Analytical method for mandestrobin (S-2200) in water

Reports: ECM: EPA MRID No.: 49068639. Wilde, N. 2011. Validation of an Analytical Method for the Determination of S-2200 in Surface Water for Post-Registration Control and Monitoring Purpose. PTRL Europe Project ID: P 2376 G. Report prepared by PTRL Europe, Ulm, Germany, sponsored by Sumitomo Chemical Co., Ltd., Tokyo, Japan, and submitted by Valent U.S.A. Corporation, Walnut Creek, California; 24 pages (pp. 3, 7). Final report issued October 19, 2011.

ILV: EPA MRID No. 49068637. Golich-Moore, R. 2013. S-2200: Independent Laboratory Validation of the Analytical Method Validation of an Analytical Method for the Determination of S-2200 in Surface Water for Post Registration Control and Monitoring Purposes. North Coast Labs Study No.: 120.026 (p. 3). Valent Study No.: VP-38491. Report prepared by North Coast Laboratories, Ltd., Arcata, California, sponsored and submitted by Valent U.S.A. Corporation, Walnut Creek, California; 61 pages, including page 1i. Final report issued October 1, 2013.

PC Code: 036603
PMRA: PMRA #s 2377854 & 2377857
MRIDs: 49068639 & 49068637
Guideline: 850.6100, European Commission SANCO/825/00 rev. 8.1 (p. 5 of MRID 49068639)

Statements: ECM: The study was conducted in compliance with the German Principles of Good Laboratory Practice (GLP) standards (p. 3). Signed and dated No Data Confidentiality, GLP, and Quality Assurance, statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with a Compliance to Guidelines statement (p. 5).
ILV: The study was conducted in compliance with USEPA GLP standards (p. 2). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 1i-3). An Authenticity Certification statement was not provided.

Reviewer: Richard Shamblen
U.S. EPA
Signature: Richard Shamblen
Date: February 20, 2015

Kim Davis
PMRA
Signature: 2181
Date: March 31, 2015

Study Classification

This analytical method is classified as **Acceptable** (USEPA)/ **Acceptable** (PMRA). The registrant failed to establish that the water matrix used in the ILV (bottled spring water) was either an equivalent, or more difficult, analytical sample condition as that used in the ECM (lake water). The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. LODs differed in the ECM and ILV. The ECM LC/MS/MS procedures should be amended to identify mandestrobin as the target analyte. The water used in the ILV was not characterized.

Executive Summary

This analytical method, PTRL Europe ID: P 2376 G, is designed for the quantitative determination of mandestrobin in water using HPLC/MS/MS. The method is quantitative for mandestrobin at the stated LOQ of 0.10 µg/L. The LOQ is less than the lowest toxicological level of concern in water (NOAEC = 49 µg/L). The independent laboratory validated the method after one trial. LODs were set at 0.02 µg/L in the ECM and 0.05 µg/L in the ILV. No major modifications were made by the independent laboratory; however, the ECM LC/MS/MS procedures should be amended to identify mandestrobin as the target analyte. The water used in the ILV was not characterized, and the registrant failed to establish that the water matrix used in the ILV (bottled spring water) was either an equivalent, or more difficult, analytical sample condition as that used in the ECM (lake water).

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						
Mandestrobin (S-2200)	49068639	49068637		Water	19/10/2011	Valent	HPLC/MS/MS	0.10 µg/L

I. Principle of the Method

Samples (1.0 mL) of water were analyzed directly for mandestrobin (S-2200) by HPLC (Phenomenex Luna C_{18}, 2.0 mm x 50 mm, 5 µm column, 40°C; Phenomenex C_{18}, 3.0 mm x 4 mm pre-column) using a mobile phase of (A) aqueous 0.1% formic acid and (B) 0.1% formic acid in acetonitrile [percent A:B at 0.0-0.2 min. 90:10, 0.7-3.8 min. 5:95, 4.0-6.0 min. 90:10] with MS/MS-ESI (electrospray ionization, positive ion mode) detection and multiple reaction monitoring (MRM; pp. 11-12; Figure 5, p. 22 of MRID 49068639). Injection volume was 20 µL. Mandestrobin was identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 314→192 (Q) and *m/z* 314→160 (C). In section **2.4 LC-MS/MS Analysis** (p. 12), the identification of MS/MS Conditions as "for chlorpyrifos-methyl" appears to be a typographical error.

The ILV was performed using an ACE 5 C18 (3 mm x 50 mm) column with Phenomenex C-12 (4 mm x 20 mm) guard column, and/or a Zorbax XDB-C18 (4.6 mm x 150 mm, 5 µm) column; there was conflicting information as to which column was used (pp. 12, 14; Appendix 3, p. 54 of MRID 49068637). Additionally, the mobile phase gradient was adjusted [percent A:B at 0.0 min. 90:10, 2.4-4.5 min. 5:95, 4.6-7.0 min. 90:10; p. 15; Appendix 3, p. 56 of MRID 49068637]. The modifications are not considered substantial changes to the ECM.

The LOQ for mandestrobin was the same in the ECM and ILV at 0.1 µg/L (p. 9 of MRID 49068639; p. 13 of MRID 49068637). The LOD was set at 0.02 µg/L (20% of LOQ) in the ECM and 0.05 µg/L (50% of LOQ) in the ILV (p. 14 of MRID 49068639; p. 13 of MRID 49068637).

II. Recovery Findings

ECM (MRID 49068639): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of mandestrobin in surface (lake) water at fortification levels of 0.10 µg/L (LOQ) and 1.0 µg/L (10x LOQ, p. 8). Mandestrobin was identified and quantified using two ion transitions. The water matrix was characterized (p. 10).

ILV (MRID 49068637): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of mandestrobin in bottled spring water at fortification levels of 0.10 µg/L (LOQ) and 1.0 µg/L (10x LOQ; Table 1, pp. 23-24). Mandestrobin was identified and quantified using two ion transitions; however, performance data from the confirmation (qualifier) ion transition were not reported (p. 15). The method was validated for the quantitation ion transition at both fortification levels using bottled spring water after one trial, with minor method and instrument parameter modifications (pp. 10, 14-15, 17). The water matrix was not characterized, and the registrant did not establish that the bottled spring water was either an equivalent, or more difficult, analytical sample condition as that used in the ECM (lake water).

Table 2. Initial Validation Method Recoveries for Mandestrobin in Surface (Lake) Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Mandestrobin (S-2200)	Quantitation ion transition *m/z* 314→192					
	0.10 (LOQ)	5	89-102	96	6	6
	1.0	5	101-107	104	2	2
	Confirmation ion transition *m/z* 314→160					
	0.10 (LOQ)	5	91-101	97	4	4
	1.0	5	102-107	104	2	2

Data (uncorrected recovery results, Average, and Relative Standard Deviation) were obtained from Table 1, p. 17 of MRID 49068639; standard deviations were reviewer-calculated (DER Attachment 2).

[1] Surface water was obtained from Waldsee (forest lake) in Senden (Southern Germany); matrix characterization was provided (p. 10 of MRID 49068639).

Table 3. Independent Validation Method Recoveries for Mandestrobin in Bottled Spring Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Mandestrobin (S-2200)	Quantitation ion transition *m/z* 314→192					
	0.10 (LOQ)	5	95.7-99.2	96.9	1.6	1.6
	1.0	5	101-106	102	1.8	1.7
	Confirmation (Qualifier) ion transition *m/z* 314→160					
	0.10 (LOQ)	5	Data were collected, but not reported.			
	1.0	5				

Data were obtained from p. 15; Table 1, pp. 23-24 of MRID 49068637.

[1] South Fork Mountain Spring Water (bottled water); matrix characterization was not provided (p. 12 of MRID 49068637).

III. Method Characteristics

In the ECM and ILV, the LOQ for mandestrobin in water was 0.10 µg/L (p. 9 of MRID 49068639; p. 13 of MRID 49068637). No justification for selection of the LOQ concentration was provided. The LOD was set at 0.02 µg/L (20% of LOQ) in the ECM and 0.05 µg/L (50% of LOQ) in the ILV (p. 14 of MRID 49068639; p. 13 of MRID 49068637). In both the ECM and ILV, the LOD appeared to be defined by the lowest standard of the calibration curve used; 0.0175 ng/L (equivalent to 0.02 µg/L) in the ECM, and 0.05 µg/L in the ILV (Table 1, p. 17; Figure 1, p. 18 of MRID 49068639; pp. 13-14 of MRID 49068637).

Table 4. Method Characteristics[1]

	Mandestrobin
Limit of Quantitation (LOQ)	0.10 µg/L
Limit of Detection (LOD)	0.05 µg/L[2]
Linearity (calibration curve r^2 and concentration range)[3]	$r^2 = 0.9979$ (0.05-2.0 µg/L)
Repeatable	Yes
Reproducible	Yes
Specific[4]	Yes

Data were obtained from pp. 10, 13, 16; Figures 8-9, pp. 34-35 of MRID 49068637 and DER Attachment 2 (r^2).

[1] The ILV was performed using bottled spring water, as approved by Valent (pp. 12 ,17 of MRID 49068637). Whereas, the ECM was performed using surface (lake) water (p. 10 of MRID 49068639). The bottled spring water was not characterized. In addition, the registrant did not establish that the bottled spring water was either an equivalent, or more difficult, analytical sample condition as that used in the ECM.

[2] LOD was set at 0.02 µg/L in ECM, based on the lowest calibration standard (Table 1, p. 17; Figure 1, p. 18 of MRID 49068639).

[3] Reviewer-calculated r^2 value (quantitation ion transition) for the provided ILV calibration standards (Figures 2-7, pp. 28-33 of MRID 49068637; DER Attachment 2). The ILV reported an r value (1/x weighting) of 0.9986 (Figure 1, p. 27). Linearity of ECM calibration curves were verified by the reviewer; $r^2 = 0.9992$ (n = 2, DER Attachment 2). ECM reported r values were 0.9996 (n = 2; Figure 1, p. 18 of MRID 49068639).

[4] Although performance data from the confirmation method (ion transition *m/z* 314→160) were not reported, OCSPP 850.6100 guidelines specify that a confirmatory procedure is not typically necessary where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

IV. Method Deficiencies and Reviewer's Comments

1. The registrant failed to establish that the water matrix used in the ILV was either an equivalent, or more difficult, analytical sample condition as that used in the ECM. The ILV was performed using purchased, bottled spring water, as approved by Valent; matrix characterization was not provided (pp. 10, 12 ,17 of MRID 49068637). The ECM was performed using surface (lake) water; matrix characterization was provided (p. 10 of MRID 49068639). For a given sample matrix, the registrant should select the most difficult analytical sample condition for use in the ILV to demonstrate how well the method performs.

2. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. In the ECM and ILV, the LOQ for mandestrobin in water was reported as 0.10 µg/L (p. 9 of MRID 49068639; p. 13 of MRID 49068637). No justification for selection of the LOQ concentration was provided. The LOD was set at 0.02 µg/L (20% of LOQ) in the ECM and 0.05 µg/L (50% of LOQ) in the ILV (p. 14 of MRID 49068639; p. 13 of MRID 49068637). In both the ECM and ILV, no justification for selection of the LOD was specified; however, the LOD appeared to be defined by the lowest standard of the calibration curve used (0.0175 ng/mL, equivalent to 0.02 µg/L, in the ECM, and 0.05 µg/L in the ILV; Table 1, p. 17; Figure 1, p. 18 of MRID 49068639; pp. 13-14 of MRID 49068637).

   Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

3. An error in the ECM LC/MS/MS procedures should be amended/corrected to identify mandestrobin as the target analyte. In section 2.4 LC-MS/MS Analysis (p. 12 of MRID 49068639), the ECM identifies the MS/MS Conditions as "for chlorpyrifos-methyl", rather than mandestrobin (S-2200). The error was also not identified/addressed by the independent laboratory (Appendix 2, pp. 52-53 of MRID 49068637).

4. For the ILV, there was conflicting information in the study report as to the LC column used for analysis. The main body of the study report specifies that an ACE 5 C18 (3 mm x 50 mm) column with Phenomenex C-12 (4 mm x 20 mm) guard column was used (pp. 10, 12, 14 of MRID 49068637), but an appendix of LC/MS/MS operating parameters identified a Zorbax XDB-C18 (4.6 mm x 150 mm, 5 µm) column (Appendix 3, p. 54). The independent laboratory should clarify which LC column was used.

5. For the ILV, performance data using the confirmation method (ion transition *m/z* 314→160) were collected, but not reported (p. 15 of MRID 49068637). However, OCSPP 850.6100 guidelines specify that a confirmatory procedure is not typically necessary where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

6. Documentation of communications between the ILV personnel and the study sponsor were not provided. However, the ILV study author reported that communication did not compromise the independent evaluation (p. 17 of MRID 49068637).

7. It was reported for the ILV that preparation and analysis time for one set of thirteen samples required approximately 1 hour (p. 16 of MRID 49068637).

Conclusion:

The results of the method validation study demonstrated that the HPLC/MS/MS analytical method can be performed successfully on water samples for mandestrobin down to a level of 0.10 µg/L with a limit of detection at 0.05 µg/L. This method was assessed to be acceptable as a post registration monitoring method.

PMRA Deficiency: MCBX, S-2200-OR and S-2200-ORC have been identified as major transformation products/metabolites in water but an analytical method(s) has not been provided for these transformation products/metabolites.

V. References

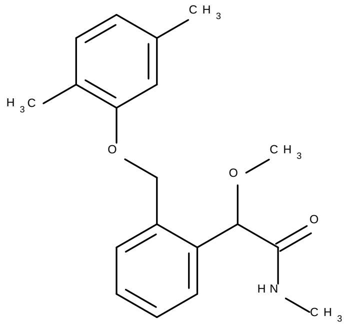
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Mandestrobin (S-2200)

IUPAC Name:	(*RS*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide
CAS Name:	2-[(2,5-Dimethylphenoxy)methyl]-α-methoxy-*N*-methylbenzeneacetamide
CAS Number:	173662-97-0
SMILES String:	Cc1ccc(c(c1)OCc2ccccc2C(C(=O)NC)OC)C



Attachment 2:

ECM Validation for Determination of Mandestrobin (S-2200) in Surface (Lake) Water

Chemical: Mandestrobin
PC: 036603
MRIDs: 49068639 & 49068637
Guideline: 850.6100
ECM Validation for Determination of Mandestrobin (S-2200) in Surface (Lake) Water

	Quantitation (ion transition m/z 314>192)							Confirmation (ion transition m/z 314>160)						
Fortified (µg a.i./L)	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Max	Min	n =	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Max	Min	n =
0.1	100							100						
LOQ	102							101						
	89							91						
	97							98						
	90	96	6	6	102	89	5	94	97	4	4	101	91	5
1.0	101							102						
	105							104						
	105							104						
	102							102						
	107	104	2	2	107	101	5	107	104	2	2	107	102	5

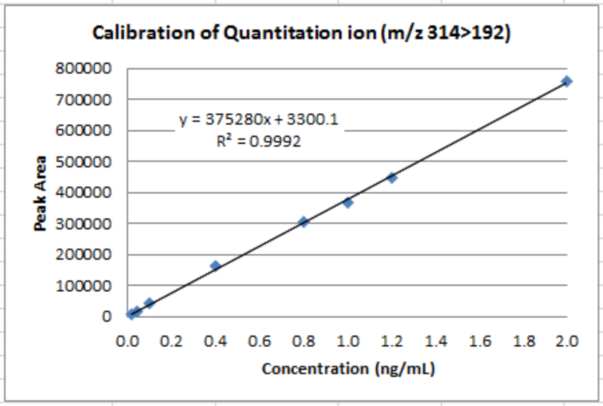
Results from Table 1, p. 17 of MRID 49068639. Surface water obtained from Waldsee (forest lake) in Senden (Southern Germany, p. 10).
Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and =STDEV(A1:A2).
Any discrepancies between reviewer calculated values and reported results most likely due to rounding.
1 SD = Standard Deviation; determined using the "unbiased" or "n-1" method.
2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.

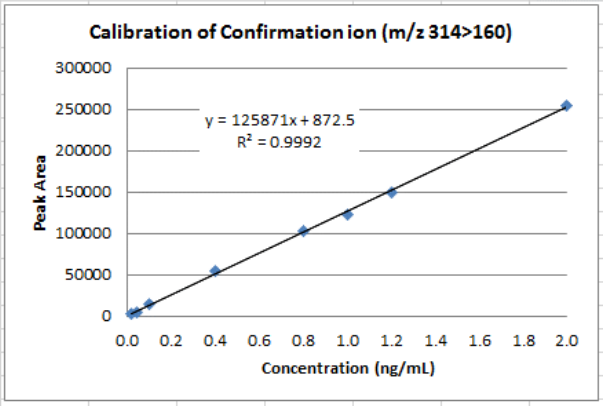
ECM Calibration Curves

Chemical: Mandestrobin
PC: 036603
MRIDs: 49068639 & 49068637
Guideline: 850.6100
ECM Calibration Curves

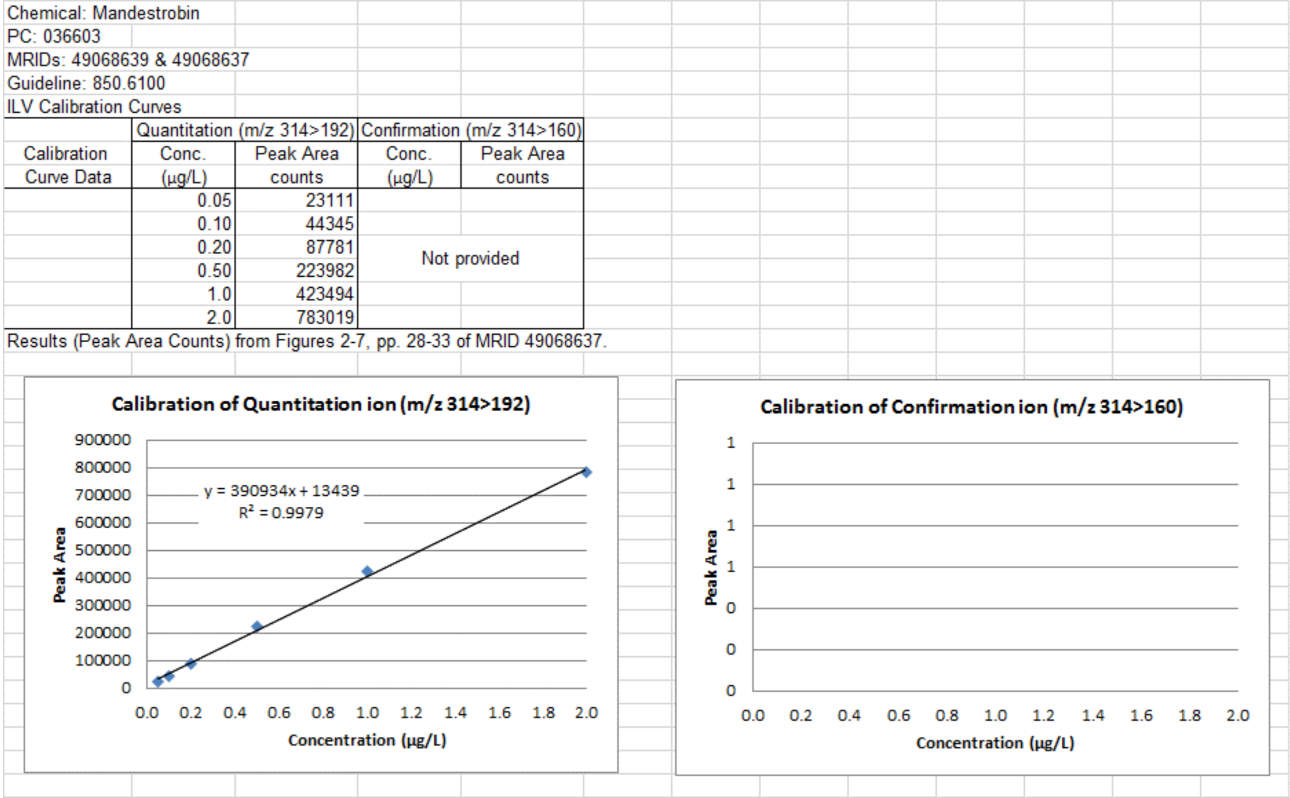
	Quantitation (m/z 314>192)		Confirmation (m/z 314>160)	
Calibration Curve Data	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts
	0.0175	8020	0.0175	2590
	0.04	18400	0.04	5520
	0.10	41600	0.10	13900
	0.40	163000	0.40	54500
	0.80	308000	0.80	103000
	1.0	366000	1.0	123000
	1.2	446000	1.2	149000
	2.0	761000	2.0	255000

Results (Peak Area Counts) from Figure 1, p. 18 of MRID 49068639.





ILV Calibration Curves



Chemical: Mandestrobin
PC: 036603
MRIDs: 49068639 & 49068637
Guideline: 850.6100
ILV Calibration Curves

	Quantitation (m/z 314>192)		Confirmation (m/z 314>160)	
Calibration Curve Data	Conc. (µg/L)	Peak Area counts	Conc. (µg/L)	Peak Area counts
	0.05	23111	Not provided	
	0.10	44345		
	0.20	87781		
	0.50	223982		
	1.0	423494		
	2.0	783019		

Results (Peak Area Counts) from Figures 2-7, pp. 28-33 of MRID 49068637.

Test Material:	Mandestrobin
MRID:	49068639
Title:	Validation of an Analytical Method for the Determination of S-2200 in Surface Water for Post-Registration Control and Monitoring Purpose
MRID:	49068637
Title:	S-2200: Independent Laboratory Validation of the Analytical Method Validation of an Analytical Method for the Determination of S-2200 in Surface Water for Post Registration Control and Monitoring Purposes
EPA PC Code:	036603
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari

Signature: Lynne Binari

Date: 9/5/14

Secondary Reviewer: Lisa Muto

Signature: Lisa Muto

Date: 9/5/14

QC/QA Manager: Joan Gaidos

Signature:

Date: 9/5/14