Batch ID:	DAH-XXXI-11
Molecular Weight:	316.401 g/mol
Structural Formula	$C_{18}H_{24}N_2O_3$
Storage Conditions:	Refrigerator
Purity:	98.8%
Expiration Date:	March 31, 2018

Compound Structure	
Syngenta Code:	NOA447204
Common Name:	NOA447204
Chemical Name:	7H-Pyrazolo[1,2-d][1,4,5]oxadiazepine-7,9(8H)-dione, 8-(2,6-diethyl-4-methylphenyl)tetrahydro-8-hydroxy-
CAS Number:	Not Assigned
IUPAC Name:	N/A
Batch ID:	KI-6385/17
Molecular Weight:	N/A
Structural Formula	$C_{18}H_{24}N_2O_4$
Storage Conditions:	Refrigerator
Purity:	99.4%
Expiration Date:	January 31, 2019

N/A: Not available

Upon receipt, the test substance Pinoxaden was stored frozen in the original container. Test substances NOA407854, and NOA447204 were stored refrigerated in the original container. Concentrations were adjusted for the purity of the test substance.

All solutions made from the test substance (analytical standard) were stored according to the method.

3.2 Test Systems

The test systems evaluated in this study were surface water and ground (drinking) water. These matrices were chosen because they are representative of the water the method is designed for. Water characterization data is listed in Tables 1-3.

Approximately 6L of surface water was collected from Pequest River near Rt 46 in New Jersey, United States on February 22, 2017. Water was collected from a depth of approximately 40 cm, approximately 1.5 M from the riverbank. Upon arrival at Symbiotic Research on the same date, the surface water was filtered with Whatman Qualitative Circles, Grade 1. Filtered water was stored refrigerated.

Groundwater (bottled natural spring water) was purchased from a local grocer in Chester, NJ on August 18, 2016, and stored room temperature in the laboratory following arrival at Symbiotic Research.

Refrigerator storage temperatures were monitored on a daily basis and were typically at *c.a.* 4.0°C. Surface water was stored refrigerated, except for the periods during which the matrix was aliquoted for analysis. Groundwater was stored at room temperature for the entire duration of the study.

3.3 Equipment and Reagents/Supplies

The equipment and reagents/supplies used for the method validation were as outlined in the method. Identical or equivalent equipment and materials were used, as permitted by the method. The equivalent equipment and reagents used were as follows:

3.3.1 Equipment

Balance:	Mettler Toledo Microbalance, Model AT20
HPLC:	Hewlett Packard Series 1100 Modular HPLC System Agilent Technology, Wilmington, DE. Equipped with Degasser, Binary Pump, Autosampler, Column Oven, DAD.
Mass Spectrometer:	SCIEX API4000

3.3.2 Reagents

Acetonitrile:	HPLC Grade (EMD Millipore)
Methanol:	HPLC Grade (EMD Millipore)
Formic Acid:	ACS Reagent (Sigma Aldrich)
Water (H_2O):	LC-MS Grade (Fluka via Sigma Aldrich)
Ammonium Formate:	MS Grade (Sigma-Aldrich)

3.4 Preparation of Standard Solutions

The preparation of Pinoxaden, NOA407854, and NOA447204 standard solutions used for this study, adjusted for purity, are described below. The solutions were stored as recommended in the method when not in use (refrigerated or frozen).

3.4.1 Stock Standard Solutions

A small amount of Pinoxaden, NOA407854, or NOA447204 test/reference substance was added to a pre-tared small glass vial and the weight was recorded. The volume of acetonitrile:methanol (90/10 v/v) needed to make stock standard solutions of Pinoxaden and its metabolites having a concentration of 200 µg/mL was calculated using the equation in section 2.3.1 of the method GRM017.07A. The calculated volumes were added to each individual vial, and the contents were mixed by vortex to give a final concentration of 200 µg/mL for each individual test/reference substance.

3.4.2 Mixed Fortification Solutions

Mixed Fortification Solutions were prepared from the Stock Standard solutions and were stored frozen when not in use.

10-µg/mL: 1 mL of a 200-µg/mL Pinoxaden, 1 mL of a 200-µg/mL NOA407854, and 1 mL of a 200-µg/mL NOA447204 stock standard solutions were transferred to an appropriate sized vial and diluted with 17 mL of acetonitrile:methanol (90/10 v/v). The final solution volume of 20 mL was mixed well.

1.0-µg/mL: 2.0 mL of a 10-µg/mL mixed fortification solution was diluted to a total volume of 20 mL with 18 mL of acetonitrile:methanol (90/10 v/v) and the solution was mixed well.

0.1-µg/mL: 2.0 mL of a 1.0-µg/mL mixed fortification solution was diluted to a total volume of 20 mL with 18 mL of acetonitrile:methanol (90/10 v/v) and the solution was mixed well.

0.01-µg/mL: 2.0 mL of a 0.1-µg/mL mixed fortification solution was diluted to a total volume of 20 mL with 18 mL of acetonitrile:methanol (90/10 v/v) and the solution was mixed well.

3.4.3 HPLC Mixed Calibration Standard Solutions

Mixed Calibration Standards were prepared from the Mixed Fortification Solutions and were stored frozen when not in use.

0.1 ng/mL: 0.030 mL of a 0.1 µg/mL Mixed Fortification Solution was diluted to a final volume of 10-mL with ultrapure water: acetonitrile (50/50, v/v) in an appropriate sized vial and mixed well. This solution was further diluted by adding 5 mL of the prepared solution to 10 mL of ultrapure water: acetonitrile (50/50, v/v) and mixed well.

0.33 ng/mL: 0.100 mL of a 0.1 µg/mL Mixed Fortification Solution was diluted to a final volume of 10-mL with ultrapure water: acetonitrile (50/50, v/v) in an appropriate sized vial and mixed well. This solution was further diluted by adding 5 mL of the prepared solution to 10 mL of ultrapure water: acetonitrile (50/50, v/v) and mixed well.

0.83 ng/mL: 0.250 mL of a 0.1 µg/mL Mixed Fortification Solution was diluted to a final volume of 10-mL with ultrapure water: acetonitrile (50/50, v/v) in an appropriate sized vial and mixed well. This solution was further diluted by adding 5 mL of the prepared solution to 10 mL of ultrapure water: acetonitrile (50/50, v/v) and mixed well.

1.67 ng/mL:	0.500 mL of a 0.1 µg/mL Mixed Fortification Solution was diluted to a final volume of 10-mL with ultrapure water: acetonitrile (50/50, v/v) in an appropriate sized vial and mixed well. This solution was further diluted by adding 5 mL of the prepared solution to 10 mL of ultrapure water: acetonitrile (50/50, v/v) and mixed well.
3.33 ng/mL:	0.100 mL of a 1.0 µg/mL Mixed Fortification Solution was diluted to a final volume of 10-mL with ultrapure water: acetonitrile (50/50, v/v) in an appropriate sized vial and mixed well. This solution was further diluted by adding 5 mL of the prepared solution to 10 mL of ultrapure water: acetonitrile (50/50, v/v) and mixed well.
4.17 ng/mL:	0.125 mL of a 1.0 µg/mL Mixed Fortification Solution was diluted to a final volume of 10-mL with ultrapure water: acetonitrile (50/50, v/v) in an appropriate sized vial and mixed well. This solution was further diluted by adding 5 mL of the prepared solution to 10 mL of ultrapure water: acetonitrile (50/50, v/v) and mixed well.
5.0 ng/mL:	0.150 mL of a 1.0 µg/mL Mixed Fortification Solution was diluted to a final volume of 10-mL with ultrapure water: acetonitrile (50/50, v/v) in an appropriate sized vial and mixed well. This solution was further diluted by adding 5 mL of the prepared solution to 10 mL of ultrapure water: acetonitrile (50/50, v/v) and mixed well.

3.5 Analytical Method

Analytical method GRM017.07A was successfully independently validated in this study. See Appendix 1 for the complete text of the method. The following is a summary of that method:

> 20 mL of water samples are acidified with formic acid. Samples are processed through an Oasis HLB SPE column and eluted with ultra-pure water/acetonitrile (50:50 v/v). An aliquot is transferred into suitable autosampler vials, and submitted for LC-MS/MS analysis. The limit of quantitation (LOQ) is 0.05 µg/L (ppb) for Pinoxaden and its Metabolites NOA407854 and NOA447204.

The analytical procedure was performed as written with the following exceptions. Surface water was filtered through Whatman Qualitative Circles, Grade 1, 110mm filter paper after collection. An additional 300 µL of formic acid was needed to acidify the samples to a pH $\leq$ 2. Autosampler injection volumes were increased to overcome detector sensitivity limits.

Residue calculations were performed as specified in the analytical method and were conducted using Analyst (version 1.4.2) to prepare the calibration curve with 1/x weighting.

3.5.1 Fortifications

Untreated surface and ground water samples were fortified using microliter amounts of the appropriate fortification standard for LOQ and 10X LOQ concentrations as per method. Fortifications used in this method validation are as follows:

Matrix	Fortification Volume (µL)	Fortification Conc. (µg/mL)	Final Volume (mL)	Final Conc. (µg/L)	Replicates
Surface Water	100	0.01	20	0.05 (LOQ)	5
Groundwater	100	0.01	20	0.05 (LOQ)	5
Surface Water	100	0.1	20	0.5 (10X LOQ)	5
Groundwater	100	0.1	20	0.5 (10X LOQ)	5

After fortification, the samples were mixed thoroughly before clean up and concentration by SPE.

3.6 Instrumentation Conditions

All samples were analyzed by LC-MS/MS detection. Typical conditions were as follows:

Chromatography Conditions (Pinoxaden & NOA407854)

HPLC System	:	Hewlett Packard Series 1100 Modular HPLC System. Equipped with Degasser, Binary Pump, Autosampler, Column Oven, DAD.
Detector	:	Applied Biosystems API 4000 triple quadrupole mass spectrometer with Analyst™ software
Column	:	ACE C18 50 x 2.1 mm 3 µm
Column Oven Temperature	:	40°C
Injection volume	:	30 µL
Stop Time	:	7.0 min
Injection protocol	:	Analyze calibration standard after 3 to 4 sample injections
Mobile phase	:	Solvent A = Ultra-Pure Water + 0.1% formic acid Solvent B = Methanol

Mobile Phase Composition (Pinoxaden & NOA407854)

Time (min)	%A	%B	Flow Rate (mL/min)
0.00	90	10	0.6
4.00	10	90	0.6
5.00	10	90	0.6
5.10	90	10	0.6
7.00	90	10	0.6

Chromatography Conditions (NOA447204)

HPLC System	:	Hewlett Packard Series 1100 Modular HPLC System. Equipped with Degasser, Binary Pump, Autosampler, Column Oven, DAD.
Detector	:	Applied Biosystems API 4000 triple quadrupole mass spectrometer with Analyst™ software
Column	:	ACE C18 50 x 2.1 mm 3 µm
Column Oven Temperature	:	40°C
Injection volume	:	50 µL
Stop Time	:	7.0 min
Injection protocol	:	Analyze calibration standard after 3 to 4 sample injections
Mobile phase	:	Solvent A = 4mM Ammonium Formate in Ultra-Pure Water Solvent B = Methanol

Mobile Phase Composition (NOA447204)

Time (min)	%A	%B	Flow Rate (mL/min)
0.00	90	10	0.6
4.00	10	90	0.6
5.00	10	90	0.6
5.10	90	10	0.6
7.00	90	10	0.6

Divert Valve Switching Times

Time (mins)	Position
0	Waste
4.2	Mass spectrometer
7.0	Waste

Under these conditions the retention times are:

Analyte	Approximate Retention Time (min)
Pinoxaden	5.7
NOA407854	4.9
NOA447204	5.1

Mass Spectrometer Conditions for Pinoxaden, NOA407854 and NOA447204

Interface	:	TurboIonSpray
Polarity	:	Positive
Curtain gas (CUR)	:	Nitrogen set at 10 (arbitrary units)
Temperature (TEM)	:	650 °C
Ionspray voltage	:	5000 V
Collision gas setting (CAD)	:	Nitrogen set at 9
Gas 1 (GS1)	:	Air set at 50 (arbitrary units)
Gas 2 (GS2)	:	Air set at 55 (arbitrary units)
Interface heater (ihe)	:	On
Scan type	:	MRM

MRM Conditions		Pinoxaden primary transition	Pinoxaden confirmatory transition	NOA407854 primary transition	NOA407854 confirmatory transition
Q1 *m/z*	:	401	401	317	317
Q3 *m/z*	:	317	57	115	91
Dwell time	:	150 ms	150 ms	150 ms	150 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	72 V	72 V	136 V	136 V
Entrance potential (EP)	:	10 V	10 V	10 V	10 V
Collision energy (CE)	:	33 V	49 V	115 V	91 V
Collision cell exit potential (CXP)	:	10 V	10 V	20 V	16 V

MRM Conditions		NOA447204 primary transition	NOA447204 confirmatory transition
Q1 *m/z*	:	333	333
Q3 *m/z*	:	149	121
Dwell time	:	300 ms	300 ms
Resolution Q1	:	Unit	Unit
Resolution Q3	:	Unit	Unit
Declustering potential (DP)	:	51 V	60 V
Entrance potential (EP)	:	10 V	10 V
Collision energy (CE)	:	19 V	35 V
Collision cell exit potential (CXP)	:	38 V	15 V

Note: The mass spectrometer tuning parameters shown here are for reference only. The analyst should always consult with instrument operation manual to obtain optimum conditions for all the analytes prior to residue analysis.

3.7 Modifications, Interpretations, and Critical Steps

The analytical procedure was performed as written with the following exceptions. Surface water was filtered through Whatman Qualitative Circles, Grade 1, 110mm filter paper after collection. An additional 300 µL of formic acid was needed to acidify the samples to a pH ≤ 2. Autosampler injection volumes were increased to overcome detector sensitivity limits.

3.8 Statistics

Statistical methods used were limited to calculations of the mean, range, standard deviation, 1/x weighting of linear regression and relative standard deviation. Software programs, Microsoft Excel® and Analyst (version 1.4.2), were employed to develop all regression analysis and statistical data.

4.0 RESULTS

4.1 Pre-Validation Evaluations

Prior to analysis of actual validation samples, the control samples initially selected for use in the study were analyzed per the method to determine if any interferences were present in the area of Pinoxaden. The result of this evaluation indicated that the control samples were free of any interference that would affect the analyte responses.

Control Suitability Evaluation (Pinoxaden)	
Matrix	**Residue (µg/L)[a]**
Surface Water	0
Ground Water	0
Control Suitability Evaluation (NOA407854)	
Matrix	**Residue (µg/L)[a]**
Surface Water	ND
Ground Water	ND
Control Suitability Evaluation (NOA447204)	
Matrix	**Residue (µg/L)[a]**
Surface Water	ND
Ground Water	ND

[a]ND = none detected, no observable chromatographic response

TABLE 1 Characterization Data

Water Type	Source	pH	Calcium (ppm)	Magnesium (ppm)	Total Hardness as CaCO3 (mg/L)	Silt Content (ppm)	Dissolved Organic Carbon (ppm)
Surface Water	Pequest River on Rt 46	8.4	58	24	246	8	5.6
Groundwater	Evian bottled mineral water	8.1	87	27	330	4	0.6