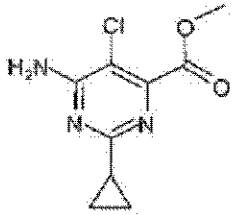
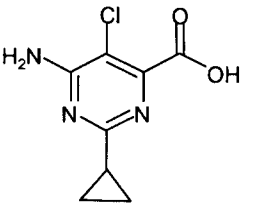
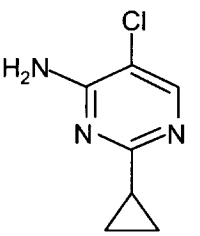
2.0 BACKGROUND INFORMATION

The chemical information for DPX-KJM44, DPX-MAT28, and IN-LXT69 are listed below.

Common Name	Aminocyclopyrachlor methyl
Structure	
DPX Number	DPX-KJM44
CAS Chemical Name	Methyl 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylate
CAS Number	858954-83-3
Formula	$C_9H_{10}ClN_3O_2$
Molecular Weight (g/mol)	227.65
Monoisotopic Weight	227 Da
pKa	none

Common Name	Aminocyclopyrachlor
Structure	
DPX Number	DPX-MAT28
CAS Chemical Name	6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
CAS Number	858956-08-8
Formula	$C_8H_8ClN_3O_2$
Molecular Weight	213.62
Monoisotopic Weight	213 Da
pKa	4.65

Common Name	Not yet available
Structure	
DPX Number	IN-LXT69
CAS Chemical Name	not yet available
CAS Number	not yet available
Formula	$C_7H_8ClN_3$
Molecular Weight	169.61
Monoisotopic Weight	169 Da
pKa	not yet available

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

Extractor

Wrist Action Shaker, Model 75 (Burrell, Pittsburgh, PA)

Instrumentation

LC system, HP1100 with temperature controlled autosampler (Agilent Technologies, Wilmington, DE)

Applied Biosystems Sciex API-5000 with ESI interface (Applied Biosystems/MDS Sciex, Foster City, CA)

Damon/IEC Division centrifuge, model HN-SII (Damon/IEC Division, Needham, Massachusetts)

VWR brand Vortex Geni 2 Mixer, 115V, 60 Hz, 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)

Balances - Mettler AG104 analytical and PM600 top-loading balances (Mettler Instrument Corp., Hightstown, NJ)

Evaporator - N-Evap® Model 111 laboratory sample evaporator/nitrogen manifold fitted with Teflon®-coated needles (Organomation Associates, South Berlin, MA) - This unit is attached to a dry, clean nitrogen source.

Solid-Phase Extraction Equipment

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

Solid-Phase Extraction Supplies

Oasis® MCX cartridge, 500 mg, 6 cc, PN 186000776 (Waters, Milford, MA) - **Do not substitute.**

Chromatographic Supplies

HPLC Column: 4.6 mm i.d. × 15 cm, Phenomenex Luna Phenyl-Hexyl analytical column with 3 µm diameter packing Part # 00F-4256-E0 (Phenomenex, Torrance, CA)

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

Labware

VWR wide mouth plastic bottle with cap, 4 oz, Cat. 16126-063 (VWR Scientific Co., Bridgeport, NJ)

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 Graduated Cylinders, Class B, 1000-mL capacity, Cat. No. 89000-260 (VWR Scientific Co., Bridgeport, NJ)

Kimax Brand Single Metric Scale Graduated Cylinders with Glass Stoppers, Cat. No. 89001-208 (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)

Sorenson™ Multifit Research Pipet Tips, 5-200 and 100-1000 µL, Catalog No. 53550-076 and 53503-076 (VWR Scientific Co., Bridgeport, NJ)

Pipet Tips, 100-5000 µL, Biohit Cat. No. 780-303, VWR Cat. No. 13502-312 (VWR Scientific Co., Bridgeport, NJ)

Propylene centrifuge tubes, 50 mL, BD Falcon®, conical bottom, BD No. 352070, VWR Cat. No. 21008-940 (VWR Scientific Co., Bridgeport, NJ)

Propylene centrifuge tubes, 15 mL, BD Falcon®, conical bottom, BD No. 352196, VWR Cat. No. 62406-200 (VWR Scientific Co., Bridgeport, NJ)

25 mm Syringe Filters with 0.45 µm PTFE Membrane, Cat. No. 28145-497 (VWR Scientific Co., Bridgeport, NJ)

5.0 mL Non-Sterile Syringes, Cat. No. BD301027 ((VWR Scientific Co., Bridgeport, NJ)

Miscellaneous

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

Carbon steel balls, 0.25 in diameter, 250/pkg, order #00073254 (MSC Industrial Supply Co., New Castle, DE)

3.2 Reagents and Standards

Equivalent reagents may be substituted for those listed below. To determine if impurities in substituted reagents interfere with analyses, a "reagent blank" should be prepared using appropriate amounts of the solvents, and using the chromatographic conditions specified in this report.

Ammonium Acetate - Baker Analyzed®, #0599-08 (J. T. Baker Inc., Danvers, MA)

Ammonium Hydroxide Solution - 28-30%, #AX-1303-13 (EM Science, Gibbstown, NJ)

Formic Acid - 98% ACS Grade, #LCFX0440-5 (EMD Chemicals, Gibbstown, NJ)

Acetonitrile (ACN) - EM Omni Solv®, HPLC-grade acetonitrile, #AX0142-1 (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)

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).

3.2.1 *Reference Analytical Standards*

Reference analytical standards of DPX-KJM44 (lot #31, 99.4% pure), DPX-MAT28 (lot #007, 91.1% pure), and IN-LXT69 (lot #002, 95.0% 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

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

A 10 g soil sample is extracted 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 5.0 mL aliquot is taken and evaporated to 1 mL under nitrogen gas flow using a N-Evap at 40°C, and then diluted with 0.2% formic acid (aq) to a volume of 6 mL. The sample is loaded into an Oasis® MCX SPE cartridge, where the analytes are retained. After washing the cartridge with 10 mL of methanol, the analytes are eluted with 15.0 mL of 50 mM ammonium hydroxide in methanol into a tube containing 1.0 mL of 0.2% formic acid (aq). The sample is evaporated to a volume of 1 mL using a N-Evap at 40°C and diluted with 0.01% formic acid. A portion of the cleaned purified is filtered through a 0.45 µm PTFE filter and analyzed by LC/MS/MS.

4.2 Analytical Procedure

4.2.1 *Glassware & Equipment Cleaning Procedures*

Due to the potential for contamination resulting from the low detection limit disposable equipment was used for sample preparation when possible. If glassware is used, cleaning should be conducted as described below.

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 & Stability of Reagent Solutions*

0.1% formic acid in water Mobile Phase A

Add 1.00 mL of concentrated formic acid to approximately 500 mL of HPLC-grade water. Dilute to 1.0 L with HPLC-grade water, cap, and homogenize. This solution is Mobile Phase A. The solution should be replaced at least once per month to avoid microbial growth.

HPLC grade methanol Mobile Phase B

Fill a glass bottle of appropriate size (e.g. 1 or 2-L) with HPLC grade methanol. This is Mobile Phase B, and should be replaced every three months.

0.15 M ammonium acetate (aq) solution

In a 1-L glass bottle, dissolve 11.55 g of ammonium acetate using approximately 300 mL of distilled/deionized water. Bring to a final volume of 1.0 L with distilled/deionized water, cap and shake to homogenize. Store at room temperature and replace monthly. This solution will be used to prepare Extraction Solution A.

0.2% v/v formic acid (aq) solution

Add approximately 500 mL of distilled/deionized water to a 1 L glass bottle. Add 2.0 mL of concentrated formic acid. Dilute to a final volume of 1.0 L with distilled/deionized water, cap and homogenize. Store at room temperature and replace every month. This solution will be used to prepare Extraction Solution B.

ACN/0.15 M ammonium acetate (aq) 70/30 Extraction Solution A

Using a 1-L graduated cylinder measure 300 mL of 0.15 M ammonium acetate (aq); then dilute to the 1.0 L mark with acetonitrile (in the same graduated cylinder), transfer to a 1 L glass bottle, cap and shake to homogenize. The solution should be stored at room temperature and replaced every month.

ACN/0.2% formic acid (aq) 80/20 Extraction Solution B

Using a 1-L graduated cylinder measure 200 mL of 0.2% v/v formic acid (aq) solution; then dilute to the 1L mark with acetonitrile (in the same graduated cylinder), transfer to a 1L glass bottle, cap and shake to homogenize. The solution should be stored at room temperature and replaced every month.

0.01% formic acid (aq)

Add approximately 500 mL of distilled/deionized water to a 1 L glass bottle. Add 50 mL of 0.2% v/v formic acid (aq) solution. Dilute to 1 L with distilled/deionized water, cap and homogenize. The solution should be stored at room temperature and replaced every month.

50 mM ammonium hydroxide in methanol Elution Solution

Add approximately 500 mL of methanol to a 1 L bottle. Add 3.50 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.

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, and IN-LXT69 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 and are stable for at least three 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 stock solutions for each analyte 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 three 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 three months.

100 ng/mL Intermediate Solution C

Transfer 1.0 mL of the 1.0 µg/mL Fortification Solution A to a 10-mL volumetric flask, dilute to volume with 0.01% formic acid, cap and mix well. This solution is the stock used to prepare the chromatographic standards. Store in a freezer (below -10 °C) and replace weekly. Let the solution warm up to room temperature and homogenize prior to each use.

Alternative or additional standard concentrations may be prepared if required.

4.2.5 *Calibration Standard Preparation and Stability*

The 100 ng/mL Intermediate Solution C is used to prepare the 2.0 ng/mL calibration standard. The remaining calibration standard concentrations are prepared from the 2.0 ng/mL calibration standard. The final solution composition for each calibration standard was about 100% 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 Std	100 ng/mL Intermediate Solution C	0.200 mL	10.0 mL
1.0 ng/mL Std	2.0 ng/mL – Calib. Std	0.500 mL	1.0 mL
0.50 ng/mL Std	2.0 ng/mL – Calib. Std	0.250 mL	1.0 mL
0.10 ng/mL Std	2.0 ng/mL – Calib. Std	0.050 mL	1.0 mL
0.050 ng/mL Std	0.50 ng/mL – Calib. Std	0.100 mL	1.0 mL
0.020 ng/mL Std	0.50 ng/mL – Calib. Std	0.040 mL	1.0 mL

Alternative or additional calibration standard concentrations may be prepared if required.

4.2.6 *Source (& Characterization) of Samples*

The method was validated on four types of soil matrices selected to cover a range of pH, percent organic matter and percent clay. The soil matrices were provided by the DuPont Discovery Soil Bank at Stine-Haskell Research Center. The characteristics of each soil matrix used for method validation are provided below:

Soil Name	Notebook Ref. No.	Country	Type	% Clay	% Sand	% Silt	pHw	OM (%)
Texas	2006-035	U.S.A.	Clay Loam	33.2	34	32.8	7.9	1.3
California	2006-026	U.S.A.	Sandy Loam	10.8	58	31.2	7.8	1.0
Sassafras	2007-004	U.S.A.	Sandy Loam	6.0	70	24.0	5.4	2.1
Lleida	2006-118	Spain	Silty Clay	44.8	0.8	54.4	7.6	3.5

4.2.7 *Storage & Preparation of Samples*

Soil samples should be stored frozen at approximately -20°C. The entire soil sample should be homogenized using a Hobart processor (or equivalent) in order to obtain a representative sample.

4.2.8 *Sample Fortification Procedure*

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*

1. Weigh (10.0 ± 0.1) g of soil into a 50mL 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 70/30 ACN/ 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 70/30 ACN/ 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 80/20 ACN/ 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). Extracts are stable for at least two weeks if stored frozen.

4.2.10 *Analyte Purification Procedure*

1. Transfer a 5.0-mL aliquot of the extract into a 15-mL propylene centrifuge tube. Evaporate to a volume of approximately 1 mL under nitrogen gas using an N-Evap set at a water bath temperature of 40°C.
2. Bring the volume of each sample to 6.0 mL with 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] A stopwatch may be used to monitor the solvent flow. For example, 5 mL of loading solvent should take more than 5 min to flow through the cartridge (7-8 min is recommended, yielding a flow ca. 0.6-0.7 mL/min). The solvent flow should go to waste until the elution of the analytes in step 9.
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 6 mL may be used (e.g. 3 × 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. Wash the cartridges with 10 mL of methanol flowing at ~5 mL/min (this can be done with two 5.0 mL rinses). Allow all the solvent to go through; then apply high vacuum for a few seconds to pull all the remaining methanol.
8. 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.
9. Elute the analytes into the propylene centrifuge tubes with 15.0 mL of 50 mM ammonium hydroxide in methanol Elution Solution (e.g. 3 x 5.0 mL). The flow should be set to **1 mL/min.**[1] A stopwatch may be used to monitor the solvent flow. For example, 5 mL of Elution Solution should take more than 5 min to flow through the cartridge (7-8 min is recommended, yielding a flow ca. 0.6-0.7 mL/min).After the 15.0 mL of Elution Solution go through, apply high vacuum to pull any remaining solvent.
10. 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.
11. Add approximately 10 mL of 0.01% formic acid (aq) to each sample and swirl to mix. Bring the volume up to 15.0 mL with 0.01% formic acid (aq), cap, shake, and vortex for about 10 seconds.[2]

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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 *Alternatively, the concentrated extract may be transferred to a 15-mL propylene centrifuge tube if a final volume of 5.0 mL is necessary to match the sensitivity of the mass spectrometer. Quantitative transfer can be achieved by rinsing the 50-mL propylene centrifuge tube with 0.01% formic acid at least twice. Bring the volume of the extract to 5.0 mL in the 15-mL propylene centrifuge tube. If sample final volume is changed, calibration standard concentrations need to be adjusted as appropriate. Dilution of final extract can increase method ruggedness.*

12. Filter a portion of the purified extracts through 0.45 µm PTFE filters. Place about 1.0 mL of each extract into amber glass autosampler vials. The samples are now ready for LC/MS/MS analysis.

4.2.11 *Derivatization Procedure*

Analyte derivatization is not required.

4.3 Instrumentation

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 ion mode with MRM detector output for quantitative and confirmatory analysis.

4.3.2 *Operating Conditions*

HPLC Operating Conditions:

Injection Volume:	10-100 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 = 4.1 min
DPX-MAT28 = 5.1 min
DPX-KJM44 = 8.8 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, positive for all analytes
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	76	100	20	50	50	325	on	8	90	10	40	15
		170	103											
2	DPX-MAT28	214	68	100										
		214	101											
3	DPX-KJM44	228	68	100										
		228	168											

4.3.3 *Calibration Procedures*

Use standard mass spectrometer tuning and calibration techniques. If confidence in the mass calibration needs to be established (modern mass spectrometers under digital control generally do not need frequent mass calibration, especially for quantitative modes), use vendor recommended calibrating solution. Optimization tuning of MS system may be accomplished by infusion of one or more of the test analytes. This method uses external standards, prepared as described in Section 4.2.5.

Instrument calibration was based on the average response factor (ARF; defined here as peak area response/analyte concentration) obtained for external calibration standards. Two ion transitions were monitored for each analyte (as shown in section 4.3.2); instrument calibration was performed using the most responsive ion transition for each compound, and using the Excel® functions AVERAGE, STDEV, and RSD. Acceptance criteria for valid quantitation are: (1) a %RSD ≤20% for the individual calibration standard response factors and (2) a RSQ (r^2) value >0.99 for linear regression analysis of the calibration standards.

The calibrated range of instrument response was 0.020 ng/mL to 2.0 ng/mL. This range of calibration is approximately equivalent to expected final extract concentrations from 0.45 × 1.0 ng/g (LOQ) to 4.5 × 10 ng/g.[3] Typically, 6 calibration standards were interspersed with samples extracts for quantitative LC/MS/MS analysis.

Net recoveries were calculated and reported only when residues in the control sample are integrable and <50% of the LOQ. When the control residues are >50% of the LOQ, the recovery samples prepared at the LOQ using that control are invalidated.

3 The relationship shown above is for samples that had a final volume (after purification) of 15.0 mL. When a final volume of 5.0 mL was used, the range of calibration is approximately equivalent to expected final extract concentrations from 0.15 × 0.0010 mg/kg (LOQ) to 1.5 × 0.010 mg/kg.

When the control residues are <50% of the LOQ, corrected ppb (ng/g) found in fortified samples are calculated by subtracting area counts found in the control from area counts found in fortified samples. If net recoveries are calculated, those results must be uniquely identified or presented in a separate spreadsheet column heading for corrected ppb (ng/g).

4.3.4 *Sample Analysis*

At least 2 preliminary runs including a calibration standard and a solvent blank are routinely made to demonstrate adequate instrument response and insure the LC/MS system is equilibrated and free of chemical interferences. If multiple sets are analyzed, a solvent blank injection should be made between the last and first injections of the sets to minimize risk of carryover from high concentration sample to a low concentration calibration standard. Calibration standard analyses should precede the first sample analysis and follow the last sample analysis. Generally, the injection sequence was organized from lowest to highest expected analyte concentrations. Calibration standard runs were intermixed with the test samples and should be analyzed before and after every 1–3 samples in each analytical set.

4.4 Calculations

4.4.1 *Methods*

DPX-KJM44, DPX-MAT28, and IN-LXT69 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 ppb (ng/g) found in residue samples using the mean 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 (15.0 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 (5.0 mL), therefore AF = 15 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, therefore DF = 1),

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.