United States Department of Agriculture

Food Safety and Inspection Service

CLG-TOX2.00

Screening of Toxins by LC-MS/MS

This method describes the laboratory procedure for extracting toxic chemicals from samples followed by analysis using liquid chromatography and quadrupole mass spectroscopy at concentrations higher than 2 ppm.

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Safety Precautions

The personnel performing the analysis are to read the Safety Data Sheets for the standards and reagents used in this method. Follow all applicable federal, state, and local regulations regarding the disposal of chemicals listed in this method.

Introduction

Plants and other organisms produce chemicals, toxins, to aid in their defense and survival. These toxins provide protection by adversely affecting an animal that is attempting to consume the plant or organism. The concentration of toxins in a plant or organism can lead to a range of effects. Humans can use toxins produced by plants and organisms to treat ailments by controlling the concentration of a toxin or by chemically modifying the compound to achieve a desired result. The presence of certain chemicals at elevated concentrations is of concern due to *sola dosis facit venenum,* the dose makes the poison, a principle of toxicology. Certain chemical residues may be present at low levels in products due to acceptable usage in the raising of livestock. However, those same chemical residues at higher concentrations may be acutely toxic and can cause adverse effects after a single exposure. The following multi-residue method is used by FSIS to test for toxins in FSIS-regulated processed products on a regular basis as deemed necessary by the agency.

Method Overview

The following method describes the laboratory procedure for screening toxin residues in processed products. This method is used to screen for 35 toxic compounds from the alkaloid, carbamate, opioid, and organophosphate classes at 2 ppm.

In brief, toxin residues are extracted from tissue using acetonitrile. The acetonitrile extract is then diluted with water and filtered. The extracted samples are then analyzed using Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS).

Decision Criteria

A sample is considered negative if the results are less the than the Minimum Level of Applicability (MLA). A sample is considered a screened positive if the results are greater than or equal to the MLA and meets the ion ratio criteria. Screened positive results will require further analysis through additional methods.

KEY DEFINITIONS

Toxin: A naturally occurring organic poison produced by metabolic activities of living cells or organisms.

LC-MS/MS: An analytical technique where there is a physical separation of target compounds followed by their mass-based detection.

MLA: Lowest level at which an FSIS method has been successfully validated for a residue in each matrix.

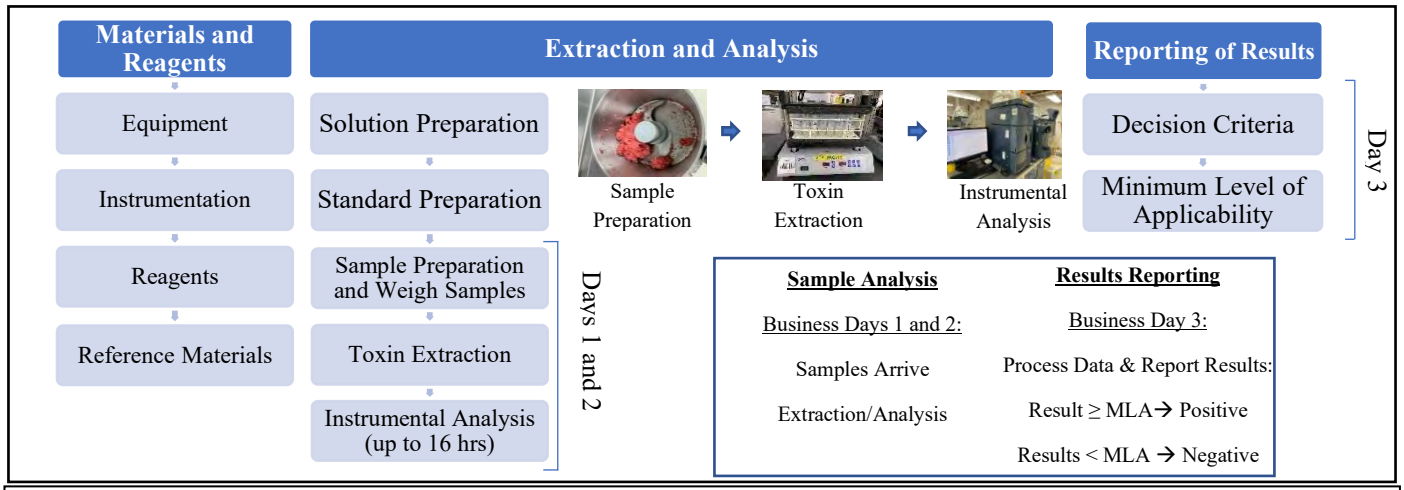

Figure 1: Overview and timeframe of Toxins by LC-MS/MS (TOX2). Materials and reagents are obtained and utilized to prepare solutions and standards. The samples arrive at the laboratory, are prepared into a homogenized mixture, weighed, extracted, and analyzed by LC-MS/MS on business days 1-2. Screening results are reported on business day 3.

Disclosure Statement

FSIS does not specifically endorse any test products listed in this method. FSIS acknowledges that equivalent equipment, reagents, or solutions may be suitable for laboratory use. The FSIS laboratory system uses method performance requirements when evaluating the equivalence of an alternative equipment, reagent, or solution for a given analyte and sample matrix pair. Significant equivalence changes would require FSIS laboratory leadership approval.

Materials and Reagents

Equipment

Table 1: Equipment Required to Perform CLG-TOX2

Equipment	Supplier and Part Number	Purpose
Cutting board and knives	General lab supplier	Preparation of sample
Food Processor	General lab supplier	Homogenizing the sample
Analytical Balance	General lab supplier	Record weight of quality controls and samples Minimum accuracy ±0.1g.
Vortex Mixer	General lab supplier	Facilitates extraction of residue from the sample
Centrifuge	General lab supplier	Separates the solid sample material from the extraction solution.
0.2 µm PVDF filter disk	Xpertek, 9474051	Removes any particulate material from the extraction solution to prevent damage to the liquid chromatograph
3 mL Luer-Lok™ Syringe	Becton Dickenson, 309657	Attaches to the syringe filter to enable filtration
Repeating pipettes and tips, 100 µL, 500 µL, 10 mL	General lab supplier	Prepare standards and spike quality control samples
Glassware, Class A	General lab supplier	Facilitates extraction of residue from the sample
Refrigerator, 2-8°C	General lab supplier	Storage of samples prior to analysis
Freezer, -20°C	General lab supplier	Storage of standards and reagents

Instrumentation

Table 2: Instrumentation

Instrument	Supplier and Model Number	Purpose
Waters UPLC-MS/MS System	Waters Xevo I-Class LC, Waters Xevo TQ-S micro Mass Spectrometer	Sample extract analysis
Zorbax SB C-18, 2.1 × 150 mm, 1.8 µm	Agilent, 859700-902	Sample extract analysis

Reagents

Table 3: Reagents

Reagent	Supplier and Part Number
Acetonitrile (ACN), LC-MS Grade	General lab supplier
Water (H_2O), Resistivity of > 18MΩ-cm	House deionized water system
Formic acid, LC-MS Grade	General lab supplier

Reference Materials

Table 4: Reference Materials

Standard	Supplier	Catalog Number
Combined Standard, 100µg/mL	o2si smart solutions	G34-140053-03

Purity and counterions are to be taken into account when calculating standard concentrations. In-house prepared standards are to be assigned an expiration date that is no later than the stability stated in the method.

Extraction and Analysis

Solution Preparation

Table 5: Preparation of Solutions

Solution	Procedure
Mobile phase A (0.1% formic acid in water) Solution expires after 1 year. Store at room temperature.	1) Using a repeating pipette add 1.0 mL of formic acid to a 1 L volumetric flask. 2) Dilute to volume with house deionized water.
Mobile phase B (0.1% formic acid in acetonitrile) Solution expires after 1 year. Store at room temperature.	1) Using a repeating pipette add 1.0 mL of formic acid to a 1 L volumetric flask. 2) Dilute to volume with LC-MS grade acetonitrile.

Standard Preparation

Table 6: Stock and Intermediate Stock Standard Solutions

Mixed Intermediate Spiking Standard (20µg/mL in acetonitrile) Solution expires after 1 year. Store at ≤ - 20°C.	1) Using a repeating pipette add 1000 µL of the commercial 100 µg/mL Combined Standard to a 5 mL volumetric flask. 2) Dilute to volume with acetonitrile.
Working Standard (2µg/mL in acetonitrile) Solution expires after 1 year. Store at ≤ - 20°C.	1) Using a repeating pipette add 500 µL of the Mixed Intermediate Spiking Standard to a 5 mL volumetric flask. 2) Dilute to volume with acetonitrile.

Sample Preparation

Samples must be kept cold before and during shipping to the laboratory. Once received at the laboratory, samples must be refrigerated (2-8°C) prior to grinding if they cannot be prepared on the day of receipt. As shown in Figure 2, trim away fat and connective tissue from the sample. As shown in Figure 3, grind sample in blender or vertical cutter-mixer until homogeneous. Store homogenized samples refrigerated (2-8°C) prior to analysis.

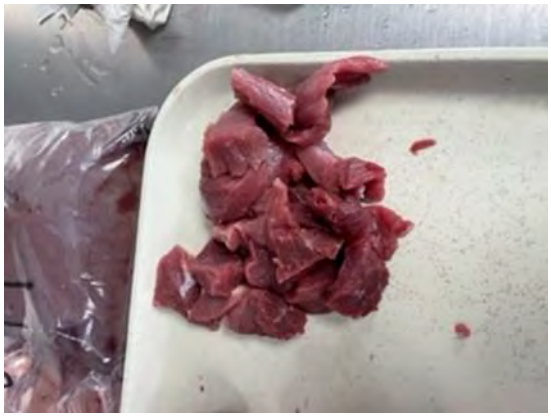

Figure 2: Prepared lean muscle sample with connective tissue removed. Photo courtesy of Hue Quach, USDA FSIS.

Figure 3: Homogenized sample. Photo courtesy of Hue Quach, USDA FSIS

Toxin Extraction

Samples

Weigh 10 ± 0.1 g of homogenized sample into labeled 50 mL polypropylene centrifuge tubes.

QUALITY CONTROL

Weigh portions of blank tissue into 50 mL polypropylene centrifuge tubes as follows (see Figure 4).

1. One blank (negative control), 10 ± 0.1 g
2. One recovery (positive control), 1 ± 0.1 g

Weigh one additional 1 ± 0.1 g portion for an intra-laboratory check sample, if necessary.

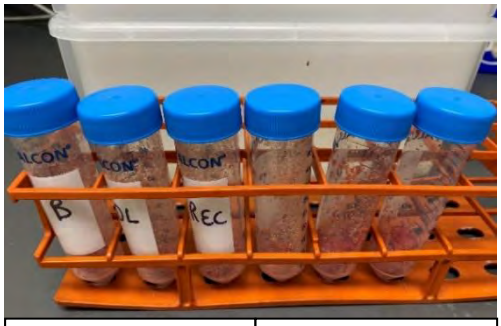

Figure 4: Weighed controls and samples. Photo courtesy of Ryan Matsuda, USDA-FSIS

KEY DEFINITIONS:

Negative control (Blank): Sample negative of all analytes. No spiking solutions are added to this sample.

Recovery (positive control): Sample is prepared with addition of analytes that have a concentration level comparable to MLA. Samples are compared to recovery.

Extraction

1) Prepare positive control (recovery) by fortifying sample with 100 µL of the 20 µg/mL Mixed Intermediate Spiking Standard.
2) Add 10 mL of acetonitrile to the negative control (blank) and all samples.
3) Add 9.90 mL of acetonitrile to the positive control (recovery) and the check sample, if present.
4) As shown in Figure 5, mix samples vigorously for 1 minute (min).
5) Centrifuge samples at 2000 x g for 10 min.
6) Check samples to determine if two liquid layers, acetonitrile and aqueous, have formed due to moisture in the sample. If separation occurs, repeat steps 4 and 5.
7) Use a pipette to remove a 500 µL aliquot of acetonitrile from the sample tube and pipet the aliquot into a syringe with PVDF filter attached.

Figure 5: Extraction of toxins from samples. Photo courtesy of Ryan Matsuda, USDA-FSIS

8) Add 500 µL of LC grade water to the syringe containing the sample extract from step 7.
9) Filter the combined acetonitrile/water mixture from step 8 through the 0.2 µm PVDF filter directly into an autosampler vial.

Figure 6: Filtration of final extracts.

Technical Note:

A lower mass of sample is used for the positive control to reduce the volume of the Mixed Intermediate Spiking Standard needed to achieve a 2 ppm concentration.

Instrumental Analysis

An example of a sample tray for an LC-MS/MS system and an example of an LC-MS/MS instrument are shown in Figure 7 and Figure 8, respectively.

Chromatographic Parameters

1) Mobile phases for TOX2 analysis
    a) Mobile Phase A – 0.1% (v/v) formic acid in water
    b) Mobile Phase B – 0.1% (v/v) formic acid in acetonitrile
2) Flow rate: 0.2 mL/min
3) Run time: 32.00 min
4) Gradient Program

Table 7: LC Gradient Program

Time (min)	% Mobile Phase A	% Mobile Phase B	Gradient
0.00	95	5	none
15.0	65	35	linear
20.0	5	95	linear
25.0	5	95	none
25.1	95	5	linear
32.0	95	5	none

5) Autosampler program
    a) Run time: 32.0 min
    b) Injection loop: 10 µL
    c) Sample injection mode: Partial loop needle overfill
    d) Injection Volume: 2 µL
    e) Strong wash solvent: 1:1 methanol:water
    f) Strong wash volume: 500 µL
    g) Sample temperature: 15°C

Instrumental Note:

Autosampler parameters can be modified or optimized to ensure that all chromatographic peaks are present.

6) Column manager

   a) Column valve position: To match column location

   b) Column manager temperature: 40°C

   c) Use divert valve to divert eluant to waste 0.25 min prior to first peak and 0.25 min after last analyte peak.

Mass Spectrometry Parameters

1) Type: MS/MS

   a) Ion Mode: ES+

2) Electrospray Source Parameters

   a) Capillary (kV): 2.00

   b) Cone (V): Variable - analyte dependent

   c) Source Temperature (°C): 150

   d) Desolvation Temperature (°C): 350

   e) Cone Gas Flow (L/hr): 40

   f) Desolvation Gas Flow (L/hr): 1000

   g) Collision Gas Flow (mL/min): 0.20

3) MS Method Parameters:

   a) Type: MRM

   b) Ion Mode: ES+

   c) Dwell (s): Auto Dwell

   d) Start time (min): 0.8

   e) End time (min): 2.6

   f) MRM Transitions

Instrumental Note:

Mass spectrometer analyzer parameters are optimized and adjusted during annual preventative maintenance and calibration.

Hardware Note:

This method is also validated using an AB SCIEX LC-MS/MS. Those MS parameters are listed beginning on page 14.

Figure 8: LC-MS/MS instrument.
Photo courtesy of Ryan Matsuda, USDA-FSIS

Table 8: MRM Transitions

Retention Time (min)	Compound	Cone (V)	Precursor ion *(m/z)*	Quant ion *(m/z)*	Collision Energy (V)	Qual ion *(m/z)*	Collision Energy (V)
18.11	Aconitine	42	646.2	526.1	36	368.1	42
15.15	Aldicarb	30	213.0	88.9	16	97.9	8
6.99	Aldicarb sulfone	15	223.1	86.0	15	148.0	8
5.50	Aldicarb-sulfoxide	10	207.1	89.0	10	132.0	10
7.71	Apomorphine	54	268.0	190.9	28	236.9	12
8.30	Atropine	72	290.0	123.9	20	76.9	52
15.15	Berberine	30	336.1	321.1	14	292.7	44
21.71	Brodifacoum	38	523.0	334.9	18	291.0	34
8.20	Brucine	60	395.1	243.9	34	324.0	28
18.45	Carbaryl	22	202.1	145.0	10	127.0	28
6.10	Codeine	30	300.0	152.1	56	164.9	38
14.68	Colchicine	26	400.2	358.0	25	152.1	76

Retention Time (min)	Compound	Cone (V)	Precursor ion *(m/z)*	Quant ion *(m/z)*	Collision Energy (V)	Qual ion *(m/z)*	Collision Energy (V)
20.88	Coumaphos	22	363.0	226.8	24	306.8	16
12.28	Digoxigenin	42	391.1	355.1	12	337.1	16
18.77	Digitoxigenin	44	375.1	339.0	12	90.9	68
7.99	Emetine	74	481.2	246.0	30	230.0	52
7.39	Eserine	38	276.0	218.9	8	161.9	24
8.61	Ethiofencarb	10	226.1	107.0	20	168.8	16
20.05	Fenamiphos	52	304.2	217.0	22	234.0	12
15.62	Fenamiphos sulfoxide	66	320.0	107.8	42	232.8	24
10.71	Heroin	32	370.2	165.1	46	268.1	24
10.78	Hydrastine	34	384.0	322.9	20	189.9	18
6.97	Hydrocodone	32	300.0	198.9	26	127.8	58
8.30	Hyoscyamine	54	290.2	124.0	20	260.1	20
3.40	Methamidophos	42	141.9	125.0	13	93.9	24
10.51	Methiocarb	30	242.1	185.0	10	122.0	10
7.50	Oxamyl	28	242.1	185.0	10	121.0	8
6.46	Oxycodone	26	316.1	241.0	28	256.0	22
13.19	Pentazocine	60	286.0	68.9	22	217.9	16
11.60	Picrotin	44	311.0	114.9	70	165.0	30
5.99	Ricinine	54	165.0	137.8	15	163.0	15
6.48	Scopolamine	28	304.0	137.9	20	156.0	16
14.45	Solanine	72	868.5	398.2	70	706.3	66
7.95	Strychnine	66	335.1	183.9	32	155.9	42
11.20	Yohimbine	72	355.1	143.8	28	211.9	20

Instrumental Note:

Retention time windows and collision energies were set and utilized at time of method validation.

- Retention time windows may be adjusted to account for aging of LC columns or for improved separation to ensure that all chromatographic peaks are present.
- Collision energies may be adjusted and optimized for improved mass spectrometry detection.
- Target masses for precursor and product ions can be optimized to a *m/z* value that falls within the unit mass resolution of the exact mass, but not to exceed the next integer value (e.g., if the exact mass is 787.5, an allowable target mass range includes 787.0-787.9).

Sample Set

The injection sequence below can be modified, as needed, but must include required controls.

1) System Suitability
2) Working Standard
3) Positive control (Recovery)
4) Negative Control (Matrix Blank)
5) Intra-laboratory check sample (if applicable)
6) Up to 20 Samples
7) Working standard or positive control

INTRA-LABORATORY CHECK SAMPLE

Defined on the CLG website here.

Reporting of Results

Decision Criteria

Screening

1) Quality Control Sample Criteria
   a) A Quant and Qual ion peak, listed in Table 8, must be present and have a signal-to-noise ratio ≥ 3. This may be verified by visual inspection.
   b) The retention time must match the reference standard retention time within ±0.25 minutes.
   c) Blank area must be less than 10% of the reference standard.
   d) Positive control areas must be greater than 10% of the reference standard.
   e) The ratio of the Qual ion to the Quant ion must meet the criteria listed below in Table 9.

Table 9: Ion ratio requirements

Relative Intensity (% of quant peak)	LC-MS^N
>50%	±20%
>20%-50%	±25%
>10%-20%	±30%
<10%	±50%

Example Ion Ratio Calculation:

Ion ratio of External Standard: 0.60

Criteria: ±20%

0.60 x 0.20 = 0.12

Upper limit: 0.60 + 0.12 = 0.72

Lower Limit: 0.60 – 0.12 = 0.48

2) Sample Criteria
   a) A Quant and Qual ion peak, listed in Table 8, must be present and have a signal-to-noise ratio ≥ 3. This may be verified by visual inspection.
   b) The retention time must match the positive control retention time within ±0.25 min.
   c) The area of the quantitation ion is greater than the area in the positive control.
   d) The ratio of Qual ion to the Quant ion must meet the criteria listed above in Table 9.

QUALITY ASSURANCE PLAN

Quality Control Procedures

1) For set acceptance, ≥95% (34/35) of the analytes in the fortified recovery (positive control) must meet screening criteria.
2) The blank (negative control) must be negative for ≥95% (34/35) using screening criteria.
3) Samples are to be reanalyzed if either of the above criteria is not achieved.

Intra-Laboratory Check Samples (If applicable)

1) Acceptability criteria.
    a. All analytes in a fortified Intra-Laboratory Check must meet screening criteria.
    b. All analytes in an unfortified Intra-Laboratory Check must be negative using the screening criteria.
    c. FSIS Field Service Laboratories are to refer to internal FSIS Quality Control Procedures, if unacceptable values are obtained:
        i. Refer to LW-Q1002, Chemistry Non-Conformance Tables, for how to proceed and whether to take corrections or corrective actions.

Minimum Level of Applicability

Table 10: Minimum Level of Applicability (MLA) for Screening level per species

Analyte	MLA (ppm)	Analyte	MLA (ppm)	Analyte	MLA (ppm)
Aconitine	2	Coumaphos	2	Methamidophos	2
Aldicarb	2	Digoxigenin	2	Methiocarb	2
Aldicarb sulfone	2	Digitoxigenin	2	Oxamyl	2
Aldicarb-sulfoxide	2	Emetine	2	Oxycodone HCl	2
Apomorphine- HCl-½ H_2O	2	Eserine	2	Pentazocine	2
Atropine	2	Ethiofencarb	2	Picrotin	2
Berberine	2	Fenamiphos	2	Ricinine	2
Brodifacoum	2	Fenamiphos sulfoxide	2	Scopolamine	2
Brucine	2	Heroin	2	Solanine	2
Carbaryl	2	Hydrastine	2	Strychnine	2
Codeine	2	Hydrocodone	2	Yohimbine HCl	2
Colchicine	2	Hyoscyamine	2		

References

FSIS CLG-TOX1 Method
https://www.fsis.usda.gov/sites/default/files/media_file/2020-09/CLG-TOX1.pdf
United States National Library of Medicine, NIH website ChemIDplus
https://chem.nlm.nih.gov/chemidplus/
Guidelines for the Validation of Chemical Methods for the FDA Foods Program, April 2015
http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM298730.pdf
USDA National Nutrient Database for Standard Reference
http://ndb.nal.usda.gov/

Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the "Ask USDA" portal at https://ask.usda.gov or please contact:

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AB SCIEX Mass Spectrometer Parameters

Mass Spectrometry Parameters

1) Type: MS/MS
    a) Ion Mode: ES+
2) Electrospray Source Parameters
    a) Curtain Gas: 30
    b) Collision Gas: 9
    c) Ion Spray Voltage (V): 4500
    d) Temperature (°C): 500
    e) Ion Source Gas 1: 40
    f) Ion Source Gas 2: 40
3) Injection Control
    a) Full MS: 14
    b) SIM: 14
    c) MSn: 14
    d) Zoom: 3000
4) Acquisition Parameters
    a) Segment windows (sec): 275 – may be narrowed once retention times are established
    b) Scan Cycle (sec): 2
    c) Duration (min): 26
    d) Setting time (ms): 50
    e) Pause between mass ranges (ms): 5.007

Hardware Note:

This method is also validated using a Waters LC-MS/MS. Those MS parameters are listed beginning on page 9.

Table 11: AB SCIEX MRM transitions

Product ions are listed with the Quant ion as *analyte-1* with the Qual ion listed as *analye-2*

Name	Retention Time (min)	Precursor Ion *(m/z)*	Fragment Ion *(m/z)*	Declustering Potential (V)	Collision Energy (V)	Cell Exit Potential (V)
Aconitine-1	17.00	646.1	526.1	180	55	10
Aconitine-2		646.1	368.2	180	93	16
Aldicarb sulfone-1	15.56	222.9	89.0	51	23	8

Name	Retention Time (min)	Precursor Ion *(m/z)*	Fragment Ion *(m/z)*	Declustering Potential (V)	Collision Energy (V)	Cell Exit Potential (V)
Aldicarb sulfone-2		222.9	59.0	51	23	8
Aldicarb-1	8.84	212.9	86.0	90	21	8
Aldicarb-2		212.9	76.0	90	13	6
Aldicarb-sulfoxide-1	7.21	206.9	132.0	80	9	10
Aldicarb-sulfoxide-2		206.9	89.0	80	19	8
Apomorphine-1	8.86	268.0	191.0	81	41	14
Apomorphine-2		268.0	236.9	81	23	16
Atropine-1	9.27	290.0	124.0	180	33	10
Atropine-2		290.0	77.1	180	87	6
Berberine-1	14.4	335.9	320.0	180	41	12
Berberine-2		335.9	291.9	180	41	10
Brodifacoum-1	22.69	522.8	80.8	-190	-86	-5
Brodifacoum-2		520.9	78.9	-190	-98	-5
Brucine-1	9.39	395.1	324.0	180	43	16
Brucine-2		395.1	243.9	180	49	18
Carbaryl-1	18.48	201.9	145.1	85	15	10
Carbaryl-2		201.9	127.0	85	37	10
Codeine-1	6.87	300.0	152.0	160	87	12
Codeine-2		300.0	165.0	160	57	12
Colchicine-1	14.98	400.0	358.1	160	31	12
Colchicine-2		400.0	152.1	160	111	10
Coumaphos-1	20.83	362.9	226.9	125	33	14
Coumaphos-2		362.9	306.8	125	25	22
Digitoxigenin-1	13.23	375.1	355.2	91	19	12
Digitoxigenin-2		375.1	373.2	91	15	10
Digoxigenin-1	18.8	391.1	329.2	96	10	19
Digoxigenin-2		391.1	91.0	96	89	10
Emetine-1	8.93	481.1	246.0	140	47	20
Emetine-2		481.1	229.9	140	71	16

Name	Retention Time (min)	Precursor Ion *(m/z)*	Fragment Ion *(m/z)*	Declustering Potential (V)	Collision Energy (V)	Cell Exit Potential (V)
Eserine-1	8.64	276.0	219.0	110	17	14
Eserine-2		276.0	162.1	56	29	16
Ethiofencarb-1	18.74	225.9	107.0	46	29	8
Ethiofencarb-2		225.9	77.0	46	59	6
Fenamiphos sulfoxide-1	19.77	319.9	216.9	170	33	14
Fenamiphos sulfoxide-2		319.9	234.1	170	23	12
Fenamiphos-1	15.69	303.9	232.8	150	33	10
Fenamiphos-2		303.9	108.1	150	55	8
Heroin-1	11.09	370.0	152.0	150	97	12
Heroin-2		370.0	165.0	150	67	8
Hydrastine-1	11.33	384.0	323.0	160	31	10
Hydrastine-2		384.0	190.0	160	31	16
Hydrocodone-1	8.15	300.0	199.1	190	39	10
Hydrocodone-2		300.0	128.1	190	75	10
Hyoscyamine-1	9.27	290.0	124.0	180	33	10
Hyoscyamine-2		290.0	77.1	180	87	6
Methamidophos-1	3.97	141.9	94.0	100	21	10
Methamidophos-2		141.9	125.0	100	19	12
Methiocarb-1	12.00	241.9	185.0	56	19	8
Methiocarb-2		241.9	122.1	56	39	10
Oxamyl-1	8.84	241.9	224.9	46	11	14
Oxamyl-2		241.9	86.0	46	27	8
Oxycodone-1	7.50	316.0	298.1	150	27	8
Oxycodone-2		316.0	240.9	150	39	16
Pentazocine-1	12.85	286.0	218.0	190	29	10
Pentazocine-2		286.0	69.0	190	41	8
Picrotin-1	14.97	310.9	165.0	191	69	14

Name	Retention Time (min)	Precursor Ion *(m/z)*	Fragment Ion *(m/z)*	Declustering Potential (V)	Collision Energy (V)	Cell Exit Potential (V)
Picrotin-2		310.9	115.0	191	93	14
Ricinine-1	11.17	164.9	163.0	226	55	16
Ricinine-2		164.9	115.0	226	51	10
Scopolamine-1	7.58	304.0	138.1	130	27	12
Scopolamine-2		304.0	156.0	130	23	12
Solanine-1	13.85	868.2	398.2	180	93	16
Solanine-2		868.2	722.3	180	93	20
Strychnine-1	9.01	335.0	184.1	50	51	10
Strychnine-2		335.0	156.0	50	61	8
Yohimbine-1	11.42	355.0	115.1	180	103	10
Yohimbine-2		355.0	117.0	180	75	10

This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.

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