



Canadian Food
Inspection Agency

Agence canadienne
d'inspection des aliments

Validation of Ergot Alkaloids in Feeds by LC-MS/MS

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Canada

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

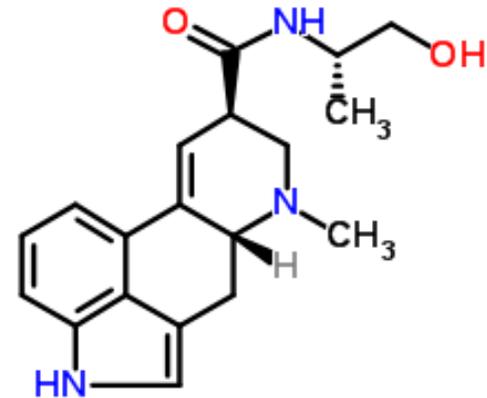


Dominique Jacquin,
<https://en.wikipedia.org/wiki/Ergotism>

What are EAs?

- The most common ergot alkaloids produced by *Claviceps purpurea*

- Ergocornine, Ergocorninine
- Ergocristine, Ergocristinine
- Ergocryptine, Ergocryptinine
- Ergometrine, Ergometrinine
- Ergosine, Ergosinine
- Ergotamine, Ergotamine



Ergometrine
(www.chemspider.com)

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

Upcoming Regulations

Proposed and current maximum levels for total ergot alkaloids

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

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

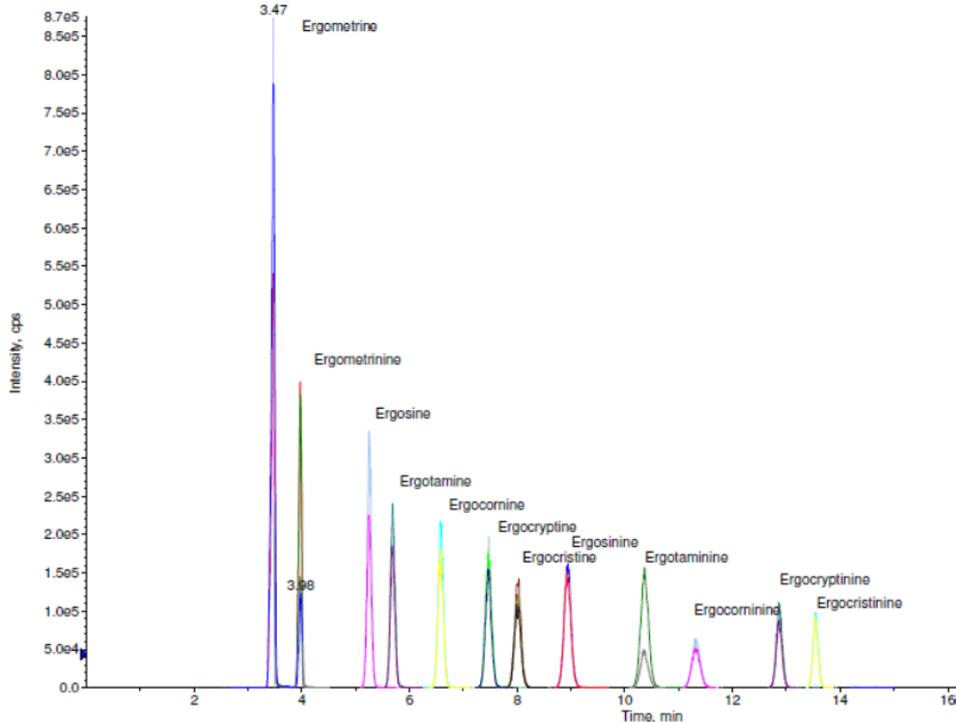
- Agilent 1200 series HPLC
- Sciex API5000 LC/MS/MS
- Electro spray ionisation



Method Summary

Example of a Wheat Spike

XIC of +MRM (26 pairs): 326.227/223.100 amu Expected RT: 3.5 ID: Metrine 1 from Sample 36 (2) 2017-0039 (Wheat) Spike2) of Erg...



- **Column:**
- Phenomenex Gemini 5 μ m C18 110 \AA , 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 μ L/min
- **Autosampler Temperature:**
- 15 $^{\circ}$ 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**

Toxins	Reporting limit µg/kg
Ergometrine + Ergometrinine	20
Ergosine + Ergosinine	20
Ergotamine + Ergotaminine	20
Ergocornine + Ergocorninine	20
Ergocryptine + Ergocryptinine	20
Ergocristine + Ergocristinine	20
Sum of Ergot Alkaloids	120

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 $\mu\text{g}/\text{kg}$ to 7 $\mu\text{g}/\text{kg}$
 - LOQ ranged from 3 $\mu\text{g}/\text{kg}$ to 16 $\mu\text{g}/\text{kg}$
- Each pair of EA
 - LOD ranged from 2 $\mu\text{g}/\text{kg}$ to 8 $\mu\text{g}/\text{kg}$
 - LOQ ranged from 5 $\mu\text{g}/\text{kg}$ to 18 $\mu\text{g}/\text{kg}$
- Sum of EA
 - LOD 29 $\mu\text{g}/\text{kg}$ and LOQ 67 $\mu\text{g}/\text{kg}$

Validation – Analytical Range & Linearity

- R values ≥ 0.995 (6 levels)

Isomer	Working standard Range (ng/ml)	Linear Sample Range ($\mu\text{g}/\text{kg}$)
-ines	0.080 to 16	4 to 1360
-inines	0.020 to 4	1 to 340

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 $\geq 10 \mu\text{g}/\text{kg}$ (ppb)
 - 75-120% for concentrations of $\geq 1000 \mu\text{g}/\text{kg}$ (ppb)
- Sum of EA all had acceptable results
 - Recoveries obtained ranged from 91% to 113%

Validation – Accuracy

- Range of recoveries

Toxins	Recovery Range %
Ergometrine + Ergometrinine	66-128
Ergosine + Ergosinine	88-110
Ergotamine + Ergotaminine	88-113
Ergocornine + Ergocorninine	93-111
Ergocryptine + Ergocryptinine	93-110
Ergocristine + Ergocristinine	94-111
Sum of Ergot Alkaloids	91-113

- PT participation :z-scores ranged from -1.0 to -0.1

Validation – Precision

- 3X3 Study - Range of Correlation of Variation

Toxins	CV %
Ergometrine + Ergometrinine	2-8
Ergosine + Ergosinine	2-5
Ergotamine + Ergotaminine	1-7
Ergocornine + Ergocorninine	1-4
Ergocryptine + Ergocryptinine	1-4
Ergocristine + Ergocristinine	1-4
Sum	2-5

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
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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

