



Amendment No. 1 to Final Report No: MR-13/100

Study Title

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

Test Item

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

Data Requirements

- Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
- European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00
- Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010
- US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method

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Reasons for the Amendment:

Correction of the used solvent mixture for fortification standard solutions and calibration standard solution. In the original report in the chapters 4.3.3, 4.3.6 and 5.7 the description of the used solvent was incorrect. The corrections are highlighted in ***bold and italic***.

OLD:

4.3.3 Fortification Standard Solutions

Secondary standard solutions were prepared from the stock solutions by dilution with acetonitrile/water/acetic acid (920/80/1; v/v/v).

Table 5: Preparation Scheme for Fortification Standards.

No.	Compound	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]**
13-SSTD_4300	BCS-CL73507	0.170	13-PSTD_3314	50	*	1998.9
13-SSTD_4307	BCS-CL73507	5	13-SSTD_4300	50	*	199.88
13-SSTD_4306	BCS-CQ63359	0.189	13-PSTD_3320	50	*	2001.2
13-SSTD_4313	BCS-CQ63359	5	13-SSTD_4306	50	*	200.12
13-PSTD_4305	BCS-CR60014	0.175	13-PSTD_3319	50	*	1998.3
13-PSTD_4312	BCS-CR60014	5	13-SSTD_4305	50	*	199.83
13-SSTD_4304	BCS-CR74541	0.202	13-PSTD_3318	50	*	2002.8
13-SSTD_4311	BCS-CR74541	5	13-SSTD_4304	50	*	200.28
13-SSTD_4301	BCS-CU81055	0.181	13-PSTD_3315	50	*	2002.4
13-SSTD_4308	BCS-CU81055	5	13-SSTD_4301	50	*	200.24
13-SSTD_4303	BCS-CT30673	0.183	13-PSTD_3317	50	*	2004.8
13-SSTD_4310	BCS-CT30673	5	13-PSTD_4303	50	*	200.48
13-SSTD_4302	BCS-CU81056	0.197	13-PSTD_3316	50	*	1995.4
13-SSTD_4309	BCS-CU81056	5	13-SSTD_4302	50	*	199.54

* acetonitrile/water/acetic acid (920/80/1; v/v/v)

** These standard concentrations were used for the fortification of the recoveries.

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4.3.6 Calibration Standard Solutions

Calibration standard solutions were prepared from the secondary and internal standard solutions by dilution with acetonitrile/water/acetic acid (920/80/1; v/v/v).

Table 8: Preparation Scheme for Calibration Standards.

No.	Reference Item	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]
13-CSTD_4219	BCS-CL73507	0.075	13-FSTD_4216	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.03
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217			
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4220	BCS-CL73507	0.125	13-FSTD_4216	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.05
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217			
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4223	BCS-CL73507	0.25	13-FSTD_4216	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.1
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217			
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						

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4.3.6 Calibration Standard Solutions (contd.)

Table 8 (contd.): Preparation Scheme for Calibration Standards.

No.	Reference Item	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]
13-CSTD_4222	BCS-CL73507	0.625	13-FSTD_4216	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.25
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217			0.25
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4224	BCS-CL73507	0.125	13-FSTD_4215	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.5
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217			0.25
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4226	BCS-CL73507	0.25	13-FSTD_4215	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	1.0
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217			0.25
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4225	BCS-CL73507	0.625	13-FSTD_4215	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	2.5
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217			0.25
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						

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4.3.6 Calibration Standard Solutions (contd.)

Table 8 (contd.): Preparation Scheme for Calibration Standards.

No.	Reference Item	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]
13-CSTD_4227	BCS-CL73507	0.125	13-FSTD_4214	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	5.0
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217	50		0.25
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4228	BCS-CL73507	0.25	13-FSTD_4214	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	10
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217	50		0.25
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4229	BCS-CL73507	0.625	13-FSTD_4214	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	25
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217	50		0.25
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4230	BCS-CL73507	1.25	13-FSTD_4214	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	50
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056	0.5	13-ISTD_4217	50		0.25
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						

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5.7 Stability in Standard Solutions

The stability of BCS-CL73507, BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056 in standard solutions was tested. The solutions were prepared in acetonitrile, acetonitrile/water (4/1, v/v) or in acetonitrile/water/acetic acid (920/80/1; v/v/v).

For this purpose aged standard solutions were quantified against freshly prepared standard solutions. The aged solutions were stored in volumetric flasks in the dark in a refrigerator at $4^{\circ}\text{C} \pm 3^{\circ}\text{C}$ until analysis.

The target value is at maximum a 6.8 percent degradation (calculated from the means) of the aged solutions.

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NEW:

4.3.3 Fortification Standard Solutions

Secondary standard solutions were prepared from the stock solutions by dilution with *water/acetonitrile/acetic acid (920/80/1; v/v/v)*.

Table 5: Preparation Scheme for Fortification Standards.

No.	Compound	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]**
13-SSTD_4300	BCS-CL73507	0.170	13-PSTD_3314	50	*	1998.9
13-SSTD_4307	BCS-CL73507	5	13-SSTD_4300	50	*	199.88
13-SSTD_4306	BCS-CQ63359	0.189	13-PSTD_3320	50	*	2001.2
13-SSTD_4313	BCS-CQ63359	5	13-SSTD_4306	50	*	200.12
13-PSTD_4305	BCS-CR60014	0.175	13-PSTD_3319	50	*	1998.3
13-PSTD_4312	BCS-CR60014	5	13-SSTD_4305	50	*	199.83
13-SSTD_4304	BCS-CR74541	0.202	13-PSTD_3318	50	*	2002.8
13-SSTD_4311	BCS-CR74541	5	13-SSTD_4304	50	*	200.28
13-SSTD_4301	BCS-CU81055	0.181	13-PSTD_3315	50	*	2002.4
13-SSTD_4308	BCS-CU81055	5	13-SSTD_4301	50	*	200.24
13-SSTD_4303	BCS-CT30673	0.183	13-PSTD_3317	50	*	2004.8
13-SSTD_4310	BCS-CT30673	5	13-PSTD_4303	50	*	200.48
13-SSTD_4302	BCS-CU81056	0.197	13-PSTD_3316	50	*	1995.4
13-SSTD_4309	BCS-CU81056	5	13-SSTD_4302	50	*	199.54

* *water/acetonitrile/acetic acid (920/80/1; v/v/v)*

** These standard concentrations were used for the fortification of the recoveries.

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4.3.6 Calibration Standard Solutions

Calibration standard solutions were prepared from the secondary and internal standard solutions by dilution with *water/acetonitrile/acetic acid (920/80/1; v/v/v)*.**Table 8: Preparation Scheme for Calibration Standards.**

No.	Reference Item	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]
13-CSTD_4219	BCS-CL73507	0.075	13-FSTD_4216	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	0.03
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673	0.5	13-ISTD_4217	50		0.25
	BCS-CU81056					
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4220	BCS-CL73507	0.125	13-FSTD_4216	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	0.05
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673	0.5	13-ISTD_4217	50		0.25
	BCS-CU81056					
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4223	BCS-CL73507	0.25	13-FSTD_4216	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	0.1
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673	0.5	13-ISTD_4217	50		0.25
	BCS-CU81056					
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						

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4.3.6 Calibration Standard Solutions (contd.)

Table 8 (contd.): Preparation Scheme for Calibration Standards.

No.	Reference Item	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]
13-CSTD_4222	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.625	13-FSTD_4216	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	0.25
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			0.25
13-CSTD_4224	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.125	13-FSTD_4215	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	0.5
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			0.25
13-CSTD_4226	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.25	13-FSTD_4215	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	1.0
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			0.25
13-CSTD_4225	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.625	13-FSTD_4215	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	2.5
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			0.25

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4.3.6 Calibration Standard Solutions (contd.)

Table 8 (contd.): Preparation Scheme for Calibration Standards.

No.	Reference Item	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]
13-CSTD_4227	BCS-CL73507	0.125	13-FSTD_4214	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	5.0
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673	0.5	13-ISTD_4217	50		0.25
	BCS-CU81056					
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4228	BCS-CL73507	0.25	13-FSTD_4214	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	10
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673	0.5	13-ISTD_4217	50		0.25
	BCS-CU81056					
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4229	BCS-CL73507	0.625	13-FSTD_4214	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	25
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673	0.5	13-ISTD_4217	50		0.25
	BCS-CU81056					
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						
13-CSTD_4230	BCS-CL73507	1.25	13-FSTD_4214	50	<i>water / acetonitrile / acetic acid (920/80/1; v/v/v)</i>	50
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673	0.5	13-ISTD_4217	50		0.25
	BCS-CU81056					
	BCS-CL73507 ISTD					
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
BCS-CR74541 ISTD						
BCS-CU81055 ISTD						
BCS-CT30673 ISTD						
BCS-CU81056 ISTD						

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5.7 Stability in Standard Solutions

The stability of BCS-CL73507, BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056 in standard solutions was tested. The solutions were prepared in acetonitrile, acetonitrile/water (4/1, v/v) or in **water/acetonitrile/acetic acid (920/80/1; v/v/v)**.

For this purpose aged standard solutions were quantified against freshly prepared standard solutions. The aged solutions were stored in volumetric flasks in the dark in a refrigerator at $4^{\circ}\text{C} \pm 3^{\circ}\text{C}$ until analysis.

The target value is at maximum a 6.8 percent degradation (calculated from the means) of the aged solutions.



Study Title

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

Test Item

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

Data Requirements

- Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
- European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00
- Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010
- US EPA Residue Chemistry Test Guideline OCSP 860.1340: Residue Analytical Method

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2 Introduction and Objective

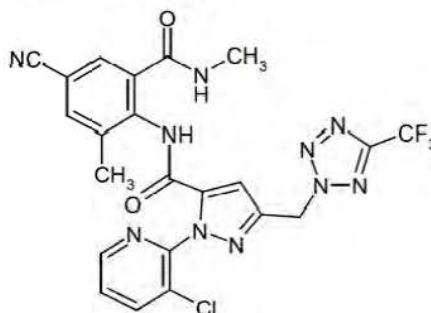
The objective of the study was to validate the analytical method 01373 for BCS-CL73507 and the metabolites BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056 in soil, the determination of the specificity, recovery experiments including repeatability, linearity of the detector, blank values of untreated control samples, determination of the stability of residues in extracts and the stability of standard solutions.

3 Compounds

3.1 Reference Items

Generally, only sufficiently characterized and certified substances were used as reference items.

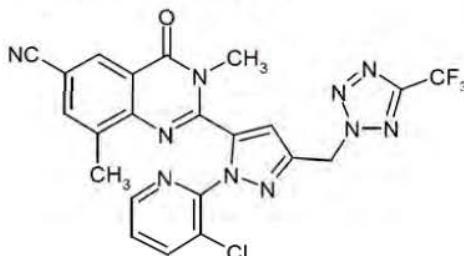
- Name of the Compound **BCS-CL73507**
- Batch Number BCS-CL73507-PU-02
- Certificate of Analysis AZ 18074, dated 2012-07-18
- Structure



- Chemical Name 1-(3-chloropyridin-2-yl)-N-[4-cyano-2-methyl-6-(methylcabamoyl)phenyl]-3-[[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl]-1H-pyrazole-5-carboxamide
- CAS Registry No. 1229654-66-3
- Empirical Formula $C_{22}H_{16}ClF_3N_{10}O_2$
- Molar Mass 544.88 g/mol
- Purity 97.9%
- Expiry Date 2014-07-12

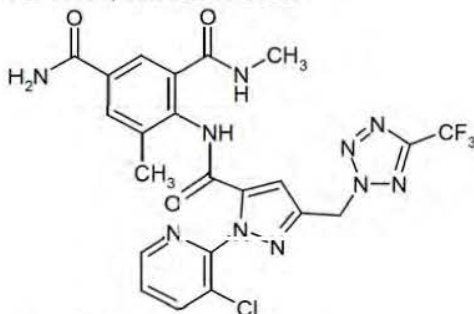
Bayer CropScience AG
BCS-R&D-D-HS-RAMethod No.: 01373
Report: MR-13/100
Page 15 (155)**3.1 Reference Item (contd.)**

- Name of the Compound **BCS-CQ63359**
- Batch Number BCS-CQ63359-01-01
- Certificate of Analysis AZ 18032, dated 2013-08-14
- Structure



- Chemical 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-dihydroquinazolin-6-carbonitrile
- Empirical Formula $C_{22}H_{14}ClF_3N_{10}O$
- Molar Mass 526.86 g/mol
- Purity 97.5%
- Expiry Date 2014-04-17

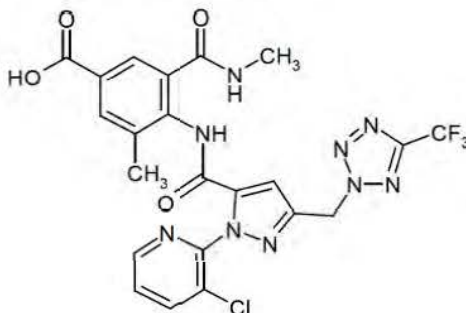
- Name of the Compound **BCS-CR60014**
- Batch Number BCS-CR60014-01-01
- Certificate of Analysis AZ 18834, dated 2013-06-28
- Structure



- Chemical Name 4-(((1-(3-chloropyridin-2-yl)-3-[[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl]-1H-pyrazol-5-yl]carbonyl)amino)-N3,5-dimethylisophthalamide
- Empirical Formula $C_{22}H_{18}ClF_3N_{10}O_3$
- Molar Mass 562.89 g/mol
- Purity 97.9%
- Expiry Date 2015-06-20

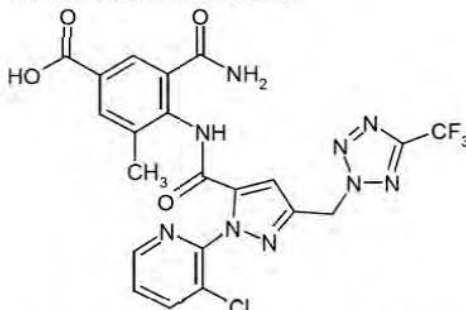
Bayer CropScience AG
BCS-R&D-D-HS-RAMethod No.: 01373
Report: MR-13/100
Page 16 (155)**3.1 Reference Item (contd.)**

- Name of the Compound **BCS-CR74541**
- Batch Number BCS-CR74541-PU-01
- Certificate of Analysis AZ 18081, dated 2012-05-31
- Structure



- Chemical 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
- Empirical Formula $C_{22}H_{17}ClF_3N_9O_4$
- Molar Mass 563.88 g/mol
- Purity 97.3%
- Expiry Date 2014-05-25

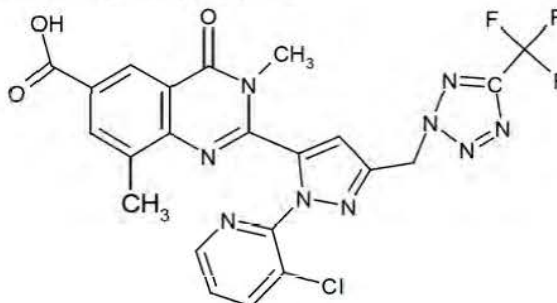
- Name of the Compound **BCS-CU81055**
- Batch Number BCS-CU81055-01-01
- Certificate of Analysis MZ 00713, dated 2013-07-24
- Structure



- Chemical 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
- Empirical Formula $C_{21}H_{15}ClF_3N_9O_4$
- Molar Mass 549.86 g/mol
- Purity 99.4%
- Expiry Date 2014-01-24

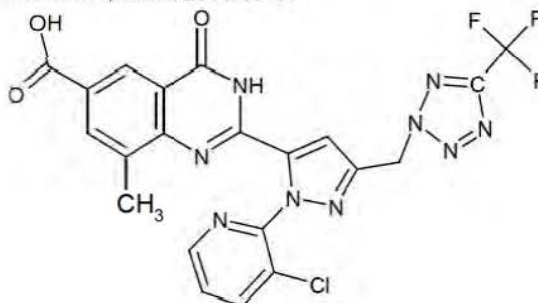
Bayer CropScience AG
BCS-R&D-D-HS-RAMethod No.: 01373
Report: MR-13/100
Page 17 (155)**3.1 Reference Item (contd.)**

- Name of the Compound **BCS-CT30673**
- Batch Number BCS-CT30673-PU-01
- Certificate of Analysis AZ 18137, dated 2012-07-06
- Structure



- Chemical 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
- Empirical Formula $C_{22}H_{15}ClF_3N_9O_3$
- Molar Mass 545.86 g/mol
- Purity 97.9%
- Expiry Date 2014-07-04

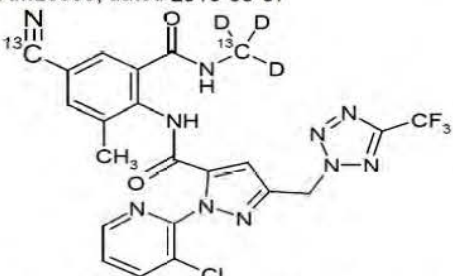
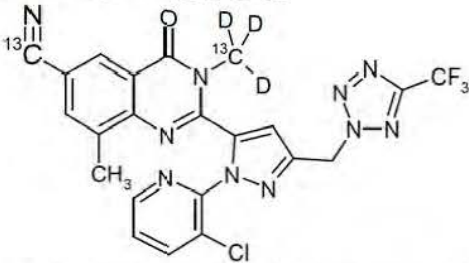
- Name of the Compound **BCS-CU81056**
- Batch Number BCS-CU81056-PU-01
- Certificate of Analysis MZ 18810, dated 2013-07-03
- Structure



- Chemical 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-dihydroquinazoline-6-carboxylic acid
- Empirical Formula $C_{21}H_{13}ClF_3N_9O_3$
- Molar Mass 531.84 g/mol
- Purity 98.3%
- Expiry Date 2015-06-19

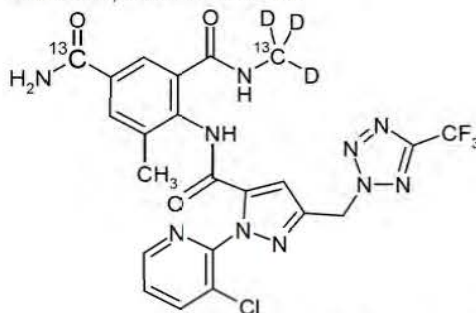
Bayer CropScience AG
BCS-R&D-D-HS-RAMethod No.: 01373
Report: MR-13/100
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3.2 Internal Standards

- Name of the Compound	[¹³C₂,D₃] BCS-CL73507 or BCS-CL73507 ISTD
- Batch Number	MXM 7015-3-2
- Certificate of Analysis	KML9603, dated 2013-05-07
- Structure	
- Chemical Name	1-(3-chloropyridin-2-yl)-N-(4-(¹³ C)cyano-2-methyl-6-[[¹³ C, ² H ₃]methylcabamoyl]phenyl)-3-[[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl]-1H-pyrazole-5-carboxamide
- Empirical Formula	¹³ C ₂ C ₂₀ D ₃ H ₁₃ Cl F ₃ N ₁₀ O ₂
- Molar Mass	549.88 g/mol
- Purity	>98%
- Name of the Compound	[¹³C₂,D₃] BCS-CQ63359 or BCS-CQ63359 ISTD
- Batch Number	MXM 7016-3-1
- Certificate of Analysis	KML9601, dated 2013-05-06
- Structure	
- Chemical 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
- Empirical Formula	¹³ C ₂ C ₂₀ D ₃ H ₁₁ Cl F ₃ N ₁₀ O
- Molar Mass	531.87 g/mol
- Purity	97%

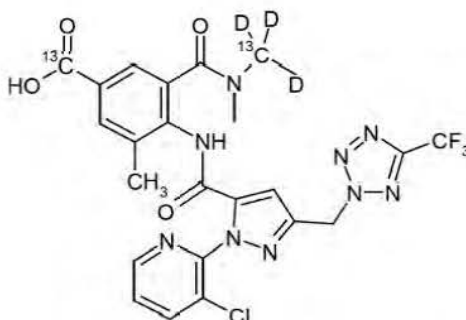
Bayer CropScience AG
BCS-R&D-D-HS-RAMethod No.: 01373
Report: MR-13/100
Page 19 (155)**3.2 Internal Standards (contd.)**

- Name of the Compound **[¹³C₂,D₃] BCS-CR60014 or BCS-CR60014 ISTD**
- Batch Number MXM7025-1-4
- Certificate of Analysis KML9602, dated 2013-05-07
- Structure



- Chemical Name **4-([[1-(3-chloropyridin-2-yl)-3-[[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl]-1H-pyrazol-5-yl]carbonyl]amino)-5-methyl-N3-(¹³C,²H₃)methylbenzene-1,3-(1-¹³C)dicarboxoamide**
- Empirical Formula **¹³C₂ C₂₀ D₃ H₁₅ Cl F₃ N₁₀ O₃**
- Molar Mass **567.9 g/mol**
- Purity **>98%**

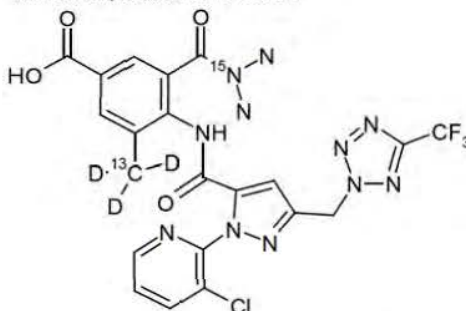
- Name of the Compound **[¹³C₂,D₃] BCS-CR74541 or BCS-CR74541 ISTD**
- Batch Number MXM 7026-1-17
- Certificate of Analysis KML9604, dated 2013-05-07
- Structure



- Chemical Name **4-([[1-(3-chloropyridin-2-yl)-3-[[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl]-1H-pyrazol-5-yl]carbonyl]amino)-3-methyl-5-[[(¹³C,²H₃)methyl]carbamoyl](carboxy-¹³C)benzoic acid**
- Empirical Formula **¹³C₂ C₂₀ D₃ H₁₄ Cl F₃ N₉ O₄**
- Molar Mass **568.88 g/mol**
- Purity **>98%**

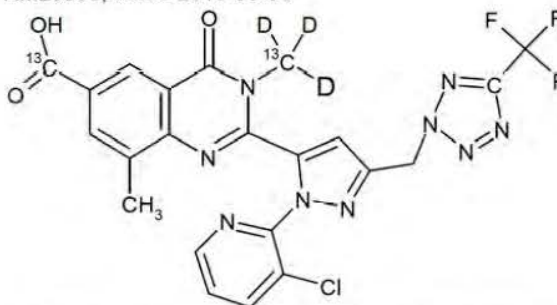
Bayer CropScience AG
BCS-R&D-D-HS-RAMethod No.: 01373
Report: MR-13/100
Page 20 (155)**3.2 Internal Standards (contd.)**

- Name of the Compound **[¹³C,^D₃, ¹⁵N] BCS-CU81055 or BCS-CU81055 ISTD**
- Batch Number **MXM7090-1-37**
- Certificate of Analysis **KML9606, dated 2013-05-22**
- Structure



- Chemical Name **3-(¹⁵N)carbamoyl-4-({[1-(3-chloropyridin-2-yl)-3-{{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]carbonyl} amino)-5-(¹³C, ²H₃)methylbenzoic acid**
- Empirical Formula **¹³C₂₀ D₃ H₁₂ Cl F₃ ¹⁵N N₈ O₃**
- Molar Mass **544.86 g/mol**
- Purity **99%**

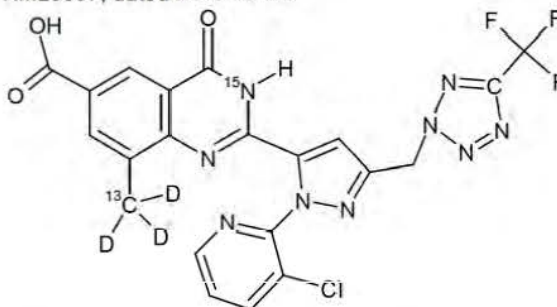
- Name of the Compound **[¹³C₂, D₃] BCS-CT30673 or BCS-CT30673 ISTD**
- Batch Number **MXM7028-6-5**
- Certificate of Analysis **KML9600, dated 2013-05-06**
- Structure



- Chemical Name **2-[1-(3-chloropyridin-2-yl)-3-{{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazol-5-yl]-8-methyl-3-(¹³C, ²H₃)methyl-4-oxo-3,4-dihydroquinazoline-6-(¹³C)carboxylic acid**
- Empirical Formula **¹³C₂ C₂₀ D₃ H₁₂ Cl F₃ N₉ O₃**
- Molar Mass **550.87 g/mol**
- Purity **>99%**

Bayer CropScience AG
BCS-R&D-D-HS-RAMethod No.: 01373
Report: MR-13/100
Page 21 (155)**3.2 Internal Standards (contd.)**

- Name of the Compound **[¹³C,^D₃, ¹⁵N] BCS-CU81056 or BCS-CU81056 ISTD**
- Batch Number **MXM7097-1-1**
- Certificate of Analysis **KML9607, dated 2013-05-22**
- Structure



- Chemical Name **2-[1-(3-chloropyridin-2-yl)-3-[[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl]-1H-pyrazol-5-yl]-8-(¹³C,²H₃)methyl-4-oxo-(3-¹⁵N)3,4-dihydroquinazoline-6-carboxylic acid**
- Empirical Formula **³C C₂₀ D₃ H₁₀ Cl F₃ ¹⁵N N₈ O₃**
- Molar Mass **536.84 g/mol**
- Purity **>98%**

Bayer CropScience AG
BCS-R&D-D-HS-RAMethod No.: 01373
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4 Experimental Section

4.1 Test System

The method was validated using the three German soils Höfchen, Dollendorf and Laacher Hof and sediment OECD 218/219. Three different soils were used in order to assess a possible influence of different soil characteristics. The soil samples were classified according to DIN and/or USDA specifications. Soil characteristics of the used soils are summarized in [Table 2](#) and [Table 3](#).

Table 2: Soil Characteristics

	Soil Höfchen	Soil Dollendorf	Soil Laacher Hof
Description	Plot 4011; 0-30 cm soil layer	ID 20100423 0-20 cm soil layer	Plot 712/718; 0-30 cm soil layer
pH (in CaCl ₂ solution)	6.7	7.3	6.8
pH (in H ₂ O)	7.4	7.4	7.4
Organic Carbon [%]	0.92	5	1.20
Organic Matter [%] *	1.58	8.6	2.06
Cation Exchange Capacity [meq / 100 g dry soil]	12.4	20.6	9.8
max. Water Holding Capacity [g / 100 g dry soil]	39.5	79.1	37.9
Textural Description according to USDA [Fraction %]	Fraction [%]	Fraction [%]	Fraction [%]
Clay (<0.002 mm)	19.4	31	12.0
Silt (0.002-0.050 mm)	76.3	38	18.3
Sand (0.050-2.000 mm)	4.3	31	69.7
Soil type	Silt loam	Clay loam	Sandy loam

* Organic matter = Organic carbon x 1.72

Table 3: Sediment Characteristics

	Sediment
Description	OECD 218/219
Peat	4 %
Kaolin	20 %
Quartz sand	75 %
Calciumcarbonat	1 %

4.2 Safety

The German guidelines for laboratories of the Employees' Liability Insurance Association, e.g. Working Safely in Laboratories [4] or comparable guidelines in other countries should be observed.

The following chemicals were used, which are classified by the hazardous material regulations. The classification is based on the Globally Harmonised System (GHS) [5].

	Signal word	Hazard statements
Acetonitrile	Danger	H225 Highly flammable liquid and vapour. H302 Harmful if swallowed. H312 Harmful in contact with skin. H319 Causes serious eye irritation. H332 Harmful if inhaled
Acetic acid	Danger	H226 Flammable liquid and vapour. H314 Causes severe skin burns and eye damage.
Formic acid	Danger	H226 Flammable liquid and vapour. H314 Causes severe skin burns and eye damage.
BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	Warning	Caution – not fully tested yet

The pertinent safety instructions must be observed when working with all compounds mentioned in this method.

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4.3 Materials

4.3.1 Apparatus and Reagents

For apparatus and reagents please see [Appendix 1](#).

4.3.2 Stock Solutions

The stock solutions were prepared by weighing a defined amount of reference item into a volumetric flask and making up to volume with acetonitrile or acetonitrile/water 4+1.

Table 4: Preparation Scheme of Reference Item Stock Solutions.

Reference Item		Mass [mg]	Volume [mL]	Solvent	Final Concentration	
No.	Compound				Required [mg/L]	Actual * [mg/L]
[13-PSTD_3314]	BCS-CL73507	12.01	20	acetonitrile	500	587.89
[13-PSTD_3320]	BCS-CQ63359	10.86	20	acetonitrile	500	529.43
[13-PSTD_3319]	BCS-CR60014	14.58	25	acetonitrile/water 4+1	500	570.95
[13-PSTD_3318]	BCS-CR74541	10.19	20	acetonitrile	500	495.74
[13-PSTD_3315]	BCS-CU81055	11.13	20	acetonitrile	500	553.16
[13-PSTD_3317]	BCS-CT30673	11.19	20	acetonitrile	500	547.75
[13-PSTD_3316]	BCS-CU81056	12.88	25	acetonitrile/water 4+1	500	506.44
[13-PSTD_3321]	BCS-CL73507 ISTD	5.28	20	acetonitrile	250	258.72
[13-PSTD_3323]	BCS-CQ63359 ISTD	5.40	20	acetonitrile	250	261.90
[13-PSTD_3324]	BCS-CR60014 ISTD	5.12	20	acetonitrile	250	250.88
[13-PSTD_3325]	BCS-CR74541 ISTD	5.06	20	acetonitrile	250	247.94
[13-PSTD_3322]	BCS-CU81055 ISTD	5.78	20	acetonitrile	250	286.11
[13-PSTD_3327]	BCS-CT30673 ISTD	5.76	20	acetonitrile	250	285.12
[13-PSTD_3326]	BCS-CU81056 ISTD	5.28	20	acetonitrile	250	258.72

* Concentrations are corrected for purity.

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Page 25 (155)**4.3.3 Fortification Standard Solutions**

Secondary standard solutions were prepared from the stock solutions by dilution with acetonitrile/water/acetic acid (920/80/1; v/v/v).

Table 5: Preparation Scheme for Fortification Standards.

No.	Compound	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [$\mu\text{g/L}$]**
13-SSTD_4300	BCS-CL73507	0.170	13-PSTD_3314	50	*	1998.9
13-SSTD_4307	BCS-CL73507	5	13-SSTD_4300	50	*	199.88
13-SSTD_4306	BCS-CQ63359	0.189	13-PSTD_3320	50	*	2001.2
13-SSTD_4313	BCS-CQ63359	5	13-SSTD_4306	50	*	200.12
13-PSTD_4305	BCS-CR60014	0.175	13-PSTD_3319	50	*	1998.3
13-PSTD_4312	BCS-CR60014	5	13-SSTD_4305	50	*	199.83
13-SSTD_4304	BCS-CR74541	0.202	13-PSTD_3318	50	*	2002.8
13-SSTD_4311	BCS-CR74541	5	13-SSTD_4304	50	*	200.28
13-SSTD_4301	BCS-CU81055	0.181	13-PSTD_3315	50	*	2002.4
13-SSTD_4308	BCS-CU81055	5	13-SSTD_4301	50	*	200.24
13-SSTD_4303	BCS-CT30673	0.183	13-PSTD_3317	50	*	2004.8
13-SSTD_4310	BCS-CT30673	5	13-PSTD_4303	50	*	200.48
13-SSTD_4302	BCS-CU81056	0.197	13-PSTD_3316	50	*	1995.4
13-SSTD_4309	BCS-CU81056	5	13-SSTD_4302	50	*	199.54

* acetonitrile/water/acetic acid (920/80/1; v/v/v)

** These standard concentrations were used for the fortification of the recoveries.

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4.3.4 Secondary Standard Solutions

Secondary standard solutions were prepared from the stock solutions by dilution with acetonitrile.

Table 6: Preparation Scheme for Secondary Standards.

No.	Compound	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]
13-FSTD_4214	BCS-CL73507	0.170	13-PSTD_3314	50	acetonitrile	2000*
	BCS-CQ63359	0.189	13-PSTD_3320			
	BCS-CR60014	0.175	13-PSTD_3319			
	BCS-CR74541	0.202	13-PSTD_3318			
	BCS-CU81055	0.181	13-PSTD_3315			
	BCS-CT30673	0.183	13-PSTD_3317			
	BCS-CU81056	0.197	13-PSTD_3316			
13-FSTD_4215	BCS-CL73507	5	13-FSTD_4214	50	acetonitrile	200*
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056					
13-FSTD_4216	BCS-CL73507	5	13-FSTD_4215	50	acetonitrile	20
	BCS-CQ63359					
	BCS-CR60014					
	BCS-CR74541					
	BCS-CU81055					
	BCS-CT30673					
	BCS-CU81056					

* These standard concentrations can be used for the fortification of the recoveries.

4.3.5 Internal Standard Solutions

Internal standard solutions were prepared from the stock solutions by dilution with acetonitrile.

Table 7: Preparation Scheme for Internal Standards.

No.	Compound	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]
13-ISTD_3506	BCS-CL73507 ISTD	3.865	13-PSTD_3321	2000	acetonitrile	500
	BCS-CQ63359 ISTD	3.818	13-PSTD_3323			
	BCS-CR60014 ISTD	3.986	13-PSTD_3324			
	BCS-CR74541 ISTD	4.033	13-PSTD_3325			
	BCS-CU81055 ISTD	3.495	13-PSTD_3322			
	BCS-CT30673 ISTD	3.507	13-PSTD_3327			
	BCS-CU81056 ISTD	3.865	13-PSTD_3326			
13-ISTD_4217	BCS-CL73507 ISTD	2.5	13-PSTD_3506	50	acetonitrile	25
	BCS-CQ63359 ISTD					
	BCS-CR60014 ISTD					
	BCS-CR74541 ISTD					
	BCS-CU81055 ISTD					
	BCS-CT30673 ISTD					

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BCS-R&D-D-HS-RAMethod No.: 01373
Report: MR-13/100
Page 27 (155)**4.3.6 Calibration Standard Solutions**

Calibration standard solutions were prepared from the secondary and internal standard solutions by dilution with acetonitrile/water/acetic acid (920/80/1; v/v/v).

Table 8: Preparation Scheme for Calibration Standards.

No.	Reference Item	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]	
13-CSTD_4219	BCS-CL73507	0.075	13-FSTD_4216	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.03	
	BCS-CQ63359						
	BCS-CR60014						
	BCS-CR74541						
	BCS-CU81055						
	BCS-CT30673						
	13-CSTD_4217	BCS-CL73507 ISTD	0.5	13-ISTD_4217	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.25
		BCS-CQ63359 ISTD					
		BCS-CR60014 ISTD					
		BCS-CR74541 ISTD					
		BCS-CU81055 ISTD					
		BCS-CT30673 ISTD					
13-CSTD_4220	BCS-CL73507	0.125	13-FSTD_4216	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.05	
	BCS-CQ63359						
	BCS-CR60014						
	BCS-CR74541						
	BCS-CU81055						
	BCS-CT30673						
	13-CSTD_4217	BCS-CL73507 ISTD	0.5	13-ISTD_4217	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.25
		BCS-CQ63359 ISTD					
		BCS-CR60014 ISTD					
		BCS-CR74541 ISTD					
		BCS-CU81055 ISTD					
		BCS-CT30673 ISTD					
13-CSTD_4223	BCS-CL73507	0.25	13-FSTD_4216	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.1	
	BCS-CQ63359						
	BCS-CR60014						
	BCS-CR74541						
	BCS-CU81055						
	BCS-CT30673						
	13-CSTD_4217	BCS-CL73507 ISTD	0.5	13-ISTD_4217	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.25
		BCS-CQ63359 ISTD					
		BCS-CR60014 ISTD					
		BCS-CR74541 ISTD					
		BCS-CU81055 ISTD					
		BCS-CT30673 ISTD					
BCS-CU81056 ISTD							

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Page 28 (155)**4.3.6 Calibration Standard Solutions (contd.)****Table 8 (contd.): Preparation Scheme for Calibration Standards.**

No.	Reference Item	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]
13-CSTD_4222	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.625	13-FSTD_4216	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.25
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			
13-CSTD_4224	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.125	13-FSTD_4215	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	0.5
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			
13-CSTD_4226	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.25	13-FSTD_4215	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	1.0
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			
13-CSTD_4225	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.625	13-FSTD_4215	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	2.5
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			

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Page 29 (155)**4.3.6 Calibration Standard Solutions (contd.)****Table 8 (contd.): Preparation Scheme for Calibration Standards.**

No.	Reference Item	Aliquot [mL]	No. of Solution	Final Volume [mL]	Solvent	Final Concentration [µg/L]
13-CSTD_4227	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.125	13-FSTD_4214	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	5.0
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			
13-CSTD_4228	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.25	13-FSTD_4214	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	10
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			
13-CSTD_4229	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	0.625	13-FSTD_4214	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	25
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			
13-CSTD_4230	BCS-CL73507 BCS-CQ63359 BCS-CR60014 BCS-CR74541 BCS-CU81055 BCS-CT30673 BCS-CU81056	1.25	13-FSTD_4214	50	acetonitrile / water / acetic acid (920/80/1; v/v/v)	50
	BCS-CL73507 ISTD BCS-CQ63359 ISTD BCS-CR60014 ISTD BCS-CR74541 ISTD BCS-CU81055 ISTD BCS-CT30673 ISTD BCS-CU81056 ISTD	0.5	13-ISTD_4217			

4.4 Sample Preparation

Extraction Procedure

1. Weigh 20 g of the soil sample into a 100-mL wide neck glass jar with screw cap containing a magnetic bar.
REMARK: For recoveries add standard solution in the appropriate height for the corresponding fortification level.
2. Add 40 mL of a mixture of acetonitrile/water/acetic acid (4000/1000/30, v/v/v).
3. Half close the 100-mL wide neck glass jar.
REMARK: Do not really close the bottle to avoid pressure build up.
4. Place ten jars with soil-solvent mixture into the microwave extractor.
5. Switch on the magnetic stirrer.
6. Extract for 15 minutes: From 0-3 minutes at 400 W (ambient temperature to 60°C) and from 3-15 minutes at 110 W (60°C).
7. Add 200 µL of internal standard solution 13-ISTD_3506 and homogenize.
8. Let the sample cool down to ambient temperature.
9. If needed centrifuge for 5 minutes at >12000 g to remove fine particles of soil.
10. Transfer an aliquot of 0.1 mL into a round bottom tube and add 0.9 mL acetic acid 0.1% and homogenize.
11. Centrifuge for 5 minutes at 13500 g and 5°C to remove fine particles of soil.
12. Determine by liquid chromatography and MS/MS.

4.5 Instrumental Analysis

4.5.1 Principle of Measurement

An aliquot of the sample solution was injected into the high performance liquid chromatograph and subjected to reversed phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. The MS/MS instrument was operated in the Multiple Reaction Monitoring mode (MRM). The pseudomolecular ions of the analytes ($[M+H]^+$, $[M-H]^-$ or any adducts) were selected by the first quadrupole. These precursor ions were impulsed with nitrogen in the collision cell (second quadrupole) and the resulting fragment ions (product ions) were separated according to their m/z ratio in the third quadrupole. Two of these product ions per analyte were selected: one product ion (MRM-transition) serving for quantitation and the second for confirmation.

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4.5.2 Variations in Instrument Conditions

Variations in equipment or sample characteristics and/or deterioration of system performance may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Instrument parameters and mobile phase may be adjusted to improve separation from unexpected interfering peaks.

Therefore, the given LC/MS/MS parameters listed may require adaptation.

4.5.3 Chromatography

Liquid Chromatograph: CTC Analytics HTC PAL Autosampler
Agilent 1290 Binary Pump
Agilent 1260 Isocratic Pump
Hot Pocket Column Oven standard version, short
(ThermoFisher)

Column: YMC Ultra HT Hydrosphere C18,
2 μ m, 12 nm, 30 x 2.00 mm i.d.

Software: Analyst 1.6.2

Injection Volume: 10 μ L into a 5 μ L loop

Oven Temperature 40 °C

Mobile Phase: A: Milli-Q-water +1.0 mL/L formic acid
B: Acetonitrile+1.0 mL/L formic acid
C: Methanol/water (4/1, v/v)

Run Time: 4.5 min

Retention Time: BCS-CL73507 / BCS-CL73507 ISTD approx. 1.9 min
BCS-CQ63359 / BCS-CQ63359 ISTD approx. 2.5 min
BCS-CR60014 / BCS-CR60014 ISTD approx. 1.3 min
BCS-CR74541 / BCS-CR74541 ISTD approx. 1.6 min
BCS-CU81055 / BCS-CU81055 ISTD approx. 1.5 min
BCS-CT30673 / BCS-CT30673 ISTD approx. 2.1 min
BCS-CU81056 / BCS-CU81056 ISTD approx. 1.9 min

HPLC Gradient:

Time [min]	0.0	3.0	3.10	3.9	4.0	4.5
% A	80	30	5	5	80	80
% B	20	70	95	95	20	20

Flow Rate (μ L/min) 600 μ L/min

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Page 32 (155)**4.5.4 Detection**

The detection by MS/MS was performed on a triple-quadrupole tandem mass spectrometer, equipped with a Turbo IonSpray (ESI) interface operated in positive ion mode and multiple reaction monitoring (MRM). Unit mass resolution was established and maintained in the mass resolving quadrupoles by maintaining a full width at half-maximum (FWHM) of about 0.7 amu. Optimal collisionally-activated dissociation (CAD) conditions for fragmentation of the pseudomolecular ions of the analytes and the corresponding matrix matched standards were applied with nitrogen as the collision gas.

Detector: Triple Quadrupole Tandem Mass Spectrometer, Applied Biosystems MDS Sciex API 6500, Windows 7, Analyst 1.6.2 software versions or any equivalent HPLC-MS/MS System

Interface: Turbo IonSpray (ESI) Gas Temperature: 400°C or as needed for the sensitivity

Scan Type: MRM (Multiple Reaction Monitoring)

Table 9: MS/MS Parameters for the Determination of BCS-CL73507, BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056.

Analyte	Precursor Ion Q1 Mass (m/z)	Product Ion Q3 Mass (m/z)	Dwell Time	Collision Energy CE (eV)	Polarity
BCS-CL73507 Quantitation	545.1	356.1	10	19	Positive
BCS-CL73507 Confirmatory	545.1	376.0	10	37	Positive
BCS-CL73507 IS	550.1	356.0	10	19	Positive
BCS-CQ63359 Quantitation	527.1	389.1	10	29	Positive
BCS-CQ63359 Confirmatory	527.1	374.1	10	35	Positive
BCS-CQ63359 IS	532.1	394.1	10	29	Positive
BCS-CR60014 Quantitation	563.0	356.1	10	21	Positive
BCS-CR60014 Confirmatory	563.0	394.1	10	37	Positive
BCS-CR60014 IS	568.0	356.0	10	21	Positive
BCS-CR74541 Quantitation	564.0	356.0	10	21	Positive
BCS-CR74541 Confirmatory	564.0	395.0	10	37	Positive
BCS-CR74541 IS	569.0	356.0	10	21	Positive
BCS-CU81055 Quantitation	550.0	395.1	10	35	Positive
BCS-CU81055 Confirmatory	550.0	356.0	10	19	Positive
BCS-CU81055 IS	555.0	399.1	10	35	Positive
BCS-CT30673 Quantitation	546.1	408.1	10	31	Positive
BCS-CT30673 Confirmatory	546.1	267.1	10	69	Positive
BCS-CT30673 IS	551.1	413.1	10	31	Positive
BCS-CU81056 Quantitation	532.1	394.1	10	31	Positive
BCS-CU81056 Confirmatory	532.1	366.1	10	47	Positive
BCS-CU81056 IS	537.1	399.1	10	31	Positive

Note: Different MS/MS-instruments may result in different MRM transitions or signal intensity.

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4.6 Calculation

The example calculation displayed below was used by the laboratory developing this method. Alternate calculation procedures appropriate to the reporting requirements may be substituted.

4.6.1 Calculation of Individual Residues and Recovery Rates using Linear Regression

For calculation of the concentrations, calibration curves were used. These curves were calculated automatically after each sequence run with the Applied Biosystem quantitation software Analyst (Version 1.6.2) using linear regressions. Further calculations were performed using the software EXCEL 2010 (Office 2010®). The quadratic equation is expressed as:

$$y = \text{Intercept} + \text{Slope} \times x$$

By means of the linear equation, the content of trifloxystrobin in wet soil or recoveries can be calculated as follows:

Area Analyte / Area Internal Standard	y	
Standard Concentration / Internal Standard Concentration	x	[(µg/L) / (µg/L)]
Sample Weight	G	[kg]
Fortified Amount	A	[µg]
Extraction Volume	VEXT	[L]
Aliquote	VALI	[L]
Final Volume	VEND	[L]
Internal Standard Concentration	STI	[µg/L]
Residue in wet Soil (only for residue samples)	R	[µg/kg]
Recovery	Rec	[%]

$$R = \frac{y - \text{Intercept}}{\text{Slope}} \times \text{STI} \times \frac{\text{VEND} \times \text{VEXT}}{\text{VALI} \times G} \quad \text{Rec} = \frac{y - \text{Intercept}}{\text{Slope}} \times \text{STI} \times \frac{\text{VEND} \times \text{VEXT}}{\text{VALI} \times A} \times 100\%$$

Example for a calculation for a BCS-CL73507 recovery 5 µg/kg in soil Höfchen (Rec Höfchen 1):

Area Analyte / Area Internal Standard	y	11517 / 28048
Sample Weight	G	0.02 kg
Fortified Amount	A	0.04 µg
Extraction Volume	VEXT	0.04 L
Aliquote	VALI	0.0001 L
Final Volume	VEND	0.001 L
Internal Standard Concentration	STI	0.25 µg/L
Recovery	Rec	91%
Intercept		0.0509
Slope		0.99

$$\text{Rec} = \frac{\frac{11517}{28048} - 0.0509}{0.99} \times 0.25 \times \frac{0.001 \times 0.04}{0.0001 \times 0.04} \times 100\% = 91\%$$

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Page 39 (155)**5.4 Limit of Detection (LOD) (contd.)****Table 19: Calculated LOD for BCS-CL73507.**

Fortification Level [µg/kg]	Residue [µg/kg]			
	Soil Höfchen	Soil Laacher Hof	Soil Dollendorf	Sediment
UTC	0	0	0	0
(Untreated control)	0	0	0	0
Replicates	2	2	2	2
Mean	0	0	0	0
2	1.82	2.05	1.62	1.88
2	1.75	2.01	1.62	1.69
2	1.89	2.14	1.69	1.29
2	1.87	2.00	2.16	1.87
2	1.56	2.16	1.78	1.46
Replicates	5	5	5	5
Mean	1.778	2.072	1.774	1.638
Standard Deviation (n-1)	0.1333	0.0740	0.2256	0.2588
Student t-factor (n-1; t _{0.99})	3.747	3.747	3.747	3.747
Calculated LOD [µg/kg]	0.499	0.277	0.845	0.970

UTC= Untreated Control

Calculated LOD = (t_{0.99} × (Standard Deviation) + average residue in UTC).**Table 20: Calculated LOD for BCS-CQ63359.**

Fortification Level [µg/kg]	Residue [µg/kg]			
	Soil Höfchen	Soil Laacher Hof	Soil Dollendorf	Sediment
UTC	0	0	0	0
(Untreated control)	0	0	0	0
Replicates	2	2	2	2
Mean	0	0	0	0
2	1.83	1.83	1.71	1.50
2	1.52	1.51	1.52	1.36
2	1.92	1.55	1.79	1.35
2	1.86	1.85	1.28	1.33
2	1.47	1.90	1.46	1.29
Replicates	5	5	5	5
Mean	1.720	1.728	1.552	1.366
Standard Deviation (n-1)	0.2087	0.1831	0.2032	0.0796
Student t-factor (n-1; t _{0.99})	3.747	3.747	3.747	3.747
Calculated LOD [µg/kg]	0.782	0.686	0.761	0.298

UTC= Untreated Control

Calculated LOD = (t_{0.99} × (Standard Deviation) + average residue in UTC).

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Page 40 (155)**5.4 Limit of Detection (LOD) (contd.)****Table 21: Calculated LOD for BCS-CR60014.**

Fortification Level [µg/kg]	Residue [µg/kg]			
	Soil Höfchen	Soil Laacher Hof	Soil Dollendorf	Sediment
UTC (Untreated control)	0	0	0	0
	0	0	0	0
Replicates	2	2	2	2
Mean	0	0	0	0
2	2.02	1.70	1.87	1.80
2	1.73	1.81	2.02	1.75
2	2.22	1.99	1.78	2.00
2	1.83	1.90	2.33	2.00
2	2.11	1.51	2.11	1.85
Replicates	5	5	5	5
Mean	1.982	1.782	2.022	1.880
Standard Deviation (n-1)	0.2007	0.1862	0.2146	0.1151
Student t-factor (n-1; t _{0.99})	3.747	3.747	3.747	3.747
Calculated LOD [µg/kg]	0.752	0.698	0.804	0.431

UTC= Untreated Control

Calculated LOD = (t_{0.99} x (Standard Deviation) + average residue in UTC).**Table 22: Calculated LOD for BCS-CR74541.**

Fortification Level [µg/kg]	Residue [µg/kg]			
	Soil Höfchen	Soil Laacher Hof	Soil Dollendorf	Sediment
UTC (Untreated control)	0	0	0	0
	0	0	0	0
Replicates	2	2	2	2
Mean	0	0	0	0
2	1.99	2.21	2.37	1.55
2	2.02	2.18	1.74	2.02
2	2.12	2.00	1.96	2.00
2	1.97	1.68	2.06	1.62
2	1.66	1.93	1.82	2.00
Replicates	5	5	5	5
Mean	1.952	2.000	1.990	1.838
Standard Deviation (n-1)	0.1731	0.2144	0.2458	0.2324
Student t-factor (n-1; t _{0.99})	3.747	3.747	3.747	3.747
Calculated LOD [µg/kg]	0.649	0.803	0.921	0.871

UTC= Untreated Control

Calculated LOD = (t_{0.99} x (Standard Deviation) + average residue in UTC).

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Page 41 (155)**5.4 Limit of Detection (LOD) (contd.)****Table 23: Calculated LOD for BCS-CU81055.**

Fortification Level [µg/kg]	Residue [µg/kg]			
	Soil Höfchen	Soil Laacher Hof	Soil Dollendorf	Sediment
UTC (Untreated control)	0	0	0	0
	0	0	0	0
Replicates	2	2	2	2
Mean	0	0	0	0
2	1.83	1.54	2.09	1.52
2	2.06	1.77	2.03	1.85
2	2.19	1.92	1.92	2.06
2	1.99	2.01	2.30	1.48
2	2.18	1.95	1.73	1.41
Replicates	5	5	5	5
Mean	2.050	1.838	2.014	1.664
Standard Deviation (n-1)	0.1488	0.1886	0.2105	0.2786
Student t-factor (n-1; t _{0.99})	3.747	3.747	3.747	3.747
Calculated LOD [µg/kg]	0.558	0.707	0.789	1.044

UTC= Untreated Control

Calculated LOD = (t_{0.99} × (Standard Deviation) + average residue in UTC).**Table 24: Calculated LOD for BCS-CT30673.**

Fortification Level [µg/kg]	Residue [µg/kg]			
	Soil Höfchen	Soil Laacher Hof	Soil Dollendorf	Sediment
UTC (Untreated control)	0	0	0	0
	0	0	0	0
Replicates	2	2	2	2
Mean	0	0	0	0
2	1.99	2.04	1.75	1.89
2	1.67	1.45	1.53	1.62
2	1.65	1.94	1.57	1.78
2	2.11	1.61	2.17	1.80
2	1.44	1.60	1.68	1.19
Replicates	5	5	5	5
Mean	1.772	1.728	1.740	1.656
Standard Deviation (n-1)	0.2726	0.2499	0.2557	0.2781
Student t-factor (n-1; t _{0.99})	3.747	3.747	3.747	3.747
Calculated LOD [µg/kg]	1.021	0.937	0.958	1.042

UTC= Untreated Control

Calculated LOD = (t_{0.99} × (Standard Deviation) + average residue in UTC).

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5.8 Confirmatory Method (contd.)

For confirmation of the individual residues a 2nd mass transition was used. Results of the confirmation procedure showed that the overall mean recovery rates for BCS-CL73507, BCS-CR60014, BCS-CR74541, and BCS-CU81055 were between 70 to 110%. The overall RSD were below 20% for all soils tested. For BCS-CQ63359, BCS-CU81056, and BCS-CT30673 the sensitivity of the instrument for the fortification level at the LOQ of 2 µg/kg was not given. For the fortification level of 20 µg/kg the recovery rates were between 70 to 110%.

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Appendix 1: Method Characteristics

Table 56: Summary Parameters for the Analytical Method Used for the Quantitation of [Chemical] Residues in [Matrices]. (DER TABLE B.1.1).

Method ID	01373	
Analyte(s)	BCS-CL73507, BCS-CQ63359, BCS-CR60014, BCS-CR74541, BCS-CU81055, BCS-CT30673 and BCS-CU81056	
Extraction solvent/technique	acetonitrile/water/acetic acid (4000/1000/30, v/v/v) Shaker	
Cleanup strategies	Extraction followed by centrifugation	
Instrument/Detector/Column	Agilent 1290 LC AB Sciex API6500 LC- MS/MS YMC Ultra HT Hydrosphere C18, 2µm, 12 nm, 30 x 2.00 mm i.d.	
Standardization method	linear regression	
Retention time	BCS-CL73507 / BCS-CL73507 ISTD	approx. 1.9 min
	BCS-CQ63359 / BCS-CQ63359 ISTD	approx. 2.5 min
	BCS-CR60014 / BCS-CR60014 ISTD	approx. 1.3 min
	BCS-CR74541 / BCS-CR74541 ISTD	approx. 1.6 min
	BCS-CU81055 / BCS-CU81055 ISTD	approx. 1.5 min
	BCS-CT30673 / BCS-CT30673 ISTD	approx. 2.1 min
	BCS-CU81056 / BCS-CU81056 ISTD	approx. 1.9 min

Appendix 2: Apparatus and Reagents

Apparatus

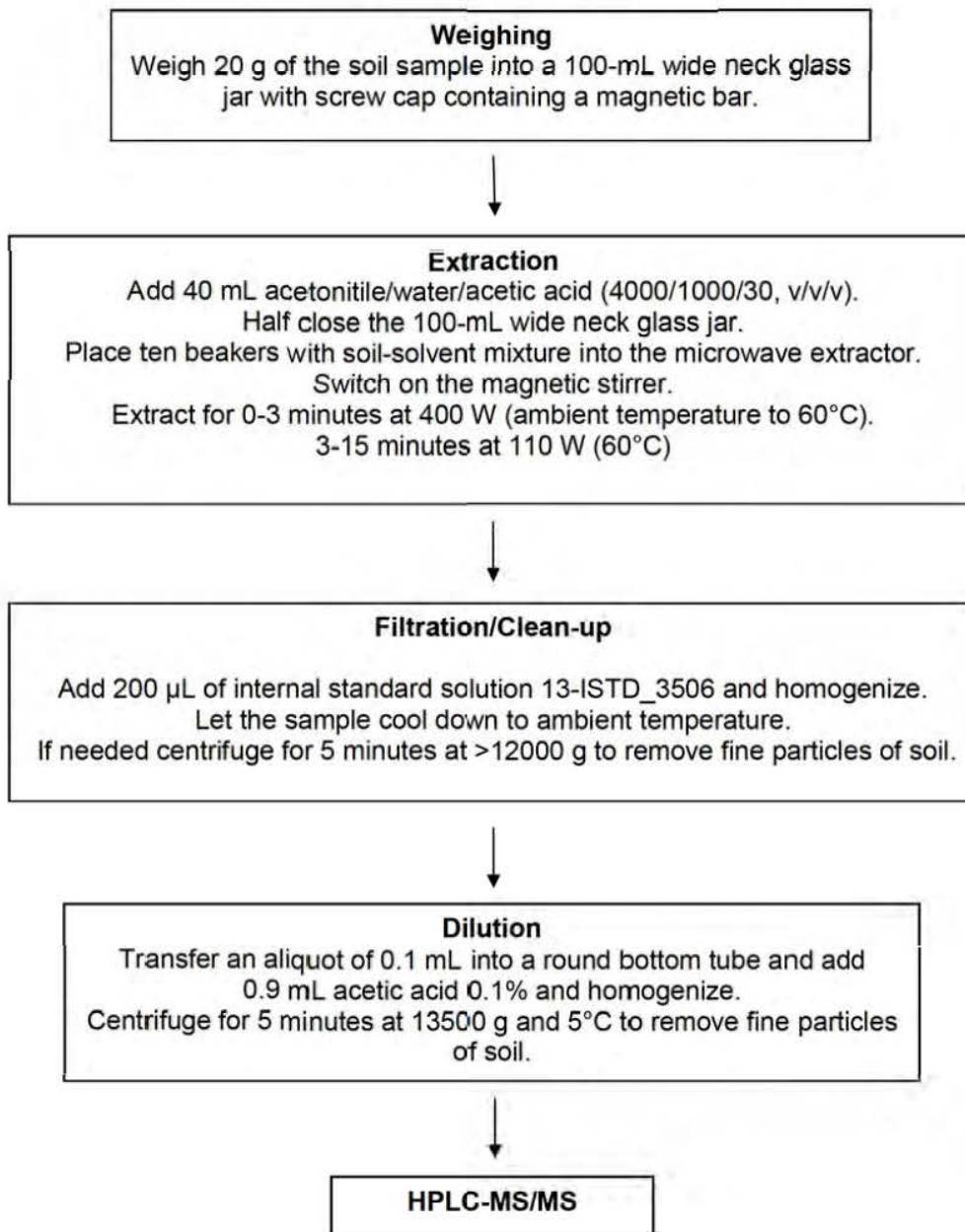
- Liquid chromatograph, Agilent 1290 Binary Pump, Agilent 1260 Isocratic Pump, Agilent Technologies, Böblingen, Germany or equivalent
- Hot Pocket Column Oven standard version, short, Thermofisher or equivalent
- Autosampler, HTC PAL, CTC Analytics, Switzerland or equivalent
- Mass spectrometer, API 6500 with ESI interface and mass spectrometric detector, Applied Biosystems, Darmstadt, Germany or equivalent
- Microwave, MLS-Ethos or equivalent
- Horizontal Shaker, SM 30, Edmund Bühler GmbH or equivalent
- Allegra X-15 Centrifuge or equivalent
- Fresco 21 Centrifuge or equivalent
- Reversed phase chromatography column, YMC Ultra HT Hydrosphere C18, 2 μ m, 12 nm, 30 x 2.00 mm i.d. or equivalent
- Volumetric flasks
- Variable dispenser, 50-mL
- Wide neck glass jars, 100-mL with caps
- Thermo Scientific Novous pipettes, 300 - 1000 μ L and 30 - 300 μ L
- Small instruments, e.g. Pasteur pipettes, autosampler vials, Uniprep tube

Reagents

- Methanol for Residue Analysis, LGC Standards GmbH, D-46485 Wesel, Germany or equivalent
- Acetonitrile for Residue Analysis, LGC Standards GmbH, D-46485 Wesel, Germany or equivalent
- Water, HPLC grade, purified with a Milli-Q-water system, Millipore Co., Eschborn, Germany or equivalent
- Formic acid, Suprapur, Merck KGaA, Darmstadt, Germany or equivalent
- Nitrogen 5.0, 99.9990% purity, as bath, nebulizer, collision, curtain, and turbo gas, Linde AG, Höllriegelskreuth, Germany or equivalent

Appendix 3:
Analytical Procedure of Method 01373

Extraction Procedure



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Page 63 (155)**Appendix 4:**
**Detailed Summary of Chromatographic and
Mass Spectrometric Conditions Quantifier**

Solvent A Binary Pump: Milli-Q-water
+ 1.0 mL/L formic acid
Solvent B Binary Pump: Acetonitril
+ 1.0 mL/L formic acid
Isocratic Pump: Methanol + Water (4+1; v+v)
Column: YMC-UltraHT Hydrosphere C18, 2 µm, 12nm, 30 x 2.00mm i.d.
Oven: HOT POCKET Column Oven standard version, short (Thermofisher)
Oventemp.: 40°C
Inject 10 µL into a 5 µL injection loop.

Comment:
Synchronization Mode: LC Sync
Auto-Equilibration: Off
Acquisition Duration: 4min30sec
Number Of Scans: 760
Periods In File: 1
Acquisition Module: Acquisition Method
Software version: Analyst 1.6.2

MS Method Properties:
Period 1:

Scans in Period: 760
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: Unit
Intensity Thres.: 0.00 cps
Settling Time: 0.0000 msec
MR Pause: 5.0000 msec
MCA: No
Step Size: 0.00 Da