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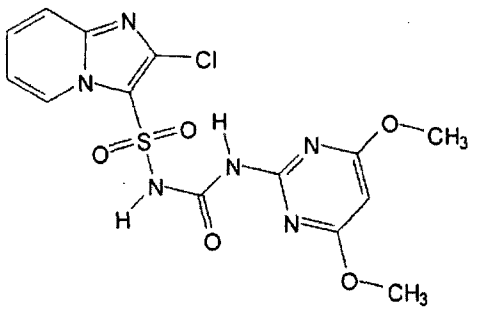
DETERMINATION OF IMAZOSULFURON, ADPM, HMS, IHOA, IPSN, AND UDPM IN WATER
Method: **RM-42W-2-1** Date: August 20, 2008

I. INTRODUCTION

This method describes the determination of imazosulfuron, 1-(2-chloroimidazo[1,2-*a*]pyridin-3-ylsulfonyl)-3-(4,6-dimethoxypyrimidin-2-yl)urea, and its degradates (ADPM, HMS, IHOA, IPSN, and UDPM) in water. Briefly, the method involves centrifuging a water sample in a glass centrifuge tube to remove solids, and then measuring a 10 mL aliquot into a 50 mL round-bottom flask. Ammonium acetate is added, and the sample is reduced slightly in volume by rotary evaporation. The residues are loaded onto an SPE cartridge, and then eluted with methanol/water. The eluant is rotary evaporated and the residues are redissolved for a final volume of 5 mL of 3:7 methanol/water (v/v). This solution is analyzed by LC/MS-MS for imazosulfuron, ADPM, HMS, IHOA, IPSN, and UDPM.

The method originally used a C18 SPE cartridge for cleanup, and the IHOA eluted after loading the sample during a deionized water rinse. This resulted a separate fraction for IHOA (in 10 mL of water) and a higher detection limit for IHOA. The method was modified to use a Strata-X cartridge as IHOA is retained on these cartridges. This allows all of the analytes to be eluted together from the cartridge with methanol/water (5:1, v/v).

II. ANALYTICAL STANDARDS



Imazosulfuron reference standard - Valent U.S.A. Corporation

Imazosulfuron Standard, 1.0 mg/mL Stock solution.

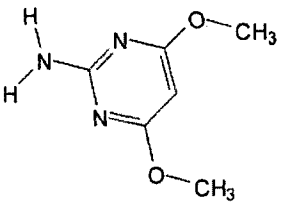
Weigh 0.100 grams into 100 mL volumetric flask (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard). Dilute to volume with acetone, and store in a freezer.

Imazosulfuron Standard, 100 µg/mL solution (in acetone).

Pipet 10.0 mL of the 1.0 mg/mL Imazosulfuron Stock solution (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store in a freezer.

Imazosulfuron Standard, 25 µg/mL solution (in acetone).

Pipet 25.0 mL of the 100 µg/mL Imazosulfuron Standard (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store in a freezer.



ADPM reference standard - Valent U.S.A. Corporation

ADPM Standard, 1.0 mg/mL Stock solution.

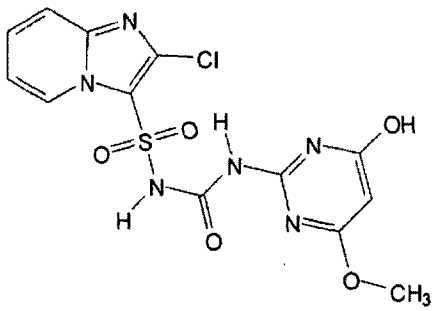
Weigh 0.100 grams into 100 mL volumetric flask (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard). Dilute to volume with acetone, and store refrigerated.

ADPM Standard, 100 µg/mL solution (in acetone).

Pipet 10.0 mL of the 1.0 mg/mL ADPM Stock solution (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

ADPM Standard, 25 µg/mL solution (in acetone).

Pipet 25.0 mL of the 100 µg/mL ADPM Standard (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.



HMS reference standard - Valent U.S.A. Corporation

HMS Standard, 1.0 mg/mL Stock solution.

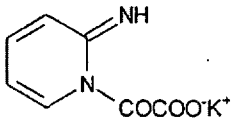
Weigh 0.100 grams into 100 mL volumetric flask (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard). Add 50 mL acetone, *add 25 mL water*, and then dilute to volume with acetone. Store refrigerated.

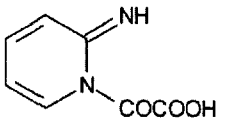
HMS Standard, 100 µg/mL solution (in methanol).

Pipet 10.0 mL of the 1.0 mg/mL HMS Stock solution into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

HMS Standard, 25 µg/mL solution (in methanol).

Pipet 25.0 mL of the 100 µg/mL HMS Standard (in methanol) into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.





IHOA (potassium salt) reference standard - Valent U.S.A. Corporation

IHOA Standard, 1.0 mg/mL Stock solution.

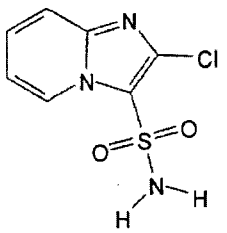
Weigh 0.123 grams of the potassium salt, to be equivalent to 0.100 grams of the acid form, into 100 mL volumetric flask (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard). *Add 10 mL water to dissolve the standard*, and dilute to volume with acetone. Store refrigerated.

IHOA Standard, 100 µg/mL solution (in water).

Pipet 10.0 mL of the 1.0 mg/mL IHOA Stock solution into a 100 mL volumetric flask. Dilute to volume with water. Store refrigerated.

IHOA Standard, 25 µg/mL solution (in water).

Pipet 25.0 mL of the 100 mg/mL IHOA Standard (in water) into a 100 mL volumetric flask. Dilute to volume with water. Store refrigerated.



IPSN reference standard - Valent U.S.A. Corporation

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IPSN Standard, 1.0 mg/mL Stock solution.

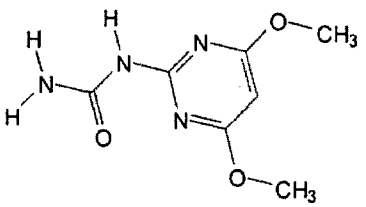
Weigh 0.100 grams into 100 mL volumetric flask (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard). Dilute to volume with acetone, and store refrigerated.

IPSN Standard, 100 µg/mL solution (in acetone).

Pipet 10.0 mL of the 1.0 mg/mL ADPM Stock solution (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

IPSN Standard, 25 µg/mL solution (in acetone).

Pipet 25.0 mL of the 100 µg/mL IPSN Standard (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.



UDPM reference standard - Valent U.S.A. Corporation

UDPM Standard, 1.0 mg/mL Stock solution.

Weigh 0.100 grams into 100 mL volumetric flask (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard). *Add 15 mL water to dissolve the standard*, and dilute to volume with acetonitrile. Store refrigerated.

UDPM Standard, 100 µg/mL solution (in acetone).

Pipet 10.0 mL of the 1.0 mg/mL UDPM Stock solution into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

UDPM Standard, 25 µg/mL solution (in acetone).

Pipet 25.0 mL of the 100 µg/mL UDPM Standard (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

IHOA/UDPM Fortification Standard, 1.0 µg/mL (in acetone).

Pipet 2.0 mL of the 25 µg/mL IHOA Standard (in water) and 2.0 mL of the 25 µg/mL UDPM Standard (in acetone) into a 50 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

Fortification Mix, 1.0 µg/mL (in acetone)

Pipet 2.0 mL of each 25 µg/mL Standard (imazosulfuron, ADPM, HMS, and IPSN) into a 50 mL volumetric flask. Dilute to volume with acetone. Store in a freezer. *This standard should be prepared bi-weekly as imazosulfuron may degrade due to the trace amount of water present.*

IHOA/UDPM Calibration Standard, 250 µg/L

Pipet 1.0 mL of the 25 µg/mL IHOA/UDPM Standard (in acetone) into a 100 mL volumetric flask. Dilute to volume with 3:7 methanol/water (v/v). Store refrigerated.

IHOA/UDPM Calibration Standard, 100 µg/L

Pipet 10.0 mL of the 250 µg/L IHOA/UDPM Calibration Standard into a 25 mL volumetric flask. Dilute to volume with 3:7 methanol/water. Store refrigerated.

IHOA/UDPM Calibration Standard, 50 µg/L

Pipet 5.0 mL of the 250 µg/L IHOA/UDPM Calibration Standard into a 25 mL volumetric flask. Dilute to volume with 3:7 methanol/water. Store refrigerated.

IHOA/UDPM Calibration Standard, 25 µg/L

Pipet 5.0 mL of the 250 µg/L IHOA/UDPM Calibration Standard into a 50 mL volumetric flask. Dilute to volume with 3:7 methanol/water. Store refrigerated.

IHOA/UDPM Calibration Standard, 5 µg/L

Pipet 5.0 mL of the 25 µg/L IHOA/UDPM Calibration Standard into a 25 mL volumetric flask. Dilute to volume with 3:7 methanol/water. Store refrigerated.

Mixed Calibration Standard, 250 µg/L

Pipet 1.0 mL each of the 25 µg/mL ADPM, HMS, IPSN, and Imazosulfuron standards into a 100 mL volumetric flask. Dilute to volume with 3:7 methanol/water (v/v). Store refrigerated. *This standard should be prepared weekly as imazosulfuron may degrade due to the water present.*

Mixed Calibration Standard, 100 µg/L

Pipet 20.0 mL of the 250 µg/L Mixed Calibration Standard into a 50 mL volumetric flask. Dilute to volume with 3:7 methanol/water. Store refrigerated. *This standard should be prepared weekly as imazosulfuron may degrade due to the water present.*

Mixed Calibration Standard, 50 µg/L

Pipet 25.0 mL of the 100 µg/L Mixed Calibration Standard into a 50 mL volumetric flask. Dilute to volume with 3:7 methanol/water. Store refrigerated. *This standard should be prepared weekly as imazosulfuron may degrade due to the water present.*

Mixed Calibration Standard, 25 µg/L

Pipet 25.0 mL of the 50 µg/L Mixed Calibration Standard into a 50 mL volumetric flask. Dilute to volume with 3:7 methanol/water. Store refrigerated. *This standard should be prepared weekly as imazosulfuron may degrade due to the water present.*

Mixed Calibration Standard, 5 µg/L

Pipet 5.0 mL of the 25 µg/L Mixed Calibration Standard into a 25 mL volumetric flask. Dilute to volume with 3:7 methanol/water. Store refrigerated. *This standard should be prepared weekly as imazosulfuron may degrade due to the water present.*

Note: Similar dilutions may also be performed to generate appropriate standards.

III. REAGENTS

Acetone - Pesticide quality

Acetonitrile - Pesticide quality

Ammonium Acetate – Reagent grade

Formic Acid, 96% - Reagent grade

Methanol - Pesticide quality

Water – Deionized

Water – HPLC grade

IV. REAGENT SOLUTIONS

Acetonitrile, with 0.05% Formic acid

Add 0.5 mL of formic acid per liter of acetonitrile. Store at room temperature.

Ammonium Acetate, 1 M.

Dissolve 7.7 g .in 100 mL of water. [This preparation may be scaled as necessary.] Store at room temperature.

Methanol, with 0.05% Formic acid

Add 0.5 mL of formic acid per liter of methanol. Store at room temperature.

Methanol/Water, 3:7 (v/v).

Combine 3 parts methanol with 7 parts water. For example, add 150 mL of methanol and 350 mL of water sequentially to a reagent bottle. Store at room temperature.

Water, with 0.05% Formic acid

Add 0.5 mL of formic acid per liter of HPLC grade water. Store at room temperature.

V. EQUIPMENT

Autosampler vials, screw-top with Teflon-coated septums

Balances, Analytical and Top Loading

Caps for Centrifuge Tubes, Qorpak Green Thermoset Screw Cap with Teflon Liner, 24-400 size, Part No. 5205 (or equivalent)

Centrifuge, Sorval Evolution RC (or equivalent)

Cork Rings (80 mm and 110 mm diameter)

Glass Centrifuge Tubes, 50 mL [Kimble Disposable Centrifuge Tube, Box of 72, Art. No. 73785-50; or equivalent]

Glass Centrifuge Tubes, Graduated - 15 mL (or equivalent)

Graduated Glass Centrifuge Tubes, 15 mL [Kimble Disposable Centrifuge Tube, Box of 72, Art. No. 73785-50; or equivalent]

Heated Water Bath (temperature <40°C)

Phenomenex Stata-X Cartridges, 33um Polymeric Reversed Phase, 500mg/12mL Giga Tubes (Part Number 8B-S100-HDG)

Pipettor(s), Automatic - capable of accurately dispensing volumes of 0.2 to 2.5 mL

Pipettes, Serological, Disposable – 5 and 10 mL

Pipettes, Volumetric – 1, 4, 5, 10, and 25 mL

Refrigerator/Freezer

Rotary Vacuum Evaporators

Round-bottom Flasks – 250 mL, 100 mL, and 50 mL

Syringes – 25 µL and 50 µL (or 100 µL)

Vacuum Manifold for SPE Cartridges, Baker (or equivalent)

VI. INSTRUMENTATION

High Performance Liquid Chromatograph with Mass Selective Detector (LC/MS-MS) - Finnigan TSQ Quantum with electrospray ionization interface. Conditions shown below are suggested for this analysis (other conditions may be used as appropriate).

IHOA/UDPM HPLC Conditions:

Column:	Luna (C18), 2.5µm, 50mm x 3.0mm (Phenomenex part # 00B-4446-Y0)
Mobile Phases:	Methanol, 0.05% formic acid Water, 0.05% formic acid
Injection Volume:	20 µL

Gradient Program:

Time, Min	Flow, µL/min	% MeOH, 0.05% HOOCH	%Water, 0.05% HOOCH
0	250	35	65
3.5	250	35	65
4	300	35	65
7	300	35	65

IHOA/UDPM LC/MS-MS Conditions:

Interface:	Electrospray Ionization
Polarity:	Positive

Segment 1 (IHOA) -

Source CID Collision Energy:	15
Q2 Collision Gas Pressure:	1.5 mTorr
Scan type:	SRM
Parent Ion Mass:	167.0
Quantitation Ion Mass	78.0
Collision Energy:	38

Segment 2 (UDPM) -

Source CID Collision Energy:	16
Q2 Collision Gas Pressure:	1.5 mTorr
Scan type:	SRM
Parent Ion Mass:	199.1
Quantitation Ion Mass	156.0
Collision Energy:	22

Retention Time:	2.6 minutes for IHOA 5.0 minutes for UDPM (*Figure 1*)

Note: Divert valve was used to direct column eluant to waste for the first minute after injection.

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Mixed Analyte HPLC Conditions:

Column:	Synergi 4µ Polar-RP, 50mm x 2.0mm (Phenomenex part # 00B-4336-B0)
Mobile Phases:	Acetonitrile, 0.05% formic acid Water, 0.05% formic acid
Flow Rate:	250 µL/minute
Injection Volume:	20 µL

Gradient Program:

Time, Min	% ACN, 0.05% HOOCH	%Water, 0.05% HOOCH
0	10	90
1.0	10	90
4.0	40	60
5.0	40	60
6.0	80	20
9.0	80	20
10.5	10	90
13	10	90

Mixed Analyte LC/MS-MS Conditions:

Interface:	Electrospray Ionization
Polarity:	Positive

Segment 1 (ADPM) -

Source CID Collision Energy:	15
Q2 Collision Gas Pressure:	1.0 mTorr
Scan type:	SRM
Parent Ion Mass:	156.0
Quantitation Ion Mass	100.0
Collision Energy:	33

Segment 2 (HMS and IPSN) -

Source CID Collision Energy:	14
Q2 Collision Gas Pressure:	0.9 mTorr
Scan type:	SRM
* Scan Event 1 (IPSN) -	
Parent Ion Mass:	232.0
Quantitation Ion Mass	152.1
Collision Energy:	38
* Scan Event 2 (HMS) -	
Parent Ion Mass:	399.0
Quantitation Ion Mass	142.1
Collision Energy:	22

Segment 3 (Imazosulfuron) -

Source CID Collision Energy:	16
Q2 Collision Gas Pressure:	0.9 mTorr
Scan type:	SRM
Parent Ion Mass:	413.0
Quantitation Ion Mass	156.1
Collision Energy:	34
Retention Times:	1.9 minutes for ADPM
	4.3 minutes for IPSN
	4.8 minutes for HMS
	8.0 minutes for Imazosulfuron (*Figure 5*)

The instrument parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography, to resolve matrix interferences, or to utilize other types of LC/MS-MS instruments. Each set of chromatograms must be clearly labeled with the LC/MS-MS parameters used.

VII. ANALYTICAL PROCEDURES

1. Sample Setup

Briefly shake the water sample. Decant approximately 40 mL into a 50 mL glass centrifuge tube [e.g.- Kimble #73785-50]. Cap the tube, and place the tube into the centrifuge. [Remember that the tubes must be placed so that the weight of the samples in the centrifuge is balanced.] It is recommended that the samples are centrifuged for 30 minutes at 3500 rpm (at ambient temperature) to separate the solids. [*Remember not to exceed the RCF or RPM maximum for the centrifuge tubes used.*] Measure 10.0 mL (with a serological pipette) into a 50 mL (or 100 mL) round-bottom flask. At this point, if required by the testing facility, a control sample to be used for method recoveries may be fortified with a the analytes (*see Note 1*).

Pipette 1 mL of ammonium acetate solution (1 M) into each sample, swirl briefly to mix, and then rotary evaporate (using a heated water bath, temperature approximately 35-40°C) for at least 15 minutes (depending on vacuum quality, 30-40 minutes may be required) to ensure complete removal of any organic solvent and reduction to 7-8 mL total volume. This removes any trace amounts of organic solvent, and reduces the volume. *Removal of all organic solvent is necessary to ensure that the analytes, especially IHOA, are retained on the SPE cartridge.*

2. SPE Cartridge Cleanup

Place the Strata-X cartridges onto a vacuum manifold or a rack (or into 250 mL round-bottom flasks, supported on the 24/40 joint), and carefully precondition them by passing through 2 x 5 mL methanol. Allow the first rinse to drain to the top of the frit, add the second rinse, and then allow the second rinse to drain to the top of the frit.

The flow through the Strata-X cartridge only requires gravity, and care should be taken to ensure that each sequential rinse passes completely through the cartridge before adding the next rinse.

Remove each cartridge and rinse the barrel of the cartridge (walls and frit) with 1 or 2 water rinses (each immediately discarded by inverting the cartridge) to ensure that no methanol is present. Place the cartridges back on the manifold or rack and pass 4 x 5 mL of water through each cartridge.

As before, care should be taken to ensure that each sequential rinse passes completely through the cartridge before adding the next rinse.

Transfer the aqueous residue from each 50 mL (or 100 mL) round-bottom flask onto the cartridge (more than one addition to the cartridge may be necessary, depending on the concentrated sample volume and the cartridge size). After the sample has been transferred, add 2.5 mL deionized water to the round-bottom flask and rotate/swirl the flask to rinse the walls of the flask.

When the sample extract in the cartridge has drained to the frit, add the water rinse to the cartridge and allow it to pass through the cartridge. Add a second 2.5-mL portion of deionized water to the round-bottom flask, and rotate/swirl to rinse the sides of the flask. When the first water rinse has drained to the frit, add the second water rinse. [*After transferring the second water rinse, a 5-mL portion of methanol may be added to the round-bottom flask for the next rinse of the flask – see below.*] When the second water rinse has drained to the frit, move the cartridge to a 100 mL round-bottom flask (with the cartridge suspended from the top of the 24/40 joint). Discard the accumulated eluant.

Add 5 mL of methanol to each 50 mL (or 100 mL) round-bottom flask [*preferably added while the second water rinse was passing through the cartridge*], and rotate/swirl the flasks to rinse the walls and dissolve any remaining the residues, and then add 1 mL of water to each flask.

Transfer the methanol/water eluant to the cartridge, and elute the residues into the 100 mL round-bottom flask. Add 5 mL of methanol again to each 50 mL (or 100 mL) round-bottom flask, rotate/swirl to rinse the sides of the flasks, and add 1 mL of water to each flask. Transfer this eluant to the each cartridge when the first methanol/water rinse has drained to the frit.

After the eluant has passed through the cartridge, the cartridge is removed and the collected eluant may be stored (in a stoppered flask) in a freezer. *Note that eluant samples should not be stored for more than overnight due to possible degradation of IHOA in 5:1 methanol/water.*

3. Concentration of Residues

Rotary evaporate the sample (temperature <40°C, typically 35 to 40°C) to reduce the volume to approximately 1 mL. Transfer the aqueous residue into a graduated vial with a Pasteur pipet. Add 1.5 mL methanol into the round-bottom flask, and rotate/swirl and sonicate to dissolve the residues. Transfer the methanol rinse with a Pasteur pipet into the graduated vial, and then add approximately 2 mL water to the round-bottom flask. Swirl the water in the flask to rinse the sides, and then transfer the water rinse to the graduated vial. Rinse the flask again with about 0.5 to 1 mL of water, adding this rinse to the graduated vial to set the volume to 5.0 mL (approximately 30% methanol in water). Mix the sample in the graduated vial using the pipet, and then transfer portions into autosampler vials for LC/MS-MS analysis for IHOA/UDPM [Step 4] and/or for imazosulfuron, ADPM, HMA and IPSN [Step 5]. The samples may be stored in a freezer. *Note that analysis of the samples within 5 days is recommended due to the possible degradation of imazosulfuron in the methanol/water solution.*

4. LC/MS-MS Analysis for IHOA/UDPM

Instrument calibration is performed using a linear fit with a non-zero intercept. The calibration is performed with linearity standards that are distributed within each analytical sequence.

Condition the instrument with at least six injections of a sample extract. Analyze five linearity standard concentrations *within the analytical sequence* to generate the linear calibration of the LC/MS-MS, including a 5 µg/L (or less) standard. A typical set of standards would include concentrations of 250, 100, 50, 25, and 5 µg/L (with an injection volume of 20 µL). The coefficient of determination (r^2) is calculated from the linearity standards (see Step 6), and this value must be greater than 0.99 for the instrument response to be considered linear over the range of concentrations. In addition, the concentration calculated from the peak area of each of the standards, using the slope and intercept from the linear fit, must be within 15% of the corresponding standard concentrations.

Additional 50 µg/L reference standards (continuing calibration standards) are also analyzed as part of the analytical sequence. Typically, the sequence is constructed with the following order: a reference standard (50 µg/L), 2 to 4 sample extracts, a reference standard or linearity standard, 2 to 4 sample extracts, ..., and a reference standard. *The sequence must begin and end with reference standards (continuing calibration standards).* The coefficient of variation of the reference standard responses must be 10% or less for the analysis set to be acceptable.

If the peak area observed for a sample is greater than the peak area of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted (with 3:7 methanol/water, v/v) such that the peaks obtained are within the documented linear response range of the LC/MS-MS.

5. LC/MS-MS Analysis for Imazosulfuron, ADPM, HMS, and IPSN

Instrument calibration is performed using a linear fit with a non-zero intercept. The calibration is performed with linearity standards that are distributed within each analytical sequence.

Condition the instrument with at least six injections of a sample extract. Analyze five linearity standard concentrations *within the analytical sequence* to generate the linear calibration of the LC/MS-MS, including a 5 µg/L (or less) standard. A typical set of standards would include concentrations of 250, 100, 50, 25, and 5 µg/L (with an injection volume of 20 µL). The coefficient of determination (r^2) is calculated from the linearity standards (see Step 6), and this value must be greater than 0.99 for the instrument response to be considered linear over the range of concentrations. In addition, the concentration calculated from the peak area of each of the standards, using the slope and intercept from the linear fit, must be within 15% of the corresponding standard concentrations

Additional 50 µg/L reference standards (or continuing calibration standards) are also analyzed as part of the analytical sequence. Typically, the sequence is constructed with the following order: a reference standard (50 µg/L), 2 to 4 sample extracts, a reference standard or linearity standard, 2 to 4 sample extracts, ..., and a reference standard. *The sequence must begin and end with reference standards.* The coefficient of variation of the reference standard responses must be 10% or less for the analysis set to be acceptable.

If the peak area observed for a sample is greater than the peak area of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted (with 3:7 methanol/water, v/v) such that the peaks obtained are within the documented linear response range of the LC/MS-MS.

6. Calculations

To calculate the linear fit, the peak area and the concentration of each of the standards is input into an Excel spreadsheet.

Excel calculates the slope for the regression line as $b = \dfrac{n\sum xy - (\sum x)(\sum y)}{n\sum x^2 - (\sum x)^2}$

and calculates the intercept for the regression line as $a = \overline{Y} - b\overline{X}$

The slope and the intercept are calculated using a linear regression weighed by 1/concentration. Typically, the linear regression is based on standard concentration and Peak Units (Area/10^6), and replicate entries are included in the data set prior to performing the linear regression in Excel [this provides weighting by 1/concentration, relative (or proportional) to the other linearity standards]. For example:

Linearity Standard	Number of Entries in Data Set
250 µg/L	2
100 µg/L	5
50 µg/L	10
25 µg/L	20
5 µg/L	100

For each analyte, the concentration in the sample is calculated as follows:

$$\text{Sample Concentration, } (\mu g/L) = \frac{[(b \times X) + a] \times C \times D}{E}$$

where:
b = slope [from regression analysis]
X = Sample response (Peak Area)
a = intercept [from regression analysis]
C = Final volume (5.0 mL)
D = Dilution factor, used if the sample extract is diluted prior to analysis
E = Sample volume (10 mL)

VIII. LIMIT OF DETECTION

The limit of detection (LOD) of this method is 2.5 ppb (µg/L). These detection limits are based on a 10-mL sample volume, a 5-mL final volume, and a 5 µg/L linearity standard as the lowest concentration in the linearity verification.

$$\text{Limit of Detection} = \frac{5 \text{ mL Final Volume} \times 5 \mu g/L}{10 \text{ mL Sample Volume}} = 2.5 \mu g/L = 2.5 \text{ ppb}$$