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Pinoxaden

Pinoxaden - Residue Method GRM017.07A for the Determination of Pinoxaden and Its Metabolites NOA407854 and NOA447204 in Water by LC-MS/MS Analysis

Analytical Method

DATA REQUIREMENT(S): EPA 850.6100 (2012)

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1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM017.07A is suitable for the determination of Pinoxaden, NOA407854 and NOA447204 (Figures 1 - 3) in water. The limit of quantification (LOQ) of the method has been established at 0.05 µg/L (or 0.05 ppb).

This method satisfies US EPA 850.6100.

1.2 Method Summary

Representative water (surface and ground) are acidified and concentrated using SPE (Oasis HLB). Final residue determination is performed by ultra-performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

The limit of quantification of the method is 0.05 µg/L (0.05 ppb).

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 2.

2.3 Preparation of Analytical Standard Solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock Solutions

Prepare individual stock solutions (50 - 200 µg/mL) for Pinoxaden, NOA407854, and NOA447204 by one of the following methods.

Note: the amount weighed out must be corrected for the purity of the analytical standard as indicated on the certificate of analysis and also any salt content, where the analytical standard is received as a salt e.g. Na^+, Cl^- etc..

Weigh out accurately, using a five figure balance, sufficient Pinoxaden, NOA407854, and NOA447204 analytical standard into separate amber "Class A" volumetric flasks (50 mL size). Dilute to the mark with acetonitrile:methanol (90/10 v/v) to yield individual stock solutions of Pinoxaden, NOA407854, and NOA447204.

Alternatively, the appropriate volume of acetonitrile:methanol (90/10 v/v) to add to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

$$V = \frac{W \times P}{C} \times 1000$$

P = Standard purity in decimal form (P(%)/100)
V = Volume of acetonitrile:methanol (90/10 v/v) required
W = Weight, in mg, of the solid analytical standard
C = Desired concentration of the final solution, (µg/mL)
1000 = Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

2.3.2 Fortification Solutions

Sample fortification solutions containing Pinoxaden, NOA407854 and NOA447204 should be prepared by serial dilution in acetonitrile:methanol (90/10, v/v). It is recommended that the following solutions are prepared: 10.0 µg/mL, 1.0 µg/mL and 0.1 µg/mL. Mixed standards of Pinoxaden, NOA407854, and NOA447204 may be prepared if desired.

2.3.3 Preparation of Calibration Standards for LC-MS/MS

No significant matrix effects, suppression or enhancement of the instrument response for Pinoxaden, NOA407854 and NOA447204 has been observed in the water types tested using the procedures described in Section 3 during method validation and non-matrix matched calibration standards should be used for quantification.

Calibration standard should be prepared in ultra-pure water:acetonitrile (50/50 v/v) using stock or fortification solutions. Serial dilutions from a standard mixture may be used. For example, dilution of 1 mL mixed 0.1 µg/mL fortification solution to 10 mL with ultra-pure water:acetonitrile (50/50 v/v) will yield a 0.01 µg/mL (or 10 ppb) calibration standard.

A calibration curve may also be generated to quantify Pinoxaden, NOA407854 and NOA447204. Standards over an appropriate concentration range should be prepared as described above, using the requisite volume of Pinoxaden, NOA407854 and NOA447204 standards in ultra-pure water:acetonitrile (50/50 v/v). A standard range of 0.1 µg/L to 5.0 µg/L is recommended.

2.3.4 Standard Solution Storage and Expiration

All stock solutions should be stored in a refrigerator or freezer when not in use to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use.

Stability of working standards of Pinoxaden, NOA407854 and NOA447204 in acetonitrile:methanol (90/10, v/v) has been demonstrated for up to one month. Fresh standards should be prepared after this period unless additional data are generated to support a longer expiration date.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S G Luxon, The Chemical Society, London (Reference 1).

Solvent and Reagent hazards

	Acetonitrile	Methanol	Formic acid
Harmful Vapour	✓	✓	✓
Highly Flammable	✓	✓	*
Harmful by Skin Absorption	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓
Causes severe burns	*	*	✓
OES Short Term (mg/m^3)	105	310	19
OES Long Term (mg/m^3)	70	260	9

N/A not known

In all cases avoid breathing vapor. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

A summary of the method is included in flow-chart form in Appendix 4.

3.1 Sample Preparation

a) If water samples are received deep frozen they should be allowed to defrost completely at room temperature. Defrosted samples should be shaken thoroughly to ensure sample homogeneity prior to analysis. Any particulates may be removed by centrifugation at a speed which visibly separates the particulates from the water.

b) Transfer water samples (20 mL) into suitable polypropylene tubes (50 mL size).

3.2 Sample Fortification & acidification

To each pre-measured control water sample (20 mL), add the appropriate amount of standard solution containing Pinoxaden, NOA407854 and NOA447204 in acetonitrile:methanol (90/10, v/v) using a volume of <1.0 mL. Mix the sample thoroughly by shaking. At least one untreated control and two fortified control samples should be analysed with each sample set.

a) Add concentrated formic acid (100 µL). Check that the pH of the samples is ≤ 2 using appropriate pH paper or a pH meter.

3.3 Solid Phase Extraction procedure

a) Take one Waters Oasis™ HLB solid phase extraction cartridge (60 mg, 3 mL size) for each sample to be analysed and place on a suitable vacuum manifold (e.g. IST Vacmaster).
Add methanol (2 mL) and allow to percolate through each cartridge under gravity or draw through under vacuum to the level of the top frit at a rate of approximately 1 mL/min, discarding the column eluate. Do not allow the cartridges to become dry.

Add ultra-pure water (2 mL) to the top of each cartridge and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate. Do not allow the cartridges to become dry.

b) Load acidified water samples from Section 3.2 (a) onto the SPE cartridges (a suitable column reservoir may be used if desired) and allow to percolate through under gravity or under low vacuum, at a rate of approximately 1 - 2 mL/min, to the level of the top frit. Do not allow cartridges to become dry. Pinoxaden, NOA407854 and NOA447204 are retained on the SPE cartridge.

c) On completion of loading, wash the empty sample tubes with ultra-pure water containing 1% formic acid (3 mL) and add the rinse to the column reservoir. Allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate. Do not allow the cartridges to become dry.

d) Wash SPE with 3 mL ultra-pure water/acetonitrile (80:20 v/v). Allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate.

e) Remove any remaining droplets of water adhering to the inside of the cartridge with absorbent tissue and dry the cartridges under high vacuum (≤500 mbar) for 15 minutes.

Note: Where achievable vacuums are less than specified or apparatus does not allow sufficient air flow through the cartridges, longer drying times may be required.

f) Place collection tubes (15 mL) under each port, as required, in the manifold rack. Add 3 mL ultra-pure water/acetonitrile (50:50 v/v) to each of the original samples tubes (50 mL) and swirl to rinse the sides of the tube to remove any residual analyte. Add rinsate to the top of each cartridge and draw through under gravity to the level of the top frit at a rate of approximately 2 mL/min. When the solvent is at the top frit, turn on the vacuum for a few seconds to expel any remaining solvent held in the cartridges.

g) Transfer 1 mL of the final fraction in ultra-pure water/acetonitrile (50:50 v/v) to an autosampler vial ready for final determination by LC-MS/MS. The final sample concentration is 6.6 mL/mL or 0.006 mL/L.

h) If sensitivity is insufficient, evaporate sample to dryness in a heating block at 40 °C under a stream of dry air or nitrogen gas and re-dissolve using ultra-pure water:acetonitrile (50/50, v/v) (1.0 mL). Ultrasonicate and transfer to an autosampler vial. Sample is ready for final determination by LC-MS/MS. The final sample concentration is 20 mL/mL or 0.02 mL/L.

3.4 Experimental Precautions

a) Some of the analytes have low solubility in acetonitrile alone, hence acetonitrile:methanol (90/10, v/v) is recommended to ensure complete dissolution of all analytes.

b) The SPE procedure has been developed using cartridges from the stated manufacturer. Similar cartridges from other manufacturers may be used. In all cases however, it is strongly recommended that the elution profile of the chosen batch of cartridges is checked prior to commencing analysis to assess any variation in manufacturers' products and between batches.

c) Bottled HPLC grade ultra-pure water is used to prepare the LC mobile phase, which produces a lower background noise in the MS/MS chromatograms than water taken from a laboratory water purification system.

d) To prevent contamination of the instrument and to minimise possible carry-over issues, it is recommended that high level recoveries (>0.1 mg/kg) and samples with expected residues greater than 1 µg/L should be diluted so that the final analyte concentration does not exceed 0.005 µg/mL. It may also be useful to include blank injections of ultra-pure water:acetonitrile (50/50, v/v) after high level samples to clear any observed carry-over greater than 10% of the LOQ.

e) Additional needle and valve washes with methanol and 0.1% formic acid solution may help to reduce any significant carry-over of analytes.

3.5 Time Required for Analysis

The methodology is normally performed with a batch of 20 samples. One person can complete the analysis of 20 samples in 1 day (8 hour working period).

3.6 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekend breaks unless otherwise specified in the analytical procedure. Acceptable precision and accuracy data obtained from the fortified control samples will validate any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

4.0 FINAL DETERMINATION

The method has been developed for use on an Applied Biosystems QTRAP® 5500. The following instrumentation and conditions have been found to be suitable for this analysis. Other instrumentation can also be used, though optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

4.1 Instrument Description

LC System	:	Waters Acquity I-Class UPLC
Detector	:	Applied Biosystems QTRAP® 5500
Gas Supply	:	PEAK Gas Generator

4.2 Chromatography Conditions for Pinoxaden and NOA407854

Column	:	ACE C18 50 x 2.1 mm 3 µm
Column Oven Temperature	:	40°C
Injection volume	:	10 µL
Stop Time	:	6.0 mins
Injection protocol	:	Analyze calibration standard after 3 to 4 sample injections
Mobile phase	:	Solvent 1: Ultra-Pure Water + 0.1% formic acid Solvent 2: Methanol

Mobile Phase Composition

Time (min)	% Solvent 1	% Solvent 2	Flow, mL/min
0	90	10	0.6
4.0	10	90	0.6
5.0	10	90	0.6
5.1	90	10	0.6
6.0	90	10	0.6

4.3 Chromatography Conditions for NOA447204

Column	:	ACE C18 50 x 2.1 mm 3 µm
Column Oven Temperature	:	40°C
Injection volume	:	10 µL
Stop Time	:	6.0 mins
Injection protocol	:	Analyze calibration standard after 3 to 4 sample injections
Mobile phase	:	Solvent 1: 4mM Ammonium Formate in UPW Solvent 2: Methanol

Mobile Phase Composition

Time (min)	% Solvent 1	% Solvent 2	Flow, mL/min
0	90	10	0.6
4.0	10	90	0.6
5.0	10	90	0.6
5.1	90	10	0.6
6.0	90	10	0.6

Under these conditions the retention times are:

Analyte	**Approximate Retention Time (min)**
Pinoxaden	3.7
NOA407854	2.7
NOA447204	2.8

Note: Retention time shift for analysis of surface water samples as observed in the Figure Section (representative chromatography) is due to instrument configuration.

4.4 Mass Spectrometer Conditions for Pinoxaden, NOA407854 and NOA447204

Interface	:	TurboIonSpray
Polarity	:	Positive
Curtain gas (CUR)	:	Nitrogen set at 20 (arbitrary units)
Temperature (TEM)	:	475 °C
Ionspray voltage	:	3800 V
Collision gas setting (CAD)	:	Nitrogen set at 20
Gas 1 (GS1)	:	Air set at 50 (arbitrary units)
Gas 2 (GS2)	:	Air set at 50 (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	:	100 ms	100 ms	100 ms	100 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	72 V	72 V	101 V	101 V
Entrance potential (EP)	:	10 V	10 V	10 V	10 V
Collision energy (CE)	:	33 V	49 V	87 V	81 V
Collision cell exit potential (CXP)	:	22 V	8 V	8 V	8 V

MRM Conditions		NOA447204 primary transition	NOA447204 confirmatory transition
Q1 *m/z*	:	333	333
Q3 *m/z*	:	149	121
Dwell time	:	100 ms	100 ms
Resolution Q1	:	Unit	Unit
Resolution Q3	:	Unit	Unit
Declustering potential (DP)	:	50 V	60 V
Entrance potential (EP)	:	10 V	10 V
Collision energy (CE)	:	17 V	32 V
Collision cell exit potential (CXP)	:	18 V	16 V

Typical chromatograms are shown in the Figures Section.

Confirmatory transition provided if further confirmation is required or interferences are observed using the primary transition.

4.5 Confirmatory Procedures for Pinoxaden, NOA407854 and NOA447204

Final determination by LC-MS/MS with two transitions is considered to be highly specific; hence no further confirmatory conditions are included.

5.0 CALCULATION OF RESULTS

5.1 Multi Point Calibration Procedure

Pinoxaden, NOA407854 and NOA447204 residues may be calculated in µg/L for each sample as follows.

a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (e.g. 30% LOQ to at least 20% above the highest fortified level as a minimum). An appropriate number of different concentrations within this range should be prepared (at least five).

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to Pinoxaden, NOA407854 and NOA447204. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions.

c) Generate calibration curve parameters using an appropriate regression package.

d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Squared" value for the regression.

Re-arrangement for x gives

$$x = \frac{y - c}{m}$$

e) Calculate the Pinoxaden, NOA407854 and NOA447204 residues in the sample, expressed as µg/L, as follows

$$\text{Residue}\ (\mu g/L) = \frac{\text{Analyte found}\ (\mu g/mL)}{\text{Sample conc.}\ (L/mL)}$$

Where analyte found (µg/mL) is calculated from the standard calibration curve and sample conc. is the final sample concentration in L/mL.

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}}\ (\mu g/L)$$

5.2 Single Point Calibration Procedure

Pinoxaden, NOA407854 and NOA447204 residues may be calculated in µg/L for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) Make repeated injections of a standard containing Pinoxaden, NOA407854 and NOA447204 at an appropriate concentration into the LC-MS/MS operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for Pinoxaden, NOA407854 and NOA447204.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to Pinoxaden, NOA407854 and NOA447204.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

d) Calculate the Pinoxaden, NOA407854 and NOA447204 residues in the sample, expressed as µg/L using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue}\ (\mu g/L) = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

Peak response for sample

Average peak response for bracketing standards

Concentration of standard (µg/mL)

Sample concentration (L/mL)

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}}\ (\mu g/L)$$

Although single point calibration may be used to quantify residues it is recommended that a calibration curve is generated with each analytical run to demonstrate the linearity of instrument response (Reference 2).

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analysed with each set of samples to verify that the sample used to prepare recovery samples is free from contamination. A minimum of one control should be analysed with each batch of samples.

The analytical method described is direct injection of the water samples and therefore recovery data cannot be calculated. The precision of the calculated concentrations from 5 replicates fortified at the LOQ and 5 replicates fortified at 10 x LOQ is presented in the Tables section.

7.0 SPECIFICITY

It is recommended that reagent blank samples be included in a sample set if contamination is suspected.

7.1 Matrix

LC-MS/MS is a highly specific detection technique. Interference arising from the matrices tested has not been observed.

7.2 Reagent and Solvent Interference

Using high purity solvents and reagents no interference has been found.

7.3 Labware Interference

This method uses mainly disposable labware. All reusable glassware should be detergent washed and then rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

8.0 METHOD VALIDATION

8.2 Limit of Quantification (LOQ)

The limit of quantification of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and acceptable precision of the data with a relative standard deviation of ≤ 20% has been obtained. Generally, for accurate quantification, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The limit of quantification has been set at 0.05 µg/L (0.05 ppb).

Aquatic plants and algae NOAEL = 3.7 ppb.
Terrestrial plants non-target NOAEL = 8.0 ppb.
Flathead minnow NOEC = >1.0 ppm.
Mysid shrimp NOEC = 0.87 ppm.

8.3 Limit of Detection (LOD)

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times background noise. Note that the LOD may vary between runs and from instrument to instrument.

The limit of detection was determined to be 0.1 µg/L.

8.4 Matrix Effects

No significant suppression or enhancement of detector response was seen for Pinoxaden, NOA407854 and NOA447204 in the presence of surface water or groundwater. Non-matrix standards should be used for quantification. A summary of the matrix effects is included in Table 5.

8.5 Detector Linearity

For accurate quantification of residue concentrations, analyses should be carried out within the linear range of the detector. For multi-point calibration, detector range and linearity will be demonstrated within each sample set.

If a residue beyond the tested concentration range is expected, dilute the sample appropriately to bring it within the tested linear range prior to quantification.

8.6 Final Fraction Stability

Final water samples in ultra-pure water:acetonitrile (50/50 v/v) retained in vials and stored at a temperature of approximately 4°C were suitable for Pinoxaden, NOA407854 and NOA447204 residue analysis, for storage periods of up to 14 days.

10.0 CONCLUSIONS

This procedure has been demonstrated to be a reliable and accurate procedure for the determination of Pinoxaden, NOA407854 and NOA447204 residues in environmental water types. Only commercially available laboratory equipment and reagents are required. The

analysis of 20 water samples for Pinoxaden, NOA407854 and NOA447204 can be completed by one person in 1 day (8 working hour period). Untreated and fortified samples should be analysed with each set of samples to demonstrate absence of any interference and that adequate accuracy and precision data can be obtained if possible. The limit of quantification of the method is 0.05 µg/L (0.05 ppb).

This method satisfies US EPA 850.6100.

CHEMICAL STRUCTURES

FIGURE 1 : Pinoxaden

Compound Code Number	:	NOA407855
IUPAC Name	:	2,2-dimethyl-propionic acid 8-(2,6-diethyl-4-methyl-phenyl)-9-oxo-1,2,4,5-tetrahydro-9.H- pyrazolo[1,2,-d] oxadiaepin-7-yl ester
Molecular Formula	:	$C_{23}H_{32}N_2O_4$
Molecular Weight	:	400.5

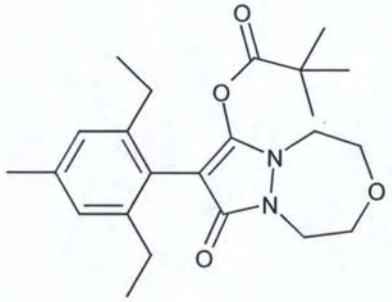


FIGURE 2 : NOA407854

Compound Code Number	:	NOA407854
IUPAC Name	:	8-(2,6-diethyl-4-methyl-phenyl)-tetrahydro-pyrazolo[1,2,-d] [1,4,5] oxadiaepine-7,9-dione
Molecular Formula	:	$C_{18}H_{24}N_2O_3$
Molecular Weight	:	316.4

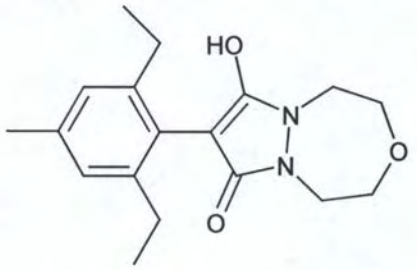
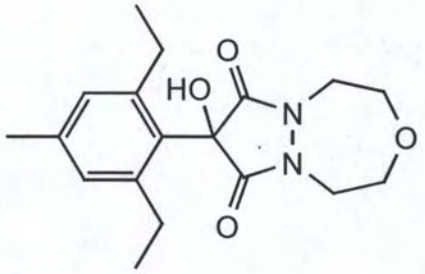


FIGURE 3 : NOA447204

Compound Code Number	:	NOA447204
Alternative code	:	CSAA783052
IUPAC Name	:	8-(2,6-diethyl-4-methyl-phenyl)-8-hydroxy-tetrahydro-pyrazolo[1,2,-d] [1,4,5] oxadiazepine-7,9-dione
Molecular Formula	:	$C_{18}H_{24}N_2O_4$
Molecular Weight	:	332.4



APPENDIX 1 : Apparatus

Recommended Suppliers

Equipment	Description	Supplier
General glassware	General glassware	Thermofisher
SPE cartridges	Waters Oasis™ HLB, 60mg 3mL	Waters
LC-MS/MS system	AB Sciex API 5500 equipped with a TurboIonSpray source	ABSciex
HPLC system	Agilent 1100 binary pump and column oven	Agilent
Autosampler	Agilent 1100 Autosampler	Agilent
HPLC column	ACE C18 50 x 2.1 mm 3 µm	Ace PN 111-0502
Nitrogen generator	Peak Scientific Genius 3030	Peak Scientific

APPENDIX 2 : Reagents

Recommended Suppliers

Reagent	Description	Supplier
Ultra-pure water	HPLC grade	Thermofisher
Acetonitrile	HPLC grade	Thermofisher
Methanol	HPLC grade	Thermofisher
Formic acid	Analytical grade	Thermofisher
Pinoxaden, NOA407854, NOA447204 analytical standards	GLP certified	GLP Testing Facility, Syngenta, CH-4333, Munchweilen, Switzerland or Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419-8300.

Preparation of Reagents

a) 1% formic acid in ultra-pure water
Add 10 mL concentrated formic acid to ultra-pure water in a 1 L volumetric flask. Adjust to the 1L mark with ultra-pure water. Stopper the flask securely and shake to mix thoroughly.

b) 1% formic acid in acetonitrile
Add 10 mL concentrated formic acid to acetonitrile in a 1 L volumetric flask. Adjust to the 1L mark with ultra-pure water. Stopper the flask securely and shake to mix thoroughly.

c) 0.2% formic acid in ultra-pure water
Add 2mL concentrated formic acid to ultra-pure water in a 1 L volumetric flask. Adjust to the 1L mark with ultra-pure water. Stopper the flask securely and shake to mix thoroughly.

d) 50/50 ultra-pure water/acetonitrile
Add 50 mL of acetonitrile in a 100 mL volumetric flask. Adjust to the 100 mL mark with ultra-pure water. Stopper the flask securely and shake to mix thoroughly.

e) 0.1% formic acid in ultra-pure water
Add 1 mL concentrated formic acid to ultra-pure water in a 1 L volumetric flask. Adjust to the 1L mark with ultra-pure water. Stopper the flask securely and shake to mix thoroughly.

APPENDIX 3 : LC-MS/MS Tuning Procedure

Calibration of Instrument

The instrument must be mass calibrated on a regular basis using polypropylene glycol (PPG) solutions according to the manufacturer's instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

Tuning Instrument for Pinoxaden, NOA407854, NOA447204

Infuse standard solutions of Pinoxaden, NOA407854, NOA447204 (0.1 to 1.0 µg/mL) in mobile phase (see section 4) directly into the mass spectrometer interface at a rate at of approximately 10-20 µL/min. Roughly adjust interface parameters (sprayer position, spray, heater/auxiliary gas flows, as well as voltages of spray, orifice, and focusing ring) for a sufficiently high parent ion signal at *m/z* 401 for Pinoxaden; *m/z* 317 for NOA407854 and *m/z* 333 for NOA447204 in positive ionisation mode.

Using the Analyst software quantitative optimisation routine, tune the instrument for Pinoxaden, NOA407854 and NOA447204 ensuring that the correct ion is selected. If desired, manual tuning of the ion optics and collision energy can be carried out to ensure maximum sensitivity.

Finally, connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injection of a Pinoxaden, NOA407854 and NOA447204 standards using mobile phase at the flow rate to be used. Tune the interface parameters (sprayer position, spray and heater gas flows, spray, orifice, and focusing ring voltages) and the collision gas flow for maximum sensitivity.

Analyte	Fragment assignment
Pinoxaden *m/z* 401 → 317	Loss of CO-C(CH_3)$_3$
NOA407854 *m/z* 317 → 115	$C_5H_{11}N_2O$ - oxadiazepin fragment
NOA447204 *m/z* 333 → 149	$C_7H_5O_2N_2$

APPENDIX 4 : Method Flow Chart

Acidify water sample (20 mL) with formic acid (100 µL). Check pH ≤ 2

↓

Load water sample (20 mL) onto a conditioned Waters Oasis™ HLB SPE cartridge (60 mg, 3 mL)

↓

Rinse sample tube with 1% formic acid (3 mL) and add rinse to SPE cartridge

↓

Wash SPE cartridges with ultra-pure water:acetonitrile (80:20 v/v) (3 mL)

↓

Elute with 3 mL ultra-pure water:acetonitrile (50:50 v/v)

↓

Analysis by LC-MS/MS