Aug 13, 2024

Analysis of sodium monofluoracetate (compound 1080) in animal kidney tissue by LC-MS/MS

DOI
dx.doi.org/10.17504/protocols.io.e6nvwdk57lmk/v1

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.e6nvwdk57lmk/v1

Protocol Citation: Jim Langston 2024. Analysis of sodium monofluoracetate (compound 1080) in animal kidney tissue by LC-MS/MS. **protocols.io https://dx.doi.org/10.17504/protocols.io.e6nvwdk57lmk/v1**

Manuscript citation:

Protocol status: Working
We use this protocol and it's working

Created: July 21, 2023

Last Modified: August 13, 2024

Protocol Integer ID: 85338

Funders Acknowledgement:
U.S. FDA/Vet-LIRN
Grant ID: 1U18FD006443-01

Disclaimer

Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the Food and Drug Administration.

Abstract

This diagnostic method provides for the identification and quantitative analysis of sodium monofluoroacetate (Compound 1080) from kidney tissue using LC-MS/MS

Sodium monofluoroacetate (Compound 1080) is an EPA Category I toxin. In the US, its use is restricted to licensed applicators who may use it in bait collars to protect small ruminants. Animal intoxication and death are reported, giving a need for a rapid, sensitive diagnostic method for detection and quantitation of Compound 1080 in animal tissues, including kidney. This SOP describes an LC-MS/MS method for the identification and quantitative analysis of Compound 1080 in kidney tissue at low ppb levels (e.g. ~5ppb). The method is rapid, sensitive, and selective.

Validation data (in-house and by an independent laboratory via collaborative study such as Blinded Method Test) are available upon request.

Guidelines

DEFINITIONS AND ACRONYMS

- Compound 1080 = Sodium monofluoroacetate
- ESI = Electrospray ionization
- UHPLC = Ultra high performance liquid chromatograph
- IS = Internal standard
- LC-MS/MS = Liquid Chromatography/Mass Spectrometry/Mass Spectrometry
- MRM = Multiple reaction monitoring
- RL = Reporting limit
- SPE = Solid phase extraction

SPECIMEN INFORMATION

5 g of kidney tissue (optimal). Sub-optimal sample amounts and quality may require modifications to the method and elevation of the method reporting limit (RL). Non-representative samples may affect results.

Quality Control and Reference Material

- Internal standard (IS) is added to each reagent blank, control sample, and sample prior to extraction. The IS used is $^{13}C_2$, d_2-sodium monofluoroacetate, added at a concentration of 10 ppb.
- Each set of samples must include a negative, unfortified control (check) sample from an appropriate control matrix.
- It is recommended, at the discretion of the analyst, to include a reagent blank with each set of samples
- Each set of samples must include a spiked (fortified) sample from an appropriate control matrix or an over spike of the sample material. The following spike levels are suggested:
  5 ppb level: add 0.005 mL of 1 µg/mL spiking standard into 1 g of control kidney
  10 ppb level: add 0.010 mL of 1 µg/mL spiking standard into 1 g of control kidney
- For quantitative results, a set of calibrations standards must be prepared and included
- The group leader, chief chemist or the toxicologist may approve deviations from these guidelines to meet the diagnostic utility of the test.

METHOD LIMITATIONS

The sample clean-up by SPE requires Compound 1080 be present as an anion. With a pKa value of 2.66, this requires that the extract be at a pH value of 4.66 or greater prior to loading onto the MAX SPE resin. It is recommended to test the pH of the extract prior to loading onto the MAX SPE resin to verify this. During validation, extracts from spiked kidneys and incurred kidneys were shown to be ~pH 7.

DOCUMENT AND RECORD REQUIREMENTS

The following information, as applicable, must be recorded in the lab notebook or other sample preparation records:

- Name of analyst prepping the samples;
- Date manipulations were started (extraction date);
- Method extraction description (ex SOP number)
- Sample ID (full accession number)
- QC sample(s) IDs (QC ID or Lot Number)
- Physical manipulations performed on samples and QC samples including:
  - amount measured
  - dilutions
  - aliquot amount
  - final volumes
  - Spiking information: ID of spiking solution used, initials and date of person performing spiking.
  - Pipet ID #
  - Lot #s of SPEs
  - Signature/Date of analyst upon completion

This is the minimum amount of information that should be recorded. If certain of the above data elements are not relevant then they may be excluded.

Materials

REAGENTS AND SUPPLIES

Chemicals

- Ammonium formate: Sigma-Aldrich
- Ammonium hydroxide: Fisher Scientific
- Methanol, water, acetonitrile, formic acid: Fisher Scientific HPLC grade or equivalent
- Trifluoroacetic acid: Fisher Scientific

Equipment and Apparatus (equivalents may be substituted)

- SciEx 7500 QTrap triple quadrupole/linear ion trap mass spectrometer or equivalent*
- Agilent Series 1290 UHPLC system or equivalent
- Waters Acquity UPLC BEH Amide column, 1.7 µm, 2.1 x 100 mm
- Beckman Coulter Avanti J-E Centrifuge
- Spex Sample Prep Genogrinder
- Stainless steel ball bearings
- Waters Oasis MAX SPE cartridges, 500 mg, 6 cc
- Pierce protein concentrator, PES, 3 kDa MWCO, 5 – 20 mL
- Whatman 1 µm GMF-150 syringe filter
- Analytical balance capable of measuring to at least two decimal places.
- 6 mL syringes, plastic
- 15 mL and 50 mL centrifuge tubes, plastic (polypropylene)
- Vortex mixer
- 2 mL autosampler vials, glass
- Millipore 0.22 µm PES syringe filters
- 1 L volumetric flasks
- Graduated cylinder
- Beakers
- Pipets, adjustable volume, volume ranges as appropriate: Eppendorf or equivalent
- Pasteur pipets, disposable: Fisher or equivalent
- Pipets, graduated, volume range as appropriate: Fisher or equivalent
- Vacuum SPE manifold

***NOTE:**

Calibration and maintenance of the SciEx QTrap 7500 triple quadrupole/linear ion trap mass spectrometer are describe in the corresponding SOP document.

Safety warnings

When working in the lab, observe all CAHFS lab safety requirements and wear appropriate personal protective equipment (PPE), including gloves, safety glasses, and lab coat.

In addition:

1. All operations which may liberate an aerosol, for example (but not limited to) pipetting and filtering, must be done in a biological safety hood up until the sample extract has passed through the protein concentrator.
2. Sodium monofluoroacetate (Compound 1080) is highly toxic
3. Formic acid, trifluoroacetic acid, and ammonium hydroxide are all highly corrosive
4. Methanol and acetonitrile are flammable

Waste disposal:

1. All chemical wastes must be disposed of following the appropriate CAHFS procedures
2. All potentially infective biological wastes must be disposed of following the appropriate CAHFS procedures

Before start

Calibration and spiking standard solutions are prepared using certified sodium monofluoroacetate standard materials, provided either neat or in solution. Certified standard materials are available from Cambridge Isotope Laboratories and other vendors. Isotopically labeled sodium monofluoroacetate ($^{13}C_2$, d_2-sodium monofluoroacetate) is available as an internal standard (IS) from Cambridge Isotope Laboratories and other vendors.

Prepare Reagents

1 5% Ammonium hydroxide in water

1.1 Combine 10 mL of ammonium hydroxide with 190 mL of water'

2 0.5% Trifluoroacetic acid in acetonitrile

2.1 Combine 5 mL of trifluoroacetic acid with 995 mL of water

3 5 mM Ammonium formate, 0.01% formic acid in water (Mobile Phase A)

3.1 Dissolve 0.315 g of ammonium formate in approximately 950 mL ofwater in a 1 L volumetric flask.

3.2 Add 0.100 mL of formic acid, and fill to a final volume of 1.00 L with water.

3.3 Store in an amber bottle.

> Note
>
> **This reagent is only usable for 30 days, and afterward a fresh reagent must be made**

Calibration standard solutions

4 Working standard solutions of sodium monofluoroacetate are prepared from neat material or solution at 1, 0.1, and 0.01 µg/mL levels.

4.1

Calibration standards are prepared as shown in Table 1 below. Calibration standard solutions are preparedin from working standard solutions, diluting into 0.5% trifluoroacetic acid in acetonitrile solvent.

Calibration Level	[Sodium Monofluoroacetate] ppb (ng/mL)
1	0.10
2	0.25
3	0.50
4	1.00
5	2.50
6	5.00
7	10.0
8	25.0
9	50.0

Table 1. Calibration standard solutions

Note

The number of calibration standards used may be adjusted depending upon the concentration range required, but at a minimum seven levels must be included.

5 Working internal standard (IS) solutions are prepared from neat material or solution at 0.5 and 0.25 µg/mL levels.

5.1

Internal standard (IS) is added to each calibration standard at a concentration of 1.25 ppb (ng/mL).

Sample Preparation

6 Ensure all samples are finely chopped

> **Note**
>
> All operations which may liberate an aerosol, for example (but not limited to) pipetting and filtering, must be done in a biological safety hood up until the sample extract has passed through the protein concentrator.

7 Weigh 1 g (+/- 0.05 g) of sample into a 50 mL plastic centrifuge tube

8 Add 0.020 mL of 0.50 µg/mL $^{13}C_2$, d_2-sodium monofluoroacetate (IS) solution

9 Add 4 mL of water and 2 ball bearings

10 Genogrind at 750 rpm for 00:05:00 5m

11 Remove ball bearings using a magnet

12 Centrifuge to pellet at 4,000 x g, 00:10:00, 10 °C 10m

13 Filter supernatant using a 6 mL syringe and a Whatman 1 µm GMF-150 syringe filter into a Pierce protein concentrator, PES, 3 kDa MWCO, 5 – 20 mL size

14 Centrifuge to obtain flow-through of a least 2 mL volume. Centrifuge set to 4,000 x g, 60 min, 10 °C. If insufficient flow-through obtained, repeat step with a new protein concentrator.

15 Further sample clean-up is next done using Waters Oasis MAX SPE cartridges, 500 mg, 6 cc:

15.1 Cartridges are positioned atop an SPE vacuum manifold

15.2 Cartridges are first conditioned by rinsing with 4 mL of methanol, followed by rinsing with 3 mL of water, and then an additional 3 mL of water

15.3 2 mL of the flow-through from the protein concentrator is next loaded onto the resin, taking care to load at a flow rate of no more than 1 drop per second

15.4 Cartridge is washed with 4 mL of 5% ammonium hydroxide in water

15.5 Cartridge is washed with 4 mL of methanol, and then pulled dry under vacuum for 00:00:30 30s

15.6 Cartridge is washed with 3 mL of 0.5% trifluoroacetic acid in acetonitrile, and then pulled dry under vacuum for 00:00:30 30s

15.7 0 mL Cartridge is washed another time with 3 mL of 0.5% trifluoroacetic acid in acetonitrile, and then pulled dry under vacuum for 00:00:30 30s

15.8 A 15 mL centrifuge is placed under each SPE column as a collection tube, and then the cartridge is eluted with 2 mL of 0.5% trifluoroacetic acid in acetonitrile at a flow rate of no more than 1 drop per second, and then pulled dry under vacuum for 00:00:30 , collecting the eluent 30s

15.9 The cartridge is eluted again with 2 mL of 0.5% trifluoroacetic acid in acetonitrile at a flow rate of no more than 1 drop per second, and then pulled dry under vacuum for 00:00:30 , collecting the eluent in the same collection tube, for a total volume of 4 mL pooled eluent 30s

15.10 Pooled eluent is next analyzed by LC-MS/MS

Instrument Parameters

16

Note

The parameters described were optimized for use with the Agilent 1290 UHPLC and SciEx 7500 QTrap mass spectrometer system described in the Materials section. These parameters are recommended settings and may be modified to optimize results as needed.

17 UHPLC Setting

17.1 Mobile Phase A: 5 mM Ammonium formate, 0.01% formic acid in water

17.2 MobilePhase B: Acetonitrile

17.3 Injection volume: 5 µL

17.4 Autosampler temperature: 10 °C

17.5 Column temperature: ambient room temperature

17.6 Gradient:

	A	B	C	D
	Time (min)	**%A**	**%B**	**Flow Rate (m L/min)**
	0	10	90	0.45
	3	10	90	0.45
	4	60	40	0.45
	5.5	60	40	0.45
	5.6	10	90	0.45
	16	10	90	0.45

18 Mass spectrometer settings

18.1 Polarity: Negative

18.2 Ionization: ESI

18.3 Curtain gas: 40 psi

18.4 Ion source gas 1: 90 psi

18.5 Ion source gas 2: 40 psi

18.6 Temperature: 500 °C

18.7 Scan type: MRM

18.8 Ion spray voltage: 4000 volts

18.9 Mass table

	A	B	C	D	E	F	G
	Compound	**Q1 mass (Da)**	**Q3 mass (Da)**	**Dwell Time (ms)**	**EP (V)**	**CE (V)**	**CXP (V)**
	Quantifier Ion	77	57	100	-10	-14	-10
	Qualifier Ion	77	33	100	-10	-17	-10
	IS Ion	81	60	100	-10	-16	-6

19 Data is acquired using SciEx OS software

20 All calculations are performed using the Analyst software within SciEx OS software

Note

Expected values- Compound 1080 in kidney may be found down to low ppb levels

Analytical Sequence

21 The sequence should begin and end with analysis of the calibration standards

22 The beginning set of standards are followed by a fortified control sample (spike) or overspiked sample

23 All sample and QC sample injections are done in duplicate

24 Sets of sample injections should be preceded by a reagent blank or an unfortified control sample (check) to demonstrate that no carryover from standards or fortified control is present

INTERPRETATION OF RESULTS

25 The analysis is acceptable if the reagent blank and/or check sample are negative, and reporting limit (RL) spike results for the analyte meets the criteria for positive identification (sec. 2, below).

26 An analyte is considered positively identified if it meets the following criteria:

26.1 The peak height signal to noise is greater than 3:1

26.2 The retention time of the analyte in the sample does not differ from that of the midpoint standard in the calibration curve by more than 0.25 min

26.3 The ratio of the peak area of the quantifier ion to that of the qualifier ion does not differ more than 20% from either that of the average of the ion ratios from the calibration standards –-or- from that of the midpoint standard from the opening calibration curve.

26.4 The group leader, chief chemist or the toxicologist may approve deviations from these guidelines to meet the diagnostic utility of the test.

27 Criteria for Data Acceptance (Quantitation)- The following conditions must be met for a concentration to be reported for the analyte:

27.1 The peak area ratio of the analyte to that of its internal standard in the sample must fall within the range of peak area ratios determined by the calibration curve

27.2 The spike recovery for the analyte should be between 70% and 120%.

27.3 If the sequence ends with a standard curve, then the two standardcurves are combined and the coefficient of determination (R^2) for the combined curve must be >0.99.

27.4 If the sequence ends with a midpoint standard from the calibration curve, then the R^2 for the analyte in the calibration curve must be 0.99 and the calculated concentration of analyte in the midpoint standard must fall within +/- 20%.

27.5 The group leader and/or chief chemist can approve any deviations from these guidelines to meet the diagnostic utility of this test.