ANALYTICAL METHOD FOR THE DETERMINATION OF DPX-Q8U80 AND METABOLITES IN SURFACE, GROUND AND DRINKING WATER USING LC/MS/MS

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1.0 SUMMARY

The analytical method described herein was developed for the detection, quantification and confirmation of residues of DPX-Q8U80 and its metabolites IN-REG72, IN-QEK31, IN-F4106, IN-A5760, IN-RYC33 and IN-VM862 surface, ground and drinking water. The method limit of quantitation (LOQ) was 0.10 µg/L (ppb) and the limit of detection (LOD) was estimated to be 0.02 µg/L (ppb). The method was validated at 0.10 and 1.0 µg/L in each matrix using a LC/MS/MS system operating with an electrospray interface (ESI) in positive/negative switching mode. This analytical method is suitable for enforcement, monitoring and data generation for regulatory studies.

The method used to determine the concentration of DPX-Q8U80 and its metabolites does not require an extraction step. Rather, the method utilized a dilute-and-shoot approach, taking advantage of the liquid sample matrix. Briefly, 5 ± 0.1 mL of each water type were placed in a 15 mL centrifuge tube and fortified at 0.10 (LOQ) or 1.0 µg/L (10x LOQ). The samples were centrifuged for 10 minutes at 3500 rpm to settle any suspended solids. Following centrifugation, 900 µL of each sample were diluted to 1.00 mL by adding 100 µL of methanol. The samples were then subjected to LC-MS/MS analysis. The LC-MS/MS method monitored two ion transitions for each analyte. Fortification of matrix blanks with DPX-Q8U80 and metabolites demonstrated that the method was free from significant matrix effects and analysis of control samples demonstrated that the method was free from interference.

Generally, a single analyst can prepare final extract solutions from at least four sets of 6 samples in a single 8 hour day, with the LC-MS/MS analysis occurring overnight and the results processed the next day.

The recoveries from the water samples fortified at 0.10 (LOQ) and 1.0 µg/L support the satisfactory performance of this method. [redacted]

2.0 BACKGROUND INFORMATION

DPX-Q8U80 is a development nematicide for DuPont Crop Protection. The purpose of this study was to develop a method to quantify DPX-Q8U80 and its associated metabolites in three different water types that meets the SANCO/825/00 rev.8.1 (16/11/2010) Guidance Document on Residue Analytical Methods (Reference 1) and the U.S. EPA Ecological Effects Test Guidelines: OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation (Reference 2). The structure and molecular weight of DPX-Q8U80 and its metabolites IN-REG72, IN-QEK31, IN-F4106, IN-A5760, IN-RYC33 and IN-VM862 can be found in Appendix 1. DPX-Q8U80 and its metabolites were analyzed by centrifuging the samples to settle the suspended solids, diluting them with methanol and subjecting them to LC/MS/MS analysis. All samples were analyzed using turbospray electrospray ionization in positive/negative switching mode. The Limit of Quantitation (LOQ) for all analytes was 0.10 µg/L (ppb). The Limit of Detection (LOD) was estimated to be 0.02 µg/L (ppb). Validation data is presented for the quantitation and confirmation ion transitions. The method was validated on surface water, ground water and drinking water.

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 Equipment

Instrumentation

LC system, Agilent 1290 with temperature controlled autosampler (Agilent Technologies, Wilmington, DE)

Mass Spectrometer System, API QTRAP 6500 triple quadrupole mass spectrometer using a Turbo Ion Spray (Applied Biosystems/ Sciex, Foster City, CA)

Biohit Proline Electronic Pipettors, Variable Volume with Tip Ejector, Vanguard, 5.0-100 µL Cat. No. 53495-200, 50-1000 µL Cat. No. 53495-205 and 0.10-5.0 mL Cat. No. 53495-290 (VWR Scientific Co., Bridgeport, NJ)

Chromatographic Supplies

HPLC Column: 2.1 mm i.d. × 50 mm, 1.8 µm particle size, Phenyl-Hexyl analytical column Part # 959757-912 (Agilent Technologies, Wilmington, DE)

HPLC Vials, Target DP Amber Kit, T/S/T Septa, 100 PK, Part # 5182-0556 (Agilent Technologies, Wilmington, DE)

Labware

Centrifuge tubes, Polypropylene 15-mL capacity, Cat. No. 21008-918 (VWR Scientific Co., Bridgeport, NJ)

Miscellaneous

VWR brand Vortex Geni 2 Mixer, Cat. No. 58815-178
(VWR Scientific Co., Bridgeport, NJ)

3.2 Reagents and Standards

Equivalent reagents may be substituted for those listed below. To determine if impurities in substituted reagents interfere with analyses, appropriate amounts of the solvents should be taken through the entire method using the chromatographic conditions specified in this report.

Acetonitrile - EM Omni Solv®, HPLC-grade acetonitrile , #AX0142-1
(EM Science, Gibbstown, NJ)

Formic Acid - Guaranteed Reagent 98% minimum, #FX0440-5
(EM Science, Gibbstown, NJ)

Methanol - EM Omni Solv®, HPLC-grade methanol, #MX0488-1
(EM Science, Gibbstown, NJ)

Water - EM Omni Solv®, HPLC-grade water, #WX0004-1
(EM Science, Gibbstown, NJ)

DPX-Q8U80-046, Purity 99.2%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-REG72-002, Purity 92.6%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-QEK31-006, Purity 98.6%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-F4106-005, Purity 97.9%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-A5760-003, Purity 98.8%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-RYC33-001, Purity 99.3%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-VM862-002, Purity 100%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

3.3 Safety and Health

Each analyst must be acquainted with the potential hazards of the reagents, products and solvents used in this method before commencing laboratory work. 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

DPX-Q8U80 and its metabolites were analyzed via a dilute-and-shoot method. Water samples were fortified at the LOQ and 10xLOQ levels, and 900 µL of each was diluted with 10 µL of acetonitrile. The samples were then analyzed using an ESI source utilizing positive/negative switching ionization and LC/MS/MS.

4.2 Analytical Procedure

4.2.1 Glassware & Equipment Cleaning Procedures

Glassware should be scrubbed with a brush using a laboratory soap solution, rinsed two to five times with tap water, rinsed with distilled or deionized water and finally rinsed with acetone or another suitable solvent and allowed to air dry prior to each use. The effectiveness of any cleaning procedure used should be demonstrated by preparation and analysis of reagent blanks. Care should be taken to avoid working with high levels of the analyte(s) being monitored in the same laboratory where samples are being extracted and analyzed.

4.2.2 Preparation & Stability of Reagent Solutions

The following procedures may be adjusted to prepare different volumes. Each solution should be prepared monthly and stored at room temperature unless stated otherwise:

Diluent for Analytical Standards, 9:1 *0.1 M Aqueous Formic Acid:Methanol*

Add 100 mL of HPLC-grade methanol to a 1L graduated cylinder. Pipette 380 µL of reagent grade formic acid into the graduated cylinder and dilute to a final volume of 1L with HPLC-grade water. Transfer solution to a clean bottle and cap. The solution may be stored at room temperature and should be prepared at least monthly.

Mobile Phase B, *0.01% Formic Acid in Methanol*

Add 100 mL of HPLC-grade methanol to a 1L graduated cylinder. Next pipette 100 µL of reagent grade formic acid into the graduated cylinder and dilute to a final volume of 1L with HPLC-grade methanol. Transfer solution to a clean bottle and cap. The solution may be stored at room temperature and should be prepared at least monthly.

4.2.3 Stock Standard Preparation and Stability

Use Class A volumetric flasks when preparing standard solutions. Prepare standard stock solutions for DPX-Q8U80, IN-REG72, IN-F4106, IN-A5760, IN-RYC33 and IN-VM862 by accurately weighing 10 ± 0.01 mg into individual 100-mL volumetric flasks using an analytical balance. Record the accurate weight of the standards. Dissolve the standards in approximately 50 mL of HPLC-grade acetonitrile. After dissolving, bring the solutions to a volume of 100 mL using HPLC-grade acetonitrile and invert the volumetric flask to mix the solution to homogeneity. The standard solutions are stable for approximately 6 months when stored in a freezer at

approximately -20°C immediately after each use. The concentration of this solution is 100 µg/mL.

Prepare the standard stock solution for IN-QEK31 by accurately weighing 10 ± 0.01 mg into a 100-mL volumetric flask using an analytical balance. Record the accurate weight of the standard. Dissolve the standard in approximately 80 mL of HPLC-grade methanol. After dissolving, bring the solution to a volume of 100 mL using HPLC-grade water and invert the volumetric flask to mix the solution to homogeneity. The standard solution is stable for approximately 6 months when stored in a freezer at approximately -20°C immediately after each use. The concentration of this solution is 100 µg/mL.

4.2.4 *Fortification Standard Preparation and Stability*

50 ng/mL Fortification Solution for DPX-Q8U80, IN-REG72, IN-F4106, IN-A5760 and IN-RYC33

Prepare an intermediate 5.0 µg/mL solution by pipetting 0.50 mL of the 100 µg/mL stock solutions of each analyte into a 10 mL volumetric flask. Dilute the standard with 5.0 mL of HPLC-grade acetonitrile and mix to homogeneity. Dilute to volume with HPLC-grade acetonitrile and mix to homogeneity. To create the 50 ng/mL fortification solution, pipette 100 µL of the 5.0 µg/mL intermediate solution into a 10 mL volumetric flask. Dilute the standard with 5.0 mL of HPLC-grade acetonitrile and mix to homogeneity. Dilute to volume with HPLC-grade acetonitrile and mix to homogeneity. This is fortification solution A-1.

5 ng/mL Fortification Solution for DPX-Q8U80, IN-REG72, IN-F4106, IN-A5760 and IN-RYC33

Pipette 1.00 mL of the previously prepared 50 ng/mL fortification solution into a 10 mL volumetric flask. Dilute the standard with 5 mL HPLC-grade acetonitrile and mix to homogeneity. Dilute to volume with HPLC-grade acetonitrile and mix to homogeneity. This is fortification solution A-2.

50 ng/mL Fortification Solution for IN-QEK31

Prepare an intermediate 5.0 µg/mL solution by pipetting 0.50 mL of the 100 µg/mL stock solution of QEK31 into a 10 mL volumetric flask. Dilute the standard with 5.0 mL of an 80/20 methanol/water solution and mix to homogeneity. Dilute to volume with 80/20 methanol/water. To create the 50 ng/mL fortification solution, pipette 100 µL of the 5.0 µg/mL stock solution of into a 10 mL volumetric flask. Dilute the standard with 5.0 mL of 80/20 methanol/water and mix to homogeneity. Dilute to volume with 80/20 methanol/water and mix to homogeneity. This is fortification solution B-1.

5 ng/mL Fortification Solution for IN-QEK31

Pipette 1.00 mL of the previously prepared 50 ng/mL fortification solution into a 10 mL volumetric flask. Dilute the standard with 5 mL 80/20 methanol/water and mix to homogeneity. Dilute to volume with 80/20 methanol/water and mix to homogeneity. This is fortification solution B-2.

4.2.5 *Calibration Standard Preparation and Stability*

Prepare the calibration standards as shown in the table below and vortex each after dilution. (Alternative or additional standards may be prepared as needed):

STANDARD USED	VOLUME TRANSFERRED (µL)	VOLUME OF DILUENT (µL)	DILUENT	FINAL CONCENTRATION (NG/ML)
5.0 µg/mL Intermediate	100 of each intermediate solution	800	9:1 0.1 M Aqueous Formic Acid:Methanol	500*
500 ng/mL	100	900	9:1 0.1 M Aqueous Formic Acid:Methanol	50.0*
50 ng/mL	100	900	9:1 0.1 M Aqueous Formic Acid:Methanol	5.0
5.0 ng/mL	200	800	9:1 0.1 M Aqueous Formic Acid:Methanol	1.0
5.0 ng/mL	100	900	9:1 0.1 M Aqueous Formic Acid:Methanol	0.50
1.0 ng/mL	100	900	9:1 0.1 M Aqueous Formic Acid:Methanol	0.10
0.50 ng/mL	100	900	9:1 0.1 M Aqueous Formic Acid:Methanol	0.05

**Note: The 500 and 50 ng/mL standards were used to create other standards. They were not utilized in the calibration curve.*

During method validation these standard solutions were freshly prepared with each sample set and stored at approximately 4°C prior to use. The standards showed stability for 4 days. Each of the calibration standards was vortex mixed for 30 seconds prior to placing them in the auto-sampler.

4.2.6 *Source (& Characterization) of Samples*

Water samples were obtained locally. All samples were assumed to have no residues of DPX-Q8U80 or its metabolites and were used for blank and fortified controls. Analysis of control samples analyzed as part of this study confirmed this assumption. No signals which matched the retention time and mass spectrum of DPX-Q8U80 or its metabolite standards were observed above the limit of detection. Characterization data for each sample may be found in Appendix 5.

4.2.7 *Storage & Preparation of Samples*

All samples were stored frozen at approximately -20°C. All samples were allowed to completely thaw before subsampling. No additional purification was performed prior to sample processing.

4.2.8 *Sample Fortification Procedure*

Fortifications were made directly to the 5.0 mL samples after weighing. Fortified samples were prepared using a 50 ng/mL and a 5.0 ng/mL standard solution.

FORTIFICATION LEVEL (µG/L)	STANDARDS USED	VOLUME OF STANDARD (ML)
0.1	5.0 ng/mL (A-2 and B-2)	0.100
1.0	50 ng/mL (A-1 and B-1)	0.100

The fortified samples were left uncapped for 10 minutes prior to extraction to allow the carrier to evaporate.

4.2.9 *Analyte Extraction Procedure*

1. Accurately pipette 5.0 ± 0.1 mL of the selected matrix into each 15-mL plastic centrifuge tube. Fortify the samples if necessary. Vortex mix samples for 10 s.
2. Transfer the samples to a room temperature centrifuge and spin for 10 minutes at 3500 rpm.
3. In an amber LC vial, dilute 900 µL of each sample to 1 mL with HPLC-grade methanol. Cap the vials and vortex mix for 10 seconds.
4. The samples are now ready for HPLC analysis.

4.3 Instrumentation

4.3.1 *Chromatography*

Reversed-phase chromatography was used to separate DPX-Q8U80 and its metabolites from co-extracts. An Agilent Zorbax Eclipse Plus® Phenyl-Hexyl column was selected. Alternative chromatographic conditions can be used, provided the analytical method is validated and provides acceptable recoveries as defined by regulatory method guidelines.

HPLC SYSTEM:	Agilent 1290 HPLC			
COLUMN:	2.1 mm x 50 mm Zorbax Eclipse Plus Phenyl-Hexyl, RRHD 1.8 μm P/N: 959757-912			
COLUMN TEMPERATURE:	50°C			
SAMPLE TEMPERATURE	10°C			
INJECTION VOLUME:	0.020 mL			
FLOW RATE:	0.500 mL/min			
CONDITIONS:	A: HPLC Grade Water			
	B: 0.01% Formic Acid in Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	90	10	0.300
	0.1	90	10	0.300
	3.5	1.0	99	0.300
	5.5	1.0	99	0.300
	5.6	90	10	0.300
	8.0	90	10	0.300
TOTAL RUN TIME:	8.0 min			

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
0.0 – 1.3	Waste
1.3 - 4.5	MS source
4.5 - End	Waste

4.3.2 *LC/MS/MS Analysis*

The quantitative analysis of DPX-Q8U80 and its metabolites was performed using an Applied Biosystem API QTRAP 6500 LC/MS/MS system. Quantitative analysis was based on the integration of a single ion transition. The instrument response during selected sample sets is provided in Appendix 2. A summary of the experimental conditions is provided in the following table:

MS SYSTEM:		ABSCIEX QTRAP 6500 W/ ESI SOURCE		
POSITIVE MODE	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-QEK31	**265→ 219 AMU**	**76**	**40**	**14**
	265→ 184 AMU	60	45	14
IN-RYC33	**264→ 157 AMU**	**76**	**61**	**10**
	264→ 184 AMU	76	45	14
IN-VM862	**197→ 141 AMU**	**70**	**40**	**10**
	197→ 114 AMU	70	45	10
Time:	0.0–8.0 minutes			
Ion Mode:	Positive	CAD:	High	
Turbospray Voltage:	5500 V	GS1:	85	
Source Temperature:	400°C	GS2:	25	
CUR:	30	Dwell	0.025 sec	

MS SYSTEM:		ABSCIEX QTRAP 6500 W/ ESI SOURCE		
NEGATIVE MODE	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-A5760	**206→ 122 AMU**	**-70**	**-20**	**-7**
	206→ 142 AMU	-70	-20	-7
IN-F4106	**220→ 156 AMU**	**-50**	**-20**	**-11**
	220→ 141 AMU	-50	-27	-11
IN-REG72	**452→ 123 AMU**	**-100**	**-37**	**-30**
	452→ 244 AMU	-100	-38	-30
DPX-Q8U80	**466→ 157 AMU**	**-110**	**-42**	**-7**
	466→ 142 AMU	-110	-48	-7
Time:	0.0–8.0 minutes			
Ion Mode:	Negative	CAD:	High	
Turbospray Voltage:	-4500 V	GS1:	85	
Source Temperatures:	400°C	GS2:	25	
CUR:	30	Dwell	0.025 sec	

A complete list of the experimental parameters is given in Appendix 3. *Typical LC/MS and LC/MS/MS full scan spectra of DPX-Q8U80 and its metabolites are shown in Figure* 1. *Peak area was used for quantitation.* ***Quantitation was performed using the ion transition displayed in bold face print.***

4.3.3 *Calibration Procedures*

A 0.05-ng/mL chromatographic standard should be analyzed prior to the start of analyses to establish that the instrument is working properly. If a signal-to-noise ratio of approximately 5-10 to 1 is not attained for each analyte, the instrument must be tuned or cleaned prior to sample analysis. Operating parameters must be tailored to the particular instrument used, especially if it is to be an alternate vendor's instrument, and should be checked daily. Note that some ion channels other than those used for development of this method may need to be added or eliminated when utilizing this method on other instrumentation. Each ion channel used for sample analysis/quantitation must be checked to insure it is free of interference. The control will be used to demonstrate that baseline interference is less than signal-to-noise 3:1. Begin each sample set by injecting a minimum of 2 calibration standards. The first injection should always be disregarded.

4.4 Calculations

4.4.1 *Methods*

Average Response Factor (RF_{Avg}) was calculated as follows:

$$RF_{Ave} = \frac{(\text{Conc. A} \div \text{Area A}) + (\text{Conc. B} \div \text{Area B}) + (\text{Conc. C} \div \text{Area C}) + (\text{Conc. D} \div \text{Area D}) + (\text{Conc. E} \div \text{Area E})}{\text{Total Number of Standards Injected}}$$

Dilution factor, used in the next equation, is calculated as follows:

$$\text{HPLC Dilution Factor} = \frac{\text{Total volume in HPLC Vial}}{\text{Volume of sample in HPLC Vial}}$$

ng/g (ppb) found was calculated as follows:

$$\text{ppb Found} = \frac{(\text{Peak Area}) \times (RF_{Ave}) \times (\text{Extract Volume}) \times (\text{HPLC Dilution Factor})}{(\text{Sample Weight})}$$

In the event a peak was detected in the control, a corrected peak area was used to calculate ppm 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:

$$\%\ \text{Recovery} = \frac{\mu\text{g/L (ppb) Found}}{\mu\text{g/L (ppb) Fortified}} \times \frac{100}{1}$$

[redacted]