2. BACKGROUND

Tetraniliprole is an anthranilic diamide insecticide with efficacy against lepidoptera, coleoptera, diptera (fruit flies and leafminers), and some sucking insects (thrips, aphids, and white flies). The mode of action of tetraniliprole is as a ryanodine receptor modulator (IRAC group28). It is currently under development by Bayer CropScience.

The purpose of this study was to demonstrate that method FV-004-W16-01 "An Analytical Method for the determination of Residues of Tetraniliprole (BCS-CL73507) and its Metabolites BCS-CQ63359, BCS-CU81055, BCS-CR74541, BCS-CR60014, BCS-CU81056, BCS-CT30673, BCS-CY28900, BCS-CY28897 and BCS-CY28906 in Water Using LC/MS/MS" [1], can be performed with acceptable recoveries using the quantitation transition.

This study was performed in accordance with US EPA Residue Chemistry Test Guidelines, OPPTS 860.1340 Residue Analytical Method, August 1996 [2], and US EPA Ecological Effects Test Guidelines, OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation[3], OECD Guidance Document on Pesticide Residue Analyticla Methods, Seiear on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 [4], Guidance document on residue analytical methods; SANCO/825/00 rev. 7, European Commission, Directorate General Health and Consumer Protection, 2004-03-17 [5], and U.S. Environmental Protection Agency, Office of Compliance Monitoring. 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule, 40 CFR, Part 160. Federal Register, Vol. 54, No. 158: pp. 34052-34074 [6].

The method extraction was performed as written. The LC/MS/MS parameter settings were as described in analytical method FV-004-W16-01.

The method was successfully validated for BCS-CL73507 and its metabolites BCS-CQ63359, BCS-CU81055, BCS-CR74541, BCS-CR60014, BCS-CU81056, BCS-CT30673, BCS-CY28900, BCS-CY28897 and BCS-CY28906 in surface water and tap water.

3.1 Test Substances

The test substances for this study were tetraniliprole (BCS-CL73507) and its metabolites BCS-CQ63359, BCS-CU81055, BCS-CR74541, BCS-CR60014, BCS-CU81056, BCS-CT30673, BCS-CY28900, BCS-CY28897 and BCS-CY28906. See Appendix 3 for complete nomenclature.

3.2 Analytical Reference Standards

The test substances also served as the analytical reference standards. See Appendix 3 for complete nomenclature and reference information for the reference standards. The test and reference substances were stored in a freezer until used to prepare fortification and calibration solutions. All stock solutions were stored in a freezer at an average temperature <-15ºC when not in use. All fortification and calibration solutions were stored in a refrigerator at an average temperature <5°C when not in use.

3.3 Test System

The test systems were a control surface water aliquot from study MELNN028 [7] and tap water obtained from Bayer CropScience, Building 108, Lab 1823 the day of the validation.

The surface water was stored in a refrigerator prior to fortification.

3.4 Method Summary

Each analytical set included three unfortified control samples, seven samples fortified at the LOQ (0.10 ng/mL or ppb), and five samples fortified at 10x LOQ (1.0 ng/g).

Residues of tetraniliprole and its metabolites are analyzed in water by taking an aliquot of water, adding isotopic internal standard and diluting with 1.25% acetic acid in acetonitrile. The samples are mixed and analyzed by LC/MS/MS with quantification based on a comparison of peak areas with those of known standards.

3.5 LC/MS/MS Conditions

Note: The following recommended conditions were used on an ABSciex 6500 mass spectrometer for the analytes with a positive polarity and an ABSciex 5500 mass spectrometer for the analytes with a negative polarity. Both mass spectrometer systems used a Shimadzu LC 20AD HPLC system and CTC auto sampler. The analyst optimized the mass spectrometer conditions to obtain satisfactory system response.

HPLC Conditions for Tetraniliprole and its metabolites analysis

Example LC/MS/MS conditions for sample 16FV-TW-LOQ-001 are shown below for BCS-CL73507, BCS-CQ63359, BCS-CU81055, BCS-CR74541, BCS-CR60014, BCS-CU81056, BCS-CT30673, BCS-CY28900, and BCS-CY28897

Mobile Phase A: 0.1% Aqueous Formic Acid
Mobile Phase B: 0.1% Formic Acid in Acetonitrile
Oven: 40 ºC
HPLC column: Phenomenex Luna C18(2)-HST 50 mm X 2.0 mm 2.5 µm particle size
Injection volume: 25 µL (Adjust as needed)

Time (min)	Mobile Phase B%	Flow rate µL/min
0.00	10	600
0.10	10	600
4.00	95	600
5.00	95	600
5.01	10	600
5.10	10	600

Analyte	Approx Retention Time (min)
BCS-CR60014	2.2
BCS-CU81055	2.4
BCS-CY28897	2.4
BCS-CR74541	2.5
BCS-CU81056	2.7
BCS-CY28900	2.7
BCS-CL73507	2.8
BCS-CT30673	2.8
BCS-CQ63359	3.2

General mass spectrometer conditions:

Instrument Used:	ABSciex 6500
Interface:	Electrospray
Software Version:	Analyst V1.6.2

Mass spectrometer compound parameter table

Ionization Mode:	Electrospray ionization (ESI) interface
Polarity	Positive
Curtain Gas (CUR)	40
Collision Gas (CAD)	4
Ion Spray Voltage (IS)	5500
Temperature (TEM)	400
Ion Source Gas 1 (GS1)	30
Ion Source Gas 2 (GS2)	30

Analyte	Q1 Mass	Q3 Mass	DP (V)	CE (V)	CXP (V)	EP (V)
BCS-CL73507	545.1	356.0	50	19	18	10
BCS-CL73507 C	545.1	376.0	50	37	18	10
BCS-CL73507-IS	550.1	356.0	50	19	18	10
BCS-CQ63359	527.0	389.0	86	29	18	10
BCS-CQ63359 C	527.0	374.1	50	35	18	10
BCS-CQ63359-IS	532.0	394.0	86	29	18	10
BCS-CR60014	563.0	356.0	50	19	18	10
BCS-CR60014 C	563.0	394.0	50	37	20	10
BCS-CR60014-IS	568.0	356.0	50	19	18	10
BCS-CR74541	564.0	356.0	80	19	18	10
BCS-CR74541 C	564.0	395.0	80	37	18	10
BCS-CR74541-IS	569.0	356.0	80	19	18	10
BCS-CU81055	550.0	395.1	116	35	18	10
BCS-CU81055 C	555.0	356.0	116	19	16	10
BCS-CU81055-IS	555.0	399.1	116	35	18	10
BCS-CT30673	545.8	408.1	91	29	20	10
BCS-CT30673 C	545.8	267.0	91	65	14	10
BCS-CT30673-IS	550.8	413.1	91	29	20	10
BCS-CU81056	532.0	394.1	146	29	18	10
BCS-CU81056 C	532.0	366.0	146	45	18	10
BCS-CU81056-IS	537.0	399.1	146	29	18	10
BCS-CY28900[1]	509.0	371.0	76	23	20	10
BCS-CY28900 C[1]	509.0	342.0	76	22	16	10
BCS-CY28897[1]	509.0	371.0	6	25	18	10
BCS-CY28897 C[1]	509.0	481.1	6	23	22	10

[1] BCS-CU81055-IS was used as the internal standard for these analytes

Note:. As the HPLC column ages, the retention times of the analytes change. A standard solution was analyzed before each set of samples to confirm the data collection parameters.

3.6 Calculations

An example calculation for BCS-CL73507 from sample 16FV-TW-LOQ-001, which was analyzed during the method validation study, is shown below. This sample was fortified with 0.10 ng/mL of BCS-CL73507 and its metabolites: BCS-CQ63359, BCS-CU81055, BCS-CR74541, BCS-CR60014, BCS-CU81056, BCS-CT30673, BCS-CY28900, BCS-CY28897 and BCS-CY28906. The chromatogram used in this example is presented in Appendix 2.

The standards were fit to the linear equation: Y = MX + B

where: X is the concentration of the reference standard
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area:isotopic peak area ratio

The example shown below is for the calculation of BCS-CL73507 residues. The residues for the other metabolites are calculated in a similar fashion.

After regression coefficients were calculated, the residue in ng/mL was determined. The ng/mL of tetraniliprole was calculated using the following equation,

$$\text{BCS-CL73507 found (ng/mL)} = \frac{(Y-B) \times D0}{}$$

$$\text{Dilution Factor } (D) = 0\frac{\text{Final volume } (V1)0}{\text{Initial sample Volume } (V0)0}$$

V0	V1	Native Peak Area	IS Peak Area	Y	M	B
4.60mL	5mL	6,624	88,038	0.0752	0.997	-0.000301

The slope and intercept were obtained from the calibration curve generated by Analyst, and is presented in Appendix 1 **(**Figure 1). The calibration points were weighted 1/x to provide better fit near the limit of detection

From the above equations:

Dilution Factor (D) = 4.60/5.00 = 1.087

$$\text{BCS-CL73507 found} = \frac{(0.0752-(-0.0\quad 301)) \times 1.0870}{.9970} = 0.082 \text{ ng/mL}$$

Therefore sample 16FV-TW-LOQ-001 contains 0.082 ng/mL BCS-CL73507. This result, using rounded numbers, agrees with the result shown in the raw data.

The % recovery was calculated using the following equation:

$$\text{Recovery (\%)} = \frac{(R-C)0}{T0} \times 1000$$

Where: R = ng/mL of target analyte found in fortified sample
C = apparent residue in the control sample
T = theoretical ng/mL in fortified sample

Therefore, for sample 16FV-TW-LOQ-001,

R = 0.082 ng/mL
C = 0.001 ng/mL
T = 0.10 ng/mL

$$\text{\% Recovery} = \frac{(0.082-0.\ 01)}{0.10} \times 1000 = 81\%$$

Remark: Example calculations shown above were performed using the LC/MS/MS software Analyst (version 1.6.2). The example calculation was performed using the area values reported by the instrument. The instrument software carries additional figures not shown in the intermediate results. Therefore, instrument software calculated values will differ slightly from the results derived using a calculator.

4. RESULTS

4.1 LC/MS/MS Verification

The ABSciex 6500 and ABSciex 5500 conditions used for this study are recorded in the raw data and example conditions are presented in Section 3.5 of this report. Calibration standards were injected prior to the method validation trial to determine the analyte retention times and instrumental sensitivity.

The calculated MDL'S for BCS-CL73507 and its metabolites: BCS-CQ63359, BCS-CU81055, BCS-CR74541, BCS-CR60014, BCS-CU81056, BCS-CT30673, BCS-CY28900, BCS-CY28897 and BCS-CY28906 for all matrices are presented below. The individual residue values are presented in Tables 3.

Method Detection Limit (MDL) for Water	
Analyte	MDL ng/mL
BCS-CL73507	0.015
BCS-CQ63359	0.020
BCS-CU81055	0.022
BCS-CR74541	0.023
BCS-CR60014	0.011
BCS-CU81056	0.028
BCS-CT30673	0.024
BCS-CY28900	0.017
BCS-CY28897	0.023
BCS-CY28906	0.016

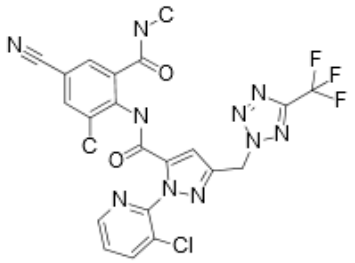
4.5 Stability of the Final Sample Extract

The stability of the analytes in the final extract was assessed by re-analyzing the control samples and 10x LOQ tap water samples 3 and 7 days after the samples were initially prepared. Between the 0 and 7 day analysis the samples were refrigerated. The final, vialed extracts removed from the refrigerator on the 3rd and 7th day after extraction and injected on the instrument. If the sample extracts have degraded by less than 15% from the initial analysis then the solutions are deemed to be stable for the specified time period. The samples did not degrade more than 15% within 7 days of storage at 1-5ºC.

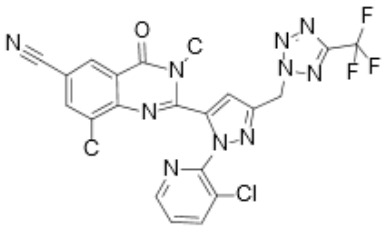
Appendix 3. Identity and Purity of the Reference Materials Used

The test and reference materials used in this study are described below:

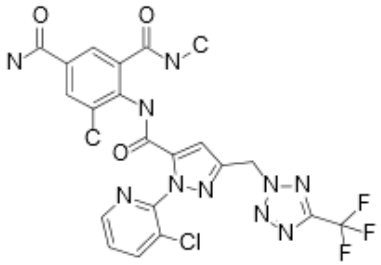
Code Name:	Tetraniliprole (BCS-CL73507)
CAS Number:	1229654-66-3
Molecular Formula:	$C_{22}H_{16}ClF_3N_{10}O_2$
Molecular Weight:	544.88 g/mol
ID No.:	K-2056
Purity:	97.9%
Expiration Date:	07/01/2017



Code Name:	BCS-CQ63359
Molecular Formula:	$C_{22}H_{14}CLF_3N_{10}O$
Molecular Weight:	526.86 g/mol
ID No.:	K-2118
Purity:	97.7%
Expiration Date:	07/24/2017

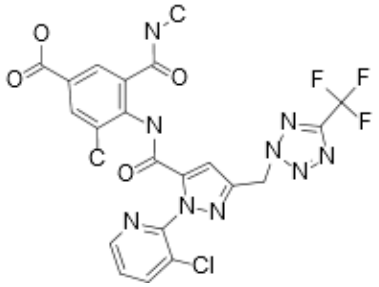


Code Name:	BCS-CR60014
Molecular Formula:	$C_{22}H_{18}ClF_3N_{10}O_3$
Molecular Weight:	562.89 g/mol
ID No.:	K-2090
Purity:	97.9%
Expiration Date:	06/18/2018

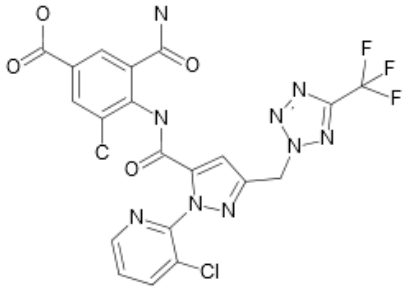


Appendix 3 Identity and Purity of the Reference Materials Used (Cont'd)

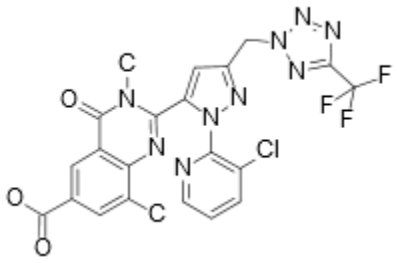
Code Name: BCS-CR74541
Molecular Formula: $C_{22}H_{17}ClF_3N_9O_4$
Molecular Weight: 563.88 g/mol
ID No.: K-2117
Purity: 97.2%
Expiration Date: 04/29/2017



Code Name: BCS-CU81055
Molecular Formula: $C_{21}H_{15}CLF_3N_9O_4$
Molecular Weight: 549.85g/mol
ID No.: K-2139
Purity: 0.11%
Expiration Date: 01/18/2019

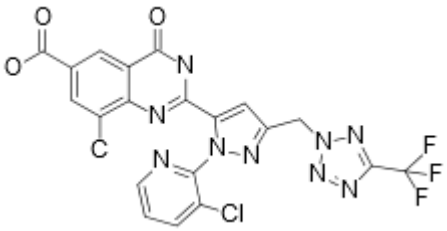


Code Name: BCS-CT30673
Molecular Formula: $C_{22}H_{15}ClF_3N_9O_3$
Molecular Weight: 545.86 g/mol
ID No.: K-2222
Purity: 97.2%
Expiration Date: 05/31/2018

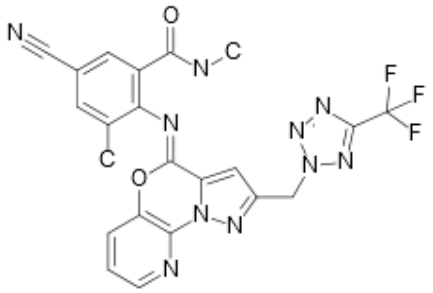


Appendix 3 Identity and Purity of the Reference Materials Used (Cont'd)

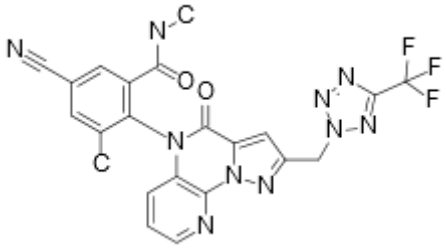
Code Name: BCS-CU81056
Molecular Formula: $C_{21}H_{13}ClF_3N_9O_3$
Molecular Weight: 531.83 g/mol
ID No.: K-2091
Purity: 98.3%
Expiration Date: 06/09/2018



Code Name: BCS-CY28900
Molecular Formula: $C_{22}H_{15}F_3N_{10}O_2$
Molecular Weight: 508.13 g/mol
ID No.: K-2196
Purity: 98.3%
Expiration Date: 01/13/2018

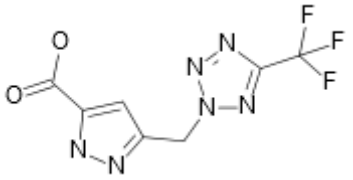


Code Name: BCS-CY28897
Molecular Formula: $C_{22}H_{15}F_3N_{10}O_2$
Molecular Weight: 508.13 g/mol
ID No.: K-2192
Purity: 97.8%
Expiration Date: 09/09/2017

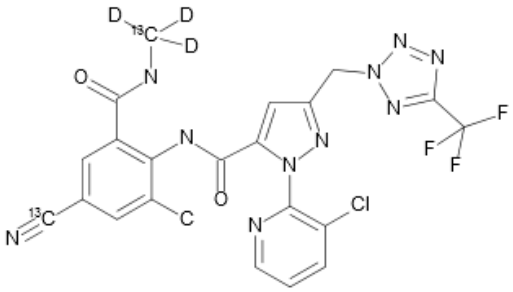


Appendix 3 Identity and Purity of the Reference Materials Used (Cont'd)

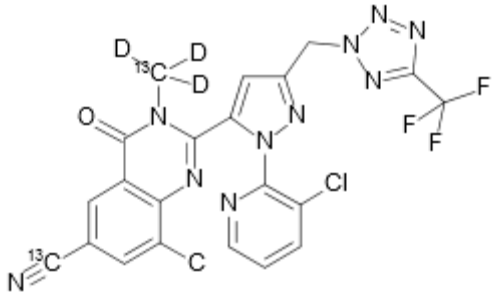
Code Name: BCS-CY28906
Molecular Formula: $C_7H_5F_3N_6O_2$
Molecular Weight: 262.04 g/mol
ID No.: K-2190
Purity: 99.9%
Expiration Date: 01/15/2018



Code Name: Tetraniliprole -$^{13}C_2,D_3$ (BCS-CL73507-$^{13}C_2,D_3$)
Molecular Formula: $^{13}C_2C_{20}D_3H_{13}ClF_3N_{10}O_2$
Molecular Weight: 549.88 g/mol
ID No.: K-2128
Purity: 99.3%
Expiration Date: 06/04/2024

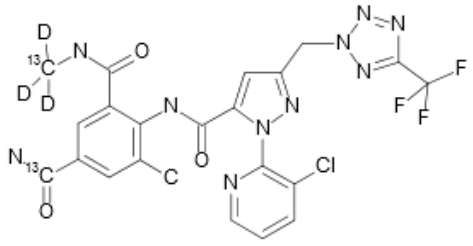


Code Name: BCS-CQ63359-$^{13}C_2,D_3$
Molecular Formula: $^{13}C_2C_{20}D_3H_{11}ClF_3N_{10}O$
Molecular Weight: 531.86 g/mol
ID No.: K-2131
Purity: 100.0%
Expiration Date: 06/04/2024

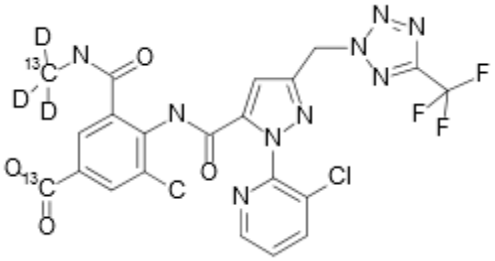


Appendix 3 Identity and Purity of the Reference Materials Used (Cont'd)

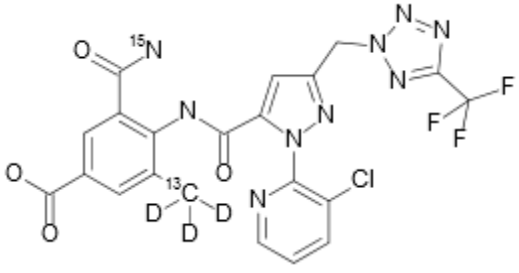
Code Name: BCS-CR60014-$^{13}C_2,D_3$
Molecular Formula: $^{13}C_2C_{20}D_3H_{15}ClF_3N_{10}O_3$
Molecular Weight: 567.90 g/mol
ID No.: K-2145
Purity: 100%
Expiration Date: 10/29/2024



Code Name: BCS-CR74541 -$^{13}C_2,D_3$
Molecular Formula: $^{13}C_2C_{20}D_3H_{14}ClF_3N_9O_4$
Molecular Weight: 568.88 g/mol
ID No.: K-2130
Purity: 100%
Expiration Date: 06/04/2024

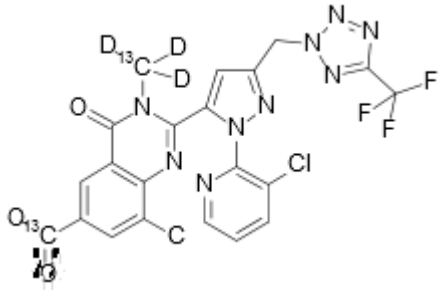


Code Name: BCS-CU81055-$^{13}C_2,D_3,^{15}N$
Molecular Formula: $^{13}CC_{20}D_3H_{12}ClF_3{}^{15}NN_8O_4$
Molecular Weight: 554.85 g/mol
ID No.: K-2129
Purity: 100%
Expiration Date: 06/04/2024



Appendix 3 Identity and Purity of the Reference Materials Used (Cont'd)

Code Name:	BCS-CT30673-$^{13}C_2,D_3$
Molecular Formula:	$^{13}C_2C_{20}D_3H_{12}ClF_3N_9O_3$
Molecular Weight:	550.86 g/mol
ID No.:	K-2146
Purity:	100%
Expiration Date:	10/29/2024



Code Name:	BCS-CU81056 -$^{13}C_2,D_3,^{15}N$
Molecular Formula:	$^{13}CC_{20}D_3H_{10}ClF_3{}^{15}NN_8O_3$
Molecular Weight:	536.84 g/mol
ID No.:	K-2147
Purity:	100%
Expiration Date:	10/29/2024