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

Division of Environmental Health Sciences
Albany, New York

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

Table of Contents

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), Subpart 55-2.15 and Chapter XIII, Section 1004.14 of the official Compilation of Codes, Rules, and Regulations, of the State of New York.

Table 1. Analyte List

Analyte	CAS Number	LOD[1] (ppb) In Tincture/Oil Matrix	LLOQ[2] (ppb) In Tincture/Oil Matrix
Aflatoxin B1 (AT-B1)	1162-65-8	0.135 / 0.227	0.68 / 1.14
Aflatoxin B2 (AT-B2)	7220-81-7	0.379 / 0.421	1.89 / 2.10
Aflatoxin G1 (AT-G1)	1165-39-5	0.330 / 0.390	5.00 / 5.00
Aflatoxin G2 (AT-G2)	7241-98-7	0.305 / 0.459	5.00 / 5.00
Ochratoxin A (OTA)	303-47-9	0.298 / 0.179	1.49 / 0.89

[1] The Limit of Detection (LOD) is the statistically calculated minimum concentration of an analyte that can be measured with 99% confidence that the value is greater than zero (Section 3.16).
[2] Lower Limit of Quantitation (LLOQ) – The minimum concentration that can be quantitatively reported for a target analyte (Section 3.17).

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 use by or under the supervision of analysts experienced in the use of Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). 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} reverse-phase column in 11 minutes using a programmed gradient of increasing organic modifier. The method limit of detection for the mycotoxins ranges from 0.1 – 0.5 ppb and the quantification ranges from 5.0 – 100 ppb.

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 matrix that is spiked with known quantities of target analytes and extracted/analyzed as a quantifiable sample. The matrix from which the portion to be spiked was taken must be analyzed separately to determine the levels of background analyte concentrations. The MS is corrected for background concentrations and used to determine whether or not the sample matrix contributes bias to the sample results. The MS is also used to determine the accuracy of the methodology in the same way that the LFB does. 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 actual sample 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. 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. Acronym: 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.

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. 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; refs. 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. These guidelines must be made available to all personnel involved in the chemical analyses.

4.4. Lab 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 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 lab 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 in a clean cabinet away from standards and syringes to prevent any 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 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 Lab 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. Instrumentation, Equipment and Supplies

(All specifications are suggested. Catalog numbers are included for illustration only.)

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.

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

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.

6.3.1.1.8. Shimadzu Labsolutions software on a compatible computer system for data collection, 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.

7.0. Reagents and Standards

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 # 3272-02 or equivalent).

7.1.4. Hemp Oil – (Hemp Meds, Real Scientific Hemp Oil, Gold Grade or equivalent).

7.1.5. Ethanol – (200 proof, Pharmco-AAPER, Catalog # 111000200, or equivalent).

7.1.6. Propylene Glycol – (Baker, Catalog # U510-07, or equivalent).

7.1.7. Olive Oil – (Sigma-Aldrich, Catalog # 01514-500mL, 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 and 35 accredited, and NIST traceable, when possible.

7.3.2. The commercial standards listed below in Tables 3 and 4 are an example of those currently used in the laboratory. This is not a fully inclusive list and any substitutions may be made as long as the criteria described above are met. At a minimum, commercial

standards are stored per the manufacturer's recommended 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 B1 (AT-B1)	Supelco	44647-U	20 µg/mL	Methanol
Aflatoxin B2 (AT-B2)	Fluka	34034-2mL-R	0.5 µg/ml	Acetonitrile
Aflatoxin G1 (AT-G1)	Fluka	34032-2mL-R	2.0 µg/ml	Acetonitrile
Aflatoxin G2 (AT-G2)	Fluka	34033-2mL-R	0.5 µg/ml	Acetonitrile
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 Standards

8.1. General Preparation Information

8.1.1. Standards labeled below are for guidance only. These 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 from.

8.1.3. All reagents, solutions and standards must be traceable to stocks and if possible, have NIST-traceable documentation. The preparation method, date of preparation, expiration date, and analyst must be also be traceable in the laboratory documentation.

8.2. Extraction Solvent Solution

8.2.1. Fill a 200 mL volumetric flask with 80 mL HPLC grade water.

8.2.2. Dilute to volume with methanol, invert 7x to mix.

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

8.2.4. 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 1.0 mL volumetric flask 3/4 full with extraction solvent (section 8.2).

8.3.2. 50 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 and invert 7x to mix.

(10 µg/ml) (50 µl) = (X µg/ml) (1000 µl)

X= 0.5 µg/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. Store 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 1.0 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) @ 0.5 µg/mL is added.

8.4.3. 100 µL of ISD @ 0.5µg/mL is added.

8.4.4. Dilute to volume with extraction solvent and invert 7x to mix.

(500 ng/ml) (100 µl) = (X ng/ml) (1000 µl)

X= 50 ng/mL

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

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

8.5. Internal Standard Working Diluent (IWD)

8.5.1. IWD is only used when preparing a calibration curve.

8.5.2. Fill a 5.0 mL volumetric flask 3/4 full with extraction solvent (section 8.2).

8.5.3. 100 µL of ISS @ 50 ng/mL is added.

8.5.4. Dilute to volume with extraction solvent and invert 7x to mix.

(50 ng/ml) (100 µl) = (X ng/ml) (5000 µl)

X= 1 ng/mL

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

8.5.6. Store at -20 °C for up to 12 months, in a sealed vial or ampule.

8.6. Stock Standard (SS) – @100 ng/ml for all five mycotoxins (Table 5).

8.6.1. SS is only used when preparing a calibration curve.

8.6.2. Fill a 1.00 ml volumetric flask volumetric with 400 µL water.

8.6.3. The following volumes of each standard are added as indicated in Table 5.

Table 5: Stock Standard preparation

Mix	Volume Added	Initial Concentration	Standard	Catalog #	Final Concentration
1	5 µl	20,000 ng/mL	Aflatoxin B1 (AT-B1)	44647-U	100 ng/ml
2	200 µl	500 ng/ml	Aflatoxin B2 (AT-B2)	34034-2mL-R	100 ng/ml
3	50 µl	2,000 ng/ml	Aflatoxin G1 (AT-G1)	34032-2mL-R	100 ng/ml
4	200 µl	500 ng/ml	Aflatoxin G2 (AT-G2)	34033-2mL-R	100 ng/ml
5	10 µl	10,000 ng/ml	Ochratoxin A (OTA)	34037-2mL-R	100 ng/ml

8.6.4. Fill to volume with methanol and invert 3X to mix.

8.6.5. This solution is filled and sealed in a 1.5 mL crimp-cap vial and labeled appropriately.

8.6.6. Store at -20 °C for up to 12 months, in a sealed vial or ampule.

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

8.7.1. WS is only used when preparing a calibration curve.

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

8.7.3. Add 200 µL of SS @ 100 ng/mL (Section 8.6).

8.7.4. Dilute to volume with extraction solvent solution and invert 3x to mix.

$$(100 \text{ ng/ml})\ (X \text{ ml}) = (20 \text{ ng/ml})\ (1.00 \text{ ml})$$

$$X = 0.20 \text{ mL} = 200 \text{ µL}$$

8.7.5. The solution is filled and sealed in a 1.5 mL crimp-cap vial and labeled appropriately.

8.7.6. Store at -20 °C for up to 12 months, in a sealed vial or ampule.

8.8. Cross Check Reference Standard Stock (CCR-S) – @ 100 ng/mL for all five mycotoxins (Table 6).

8.8.1. The CCR-S should be from a different source than the SS as described in the definition of the CCR (Section 3.15).

8.8.2. Fill a 1.00 mL volumetric flask with 400 µL water.

8.8.3. The following volumes of each standard are added as indicated in Table 6.

Table 6: Cross Check Reference Standard Stock Preparation

Mix	Volume Added	Initial Concentration	Standard	Catalog #	Final Concentration
1	400 µl	250 ng/mL	Mix 5 (Aflatoxin B1,B2,G1,G2)	002022	100 ng/ml
5	10 µl	10,000 ng/ml	Ochratoxin A (OTA)	002023	100 ng/ml

8.8.4. Fill to volume with methanol and invert 3X to mix.

8.8.5. This solution is filled and sealed in a 1.5 mL crimp-cap vial and labeled appropriately.

8.8.6. Store at -20 °C for up to 12 months, in a sealed vial or ampule.

8.9. Cross Check Reference Working Standard (CCR-WS) – @ 20.0 ng/mL for all five mycotoxins.

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

8.9.2. Add 200 µL of CCR-S @ 100 ng/mL (Section 8.8).

8.9.3. Dilute to volume with extraction solvent solution and invert 3x to mix.

$$(100 \text{ ng/ml})\ (X \text{ ml}) = (20 \text{ ng/ml})\ (1.00 \text{ ml})$$

$$X = 0.20 \text{ mL} = 200 \text{ µL}$$

8.9.4. The solution is filled and sealed in a 1.5 mL crimp-cap vial and labeled with contents, concentration, notebook ID, preparation and expiration dates.

8.9.5. Store at -20 °C for up to 12 months, in a sealed vial or ampule.

8.10. Mobile Phases

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

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

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

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

8.10.1.4. This solution must be prepared monthly.

8.10.2. Mobile phase B: Methanol

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

8.11. Matrices

8.11.1. Olive Oil Matrix (stored in -20 °C for up to 6 months)

8.11.1.1. Weigh 200 ± 20 mg hemp oil extract into 1.5-mL centrifuge tube.

8.11.1.1.1. Hemp oil – Hemp Meds, Real Scientific Hemp Oil, Gold Grade or equivalent.

8.11.1.2. Add 800 ± 80 mg olive oil.

8.11.1.2.1. Olive oil – Sigma-Aldrich, Catalog # 01514-500 mL, or equivalent.

8.11.1.3. Sonicate for 5 minutes.

8.11.1.4. Vortex for 1 minute.

8.11.1.5. Sonicate for an additional 5 minutes.

8.11.2. Tincture Matrix (stored in -20 °C for up to 6 months)

8.11.2.1. Weigh 60 ± 6 mg hemp oil extract into 1.5-mL centrifuge tube.

8.11.2.1.1. Hemp oil – Hemp Meds, Real Scientific Hemp Oil, Gold Grade or equivalent.

8.11.2.2. Add 500 ± 50 mg propylene glycol.

8.11.2.2.1. Propylene glycol – Baker, Catalog # U510-07, or equivalent.

8.11.2.3. Add 440 ± 44 mg ethanol.

8.11.2.3.1. Ethanol, 200 proof – Pharmco-AAPER, Catalog # 111000200, or equivalent.

8.11.2.4. Sonicate for 5 minutes.

8.11.2.5. Vortex for 1 minute.

8.11.2.6. Sonicate for an additional 5 minutes.

9.0. Shipping Conditions, Receiving, Preservation, Analysis and Storage

9.1. Sample shipping conditions:

9.1.1. The medical marijuana products from Registered Organizations (ROs) are shipped as per manufacturer's specifications and must adhere to all regulatory requirements.

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. The storage is documented.

9.3. Preservation:

9.3.1. All samples prepared for analysis should be put in a 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. Analysis samples 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. 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. The current optimal MS/MS conditions are described in Tables 7 and 8.

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 9, analyze a mid-level calibration standard to obtain retention times (Table 10) 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: 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 8: 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

Table 9: 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 10: Analyte Retention Times

Analyte	Average Retention Time (min.)	Accepted 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

10.2. Calibration Curve Preparation (CalS)

10.2.1. Serial dilutions are made from the Working Standard (Section 8.7). 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 11 for a summary of the dilutions. All calibration standards are prepared in extraction solvent solution (Section 8.2). Suggested storage for CalS standards without IWD is -20 °C for up to 6 months, in a sealed vial or ampule.

10.2.2. ***CalS 6a*** (20 ng/ml)

10.2.2.1. CalS 6a is the same as the Working Standard (Section 8.7).

10.2.3. ***CalS 5a*** (6 ng/ml)

10.2.3.1. Pipet 300 µl of Working Standard (Section 8.7) 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 ng/ml) (300 µL) = (X ng/ml) (1000 µl)

X= 6 ng/mL

10.2.4. ***CalS 4a*** (1.8 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 ng/ml) (300 µL) = (X ng/ml) (1000 µl)

X= 1.8 ng/mL

10.2.5. ***CalS 3a*** (0.54 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.

(1.8 ng/ml) (300 µL) = (X ng/ml) (1000 µl)

X= 0.54 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.

(0.54 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 11. – 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	6.0	10.2.3	300 µL	700 µL
CalS 4a	1.8	10.2.4	300 µL	700 µL
CalS 3a	0.54	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 50 µL of each CalS (Section 10.2.2 – 10.2.7) with 50 µL of IWD @ 1.0 ng/mL (Section 8.5) and mixing well. 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 12.

Table 12. – Calibration Curve with Internal Standard

Cal STD w/IWD	Mycotoxin Final Concentration (ng/mL)	Mycotoxin Std Ref Section ID	Volume of Mycotoxin STD without IS	IWD Reference ID	Volume of IWD	IS Final Concentration (ng/mL)
CalS 6b	10.0	10.2.2	50 µL	8.3	50 µL	0.5
CalS 5b	3.0	10.2.3	50 µL	8.3	50 µL	0.5
CalS 4b	0.90	10.2.4	50 µL	8.3	50 µL	0.5
CalS 3b	0.27	10.2.5	50 µL	8.3	50 µL	0.5
CalS 2b	0.081	10.2.6	50 µL	8.3	50 µL	0.5
CalS 1b	0.0243	10.2.7	50 µL	8.3	50 µL	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 absolute IS response in each chromatographic run must not deviate by more than 20% from its average value for each matrix analyzed.

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

10.3.5. 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 is the lowest calibration point, which may be within 70-130% of the true value for each analyte.

10.3.6. 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).

10.4.2. CCR @ 1.0 ng/mL

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 @ 20 ng/mL (Section 8.9).

10.4.2.3. Dilute to volume with extraction solvent.

10.4.2.4. Invert 3X to mix. Storage is at -20 °C for up to 12 months, in a sealed vial or ampule labelled CCR-a.

10.4.2.5. Dilute 50 µL from Section 10.4.2.4, with 50 µL of IWD @ 1.0 ng/mL (Section 8.5) and mix well. Be sure to rinse solution down the sides of the container.

10.4.2.6. Label as CCR-b. Store at -20 °C for up to 12 months in a sealed vial or ampule

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 and average response factors 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 ±30% of the predicted response. The CCVs must be within ± 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 ng/mL, 0.90 ng/mL, 0.27 ng/mL, and 0.081 ng/mL (same as CalS 5b, 4b, 3b and 2b, respectively – (Section 10.2.8, Table 12)

10.5.2.2. **Lower Limit of Quantitation** (LLOQ) 0.0243 ng/mL in extraction solvent (same as CalS 1b) – (Section 102.8, Table 12).

11.0. Quality Control and 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 method 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 ± 20%, except within 25% of the LLOQ, 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 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 ± 20%, except within 25% of the LLOQ, 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 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 of a blind sample, such as an external proficiency test (PT) sample.

11.1.1.2.2. Internally prepared PT sample in which all analytes concentration have been accurately measured.

11.1.1.2.3. Acceptable performance of an initial DOC as described above in section 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 CCR 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 1.0 ppb sample. The fortified samples must be treated as if they were real samples and processed through all of the applicable method procedures (Section 12.0). 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, but it is not a requirement.

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

LOD = SD of n 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 13)

Table 13. 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 analysis, a vial of extraction solvent (Section 8.2) will be injected.

11.3.2. Background contamination found, which could interfere with the measurement of target analytes, must be < 1/3 the LLOQ for routine samples.

11.3.3. Background contamination is defined as a peak with a signal greater than (>) 3 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 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 (LRB) 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 ppb 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 5.0 ppb.

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 within 25% of the 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).

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. Spike 0.5 ng (10 µL, 50 ng/mL) ISS (Section 8.4).

12.1.3. Add 1000 µL extraction solvent (Section 8.2).

12.1.4. Sonicate for 15 minutes.

12.1.5. Vortex for 30 seconds.

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 8.11) directly into a tared 1.5–mL centrifuge tube.

12.2.2. Spike 0.5 ng (25.0 µL, 20 ng/mL) WS (Section 8.7).

12.2.3. Spike 0.5 ng (10 µL, 50 ng/mL) ISS (Section 8.4).

12.2.4. Add 1000 µL extraction solvent (Section 8.2).

12.2.5. Sonicate for 15 minutes.

12.2.6. Vortex for 30 seconds.

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.

12.3. 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.

12.4. 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 12.2.9.

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.

12.6. 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 Section 10.1.4, Table 9.

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, then 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 14 lists an example of a full 20-sample batch, with a full calibration curve.

Table 14: 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 11.3).
2	SBLK	
3	SBLK	
4	LRB	
5	CalS-1b	This example uses a full calibration curve. If a full calibration curve is not needed (see Section 12.6.5), only the LLOQ (CalS-1b) and a CCV (CalS-2b, 3b, 4b, or 5b) is needed.
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-2b – 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 15 and Table 16 list the ideal 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 15: 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	0	0	0	0	0
Base Sub Window (min)	0.2	0.2	0.2	0.2	0.2
Report Largest Peak	No	No	No	No	No

Table 16: 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	0	0	0	0	0
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 16 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 15. 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 16 are from a representative injection, and do not represent required retention times.

13.0. Data Acquisition, Reduction, Analysis, and Calculations

13.1. HPLC

13.1.1. Table 9 (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 ^{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 10 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 17.

Table 17: 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 15 and 16).

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.

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

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. Calculate the average IS peak area for each matrix (solvent, tincture, olive oil). Evaluate the system stability by using the following equation on every injection, and comparing to the criteria set in Section 10.3.

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

Where: IS_I = IS peak area for individual injection
IS_A = IS peak area average for given matrix

13.3.1.4. Using the weighted 1/X linear regression curve for all calibration standards, check the curve linearity and calculate the standards recovery at each levels. 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 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 real medical marijuana samples, this value must then be converted to a sample concentration in ng/g using the following equation:

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

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. 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 at or above the LLOQ and at or below the ULOQ are reported using 2 significant figures.

13.4.3. Analytes detected at a concentration at or above the LOD and below the LLOQ are reported as less than (<) the LLOQ as specified in Section 1.1.

13.4.4. Analytes detected at a concentration below the LOD 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.

13.4.5. 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 ppb) 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 10.3.4.

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.

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 10.3.4), calibration curve response (Section 10.3.5), 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 10.3.5 and Section 10.5.1, respectively.

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 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 Lower Limit of quantification (LLOQ) of 70% to 130% recovery for routine sample batches as required in Section 10.3.5 and Section 10.5.1, respectively.

14.6.1. A new Initial Calibration and/or CalS-1b sample 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 CalS-1b sample 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 CalS-1b sample 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 of < 1/3 the LLOQ for target analyte(s) in routine sample batches.

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 11.7.3.

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 11.7.4**.**

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 10.3.

14.12.1. Check if there is a sample preparation error.

14.12.2. Check if there is an interference peak co-eluting with IS.

14.12.3. If IS variation is higher than 20%, problem investigation must be performed until this problem is solved. Otherwise, the data should not be reported as a qualified data. Samples need to be reanalyzed and reported.

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 water 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

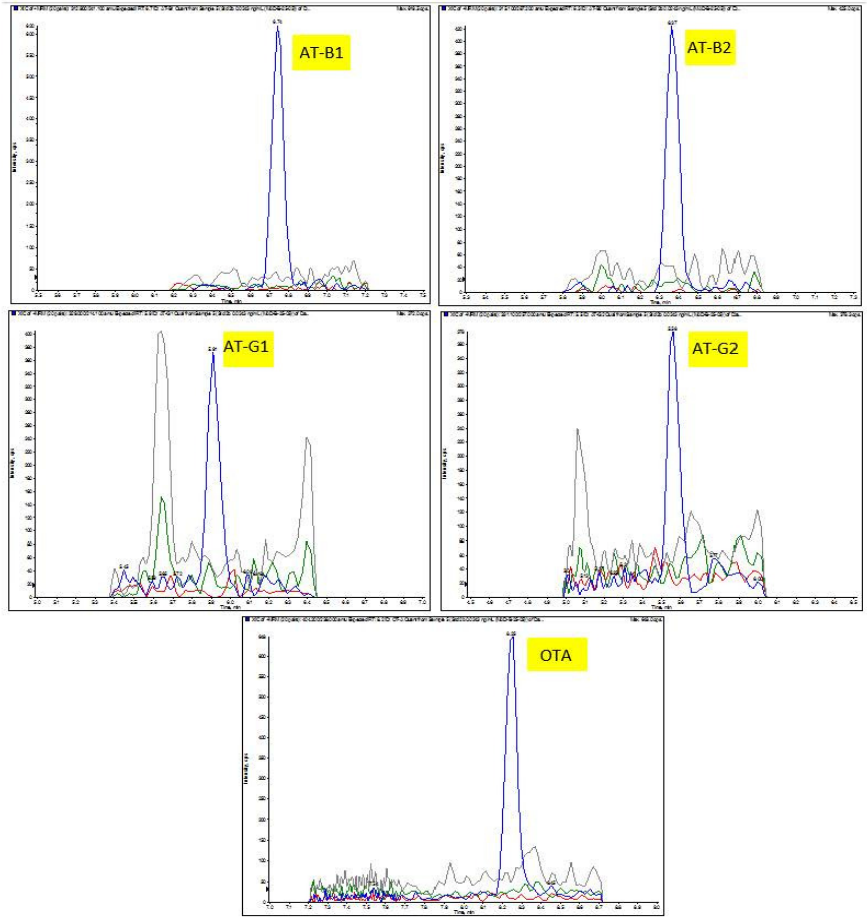
17.5. Shimadzu HPLC Module Manuals http://store.shimadzu.com/s-1003-liquid-chromatography.aspx?pagenum=1

18.0. Supporting Documents

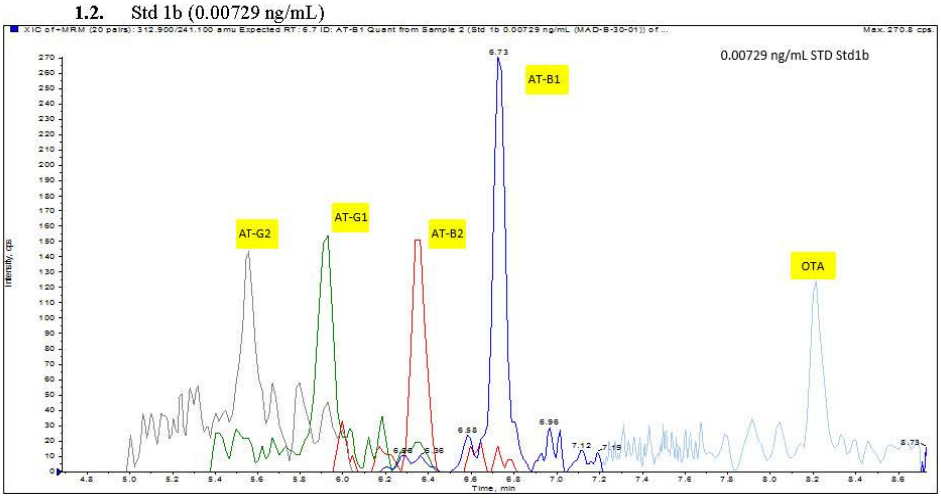
18.1. Appendix A – Tables and figures

1.0. Example Chromatography

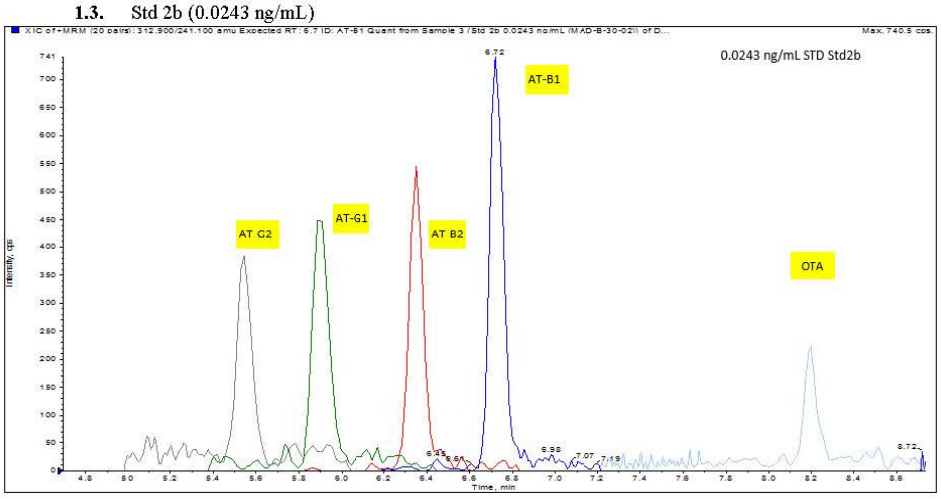
1.1. Individual mycotoxin MRM transitions overlaying 0.0243 ng/mL standard 2b (blue), system blank (red), olive oil matrix blank (grey), and tincture matrix blank (green). Notice that the matrix peaks in aflatoxin G1 and G2 do not interfere with the analyte peak and are outside of the expected retention time window.



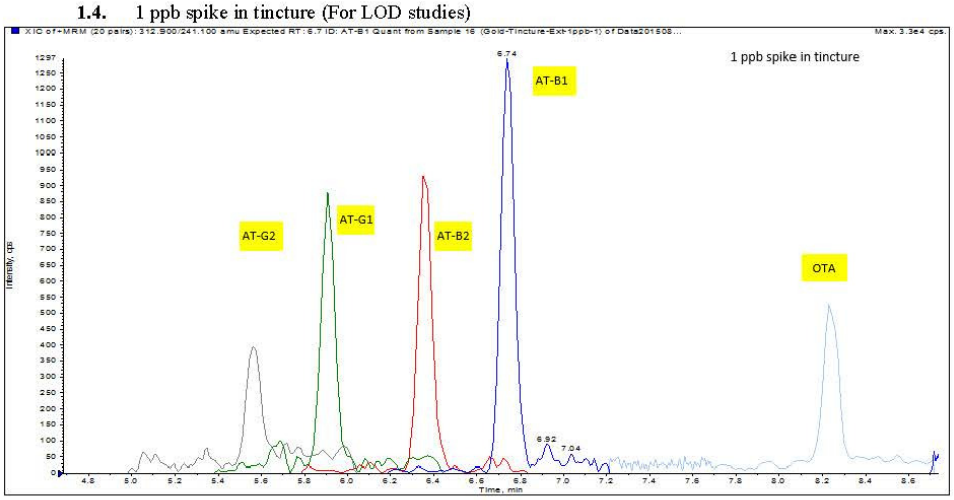
1.2. Std 1b (0.00729 ng/mL)



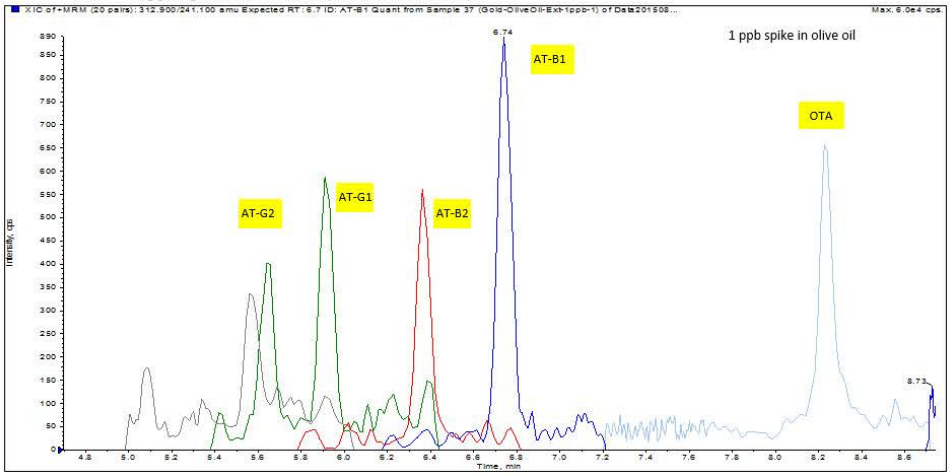
1.3. Std 2b (0.0243 ng/mL)



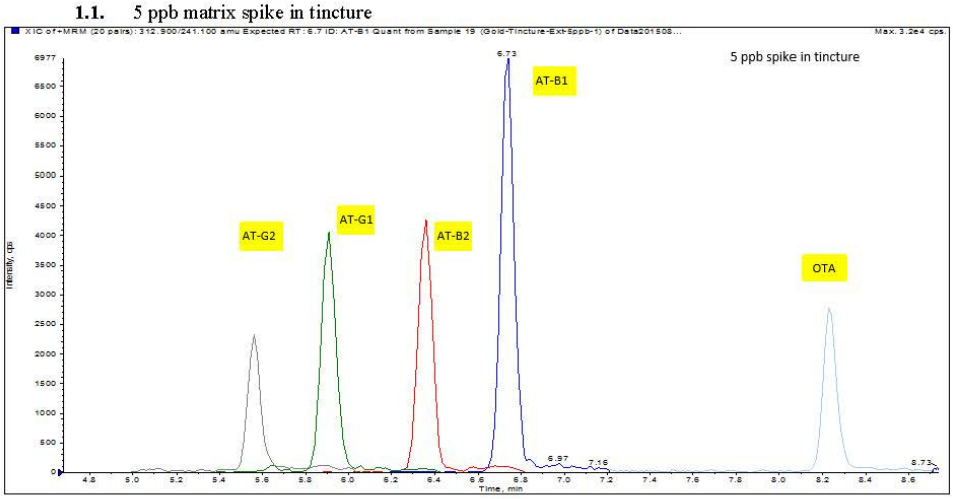
1.4. 1 ppb spike in tincture (For LOD studies)



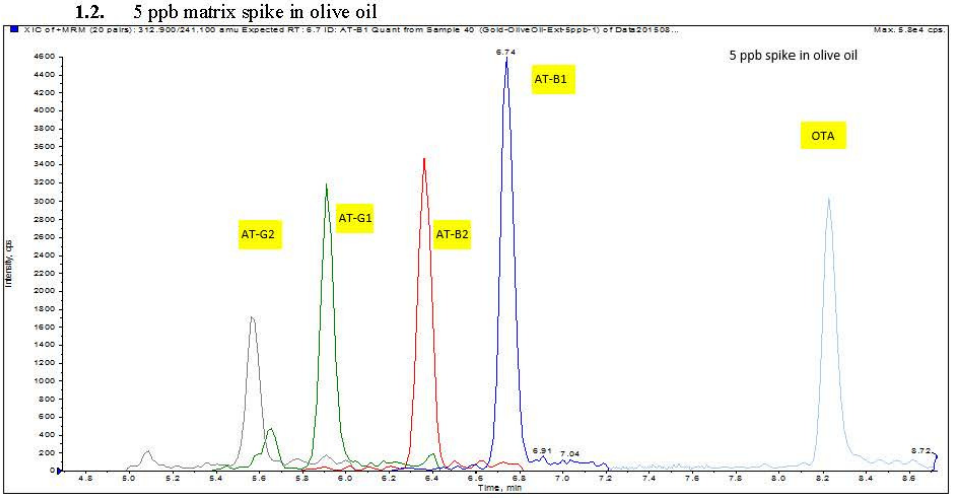
1.5. 1 ppb spike in olive oil (For LOD studies)



1.1. 5 ppb matrix spike in tincture



1.2. 5 ppb matrix spike in olive oil



2.0. Example Analysis Parameters

2.1. MS/MS Non-specific parameters:

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

2.2. MS/MS Analyte-specific parameters.

Quantifying product ions and CE are bold, qualifying product ions and CE are in (parenthesis).

Analytes	**Polarity**	**Precursor ion**	**Product ion**	**Collision energy (eV)**	**Scheduled MRM collection 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

2.3. 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 (MPA):	15 mM Ammonium Formate in H_2O
Mobile Phase B (MPB):	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**
Initial:	20%
1.0 min:	50%
11.0 min:	90%
11.1 min:	95%
20.0 min:	95%
20.1 min:	20%
25.0 min:	20% (End of Run)

3.0. Example Integration / Quantitation Parameters

3.1. 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	0	0	0	0	0
Base Sub Window (min)	0.2	0.2	0.2	0.2	0.2
Report Largest Peak	No	No	No	No	No

3.2. 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	0	0	0	0	0
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