

Test Material: Spinetoram (XDE-175)

MRID: 46695146

Title: Method Validation Report for the Determination of XDE-175 and its Metabolites in Water using Dow AgroSciences Method GRM 05.12

MRID: 46695145

Title: Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 05.12 - Determination of Residues of XDE-175 and its Metabolites in Water by Liquid Chromatography with Tandem Mass Spectrometry

EPA PC Code: 110009

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

Signature: 

Date: 9/8/15

Secondary Reviewer: Kathleen Ferguson

Signature: 

Date: 9/8/15

QC/QA Manager: Joan Gaidos

Signature: 

Date: 9/8/15

Analytical method for spinetoram [XDE-175 (XDE-175-J and XDE-175-L)] and its transformation products, XDE-175-N-demethyl-J and XDE-175-N-demethyl-L, in water

Reports: ECM: EPA MRID No.: 46695146. Schelle, G.E. and M.J. Hastings. 2005. Method Validation Report for the Determination of XDE-175 and its Metabolites in Water using Dow AgroSciences Method GRM 05.12. Laboratory Study ID: 051017. Report prepared, sponsored and submitted by Regulatory Laboratories – Indianapolis Lab, Dow AgroSciences LLC, Indianapolis, Indiana; 77 pages (including page 4.1R1). Final report and Method dated June 22, 2005 (pp. 1, 21). Amended report dated July 26, 2005 (p. 1).
ILV: EPA MRID No. 46695145. Richter, M. 2005. Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 05.12 - Determination of Residues of XDE-175 and its Metabolites in Water by Liquid Chromatography with Tandem Mass Spectrometry. PTRL Europe Study No.: P 865 G. Dow AgroSciences Protocol No.: 050057. Report prepared by PTRL Europe GmbH, Ulm, Germany, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 56 pages. Final report issued August 30, 2005.

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Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA and OECD Good Laboratory Practice (GLP) standards (1998; p. 3R1 of MRID 46695146). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2, 3R1-4R1). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4R1). A summary of amendment was provided (p. 4.1R1).
ILV: The study was conducted in accordance with USEPA and OECD GLP standards (1998; p. 3 of MRID 46695145). 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 the quality assurance statement (p. 4).

Classification: This analytical method is classified as supplemental. In the ECM, samples were not prepared at 10×LOQ, and the number of samples was insufficient for all analyses. ECM representative chromatograms were inadequate to support the method. Sample recoveries were corrected in the ECM.

PC Code: 110009

Reviewer: Larry Liu



Date: 5/3/17

All page numbers refer to those listed in the upper right-hand corner of the MRIDs.

Executive Summary

The analytical method, Dow AgroSciences Method GRM 05.12, is designed for the quantitative determination of XDE-175 (XDE-175-J and XDE-175-L) and its transformation products (XDE-175-N-demethyl-J and XDE-175-N-demethyl-L) in water at the LOQ of 0.05 µg/L using LC/MS/MS. GRM 05.12 employed a quantitative and a confirmatory HPLC analysis. The LOQ is less than the lowest toxicological level of concern in water for all analytes. Method GRM 05.12 was validated by the ILV in the first trial using pond water. Sample recoveries were corrected in the ILV. In the ECM, the number of samples was insufficient for all analyses, and samples were not prepared at 10×LOQ.

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 | | | | | | |
| XDE-175- J | 46695146 (GRM 05.12) | 46695145 | | Water ^{1,2} | 22/06/2005 (Original report) 26/07/2005 (Amended report) | Dow AgroSciences LLC | LC/MS/MS | 0.05 µg/L |
| XDE-175- L | | | | | | | | |
| XDE-175- N- demethyl-J | | | | | | | | |
| XDE-175- N- demethyl- L | | | | | | | | |

1 For the ECM, drinking water 189 (pH 7.9, dissolved organic carbon 9.1 ppm), drinking water 194 (pH 8.2, dissolved organic carbon 2.3 ppm), ground water 190 (pH 7.9, dissolved organic carbon 1.3 ppm), ground water 193 (pH 8.0, dissolved organic carbon 5.3 ppm), surface water 191 (pH 8.3, dissolved organic carbon 7.3 ppm) and surface water 192 (pH 7.9, dissolved organic carbon 9.4 ppm) were used (p. 10 of MRID 46695146). The sources were reported, but not specified for the sample numbers.

2 For the ILV, pond (surface) water was characterized (pH 7.9, dissolved organic carbon 29 mg/L); the pond water was collected from a pond in Ulm, Germany (yellowish color, no odor; p. 10 of MRID 46695145).

I. Principle of the Method

Samples (10 mL) of water in 40-mL vials were thawed in the dark and fortified, as necessary (pp. 10-12; Appendix A, pp. 29-30 of MRID 46695146). The method noted that XDE-175 has low water solubility and readily adsorbs from water samples onto glass or plastic containers. Each sample was mixed with 10 mL of acetonitrile and 100 μ L of the 0.1 μ g/mL mixed XDE-175 and metabolites stable isotope internal standard solution. After mixing via vortex for *ca.* 10 seconds, an aliquot was transferred to a 96-well plate. Samples containing high concentrations (>10 ng/mL, equivalent to >20 μ g/L) were diluted with acetonitrile:water (80:20, v:v) containing 0.5 ng/mL mixed XDE-175 and metabolites stable isotope internal standard solution. XDE-175 and its metabolites were analyzed by liquid chromatography with positive-ion electrospray ionization (ESI) tandem mass spectrometry.

Samples were analyzed for XDE-175-J, XDE-175-L, XDE-175-N-demethyl-J and XDE-175-N-demethyl-L using a MDS/SCIEX API 4000 LC/MS/MS (Appendix A, pp. 27-29 of MRID 46695146). The instrumental conditions consisted of a YMC ODS-AM column (4.6 x 50 mm, 5- μ m; column temperature ambient), a mobile phase gradient of (A) acetonitrile:methanol (1:1, v:v) containing 2 mM ammonium acetate and (B) water containing 2 mM ammonium acetate [percent A:B (v:v) at 0:01 min. 70:30, 3:00-5:30 min. 100:0, 5:45-7:00 min. 70:30] and MS/MS detection in positive ESI ionization mode (temperature, 425°C), and injection volume 100 μ L. One parent-daughter ion transition was monitored per analyte or IS: m/z 748.6 \rightarrow 142.2 for XDE-175-J, m/z 760.9 \rightarrow 142.2 for XDE-175-L, m/z 734.9 \rightarrow 128.2 for XDE-175-N-demethyl-J, and m/z 746.7 \rightarrow 128.2 for XDE-175-N-demethyl-L; and m/z 757.9 \rightarrow 146.2 for XDE-175-J IS, m/z 769.9 \rightarrow 146.2 for XDE-175-L IS, m/z 739.9 \rightarrow 128.2 for XDE-175-N-demethyl-J IS, and m/z 751.7 \rightarrow 128.2 for XDE-175-N-demethyl-L IS. Confirmatory HPLC analysis was performed using a Synergi Polar RP column (4.6 x 75 mm, 4- μ m). Retention times for XDE-175-J, XDE-175-L, XDE-175-N-demethyl-J and XDE-175-N-demethyl-L were observed at 4.1 (4.0), 4.2, 3.4 and 3.6 min., respectively, for quantitative HPLC and at 3.8, 3.9, 4.0-4.1 and 4.1-4.2 min., respectively, for confirmatory HPLC (RT for the IS was reported in parenthesis if it differed from the RT of the analyte; Appendix A, Figures 21-28, pp. 69-76).

ILV

In the ILV, Method GRM 05.12 was performed exactly as written, except for slight differences of laboratory equipment and practices (pp. 12-14 of MRID 46695145). Samples were analyzed for XDE-175-J, XDE-175-L, XDE-175-N-demethyl-J and XDE-175-N-demethyl-L and their internal stable isotope standards (IS) using a MDS/SCIEX API 3000 LC/MS/MS with TurboIonSpray (MRM). Several instrumental conditions differed from that in the ECM. The LC columns (quantitative and confirmatory) were the same as the ECM; however, a Securityguard column was used for both the quantitative and confirmatory HPLC analysis [Waters Hypersil Gold (10 x 3 mm, 5 μ m) and Phenomenex Polar-RP (4 x 3 mm, 4 μ m), respectively]. The mobile phase gradient was [percent A:B (v:v) at 0:00-1.00 min. 30:70, 3:00-13:00 min. 100:0, 13:10-16:00 min. 30:70]. Injection volume was 95 μ L. One parent-daughter ion transition was monitored per analyte or IS: m/z 748.7 \rightarrow 142.1 for XDE-175-J, m/z 760.7 \rightarrow 142.1 for XDE-175-L, m/z 734.7 \rightarrow 127.9 for XDE-175-N-demethyl-J, and m/z 746.8 \rightarrow 127.9 for XDE-175-N-demethyl-L; and m/z 757.9 \rightarrow 146.2 for XDE-175-J IS, m/z 769.9 \rightarrow 146.2 for XDE-175-L IS,

m/z 739.9 \rightarrow 128.2 for XDE-175-N-demethyl-J IS, and m/z 751.7 \rightarrow 128.2 for XDE-175-N-demethyl-L IS. Retention times for XDE-175-J, XDE-175-L, XDE-175-N-demethyl-J and XDE-175-N-demethyl-L were observed at *ca.* 9.05-9.2, 9.35-9.5, 8.15-8.2 and 8.4 min., respectively, for quantitative HPLC and at *ca.* 9.6, 9.7, 12 and 8.4-8.5 min., respectively, for confirmatory HPLC (IS and analyte; Figures 8-13, pp. 30-35).

LOQ/LOD

The LOQ and LOD for all analytes was the same in the ECM and ILV at 0.05 $\mu\text{g/L}$ and 0.015 $\mu\text{g/L}$, respectively (pp. 7, 15-17; Appendix A, p. 35; Appendix A, Tables 17-19, pp. 55-56 of MRID 46695146; pp. 8, 18, 20 of MRID 46695145).

II. Recovery Findings

ECM (MRID 46695146: Method GRM 05.12): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of XDE-175-J, XDE-175-L, XDE-175-N-demethyl-J and XDE-175-N-demethyl-L in drinking, ground and surface waters at fortification levels of 0.05 $\mu\text{g/L}$ (LOQ), 5.0 $\mu\text{g/L}$ (100 \times LOQ) and 50 $\mu\text{g/L}$ (1000 \times LOQ; Appendix A, Tables 2-13, pp. 41-52; DER Attachment 2). Samples were not prepared at 10 \times LOQ. The number of samples ($n = 4$) was insufficient for all analyses. Performance data (recovery results) were only provided for quantitative HPLC analysis; recovery results from the confirmatory HPLC analysis were not reported. Confirmation of residue identity was performed by comparing the retention times of the analytes in the samples to the analytes in the calibration standards; the method noted that the confirmatory HPLC analysis could be performed, if necessary, since only one ion transition was monitored per analyte (p. 17; Appendix A, p. 35). Method GRM 05.12 allowed for recovery data to be corrected for residues found in the control samples; however, residues were only quantified in the control samples of XDE-175-N-demethyl-J (all matrices, except ground water 193; 0.0013-0.0020 $\mu\text{g/L}$) and XDE-175-N-demethyl-L (all matrices; 0.0003-0.0027 $\mu\text{g/L}$; p. 13; Appendix A, pp. 30-34; Appendix A, Tables 2-13, pp. 41-52). Recoveries from samples fortified at 0.015 $\mu\text{g/L}$ (LOD) ranged (methods and matrices combined) from 61-90% for XDE-175-J, 53-97% for XDE-175-L, 74-104% for XDE-175-N-demethyl-J and 53-105% for XDE-175-N-demethyl-L ($n = 2$ for each matrix/analyte); mean recoveries and relative standard deviations (RSDs) of statistical significance could not be calculated (DER Attachment 2). The water matrices were characterized (p. 10). Drinking water samples were obtained from the Dow AgroSciences Research and Development Center, Indianapolis, Indiana, and from a residence in Fishers, Indiana. The untreated ground water samples were obtained from residential wells in Cicero and Greenfield, Indiana. The surface water samples were obtained from a residential pond in Cicero, Indiana and from a pond within the Dow AgroSciences Research and Development Center, Indianapolis, Indiana. Drinking water 189 (pH 7.9, dissolved organic carbon 9.1 ppm), drinking water 194 (pH 8.2, dissolved organic carbon 2.3 ppm), ground water 190 (pH 7.9, dissolved organic carbon 1.3 ppm), ground water 193 (pH 8.0, dissolved organic carbon 5.3 ppm), surface water 191 (pH 8.3, dissolved organic carbon 7.3 ppm) and surface water 192 (pH 7.9, dissolved organic carbon 9.4 ppm) were used. The sources were not specified for the sample numbers.

ILV (MRID 46695145): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of XDE-175-J, XDE-175-L, XDE-175-N-demethyl-J and XDE-175-N-demethyl-L in pond water at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ) using the quantitative and confirmatory HPLC analyses (uncorrected recovery results; pp. 14-16; Tables 1-2, pp. 21-22). Performance data (recovery results) from quantitative HPLC analyses yielded slightly higher mean recoveries than confirmatory HPLC analyses, but RSDs were comparable between the quantitative and confirmatory HPLC analyses. The pond water was characterized (pH 7.9, dissolved organic carbon 29 mg/L); the pond water was collected from a pond in Ulm, Germany (yellowish color, no odor; p. 10). Method GRM 05.12 was validated in the first trial (p. 18).

Table 2. Initial Validation Method Recoveries for XDE-175 and Its Transformation Products in Drinking, Ground and Surface Water^{1,2}

| Analyte | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|-------------------------------------|----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| GRM 05.12– Quantitative HPLC | | | | | | |
| Drinking Water - 189 | | | | | | |
| XDE-175-J | 0.015 (LOD) | 2 | 61, 87 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 76-89 | 82 | 6 | 7 |
| | 5.0 | 4 | 94-98 | 96 | 2 | 2 |
| | 50 | 4 | 95-101 | 97 | 3 | 3 |
| XDE-175-L | 0.015 (LOD) | 2 | 61, 79 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 69-81 | 73 | 5 | 7 |
| | 5.0 | 4 | 80-96 | 88 | 8 | 9 |
| | 50 | 4 | 88-102 | 96 | 8 | 8 |
| XDE-175-N-demethyl-J | 0.015 (LOD) | 2 | 74, 83 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 83-89 | 85 | 3 | 3 |
| | 5.0 | 4 | 92-101 | 97 | 4 | 4 |
| | 50 | 4 | 89-94 | 92 | 2 | 3 |
| XDE-175-N-demethyl-L | 0.015 (LOD) | 2 | 57, 90 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 76-89 | 83 | 5 | 6 |
| | 5.0 | 4 | 89-97 | 94 | 4 | 4 |
| | 50 | 4 | 87-94 | 91 | 3 | 3 |
| Drinking Water - 194 | | | | | | |
| XDE-175-J | 0.015 (LOD) | 2 | 77 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 82-88 | 85 | 2 | 3 |
| | 5.0 | 4 | 97-100 | 99 | 2 | 2 |
| | 50 | 4 | 98-99 | 99 | 1 | 1 |
| XDE-175-L | 0.015 (LOD) | 2 | 53, 96 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 67-84 | 76 | 8 | 11 |
| | 5.0 | 4 | 89-92 | 90 | 2 | 2 |
| | 50 | 4 | 92-93 | 92 | 1 | 1 |
| XDE-175-N-demethyl-J | 0.015 (LOD) | 2 | 75 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 64-77 | 71 | 6 | 8 |
| | 5.0 | 4 | 82-94 | 88 | 6 | 7 |
| | 50 | 4 | 83-89 | 87 | 3 | 3 |
| XDE-175-N-demethyl-L | 0.015 (LOD) | 2 | 53, 67 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 75-82 | 79 | 3 | 4 |
| | 5.0 | 4 | 82-90 | 86 | 4 | 5 |
| | 50 | 4 | 84-90 | 88 | 3 | 3 |
| Ground Water - 190 | | | | | | |
| XDE-175-J | 0.015 (LOD) | 2 | 70, 72 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 84-92 | 88 | 3 | 4 |
| | 5.0 | 4 | 92-102 | 96 | 4 | 5 |
| | 50 | 4 | 96-97 | 96 | 1 | 1 |
| XDE-175-L | 0.015 (LOD) | 2 | 59, 91 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 76-87 | 81 | 6 | 7 |
| | 5.0 | 4 | 82-97 | 88 | 6 | 7 |
| | 50 | 4 | 93-102 | 96 | 4 | 4 |

| Analyte | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|----------------------|----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| XDE-175-N-demethyl-J | 0.015 (LOD) | 2 | 83, 90 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 96-108 | 101 | 5 | 5 |
| | 5.0 | 4 | 100-102 | 102 | 1 | 1 |
| | 50 | 4 | 99-100 | 99 | 1 | 1 |
| XDE-175-N-demethyl-L | 0.015 (LOD) | 2 | 90, 105 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 93-101 | 96 | 4 | 4 |
| | 5.0 | 4 | 96-100 | 98 | 2 | 2 |
| | 50 | 4 | 93-98 | 96 | 2 | 2 |
| Ground Water - 193 | | | | | | |
| XDE-175-J | 0.015 (LOD) | 2 | 72, 83 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 79-89 | 85 | 4 | 5 |
| | 5.0 | 4 | 97-101 | 99 | 2 | 2 |
| | 50 | 4 | 98-101 | 99 | 2 | 2 |
| XDE-175-L | 0.015 (LOD) | 2 | 59, 82 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 70-81 | 75 | 5 | 6 |
| | 5.0 | 4 | 84-90 | 87 | 3 | 3 |
| | 50 | 4 | 91-98 | 95 | 3 | 3 |
| XDE-175-N-demethyl-J | 0.015 (LOD) | 2 | 99, 104 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 92-104 | 100 | 5 | 5 |
| | 5.0 | 4 | 100-102 | 101 | 1 | 1 |
| | 50 | 4 | 99-101 | 100 | 1 | 1 |
| XDE-175-N-demethyl-L | 0.015 (LOD) | 2 | 82, 90 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 88-93 | 90 | 2 | 3 |
| | 5.0 | 4 | 96-99 | 98 | 2 | 2 |
| | 50 | 4 | 95-100 | 98 | 2 | 2 |
| Surface Water - 191 | | | | | | |
| XDE-175-J | 0.015 (LOD) | 2 | 67, 73 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 89-92 | 91 | 1 | 1 |
| | 5.0 | 4 | 97-103 | 99 | 3 | 3 |
| | 50 | 4 | 96-99 | 98 | 1 | 1 |
| XDE-175-L | 0.015 (LOD) | 2 | 66, 96 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 88-94 | 91 | 3 | 3 |
| | 5.0 | 4 | 87-104 | 95 | 9 | 9 |
| | 50 | 4 | 91-101 | 96 | 5 | 5 |
| XDE-175-N-demethyl-J | 0.015 (LOD) | 2 | 82, 94 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 94-97 | 95 | 1 | 1 |
| | 5.0 | 4 | 98-105 | 101 | 3 | 3 |
| | 50 | 4 | 97-102 | 100 | 2 | 2 |
| XDE-175-N-demethyl-L | 0.015 (LOD) | 2 | 80, 97 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 91-100 | 96 | 4 | 5 |
| | 5.0 | 4 | 95-99 | 97 | 2 | 2 |
| | 50 | 4 | 93-97 | 96 | 2 | 2 |
| Surface Water - 192 | | | | | | |
| XDE-175-J | 0.015 (LOD) | 2 | 79, 90 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 85-100 | 93 | 6 | 7 |
| | 5.0 | 4 | 99-101 | 100 | 1 | 1 |

| Analyte | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|----------------------|----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| | 50 | 4 | 98-101 | 99 | 1 | 1 |
| XDE-175-L | 0.015 (LOD) | 2 | 53, 97 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 83-91 | 87 | 3 | 4 |
| | 5.0 | 4 | 92-101 | 96 | 5 | 5 |
| | 50 | 4 | 93-97 | 95 | 2 | 2 |
| XDE-175-N-demethyl-J | 0.015 (LOD) | 2 | 74, 92 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 87-99 | 95 | 5 | 6 |
| | 5.0 | 4 | 101-104 | 102 | 1 | 1 |
| | 50 | 4 | 98-101 | 100 | 1 | 1 |
| XDE-175-N-demethyl-L | 0.015 (LOD) | 2 | 95, 98 | -- | -- | -- |
| | 0.05 (LOQ) | 4 | 80-100 | 91 | 9 | 10 |
| | 5.0 | 4 | 97-100 | 98 | 2 | 2 |
| | 50 | 4 | 94-97 | 96 | 2 | 2 |

Data (recovery results were corrected when residues were quantified in the controls; p. 13; Appendix A, pp. 30-34; Appendix A, Tables 2-13, pp. 41-52) were obtained from Appendix A, Tables 2-13, pp. 41-52 of MRID 46695146 and DER Attachment 2 (mean, s.d. and RSD values and % recovery at LOD).

- The water matrices were characterized (p. 10). Drinking water 189 (pH 7.9, dissolved organic carbon 9.1 ppm), drinking water 194 (pH 8.2, dissolved organic carbon 2.3 ppm), ground water 190 (pH 7.9, dissolved organic carbon 1.3 ppm), ground water 193 (pH 8.0, dissolved organic carbon 5.3 ppm), surface water 191 (pH 8.3, dissolved organic carbon 7.3 ppm) and surface water 192 (pH 7.9, dissolved organic carbon 9.4 ppm) were used. The sources were reported, but not specified for the sample numbers.
- One ion transition was monitored for each of the analytes (p. 14; Appendix A, pp. 27-29). Analytes were analyzed by liquid chromatography with positive-ion ESI tandem mass spectrometry for both quantitative and confirmatory HPLC; different LC columns were used for quantitation and confirmation. Only results for the quantitative HPLC were reported (p. 17; Appendix A, p. 35).

Table 3. Independent Validation Method Recoveries for XDE-175 and Its Transformation Products in Pond Water^{1,2}

| Analyte | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|----------------------|----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| Quantitative HPLC | | | | | | |
| XDE-175-J | 0.05 (LOQ) | 5 | 90-100 | 94 | 4 | 4 |
| | 0.5 | 5 | 90-97 | 94 | 3 | 3 |
| XDE-175-L | 0.05 (LOQ) | 5 | 82-92 | 88 | 4 | 5 |
| | 0.5 | 5 | 94-100 | 97 | 2 | 2 |
| XDE-175-N-demethyl-J | 0.05 (LOQ) | 5 | 87-96 | 92 | 3 | 3 |
| | 0.5 | 5 | 96-98 | 97 | 1 | 1 |
| XDE-175-N-demethyl-L | 0.05 (LOQ) | 5 | 92-103 | 98 | 4 | 4 |
| | 0.5 | 5 | 97-102 | 98 | 2 | 2 |
| Confirmatory HPLC | | | | | | |
| XDE-175-J | 0.05 (LOQ) | 5 | 98-109 | 105 | 4 | 4 |
| | 0.5 | 5 | 96-106 | 101 | 4 | 4 |
| XDE-175-L | 0.05 (LOQ) | 5 | 96-105 | 101 | 4 | 4 |
| | 0.5 | 5 | 101-107 | 105 | 2 | 2 |
| XDE-175-N-demethyl-J | 0.05 (LOQ) | 5 | 101-106 | 104 | 2 | 2 |
| | 0.5 | 5 | 98-102 | 100 | 2 | 2 |
| XDE-175-N-demethyl-L | 0.05 (LOQ) | 5 | 96-109 | 103 | 5 | 6 |
| | 0.5 | 5 | 96-106 | 102 | 4 | 4 |

Data (uncorrected results; pp. 14-16) were obtained from Tables 1-2, pp. 21-22 of MRID 46695145 and DER Attachment 2 (s.d.). The recovery results for the first replicate were the means of two injections. The standard deviations were reviewer-calculated based on recovery results provided in the study report since the study author only provided means and RSDs.

1 The pond (surface) water was characterized (pH 7.9, dissolved organic carbon 29 mg/L); the pond water was collected from a pond in Ulm, Germany (yellowish color, no odor; p. 10).

2 One ion transition was monitored for each of the analytes (pp. 12-14). Analytes were analyzed by liquid chromatography with positive-ion ESI tandem mass spectrometry for both quantitative and confirmatory HPLC; different LC columns were used for quantitation and confirmation.

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD values for XDE-175-J, XDE-175-L, XDE-175-N-demethyl-J and XDE-175-N-demethyl-L were established at 0.05 µg/ and 0.015 µg/L, respectively (pp. 7, 15-17, 37; Appendix A, p. 35; Appendix A, Tables 17-19, pp. 55-56 of MRID 46695146; pp. 8, 18, 20 of MRID 46695145). Following the method of Keith, L. H., *et al.* (see section V. **References** below), the LOD and LOQ for determination of XDE-175 and its transformation products in water were calculated in the ECM using the standard deviation from the 0.05 µg/L recovery results. The LOD was calculated as three times the standard deviation (3s), and the LOQ was calculated as ten times the standard deviation (10s) of the recovery results. The calculated values support the LOQ and LOD established for the study and are presented in **Table 4** below.

Table 4. Method Characteristics – GRM 05.12

| | | XDE-175-J | XDE-175-L | XDE-175-N-demethyl-J | XDE-175-N-demethyl-L |
|---|---------------------|--|--|---|-------------------------|
| Limit of Quantitation (LOQ) | Established | 0.05 µg/L | | | |
| | Calculated (ECM) | 0.0207-0.0220 | 0.0166-0.0325 | 0.0186-0.0428 | 0.0212-0.0351 |
| Limit of Detection (LOD) | Established | 0.015 µg/L | | | |
| | Calculated (ECM) | 0.0062-0.0066 | 0.0050-0.0097 | 0.0056-0.0128 | 0.0063-0.0105 |
| Linearity (Least squares calibration curve r and concentration range) | ECM ¹ | r ² = 0.9996 | r ² = 0.9990 | r ² = 0.9999 | r ² = 0.9998 |
| | ILV ² | r ² = 1.0000 | | | |
| | Concentration range | 0.0075-10 ng/mL | | | |
| Repeatable | ECM ³ | Yes at LOQ, 100×LOQ and 1000×LOQ, but n = 4. No samples were prepared at 10×LOQ. | | | |
| | ILV ⁴ | Yes at LOQ and 10×LOQ (n = 5). | | | |
| Reproducible | | Yes at the LOQ and 10×LOQ. | | | |
| Specific | ECM | Only chromatograms of drinking water 189 were provided for each HPLC method: the quantitative and confirmatory HPLC. | | | |
| | | Yes, no interferences were observed in the matrix control. | Yes, matrix interferences were <20% of the LOD, based on recovery results. | | |
| | ILV | Yes, no interferences were observed in the matrix control. | Yes, minor matrix interferences at the retention times of the analyte (estimated at <5% of the LOQ) for the quantitative HPLC. | Yes, some minor baseline interference (estimated at <5% of the LOQ) interfered with peak attenuation. | |

Data were obtained from pp. 7, 15-17, 37; Appendix A, p. 35; Appendix A, Tables 2-13, pp. 41-52; Appendix A, Tables 17-19, pp. 55-56; Appendix A, Figures 17-28, pp. 65-76 of MRID 46695146; pp. 8, 18, 20; Tables 1-2, pp. 21-22; Figures 1-13, pp. 23-35 of MRID 46695145.

1 Only one set of calibration curves (for “water” – water matrix not specified) was provided in the ECM.

2 Only one set of calibration curves was provided in the ILV; the set of calibration curves was not specified as quantitative or confirmatory HPLC analysis.

3 Only quantitative HPLC results were provided in the ECM.

4 For the ECM, drinking water 189 (pH 7.9, dissolved organic carbon 9.1 ppm), drinking water 194 (pH 8.2, dissolved organic carbon 2.3 ppm), ground water 190 (pH 7.9, dissolved organic carbon 1.3 ppm), ground water 193 (pH 8.0, dissolved organic carbon 5.3 ppm), surface water 191 (pH 8.3, dissolved organic carbon 7.3 ppm) and surface water 192 (pH 7.9, dissolved organic carbon 9.4 ppm) were used (p. 10 of MRID 46695146). The sources were reported, but not specified for the sample numbers.

5 For the ILV, pond (surface) water was characterized (pH 7.9, dissolved organic carbon 29 mg/L); the pond water was collected from a pond in Ulm, Germany (yellowish color, no odor; p. 10 of MRID 46695145).

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, samples were not prepared at 10×LOQ. 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.
2. In the ECM, the number of samples was insufficient for all analyses at the LOQ, 100×LOQ and 1000×LOQ (n = 4; Appendix A, Tables 2-13, pp. 41-52 of MRID 46695146). OSCPP guidelines recommend a minimum of five samples spiked at each fortification level.
3. In the ECM, representative chromatograms were only provided for the quantitative and confirmatory HPLC analysis for drinking water (189; Appendix A, Figures 21-28, pp. 69-76 of MRID 46695146). One standard, the control, the LOQ and the 1000×LOQ chromatogram was provided. No 10×LOQ chromatograms were included since these samples were not included in the validation. OCSPP guidelines recommend that chromatograms are provided for all matrices and HPLC methods which were included in the validation. Also, a reagent blank was not included.
4. Recoveries were corrected in the ECM. Calculations allowed for recovery data to be corrected for residues found in the control samples; however, residues were only quantified in the control samples of XDE-175-N-demethyl-J (all matrices, except ground water 193; 0.0013-0.0020 µg/L) and XDE-175-N-demethyl-L (all matrices; 0.0003-0.0027 µg/L; p. 13; Appendix A, pp. 30-34; Appendix A, Tables 2-13, pp. 41-52). Recoveries were not corrected in the ILV.
5. In the ILV, Method GRM 05.12 was reportedly performed exactly as written, except for slight differences of laboratory equipment and practices (pp. 12-14 of MRID 46695145). Two notable differences in the ILV analytical method was 1) the addition of the Securityguard columns and 2) that the mobile phase gradient [percent A:B (v:v) at 0:00-1:00 min. 30:70, 3:00-13:00 min. 100:0, 13:10-16:00 min. 30:70] differed from the ECM mobile phase gradient [percent A:B (v:v) at 0:01 min. 70:30, 3:00-5:30 min. 100:0, 5:45-7:00 min. 70:30]. Overall, the HPLC system and parameters of the ILV varied from that used in the ECM (AB API 3000 LC/MS/MS versus MDS/Sciex API 4000 LC/MS/MS). The reviewer assumed that all of the changes were system optimization of the ILV equipment and were not a significant modification of the ECM.
6. Drinking (pH 7.9-8.2, dissolved organic carbon 2.3-9.1 ppm), ground (pH 7.9-8.0, dissolved organic carbon 1.3-5.3 ppm) and surface (pH 7.9-8.3, dissolved organic carbon 7.3-9.4 ppm) waters were used in the ECM; pond (surface) water (pH 7.9, dissolved organic carbon 29 mg/L) was used in the ILV (p. 10 of MRID 46695146; p. 10 of MRID 46695145). The ILV study author noted that "surface water typically represents the most difficult type of water sample" compared to drinking and well water due to the amount of suspended solids and impurities (p. 20 of MRID 46695145).

7. The toxicological level of concern was not reported for the analytes in water.
8. Recovery results in the ECM were calculated for each water matrix combining both of the sample sets (n = 8; Appendix A, Tables 14-16, pp. 53-55 of MRID 46695146).
9. In the ILV, the study author reported that communications did not occur and were not necessary between the ILV and the sponsor (p. 19 of MRID 46695145).
10. It was reported for the ILV that the analytical procedure for one set of 13 samples required approximately three person hours for preparation (approximately a half-work day; p. 18 of MRID 46695145). The HPLC analyses were conducted overnight unattended; results analysis required approximately 2 hours.
11. The reviewer noted that spinetoram is a mixture of XDE-175-J and XDE-175-L [3:1 ratio (J:L); p. 3; http://epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-110009_12-Oct-10_a.pdf]. Additionally, the reviewer found that PC code 110008 is also associated with spinetoram (pp. 1, 38).

V. References

- Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, 55, 2210-2218 (Appendix A, p. 49; Appendix B, p. 107 of MRID 46695146).
- 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.

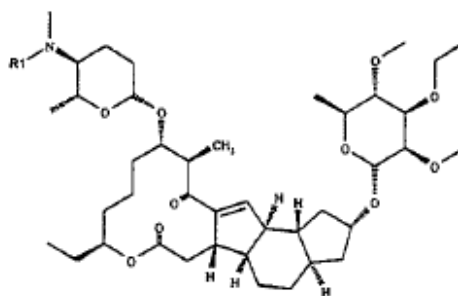
Attachment 1: Chemical Names and Structures**Spinetoram (XDE-175-J)**

IUPAC Name: (2R,3aR,5aR,5bS,9S,13S,14R,16aS,16bR)-13-[[[(2R,5S,6R)-5-(dimethylamino)-6-methyltetrahydro-2H-pyran-2-yl]oxy]-9-ethyl-14-methyl-7,15-dioxo-2,3,3a,4,5,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-octadecahydro-1-H-as-indaceno[3,2-d]oxacyclododecin-2-yl 6-deoxy-3-O-ethyl-2,4-di-O-methyl- α -L-mannopyranoside

CAS Name: 1-H-as-indaceno[3,2-d]oxacyclododecin-7,15-dione, 2-[(6-deoxy-3-O-ethyl-2,4-di-O-methyl- α -L-mannopyranosyl)oxy]-13-[[[(2R,5S,6R)-5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,4,5,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro 14-methyl-(2R,3aR,5aR,5bS,9S,13S,14R,16aS,16bR)

CAS Number: 187166-40-1

SMILES String:



XDE-175-J, R1 = CH₃

XDE-175-N-Demethyl-J, R1 = H

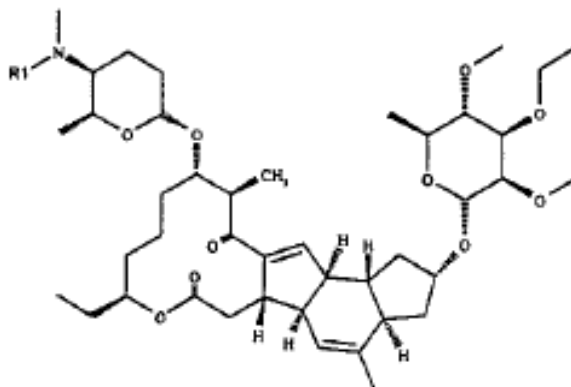
Spinetoram (XDE-175-L)

IUPAC Name: (2S,3aR,5aS,5bS,9S,13S,14R,16aS,16bS)-13-[[[(2R,5S,6R)-5-(dimethylamino)-6-methyltetrahydro-2H-pyran-2-yl]oxy]-9-ethyl-4,14-dimethyl-7,15-dioxo-2,3,3a,4,5,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-1-H-as-indaceno[3,2-d]oxacyclododecin-2-yl 6-deoxy-3-O-ethyl-2,4-di-O-methyl- α -L-mannopyranoside

CAS Name: 1-H-as-indaceno[3,2-d]oxacyclododecin-7,15-dione, 2-[(6-deoxy-3-O-ethyl-2,4-di-O-methyl- α -L-mannopyranosyl)oxy]-13-[[[(2R,5S,6R)-5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,4,5,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-tetradecahydro 4,14-dimethyl-(2S,3aR,5aS,5bS,9S,13S,14R,16aS,16bS)

CAS Number: 187166-15-0

SMILES String:



XDE-175-L, R1 = CH₃

XDE-175-N-Demethyl-L, R1 = H

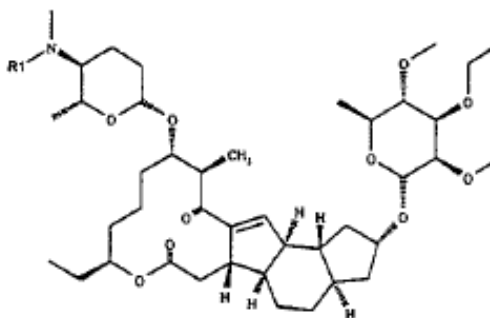
XDE-175-N-Demethyl-J

IUPAC Name: (2R,3aR,5aR,5bS,9S,13S,14R,16aS,16bR)- 9-ethyl-14-methyl-13-[[[(2S,5S,6R)- 6-methyl-5-(methylamino)tetrahydro-2H-pyran-2-yl]oxy]-7,15-dioxo-2,3,3a,4,5,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-octadecahydro-1-H-as-indaceno[3,2-d]oxacyclododecin-2-yl 6-deoxy-3-O-ethyl-2,4-di-O-methyl-beta-L-mannopyranoside

CAS Name:

CAS Number:

SMILES String:



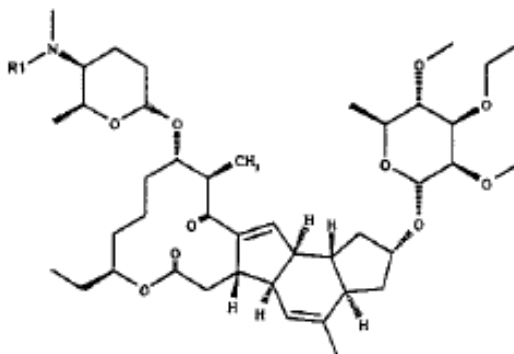
XDE-175-J, R1 = CH₃

XDE-175-N-Demethyl-J, R1 = H

XDE-175-N-Demethyl-L

IUPAC Name: (2S,3aR,5aS,5bS,9S,13S,14R,16aS,16bS)- 9-ethyl-4,14-dimethyl-13-[[[(2S,5S,6R)- 6-methyl-5-(methylamino)tetrahydro-2H-pyran-2-yl]oxy]-7,15-dioxo-2,3,3a,4,5,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-1-H-as-indaceno[3,2-d]oxacyclododecin-2-yl 6-deoxy-3-O-ethyl-2,4-di-O-methyl-beta-L-mannopyranoside

CAS Name:
CAS Number:
SMILES String:



XDE-175-L, R₁ = CH₃

XDE-175-*N*-Demethyl-L, R₁ = H