ANALYTICAL METHOD FOR THE DETERMINATION OF DPX-QGU42 AND METABOLITES IN-E8S72, IN-QPS10, IN-RDT31 AND IN-RAB06 IN SOIL USING LC/MS/MS

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1.0 SUMMARY

The purpose of this study was to develop an analytical method for the detection, quantitative analysis, and confirmation of DPX-QGU42 and metabolites IN-E8S72, IN-QPS10, IN-RDT31, and IN-RAB06 in soil.

DPX-QGU42, IN-E8S72, IN-QPS10, IN-RDT31, and IN-RAB06 were extracted from soil samples using a solution of formic acid, water and acetonitrile. An aliquot of the extract was removed, volume reduced under a stream of nitrogen and diluted with water. An aliquot of the diluted extract was transferred to an auto-sampler vial for analysis. DPX-QGU42, IN-E8S72, IN-QPS10, IN-RDT31, and IN-RAB06 were separated from co-extracts by reversed phase liquid chromatography (LC). DPX-QGU42, IN-QPS10, and IN-RDT31 were detected by positive ion electrospray mass spectrometry/mass spectrometry (MS/MS) and IN-RAB06 was detected by negative ion electrospray MS/MS. Additional validation sets were completed adding IN-E8S72 to the method. During these additional sets IN-RAB06 was analyzed in the positive ion mode. The Limit of Quantitation (LOQ) for each analyte was 1.0 μg/kg (ppb). The Limit of Detection (LOD) was estimated to be 0.3 μg/kg (ppb) based on the least responsive analyte, IN-QPS10.

2.0 INTRODUCTION

The structure, CAS name, CAS registry number, and various physical properties of DPX-QGU42 and metabolites IN-E8S72, IN-QPS10, IN-RD31, and IN-RAB06 can be found in Appendix 1. The method was validated on soil from Germany and the United States.

DPX-QGU42, IN-E8S72, IN-QPS10, IN-RDT31, and IN-RAB06 were extracted from soil samples using a solution of formic acid, water and acetonitrile. An aliquot of the extracts were evaporated under a stream of nitrogen, diluted and analyzed using reversed phase liquid chromatography (LC) and electrospray mass spectrometry/mass spectrometry (MS/MS). The Limit of Quantitation (LOQ) was 1.0 µg/kg (ppb). The Limit of Detection (LOD) was estimated to be 0.3 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)

Carbon Steel Balls, 1/4 inch, Catalog No. 00073254 (MSC Industrial Supply, Melville, NY)

Carbon Steel Balls, 5/16 inch, Catalog No. 00073262 (MSC Industrial Supply, Melville, NY)

Genogrinder : Spex SamplePrep Model number 2000

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)

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)

IN-E8S72-001, non-GLP characterized material used, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-QPS10-002, non-GLP characterized material used, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-RDT31-001, non-GLP characterized material used, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-RAB06-001, non-GLP characterized material used, prepared by 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

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-QPS10, IN-RDT31, and IN-RAB06 were extracted from soil samples using a solution of water / acetonitrile. An aliquot of the extracts were diluted and analyzed using reversed phase liquid chromatography (LC) and electrospray mass spectrometry/mass spectrometry (MS/MS).

An additional solid phase extract step was also tested in the event of a matrix interference or matrix suppression. The SPE step and analysis is provided in Appendix 4.

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 990 mL of water and 10 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-QPS10, IN-RDT31, and IN-RAB06 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-QPS10, IN-RDT31, and IN-RAB06 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-QPS10, IN-RDT31, and IN-RAB06 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	10.0	990	1.00
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*

Soil control samples were obtained from a field test site located in Goch in Germany and Drummer in the USA. The soil characteristics are shown in the following table:

Soil Name	Country	Type	% Clay	% Sand	% Silt	pH_w	OM (%)	Notebook
Drummer	USA	Clay Loam	33	24	43	6	4.8	2004-073
Goch	Germany	Silt Loam	8.4	29.2	62.4	6.3	2.4	2004-042A

4.2.7 *Storage and Preparation of Samples*

Soil samples should be stored frozen at approximately -20°C until use. The soil core was dived into segments based on depth. For method development purposes only the 0-5 cm cores were selected. Both cores were mixed by hand prior to analysis.

4.2.8 *Sample Fortification Procedure*

All fortifications were made directly to the 5.0-g soil sample after weighing the sample. Fortified samples were prepared using a 0.10-µg/mL standard solution.

Fortification Level (µg/kg)	Volume of Standard (mL)
1.00	0.050
10.0	0.500

4.2.9 *Analyte Extraction and Purification Procedures*

1. Accurately measure 5.0-g (± 1%) of soil into a 50-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 2-mL of water to each sample and let the sample soak for approximately 5-minutes. Add three 1/4" steel balls, 15-mL of acetonitrile and 0.5-mL of formic acid to each sample.
3. Place samples on a genogrinder and homogenize for 2 minutes at a rate of approximately 1200 strokes per minute.
4. Centrifuge the samples for 5 minutes to drive the particulates to the bottom of the tube at a rate of approximately 3000 RPM.
5. Transfer the supernatants into a clean 50-mL centrifuge tubes. Repeat steps 2-4 using 15-mL of acetonitrile and 0.5-mL of formic acid. Do not add water for the second extraction. Combining the two extracts into the same 50-mL centrifuge tube.
6. Repeat steps 2-4 using 15-mL of acetonitrile and 0.5-mL of formic acid a third time. Do not add water for the third extraction. Combining the three extracts and adjust the volume of the extracts from each sample to 50-mL using acetonitrile. Mix the extract using a vortex mixer for approximately 30 seconds.
7. Pipette 2.0-mL of each extract into a clean 14-mL centrifuge tubes. Add 1-mL of HPLC grade water to each centrifuge tube. Evaporate using an N-EVAP set to 30 °C to a volume just under 2-mL. Dilute to 2-mL using HPLC grade water. Mix the extract using a vortex mixer for approximately 30 seconds. 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.

An additional solid phase extraction procedure (SPE) was tested in the event a co-eluting interference or LC/MS/MS matrix effects were observed. The SPE procedure is provided in Appendix 4.

During method transfer and validation at ABC Laboratories in Columbia Missouri this procedure was validated with a minor modification. The alternative procedure is provided in Appendix 6.

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.

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:	0.025 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
	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-E8S72 RETENTION TIME:	4.8 minutes			
IN-QPS10 RETENTION TIME:	6.2 minutes			
IN-RDT31 RETENTION TIME:	9.5 minutes			
IN-RAB06 RETENTION TIME:	10.2 minutes			
DPX-QGU42 RETENTION TIME:	10.6 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
0.00-2.5	Waste
2.5-11.0	MS source
11.0-End	Waste

During method transfer and validation at ABC Laboratories in Columbia Missouri alternative chromatography using UPLC was validated. The alternative chromatography is provided in Appendix 6.

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-QPS10, IN-RDT31, IN-RAB06 and DPX-QGU42

PERIOD 1 **ANALYTES**	**IONS MONITORED**	**DECLUSTERING POTENTIAL (DP)**	**COLLISION ENERGY (CE)**	**EXIT POTENTIAL (CXP)**
IN-QPS10	**349.8→ 209.8 AMU**	**21**	**35**	**54**
	349.8→ 81.9 AMU	21	41	10
IN-RDT31	**556.1→ 537.9 AMU**	**11**	**29**	**32**
	556.1→ 330.9 AMU	11	45	46
Time:	0-9.8 minutes			
Ion Mode:	Positive			
Turbopray Voltage:	4500 V			
Source Temperatures:	600 C			
CUR:	30			
CAD:	4			
GS1:	40			
GS2:	50			
Dwell	0.15 Seconds			
PERIOD 2 **ANALYTE**	**IONS MONITORED**	**DECLUSTERING POTENTIAL (DP)**	**COLLISION ENERGY (CE)**	**EXIT POTENTIAL (CXP**
IN-RAB06	**567.9→ 523.7 AMU**	**-115**	**-20**	**-33**
	567.9→ 134.9 AMU	-115	-54	-15
Time:	9.8 – 10.4 minutes			
Ion Mode:	Negative			
Turbopray Voltage:	-4500 V			
Source Temperatures:	600 C			
CUR:	30			
CAD:	4			
GS1:	40			
GS2:	50			
Dwell	0.15 Seconds			

Conditions used for the Analysis of IN-QPS10, IN-RDT31, IN-RAB06 and DPX-QGU42 (continued)

PERIOD 3 ANALYTE	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-QGU42	**540.0→ 499.9 AMU**	**1**	**37**	**20**
	540.0→ 163.0 AMU	1	69	28
Time:	10.4-20 minutes			
Ion Mode:	Positive			
Turbopray Voltage:	4500 V			
Source Temperatures:	600 C			
CUR:	30			
CAD:	4			
GS1:	40			
GS2:	50			
Dwell	0.15 Seconds			

Conditions used for the Analysis of IN-E8S72, IN-QPS10, IN-RDT31, IN-RAB06 (Positive) and DPX-QGU42

PERIOD 1 ANALYTE	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-E8S72	**178.8→ 65.0 AMU**	**-25**	**-26**	**-13**
	178.8→ 134.9 AMU	-25	-16	-11
Time:	0-6.0 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-E8S72, IN-QPS10, IN-RDT31, IN-RAB06 (Positive) and DPX-QGU42 (continued)

PERIOD 2 ANALYTES	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-QPS10	**350.3→ 210.2 AMU**	**85**	**40**	**14**
	350.3→ 82.0 AMU	90	55	13
IN-RDT31	**556.5→ 191.2 AMU**	**60**	**60**	**15**
	556.5→ 331.3 AMU	77	40	30
IN-RAB06	**570.4→ 167.4 AMU**	**130**	**40**	**20**
	570.4→ 177.1 AMU	98	68	22
IN-QGU42	**540.0→ 500.5 AMU**	**116**	**34**	**18**
	540.0→ 163.0 AMU	95	62	11
Time:	6.0-20.0 minutes			
Ion Mode:	Positive			
Turbopray Voltage:	5500 V			
Source Temperatures:	600 C			
CUR:	15			
CAD:	10			
GS1:	70			
GS2:	70			
Dwell	0.15 Seconds			

A complete list of the experimental parameters is given in Appendix 5 including the conditions from the analysis of IN-E8S72 and IN-RAB06 in the positive ion mode. The MS conditions were different due to retuning the instrument in-between the generation of the original conditions and the addition of IN-E8S72 to the method. 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.

During method transfer and validation at ABC Laboratories in Columbia Missouri alternative LC/MS/MS conditions were validated. The alternative conditions are provided in Appendix 6.

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:

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

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 soil sample fortified with DPX-QGU42 at 1.0 ppb) [Date analyzed 28-June-10, 1.0 ppb Fortification (1)], the concentration found was calculated as follows:

Average Response Factor was calculated as follows:

$$RF_{Ave} = \frac{(0.050\text{ng/mL} \div 12100) + (0.10\text{ng/mL} \div 23200) + (0.20\text{ng/mL} \div 44200) + (0.50\text{ng/mL} \div 105000) + (0.80\text{ng/mL} \div 170000) + (0.10\text{ng/mL} \div 221000)}{6}$$

(AC ≡ Area Counts)

$RF_{Avg} = 4.49336^{-6}$ ng/mL/AC

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

$$\text{ng/g Found} = \frac{(20900\ \text{AC}) \times (4.49336\text{e-}6\ n\text{g/mL/AC}) \times (2\ \text{mL}) \times (25)}{(5\ \text{grams})}$$

ng/g Found = 0.939

(ppb values are reported to two significant figures in Table 1 of this report. Rounding was performed using the Microsoft Excel version 7.0 for Windows 95 rounding function)

The percent recovery found was calculated as follows:

$$\%\ \text{Recovery} = \frac{(0.939\ \text{ng/g})}{(1.00\ \text{ng/g})}\ \text{x}\ 100$$

% Recovery = 94%

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

6.0 CONFIRMATION OF DETECTED RESIDUES

6.1 Method

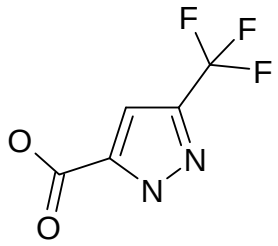
The confirmation method is based on evaluating the ion ratios collected during method validation. During the quantitative analysis of possible residues, two ion transitions were monitored. The ion ratio from the transitions monitored was used to establish criteria against which possibly detected residues 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

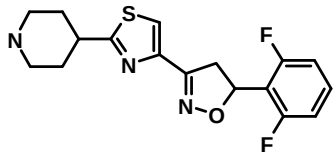
parameters. Therefore, the ratio is expected to vary from day to day and when different vendor's instrumentation is used. For every sample set, the ion ratio data must be calculated based on the calibration standards and compared to actual sample data.

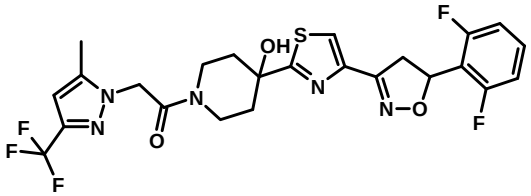
6.2 Confirmation Criteria

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 soil 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.

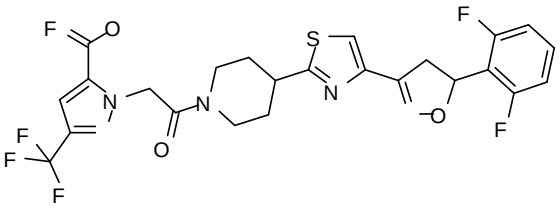
APPENDIX 1 STRUCTURE AND PROPERTIES OF DPX-QGU42 AND METABOLITES

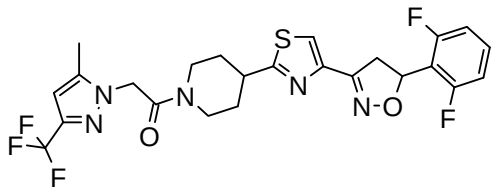
Common Name	IN-E8S72
Structure	
DPX Number	IN-E8S72
Formula	$C_5H_3F_3N_2O_2$
Molecular Weight	180.09
Monoisotopic Weight	180.01

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

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

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

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	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

APPENDIX 4 OPTIONAL SOLID PHASE EXTRACTION PROCEDURE

Analyte Extraction and Purification Procedure with Optional SPE Clean-up

1. Accurately measure 5.0-g (± 1%) of soil into a 50-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 2-mL of water to each sample and let the sample soak for approximately 5-minutes. Add three 1/4" steel balls, 15-mL of acetonitrile and 0.5-mL of formic acid to each sample.
3. Place samples on a genogrinder and homogenize for 2 minutes at a rate of approximately 1200 strokes per minute.
4. Centrifuge the samples for 5 minutes to drive the particulates to the bottom of the tube at a rate of approximately 3000 RPM.
5. Transfer the supernatants into a clean 50-mL centrifuge tubes. Repeat steps 2-4 using 15-mL of acetonitrile and 0.5-mL of formic acid. Do not add water for the second extraction. Combining the two extracts into the same 50-mL centrifuge tube.
6. Repeat steps 2-4 using 15-mL of acetonitrile and 0.5-mL of formic acid a third time. Do not add water for the third extraction. Combining the three extracts and adjust the volume of the extracts from each sample to 50-mL using acetonitrile. Mix the extract using a vortex mixer for approximately 30 seconds.
7. Attach a 6-cc, 0.5-g SAX[1] SPE cartridge to an SPE manifold[2]. Condition the cartridges with 5-mL of methanol followed by 10-mL of a 3% formic acid in acetonitrile solution[3]. Place a 14-mL graduated centrifuge tube under each cartridge. **Do not let the cartridge go to dryness**.
8. Pipette 2.0-mL of each extract into an SAX SPE cartridge. Using light vacuum, allow the sample to pass through the SAX cartridges at a flow rate of 2-5 mL/min. Rinse the SPE cartridges with 2-mL of 3% formic acid in acetonitrile solution and allow the wash to pass through the SAX cartridges at a flow rate of 2-5 mL/min using vacuum dry the cartridges for 5.0 minutes.
9. Add 1-mL of HPLC grade water to each centrifuge tube. Evaporate using an N-EVAP set to 30 °C to a volume just under 2-mL. Dilute to 2-mL using HPLC grade water. Mix the extract using a vortex mixer for approximately 30 seconds. 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.

1 Varian part number 12102144 (Palo Alto, CA)

2 Visiprep 12 port SPE vacuum manifold, PN 5-7030 (Supelco, Bellefonte, PA)

3 Solution prepared by adding 3-mL of concentrated formic acid to 97-mL of acetonitrile and mixing to homogeneity.

APPENDIX 6 ALTERNATIVE CONDITIONS

Sample Extraction and Preparation

1. Weigh five grams of sample into 50-mL centrifuge tube.
2. Fortified appropriate samples, let dry for approximately 15 minutes, cap and shake.
3. Add 2 mL of water and let the sample soak for approximately 5 minutes. After soak add three 1/4" steel balls.
4. Add 15 mL of acetonitrile, and 0.5 mL of formic acid to each sample. Placed samples on a genogrinder and homogenize for 2 minutes at approximately 1200 strokes/min.
5. Centrifuge samples for 5 minutes at approximately 3000 RPM. Decanted supernatant into a 50 mL centrifuge tube.
6. Repeat steps 4 and 5. Decanted the supernatant into the 50 mL centrifuge tube from step 5.
7. Repeat steps 4 and 5 a third time. Decanted the supernatant into the 50 mL centrifuge tube from step 5. Adjusted the volume to 50 mL using acetonitrile and mix using a vortex mixer.
8. Pipette 2.0 mL of each extract into 15 mL polypropylene centrifuge tubes. Using an N-EVAP with heated water 30 °C, bath evaporated samples to dryness.
9. Reconstitute samples to 2 mL using 70:30 water: ACN.
10. Sonicated and vortexed the samples. Transferred an aliquot into an auto-sampler vial for LC/MS/MS analysis.

Instrumental Analysis

SYSTEM:	Waters Acquity UPLC
COLUMN:	2.1 mm × 10 cm, 1.7 μm Waters Acquity HSS T3
COLUMN TEMPERATURE:	40 °C
SAMPLE TEMPERATURE	20 °C
INJECTION VOLUME:	0.025 mL
FLOW RATE:	0.500 mL/min
CONDITIONS:	A: 0.05 % aqueous Formic Acid B: 0.01 % Formic Acid in Methanol

Time	%A	%B	Flow (mL/Min.)
0.0	85	15	0.50
5.0	5	95	0.50
6.0	5	95	0.50
6.1	85	15	0.50
7.0	85	15	0.50

IN-QPS10 RETENTION TIME:	2.00 minutes
IN-RDT31 RETENTION TIME:	3.72 minutes
IN-RAB06 RETENTION TIME:	3.79 minutes
DPX-QGU42 RETENTION TIME:	3.88 minutes
TOTAL RUN TIME:	7.0 minutes

ANALYTES	**IONS MONITORED**	**DECLUSTERING POTENTIAL (DP)**	**COLLISION ENERGY (CE)**	**EXIT POTENTIAL (CXP)**
IN-QPS10	**350.3→ 210.2 AMU**	**85**	**40**	**14**
	350.3→ 82.0 AMU	90	55	13
IN-RDT31	**556.5→ 191.2 AMU**	**60**	**60**	**15**
	556.5→ 331.3 AMU	77	40	30
IN-RAB06	**570.4→ 177.1 AMU**	**98**	**68**	**22**
	570.4→ 167.4 AMU	130	40	20
DPX-QGU42	**540.0→ 163.1 AMU**	**95**	**62**	**11**
	540.0→ 500.5 AMU	116	34	18

Ion Mode:	Positive
Turbospray Voltage:	5500 V
Source Temperature:	600 C
CUR:	15
CAD:	10
GS1:	70
GS2	70