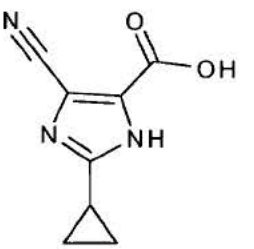
[redacted]

2.0 BACKGROUND INFORMATION

The chemical information for DPX-KJM44, DPX-MAT28, and IN-LXT69 appear in the original method report (Reference 1). Chemical information about the degradation product IN-QFH57 is provided below.

Structure	
DPX Number	IN-QFH57
CAS Chemical Name	not yet available
CAS Number	not yet available
Formula	$C_8H_7N_3O_2$
Molecular Weight	177.16
Monoisotopic Weight	177 Da
pKa	not yet available

3.0 MATERIALS

See original report.

3.1 Equipment

See original report.

3.2 Reagents and Standards

See original report.

3.2.1 Reference Analytical Standards

Reference analytical standards of DPX-KJM44 (lot #31, 99.4% pure), DPX-MAT28 (lot #007, 91.1% pure), IN-LXT69 (lot #002, 95.0% pure), and IN-QFH57 (lot #001, 95.07% pure) were used. All analytical reference substances used for sample analysis were analytical standard grade reagents (prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company).

3.3 Safety and Health

See original report.

4.0 METHODS

4.1 Principle of the Analytical Method

The analytes were extracted from a 10-g soil sample twice with 25 mL of ACN/0.15 M ammonium acetate (aq) 70/30, and once with 25 mL of ACN/0.2% formic acid (aq) 80/20 by shaking at high speed on a wrist action shaker. A 15.0-mL aliquot was taken and evaporated to 1 mL using a nitrogen evaporator with water bath temperature of 40°C, and then 5 mL of 0.2% formic acid (aq) were added. The extract was loaded into an Oasis® MCX SPE cartridge, where the analytes were retained. The analytes were eluted from the SPE cartridge with 15.0 mL of 75 mM ammonium hydroxide in methanol into a tube containing 1.0 mL of 0.2% formic acid (aq). The extract was evaporated under nitrogen gas flow to 1 mL using a nitrogen evaporator with water bath temperature of 40°C and diluted to 5.0 mL with 0.01% formic acid (aq). The extract was filtered through a 0.45 µm PTFE filter and placed in a clean 15-mL propylene centrifuge tube. Approximately 1 mL of the purified sample was placed into a glass autosampler vial and analyzed for IN-QFH57 by LC/MS/MS. A 10-fold dilution of the purified sample was made by mixing 900 µL of 0.01% formic acid (aq) with100 µL of each sample in a clean glass autosampler vial. The 10-fold diluted sample was analyzed for DPX-KJM44, DPX-MAT28, and IN-LXT69 by LC/MS/MS.

4.2 Analytical Procedure

4.2.1 Glassware & Equipment Cleaning Procedures

See original report.

4.2.2 Preparation & Stability of Reagent Solutions

75 mM ammonium hydroxide in methanol Elution Solution

Add approximately 500 mL of methanol to a 1 L bottle. Add 5.25 mL of concentrated (28-30%) ammonium hydroxide. Dilute to 1 L with methanol, cap and

shake to homogenize. Store this solution at room temperature and replace every month.[1]

See original report for information regarding how to prepare the remaining reagent solutions.

4.2.3 *Stock Standard Preparation and Stability*

If possible, use standards with purity greater than 95% and determine sample weights to 3 significant figures. The analytical balance must provide a weight precision to 3 significant figures, or the amount and volume must be adjusted to meet this condition. Clearly label as stock solutions with date prepared, analyte, and concentration.

Prepare individual 100 µg/mL stock standards solutions for DPX-KJM44, DPX-MAT28, IN-LXT69, and IN-QFH57 by weighing 10.0 mg of standard (adjusted for purity) into a tared 100-mL volumetric flask, mixing and dissolving the analyte in methanol, then diluting to final volume in methanol. These solutions are stored at or below -10°C. Stock solutions of DPX-KJM44, DPX-MAT28, and IN-LXT69 are stable for at least three months. The stock solution of IN-QFH57 is stable for at least two months.

4.2.4 *Fortification and Intermediate Standard Preparation and Stability*

1.0 µg/mL Fortification Solution A

Combine 1.0 mL of each of the individual 100 µg/mL stock solutions for each analyte (DPX-KJM44, DPX-MAT28, IN-LXT69, and IN-QFH57) into a common 100-mL volumetric flask, dilute to volume with methanol, cap and mix well. This solution should be stored at or below -10°C and is stable for at least two months.

100 ng/mL Fortification Solution B

Transfer 10.0 mL of the 1.0-µg/mL Fortification Solution A to a 100-mL volumetric flask, dilute to volume with methanol, cap and mix well. Store in a freezer (below -10°C) and replace every two months.

1.0 µg/mL DPX-KJM44, DPX-MAT28, IN-LXT69, and 10 µg/mL IN-QFH57 Mixed Stock Standard A

Combine 1.00 mL of the stock solutions of DPX-KJM44, DPX-MAT28 and IN-LXT69, and 10.0 mL of the stock solution of IN-QFH57 into a common 100-mL volumetric flask, dilute to volume with methanol, cap and mix well. This solution should be stored at or below -10°C and is stable for at least two months.

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1 Note that this solution replaces the 50 mM ammonium hydroxide Elution Solution used in the original method. The increased concentration of ammonium hydroxide facilitates the elution of the analytes from the SPE cartridges.

20 ng/mL DPX-KJM44, DPX-MAT28, IN-LXT69, and 200 ng/mL IN-QFH57 Mixed Stock Standard B

Transfer 200 µL of the Mixed Stock Standard A into a 10-mL volumetric flask, dilute to volume with methanol, cap and mix well. Store in a freezer (below -10°C) and replace weekly. This solution is used to prepare the chromatographic calibration standards.

Alternative or additional standard concentrations may be prepared if required. Stock standards are compatible with analytical method DuPont-22042 (Reference 2).

4.2.5 *Calibration Standard Preparation and Stability*

The 20 ng/mL DPX-KJM44, DPX-MAT28, IN-LXT69 and 200 ng/mL IN-QFH57 Mixed Stock Standard B is used to prepare the 2.0 ng/mL DPX-KJM44, DPX-MAT28, IN-LXT69 20 ng/mL IN-QFH57 calibration standard, which is serially diluted to prepare the remaining calibration standards. The final solution composition for each calibration standard was about 0.01% formic acid (aq). Calibration standards were generally prepared the day of analysis and were stable for at least 5 days if maintained refrigerated. All calibration standards were prepared by diluting with 0.01% formic acid (aq). The following table provides guidance for the preparation of calibration standards.

CALIBRATION STANDARD ID	STANDARD SOLUTION USED	STANDARD SOLUTION AMOUNT	FINAL VOLUME
2.0 ng/mL KJM44, MAT28, LXT69; 20 ng/mL QFH57	Mixed Stock Standard B	1.00 mL	10.0 mL
1.0 ng/mL KJM44, MAT28, LXT69; 10 ng/mL QFH57	2.0 ng/mL KJM44, MAT28 LXT69; 20 ng/mL QFH57	0.500 mL	1.0 mL
0.50 ng/mL KJM44, MAT28, LXT69; 5.0 ng/mL QFH57	2.0 ng/mL KJM44, MAT28 LXT69; 20 ng/mL QFH57Std	0.250 mL	1.0 mL
0.10 ng/mL KJM44, MAT28, LXT69; 1.0 ng/mL QFH57	2.0 ng/mL KJM44, MAT28 LXT69; 20 ng/mL QFH57	0.050 mL	1.0 mL
0.050 ng/mL KJM44, MAT28, LXT69; 0.50 ng/mL QFH57	0.50 ng/mL KJM44, MAT28 LXT69; 5.0 ng/mL QFH57	0.100 mL	1.0 mL
0.020 ng/mL KJM44, MAT28, LXT69; 0.20 ng/mL QFH57	0.50 ng/mL KJM44, MAT28 LXT69; 5.0 ng/mL QFH57	0.040 mL	1.0 mL

Alternative or additional calibration standard concentrations may be prepared if required. Calibration standards are compatible with analytical method DuPont-22042 (Reference 2).

4.2.6 *Source (& Characterization) of Samples*

The analytical method was validated in California soil, notebook reference number 2006-026. See original report for information regarding soil characterization.

4.2.7 *Storage & Preparation of Samples*

See original report.

4.2.8 *Sample Fortification Procedure*

The Sample Fortification Procedure was followed exactly as described in the original report. The procedure is provided below for convenience.

Sample fortification was done after weighing the soil samples in the 50-mL propylene centrifuge tubes. LOQ-level fortification was done by adding 0.100 mL of the 100 ng/mL Fortification Solution B to 10 g of soil; while 10 × LOQ fortification was achieved by adding 0.100 mL of the 1.0 µg/mL Fortification Solution A to 10-g soil samples.

Sample Identification	Amount (g)	Fortification Solution		Fortification (ng/g, ppb)
		µg/mL	mL	
LOQ Fort	10.0 ± 0.1	0.100	0.100	1.0
10×LOQ Fort	10.0 ± 0.1	1.00	0.100	10

4.2.9 *Analyte Extraction Procedure*

The Analyte Extraction Procedure was followed exactly as described in the original report. The procedure is provided below for convenience.

1. Weigh (10.0 ± 0.1) g of soil into a 50-mL propylene centrifuge tube.
2. If necessary, fortify samples at this point (does not apply to treated samples), and allow 15 minutes for the solvent to dry in the hood.
3. Add 3 metal beads to the tube to improve agitation during the extraction process.
4. Add 25 ± 1 mL of 70 ACN/30 0.15 M ammonium acetate (aq) Extraction Solution A. Cap the propylene centrifuge tube and vortex for few seconds to mix.
5. Extract analytes for 15 min using a wrist action shaker set at maximum deflection.
6. Centrifuge samples for 10 min at 3,000 rpm.
7. Carefully transfer the extracts to clean 100 mL graduated cylinders.
8. Add 25 ± 1 mL of 70 ACN/ 30 0.15 M ammonium acetate (aq), Extraction Solution A to the sample. Cap the propylene centrifuge tube and vortex for few seconds to redisperse the pellet. Then repeat steps 5 through 7, combining the extracts in the corresponding graduated cylinders.
9. Add 25 ± 1 mL of 80 ACN/ 20 0.2% formic acid (aq), Extraction Solution B to the sample. Cap the propylene centrifuge tube and vortex for few seconds to redisperse the pellet. Then repeat steps 5 through 7, combining the extracts in the corresponding graduated cylinders.
10. Bring the extracts to a final volume of 75 mL with ACN/0.2% formic acid (aq) 80/20 Extraction Solution B, cap and homogenize.
11. Transfer the extracts to plastic bottles of a convenient size (*e.g.* 100-125 mL). The samples may be stored overnight at this point, if necessary, in a freezer (approximately -20°C). The extracts are stable for at least two weeks when stored frozen.

4.2.10 *Analyte Purification Procedure*

Minimal modifications were made to the Analyte Purification Procedure to allow the analysis of IN-QFH57 and these appear in italics.

1. Transfer a 15.0-mL aliquot of the extract into a 50-mL propylene centrifuge tube. Evaporate to a volume of approximately 2 mL under nitrogen gas using an N-Evap set at a water bath temperature of 40°C.
2. Add 5.0 mL of 0.2% formic acid (aq). Cap the propylene centrifuge tubes, vortex for ten seconds and shake by hand vigorously to dissolve any portion of the analytes on the inner wall of the tube. The extracts are now ready for SPE purification.
3. Place the Oasis® MCX cartridges in the SPE manifold. Condition each cartridge with 3 mL of methanol flowing at ~5 mL/min. If necessary, use light vacuum to start the flow. Stop vacuum before the columns are dry. Then, allow the methanol to go through the cartridges by gravity until the flow stops.
4. Equilibrate each cartridge with two sequential column volumes (approximately 6 mL each) of 0.01% formic acid (aq). Keep the flow at approximately 5 mL/min. Do not let the cartridges go to dryness.
5. Load the extracts into the Oasis® MCX cartridges at a flow rate ≤1 mL/min.[1] The solvent flow should go to waste until the elution of the analytes in step 8.
6. Complete quantitative transfer by rinsing the propylene centrifuge tubes with 0.2% formic acid (aq) and loading the rinsate into the corresponding Oasis® MCX cartridges. A maximum volume of 4 mL may be used *(e.g. 2 × 2 mL)*. Maintain the flow at or below 1 mL/min.[1] Allow all the solution to go through; then apply vacuum for a few seconds to slowly pull the remaining solvent.
7. Dispose of any waste solvents and prepare for the elution of the analytes by adding 1.0 mL of 0.2% formic acid into 50-mL propylene centrifuge tubes (one tube per sample). Place the 50-mL propylene centrifuge tubes in the SPE manifold.[2]
8. Elute the analytes into the propylene centrifuge tubes with 15.0 mL of 75 mM ammonium hydroxide in methanol Elution Solution (e.g. 3 x 5.0 mL). The flow should be set to 1 mL/min.[1] After the 15.0 mL of Elution Solution go through, apply high vacuum to pull any remaining solvent.
9. Remove the tubes containing the samples from the SPE manifold and swirl gently the contents to homogenize. Place the samples in the N-Evap set at a water bath temperature of 40°C. Evaporate under nitrogen gas to a volume of about 1 mL.

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1 Analytes are retained in the Oasis® MCX SPE cartridges by mixed-mode, i.e. cation exchange and reverse phase. High flow (i.e. >1 mL/min) during the analyte loading and elution steps may affect recoveries and overall method performance.

2 Note that the methanol rinse (step 4.2.10.7 in the original report) has been removed from the procedure.

10. Add approximately 3 mL of 0.01% formic acid (aq) to each sample and swirl to mix. Bring the volume up to 5.0 mL with 0.01% formic acid (aq), cap, shake, and vortex for about 10 seconds.

11. Filter the purified extracts through 0.45 µm PTFE filters into clean 15-mL propylene centrifuge tubes.

12a. Samples for quantification of IN-QFH57: Place about 1.0 mL of each preconcentrated/filtered soil extract into amber glass autosampler vials. These samples are now ready for LC/MS/MS analysis of IN-QFH57.

12b. Samples for quantification of DPX-KJM44, DPX-MAT28, IN-LXT69: Add 900 µL of 0.01% formic acid (aq) into clean amber glass autosampler vials (one vial per sample). Add 100 µL of each preconcentrated/filtered soil extract into the corresponding amber glass autosampler vial, cap and vortex for few seconds. These 10-fold diluted samples are now ready for LC/MS/MS analysis of DPX-KJM44, DPX-MAT28, and IN-LXT69.

4.3 Instrumentation

The instrumentation and HPLC operating conditions used were exactly as described in the original report. The parameters for detection of IN-QFH57 were added to the LC/MS/MS acquisition program and only the ESI source voltage was increased to 2.0 kV for DPX-MAT28 since an improvement in sensitivity was observed at the time validation sets were analyzed. The complete description of the instrumentation and conditions used are provided below for convenience.

4.3.1 Description

An Agilent 1100 Series HPLC connected to an Applied Biosystems API-5000 triple quadrupole mass spectrometer with an electrospray interface (ESI) was used for instrumental analysis. HPLC components were: G1379A vacuum degasser, G1312A binary pump, G1316A column compartment, and G1367A refrigerated autosampler. Data acquisition and system was controlled by Analyst 1.4.2 software. The Applied Biosystems API-5000 was operated in LC/MS/MS positive (IN-LXT69, DPX-MAT28, and DPX-KJM44) and negative (IN-QFH57) ion mode with MRM detector output for quantitative and confirmatory analysis.

4.3.2 Operating Conditions

HPLC Operating Conditions:

Injection Volume:	60 µL
Column:	Luna Phenyl-Hexyl, 4.6 mm × 150 mm, 3-µm diameter particulate
Column Temperature:	30°C
Solvent A:	0.1% formic acid in HPLC-grade water
Solvent B:	HPLC-grade methanol

Time	Flowrate (mL/min)	%A	%B	Comments
0.00	1.000	95	5	
5.00	1.000	41	59	
8.00	1.000	1	99	
10.00	1.000	1	99	
10.10	1.000	95	5	
14.50	1.000	95	5	End Run

Approximate Analyte Retention Times:

IN-LXT69 = 3.8 min
DPX-MAT28 = 5.1 min
IN-QFH57 = 8.3 min
DPX-KJM44 = 8.9 min

Post-column Split:

~100 µL/min to MS and ~900 µL/min to waste

Triple Quadrupole MS Operating Conditions:

Interface: electrospray (ESI)
Mode: MRM
Resolution Q1: Unit
Resolution Q3: Unit
ESI Source Voltage: 1.5 kV for IN-LXT69 and DPX-KJM44, 2.0 kV for DPX-MAT28, and -4.0 kV for IN-QFH57
Divert Valve: 0.0–3.0 min to waste
3.0–9.5 min to source
9.5–14.5 min to waste

AB SCIEX API-5000 ACQUISITION PARAMETERS (ESI INTERFACE, MRM MODE)														
Period	Analyte	Q1 m/z	Q3 m/z	Dwell (msec)	CUR (psi)	GS1 (psi)	GS2 (psi)	TEM (°C)	IHE	CAD (psi)	DP (V)	EP (V)	CE (V)	CXP (V)
1	IN-LXT69	170.0	76.0	100	20	50	50	325	on	8	90	10	40	15
		170.0	103.0	100										
2	DPX-MAT28	214.0	68.0	100										
		214.0	101.0	100										
3	IN-QFH57	176.27	131.9	100	50	60	0	325	on	7	-45	-10	-18	-17
		176.27	105.0	100	50	60	0	325	on	7	-45	-10	-30	-17
4	DPX-KJM44	228.0	68.0	100	20	50	50	325	on	8	90	10	40	15
		228.0	168.0	100										

4.3.3 *Calibration Procedures*

The calibrated range of instrument response was 0.020 ng/mL to 2.0 ng/mL for DPX-KJM44, DPX-MAT28, and IN-LXT69, and 0.20 ng/mL to 20 ng/mL for IN-QFH57. These calibration ranges are approximately equivalent to expected final solution (step 4.2.9.12) concentrations from 0.5 ng/g (0.5 × LOQ) to 50 ng/g (50 × LOQ). Typically, 6 calibration standards were interspersed with soil samples for quantitative LC/MS/MS analysis.

See original report for information regarding mass spectrometer tuning and calibration, standard calibration, average response factor, and calculation of net recoveries.

4.3.4 *Sample Analysis*

See original report.

4.4 Calculations

4.4.1 *Methods*

DPX-KJM44, DPX-MAT28, IN-LXT69, and IN-QFH57 residues found at or above the LOQ are reported to 2 significant figures. Detected residues equal to or above the limit of detection (LOD), but below the LOQ, are reported to 1 significant figure. Recoveries for fortified samples are reported to the nearest whole number percentage (%).

The calculation to determine ng/g (ppb) found in residue samples using the average response factor calculated from calibration standards follows:

$$\text{ng/g (ppb) found} = \frac{(PA)(FV)(AF)(DF)}{(SW)(ARF)}$$

where,

PA is Analyte **P**eak **A**rea,

FV is the purified extract **F**inal **V**olume just before LC/MS/MS analysis (5.0 mL for all soil samples in validation sets),

AF is **A**liquot **F**actor, which is equal to the Extract Volume (75.0 mL) divided by the Aliquot Volume (15.0 mL), therefore AF = 5 for all soil validations sets,

DF is **D**ilution **F**actor used just prior to LC/MS/MS analysis (no dilution was done in soil validation sets for IN-QFH57, therefore DF = 1 for that analyte, while DF = 10 for DPX-KJM44, DPX-MAT28 and IN-LXT69),

SW is **S**ample **W**eight (in grams) of sample aliquot extracted,

RF is **R**esponse **F**actor, $RF = \left(\frac{\text{Peak Area}}{\text{Analyte Concentration (ng/mL)}}\right)$ obtained for calibration standards, and

ARF is the **A**verage **R**esponse **F**actor calculated by averaging the Response Factor obtained in all calibration standard injections.

If significant residues are found in control samples, and the analyte concentration is less than 50% the LOQ (0.50 × 1.0 ng/g), a corrected ng/g value should be calculated for fortified samples as described below:

$$\text{Corrected ng/g (ppb)} = \frac{(PA - CPA)(FV)(AF)(DF)}{(SW)(ARF)}$$

where,

CPA is Control Peak Area.

Note that CPA = 0 if control samples are not contaminated with the analyte(s), and therefore the corrected ng/g can still be used to calculate recoveries. The % Recovery of DPX-KJM44, DPX-MAT28, IN-LXT69 and IN-QFH57 in fortified samples is determined as follows:

% Recovery = (Corrected ng/g (ppb)) × 100 / (ng/g applied)

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