2.0 INTRODUCTION

The structure, CAS name, CAS registry number, and various physical properties of DPX-QGU42 and metabolites IN-E8S72, IN-RAB06, IN-QPS10, IN-RDT31, IN-Q7D41, and IN-P3X26 can be found in Appendix 1. The method was validated on ground, surface and drinking water.

DPX-QGU42, IN-E8S72, IN-RAB06, IN-QPS10, IN-RDT31, IN-Q7D41, and IN-P3X26 were extracted from water samples using a liquid/ liquid partition. The extract was evaporated under a stream of nitrogen to dryness. The extracts were reconstituted using acetonitrile and diluted with water. An aliquot of the extract was transferred to an auto-sampler vial for analysis. DPX-QGU42, IN-E8S72, IN-RAB06, IN-QPS10, IN-RDT31, IN-Q7D41, and IN-P3X26 were separated from co-extracts by reversed phase liquid chromatography (LC) and detected using electrospray ionization and MS/MS detection. The Limit of Quantitation (LOQ) for each analyte was 0.10 µg/kg (ppb). The Limit of Detection (LOD) was estimated to be 0.03 µg/kg (ppb) based on the least responsive analyte, IN-QPS10

Due to the selective nature of the LC/MS/MS method, a separate confirmation method was not necessary. Confirmation using LC/MS/MS of possible residues were based on the detection and relative ratios of two MS/MS ion fragments. Confirmation criteria and examples are discussed in this report.

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified. Note any specification 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, HP1200 with temperature controlled autosampler (Agilent Technologies, Wilmington, DE)

Mass Spectrometer System, API 5000 triple quadrupole mass spectrometer using a Turbo Ion Spray and Analyst version 1.4 software (Applied Biosystems/MDS Sciex, Foster City, CA)

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

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: 3.0 mm i.d. × 15 cm, Zorbax SB Phenyl analytical column with 3.5-µm diameter packing Part # 863954.312 (Agilent Technologies, Wilmington, DE)

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

Low Flow Mixer Assembly, Part# 411-0050 (Analytical Scientific Instruments)

Labware

Pyrex Brand Single Metric Scale Graduated Cylinders, 10-mL and 100-mL capacity, Cat. No. 24709-715 and 24709-748, respectively (VWR Scientific Co., Bridgeport, NJ)

VWR brand Disposable Pasteur Pipettes, Borosilicate Glass, 9 in, Cat. No. 53283-914 equipped with 2 mL, 13 X 32 mm rubber bulbs, Cat. No. 56310-240 (VWR Scientific Co., Bridgeport, NJ)

Centrifuge tubes, Polystyrene 50-mL capacity, Cat. No. 21008-939 (VWR Scientific Co., Bridgeport, NJ)

Centrifuge tubes, Polystyrene 15-mL capacity, Cat. No. 21008-930 (VWR Scientific Co., Bridgeport, NJ)

Miscellaneous

6 Port Electrically Actuated Valve, Valco Instruments Co. Inc., PN 1384 (Alltech, Deerfield, IL)

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

Ethyl Acetate - EM Omni Solv®, HPLC-grade ethyl acetate, #EX0241-1 (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)

Sodium Chloride - EMD, #SX0420-1 (EM Science, Gibbstown, NJ)

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

IN-E8S72 reference substance (Dash 000, 99.7% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

IN-RAB06 reference substance (Dash 001, 97.3% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

IN-QPS10 reference substance (Dash 003, 99.3% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

IN-RDT31 reference substance (Dash 001, 94% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

DPX-QGU42-028, non-GLP characterized material used, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-Q7D41 reference substance (Dash 002, 91.5% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

IN-P3X26 reference substance (Dash 002, 95.4% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

3.3 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 used. An MSDS sheet for the analytes is available from DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company.

4.0 METHOD

4.1 Principles of the Analytical Method

DPX-QGU42, IN-E8S72, IN-RAB06, IN-QPS10, IN-RDT31, IN-Q7D41, and IN-P3X26 were extracted from water samples using a liquid/ liquid partition. The extract was evaporated under a stream of nitrogen to dryness. The extracts were reconstituted using acetonitrile and diluted with water. An aliquot of the extract was transferred to an auto-sampler vial for analysis. DPX-QGU42, IN-E8S72, IN-RAB06, IN-QPS10, IN-RDT31, IN-Q7D41, and IN-P3X26 were separated from co-extracts by reversed phase liquid chromatography (LC) and detected using electrospray ionization and MS/MS detection.

4.2 Analytical Procedure

4.2.1 Glassware and Equipment Cleaning

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.

4.2.2 *Preparation of Solutions*

The following solutions should be prepared monthly and stored at room temperature unless stated otherwise:

Mobile Phase A: 0.05 % aqueous formic acid solution - Add 500 µL of formic acid to 999 mL of water and 1 mL of methanol mix the resulting solution to homogeneity.

Mobile Phase B: 0.01 % formic acid solution - Add 100 µL of formic acid to 1000 mL of methanol and mix the resulting solution to homogeneity.

70% Water/ 30% Acetonitrile: Add 300 mL of acetonitrile to 700 mL of water and mix the resulting solution to homogeneity.

4.2.3 *Preparation and Stability of Stock Standard*

Use Class A volumetric flasks when preparing standard solutions.

Prepare standard stock solutions by accurately weighing 10 ± 0.01 mg of each analyte into individual 100-mL volumetric flask using an analytical balance. Record the accurate weight of the standard. Dissolve the standards in approximately 50 mL of HPLC-grade acetonitrile. After dissolving, bring the solution 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 3 months when stored in a freezer at approximately -20°C immediately after each use. The concentration of each analyte in solution is 100 µg/mL.

4.2.4 *Preparation and Stability of Intermediate and Fortification Standards*

Use Class A volumetric flasks when preparing standard solutions.

Prepare a 1.0-µg/mL DPX-QGU42, IN-E8S72, IN-RAB06, IN-QPS10, IN-RDT31, IN-Q7D41, and IN-P3X26 intermediate standard in acetonitrile by pipetting 1.00 mL of each 100.0-µg/mL stock standard into a 100-mL volumetric flask. Dilute the standard to approximately 50-mL with acetonitrile and add 1.0-mL of concentrated formic acid. Bring to volume using HPLC-grade acetonitrile and mix to homogeneity.

Prepare a 0.10-µg/mL DPX-QGU42, IN-E8S72, IN-RAB06, IN-QPS10, IN-RDT31, IN-Q7D41, and IN-P3X26 standard in acetonitrile by pipetting 1.00 mL of the 1.0-µg/mL standard into a 10-mL volumetric flask. Dilute the standard to approximately 5-mL with acetonitrile and add 0.10-mL of concentrated formic acid. Bring to volume using HPLC-grade acetonitrile and mix to homogeneity.

Prepare a 0.010-µg/mL DPX-QGU42, IN-E8S72, IN-RAB06, IN-QPS10, IN-RDT31, IN-Q7D41, and IN-P3X26 standard in acetonitrile by pipetting 1.00 mL of the 0.10-µg/mL standard into a 10-mL volumetric flask. Dilute the standard to approximately 5-mL with acetonitrile and add 0.10-mL of concentrated formic acid. Bring to volume using HPLC-grade acetonitrile and mix to homogeneity.

Alternate or additional solutions may be prepared as needed. All standard solutions prepared in acetonitrile or acetonitrile are stable for approximately 3 months if stored in a freezer at approximately -20°C immediately after each use.

4.2.5 *Preparation and Stability of Calibration Standards*

Prepare the calibration standards as showed in the table below (alternative or additional standards may be prepared as needed):

STANDARD USED (µG/ML)	VOLUME PIPPETTED (µL)	VOLUME OF 70%WATER/ 30% ACETONITRILE ADDED (µL)	FINAL CONCENTRATION (NG/ML)
0.10	50.0	950	5.0
0.10	25.0	975	2.5
0.010	100.0	900	1.0
0.010	50.0	950	0.50
0.010	25.0	975	0.25
0.010	10.0	990	0.10
0.010	5.0	995	0.050

These standard solutions should be freshly prepared with each sample set and stored approximately 4°C prior to use. Each of the calibration standards was vortex mixed for 30 seconds prior to filling the auto-sampler vials.

4.2.6 *Source of Samples*

Water control samples were intended to represent the three water types: ground surface and drinking. The waters selected were:

WATER TYPE	IDENTIFICATION
Surface	White Clay Creek, DE
Ground	Kimbelsville Well Water, PA
Drinking	Tap Water, Newark DE

Water characterization information for the surface and ground water are provided in Appendix 5.

4.2.7 *Storage and Preparation of Samples*

Water samples should be in a refrigerator at -4°C until use. Samples were vigorously shaken prior to sub-sampling

4.2.8 *Sample Fortification Procedure*

All fortifications were made directly to the 5.0-mL water sample. Fortified samples were prepared using a 0.010-µg/mL standard solution.

FORTIFICATION LEVEL (µG/KG)	VOLUME OF STANDARD (ML)
0.10	0.050
1.0	0.500

4.2.9 *Analyte Extraction Procedure*

1. Accurately measure 5.0-mL (± 1%) of water into a 15-mL plastic centrifuge tubes. Fortify samples if necessary and allow the fortification to dry in a fume hood for approximately 15-minutes. Cap and shake the samples vigorously.
2. Add 0.10-mL of concentrated formic acid, 1.5 grams of sodium chloride and 5.0-mL of ethyl acetate and to each sample. Mix thoroughly using a vortex mixer for 30-seconds .
3. Allow the sample to sit until the two phases separate. If a clear separation is not observed centrifuge the sample at 3000 rpm for 5-minutes.
4. Using a pipette transfer the upper layer into a clean 15-mL centrifuge tube.
5. To the samples add an additional 5-mL of acetonitrile and mix thoroughly using a vortex mixer for 30-seconds. Allow the sample to sit until the two phases separate. If a clear separation is not observed centrifuge the sample at 3000 rpm for 5-minutes.
6. Using a pipette combine the upper layers from the two liquid/ liquid partitions.
7. To the samples add an additional 5-mL of ethyl acetate and mix thoroughly using a vortex mixer for 30-seconds. Allow the sample to sit until the two phases separate. If a clear separation is not observed centrifuge the sample at 3000 rpm for 5-minutes.
8. Using a pipette combine the upper layers from the three liquid/ liquid partitions. Dilute the extracts to 15-mL using ethyl acetate.
9. Evaporate the extracts using an N-EVAP set to 30 °C to dryness. Reconstitute the extracts using 0.90-mL of acetonitrile. Mix the extract using a vortex mixer for approximately 30 seconds and sonicate for 5 minutes.
10. Dilute the extracts to 3-mL by adding 2.1-mL of HPLC grade water and mix using a vortex mixer for approximately 30 seconds.
11. Transfer an aliquot of each extract into an auto-sampler vial for LC/MS/MS analysis.

Extracts will be stable for approximately 48 hours if stored at 20°C.

4.3 Instrumentation for the Method

4.3.1 *Chromatography*

Reversed-phase chromatography was used to separate DPX-QGU42 and metabolites from co-extracts. An Agilent SB-phenyl column was selected. The column choice reflected experimental results indicating preferred separation from co-extractants.

Alternative chromatographic conditions can be used, provided the analytical method is validated and provides acceptable recoveries as defined by regulatory method guidelines.

For this method the HPLC is operating at a flow rate of 0.60 mL/min. To accommodate the low flow rate the solvent mixing chamber (Agilent part no. G1312-87330) is replaced with a low flow mixer assembly from Analytical Scientific Instruments (ASI part no. 411-0050). This reduces the volume of the mixing chamber from 450 to 50 microliters.

Conditions used for the analysis of IN-E8S72 and IN-RAB06

SYSTEM:	Agilent 1200 HPLC			
COLUMN:	3.0 mm i.d. × 15 cm, 3.5 μm Agilent SB-phenyl			
COLUMN TEMPERATURE:	40 °C			
SAMPLE TEMPERATURE	4 °C			
INJECTION VOLUME:	0.010 mL			
FLOW RATE:	0.600 mL/min			
CONDITIONS:	A: 0.05 % aqueous Formic Acid			
	B: 0.01 % Formic Acid in Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	75	25	0.60
	0.3	75	25	0.60
	4.0	40	60	0.60
	6.0	1.0	99	0.60
	11.0	1.0	99	0.60
	11.1	75	25	0.60
	15.0	75	25	0.60
IN-E8S72 RETENTION TIME:	4.75 minutes			
IN-RAB06 RETENTION TIME:	8.61 minutes			
TOTAL RUN TIME:	15.0 minutes			

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
0.00-3.0	Waste
3.0-10.0	MS source
10.0-End	Waste

Conditions used for the analysis of IN-QPS10, DPX-QGU42, IN-RDT31, IN-Q7D41 and IN-P3X26

SYSTEM:	Agilent 1200 HPLC			
COLUMN:	3.0 mm i.d. × 15 cm, 3.5 μm Agilent SB-phenyl			
COLUMN TEMPERATURE:	40 °C			
SAMPLE TEMPERATURE	4 °C			
INJECTION VOLUME:	0.010 mL			
FLOW RATE:	0.600 mL/min			
CONDITIONS:	A: 0.05 % aqueous Formic Acid			
	B: 0.01 % Formic Acid in Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	75	25	0.60
	0.3	75	25	0.60
	4.0	40	60	0.60
	5.0	30	70	0.60
	10.0	20	80	0.60
	10.2	1.0	99	0.60
	14.0	1.0	99	0.60
	15.0	75	25	0.60
	20.0	75	25	0.60
IN-QPS10 RETENTION TIME:	6.91 minutes			
IN-P3X26 RETENTION TIME:	8.11 minutes			
IN-RDT31 RETENTION TIME:	9.58 minutes			
DPX-QGU42 RETENTION TIME:	10.59 minutes			
IN-Q7D41 RETENTION TIME:	12.01 minutes			
TOTAL RUN TIME:	20.0 minutes			

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
0.00-4.0	Waste
4.0-16.0	MS source
16.0-End	Waste

4.3.2 *LC/MS/MS Analysis*

The quantitative analysis of DPX-QGU42 and metabolites was performed using an Applied Biosystem API 5000 LC/MS/MS system. Quantitative analysis was based on the integration of a single ion transition. The system parameters were adjusted while a solution of each analyte was infused directly into the ion source. The solution composition was 85% methanol/15% water, so that it would approximate the composition of the mobile phase at the retention time of the analyte. The solution concentration was approximately 2 μg/mL. A summary of the experimental conditions is provided in the following table:

Conditions used for the analysis of IN-E8S72 and IN-RAB06 (Negative)

PERIOD 1 **ANALYTES**	**IONS MONITORED**	**DECLUSTERING POTENTIAL (DP)**	**COLLISION ENERGY (CE)**	**EXIT POTENTIAL (CXP)**
IN-QPS10	**178.77→ 65.01 AMU**	**-25**	**-26**	**-11**
	178.77→ 134.85 AMU	-25	-16	-13
IN-RAB06	**567.9→ 523.7 AMU**	**-115**	**-20**	**-33**
	567.9→ 134.9 AMU	-115	-54	-15
Time:	0-15 minutes			
Ion Mode:	Negative			
Turbopray Voltage:	-4500 V			
Source Temperatures:	600 C			
CUR:	15			
CAD:	10			
GS1:	70			
GS2:	70			
Dwell	0.15 Seconds			

Conditions used for the analysis of IN-QPS10, DPX-QGU42, IN-RDT31, IN-Q7D41 and IN-P3X26 (Positive)

Period 2 Analytes	Ions Monitored	Declustering Potential (DP)	Collision Energy (CE)	Exit Potential (CXP)
IN-QPS10	**349.8→ 81.9 AMU**	**21**	**41**	**10**
	349.8→ 209.8 AMU	21	35	54
DPX-QGU42	**540.1→ 499.9 AMU**	**196**	**37**	**34**
	540.1→ 522.0 AMU	196	37	34
IN-RDT31	**566.1→ 330.9 AMU**	**11**	**45**	**46**
	566.1→ 537.9 AMU	11	29	32
IN-Q7D41	**538.1→ 498.0 AMU**	**196**	**33**	**20**
	538.1→ 141.0 AMU	196	59	24
IN-P3X26	**403.09→ 362.9 AMU**	**51**	**29**	**34**
	403.09→ 384.9 AMU	51	29	26
Time:	0.0-20.0 minutes			
Ion Mode:	Positive			
Turbopray Voltage:	4500 V			
Source Temperatures:	600 C			
CUR:	30			
CAD:	4			
GS1:	40			
GS2:	50			
Dwell	0.15 Seconds			

A complete list of the experimental parameters is given in Appendix 4 . A typical LC/MS and LC/MS/MS full scan spectrum of each analyte is shown in Figure 1 and Figure 2, respectively.

The instrument was operated in MS/MS-(MRM) positive and negative ion modes for quantitative analysis. Peak area was used for quantitation. **Quantitation was performed using the ion transition displayed in bold face print.** The relative ratio of the fragment ions was evaluated to confirm the presence of an analyte in an unknown sample.

4.3.3 *Calibration Procedure and Sample Analysis*

A 0.050-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, 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:

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

Corrected Area = (Area in the standard – Area on the control)

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

$$\text{ng/g Found} = \frac{(\text{Corrected Peak Area}) \times (RF_{Ave}) \times (\text{Final Volume}) \times (\text{Aliquot Factor})}{(\text{grams of Sample})}$$

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:

$$\text{\% Recovery} = \frac{(\text{ng/g Found})}{(\text{ng/g Fortified})} \text{ x } 100$$

4.4.2 *Example*

For a Water sample fortified with DPX-QGU42 at 0.10 ppb) [Date analyzed 15-Nov-10, 0.10 ppb Fortification (LOQ 1 Well)], the concentration found was calculated as follows:

Average Response Factor was calculated as follows:

$$RF_{Ave} = \frac{(0.050\text{ng/mL} \div 11800) + (0.10\text{ng/mL} \div 26100) + (0.20\text{ng/mL} \div 54700) + (0.50\text{ng/mL} \div 127000) + (1.0\text{ng/mL} \div 257000) + (2.0\text{ng/mL} \div 519000)}{6}$$

(AC ≡ Area Counts)

$RF_{Avg} = 3.90111e^{-6}$ ng/mL/AC

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

$$\text{ng/g Found} = \frac{(44100 - 1660\ \text{AC}) \times (3.90111\text{e}-6\ \text{ng/mL/AC}) \times (3.0\ \text{mL}) \times (1)}{(5\ \text{grams})}$$

ng/g Found = 0.099

The percent recovery found was calculated as follows:

$$\%\ \text{Recovery} = \frac{(0.099\ \text{ng/g})}{(0.10\ \text{ng/g})}\ \text{x}\ 100$$

% Recovery = 99%

(percent recoveries are rounded to the nearest whole number in Table 1, without rounding the concentration or ppb found)

5.3 Modifications or Special Precautions

The addition of concentrated formic acid was required to completely partition IN-E8S72 and IN-RAB06 into the organic phases. Special care should always be taken when using concentrated acids.

6.0 CONFIRMATION OF DETECTED RESIDUES

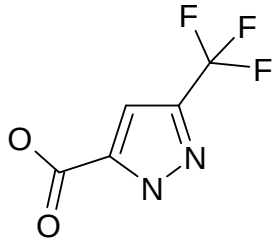
6.1 Method

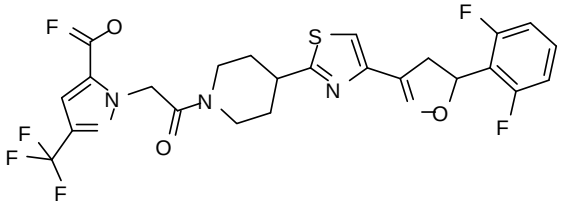
The confirmation method is based on evaluating the ion ratios collected during method validation. During the quantitative analysis of possible residues, two ion transitions were monitored. The ion ratio from the transitions monitored was used to establish criteria against which possibly detected residues are compared. The ratio of the ion intensity (area) of (A→B/A→C) was used to positively confirm the identity of an unknown compound. Since the ions detected originate by collision-induced fragmentation in an MS/MS system, the absolute intensity is dependent on gas cell pressure, gas cell size, storage time, system geometry, and other instrument specific parameters. Therefore, the ratio is expected to vary from day to day and when different vendor's instrumentation is used. For every sample set, the ion ratio data must be calculated based on the calibration standards and compared to actual sample data.

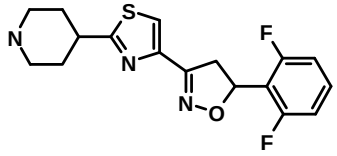
6.2 Confirmation Criteria

In order for a sample set to be valid, the relative standard deviation of the ion ratios calculated from the calibration standards analyzed must be less than 20%. For the confirmation of possible DPX-QGU42 and metabolite residues in a Water sample, the ion ratio must fall within ±30% of the average ratio for all calibration standards for a specific sample set. If the ion ratio is outside the ±30% range, the signal was most likely generated from a compound that is unrelated to DPX-QGU42. The unknown compound also has the same ion by LC/MS and a similar fragmentation pattern. In addition to meeting the defined ion ratio criteria, the elution time of the compound of interest must fall within 2% of the elution time of the standards analyzed for that sample set.

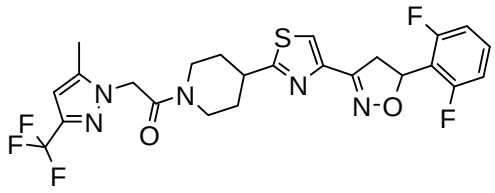
APPENDIX 1 STRUCTURE AND PROPERTIES OF DPX-QGU42 AND METABOLITES

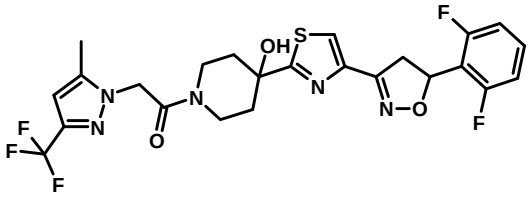
Common Name	IN-E8S72
Structure	
DPX Number	IN-E8S72
Formula	$C_5H_3F_3N_2O_2$
Molecular Weight	180.09
Monoisotopic Weight	180.01

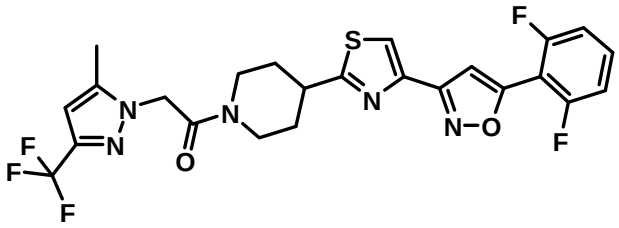
Common Name	None
Structure	
DPX Number	IN-RAB06
Formula	$C_{24}H_{20}F_5N_5O_4S$
Molecular Weight	569.51
Monoisotopic Weight	569.12

Common Name	IN-QPS10
Structure	
DPX Number	IN-QPS10
Formula	$C_{17}H_{17}F_2N_3OS$
Molecular Weight	349.40
Monoisotopic Weight	349.11

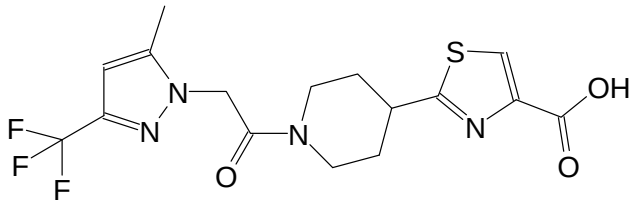
APPENDIX 1 STRUCTURE AND PROPERTIES OF DPX-QGU42 AND METABOLITES (CONTINUED)

Common Name	DPX-QGU42
Structure	
DPX Number	DPX-QGU42
Formula	$C_{24}H_{22}F_5N_5O_2S$
Molecular Weight	539.53
Monoisotopic Weight	539.14
CAS Number	1003318-67-9

Common Name	None
Structure	
DPX Number	IN-RDT31
Formula	$C_{24}H_{22}F_5N_5O_3$ S
Molecular Weight	555.53
Monoisotopic Weight	555.14

Common Name	None
Structure	
DPX Number	IN-Q7D41
Formula	$C_{24}H20F_5N_5O_2S$
Molecular Weight	537.51
Monoisotopic Weight	537.13

APPENDIX 1 STRUCTURE AND PROPERTIES OF DPX-QGU42 AND METABOLITES (CONTINUED)

Common Name	None
Structure	
DPX Number	IN-P3X26
Formula	$C_{16}H_{17}F_3N_4O_3S$
Molecular Weight	402.397
Monoisotopic Weight	402.097