Sodium Fluoroacetate by LC-MS/MS

1 SCOPE OF APPLICATION

Description of an in-house method for the quantitative determination of sodium fluoroacetate in liquid and powdered milk- and soy-based infant formulas by liquid chromatography tandem mass spectrometry (LC-MS/MS).

The limit of quantification (LOQ) of sodium fluoroacetate is 1 µg/kg by this method.

An application of this method to matrices not covered by the scope of application requires an additional validation.

2 DEFINITION AND ABBREVIATIONS

2.1 Definition

Sodium fluoroacetate (Figure 1) is a synthetic pesticide known as “1080” and used to fight mammalian pest species. Farmers and graziers use the poison to protect pastures and crops from various herbivorous mammals. It is used as well to protect sheep and goats from predatory coyotes (predacide). In New Zealand and Australia it is employed to control invasive non-native mammals that prey on or compete with native wildlife and vegetation. Sodium fluoroacetate is highly toxic to mammals, including humans. This pesticide is approved for use in the following countries: USA, Canada, Mexico, Australia, New Zealand, Korea, Japan and Israel. New Zealand has used “1080” for pest control since the 1950’s, while the United States began use in the 1940’s.

Sodium fluoroacetate is also a naturally occurring poison found in at least 40 plants native in Australia, South and West Africa and Brazil.

![Figure 1. Chemical structure of sodium fluoroacetate](NaFC_2H_2O_2; CAS 62-74-8; MW 100 g/mol)
2.2 Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-MS/MS</td>
<td>Liquid Chromatography tandem Mass Spectrometry</td>
</tr>
<tr>
<td>ESI</td>
<td>Electro Spray Ionisation</td>
</tr>
<tr>
<td>SRM</td>
<td>Selected Reaction Monitoring</td>
</tr>
<tr>
<td>IS</td>
<td>Internal Standard</td>
</tr>
</tbody>
</table>

3 PRINCIPLE OF METHOD

Milk powder is first re-constituted in water. Acetonitrile is added to precipitate proteins. After centrifugation, the supernatant is washed with hexane and then acidified with concentrated sulphuric acid. QuEChERS salts (MgSO₄ and NaCl) are added for phase separation and the mixture is centrifuged. The resulting supernatant is evaporated to 0.5 mL remaining volume and centrifuged before LC-MS/MS analysis in selected reaction monitoring (SRM) by electrospray ionisation (ESI) in negative mode. The compound is analysed as its fluoroacetate anion.

Quantification is performed by the isotopic dilution approach using ¹³C labelled sodium fluoroacetate.

Positive identification of fluoroacetate in samples is conducted according to the confirmation criteria defined in EU Commission Decision 2002/657/EC [1].

4 SAFETY PRECAUTIONS

Material Safety Data Sheets (MSDS) should be available for all chemicals, inherent risks and corresponding safety precautions shall be identified.
Follow general safety precautions and environmental aspects as described in the local Safety, Health & Environment rules in place.

**WARNING** - Sodium fluoroacetate is highly toxic to humans. Take all necessary precautions especially when working with concentrated stock standard solutions.

5 CHEMICALS AND MATERIALS

*Commercial references are only a guideline. Use equivalent chemicals or materials when listed items are not locally available.*
5.1 Chemicals

Before using chemicals, refer to the Sigma/Aldrich Guide to Chemical Safety and/or other adequate manuals or safety data sheets approved by your local authorities and ensure that the safety guidelines are applied.

<table>
<thead>
<tr>
<th>CAS number</th>
<th>Chemical or reagent, purity (e.g. supplier, article number and website)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7732-18-5</td>
<td>Water for chromatography (e.g. Merck LiChrosolv® art. 1.15333.1000, <a href="http://www.emdmillipore.com">www.emdmillipore.com</a>)</td>
</tr>
<tr>
<td>75-05-8</td>
<td>Acetonitrile, hypergrade for LC-MS (e.g. Merck LiChrosolv® art. 100029, <a href="http://www.chemdat.info">www.chemdat.info</a>)</td>
</tr>
<tr>
<td>7487-88-9</td>
<td>QuEChERS extraction packets, 10 g, 200 foil packs per box, each pack containing 4 g of magnesium sulphate (MgSO₄) and 1 g of sodium chloride (NaCl) (e.g. Agilent art. 5982-7550, <a href="http://www.agilent.com">www.agilent.com</a>)</td>
</tr>
<tr>
<td>7647-14-5</td>
<td>Ammonium formate, LC-MS ultra, eluent additive for UHPLC-MS (e.g. Fluka art. 14266, <a href="http://www.sigmaaldrich.com">www.sigmaaldrich.com</a>)</td>
</tr>
<tr>
<td>540-69-2</td>
<td>Sulfuric acid, concentrated, w = 95 – 97 % (e.g. Merck art. 100731, <a href="http://www.merckmillipore.com">www.merckmillipore.com</a>)</td>
</tr>
<tr>
<td>7664-93-9</td>
<td>Formic acid, concentrated (e.g. Merck art. 100264, <a href="http://www.chemdat.info">www.chemdat.info</a>)</td>
</tr>
<tr>
<td>64-18-6</td>
<td>Sodium fluoroacetate, w = 99 %, 10 µg/mL in water (e.g. Dr Ehrenstorfer art. DRE-L13772000AL, <a href="http://www.lgcstandards.com">www.lgcstandards.com</a>)</td>
</tr>
<tr>
<td>62-74-8</td>
<td>¹³C₂-Sodium fluoroacetate, w = 99 %, isotopic purity &gt; 99.5 % (e.g. BDG Synthesis art. 130042-10, <a href="http://bdg.co.nz">http://bdg.co.nz</a>)</td>
</tr>
</tbody>
</table>

5.2 Materials

- Falcon tubes, conical, polypropylene, 50-mL (e.g. Becton Dickinson Labware, art. 352070, wwwbdbiosciences.com)
- Falcon tubes, conical, polypropylene, 15-mL (e.g., Becton Dickinson Labware, art. 352097, http://wwwbdbiosciences.com)
- Centrifuge with rotors adapted for 50-mL and 15-mL tubes, 4000 x g, temperature controlled (e.g. Multifuge Heraeus, www.thermo.com)
- Vortex (e.g. Millian Genie 2, http://www.milian.com)
- Centrifuge with rotor adapted for 2-mL tubes, 17’000 x g, (e.g. Heraeus Frisco 17, www.thermoscientific.com)
- Microcentrifuge tubes, polypropylene, 2 mL (e.g. Trefflab, art. 9607246901, www.treff-ag.ch)
- Analytical balance with precision range 0.01 mg

5.3 Special equipment and instrumentation

Where a specific model is cited, an alternative may be used if it has the same characteristics.

HPLC Agilent 1200 SL (www.agilent.com) coupled to a Sciex 5500 triple stage quadrupole mass spectrometer equipped with a TurbolonSpray® ionization source (www.abschiex.com).
5.3.1 HPLC column
Acquity UPLC BEH Amide, 2.1 mm x 100 mm, 1.7 µm (Waters art. 186004801, www.waters.com).

5.4 Glassware decontamination
No specific requirement.

6 PREPARATION OF REAGENTS

Volumes of glassware are purely indicative and may be modified as long as the proportion of reagents is maintained.

6.1 Sodium fluoroacetate stock standard solution, 10 µg/mL in water
The stock standard solution is available as ready-to-use 10-mL solution. Store at room temperature for the time given in the certificate of analysis.

6.2 Sodium fluoroacetate working standard solution, 1.0 µg/mL in acetonitrile:water (9 + 1)
Into a 10-mL volumetric flask, pipette 1.0 mL of the stock standard solution 10 µg/mL (6.1). Complete to volume with acetonitrile. Store at -20 °C for no longer than 6 months. Allow warming at room temperature before use.

6.3 Sodium fluoroacetate working standard solution, 0.2 µg/mL in acetonitrile
Into a 10-mL volumetric flask, pipette 2.0 mL of the stock standard solution 1 µg/mL (6.2). Complete to volume with acetonitrile. Store at -20 °C for no longer than 6 months. Allow warming at room temperature before use.

6.4 Sodium fluoroacetate working standard solution, 0.05 µg/mL in acetonitrile
Into a 10-mL volumetric flask, pipette 2.5 mL of the stock standard solution 0.2 µg/mL (6.3). Complete to volume with acetonitrile. Store at -20 °C for no longer than 6 months. Allow warming at room temperature before use.

6.5 13C2-Sodium fluoroacetate (IS) stock standard solution, 1000 µg/mL in water
Into a 10-mL volumetric flask, weigh 10 mg ± 0.1 mg of standard. Dissolve and complete to the mark with water. Alternatively (to minimize analyst exposure during weighing) weigh the container containing the analyte first (w1, in mg), then transfer its whole content into a 10-mL volumetric flask.
Dissolve and complete to mark with water for chromatography. Weigh again the empty original container once dried ($w_2$, in mg). Concentration of this solution in $\mu$g/mL is $(w_1- w_2)/(10 \times 1000)$. Store at -20 °C for no longer than 6 months. Allow warming at room temperature before use.

6.6 $^{13}$C$_2$-Sodium fluoroacetate (IS) working standard solution, 10 $\mu$g/mL in acetonitrile

Into a 10-mL volumetric flask, pipette 100 $\mu$L of the stock solution 1000 $\mu$g/mL (6.5). Complete to volume with acetonitrile. Store at -20 °C for no longer than 6 months. Allow warming at room temperature before use.

6.7 $^{13}$C$_2$-Sodium fluoroacetate (IS) working standard solution, 0.2 $\mu$g/mL in acetonitrile

Into a 50-mL volumetric flask, pipette 1000 $\mu$L of the working standard solution 10 $\mu$g/mL (6.6). Complete to volume with acetonitrile. Store at -20 °C for no longer than 6 months. Allow warming at room temperature before use.

6.8 Standard solutions for calibration curve

Into six separate 5-mL volumetric flasks, transfer the volumes of working standard solutions as described in the following table. Complete to the mark with acetonitrile. Store at -20 °C for no longer than 6 months. Allow warming at room temperature before use.

<table>
<thead>
<tr>
<th>Working standard solution of sodium fluoroacetate, 0.2 $\mu$g/mL (6.3) ($\mu$L)</th>
<th>STD 1</th>
<th>STD 2</th>
<th>STD 3</th>
<th>STD 4</th>
<th>STD 5</th>
<th>STD 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>150</td>
<td>300</td>
<td>500</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>Working standard solution of IS, 0.2 $\mu$g/mL (6.7) ($\mu$L)</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>Complete to the 5-mL mark</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This corresponds to:

<table>
<thead>
<tr>
<th>Concentration of sodium fluoroacetate (ng/mL)</th>
<th>0</th>
<th>2</th>
<th>6</th>
<th>12</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of IS (ng/mL)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

6.9 Solutions for LC-MS/MS

6.9.1 Mobile phase A, water containing 5 mM of ammonium formate and 0.01 % (v/v) formic acid

Into a weighing boat, weigh 315 mg ± 5 mg of ammonium formate. Transfer this mass into a 1000-mL volumetric flask. Add ca. 300 mL of water for chromatography and mix to dissolve. Add 100 $\mu$L of concentrated formic acid. Complete to volume with water for chromatography. Mix. Store at room temperature for no longer than 1 month.
6.9.2 Mobile phase B, acetonitrile

Use acetonitrile gradient grade for liquid chromatography.

6.9.3 Solution for flushing injection port, acetonitrile – water (1 + 1)

Into a 1000-mL volumetric flask, transfer by means of graduated cylinder, 500 mL of acetonitrile gradient grade for chromatography. Complete to volume with water for chromatography. Transfer into a HPLC bottle. Store at room temperature for no longer than 1 month.

7 SAMPLING AND PREPARATION OF TEST SAMPLES

7.1 Sampling procedure

A representative sample (min 100 g or 100 mL) should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

7.2 Laboratory sample

Store in the laboratory at room temperature until analysis, unless otherwise mentioned.

7.3 Test sample preparation

**Powdered sample:** Mix well the powdered laboratory sample by means of a spoon before taking a test portion. Alternatively, transfer the whole sample into a container of capacity about twice that of the laboratory sample volume. Close the container immediately. Mix thoroughly by repeatedly shaking and inverting the container.

**Liquid sample:** Shake thoroughly the container containing the sample.

8 PREPARATION OF TEST PORTIONS & EXTRACTION PROCEDURE

QC samples (certified, P-test, in-house reference samples or spiked samples) must be regularly included and analysed in duplicate. Different product types should be analysed regularly in duplicate. If necessary, different sized glassware may be substituted for specific volumes listed during the preparation of test solutions as long as the proper dilutions ratios are maintained.

8.1 Test portion preparation

8.1.1 Powdered sample

- Into a 50-mL polypropylene Falcon tube, weigh 5.0 g ± 0.1 g of powdered sample (7.3). Record the mass to 0.1 g.
• Add 20 mL of water for chromatography. Mix thoroughly by inversion and place onto a GenoGrinder shaker. Shake for 1.5 min at 1500 rpm. No lump should be visible.

• Transfer 5.0 g ± 0.1 g of this slurry into a 15-mL polypropylene Falcon tube. Record the mass to 0.1 g.

• Add 50 µL of the IS working solution 0.2 µg/mL (6.7). Mix thoroughly and make sure that the spiked volume is totally absorbed by the matrix. This spike corresponds to 10 µg/kg equivalent-in-sample concentration of IS.

8.1.2 Liquid sample

• Into a 15-mL polypropylene Falcon tube, weigh 5.0 g ± 0.1 g of liquid sample (7.3).

• Add 250 µL of the IS working solution 0.2 µg/mL (6.7). Mix thoroughly and make sure that the spiked volume is totally absorbed by the matrix. This spike corresponds to 10 µg/kg equivalent-in-sample concentration of IS.

8.2 Extraction procedure

• To the test portion prepared as described in 8.1.1 or 8.1.2, add 8 mL of acetonitrile. Mix thoroughly. Place onto a GenoGrinder shaker and shake for 1.5 min at 1500 rpm.

• Centrifuge at 4000 g at room temperature for 5 min and transfer the supernatant (ca. 9 mL to 10 mL) into a 50-mL Falcon tube.

• Add 10 mL of hexane. Place onto a GenoGrinder shaker and shake for 1.5 min at 1500 rpm.

• Centrifuge at 4000 g at room temperature for 5 min. Pipette the upper hexane phase and discard it to waste.

• Add 100 µL of concentrated sulphuric acid (H₂SO₄) to the solution containing the analyte. Mix thoroughly. The resulting pH must be ≤ 1 to have the analyte in its acidic form (pKa of fluoroacetic acid is 2.39).

• Add a buffer salt mixture (Agilent QuEChERS ready-to-use mix) containing 4.0 g ± 0.4 g of MgSO₄ and 1.0 g ± 0.1 g of NaCl. Immediately hand-shake by inversion or by vortexing to prevent any lump formation. Place onto a GenoGrinder shaker and shake for 1.5 min at 1500 rpm.

• Centrifuge at 4000 g at room temperature for 5 min and transfer the supernatant (ca. 5 mL) into a 15-mL Falcon tube.

• Evaporate the collected supernatant under a stream of nitrogen at 40 °C ± 2 °C until a 0.5 mL remaining volume. A mark at the 0.5 mL level is visible onto the tube. Do not evaporate to lower volumes to prevent loss on evaporation.
• Transfer the 0.5-mL remaining volume into a 2-mL tube and centrifuge at 17'000 g at room temperature for 5 min.

• Transfer the clear supernatant into a HPLC vial for further LC-MS/MS analysis.

8.3 Reagent blank

In order to control any contamination during the sample workup, a reagent blank must be analysed along with each series of routine samples. Water is used instead of milk. Proceeded exactly as described in 8.1 and 8.2.

9 INSTRUMENTAL CONDITIONS

9.1 LC-MS/MS analysis

Where a specific instrument is cited, an alternative may be used provided it has the same or better characteristics. As well, an alternative HPLC column may be used provided it allows a retention time of the first eluting analyte that is at least twice the retention time corresponding to the void volume of the column.

9.1.1 HPLC conditions (using an Agilent 1200SL HPLC system)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase A</td>
<td>Water containing 5 mM of ammonium formate and 0.01 % formic acid (6.9.1)</td>
</tr>
<tr>
<td>Mobile phase B</td>
<td>Acetonitrile (6.9.2)</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 µL</td>
</tr>
<tr>
<td>Column</td>
<td>Waters Acquity UPLC BEH Amide, 2.1 mm x 100 mm, 1.7 µm</td>
</tr>
<tr>
<td>Column oven temp</td>
<td>45 °C</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.45 mL/min</td>
</tr>
<tr>
<td>Needle wash</td>
<td>In flush port for 20 s using acetonitrile : water (1+1) solution (6.9.3)</td>
</tr>
<tr>
<td>Diverter valve</td>
<td>HPLC flow is directed into the MS detector between 1.0 min and 2.5 min</td>
</tr>
<tr>
<td>Gradient</td>
<td>LC gradient is described in Table 1</td>
</tr>
</tbody>
</table>

Table 1. LC gradient used for the analysis sodium fluoroacetate

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A (%)</th>
<th>B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>2.0</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>3.0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>4.5</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>4.6</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>8.0</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

Using these conditions, the compound elutes at ca. 1.7 min (see Enclosures 1 to 4).
9.1.2 MS parameters

MS parameters (Tables 2 and 3) are obtained by separately syringe-infusing standard solution (ca. 1 µg/mL) of each unlabelled and labelled compounds (syringe flow rate of 10 µL/min) along with the HPLC flow at 0.45 mL/min using a T connector. The HPLC flow is constituted with 10 % A (6.9.1) and 90 % B (6.9.2).

Table 2. Typical MS parameters for the analysis of sodium fluoroacetate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Applied Biosystems Sciex 5500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionisation type</td>
<td>Electrospray (ESI)</td>
</tr>
<tr>
<td>Polarity</td>
<td>Negative ionisation</td>
</tr>
<tr>
<td>Spray voltage</td>
<td>-4500 V</td>
</tr>
<tr>
<td>Source block temperature</td>
<td>500 °C</td>
</tr>
<tr>
<td>Gas</td>
<td>Curtin Gas: 30 psi</td>
</tr>
<tr>
<td></td>
<td>Ion Source Gas 1 (GS1): 40 psi</td>
</tr>
<tr>
<td></td>
<td>Ion Source Gas 2 (GS2): 40 psi</td>
</tr>
<tr>
<td>Source position adjustments</td>
<td>Vertical micrometer value: 5.0</td>
</tr>
<tr>
<td></td>
<td>Horizontal micrometer value: 5.0</td>
</tr>
<tr>
<td></td>
<td>Electrode protusion: 1.0 mm</td>
</tr>
<tr>
<td>Collision Energy (CE)</td>
<td>-15</td>
</tr>
<tr>
<td>Entrance potential (EP)</td>
<td>-10 V</td>
</tr>
<tr>
<td>Collision exit potential (CXP)</td>
<td>-9 V</td>
</tr>
<tr>
<td>Declustering potential (DP)</td>
<td>-45 V</td>
</tr>
<tr>
<td>CAD gas pressure (MRM)</td>
<td>Medium (8)</td>
</tr>
<tr>
<td>Resolution</td>
<td>High on each quadrupole</td>
</tr>
<tr>
<td>Scan time (for each transition)</td>
<td>100 ms</td>
</tr>
</tbody>
</table>

Table 3. Transition reactions monitored for the analysis of sodium fluoroacetate (as its fluoroacetate anion) and its corresponding IS and peak area ratios along with their limit of acceptance according to CD 2002/657/EC [1]

<table>
<thead>
<tr>
<th>Transition reactions (m/z) used for</th>
<th>Peak area ratio ± limit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quantification</td>
</tr>
<tr>
<td>Fluoroacetate</td>
<td>77.0 → 33.0</td>
</tr>
<tr>
<td>13C2- Fluoroacetate (IS)</td>
<td>79.0 → 34.0</td>
</tr>
</tbody>
</table>

Note: m/z 57 corresponds to the loss of hydrofluoric acid [M-HF]- and m/z 33 to the loss of carbone dioxide [M-CO2]-.
9.2 Instrument check test

Before routine analysis, ensure that your LC-MS/MS apparatus is working in conditions such as the method stays fit for purposes. This involves to inject a low concentration calibrant (e.g. STD2, 6.8) to check that sensitivity of the instrument is adequate.

10 OPERATING PROCEDURE & DETERMINATION

10.1 Sequence set up

Inject solutions in the following order: acetonitrile (as blank solvent) at least three times, standard solutions (6.8), acetonitrile at least three times, reagent blank (8.3), extract solutions (8.2) and standard solutions (6.8) again. Inject acetonitrile after each three to four extract solutions to check for any carry-over.

10.2 Calibration

Construct a calibration curve by plotting peak area ratio of the analyte and its IS (= y axis) against concentration ratio of the analyte and its IS (= x axis). Calculate the slope and the intercept by linear regression. Check the linearity of the calibration (regression coefficient R² should be higher than 0.98 and relative standard deviation of the average of response factors (= y/x) should be < 15 %).

10.3 Identification and confirmation

Sodium fluoroacetate is identified and confirmed when the following criteria are fulfilled [1]:

a) The ratio of the chromatographic retention time of the analyte to that of its IS, i.e. the relative retention time, corresponds to that of the averaged relative retention time of the calibration solutions within a ± 2.5 % tolerance.

b) The peak area ratios from the different transition reactions recorded for the analyte and its IS are within the tolerances fixed by the EU criteria [1] as shown in Table 3.

10.4 Time of analysis

Following this procedure, 20 samples can be analysed within 24 h.

11 CALCULATIONS AND EXPRESSION OF RESULTS

11.1 Calculation

Calculate the mass fraction, w, of sodium fluoroacetate in microgram per kilogram of sample (µg/kg), using the equation:
\[ w = \left( \frac{A_a}{A_{is}} \right) - I \times \frac{m_{is}}{m_a} \]

where \( A_a \) = peak area of the analyte in the sample (transition reaction used for quantification); \( A_{is} \) = peak area of the IS in the sample (transition reaction used for quantification); \( I \) = intercept of the regression line for the transition reaction used for quantification; \( S \) = slope of the regression line for the transition reaction used for quantification; \( m_{is} \) = mass of IS added to the test portion, in ng (i.e. 10 ng for powdered sample and 50 ng for liquid sample); \( m_a \) = mass of the test portion, in g (i.e. 1 g for powdered sample and 5 g for liquid sample)

11.2 Expression of Results

Report the result of sodium fluoroacetate in µg/kg with one significant figure. Non detected amount must be expressed as < 1 µg/kg.

12 PERFORMANCE CHARACTERISTICS

12.1 Linearity

Linearity was verified over the 0 – 2 area ratio range, corresponding to 0 – 0.8 ng of sodium fluoroacetate (0.4 ng of IS) injected on-column. The calibration follows a linear model with \( R^2 > 0.99 \) and relative standard deviation of the average of response factors < 15 %.

12.2 Limit of quantification (LOQ)

LOQ has been estimated by considering a signal-to-noise (S/N) ratio of 10 at the transition reaction used for quantification while having S/N of at least 3 at the transition reaction used for peak confirmation. This LOQ is 1 µg/kg.

12.3 Recovery

Recovery data have been obtained by spiking four powdered milk-based infant formulas, two soy-based infant formula and three liquid milk-based infant formula at the 1 µg/kg level in duplicate. Samples were previously known to be free of sodium fluoroacetate.

Average recovery data were 113 % ± 5 % (n=9). Chromatograms are shown in Enclosures 1 to 4.

13 INTERNAL CONTROL PLAN

QC samples (certified, P-test, in-house reference samples or spiked samples) must be regularly included and analysed in duplicate.
13.1 Spiked experiment

Calculate the recovery rate (Rec) of the spiked sample using the following equation:

\[
Rec = \frac{\rho_T - \rho_N}{\rho_{Spiked}} \times 100
\]

where \(\rho_T\) is the total concentration of sodium fluoroacetate measured in the spiked sample in micrograms per kg, \(\rho_N\) is the native concentration of sodium fluoroacetate measured in the non-spiked sample in micrograms per kg, \(\rho_{Spiked}\) is the concentration of sodium fluoroacetate spiked in the sample in micrograms per kg (calculated value).

The recovery rate should be between 90 – 120% when spiked at the 1 µg/kg level.

14 LITERATURE


15 ENCLOSURES

1. **Enclosure 1** – LC-MS/MS chromatograms of a powdered milk-based (lactose free) infant formula unspiked and spiked at the 1 µg/kg (IS 10 µg/kg) level.
2. **Enclosure 2** – LC-MS/MS chromatograms of a liquid milk-based infant formula (ready-to-feed) unspiked and spiked at the 1 µg/kg (IS 10 µg/kg) level.
3. **Enclosure 3** – LC-MS/MS chromatograms of a powdered soya-based infant formula unspiked and spiked at the 1 µg/kg (IS 10 µg/kg) level.
4. **Enclosure 4** – LC-MS/MS chromatograms of a powdered milk infant formula unspiked and spiked at the 1 µg/kg (IS 10 µg/kg) level.
Enclosure 1 – LC-MS/MS chromatograms of a powdered milk-based (lactose free) infant formula unspiked and spiked at the 1 µg/kg (IS 10 µg/kg) level
Enclosure 2 – LC-MS/MS chromatograms of a liquid milk-based infant formula (ready-to-feed) unspiked and spiked at the 1 µg/kg (IS 10 µg/kg) level
Enclosure 3 – LC-MS/MS chromatograms of a powdered soya-based infant formula unspiked and spiked at the 1 µg/kg (IS 10 µg/kg) level
Enclosure 4 – LC-MS/MS chromatograms of a powdered milk infant formula unspiked and spiked at the 1 µg/kg (IS 10 µg/kg) level