TITLE

Independent Laboratory Validation of “Analytical Method 01373 for the Determination of BCS-CL73507 and the Metabolites BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056 in Soil and Sediment by HPLC-MS/MS”

- Final Report -

Test Item

BCS-CL73507, BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056

Data Requirement

US EPA OCSPP 860.1340, Residue Analytical Method
US EPA OCSPP 850.6100, Independent Laboratory Validation

Completion Date

2016-05-04

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1.0 SUMMARY

The purpose of this study was to conduct an independent laboratory validation (ILV) of Bayer CropScience analytical method 01373 entitled "Analytical Method 01373 for the determination of BCS-CL73507 and the metabolites BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056 in soil and sediment by HPLC-MS/MS", as written.

The method was validated using sub-samples of bulk untreated soil collected from two terrestrial field dissipation studies performed in the United States in the states of Washington and Florida. Bulk soil samples from each test system were stored at room temperature prior to fortification. After fortification, the soil extracts were stored in a refrigerator until they were prepared for analysis using BCS Analytical Method 01373.

In brief, the method used for analysis was as follows:

Soil samples (20 g) were extracted in a microwave extractor with a mixture of acetonitrile/water/acetic acid (400/100/3, v/v/v). The extracts were centrifuged to remove fine particulates of the soil. Possible matrix effects of BCS-CL73507 and the metabolites BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056 are eliminated by using an internal standard solution of isotopically labeled reference items. Identification and quantitation of the active substance was performed by high performance liquid chromatography using tandem MS/MS detection (LC-MS/MS) in the Multiple Reaction Monitoring (MRM) mode using the following MRM transitions:

Analyte	Quantitation MRM	Confirmation MRM
BCS-CL73507	545.1 → 356.0	545.1 → 376.0
BCS-CQ63359	527.0 → 389.0	527.0 → 374.1
BCS-CR60014	563.0 → 356.0	563.0 → 394.0
BCS-CR74541	564.0 → 356.0	564.0 → 395.0
BCS-CU81055	550.0 → 395.1	550.0 → 356.0
BCS-CT30673	545.8 → 408.1	545.8 → 267.0
BCS-CU81056	532.0 → 394.1	532.0 → 366.0

The limit of quantitation (LOQ) for each single analyte was 2 ppb in soil. The limit of determination (LOD) ranged from 0.4 to 0.9ppb

Apparent residues in control samples were below the method LOD. The recoveries were not corrected for interferences. The correlation between the injected amount of substance and the detector response was linear for standards ranging from 0.03 ppb to 50 ppb, with correlation coefficients of >0.99 for both the Washington soil sample and the Florida soil sample for all test items.

2.0 INTRODUCTION

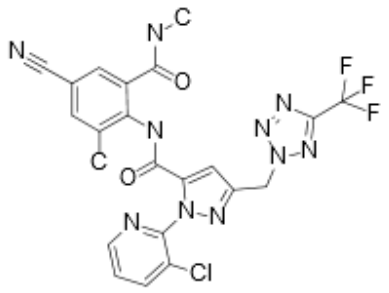
The purpose of this study was to validate the analytical method 01373 for BCS-CL73507 and the metabolites BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056 in soil. This study was conducted to satisfy guideline requirements described in the US EPA guidance documents Ecological Effects Test Guidelines OCSPP 850.6100 and Residue Chemistry Test Guidelines OCSPP 860.1340. In addition, this study will be conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

3.0 MATERIALS AND METHODS

3.1 TEST ITEMS/REFERENCE SUBSTANCES

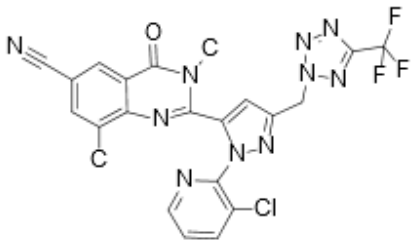
Only sufficiently characterized and certified substances were used as reference items.

BCS-CL73507:



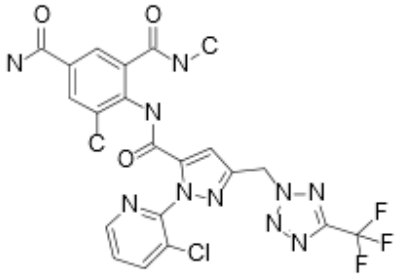
CAS Name:	1-(3-chloropyridin-2-yl)-N-[4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-3-{[5-(trifluoromethyl-2H-tetrazol-2-yl]methyl}-1H-pyrazole-5-carboxamide
CAS Number	1229654-66-3
Molecular Formula:	$C_{22}H_{16}ClF_3N_{10}O_2$
Molecular Weight:	544.88 g/mol
Standard No.:	K-2056
Purity:	97.9%
Expiration Date:	07/01/2017
Date of Analysis:	07/01/2014
Storage Conditions:	Frozen
Source:	Bayer CropScience, Frankfurt, Germany

BCS-CQ63359:



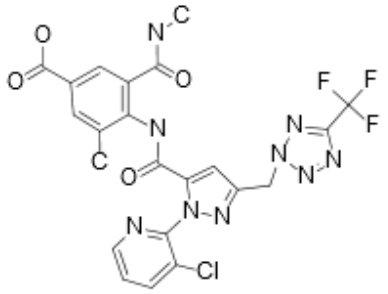
CAS Name:	2-[1[-(3-chloropyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]-3,8-dimethyl-4-oxo-3,4-dihydroquinazoline-6-carbonitrile
Molecular Formula:	$C_{22}H_{14}ClF_3N_{10}O$
Molecular Weight:	526.86 g/mol
Standard No.:	K-2118
Purity:	97.7%
Expiration Date:	08/01/2015
Date of Analysis:	08/01/2013
Storage Conditions:	Frozen
Source:	Bayer CropScience, Frankfurt, Germany

BCS-CR60014:



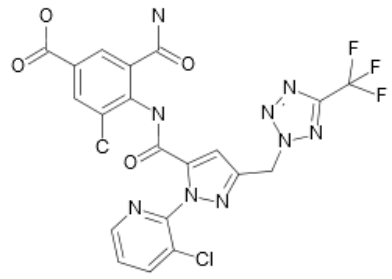
CAS Name:	4-({[1-(3-chloropyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl-1H-pyrazol-5-yl]carbonyl}amino)-N3,5-dimethylisophthalimide
Molecular Formula:	$C_{22}H_{18}ClF_3N_{10}O_3$
Molecular Weight:	562.89 g/mol
Standard No.:	K-2090
Purity:	97.9%
Expiration Date:	06/18/2018
Date of Analysis:	06/20/2013
Storage Conditions:	Frozen
Source:	Bayer CropScience, Frankfurt, Germany

BCS-CR74541:



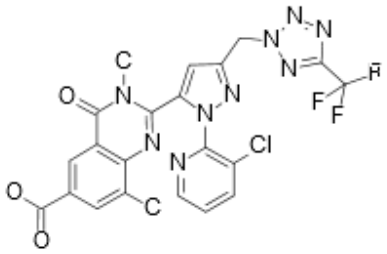
CAS Name:	4-({[1-(3-chloropyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]carbonyl}amino)-3-methyl-5-(methylcarbamoyl)benzoic acid
Molecular Formula:	$C_{22}H_{17}ClF_3N_9O_4$
Molecular Weight:	563.88 g/mol
Standard No.:	K-2117
Purity:	97.2%
Expiration Date:	04/29/2017
Date of Analysis:	05/28/2013
Storage Conditions:	Frozen
Source:	Bayer CropScience, Frankfurt, Germany

BCS-CU81055:



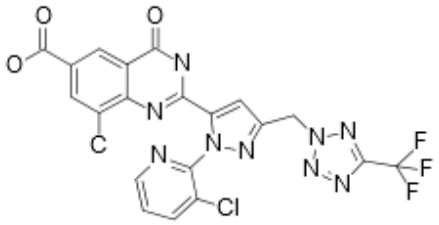
CAS Name:	3-carbamoyl-4-({[1-(3-chloropyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]carbonyl}amino)-5-methylbenzoic acid
Molecular Formula:	$C_{21}H_{15}ClF_3N_9O_4$
Molecular Weight:	549.85 g/mol
Standard No.:	K-2139
Purity:	0.11% (solution 1.021 mg/mL in 1:1 ACN/water)
Expiration Date:	01/16/2016
Date of Analysis:	01/16/2014
Storage Conditions:	Frozen
Source:	Bayer CropScience, Frankfurt, Germany

BCS-CT30673:



CAS Name:	2-[1-(3-chloropyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]-3,8-dimethyl-4-oxo-3,4-dihydroquinazoline-6-carboxylic acid
Molecular Formula:	$C_{22}H_{15}ClF_3N_9O_3$
Molecular Weight:	545.86 g/mol
Standard No.:	K-2066
Purity:	97.9%
Expiration Date:	07/24/2016
Date of Analysis:	07/04/2012
Storage Conditions:	Frozen
Source:	Bayer CropScience, Frankfurt, Germany

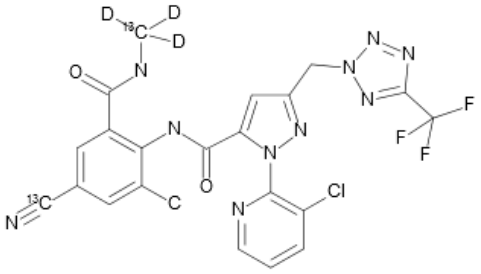
BCS-CU81056:



CAS Name:	2-[1-(3-chloropyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]-8-methyl-4-oxo-3,4-dihydroquinaziline-6-carboxylic acid
Molecular Formula:	$C_{21}H_{13}ClF_3N_9O_3$
Molecular Weight:	531.83 g/mol
Standard No.:	K-2091
Purity:	98.3%
Expiration Date:	06/09/2018
Date of Analysis:	06/19/2013
Storage Conditions:	Frozen
Source:	Bayer CropScience, Frankfurt, Germany

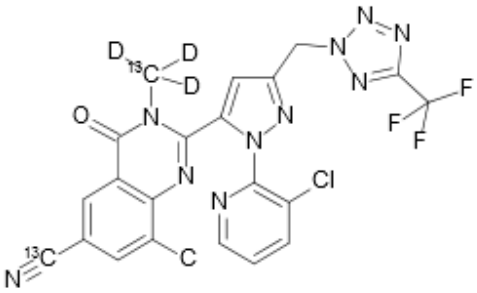
3.2 INTERNAL STANDARDS

[$^{13}C_2$, D_3] BCS-CL73507 (BCS-CL73507 ISTD)



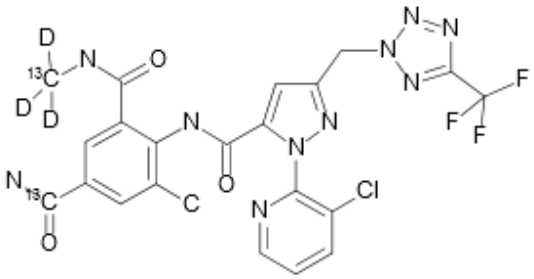
CAS Name:	1-(3-chloropyridin-2-yl)-N-{4-(^{13}C)cyano-2-methyl-6-[(^{13}C,$^{2}H_3$)methylcarbamoyl]phenyl}-3-{[5-(trifluoromethyl)-2H-tetrazol-3-yl]methyl}-1H-pyrazole-5-carboxamide
Molecular Formula:	$^{13}C_2C_{20}D_3H_{13}ClF_3N_{10}O_2$
Molecular Weight:	549.88 g/mol
Standard No.:	K-2128
Purity:	99.3%
Date of Analysis:	06/04/2014
Expiration Date:	06/04/2024
Storage Conditions:	Frozen
Source:	Bayer CropScience, Wuppertal, Germany

[$^{13}C_2$, D_3] BCS-CQ63359 (BCS-CQ63359 ISTD)



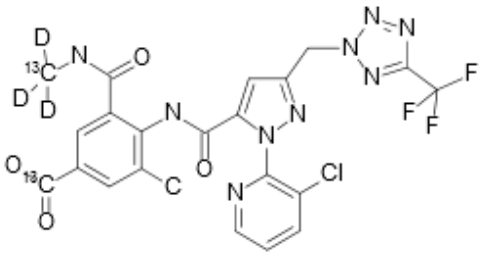
CAS Name:	2-[1-(3-chloropyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]-8-methyl-3-(^{13}C,$^{2}H_3$)methyl-4-oxo-3,4-dihydroquinazoline-6-(^{13}C)carbonitrile
Molecular Formula:	$^{13}C_2C_{20}D_3H_{11}ClF_3N_{10}O$
Molecular Weight:	531.86 g/mol
Standard No.:	K-2131
Purity:	100%
Date of Analysis:	06/04/2014
Expiration Date:	06/04/2024
Storage Conditions:	Frozen
Source:	Bayer CropScience, Wuppertal, Germany

[$^{13}C_2$, D_3] BCS-CR60014 (BCS-CR60014 ISTD)



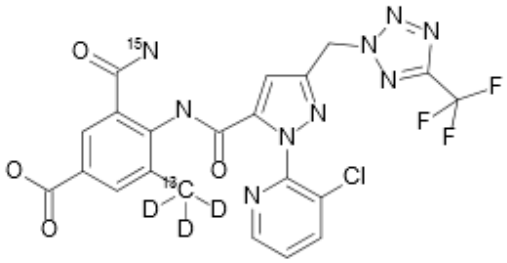
CAS Name:	4-({[1-(3-chloropyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl-1H-pyrazol-5-yl]carbonyl}amino)-5-methyl-N3-(^{13}C,$^{2}H_3$)methylbenzene-1,3-(1-^{13}C)dicarboxoamide
Molecular Formula:	$^{13}C_2C_{20}D_3H_{15}ClF_3N_{10}O_3$
Molecular Weight:	567.90 g/mol
Standard No.:	K-2145
Purity:	100%
Date of Analysis:	10/01/2014
Expiration Date:	10/29/2024
Storage Conditions:	Frozen
Source:	Bayer CropScience, Wuppertal, Germany

[$^{13}C_2$, D_3] BCS-CR74541 (BCS-CR74541 ISTD)



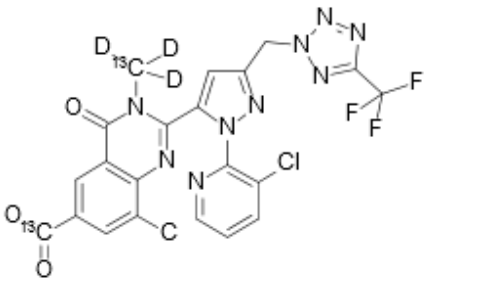
CAS Name:	4-({[1-(3-chloropyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl-1H-pyrazol-5-yl]carbonyl}amino)-5-methyl-N3-(^{13}C,$^{2}H_3$)methylbenzene-1,3-(1-^{13}C)dicarboxoamide
Molecular Formula:	$^{13}C_2C_{20}D_3H_{14}ClF_3N_9O_4$
Molecular Weight:	568.88 g/mol
Standard No.:	K-2130
Purity:	100%
Date of Analysis:	06/04/2024
Expiration Date:	06/04/2014
Storage Conditions:	Frozen
Source:	Bayer CropScience, Wuppertal, Germany

[^{13}C, D_3, ^{15}N] BCS-CU81055 (BCS-CU81055 ISTD)



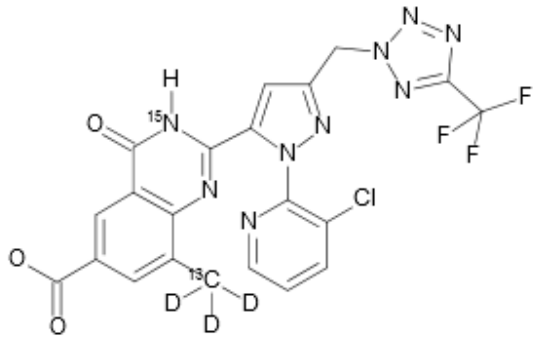
CAS Name:	3-(^{15}N)carbamoyl-4-({[1-(3-chloropyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]carbonyl}amino)-5-(^{13}C,$^{2}H_3$)methylbenzoic acid
Molecular Formula:	$^{13}CC_{20}D_3H_{12}ClF_3{}^{15}NN_8O_4$
Molecular Weight:	544.85 g/mol
Standard No.:	K-2129
Purity:	100%
Date of Analysis:	06/04/2024
Expiration Date:	06/04/2014
Storage Conditions:	Frozen
Source:	Bayer CropScience, Wuppertal, Germany

[$^{13}C_2$, D_3,] BCS-CT30673 (BCS-CT30673 ISTD)



CAS Name:	2-[1-(3-chlorpyridin-2-yl)-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]-8-methyl-3-(^{13}C,^{2}
Molecular Formula:	$^{13}C_2C_{20}D_3H_{12}ClF_3N_9O_3$
Molecular Weight:	550.86 g/mol
Standard No.:	K-2146
Purity:	100%
Date of Analysis:	10/29/2014
Expiration Date:	10/29/2024
Storage Conditions:	Frozen
Source:	Bayer CropScience, Wuppertal, Germany

[^{13}C, D_3, ^{15}N] BCS-CU81056 (BCS-CU81056 ISTD)



CAS Name:	2-[1-(3-chloropyridin-2-yl)-3-{[5-trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]-8-(^{13}C,$^{2}H_3$)methyl-4-oxo-(3-^{15}N)3,4-dihydroquinazoline-6-carboxylic acid
Molecular Formula:	$^{13}CC_{20}D_3H_{10}ClF_3{}^{15}NN_8O_3$
Molecular Weight:	536.84 g/mol
Standard No.:	K-2147
Purity:	100%
Date of Analysis:	10/29/2014
Expiration Date:	10/29/2024
Storage Conditions:	Frozen
Source:	Bayer CropScience, Wuppertal, Germany

Characterization data for the reference substances (analytical standards) and internal standards are maintained by Bayer CropScience.

The test/reference substances (analytical standards) and internal standard used in this study were procured from Bayer CropScience and stored as directed on “Analytical Standards Chain of Custody” and “Certificate of Analysis” documents. All solutions made from the reference substances (analytical standards) were stored according to the method

3.3 TEST SYSTEMS

The analytical method 01373 was evaluated in two different soils, both of which originated in the United States. The two soils were collected as sub-samples of bulk untreated samples from soil dissipation studies done in the United States in the states of Washington (WA) and Florida (FL). The soil samples were classified according to USDA specifications and the characteristics are summarized below:

Description	Washington Soil (WA) (Study MEFVN015)	Florida Soil (FL) (Study MEFVP115)
pH ($CaCl_2$ solution)	7.1	5.8
pH (aqueous solution)	7.7	6.3
Organic carbon (%)[a]	0.7	1.5
Organic matter (%)	1.2	2.5
Cation Exchange Capacity (CEC) (meq/100 g dry soil)	8.9	4.9
Maximum water holding capacity (g water/100 g dry soil)	NR	NR
USDA Textural Description (Fraction %)		
Clay (<0.002 mm)	1	1
Silt (0.002 – 0.050 mm)	11	2
Sand (0.050 - 2.000 mm)	88	97
Soil type	Sand	Sand

[a] Organic carbon = Organic matter ÷ 1.72
NR = Not reported

3.4 SAMPLE PREPARATION AND EXTRACTION

Bayer Analytical Method 01373 was used for the analysis of BCS-CL73507 and the soil metabolites BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673, and BCS-CU81056. The preparation of the soil residues was as follows:

Soil samples were weighed into glass jars and were then taken up in an extraction solvent mixture of acetonitrile/water/acetic acid (4000/1000/30, v/v/v). The slurries were then extracted for 15 minutes using a microwave extractor. Once the extraction was complete, internal standard solutions were added and the mixtures homogenized. The samples were then cooled to ambient temperatures and centrifuged for five minutes at >12000 rpm to remove fine particles of soil. An aliquot of the supernatant was then removed, diluted with acetic acid, and centrifuged for another five minutes at 13500 rpm at 5°C to further remove fine particles of soil. The samples were then analyzed by liquid chromatography and tandem mass spectroscopy (LC/MS/MS) using electrospray ionization (ESI) operating in the Multiple Reaction Monitoring (MRM) mode.

Two product ions per analyte were selected for monitoring; one ion mass transition served as quantitation and the other ion mass transition was used for confirmation. The mass transitions for both the quantitation as well as the confirmation for all substances are presented below:

MASS TRANSITIONS FOR PARENT BCS-CL73507 AND ITS METABOLITES

Analyte	Quantitation[a]	Confirmation[a]
BCS-CL73507	545.1 → 356.0	545.1 → 376.0
BCS-CQ63359	527.0 → 389.0	527.0 → 374.1
BCS-CR60014	563.0 → 356.0	563.0 → 394.0
BCS-CR74541	564.0 → 356.0	564.0 → 395.0
BCS-CU81055	550.0 → 395.1	550.0 → 356.0
BCS-CT30673	545.8 → 408.1	545.8 → 267.0
BCS-CU81056	532.0 → 394.1	532.0 → 366.0

[a] All masses reported in amu

3.5 INSTRUMENTATION

Standard Method for all Analytes

The HPLC conditions employed were as follows:

HPLC Systems: Shimadzu 20ADXR HPLC Pumps
Shimadzu CBM20A Controller
Shimadzu CTO-20A Column Oven
PAL RTC-xt Autosampler

HPLC analytical Column: YMC Ultra HT Hydrosphere C18,
2 µm, 12 nm, 30 x 2.00 mm i.d.

Mobile phase: Solvent A: Water + 0.1% formic acid
Solvent B: Acetonitrile + 0.1% formic acid

Gradient:

Time (min)	% Solvent A	% Solvent B
0.0	80	20
0.1	80	20
3.0	30	70
3.1	5	95
3.9	5	95
4.0	80	20
4.5	80	20

Divert Valve: Programmed to divert LC flow from column to waste (bypassing detector) from 0 to 1.4 minutes and again from 3.0 to end. LC flow is directed to detector during the 1.4 to 3.0 minute window.

Flow rate: 0.600 mL/min

Column Temperature: 40 ºC

Injection Volume: 25 µL

Retention Times:

BCS-CL73507:	~2.22 min.
BCS-CQ63359:	~2.80 min.
BCS-CR60014:	~1.65 min.
BCS-CR74541:	~1.95 min.
BCS-CU81055:	~1.80 min.
BCS-CT30673:	~2.37 min.
BCS-CU81056:	~2.21 min.

The MS/MS conditions employed were as follows:

MS System: AB Sciex Triple Quad API 6500 LC/MS/MS System with Analyst Software (ver 1.6.2)

Interface: Electrospray (ESI)
Ionization Mode: Positive (+)
Acquisition mode: SRM (Single Reaction Monitoring)

Ion Spray Voltage (IS): 5500 v
Temperature (TEM): 400 ºC
Ion Source Gas 1(GS1): 70
Ion Source Gas 2(GS2): 70
Curtain Gas(CUR): 40
Collision Gas (CAD): 4
Resolution Q1: Unit
Resolution Q3: Low

Analyte	Q1 Mass	Q3 Mass	Dwell Time (ms)	DP (V)	CE (V)	CXP (V)	EP (V)
BCS-CL73507	545.1	356.0	10	50	19	18	10
BCS-CL73507 C	545.1	376.0	10	50	37	18	10
BCS-CL73507-IS	550.1	356.0	10	50	19	18	10
BCS-CQ63359	527.0	389.0	10	86	29	18	10
BCS-CQ63359 C	527.0	374.1	10	50	35	18	10
BCS-CQ63359-IS	532.0	394.0	10	86	29	18	10
BCS-CR60014	563.0	356.0	10	50	19	18	10
BCS-CR60014 C	563.0	394.0	10	50	37	20	10
BCS-CR60014-IS	568.0	356.0	10	50	19	18	10
BCS-CR74541	564.0	356.0	10	80	19	18	10
BCS-CR74541 C	564.0	395.0	10	80	37	18	10
BCS-CR74541-IS	569.0	356.0	10	80	19	18	10
BCS-CU81055	550.0	395.1	10	116	35	18	10
BCS-CU81055 C	555.0	356.0	10	116	19	16	10
BCS-CU81055-IS	555.0	399.1	10	116	35	18	10
BCS-CT30673	545.8	408.1	10	91	29	20	10
BCS-CT30673 C	545.8	267.0	10	91	65	14	10
BCS-CT30673-IS	550.8	413.1	10	91	29	20	10
BCS-CU81056	532.0	394.1	10	146	29	18	10
BCS-CU81056 C	532.0	366.0	10	146	45	18	10
BCS-CU81056-IS	537.0	399.1	10	146	29	18	10

Example LC/MS/MS conditions are presented in Appendix 3.

3.6 CALCULATIONS

The example calculation displayed below was used by the laboratory performing the independent laboratory validation. Alternate calculation procedures appropriate to the reporting requirements may be substituted.

Residue concentrations were determined using calibration curves which were generated after each analysis using ABSciex quantitation software Analyst (Version 1.6.2) using linear regression with 1/x weighting.

The standards were fit to the linear equation:

$$Y = MX + B \text{ with } 1/x \text{ weighting.}$$

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

After regression coefficients were calculated, the residue in ng/g was determined using the following equation,

$$\text{Residue found (ng/g)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume } (V_1)}{\text{Initial sample wt. (W)}} \times \frac{\text{Final dilution volume } (V_3)}{\text{Aliquot taken } (V_2)}$$

Where: W = 20g
V_1 = 40mL
V_2 = 0.1mL
V_3 = 1.0mL

Analyst software was used to calculate the amount of BCS-CL73507 and its metabolites in ng/g for each sample and the percent recovery for the spiked samples.

The percent recovery was calculated as follows:

$$\%\,\text{Recovery} = \frac{\text{Mean residue R X 100}}{\text{Fortification Level}}$$

where:
Recovery Rate: Recovered amount found in fortified sample (%)
Mean Residue R: Average residue in fortified sample determined (ppb)
Fortification Level: Fortified concentration of the test compound (ppb)

4.0 RESULTS AND DISCUSSION

4.1 SELECTIVITY

Separation of analytes by HPLC followed by detection using tandem MS/MS resulted in high selectivity for the method.

4.2 LINEARITY

The correlation between the injected amount of substance and the detector response was linear for standards ranging from 0.03 ppb to 50 ppb, with correlation coefficients of >0.99 for both soil sample for all test items. All correlation coefficients are presented for all test substances in both soils along with their representative SADRS are presented in Appendix 1.

4.3 UNTREATED CONTROL SAMPLES

Untreated control samples of soil from both test locations were examined. All residues of parent BCS-CL37507 and its metabolites were below one third of the limit of quantitation (LOD), which was 0.7 ppb. Associated chromatograms are presented are presented in Appendix 2.

4.4 LOQ, RECOVERY RATES (ACCURACY) AND REPEATABILITY (PRECISION)

The Limit of Quantitation (LOQ) is defined as the lowest fortification level experimentally providing a mean recovery between 70 and 120% with a relative standard deviation of ≤20%, provided that the blank values were below 30% at this level. The LOQ was set at 2 ppb.

4.5 METHOD DETECTION LIMIT (MDL)

An estimate of the potential MDL was determined by examining the variability in the recovery as measured by the standard deviation of the amount found at the target LOQ (2.0ng/g) fortification. The estimated potential method detection limit for each analyte was calculated using the equation shown below.

$$MDL(calculated) = (standard\ deviation \times t_{0.99})$$

Where $t_{0.99}$ = one-tailed t-statistic at the 99% confidence level for n-1 replicates.

As 10 replicate analyses were performed during the soil verification, from the Student t-tables, $t_{0.99}$ = 2.821.

The calculated MDL's (ng/g) for BCS-CL73507 and its metabolites determined during the method validation study[2] in soil were found to range between 0.4 to 0.9ng/g as presented in Table 15.

4.7 TIME REQUIREMENTS

A single analyst completed sample sets consisting of 13 samples in three to four hours with HPLC-MS/MS analysis performed overnight.

4.8 PROTOCOL/SOP/METHOD DEVIATIONS

No protocol, SOP or method deviations were generated during the study.

5.0 CONCLUSION

Bayer CropScience in the U.S. successfully independently validated Bayer CropScience analytical method "Analytical Method 01373 for the Determination of BCS-CL73507 and the Metabolites BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056 in Soil and Sediment by HPLC-MS/MS ". The method was validated on the first validation set.

The method was demonstrated to be suitable for the determination of all targeted analytes in two separate soils collected from sites in the United States in the states of Washington (WA) and Florida (FL). A LOQ of at least 2 ppb was demonstrated for each analyte in each matrix evaluated. In both of the soil samples tested, none of the parent compound or any of its metabolites were found in the untreated control samples in excess of the LOD (0.7 ppb, one third of the LOQ).

Appendix 3. Typical HPLC-MS/MS Parameters

File Information for Sample RAFVP017-FL-05 of FV071415-FJ1.wiff

File Name:	FV071415-FJ1.wiff
File Path:	\\ausresc9913\Analyst Data\Projects\BCS-CL73507\RAFVP017\Data\
Original Name:	FV071415-FJ1.wiff
Software Version:	Analyst 1.6.2

Time from start =5.9833 min

Acquisition Info

Acquisition Method:	\507 soil ILV.dam
Acquisition Path:	D:\Analyst Data\Projects\BCS-CL73507\Acquisition Methods\
First Sample Started:	Tuesday, July 14, 2015 9:52:28 AM
Last Sample Finished:	Tuesday, July 14, 2015 1:49:40 PM
Sample Acq Time:	Tuesday, July 14, 2015 11:54:05 AM
Sample Acq Duration:	5min0sec
Periods in File:	1
Batch Name:	\AutomationBatch
Batch Path:	D:\Analyst Data\Projects\BCS-CL73507\Batch\
Submitted by:	AUSRESC9913\sciex()
Logged-on User:	sciex
Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Software Version:	Analyst 1.6.2
Set Name:	Set1
Sample Name	RAFVP017-FL-05
Autosampler Vial:	17
Plate Code:	Drawer 3:Slot2
Plate Position	0

Valco Valve	Diverter	
	Total Time (min)	Position
1	1.4	MS
2	3.0	waste

Shimadzu LC Method Properties

Shimadzu LC system Equlibration time = 0.00 min
Shimadzu LC Method Parameters

Pumps
Pump A Model: LC-20ADXR
Pump B Model: LC-20ADXR
Pump C Model: LC-20ADXR
Pumping Mode: Binary Flow
Total Flow: 0.6000 mL/min
Pump B Conc: 20.0 %
B Curve: 0
Pump C Flow: 0.0000 mL/min
Pressure Range (Pump A/B): 0 - 9000 psi
Pressure Range (Pump C): 0 - 9000 psi

Oven
Model: CTO-20A
Temperature Control: Enabled
Temperature: 40 deg. C
Max. Temperature: 85 deg. C

System Controller
Model: CBM-20A
Power: On

Port 1 Valve Position: B - Water +1mL/L formic acid
Port 2 Valve Position: B - ACN +1mL/L formic acid
Port 3 Valve Position: A Time Program

Time	Module	Events	Parameter
0.10	Pumps	Pump B Conc.	20
3.00	Pumps	Pump B Conc.	70
3.10	Pumps	Pump B Conc.	95
3.90	Pumps	Pump B Conc.	95
4.00	Pumps	Pump B Conc.	20
4.50	Pumps	Pump B Conc.	20
4.51	System Controller	Stop	

Quantitation Information:
Sample Type: QC
Dilution Factor: 20.000000

Custom Data:

Quantitation Table:

Period 1:
Scans in Period: 952
Relative Start Time: 0.00 msec
Experiments in Period: 1

Period 1 Experiment 1:
Scan Type: MRM (MRM)
Scheduled MRM: No
Polarity: Positive
Scan Mode: N/A
Resolution Q1: Unit
Resolution Q3: Low
Intensity Thres.: 0.00 cps
Settling Time: 20.0000 msec
MR Pause: 5.0070 msec
MCA: No
Step Size: 0.00 Da

Appendix 4 Example Calculations

BCS-CL73507residues were quantified using internal standard linear regression analysis. A separate calibration curve was produced for each set of samples analyzed on the LC/MS/MS. A calibration curve was generated by linear regression of the ratio of standard peak/internal standard peak areas versus the standard concentrations in ng/mL using Analyst Software, a computer-programmed data capturing system. The Analyst Software uses the MS/MS standard responses to calculate the regression coefficients a and b, respectively called slope and intercept, for each analytical set.

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

where: X is the concentration of the reference standard in ng/mL
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 for sample RAFVP017-WA-04 which was fortified with 10ng/g of BCS-CL73507. This chromatogram is presented in Appendix 2, page 74.

After regression coefficients were calculated, the residue in ng/g was determined. The ppb of BCS-CL73507 in soil was calculated using the following equation,

$$\text{BCS-CL73507 found (ppb)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume}(V_1)}{\text{Initial sample wt.}(W)} \times \frac{\text{Final volume}(V_3)}{\text{Aliquot taken}(V_2)}$$

W	V_1	V_2	V_3	Native Peak Area	IS Peak Area	Y	M	B
20g	40mL	0.1mL	1.0mL	27076	59627	0.4549	3.84	0.0169

The slope and intercept were obtained from the calibration curve generated by Analyst, and is presented in Appendix 5. The calibration points were weighted 1/x to provide better fit near the limit of detection

From the above equations:

$$\text{Dilution Factor (D)} = \frac{40}{20} = 2$$

$$\text{BCS-CL73507 found} = \frac{(0.4549 - 0.0169) \times 20}{3.84} = 2.28\text{ppb}$$