LC-MS/MS Analysis of Emerging Food Contaminants

Detection of Pesticide 1080 in Milk and Infant Formula using the SCIEX QTRAP® 6500+ System

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Introduction

Recently (November 2014), threats in the form of letters were sent to farming and dairy industry leaders in New Zealand. The letters were accompanied by small packages of milk powder that were shown to contain a concentrated form of the pesticide 1080 (sodium fluoroacetate). The sender demanded that the New Zealand government stop using 1080 for pest control. Sodium fluoroacetate is used to protect New Zealand's native flora and fauna against introduced pests like possums and ferrets. Opponents, however, argue that it also kills native animals and contaminates the environment.[1-2]

Such criminal threats are a potential danger and weaken consumers' trust in the food supply chain. Accurate and reliable analytical methods are needed to monitor food ingredients and final products to ensure food safety in light of this threat.

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is an ideal analytical technique to detect polar analytes in complex food samples.

Here we present first results of method development to detect sodium fluoroacetate in milk and infant formula. The sample preparation protocol and LC conditions were adopted from the AOAC First Action Official Method 2015.03.[3] Samples were extracted using acetonitrile followed by a fat removal step using hexane. LC separation was achieved using a HILIC column in normal phase mode. The mass spectrometer was operated in negative polarity using Electrospray Ionization (ESI) and Multiple Reaction Monitoring (MRM). In MRM mode the transition of a molecular ion into a characteristic fragment ion is monitored. The monitoring of multiple fragment ions allows not only quantitation, but also highly confident identification based on the ratio between quantifier and qualifier transitions.

Initial studies show that sodium fluoroacetate can be detected at concentrations below 0.1 ng/mL (below 10 parts-per-billion, ppb in matrix samples) using the SCIEX QTRAP® 6500+ LC-MS/MS system, with good accuracy and repeatability. Linearity for quantitation was achieved over 4 orders of magnitude (0.02 to 500 ng/mL).

Experimental

Standards

Sodium fluoroacetate (Pestanal, analytical standard, Sigma-Aldrich #31220) was purchased from Sigma Aldrich.

The internal standard, sodium fluoroacetate-$^{13}C_2$ (99%) D_2 (98%), was purchased from Cambridge Isotope Laboratories.

Sample preparation

The sample preparation protocol was adopted from the AOAC First Action Official Method 2015.03.[3]

5g of infant formula was thoroughly mixed with 20 mL of water and a 5 g aliquot of the slurry was extracted. 5 mL of milk samples were extracted directly. Each sample was spiked with the internal standard before extraction. Samples were extracted with 10 mL of acetonitrile. After centrifugation the supernatant was washed using 10 mL of hexane. After pH adjustment with H_2SO_4 the extract was phase-separated using QuEChERS salts ($MgSO_4$ and NaCl), diluted and analyzed by LC-MS/MS in negative polarity.

LC Separation

LC separation was performed using a SCIEX ExionLC™ AC system with an BEH Amide column (100 x 2.1 mm, 1.7 µm) and a normal phase gradient consisting of (A) water with 5 mM

ammonium formate and 0.01% formic and (B) acetonitrile (Table 1).

The injection volume was 20 µL.

Table 1. LC gradient conditions

Time (min)	Flow Rate (µL/min)	A (%)	B (%)
0.0	450	10	90
2.0	450	10	90
3.0	450	60	40
4.5	450	60	40
4.6	450	10	90
8.0	450	10	90

MS/MS Detection

The SCIEX QTRAP® 6500+ system with IonDrive™ source was operated using an ESI probe in negative polarity. The MRM transitions monitored were 77/57and 77/33 for the target analyte and 81/60 and 81/35 for the internal standard, respectively.

Ion source parameters were set to the following values: CUR = 30 psi; Gas1 = 50 psi; Gas2 = 60 psi; TEM = 650ºC; and IS = -4500 V. The collision gas (CAD) was set to 10.

Results and Discussion

An example chromatogram is shown in Figure 1. The selected LC conditions guaranteed separation from the majority of matric components (retention time 1.8 min) to minimize potential matrix effects (i.e. ion suppression).

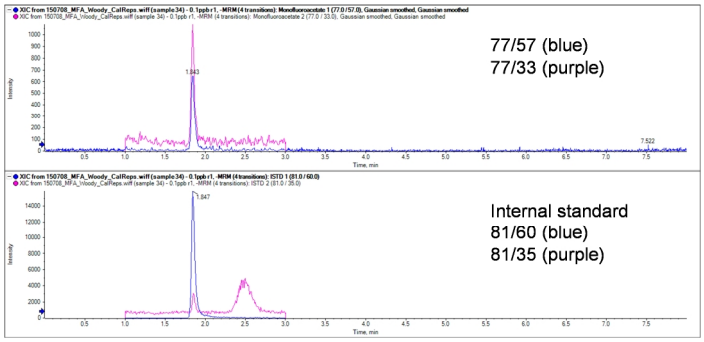

Figure 1. Example chromatogram of a 0.1 ng/mL standard of sodium fluoroacetate with internal standard

Sodium fluoroacetate was accurately and reproducibly identified and quantified. Results of the repeat analysis of a 0.1 ng/mL standard and coefficients (%CV) of variation (n=3) are shown in Figure 2.

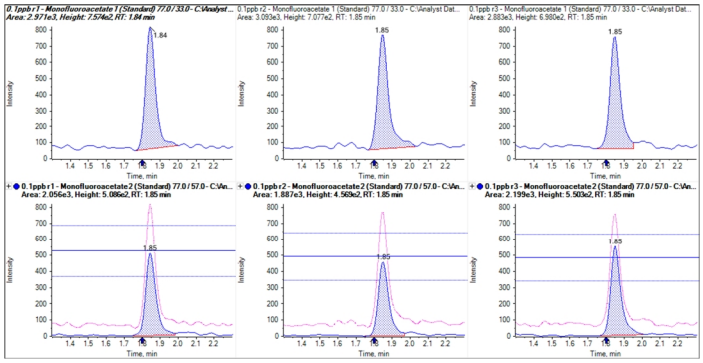

Figure 2. Repeat analysis at 0.1 ng/mL standard, 2 MRM transitions were monitored and the ratio of quantifier (top) and qualifier transition (bottom) was used for compound identification, 30% MRM tolerances are displayed in the peak review following SANCO/12571/2013)

Identification was achieved using the ratio of quantifier and qualifier ion. MRM ratios were well within the ±30% tolerance set by food testing guidelines (i.e. SANCO/12571/2013).[4] The MRM ratio was automatically calculated in MultiQuant™ software (version 3.0.2) and tolerance levels are displayed in the peak review window for easy data review (Figure 2).

Calibration lines for both MRM transitions are shown on Figure 3. Good linearity was achieved for the quantifier transition from 0.02 to 500 ng/mL and for the qualifier transition from 0.05 to 500 ng/mL with r > 0.999 using linear fit and 1/x weighting.

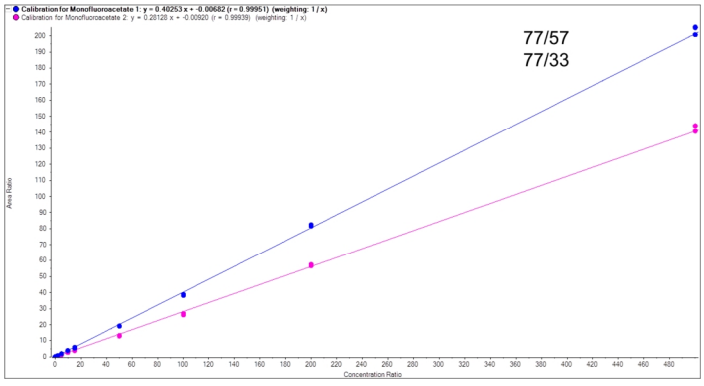

Figure 3. Calibration lines from 0.02 to 500 ng/mL for the quantifier ion (blue) and 0.05 to 500 for the qualifier ion (purple)

Repeatability was excellent at all concentration levels and well below 10% (Table 2).

Table 2. Repeatability of sodium fluoroacetate detection using the SCIEX QTRAP® 6500+ system

Concentration (ng/mL)	%CV of Quantifier MRM	%CV of Qualifier MRM
0.1	4.37%	5.17%
1.0	0.66%	2.53%
10.0	1.62%	1.10%
100.0	0.64%	1.89%

Application of the Developed Method to Food Samples

Initial studies show that the developed method can detect sodium fluoroacetate in matrix samples at 10 ppb. Figures 4a and 4b show the pre-extraction and post-extraction spike of sodium fluoroacetate into milk (2% fat) and NIST infant formula at 10 ppb.

The post-extraction spike indicates ion suppression of ~40-50% for both matrices, which was compensated by the internal standard. The pre-extraction spike shows an additional recovery loss of ~30% for milk and ~45% for infant formula.

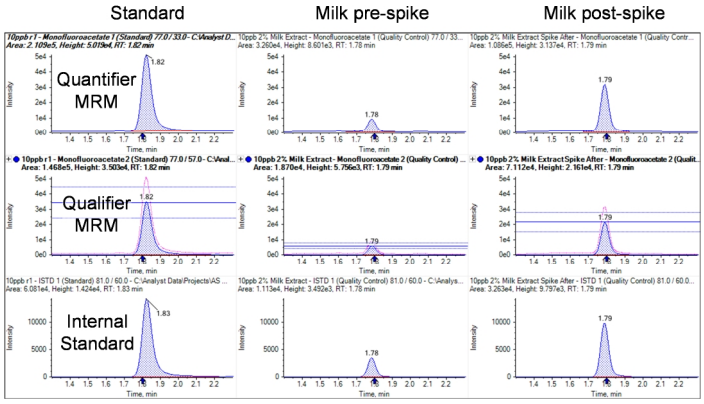

Figure 4a. Chromatograms of a standard in comparison to the pre-extraction and post-extraction spike of milk at 10 ng/mL; quantifier ion (top), qualifier ion with ratio tolerances (middle), and internal standard (bottom)

Summary

First results of method development were presented to detect sodium fluoroacetate by LC-MS/MS using the SCIEX QTRAP® 6500+ system. Samples were prepared by simple acetonitrile extraction and defatting using hexane. LC separation was achieved using a HILIC column and normal phase chromatography. The MS/MS was operated in MRM mode, enabling limits of quantitation below 0.1 ng/mL (below 10 ppb in milk and infant formula samples). Good accuracy, repeatability, and linearity for quantitation were achieved over 4 orders of magnitude.

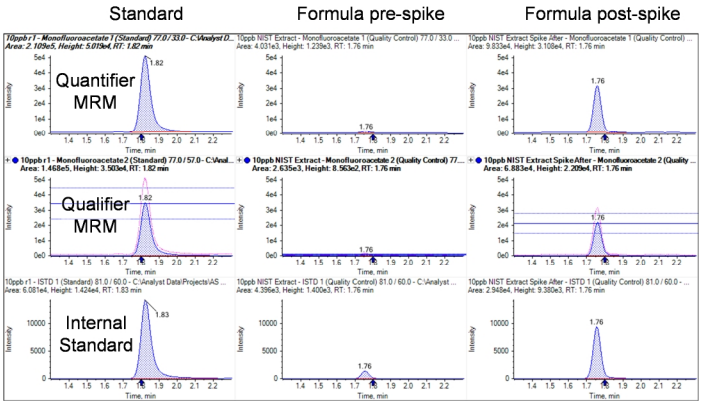

Figure 4b. Chromatograms of a standard in comparison to the pre-extraction and post-extraction spike of infant formula at 10 µg/kg; quantifier ion (top), qualifier ion with ratio tolerances (middle), and internal standard (bottom)

Further experiments are planned to simplify sample extraction and utilize extensive dilution to improve recoveries and further reduce ion suppression.

References

1 http://www.nzherald.co.nz/business/news/article.cfm?c_id=3&objectid=11414980

2 http://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=11415012

3 AOAC INTERNATIONAL: 'Sodium Fluoroacetate by LC-MS/MS' First Action Official Method 2015.03 (3/30/2015) Rev 2

4 SANCO Document: 'Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed' SANCO/12571/2013

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