2.0 INTRODUCTION

This method was developed to support the registration effort for famoxadone (DPX-JE874), an active ingredient in several DuPont fungicide products used for the control of various fungal diseases in selected crops. This method satisfies requirements in Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market, and U.S. EPA Ecological Effects Test Guidelines: OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation Guidelines for the determination of residues of famoxadone, IN-H3310, IN-JS940, IN-KF015, and IN-KZ007 in soil and water (ground and surface).

This method determines residues of famoxadone (DPX-JE874) and famoxadone metabolites (IN-H3310, IN-JS940, IN-KF015 and IN-KZ007) in soil and water. The LOQ for each analyte in soil is 10 ppb (10 µg/kg) based on fresh weight for soil. The LOQ for each analyte in water is 0.10 ppb (0.10 µg/L).

In this method, LC/MS/MS analysis conditions were applied to the analysis of famoxadone, IN-H3310, IN-JS940, IN-KF015 and IN-KZ007 for soil and water matrices.

1) For soil: Samples were extracted in methanol / buffered, acidic water solution (4/1, v/v).

2) For water: Samples were adjusted to approximately 5% acetonitrile and 0.01% formic acid. The analytes were extracted from the solution onto a C_{18}-SPE cartridge and recovered in acidified acetonitrile and acidified methanol.

3) For all matrices: Aliquots of the acidified acetonitrile/methanol final sample extract were diluted for quantitative analysis by LC/MS/MS using an ESI in negative and positive ion mode to detect the molecular ions (M-1 or M+1) for each analyte.

Chromatographic separation of the analytes was achieved using an Eclipse XDB C18 column (4.6mm x 50 mm, 1.8 µm diameter particle size) and 0.01M aqueous formic acid and 0.01M formic acid in methanol as mobile phases for gradient elution of the analytes. Validation data is presented for the quantitation and confirmation ions transitions.

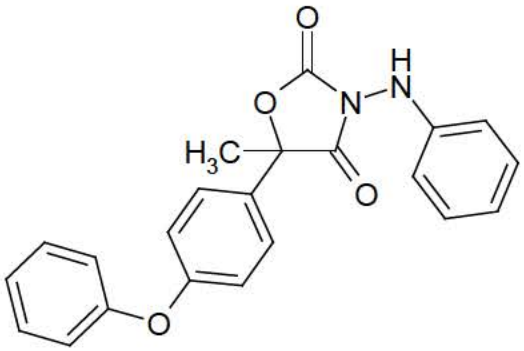
3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified; note any specifications in the following descriptions before making substitutions. Substitutions should only be made if equivalency/suitability has been verified with acceptable control and fortification recovery data.

3.1 Test Substances

The test substances examined in this study were famoxadone (DPX-JE874), IN-H3310, IN-JS940, IN-KF015 and IN-KZ007. The test substances were supplied by E. I. du Pont de Nemours and Company, DuPont Crop Protection, Newark, DE. Information pertaining to the characterization and stability of the test substance is archived by DuPont Crop Protection, E. I. du Pont de Nemours and Company, Newark, Delaware.

The structures and specific information for the test substances follow:



Famoxadone

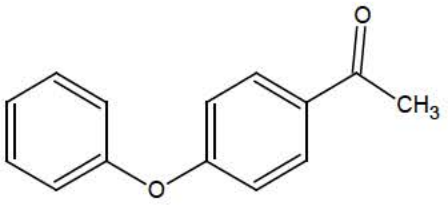
DuPont Code: DPX-JE874

CAS Chemical Name: 5-methyl-5-(4-phenoxyphenyl)-3-(phenylamino)-2,4-oxazolidinedione

CAS Registry Number: 131807-57-3

IUPAC Chemical Name: 3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione

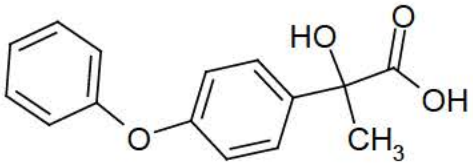
Molecular Weight: 374.40 g/mole
Monoisotopic Mass: 374.13 g/mole



DuPont Code: IN-H3310

IUPAC Chemical Name: 1-(4-phenoxyphenyl)ethanone

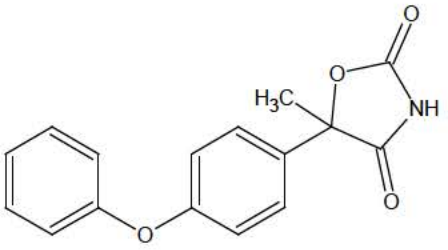
Molecular Weight: 212.24 g/mole



DuPont Code: IN-JS940

CAS Chemical Name: α-hydroxy- α - methyl-4-phenoxybenzeneacetic acid

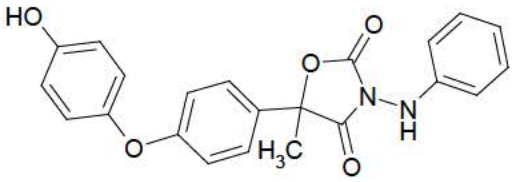
Molecular Weight: 258.28 g/mole
Monoisotopic Mass: 258.09 g/mole



DuPont Code: IN-KF015

IUPAC Chemical Name: 5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione

Molecular Weight: 283.28 g/mole



DuPont Code: IN-KZ007

CAS Chemical Name: 5-[4-(4-hydroxyphenoxy)phenyl]-5-methyl-3-(phenylamino)-2,4,-oxazolidinedione

Molecular Weight: 390.40 g/mole
Monoisotopic Mass: 390.12 g/mole

3.2 Equipment

Instrumentation

LC system, 1200 Series HPLC system with temperature controlled autosampler (Agilent Technologies, Wilmington, DE)

Mass Spectrometer System, API 4000 triple quadrupole mass spectrometer using a Turbo Ion Spray and Analyst version 1.4 software (Applied Biosystems/MDS Sciex, Foster City, CA)

Mettler AT261 Delta Range analytical balance (Mettler Instrument Corp., Hightstown, N.J.)

Centrifuge: Thermo Scientific Heraeus Multifuge X3

Digital pipettors, adjustable volume, 10-300 µL, 50-1000 µL, and 100-5000 µL (Biohit Oyi, Helsinki, Finland)

Ultrasonicator – Bransonic® Ultrasonic Cleaner, Model 3210 (Branson Ultrasonics Corp., Danbury, CT)

Vortex Mixer – VWR Vortex-2 Genie® Model G-560 (VWR Scientific)

Chromatographic Supplies

HPLC column: Eclipse XDB C18 HPLC column, 50 mm × 4.6 mm, 1.8-µm diameter particle size, Part No. 922975-902

HPLC Sample Vials: amber vials, 2.0-mL capacity, Part No. 5182-0716 (Agilent Technologies)

Labware

Transfer pipets – Samco® B/B PET transfer pipets, Catalog No. 336 (VWR Scientific)

1000 µL pipet tips (Eppendorf)

300 µL and 5000 µL pipet tips (Biohit Oyi, Helsinki, Finland)

Centrifuge Tubes: BD Falcon polypropylene 15-mL (Catalog No. 62406-200) and 50-mL (Catalog No. 21008-940) conical tubes (VWR Scientific Co., Bridgeport, NJ)

Plastic 250-mL centrifuge bottles (Part No. 3120-0250) (Thermo Scientific)

Solid Phase Extraction Cartridge (**do not substitute**): Isolute®, C_{18} bonded phase, 3-cc/500mg, Part No. 220-0050-B (Biotage)

Solid Phase Extraction Apparatus – Visiprep™ DL SPE Manifold, Catalog No. 5-7044 (Supelco, Inc., Bellefonte, PA)

Solid Phase Extraction Column Adapters – for 1-, 3-, and 6-mL Bond Elut Columns, Solid Phase Extraction Plastic Reservoirs – 60-mL size, Part No. 1213-1012 (Varian, Inc.)

Disposable flow control valve liners for SPE, Catalog No. 57059 (Supelco, Bellefonte, Penn.)

3.3 Reagents and Standards

Acetonitrile (ACN) – OmniSolv HPLC grade AX0142-1 (EMD Chemicals)

Formic Acid (HCOOH) – EMD Chemicals, 98%, ACS Reagent, Catalog No. FX0440-5

Methanol (MeOH) – OmniSolv HPLC grade MX0488-1 (EMD Chemicals)

Water (H_2O) – In-house Milli-Q system (Millipore)

DPX-JE874-313 (famoxadone) analytical standard, 99.8% Purity, Lot No. AG0429-181, Stock No. 1001890, Expiration: 28-Feb-2019 (DuPont Crop Protection, E. I. du Pont de Nemours and Company, Newark, DE).

IN-H3310-003 analytical standard, 98.8% Purity, Lot No. E87531-42, Expiration: 15-Feb-2020 (DuPont Crop Protection).

IN-JS940-003 analytical standard, 95.9% Purity, Lot No. GVK-DU-P554-1, Expiration: 14-Sep-2015 (DuPont Crop Protection).

IN-KF015-003 analytical standard, 99.7% Purity, Lot No. GVK-DU-P555-1, Expiration: 17-Dec-2019 (DuPont Crop Protection).

IN-KZ007-004 analytical standard, 99.7% Purity, Lot No. GVK-DU-P556-1, Expiration: 02-Jul-2020 (DuPont Crop Protection).

3.4 Safety and Health

No unusually hazardous materials are used in this method. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment should be used.

4.0 METHODS

4.1 Principle of the Analytical Method

The analytes (famoxadone, IN-H3310, IN-JS940, IN-KF015, and IN-KZ007) were extracted from soil samples using a solution of methanol/30 mM aqueous sodium acetate (adjusted to pH 2.5-3) (4/1, v/v,). Extracts were diluted to 50 mL with 0.01M aqueous formic acid and then centrifuged to separate particulate matter.

Water samples were adjusted to approximately 5% acetonitrile and 0.01% formic acid. The analytes (famoxadone, IN-H3310, IN-JS940, IN-KF015, and IN-KZ007) were extracted from solution onto a C_{18}-SPE cartridge and recovered in 2.5 mL 0.01% formic acid in acetonitrile and 2.5 mL 0.01% formic acid in methanol. Final extracts were diluted 1:1 with 0.01M aqueous formic acid for analysis

Aliquots from each extract were analyzed using reversed phase liquid chromatography (LC) and electrospray mass spectrometry/ mass spectrometry (MS/MS).

4.1.1 Soil Samples

A soil sample size of 10.0±0.1 g was selected to provide sufficient sample to detect and quantify famoxadone and metabolites at an LOQ of 10 ppb (10 µg/kg). Samples were extracted with 2 volumes (20 and 15 mL) of methanol/buffered, acidic water solution (4/1**,** v/v,). The aqueous component of the extraction solution was 30 mM aqueous sodium acetate adjusted to pH 2.5-3 and contributes counter ions in the extraction to improve analyte recoveries in soil matrices. Extracts were diluted to 50 mL with 0.01M aqueous formic acid. Final extracts were centrifuged to separate particulate matter. An aliquot from each extract was added to LC autosampler vials for analysis.

4.1.2 Water Samples

A water sample size of 0.2 L was selected to provide sufficient sample to detect and quantify famoxadone and metabolites at an LOQ of 0.1 ppb (0.1 µg/L). Samples

were adjusted to approximately 0.01% formic acid and 5% acetonitrile to promote solubility and stability of the analytes. The sample solutions were filtered through a C_{18}-SPE (3cc/500mg) cartridge. The analytes are eluted from the SPE cartridge with 0.01% formic acid in acetonitrile (2.5 mL) and 0.01% formic acid in methanol (2.5 mL). Final extracts were diluted 1:1 with corresponding solution to make analysis samples.

4.1.3 *LC/MS/MS Analysis*

An endcapped C_{18} stationary phase with 1.8 µm diameter particle size was used for best resolution and peak shape of the analytes. IN-H3310 has only positive fragments with a retention time between famoxadone and IN-KF015. Samples were injected twice and analyzed in both negative and positive ion modes.

4.2 Analytical Procedure

4.2.1 *Glassware & Equipment Cleaning Procedures*

Due to the potential for contamination resulting from the low detection limit, disposable equipment was used for sample preparation when possible. If glassware is used, care should be taken to minimize the potential for contamination due to insufficient cleaning of the glassware. Care should be taken to avoid working with high levels of the analyte being monitored in the same laboratory where samples are being extracted and analyzed.

4.2.2 *Preparation & Stability of Reagent Solutions*

30 mM aqueous sodium acetate, pH 2.5-3

Dilute 2.46 g of sodium acetate per liter of distilled, deionized water and adjust pH to 2.5-3 with concentrated phosphoric acid in beaker with stir bar mixing. Transfer solution to capped, labeled bottle for use. It may be stored at room temperature and should be prepared at least monthly.

Soil Extraction Solution - Methanol/30 mM aqueous sodium acetate, pH 2.5-3 (4/1, v/v)

Per liter volume, add 200 mL of 30 mM aqueous sodium acetate (pH 2.5-3) and dilute to final volume with methanol. Mix thoroughly. The solution is ready for use. It may be stored at room temperature and should be prepared at least every 2 weeks.

0.01% formic acid in acetonitrile (SPE Eluting Solution)

Per liter volume, add 100 µL of concentrated formic acid to acetonitrile and dilute to final volume. Mix thoroughly. This solution may be stored at room temperature and should be prepared weekly.

0.01% formic acid in methanol (SPE Eluting Solution)

Per liter volume, add 100 µL of concentrated formic acid to methanol and dilute to final volume. Mix thoroughly. This solution may be stored at room temperature and should be prepared weekly.

0.01 M Aqueous formic acid Mobile Phase

Per 2-liter volume, add 0.76 mL of concentrated formic acid to pre-filtered distilled, deionized water and dilute to final volume. Mix thoroughly. This solution may be stored at room temperature and should be prepared weekly. Recommend replacing bottle and solution to maintain consistent solution for analysis.

0.01 M formic acid in methanol Mobile Phase

Per 2-liter volume, add 0.76 mL of concentrated formic acid to pre-filtered or glass distilled methanol and dilute to final volume. Mix thoroughly. This solution may be stored at room temperature and should be prepared weekly. Recommend replacing bottle and solution to maintain consistent solution for analysis.

4.2.3 *Stock Standard Solutions Preparation and Stability*

Weigh 12.5 mg ± 0.50 mg (recorded to the nearest 0.01 mg) of each analytical standards of famoxadone (DPX-JE874), IN-H3310, IN-JS940, IN-KF015, and IN-KZ007 in separate 25-mL volumetric flasks. Dissolve and dilute to the mark with methanol to make a stock standard solutions of approximately 500 µg/mL of famoxadone (DPX-JE874), IN-H3310, IN-JS940, IN-KF015, and IN-KZ007.

These stock standard solutions are stable for at least 3 months when stored capped at ≤ -10°C.

4.2.4 *Fortification Solutions Preparation and Stability*

Prepare a 2.5-µg/mL MIX fortification solution in methanol by adding 50 µL of each 500.0-µg/mL stock standards of DPX-JE874, IN-H3310, IN-JS940, IN-KF015, and IN-KZ007 into a 10-mL volumetric flask. Dilute to the mark with methanol, cap and mix to homogeneity.

Prepare a 0.25 µg/mL MIX fortification standard in acetonitrile by adding 1.0 mL of the 2.5-µg/mL MIX fortification solution of DPX-JE874, IN-H3310, IN-JS940, IN-KF015, and IN-KZ007 into a 10-mL volumetric flask. Dilute to the mark with acetonitrile and mix to homogeneity.

These solutions are stable for at least 3 months when stored capped at ≤-10°C.

4.2.5 *Calibration Standards Preparation and Stability*

Two sets of calibration curves were used during this study; one for soil samples and one for water samples. Using measuring pipets (0.010 – 1000 µL), prepare calibration standards ranging from 1.50 to 25.0 ng/mL for soil samples and from 1.80 to 30.0 ng/mL for water samples (or in concentrations expected to cover the range of famoxadone and its metabolites in the investigative samples).

Calibration standards were prepared concurrently with sample sets for LC/MS/MS analysis. The calibration standards for soil samples were prepared from dilutions of the 0.250 µg/mL Fortification Solution and the 25.0 ng/mL Calibration Standard. The calibration standards for water samples were prepared from dilutions of the 0.250 µg/mL Fortification Solution and the 30.0ng/mL Calibration Standard. A 50:50 mixture of 0.01% formic acid in methanol and 0.01% formic acid in acetonitrile is

used to make the calibration standards. The final solutions are approximately 1:1, methanol/ACN:water, and 0.01M formic acid by volume which is consistent with sample extract analysis solutions. Calibration Standards should be used promptly within a 48-hour period.

Keep all chromatographic standards at or below 4°C after preparation. The tables below describe how standards were prepared for the validation work presented in this report:

SOIL CALIBRATION STANDARDS					
FORT. SOLUTIONN. USED	FORT. SOLUTION ADDED (ML)	0.01% FORMIC ACID IN MEOH/ACN MIX ADDED (ML)	0.01M FORMIC ACID IN H_2O ADDED (ML)	FINAL VOLUME (ML)	CALIBRATION STANDARD NG/ML)
0.250 µg/mL fortification	0.10	0.40	0.50	1.0	25.0
0.250 µg/mL fortification	0.08	0.42	0.50	1.0	20.0
0.250 µg/mL fortification	0.04	0.46	0.50	1.0	10.0
25.0 ng/mL cal. std.	0.08	0.42	0.50	1.0	2.0
25.0 ng/mL cal. std.	0.06	0.44	0.50	1.0	1.5

WATER CALIBRATION STANDARDS					
FORT. SOLUTION. USED	FORT. SOLUTION. ADDED (ML)	0.01% FORMIC ACID IN MEOH/ACN MIX ADDED (ML)	0.01M FORMIC ACID IN H_2O ADDED (ML)	FINAL VOLUME (ML)	CALIBRATION STANDARD NG/ML)
0.250 µg/mL fortification	0.12	0.38	0.50	1.0	30.0
0.250 µg/mL fortification	0.08	0.42	0.50	1.0	20.0
0.250 µg/mL fortification	0.04	0.46	0.50	1.0	10.0
30.0 ng/mL cal. std.	0.08	0.42	0.50	1.0	2.4
30.0 ng/mL cal. std.	0.06	0.44	0.50	1.0	1.8

4.2.6 *Source of Samples*

Listed below are sources and physical characteristics of the soil and water samples tested in this study:

MATRIX	SOURCE	TYPE	pH	% OM	% SAND	% SILT	% CLAY	CEC (MEQ/100G)
Drummer soil	Rochelle, IL	Clay Loam	6.5	5.6	25	41	34	28.8
Nambsheim soil	Nambsheim, Germany	Sandy Loam	7.5	3.8	60	30	10	10.4

MEASUREMENT	WHITE CLAY CREEK WATER*	KEMBLESVILLE WELL WATER*
pH	8.1	7.6
Calcium (ppm)	35	17
Magnesium (ppm)	13	6.3
Sodium (ppm)	18	12
Hardness (mg equivalent $CaCO_3$/L)	144	68
Conductivity (mmhos/cm)	0.35	0.20
Sodium Adsorption Ratio (SAR)	0.67	0.63
Total Dissolved Solids (ppm)	254	222
Turbidity (NTU)	0.59	0.81

* Sources: Surface/Stream water, White Clay Creek, Newark, DE, U.S.A.
Ground/Well Water: Kemblesville, PA, U.S.A.

4.2.7 *Preparation & Storage of Samples*

4.2.7.1 *Soil Samples*

Subsamples (10.0 g) of the individual soil types were weighed into tared 50-mL polypropylene centrifuge tubes for analysis.

4.2.7.2 *Water Samples*

Well and stream water samples were collected in individual clean 1.0-gallon Carboys and tightly sealed. The pH of each of the collected water samples was measured. 200-mL water subsamples were prepared for analysis by adjusting to approximately 5% acetonitrile and 0.01% formic acid by volume. 10 mL of acetonitrile and 20 µL of concentrated formic acid were added to 250-mL plastic centrifuge bottles and diluted to a total volume of 200 mL with the water sample.

4.2.8 *Sample Fortification Procedure*

4.2.8.1 *Soil Samples*

The 2.5-µg/mL Fortification Solution containing a mixture of the 5 analytes was used to fortify 10.0-g soil (fresh weight) subsamples at 10 µg/kg (LOQ) and 100 µg/kg

(10×LOQ), respectively. Soil subsample fortifications were made according to the following table:

SAMPLE IDENTIFICATION	SAMPLE WEIGHT (KG)	FORT. SOLUTION. (µG/ML)	FORT. SOLUTION. ADDED (ML)	FORT. LEVEL (PPB, µG/KG)
LOQ	0.010	2.5	0.040	10
10×LOQ	0.010	2.5	0.400	100

4.2.8.2 Water Samples

2.5-µg/mL and 0.25-µg/mL Fortification Solutions containing a mixture of the 5 analytes were used to fortify 200-mL water subsamples at 0.10 µg/L (LOQ) and 1.0 µg/L (10×LOQ), respectively. Fortified samples were gently swirled prior to extraction. Water subsample fortifications were made according to the following table.

SAMPLE IDENTIFICATION	SAMPLE VOLUME (L)	HCOOH ADDED (ML)	ACN ADDED (ML)	FORT. SOLUTION. (µG/ML)	FORT. SOLUTION. ADDED (ML)	FORT. LEVEL (PPB, µG/L)
LOQ	0.200	0.10	10	0.25	0.08	0.10
10×LOQ	0.200	0.10	10	2.5	0.08	1.0

4.2.9 Analyte Extraction/Purification Procedures

4.2.9.1 Soil

Extraction Procedure

1. Add 20 mL of MeOH/30 mM NaOAc (80%/20%, v/v, adjusted to pH 2.5-3) Extraction Solution, vortex mix for ≥30 sec, sonicate for 5 min, vortex mix momentarily, and centrifuge for ≥5 min at ≥2500 rpm.
2. Decant solution into clean 50-mL polypropylene centrifuge tube.
3. Repeat Steps 1-2 with 15 mL for a total of 35-mL extraction solution used
4. Dilute to 50 mL with 0.01M aqueous formic acid, cap, and mix thoroughly.

Purification Procedure

5. Centrifuge for ≥5 min at ≥2500 rpm to separate extract from particulate matter.
6. An aliquot from each extract was added to LC autosampler vial for analysis

4.2.9.2. Water

1. Attach a C_{18} SPE (3-cc/500-mg) cartridge to an extraction manifold. Condition with 5 mL of methanol, and 5 mL of Milli-Q H_2O. STOP FLOW as last solution reaches top of sorbent.
   [***SPE sorbent must not be allowed to dry through Step 2***]

2. Add 2.5 mL of sample to SPE cartridge packing and, attach SPE reservoir using an adapter, and begin addition of rest of sample. [Once all samples are flowing START VACUUM FLOW and adjust vacuum to a fast drip rate (2–3 mL/min).]
3. Continue full vacuum after solution passes through SPE Cartridge to dry. BREAK VACUUM.
4. Place 15-mL graduated centrifuge tubes under SPE cartridges.
5. Add 2.5 mL of 0.01% formic acid in acetonitrile to headspace of respective SPE cartridge. Open stopcock under SPE cartridge and allow solvent to flow at slow drip rate through cartridge into collection tube. If necessary, apply gentle vacuum to get flow started and then allow gravity flow for the remainder of the solution.
6. Add 2.5 mL of 0.01% formic acid in methanol to headspace of respective SPE cartridge. Open stopcock under SPE cartridge and allow solvent to flow at slow drip rate through cartridge into collection tube.
7. Recover collection tubes, and vortex mix.
8. For all extracts, add 0.40 mL of 0.01M aqueous formic acid to autosampler vials labeled for samples, then transfer 0.60 mL of the final extract to respective autosampler vials, cap, and vortex mix for LC/MS analysis.

4.3 Instrumentation

4.3.1 Description

LC/MS/MS system:

1. An Agilent 1200 Series HPLC system connected to an AB Sciex API 4000 Triple quadrupole mass spectrometer using a Turbo Ion Spray interface (TIS) at atmospheric pressure was used for this study. Instrument operation and data acquisition were controlled using Analyst 1.4.1 software. The mass spectrometer was operated in MRM mode in both negative and positive ion mode for quantitative analysis. Two fragments are monitored for all three compounds, one fragment for quantitation and the other for confirmation.

4.3.2 *Operating Conditions*

The HPLC and MS operating conditions used during method validations are summarized in the following tables:

HPLC Conditions

SYSTEM:	AGILENT 1200 HPLC			
Column:	Zorbax® XDB C18, 4.6 mm × 50 mm, 1.8-μm dp			
Column Temperature:	40°C			
Injection Volume:	100 μL			
Autosampler Temperature	4°C			
Flow Rate:	0.5 mL/min			
Conditions:	A: 0.01M aqueous formic acid			
	B: 0.01M formic acid in methanol			
	Time	%A	%B	Flow (mL/min)
	0.00	35.0	65.0	0.50
	1.00	35.0	65.0	0.50
	10.0	12.5	87.5	0.50
	10.4	35.0	65.0	0.50
	14.0	35.0	65.0	0.50
ANALYTE	RETENTION TIMES (MINUTES)			
Famoxadone	~7.06			
IN-H3310	~5.10			
IN-JS940	~2.99			
IN-KF015	~4.54			
IN-KZ007	~3.51			
Total Run Time:	14.0			

A six port electronically activated switching valve was used to direct the flow to waste prior to and following the elution of the compounds of interest. The use of this valve reduces source contamination and enables additional samples to be analyzed prior to source cleaning. The valve switching times are given in the following table.

TIME (MINUTES)	COLUMN ELUATE FLOW
0.1-2.5	Waste
2.5-10.0	MS source
10.0-End	Waste

MS Conditions

MS SYSTEM:		APPLIED BIOSYSTEMS SCIEX API4000 LC/MS/MS						
ANALYTE MONITORED	IONS MONITORED (AMU)		DP[a] (V)	EP[b] (V)	CE[c] (V)	CXP[d] (V)	DWELL TIME (MS)	ACQUISITION TIME (MIN)
Famoxadone	Q	373.1 → 281.9 ± 0.1	-65	-10	-26	-19	150	0.0 – 14
	C	373.1 → 329.1 ± 0.1			-22	-9		
	C**	373.1 → 133.0 ± 0.1			-22	-9		
IN-JS940	Q	256.9 → 211.0 ± 0.1	-50	-10	-26	-3	150	
	C	256.9 → 93.0 ± 0.1			-54	-3		
IN-KF015	Q	282.2 → 195.0 ± 0.1	-55	-10	-24	-11	150	
	C	282.2 → 239.2 ± 0.1			-21	-15		
IN-KZ007	Q	389.1 → 345.0 ± 0.1	-65	-10	-16	-9	150	
	C	389.1 → 132.7 ± 0.1			-30	-23		
IN-H3310*	Q	213.2 → 170.9 ± 0.1	65	10	21	10	150	0.0 – 14
	C	213.2 → 153.0 ± 0.1			27	8		
	C**	213.2 → 153.0 ± 0.1			35	8		

Scan type/Polarity:	MRM/Negative/Positive
Ion Source Voltage:	ESI, -4500/4500
Collision Gas (CAD):	6 psig
Curtain Gas (CUR):	15 psig
Nebulizer Gas (GS1):	65 psig
Heater Gas (GS2):	65 psig
Source Heater (TEM):	650°C
Interface Heater (ihe):	ON
Resolution Q1	Unit
Resolution Q2	Unit

a Declustering Potential
b Entrance Potential
c Collision Energy
d Collision Exit Potential
(Q) Quantitation Ion
I Confirmatory Ion

* DPX-JE874, IN-JS940, IN-KF015, IN-KZ007 were analyzed together by LC/MS/MS under negative polarity; IN-H3310 was injected and analyzed separately by LC/MS/MS under positive polarity

** These confirmatory transitions for famoxadone and IN-H3310 were used for soil analysis.

4.3.3 *Calibration Procedures*

Use standard mass spectrometer tuning and calibration techniques. If confidence in the mass calibration needs to be established (modern mass spectrometers under digital control generally do not need frequent mass calibration, especially for quantitative modes), use vendor recommended calibrating solution. Initial optimization of the MS response of each analyte may be accomplished by infusing the analyte into the ion source using the mobile phase (50% aqueous/50% organic mobile phase) as the solvent. Further optimization maybe accomplished by flow injection analysis with the flow rate and mobile phase adjusted to the elution conditions of the analytes from the HPLC column. For each analyte, at least two parent-daughter ion transitions (if possible) should be generated and each should have a signal-to-noise ratio ≥ 3.

Instrument calibration was based on the average response factor (RF; defined here as analyte peak area response divided by concentration) obtained for external calibration standards of famoxadone and its metabolites. Two ion transitions per analyte were monitored, as shown in Section 4.3.1; instrument calibration was performed using the Excel® functions AVERAGE, STDEV, and RSD. Acceptance criteria for valid quantitation are: (1) a %RSD ≤20% for the individual calibration standard response factors and (2) an r^2 value >0.99 for linear regression analysis of the calibration standards.

The calibrated range of instrument response was 0.0015 µg/mL to 0.025 µg/mL for soil samples and 0.0018 µg/mL to 0.030 µg/mL for water samples. Typically, five calibration standards were interspersed with sample extracts for quantitative LC/MS/MS analysis.

4.3.4 *Sample Analysis*

Preliminary runs of 1–2 calibration standards are necessary to check the period windows to be correct (if not, they should be adjusted before run). If multiple sets are analyzed, a solvent blank injection should be made between the last and first injections of the sets to minimize risk of carryover from high concentration sample to a low concentration calibration standard. Generally, the injection sequence was organized from lowest to highest expected analyte concentrations. Calibration standard runs were intermixed with the test samples and analyzed before and after every 1-3 samples in each analytical set.

4.4 Calculations

4.4.1 *Methods*

Famoxadone (DPX-JE874), IN-H3310, IN-JS940, IN-KF015, and IN-KZ007 residues were measured as ppb in soil (µg/kg) and water (µg/L). Quantitation was based on analyte response in Calibration Standard and Sample Extract analyses determined as µg/mL for the calculation of ppb residue concentrations in commodity.

Determination of residues from external calibration standards using the average response factor method are as follows:

$$\text{Standard Response Factor} = \frac{\text{Peak Area counts of Standard (ac)}}{\text{Concentration (ng/mL)}}$$

$$\text{RF}_{\text{Avg}} = \frac{\sum \text{Response Factors}}{\text{n}}$$

where:

Rf_{avg} = Average Response Factor

n = total number of standards analyzed in a sample set

µg/kg (ppb) found was calculated as follows:

$$\text{ppb found} = \frac{(\text{Peak Area}) \times (\text{RF}_{\text{Avg}}) \times (\text{Final Volume (mL)})}{(\text{Sample Weight (g)}) \times (\text{Dilution Factor})}$$

µg/L (ppb) found was calculated as follows:

$$\text{ppb found} = \frac{(\text{Peak Area}) \times (\text{RF}_{\text{Avg}}) \times (\text{Final Volume (mL)})}{(\text{Sample Volume (mL)}) \times (\text{Dilution Factor})}$$

In the event a peak was detected in the control, a corrected peak area was used to calculate ppb found for freshly fortified samples. The corrected peak area is the area of the fortified sample minus the area of the control sample.

The percent recovery found was calculated as follows:

$$\%\ \text{Recovery} = \frac{\text{ppb Found}}{\text{ppb Fortified}} \times \frac{100}{1}$$