



**Department
of Health**

ANDREW M. CUOMO
Governor

HOWARD A. ZUCKER, M.D., J.D.
Commissioner

SALLY DRESLIN, M.S., R.N.
Executive Deputy Commissioner

**New York State Department of Health - Wadsworth Center
Laboratory of Organic and Analytical Chemistry—
NYS ELAP Laboratory ID 10763**

**Division of Environmental Health Sciences
Albany, New York**

**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 MCT² Matrix | LLOQ¹ (ng/g) in MCT² Matrix | LOD (ng/mL) in MCT²/ Solvent Matrix | LLOQ¹ (ng/mL) in MCT²/ 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 Lower Limit of Quantitation (LOQ) is the lowest concentration that can be accurately quantified for a target analyte (**Section 3.17**).

²LOQs were determined with medium-chain triglycerides (MCT) as the matrix

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 Lower Limit of Quantitation (LLOQ) calculations are performed annually on every instrument, and are subject to change (see **Section 11.2**). The above values are examples only. 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 C₁₈ reversed-phase column in 11 minutes using a programed gradient of increasing organic modifier. See Table 1 for LOD and LLOQ 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 ¹³C-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 (CaIS) – 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. Lower Limit of Quantitation (LLOQ) – The minimum concentration that can be quantitatively reported for a target analyte. For routine analyses, the lowest calibration standard must be at or below the **LLOQ** for each analyte. **LLOQ** is at least 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 **CaIS** standard.
- 3.19. Sample Batch – A group of samples that are processed together as a unit using the same procedure and materials. A typical batch consists of 20 samples. A batch includes the necessary quality controls, including method blanks, laboratory fortified blanks, matrix spikes, duplicates, and quality control 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; ref. s 17.1 and 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 has 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 coat, 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 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 and 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 needle 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 (calculated concentration < LOD), 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 be 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. These 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, or equivalent.
 - 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.

6.3. 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).

7.2. Gases

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

7.3. Stock Analytical and Internal Standard Solutions

- 7.3.1. Stock standard solutions may be purchased from any vendor. Standards should preferably be ISO Guide 31 or 35 accredited, and NIST traceable, where available.
- 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. Standards and 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 ¹³C₂₀-Ochratoxin A (¹³C₂₀-OTA) @ 10 µg/mL is added.
- 8.3.3. Dilute to volume with extraction solvent, invert 7x, and sonicate for 1 minute to mix.

$$(10.0 \mu\text{g/ml}) (50.0 \mu\text{l}) = (X \mu\text{g/ml}) (1000 \mu\text{l})$$

$$X = 0.500 \mu\text{g/mL}$$

- 8.3.4. 1.0 mL of the solution is transferred and sealed in a 2.0 mL crimp-cap vial and labeled appropriately.
- 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 (¹³C₁₇-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.

$$(500 \text{ ng/ml}) (100 \text{ }\mu\text{l}) = (X \text{ ng/ml}) (1000 \text{ }\mu\text{l})$$

$$X = 50.0 \text{ ng/mL}$$

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

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.

$$(50.0 \text{ ng/ml}) (100 \text{ }\mu\text{l}) = (X \text{ ng/ml}) (5000 \text{ }\mu\text{l})$$

$$X = 1.00 \text{ ng/mL}$$

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

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

8.6. Stock Standard – Ochratoxin A (**SSO**) – @1,000 ng/mL

8.6.1. Fill a 1.00 mL volumetric flask 1/2 full with acetonitrile.

8.6.2. 100 µL of Ochratoxin A (OTA) @ 10.0 µg/mL is added from Romer Labs, # 002023.

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

$$(10 \text{ }\mu\text{g/ml}) (100 \text{ }\mu\text{l}) = (X \text{ }\mu\text{g/ml}) (1000 \text{ }\mu\text{l})$$

$$X = 1.00 \text{ }\mu\text{g/mL} = 1,000 \text{ ng/mL}$$

8.6.4. 1.0 mL of the solution is transferred and sealed in a 2.0 mL crimp-cap vial and labeled appropriately.

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



- 8.7.** Stock Standard Mix (**SSM**) – @100 ng/ml for all five mycotoxins.
- 8.7.1.** Fill a 1.00 ml volumetric flask with 400 µL water.
 - 8.7.2.** Add 100 µL of **SSO** @ 1,000 ng/mL (**Section 8.6**)
 - 8.7.3.** Add 400 µL of Mix 5 @ 250 ng/mL from Romer Labs, # 002022.
 - 8.7.4.** Fill to volume with methanol, invert 7x, and sonicate for 1 minute to mix.
 - 8.7.5.** This solution is filled and sealed in a 1.5 mL crimp-cap vial and labeled appropriately.
 - 8.7.6.** Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule.
- 8.8.** Working Standard (**WS**) – @ 20.0 ng/mL for all five mycotoxins.
- 8.8.1.** Fill a 1.00 mL volumetric flask 1/2 full with extraction solvent (**Section 8.2**).
 - 8.8.2.** Add 200 µL of **SSM** @ 100 ng/mL (**Section 8.7**)
 - 8.8.3.** Dilute to volume with extraction solvent, invert 7x, and sonicate for 1 minute to mix.
$$(100 \text{ ng/ml}) (X \text{ ml}) = (20 \text{ ng/ml}) (1.00 \text{ ml})$$
$$X = 0.20 \text{ mL} = 200 \text{ µL}$$
 - 8.8.4.** The solution is filled and sealed in a 1.5 mL crimp-cap vial and labeled appropriately.
 - 8.8.5.** Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule.
- 8.9.** Cross Check Reference – Stock Standard Ochratoxin A (**CCR-SSO**) – @1,000 ng/mL
- 8.9.1.** The **CCR-SSO** should be from a different source than the **SSO** as described in the definition of the **CCR** (**Section 3.15**)
 - 8.9.2.** Fill a 1.00 mL volumetric flask 1/2 full with acetonitrile.
 - 8.9.3.** 100 µL of Ochratoxin A (OTA) @ 10.0 µg/mL is added from Fluka, # 34037-2mL-R.
 - 8.9.4.** Dilute to volume with extraction solvent, invert 7x, and sonicate for 1 minute to mix.
$$(10.0 \text{ µg/ml}) (100.0 \text{ µl}) = (X \text{ µg/ml}) (1000 \text{ µl})$$
$$X = 1.00 \text{ µg/mL} = 1,000 \text{ ng/mL}$$
 - 8.9.5.** 1.0 mL of the solution is transferred and sealed in a 2.0 mL crimp-cap vial and labeled appropriately.
 - 8.9.6.** Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule.



- 8.10.** Cross Check Reference – Stock Standard Mix (**CCR-SSM**) – @ 100 ng/mL for AT-B1, AT-G1, and OTA @ 30.0 ng/mL for AT-B2, and AT-G2.
- 8.10.1.** The **CCR-SSM** should be from a different source than the **SSM** as described in the definition of the **CCR** (**Section 3.15**)
 - 8.10.2.** Fill a 1.00 mL volumetric flask with 400 µL water.
 - 8.10.3.** Add 100 µg/mL of **CCR-SSO** @ 1,000 ng/mL (**Section 8.9**).
 - 8.10.4.** Add 100 µg/mL of Aflatoxin Mix (Aflatoxin B1, G2, G1, G2) @ 1,000/300 ng/mL from Supelco, CRM46304.
 - 8.10.5.** Fill to volume with methanol, invert 7x, and sonicate for 1 minute to mix.
 - 8.10.6.** This solution is filled and sealed in a 1.5 mL crimp-cap vial and labeled appropriately.
 - 8.10.7.** Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule.
- 8.11.** 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.
- 8.11.1.** Fill a 1.00 mL volumetric flask 1/2 full with extraction solvent (**Section 8.2**).
 - 8.11.2.** Add 200 µL of **CCR-SSM** @ 100 ng/mL (**Section 8.10**)
 - 8.11.3.** Dilute to volume with extraction solvent, invert 7x, and sonicate for 1 minute to mix.
$$(100 \text{ ng/ml}) (0.200 \text{ ml}) = (X \text{ ng/ml}) (1.00 \text{ ml})$$
$$X = 20.0 \text{ ng/mL}$$
$$(30 \text{ ng/ml}) (0.200 \text{ ml}) = (X \text{ ng/ml}) (1.00 \text{ ml})$$
$$X = 6.00 \text{ ng/mL}$$
 - 8.11.4.** 1.0 mL of the solution is transferred and sealed in a 2.0 mL crimp-cap vial and labeled appropriately.
 - 8.11.5.** Suggested storage is at -20 °C for up to 12 months, in a sealed vial or ampule.
- 8.12.** Mobile Phases
- 8.12.1.** Mobile phase A: 15 mM ammonium formate (aq).
 - 8.12.1.1.** Fill 1.0 L volumetric flask half full of water.
 - 8.12.1.2.** Add 945.9 ± 15 mg of ammonium formate to the flask.



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

8.12.1.4. This solution must be prepared monthly.

8.12.2. Mobile phase B: Methanol.

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

8.13. Matrices

8.13.1. MCT as a representative matrix.

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

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 $\leq 4^{\circ}\text{C}$ 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 $\leq -20^{\circ}\text{C}$ until analysis. Analysis must be completed within 7 days of extraction. After testing is complete, the remaining extract is stored at $\leq -20^{\circ}\text{C}$ 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 production 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. 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.

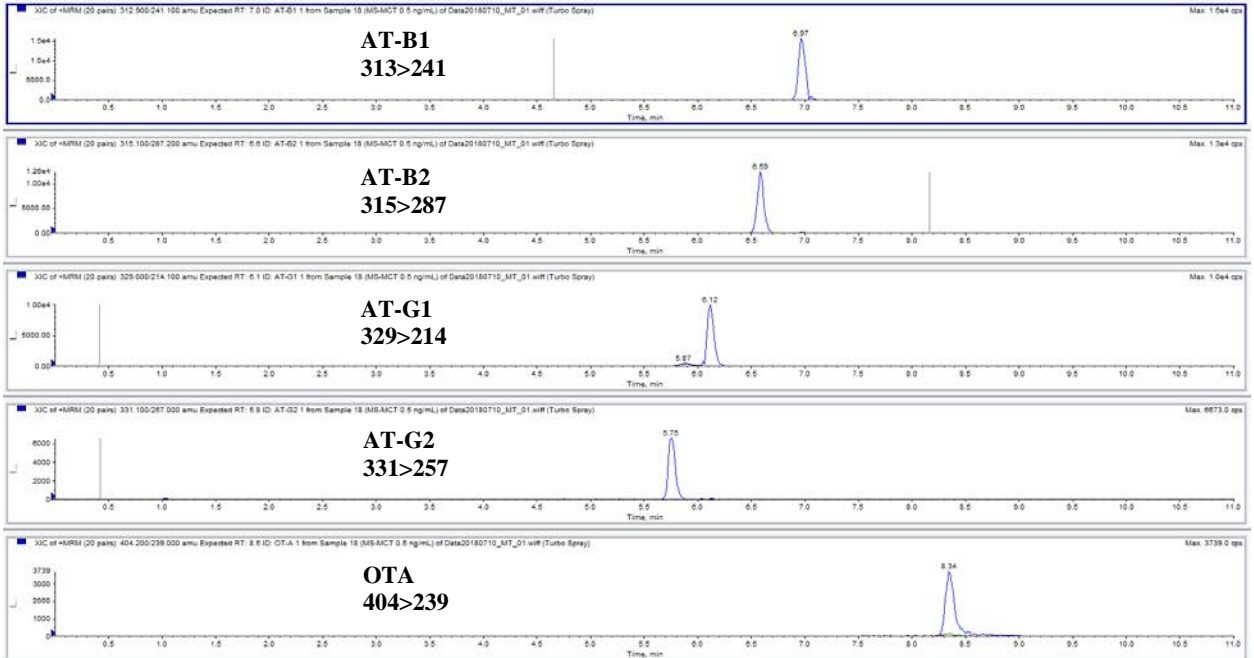
Table 7: HPLC parameters

| 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 ₂ O |
| 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 – 6.39 |
| 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 – 6.37 |
| 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 solvent 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 8.8**). An example of the current standard levels is provided as follows, but higher or lower levels or volumes may be prepared and analyzed, provided they are within the concentration range of 10 ng/ml to 0.0243 ng/ml. See **Table 9** in **Section 10.2.8** 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. CalS 6a (20.0 ng/ml)

10.2.2.1. **CalS 6a** is the same as the Working Standard (**Section 8.8**).

10.2.3. CalS 5a (6.00 ng/ml)

10.2.3.1. Pipet 300 µl of Working Standard (**Section 8.8**) into vial labeled **CalS 5a** containing 700 µl of extraction solvent.

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

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

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



10.2.4. CalS 4a (1.80 ng/ml)

10.2.4.1. Pipet 300 µl of **CalS 5a (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.

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

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

10.2.5. CalS 3a (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.

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

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

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.

$$(0.540 \text{ ng/ml}) (300 \text{ }\mu\text{L}) = (X \text{ ng/ml}) (1000 \text{ }\mu\text{l})$$

$$X = 0.162 \text{ ng/mL}$$

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.

$$(0.162 \text{ ng/ml}) (300 \text{ }\mu\text{L}) = (X \text{ ng/ml}) (1000 \text{ }\mu\text{l})$$

$$X = 0.0486 \text{ ng/mL}$$

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 (8.1) |
|----------|----------------------------|------------------------------|-------------------------|--------------------------|
| 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 | 8.3 | 0.5 |
| CalS 5b | 3.00 | 10.2.3 | 8.3 | 0.5 |
| CalS 4b | 0.900 | 10.2.4 | 8.3 | 0.5 |
| CalS 3b | 0.270 | 10.2.5 | 8.3 | 0.5 |
| CalS 2b | 0.0810 | 10.2.6 | 8.3 | 0.5 |
| CalS 1b | 0.0243 | 10.2.7 | 8.3 | 0.5 |

10.2.9. After at least one system blank 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).

10.3. Initial Calibration Criteria

10.3.1. The solutions prepared in Section 10.2 are used to prepare a calibration curve for the mycotoxins at concentrations appropriate for the instrument’s range and sample content. A minimum of 5 calibration concentrations is used for each mycotoxin.

10.3.2. For routine analyses, the lowest calibration standard must be at or below the LLOQ listed in Section 1.1 for each analyte, or the LLOQ 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 \leq LLOQ, which may be within 70-130% of the true value for each analyte.
- 10.3.5. If all of these criteria cannot be met a new calibration must be established.

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 or volumes 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 μ L from CCR-WS (Section 8.11)
 - 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.
 - 10.4.2.5. Dilute 10.4.2.4, with an equal amount of IWD @ 1.00 ng/mL (Section 8.5) and mix well (e.g., 200 μ L CalS with 200 μ L IWD). Be sure to rinse solution down the sides of the container.
 - 10.4.2.6. Label CCR-b.
- 10.4.3. Measured recovery value of CCR-b should be within 80 – 120% of the true value for all mycotoxins.

10.5. Continuing Calibration Verification

- 10.5.1. The calibration curve must be verified on each working day by the measurement of a LLOQ standard and a minimum of two continuing CCVs, one at the beginning and one at the end of the analysis day. The beginning CCV and LLOQ standard may be substituted by a full initial calibration. For extended periods of analysis (greater than 8 hrs.), it is strongly recommended that CCVs be interspersed with samples at regular intervals during the course of the analysis at varying concentrations. The response for any analyte in the LLOQ standard must be within $\pm 30\%$ of the predicted response. The CCVs must be within $\pm 20\%$ of the predicted response for routine sample batches.



10.5.2. The **CCV** and **LLOQ** standards are prepared at the following concentrations but higher or lower levels may be prepared as necessary. The **CCV** standard must be at a concentration within the calibration curve and the **LLOQ** standard must be at a concentration at or below the **LLOQ** as listed in (Section 1.1).

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

10.5.2.1.1. 3.0 ng/mL, 0.90 ng/mL, and 0.27 ng/mL (same as **CalS 3b** thru **5b** – (Section 10.2.8))

10.5.2.2. **Lower Limit of Quantitation (LLOQ)** 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/Assurance

11.1. Demonstration of Capability (DOC)

11.1.1. Each analyst must perform an initial demonstration of capability in 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 have been fortified with all analytes of interest at a low-level concentration. The spiking solution used should be from an independent prepared source used to calibrate if available.

11.1.1.1. For each individual analyte, the recovery value for all replicates must fall in the range of $\pm 20\%$, except \leq **LLOQ**, where the value must fall in the range of $\pm 30\%$. 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 until satisfactory performance has been demonstrated.

11.1.1.2. Annually, each analyst must complete a continuing DOC. For each individual analyte, the recovery value for all replicates must fall in the range of $\pm 20\%$, except \leq **LLOQ**, where the value must fall in the range of $\pm 30\%$. 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 until satisfactory performance has been demonstrated. The continuing DOC may be completed by one of the following techniques:

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

11.1.1.2.2. Acceptable performance of an initial DOC as described above in 11.1.1.

11.1.2. 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** for routine samples or the cross-check reference standard per **Section 10.4**.

11.2. Method Detection Limits

11.2.1. An **LOD** study must be completed for all target analytes, as listed in **Section 1.1**, on each instrument used to analyze sample extracts, and for each matrix. An **LOD** study consists of the analysis of seven to ten low level matrix spike samples that have been fortified with all target analytes at no more than twice the laboratory’s minimum reporting limit. In this study, all analytes were spiked into matrix to form a 0.1 ng/mL (or 1.0 ng/g) sample. The fortified samples must be treated as if they were real samples and processed through all of the applicable method procedures (**Section 12.1**). It is recommended that the low-level matrix spike samples be prepared and analyzed over a period of several days, so that day-to-day variations are reflected in the precision data.

11.2.2. Calculate the mean recovery and standard deviation for each analyte. Use the following equation to calculate the **LOD**:

$$LOD = SD \text{ of } n \text{ samples} * t$$

SD = standard deviation

n = sample size (7-10)

t = student’s t value for the 99% confidence level with n-1 degrees of freedom where n is the number of replicates. (single-tailed)

Table 11. Student’s t value for calculating LOD

| n | t |
|----|-------|
| 7 | 3.143 |
| 8 | 2.998 |
| 9 | 2.896 |
| 10 | 2.821 |

11.2.3. The **LOD** for each mycotoxin is then used to calculate a **LLOQ** for each mycotoxin by multiplying the **LOD** by a factor of five.

11.2.4. The **LLOQ** for each mycotoxin in each matrix must then be verified by extracting and analyzing a **MS** that has been spiked at no more than twice the **LLOQ**. The measured value should be within 70-130% of expected value and have a signal-to-noise ratio >3.

11.2.5. A new **LOD** study must be verified annually on each instrument, for each analyte, in each matrix. **LOD**’s are also determined each time that there is a significant change in the test method or instrument type.

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. For our analysis, a vial of extraction solvent (**Section 8.2**) will be injected.

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, a **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 found must be < 1/3 the **LLOQ** 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 found must be < 1/3 the **LLOQ** for each target analyte.

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

11.7.1. A matrix spike sample must be analyzed every twenty samples or one per sample set, 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).

11.7.2. A duplicate matrix spike 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 \leq LLOQ, 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 25-125% 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:

- 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 μ L, 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 μ L extraction solvent (**Section 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 μ L 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 8.13**) directly into a tared 1.5-mL centrifuge tube.
- 12.2.2. Add 0.5 ng (25.0 μ L, 20 ng/mL) **WS (Section 8.8)** to the tube wall.



- 12.2.3. Add 0.5 ng (10.0 μ L, 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 μ L extraction solvent (**Section 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 μ L of supernatant to centrifuge tube for storage.
- 12.2.10. Suggested storage is -20 $^{\circ}$ 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 $^{\circ}$ 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 $^{\circ}$ C for up to 6 months, in a sealed vial or tube.
- 12.6. LC-MS/MS Analysis Procedure.
 - 12.6.1. Perform initial LC-MS/MS calibration (**Section 10.1**) if needed.
 - 12.6.2. Equilibrate the LC-MS/MS system by flowing at initial parameters described in **Table 7** in **Section 10.1.4**.



- 12.6.3.** Analyze at least one **SBLK**, followed by a **LRB**. The **SBLK** must pass criteria in **Section 11.3**.
- 12.6.4.** If it has been more than one month (>31 days) since running the last calibration curve, or if the mobile phase A was changed since the last curve, a full calibration curve needs to be analyzed:
- 12.6.4.1.** Analyze a full initial calibration (**Section 10.2**), followed by a **LRB** to prevent carry-over.
 - 12.6.4.2.** Analyze a **CCR** followed by a **LRB**.
 - 12.6.4.3.** The calibration curve and **CCR** must pass the criteria set in **Section 10.3 and 10.4.3** respectively before samples can analyzed and reported.
- 12.6.5.** If it has been one month or less (≤ 31 days) since running the last calibration curve and the mobile phase A has not been changed, the previous curve still needs to be verified as viable. If it fails verification, a new curve needs to be analyzed (see **Section 12.6.4**).
- 12.6.5.1.** Analyze an **LLOQ**, and **CCV**, followed by a **LRB** to prevent carry-over.
 - 12.6.5.2.** Analyze a **CCR**, followed by a **LRB**.
 - 12.6.5.3.** The **LLOQ**, **CCV**, and **CCR** must pass criteria set in **Section 10.5.1** and **Section 10.4.3** before any samples can be analyzed and reported.
- 12.6.6.** Analyze up to 20 samples, including **LFB**, **MB**, **MS**, and **MSD QC** samples.
- 12.6.7.** Analyze in the following order a **LRB**, **CCV**, and **LRB** at the end of the 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 full 20-sample batch, with a full calibration curve.



Table 12: Batch submission example

| Injection # | Sample | Comments |
|-------------|-------------------------|--|
| 1 | SBLK | 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 | SBLK | |
| 3 | SBLK | |
| 4 | LRB | This example uses a full calibration curve. If a full calibration curve is not needed (see Section 12.6.5), only an LLOQ (CalS-1b or 2b) and a CCV (CalS-3b, 4b, or 5b) is needed. |
| 5 | CalS-1b | |
| 6 | CalS-2b | |
| 7 | CalS-3b | |
| 8 | CalS-4b | |
| 9 | CalS-5b | |
| 10 | CalS-6b | |
| 11 | LRB | Blank to check for carry-over. |
| 12 | CCR | Calibration curve cross-check. |
| 13 | LRB | Blank to check for carry-over. |
| 14 | LFB | Sample 1 |
| 15 | MB | Sample 2 |
| 16 | MS | Sample 3 |
| 17 | MSD | Sample 4 |
| 18 | Unknown 1 | Sample 5 |
| 19 | Unknown 2 | Sample 6 |
| 20 | Unknown 3 | Sample 7 |
| 21 | Unknown 4 | Sample 8 |
| 22 | Unknown 5 | Sample 9 |
| 23 | Unknown 6 | Sample 10 |
| 24 | Unknown 7 | Sample 11 |
| 25 | Unknown 8 | Sample 12 |
| 26 | Unknown 9 | Sample 13 |
| 27 | Unknown 10 | Sample 14 |
| 28 | Unknown 11 | Sample 15 |
| 29 | Unknown 12 | Sample 16 |
| 30 | Unknown 13 | Sample 17 |
| 31 | Unknown 14 | Sample 18 |
| 32 | Unknown 15 | Sample 19 |
| 33 | Unknown 16 | Sample 20 |
| 34 | LRB | Blank to check for carry-over. |
| 35 | CCV (CalS-3b – CalS-5b) | Checks calibration still valid. |
| 36 | LRB | Blank to check for carry-over. |

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 (¹³C-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**. For routine analyses 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 ¹³C-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 quant/qual 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**. The calculation does not need to be done by hand if the software can calculate it.

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

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 B1-¹³C₁₇.

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 standard, QC sample, and real sample. The calculation does not need to be done by hand if the software can calculate it. The ratio is calculated using the following formula:

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

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

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

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

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 ppb.

MSD = Matrix Spike Duplicate concentration in 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**.

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

Where: IS_I = IS peak area for individual injection

IS_A = IS peak area average from calibration curve



- 13.3.5. Do not use daily calibration verification standards 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 **LLOQ** 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 **LLOQ**, that also match identification requirements in Section 13.2, are reported as less than (<) the **LLOQ** 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. If so, re-prepare that standard and analyze. If more than eight (8) hours has elapsed since the original failing calibration standard was analyzed, then all calibration standards must be re-analyzed. If the standard is the highest or lowest standard (CalS 1b, or CalS 6b), it may be dropped from the calibration as long as there are five (5) consecutive points on the curve. The LOQ may need to be adjusted.



- 14.3.2. If more than one standard appears to be prepared incorrectly, or the calibration curve is erratic, re-prepare all calibration standards and analyze. This may involve re-preparing the working standard solution or opening new stock standard solutions.
 - 14.3.3. If necessary, perform instrument maintenance.
 - 14.3.4. 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 80-120% 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 curve will need to be made.
 - 14.4.4.1. If the curve that failed comes from a freshly prepared SS or WS, one or both may need to be remade.
- 14.5. Failure to meet required QC criteria for Initial Calibration and/or Continuing Calibration Verification standard (CCV) of 80% to 120% recovery for routine sample batches as required in Section 10.3.4 and Section 10.5.1.
- 14.5.1. A new Initial Calibration curve and/or CCV is prepared and re-analyzed; may need to prepare from new working or stock solutions.
 - 14.5.2. If a newly prepared Initial Calibration curve and/or CCV 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 calibration curve is set up on the instrument and verified with a CCR using the new curve.
 - 14.5.3. All samples must be 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 calibration is established. If reanalysis is 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 Lower Limit of quantification (**LLOQ**) of 70% to 130% recovery for routine sample batches as required in **Section 10.3.4** and **Section 10.5.1**.
- 14.6.1.** A new Initial Calibration and/or a standard \leq LLOQ is prepared and re-analyzed; may need to prepare from new working or stock solutions.
- 14.6.2.** If a newly prepared Initial Calibration curve and/or a standard \leq LLOQ 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 calibration curve is setup on the instrument and verified with a **CCR** using the new curve.
- 14.6.3.** A standard \leq LLOQ within 70-130% recovery must be achieved before quantification analysis can begin. If samples were analyzed before an acceptable **LLOQ** was achieved, all affected samples must be re-analyzed after an acceptable **LLOQ** 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 manufacture'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.
- Exception: If the samples do not contain target analytes at or above the **LLOQ**, 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 **LLOQ** 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, request that a new **LRB** be extracted 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.

Exception: If the samples do not contain target analytes at or above the **LLOQ**, 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** 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**.

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 see if it's 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 11.6.**
- 14.11.1.** Compare the **MS** and **MSD** for **IS** Peak Area Deviation (**Section 13.3.1.3**) 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).