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Measurement of Mycotoxins in Medical Marijuana by LC-MS/MS
NYS DOH MML-303

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1.0. Scope and Application

1.1. This method (NYS ELAP Method ID 9982) is to be used for the analysis of medical marijuana (MM) products for the determination of concentrations of the following analytes (listed in **Table 1**) as required in Title 10 (Health), Chapter XIII, Part 1004.14(g) of the official Compilation of Codes, Rules, and Regulations, of the State of New York.

Table 1. Analyte List

Analyte	CAS Number	LOD (ng/g) in MCT[2] Matrix	LOQ[1] (ng/g) in MCT[2] Matrix	LOD (ng/mL) in MCT[2]/ Solvent Matrix	LOQ[1] (ng/mL) in MCT[2]/ Solvent Matrix
Aflatoxin B1 (AT-B1)	1162-65-8	0.50	2.5	0.050	0.25
Aflatoxin B2 (AT-B2)	7220-81-7	0.50	2.5	0.050	0.25
Aflatoxin G1 (AT-G1)	1165-39-5	0.50	2.5	0.050	0.25
Aflatoxin G2 (AT-G2)	7241-98-7	0.50	2.5	0.050	0.25
Ochratoxin A (OTA)	303-47-9	0.50	2.5	0.050	0.25

[1] The Limit of Quantitation (LOQ) is the lowest concentration that can be accurately quantified for a target analyte (**Section 3.17**).

[2] LOQs were determined with medium-chain triglycerides (MCT) as the matrix. LOQs referenced within Table 1 are subject to change based on LOD/LOQ determinations detailed within section 11.2.

Table 2. Internal Standard List

Analyte	CAS Number
Aflatoxin B1-$^{13}C_{17}$ (AT-B1-$^{13}C_{17}$)	1217449-45-0
Aflatoxin B2-$^{13}C_{17}$ (AT-B2-$^{13}C_{17}$)	1217470-98-8
Aflatoxin G1-$^{13}C_{17}$ (AT-G1-$^{13}C_{17}$)	1217444-07-9
Aflatoxin G2-$^{13}C_{17}$ (AT-G2-$^{13}C_{17}$)	1217462-49-1
Ochratoxin A-$^{13}C_{20}$ (OTA-$^{13}C_{20}$)	911392-42-2

1.2. This method is restricted to analytical chemists experienced in the use of Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Limit of Detection (**LOD**) and Limit of Quantitation (**LOQ**) studies and/or verifications are performed on every instrument and are subject to change (see **Section 11.2**). Each analyst must demonstrate the ability to generate acceptable results with this method using the procedures described in **Section 11.1**.

2.0. Summary of the Method

2.1. After liquid extraction with an aqueous-organic solvent mixture, the samples are analyzed using an LC-MS/MS system equipped with an electrospray ionization (ESI) source which is operated in the multiple reaction monitoring (MRM) mode. The baseline separation of 5 mycotoxins was achieved on a C_{18} reversed-phase column in 11 minutes using a programed gradient of increasing organic modifier. See Table 1 for **LOD** and **LOQ** for the mycotoxins in MCT.

3.0. Definitions

3.1. Internal Standard (**IS) -** Pure compounds, that are not be found in any sample. In this procedure a mixture of ^{13}C-labeled analogs of the mycotoxins are used as **IS**. These compounds are added to a sample in a known amount before the extraction and are measured in a similar manner as the samples. The internal standards allow for the determination of area ratios in the quantitation and adjust for any matrix ion suppression or low recovery.

3.2. Internal Standard Stock Diluent **(ISD)** – A solution of high-concentration **IS** in extraction solvent. This diluted solution will be the precursor to the **ISS**.

3.3. Internal Standard Spiking Solution (**ISS**) – A mixture of mid-concentration **IS** in extraction solvent. This solution is spiked into samples to adjust for matrix ion suppression or low recovery, and to monitor the integrity of the sample injections. This solution will be the precursor to the **IWD**.

3.4. Internal Standard Working Diluent (**IWD**) – A mixture of low-concentration **IS** in extraction solvent. This solution is used to dilute the standards and monitor the integrity of the standard injections.

3.5. System Blank **(SBLK)** – A portion of appropriate clean solvent that is analyzed to verify that the instrument is free from background contamination.

3.6. Laboratory Reagent Blank (**LRB)** – An aliquot of extraction solvent that is treated exactly as if it were a sample including exposure to all glassware, equipment, solvents, internal standards, and reagents that are used with the samples. The **LRB** is used to determine whether method analytes or other interferences are present in the laboratory environment, reagents or apparatus.

3.7. Laboratory Fortified Blank (**LFB**) – An aliquot of extraction solvent that is spiked with known quantities of target analytes and analyzed as a sample. The **LFB** measures the accuracy of the methodology.

3.8. Method Blank (**MB**) – An aliquot of matrix that is treated exactly as if it were a sample including exposure to all glassware, equipment, solvents, internal standards, and reagents that are used with the samples. The **MB** is analyzed to verify that there are no interfering peaks in the matrix.

3.9. Matrix Spike (**MS**) – An aliquot of sample prepared, taken through all sample preparation and analytical steps of the procedure unless otherwise noted in a referenced method, by adding a known amount of target analyte to a specified amount of sample for which an independent test result of target analyte concentration is available. Matrix spikes are used, for example, to determine the effect of the matrix on a method's recovery efficiency. When sample is not suitable, a "representative" matrix may be used instead. Synonym: Laboratory Fortified Sample Matrix (LFM) and Laboratory Control Sample (LCS).

3.10. Matrix Spike Duplicate (**MSD**) – Prepared identically to the **MS**, the Matrix Spike Duplicate is a second portion of matrix used to prepare the **MS** that is spiked and processed just like the **MS**. The **MS** and **MSD** are used together to measure the precision of the methodology.

3.11. Stock Standard (**SS**) – A concentrated solution of method analyte(s) prepared in the laboratory from referenced and certified analyte standards or a concentrated solution of method analyte(s) purchased directly from a referenced and certified source.

3.12. Working Standard (**WS**) – A solution of method analytes prepared from stock standard solutions that is diluted as necessary to prepare calibration standards or other necessary analyte solutions.

3.13. Calibration Standard (**CalS**) – A solution of method analytes prepared from stock or working standard solutions that is used to calibrate the instrument response with respect to analyte concentration.

3.14. Continuing Calibration Verification Standard (**CCV**) – One of the calibration standards used to verify the acceptability of an existing calibration. Synonyms: Continuing Calibration Check Standard (CCC).

3.15. Cross Check Reference Standard (**CCR**) – A solution of method analytes prepared from a stock standard solution that is different (different vendor, different lot, or different preparation) from the solution used to prepare calibration standards. The **CCR** verifies that the original calibration source is acceptable and measures the accuracy of laboratory performance on outside sources. The final concentration of the CCR should be near the middle of the calibration curve range. Synonym: Secondary Source Standard (SSS).

3.16. Limit of Detection **(LOD)** – The statistically calculated minimum concentration of an analyte that can be measured with 99% confidence that the value is greater than zero. Synonym: Method Detection Limit (MDL).

3.17. Limit of Quantitation (**LOQ)** – The minimum concentration that can be quantitatively reported for a target analyte. The lowest calibration standard must be at or below the **LOQ** for each analyte. **LOQ** is typically 3-5 times the **LOD**. Synonym: Method Reporting Limit (MRL).

3.18. Upper Limit of Quantitation (**ULOQ**) – The maximum acceptable point on the calibration curve. The **ULOQ** is the concentration of the most concentrated **CalS** standard.

3.19. Preparation Batch – Samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A preparation batch consists of one to twenty samples (not including method blanks, LCS, matrix spikes and matrix duplicates) of the same matrix with a maximum processing time of twenty-four (24) hours between the first and last sample.

3.20. Analytical Batch - An analytical batch consists of prepared samples which are analyzed together as a group. An analytical batch can include prepared samples originating from different matrices and can exceed twenty samples.

4.0. Health and Safety Warnings

4.1. Caution must be used when working with mycotoxins. According to the International Agency for Research on Cancer (IARC; See ref. 17.1, 17.2), aflatoxins are carcinogenic to humans and ochratoxin A is possibly carcinogenic to humans. Always use gloves and operate in a well-ventilated hood when working with mycotoxins. Immediate handwashing following the handling of standards and samples is greatly encouraged.

4.2. The toxicity and carcinogenicity of each chemical used in this method have not been thoroughly investigated. Each chemical compound must be treated as a potential health hazard and exposure must be limited to the lowest possible level.

4.3. Always follow guidelines listed in Safety Data Sheets (SDS) for proper storage, handling, and disposal of solvents, reagents, and standards. SDSs are located within the laboratory in labeled, yellow binders. These guidelines must be made available to all personnel involved in the chemical analyses.

4.4. Appropriate laboratory coats, safety glasses and gloves must be worn when performing standard or sample preparations, working on instrumentation, disposing of waste, and cleaning glassware.

4.5. The chemical fume hood must be used when using or preparing standards, reagents, or samples that require proper ventilation.

5.0. Interferences

5.1. Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in the chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running a **LRB** as described in **Section 11.4.**

5.1.1. Glassware and syringes used in the medical marijuana laboratory must be thoroughly cleaned to prevent contamination. After use, rinse with the last solvent used, then rinse three times with dichloromethane, three times with acetone, and three times with methanol.

5.1.2. The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in the laboratory is not performed, nor required.

5.1.3. After cleaning, glassware is stored away from standards and syringes to prevent cross-contamination.

5.2. When interferences or contamination are evident in a sample, the re-preparation of the original sample is recommended after the source of contamination is identified and removed.

5.3. Interfering contamination due to "carry-over" may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Rinsing of the autosampler syringe and associated equipment with solvent wash (methanol) can minimize sample cross contamination.

5.3.1. If target analytes are present in at an unusually high concentration of extracted sample, the analyst must demonstrate that the analytes in the subsequent samples are not due to carry over. In addition, after analysis of a sample containing high concentrations of analytes, one or more injections of Laboratory Reagent Blank (**LRB; Section 12.3**) should be made to ensure that there is no carry-over, and that accurate values are obtained for the next sample. The LRB must pass contamination criteria set in **Section 11.4.2**.

5.3.2. Alternately, if the samples immediately following the high concentration sample do not contain the analytes that were at high concentration and have a calculated concentration <**LOQ**, freedom from contamination has been established.

5.4. Matrix interferences may occur as a result of contaminants present in the sample. If matrix interference is believed to have occurred, it is recommended that a matrix spike is analyzed with the sample to verify results. This may not always be possible given the amount of sample that is received for analysis.

5.5. Samples, QC samples, and standards must be prepared in the same final solvent to allow for chromatographic comparability of samples and standards.

6.0. Equipment and Supplies

(Vendors and catalog numbers are included for illustration only. The following are examples of the products currently used in the laboratory. This is not a fully inclusive list, and inclusion should not imply product endorsement. Instrumentation, equipment and supply substitutions may be made provided that the substitutions meet the method criteria.)

6.1. Standard and Sample Preparation Equipment

6.1.1. Syringes, various sizes.

6.1.2. Class A volumetric flasks with stoppers, various sizes.

6.1.3. Disposable pipettes.

6.1.4. Pipette bulbs.

6.1.5. 2-mL auto-sampler vials with 0.3-mL target poly-spring inserts and Teflon-lined and/or Crimp top caps, or equivalent.

6.1.6. Micro pipette controller, Various Sizes, Eppendorf, Research Plus, or equivalent.

6.1.7. Repeating Pipette (optional), various tip sizes, Brand, HandyStep S, or equivalent.

6.2. Sample Extraction Equipment

6.2.1. Analytical balance, Mettler-Toledo, model # XSE205DU.

6.2.2. 1.5-mL centrifuge tubes, or equivalent.

6.2.3. Sonicator – Branson, model # 2510R-DTH, or equivalent.

6.2.4. Vortex – ThermoLyne, Maxi Mix 11, model #37615, or equivalent.

6.2.5. Centrifuge – Eppendorf, model # 5415D, or equivalent.

6.2.6. Biotage TurboVap LV, or equivalent

6.3. Instrumentation

6.3.1. An LC-MS/MS system that is suitable for use with all required accessories including: syringes, analytical columns, mobile phases, detectors, and data system (e.g. Shimadzu HPLC interfaced with an AB Sciex triple quadrupole mass spectrometer, or equivalent). The mass spectrometer system must be capable of running multiple reaction monitoring (MRM) instrument methods, or an equivalent type of method.

6.3.1.1. Shimadzu HPLC system includes:

6.3.1.1.1. Micro vacuum degasser, model # DGU-20A5 or equivalent

6.3.1.1.2. Pumps, model # LC-20ADxR, or equivalent

6.3.1.1.3. Column Oven, model # CTO-20A or equivalent

6.3.1.1.4. Autosampler, model # SIL-20ACxR or equivalent

6.3.1.1.5. Solvent Selector model# FCV-11AL or equivalent

6.3.1.1.6. System Controller model # CBM-20A or equivalent

6.3.1.1.7. HPLC Column: Agilent Poroshell 120 EC-C18, 2.7 µm, 3.0 x 150 mm column, #693975-302, or equivalent.

6.3.1.2. Triple Quadrupole Mass Spectrometer system includes:

6.3.1.2.1. AB Sciex 4500 Mass Spectrometer (MS/MS), model # API-4500, or equivalent.

6.3.1.2.2. Analyst Software, version 1.6.1, or equivalent.

6.3.1.2.3. Nitrogen gas as the nebulizing gas, turbo gas, and collision cell gas, or equivalent gas necessary for the instrument.

7.0. Reagents and Standards (Consumables)

7.1. Solvents and Reagents

7.1.1. Methanol – (HPLC grade, J.T. Baker, catalog # 9830-03 or equivalent).

7.1.2. HPLC Water – (HPLC grade, Sigma Aldrich, catalog # 34877-4L or equivalent).

7.1.3. Ammonium Formate – (High purity (98 % +), Fluka catalog # 70221 or equivalent).

7.1.4. Dichloromethane – (Sigma-Aldrich, Catalog # 650463-4L, or equivalent)

7.1.5. Acetone – (Mallinckrodt, Catalog # 2432, or equivalent).

7.1.6. Medium Chain Triglycerides (MCT Oil) – (Warner Graham, Miglyol 812, or equivalent).

7.1.7. Ethyl acetate

7.2. Gases

7.2.1. Nitrogen Gas – (Airgas, Cryogenic Liquid Nitrogen, NI 265LT350, or equivalent).

7.3. Analytical and Internal Standard Solutions

7.3.1. Analytical standard solutions may be purchased from any vendor. Standards must be NIST traceable, where available, and preferably produced in accordance with ISO Guide 31 or 35 requirements.

7.3.2. The commercial standards listed below are an example of those currently used in the laboratory. This is not a fully inclusive list and substitutions may be made as long as the criteria described above are met. At a minimum, commercial standards are stored per the manufacturer's recommendation storage conditions and expiration dates of commercially prepared standards are as prescribed by the vendor on their Certificate of Analysis.

Table 3. Analytical Standards

Standard	Manufacturer	Catalog #	Concentration	Solvent
Aflatoxin Mix (Aflatoxin B1, B2, G1, G2)	Supelco	CRM46304	1.0 µg/mL (B1, G1) 0.3 µg/mL (B2, G2)	Methanol
Ochratoxin A (OTA)	Fluka	34037-2mL-R	10 µg/mL	Acetonitrile
Mix 5 (Aflatoxin B1, B2, G1, G2)	Romer Labs	002022	0.25 µg/mL	Acetonitrile
Ochratoxin A (OTA)	Romer Labs	002023	10 µg/mL	Acetonitrile

Table 4. Internal Standards

Standard	Manufacturer	Catalog #	Concentration	Solvent
Mix 11 ($^{13}C_{17}$-Aflatoxin B1, B2, G1, G2)	Romer Labs	ILM—024-1.2mL	0.50 µg/mL	Acetonitrile
$^{13}C_{20}$-Ochratoxin A ($^{13}C_{20}$-OTA)	Romer Labs	ILM-007	10 µg/mL	Acetonitrile

8.0. Preparation of Reagents, Solutions, and Standard

8.1. General Preparation Information

8.1.1. Analytical stock standards and related preparations referenced in **Table 3, Table 4** and below are offered for guidance only. Comparable standards may be interchanged. In addition, different concentrations or analyte stock mixtures may be prepared as necessary.

8.1.2. To ensure an accurate amount of diluent is transferred, mix each solution in the Eppendorf tip by drawing up and dispensing diluent at least 5 times before transferring. Diluent can be dispensed back to the container from which it was drawn.

8.2. Extraction Solvent Solution

8.2.1. Add an appropriate amount of HPLC grade water to a volumetric flask such that the water makes up 40% of the final volume.

  8.2.1.1. For example, fill a 200 mL volumetric flask with 80 mL HPLC grade water.

8.2.2. Dilute to volume with methanol, invert 7x, and sonicate for 1 minute to mix.

8.2.3. Dilute to volume again, invert 7x to mix.

8.2.4. Suggested storage is at -20 °C for up to 6 months, or room temperature for up to 1 month.

8.3. Internal Standard Stock Diluent **(ISD)**

8.3.1. Fill a 1.00 mL volumetric flask 3/4 full with extraction solvent (**Section 8.2**).

8.3.2. 50.0 µL of **IS** $^{13}C_{20}$-Ochratoxin A ($^{13}C_{20}$-OTA) @ 10 µg/mL is added.

8.3.3. Dilute to volume with extraction solvent, invert 7x, and sonicate for 1 minute to mix.

$$(10.0\ \mu g/mL)\ (50.0\ \mu l) = (X\ \mu g/mL)\ (1000\ \mu L)$$

$$X= 0.500\ \mu g/mL$$

8.3.4. 1.0 mL of the solution is transferred and sealed in a 2.0 mL crimp-cap vial and labeled.

8.3.5. Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule.

8.4. Internal Standard Spiking Solution **(ISS)**

8.4.1. Fill a 1.00 mL volumetric flask 1/2 full with extraction solvent (**Section 8.2**).

8.4.2. 100 µL of Internal Standard Mix 11 ($^{13}C_{17}$-Aflatoxin B1, B2, G1, G2) @ 0.500 µg/mL is added.

8.4.3. 100 µL of **ISD** @ 0.500 µg/mL is added.

8.4.4. Dilute to volume with extraction solvent, invert 7x, and sonicate for 1 minute to mix.

$$(500\ ng/mL)\ (100\ \mu l) = (X\ ng/mL)\ (1000\ \mu L)$$

$$X= 50.0\ ng/mL$$

8.4.5. Solution is transferred and sealed in 1.5 mL crimp-cap vial and labeled.

8.4.6. Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule.

8.5. Internal Standard Working Diluent **(IWD)**

8.5.1. Fill a 5.00 mL volumetric flask 3/4 full with extraction solvent (**Section 8.2**).

8.5.2. 100 µL of **ISS** @ 50.0 ng/mL is added.

8.5.3. Dilute to volume with extraction solvent, invert 7x, and sonicate for 1 minute to mix.

$$(50.0\ ng/mL)\ (100\ \mu l) = (X\ ng/mL)\ (5000\ \mu L)$$

$$X= 1.00\ ng/mL$$

8.5.4. Transfer 1.0 mL aliquots of the solution, and seal in 1.5 mL crimp-cap vials and labeled.

8.5.5. Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule.

8.6. Working Standard (**WS**) – @ 20.0 ng/mL for all five mycotoxins.

8.6.1. Fill a 10.00 mL volumetric flask with about 4 mL methanol.

8.6.2. Add 20 µL of Ochratoxin A (OTA) @ 10.0 µg/mL from Romer Labs, # 002023.

8.6.3. Add 0.80 mL of Mix 5 @ 250 ng/mL from Romer Labs, # 002022.

8.6.4. Add 4.00 mL HPLC grade water.

8.6.5. Dilute to volume with methanol, invert 7x, and sonicate for 1 minute to mix.

(10,000 ng/mL) (0.02 mL) = (X ng/mL) (10.00 mL)

X= 20 ng/mL OTA

(250 ng/mL) (0.80 mL) = (X ng/mL) (10.00 mL)

X= 20 ng/mL Aflatoxins

8.6.6. The solution is filled and sealed in multiple 1.5 mL crimp-cap vials and labeled.

8.6.7. Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule.

8.7. Cross Check Reference Working Standard (**CCR-WS**) – @ 20.0 ng/mL for AT-B1, AT-G1, and OTA @ 6.00 ng/mL for AT-B2, and AT-G2.

8.7.1. Fill a 10.00 mL volumetric flask with about 4 mL methanol.

8.7.2. Add 20 µL of Ochratoxin A (OTA) @ 10.0 µg/mL from Fluka, # 34037-2mL-R.

8.7.3. Add 200 µL of Aflatoxin Mix (Aflatoxin B1, G1 @ 1,000 ng/mL / Aflatoxin B2, G2 @ 300 ng/mL) from Supelco, CRM46304.

8.7.4. Add 4.00 mL HPLC grade water.

8.7.5. Dilute to volume with methanol, invert 7x, and sonicate for 1 minute to mix.

<u>Ochratoxin A (see section 8.7.2)</u>

(10,000 ng/mL) (0.020 mL) = (X ng/mL) (10.00 mL)

X= 20.0 ng/mL

Aflatoxin B1 and G1 (see section 8.7.3)

(1,000 ng/mL) (0.200 mL) = (X ng/mL) (10.00 mL)

X= 20.0 ng/mL

Aflatoxin B2 and G2 (see section 8.7.3)

(300 ng/mL) (0.200 mL) = (X ng/mL) (10.00 mL)

X= 6.00 ng/mL

8.7.6. 1.0 mL of the solution is transferred and sealed in a 2.0 mL crimp-cap vial and labeled.

8.7.7. Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule.

8.8. Mobile Phases

8.8.1. Mobile phase A: 15 mM ammonium formate (aq).

8.8.1.1. Fill 1.0 L volumetric flask half full of water.

8.8.1.2. Add 945.9 ± 15 mg of ammonium formate to the flask.

8.8.1.3. Dilute to volume with water and invert 7x to mix.

8.8.1.4. This solution must be prepared monthly.

8.8.2. Mobile phase B: Methanol.

8.8.2.1. This solution must be changed at least every 6 months.

8.9. Matrices

8.9.1. MCT as a representative matrix.

8.9.1.1. MCT matrix will be used for the Demonstration of Capability (DOC), LOD and matrix spike/ matrix spike duplicate.

9.0. Sample Transport, Receipt, Preservation, Handling, and Storage

9.1. Sample transport conditions:

9.1.1. The medical marijuana products from Registered Organizations (ROs) are shipped as per manufacturer’s specifications.

9.2. Sample receipt:

9.2.1. Medical marijuana products from the RO are received, verified and documented ensuring that method, regulatory and Accreditation Body requirements are met.

9.2.2. All medical marijuana products must be stored under the conditions based on the manufacturer's recommendation.

9.3. Preservation:

9.3.1. All samples prepared for analysis should be put in sealed container and refrigerated at ≤ 4°C for storage no more than one week and kept away from light until analysis.

9.4. Sample Analysis:

9.4.1. Samples for analysis are placed in the auto sampler which is set to a temperature of 4°C.

9.5. Extract Storage:

9.5.1. Sample extracts are stored in a freezer at -20°C until analysis. Analysis must be completed within 7 days of extraction. After testing is complete, the remaining extract is stored at -20 °C for one month if necessary.

10.0. Calibration

10.1. LC-MS/MS

10.1.1. The mass scale and resolution of the ESI mass spectrometer must be periodically calibrated with the tuning solution and procedures prescribed by the manufacturer.

10.1.2. If setting up the instrument for the first time, directly infuse approximately 0.1 µg/mL of each compound into the MS using the integrated syringe pump, or an equivalent pump. Observe the ion representing the protonated molecule, $[M + H]^+$, and record the product-ion spectra to verify the choice of product ions for MRM analysis.

10.1.3. Optimize the MS/MS parameters including collision energies (CE), declustering potential, gas flows, and temperature using the flow injection analysis (FIA) optimization method. An example of the optimal MS/MS conditions are described in **Tables 5 and 6**. The scheduled MRM recording window may vary from column to column, and instrument to instrument. Once the retention time of each analyte is determined, select a window at least one-minute wide, centered on the measured retention time.

Table 5: MS/MS Non-specific parameters:

Parameter	Value
MS Acquisition Time:	0 – 11.0 min.
Curtain Gas Flow (CUR):	30.00 psi
Collision Gas Pressure (CAD Gas):	Medium (unitless)
Ion Transfer Voltage (IS):	4500.00 V
Temperature of Turbo Gas (TEM):	400.00 °C
Gas 1 – Nebulizer Gas (GS1):	45.00 psi
Gas 2 – Turbo Gas (GS2):	35.00 psi
Declustering Potential (DP):	80.00 V
Entrance Potential (EP):	10.00 V
Collision Cell Exit Potential (CXP):	13.00 V

Table 6: MS/MS Analyte-specific parameters.
Quantifying product ions and CE are **bold**, qualifying product ions and CE are in (parenthesis).

Analytes	**Polarity**	**Precursor ion (Da)**	**Product ion (Da)**	**Collision energy (eV)**	**Scheduled MRM recording window (min.)**
Aflatoxin B1	Positive	312.9	**241.1** (269.0)	**60** (50)	6.20 – 7.20 6.20 – 7.20
Aflatoxin B2	Positive	315.1	**287.2** (259.1)	**45** (45)	5.80 – 6.80 5.80 – 6.80
Aflatoxin G1	Positive	329.0	**214.1** (243.1)	**45** (35)	5.40 – 6.40 5.40 – 6.40
Aflatoxin G2	Positive	331.1	**257.0** (189.1)	**45** (55)	5.00 – 6.00 5.00 – 6.00
Ochratoxin A	Positive	404.2	**239.0** (221.0)	**30** (50)	7.70 – 8.70 7.70 – 8.70
Aflatoxin B1-$^{13}C_{17}$	Positive	330.1	**255.1** (284.2)	**55** (55)	6.20 – 7.20 6.20 – 7.20
Aflatoxin B2-$^{13}C_{17}$	Positive	332.2	**303.2** (273.1)	**35** (35)	5.80 – 6.80 5.80 – 6.80
Aflatoxin G1-$^{13}C_{17}$	Positive	346.1	**227.2** (257.2)	**55** (35)	5.40 – 6.40 5.40 – 6.40
Aflatoxin G2-$^{13}C_{17}$	Positive	348.1	**200.2** (272.3)	**55** (35)	5.00 – 6.00 5.00 – 6.00
Ochratoxin A-$^{13}C_{20}$	Positive	424.2	**250.1** (232.1)	**30** (50)	7.70 – 8.70 7.70 – 8.70

10.1.3.1.1. Please note that the parameters above have been optimized for the best signal-to-noise ratio in matrices, not necessarily the best signal-to-noise ratio in solvent.

10.1.4. Using the MS/MS parameters obtained from the FIA optimization above and the LC operating parameters described in **Table 7**, analyze a mid-level calibration standard to obtain retention times for each target analyte using an MRM instrument method, or equivalent. For optimum MS/MS precision there must be at least 10 scans across each peak.

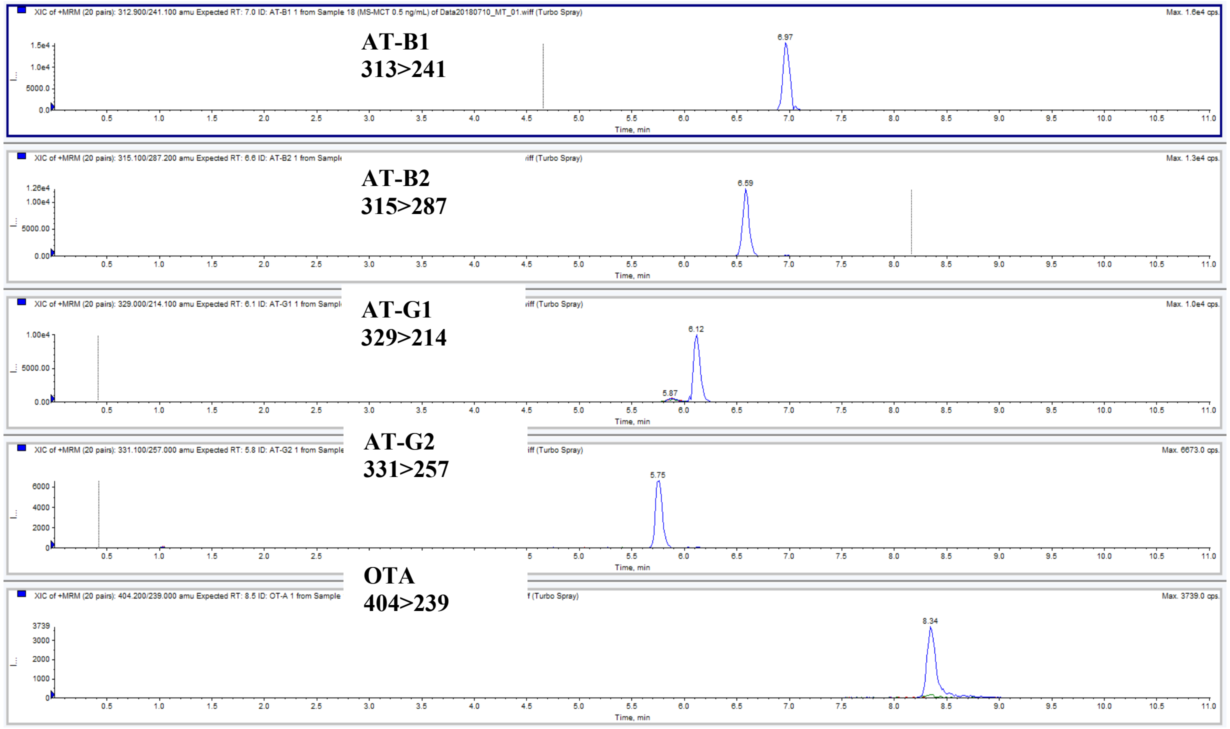
Table 7: HPLC parameters

Column	**Value**
Column:	Agilent Poroshell 120 EC-C18, 2.7 µm, 3.0 x 150 mm, # 693975-302
Guard Column:	Phenomenex SecurityGuard, Analytical Guard Cartridge System; # KJ0-4282
Column Temperature:	40.0 °C
Autosampler	**Value**
Temperature:	4.0 °C
Injection Volume:	10.0 µL
Injection Loop Volume:	50.0 µL
Rinse Mode:	Before and after aspiration
Rinse Volume:	200 µL
Rinse Solvent:	Methanol
Pumps	**Value**
Mobile Phase A:	15 mM Ammonium Formate in H_2O
Mobile Phase B:	Methanol
Flow Rate:	0.300 mL/min
Initial Percentage MPB:	20%
Diverter Valve - Time	**Flow Destination (Position)**
Initial:	Divert to waste (Position A)
1.0 min:	Divert to MS detector (Position B)
11.0 min:	Divert to waste (Position A)
Pump Gradient - Time	**Percentage Mobile Phase B**
0.0 min: (data collection begins)	20%
1.0 min:	50%
11.0 min: (data collection ends)	90%
11.1 min: (column flush begins)	95%
20.0 min: (flush ends)	95%
20.1 min: (inter-sample equilibration begins)	20%
25.0 min: (equilibration ends)	20%

Table 8: Analyte Retention Times

Analyte	Average Retention Time (min.)	Example Retention Time Range (min.)
Aflatoxin B1 (AT-B1)	6.73	6.68 – 6.77
Aflatoxin B2 (AT-B2)	6.35	6.31 – 3.39
Aflatoxin G1 (AT-G1)	5.90	5.87 – 5.94
Aflatoxin G2 (AT-G2)	5.56	5.52 – 5.59
Ochratoxin A (OTA)	8.24	8.17 – 8.31
Aflatoxin B1-$^{13}C_{17}$ (AT-B1-$^{13}C_{17}$)	6.71	6.67 – 6.75
Aflatoxin B2-$^{13}C_{17}$ (AT-B2-$^{13}C_{17}$)	6.34	6.30 – 3.37
Aflatoxin G1-$^{13}C_{17}$ (AT-G1-$^{13}C_{17}$)	5.89	5.86 – 5.92
Aflatoxin G2-$^{13}C_{17}$ (AT-G2-$^{13}C_{17}$)	5.55	5.51 – 5.58
Ochratoxin A-$^{13}C_{20}$ (OTA-$^{13}C_{20}$)	8.22	8.15 – 8.30

Figure 1: Chromatogram of the spiked MCT matrix at 0.5 ng/mL in MCT matrix. The matrix spike (blue) is shown overlaid on the chromatogram recorded for the system blank (red) and the MCT matrix blank (green).



10.2. Calibration Curve Preparation (**CalS**)

10.2.1. Serial dilutions are made from the Working Standard (**Section 8.6**). An example of the current standard levels is provided as follows, but higher or lower levels or volumes may be prepared and analyzed. See **Table 9** and **Table 10** for a summary of the dilutions. All calibration standards are prepared in extraction solvent solution (**Section 8.2**). Suggested storage for standards is -20 °C for up to 6 months, in a sealed vial or ampule.

10.2.2. ***<u>CalS 6a</u>*** (20.0 ng/mL)

10.2.2.1. **CalS 6a** is the same as the Working Standard (**Section 8.6**).

10.2.3. ***<u>CalS 5a</u>*** (6.00 ng/mL)

10.2.3.1. Pipet 300 µl of Working Standard (**Section 8.6**) into vial labeled **CalS 5a** containing 700 µL of extraction solvent.

10.2.3.2. Mix well using Eppendorf tip, at least 10 times.

$$(20.0\ \text{ng/mL})\ (300\ \mu\text{L}) = (X\ \text{ng/mL})\ (1000\ \mu\text{L})$$

$$X = 6.00\ \text{ng/mL}$$

10.2.4. ***<u>CalS 4a</u>*** (1.80 ng/mL)

10.2.4.1. Pipet 300 µL of **CalS 5**a (**Section 10.2.3**) into vial labeled **CalS 4a** containing 700 µL of extraction solvent.

10.2.4.2. Mix well using Eppendorf tip, at least 10 times.

$$(6.00\ \text{ng/mL})\ (300\ \mu\text{L}) = (X\ \text{ng/mL})\ (1000\ \mu\text{L})$$

$$X = 1.80\ \text{ng/mL}$$

10.2.5. ***<u>CalS 3a</u>*** (0.540 ng/mL)

10.2.5.1. Pipet 300 µL of **CalS 4a** (**Section 10.2.4**) into vial labeled **CalS 3a** containing 700 µL of extraction solvent.

10.2.5.2. Mix well using Eppendorf tip, at least 10 times.

$$(1.80\ \text{ng/mL})\ (300\ \mu\text{L}) = (X\ \text{ng/mL})\ (1000\ \mu\text{L})$$

$$X = 0.540\ \text{ng/mL}$$

10.2.6. ***CalS 2a*** (0.162 ng/mL)

10.2.6.1. Pipet 300 µL of **CalS 3a** (**Section 10.2.5**) into vial labeled **CalS 2a** containing 700 µL of extraction solvent.

10.2.6.2. Mix well using Eppendorf tip, at least 10 times.

(0.540 ng/mL) (300 µL) = (X ng/ml) (1000 µL)

X= 0.162 ng/mL

10.2.7. ***CalS 1a*** (0.0486 ng/mL)

10.2.7.1. Pipet 300 µL of **CalS 2a** (**Section 10.2.6**) into vial labeled **CalS 1a** containing 700 µL of extraction solvent.

10.2.7.2. Mix well using Eppendorf tip, at least 10 times.

(0.162 ng/mL) (300 µL) = (X ng/mL) (1000 µL)

X= 0.0486 ng/mL

Table 9. – Calibration Curve without Internal Standard

CalS STD	Mycotoxin Std Conc (ng/mL)	Mycotoxin Std Ref Section ID	Volume of Mycotoxin STD	Extraction Solvent (8.2)
CalS 6a	20.0	10.2.2	-	-
CalS 5a	6.00	10.2.3	300 µL	700 µL
CalS 4a	1.80	10.2.4	300 µL	700 µL
CalS 3a	0.540	10.2.5	300 µL	700 µL
CalS 2a	0.162	10.2.6	300 µL	700 µL
CalS 1a	0.0486	10.2.7	300 µL	700 µL

10.2.8. Prepare calibration standard mixtures with **IWD** by diluting each **CalS** (**Section 10.2.2 – 10.2.7**) with equal amounts of **IWD** @ 1.0 ng/mL (**Section 8.5**) and mixing well (e.g., 200 µL CalS with 200 µL IWD). Be sure to rinse solution down the sides of the container. Failure to mix well will fail linearity requirements. The final concentrations can be seen in **Table 10**.

Table 10. – Calibration Curve with Internal Standard

Cal STD w/IWD	Mycotoxin Final Concentration (ng/mL)	Mycotoxin Std Ref Section ID	IWD Reference ID	IS Final Concentration (ng/mL)
CalS 6b	10.0	10.2.2	8.5	0.5
CalS 5b	3.00	10.2.3	8.5	0.5
CalS 4b	0.900	10.2.4	8.5	0.5
CalS 3b	0.270	10.2.5	8.5	0.5
CalS 2b	0.0810	10.2.6	8.5	0.5
CalS 1b	0.0243	10.2.7	8.5	0.5

10.2.9. After at least one system blank injection and one LRB injection, start with the lowest standard concentration (**CalS 1b**) and analyze each calibration standard. Tabulate the response (peak area/internal standard peak area ratio) and use the result to prepare a calibration curve for each target analyte (weighted 1/X linear regression).

10.2.10. See **Section 11.8** for IS acceptance criteria.

10.3. Initial Calibration Criteria

10.3.1. The solutions prepared in **Section 10.2** are used to prepare an initial calibration curve for the mycotoxins at concentrations appropriate for the instrument's range and sample content. A minimum of 5 calibration concentrations is required for each mycotoxin. See **Section 12.6** for frequency requirements related to establishing a new initial calibration.

10.3.2. The lowest calibration standard must be at or below the **LOQ** listed in **Section 1.1** for each analyte, or the **LOQ** must be adjusted accordingly.

10.3.3. The correlation coefficient (R) of the calibration curve for each analyte must be ≥ 0.995 before any analysis of samples can begin.

10.3.4. Each calibration standard, processed under the new initial calibration, must be within 80-120% of the true value for each analyte for the initial calibration to be considered valid. The exception are standards with concentrations ≤**LOQ**, which may be within 70-130% of the true value for each analyte. Evaluation of each standard also serves as the measure of % Relative Error with the same acceptance criteria.

10.3.5. If all of these criteria cannot be met a new calibration must be established.

10.3.6. Only the quantifying transitions are used for the calibration curve. The qualifying transitions do not need to meet the initial calibration requirements. The qualifying transitions are only used for identification of analytes (**Section 13.2.2**.).

10.4. Initial Verification of Calibration

10.4.1. The initial calibration for each mycotoxin must be verified by analyzing a Cross Check Reference Standard (**CCR**). An example of the current CCR levels is provided as follows, but higher or lower concentrations may be prepared and analyzed, provided they are within the concentration range of 10 ng/mL to 0.0243 ng/mL. Typically, a mid-level concentration is used.

10.4.2. **CCR** @ 1.00 ng/mL AT-B1, AT-G1, and OTA / @ 0.300 ng/mL AT-B2, and AT-G2.

10.4.2.1. Fill a 1.0 mL volumetric flask 1/2 full with extraction solvent (**Section 8.2**)

10.4.2.2. Add 100 µL from **CCR-WS** (**Section 8.7**)

10.4.2.3. Dilute to volume with extraction solvent.

10.4.2.4. Invert 7X and sonicate for 1 minute to mix. Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule labelled **CCR-a**.

Aflatoxin B1, G1 and Ochratoxin A (see section 8.7)

(20.0 ng/mL) (100 uL) = (X ng/mL) (1000 uL)

X= 2.00 ng/mL

Aflatoxin B2 and G2 (see section 8.7)

(6.00 ng/mL) (100 uL) = (X ng/mL) (1000 uL)

X= 0.60 ng/mL

10.4.2.5. Mix solution from **Section 10.4.2.4**, with an equal amount of **IWD** @ 1.00 ng/mL (**Section 8.5**) and mix well (e.g., 200 µL CalS with 200 µL IWD). Be sure to rinse solution down the sides of the container.

Aflatoxin B1, G1 and Ochratoxin A (see section 10.4.2.4)

(2.00 ng/mL) (200 uL) = (X ng/mL) (400 uL)

X= 1.00 ng/mL

Aflatoxin B2 and G2 (see section 10.4.2.4)

(0.60 ng/mL) (200 uL) = (X ng/mL) (400 uL)

X= 0.30 ng/mL

10.4.2.6. Label **CCR-b**.

10.4.3. Measured recovery value of **CCR-b** must be within 70 – 130% of the true value for all mycotoxins.

10.5. Continuing Calibration Verification

10.5.1. After verifying the initial calibration, a **CCV** that is ≤ 1/2 the highest calibration standard must be analyzed with each analytical batch (typically 20 samples). It is also recommended that a **CCV** at the **LOQ** is analyzed.

10.5.2. The response for any analyte in the **LOQ** standard must be within ±30% of the predicted response. All other **CCVs** must be within ± 20% of the predicted response.
CCVs may also be interspersed throughout the analytical batch at varying concentrations provided that the CCVs analyzed at the beginning and end (for external calibration) of each analytical batch are equal to or less than half the highest calibration level. Additional CCVs may also be run at higher levels to evaluate the upper end of the calibration curve.

10.5.3. The **CCV** and **LOQ** standards are prepared at the following concentrations but additional higher or lower levels may be prepared as necessary.

10.5.3.1. **Continuing Calibration Verifications (CCVs)** are prepared at the following levels:

10.5.3.1.1. 3.0 ng/mL, 0.90 ng/mL, and 0.27 ng/mL (same as **CalS 3b** thru **5b** – (**Section 10.2.8**)

10.5.3.2. **Limit of Quantitation (LOQ)** standard is prepared at 0.024 ng/mL or 0.081 ng/mL in extraction solvent (same as **CalS 1b** or **CalS 2b**).

11.0. Quality Control/ Quality Assurance

11.1. Demonstration of Capability (DOC)

11.1.1. Initial Demonstration of Capability

11.1.1.1. Each analyst must perform an initial demonstration of capability using the procedures described in this SOP for each target analyte. The initial DOC must consist of the analysis of four or five matrix spike samples that has been fortified with all analytes of interest at a low-level concentration (not higher than 4 times the LOQ). If available, the spiking solution should be from a source independent of that used for the calibration standards.

11.1.1.2. For each individual analyte, the recovery value for all replicates must fall in the range of ± 20%, except ≤ **LOQ**, where the value must fall in the range of ± 30%. The precision of the measurements, calculated as relative standard deviation (RSD), must be 20% or less. For those compounds that fail these criteria, this procedure must be repeated (and corrective action performed where required) until satisfactory performance has been demonstrated.

11.1.2. Continuing Demonstration of Capability

11.1.2.1. Annually, each analyst must complete a continuing DOC. The continuing DOC may be completed by one of the following techniques:

11.1.2.1.1. Acceptable performance on the analysis of a blind sample, such as an external proficiency test, when available.

11.1.2.1.2. Acceptable performance of an initial DOC as described above in **Section 11.1.1 1** at any concentration within the calibration range.

11.1.3. If major changes to the method or instrument are made, or the laboratory/analyst has not performed the method in a twelve (12) month period, each analyst must complete an initial DOC as described in **Section 11.1.1**. Minor changes to the method are evaluated using the matrix spike per **Section 11.7** or the cross-check reference standard per **Section 10.4**.

11.2. Limit of Detection (**LOD**) and Limit of Quantitation (**LOQ**)

11.2.1. An initial **LOD** study for each method must be completed and documented for all target analytes in each representative matrix (see **MML-301-SOP**), on each instrument used to analyze sample extracts. If the laboratory intends to report results below the **LOQ**, an ongoing **LOD** verification is also required.

11.2.2. Based on the **LOD**, the laboratory shall select an **LOQ** that is greater than the **LOD** (typically 3-5x the **LOD**) and consistent with the needs of its client. An **LOQ** is required for each representative matrix, method and analyte combination. For each method, the lowest calibration standard concentration must be at or below the corresponding **LOQ**.

11.2.3. An initial **LOQ** study for each method must be completed and documented for all target analytes in each representative matrix. The initial **LOD** samples may be used for this purpose as long as the concentration used is at or below the **LOQ**. The mean recovery shall be within 70-130% of the spiked value.

11.2.4. On an ongoing basis, the laboratory shall prepare and analyze a minimum of one **LOQ** verification sample spiked at the same concentration as the initial **LOQ** verification study on each instrument during each quarter in which samples are being analyzed for each representative matrix, method, and analyte combination. The recovery of the **LOQ** verification samples shall be within 70-130%.

11.2.5. The 2017 Method Update Rule finalized in the Environmental Protection Agency's (EPA's) Federal Register on August 28, 2017, prescribes a revised approach to Method Detection Limit (MDL)/LOD data collection and calculation per Part 136 Appendix B. The New York State (NYS) Environmental Laboratory Program (ELAP) requires that the revised procedure detailed within the EPA's document *Definition and Procedure for the Determination of the Method Detection Limit, Revision 2, December 2016* be implemented for all NYS ELAP accredited methods.

11.3. System Blank (**SBLK**)

11.3.1. Before processing samples, the analyst must demonstrate that the instrument is free from background interference by analyzing a system blank (**SBLK**).

11.3.1.1. A vial of extraction solvent (**Section 8.2**) is injected and evaluated.

11.3.2. Background interference is defined as a peak with a signal greater than >10 times the noise signal, and a retention time within 0.1 minutes of the target peak.

11.4. Laboratory Reagent Blank (**LRB**)

11.4.1. Before processing samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted, or reagents are changed, an **LRB** must be prepared and analyzed. If, within the relative retention time window of any target analyte, the **LRB** produces a peak that would prevent the determination of the analyte, determine the source of contamination and eliminate the interference before processing the samples.

11.4.2. Background contamination must be < 1/3 the **LOQ** for each target analyte.

11.5. Laboratory Fortified Blank (**LFB**)

11.5.1. The laboratory must analyze at least one laboratory fortified blank (**LFB**) with every twenty samples or one per sample set (all samples extracted within a 24-hr period), whichever is greater. The **LFB** is fortified with each target analyte at a low-level concentration, typically 0.5 ng/mL (equivalent to a 5.0 ng/g sample).

11.5.2. The accuracy is calculated as percent recovery. The recovery for individual analytes must be 80 – 120% of the true value.

11.6. Method Blank (**MB**)

11.6.1. The laboratory must analyze at least one method blank (**MB**) with every twenty samples or one per sample set (all samples extracted within a 24-hour period), whichever is greater. If, within the relative retention time window of any target analyte, the **MB** produces a peak that would interfere with the determination of the analyte, consult your supervisor on how to move forward.

11.6.1.1. Typically, adjusting the mobile phase A pH by modifying the concentration of ammonium formate, or adding formic acid, will shift the matrix interfering peaks outside of the retention time window. This will not affect the aflatoxin retention times, but ochratoxin A will elute sooner as the concentration of ammonium formate increases, and later as the concentration of formic acid increases.

11.6.2. Background contamination must be < 1/3 the **LOQ** for each target analyte.

11.7. Matrix Spike (**MS)** and Matrix Spike Duplicate (**MSD**)

11.7.1. A **MS** sample must be analyzed every twenty samples or one per sample set (all samples extracted within a 24-hour period), whichever is more frequent. The **MS** is fortified with each target analyte at a low-level concentration, typically 0.5 ng/mL (equivalent to a 5.0 ng/g sample).

11.7.1.1. A MS (or LCS) may be used in place of a continuing calibration verification (CCV) (but not as a replacement for a failing CCV) for methods where the calibration goes through the same process as the LCS. Note that the more stringent acceptance criteria must be met.

11.7.1.2. The matrix spike may be used in place of the LCS as long as the acceptance criteria are as stringent as for the LCS.

11.7.2. A **MSD** will be prepared and compared against the original **MS** sample.

11.7.3. To determine the accuracy, calculate the percent recovery of the concentration for each analyte in the **MS**. Recovery must be within 80 – 120% of the true value, except ≤ **LOQ**, where the value must fall in the range of 70 – 130%.

11.7.4. To determine the precision, calculate the relative percent difference (RPD). The RPD must be <20% (**Section 13.3.3**).

11.8. Internal Standards

11.8.1. The five internal standards listed in **Section 7.3** are added to all standards, quality control samples, and samples.

11.8.2. The internal standard peak area for each injection is compared against the average peak area from the calibration curve.

11.8.3. The recovery of each internal standard must be 25-130% of the average peak area from the calibration curve.

11.8.4. If these criteria cannot be met (e.g., a sample with a complex matrix), the data for such a sample must be reported with an appropriate qualifier, after approval from the laboratory supervisor, or equivalent senior staff member.

12.0. Procedure

12.1. Sample Preparation (see MML-303-AppB for alternate preparation procedure):

12.1.1. Weigh 100 ± 5 mg material directly into a tared 1.5–mL centrifuge tube.

12.1.2. Add 0.5 ng (10.0 µL, 50 ng/mL) **ISS** (**Section 8.4**) to the tube wall, and centrifuge at 5,000 RPM for 1 minute to mix.

12.1.3. Add 1000 µL extraction solvent (S**ection 8.2**).

12.1.4. Sonicate for 15 minutes.

12.1.5. Vortex to thoroughly mix.

12.1.6. Sonicate for an additional 15 minutes.

12.1.7. Centrifuge for 5 minutes at 12,000 RPM (13,362 RCF).

12.1.8. Transfer approximately 900 µL of supernatant to centrifuge tube for storage.

12.2. Matrix Spike and Matrix Spike Duplicate Preparation (**MS + MSD**):

12.2.1. Weigh 100 ± 5 mg matrix (**Section 8.9**) directly into a tared 1.5–mL centrifuge tube.

12.2.2. Add 0.5 ng (25.0 µL, 20 ng/mL) **WS** (**Section 8.6**) to the tube wall.

12.2.3. Add 0.5 ng (10.0 µL, 50 ng/mL) **ISS** (**Section 8.4**) to the tube wall, and centrifuge at 5,000 RPM for 1 minute.

12.2.4. Add 1000 µL extraction solvent (S**ection 8.2**).

12.2.5. Sonicate for 15 minutes.

12.2.6. Vortex to thoroughly mix.

12.2.7. Sonicate for an additional 15 minutes.

12.2.8. Centrifuge for 5 minutes at 12,000 RPM (13,362 RCF).

12.2.9. Transfer approximately 900 µL of supernatant to centrifuge tube for storage.

12.2.10. Suggested storage is -20 °C for up to 6 months, in a sealed vial or tube.

12.3. Laboratory Reagent Blank (**LRB**)

12.3.1. Label a 1.5–mL centrifuge tube.

12.3.2. Follow the same steps in **Section 12.1.2** through **Section 12.1.8**.

12.3.3. Must be prepared fresh for each batch (See **Section 11.4.1**).

12.4. Laboratory Fortified Blank (**LFB**)

12.4.1. Label a 1.5–mL centrifuge tube.

12.4.2. Follow the same steps in **Section 12.2.2** through **Section 12.2.10.**

12.4.3. Suggested storage is -20 °C for up to 6 months, in a sealed vial or tube.

12.5. Method Blank (**MB**)

12.5.1. Label a 1.5–mL centrifuge tube.

12.5.2. Follow the same steps in **Section 12.2**, omitting line **12.2.2** where the **WS** is spiked. All other steps are identical.

12.5.3. Suggested storage is -20 °C for up to 6 months, in a sealed vial or tube.

12.6. LC-MS/MS Analysis Procedure.

12.6.1. Establish initial LC-MS/MS parameters (**Section 10.1**) if needed.

12.6.2. Equilibrate the LC-MS/MS system by flowing at initial parameters described in **Table 6** and **Table 7**.

12.6.3. Analyze at least one **SBLK**, followed by a **LRB**. The **SBLK** must pass criteria in **Section 11.3** and the **LRB** must pass the criteria in **Section 11.4.**

12.6.4. If it has been more than one month (>31 days) since running the last initial calibration curve, or if the mobile phase A was changed since the last curve, a new initial calibration curve needs to be analyzed:

12.6.4.1. Analyze an initial calibration (**Section 10.2**), followed by an **LRB** to evaluate carry-over.

12.6.4.2. Analyze a **CCR**.

12.6.4.3. The initial calibration curve and **CCR** must pass the criteria set in **Section 10.3 and 10.4** respectively before samples can analyzed and reported.

12.6.5. If it has been one month or less (≤ 31 days) since running the last initial calibration curve and the mobile phase A has not been changed, the most recent initial calibration curve must be verified as viable by passing a continuing calibration as described below. If it fails verification, a new initial calibration curve needs to be analyzed (see **Section 12.6.4**).

12.6.5.1. Analyze an **LOQ**, and **CCV**, followed by an **LRB** to evaluate carry-over.

12.6.5.2. The **LOQ** and **CCV** must pass criteria set in **Section 10.5.** before any samples can be analyzed and reported.

12.6.6. Analyze the **LFB**, **MB**, **MS**, and **MSD** QC samples, and up to 20 medical marijuana samples.

12.6.7. While not required for internal standard calibration, it is also recommended to analyze a **CCV** at the end of each analytical batch. This confirms that the calibration was valid throughout the entire run.

12.7. LC-MS/MS Batch

12.7.1. **Table 12** lists an example of a 20-sample batch, with an initial calibration curve.

Table 12: Example of Batch with Initial Calibration Curve

Injection #	Sample	Comments
1	RINSE	While only 1 blank is needed to check the instrument for interference, multiple injections may be needed to reduce noise (See **Section 14.7.1.4**).
2	RINSE	
3	SBLK	
4	LRB	Background contamination found must be < 1/3 the **LOQ** for each target analyte **(See Section 11.4.2)**
5	CalS-1b	This example uses an initial calibration curve. If an initial calibration curve is not needed (see **Section 12.6.5**), analyze a LOQ (CalS-1b or 2b) and a CCV (CalS-3b, 4b, or 5b) to verify the initial calibration.
6	CalS-2b	
7	CalS-3b	
8	CalS-4b	
9	CalS-5b	
10	CalS-6b	
11	LRB	Blank to evaluate for carry-over.
12	CCR	Calibration curve cross-check.
13	LFB	QC
14	MB	QC
15	MS	QC
16	MSD	QC
17	Unknown 1	Sample 1
18	Unknown 2	Sample 2
19	Unknown 3	Sample 3
20	Unknown 4	Sample 4
21	Unknown 5	Sample 5
22	Unknown 6	Sample 6
23	Unknown 7	Sample 7
24	Unknown 8	Sample 8
25	Unknown 9	Sample 9
26	Unknown 10	Sample 10
27	Unknown 11	Sample 11
28	Unknown 12	Sample 12
29	Unknown 13	Sample 13
30	Unknown 14	Sample 14
31	Unknown 15	Sample 15
32	Unknown 16	Sample 16
33	Unknown 17	Sample 17
34	Unknown 18	Sample 18
35	Unknown 19	Sample 19
36	Unknown 20	Sample 20
37	CCV (CalS-3b – CalS-5b)	Verifies initial calibration is still valid. (Recommended, see Section 12.6.7)
38	RINSE	One or more injections of solvent may be utilized to clean the column prior to the next analysis.

12.8. LC-MS/MS Integration/Quantitation Parameters

12.8.1. The integration of peaks should be done by the software whenever possible. **Table 13** and **Table 14** list example parameters for integrating peaks using Analyst 1.6.1 software.

12.8.2. When the software inadequately integrates peaks, manual integration is necessary. A laboratory specific procedure must be available for manual peak integration.

Table 13: Internal Standards (Quantifying transitions only)

Mycotoxin (^{13}C-labelled)	**AT-B1**	**AT-B2**	**AT-G1**	**AT-G2**	**OTA**
Q1/Q3	330.1 / 255.1	332.2 / 303.2	346.1 / 227.2	348.1 / 200.2	424.2 / 250.1
Min. Peak Height	0	0	0	0	0
Min. Peak Width	0	0	0	0	0
RT window (sec)	30.0	30.0	30.0	30.0	30.0
Expected RT (min)	6.68	6.31	5.86	5.52	8.22
Smoothing Width	3	3	3	3	5
Use Relative RT	No	No	No	No	No
Automatic – IQAIII	No	No	No	No	No
Specify Parameters-MQ III	Yes	Yes	Yes	Yes	yes
Noise Percent	50	50	50	50	50
Peak Splitting Factor	1	1	1	1	1
Base Sub Window (min)	0.2	0.2	0.2	0.2	0.2
Report Largest Peak	No	No	No	No	No

Table 14: Standards (Quantifying transitions only)

Mycotoxin	**AT-B1**	**AT-B2**	**AT-G1**	**AT-G2**	**OTA**
Associated Internal Standard	AT-B1	AT-B2	AT-G1	AT-G2	OTA
Q1/Q3	312.9 / 241.1	315.1 / 287.2	329.0 / 214.1	331.1 / 257.0	404.2 / 239.0
Min. Peak Height	0	0	0	0	0
Min. Peak Width	0	0	0	0	0
RT window (sec)	3.0	3.0	3.0	3.0	3.0
Expected RT (min)*	6.69	6.33	5.88	5.53	8.24
Smoothing Width	3	3	3	3	5
Use Relative RT	Yes	Yes	Yes	Yes	Yes
Automatic – IQAIII	No	No	No	No	No
Specify Parameters-MQ III	Yes	Yes	Yes	Yes	Yes
Noise Percent	50	50	50	50	50
Peak Splitting Factor	1	1	1	1	1
Base Sub Window (min)	0.2	0.2	0.2	0.2	0.2
Report Largest Peak	No	No	No	No	No
Fit Type	Linear	Linear	Linear	Linear	Linear
Parameter	Area	Area	Area	Area	Area
Weighting	1/X	1/X	1/X	1/X	1/X
Iterate	No	No	No	No	No

*The expected standard retention time in **Table 14** will vary as the associated IS retention time (RT) varies for every injection. First, the software searches for the IS peak within the expected IS RT from **Table 13**. Then, the software uses the actual IS RT found in that injection, and searches for the standard peak within a RT window relative to the IS RT (currently at ± 3.0 sec). The RT's listed in **Table 14** are from a representative injection only, and do not represent required retention times.

13.0. Data Acquisition, Reduction, Analysis, and Calculations

13.1. HPLC

13.1.1. **Table 7** (**Section 10.1.4**) summarizes the recommended operation conditions for the HPLC.

13.1.2. Calibrate or verify the calibration on each day of analysis as described in **Section 12.6.4** and **Section 12.6.5**. The standards and sample extracts must be in extraction solvent (**Section 8.2**).

13.2. Identification of Analytes

13.2.1. Identify a sample component using relative retention time by comparing its retention time to the retention time of the ^{13}C-labelled internal standard. If the retention time of an unknown compound corresponds, within limits, to the relative retention time when compared to the internal standard, then initial identification is positive.

13.2.1.1. The width of the relative retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of an analytical sequence. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of the chromatograms.

13.2.1.2. Current relative retention time windows are set at 0.990 – 1.010 the retention time of the internal standard. This is true for all analytes. See **Section 10.1.4**, **Table 8** for additional retention time information.

13.2.2. Confirm a sample component after initial identification using the ratio of quantifying and qualifying peak areas.

13.2.2.1. The area ratio of quantifying to qualifying transitions used to make identifications should be based upon measurements of actual ratio variations over the course of multiple runs and concentration levels. Three times the standard deviation of a ratio can be used to calculate a suggested window size for a compound.

13.2.2.2. Current quantifying/qualifying ratios are listed in **Table 15**.

Table 15: Quantifying / Qualifying transition ion peak area ratios.

Mycotoxin	**Quantifying Transition (Da)**	**Qualifying Transition (Da)**	**Average Ratio**	**± 3 Standard Deviation**	**Ratio Range**
AT-B1	312.9 / 241.1	312.9 / 269.0	1.513	0.294	1.219 – 1.807
AT-B2	315.1 / 287.2	315.1 / 259.1	0.637	0.199	0.438 – 0.836
AT-G1	329.0 / 241.1	329.0 / 243.1	0.447	0.186	0.261 – 0.633
AT-G2	331.1 / 257.0	331.1 / 189.1	0.905	0.499	0.406 – 1.404
OTA	404.2 / 239.0	404.2 / 221.0	1.988	0.740	1.248 – 2.728

13.2.3. Identification requires expert judgment when sample components are not resolved chromatographically. When chromatographic peaks obviously represent more than one sample component (*i.e.* broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak on a chromatogram, appropriate alternate techniques to help confirm peak identification need to be employed.

13.3. Calculations

13.3.1. Initial Calibration

13.3.1.1. Use the instrument software and specified parameters to perform peak integration for all identified peaks (**Tables 13 and 14**).

13.3.1.2. Calculate the relative retention time for each standard, by comparing the standard retention time to the equivalent **IS** retention time using the following formula. The relative retention time must pass the criteria set in **Section 13.2.1**. Relative retention times are calculated as follows:

$$Relative\ Retention\ Time = \frac{RT_{Std}}{RT_{IS}}$$

Where: RT_{Std} = retention time of the standard
RT_{IS} = retention time of the corresponding **IS**

For example, compare the RT of Aflatoxin B1 to the RT of Aflatoxin B1-$^{13}C_{17}$.

13.3.1.3. Using the weighted 1/X linear regression curve for all calibration standards, check the curve linearity and calculate the standards recovery at each level. Evaluate the linearity and recovery based on the criteria set in **Section 10.3**.

13.3.2. QC and unknown samples

13.3.2.1. Apply the linear regression calibration curve generated from the calibration standards to all QA/QC and real samples to calculate the concentration (ng/mL) of each mycotoxin using the instrument quantification software.

13.3.2.2. Calculate the area ratio of the quantifying and qualifying transition of all analytes in each medical marijuana sample. The ratio is calculated using the following formula:

$$Area\ Ratio = \frac{A_{Quant}}{A_{Qual}}$$

Where: A_{Quant} = Area of quantifying transition
A_{Qual} = Area of qualifying transition

13.3.2.3. For medical marijuana samples, this value must then be converted to a sample concentration in ng/g using the following equation:

$$C_s\left(\frac{ng}{g}\right) or\ (ppb) = \frac{C_E\left(\frac{ng}{mL}\right) * V_F(mL) * D}{M_I(mg) * 0.001(\frac{g}{mg})}$$

Where:
C_S = Concentration of analyte in sample (ng/g) or (ppb)
C_E = Concentration of analyte in solvent (ng/mL) (from software)
V_F = Final volume of extract (mL)
M_I = Initial mass of sample (mg)
0.001 g/mg= Conversion from mg to g
D = Dilution factor, if applicable.

13.3.3. Matrix Spike and Matrix Spike Duplicate (**MS + MSD**)

13.3.3.1. To determine the precision, calculate the relative percent difference (RPD). The RPD must be <20%.

$$RPD = \frac{|MS - MSD|}{\left(\frac{|MS + MSD|}{2}\right)} * 100$$

Where: RPD is in percent (%).
MS = Matrix Spike concentration in ng/g or ppb.
MSD = Matrix Spike Duplicate concentration in ng/g or ppb.

13.3.4. Calculate the average **IS** peak area from the calibration curve for each **IS** analyte. Evaluate the system stability by using the following equation on every injection and comparing to the criteria set in **Section 11.8**.

$$IS\ Peak\ Area\ Deviation\ (\%) = \frac{IS_I}{IS_A} * 100$$

Where: IS_I = IS peak area for individual injection
IS_A = IS peak area average from calibration curve

13.3.5. Continuing calibration verification standards must not be used to calculate the concentration of analytes in samples.

13.4. Reporting of Results

13.4.1. Non-detected analytes are reported as less than (<) the **LOD** as specified in **Section 1.1**.

13.4.2. Analytes detected at a concentration below the **LOD**, or that fail the identification requirements in **Section 13.2**, are considered non-detects due to the uncertainty of the actual presence of the analyte and are reported as less than (<) the **LOD** as specified in **Section 1.1**.

13.4.3. Analytes detected at a concentration at or above the **LOQ** and at or below the **ULOQ** are reported using 2 significant figures.

13.4.4. Analytes detected at a concentration at or above the **LOD** and below the **LOQ**, that also match identification requirements in **Section 13.2**, must be qualified and are reported as less than (<) the **LOQ** as specified in **Section 1.1**.

13.4.5. Analytes detected at a concentration above the **ULOQ** cannot be accurately reported. A new sample must be prepared using a smaller amount of sample. Use the approximate concentration to adjust the sample size. If there is not enough for a new preparation, report as greater than (>) the **ULOQ**.

13.4.5.1. For example, if a concentration of 15.2 ng/mL (152 ng/g) is measured, prepare a new sample using 50 mg of sample instead of 100 mg and follow Section 12.1.2 – 12.1.8 as before. The newly prepared sample should be about 7.6 ng/mL.

14.0. Data Assessment, Acceptance Criteria, and Corrective Actions for Out-of-Control Data

14.1. All analytical batches must meet all quality control criteria as described within this procedure and all quality control results must be documented.

14.2. The acceptance criteria for standards and quality control samples are defined in **Section 10**, and **Section 11**. The sections below (**Sections 14.3 – 14.14**) outline the most common corrective action procedures for nonconforming data and inconsistent chromatograms. Since re-injection of a standard or sample is a routine corrective action for most nonconformities, it is not included in each individual section below, but may be used whenever applicable.

14.3. Failure to meet QC criteria for a Calibration Curve Correlation Factor of ≥0.995 required in **Section 10.3.3**.

14.3.1. Assess the calibration curve to determine if there is one particular standard that appears to be prepared incorrectly.

14.3.2. If necessary, perform instrument maintenance.

14.3.3. A correlation factor of ≥ 0.995 must be achieved before sample analysis can begin. If samples were analyzed before an acceptable calibration curve was established, all affected samples must be re-analyzed under an acceptable curve or the results will be appropriately qualified.

14.4. Cross Check Reference Standard (**CCR**) failure to meet the 70-130% recovery criteria required in **Section 10.4.3**.

14.4.1. Check the calibration curve linearity (**Section 10.3.3**), calibration curve response (**Section 10.3.4**), and internal standards response (**Section 13.3.1**).

14.4.2. Check **LFB** recovery value (**Section 11.5**).

14.4.3. If the **LFB** and other responses of standards curve appear normal, then the current **CCR** is likely compromised and a new **CCR** will be prepared and re-analyzed; may need to prepare new stock solution or working solution.

14.4.4. If the criteria fail for **LFB** recovery, and/or the curve linearity and/or the curve response, the calibration curve is likely compromised, and a new initial calibration curve will need to be analyzed.

14.4.4.1. If the curve that failed comes from a freshly prepared **SS** or **WS**, one or both may need to be re-prepared.

14.5. Failure to meet required QC criteria for Initial Calibration and/or Continuing Calibration Verification standard (**CCV**) of 80% to 120% recovery as required in **Section 10.3.4** and **Section 10.5.1**.

14.5.1. If an Initial Calibration curve and/or **CCV** doesn't meet the required criteria, prepare calibration standards from new or existing working standard solutions or stock standard solutions. A new initial calibration curve is then analyzed and verified with a **CCR**.

14.5.2. It is recommended that all samples are bracketed by an acceptable **CCV**. Any samples that are analyzed without an acceptable bracket must be reanalyzed when an acceptable **CCV** is achieved or a new initial calibration is established. If reanalysis in not possible due to lack of remaining extract or sample, the original sample results will be appropriately qualified.

14.6. Failure to meet required QC criteria for Initial Calibration and/or Limit of Quantification (**LOQ**) of 70% to 130% recovery as required in **Section 10.3.4** and **Section 10.5.1**.

14.6.1. A new Initial Calibration and/or a standard **≤LOQ** is prepared and re-analyzed. Prepare from new working or stock solutions, if necessary.

14.6.2. If a newly prepared Initial Calibration curve and/or a standard **≤LOQ** still doesn't meet the required criteria. The instrument is recalibrated with new calibration standards which may be prepared from new or existing working standard solutions or stock standard solutions. A new initial calibration curve is analyzed and verified with a **CCR** using the new curve.

14.6.3. A standard **≤LOQ** within 70-130% recovery must be achieved before analysis can begin. If samples were analyzed before an acceptable **LOQ** was achieved, all affected samples must be re-analyzed after an acceptable **LOQ** is achieved.

14.7. Failure to meet required QC criteria for System Blank free from interference peaks.

14.7.1. Try one or more of the following:

14.7.1.1. Replace the mobile phase with freshly made mobile phase.

14.7.1.2. Change the pre-column.

14.7.1.3. Clean the column at an appropriate temperature by extended flow of a strong solvent such as isopropanol until such time as contaminants are removed from the column based on column manufacturer's recommendations.

14.7.1.4. Inject multiple system blanks and run through the system until background contamination is removed or reduced to an acceptable level.

14.7.2. An acceptable system blank must be achieved before sample analysis begins. If samples have already been analyzed, then any samples containing target analytes must be re-analyzed. If re-analysis of suspect samples is not possible due to lack of remaining extract or sample, the original sample results will be appropriately qualified.

<u>Exception:</u> If the samples do not contain target analytes at or above the **LOQ**, the original results may be reported without re-analysis and qualification is not necessary.

14.8. Failure to meet required QC criteria for Laboratory Reagent Blank of < 1/3 the **LOQ** for target analyte(s).

14.8.1. Analyze a system blank to ensure that the system is free from background contamination. If background contamination is discovered in the system blank, follow the corrective actions described above (**Section 14.7.1**)

14.8.2. Re-inject the **LRB** once a contaminant-free system is achieved.

14.8.3. If the re-injection still fails, extract a new **LRB** and analyze to ensure that a systemic problem does not exist. If a new **LRB** has already been extracted with a subsequent batch, then the extraction of an additional **LRB** is not required.

14.8.4. An acceptable **LRB** must be achieved before sample analysis begins. If samples have already been analyzed, then any samples containing target analytes must be re-analyzed (if system contamination is suspected) or re-extracted and analyzed (if extraction contamination is suspected). If re-analysis or re-extraction of suspect samples is not possible due to lack of remaining extract or sample, the original sample results will be appropriately qualified.

<u>Exception:</u> If the samples do not contain target analytes at or above the **LOQ**, the original results may be reported without re-analysis and qualification is not necessary.

14.8.5. If subsequent **LRB**'s continue to show unacceptable levels of background contamination, the extraction of additional samples must be halted until the source of the contamination can be determined and eliminated or reduced to acceptable levels.

14.9. Failure to meet required QC criteria for Laboratory Fortified Blank (**LFB**) as described in **Section 11.5**.

14.9.1. Check if there is an interference peak which was not identified.

14.9.2. Reanalyze the **LFB** sample, if still out of the range, check the **MS/MSD** for a similar problem.

14.9.3. If the **MS** also fails, the problem is related to the spiking solution. Discard the problematic solution and re-prepare the spiking solution, **LFB**, and **MS/MSD**.

14.9.4. If the **MS** passes, there is no problem with the spiking solution, but the **LFB** must be re-prepared.

14.10. Failure to meet required QC accuracy (recovery) criteria for Matrix Spike (**MS**) as described in **Section 11.7**.

14.10.1. Check the **LFB** recovery, to determine if the failure is related to the spiking solution.

14.10.2. If the **LFB** also fails, discard problematic spiking solution and re-prepare the spiking solution, **LFB**, **MS,** and **MSD**.

14.10.3. If the subsequent **MS** is prepared with a newly prepared spiking solution and meets acceptance criteria, no further action is required.

14.10.4. If the **MS** fails to meet the acceptance criteria, but the **LFB** is acceptable, then it is recommended that the **MS** and/or **MSD** be re-prepared and analyzed if sufficient sample remains; this may require preparation from a new stock standard.

14.10.5. If re-analysis is performed and the new **MS** and/or **MSD** meets the acceptance criteria, only report those results.

14.10.6. If the **MS** and/or **MSD** cannot be reanalyzed, or if the re-analyzed **MS** and/or **MSD** still fails, the original **MS** and/or **MSD** and all corresponding sample results will be appropriately qualified on the report.

14.11. Failure to meet required QC precision (RPD) criteria for Matrix Spike as described in **Section 11.7.**

14.11.1. Compare the **MS** and **MSD** for **IS** Peak Area Deviation (**Section 13.3.4**) using only the **IS** peak area from these two samples.

14.11.2. If the **IS** deviation check fails, both **MS** and **MSD** must be re-prepared because the **IS** was not accurately spiked in.

14.11.3. If the deviation check passes, repeat the calculation using the peak areas of both samples in place of **IS** peak area.

14.11.4. If this deviation check fails, both **MS** and **MSD** must be re-prepared, because the analyte spike was not accurately spiked in.

14.11.5. If samples do not deviate from peak area or **IS** peak area, yet still fail precision criteria, both **MS** and **MSD** must be re-prepared. In addition, notify immediate supervisor of the issue.

14.11.6. If the re-prepared **MS** and/or **MSD** still fail RPD, the original **MS** and/or **MSD** and all corresponding sample results will be appropriately qualified on the report.

14.12. Failure to meet required QC criteria for IS peak area variation described in **Section 11.8**.

14.12.1. See **Section 11.8.4**.

14.13. Inconsistent baseline

14.13.1. Try one or more of the following:

14.13.1.1. Replace the mobile phase with freshly made mobile phase.

14.13.1.2. Perform appropriate instrument maintenance, if applicable.

14.13.2. Repeat the sequence using the same standards/samples. If repeat analysis is acceptable, report only those results.

14.13.3. If instrument maintenance and repeat analysis fails to produce acceptable data, the sample results will be appropriately qualified.

14.14. All other nonconforming data, not addressed within this procedure, requires the completion of a nonconformance/corrective action report.

15.0. Method Performance

15.1. Detection limit study results and demonstration of capability study results are maintained by the laboratory.

16.0. Waste Management/Pollution Prevention

16.1. It is the responsibility of the laboratory to comply with all federal, state and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions.

16.2. Minimize solvent, chemical, reagent, and standard use whenever possible to reduce the amount of hazardous waste generated.

16.3. Dispose of solvent waste in an appropriate solvent waste container, properly labeled.

16.3.1. Acetonitrile and Methanol must be disposed of separately from all other solvents in a container no larger than 4-Liters (1-Gallon).

16.3.2. All other solvents are separated into two categories, chlorinated and non-chlorinated, and are disposed of in red, 5-Gallon solvent cans.

16.4. Dispose of non-hazardous aqueous waste in the laboratory sink followed by flushing with tap water.

16.5. Dispose of glassware in appropriately labeled boxes. Be sure that, whenever possible, the glass has been thoroughly rinsed and is contaminant-free before disposal.

17.0. References

17.1. IARC (2012) Chemical agents and related occupations: a review of human carcinogens. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Volume 100F: 225 – 248

17.2. IARC (1993) Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Volume 56: 489 – 529

17.3. Eppendorf Relative Centrifugal Force Calculator for model 5415D, 24-place fixed-angle rotor.
http://www.eppendorf.com/int/index.php?l=1&action=products&contentid=169

17.4. AB Sciex 4500 Series of Instruments – System User Guide
http://sciex.com/Documents/Downloads/Literature/4500-system-user-guide-en.pdf

17.5. Shimadzu HPLC Module Manuals
http://store.shimadzu.com/s-1003-liquid-chromatography.aspx?pagenum=1

17.6 Public Health Law, section 502 of the Public Health Law (“PHL”), Title 10 (Health) of The Official Compilation of Codes, Rules and Regulations of the State of New York (NYCRR) subpart 55-2 (Approval of Laboratories Performing Environmental Analysis).

18.0. Appendices

Appendix B (MML-303-AppB) – Alternate Approved Preparation Steps/ Method Validation Summary by Brand/Form (see MML-303-SOP, section 12.0)

1. **Product/Form –Water Soluble Matrix exhibiting suppressed isotopically labeled internal standard transitions.**

   a. **Method Development Narrative/Background**

      i. Some matrices contain water soluble impurities which can present a challenge for the laboratory to achieve acceptable recoveries of the isotopically labeled internal standards. The laboratory proceeded to develop an alternate extraction procedure to address when an unknown impurity fully suppresses the isotopically labeled internal standard signal for all analytes (AT-B1, AT-B2, AT-G1, AT-G2, and Ochratoxin). This is typically < 25 % internal standard recovery and a signal to noise of less than 15. A liquid-liquid extraction method was developed to improve the internal standard recovery and coordinated with the current instrumental analysis portion of the MML-303 method.

      ii. As per MML-303 method, a matrix blank (MB) and LFB (laboratory fortified blank) are also included as controls as designated in MML-303.

   b. **Procedure:**

      1. Weigh 100±5 mg as described in MML-303-SOP (**See section 12.1.1.**) substituting a 2.0-mL centrifuge tube.
      2. Spike with ISS as described in MML-303-SOP **(See section 12.1.2.)**
      3. Replace extraction solvent in MML-303-SOP **(See section 12.1.3.)** with 1,000 µL of ethyl acetate followed by 800 µL water to the tube.
      4. Vortex sample 20 seconds, repeatedly to mix until both layers are clear of visible solids.
      5. Centrifuge for 5 minutes at 12,000 rpm (~13,400 RCF) (**See section 12.1.7.**)
      6. Separate the top, organic layer by pipette and transfer into a glass culture tube (16x125 mm, or equivalent).
      7. Remove solvent by passing air over the tops of the tubes at 26 °C temperature setting with Biotage TurboVap LV, or equivalent (approx. 20 minutes).
      8. Reconstitute residue with MML-303 extraction solvent (1,000 µL) and continue with solution transfer described in MML-303-SOP **(See section 12.1.8.)**

19.0. Addendum

GUIDANCE REGARDING FLEXIBILITY INHERENT TO MM METHODS AND THE PRECEDENCE OF QUALITY CONTROL CRITERIA

The specific products and instrument settings cited in the DOH methods represent those products and settings used during method development or subsequently evaluated by the Department for use in the method. Glassware, reagents, supplies, equipment and settings other than those listed in this method may be employed, provided that method performance appropriate for the intended application has been documented. Such performance includes consideration of precision, accuracy (or bias), recovery, representativeness, comparability, and sensitivity (quantitation or reporting limits, now referred to as limit of quantitation (LOQ) relative to the reporting or regulatory limits) for the intended use of the analytical results.

In response to this inherent flexibility, if an alternative analytical procedure is employed, then the Department expects the laboratory to demonstrate and document that the procedure is capable of providing appropriate performance for its intended application. This demonstration must not be performed after the fact, but as part of the laboratory’s initial demonstration of proficiency (or capability; iDOC) with the method. The documentation should be in writing, maintained in the laboratory, and available for inspection upon request by the Department. The documentation should include the performance data as well as a detailed description of the procedural steps as performed (i.e., a written standard operating procedure).