Rapid and Sensitive Detection of Ergot Alkaloids in Wheat Using the Agilent 6460 Triple Quadrupole LC/MS with Jet Stream Technology

Application Note

Food Testing

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Abstract

A facile method for the extraction and sensitive detection of ergot alkaloids in cereals has been developed on the Agilent 1290 Infinity LC System coupled to an Agilent 6460 Triple Quadrupole LC/MS. It provides Limits of Quantitation (LOQs) well below 0.1 µg/kg in wheat with a chromatographic run time of 16 minutes.

Introduction

The term ergot refers to fungal structures from *Claviceps* species replacing kernels on grain ears or seeds on grass heads, being visible as large discolored sclerotia. These sclerotia contain different classes of alkaloids, the most prominent being ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, and ergocornine. Ergot alkaloids (ergolines) exert toxic effects in all animal species, including vaso-constriction that may progress into vaso-occlusion and gangrenous changes. The neurotropic activities of the ergot alkaloids may also cause hallucinations and attendant irrational behavior, convulsions, and even death.

A European Union commission recommendation published in March 2012 states that the physical determination of the contamination rate of cereals by rye ergot is often inaccurate, as size and weight of the sclerotia may vary considerably. Moreover, this physical determination is impossible in processed feed and food. Hence, it has been suggested to provide in addition to control by physical methods also the possibility to control by chemical analysis of potentially contaminated feed and food. Chromatographic methods are suggested.

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The Commission recommendation also states that it is necessary to generate more data on the presence of these ergot alkaloids, not only in unground cereals but also in cereal products and compound feed and food. Reliable data on the ergot alkaloid pattern in feed and food is also necessary, as well as the ability to relate the presence of ergot alkaloids to the amount of sclerotia present. It is appropriate to focus this monitoring on the six predominantly present ergot alkaloids, that is, ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, and ergocornine and their related -inines,

Per the Commission recommendation, member States should also work closely with feed and food business operators to monitor the presence of ergot alkaloids in cereals and cereal products intended for human consumption or for animal feeding, in pasture/forage grasses for animal feeding, and in compound feed and food. The analytical results should be provided, on a regular basis, to the European Food Safety Authority (EFSA) for compilation into a database.

This application note describes a simple, rapid and easy to perform analytical technique to determine six ergot alkaloids and their inine epimers in wheat. It combines an inexpensive solvent extraction and cleanup procedure with high sensitivity analysis using the Agilent 1290 Infinity LC System coupled to the 6460 Triple Quadrupole LC/MS with Jet Stream Technology. Reporting limits for ergot alkaloids are not currently required by any legislation. However, it is typical to show calibrated results starting from 5 µg/kg upwards. The lowest calibration concentration for this method was 1 ng/mL, which is equivalent to 5 µg/kg. However, the sensitivity of the 6460 Triple Quadrupole LC/MS would allow the method to be implemented at an order of magnitude lower concentration if necessary, providing LODs ≤ 0.021 µg/kg and LOQs ≤ 0.071 µg/kg.

Experimental

Reagents and Standards

Ergot alkaloids and their epimers were obtained from Alfarma (Czech Republic). The standards were used to make up a working solution at 50 ng/mL in acetonitrile of all six alkaloids and their epimers. These were then used to construct calibration curves in acetonitrile.

Sample Preparation

A wheat flour sample (5 g) was added to a dark tube, and 25 mL of extraction solvent (acetonitrile:200 mg/L ammonium carbonate (84:16)) was then added and spiked with the ergot alkaloid standard mix. The mixture was then shaken for 30 minutes and filtered through Whatman 54 filter paper. PSA (100 mg) was added to a 2-mL aliquot of this sample, which was vortexed and filtered through a 0.02-µm PTFE membrane filter. The sample was then ready for injection into the LC .

Instruments

This method was developed on the Agilent 1290 Infinity LC System coupled to the Agilent 6460 Triple Quadrupole LC/MS with Jet Stream Technology. The instrument conditions are listed in Table 1.

Table 1. Agilent 1290 Infinity LC System and Agilent 6460 Triple Quadrupole LC/MS Instrument Conditions

LC conditions		
Analytical column	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 mm × 150 mm, 1.8 um (p/n 959759-902)	
Column temperature	30 °C	
Injection volume	5 µL	
Mobile phase	A = 3 mM ammonium carbonate in water B = Acetonitrile	
Flow rate	0.2 mL/min	
Gradient program	Time (min)	% Solvent B
	0	5
	2	5
	4	50
	8	90
	14	90
	15	5
	Stop time: 16 minutes	
MS conditions		
Acquisition parameters	ESI with Jet Stream, positive ionization, Dynamic MRM	
Sheath gas temperature	400 °C	
Sheath gas flow rate	12 L/min	
Drying gas	Nitrogen, 8 L/min	
Drying gas temperature	200 °C	
Nebulizer pressure	45 psig	
Nozzle voltage	+500 V	
Vcap voltage	+3,000 V	
Delta EMV	+500 V	
Resolution	Unit, unit	

Acquisition Parameters

The Triple Quadrupole MRM acquisition parameters for the ergot alkaloids and their epimers are shown in Table 2.

Table 2. Acquisition Parameters for the Ergot Alkaloids and Their Epimers on the Agilent 6490 Triple Quadrupole LC/MS

Compound name	Precursor ion	Product ion	Dwell time	Fragmentor voltage	Collision energy
Ergometrine	326	223.1	40	147	20
Ergometrine	326	208	40	147	36
Ergometrine	326	197.1	40	147	20
Ergometrinine	326	223.1	40	152	24
Ergometrinine	326	208	40	152	28
Ergometrinine	326	265.1	40	152	16
Ergocryptine	576	223.1	40	152	36
Ergocryptine	576	305.1	40	152	24
Ergocryptine	576	291.1	40	152	24
Ergocryptinine	576	223.1	40	201	36
Ergocryptinine	576	305.1	40	201	28
Ergocryptinine	576	291.1	40	201	24
Ergocristine	610	223.1	40	172	36
Ergocristine	610	305.1	40	172	24
Ergocristine	610	268	40	172	24
Ergocristinine	610	223.1	40	162	36
Ergocristinine	610	305.1	40	162	28
Ergocristinine	610	268	40	162	24
Ergocornine	562	223.1	40	147	36
Ergocornine	562	277.1	40	147	24
Ergocornine	562	305.1	40	147	24
Ergocorninine	562	223.1	40	147	36
Ergocorninine	562	277.1	40	147	24
Ergocorninine	562	305.1	40	147	24
Ergotamine	582	223.1	40	167	32
Ergotamine	582	277.1	40	167	24
Ergotamine	582	208	40	167	50
Ergotaminine	582	223.1	40	167	36
Ergotaminine	582	208	40	167	50
Ergotaminine	582	277.1	40	167	24
Ergosine	548	223.1	40	152	28
Ergosine	548	530.2	40	152	12
Ergosine	548	208	40	152	50
Ergosinine	548	223.1	40	196	32
Ergosinine	548	208	40	196	50
Ergosinine	548	530.2	40	196	12

Results and Discussion

Chromatographic Separation

The chromatographic method easily resolves each of the six ergot alkaloids from its epimer inine (Figure 1). It also provides at least partial resolution of all 12 compounds (Figure 4). The total separation time is only 16 minutes.

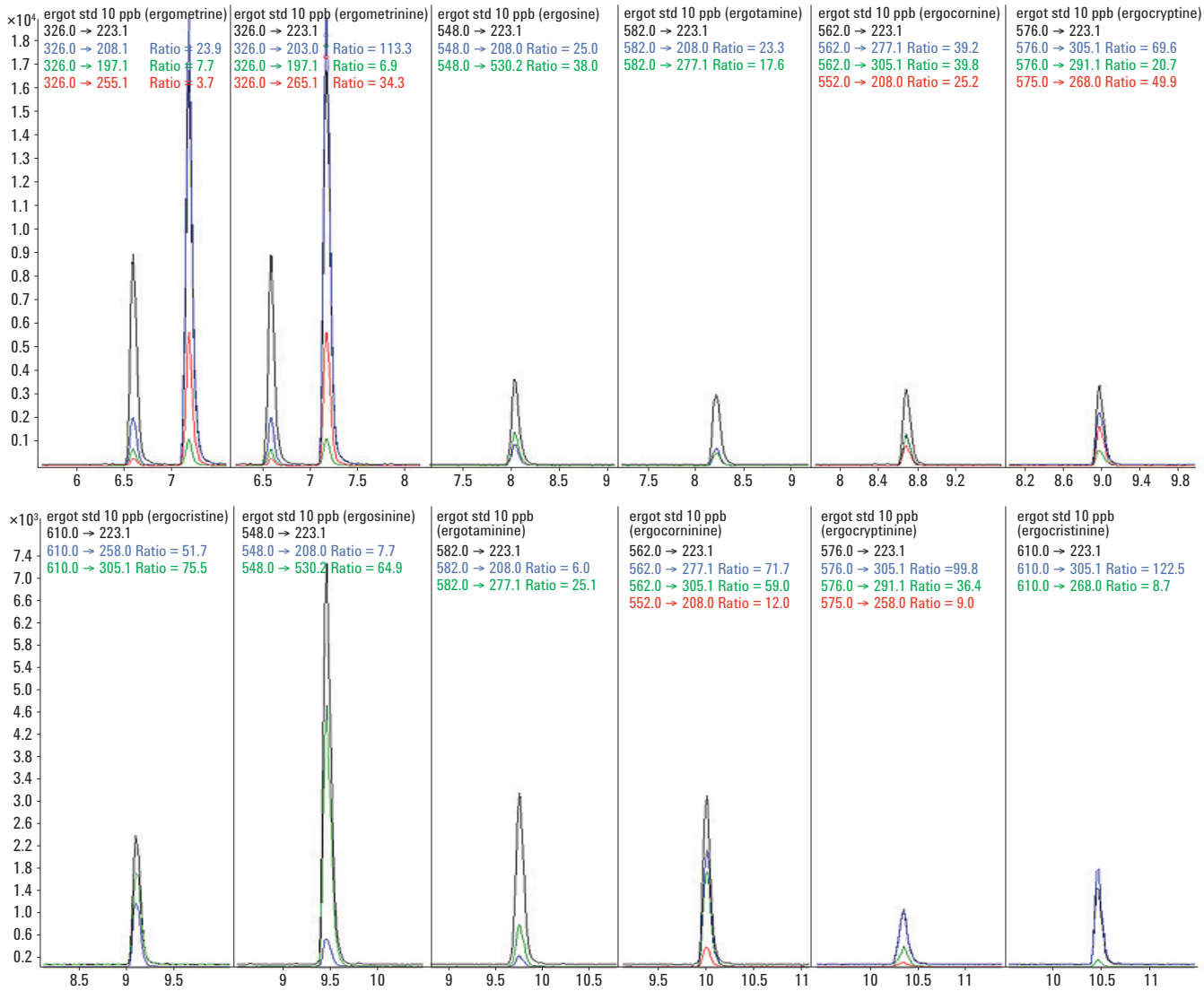


Figure 1. Six ergot alkaloids and their inine epimers at a concentration of 10 ng/mL in a standard. Ratios between quantifier and qualifier traces can be set into the method and used to assess reliability of sample results. Those results outside predetermined limits of acceptance are flagged.

Qualification

MS/MS detection confers considerable qualitative verification of the result through the structurally significant mass transitions of the various compounds. This advantage is further leveraged by employing additional qualifying mass transitions per compound which can be used to flag any suspect result if the ratio of peak areas (quantifier versus qualifier) is outside an expected ratio as found in a standard, as shown in Figure 1. These ratios can also be compared against sample results either as chromatographic overlay or as an MRM composite spectrum, as shown in Figure 2.

Quantitation

The calibration curves in acetonitrile demonstrate excellent linearity of quantitation between 1 and 50 ng/mL, with R^2 values typically >0.9995 (Figure 3). Recoveries in spiked samples were also good, ranging from 80 to 107%, with relative standard deviations (RSDs) ≤ 17.7% (Table 3).

Batches of samples of a given commodity that arrive at the lab for analysis are always checked for recovery for the target compounds by spiking 10% of the samples in the batch. This approach checks for matrix effects coming from a particular set of samples.

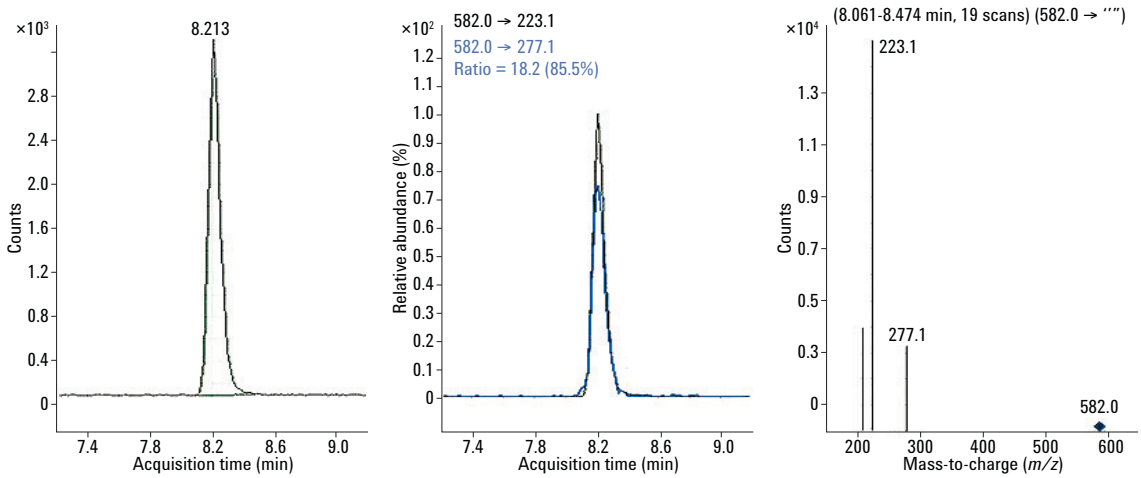
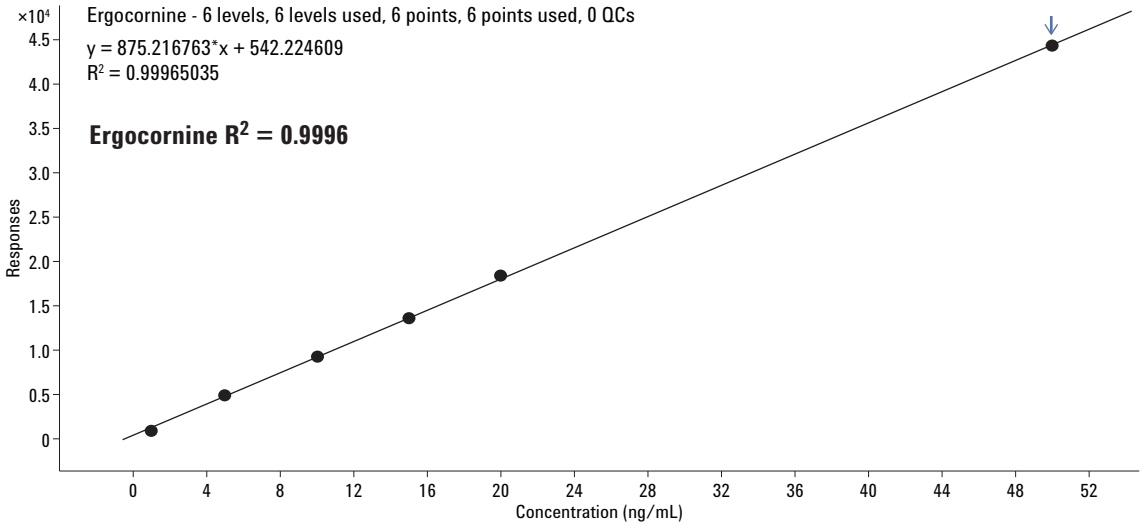
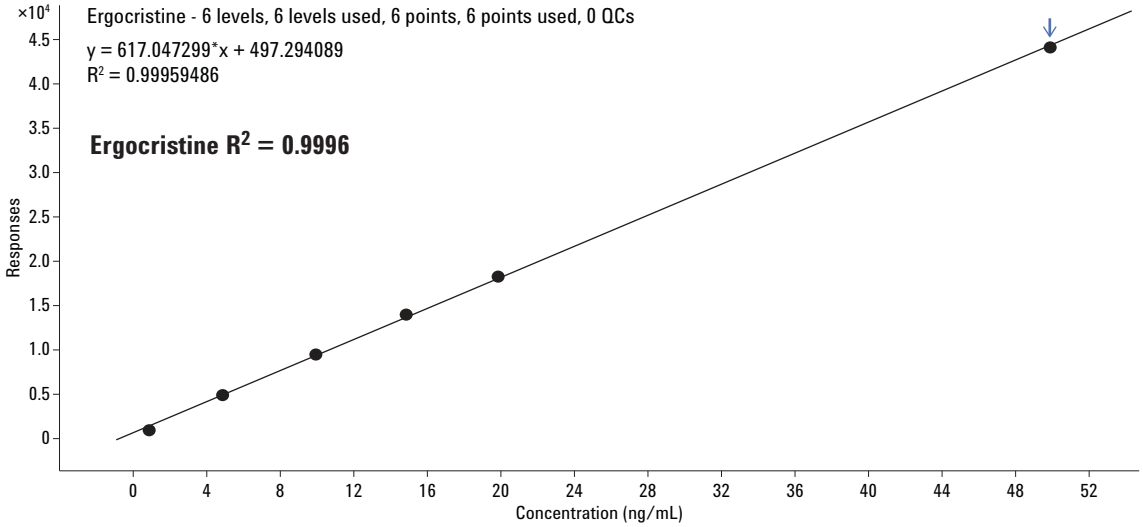


Figure 2. Extracted MRM results for ergotamine spiked into wheat flour at 50 ppb, illustrating the quantifier and qualifier transitions.





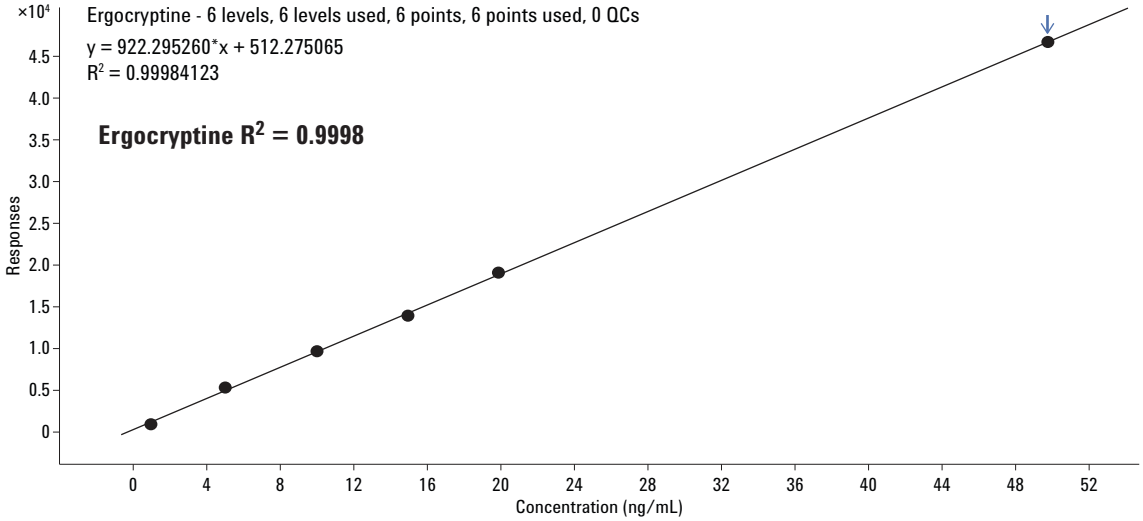


Figure 3. Calibration curves for three ergot alkaloids in acetonitrile.

Table 3. Recoveries for Wheat Flour Samples Spiked with Ergot Alkaloids at 50 µg/kg (ppb)

Analyte	Retention time (min)	Recovery (%)	RSD (%)
Ergometrine	6.6	80.1	16.6
Ergometrinine	7.2	89.6	14.6
Ergotamine	8.2	97.4	11.0
Ergotaminine	9.8	100	17.2
Ergosine	8.1	102	11.1
Ergosinine	9.5	97.1	6.7
Ergocristine	9.1	107.2	7.8
Ergocristinine	10.5	99.1	17.7
Ergocryptine	9.0	105.8	6.8
Ergocryptinine	10.4	91.3	16.9
Ergocornine	8.7	104.9	8.3
Ergocorninine	10.1	103.8	12.8

RSD = Relative standard deviation

Table 4. Limits of Detection and Quantitation of Six Ergot Alkaloids and Their Epimers Spiked into Wheat Flour

Analyte	LOD (µg/kg)	LOQ (µg/kg)
Ergometrine	0.0034	0.011
Ergometrinine	0.0017	0.0056
Ergotamine	0.0093	0.031
Ergotaminine	0.012	0.041
Ergosine	0.0063	0.021
Ergosinine	0.0030	0.0098
Ergocristine	0.017	0.056
Ergocristinine	0.021	0.071
Ergocryptine	0.0023	0.0075
Ergocryptinine	0.0081	0.027
Ergocornine	0.0060	0.020
Ergocorninine	0.0055	0.018

LOD = Limit of Detection (S/N>3)
LOQ = Limit of Quantitation (S/N>10)

Sensitivity

Due to the low chromatographic background of this method (Figure 4), LODs and LOQs were below 0.1 µg/kg (Table 4).

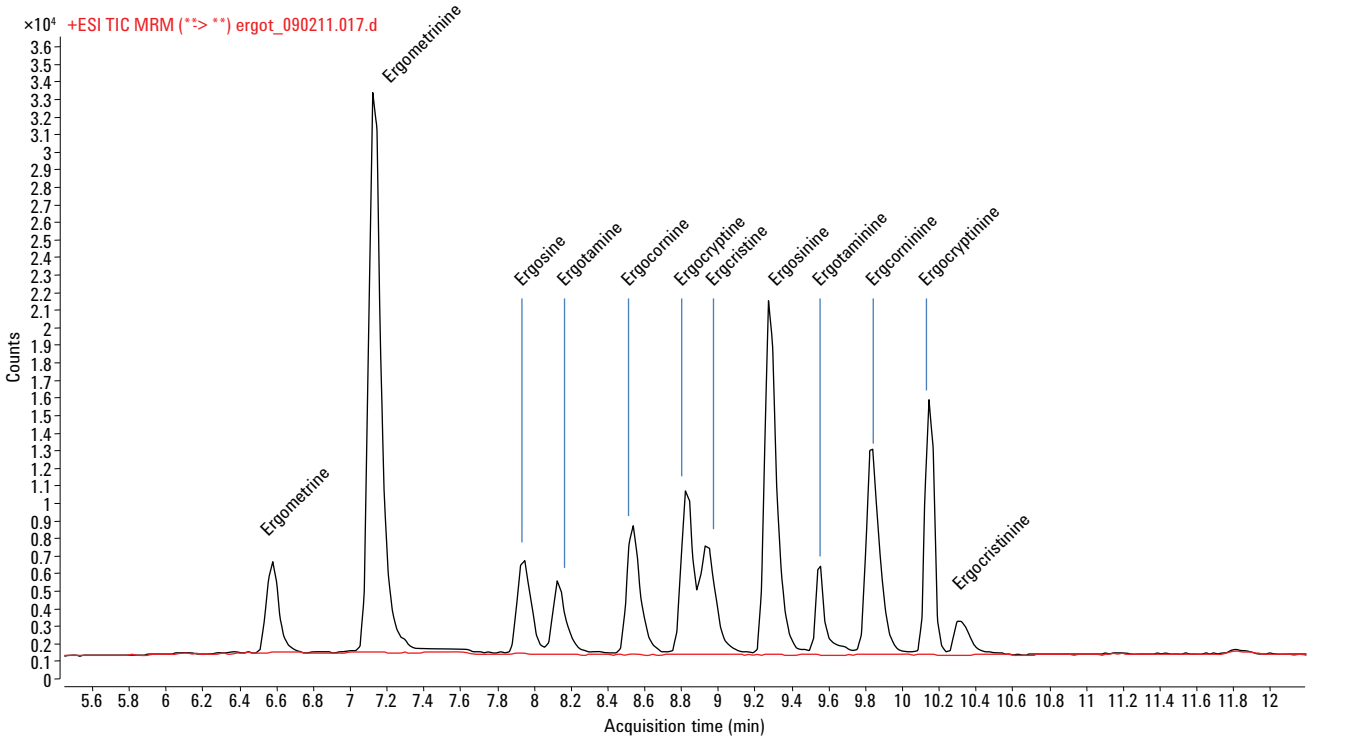


Figure 4. Total ion current (TIC) traces showing blank wheat sample (red) compared to a sample spiked at 50 µg/kg with the ergot alkaloid mix (black).

Conclusions

A simple, quick and easy-to-perform analytical technique has been developed to determine six ergot alkaloids and their epimers in wheat. It combines an inexpensive solvent extraction and cleanup procedure with high sensitivity UHPLC/MS/MS determination on the 6460 Triple Quadrupole LC/MS with Jet Stream Technology. The simplicity and flexibility of the extraction procedure have enabled these analytes to be determined in other cereal matrices that Campden is required to analyze.

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