Determination of Sodium Monofluoroacetate in Dairy Powders by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

1. INTRODUCTION AND OVERVIEW

1.1 INTRODUCTION

Sodium monofluoroacetate is a rodenticide used in New Zealand to control rats, possums and rabbits. It is commonly known by its original registration number as compound 1080, but may also be known as sodium fluoroacetate and fluoroacetic acid sodium salt.

1.2 SCOPE

This method has been validated in cow, goat and sheep dairy powders and dairy powder formulations. It can be used for other similar matrices provided that it is demonstrated that the method performance values are met. Additional validation and records that may be required will be according to the relevant validation procedure of the laboratory.

1.3 METHOD SUMMARY

The samples are dissolved in water and extracted into acetone. The solutions are passed through an anion exchange column and eluted with acid. The resulting monofluoroacetic acid is then converted to 2-fluoro-3'-nitroacetanilide. The derivative is then subjected to a SPE clean-up, eluting with TBME/n-Hexane (70:30), concentrated and quantified by LC-MS/MS using derivatised isotopically substituted sodium monofluoroacetate as an internal standard.

The method reports the analyte as sodium monofluoroacetate.

1.4 ANALYTES

Analytes and Internal Standards are summarised in Table 1.

TABLE 1: Test Articles (Analytes)

<table>
<thead>
<tr>
<th>Test Articles</th>
<th>CAS Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte</td>
<td></td>
</tr>
<tr>
<td>Sodium Monofluoroacetate</td>
<td>62-74-8</td>
</tr>
<tr>
<td>Internal Standards</td>
<td></td>
</tr>
<tr>
<td>Sodium Monofluoroacetate -13C2, 2,2-D2</td>
<td>Not Available</td>
</tr>
</tbody>
</table>
1.5 STRUCTURE

![Sodium monofluoroacetate structure]

Sodium monofluoroacetate

2. REFERENCED DOCUMENTS


3. SAFETY AND HEALTH

SDS of all chemicals, solvents and standards must be understood by the analyst before commencing the method. Avoid inhaling vapours or chemicals. Use the assigned protective clothing and equipment at all times. Ensure that you are familiar with the location of fire extinguishers, first aid kit and any safety equipment that may be required.

Refer to the Laboratory’s Hazard Register for Significant Hazards identified and the control methods specified to minimise these hazards.

3.1 Specific Hazards and Control Methods

- **5 M Hydrochloric Acid. CAUTION:** Wear PPE and handle in a fume cupboard.
- **Sulphuric Acid in Water 30% w/v. CAUTION:** Wear PPE and handle in a fume cupboard.
- **Sodium Monofluoroacetate. CAUTION:** Wear PPE, including safety glasses and a dust mask when weighing out the primary material.

4. DEFINITIONS

*See Appendix for definitions.*

5. REAGENTS

**DO NOT USE EXPIRED CHEMICALS, REAGENTS OR SOLUTIONS.**

All reagents and chemicals must be of such a grade that they do not interfere with the analytical process. Use the stated grade or equivalent.
5.1 CHEMICALS

- Acetone (Pesticide grade)
- Acetonitrile (Pesticide grade)
- AG 1-X8 Resin, 100-200 mesh chloride form (ACS reagent grade)
- Ammonium Acetate (ACS reagent grade)
- Deionised Water
- Hydrochloric Acid (concentrated) (ACS reagent grade) (37 – 38%)
- Methanol (Pesticide grade)
- $n$-Hexane (Pesticide grade)
- Phosphoric Acid (concentrated) (ACS reagent grade)
- Potassium Dihydrogen Phosphate (ACS reagent grade)
- Potassium Hydroxide (ACS reagent grade)
- Sodium Sulphate, Anhydrous (ACS reagent grade)
- Sodium Hydrogen Carbonate (ACS reagent grade)
- Sulphuric Acid (concentrated) (ACS reagent grade)
- $t$-Butyl Methyl Ether (TBME, Pesticide grade)
- 1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide Hydrochloride (EDAC, ACS reagent grade)
- 3-Nitroaniline (ACS reagent grade)

5.2 SOLUTIONS

- **5 M Hydrochloric Acid (2000 mL)**
  
  **CAUTION:** Preparation of solution should be performed inside a fume cupboard.

  Into a 2000 mL volumetric flask add 800 mL of deionised water. To this add 832 mL of 37-38% (12 M) concentrated hydrochloric acid. Mix and allow to cool. Bring to volume with deionised water.

  Store at room temperature. Stable for three years.

- **0.2 M Hydrochloric Acid (2000 mL)**

  Into a 2000 mL volumetric flask add 800 mL of deionised water. To this add 80 mL of 5 M hydrochloric acid. Mix and allow to cool and bring to volume with deionised water.

  Store at room temperature. Stable for three years.

- **2 M Potassium Hydroxide (2000 mL)**
CAUTION: Preparation of solution should be performed inside a fume cupboard.

Place 1600 mL of deionised water in a 2000 mL beaker and place on a magnetic stirrer with follower. Weigh 224.4 g of potassium hydroxide into a 500 mL beaker. Add the potassium hydroxide, a few pellets at a time, to the stirred solution. Do not allow the temperature to rise above warm to the touch. When all the pellets have dissolved allow the solution to cool then transfer quantitatively through a glass funnel into a 2000 mL measuring cylinder and bring to volume with deionised water.

Store at room temperature. Stable for three years.

- 20 mg/mL 3-Nitroaniline (100 mL)

CAUTION: Wear gloves when working with this chemical

Weigh 2.0 g aliquots of 3-nitroaniline into 100 mL Schott bottles and cap tightly.

Store at room temperature. Stable for five years.

To one pre-weighed bottle of 3-nitroaniline add 100 mL of acetonitrile using a graduated measuring cylinder. This is sufficient for two batches of 36 sample tubes.

Prepare fresh daily.

- 100 mg/mL EDAC (25 mL)

CAUTION: Exposure to moisture degrades this reagent

Buy only a 25 g bottle and open it only 2 – 3 times. Weigh enough tubes when opened and store the tubes in the freezer at or below -10ºC.

Pre-weigh 2.5 g aliquots of EDAC (inside a nitrogen filled glove bag) into 50 mL polypropylene tubes and cap tightly.

Store in a freezer at or below -10ºC in a desiccated container. Stable for two years.

To one pre-weighed tube of EDAC add 25 mL of deionised water. Prepare fresh daily.

- TBME:Hexane 70:30 (v/v) (2000 mL)

Measure 1400 mL of TBME into a 2000 mL Schott bottle and add 600 mL of n-hexane. Cap and swirl to mix.

Store at room temperature. Stable for three months.

- Sulphuric Acid in Water 25% v/v (2000 mL)

CAUTION: Preparation of solution should be performed inside a fume cupboard.

Into a 2 L volumetric flask add about 1200 mL of deionised water and then slowly add 500 mL of sulphuric acid. Swirl to mix and allow to cool. Bring to 2 L volume, then store in a Schott bottle.

Store at room temperature. Stable for three years.

- 0.05 M Potassium Dihydrogen Phosphate, pH 2.3 (1000 mL)

Weigh 6.80 g of potassium dihydrogen phosphate into a 500 mL beaker. Add 300 mL of deionized water to dissolve the potassium dihydrogen phosphate and decant into a 1 L Schott bottle. Add a further 300 mL of deionized water to the beaker to dissolve any
remaining potassium dihydrogen phosphate, decant into the Schott bottle and make up to 1 L. Cap, swirl, mix and adjust the pH to 2.3 ± 0.1 with concentrated phosphoric acid. Store at room temperature. Stable for three months.

- **0.1 M Sodium Hydrogen Carbonate (1000 mL)**

Weigh 8.40 g of sodium hydrogen carbonate into a 500 mL beaker. Add 300 mL of deionized water to dissolve the sodium hydrogen carbonate and decant into a 1 L Schott bottle. Add a further 300 mL of deionized water to the beaker to dissolve any remaining sodium hydrogen carbonate, decant into the Schott bottle, make up to 1 L, cap and swirl ensure full solubility.

Store at room temperature. Stable for one month.

- **AG 1-X8 Anion Exchange Resin**

Before use, soak the AG 1-X8 anion exchange resin in deionised water for 18-24 hours, then leave in deionised water.

Store in a fridge. Stable for one year.

- **HPLC Mobile Phase A: 10 mM Ammonium Acetate in Water (1000 mL)**

Weigh 0.77 g of ammonium acetate into a 1 L Schott bottle followed by 1000 mL deionised water. Cap and swirl ensure full solubility.

Store at room temperature. Stable for two weeks.

- **HPLC Mobile Phase B: 10 mM Ammonium Acetate in 97% Acetonitrile (1000 mL)**

Weigh 0.77 g of ammonium acetate into a 50 mL beaker. Add 30 mL of deionised water and stir to fully dissolve. Decant into 1 L Schott bottle and add 970 mL of acetonitrile. Sonicate for 10 minutes to ensure full solubility.

Store at room temperature. Stable for two weeks.

### 6. STANDARDS

#### NOTE:

Do not use expired primary standards or standard solutions unless they have been released/approved for use by a Senior Scientist, Scientist (approved by the Technical Manager) or the Technical Manager.

#### 6.1 PRIMARY STANDARDS

Primary standards are stored in a refrigerator between 2-8°C in the dark and are stable for five (5) years from QC date on the certificate of analysis. Sources of standards other than those listed may be used provided they have a satisfactory purity and are accompanied by a certificate of analysis.

#### 6.1.1 Analytes

6.1.2 Internal Standard

- Sodium monofluoroacetate -\(^{13}\)C\(_2\), 2,2-D\(_2\) was purchased from Cambridge Isotope Laboratories. **CAUTION:** Wear PPE, including safety glasses and a dust mask when weighing out the primary material.

6.2 SECONDARY STANDARDS

Suitably diluted solutions of the new secondary standards are to be compared to those of the previous secondary standards. The comparison of the response of the solution should not exceed a difference of 20%.

6.2.1 Analytes

- Sodium Monofluoroacetate Standard (1000 mg/L)

Weigh 10 mg of sodium monofluoroacetate into a calibrated 10 mL volumetric flask. Add deionised water and make to volume. Place in an ultrasonic bath for 2 minutes or until solid is completely dissolved. Transfer to a 15 mL screw cap test tube and cap tightly.

Store in a freezer at less than -10°C. Stable for three years.

6.2.2 Internal Standard

- Sodium Monofluoroacetate – \(^{13}\)C\(_2\), 2,2-D\(_2\) Standard (500 mg/L)

Weigh 5.0 mg of sodium monofluoroacetate – \(^{13}\)C\(_2\), 2,2-D\(_2\) into a calibrated 10 mL volumetric flask. Add deionised water and make to volume. Place in an ultrasonic bath for 2 minutes or until solid is completely dissolved. Transfer to a 10 mL Teflon lined screw-top tube for storage in the freezer.

Store in the freezer at -10°C or below. Stable for five years.

6.3 WORKING SOLUTIONS

6.3.1 Analyte

- Sodium Monofluoroacetate Intermediate Standard (50 mg/L)

Dilute 2.5 mL of Sodium Monofluoroacetate secondary standard (1000 mg/L) to 50 mL with deionized water in a calibrated volumetric flask. Use a calibrated positive displacement pipette.

Dispense aliquots of the standard into 15 mL polypropylene tubes for frozen storage.

Store in the freezer at -10°C or below. Stable for three years.

From the Intermediate standard prepare the analyte Working Standard (WS1) as summarised in Table 2 using a calibrated 50 mL volumetric flask and calibrated positive displacement pipette. Make up to volume with deionised water.
Dispense aliquots (about 10-15 mL) of the WS1 standard into 15 mL polypropylene tubes for frozen storage.

Store in the freezer at -10°C or below. Stable for one year.

A thawed and opened tube WS1 can be stored for up to two months in a refrigerator between 2-8°C, provided it is resealed and immediately refrigerated after each use. **NOTE:** Write a two month expiry date on the tube when it is removed from the freezer.

A more dilute working solution, WS2, is prepared from WS1 as described in Table 3 using a calibrated 100 mL volumetric flask and calibrated positive displacement pipette.

### TABLE 3: Composition of Analyte Working Solution WS2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of WS1 (mg/L)</th>
<th>Volume of WS1 in 100 mL (mL)</th>
<th>Final Concentration (mg/L) WS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Monofluoroacetate</td>
<td>1</td>
<td>10</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Dispense aliquots (about 10-15 mL) of WS2 into 15 mL polypropylene tubes for frozen storage.

Store in the freezer at -10°C or below. Stable for 6 months.

A thawed and opened tube of WS2 can be stored for up to two months in a refrigerator between 2-8°C, provided it is resealed and immediately refrigerated after each use. **NOTE:** Write a two month expiry date on the tube when it is removed from the freezer.

### 6.3.2 Internal Standard

- Sodium Monofluoroacetate–13C2,2,2-D2 Standard (50 mg/L)

Dilute 2500 µL of Sodium Monofluoroacetate - 13C2,2,2-D2 secondary standard (500 mg/L) to 25 mL with water in a calibrated volumetric flask. Use a calibrated positive displacement pipette.

Dispense aliquots of the standard into 15 mL polypropylene tubes for frozen storage.
Store in the freezer at -10°C or below. Stable for five years or until no longer fit for purpose.

The Internal Standard working solution (ISWS) preparation is summarised in Table 4. Dilute the volume of the secondary standard given in Table 4 to 500 mL with deionised water, using a calibrated positive displacement pipette.

**TABLE 4: Composition of Internal Standard Working Solution (ISWS)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of Secondary Standard (mg/L)</th>
<th>Volume of Secondary Standard Used per 500 mL (mL)</th>
<th>Final Concentration of Working Solution (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Monofluoroacetate</td>
<td>50</td>
<td>5.0</td>
<td>0.5</td>
</tr>
<tr>
<td>$\text{-}^{13}\text{C}_2\text{, 2,2-D}_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pipette aliquots of the Internal Standard Working Solution (ISWS) into 50 mL polypropylene containers for storage in a freezer.

Store in the freezer at -10°C or below. Unopened containers contents stable for five years

A sub-sample can be kept in use in a refrigerator at between 2-8°C. A thawed and opened tube of ISWS can be stored for up to two months in a refrigerator between 2-8°C, provided it is resealed and immediately refrigerated after each use.

**NOTE:** Write a two month expiry date on the tube when it is removed from the freezer.

### 7. APPARATUS

**NOTE:** Where specific equipment is listed, other brands or models may be used provided that they have equivalent performance.

#### 7.1 LABORATORY EQUIPMENT

- Air displacement pipettes
- Air displacement pipettes, 5 mL, fitted with long tips
- Auto-sampler vials (2 mL) with tapered glass inserts
- Balance, 2 or 3 decimal top pan
- Balance, 5 decimal place analytical
- Beakers, measuring cylinders, volumetric flasks
- Bio Rad 10 mL plastic reservoir
- Centrifuge capable of centrifuging 15 mL tubes at 4000 rpm
- Condenser tubes, glass, 50 mL
- Eppendorf or equivalent multi – step dispenser with appropriate tips
7.2 ANALYTICAL INSTRUMENTATION

- LC-MS/MS instrument: ABSciex 5500 QTRAP coupled with Agilent 1290 Series HPLC (or equivalent)
- HPLC Guard Column: Phenomenex Security C18, 4 x 2 mm
- HPLC column: Agilent XDB-C18 100 x 4.6 mm, (or equivalent)

8. SAMPLING AND SAMPLE PREPARATION

8.1 GENERAL

Each batch is uniquely identified.

Arrange and check the identity of the samples to be tested then generate a batch form.

8.2 PREPARATION OF A TEST SAMPLE

The samples are brought to room temperature on a laboratory bench.

8.3 PREPARATION OF A TEST PORTION

Accurately weigh 2.5 ± 0.03 g of sample into a labelled 50 mL polypropylene tube. In addition to the analytical samples, there are four recovery samples per batch, a reagent blank, and an extra blank for the matrix standard.

NOTE 1: Unless values for weight and volume have either a ‘±’ sign or the word ‘exactly’ then they are to be taken as approximate values.
NOTE 2: All samples are tested singly as 2.5 g test-portions of the supplied sample. Deviations from this may be where on repeat testing there was insufficient sample, or a confirmation that require less sample to minimise background interferences.

9. PROCEDURE

9.1 DETERMINATION

9.1.1 Fortification of Recoveries

Fortify the recoveries as set out in Table 5 using the working solutions prepared in Section 6.3.

TABLE 5: Fortification of Recovery Samples

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Name</th>
<th>Volume WS2 (µL)</th>
<th>Volume ISWS (µL)</th>
<th>Concentration Sodium Monofluoroacetate (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>Matrix Standard</td>
<td>0*</td>
<td>0*</td>
<td>0.0050*</td>
</tr>
<tr>
<td>2</td>
<td>Recovery 1</td>
<td>0</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Recovery 2</td>
<td>2.5</td>
<td>40</td>
<td>0.00010</td>
</tr>
<tr>
<td>4</td>
<td>Recovery 3</td>
<td>12.5</td>
<td>40</td>
<td>0.00050</td>
</tr>
<tr>
<td>5</td>
<td>Recovery 4</td>
<td>25</td>
<td>40</td>
<td>0.0010</td>
</tr>
<tr>
<td>6</td>
<td>Recovery 5</td>
<td>125</td>
<td>40</td>
<td>0.0050</td>
</tr>
<tr>
<td>7</td>
<td>Reagent Blank</td>
<td>0</td>
<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>

* The matrix standard is fortified at step 9.2.9.

NOTE: Fortify onto test portion and allow recoveries to equilibrate for 10 minutes after fortification.

9.1.2 Internal Standard

With an Eppendorf multipette add 40 µL of ISWS to all unknown and recovery samples.

NOTE: Do not add any ISWS to the matrix blank to be used for the matrix standard.

9.2 ANALYTICAL DETERMINATION

9.2.1 Place the Bio Rad columns onto a vacuum manifold and fill with 1.4 (± 0.2) mL of resin. Add 2.5 mL of deionised water above the resin bed and close tap. Fit suitable reservoirs above the columns.

9.2.2 To each test portion add 5 mL of water and briefly shake vigorously by hand, cap and then shake tubes at medium speed on a reciprocating shaker for 5 minutes to dissolve. Variation to this procedure may be required and will be specified for atypical matrices.

9.2.3 Add 10 mL of acetone to each tube and briefly shake vigorously by hand followed by 2 minutes on a reciprocating shaker at medium speed.
9.2.4 Centrifuge at 4000 rpm for 10 minutes.

9.2.5 Carefully pour the top solvent layer into the corresponding reservoirs taking care not to transfer any precipitate.

9.2.6 Allow the samples to pass through the columns under gravity or gentle vacuum, if required.

9.2.7 After the samples have passed through the columns, remove the reservoirs and wash the columns with 1 mL of 0.2 M hydrochloric acid. Close tap. Do not allow the resin to dry.

9.2.8 Place 15 mL polypropylene tubes beneath each column. Elute the samples with one 5 mL volume of 0.2 M hydrochloric acid at about 30 drops per minute. Suck the remaining hydrochloric acid solution into the collecting tubes under vacuum.

9.2.9 To the matrix standard tube only, add 125 µL of WS2 and 40 µL of ISWS, cap and vortex mix.

9.2.10 Add 1.25 mL of 20 mg/mL 3-nitroaniline and 0.25 mL of 100 mg/mL EDAC solution followed by 0.5 mL of 2 M potassium hydroxide and 1 mL of 0.05 M potassium dihydrogen phosphate buffer to all tubes. Cap and mix.

9.2.11 Place tubes in a 40 ± 2°C waterbath for 20 minutes.

9.2.12 Remove tubes, cool to room temperature and proceed without delay with SPE clean up.

9.2.13 Set up a vacuum manifold with Oasis HLB, 60 mg 3 mL cartridges.

9.2.14 Condition the cartridge with 1 mL methanol. Close the tap when the methanol reaches the top frit.

9.2.15 Load a portion of the derivatised extract onto the conditioned SPE cartridge.

9.2.16 Place an adapter and 10 mL reservoir on top of the cartridge.

9.2.17 Transfer the remaining derivatised extract into the reservoir and open the tap. Allow to drip slowly at about 30-40 drops per minute.

9.2.18 When the extract has passed through the cartridge, remove the adapter and reservoir.

9.2.19 Wash the cartridge with 2 mL 25% (v/v) sulphuric acid, 1 mL deionized water, 1 mL 0.1 M sodium hydrogen carbonate and a further 2 mL of deionized water.

9.2.20 Dry the cartridge by applying full vacuum for 5 minutes.

9.2.21 Place 15 mL polypropylene tubes beneath each SPE. Elute the derivatised extract with 2 x 2.5 mL of TBME/n-Hexane (70:30 v/v) into the test tubes.

9.2.22 Dry the cartridge by briefly applying a full vacuum.

9.2.23 Check the tubes for remaining water. There should be minimal water present. The presence of more than about 50 µL of water would indicate inadequate vacuum.
9.2.24 Add approximately 200 mg of sodium sulphate, anhydrous, to each tube and vortex mix.

9.2.25 Centrifuge at 3000 rpm for one minute.

9.2.26 Decant the supernatant into a clean 15 mL tapered, polypropylene tube.

9.2.27 Evaporate the solvent to incipient dryness under nitrogen at 40 ± 10°C.

**NOTE:** Do not leave on heating block as excess heating may degrade derivatised analyte.

9.2.28 Allow the tubes to return to near room temperature then re-dissolve residue in 150 µL of acetonitrile.

9.2.29 Carefully vortex mix at low speed.

9.2.30 Centrifuge at 3000 rpm for one minute.

9.2.31 Transfer clear solvent layer to a LC vial with a tapered insert making sure not to transfer any solid and/or particulate matter. Cap firmly.

**NOTE:** The final extracts have been shown to be stable at least 5 days when stored in the freezer at -10°C or below.

### 9.3 INSTRUMENT DETERMINATION

#### 9.3.1 Identification Parameters

Mass spectral and chromatographic parameters for the analysis of sodium monofluoroacetate are given in Table 6.

**TABLE 6: Identification Parameters for Compounds Analysed as Negative Ions**

<table>
<thead>
<tr>
<th>Compound (3-nitroaniline derivatives of analyte and internal standard)</th>
<th>Expected Retention Time* (minutes)</th>
<th>Quantifying Transition</th>
<th>Qualifier Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Monofluoroacetate</td>
<td>2.05</td>
<td>196.9 → 122.0</td>
<td>196.9 → 146.9</td>
</tr>
<tr>
<td>Sodium Monofluoroacetate - $^{13}$C$_2$2,2-D$_2$</td>
<td>2.05</td>
<td>201 → 134.9</td>
<td>201 → 45.9</td>
</tr>
</tbody>
</table>

*Retention time data from a representative matrix standard. Determined for each batch from matrix standards.

#### 9.3.2 Analytical Instrumentation

##### 9.3.2.1 General

The instrument used is an Agilent 1290 HPLC system coupled with a 5500 QTRAP Triple Quad Mass Spectrometer. The system is controlled by ABSciex LC-MS/MS software. Peak integration is handled with ABSciex MultiQuant Analysis software.
9.3.2.2 LC Parameters:
- Column: Agilent XDB-C18 100 x 4.6 mm
- Guard column: Phenomenex Security C18, 4 x 2mm
Refer to appendix 15.2 ‘Instrument Parameters for ABSciex LC-MS/MS System’ for full analytical parameters.

9.3.2.3 Mass Spectrometer Parameters:
Refer to appendix 15.2 ‘Instrument Parameters for ABSciex LC-MS/MS System’ for full analytical parameters.

10. MONITORED CONTROL POINTS

TABLE 7: Factors that Must be Monitored During the Analysis

<table>
<thead>
<tr>
<th>Control Point</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary and Working Standard Solutions</td>
<td>Store secondary standards in the dark in the freezer at less than -10°C. Minimise the exposure of standard solutions to fluorescent light or direct sunlight. Record the relevant ID number and expiry dates on batch form/worksheet.</td>
</tr>
<tr>
<td>Blind Spikes</td>
<td>At least one per month, or as required. Record blind spike details in blind spike form.</td>
</tr>
<tr>
<td>Reagents, Pipette and Balance ID</td>
<td>Record the relevant ID numbers and expiry dates on worksheet.</td>
</tr>
<tr>
<td>Control Charts</td>
<td>Plot relative recoveries of sodium monofluoroacetate and absolute recoveries for internal standards and QC samples where applicable.</td>
</tr>
<tr>
<td>Standard Comparison</td>
<td>As standards are replaced, dilutions of new and old standards are compared. The comparison of the response of the solution should not exceed a difference of 20%. Do not use expired primary standards or standard solutions unless they have been released/approved for use by a Senior Scientist, Scientist (Approved by the Technical Manager) or the Technical Manager.</td>
</tr>
<tr>
<td>Sample Identification</td>
<td>Ensure ID number is on batch form/worksheet.</td>
</tr>
<tr>
<td>Water Bath</td>
<td>Ensure temperature does not exceed 40 ± 2°C.</td>
</tr>
</tbody>
</table>
TABLE 8: Other Assay Actions Important to Performance

<table>
<thead>
<tr>
<th>Assay Area</th>
<th>Precautionary Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Storage</td>
<td>Store samples at -10°C or below.</td>
</tr>
<tr>
<td>Nitrogen Blow-Down</td>
<td>Ensure temperature does not exceed 40 ± 10°C.</td>
</tr>
<tr>
<td>Storage of sample extracts</td>
<td>If sample extracts cannot be analysed on the day of extraction then extracts should be stored frozen. Extracts are stable for at least 5 days.</td>
</tr>
</tbody>
</table>

11. CALCULATIONS

The quantification of sodium monofluoroacetate is carried out by Microsoft Excel calculations and is based on peak area obtained from ABSciex MultiQuant software.

Matrix recoveries are used to generate calibration curves. An unknown peak that falls within the evaluation window (as calculated by recoveries and internal standard) is quantified from the appropriate calibration curve and the value tabulated, together with peak identification information. Each potential unknown is then manually assessed for the quality of identification by viewing integrated chromatograms and those of any qualifying ions.

11.1 FORMULA

\[
\text{Concentration}_{\text{(Unknown)}} = \frac{\text{Relative Response}_{\text{(Unknown)}}}{\text{Slope}_{\text{(Calibration Curve)}}}
\]

12. METHOD PERFORMANCE AND QUALITY CONTROL

12.1 REAGENT BLANK TEST

A Reagent Blank (deionised water) test is performed with each batch.

12.2 MATRIX BLANK TEST (RECOVERY 1)

A Matrix Blank test is performed with each batch.

12.3 MATRIX RECOVERY TEST (RECOVERY SAMPLES)

Are performed with each batch according to the schedule in Table 5.

12.4 CERTIFIED REFERENCE MATERIALS

No CRM is available. In practice, external checks of the method are performed by participation in inter-laboratory calibration studies when available.

12.5 PERFORMANCE VALUES

Values found in Table 9 are calculated from the in-house validation completed by AsureQuality Limited.
TABLE 9: Performance Values of Analytes

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD (mg/kg)</th>
<th>LOQ (mg/kg)</th>
<th>LOR (mg/kg)</th>
<th>Within Day CV</th>
<th>Between-Day CV (WLR)</th>
<th>U (for 95% CI)</th>
<th>Percent Recovery % (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Monofluoroacetate</td>
<td>0.00018</td>
<td>0.00054</td>
<td>0.0010</td>
<td>8.8</td>
<td>9.1</td>
<td>18</td>
<td>97*</td>
</tr>
<tr>
<td>Sodium Monofluoroacetate - 13C2, 2,2-D2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>70 (12)**</td>
</tr>
</tbody>
</table>

*Relative recovery  
**Absolute recovery.

12.6 ACCEPTANCE CRITERIA

12.6.1 Individual Sample Acceptance Criteria
The internal standard response for an individual sample should exceed 33% of the mean internal standard response of the recovery samples.

12.6.2 Batch Acceptance Criteria
12.6.2.1 Analyte relative recoveries for the recovery samples should be within 3 SD of the mean relative recovery established from control charts.

12.6.2.2 Where Quality Control (QC) samples are used for batch acceptance then the recovery of the analyte should be within 3 SD of the quoted mean recovery for the respective concentration of QC samples.

12.6.2.3 Calibration curves should have a coefficient of determination R^2 > 0.95.

12.6.3 Positive Sample Acceptance Criteria
12.6.3.1 Retention Time Acceptance Criteria are given in Table 10.

TABLE 10: Relative Retention Time (RRT) and Limits of Acceptance

<table>
<thead>
<tr>
<th>Compound</th>
<th>Monitored Compounds</th>
<th>RRT</th>
<th>Acceptance Limit^1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Monofluoroacetate</td>
<td>Analyte / Internal Standard</td>
<td>1.004* RRT ± 2.5 %</td>
<td></td>
</tr>
</tbody>
</table>

* Representative Relative Retention Time.

NOTE: These values are indicative and should be measured for each individual batch.

12.6.3.2 Ion ratio acceptance limits are given in Table 11.
TABLE 11: Ion Ratios and Limits of Acceptance

<table>
<thead>
<tr>
<th>Compound</th>
<th>Transitions</th>
<th>MRM Ratio*</th>
<th>Acceptance Limit¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Monfluoroacetate</td>
<td>196.9 → 146.9 / 196.9 → 122.0</td>
<td>1.01</td>
<td>± 20 %</td>
</tr>
<tr>
<td></td>
<td>196.9 → 117.8 / 196.9 → 122.0</td>
<td>0.75</td>
<td>± 20 %</td>
</tr>
</tbody>
</table>

* Representative MRM Ratio.

**NOTE:** These values are indicative and should be measured for each individual batch.

Quality control data must be scrutinised and when found to be outside the method acceptance criteria, planned action must be taken (via the laboratories Corrective Action Request process) to correct the problem and prevent incorrect results from being reported.

12.7 CONTROL CHARTS

Control charts will be maintained for this method:

1. A plot of the relative recovery of the matrix standard quantified from the slope of the recovery curve.
2. The estimated concentrations of any Quality Control samples. The Quality Control samples are usually fortified at the LOR (0.0010 mg/kg).

12.8 BLIND SPIKES

Blind spike samples are included with a batch of samples and are performed at least every month or when required.

**NOTE:** The blind spike sample, recoveries and matrix standard must be of the same matrix type.

For blind spike results to be accepted, the concentration of the spiked analyte needs to be 55 - 145% of the expected concentration.

13. REPORTING

13.1 RESULTS

The reporting of results is carried out in accordance with laboratory policy.

A summary of the data from ABSciex MultiQuant software is generated using Microsoft Excel. Both raw and reviewed data and summary reports are stored and archived appropriately in line with relevant laboratory policies and procedures.

If the analyte does not recover in recovery samples, it is a failed batch and must be repeated.

**NOTE:** Results must be entered and reported in mg/kg units to two significant figures.
The Authorised Signatory or a nominated staff member issues the report to the customer in accordance with the requirements of the customer and a copy stored as per Laboratory policy.

13.2 CONFIRMATION METHOD

A sample found to be non-compliant for sodium monofluoroacetate would be re-tested in duplicate from new test portions to confirm the result.

It is also preferable to re-analyse extracts using a different polarity LC column and/or alternative instrumental technique.

14. REFERENCES


3H. Ozawa, T. Tsukioka, Determination of Sodium Monofluoroacetate in soil and biological samples as the dichloroanilide derivative. J Chrom. 473 (1989) 251 – 259

15. APPENDICES

15.1 TYPICAL CHROMATOGRAMS

Figure 1. Chromatograms of weaker confirmation ion of Matrix Blank (top), Recovery 3 (0.0005 mg/kg, middle) and Recovery 4 (0.001 mg/kg, bottom)
15.2 INSTRUMENT PARAMETERS FOR ABSCIEX LC-MS/MS SYSTEM

5500QT1_TSMF05-M01
Comment: Zorbax XDB-C18 100mm x 4.6mm 10mM NH4Ac in H2O(A) and 10mM NH4Ac in 97% ACN(B)
Synchronization Mode: LC Sync
Auto-Equilibration: Off
Acquisition Duration: 4min0sec
Number Of Scans: 436
Periods In File: 1
Acquisition Module: Acquisition Method
Software version Analyst 1.6

Agilent LC Pump Method Properties
Pump Model: Agilent 1290 Binary Pump
Minimum Pressure (psi): 0.0
Maximum Pressure (psi): 17404.0
Dead Volume (µl): 40.0
Stroke Volume (µl): -1.0
Maximum Flow Ramp (ml/min²): 100.0
Maximum Pressure Ramp (psi/sec): 290.0
Max Flow Ramp Up (ml/min²): 100.0
Max Flow Ramp Dn (ml/min²): 100.0

Step Table:
@Step Total Time(min) Flow Rate(µl/min) A (%) B (%)
0 0.00 1000 80.0 20.0
1 2.50 1000 0.0 100.0
2 3.00 1000 0.0 100.0
3 3.01 1000 80.0 20.0
4 4.00 1000 80.0 20.0

Left Stroke Volume (µl): -1.0
Right Stroke Volume (µl): -1.0
Left Solvent: A2
Right Solvent: B2

Agilent Autosampler Properties
Autosampler Model: Agilent 1290 Infinity Autosampler
Syringe Size (µl): 20
Injection Volume (µl): 5.00
Draw Speed (µl/min): 100.0
Eject Speed (µl/min): 200.0
Needle Level (mm): 0.00
Temperature Control Enabled
Setpoint (4 - 40 C): 10
Wash Location: Flush Port
Wash Time (1 - 999 sec): 3
Automatic Delay Volume Reduction: Not Used
Equilibration Time (sec): 2
Enable Vial/Well Bottom Sensing: No
Use Custom Injector Program: No

Agilent Column Oven Properties
Left Temperature (°C): 60.00
Right Temperature (°C): 60.00
Temperature Tolerance +/- (°C): 1.00
Start Acquisition Tolerance +/- (°C): 0.50
Time Table (Not Used)
Column Switching Valve Installed: 10Port2Pos SN#: 0003096674
Position for first sample in the batch: Left
Use same position for all samples in the batch

MS Method Properties:
Period 1:
---------------------
Scans in Period: 436
Relative Start Time: 0.00 msec
Experiments in Period: 1

Period 1 Experiment 1:
----------------------
Scan Type: MRM (MRM)
Scheduled MRM: No
Polarity: Negative
Scan Mode: N/A
Ion Source: Turbo Spray
Resolution Q1: Unit
Resolution Q3: Unit
Intensity Thres.: 0.00 cps
Settling Time: 0.0000 msec
MR Pause: 5.0070 msec
MCA: No
Step Size: 0.00 Da

@Q1 Mass (Da) Q3 Mass (Da) Dwell(msec) Param Start Stop ID
196.931 122.000 50.00 DP -120.00 -120.00 1080D
CE -24.00 -24.00
CXP -17.00 -17.00

@Q1 Mass (Da) Q3 Mass (Da) Dwell(msec) Param Start Stop ID
196.931 146.900 50.00 DP -120.00 -120.00 1080D
CE -22.00 -22.00

---

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Bob Rathbone, Senior Director, Publications, rrathbone@aoac.org
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<table>
<thead>
<tr>
<th>@Q1 Mass (Da)</th>
<th>Q3 Mass (Da)</th>
<th>Dwell(msec)</th>
<th>Param</th>
<th>Start</th>
<th>Stop</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>196.931</td>
<td>117.800</td>
<td>-120.00</td>
<td>-120.00</td>
<td>1080D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>-28.00</td>
<td>-28.00</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXP</td>
<td>-17.00</td>
<td>-17.00</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>@Q1 Mass (Da)</th>
<th>Q3 Mass (Da)</th>
<th>Dwell(msec)</th>
<th>Param</th>
<th>Start</th>
<th>Stop</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>196.931</td>
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<td>-120.00</td>
<td>1080D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>-30.00</td>
<td>-30.00</td>
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<td></td>
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<td></td>
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<tr>
<td>CXP</td>
<td>-19.00</td>
<td>-19.00</td>
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<th>Q3 Mass (Da)</th>
<th>Dwell(msec)</th>
<th>Param</th>
<th>Start</th>
<th>Stop</th>
<th>ID</th>
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<tbody>
<tr>
<td>201.001</td>
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<tr>
<td>13C2D2</td>
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<td>CXP</td>
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<td>-1.00</td>
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<th>@Q1 Mass (Da)</th>
<th>Q3 Mass (Da)</th>
<th>Dwell(msec)</th>
<th>Param</th>
<th>Start</th>
<th>Stop</th>
<th>ID</th>
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<td>CXP</td>
<td>-21.00</td>
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<table>
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<tr>
<th>@Q1 Mass (Da)</th>
<th>Q3 Mass (Da)</th>
<th>Dwell(msec)</th>
<th>Param</th>
<th>Start</th>
<th>Stop</th>
<th>ID</th>
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</thead>
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<tr>
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<td>1080D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13C2D2</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>CE</td>
<td>-22.00</td>
<td>-22.00</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CXP</td>
<td>-23.00</td>
<td>-23.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Parameter Table (Period 1 Experiment 1):
CUR: 30.00
TEM: 750.00
GS1: 60.00
GS2: 60.00
IS: -4500.00

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Valco Valve Diverter

<table>
<thead>
<tr>
<th>Total Time (min)</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6</td>
<td>A</td>
</tr>
<tr>
<td>2.6</td>
<td>B</td>
</tr>
</tbody>
</table>
APPENDIX

DEFINITIONS

ABBREVIATIONS

CI  Confidence Interval
CRM Certified Reference Material
CV  Coefficient of Variation
EDAC 1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide
LC Liquid Chromatography
LC-MS/MS Liquid Chromatography Tandem Mass Spectrometry
IS  Internal Standard
ISWS Internal Standard Working Solution
LIMS Laboratory Information Management System
LOD  Limit of Detection
LOQ  Limit (or Level) of Quantification
LOR Limit of Reporting
MRL Maximum Residue Level
PPE Personal Protective Equipment
QC Quality Control
RR Relative Response
SD Standard Deviation
SDS Safety Data Sheet
SOP Standard Operating Procedure
SPE Solid Phase Extraction
TBME t-Butyl Methyl Ether
U Uncertainty of Measurement with a 95% Confidence Interval
VR Validation Report
WLR Within Laboratory Reproducibility
WS Working Standard
DEFINITION OF TERMS

Absolute Recovery: Percentage recovery calculated from the response factor of a fortified sample divided by the response factor of a matrix standard.

Analyte: This is the component of a sample that has to be detected, identified and quantified. The term “analyte” includes, where appropriate, derivatives formed from the analyte during analysis.

Calibration (Standard) Curve: Is the relationship between instrument response and known concentrations of an analyte.

Certified Reference Material (CRM): A material that has a specified analyte content assigned to it.

Coefficient of Determination ($R^2$): The square of the correlation coefficient ($r$) for a linear curve.

Coefficient of Variation (CV): The standard deviation divided by the mean, expressed as a percentage.

Fortified Sample: A sample enriched (spiked) with a known amount of analyte.

Incurred Sample: A sample that contains a measurable concentration of the analyte of interest.

Intermediate Precision: The same definition as precision, but on data collected in the same laboratory over an extended period, preferably using different analysts and different equipment. The symbol for the intermediate precision standard deviation is $S_I$. Sometimes called within-laboratory reproducibility.

Internal Standard (IS): A known amount of a non-analyte compound that is added at the beginning of the analysis against which the relative response (RR) of an analyte can be determined.

Lab QC: Blank or sample associated with testing a laboratory procedure.

Limit (or Level) of Quantification (LOQ): Regarded as the lower limit for precise quantitative measurements. Commonly represented by the lowest concentration calibration standard, so that samples cannot be quantified below this standard (by definition).

Limit of Detection (LOD): Concentration of analyte which gives an instrument signal significantly different from the blank.

Limit of Reporting (LOR): The practical limit of residue quantification at or above the LOQ. The LOR must be no lower than the lowest calibration standard; a limit specified by a regulatory body or customer.

Matrix Blank Determination: This is the complete analytical procedure applied to a test portion taken from a sample from which the analyte is absent.

Matrix Recovery Sample: A sample prepared from the matrix blank to which known amounts of analytes and internal standards are added before extraction in order to establish the efficacy of the method.
Matrix Standard: A sample prepared from an extract of a matrix blank to which known amounts of analyte and internal standards are added after extraction.

Precision: The closeness of agreement between independent test results under stipulated conditions expressed as a standard deviation.

Primary Standard: A solution or pure compound as received from the supplier or manufacturer.

Reagent Blank Determination: This is the complete analytical procedure applied with the omission of the test portion or using an equivalent amount of suitable solvent in place of the test portion.

Relative Internal Standard Method: Calibration curve is a plot of RR times the concentration of internal standard versus concentration of analyte in calibration standards. The calibration standards contain both the analyte (at varying concentrations) and the internal standard at (normally) equal concentrations.

Relative Recovery: Percentage recovery calculated from the fortification concentration divided by the measured concentrations of the samples.

Relative Response (RR): Ratio of the response of an analyte to its internal standard. The test solution contains the internal standard only.

Secondary Standard: A solution of a compound or compounds derived from the primary standards by dilution and/or mixing.

Uncertainty of Measurement: The combination of precision and bias initially derived from precision and recovery experiments.

Working Standard: A solution used for the analysis of a sample. In some cases a working standard is the same as a secondary standard. In other cases, the working standard is a further dilution and/or mixing of secondary standards.