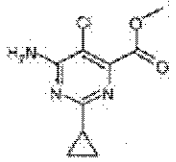
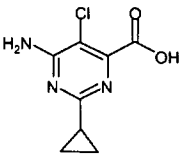
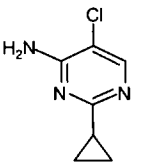
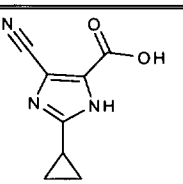
2.0 BACKGROUND INFORMATION

The chemical information for the analytes of interest is listed below.

Common Name	Aminocyclopyrachlor methyl ester
Structure	
DPX Number	DPX-KJM44
CAS Chemical Name	Methyl 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylate
CAS Registry 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 Registry 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 Registry Number	not yet available
Formula	$C_7H_8ClN_3$
Molecular Weight	169.61
Monoisotopic Weight	169 Da
pKa	not yet available

Common Name	Not yet available
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

Equivalent equipment and materials may be substituted unless otherwise specified; note any specifications in the following descriptions before making substitutions. Substitutions should only be made if equivalency/suitability has been verified with acceptable control and fortification recovery data.

3.1 Equipment

Instrumentation

LC System, HP1100 with Temperature-Controlled Autosampler
(Agilent Technologies, Wilmington, DE)

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

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)

Balance - Mettler AG104 Analytical Top-Loading Balance
(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, Part No. 5-7030 (Supelco, Bellefonte, PA)

Solid-Phase Extraction Supplies

Oasis® MCX Cartridge, 500 mg, 6 cc, Part No. 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 No. 00F-4256-E0
(Phenomenex, Torrance, CA)

HPLC Vials, Target DP Amber Kit, T/S/T Septa, 100 PK, Part No. 5182-0556
(Hewlett-Packard, Wilmington, DE)

Labware

250 mL, Nalgene® Cat. No. 16129 028 Polypropylene Centrifuge Bottles; VWR Brand (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)

Sorenson™ Multifit Research Pipet Tips, 5-200 and 100-1000 µL, Cat. 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) – *May be substituted with plasticware only; the use of* ***glass*** *tubes during blow down (solvent evaporation) steps may reduce analyte recoveries.*

Propylene Centrifuge Tubes, 15 mL, BD Falcon®, Conical Bottom, BD No. 352196, VWR Cat. No. 62406-200 (VWR Scientific Co., Bridgeport, NJ) – *May be substituted with plasticware only; the use of* ***glass*** *tubes during blow down (solvent evaporation) steps may reduce analyte recoveries.*

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., Part No. 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, a "reagent blank" should be prepared using appropriate amounts of the solvents, and using the chromatographic conditions specified in this report.

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

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

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

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

3.2.1 *Reference Analytical Standards*

Reference analytical standards of DPX-KJM44 (Lot No. 31, 99.4% pure), DPX-MAT28 (Lot No. 007, 91.1% pure), IN-LXT69 (Lot No. 002, 95.0% pure), and IN-QFH57 (Lot No. 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

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 20-mL water aliquot was measured into a 50-mL propylene centrifuge tube. The sample was acidified by adding 60 µL of concentrated formic acid. The sample was loaded into an Oasis® MCX solid phase extraction (SPE) cartridge, where the analytes were retained. The analytes were eluted 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 sample was evaporated under nitrogen gas flow to 2 mL with the water bath at 40°C and diluted with 0.01% formic acid (aq) to a final volume of 5.0 mL. The purified sample was filtered through a 0.45-µm PTFE filter and placed in a clean 15-mL propylene centrifuge tube. Approximately 1 mL of the concentrated sample was placed into a glass autosampler vial and analyzed for IN-QFH57 by LC/MS/MS. A 10-fold dilution was made by mixing 900 µL of 0.01% formic acid (aq) with100 µL of each concentrated/filtered water 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*

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 approximately 500 mL of HPLC-grade water to a 1 L glass bottle. Add 1.00 mL of concentrated formic acid. Dilute to a final volume of 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.

0.2% formic acid (aq) solution

Add approximately 500 mL of HPLC-grade 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 HPLC-grade water, cap, and homogenize. Store at room temperature and replace every month.

0.01% formic acid (aq)

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

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.

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 Intermediate Fortification Solution

Combine 1.00 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 one month.

100 ng/mL Fortification Solution A

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

10 ng/mL Fortification Solution B

Transfer 1.00 mL of the 1.0 µg/mL Intermediate Fortification Solution to a 100-mL volumetric flask, dilute to volume with methanol, cap, and mix well. Store in a freezer (below -10°C) and replace monthly.

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 one month.

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 **0.01% formic acid**, 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.

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.

4.2.6 *Source (& Characterization) of Samples*

The method was validated on water from four different sources in the USA: White Clay Creek, Newark, Delaware collected on 21-Nov-2007; Lums Pond, Bear, Delaware collected on 21-Nov-2007; drinking (tap) water from Stine-Haskell Research Center, Newark, Delaware collected on 29-Nov-2007, and well water from Kemblesville, Pennsylvania collected on 20-Nov-2007. A subsample of each type of water used for method validation was sent to Agvise Laboratories (Northwood, North Dakota, U.S.A.) for characterization. A summary of the characterization data is provided in the table below. A copy of Agvise Laboratories' water characterization report is provided in Appendix 4.

Measurement	White Clay Creek Water	Lums Pond Water	Newark Drinking Water	Kemblesville Well Water
pH	7.3	6.8	7.7	7.4
Calcium (ppm)	31	8.1	41	10
Magnesium (ppm)	12	5.2	12	7.5
Sodium (ppm)	13	14	18	11
Hardness (mg equivalent $CaCO_3$/L)	126	42	153	57
Conductivity (mmhos/cm)	0.31	0.17	0.39	0.17
Sodium Adsorption Ratio (SAR)	0.49	0.93	0.62	0.62
Total Dissolved Solids (ppm)	322	340	456	210
Turbidity (NTU)	1.35	6.80	0.23	2.98

4.2.7 *Storage & Preparation of Samples*

Water samples should be stored frozen at approximately -20°C. The samples should be thawed and shaken by hand to ensure homogeneity before aliquots are taken for purification and analysis.

4.2.8 *Sample Fortification Procedure*

Sample fortification was done after transferring a 20 mL aliquot of control water sample into 50-mL propylene centrifuge tube. LOQ-level fortification was done by adding 0.200 mL of the 10 ng/mL Fortification Solution B to the 20 mL water aliquot; while 10 × LOQ fortification was achieved by adding 0.200 mL of the 100 ng/mL Fortification Solution A to the 20.0-mL water sample aliquot.

Sample Identification	Amount (mL)	Fortification Solution		Fortification (ng/g, ppb)
		ng/mL	mL	
LOQ Fort	20.0 ± 0.1	10	0.200	0.10
10×LOQ Fort	20.0 ± 0.1	100	0.200	1.0

4.2.9 *Analyte Purification Procedure*

1. Carefully measure a 20 mL aliquot of the water sample in a 50-mL propylene centrifuge tube.
2. Add 0.060 mL of concentrated formic acid to each propylene centrifuge tube containing the water sample aliquots, cap, and mix using a vortex mixer for about three seconds. The samples now contain approximately 0.3% formic acid.
3. Fortify samples, if necessary, cap, and mix using a vortex mixer for about three seconds. The samples are now ready for the Oasis® MCX SPE cartridges.
4. Place the Oasis® MCX cartridges in the SPE manifold. Condition each cartridge with 5 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.
5. 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.
6. 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 9.
7. After all 20 mL of sample have been loaded, complete quantitative transfer by rinsing the propylene centrifuge tubes with 2 mL of 0.2% formic acid (aq) and loading the rinsate into the corresponding Oasis® MCX cartridges. Maintain the

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1 ***Three of the 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.***

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.

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 75 mM ammonium hydroxide in methanol Elution Solution (e.g. 3 × 5.0 mL). Maintain the flow at or below **1 mL/min.**[1] 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, cap and homogenize using a vortex mixer for few seconds. Place the samples in the nitrogen evaporator with water bath temperature of 40°C. Evaporate under nitrogen gas to a volume of approximately 2 mL.
11. Add approximately 2 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 mix using a vortex mixer for about 10 seconds. Then, vigorously shake the samples by hand to re-dissolve any analytes from the walls of the tube.
12. Filter the samples through 0.45-µm PTFE filters into clean 15-mL propylene centrifuge tubes.

13a. **Samples for quantification of IN-QFH57:** Place about 1.0 mL of each filtered water sample into clean glass autosampler vials. These samples are now ready for LC/MS/MS analysis of IN-QFH57.

13b. **Samples for quantification of DPX-KJM44, DPX-MAT28, and IN-LXT69:** Add 900 µL of 0.01% formic acid (aq) into clean glass autosampler vials (one vial per water sample). Add 100 µL of each filtered sample into the corresponding glass autosampler vial, cap, and mix using a vortex mixer for few seconds. These 10-fold diluted samples are now ready for LC/MS/MS analysis of DPX-KJM44, DPX-MAT28, and IN-LXT69.

4.2.10 *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

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1 ***Three of the 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.***

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*

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 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 analyte concentration in water samples from 0.05 ng/g (0.5 × LOQ) to 5 ng/g (50 × LOQ). Typically, 6 calibration standards were interspersed with water samples 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. When the control residues are <50% of the LOQ, corrected ng/g (ppb) 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 ng/g (ppb).

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 1–4 samples in each analytical set.

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 preconcentrated sample **F**inal **V**olume just before LC/MS/MS analysis (5.0 mL for all water samples in validation sets),

AF is **A**liquot **F**actor, which is equal to the Sample Volume (20.0 mL) divided by the Aliquot Volume (20.0 mL), therefore AF = 1 for all water validations sets,

DF is **D**ilution **F**actor used just prior to LC/MS/MS analysis (no dilution was done in water validation sets for IN-QFH57, therefore DF = 1; while a 10-fold dilution was done for DPX-KJM44, DPX-MAT28, and IN-LXT69, thus DF = 10 for those analytes),

SW is Sample **W**eight in grams of sample aliquot (assuming a water density of 1.0 g/mL: 20 mL = 20 g),

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.