Analytical method for R182281, a metabolite of chlorothalonil, in air from air sampling tubes

Reports: ECM: EPA MRID No.: 50826505. Mayer, L.C. 2016. R182281. R182281 - Method GRM005.17A for the Determination of R182281 in OVS Silica Gel Air Sampling Tubes By LC-MS/MS. Analytical Method. Report No.: GRM005.17A. Task No.: TK0290289. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC., Greensboro, North Carolina; 41 pages. Final report issued May 19, 2016.

ILV: EPA MRID No. 50826506. Xu, A. 2016. Chlorothalonil. Independent Laboratory Validation of Analytical Method (GRM005.17A) for the Determination of R182281 in OVS Silica Gel Air Sampling Tubes by LC-MS/MS. ILV Final Report. Report No.: PASC-REP-0762. PASC Project No.: 141-1339. Task No.: TK0290290. Report prepared by Primera Analytical Solutions Corp., Princeton, New Jersey, sponsored and submitted by Syngenta Crop Protection, LLC., Greensboro, North Carolina; 100 pages. Final report issued April 5, 2016.

Document No.: MRIDs 50826505 & 50826506

Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (p. 3 of MRID 50826505). Signed and dated GLP statement was provided (p. 3). A No Data Confidentiality report page was left intentionally blank (p. 2). A certification of authenticity and Quality Assurance statement were not included. A signed summary of revisions to previous method version was included (p. 4).

ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50826506). Signed and dated GLP and Quality Assurance statements were provided (pp. 3-4). A No Data Confidentiality report page was left intentionally blank (p. 2). An authenticity statement was not included.

Classification: This analytical method is classified as Supplemental. It could not be determined if the ILV was performed independently from the ECM since the ILV study author and ECM study author communicated directly via email. In the ECM, an inadequate number of samples was prepared for both fortifications.

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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

This analytical method, Syngenta Method GRM005.17A, is designed for the quantitative determination of R182281, a metabolite of chlorothalonil, in air from air sampling tubes at the LOQ of 0.01 µg/air sample tube using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in air from air sampling tube. The ECM and ILV test matrices were OVS silica gel sorbent (520/260 mg) air sampling tube. The ILV validated the method in the first trial as written with insignificant analytical instrument modifications. **It could not be determined if the ILV was performed independently from the ECM since the ILV study author and ECM study author communicated directly with one another via email; however, the emails were not provided for review.** R182281 was identified via LC/MS/MS using two ion transitions. All ILV and ECM data regarding repeatability, accuracy, and precision were satisfactory for R182281; however, an inadequate number of samples (n = 3) was prepared for both fortifications in the ECM. No LOD was reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
R182281[1]	50826505	50826506		Air from air sampling tube[2]	19/05/2016	Syngenta Crop Protection, LLC	LC/MS/MS	0.01 µg/air sample tube

1 2,4,5-Trichloro-6-hydroxy-isophthalonitrile (metabolite of chlorothalonil; p. 11 of MRID 50826506).

2 OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99) used in ECM and ILV (p. 10 of MRID 50826505; Appendix 1, p. 49 of MRID 50826506).

I. Principle of the Method

Air samples were collected from OVS silica gel sorbent (520/260 mg) air sampling tubes (SKC Inc. Cat No. 226-99; pp. 10-14; Appendix 4, p. 41 of MRID 50826505). After the sample tubes were fortified, if necessary, with the appropriate amount of R182281 in methanol (10, 2.0 or 0.20 µg/mL fortification solutions, each sample stood for at least five minutes before extraction. For extraction, remove all contents of the tube into a 50 mL polypropylene tube; the glass tube is not included in the extraction. Extract sample using 10 mL of acidified acetone (1% formic acid) via sonication for 10 minutes. An aliquot (0.5 mL) of the extract was transferred to an LC autosampler vial with 0.1 mL ultra-pure water (as a keeper), and the solvent was evaporated to near dryness. The method noted that loss of analyte would occur if the sample was evaporated to complete dryness. The residue was reconstituted with 1.0 mL of ultra-pure water:acetonitrile (50:50, v:v). After vortexing, the sample was analyzed by LC/MS/MS directly. The method noted that samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

Samples were analyzed for R182281 using an Aquity UPLC coupled with a Sciex API 4000 MS (pp. 14-15 of MRID 50826505). The following conditions were used: ACE 3 C18 column (50 x 3 mm, 3 µ; column temperature 30°C), gradient mobile phase of A) 0.1% formic acid in Optima water and B) 0.1% formic acid in Optima acetonitrile [percent A:B; 0.0-0.5 min. 70:30, 2.5-3.5 min. 10:90, 4.0-5.0 min. 70:30], and negative polarity ESI mode (source temperature 650°C) with MRM. Injection volume was 10 µL. R182281 was identified with the following ion transitions (primary and confirmatory, respectively): *m/z* 244.8→181.8 and *m/z* 244.8→174.8. Retention time was 1.3 minutes for R182281.

In the ILV, the ECM was performed as written with insignificant analytical instrument modifications, also using the OVS silica gel sorbent (520/260 mg) air sampling tubes (SKC Inc. Cat No. 226-99; pp. 10-16; Appendix 1, p. 49 of MRID 50826506). Samples were analyzed for R182281 using a Shimadzu HPLC coupled with an Applied Biosystems API 4000 Triple Quadrupole MS; all parameters were the same as those of the ECM. Retention time was 1.5 minutes for R182281. The same MRM ion transitions were monitored. No other modifications of the ECM were reported.

The Limit of Quantification (LOQ) for R182281 was reported as 0.01 µg/air sample tube in the ECM and the ILV (pp. 10, 18-19 of MRID 50826505; pp. 10, 17 of MRID 50826506). The Limit of Detection (LOD) for R182281 was 0.20 pg/µL, equivalent to 2 pg injected on column when using a 10 µL injection volume by LC/MS/MS in the ECM. No LOD was reported in the ILV.

II. Recovery Findings

ECM (MRID 50826505): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of R182281 from air sampling tubes at fortification levels of 0.01 µg/tube (LOQ) and 0.10 µg/tube (10×LOQ); however, an inadequate number of samples was prepared for both fortifications (n = 3; Tables 1-2, p. 22). R182281 was identified with two ion transitions using LC/MS/MS; recovery results of the primary and confirmatory analyses were comparable, except that the LOQ recovery results had better reproducibility in the primary ion transition. The matrix was OVS silica gel sorbent (140/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; p. 10).

ILV (MRID 50826506): Mean recoveries and RSDs were within guideline requirements for analysis of R182281 from air sampling tubes at fortification levels of 0.01 µg/tube (LOQ) and 0.10 µg/tube (10×LOQ; Table 2, p. 23). R182281 was identified with two ion transitions using LC/MS/MS; recovery results of the primary and confirmatory analyses were comparable. The matrix was OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; Appendix 1, p. 49). The method was validated in the first trial as written with insignificant analytical instrument modifications (pp. 14-17).

Table 2. Initial Validation Method Recoveries for R182281, a Metabolite of Chlorothalonil, in Air[1,2]

Analyte	Fortification Level (µg/tube)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	OVS Silica Gel Sorbent					
	Primary Ion Transition					
R182281	0.01 (LOQ)	3	102-107	104	--	2.7
	0.10	3	99-115	107	--	7.5
	Confirmation Ion Transition					
R182281	0.01 (LOQ)	3	90-112	101	--	10.9
	0.10	3	104-114	110	--	5.0

Data (recovery results were corrected for residues quantified in the controls, pp. 16-17) were obtained from Tables 1-2, p. 22 of MRID 50826505.

1 The matrix was OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; p. 10).

2 R182281 was identified with the following ion transitions (primary and confirmatory, respectively): *m/z* 244.8→181.8 and *m/z* 244.8→174.8.

3 Standard deviations were not reported since the study author did not report these values and raw data was not provided in the study report.

Table 3. Independent Validation Method Recoveries for R182281, a Metabolite of Chlorothalonil, in Air[1,2]

Analyte	Fortification Level (µg/tube)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	OVS Silica Gel Sorbent					
	Primary Ion Transition					
R182281	0.01 (LOQ)	5	68.8-98.6	80.4	11	14
	0.10	5	76.2-94.4	86.0	6.8	7.9
	Confirmation Ion Transition					
R182281	0.01 (LOQ)	5	68.2-91.6	78.5	9.4	12
	0.10	5	76.4-103	84.4	11	13

Data (uncorrected recovery results, Appendix 3, pp. 99-100) were obtained from Table 2, p. 23 of MRID 50826506.

1 The matrix was OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; Appendix 1, p. 49). This was the same matrix as the ECM.

2 R182281 was identified with the following ion transitions (primary and confirmatory, respectively): *m/z* 244.8→181.8 and *m/z* 244.8→174.8.

III. Method Characteristics

The LOQ for R182281 was reported as 0.01 µg/air sample tube in the ECM and the ILV (pp. 10, 18-19 of MRID 50826505; pp. 10, 17 of MRID 50826506). In the ECM, the LOQ was defined as the lowest analyte concentration which was demonstrated to have acceptable mean recovery (70 to 120%) and precision (relative standard deviation of ≤20%). The ECM also stated that the response of the LOQ analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No justification for LOQ was reported in the ILV. The LOD for R182281 in the ECM was 0.20 pg/µL, equivalent to 2 pg injected on column when using a 10 µL injection volume by LC/MS/MS in the ECM. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The ECM also stated that an estimate of the LOD can be taken as three times background noise. No LOD was reported in the ILV.

Table 4. Method Characteristics

Analyte		**R182281**[1]	
Matrix		**OVS Silica Gel Sorbent**	
		Primary Ion Transition	Confirmatory Ion Transition
Limit of Quantitation (LOQ)	ECM	0.01 µg/air sample tube	
	ILV		
Limit of Detection (LOD)	ECM	0.20 pg/µL (2 pg injected on column when using a 10 µL injection volume)	
	ILV	**Not reported**	
Linearity (calibration curve r^2 and concentration range)[2]	ECM	$r^2 = 0.9996$	$r^2 = 0.9998$
	ILV	$r^2 = 0.9966$	$r^2 = 0.9976$
	Range	(0.2 pg/µL to 10 pg/µL, equivalent to 2 pg to 100 pg on column)	
Repeatable	ECM[3]	Yes at LOQ and 10×LOQ, but n = 3.	
	ILV[4,5]	Yes at LOQ and 10×LOQ.	
Reproducible		Yes at LOQ and 10×LOQ.	
Specific	ECM	Yes, matrix interferences were <5% of the LOQ (based on peak height estimation). Some minor baseline interference was observed.	
	ILV	Yes, matrix interferences were <2% of the LOQ (based on peak area). Some minor peak tailing was observed.	

Data were obtained from pp. 10, 18-19 (LOQ/LOD); Tables 1-2, p. 22 (recovery data); Figures 2-21, pp. 27-36 (chromatograms); Figure 7, p. 29 and Figure 17, p. 34 (calibration curve) of MRID 50826505; pp. 10, 17 (LOQ/LOD); Table 2, p. 23 (recovery data); Figures 2-39, pp. 26-45 (chromatograms); Figure 7, p. 28 and Figure 26, p. 38 (calibration curve) of MRID 50826506; and DER Attachment 2.

[1] Metabolite of chlorothalonil. 2,4,5-Trichloro-6-hydroxy-isophthalonitrile. R182281 was identified using LC/MS/MS with the following ion transitions (primary and confirmatory, respectively): *m/z* 244.8→181.8 and *m/z* 244.8→174.8.

[2] For the ECM and ILV, the correlation coefficients were reviewer-calculated from r values provided in the study report (Figure 7, p. 29 and Figure 17, p. 34 of MRID 50826505; Figure 7, p. 28 and Figure 26, p. 38 of MRID 50826506; DER Attachment 2).

3 The ECM matrix was OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; p. 10 of MRID 50826505).

4 The ILV matrix was OVS silica gel sorbent (520/260 mg) air sampling tube (SKC Inc. Cat No. 226-99; Appendix 1, p. 49 of MRID 50826506). This was the same as that used in the ECM.

5 The ILV validated the method in the first trial as written with insignificant analytical instrument modifications (pp. 14-17 of MRID 50826506).

IV. Method Deficiencies and Reviewer's Comments

1. It could not be determined if the ILV was performed independently from the ECM since the ILV Study Monitor was the ECM study author (Louis Mayer; p. 1 of MRID 50826505; p. 5 of MRID 50826506). The ILV study author and ECM study author communicated directly with one another via email; however, the emails were not provided for review (included in raw data; p. 19 of MRID 50826506). Communications between the ILV study author and ECM study author were summarized as 1) clarification/approval of the protocol and method and 2) acquisition of analytical standard and control sample. OCSPP guidelines state that the analysts, study director, equipment, instruments, and supplies of the two laboratories must be distinct and operated separately and without collusion.

2. In the ECM, an inadequate number of samples (n = 3) was prepared for both fortifications (Tables 1-2, p. 22 of MRID 50826505). A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix.

3. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10, 18-19 of MRID 50826505; pp. 10, 17 of MRID 50826506). In the ECM, the LOQ was defined as the lowest analyte concentration which was demonstrated to have acceptable mean recovery (70 to 120%) and precision (relative standard deviation of ≤20%). The ECM also stated that the response of the LOQ analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No justification for LOQ was reported in the ILV. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The ECM also stated that an estimate of the LOD can be taken as three times background noise. No LOD was reported in the ILV. Detection limits should not be based on arbitrary values.

4. In the ECM, no significant matrix effects have been observed for the air sample sorbent type tested (p. 19; Table 5, p. 24 of MRID 50826505). Non-matrix-matched standards were recommended, unless effects were observed.

5. In the ECM, the final R182281 extracts in ultra-pure water:acetonitrile (50:50, v:v) were determined to stable up to 7 days of storage at 4°C (p. 19; Table 6, p. 24 of MRID 50826505).

6. It was reported for the ILV that one batch of 13 samples required one working day with LC/MS/MS analysis performed overnight (p. 19 of MRID 50826506).

7. The reviewer noted that the ECM data tables reported the fortification level as “mg/kg” (Tables 1-4, pp. 22-23 of MRID 50826505). The reviewer did not use this labeling in the DER since it did not seem appropriate. Additionally, the reviewer noted that the ECM reported the ILV recovery data in Tables 3-4.

V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

R182281 (SDS3701; CSCA105253)

IUPAC Name:	2,4,5-Trichloro-6-hydroxy-benzene-1,3-dicarbonitrile 2,4,5-Trichloro-6-hydroxy-isophthalonitrile
CAS Name:	Not reported
CAS Number:	28343-61-5
SMILES String:	C(#N)c1c(c(c(c(c1Cl)C#N)Cl)Cl)O