2.0 INTRODUCTION

This report describes the independent laboratory validation (ILV) of Bayer Analytical Method FV-004-W16-01, "Independent Laboratory Validation (ILV) of Bayer Method FV-004-W16-01 for the Determination of Residues of Tetraniliprole (BCS-CL73507) and its Metabolites BS-CQ63359, BCS-CU81055, BCS-CR74541, BCS-CR60014, BCS-CU81056, BCS-CT30673, BCS-CY28900, BCS-CY-28897 and BCS-CY28906 in Water Using LC/MS/MS" performed by ADPEN Laboratories, Inc. The analytical method submitted by Bayer is presented in Appendix 2.

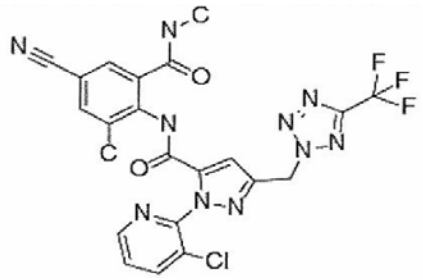
This study was designed to satisfy harmonized guideline requirements described in US EPA Test Guidelines OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation (Reference 2) and OPPTS (OCSPP) 860.1340(c) (6) (Reference 3). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (Reference 4).

3.0 MATERIALS AND METHODS

3.1 Reference Substances

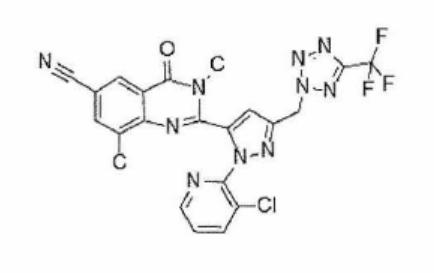
The following reference substances were obtained from Bayer CropScience:

Standard Name:	**Tetraniliprole (BCS-CL73507)**
Standard Number:	K-2056
CAS Name:	1-(3-chloro-2-pyridinyl)-N-[4-cyano-2-methyl-6-[(methylamino)carbonyl]phenyl]-3-[[(5-trifluoromethyl)-2H-tetrazol-2-yl]methyl]-1H-pyrazole-5-carboxamide
CAS Number:	1229654-66-3
Lot Number:	1009201201
Molecular Formula:	$C_{22}2H_{16}ClF_3N_{10}O_2$
Molecular Weight:	544.88 g/mol
GLP Purity:	97.9%
Expiration Date:	7/1/2017
Storage Conditions:	Freezer

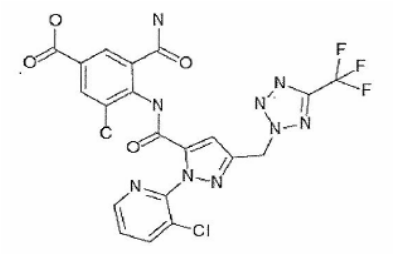


Reference Substances (continued)

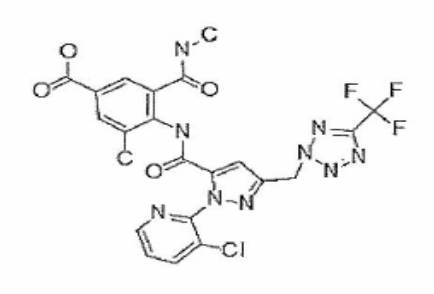
Standard Name: **BCS-CQ63359**
Standard Number: K-2118
Molecular Formula: $C_{22}H_{14}ClF_3N_{10}O$
Molecular Weight: 526.86 g/mol
GLP Purity: 97.7%
Expiration Date: 7/24/2017
Storage Conditions: Freezer



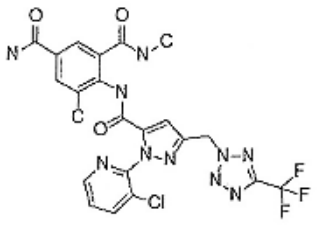
Standard Name: **BCS-CU81055**
Standard Number: K-2139
Molecular Formula: $C_{21}H_{15}ClF_3N_9O_4$
Molecular Weight: 549.85 g/mol
GLP Purity: 0.11%
Expiration Date: 1/18/2019
Storage Conditions: Freezer



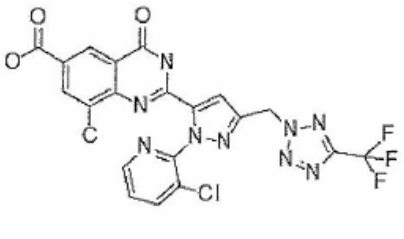
Standard Name: **BCS-CR74541**
Standard Number: K-2117
Molecular Formula: $C_{22}H_{17}ClF_3N_9O_4$
Molecular Weight: 563.88 g/mol
GLP Purity: 97.2%
Expiration Date: 4/29/2017
Storage Conditions: Freezer



Standard Name: **BCS-CR60014**
Standard Number: K-2090
Molecular Formula: $C_{22}H_{18}ClF_3N_{10}O_3$
Molecular Weight: 562.89 g/mol
GLP Purity: 97. 9%
Expiration Date: 06/18/2018
Storage Conditions: Freezer

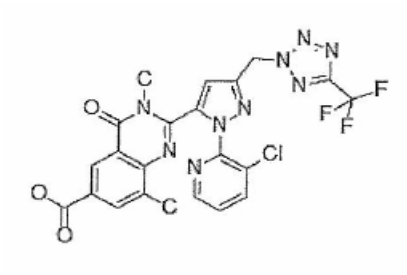


Standard Name: **BCS-CU81056**
Standard Number: K-2091
Molecular Formula: $C_{21}H_{13}ClF_3N_9O_3$
Molecular Weight: 531.83 g/mol
GLP Purity: 98.3%
Expiration Date: 6/9/2018
Storage Conditions: Freezer

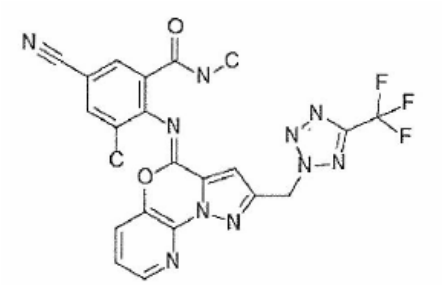


Reference Substances (continued)

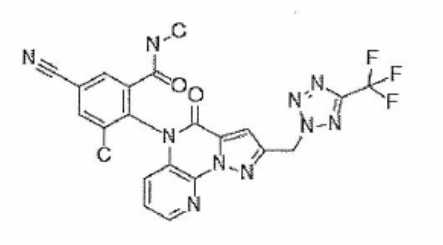
Standard Name:	**BCS-CT30673**
Standard Number:	K-2222
Molecular Formula:	$C_{22}H_{15}ClF_3N_9O_3$
Molecular Weight:	545.86 g/mol
GLP Purity:	97.2%
Expiration Date:	5/31/2018
Storage Conditions:	Freezer



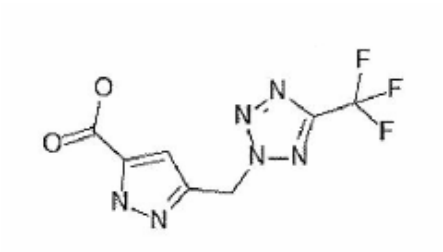
Standard Name:	**BCS-CY28900**
Standard Number:	K-2196
Molecular Formula:	$C_{22}H_{15}F_3N_{10}O_2$
Molecular Weight:	508.13 g/mol
GLP Purity:	98.3%
Expiration Date:	1/13/2018
Storage Conditions:	Freezer



Standard Name:	**BCS-CY28897**
Standard Number:	K-2192
Molecular Formula:	$C_{22}H_{15}F_3N_{10}O_2$
Molecular Weight:	508.13 g/mol
GLP Purity:	97.8%
Expiration Date:	9/9/2017
Storage Conditions:	Freezer

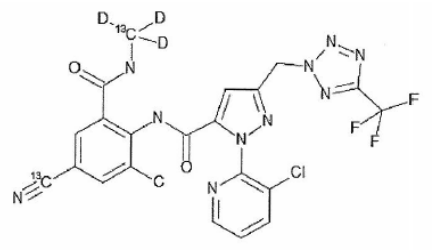


Standard Name:	**BCS-CY28906**
Standard Number:	K-2190
Molecular Formula:	$C_7H_5F_3N_6O_2$
Molecular Weight:	262.04 g/mol
GLP Purity:	99.9%
Expiration Date:	1/15/2018
Storage Conditions:	Freezer

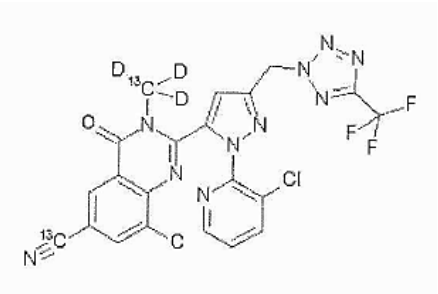


The following internal standard substances were obtained from Bayer CropScience:

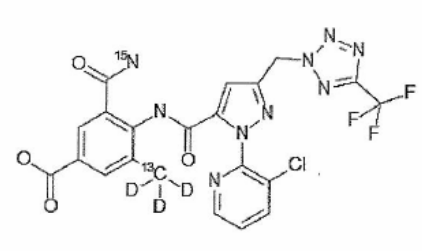
Standard Name: **BCS-CL73507-13C2,D3**
Standard Number: K-2128
Molecular Formula: $_{13}C_2C_{20}D_3H_{13}ClF_3N_{10}O_2$
Molecular Weight: 549.88 g/mol
GLP Purity: 99.3%
Expiration Date: 6/4/2024
Storage Conditions: Freezer



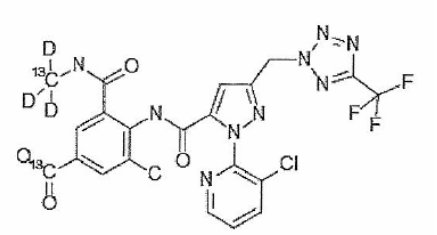
Standard Name: **BCS-CQ63359-13C2,D3**
Standard Number: K-2131
Molecular Formula: $_{13}C_2C_{20}D_3H_{11}ClF_3N_{10}O$
Molecular Weight: 549.88 g/mol
GLP Purity: 100%
Expiration Date: 6/4/2024
Storage Conditions: Freezer



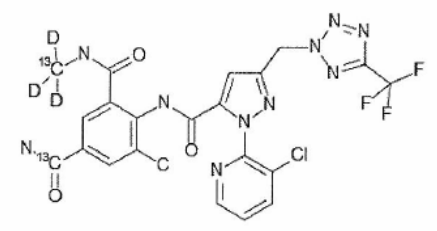
Standard Name: **BCS-CU81055-13C2,D3, 15N**
Standard Number: K-2129
Molecular Formula: $_{13}CC_{20}D_3H_{12}ClF_3N_8O_4$
Molecular Weight: 554.85 g/mol
GLP Purity: 100%
Expiration Date: 6/4/2024
Storage Conditions: Freezer



Standard Name: **BCS-CR74541-$^{13}C_2$,D_3**
Standard Number: K-2130
Molecular Formula: $_{13}C_2C_{20}D_3H_{14}ClF_3N_9O_4$
Molecular Weight: 568.88 g/mol
GLP Purity: 100%
Expiration Date: 6/4/2024
Storage Conditions: Freezer

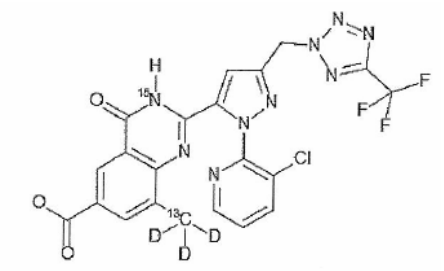


Standard Name: **BCS-CR60014-13C2,d3**
Standard Number: K-2145
Molecular Formula: $_{13}C_2C_{20}D_3H_{15}ClF_3N_{10}O_3$
Molecular Weight: 567.9 g/mol
GLP Purity: 100%
Expiration Date: 10/29/2024
Storage Conditions: Freezer

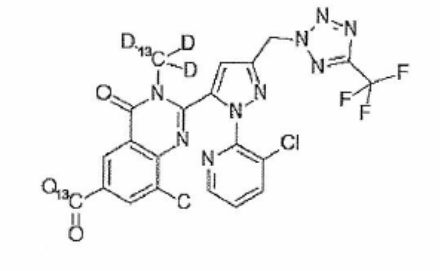


Internal Standard Substances (continued)

Standard Name: **BCS-CU81056-13C,d3,15N**
Standard Number: K-2147
Molecular Formula: $_{13}CC_{20}D_3H_{10}ClF_{315}NN_8O_3$
Molecular Weight: 536.84 g/mol
GLP Purity: 100%
Expiration Date: 10/29/2024
Storage Conditions: Freezer



Standard Name: **BCS-CT30673-13C2,d_3**
Standard Number: K-2146
Molecular Formula: $_{13}C_2C_{20}D_3H_{12}ClF_3N_9O_3$
Molecular Weight: 550.86 g/mol
GLP Purity: 100%
Expiration Date: 10/29/2024
Storage Conditions: Freezer



The reference substances used in this study were shipped from Bayer and were received at ADPEN Laboratories, Inc. on September 15, 2016 and were stored frozen as recommended on their respective certificates of analysis. Upon receipt, the reference standards were stored in freezer E-119 which operated at an average temperature of −22 °C.

The certificates of analysis are presented in Appendix 3. All fortification and calibration solutions made from the reference substances were stored according to the method.

3.2 Test System

The test system used in this study was a water sample. The control sample was provided by Bayer and stored refrigerated prior to analysis. The following table is a description of the sample.

SAMPLE	MATRIX	SAMPLE DESCRIPTION
Bayer CropScience tap water, Lab 1823	Water	Tap water

The sample was logged into the Laboratory Information Management System (LIMS) and assigned a unique laboratory code, which is cross-referenced to the sample identification (ID) on raw data and detailed residue reports. During the course of this study, the sample was stored in refrigerator E-57, which operates at an average temperature of 4 °C.

3.3 Analytical Procedures

Analytical Method FV-004-W16-01was independently validated as written and submitted by Bayer CropScience. The apparatus and reagents were used as outlined in the analytical method with equivalent apparatus or reagents substituted as necessary.

3.3.1 Fortifications

Untreated control water samples were fortified at LOQ (0.10 ppb) and 10× LOQ (1.0 ppb) using the appropriate fortification standard concentrations as per the method. The fortification standard consisted of a mix of tetraniliprole and its nine metabolites. Fortifications were prepared as described below:

Matrix	Parent Number	Concentration (ng/µL)	Aliquot Volume (mL)	Dilution Volume (mL)[1]	Final Concentration (ng/µL)
Water	I9060	2.0	5.0	50.0	0.2
	W13399-1	0.2	5.0	50.0	0.02

[1] Dilution solvent: Water:Acetonitrile:Acetic Acid (920:80:1, v/v)

3.3.2 Extraction Procedure for Water

(The sample was equilibrated to room temperature and hand mixed for homogeneity prior to extraction procedure.)

1. A 4.6-mL aliquot of the water sample was transferred to a glass graduated centrifuge tube.
2. Recovery samples were fortified at LOQ and 10x LOQ.
3. A 0.01 mL aliquot of the 0.5 µg/mL mixed-analyte internal standard solution was added to each sample and mixed well.
4. The sample volume was adjusted to 5 mL using 1.25% acetic acid in acetonitrile.
5. The samples were vortexed for homogeneity.
6. An aliquot was transferred to an HPLC vial for analysis by LC-MS/MS.

3.3.3 Modifications

No modifications were made to the analytical procedure.

Instrument Operating Conditions – Positive Polarity (continued)

MRM Conditions					
Analyte[1]	**Q1**	**Q3**	**DP (V)**	**CE (V)**	**CXP (V)**
Tetraniliprole (1)	545.0	356.0	66	19	18
Tetraniliprole (2)	545.0	376.0	66	35	18
Tetraniliprole-IS	550.0	355.9	51	19	18
BCS-CQ63359 (1)	527.0	389.0	21	29	18
BCS-CQ63359 (2)	527.0	374.0	21	33	18
BCS-CQ63359-IS	532.0	394.1	86	27	20
BCS-CR60014 (1)	563.1	356.0	71	19	18
BCS-CR60014 (2)	563.1	394.0	71	37	20
BCS-CR60014-IS	568.1	355.9	66	19	18
BCS-CR74541 (1)	564.0	356.0	61	17	18
BCS-CR74541 (2)	564.0	395.0	61	33	20
BCS-CR74541-IS	569.1	356.0	61	19	18
BCS-CU81055 (1)	550.0	395.0	61	31	20
BCS-CU81055 (2)	550.0	356.0	61	30	18
BCS-CU81055-IS	555.0	399.0	56	33	20
BCS-CT30673 (1)	546.0	408.0	41	27	12
BCS-CT30673 (2)	546.0	267.1	41	63	20
BCS-CT30673-IS	551.0	413.0	91	29	22
BCS-CU81056 (1)	532.0	394.0	21	27	18
BCS-CU81056 (2)	532.0	366.0	21	43	18
BCS-CU81056-IS	537.0	399.0	101	27	20
BCS-CY28900 (1)[2]	509.0	371.1	76	21	18
BCS-CY28900 (2)[2]	509.0	342.1	76	31	18
BCS-CY28897 (1)[2]	509.1	371.1	11	25	6
BCS-CY28897 (2)[2]	509.1	481.1	11	23	8

[1] Primary transition is indicated by "(1)" and the confirmatory transition by "(2)"
[2] BCS-CU81055-IS was used as the internal standard.

3.5 Instrument Operating Parameters – Negative Polarity

Instrument conditions for BCS-CY28906

Chromatographic System:	Agilent 1290 UPLC			
Analytical Column:	Phenomenex Luna C18(2)-HST, 50 mm X 2.0 mm, 2.5 µm; part number: 00B-4496-B0			
Column Temperature:	40 °C			
Injection Volume:	40.0 µL			
Mobile Phase A:	0.1% Formic Acid in Water (Optima grade)			
Mobile Phase B:	0.1% Formic Acid in Acetonitrile (Optima grade)			
Gradient:	Time (min.)	Flow Rate (µL/min)	A (%)	B (%)
	0.00	600	85	15
	0.50	600	85	15
	1.50	600	50	50
	2.00	600	50	50
	3.50	600	40	60
	4.00	600	5	95
	5.01	600	85	15
	6.00	600	85	15

Instrument Operating Conditions – Negative Polarity (continued)

Interface:	AB SCIEX 5500 Triple Quad
Ionization Mode:	Electrospray ionization (ESI) interface
Ionization Spray Voltage (IS):	-4500
Polarity:	Negative
Curtain gas (CUR):	20
Temperature (TEM):	500 °C
Collision gas setting (CAD):	7
GS1:	40
GS2:	40
Entrance potential (EP):	-10
Scan Type:	MRM

MRM Conditions					
Analyte[1]	**Q1**	**Q3**	**DP (V)**	**CE (V)**	**CXP (V)**
BCS-CY28906 (1)	261.0	136.9	-110	-18	-21
BCS-CY28906 (2)	261.0	108.8	-110	-42	-11
BCS-CU81055-IS	553.0	137.0	-200	-34	-13

[1] Primary transition is indicated by "(1)" and the confirmatory transition by "(2)"

3.6 Data Acquisition

Peak integration and peak area count quantitation were performed by Analyst® (version 1.6.2) data processing software. A best-fit, linear regression equation with 1/x weighting was

derived and used in conjunction with the analyte response in each sample to calculate the concentration of the analyte. The square of correlation coefficients (r^2) for the calibration curves for each analytical set was greater than 0.99.

Statistical treatment of the data including the calculation of percent recovery, means, and standard deviations were calculated within LIMS and reported using Microsoft® Office Excel spreadsheets. Example calculations are presented in Appendix 5.

4.0 RESULTS AND DISCUSSION

4.1 Method Establishment/Pre-Validation Evaluation

Prior to conducting the ILV, control of the method was established by performing instrument optimization. Optimization was performed on both an ABSciex 5500 and ABSciex 6500 LC-MS/MS system. Instrument optimization included infusing individual standard solutions onto the mass spectrometer and injecting standards onto the column under the conditions outlined in the method. Therefore, retention times, detection limits and linearity were also established. Prior to analysis of the validation set, an aliquot of the sample was evaluated according to the analytical procedure to determine if there was any analyte contamination and interferences. The test control water sample was free of residues.

4.2 Independent Laboratory Results

The method was successfully validated during the first trial at LOQ (0.10 ppb) and at 10× LOQ (1.0 ppb) as written. Overall mean recoveries at LOQ and 10× LOQ were within the acceptable range (70–120%). A brief summary of the overall results are presented on the next page. Details of the results are presented in Tables 2 through 11.

4.4 Time Required for Analysis

A single analyst prepared a set of 13 water samples in 1 hour, not including integration and reporting. Analysis by LC-MS/MS can be performed overnight.

4.5 Recommendations

No recommendations to the analytical procedure were documented for this study.

5.0 PROTOCOL DEVIATIONS

No deviations to the study protocol were documented for this study.

6.0 CONCLUSIONS

In summary, ADPEN Laboratories successfully and independently validated Bayer Analytical Method FV-004-W16-01 during the first trial.

The method was demonstrated to be suitable for the determination of Tetraniliprole (BCS-CL73507) and its metabolites BCS-CQ63359, BCS-CU81055, BCS-CR74541, BCS-CR60014, BCS-CU81056, BCS-CT30673, BCS-CY28900, BCS-CY-28897 and BCS-CY28906 in water at a LOQ of 0.10 ppb and 10× LOQ of 1.0 ppb for all validated transition ions. The extraction procedures are clear and contain sufficient information to guide an analyst.

TABLE 1. Fortification Levels

Lab Code	Fortification Level (ppb)	Number of Samples
16101702-MB-1	Method Blank	1
161011001-001B, 161011001-001C	Control	2
16101702-Recovery1-1, 16101702-Recovery1-2, 16101702-Recovery1-3, 16101702-Recovery1-4, 16101702-Recovery1-5	0.10	5
16101702-Recovery2-1, 16101702-Recovery2-2, 16101702-Recovery2-3, 16101702-Recovery2-4, 16101702-Recovery2-5	1.0	5