Environmental Chemistry Method/Independent Laboratory Validation for CSCD465008 and CSAA798670 (Solatenol Degradates) in Soil (Method GRM023.05A)

Reports:

ECM: Hargreaves S (2009). SYN524464 – Analytical Method for the Determination of Residues of the Metabolites CSCD465008 and CSAA798670 in Soil – Final Determination by LC-MS/MS. Syngenta Ltd., Jealott's Hill International Research Centre, Bracknell, United Kingdom. Issued: November 2009. Unpublished Report No. GRM023.05A. (Syngenta Regulatory Document No. R958945_11258), EPA MRID No 47473343.

Mewis A (2010). SYN524464 – Validation of an Analytical Method for Determination of CSCD465008 and CSAA798670 in Soil – Validation Report Amendment 1.Eurofins-GAP GmbH, Pforzheim, Germany. Amendment Date: February 2010. Unpublished Report No. S09-00917 (Syngenta Regulatory Document No. SYN524464_11172), EPA MRID No 47473347.

ILV: Miska, J. (2012) SYN524464 - Independent Laboratory Validation of Residue Method (GRM023.05A) for the Determination of Metabolites CSCD465008 and CSAA798670 in Soil by LC-MS/MS: Final Report. Project Number: TK0160660/OCR, GRM023/05A. Unpublished study prepared by ADPEN Laboratories, Inc,), EPA MRID No 48604559.

Document No.: MRIDs 47473343, 47473347, & 48604559

Guideline: USEPA 850.6100
PMRA 8.2.2.1

Statements: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) Standards, 40 CFR Part 160 (pp. 3, 16). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3).

Classification: This analytical method is classified as **Supplemental**. The independent laboratory validation did not attempt to reproduce the initial method validation for CSCD465008 and CSAA798670 using the two soils textures evaluated, clay and loamy sand soils. Rather, the results were reproduced evaluating a different soil texture, sandy loam soil. Overall mean recoveries of CSCD465008 and CSAA798670 from the initial validation of the method were less than 90 percent but always greater than 77 percent in loamy sand soil for all cases. In the independent laboratory validation, overall mean recoveries of CSCD465008 and CSAA798670 were less than 90 percent but always greater than 75 percent in sandy loam soil for all cases. Furthermore, there are two reports provided for the method which appear to convey identical information (MRID Nos. 47447343 and 47473347).

PC Code: 122305

Reviewer:

Gabe Rothman
Environmental Scientist

Signature:

Date: August 29, 2013

Executive Summary

Soil matrices are extracted with 0.2% formic acid in ultra-pure water and the extracts centrifuged. Aliquots are acidified with hydrochloric acid to pH <2 and the samples are taken through a solid-phase extraction (SPE) procedure using Oasis TM HLB cartridges. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

I. Recovery Findings

Initial Validation of Method

Table 1. Initial Validation Method Recoveries for CSCD465008 in soil (primary transition *m/z* = 161/141).

Soil Matrix (type)	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
6S (clay)	CSCD465008	0.0005	5	97	8	88 – 108
		0.005	5	84	5	78 - 88
		Overall	10	91	10	78 - 108
2.2 (loamy sand)	CSCD465008	0.0005	5	81	3	79 - 84
		0.005	5	73	6	66 - 77
		Overall	10	77	7	66 - 84

Table 2. Initial Validation Method Recoveries for CSAA798670 in soil (primary transition, *m/z* 175/91).

Soil Matrix (type)	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
6S (clay)	CSAA798670	0.0005	5	92	6	88 - 101
		0.005	5	90	2	88 - 93
		Overall	10	91	4	88 - 101
2.2 (loamy sand)	CSAA798670	0.0005	5	84	5	79 - 90
		0.005	5	81	5	75 - 85
		Overall	10	83	5	75 - 90

Table 3. Initial Validation Method Recoveries for CSCD465008 in soil (primary transition *m/z* = 161/66).

Soil Matrix (type)	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
6S (clay)	CSCD465008	0.0005	5	96	10	81 - 103
		0.005	5	87	6	79 - 92
		Overall	10	91	10	79 - 103
2.2 (loamy sand)	CSCD465008	0.0005	5	84	4	80 - 88
		0.005	5	75	7	67 - 80
		Overall	10	80	8	67 - 88

Table 4. Initial Validation Method Recoveries for CSAA798670 in soil (primary transition *m/z* = 175/131).

Soil Matrix (type)	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
6S (clay)	CSAA798670	0.0005	5	94	7	85 - 101
		0.005	5	88	4	84 - 94
		Overall	10	91	6	84 - 101
2.2 (loamy sand)	CSAA798670	0.0005	5	82	5	77 - 87
		0.005	5	79	6	72 - 84
		Overall	10	81	5	72 - 87

Independent Validation of Method

Table 5. Independent Validation Method Recoveries for CSCD465008 in soil (primary transition, *m/z* = 161/148).

Soil Matrix (type)	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Sandy loam	CSCD465008	0.0005	5	77	3.8	73-81
		0.005	5	72	4.5	67-75
		Overall	10	75	5.4	67-81

Table 6. Indpendent Validation Method Recoveries for CSCD465008 in soil (primary transition *m/z* = 175/131).

Soil Matrix (type)	Analyte	Fortification (mg/kg)	Number of analyses (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Sandy loam	CSAA798670	0.0005	5	90	3.4	86-94
		0.005	5	78	2.2	76-80
		Overall	10	84	7.7	76-94

II. Method Characteristics

Extractability

SYN545720 (CSCD465008) has been shown to be efficiently extracted from soil under the conditions of method GRM023.05A in a radio-labelled soil metabolism study (Kuet & Oliver, 2007).

Specificity

LC-MS/MS as a detection technique with two transitions is considered to be highly specific and therefore according to the guidance (see guidance section of this summary) further confirmation is not required. No significant interferences arising from the soil matrices, the lab ware, reagents or solvents tested have been observed at the retention time of interest for any of the analytes.

Linearity

The linearity of the MS/MS detector responses was confirmed for both primary quantification and confirmatory transitions by generating calibration curves.
The linearity of the detector response was assessed by analysis of a minimum of 7 standard solutions covering the working range of at least 30 % of the LOQ to 50 times the LOQ concentrations (0.6 ng/mL – 100 ng/mL) in the final extracts. The correlation coefficients (r) of all calibration plots were found to be $\geq$ 0.999.

Accuracy

The mean CSCD465008 and CSAA798670 recoveries for both primary quantification and confirmatory transitions at each fortification level and overall for each soil type tested during method validation were between 73% – 97%. These values are all between 70% and 110% and therefore according to the guidance (see guidance section of this summary) these results demonstrate the method has satisfactory accuracy.
Precision
The relative standard deviations (RSDs) of CSCD465008 and CSAA798670 recoveries for both primary quantification and confirmatory transitions at each fortification level and overall for each soil type tested during method validation were between 2% – 10%. These values are all

below 20% and therefore according to the guidance (see guidance section of this summary) these results demonstrate the method has satisfactory repeatability.

Limit of Quantification

The limit of quantification (LOQ) of a method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and for which a mean recovery of 70% – 110% with a relative standard deviation (RSD) of ≤ 20% has been obtained.
The limit of quantification for CSCD465008 and CSAA798670 residues in soil using method GRM023.05A was established at 0.0005 mg/kg. Residues of all analytes measured in the control samples were always below 30% of the LOQ during method validation.

Matrix Effect

The effect of soil matrices on the LC-MS/MS response was assessed by preparing standards in the presence of matrix and comparing the peak areas of the analytes CSCD465008 and CSAA798670 against non-matrix standards at an equivalent concentration. Matrix effects were observed and ranged from 16% to 27% suppression for CSCD465008 and between 10% suppression to 1% enhancement for CSAA798670. Matrix matched standards were used for sample quantification.

Final Extract Stability

Analysis of CSCD465008 and CSAA798670 in final sample extracts after storing for a period of at least 13 days at a nominal temperature of 4□C, gave acceptable recoveries between 70% and 110%, demonstrating that CSCD465008 and CSAA798670 in soil extracts are stable on storage under these conditions.

Reproducibility

Method GRM023.05A is not intended for post registration monitoring and control purposes and therefore according to the guidance (see guidance section of this summary) an independent laboratory validation to demonstrate reproducibility is not required.

III. Method Deficiencies and Reviewer's Comments

1. The independent laboratory validation did not attempt to reproduce the initial method validation for CSCD465008 and CSAA798670 using the two soils textures evaluated, clay and loamy sand soils. Rather, the results were reproduced evaluating one different soil texture, sandy loam soil.

2. Overall mean recoveries of CSCD465008 and CSAA798670 from loamy sand soil was less than 90 percent in all cases, but always greater than 77 percent in the initial validation of the method.

3. In the independent laboratory validation, overall mean recoveries of CSCD465008 and CSAA798670 were less than 90 percent but always greater than 75 percent in sandy loam soil for all cases.

4. Furthermore, there are two reports provided for the method which appear to convey identical information (MRID Nos. 47447343 and 47473347).

IV. References

1. Luxon S G (1992): Hazards in the Chemical Laboratory 5th Edition. The RoyalSociety of Chemistry. Thomas Graham House, The Science Park, CambridgeCB4 4WF, UK.ISBN 0-85186-229-2.

2. Cardone M J, Palermo P J and Sybrand L B: Potential error in single point ratiocalculations based on linear calibration curves with a significant intercept. Anal. Chem., 52 pp 1187-1191, 1980.

3. Fitzmaurice M and Mackenzie E (2009). SYN524464 : [14C]-SYN524464 - Rate of Degradation in Three Soils at 20°C. Battelle UK Ltd. Report Number NC/07/015.4. Mewis A (2009): SYN524464 – Validation of a Method for the Determination of CSCD465008 and CSAA798670 in Soil. eurofins-GAB GmbH report number S09-00917.