APPLICATION BRIEF

Transferring a HPLC Fluorescence Method for Analyzing Ergot Alkaloids to a UHPLC/SQ MS Method

Food/Nutraceutical

Ergot alkaloids are mycotoxins produced by fungi such as *Claviceps* that live on grass and grain. Cattle that consume these alkaloids in grass can experience severe poisoning that can affect their central nervous system, immune and reproductive systems. In the United States alone, ergot alkaloid poisoning results in a billion dollars of annual damage to livestock. We describe a UHPLC/SQ MS method for measuring 8 ergot alkaloids. The method was transferred from HPLC to UHPLC conditions using a PerkinElmer Flexar UHPLC pump with a sub-2 micron column.

Experimental Conditions

Target Analytes: Lysergic acid, ergonovine, lysergol, ergovaline, ergotamine, ergocornine, α-ergocryptine, ergocristine

Standard Concentrations

A sample of ergot alkaloids, 10 µg/mL of each standard in methanol, was diluted in mobile phase A to a concentration of 2 µg/mL each.

Liquid Chromatography Conditions

Pump Type:	PerkinElmer® Flexar™ FX-15
Column:	PerkinElmer Brownlee™ HRes C18 column (2.1 mm x 50 mm, 1.9 µm)
Mobile Phase:	A: 2.5 mM ammonium bicarbonate in water, pH 7.7 B: acetonitrile
Flow Rate:	0.5 mL/min
Injection Volume:	2 µL in partial fill mode

Gradient:	Time (min)	%A	%B
	0	90	10
	1	90	10
	0.5	70	30
	4	30	70

Mass Spectrometer Conditions

Ionization:	Ultraspray™ ESI – Positive mode
Scan Range:	150-700 m/z
Scan Rate:	1000 u/sec
Capillary Exit Voltage:	80 V

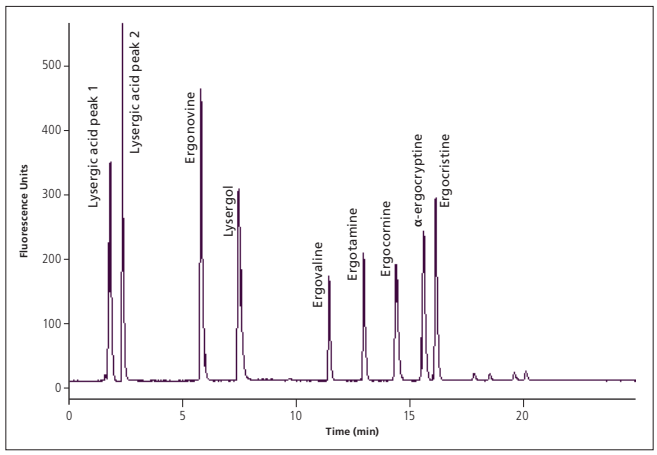

Figure 1. HPLC separation of ergot alkaloids on a 4.6 mm x 150 mm, 3 µm column (Phenomenex®) with fluorescence detection. The analysis time including equilibration time was 30 min.

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Results

A HPLC method for analyzing ergot alkaloids uses a PerkinElmer Series 200 pump with a PerkinElmer fluorescence detector on a Phenomenex® C18 column (4.6 mm x 150 mm, 3 µm). The analysis time including column equilibration was 30 min (Figure 1). The method transferred to UHPLC conditions resulted in a 9 min analysis time including column equilibration time (Figure 2; concentrations injected different from Figure 1 sample). A total savings of 21 min per run was achieved by the UHPLC separation. The flow rate used for the HPLC sample analysis was 1 mL/min as opposed to a flow of 0.5 mL/min used for UHPLC separation, resulting in a solvent saving of ~26 mL per sample analysis.

Conclusions

The mass spectrometer detector offers excellent specificity and sensitivity, as it is based on measuring ions characteristic of the analytes. The extracted ion chromatograms (EICs) of the $[M+H]^+$ ions of each of the alkaloids are shown in Figure 3. When further sensitivity is required Selective Ion Monitoring (SIM) is the acquisition mode of choice.

By transferring a HPLC method to UHPLC conditions, we can significantly scale down analysis time, solvent consumption and improve overall sample throughput.

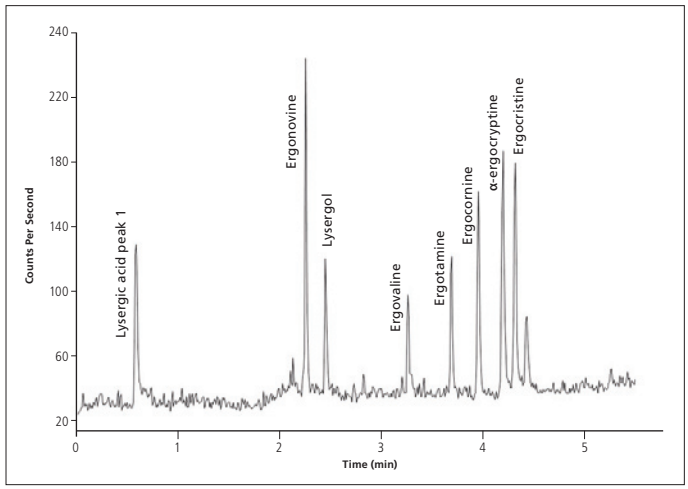

Figure 2. UHPLC separation of ergot alkaloids on a 2.1 mm x 50 mm, 1.9 µm column (PerkinElmer, Brownlee HRes C18) with MS detection in Full Scan mode. The analysis time including equilibration time was 9 min.

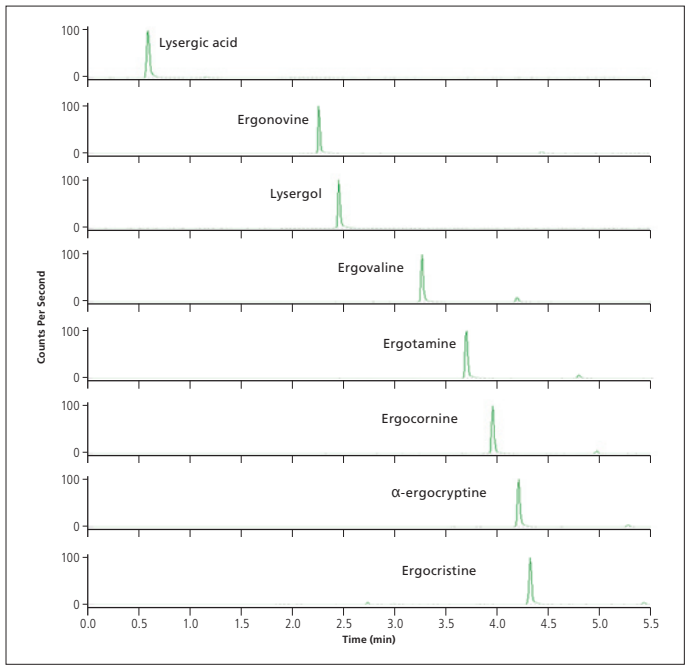

Figure 3. Extracted ion chromatograms of the molecular ion of each of the ergot alkaloids.

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