

# HPLC/MS method for the determination of ergot alkaloids in cereals and cereal products

Area of research interest: [Chemical hazards in food and feed](#)

Project code: C03057

Conducted by: Central Science Laboratory (CSL)

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## Background

Ergot alkaloids are mycotoxins produced by the ergot causing fungi of all species of the *Claviceps* genus, most notably by *C. purpurea*, and mainly affect cereals and grasses. The fungus replaces the developing grain or seed with an ergot sclerotium. The sclerotia are harvested together with the cereal crop and can thus lead to contamination of cereal based food and feed products. Ergotism was common place in the middle ages and can cause hallucinations and in extreme cases even loss of limbs. Fungal infections are most prevalent in rye and triticale that have open florets but also wheat, sorghum and other small grains can be affected.

Ingestion of the ergot alkaloids can cause ergotism in humans and animals. In some severe cases symptoms can include intense pain resulting from vasoconstriction and subsequent gangrene with possible loss of fingers, hands, feet and even entire limbs. Other symptoms can include abdominal pains, vomiting, burning sensations of the skin, insomnia and hallucinations (ergotamine is a precursor of LSD). Ergotism was common place in the middle ages (it was known as 'St Anthony's fire') but has practically been eliminated as a significant human disease in the developed world. It remains an important veterinary problem, particularly in cattle, horses, sheep, pigs and chicken.

In the EU no regulatory limits apply to ergot alkaloids in grain or processed products. The EU Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) has reviewed the ergot issue recently (EFSA, 2005). EFSA concluded that the alkaloid concentrations are very variable and a consistent relationship between the amount of sclerotia and the total ergot alkaloid (ergoline) concentration cannot be established.

The aim of this project was to develop a validated analytical method covering the major ergot alkaloids found in grains this would enable further research and study of the ergot problem, providing the means for the acquisition of data on the contamination levels of feed and food and characterising ergot toxins. Currently the visible sclerotia themselves are used to determine contamination. In the long term the method is likely to help in the development of a method suitable for use by official control laboratories, should limits be set by the EU Commission.

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## Research Approach

The method development involved the optimisation of extraction and clean-up procedures using spiked and naturally contaminated samples. Owing to the potential for very high levels of ergot alkaloids in mixed grain samples, all naturally contaminated sample analyses were conducted on individual grain types (i.e. wheat or barley or oats or rye). The matrix effects of naturally contaminated raw cereal and processed cereal food samples were investigated. The validation studies revealed the precision, recovery, LOD and LOQ and the measurement uncertainty of the method was also calculated. Due to the lack of appropriate ergot alkaloid reference materials a comparison study was carried out between three laboratories to check for systematic errors and to reveal the comparability of the developed method.

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## Results

A method has been developed and validated for 10 different cereal and food samples which enabled the quantification of the 6 major ergot alkaloids defined by EFSA (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine) and their corresponding epimers (-inines). This is of considerable importance in terms of the differences in toxicity of the isomeric forms. The method performance was satisfactory when tested in a mini inter-comparison study with two other laboratories that used alternative methods. The newly developed method showed good comparability with the results obtained from the other two participants.

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## Additional Info

The extraction and clean-up procedure is simple and the analysis time short. The limits of quantification were 0.17 to 2.78 µg/kg depending on the analyte and matrix. Recovery values for the 12 ergot alkaloids spiked into 10 different matrices at levels of 5, 50 and 100 µg/kg were between 70 and 105% for 85 of 90 recovery measurements made over six days.

Measurement uncertainty values were highly satisfactory. At a concentration level of 5 µg/kg the expanded measurement uncertainty ranged from  $\pm 0.56$  to  $\pm 1.49$  µg/kg, at a concentration level of 100 µg/kg the expanded measurement uncertainty ranged from  $\pm 8.9$  to  $\pm 20$  µg/kg. Both LOQs and measurement uncertainties were dependent on the analyte but almost independent of the matrix. The method performance was satisfactory when tested in a mini-intercomparison study with two other laboratories that used alternative methods.

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## Published Papers

- R. Krska, G. Stubbings, R. McArthur, C. Crews. A rapid LC/MS/MS method for the determination of 6 major ergot alkaloids and their epimers. Anal. Bioanal. Chem. (submitted in January 2008).
- M. Hafner, R. Krska, C. Crews, K. Fog-Nielsen. Stability of ergot alkaloid calibrants. Journal of AOAC International (in preparation).
- Krska, R., Berthiller, F., Schuhmacher, R., Nielsen, K. F. and Crews, C. (2007). Determination of ergot alkaloids: Purity assessment of standards and optimisation of extraction and clean-up conditions for food samples. In preparation for submission to: J. AOAC Int.
- Krska, R., Stubbings, G., Macarthur, R. and Crews, C. (2007). Rapid simultaneous determination of 6 major ergot alkaloids and their epimers in cereals and food stuffs by LC/MS/MS. In preparation for submission to: Analytical and Bioanalytical Chemistry.

- Krska, R and Crews, C. (2007) Significance, chemistry and determination of ergot alkaloids. Food Additives and Contaminants (in press).
- Crews, C., Krska, R and Berthiller, F. (2007) Determination of Pyrrolizidine alkaloids in ragwort (*Senecio jacobaea*) by liquid chromatography time-of-flight mass spectrometry (LC-TOFMS) supported by tandem mass spectrometry (LC-MS/MS). In preparation for submission to: Journal of the American Society for Mass Spectrometry.
- Hafner, M., Sulyok, M., Schuhmacher, R., Crews, C. and Krska, R. (2007) Stability and epimerisation behaviour of ergot alkaloids in various solvents. Submitted to: The World Mycotoxin Journal.
- R. Krska, G. Stubbings, C. Crews Rapid simultaneous determination of 6 major ergot alkaloids and their epimers in cereals and food stuffs by LC/MS/MS. Lecture at the Prague Food Symposium, Nov. 7-9, 2007.

Research report

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