

Analytical method for famoxadone (DPX-JE874) and its metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007, in soil and water

Reports: ECM: EPA MRID No.: 49970601. Fett, L.K. 2015. Analytical Method for the Determination of Famoxadone (DPX-JE874) and its Metabolites in Soil and Water by LC-ESI-MS/MS. Project Identification No.: DuPont-43971. Report prepared by E. I. du Pont de Nemours and Company, DuPont Crop Protection, Newark, Delaware, and sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 94 pages. Final report issued November 3, 2015.

ILV: EPA MRID No. 49970602. Black, M. 2015. Independent Laboratory Validation of DuPont-43971, "Analytical Method for the Determination of Famoxadone (DPX-JE874) and its Metabolites in Soil and Water by LC-ESI-MS/MS". Project Identification No.: DuPont-43972. Alliance Pharma Project No.: 150813. Report prepared by Alliance Pharma, Malvern, Pennsylvania, sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 175 pages. Final report issued November 18, 2015.

Document No.: MRIDs 49970601 & 49970602

Guideline: 850.6100

Statements: ECM: The study was not conducted in accordance with USEPA FIFRA and OECD Good Laboratory Practice (GLP) standards; however, the study was conducted at a GLP compliant facility (p. 3 of MRID 49970601). Signed and dated No Data Confidentiality, GLP and Authenticity statements were provided (pp. 2-4). A Quality Assurance of the study report was not included.

ILV: The study was conducted in accordance with USEPA FIFRA and OECD GLP standards (p. 3 of MRID 49970602). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as **Acceptable**. However, the LOQ in water (0.10 µg/L) is near but greater than the lowest toxicological level of concern in water (0.085 µg/L). The ILV linearity was unsatisfactory for famoxadone in water. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. The LODs for soil and water matrices were not reported in the ILV.

PC Code: 113202

Final EPA Reviewer: Lewis R. Brown, III,
Environmental Biologist

Signature: Lewis Ross Brown, III

Date: 4/5/17

CDM/CSS-Dynamac JV Reviewers: Lisa Muto,
Environmental Scientist

Signature: 

Date: 1/10/17

Reviewers: Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: 

Date: 1/10/17

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

This analytical method, DuPont-43971, is designed for the quantitative determination of famoxadone (DPX-JE874) and its metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007, in soil at the LOQ of 10 ppb (10 µg/kg) and in water at the LOQ of 0.10 ppb (0.1 µg/L) using LC/MS/MS. The LOQ in soil (10 µg/kg) is less than the lowest toxicological level of concern in soil (370 µg/kg). The LOQ in water (0.10 µg/L) is near but greater than the lowest toxicological level of concern in water (0.085 µg/L). Characterized clay loam and sandy loam soils were used in the ECM soil validation; characterized ground and surface waters were used in the ECM water validation. The extraction procedure for the soil and water validations differed, but all analytes were identified using two ion transitions. The ILV validated the method in the first trial with insignificant modifications to the analytical instrumentation. Characterized sandy loam soil and surface water were used for the ILV validation; however, ILV may not have been provided with the most difficult matrices with which to validate the method. The ILV linearity was unsatisfactory for famoxadone in water, and the LODs for soil and water matrices were not 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						
Famoxadone (DPX-JE874)	49970601	49970602		Soil ^{1,2}	03/11/2015	E. I. du Pont de Nemours and Company	LC/MS/MS	10 µg/kg
IN-H3310								
IN-JS940								
IN-KF015								
IN-KZ007								
Famoxadone (DPX-JE874)				Water ^{3,4}				0.10 µg/L
IN-H3310								
IN-JS940								
IN-KF015								
IN-KZ007								

1 In the ECM, Drummer clay loam soil (25% sand, 41% silt, 34% clay; pH 6.5; 5.6% organic matter), collected from Rochelle, Illinois, and Nambenheim sandy loam soil (60% sand, 30% silt, 10% clay; pH 7.5; 3.8% organic matter), collected from Nambenheim, Germany, were used (p. 19 of MRID 49970601). Soil characterization was not specified as USDA.

2 In the ILV, Sassafras sandy loam soil (62% sand, 29% silt, 9% clay; pH 5.1 in 1:1 soil:water; 2.2% organic matter), collected from Pike Creek in Newark, Delaware, was used (USDA soil characterization; p. 21; Appendix 2, pp. 170-173 of MRID 49970602).

3 In the ECM, Kemblesville well water (pH 7.6; 68 mg equiv. CaCO₃/L; 222 ppm total dissolved solids), collected from Kemblesville, Pennsylvania, and White Clay Creek Water (pH 8.1; 144 mg equiv. CaCO₃/L; 254 ppm total dissolved solids), collected from White Clay Creek, Newark, Delaware, were used (p. 19 of MRID 49970601).

4 In the ILV, Pike Creek surface water (pH 7.6; 114 mg equiv. CaCO₃/L; 184 ppm total dissolved solids), collected from Pike Creek in Newark, Delaware, was used (p. 21; Appendix 2, pp. 174-175 of MRID 49970602).

I. Principle of the Method

Soil samples (10.0 ± 0.1 g) in 50-mL polypropylene centrifuge tubes were fortified and extracted twice with methanol:30 mM aqueous sodium acetate adjusted to pH 2.5-3 (4:1, v:v; 20 mL and 15 mL; pp. 15, 19-20 of MRID 49970601). For each extraction, the mixture was vortexed for ≥ 30 seconds, sonicated for 5 minutes, vortexed briefly then centrifuged for ≥ 5 minutes at ≥ 2500 rpm. The supernatant was decanted into a clean 50-mL polypropylene centrifuge tube. The combined extract solution was diluted to 50 mL with 0.01M aqueous formic acid then centrifuged (≥ 5 minutes at ≥ 2500 rpm) to separate extract from particulate matter. An aliquot from the extract was analyzed using reverse-phase LC/MS/MS.

Water samples (0.2 L) were fortified and adjusted to *ca.* 0.01% formic acid and 5% acetonitrile (10 mL of acetonitrile and 20 μ L of concentrated formic acid; pp. 15-16, 19-21 of MRID 49970601). A C₁₈-solid phase extraction (SPE) cartridge (3 cc/500 mg) was pre-conditioned with 5 mL each of methanol and Milli-Q water. The cartridge was not allowed to dry before the water sample was added. After all of the sample was applied to the cartridge, a vacuum was applied to achieve a fast drip rate (2-3 mL/min) until all of the solution passed through the cartridge. The analytes were eluted with 2.5 mL of 0.01% formic acid in acetonitrile and 2.5 mL of 0.01% formic acid in methanol using a slow drip rate with vacuum, as necessary. The 0.60 mL of the final extracts were combined with 0.40 mL of 0.01M aqueous formic acid in autosampler vials prior to analysis by reverse-phase LC/MS/MS.

Soil and water samples were analyzed for famoxadone and its metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007, using Agilent 1200 HPLC system (Zorbax® XDB C18 column, 4.6 mm x 50 mm, 1.8 μ m column; column temperature 40°C) using a gradient mobile phase of (A) 0.01M aqueous formic acid and (B) 0.01M formic acid in methanol [time ratio A:B; 0.0-1.0 min. 35.0:65.0, 10.0 min. 12.5:87.5, 10.4-14.0 min. 35.0:65.0] coupled with a AB Sciex API Triple quadrupole mass spectrometer using a Turbo Ion Spray interface (TIS) in Multiple Reaction Monitoring (MRM) mode in positive and negative ion mode (pp. 21-23 of MRID 49970601). Injection volume was 100 μ L. Negative mode was employed for famoxadone, IN-JS940, IN-KF015 and IN-KZ007; positive mode was employed for IN-H3310. Two ion transitions (± 0.1) were monitored (quantitation and confirmatory, respectively) for each analyte as follows: *m/z* 373.1 \rightarrow 281.9 and *m/z* 373.1 \rightarrow 329.1 (water)/ *m/z* 373.1 \rightarrow 133.0 (soil) for famoxadone, *m/z* 256.9 \rightarrow 211.0 and *m/z* 256.9 \rightarrow 93.0 for IN-JS940, *m/z* 282.2 \rightarrow 195.0 and *m/z* 282.2 \rightarrow 239.2 for IN-KF015, *m/z* 389.1 \rightarrow 345.0 and *m/z* 389.1 \rightarrow 132.7 for IN-KZ007, and *m/z* 213.2 \rightarrow 170.9 and *m/z* 213.2 \rightarrow 153.0 (water)/ *m/z* 213.2 \rightarrow 153.0 (soil) for IN-H3310. Injection volumes were 50 μ L. Retention times were *ca.* 7.06, 5.10, 2.99, 4.54 and 3.51 minutes for famoxadone, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007, respectively.

In the ECM, the following precautions for the extraction procedure were reported: 1) glassware should be rigorously cleaned to avoid contamination since the analytes readily adsorb to the surfaces of containers when in mostly aqueous solution; 2) filtration should not be used; and 3) methanol should be used to rinse containers for environmental water samples since loss of analytes occurs in aqueous solutions (p. 30 of MRID 49970601).

In the ILV, the ECM was performed as written, except for the use of a different LC/MS/MS system (pp. 22-26 of MRID 49970602). A Shimadzu LC-30AD HPLC system (Agilent XDB-C18 column, 4.6 mm x 50 mm, 1.8 μ m column; column temperature 40°C) was coupled to an AB Sciex API 400 Triple Quad MS/MS system. Retention times were *ca.* 7.2, 5.2, 3.2, 4.7 and 3.7 minutes for famoxadone, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007, respectively. The monitored ions were the same as those of the ECM, \pm 0.2. No other modifications of the ECM were reported.

The Limits of Quantification (LOQs) for soil and water were 10 ppb (10 μ g/kg) and 0.10 ppb (0.1 μ g/L), respectively, in the ECM and ILV (pp. 10, 29 of MRID 49970601; p. 13 of MRID 49970602). The Limits of Detection (LODs) for soil and water were 3 ppb (3 μ g/kg) and 0.03 ppb (0.03 μ g/L), respectively, in the ECM (See Reviewer's Comment #2). The LODs were not reported in the ILV.

II. Recovery Findings

ECM (MRID 49970601) - Soil: Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of famoxadone and its metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007 in clay loam and sandy loam soil matrices at fortification levels of 10 ppb (10 μ g/kg; LOQ) and 100 ppb (100 μ g/kg; 10 \times LOQ; Table 3, pp. 32-35). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Drummer clay loam soil (25% sand, 41% silt, 34% clay; pH 6.5; 5.6% organic matter) was collected from Rochelle, Illinois, and Nambshiem sandy loam soil (60% sand, 30% silt, 10% clay; pH 7.5; 3.8% organic matter) was collected from Nambshiem, Germany (p. 19). Soil characterization was not specified as USDA.

ECM (MRID 49970601) - Water: Mean recoveries and RSDs were within guideline requirements for analysis of famoxadone and its metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007 in ground and surface water matrices at fortification levels of 0.10 ppb (0.10 μ g/L; LOQ) and 1.0 ppb (1.0 μ g/L; 10 \times LOQ; Table 4, pp. 36-39). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Kemblesville well water (pH 7.6; 68 mg equiv. CaCO₃/L; 222 ppm total dissolved solids) was collected from Kemblesville, Pennsylvania, and White Clay Creek Water (pH 8.1; 144 mg equiv. CaCO₃/L; 254 ppm total dissolved solids) was collected from White Clay Creek, Newark, Delaware (p. 19).

ILV (MRID 49970602) - Soil: Mean recoveries and RSDs were within guideline requirements for analysis of famoxadone and its metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007 in a sandy loam soil matrix at fortification levels of 10 ppb (10 μ g/kg; LOQ) and 100 ppb (100 μ g/kg; 10 \times LOQ; pp. 28-30). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Sassafras sandy loam soil (62% sand, 29% silt, 9% clay; pH 5.1 in 1:1 soil:water; 2.2% organic matter) was collected from Pike Creek in Newark, Delaware and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; p. 21; Appendix 2, pp. 170-173). The

method was validated in the first trial with insignificant modifications to the analytical instrumentation (p. 31).

ILV (MRID 49970602) - Water: Mean recoveries and RSDs were within guideline requirements for analysis of famoxadone and its metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007 in a surface water matrix at fortification levels of 0.10 ppb (0.10 µg/L; LOQ) and 1.0 ppb (1.0 µg/L; 10×LOQ; pp. 28-30). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Pike Creek surface water (pH 7.6; 114 mg equiv. CaCO₃/L; 184 ppm total dissolved solids) was collected from Pike Creek in Newark, Delaware and characterized by Agvise Laboratories, Northwood, North Dakota (p. 21; Appendix 2, pp. 174-175). The method was validated in the first trial with insignificant modifications to the analytical instrumentation (p. 31).

Table 2a. Initial Validation Method Recoveries for Famoxadone (DPX-JE874) and its Metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007, in Soil^{1,2}

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Clay Loam Soil						
Quantitation ion						
Famoxadone (DPX-JE874)	10 (LOQ)	5	70.4-85.7	77.5	7.0	9.0
	100	5	75.5-78.4	76.7	1.3	1.7
IN-H3310	10 (LOQ)	5	81.2-84.7	83.3	1.4	1.7
	100	5	80.2-82.0	81.5	0.7	0.8
IN-JS940	10 (LOQ)	5	85.3-94.6	88.0	3.7	4.3
	100	5	89.7-92.2	90.9	1.1	1.2
IN-KF015	10 (LOQ)	5	102.8-108.6	107.0	2.4	2.3
	100	5	92.4-99.5	96.1	2.3	2.4
IN-KZ007	10 (LOQ)	5	75.3-77.4	75.9	0.9	1.1
	100	5	71.8-75.7	73.8	1.7	2.3
Confirmatory ion						
Famoxadone (DPX-JE874)	10 (LOQ)	5	73.8-91.6	83.0	6.8	8.1
	100	5	74.2-80.5	77.0	2.3	3.0
IN-H3310	10 (LOQ)	5	89.5-97.4	94.1	3.2	3.5
	100	5	82.2-86.2	83.7	1.4	1.7
IN-JS940	10 (LOQ)	5	85.0-96.9	88.2	4.9	5.6
	100	5	92.6-97.5	95.0	2.0	2.1
IN-KF015	10 (LOQ)	5	93.6-118.8	106.1	8.9	8.4
	100	5	90.4-96.5	93.5	2.7	2.9
IN-KZ007	10 (LOQ)	5	72.4-75.6	73.8	1.2	1.7
	100	5	72.9-76.7	74.6	1.5	1.9
Sandy Loam Soil						
Quantitation ion						
Famoxadone (DPX-JE874)	10 (LOQ)	5	72.2-87.5	81.0	6.3	7.8
	100	5	90.1-94.9	92.2	2.2	2.3
IN-H3310	10 (LOQ)	5	96.6-100.0	98.7	3.2	3.2
	100	5	97.6-99.5	98.5	0.7	0.7
IN-JS940	10 (LOQ)	5	83.1-85.4	84.5	1.0	1.2
	100	5	88.5-90.8	89.7	0.9	1.0

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-KF015	10 (LOQ)	5	101.0-115.1	108.3	5.6	5.2
	100	5	97.7-106.8	102.4	3.3	3.3
IN-KZ007	10 (LOQ)	5	80.1-83.9	81.8	1.6	2.0
	100	5	80.7-82.7	81.6	0.9	1.1
Confirmatory ion						
Famoxadone (DPX-JE874)	10 (LOQ)	5	63.0-100.7	91.6	16.1	17.6
	100	5	74.9-83.4	80.3	3.6	4.4
IN-H3310	10 (LOQ)	5	90.7-112.4	101.0	8.0	7.9
	100	5	95.2-100.7	98.1	2.2	2.2
IN-JS940	10 (LOQ)	5	81.7-84.9	83.8	1.4	1.7
	100	5	90.5-93.9	92.3	1.2	1.3
IN-KF015	10 (LOQ)	5	88.8-107.1	97.7	7.2	7.4
	100	5	98.2-101.9	100.4	1.6	1.5
IN-KZ007	10 (LOQ)	5	76.7-84.2	80.5	3.2	3.9
	100	5	79.9-83.7	81.6	1.4	1.7

Data (uncorrected recovery results, pp. 24-26) were obtained from Table 3, pp. 32-35 of MRID 49970601.

1 The Drummer clay loam soil (25% sand, 41% silt, 34% clay; pH 6.5; 5.6% organic matter) was collected from Rochelle, Illinois, and Nambshiem sandy loam soil (60% sand, 30% silt, 10% clay; pH 7.5; 3.8% organic matter) was collected from Nambshiem, Germany (p. 19). Soil characterization was not specified as USDA.

2 Two ion transitions (± 0.1) were monitored (quantitation and confirmatory, respectively) for each analyte as follows: m/z 373.1 \rightarrow 281.9 and m/z 373.1 \rightarrow 133.0 for famoxadone, m/z 256.9 \rightarrow 211.0 and m/z 256.9 \rightarrow 93.0 for IN-JS940, m/z 282.2 \rightarrow 195.0 and m/z 282.2 \rightarrow 239.2 for IN-KF015, m/z 389.1 \rightarrow 345.0 and m/z 389.1 \rightarrow 132.7 for IN-KZ007, and m/z 213.2 \rightarrow 170.9 and m/z 213.2 \rightarrow 153.0 for IN-H3310 (p. 23).

Table 2b. Initial Validation Method Recoveries for Famoxadone (DPX-JE874) and its Metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007, in Water^{1,2}

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground/Well Water						
Quantitation ion						
Famoxadone (DPX-JE874)	0.10 (LOQ)	5	70.9-91.5	77.1	8.2	10.6
	1.0	5	73.6-95.8	80.3	8.9	11.1
IN-H3310	0.10 (LOQ)	5	88.7-93.8	90.8	1.9	2.1
	1.0	5	84.3-101.2	89.5	6.8	7.6
IN-JS940	0.10 (LOQ)	5	70.3-94.0	76.3	10.0	13.1
	1.0	5	80.4-94.0	83.5	5.9	7.1
IN-KF015	0.10 (LOQ)	5	88.9-114.6	96.4	10.4	10.8
	1.0	5	88.5-104.4	93.1	6.5	7.0
IN-KZ007	0.10 (LOQ)	5	75.5-100.1	83.2	9.7	11.6
	1.0	5	79.3-98.7	94.4	8.1	9.5
Confirmatory ion						
Famoxadone (DPX-JE874)	0.10 (LOQ)	5	75.0-97.8	85.6	9.7	11.3
	1.0	5	78.4-96.9	84.1	7.5	8.9
IN-H3310	0.10 (LOQ)	5	80.7-100.6	92.6	7.2	7.8
	1.0	5	86.7-95.6	90.8	4.2	4.6
IN-JS940	0.10 (LOQ)	5	70.3-88.3	75.7	7.3	9.6
	1.0	5	80.7-95.8	85.1	6.1	7.1

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-KF015	0.10 (LOQ)	5	81.2-114.9	93.2	13.1	14.1
	1.0	5	85.5-100.9	89.8	6.4	7.1
IN-KZ007	0.10 (LOQ)	5	75.2-99.2	82.2	9.7	11.9
	1.0	5	79.6-97.8	94.4	7.5	8.9
Surface/Stream Water						
Quantitation ion						
Famoxadone (DPX-JE874)	0.10 (LOQ)	5	74.5-81.6	77.3	3.0	3.8
	1.0	5	83.5-85.2	84.3	0.7	0.8
IN-H3310	0.10 (LOQ)	5	92.9-103.6	99.3	4.5	4.6
	1.0	5	87.2-95.1	91.4	2.9	3.2
IN-JS940	0.10 (LOQ)	5	85.2-95.0	88.0	4.2	4.8
	1.0	5	96.1-101.1	98.1	2.1	2.1
IN-KF015	0.10 (LOQ)	5	106.3-114.5	111.4	3.3	2.9
	1.0	5	98.6-104.2	101.1	2.4	2.4
IN-KZ007	0.10 (LOQ)	5	71.5-78.0	74.2	2.7	3.7
	1.0	5	72.9-75.2	73.8	0.9	1.3
Confirmatory ion						
Famoxadone (DPX-JE874)	0.10 (LOQ)	5	72.2-109.2	88.0	15.9	18.0
	1.0	5	81.2-85.5	83.0	1.8	2.2
IN-H3310	0.10 (LOQ)	5	70.5-108.2	88.9	17.3	19.5
	1.0	5	85.1-94.2	89.9	3.9	4.4
IN-JS940	0.10 (LOQ)	5	84.5-92.9	87.7	3.5	4.0
	1.0	5	96.2-101.2	98.2	1.9	2.0
IN-KF015	0.10 (LOQ)	5	104.4-116.9	111.7	5.2	4.7
	1.0	5	97.6-102.0	100.0	1.7	1.7
IN-KZ007	0.10 (LOQ)	5	71.4-78.2	74.2	2.5	3.4
	1.0	5	72.6-74.9	73.7	1.0	1.3

Data (uncorrected recovery results, pp. 24-26) were obtained from Table 4, pp. 36-39 of MRID 49970601.

1 The Kemblesville well water (pH 7.6; 68 mg equiv. CaCO₃/L; 222 ppm total dissolved solids) was collected from Kemblesville, Pennsylvania, and White Clay Creek Water (pH 8.1; 144 mg equiv. CaCO₃/L; 254 ppm total dissolved solids) was collected from White Clay Creek, Newark, Delaware (p. 19).

2 Two ion transitions (± 0.1) were monitored (quantitation and confirmatory, respectively) for each analyte as follows: m/z 373.1 \rightarrow 281.9 and m/z 373.1 \rightarrow 329.1 for famoxadone, m/z 256.9 \rightarrow 211.0 and m/z 256.9 \rightarrow 93.0 for IN-JS940, m/z 282.2 \rightarrow 195.0 and m/z 282.2 \rightarrow 239.2 for IN-KF015, m/z 389.1 \rightarrow 345.0 and m/z 389.1 \rightarrow 132.7 for IN-KZ007, and m/z 213.2 \rightarrow 170.9 and m/z 213.2 \rightarrow 153.0 for IN-H3310 (p. 23).

Table 3a. Independent Validation Method Recoveries for Famoxadone (DPX-JE874) and its Metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007, in Soil^{1,2}

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantitation ion						
Famoxadone (DPX-JE874)	10 (LOQ)	5	84-89	86	2.2	3
	100	5	88-94	92	2.4	3
IN-H3310	10 (LOQ)	5	90-98	95	3.2	3
	100	5	93-99	97	2.8	3
IN-JS940	10 (LOQ)	5	84-92	89	3.6	4
	100	5	83-87	86	1.5	2
IN-KF015	10 (LOQ)	5	93-99	97	2.6	3
	100	5	93-100	96	2.7	3
IN-KZ007	10 (LOQ)	5	80-87	84	2.5	3
	100	5	80-86	84	2.2	3
Confirmatory ion						
Famoxadone (DPX-JE874)	10 (LOQ)	5	70-104	84	12.6	15
	100	5	84-92	87	3.2	4
IN-H3310	10 (LOQ)	5	91-109	97	7.8	8
	100	5	93-100	97	2.6	3
IN-JS940	10 (LOQ)	5	81-89	85	2.8	3
	100	5	87-90	88	1.7	2
IN-KF015	10 (LOQ)	5	95-112	102	7.4	7
	100	5	89-96	93	2.9	3
IN-KZ007	10 (LOQ)	5	73-85	80	4.4	5
	100	5	83-85	84	0.8	1

Data (uncorrected recovery results, pp. 26-27) were obtained from pp. 28-30 of MRID 49970602.

1 The Sassafras sandy loam soil (62% sand, 29% silt, 9% clay; pH 5.1 in 1:1 soil:water; 2.2% organic matter) was collected from Pike Creek in Newark, Delaware, and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; p. 21; Appendix 2, pp. 170-175).

2 Two ion transitions (± 0.1) were monitored (quantitation and confirmatory, respectively) for each analyte as follows: m/z 373.1 \rightarrow 282.1 and m/z 373.1 \rightarrow 133.0 for famoxadone, m/z 256.9 \rightarrow 211.0 and m/z 256.9 \rightarrow 93.0 for IN-JS940, m/z 282.2 \rightarrow 195.0 and m/z 282.2 \rightarrow 239.2 for IN-KF015, m/z 389.1 \rightarrow 345.0 and m/z 389.1 \rightarrow 132.7 for IN-KZ007, and m/z 213.2 \rightarrow 170.9 and m/z 213.2 \rightarrow 153.1 for IN-H3310 (pp. 28-30).

Table 3b. Independent Validation Method Recoveries for Famoxadone (DPX-JE874) and its Metabolites, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007, in Water^{1,2}

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
Quantitation ion						
Famoxadone (DPX-JE874)	0.10 (LOQ)	5	67-87	76	8.8	12
	1.0	5	63-87	72	9.4	13
IN-H3310	0.10 (LOQ)	5	86-103	93	7.9	8
	1.0	5	80-104	90	8.6	10
IN-JS940	0.10 (LOQ)	5	75-97	89	8.6	10
	1.0	5	85-106	100	5.1	5
IN-KF015	0.10 (LOQ)	5	91-109	98	9.5	10
	1.0	5	81-110	91	11.3	12
IN-KZ007	0.10 (LOQ)	5	73-92	85	8.6	10
	1.0	5	67-101	79	13.2	17
Confirmatory ion						
Famoxadone (DPX-JE874)	0.10 (LOQ)	5	66-80	73	5.0	7
	1.0	5	62-92	71	12.0	17
IN-H3310	0.10 (LOQ)	5	71-97	84	10.1	12
	1.0	5	85-100	88	7.5	9
IN-JS940	0.10 (LOQ)	5	79-101	92	8.5	9
	1.0	5	95-109	99	5.9	6
IN-KF015	0.10 (LOQ)	5	84-104	93	9.5	10
	1.0	5	83-112	93	11.6	12
IN-KZ007	0.10 (LOQ)	5	71-95	82	10.2	12
	1.0	5	70-97	79	10.8	14

Data (uncorrected recovery results, pp. 26-27) were obtained from pp. 28-30 of MRID 49970602.

1 The Pike Creek surface water (pH 7.6; 114 mg equiv. CaCO₃/L; 184 ppm total dissolved solids) was collected from Pike Creek in Newark, Delaware, and characterized by Agvise Laboratories, Northwood, North Dakota (p. 21; Appendix 2, pp. 170-175).

2 Two ion transitions (± 0.1) were monitored (quantitation and confirmatory, respectively) for each analyte as follows: m/z 373.1 \rightarrow 282.1 and m/z 373.1 \rightarrow 329.1 for famoxadone, m/z 256.9 \rightarrow 211.0 and m/z 256.9 \rightarrow 93.0 for IN-JS940, m/z 282.2 \rightarrow 195.0 and m/z 282.2 \rightarrow 239.2 for IN-KF015, m/z 389.1 \rightarrow 345.0 and m/z 389.1 \rightarrow 132.7 for IN-KZ007, and m/z 213.2 \rightarrow 170.9 and m/z 213.2 \rightarrow 153.0 for IN-H3310 (p. 23).

III. Method Characteristics

The LOQs for soil and water were 10 ppb (10 µg/kg) and 0.10 ppb (0.1 µg/L), respectively, in the ECM and ILV (pp. 10, 29 of MRID 49970601; p. 13 of MRID 49970602). In the ECM, the LOQ was defined as the lowest fortification level at which average recoveries of 70-120% and a RSD of <20% was achieved. No justification of the LOQ was reported in the ILV. The LODs for soil and water were 3 ppb (3 µg/kg) and 0.03 ppb (0.03 µg/L), respectively, in the ECM (See Reviewer's Comment #2). In the ECM, the LOD was reported as approximately one-third of the LOQ. The LODs were not reported in the ILV. No calculations or comparisons to background levels were reported to justify the LOQ and LOD for the method.

Table 4a. Method Characteristics - Soil

Analyte		Famoxadone (DPX-JE874)	IN-H3310	IN-JS940	IN-KF015	IN-KZ007
Limit of Quantitation (LOQ)	ECM	10 ppb (10 µg/kg)				
	ILV					
Limit of Detection (LOD)	ECM	3 ppb (3 µg/kg)				
	ILV	Not reported				
Linearity (calibration curve r ² and concentration range)	ECM	r ² = 0.9990 (Q) r ² = 0.9978 (C)	r ² = 0.9999 (Q) r ² = 0.9996 (C)	r ² = 0.9999 (Q) r ² = 0.9998 (C)	r ² = 0.9989 (Q) r ² = 0.9998 (C)	r ² = 1.0000 (Q) r ² = 0.9992 (C)
	ILV ¹	r ² = 0.9966 (Q) r ² = 0.9916 (C)	r ² = 0.9970 (Q) r ² = 0.9815 (C)	r ² = 0.9970 (Q) r ² = 0.9968 (C)	r ² = 0.9976 (Q) r ² = 0.9972 (C)	r ² = 0.9988 (Q) r ² = 0.9958 (C)
	Concentration Range	(1.50-25.0 ng/mL)				
Repeatable	ECM ²	Yes at LOQ and 10×LOQ				
	ILV ^{3,4}	Yes at LOQ and 10×LOQ				
Reproducible	Yes at LOQ and 10×LOQ					
Specific	ECM	Yes, no matrix interferences were observed. Baseline was highly irregular in LOQ C ion chromatogram. ⁵	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed. Minor peak tailing was observed in most chromatograms.	Yes, no matrix interferences were observed. Baseline was irregular in LOQ C ion chromatogram. ⁵	Yes, no matrix interferences were observed.
	ILV	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed. The LOQ C ion peak was small but clearly resolved.	Yes, no matrix interferences were observed.		

Data were obtained from pp. 10, 17, 29; Table 3, pp. 32-35 (recovery data); Figure 6, pp. 45-46 (calibration curves); Figures 9-10, pp. 53-64 (chromatograms) of MRID 49970601; pp. 13, 28-30 (recovery data); Figure 11, pp. 113-122 (calibration curves); Figures 12-16, pp. 123-137 (chromatograms); Figure 22, pp. 153-162 (reagent blank chromatograms) of MRID 49970602. Q = Quantitation ion transition; C = Confirmatory ion transition.

1 Correlation coefficients (r²) values were reviewer-calculated from r values provided in the study report (Figure 11, pp. 113-122 of MRID 49970602; DER Attachment 2).

2 In the ECM, Drummer clay loam soil (25% sand, 41% silt, 34% clay; pH 6.5; 5.6% organic matter), collected from Rochelle, Illinois, and Nambshiem sandy loam soil (60% sand, 30% silt, 10% clay; pH 7.5; 3.8% organic matter), collected from Nambshiem, Germany, were used (p. 19 of MRID 49970601). Soil characterization was not specified as USDA.

3 In the ILV, Sassafras sandy loam soil (62% sand, 29% silt, 9% clay; pH 5.1 in 1:1 soil:water; 2.2% organic matter), collected from Pike Creek in Newark, Delaware, was used (USDA soil characterization; p. 21; Appendix 2, pp. 170-173 of MRID 49970602).

4 The ILV validated the method after one trial with insignificant modifications to the analytical instrumentation (p. 31 of MRID 49970602).

5 Based on Figure 9, p. 56 and Figure 10, p. 62 of MRID 49970601.

Linearity is satisfactory when $r^2 \geq 0.995$.

Table 4b. Method Characteristics - Water

Analyte		Famoxadone (DPX-JE874)	IN-H3310	IN-JS940	IN-KF015	IN-KZ007
Limit of Quantitation (LOQ)	ECM	0.10 ppb (0.1 µg/L)				
	ILV					
Limit of Detection (LOD)	ECM	0.03 ppb (0.03 µg/L)				
	ILV	Not reported				
Linearity (calibration curve r^2 and concentration range)	ECM	$r^2 = 0.9985$ (Q) $r^2 = 0.9951$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9989$ (C)	$r^2 = 0.9986$ (Q) $r^2 = 0.9991$ (C)	$r^2 = 0.9990$ (Q) $r^2 = 0.9993$ (C)	$r^2 = 0.9998$ (Q) $r^2 = 0.9992$ (C)
	ILV ¹	$r^2 = 0.9874$ (Q) $r^2 = 0.9831$ (C)	$r^2 = 0.9980$ (Q) $r^2 = 0.9968$ (C)	$r^2 = 0.9986$ (Q) $r^2 = 0.9992$ (C)	$r^2 = 0.9986$ (Q) $r^2 = 0.9958$ (C)	$r^2 = 0.9984$ (Q) $r^2 = 0.9960$ (C)
	Concentration Range	(1.80-30.0 ng/mL)				
Repeatable	ECM ²	Yes at LOQ and 10×LOQ				
	ILV ^{3,4}	Yes at LOQ and 10×LOQ				
Reproducible		Yes at LOQ and 10×LOQ				
Specific	ECM	Yes, no matrix interferences were observed. The LOQ C ion peak was very small; peak height was equivalent to baseline peak heights. ⁵	Yes, matrix interferences were <12% of the LOQ in the Q ion chromatograms (based on peak area). The LOQ C ion peak was small and peak integration was irregular. ⁵	Yes, no matrix interferences were observed. Minor peak tailing was observed in most chromatograms.	Yes, no matrix interferences were observed.	
	ILV	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed. The LOQ C ion peak was small but clearly resolved.	Yes, no matrix interferences were observed.		

Data were obtained from pp. 10, 17, 29; Table 4, pp. 36-39 (recovery data); Figure 6, pp. 47-48 (calibration curves); Figures 11-12, pp. 65-76 (chromatograms) of MRID 49970601; pp. 13, 28-30 (recovery data); Figure 11, pp. 113-122 (calibration curves); Figures 17-22, pp. 138-162 (chromatograms & reagent blank chromatograms) of MRID 49970602. Q = Quantitation ion transition; C = Confirmatory ion transition.

1 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Figure 11, pp. 113-122 of MRID 49970602; DER Attachment 2).

2 In the ECM, Kemblesville well water (pH 7.6; 68 mg equiv. CaCO_3/L ; 222 ppm total dissolved solids), collected from Kemblesville, Pennsylvania, and White Clay Creek Water (pH 8.1; 144 mg equiv. CaCO_3/L ; 254 ppm total dissolved solids), collected from White Clay Creek, Newark, Delaware, were used (p. 19 of MRID 49970601).

3 In the ILV, Pike Creek surface water (pH 7.6; 114 mg equiv. CaCO_3/L ; 184 ppm total dissolved solids), collected from Pike Creek in Newark, Delaware, was used (p. 21; Appendix 2, pp. 174-175 of MRID 49970602).

4 The ILV validated the method after one trial with insignificant modifications to the analytical instrumentation (p. 31 of MRID 49970602).

5 Based on Figure 11, p. 68 and Figure 12, p. 74 of MRID 49970601.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The LOQ in water (0.10 µg/L) is near but greater than the lowest toxicological level of concern in water (0.085 µg/L), which is the daphnid chronic toxicity NOEC (MRID 44946313).
2. In the ILV, linearity was unsatisfactory ($r^2 < 0.995$) for the quantitative and confirmatory ion analyses of famoxadone [$r^2 = 0.9874$ (Q); $r^2 = 0.9831$ (C)] in water and the confirmatory ion analyses of famoxadone ($r^2 = 0.9916$) and IN-H3310 ($r^2 = 0.9815$) in soil (Figure 6, pp. 45-48 of MRID 49970601). The reviewer noted that a confirmatory method is not always required when LC/MS/MS or GC/MS/MS is the primary identification method.
3. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method. For the ILV soil and water validations, only one matrix was tested versus two matrices tested in ECM. The ILV soil matrix, sandy loam soil, had a lower clay percentage than either of the ECM soil matrices. The hardness of the ILV surface water matrix was equivalent to the ECM surface water matrix, but the total dissolved solids was less than that of the ECM surface water matrix.
4. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 ILV (pp. 10, 29 of MRID 49970601; p. 13 of MRID 49970602). In the ECM, the LOQ was defined as the lowest fortification level at which average recoveries of 70-120% and a RSD of <20% was achieved. No justification of the LOQ was reported in the ILV. In the ECM, the LOD was reported as approximately one-third of the LOQ. The LODs were not reported in the ILV. No calculations or comparisons to background levels were reported to justify the LOQ and LOD for the method.
5. In the ECM representative chromatograms for the soil analyses, the baseline was highly irregular in LOQ confirmatory ion chromatogram for famoxadone, especially in the case of clay loam soil (Figure 9, p. 56; Figure 10, p. 62 of MRID 49970601). Also, the baseline was also irregular in LOQ confirmatory ion chromatogram for IN-KF015, especially in the case of sandy loam soil. The reviewer noted that a confirmatory method is not always required when LC/MS/MS or GC/MS/MS is the primary identification method. Minor peak tailing was observed for IN-JS940 in most soil chromatograms.

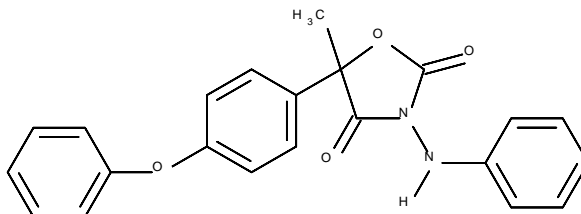
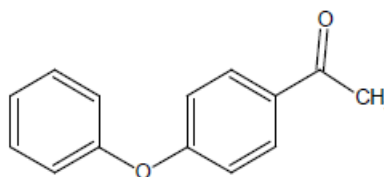
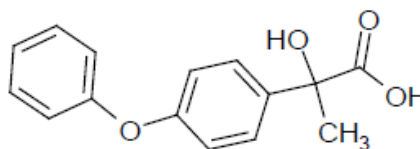
In the ECM representative chromatograms for the water analyses, the famoxadone LOQ confirmatory ion peak was very small; the analyte peak height was equivalent to baseline peak heights (Figure 11, p. 68; Figure 12, p. 74 of MRID 49970601). Also, the IN-H3310 LOQ confirmatory ion peak was small and peak integration was irregular. The reviewer noted that a confirmatory method is not always required when LC/MS/MS or GC/MS/MS is the primary identification method.

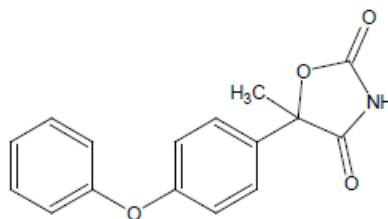
6. In the ECM, the LODs for soil and water were reported as 0.3 µg/kg and 0.03 µg/L, respectively; however, the reviewer reported the LOD for soil as 3 µg/kg since the ECM

- reported that the LOD was considered to be approximately one-third of the LOQ (p. 29 of MRID 49970601). The reviewer determined that 0.3 µg/kg was a typographical error.
7. The reviewer noted that the RSDs for the famoxadone LOQ confirmation ion transitions in sandy loam soils differed significantly from the RSDs for the famoxadone LOQ quantitative ion transitions in the ECM (RSD 7.8% Q, 17.6% C) and ILV (RSD 3% Q, 15% C; Table 3, pp. 32-35 of MRID 49970601; pp. 28-30 of MRID 49970602).
 8. Communications between the ILV and study monitor were summarized as 1) clarification/approval of the protocol and method, 2) acquisition of analytical standard, and 3) approval of final ILV results (p. 31 of MRID 49970602). The full list of communications was maintained with the study raw data.
 9. In the ECM, it was reported that the standards and samples were stable in organic solutions for up to 1 month of refrigerated storage ($4 \pm 2^\circ\text{C}$; p. 30 of MRID 49970601). Extracts for LC/MS/MS should be used within 48 hours or prepared again.
 10. It was reported for the ILV that one sample set (two controls, five LOQ fortifications, and five 10×LOQ fortifications) required one working day (8 hours) with LC-ESI-MS/MS performed unattended (p. 31 of MRID 49970602). The reported time requirement was the same in the ECM (pp. 29-30 of MRID 49970601).

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**Famoxadone (DPX-JE874)****IUPAC Name:** (RS)-3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione**CAS Name:** 5-Methyl-5-(4-phenoxyphenyl)-3-(phenylamino)-2,4-oxazolidinedione**CAS Number:** 131807-57-3**SMILES String:** c1cc(Oc2ccccc2)ccc1C3(C)C(=O)N(Nc4ccccc4)C(=O)O3**IN-H3310****IUPAC Name:** 1-(4-Phenoxyphenyl)ethanone**CAS Name:** Not reported**CAS Number:** Not available**SMILES String:** Not found**IN-JS940****IUPAC Name:** Not reported**CAS Name:** α -Hydroxy- α -methyl-4-phenoxybenzeneacetic acid**CAS Number:** Not available**SMILES String:** Not found

IN-KF015**IUPAC Name:** 5-Methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione**CAS Name:** Not reported**CAS Number:** Not available**SMILES String:** Not found**IN-KZ007****IUPAC Name:** Not reported**CAS Name:** 5-[-(4-Hydroxyphenoxy)phenyl]-5-methyl-3-(phenylamino)-2,4-oxazolidinedione**CAS Number:** Not available**SMILES String:** Not found