Cyantraniliprole

SYN545377 – Independent Laboratory Validation of Residue Method (GRM073.01A) for the Determination of Cyantraniliprole Aqueous Photo-Products in Surface Water by LC-MS/MS

Final Report

DATA REQUIREMENT(S): OECD ENV/JM/MONO17
EPA 850.6100
EC SANCO/3029/99 rev 4
EC SANCO/825/00 rev 8.1

-

2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Syngenta Analytical Method GRM073.01A entitled "SYN545377 - Residue Method GRM073.01A for the Determination of Photolysis Products IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in Surface Water.

This study was designed to satisfy harmonized guideline requirements described in EPA 850.6100 (Data Reporting for Environmental Chemistry Methods) (2), EC SANCO/3029/99 Rev.4, and EC SANCO/825/00 Rev.8.1. This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (3).

The residue analytical method is suitable for the determination of Cyantranilprole aqueous photo-products IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in surface water.

Due to potential degradation of IN-NXX69, it was analyzed separately from IN-NXX70, IN-QKV54, and IN-RUNU71.

For IN-NXX69 analysis, water samples were acidified to pH 4 prior to fortification. Acidified samples were passed through a 6 mL/500 mg ENV solid phase extraction (SPE) cartridge where IN-NXX69 is retained. The cartridge was washed with water prior to eluting the compound with 0.1 % formic acid in acetonitrile. The column eluates were evaporated to dryness and then re-dissolved in equal portions of methanol and 0.02 M formic acid (aq) with sonication and vortex-mixing.

For IN-NXX70, IN-QKV54, and IN_RNU71 analysis, water samples were passed through a 6 mL/500 mg ENV solid phase extraction (SPE) cartridge where the analytes were retained. The cartridges were washed with water prior to eluting the compounds with 0.02 M ammonium hydroxide in acetonitrile. The column eluates were evaporated to dryness and then re-dissolved in equal portions of methanol and 0.02 M formic acid (aq) with sonication and vortex-mixing.

All samples were analyzed by liquid chromatography/mass spectrometry (LC-MS/MS).

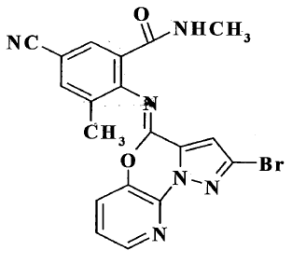
The limit of quantification of the method is 0.10 ppb (or 0.10 µg/L) for all compounds.

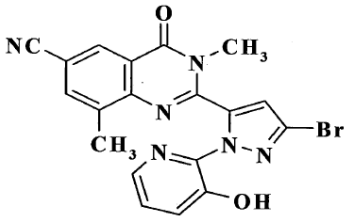
The analytical method extraction schematic is provided in Appendix 2.

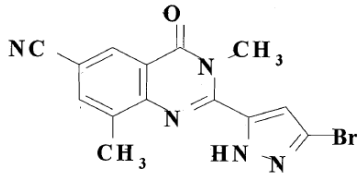
3.0 MATERIALS AND METHODS

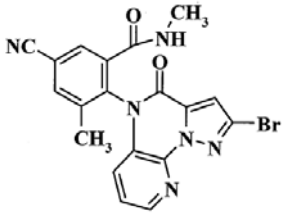
3.1 Test/Reference Substance

The test/reference substances were obtained from ABC Laboratories, 7200 E ABC Lane, Columbia, MO 65202 on September 22, 2015 and DuPont Crop Protection, Stine Haskell Research Center Sample Management Facility, S210/ Room 186, 1090 Elkton Road Newark, DE 19714 on September 22, 2015. The following test/reference substances were used:

Compound Structure	
Syngenta Code:	IN-NXX69
PTRL West Identification:	2803W-004
Lot Number:	80378-1-11-1
Common Name:	Degradate of Cyantraniliprole
Molecular Weight:	437.25 g/mole
Storage Conditions:	Frozen
Purity:	99.4%
Expiration Date:	11-14-2015

Compound Structure	
Syngenta Code:	**IN-NXX70-001**
PTRL West Identification:	**2803W-001**
Lot Number:	**E112180-119F**
Common Name:	**Degradate of Cyantraniliprole**
Molecular Weight:	**437.26 g/mole**
Storage Conditions:	**Ambient**
Purity:	**98.2%**
Expiration Date:	**5-8-2018**

Compound Structure	
Syngenta Code:	**IN-QKV54-002**
PTRL West Identification:	**2803W-003**
Lot Number:	**GF1001133**
Common Name:	**Degradate of Cyantraniliprole**
Molecular Weight:	**344.17 g/mole**
Storage Conditions:	**Ambient**
Purity:	**98.1%**
Expiration Date:	**1-17-2016**

Compound Structure	
Syngenta Code:	**IN-RNU71-002**
PTRL West Identification:	**2803W-002**
Lot Number:	**D100877-122**
Common Name:	**Degradate of Cyantraniliprole**
Molecular Weight:	**437.26 g/mole**
Storage Conditions:	**Ambient**
Purity:	**95.1%**
Expiration Date:	**2-4-2016**

Characterization data for the test/reference standards are maintained by the Sponsor, Syngenta Crop Protection, LLC.

The test/reference substances (analytical standards) used in this study were procured from the Sponsor or their representative and stored as directed on their Certificates of Analysis. All solutions made from the reference substances (analytical standards) were stored according to the method.

3.2 Test System

The test system evaluated in this study was surface water. The matrix was chosen because it is representative of the matrix the method was designed for. Control samples used in this study were provided by PTRL West (a division of EAG). These control water samples were characterized by AGVISE Laboratories of Northwood, North Dakota and reported to PTRL West Study Number 2706W. GLP characterization reports are presented in Appendix 3 and results are provided in Table 1 and summarized below:

Sample ID	pH	Calcium (ppm)	Magnesium (ppm)	Hardness CaCO3 (mg/L)	TDS (ppm)	TOC (ppm)
Surface 2706W-034)	8.3	124	69	598	1138	20.2
Surface water 2706W-029	8.3	136	81	678	1846	20.7

Test system was stored under refrigerated conditions when not in use.

Unfortified control samples were checked during the ILV for contamination by employing the same extraction and detection method as described in Syngenta Method GRM073.01A.

3.3 Equipment and Reagents

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

3.3.1 Equipment

Instrument	AB Sciex API 5500 Series Tandem Mass Spectrometer with Agilent 1200 HPLC system (LC-MS/MS)
Laboratory Balance	OHAUS AP250D
Pipetman with plastic disposable tips	Eppendorf Automatic Pipet: P1000, P200
Vortex Mixer	MaxiMixI Type 16700 Mixer
Ultrasonic Bath	Branson 2800/ Branson 2510
Centrifuge Tubes	15 mL glass tubes
N_2 Turbo-Vap	Zymark TurboVap LV Evaporator
pH Meter	Thermo Scientific Orion Star A321

3.3.2 Reagents

HPLC grade water, methanol, acetonitrile, and dimethyl sulfoxide were obtained from Burdick & Jackson; LC-MS grade formic acid was obtained from Sigma Aldrich. ACS reagent grade ammonium hydroxide was obtained from Fisher Scientific.

3.3.3 Preparation of Reagents

- 1 M Formic Acid (aq) was typically prepared by adding 0.381 mL of formic acid (99%) to a 10 mL volumetric flask and diluted to volume with HPLC water and thoroughly mixed.
- 1mM Formic Acid (aq) was typically prepared by adding 100 µL of 1 M Formic Acid (aq) to 100 mL volumetric flask and diluted to volume with HPLC water and mixed well.
- 0.02 M Formic Acid (aq) was typically prepared by diluting 2.0 mL of 1 M Formic Acid (aq) to 100 mL volumetric flask and diluted to volume with HPLC water and mixed well.
- 1 M Ammonium Hydroxide (aq) was typically prepared by adding 7.0 mL of ammonium hydroxide (28%) to 93 mL of HPLC water and mixed well.
- 0.02 M Ammonium Hydroxide in ACN was typically prepared by diluting 10 mL of 1 M Ammonium Hydroxide (aq) to 490 mL of acetonitrile and mixed well.
- 0.1% Formic Acid in ACN was typically prepared by adding 0.5 mL of formic acid (99%) to a 500 mL volumetric flask and diluted to volume with acetonitrile and mixed well.
- 50:50 MeOH:0.02 M Formic Acid (v:v) was typically prepared by adding 50 mL of methanol to 50 mL of 0.02 M Formic Acid (aq) and mixed well.

3.4 Preparation of Standard Solutions

Standard solutions were prepared and stored as recommended in the method.

3.4.1 Stock Standards

Individual 100 µg/mL stock solutions for IN-NXX69, IN-NXX70, IN QKV54, and IN-RNU71 were prepared by weighing an aliquot of each reference standard into separate appropriately sized volumetric flask. The stock solutions were dissolved with acetonitrile to yield approximate concentrations of 100 µg/mL after adjusting for purities. The stock solutions were mixed well, transferred to amber glass bottles and stored in freezer (typically < -10°C) when not in use.

Ptrl West No.	Standard Name	Weight (mg)	volume (mL)	Purity (%)	Theoretical Conc. (µg/mL)
2803W-004	IN-NXX69	5.11	50	99.4	101.59
2803W-001	IN-NXX70	2.41	25	98.2	94.66
2803W-003	IN-QKV54	5.34	50	98.1	104.77
2803W-002	IN-RNU71	5.20	50	95.1	98.90

3.4.2 Fortification Standard

Two separate sets of fortification solutions were prepared at 1 µg/mL, 100 ng/mL and 10 ng/mL. The first set included only IN-NXX69, with the second set including the remaining standards, IN-NXX70, IN-QKV54, and IN-RNU71. All fortification solutions were prepared by performing serial dilutions of the stock solutions. Appropriate aliquots were removed from each stock standard using automatic calibrated pipets and diluted to volume with acetonitrile into 10 mL volumetric flasks. Fortification solutions were mixed well, transferred to amber glass storage bottles and stored in the freezer (typically < -10°C) when not in use. Details for the dilutions are provided below and presented in Table 2.

Fortification Solution Identification	Theoretical Conc. (ng/mL)	Solution Used (µg/mL)	Volume of Solution (mL) (mL)	Final Volume (mL)
Set 1				
IN-NXX69	1000	101.59	0.0986	10
	100	1.0	1.0	10
	10	1.0	0.1	10
Set 2				
*IN-NXX70	1000	94.66	0.1058	10
	100	1.0	1.0	10
	10	1.0	0.1	10
*IN-QKV54	1000	104.77	0.0956	10
	100	1.0	1.0	10
	10	1.0	0.1	10
*IN-RNU71	1000	98.90	0.1012	10
	100	1.0	1.0	10
	10	1.0	0.1	10

*Prepared as mixed fortification solutions

3.4.3 Calibration Standard

Two sets of calibration standard solutions were prepared; set one contained only the IN-NXX69 standard and set two contained the remaining standards, IN-NXX70, IN-QKV54, and IN-RNU71. Prior to preparing the two sets of calibration standard solutions an intermediate solution was prepared for each set. An intermediate solution containing only IN-NXX69 was prepared by diluting 100 µL of the 1000 ng/mL fortification solutions into a 10 mL volumetric flask and diluting to volume with a 0.02 M formic acid: methanol (1:1, v:v). A second intermediate solution containing IN-NXX70, IN-QKV54, and IN-RNU71 was prepared by diluting 100 µL of each of the 1000 ng/mL mixed fortification solution into the same 10 mL volumetric flask and diluting to volume with a 0.02 M formic acid: methanol (1:1, v:v). The intermediate solutions were mixed well and then transferred to amber glass bottles. From the intermediate solutions, dilutions were made to prepare two sets of seven

calibration standards. Appropriate volumes were removed using automatic calibrated pipets and diluted in 5 mL volumetric flasks with a 0.02 M formic acid: methanol (1:1, v:v) solution as described below. Final calibrants were transferred into glass amber bottles and stored in the freezer (typically < -10°C) when not in use.

Theoretical Conc. (ng/mL)	Solution Used (ng/mL)	Volume of Solution (mL) (mL)	Final Volume (mL)
4	10	2.0	5
3	10	1.5	5
2	10	1.0	5
1	10	0.5	5
0.8	10	0.4	5
0.6	10	0.3	5
0.5	10	0.25	5

Note: 1 set contained only IN-NXX69, second set contained IN-NXX70, IN-QKV54, IN-RNU71

3.5 Analytical Procedures and Modifications

Analytical Method GRM073.01A was independently validated as written.

3.5.1 Modifications

Syngenta Method GRM073.01A was followed as written with no exceptions.

3.5.2 Fortifications

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

Matrix	Test Substance	Fortification Volume (µL)	Fortification Conc. (ng/mL)	Final Volume (mL)	Final Conc. (ng/mL)	Replicates
Surface	IN-NXX69	100	10	10	0.1	5
Surface		100	100	10	1.0	5
Surface	IN-NXX70, IN-QKV54, IN-RNU71	200	10	20	0.1	5
Surface		200	100	20	1.0	5

3.5.3 Extraction Procedure

As indicated by method GRM073.01A the following extraction steps were performed for IN-NXX69:

1. Transfer 10 mL of test system water into 50 mL plastic centrifuge tubes. Adjust pH to approximately 4 with formic acid.
2. Fortify samples, if appropriate; five at LOQ and five at 10 X LOQ
3. Condition Bond Elut-ENV (500 mg/6 mL) cartridge as follows: 6 mL methanol, 6 mL 1 mM formic acid solution, and 5 mL 1 mM formic acid solution (no vacuum)
4. Add samples and allow to go to waste, after loaded apply slight vacuum to dry the cartridge of excess water.
5. Elute IN-NXX69 into 15 mL glass centrifuge tubes with 2 times 5 mL of 0.1 formic acid in acetonitrile, after loaded strong vacuum applied to collect all acetonitrile.
6. Concentrate eluates to dryness in turbo-vap at 40 °C.
7. Add 0.5 mL of methanol, vortex and sonicate for approximately 2 minutes, then add 0.5 mL of 0.02 M formic acid (aq) solution, vortex to mix, sonicate 2 minutes.
8. Filter samples through PTFE filter
9. Add filtered extracts to auto-sampler vials for controls and LOQ samples.
10. Perform 10x dilution on 10x LOQ samples by combing 0.1 mL of sample with .0.9 mL of methanol: formic acid solution (aq) (50:50, v:v)

As indicated by method GRM073.01A the following extraction steps were performed for IN-NXX70, IN-QKV54, and IN-RNU71:

1. Transfer 20 mL of test system water into 50 mL plastic centrifuge tubes.
2. Fortify samples, if appropriate; five at LOQ and five at 10 X LOQ
3. Condition Bond Elut-ENV (500 mg/6 mL) cartridge as follows: 6 mL methanol, 6 mL 1 mM formic acid solution, and an additional 5 mL of 1 mM formic acid solution (no vacuum, to not let cartridge go to dryness)
4. Add samples and allow to go to waste, after loaded apply additional 5 mL HPLC water and allow to go to waste.
5. Elute IN-NXX70, IN-QKV54, and IN-RNU71 into 15 mL glass centrifuge tubes by adding three times 5 mL aliquots of 0.02 M ammonium hydroxide: acetonitrile solution. After elution, apply strong vacuum to collect all acetonitrile.
6. Concentrate eluates to dryness in turbo-vap at 40 °C.
7. Add 1 mL of methanol, vortex and sonicate for approximately 2 minutes, then add 1 mL of 0.02 M formic acid (aq) solution, vortex and then sonicate to mix.
8. Filter samples through PTFE filter
9. Add filtered extracts to auto-sampler vials for controls and LOQ samples.
10. Perform 10x dilution on 10x LOQ samples by combing 0.1 mL of sample with .0.9 mL of methanol: formic acid solution (aq) (50:50, v:v)

3.6 Instrumentation

LC System	:	1200 HPLC System
MS Detector	:	API 5500 tandem mass spectrometer

Flow Rate: 0.5 mL/min
Column: Phenomenex Synergi 2.5 µ Polar – RP 50 x 3 mm
Column Oven Temp: 40 °C
Injection Vol. 10 µL
Run Time: 10 minute
Retention Time: IN-NXX69 = 4.5, IN-NXX70 = 5.2, IN-QKV54 = 5.4, and IN-RNU71 = 3.2 minutes
Mobile Phase A: 0.1 % Formic Acid in Water
Mobile Phase B: 0.1 % Formic Acid Methanol

Gradient Program:

Time	A%	B%
0	50	50
3	30	70
6	30	70
7	5	95
8	5	95
8.1	50	50
10	50	50

Mass Spectrometer Conditions

Interface	:	Turbo Ion Spray (ESI)
Polarity	:	Positive
Curtain gas (CUR)	:	15
Temperature (TEM)	:	600
Ionspray voltage	:	5500
Collision gas setting (CAD)	:	10
Gas 1 (GS1)	:	60
Gas 2 (GS2)	:	40
Scan type	:	MRM

MRM Conditions		IN-NXX69 Primary Transition	IN-NXX69 Confirmatory Transition	IN-NXX70 Primary Transition	IN-NXX70 Confirmatory Transition
Q1 *m/z*	:	437.0	437.0	437.0	439.0
Q3 *m/z*	:	406.1	343.9	344.0	346.0
Dwell time	:	100	100	100	100
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Low	Low	Low	Low
Declustering potential (DP)	:	70.0	70.0	110.0	110.0
Entrance potential (EP)	:	10.0	10.0	10.0	10.0
1Collision energy (CE)	:	50.0	50.0	45.0	45.0
Collision cell exit potential (CXP)	:	30.0	30.0	30.0	23.0

MRM Conditions		IN-QKV54 Primary Transition	IN-QKV54 Confirmatory Transition	IN-RNU71 Primary Transition	IN-RNU71 Confirmatory Transition
Q1 *m/z*	:	344.0	344.0	437.0	437.0
Q3 *m/z*	:	236.0	186.0	406.0	300.0
Dwell time	:	100	100	100	100
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Low	Low	Low	Low
Declustering potential (DP)	:	114.0	114.0	150.0	150.0
Entrance potential (EP)	:	10.0	10.0	10.0	10.0
Collision energy (CE)	:	44.0	49.0	38.0	52.0
Collision cell exit potential (CXP)	:	15.0	15.0	14.0	14.0

3.7 Data Acquisition

Peak integration and peak area count quantitation were performed by Analyst Software version 1.6.2. A best-fit, linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of analyte. The square of correlation coefficients (R^2) for the calibration curves for each analytical set was greater than 0.99. Recovery results were computed for each sample.

A statistical treatment of the data includes the calculation of averages, standard deviations, relative standard deviations. Mean percent recoveries, standard deviations, and relative standard deviations were calculated using a current Microsoft Office Excel package. Example calculations are provided in Appendix 4.

4.0 RESULTS AND DISCUSSION

4.1 Method Establishment/Pre-Validation Evaluation

Using the instrument parameters as described in the method, the retention times of the analytes, instrument detection limits and response linearity were established by injecting a series of calibration reference standards.

In addition, matrix assessment was performed on validation sample sets by analyzing the reagent blank and untreated control samples prior to analysis of the fortified samples. No detectable residues were observed and no peaks were present that might interfere with the quantitation of IN-NXX69, IN-NXX70, IN-QKV54 and IN-RNU71 in untreated control and reagent blank samples. Therefore, no further matrix assessment was initiated.

4.2 Independent Laboratory Results

The method was successfully validated on the first attempt for IN-NXX70, IN QKV54, and IN-RNU71 test substances on surface water at the method LOQ and 10 X LOQ concentration levels, using the method as written. However, IN-NXX69 (2803W-004), required a second attempt due to low recoveries, which may be attributed to the pH adjustment of samples prior to fortification. The stated LOQ of the method for all is 0.1 µg/L

4.6 Protocol Amendments and Deviations

One protocol amendment and one protocol deviation occurred over the course of the study.

Protocol Deviation 1:

The protocol states:
Prior to conducting the ILV, the performing laboratory will need to establish method control not limited to but including analyte retention time, linearity, instrument response, instrument detection limits, procedures and verification that the control matrix is free of interferences. The performing laboratory should demonstrate method control by performing assessment tests before proceeding to method validation trials. More than one assessment test may be made depending on the number and type of substitutions. Data and results of any assessment test shall be included in the study records, but not in the final report.

Prior to conducting the ILV, matrix interference was not assessed, however during the conduct of the ILV, matrix interferences were not observed in the control samples, therefore this deviation did not negatively impact the results of the study.

No SOP, or method deviations were generated during this validation.

4.7 Circumstances Affecting Data

No circumstances occurred during this validation that affected quality or integrity of the data.

4.8 Matrix Effects

No significant interfering peaks were observed in the surface water control samples tested for IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 during method validation, and non-matrix standards should generally be used for quantification as recommended by the method. A summary of comparisons between non-matrix based calibrants and fortified control samples is provided below.

4.9 Final Fraction Stability

Final fraction residues in methanol: 0.02 M formic acid (aq) (50:50 v:v) retained in vials were found to be stable for at least 7 days upon storage at a temperature of approximately 4°C during method validation.

5.0 CONCLUSIONS

PTRL West (a division of EAG) successfully independently validated Syngenta Analytical Method GRM073.01A entitled "SYN545377 - Residue Method GRM073.01A for the Determination of Photolysis Products IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in Surface Water. Final Determination by LC-MS/MS".

The method was demonstrated to be suitable for the determination of cyantraniliprole aqueous photo-products in surface water at a LOQ of 0.1 µg/L and at 10X the LOQ 1.0 µg/L.

TABLE 1 Characterization Data of Water Samples used for Method Validation

Water Type	Sample ID	AGVISE Lab #	Sample Description	pH	Calcium (ppm)	Magnesium (ppm)	Hardness CaCO3 (mg/L)	TDS (ppm)	DOC (ppm)
Surface	2706W-034	15-210	Goose River Water	8.3	124	69	598	1138	20.2
Surface	2706W-029	15-138	Goose River Water	8.3	136	81	678	1846	20.7

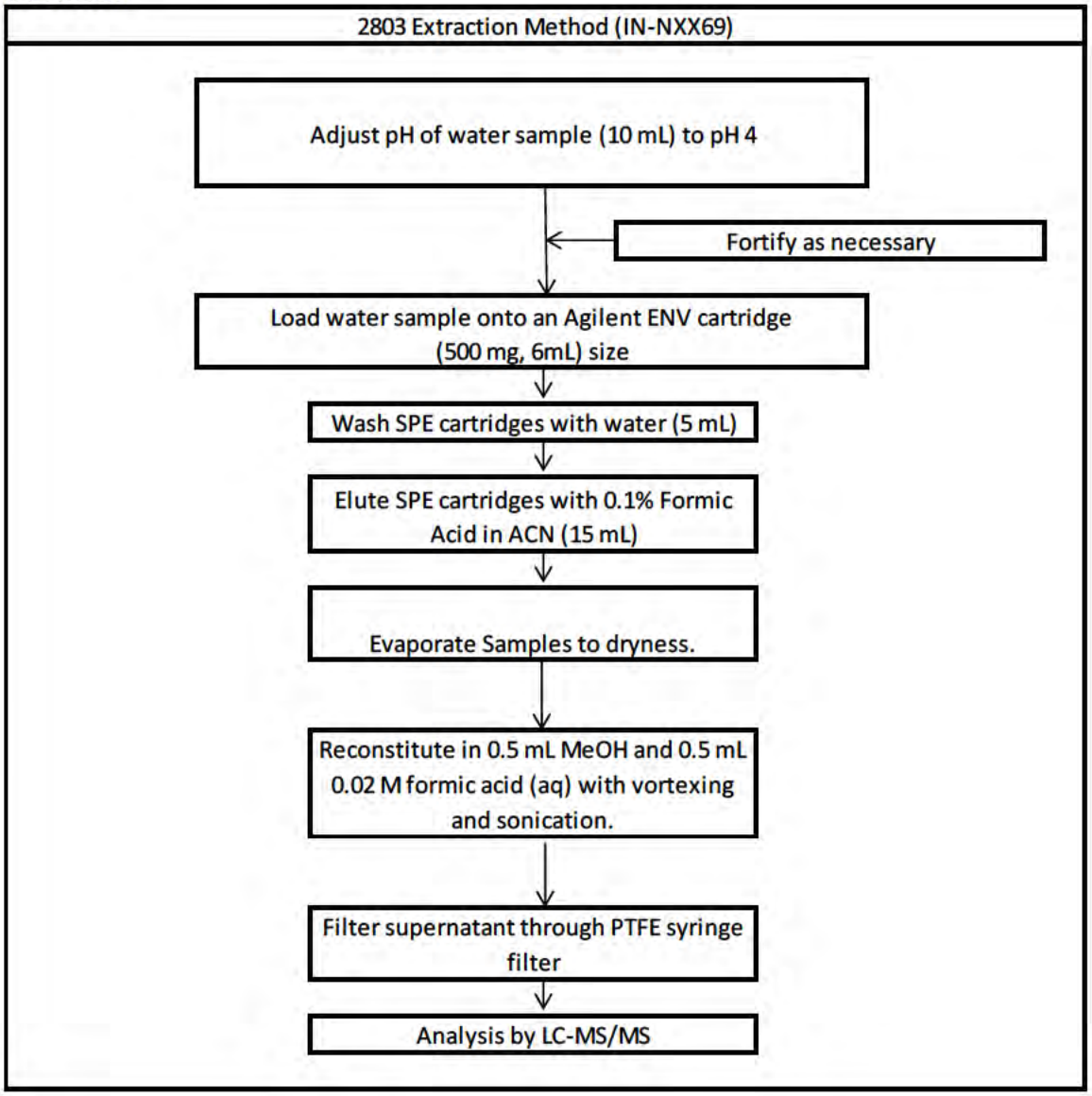
TDS (Total Dissolved Solids), DOC (Dissolved Organic Carbon)
Note: The GLP characterization of this water samples was performed by AGVISE Laboratories, 604 Highway 15, P. O. Box 510, Northwood, ND 58267.

TABLE 2 Water Fortification Levels

Water Type	Fortification Level (ppb)	Number of Recovery Samples
Surface	Control	2
LOQ	0.1	5
10X LOQ	1.0	5

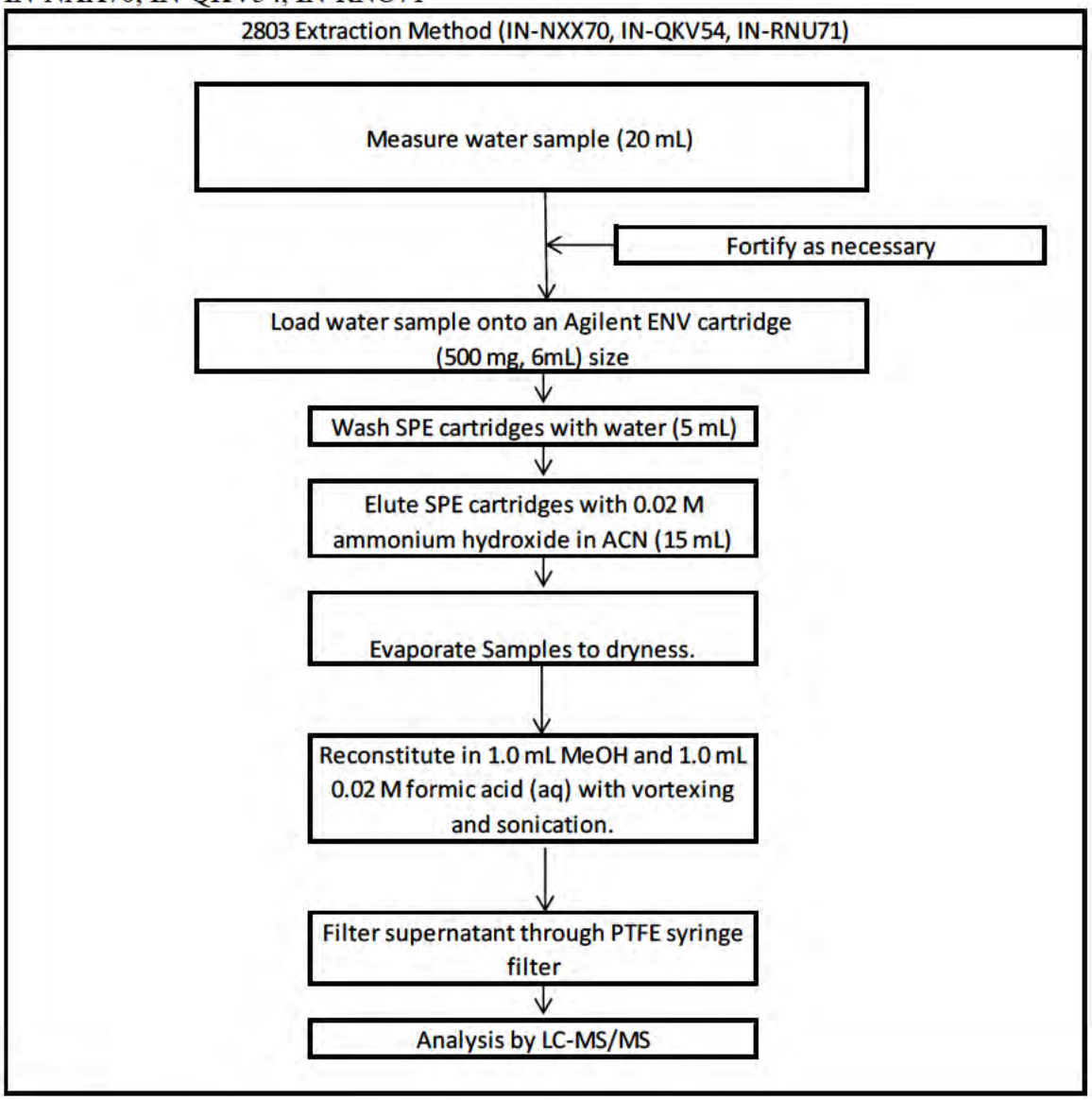
APPENDIX 2 Analytical Extraction Method Flow-Chart

IN-NXX69



APPENDIX 2 cont. **Analytical Extraction Method Flow-Chart**

IN-NXX70, IN-QKV54, IN-RNU71



APPENDIX 5 Example Calculations

IN-NXX69

The quantitation of IN-NXX69 was conducted using peak area relative to the theoretical concentrations of the calibrants. The content of IN-NXX69 in sample was quantitated against 1/x weighted linear curve of IN-NXX69 calibrants where:

$$\text{ng/mL analyte} = \frac{y - b}{m}$$

y = peak area
x = ng/mL compound injected
m = slope
b = intercept

Weighting of the calibration curve was applied to provide better curve fit at the lower concentration levels of IN-NXX69.

The calculation of weighted curve equations (linear regression) and concentrations (ng/mL) present in samples and calibrants was conducted using Analyst® software.

The residue of the analyte in the sample is determined as follows:

$$\text{Residue (ng/mL)} = \frac{\text{ng/mL analyte x final sample volume (mL) x Dil. Factor}}{\text{initial sample volume (mL)}}$$

where ng/mL = µg/L or ppb and

Initial Sample Volume = 10 mL (volume of test system)

The Percent Recovery of a fortified sample is determined as follows:

$$\frac{\text{Residue (ng/mL) –Average Residue of Controls (ng/mL)}}{\text{Fortification Level (ng/mL)}} \times 100$$

An example calculation from the second analysis for the recovery of IN-NXX69 (m/z 437/406 ion transition) in surface water fortified at 0.1 ng/mL (sample designated F1-A) is given in following: