Validation of Ergot Alkaloids in Feeds by LC-MS/MS
AAFCO Annual Meeting 2018
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Overview

• CFIA - OLC - FFC
• What are Ergot Alkaloids (EA)?
• Upcoming Regulations
• Method Summary
• Validation Results
• Challenge - Standards
• Next Steps
CFIA - OLC - FFC

• Regulatory analytical testing for CFIA enforcement and monitoring programs

• Accredited for method development, validation and evaluation to meet CFIA’s changing needs

• Expert scientific advice to Program officials, industry, other laboratories, national and international regulatory and scientific organizations
What are Ergot Alkaloids?

- Ergot Infection
  - Rye, triticale, wheat, barley, oats and grasses

- Ergotism
  - What causes ergotism
  - Effect on humans
  - Effect on animals

What are EAs?

• The most common ergot alkaloids produced by Claviceps purpurea
  
  • Ergocornine, Ergocorninine
  • Ergocristine, Ergocristinine
  • Ergocryptine, Ergocryptinine
  • Ergometrine, Ergometrinine
  • Ergosine, Ergosinine
  • Ergotamine, Ergotamine

• Epimers r-isomers (-ine) are biologically active
• Epimers s-isomers (-inine) are less active

Ergometrine (www.chemspider.com)
## Upcoming Regulations

<table>
<thead>
<tr>
<th>Species/Class of Animal</th>
<th>Proposed Maximum Limit: Single Ingredient Feeds (e.g., cereals and cereal by-products), in an amount not exceeding (ppm)</th>
<th>Proposed Maximum Limit: Total Diet in an amount not exceeding (ppm)</th>
<th>Current Action Level: Complete diets, in an amount not exceeding (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaned piglets</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Growing-finishing pigs and sows</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Poultry</td>
<td>6</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Cattle</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.6</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>Horses</td>
<td>0.3</td>
<td>0.15</td>
<td>2</td>
</tr>
</tbody>
</table>
Method Summary

• FD-TOXINS-ERG - Determination of EAs in Feed and Feed Ingredients by Liquid Chromatography with Tandem Mass Spectrometer Detection

• 50g samples are extracted by shaking with acetonitrile/ammonium carbonate (2.08 mmol/L) (84 + 16) solution.

• The extract is filtered and purified using commercially available mycosep column.

• The purified extract is then diluted with acetonitrile prior to determination by reversed phase LC-MS/MS.

• Results are quantified using a product ion for each analyte and confirmed by another product ion of the same analyte.
Method Summary

- Agilent 1200 series HPLC
- Sciex API5000 LC/MS/MS
- Electro spray ionisation
Method Summary

Example of a Wheat Spike

- **Column:**
  - Phenomenex Gemini 5μm C18 110Å, 150 x 2.0 mm

- **Mobile phase**
  - A 2.08 mM ammonium carbonate
  - B Acetonitrile

- **Gradient:**
  - 95% A to 20% in 15 min.

- **Flow rate:**
  - 500 µL/min

- **Autosampler Temperature:**
  - 15°C
Method Summary

• It is important to use indirect incandescent lighting in fume hoods or no lighting. Do not leave solution at room temperature for prolonged periods of time.

• Stock solutions are kept in -80°C and all intermediate/spike/working solution are kept at -20°C and only taken out when needed.
Method Summary

• Analyte Stability - Sample
  • Extraction solution at room temperature
    – Epimerization was observed after 3 hours

• Cleaned-up fraction and final solution
  – Stable for at least 4 days in freezer
## Method Summary

### FD-TOXINS-ERG Reporting Limit

<table>
<thead>
<tr>
<th>Toxins</th>
<th>Reporting limit µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergometrine + Ergometrinine</td>
<td>20</td>
</tr>
<tr>
<td>Ergosine + Ergosinine</td>
<td>20</td>
</tr>
<tr>
<td>Ergotamine + Ergotaminine</td>
<td>20</td>
</tr>
<tr>
<td>Ergocornine + Ergocorninine</td>
<td>20</td>
</tr>
<tr>
<td>Ergocryptine + Ergocryptinine</td>
<td>20</td>
</tr>
<tr>
<td>Ergocristine + Ergocristinine</td>
<td>20</td>
</tr>
<tr>
<td>Sum of Ergot Alkaloids</td>
<td>120</td>
</tr>
</tbody>
</table>
Validation – Matrices & Analyte Confirmation

• Validated in the following matrices
  – Feed Ingredients:
    • Wheat, Barley, Triticale, Oats, Rye and Wheat Dried Distiller’s Grain (DDG)
  – Complete Feeds:
    • Cattle, Swine, Layer Mash, Ewe Ration, Horse

• Confirmation of analyte
  – Retention time ratio (±0.2 min from standards)
  – Ion ratio EU criteria
Validation – LOD/LOQ

• Each individual EA
  – LOD ranged from 1 µg/kg to 7 µg/kg
  – LOQ ranged from 3 µg/kg to 16 µg/kg

• Each pair of EA
  – LOD ranged from 2 µg/kg to 8 µg/kg
  – LOQ ranged from 5 µg/kg to 18 µg/kg

• Sum of EA
  – LOD 29 µg/kg and LOQ 67µg/kg
Validation – Analytical Range & Linearity

- **R values ≥ 0.995 (6 levels)**

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Working standard Range (ng/ml)</th>
<th>Linear Sample Range (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-ines</td>
<td>0.080 to 16</td>
<td>4 to 1360</td>
</tr>
<tr>
<td>-inines</td>
<td>0.020 to 4</td>
<td>1 to 340</td>
</tr>
</tbody>
</table>
Validation – Accuracy and Precision

• Validation Plan:
  – 3 X 3 Study
  – 6 feed ingredients and 5 complete feeds
  – 3 levels
    • -ines from 100 µg/kg to 800 µg/kg
    • -inines from 25 µg/kg to 200 µg/kg
    • Sum of –ine + -inine 125 µg/kg to 1000µg/kg
  – 3 replicates
Validation – Accuracy

• Criteria for validation
  – For each analyte pair (-ine + -inine)
    • 70-125% for concentrations of ≥10 µg/kg (ppb)
    • 75-120% for concentrations of ≥1000 µg/kg (ppb)

• Sum of EA all had acceptable results
  – Recoveries obtained ranged from 91% to 113%
Validation – Accuracy

- Range of recoveries

<table>
<thead>
<tr>
<th>Toxins</th>
<th>Recovery Range %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergometrine + Ergometrinine</td>
<td>66-128</td>
</tr>
<tr>
<td>Ergosine + Ergosinine</td>
<td>88-110</td>
</tr>
<tr>
<td>Ergotamine + Ergotaminine</td>
<td>88-113</td>
</tr>
<tr>
<td>Ergocornine + Ergocorninine</td>
<td>93-111</td>
</tr>
<tr>
<td>Ergocryptine + Ergocryptinine</td>
<td>93-110</td>
</tr>
<tr>
<td>Ergocristine + Ergocristinine</td>
<td>94-111</td>
</tr>
<tr>
<td>Sum of Ergot Alkaloids</td>
<td>91-113</td>
</tr>
</tbody>
</table>

- PT participation: z-scores ranged from -1.0 to -0.1
Validation – Precision

- 3X3 Study - Range of Correlation of Variation

<table>
<thead>
<tr>
<th>Toxins</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergometrine + Ergometrinine</td>
<td>2-8</td>
</tr>
<tr>
<td>Ergosine + Ergosinine</td>
<td>2-5</td>
</tr>
<tr>
<td>Ergotamine + Ergotaminine</td>
<td>1-7</td>
</tr>
<tr>
<td>Ergocornine + Ergocorninine</td>
<td>1-4</td>
</tr>
<tr>
<td>Ergocryptine + Ergocryptinine</td>
<td>1-4</td>
</tr>
<tr>
<td>Ergocristine + Ergocristinine</td>
<td>1-4</td>
</tr>
<tr>
<td><strong>Sum</strong></td>
<td><strong>2-5</strong></td>
</tr>
</tbody>
</table>
Validation – Precision

• Incurred Sample
• CV obtained in Control sample (n=4)
  – Each analyte pair ranged from 1% to 3%
  – Sum of EA 2%

• CV obtained in 3 Lab Samples (n=4)
  – Each analyte pair ranged from 1% to 26%
  – Sum of EA ranged from 1% to 11%
Challenges - Standards

• 4 of the 12 ergot alkaloids standards are controlled substances
  – Ergometrine and ergometrinine
  – Ergotamine and ergotaminine

• Standards are unstable once in solution
  – 2 months expiry date from manufacturer
  – Storage condition
  – Manipulation
Challenges - Standard

• Standard Stability Study
  – QC every 2 months
  – New standard preparation after 6 months

• Finding commercially available QC

• Controlling Epimerization
Next Steps

• Add the method to the scope of accreditation, ISO 17025:2017
• Scope expansion for the Ergovaline/Ergovalinine
• Continue looking for affordable internal standard and reference material
Acknowledgment

• Mélanie Titley & Andrée Demers