

Analytical method for novaluron and its metabolites CPU and CLA in soil

Reports: ECM: EPA MRID No.: MRID 50610216. Reibach, P. 2018. Validation of the Analytical Method for the Determination of Novaluron and its Degradates in Soil Matrices by LC-MS/MS. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored by ADAMA Agricultural Solutions, Ltd., Israel, and submitted by Agan Chemical Manufacturers, Ltd., c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA), Raleigh, North Carolina; 134 pages. Smithers Viscient Study No.: 14125.6101. Sponsor Protocol/Project No.: R-38893. Final report issued May 21, 2018.

ILV: EPA MRID No.: MRID 50691104. Cashmore, A. 2018. Novaluron – Independent Laboratory Validation of Analytical Method 14125.6101 for the Determination of Novaluron and its Degradates in Soil. Report prepared by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom, and sponsored by ADAMA Makhteshim Ltd., Beer-Sheva, Israel, and submitted by Agan Chemical Manufacturers, Ltd., c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA), Raleigh, North Carolina; 105 pages. Study No.: 3201700. Sponsor Study ID: R-38894. Final report issued July 25, 2018 (p. 3).

Document No.: MRIDs 50610216 & 50691104

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50610216). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was included with the Quality Assurance statement.

ILV: The study was conducted in compliance with UK and OECD GLP standards (p. 3; Appendix 3, p. 91 of MRID 50691104). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5; Appendix 3, p. 91).

Classification: This analytical method is classified as **Acceptable**. However, it could not be determined if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies. Linearity was not satisfactory for CPU in some matrices.

PC Code: 124002

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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, Smithers Viscient Analytical Method 14125.6101, is designed for the quantitative determination of novaluron and its metabolites CPU and CLA in soil at the stated LOQ of 50.0 µg/kg. The LOQ is less than the lowest toxicological level of concern (>160 µg/kg, ; MRID 50610208, -09) in soil for all three analytes. The ECM used one characterized sediment and one characterized soil; the ILV used two characterized soils. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies. The ILV validated the ECM in the first trial for all three analytes in both soil matrices with the addition of sample centrifugation prior to LC/MS analysis, the use of one set of LC/MS parameters for all analyses, and insignificant modifications to the analytical equipment. All submitted ILV and ECM data pertaining to precision, repeatability, reproducibility, and specificity were acceptable at the LOQ and 10×LOQ for all three analytes in both matrices. Based on quantitation ion transition results, ILV linearity was acceptable for all analytes/matrices, except for CPU in one soil. ECM linearity was acceptable for all analytes/matrices, except for CPU in the sediment using solvent-based calibration standards.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Novaluron	MRID 50610216 ¹	MRID 50691104 ²		Soil	21/05/2018	Agan Chemical Manufacturers, Ltd. ³	LC/MS/MS	50.0 µg/kg
CPU								
CLA								

1 In the ECM, the sandy loam sediment [SMV Lot No.s 060517 and 060717, artificial sediment; pH 7.6 (in 1:1 soil:water), 78% sand 6% silt 16% clay, 2.1% organic matter (Walkley Black)] and loamy sand soil [SMV Lot No. 041917b; pH 6.6 (in 1:1 soil:water), 83% sand 16% silt 1% clay, 13.5% organic matter (Walkley Black)] were collected from Sunnynook Farm in Rochester, Massachusetts, and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification not specified; p. 16 of MRID 50610216).

2 In the ILV, Brierlow silt loam soil [CS 30/16; pH 5.6 (in 0.01M CaCl₂), 26% sand 58% silt 16% clay, 2.5% organic carbon] and Speyer 5M sandy loam soil [CS 27/16; pH 8.5 (in 0.01M CaCl₂), 59% sand 30% silt 11% clay, 1.0% organic carbon] were sourced by Smithers Viscient (ESG) and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; pp. 14-16; Appendix 2, pp. 89-90 of MRID 50691104). Soil source information was provided.

3 c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA).

I. Principle of the Method

Soil (5.00 g dry wt.) was fortified with fortification solutions (0.025 or 0.25 mL of 10.0 mg/L solution; CPU and CLA mixed, novaluron separate) in 50-mL Nalgene centrifuge tubes (pp. 17-21 of MRID 50610216). The soil samples were extracted twice with 20-mL aliquots of methanol via shaking on a shaker table for 30 minutes at 150 rpm. The combined extracts were taken to volume (50.0 mL) with methanol and mixed well. An aliquot (0.2 mL) was diluted to 10 mL with acetonitrile:purified reagent water (50:50, v:v). LOQ samples were directly analyzed by LC/MS/MS; the 10×LOQ samples were further diluted 10x with acetonitrile:purified reagent water (50:50, v:v) before aliquots were analyzed by LC/MS/MS.

Samples were analyzed for novaluron, CPU, and CLA using a Shimadzu LC-20ADXR coupled to a Sciex 6500+ QTRAP mass spectrometer equipped with electrospray ionization (ESI) interface in the positive ion, multiple reaction monitoring (MRM) mode (pp. 15, 21-25 of MRID 50610216). The following LC conditions were used for CPU and CLA in sandy loam sediment: Waters XBridge BEH C18 column (2.1 mm x 50 mm, 2.5 μ m; oven temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.01-0.50 min. 70.0:30.0, 1.50 min. 40.0:60.0, 4.00-5.00 min. 0.00:100.00, 5.10-6.10 min. 70.0:30.0], MS temperature 500°C, and injection volume of 20 μ L. Expected retention times were *ca.* 3.0, and 3.3 minutes for CPU, and CLA, respectively. The following LC conditions were used for novaluron in sandy loam sediment: Waters XBridge BEH C18 column (2.1 mm x 50 mm, 2.5 μ m; oven temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 1.00-1.10 min. 60.0:40.0, 3.00-4.60 min. 0.0:100.0, 4.70-6.00 min. 60.0:40.0], MS temperature 550°C, and injection volume of 25 μ L. Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 493.1→158.0 and m/z 493.1→140.9 for novaluron, m/z 353.0→275.2 and m/z 353.0→108.1 for CPU, and m/z 310.1→108.0 and m/z 310.1→127.2 for CLA. Expected retention time was *ca.* 2.5 minutes for novaluron. The following LC conditions were used for all three analytes in loamy sand soil: Waters XBridge BEH C18 column (2.1 mm x 50 mm, 2.5 μ m; oven temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 1.00 min. 60.0:40.0, 1.10 min. 20.0:80.0, 3.00-4.60 min. 0.0:100.0, 4.70-6.00 min. 60.0:40.0], MS temperature 550°C, and injection volume of 25 μ L. Expected retention times were *ca.* 2.4, 2.1, and 2.2 minutes for novaluron, CPU, and CLA, respectively. Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 493.1→158.0 and m/z 493.1→140.9 for novaluron, m/z 353.0→275.0 and m/z 353.0→309.9 for CPU, and m/z 310.1→108.0 and m/z 310.1→127.2 for CLA.

The ILV performed the ECM method as written, except for centrifugation (13,000 rpm for 10 minutes) of the sample prior to LC/MS analysis, the use of one set of LC/MS parameters for all analyses, and insignificant modifications to the analytical equipment (pp. 15, 17-20 of MRID 50691104). Samples were analyzed for novaluron, CPU, and CLA using a Shimadzu Nexera series HPLC system coupled with an AB Sciex API 5000 Triple Quadrupole Mass Spectrometer. The LC/MS/MS parameters were the same as those of the ECM for all three analytes in loamy sand soil. Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 493.1→158.0 and m/z 493.1→141.1 for novaluron, m/z 353.0→275.4 and m/z 353.0→310.2 for CPU, and m/z 310.1→108.0 and m/z 310.1→127.1 for CLA. Expected retention times were *ca.* 1.9, 1.6, and 1.8 minutes for novaluron, CPU, and CLA, respectively. The ILV noted that novaluron stocks should be prepared directly into disposable glass vials (p. 24).

In the ECM and ILV, the Limit of Quantification (LOQ) was 50.0 µg/kg for novaluron, CPU and CLA in soil (pp. 13, 26-29 of MRID 50610216; pp. 21, 23-25 of MRID 50691104). In the ECM, the Limit of Detection (LOD) was calculated to be 0.1-0.7 µg/kg (solvent-based and matrix-matched calibration) for novaluron, 0.6-0.8 µg/kg (solvent-based calibration) and 1-3 µg/kg (matrix-matched calibration) for CPU, and 0.1-0.6 µg/kg (solvent-based calibration) and 1 µg/kg (matrix-matched calibration) for CLA. In the ILV, the LOD was calculated to be 0.231-1.210 µg/kg for novaluron, 1.892-4.353 µg/kg for CPU, and 0.231-1.241 µg/kg for CLA.

II. Recovery Findings

ECM (MRID 50610216): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%) for analysis of novaluron, CPU, and CLA at fortification levels of 50.0 µg/kg (LOQ) and 500 µg/kg (10×LOQ) in two soil matrices (pp. 28-29; Tables 1-17, pp. 41-57). Matrix-matched and solvent-based calibration standards were used for the sandy loam sediment; for the loamy sand soil, matrix-matched calibration standards were used for novaluron and CLA, and non-matrix matched calibration standards were used for CPU (p. 30). Two ion pair transitions were monitored for novaluron, CPU and CLA using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable for all analytes/matrices, except CPU in sandy loam sediment using solvent-based calibration standards for which the confirmation ion data was not reported due to acceptability. The sandy loam sediment [SMV Lot No.s 060517 and 060717, artificial sediment; pH 7.6 (in 1:1 soil:water), 78% sand 6% silt 16% clay, 2.1% organic matter (Walkley Black)] and loamy sand soil [SMV Lot No. 041917b; pH 6.6 (in 1:1 soil:water), 83% sand 16% silt 1% clay, 13.5% organic matter (Walkley Black)] were collected from Sunnynook Farm in Rochester, Massachusetts, and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification not specified; p. 16).

ILV (MRID 50691104): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%) for analysis of novaluron, CPU, and CLA at fortification levels of 50.0 µg/kg (LOQ) and 500 µg/kg (10×LOQ) in two soil matrices (Tables 1-12, pp. 29-39). The report stated that matrix effects were insignificant for all analytes/transitions/soils; however, matrix-matched calibration standards were used for novaluron and CLA, and non-matrix matched calibration standards were used for CPU, in line with the primary method (p. 24). Two ion pair transitions were monitored for novaluron, CPU and CLA using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable. Brierlow silt loam soil [CS 30/16; pH 5.6 (in 0.01M CaCl₂), 26% sand 58% silt 16% clay, 2.5% organic carbon] and Speyer 5M sandy loam soil [CS 27/16; pH 8.5 (in 0.01M CaCl₂), 59% sand 30% silt 11% clay, 1.0% organic carbon] were sourced by Smithers Viscient (ESG) and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; pp. 14-16; Appendix 2, pp. 89-90). Soil source information was provided. The ILV validated the ECM in the first trial for all three analytes in both soil matrices with the addition of sample centrifugation prior to LC/MS analysis, the use of one set of LC/MS parameters for all analyses, and insignificant modifications to the analytical equipment (pp. 15, 17-20, 24).

Table 2. Initial Validation Method Recoveries for Novaluron, CPU and CLA in Soil

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Sediment¹						
Matrix-matched Calibration Standards						
Quantitation ion ²						
Novaluron	50.0 (LOQ)	5	79.9-94.5	88.5	6.85	7.74
	500	5	97.2-105	100	3.04	3.03
CPU	50.0 (LOQ)	5	69.0-96.4	86.0	11.2	13.0
	500	5	98.5-105	103	2.66	2.58
CLA	50.0 (LOQ)	5	70.5-96.8	87.5	11.3	12.9
	500	5	97.7-106	103	3.22	3.13
Confirmation ion ²						
Novaluron	50.0 (LOQ)	5	81.9-93.1	87.5	4.53	5.18
	500	5	96.8-101	99.4	1.70	1.71
CPU	50.0 (LOQ)	5	70.7-97.2	86.2	11.1	12.8
	500	5	97.0-105	102	3.61	3.54
CLA	50.0 (LOQ)	5	71.6-96.6	87.4	10.4	12.0
	500	5	99.0-105	102	3.05	3.00
Solvent-based Calibration Standards						
Quantitation ion ²						
Novaluron	50.0 (LOQ)	5	79.6-94.2	88.1	6.83	7.75
	500	5	96.8-104	99.9	3.03	3.03
CPU	50.0 (LOQ)	5	66.8-93.9	83.6	11.1	13.2
	500	5	96.0-102	101	2.63	2.62
CLA	50.0 (LOQ)	5	69.7-96.0	86.7	11.3	13.0
	500	5	97.0-105	102	3.23	3.16
Confirmation ion ²						
Novaluron	50.0 (LOQ)	5	80.9-92.1	86.5	4.53	5.23
	500	5	95.8-100	98.4	1.70	1.73
CPU	50.0 (LOQ)	5	-- ³	--	--	--
	500	5	--	--	--	--
CLA	50.0 (LOQ)	5	71.8-97.1	87.7	10.6	12.0
	500	5	99.5-106	102	3.09	3.02
Loamy Sand Soil¹						
Matrix-matched Calibration Standards						
Quantitation ion ⁴						
Novaluron ⁵	50.0 (LOQ)	5	73.2-91.3	81.2	8.34	10.3
	500	5	100-109	104	4.08	3.90
CPU ⁶	50.0 (LOQ)	5	64.7-85.6	77.8	8.82	11.3
	500	5	85.2-103	93.6	7.37	7.88
CLA ⁵	50.0 (LOQ)	5	70.9-88.8	78.7	8.25	10.5
	500	5	90.9-103	96.0	4.67	4.87
Confirmation ion ⁴						
Novaluron ⁵	50.0 (LOQ)	5	72.2-91.1	80.8	9.24	11.4
	500	5	99.4-107	103	3.09	3.01
CPU ⁶	50.0 (LOQ)	5	68.2-87.0	77.3	7.95	10.3
	500	5	83.1-98.4	92.0	6.16	6.69

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
CLA ⁵	50.0 (LOQ)	5	70.8-85.8	77.4	7.63	9.87
	500	5	93.2-101	96.4	3.00	3.11

Data (uncorrected recovery results; pp. 26-27) were obtained from pp. 28-29; Tables 1-17, pp. 41-57 of MRID 50610216.

- 1 The sandy loam sediment [SMV Lot No.s 060517 and 060717, artificial sediment; pH 7.6 (in 1:1 soil:water), 78% sand 6% silt 16% clay, 2.1% organic matter (Walkley Black)] and loamy sand soil [SMV Lot No. 041917b; pH 6.6 (in 1:1 soil:water), 83% sand 16% silt 1% clay, 13.5% organic matter (Walkley Black)] were collected from Sunnynook Farm in Rochester, Massachusetts, and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification not specified; p. 16).
- 2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 493.1→158.0 and m/z 493.1→140.9 for novaluron, m/z 353.0→275.2 and m/z 353.0→108.1 for CPU, and m/z 310.1→108.0 and m/z 310.1→127.2 for CLA.
- 3 Not reported. Data for the confirmatory transition with solvent-based calibration standards for CPU is data not used, and therefore, is not reported as the standard curve failed to meet acceptance criteria. Matrix matched curve used.
- 4 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 493.1→158.0 and m/z 493.1→140.9 for novaluron, m/z 353.0→275.0 and m/z 353.0→309.9 for CPU, and m/z 310.1→108.0 and m/z 310.1→127.2 for CLA.
- 5 Matrix-matched calibration standards were reportedly used for analysis (p. 30).
- 6 Solvent-based calibration standards were reportedly used for analysis (p. 30).

Table 3. Independent Validation Method Recoveries for Novaluron, CPU, and CLA in Soil

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Speyer 5M Sandy Loam Soil¹						
Quantitation ion ²						
Novaluron ³	50.0 (LOQ)	5	105-113	109	3.6	3.4
	500	5	100-116	110	6.4	5.8
CPU ⁴	50.0 (LOQ)	5	84-106	96	8.1	8.5
	500	5	82-121	101	14.0	13.8
CLA ³	50.0 (LOQ)	5	102-109	106	3.5	3.3
	500	5	95-109	105	5.8	5.6
Confirmation ion ²						
Novaluron ³	50.0 (LOQ)	5	103-109	105	2.8	2.7
	500	5	99-120	113	8.4	7.4
CPU ⁴	50.0 (LOQ)	5	93-113	104	7.7	7.4
	500	5	84-126	101	17.7	17.6
CLA ³	50.0 (LOQ)	5	92-106	97	6.4	6.6
	500	5	96-111	104	6.0	5.8
Brierlow Silt Loam Soil¹						
Quantitation ion ²						
Novaluron ³	50.0 (LOQ)	5	97-106	103	3.6	3.5
	500	5	98-105	103	2.7	2.6
CPU ⁴	50.0 (LOQ)	5	82-102	95	8.9	9.3
	500	5	95-109	100	6.8	6.7
CLA ³	50.0 (LOQ)	5	91-98	95	2.7	2.9
	500	5	96-100	98	1.6	1.6
Confirmation ion ²						
Novaluron ³	50.0 (LOQ)	5	99-104	102	1.9	1.9
	500	5	102-109	105	3.0	2.9
CPU ⁴	50.0 (LOQ)	5	94-104	100	3.8	3.8
	500	5	92-105	100	5.1	5.1
CLA ³	50.0 (LOQ)	5	90-98	95	3.2	3.4
	500	5	97-99	98	1.0	1.0

Data (uncorrected recovery results; pp. 20-21) were obtained from Tables 1-12, pp. 28-39 of MRID 50691104.

1 The Brierlow silt loam soil [CS 30/16; pH 5.6 (in 0.01M CaCl₂), 26% sand 58% silt 16% clay, 2.5% organic carbon] and Speyer 5M sandy loam soil [CS 27/16; pH 8.5 (in 0.01M CaCl₂), 59% sand 30% silt 11% clay, 1.0% organic carbon] were sourced by Smithers Viscient (ESG) and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; pp. 14-16; Appendix 2, pp. 89-90). Soil source information was provided.

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 493.1→158.0 and *m/z* 493.1→141.1 for novaluron, *m/z* 353.0→275.4 and *m/z* 353.0→310.2 for CPU, and *m/z* 310.1→108.0 and *m/z* 310.1→127.1 for CLA; these were similar to those of the ECM for loamy sand soil analysis.

3 Matrix-matched calibration standards were reportedly used for analysis (p. 24).

4 Solvent-based calibration standards were reportedly used for analysis (p. 24).

III. Method Characteristics

In the ECM and ILV, the LOQ was 50.0 µg/kg for novaluron, CPU, and CLA in soil (pp. 13, 26-29 of MRID 50610216; pp. 21, 23-25 of MRID 50691104). In the ECM, the LOQ was defined as the lowest fortification level. In the ILV, the LOQ was reported as the lowest level validated. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was calculated using three times the signal-to-noise value of the control samples. The LOD was calculated for each analyte using the following equation:

$$\text{LOD} = (3 \times (\text{SN}_{\text{ctl}}) / (\text{Resp}_{\text{LS}}) \times \text{Conc}_{\text{LS}} \times \text{DF}_{\text{CTRL}})$$

Where, LOD is the limit of detection of the analysis, SN_{ctl} is the mean signal to noise in height of the control samples (or Blanks), Resp_{LS} is the mean response in height of the two low calibration standards, Conc_{LS} is the concentration of the low calibration standard, and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 10).

In the ECM, the LOD was calculated to be 0.1-0.7 µg/kg (solvent-based and matrix-matched calibration) for novaluron, 0.6-0.8 µg/kg (solvent-based calibration) and 1-3 µg/kg (matrix-matched calibration) for CPU, and 0.1-0.6 µg/kg (solvent-based calibration) and 1 µg/kg (matrix-matched calibration) for CLA.

In the ILV, the LOD based upon the sample concentration equivalent to three times the baseline noise of a control sample was calculated as follows:

$$\text{LOD} = 3 \times \text{height of control baseline noise} \times \text{control dilution factor} \times \text{calibration standard concentration } (\mu\text{g/mL}) / \text{height of calibration standard peak.}$$

In the ILV, the LOD was calculated to be 0.231-1.210 µg/kg for novaluron, 1.892-4.353 µg/kg for CPU, and 0.231-1.241 µg/kg for CLA.

Table 4. Method Characteristics for Novaluron, CPU and CLA in Soil

Parameter		Novaluron (HEC-5725)	CPU	CLA	
Limit of Quantitation (LOQ)	ECM	50.0 µg/kg			
	ILV				
Limit of Detection (LOD) ¹	ECM	Matrix	0.1-0.5 µg/kg	1-3 µg/kg	1 µg/kg
		Solvent	0.3-0.7 µg/kg	0.6-0.8 µg/kg	0.1-0.6 µg/kg
	ILV	0.231-1.210 µg/kg	1.892-4.353 µg/kg	0.231-1.241 µg/kg	
Linearity (calibration curve r ² and concentration range)	ECM ²	Matrix	r ² = 0.999 (Q) r ² = 0.997-0.999 (C)	r ² = 0.999 (Q) r ² = 0.996 (C)	r ² = 0.998-0.999 (Q) r ² = 0.996-0.999 (C)
		Solvent	r ² = 0.997 (Q) r ² = 0.996 (C)	r ² = 0.993 -0.996 (Q) r ² = 0.995 (C)	r ² = 0.999 (Q) r ² = 0.998 (C)
	0.01-0.20 µg/kg				
	ILV ^{3,4}	Matrix	r ² = 0.9978-0.9990 (Q) r ² = 0.9932 -0.9976 (C) ⁵	Not performed	r ² = 0.9982-0.9998 (Q) r ² = 0.9978-0.9990 (C)
		Solvent	Not performed	r ² = 0.9900 -0.9968 (Q) r ² = 0.9942-0.9946 (C) ⁵	Not performed
	0.01-0.20 µg/kg				
Repeatable	ECM ⁶	Yes at LOQ and 10×LOQ. (one characterized sediment matrix and one uncharacterized soil matrix used)			
	ILV ^{7,8}	Yes at LOQ and 10×LOQ. (two characterized soil matrices used)			
Reproducible		Yes at LOQ and 10×LOQ.			
Specific	ECM	Yes, no matrix interferences were observed, but some minor baseline noise was observed at LOQ.	Yes, matrix interferences were <i>ca.</i> 4% of the LOQ (based on peak area), but some minor baseline noise was observed at LOQ.	Yes, no matrix interferences were observed, but some minor baseline noise was observed at LOQ.	
	ILV	Yes, matrix interferences were <i>ca.</i> 1% of the LOQ (based on peak area).	Yes, no matrix interferences were observed, but some peak splitting and minor baseline noise was observed at LOQ.	Yes, matrix interferences were <i>ca.</i> 1% of the LOQ (based on peak area).	

Data were obtained from pp. 13, 26-29 (LOQ/LOD); pp. 28-29; Tables 1-17, pp. 41-57 (recovery results); p. 30 (calibration data); Figures 1-42, pp. 58-99 (chromatograms); Figures 43-59, pp. 100-116 (calibration curve) of MRID 50610216; pp. 21, 23-25 (LOQ/LOD); Tables 1-12, pp. 28-39 (recovery results); p. 23 (calibration data); Figures 1-62, pp. 47-82 (calibration curves & chromatograms) of MRID 50691104; DER Attachment 2. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise. All results reported for both test matrices in each study unless specified otherwise.

1 Ranges include Q and C LODs unless separated.

2 Matrix-matched and solvent-based calibration standards were used for the sandy loam sediment; for the loamy sand soil, matrix-matched calibration standards were used for novaluron and CLA, and non-matrix matched calibration standards were used for CPU (p. 30 of MRID 50610216).

3 Reported r² values were reviewer-calculated from r values provided in the study report (p. 23 of MRID 50691104; DER Attachment 2). Reported ranges include sample set correlation data from both soils.

4 The report stated that matrix effects were insignificant for all analytes/transitions/soils; however, matrix-matched calibration standards were used for novaluron and CLA, and non-matrix matched calibration standards were used for CPU, in line with the primary method (p. 24 of MRID 50691104).

- 5 Does not affect the validity of the linearity/specificity of the method since a confirmation method is not usually required when LC/MS is the primary method used to generate study data.
- 6 In the ECM, the sandy loam sediment [SMV Lot No.s 060517 and 060717, artificial sediment; pH 7.6 (in 1:1 soil:water), 78% sand 6% silt 16% clay, 2.1% organic matter (Walkley Black)] and loamy sand soil [SMV Lot No. 041917b; pH 6.6 (in 1:1 soil:water), 83% sand 16% silt 1% clay, 13.5% organic matter (Walkley Black)] were collected from Sunnynook Farm in Rochester, Massachusetts, and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification not specified; p. 16 of MRID 50610216).
- 7 In the ILV, Brierlow silt loam soil [CS 30/16; pH 5.6 (in 0.01M CaCl₂), 26% sand 58% silt 16% clay, 2.5% organic carbon] and Speyer 5M sandy loam soil [CS 27/16; pH 8.5 (in 0.01M CaCl₂), 59% sand 30% silt 11% clay, 1.0% organic carbon] were sourced by Smithers Viscient (ESG) and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; pp. 14-16; Appendix 2, pp. 89-90 of MRID 50691104). Soil source information was provided.
- 8 The ILV validated the ECM in the first trial for all three analytes in both soil matrices with the addition of sample centrifugation prior to LC/MS analysis, the use of one set of LC/MS parameters for all analyses, and insignificant modifications to the analytical equipment (pp. 15, 17-20, 24 of MRID 50691104).
Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. ILV linearity was not satisfactory for the quantitation ion analysis of CPU in Speyer soil using matrix-matched calibration standards ($r^2 = 0.9900$; p. 23 of MRID 50691104; DER Attachment 2). ECM linearity was not satisfactory for the quantitation ion analysis of CPU in the sediment using solvent-based calibration standards ($r^2 = 0.993$; p. 30 of MRID 50610216; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$.

ILV linearity was not satisfactory for the confirmation ion analysis of novaluron in Speyer soil using matrix-matched calibration standards ($r^2 = 0.9932$) and CPU in both soils using matrix-matched calibration standards ($r^2 = 0.9942-0.9946$; p. 23 of MRID 50691104; DER Attachment 2). These deviations do not affect the validity of the linearity of the method since a confirmation method is not usually required when LC/MS is the primary method used to generate study data.

2. The ECM validated the method using a sediment and a soil matrix; the ILV only used soil matrices. While the ILV soil matrices were diverse in sand/silt/clay ratios, the percent organic carbon was similar between the two ILV soils (2.5% and 1.0%; pp. 14-16; Appendix 2, pp. 89-90 of MRID 50691104).

In the ECM, the sediment and soil matrices were characterized, but the soil characterization was not specified as USDA soil texture classification (p. 16 of MRID 50610216). Particle distribution sizes were not reported.

3. The ECM was performed by Smithers Viscient, Wareham, Massachusetts, and the ILV was performed by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom (pp. 1, 5 of MRID 50610216; pp. 1, 6 of MRID 50691104). No personnel overlapped between the ECM and ILV.

The communications between the ECM and ILV were reportedly limited to study progress and trial results (Appendix 5, p. 93 of MRID 50691104). Detailed communication records were not provided.

4. In the ILV, it was reported that matrix effects were insignificant (< 20% difference from non-matrix standards) for novaluron, CLA and CPU for each mass transition for the two soils tested (p. 24 of MRID 50691104). However, matrix-matched calibration standards were used for novaluron and CLA, and non-matrix matched calibration standards were used for CPU, in line with the primary method.

In the ECM, it was determined that for artificial sediment, solvent-based calibration standards could be used for all analytes/transitions, except for the CPU confirmatory transition (p. 29 of MRID 50610216). In this case, matrix-matched calibration standards were used. For loamy sand, acceptable results were obtained for CPU with solvent-based calibration standards, but matrix-matched calibration standards were needed for both novaluron and CLA. Overall, the ECM determined that matrix-matched calibration standards will be utilized for future testing with this method for novaluron and CLA for both artificial sediment and loamy sand; solvent-based calibration standards will be utilized for future testing with CPU for both artificial sediment and loamy sand.

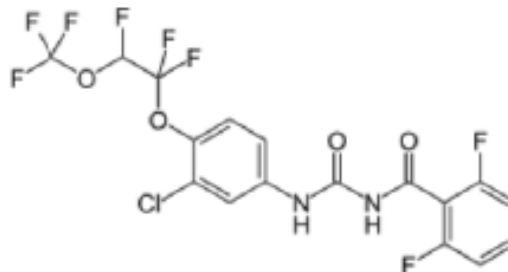
5. The reported limit of quantification (LOQ) was determined as the lowest level of method validation (LLMV) in the ECM and ILV (pp. 13, 26-29 of MRID 50610216; pp. 21, 23-25 of MRID 50691104). Further work could have been done to explore the actual LOQ. This means that concentrations can be reliably quantified at the LOQ (i.e., LLMV), but whether lower concentrations may also be reliably quantified is uncertain. In the ECM, the LOQ was defined as the lowest fortification level. In the ILV, the LOQ was reported as the lowest level validated. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was calculated using three times the signal-to-noise value of the control samples. In the ILV, the LOD was based upon the sample concentration equivalent to three times the baseline noise of a control sample.
6. The time required to complete the method was not reported in the ECM or ILV.

V. References

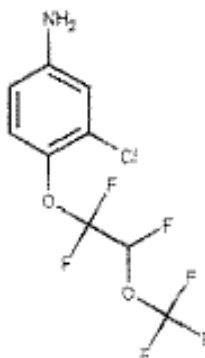
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- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.
- USEPA. 2014. Aquatic Life Benchmarks and Ecological Risk Assessments for Registered Pesticides. http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm.
- USEPA 2015. Registration Review Problem Formulation for Novaluron. DP barcode 447941, 449151. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Environmental Fate and Effects Division. Memorandum to the Pesticide Re-Evaluation Division. Feb. 26, 2015.

Attachment 1: Chemical Names and Structures**Novaluron**

IUPAC Name: Not reported
CAS Name: Not reported
CAS Number: 116714-46-6
SMILES String: Not found

**CLA**

IUPAC Name: Not reported
CAS Name: 3-Chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)aniline
CAS Number: 554-136-01
SMILES String: Not found



CPU

IUPAC Name: Not reported
CAS Name: 1-[3-Chloro-4-(1,1,2-trifluoro-2 trifluoromethoxyethoxy)phenyl]urea
CAS Number: 554-187-04
SMILES String: Not found

