Environmental Chemistry Method/Independent Laboratory Validation for Solatenol in Soil (Method GRM042.02A)

Reports: ***ECM:*** Braid S and Lin K (2011). SYN545192 – Analytical Method for the Determination of SYN545192 in Soil. Syngenta Ltd., Jealott's Hill International Research Centre, Bracknell, United Kingdom. Issued: March 2011. Unpublished Method No. GRM042.02A (Syngenta Regulatory Document No. SYN545192_10095), EPA MRID No 48604413.

Lin K (2010). SYN545192 – Validation of Residue Method GRM42.02A for the Determination of SYN545192 in Soil. Syngenta Crop Protection, Inc., Greensboro, NC, USA.. Study Dates: June 2010 to December 2010. Unpublished Report No. TK0002508 (Syngenta Regulatory Document No. SYN545192_50018), EPA MRID No 48604412.

ILV: Amic S (2012). SYN545192 – Independent Laboratory Validation of Residue Method GRM42.02A for the Determination of SYN545192 in Soil. Eurofins/ADME BIOANALYSES, Vergeze, France. Study Dates: December 2011 to February 2012. Unpublished Report No. S11-03840 (Syngenta Regulatory Document No. SYN545192_10194), EPA MRID No 48604557.

Document No.: MRIDs 48604413, 48604412, & 48604557

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. 2-4).

Classification: This analytical method is classified as **Supplemental**. The independent laboratory validation did not attempt to reproduce the initial method validation for SYN545192 using the two soils textures evaluated, clay loam and sandy loam soils. Rather, the results were reproduced evaluating a different soil texture, loamy sand soil. . Overall mean recoveries of SYN545192 from sandy loam soil and clay loam soil was less than 90 percent in all cases, but always greater than 84 percent in the initial and independent validations of the method. Furthermore, there are two reports provided for the method which appear to convey identical information (MRID Nos. 48604413 and 48604412).

PC Code: 122305

Reviewer:
Gabe Rothman
Environmental Scientist

Signature: Gabriel J Rothman

Date: August 29, 2013

Executive Summary

Sub-samples of soil are extracted by reflux in 80/20 v/v acetonitrile/ultra-pure water. After cooling to room temperature, extracts are decanted and centrifuged. Aliquots of the extract are diluted with ultra-pure water and purified using solid phase extraction. Samples at an equivalent concentration of 0.01 mg/kg are diluted 10 fold in acetone/ultra-pure water (50/50 v/v) for analysis by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). A primary transition m/z 396.0 → 368.0 and a confirmatory transition m/z 396.0 → 91.0 is used for quantification.

The validated limit of quantification is 0.001 mg/kg.

I. Recovery Findings

Initial Validation of Method

Table 1: Recoveries of SYN545192 from soils.

Transition	Fortification level (mg/kg)	Recovery (%)*	Number of analyses (n)	Mean recovery (%)	RSD (%)	Recovery range (%)
Clay loam						
m/z 396 →368	0.001	85, 89, 81, 88, 91	5	87	4	81-91
	0.01	89, 100, 88, 94, 81	5	90	8	81-100
	Overall		10	89	6	81-100
m/z 396 →91.0	0.001	88, 85, 79, 88, 91	5	86	5	79-91
	0.01	88, 95, 92, 95, 85	5	91	5	85-95
	Overall		10	89	6	79-95
Sandy loam						
m/z 396 →368	0.001	80, 80, 73, 80, 75	5	78	3	75-80
	0.01	93, 95, 94, 94, 81	5	91	6	81-95
	Overall		10	85	10	75-95
m/z 396 →91.0	0.001	82, 76, 77, 75, 80	5	78	4	75-82
	0.01	102, 98, 94, 95, 85	5	95	7	85-102
	Overall		10	86	12	75-102

*Residues in control samples and reagent blanks were less than 30% of the LOQ

Independent Validation of Method

Table 2. Recoveries of SYN545192 and metabolites from loamy sand soil.

Transition	Fortification level (mg/kg)	Recovery (%)	Number of analyses (n)	Mean recovery (%)	RSD (%)	Recovery range (%)
m/z 396.1 →368.0	0.001	77, 86, 94, 84, 93	5	87	8	77-94
	0.01	95, 94, 91, 96, 84	5	92	5	84-96
	Overall		10	89	7	77-96
m/z 396.1 →91.0	0.001	83, 85, 83, 82, 84	5	83	1	82-85
	0.01	86, 88, 87, 80, 84	5	85	4	80-88
	Overall		10	84	3	80-88

II. Method Characteristics

Specificity

LC-MS/MS is a highly specific detection technique and therefore a further confirmatory technique is not required. The method includes two MS/MS transitions, both of which have been validated.

No interferences were found higher than 30% of LOQ at or near the retention time of SYN545192 in control samples or reagent blanks.

Linearity

The response of the LC-MS/MS detector was shown to be linear ($r > 0.99$) for both primary and secondary transitions for SYN545192 over a concentration range equivalent to 1.0 pg to 50 pg of analyte injected on the column.

Accuracy

Clay loam: The overall SYN545192 mean recovery values for both the *m/z* 396→368 transition and the *m/z* 396 → 91 transition was 89% demonstrating acceptable accuracy of the method for both transitions.

Sandy loam: The overall SYN545192 mean recovery values for the *m/z* 396→368 transition was 85% and for the *m/z* 396 → 91 transition was 86% demonstrating acceptable accuracy of the method for both transitions.

Precision

Clay loam: The overall SYN545192 relative standard deviation for both the *m/z* 396→368 transition and the *m/z* 396 → 91 transition was 6%. The precision of the method is therefore acceptable for both transitions.

Sandy loam: The overall SYN545192 relative standard deviation for the *m/z* 396→368 transition was 10% and for the *m/z* 396 → 91 transition was 12%. The precision of the method is therefore acceptable for both transitions.

Limit of Quantification

The validated limit of quantification of the method for both soil types was 0.001 mg/kg quantified on both the *m/z* 396→91 m/z transition and the *m/z* 396 → 368 transition.

Matrix effects

Matrix effects were assessed for each matrix type and are shown in Table 3. They are all less than < 20% enhancement or suppression.

Table 3. LC-MS/MS Matrix Effects.

Analyte/Transition	Matrix Effect*	
	Clay loam	Sandy loam
SYN545192 *m/z* = 396→368	-11%	-15%
SYN545192 *m/z* = 396→91	-10%	-25%

*Matrix effect = (peak area standard in matrix – peak area standard in solvent) x 100%/(peak area standard in solvent)

Stability of SYN545192 in sample extracts and sample final solutions

Sample extracts at 0.001 mg/kg fortification were injected, then stored refrigerated at 4°C for 7 days and re-injected. The comparison between the zero and seven day analyses is shown in Table 4. Residues of SYN545192 were found to be stable in sample extracts from both soil types of at least 7 days when stored at 4°C.

Table 4: Stability of SYN545192 in sample extracts before and after 7 days storage at 4°C (determined using primary transition).

Soil type	Fortification (mg/kg)	Storage time (days)	Recoveries (%)	n	Mean recovery (%)	RSD (%)
Clay loam	0.001	0	85, 89, 81, 88, 91	5	87	4
Clay loam	0.001	7	90, 90, 82, 84, 86	5	86	4
Sandy loam	0.001	0	80, 80, 73, 80, 75	5	78	4
Sandy loam	0.001	7	76, 76, 73, 78, 70	5	75	4

Sample final solutions from extracts of both soil types fortified at 0.001 mg/kg were injected, stored at 4°C and re-injected after 16 days (sandy loam) or 19 days (clay loam). The comparison data is shown in Table 5. Residues of SYN545192 were found to be stable in final solutions for at least 16 days when stored at 4°C.

Table 5: Stability of SYN545192 in final extracts before and after 16 or 19 days storage at 4°C (determined using primary transition).

Soil type	Fortification (mg/kg)	Storage time (days)	Recoveries (%)	n	Mean recovery (%)	RSD (%)
Clay loam	0.001	0	90, 90, 82, 84, 86	5	86	4
Clay loam	0.001	7	82, 87, 80, 96, 89	5	87	7
Sandy loam	0.001	0	76, 76, 73, 78, 70	5	75	4
Sandy loam	0.001	7	76, 77, 72, 77, 84	5	77	6

III. Method Deficiencies and Reviewer's Comments

1. The independent laboratory validation did not attempt to reproduce the initial method validation for SYN545192 using the two soils textures evaluated, clay loam and sandy loam soils. Rather, the results were reproduced evaluating a different soil texture, loamy sand soil.

2. Overall mean recoveries of SYN545192 from sandy loam soil and clay loam soil was less than 90 percent in all cases in the initial and independent validations, but always greater than 84 percent in the initial and independent validations of the method.

3. There are two reports provided for the method which appear to convey identical information (MRID Nos. 48604413 and 48604412).

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. Lowrie, C. And Phillips, M. (2011). Rate and route of degradation of [14C]-phenyl labelled SYN545192 under aerobic conditions in one soil at 20°C. Charles River, Tranent, Edinburgh, EH33 2NE. Report number 31530.

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

4. Lin K (2010): SYN545192 - Validation of Residue Method GRM042.02A for the Determination of SYN545192 in Soil. Syngenta Report No. TK0002508