Residue Method for the Determination of NOA449280 and Metabolite SYN503780 in soils.

Data Requirement: EPA Guideline: 850.6100
OECD Data Point: IIA 4.5

Reports: Analytical Method: MRID No. 47841949. Hargreaves, S. 2007. NOA449280: Residue Method for the Determination of NOA449280 and Metabolite SYN503780 in soil. Unpublished study prepared by Syngenta Ltd., Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK. Submitted by Syngenta Crop Protection, LLC, Greensboro, NC.

Independent Laboratory Validation: MRID No. 47841951. Eversfield, S. 2007. NOA449280 – Validation of a Method (Draft GRM030.02A) for the Determination of NOA449280 and SYN503780 in Soil. Unpublished study prepared by CEM Analytical Service (CEMAS), Glendale Park, Fernbank Road, North Ascot, Berkshire SL5 8JB. Submitted by Syngenta Crop Protection, LLC, Greensboro, NC.

Independent Laboratory Validation: MRID No. 47842137. McDonald, T.J., 2010. NOA449280: Independent Laboratory Validation - Analytical Method GRM030.02A for the Determination of NOA449280 and Metabolite SYN503780 in Soil. Final Report. Unpublished study prepared by Syngenta Crop Protection, Inc., 410 Swing Road Greensboro, NC 27409 USA. Submitted by Syngenta Crop Protection, LLC, Greensboro, NC.

Statements: The reports stated that these studies were conducted in compliance with GLP practices.

Classification: This environmental chemistry method is classified, overall, as **Fully Reliable** (EPA classification: Acceptable) for the determination of NOAA449280 and metabolite SYN503780 in soil. While the initial method validation demonstrates that the analytical chemistry method for the analysis the parent and one metabolite in soil is acceptable, these reports address only the parent and one of the major degradates. Analytical method validation data and ILV data for the other major degradates of bicyclopyrone have been submitted separately in MRIDs 47841950, 47841952 and 47841953, and have also been classified as **Fully Reliable** (EPA classification: Acceptable).

PC Code: 018986

Primary Reviewer: Paul Mastradone, Ph.D. Chemist (USEPA)
Signature: Paul J Mastradone
Date: June 30, 2014

Secondary Reviewer: Cheryl Sutton, Ph.D. Environmental Scientist (USEPA)
Signature: Cheryl Sutton
Date: June 30, 2014

Digitally signed by Sutton, Cheryl
DN: cn=Sutton, Cheryl, email=sutton.cheryl@epa.gov
Date: 2014.11.19 12:45:31 -05'00'

EXECUTIVE SUMMARY

This analytical method is designed for the quantitative determination of NOA449280 (parent bicyclopyrone; CAS# 352010-65-5) and its metabolite SYN503780 (CAS# 380355-55-5) in soil using a method that involves successive extraction with three solvents which are then combined and a subsample analyzed by LC-MS/MS (see Table 1). The method is quantitative for NOA449280 and Metabolite SYN503780 at the stated LOQ of 0.001 mg/kg and an LOD estimated to be 0.5 µg/kg. An Independent Lab Validation (ILV) of the method indicated no changes or special practices other than those described in the Materials and Methods were needed to achieve the stated LOQ.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID Environmental Chemistry Method	MRID Independent Laboratory Validation	EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
NOA449280 SYN503780	47841949	47841951 47842137	None	Soil	09/04/2007	Syngenta	LC/MS-MS	0.001 mg kg^{-1}

I. PRINCIPLE OF THE METHOD

The method involves successive extraction of a 10-g soil samples with three extractants. Each sub-extraction involves the addition of a 20-ml aliquot of the extractant followed by shaking, centrifugation, decanting. The extractants are 0.05M ammonium hydroxide, 0.05M ammonium hydroxide/acetone 50:50 v/v, and then acetone. The extractants are combined and checked for clarity (additional centrifugation may be necessary). An approximate 5 ml aliquot of the combined extractants is then drawn into a disposable syringe and filtered through a 25mm GHP, 0.45 µm filter disc. After appropriate dilution a subsample of this then analyzed by HPLC-MS/MS.

II. RECOVERY FINDINGS

Table 2. Initial Validation Method Recoveries for Analytes in Soil (Sandy Loam)

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA449280 Primary transition	0.001	5	73-88	81	n/a	6.7
	0.01	5	83-88	85	n/a	5.2
SYN503780 Primary transition	0.001	5	90-97	95	n/a	3.0
	0.01	5	106-111	109	n/a	1.8
NOA449280 Confirmatory transition	0.001	5	95-125	108	n/a	11.5
	0.01	5	88-104	95	n/a	7.3
SYN503780	0.001	5	76-115	96	n/a	16.9

Confirmatory transition	0.01	5	115-118	117	n/a	1.1

Table 3. Initial Validation Method Recoveries for Analytes in Soil (Clay)

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA449280 Primary transition	0.001	5	74-86	82	n/a	4.1
	0.01	5	74-86	78	n/a	6.5
SYN503780 Primary transition	0.001	5	98-115	104	n/a	6.6
	0.01	5	105-114	109	n/a	3.5
NOA449280 Confirmatory transition	0.001	5	95-125	108	n/a	11.5
	0.01	5	88-104	95	n/a	7.3
SYN503780 Confirmatory transition	0.001	5	76-108	92	n/a	15.2
	0.01	5	88-108	98	n/a	7.3

Table 4. Independent Validation Method Recoveries for Analytes in Soil (Loamy Sand)

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA449280 Primary transition	0.001	5	86-100	92	n/a	6.0
	0.01	5	88-95	94	n/a	3.3
SYN503780 Primary transition	0.001	5	95-103	98	n/a	3.9
	0.01	5	97.8-99.8	98	n/a	0.8
NOA449280 Confirmatory transition	0.001	5	95-103	98	n/a	3.2
	0.01	5	92-97	94	n/a	2.0
SYN503780 Confirmatory transition	0.001	5	87-105	96	n/a	7.0
	0.01	5	97-99	98	n/a	1.0

Table 5. Independent Validation Method Recoveries for Analytes in Soil (Loamy Silt)

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA449280 Primary transition	0.001	5	81-99	88	n/a	9.2
	0.01	5	92-95	94	n/a	1.5
SYN503780 Primary transition	0.001	5	87-103	98	n/a	6.8
	0.01	5	96-100	99	n/a	1.5
NOA449280 Confirmatory transition	0.001	5	80-95	91	n/a	7
	0.01	5	91-95	94	n/a	1.6
SYN503780	0.001	5	86-109	101	n/a	8.7

Confirmatory transition	0.01	5	98-101	100	n/a	1.3

Table 6. Independent Validation Method: Final Report Recoveries for Analytes in Soil (Sandy Loam)

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA449280	0.001	5	94-101	98	n/a	3.1
	0.01	5	94-99	97	n/a	2.0
SYN503780	0.001	5	82-95	88	n/a	5.6
	0.01	5	96-100	88.2	n/a	6.3

Table 7. Independent Validation Method: Final Report Recoveries for Analytes in Soil (Clay Loam)

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA449280	0.001	5	91-104	97	n/a	5.2
	0.01	5	95-102	96	n/a	4.0
SYN503780	0.001	5	85-101	91	n/a	7.3
	0.01	5	82-97	92	n/a	6.2

III. METHOD CHARACTERISTICS

The limit of detection (LOD) of the method for the initial validation study was 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 for this procedure in the soils tested is estimated at 0.08 $\mu g\ kg^{-1}$ for NOA449280 and 0.1 $\mu g\ kg^{-1}$ for SYN503780 using the primary transition and 0.2 $\mu g\ kg^{-1}$ for NOA449280 and 0.4 $\mu g\ kg^{-1}$ for SYN503780 using the confirmatory ion.

Similarly, for the ILV study the LOD 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.

Table 8. Method Characteristics

	NOA449280	SYN503780
Limit of Quantitation (LOQ)*	0.001 mg kg^{-1}	0.001 mg kg^{-1}
Limit of Detection (LOD) primary ion	0.08 µg kg^{-1}	0.1 µg kg^{-1}
Limit of Detection (LOD) confirmatory ion	0.2 µg kg^{-1}	0.4 µg kg^{-1}
Linearity (calibration curve r^2 and spike concentration)	$r^2 = 0.9999$ 0.001 mg kg^{-1}	$r^2 = 0.9999$ 0.001 mg kg^{-1}
Repeatable	Yes	Yes
Reproducible	Yes	Yes
Specific	Yes	Yes

* The limit of quantitation of the method is 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 relative standard deviation of ≤ 20% has been obtained.

IV. METHOD DEFICIENCIES AND REVIEWER'S COMMENTS:

Although submitted and titled as an independent laboratory validation ("Final Report," MRID 47842137) one of the ILV studies appears to have been conducted at a Syngenta laboratory rather than an outside (independent) laboratory. However, this study reports the same data as presented in the other submitted ILV, which was reportedly conducted by an independent laboratory.

All studies report excellent detector linearity with ($R^2 = 0.9993$ to 0.9999) for both the primary and confirmatory ions.

Both the LOQ and the LOD are acceptable and within ranges of 70-120 %. The wide range of the LOD is of note. It does not appear to be directly linked to an obvious factor such as soil type or organic matter content.

The method seems to demonstrate lowest recoveries at the 10 ppb (higher) fortifications in finer soils.

The only reported method modifications were for optimization of equipment for different bands.

This method is designed for the quantitative determination of residues of bicyclopyrone (NOA449280) and its metabolite SYN503780 in soil. The initial validation of the environmental chemistry method is acceptable and meets the criteria for a scientifically valid method for the parent and the single metabolite studied.

Attachment 1: Chemical Names and Structures:

IUPAC Name:	**4-Hydroxy-3-[2-(2-methoxy-ethoxymethyl)-6-trifluoromethyl-pyridine-3-carbonyl]-bicyclo[3.2.1]oct-3-en-2-one**
CAS Name:	**N/A**
CAS Number:	**352010-65-5**

IUPAC Name:	**2-(2-Methoxy-ethoxymethyl)-6-trifluoromethyl-nicotinic acid**
CAS Name:	**N/A**
CAS Number:	**380355-55-5**