Analytical method for cyantraniliprole photolysis metabolites IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in water

Reports: ECM: EPA MRID No.: MRID 50234306. Vogl, E. 2015. Cyantraniliprole – SYN545377 - Residue Method GRM073.01A for Determination of the Photolysis Products IN-NXX69, IN-NXX70, IN-QKV54, IN-RNU71 in Surface Water. Final Determination by LC-MS/MS – Analytical Method. Syngenta Report No.: GRM073.01A and Task No.: TK0261486. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, and sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 79 pages. Final report issued August 5, 2015.

ILV: EPA MRID No. 50234307. Keenan, D. 2016. Cyantraniliprole – SYN545377 - Independent Laboratory Validation of Residue Method (GRM073.01A) for Determination of Cyantraniliprole Aqueous Photo-Products in Surface Water by LC-MS/MS – Final Report. EAG Project No. 2812W. FMC Tracking No. 2015AMT-FLU2297. Report prepared by PTRL West (a division of EAG), Hercules, California, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 107 pages. Final report issued February 5, 2016.

Document No.: MRIDs 50234306 & 50234307

Guideline: 850.6100

Statements: ECM: The study was not conducted in accordance with the USEPA FIFRA or OECD Good Laboratory Practice (GLP) standards (p. 3 of MRID 50234306). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. A signed and dated Summary of Revisions to Pervious Version was included (p. 4).

ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50234307). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of authenticity was included with the Quality Assurance statement.

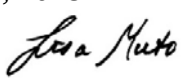
Classification: This analytical method is classified as **Supplemental**. An updated ECM should be submitted for the IN-NXX69 portion of the method. ILV communication report should have been more detailed to assure independence of the ILV from the ECM. In the ECM, an insufficient number of samples (3) was prepared for all analyses, the purities of the test materials were not reported, and no 10×LOQ representative chromatograms were provided.

PC Code: 090098

EFED Final Reviewer: Christopher M. Koper, M.S., Chemist

Signature: 

Date: July 13, 2018

CDM/CSS- Lisa Muto, M.S., Signature: 

Dynamac JV Reviewers:	Environmental Scientist	Date:	4/5/18
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature:	Kathleen P. Ferguson
		Date:	4/5/18

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Syngenta Method GRM073.01A, is designed for the quantitative determination of cyantraniliprole photolysis metabolites IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in water at the LOQ of 0.10 µg/L using LC/MS/MS. **Currently, there is no aquatic toxicity data available for the cyantraniliprole photolysis metabolites IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71. However, the LOQ in water of 0.10 µg/L is currently less than the lowest toxicological level of concern for aquatic organisms for the parent cyantraniliprole (*Daphnia magna* 48-hour EC_{50} = 20.4 ug a.i./L; MRID 48120114).** The ECM and ILV validated the method using one characterized surface water matrix; matrices differed between the ECM and ILV. The ILV validated the method for IN-NXX70, IN-QKV54, and IN-RNU71 at both fortification levels after the first trial, with insignificant analytical instrument modifications. The method was validated for IN-NXX69 at both fortification levels after the second trial, with insignificant analytical instrument modifications; the first trial failed due to low recoveries due to inadequate acidification. An updated ECM should be submitted identifying the adjustment of the pH to exactly 4 as a critical step. All ILV data was satisfactory regarding accuracy, precision, linearity, and specificity; however, the ILV communication report should have been more detailed to assure independence of the ILV from the ECM. All ECM data was satisfactory regarding accuracy, precision, linearity, and specificity, but an insufficient number of samples was prepared for all analyses, the purities of the test materials were not reported, and no 10×LOQ representative chromatograms were provided. The LOD of the ECM differed from that of the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
IN-NXX69	50234306	50234307		Water[2,3]	05/08/2015	Syngenta Crop Protection	LC/MS/MS	0.10 µg/L
IN-NXX70								
IN-QKV54								
IN-RNU71								

1 IN-NXX70 = 2-[3-Bromo-1-(3-hydroxypyridin-2-yl)-1H-pyrazol-5-yl]-3,8-dimethyl-4-oxo-3,4-dihydroquinazoline-6-carbonitrile; IN-QKV54= 2-(5-Bromo-1H-pyrazol-3-yl)-3,4-dihydro-3,8-dimethyl-4-oxo-6-quinazolinecarbonitrile; and IN-RNU71 = 2-(2-Bromo-4-oxopyrazolo[1,5-a]pyrido[3,2-e]pyrazin-5(4H)-yl)-5-cyano-N,3-dimethylbenzamide (no chemical name was provided for IN-NXX69).

2 In the ECM, the surface (rice paddy) water (pH 7.8, 379 mg/L hardness as $CaCO_3$, 450 ppm total dissolved solids, 5.2 mg/L dissolved oxygen) was obtained from Rapides Parish, Louisiana, and used in the study (Appendix I, p. 103 of MRID 50234306). The water characterization laboratory was not reported.

3 In the ILV, the surface (river) water (Sample ID: 2706W-034; pH 8.3, 598 mg equivalent $CaCO_3$/L hardness, 1138 ppm total dissolved solids, 20.2 ppm total organic carbon) was obtained from Goose River, North Dakota, and characterized by Agvise Laboratories, Northwood, North Dakota (p. 16; Appendices 4-6, pp. 96, 99-102, 107 of MRID 50234307).

I. Principle of the Method

Syngenta Method GRM073.01A

For IN-NXX69, water (10 mL) was measured into a polypropylene centrifuge tube, acidified to *ca*. pH 4 via addition of formic acid dropwise, and fortified with the standard solution of IN-NXX69 in acetonitrile for procedural recoveries (pp. 12, 14, 16-17; Appendix 1, p. 75 of MRID 50234306). The samples were applied to a Bond Elut ENV Solid Phase Extraction (SPE) cartridge (500 mg, 6 mL; pre-conditioned with 6 mL of methanol, 6 mL of 1 mM aqueous formic acid, then 5 mL of 1 mM aqueous formic acid) via a 20-mL reservoir on top of the SPE cartridge. The column should not be allowed to run dry. The column reservoir and connector were rinsed with 5 mL of water; the water was drawn through the column via applied vacuum. The analyte was eluted from the column using 2 x 5 mL 0.1% formic acid in acetonitrile via gravity (vacuum may be applied for 5 seconds to collect excess solvent from the cartridge). The solvent was removed from the sample to dryness via a stream of nitrogen in an N-Evap with a water bath temperature of *ca*. 40°C. The residue was reconstituted in 0.50 mL methanol and vortex-mixed for *ca*. 20 seconds then ultrasonicated for *ca*. 2 min. 0.50 mL of 0.02 M aqueous formic acid was added, then the sample was vortex-mixed for *ca*. 20 seconds then ultrasonicated for *ca*. 2 min. The samples were filtered (0.2 µm PTFE filter), then an aliquot was transferred to an autosampler vial for analysis by LC/MS/MS.

For IN-NXX70, IN-QKV54, and IN-RNU71, water (20 mL) was measured into a polypropylene centrifuge tube and fortified with the mixed standard solution of IN-NXX70, IN-QKV54, and IN-RNU71 in acetonitrile for procedural recoveries (pp. 12, 14, 17-18; Appendix 1, p. 75 of MRID 50234306). The samples were applied to a Bond Elut ENV Solid Phase Extraction (SPE) cartridge (500 mg, 6 mL; pre-conditioned with 6 mL of methanol, 6 mL of 1 mM aqueous formic acid, then 5 mL of 1 mM aqueous formic acid) via a 20-mL reservoir on top of the SPE cartridge. The column should not be allowed to run dry. The column reservoir and connector were rinsed with 5 mL of water; the water was drawn through the column via applied vacuum. The analyte was eluted from the column using 3 x 5 mL 0.02 M ammonium hydroxide in acetonitrile via gravity (vacuum may be applied for 5 seconds to collect excess solvent from the cartridge). The solvent was removed from the sample to dryness via a stream of nitrogen in an N-Evap with a water bath temperature of *ca*. 40°C. The residue was reconstituted in 1.0 mL methanol and vortex-mixed for *ca*. 20 seconds then ultrasonicated for *ca*. 2 min. 1.0 mL of 0.02 M aqueous formic acid was added, then the sample was vortex-mixed for *ca*. 20 seconds then ultrasonicated for *ca*. 2 min. The samples were filtered (0.2 µm PTFE filter), then an aliquot was transferred to an autosampler vial for analysis by LC/MS/MS.

The method noted that 1) HPLC grade ultra-pure water should be used in the LC mobile phase; 2) it is critical that the pH of the samples with IN-NXX69 is adjusted to 4 to prevent decomposition (pp. 18-19 of MRID 50234306). Additionally, statements were made which indicated that the use of laboratory SPE columns could require optimization of the method parameters and that dilutions of samples may require additional control aliquots for SPE procedures.

Method Flow Diagrams were included (Appendices 4-5, pp. 78-79 of MRID 50234306).

Samples were analyzed using an Applied Biosystems Sciex API 5000 triple quadrupole LC/MS/MS system and a Waters Acquity LC column oven and binary pump (pp. 19-22; Appendix 1, p. 75; Appendix 3, p. 77 of MRID 50234306). The following LC conditions were used: Phenomenex

Synergi Polar RP column (3.0 mm x 50 mm, 2.5 µm, column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol [percent A:B (v:v) at 0.00 min. 50:50, 3.00-6.00 min. 30:70, 7.00-8.00 min. 5:95, 8.10-10.00 min. 50:50], and injection volume of 10 µL. The following MS/MS conditions were used: positive Turbo Ion Spray (ESI) mode, temperature 600°C, and multiple reaction monitoring (MRM). Analytes were identified using two ion pair transitions as follows (quantitation and confirmation, respectively): *m/z* 437.0→406.1 and *m/z* 437.0→343.9 for IN-NXX69, *m/z* 437.0→344.0 and *m/z* 439.0→346.0 for IN-NXX70, *m/z* 344.0→236.0 and *m/z* 344.0→186.0 for IN-QKV54, and *m/z* 437.0→406.0 and *m/z* 437.0→300.0 for IN-RNU71. Expected retention times are *ca*. 3.49, 3.97, 4.17, and 2.49 minutes for IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71, respectively. The method noted that IN-NXX69 should be injected in separate runs from IN-NXX70, IN-QKV54, and IN-RNU71, especially when matrix-matched standards are used for IN-NXX70, IN-QKV54, and IN-RNU71.

ILV

The independent laboratory performed the ECM as written, except insignificant modifications of analytical instrumentation (pp. 16-22 of MRID 50234307). An AB Sciex API 5500 Series Tandem MS and an Agilent 1200 HPLC were used. All LC and MS parameters were the same as the ECM. The same two ion pair transitions were used as those of the ECM. Approximate retention times were 4.5, 5.2, 5.4, and 3.2 minutes for IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71, respectively. The ILV modifications did not warrant an updated ECM; however, based on the failure of the first trial for IN-NXX69 due to inadequate acidification, the reviewer believed that an updated ECM should be submitted identifying this as a critical step.

LOQ and LOD

In the ECM and ILV, Limit of Quantification (LOQ) for cyantraniliprole metabolites IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in water was 0.10 µg/L (pp. 12, 26 of MRID 50234306; pp. 11, 23; Appendix 5, pp. 99-102 of MRID 50234307). The Limit of Detection (LOD) for all analytes in water was 0.03 µg/L (30% of the LOQ) in the ECM. In the ILV, the LOD was 0.25 ng/mL for IN-NXX69 and 0.20 ng/mL for IN-NXX70, IN-QKV54, and IN-RNU71.

II. Recovery Findings

ECM (MRID 50234306): For Syngenta Method GRM073.01A, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of cyantraniliprole photolysis transformation products IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in one water matrix at fortification levels of 0.10 µg/L (LOQ), 1.0 µg/L (10×LOQ), and 10 µg/L (100×LOQ); however, an insufficient number of samples was prepared for all analyses (n = 3; Tables 2-9, pp. 32-34; DER Attachment 2). Analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The surface (rice paddy) water (pH 7.8, 379 mg/L hardness as $CaCO_3$, 450 ppm total dissolved solids, 5.2 mg/L dissolved oxygen) was obtained from Rapides Parish, Louisiana, and used in the study (Appendix I, p. 103). The water characterization laboratory was not reported.

ILV (MRID 50234307): For Syngenta Method GRM073.01A, mean recoveries and RSDs were within guidelines for analysis for analysis of cyantraniliprole photolysis transformation products IN-

NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in one water matrix at fortification levels of 0.10 µg/L (LOQ) and 1.0 µg/L (10×LOQ; p. 24; Tables 3-10, pp. 33-40). Analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The surface (river) water (Sample ID: 2706W-034; pH 8.3, 598 mg equivalent $CaCO_3$/L hardness, 1138 ppm total dissolved solids, 20.2 ppm total organic carbon) was obtained from Goose River, North Dakota, and characterized by Agvise Laboratories, Northwood, North Dakota (p. 16; Appendices 4-6, pp. 96, 99-102, 107). The other sample of surface (river) water (Sample ID: 2706W-029) was not used in the study. The method was validated for IN-NXX70, IN-QKV54, and IN-RNU71 at both fortification levels in surface water after the first trial, with insignificant analytical instrument modifications (pp. 11, 16-23). The method was validated for IN-NXX69 at both fortification levels in surface water after the second trial, with insignificant analytical instrument modifications; the first trial failed due to low recoveries. According to the communication summary, it appeared that the water samples needed to be acidified to exactly pH 4, instead of approximately pH 4 (Appendix 6, p. 107). The reviewer believed that an updated ECM should be submitted identifying this as a critical step.

Table 2. Initial Validation Method Recoveries for Cyantraniliprole Transformation Products IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in Water[1,2]

Analyte[3]	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[4]	Relative Standard Deviation (%)
	Surface (Rice Paddy) Water					
	Quantitation Ion Transition					
IN-NXX69	0.10	3	80-84	82	2	2.5
	1.0	3	92-100	96	4	4.2
	10	3	80-91	87	6	7.0
IN-NXX70	0.10	3	90-92	92	1	1.3
	1.0	3	93-96	94	2	1.6
	10	3	88-93	91	3	2.8
IN-QKV54	0.10	3	70-75	72	3	3.7
	1.0	3	84-91	87	4	4.1
	10	3	82-87	85	3	3.1
IN-RNU71	0.10	3	95-99	97	2	2.1
	1.0	3	98-101	99	2	1.5
	10	3	89-98	95	5	5.2
	Confirmation Ion Transition					
IN-NXX69	0.10	3	80-86	82	3	3.9
	1.0	3	93-95	94	1	1.1
	10	3	76-90	85	8	9.5
IN-NXX70	0.10	3	90-96	93	3	3.3
	1.0	3	94-98	96	2	2.1
	10	3	85-94	91	5	5.4
IN-QKV54	0.10	3	72-75	74	2	2.1
	1.0	3	84-88	86	2	2.4
	10	3	82-86	85	2	2.7
IN-RNU71	0.10	3	93-103	98	5	5.1
	1.0	3	97-100	98	2	1.8
	10	3	88-97	94	5	5.5

Data (uncorrected recovery results; pp. 23-25) were obtained from Tables 2-9, pp. 32-34 of MRID 50234306 and DER Attachment 2.

1 The surface (rice paddy) water (pH 7.8, 379 mg/L hardness as $CaCO_3$, 450 ppm total dissolved solids, 5.2 mg/L

dissolved oxygen) was obtained from Rapides Parish, Louisiana, and used in the study (Appendix I, p. 103). The water characterization laboratory was not reported.

2 Analytes were identified using two ion pair transitions as follows (quantitation and confirmation, respectively): *m/z* 437.0→406.1 and *m/z* 437.0→343.9 for IN-NXX69, *m/z* 437.0→344.0 and *m/z* 439.0→346.0 for IN-NXX70, *m/z* 344.0→236.0 and *m/z* 344.0→186.0 for IN-QKV54, and *m/z* 437.0→406.0 and *m/z* 437.0→300.0 for IN-RNU71.

3 IN-NXX70 = 2-[3-Bromo-1-(3-hydroxypyridin-2-yl)-1H-pyrazol-5-yl]-3,8-dimethyl-4-oxo-3,4-dihydroquinazoline-6-carbonitrile; IN-QKV54= 2-(5-Bromo-1H-pyrazol-3-yl)-3,4-dihydro-3,8-dimethyl-4-oxo-6-quinazolinecarbonitrile; and IN-RNU71 = 2-(2-Bromo-4-oxopyrazolo[1,5-a]pyrido[3,2-e]pyrazin-5(4H)-yl)-5-cyano-N,3-dimethylbenzamide (no chemical name was provided for IN-NXX69).

4 Standard deviations were reviewer-calculated based on data provided in the study report since the study author did not provide them (see DER Attachment 2). Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Cyantraniliprole Transformation Products IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in Water[1,2]

Analyte[3]	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface (River) Water					
	Quantitation Ion Transition					
IN-NXX69	0.10 (LOQ)	5	81-92	86	5.5	6.4
	1.0	5	85-94	90	3.6	4.0
IN-NXX70	0.10 (LOQ)	5	81-96	91	5.8	6.4
	1.0	5	83-89	85	2.1	2.5
IN-QKV54	0.10 (LOQ)	5	87-93	89	2.5	2.8
	1.0	5	93-97	95	1.7	1.8
IN-RNU71	0.10 (LOQ)	5	86-92	90	2.3	2.6
	1.0	5	92-101	96	3.3	3.4
	Confirmation Ion Transition					
IN-NXX69	0.10 (LOQ)	5	77-87	81	4.1	5.1
	1.0	5	85-90	87	2.0	2.3
IN-NXX70	0.10 (LOQ)	5	88-95	91	2.6	2.9
	1.0	5	86-93	90	2.6	2.9
IN-QKV54	0.10 (LOQ)	5	89-100	93	4.2	4.5
	1.0	5	91-98	94	2.9	3.1
IN-RNU71	0.10 (LOQ)	5	85-96	88	4.4	5.0
	1.0	5	92-96	94	1.9	2.0

Data (uncorrected recovery results; Appendices 5-6, pp. 97-102) were obtained from p. 24; Tables 3-10, pp. 33-40 of MRID 50234307.

1 The surface (river) water (Sample ID: 2706W-034; pH 8.3, 598 mg equivalent $CaCO_3$/L hardness, 1138 ppm total dissolved solids, 20.2 ppm total organic carbon) was obtained from Goose River, North Dakota, and characterized by Agvise Laboratories, Northwood, North Dakota (p. 16; Appendices 4-6, pp. 95-96, 99-102, 107). The other sample of surface (river) water (Sample ID: 2706W-029; pH 8.3, 678 mg equivalent $CaCO_3$/L hardness, 1846 ppm total dissolved solids, 20.7 ppm total organic carbon) was not used in the study.

2 Analytes were identified using two ion pair transitions as follows (quantitation and confirmation, respectively): *m/z* 437.0→406.1 and *m/z* 437.0→343.9 for IN-NXX69, *m/z* 437.0→344.0 and *m/z* 439.0→346.0 for IN-NXX70, *m/z* 344.0→236.0 and *m/z* 344.0→186.0 for IN-QKV54, and *m/z* 437.0→406.0 and *m/z* 437.0→300.0 for IN-RNU71 (the ion transitions were the same as those of the ECM).

3 IN-NXX70 = 2-[3-Bromo-1-(3-hydroxypyridin-2-yl)-1H-pyrazol-5-yl]-3,8-dimethyl-4-oxo-3,4-dihydroquinazoline-6-carbonitrile; IN-QKV54= 2-(5-Bromo-1H-pyrazol-3-yl)-3,4-dihydro-3,8-dimethyl-4-oxo-6-quinazolinecarbonitrile; and IN-RNU71 = 2-(2-Bromo-4-oxopyrazolo[1,5-a]pyrido[3,2-e]pyrazin-5(4H)-yl)-5-cyano-N,3-dimethylbenzamide (no chemical name was provided for IN-NXX69).

III. Method Characteristics

In the ECM and ILV, LOQ for cyantraniliprole metabolites IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in water was 0.10 µg/L (pp. 12, 26 of MRID 50234306; pp. 11, 23; Appendix 5, pp. 99-102 of MRID 50234307). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-120% and relative standard deviation of ≤20%. Also, the ECM noted that the response of the LOQ peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time for accurate quantification. No LOQ calculations were provided in the ECM or ILV. No justifications of the LOQ were provided in the ILV. The LOD for all analytes in water was 0.03 µg/L (30% of the LOQ) in the ECM. In the ILV, the LOD was 0.25 ng/mL for IN-NXX69 and 0.20 ng/mL for IN-NXX70, IN-QKV54, and IN-RNU71. In the ECM, the LOD 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. The ECM study author noted that the LOD may vary from instrument to instrument. No LOD calculations were reported in ECM or ILV. No justifications of the LOD were provided in the ILV.

Table 4. Method Characteristics for Cyantraniliprole Transformation Products IN-NXX69, IN-NXX70, IN-QKV54, and IN-RNU71 in Water

<table>
<tr><th colspan="2">Analyte[1]</th><th>IN-NXX69</th><th>IN-NXX70</th><th>IN-QKV54</th><th>IN-RNU71</th></tr>
<tr><td rowspan="2">Limit of Quantitation (LOQ)</td><td>ECM</td><td colspan="4" rowspan="2">0.10 µg/L</td></tr>
<tr><td>ILV</td></tr>
<tr><td rowspan="2">Limit of Detection (LOD)[2]</td><td>ECM</td><td colspan="4">0.03 µg/L (30% of the LOQ)</td></tr>
<tr><td>ILV</td><td>0.25 ng/mL</td><td colspan="3">0.20 ng/mL</td></tr>
<tr><td rowspan="4">Linearity (calibration curve r^2 and concentration range)[3]</td><td rowspan="2">ECM</td><td>r^2 = 0.9997 (Q)
r^2 = 0.9995 (C)</td><td>r^2 = 0.9991 (Q)
r^2 = 0.9992 (C)</td><td>r^2 = 0.9990 (Q)
r^2 = 0.9997 (C)</td><td>r^2 = 0.9997 (Q & C)</td></tr>
<tr><td colspan="3">0.50-10 ng/mL</td></tr>
<tr><td rowspan="2">ILV</td><td>r^2 = 0.9950 (Q)
r^2 = 0.9962 (C)</td><td>r^2 = 0.9987 (Q)
r^2 = 0.9995 (C)</td><td>r^2 = 0.9977 (Q)
r^2 = 0.9984 (C)</td><td>r^2 = 0.9992 (Q)
r^2 = 0.9994 (C)</td></tr>
<tr><td>0.25-4 ng/mL</td><td colspan="3">0.2-4 ng/mL</td></tr>
<tr><td rowspan="2">Repeatable</td><td>ECM[4]</td><td colspan="4">Yes at LOQ and 10×LOQ, but n = 3
[characterized surface (rice paddy) water]</td></tr>
<tr><td>ILV[5,6]</td><td colspan="4">Yes at LOQ and 10×LOQ
[characterized surface (river) water]</td></tr>
<tr><td colspan="2">Reproducible</td><td colspan="4">Yes at LOQ and 10×LOQ</td></tr>
<tr><td rowspan="2">Specific</td><td>ECM</td><td colspan="4">Yes, no matrix interferences were observed.
No 10×LOQ representative chromatograms were provided.</td></tr>
<tr><td>ILV</td><td>Yes, matrix interferences were ca. 3% of the LOQ (based on peak area). Minor baseline noise interfered with LOQ peak attenuation and integration.</td><td colspan="3">Yes, no matrix interferences were observed.</td></tr>
</table>

Data were obtained from pp. 12, 26; Tables 2-9, pp. 32-34 (recovery results); Figures 21-44, pp. 47-58 (chromatograms); Figures 45-52, pp. 60-67 (calibration curves) of MRID 50234306; pp. 11, 23-24; Appendix 5, pp. 99-102; Tables 3-10, pp. 33-40 (recovery results); Figures 57-64, pp. 74-77 (calibration curves); Figures 13-28, pp. 52-59 and 41-56, pp. 66-73 (chromatograms) of MRID 50234307; DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

1 IN-NXX70 = 2-[3-Bromo-1-(3-hydroxypyridin-2-yl)-1H-pyrazol-5-yl]-3,8-dimethyl-4-oxo-3,4-dihydroquinazoline-6-carbonitrile; IN-QKV54= 2-(5-Bromo-1H-pyrazol-3-yl)-3,4-dihydro-3,8-dimethyl-4-oxo-6-quinazolinecarbonitrile;

and IN-RNU71 = 2-(2-Bromo-4-oxopyrazolo[1,5-a]pyrido[3,2-e]pyrazin-5(4H)-yl)-5-cyano-N,3-dimethylbenzamide (no chemical name was provided for IN-NXX69).

2 LOD reported in the ILV differed from that reported in the ECM.

3 ECM and ILV coefficient of determination (r^2) values are reviewer-generated from reported correlation coefficient (r) values (1/x weighting; Figures 45-52, pp. 60-67 of MRID 50234306; p. 25; Figures 57-64, pp. 74-77 of MRID 50234307; DER Attachment 2). Matrix-based standards were used for all analytes, except IN-NXX69. Solvent-based calibration standards were used in the ILV. The reviewer limited the calculated r^2 to 4 significant figures although 7+ significant figures were reported in the ECM and ILV for r.

4 In the ECM, the surface (rice paddy) water (pH 7.8, 379 mg/L hardness as $CaCO_3$, 450 ppm total dissolved solids, 5.2 mg/L dissolved oxygen) was obtained from Rapides Parish, Louisiana, and used in the study (Appendix I, p. 103 of MRID 50234306). The water characterization laboratory was not reported.

5 In the ILV, the surface (river) water (Sample ID: 2706W-034; pH 8.3, 598 mg equivalent $CaCO_3$/L hardness, 1138 ppm total dissolved solids, 20.2 ppm total organic carbon) was obtained from Goose River, North Dakota, and characterized by Agvise Laboratories, Northwood, North Dakota (p. 16; Appendices 4-6, pp. 96, 99-102, 107 of MRID 50234307). The other sample of surface (river) water (Sample ID: 2706W-029) was not used in the study.

6 The ILV validated for the method for IN-NXX70, IN-QKV54, and IN-RNU71 at both fortification levels in surface water after the first trial, with insignificant analytical instrument modifications (pp. 11, 16-23 of MRID 50234307). The method was validated for IN-NXX69 at both fortification levels in surface water after the second trial, with insignificant analytical instrument modifications; the first trial failed due to low recoveries. According to the communication summary, it appeared that the water samples needed to be acidified to exactly pH 4, instead of approximately pH 4 (Appendix 6, p. 107).

IV. Method Deficiencies and Reviewer's Comments

1. ILV modifications of analytical instrumentation did not warrant an updated ECM; however, based on the failure of the first trial for IN-NXX69 due to inadequate acidification, the reviewer believed that an updated ECM should be submitted identifying this as a critical step (p. 16 of MRID 50234306; Appendix 6, p. 107 of MRID 50234307). The reviewer ascertained that the ECM direction should also be modified from "approximately" pH 4 to "exactly" pH 4.

2. ILV communication report/discussion should have been more detailed to assure independence of the ILV from the ECM. The ILV study author provided a communication log between the ILV laboratory personnel and the Study Sponsor Monitor (Lula Ghebremichael of Syngenta) "or others familiar with the method" (pp. 5, 24; Appendix 6, p. 107 of MRID 50234307). These communications included 1) protocol approval; 2) acquisition of analytical standard and control sample; 3) questions regarding preparation of reagents; 4) pre-validation evaluation and method establishment including calibration curve linearity; 5) communication of trial success or failure; and 6) guidance for trial 2. The reviewer noted that the others familiar with the method were not specified in the ILV, so it could not be determined which personnel advised the ILV to adjust the pH to exactly pH 4 for future analyses of IN-NXX69.

3. In the ECM, an insufficient number of samples was prepared for all analyses, n = 3 (Tables 2-9, pp. 32-34 of MRID 50234306). OCSPP guidelines state that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

4. The purities of the test materials were not reported in the ECM (Figures 1-4, pp. 36-37 of MRID 50234306).

5. In the ECM, no 10×LOQ representative chromatograms were provided for review, only LOQ

and 100×LOQ were provided (Figures 21-44, pp. 47-58 of MRID 50234306). Representative chromatograms from all fortification levels should be provided to allow the evaluation of the specificity of the method.

6. Two ILV surface water matrices were reported in the Test System section of the study report: Sample ID: 2706W-034 and Sample ID: 2706W-029 (p. 16 of MRID 50234307). The reviewer determined that only Sample ID: 2706W-034 was used as a test water in the ILV based on the raw data provided in Appendix 5 Spreadsheets (Appendix 5, pp. 99-102). The water characterization for Sample ID: 2706W-029 was as follows: pH 8.3, 678 mg equivalent $CaCO_3$/L hardness, 1846 ppm total dissolved solids, 20.7 ppm total organic carbon (p. 16; Appendix 4, p. 95).

7. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 12, 26 of MRID 50234306; pp. 11, 23; Appendix 5, pp. 99-102 of MRID 50234307). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-120% and relative standard deviation of ≤20%. Also, the ECM noted that the response of the LOQ peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time for accurate quantification. No LOQ calculations were provided in the ECM or ILV. No justifications of the LOQ were provided in the ILV. In the ECM, the LOD 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. The ECM study author noted that the LOD may vary from instrument to instrument. No LOD calculations were reported in ECM or ILV. No justifications of the LOD were provided in the ILV. The LOD of the ECM differed from that of the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

8. The matrix effects were determined to be insignificant in the ILV; solvent standards were used (pp. 25-27 of MRID 50234307). In the ECM, matrix effects were also determined to be insignificant; however, matrix-matched standards were used for IN-NXX70, IN-QKV54, and IN-RNU71 since they were analyzed along with eight other compounds which were quantitated with matrix-matched standards (p. 27 of MRID 50234306). Solvent standards were used to quantitate IN-NXX69.

9. It was reported for the ILV that a single analyst can complete a set of thirteen samples (one reagent blank, two matrix controls, and ten fortified samples) in one working day with LC/MS/MS analysis performed overnight (p. 24 of MRID 50234307).

10. In the ILV, the storage stability of the final fraction residues in methanol:0.02 M aqueous formic acid (50:50, v:v) was determined to be up to 7 days under refrigeration (4°C; p. 28; Tables 11-14, pp. 41-44 of MRID 50234307). Extract stability was not determined in the ECM (p. 28 of MRID 50234306).

V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP

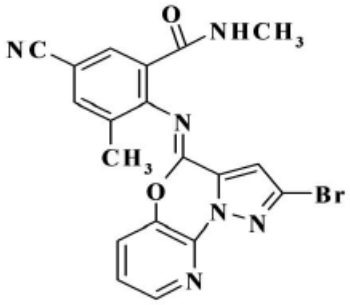
850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

DER Attachment 1: Chemical Names and Structures

IN-NXX69

IUPAC Name: None given
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



IN-NXX70

IUPAC Name: 2-[3-Bromo-1-(3-hydroxypyridin-2-yl)-1H-pyrazol-5-yl]-3,8-dimethyl-4-oxo-3,4-dihydroquinazoline-6-carbonitrile
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CAS Number: Not reported
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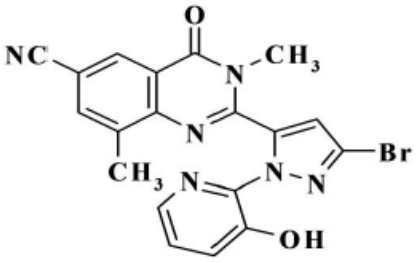

IN-QKV54

IUPAC Name: 2-(5-Bromo-1H-pyrazol-3-yl)-3,4-dihydro-3,8-dimethyl-4-oxo-6-quinazolinecarbonitrile

CAS Name: Not reported

CAS Number: Not reported

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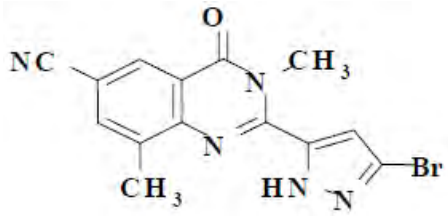


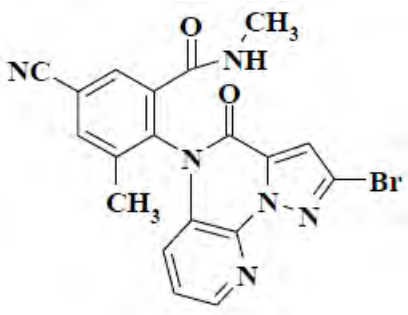
IN-RNU71

IUPAC Name: 2-(2-Bromo-4-oxopyrazolo[1,5-a]pyrido[3,2-e]pyrazin-5(4H)-yl)-5-cyano-N,3-dimethylbenzamide

CAS Name: Not reported

CAS Number: Not reported

SMILES String: Not found



DER Attachment 2: Calculations

DER calcs