Simultaneous Determination of Alternaria Toxins, Ergot Alkaloid Epimers and other major Mycotoxins in various Food Matrices by LC-MS/MS

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Outline

Analyte List of 37 Mycotoxins (incl. Alternaria Toxins and Ergot Alkaloid)

LC Method Development

Food Matrices and Sample Preparation

Method Accuracy & Precision

Conclusion

Analyte List

Aspergillus	Fusarium	Penicillum	Alternaria	Claviceps
Aflatoxin B1	Fumonisin B1	Citrinin	Alternariol	Ergocornine
Aflatoxin B2	Fumonisin B2	Patulin	Alternariol MME	Ergocristine
Aflatoxin G1	Fumonisin B3		Altenuene	Ergocryptine
Aflatoxin G2	Fusarenon X		Tentoxin	Ergotamine
Ochratoxin A	Nivalenol		Tenuazonic Acid	Ergosine
	Deoxynivalenol (DON)			+
	3-Acetyl – DON			Epimers
	15-Acetyl – DON			
	Diacetoxyscirpenol			
	T-2 toxin			
	HT-2 toxin			
	Zearalenone			
	A-Zearalenol			

Alternaria Toxins & Ergot Alkaloids

Alternarias exist in Cereals, Fruit and Vegetables

Ergot alkaloids exist in Cereal Crops

Both co-exist in grain-based food!

It´s valuable to analyze AT and EA together!

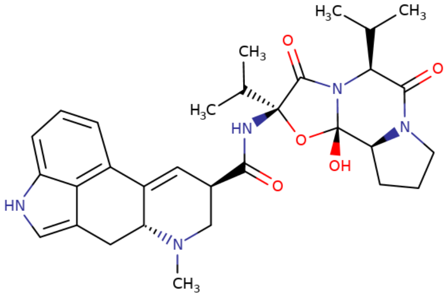
Alternaria Toxins & Ergot Alkaloids – current status

Seperate methods for AT and EA analysis

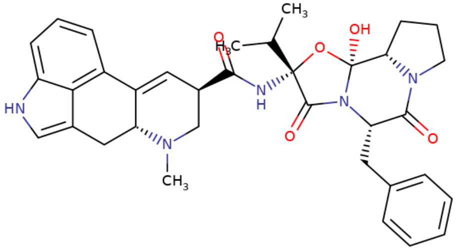
Alkaline pH LC condition for both AT and EA analysis

Restricted analytical condition hindered the simultaneous analysis of AT/ET and major mycotoxins

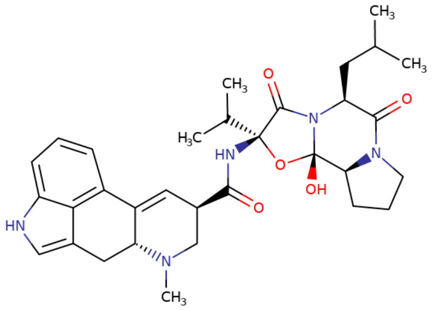
Ergot Alkaloids - Structures



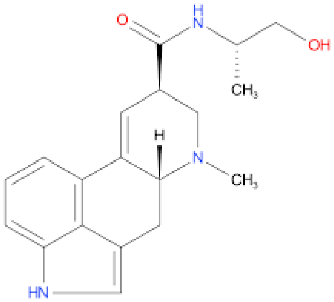
Ergocornine



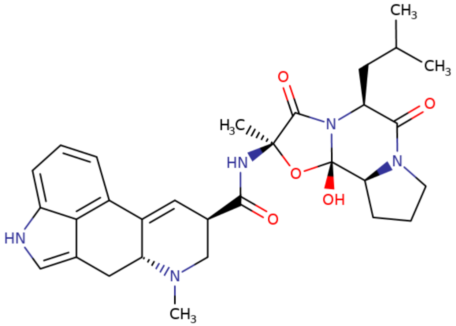
Ergocrystine



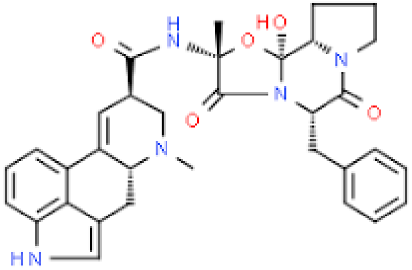
Ergocryptine



Ergometrine



Ergosine



Ergotamine

Alternaria Toxins & Ergot Alkaloids – current status

Seperate methods for AT and EA analysis

Alkaline pH LC condition for both AT and EA analysis

Restricted analytical condition hindered the simultaneous analysis of AT/ET and major mycotoxins

Our solution is unique to analyze AT and EA together with other major Mycotoxins!

Analyte List – legal limits

EU June 2019 Stakeholder Update on Rapidly Developing Policy on Food Contaminants

Food	Alternariol (AOH) (µg/kg)	Alternariol monomethyl ether (AME) (µg/kg)	Tenuazonic acid (TeA) (µg/kg)
Processed tomato products	10	5	500
Paprika powder	-	-	10000
Sesame seeds	30	30	100
Sunflower seeds	30	30	1000
Sunflower oil	10	10	100
Tree nuts	-	-	100
Dried figs	-	-	1000
Cereal based foods for infants and young children	5	5	500

EU Regulation Limits for ergot Alkaloids

Products	Total Amount µg/kg
Milling products of barley, wheat, spelt, oats (with an ash content lower than 900mg/100g)	100 (50 from 1/7/2022)
Barley, wheat, spelt and oats placed on the market for the final consumer	150
Rye milling products and rye placed on the market for the final consumer	500 (250 from 1/7/2022)
Processed cereal based food for infant and young children	20

LC Method Development

Biphenyl phase is the most suitable analytical column

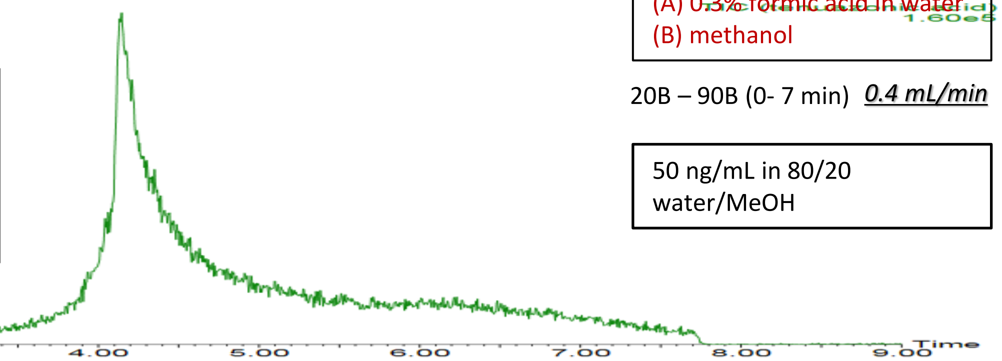
- → The Tenuazonic Acid peak could only be integrated and quantified on the BiPh phase

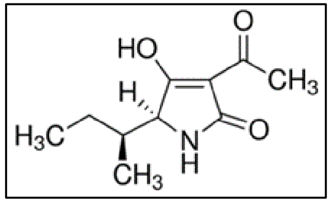
(A) 0.3% formic acid in water
(B) methanol

20B – 90B (0- 7 min) ***0.4 mL/min***

50 ng/mL in 80/20 water/MeOH

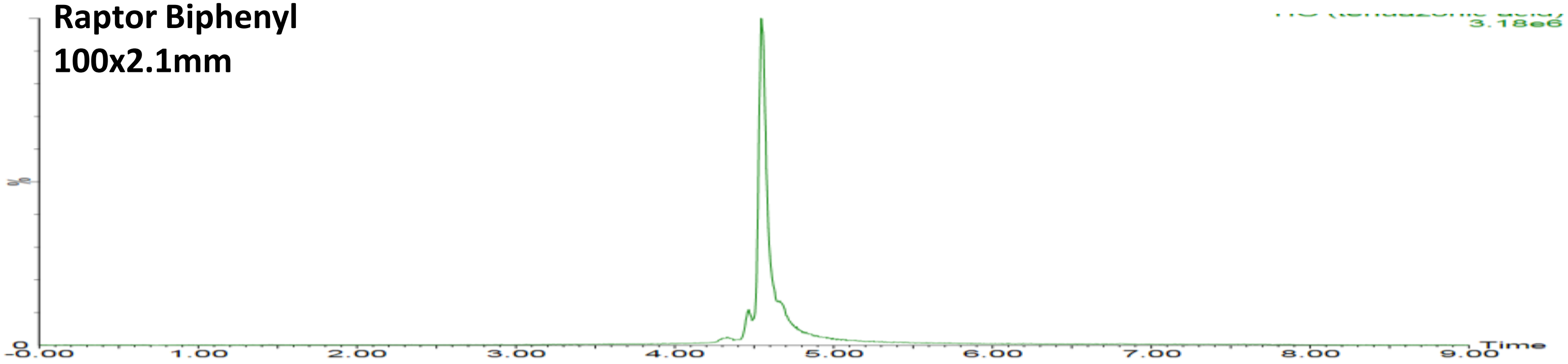
Raptor FluoroPhenyl 100x2.1mm



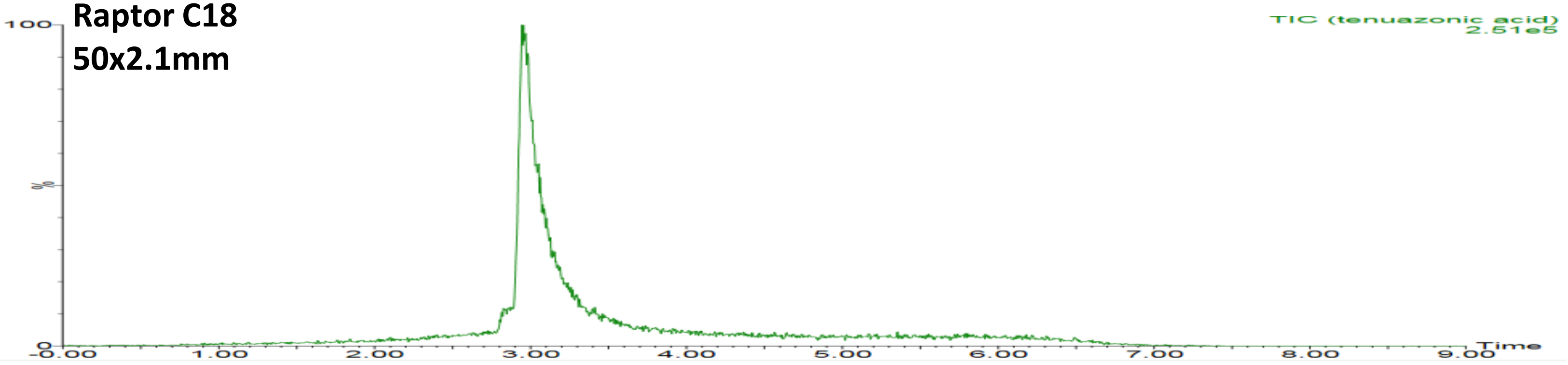


(TeA)

Raptor Biphenyl 100x2.1mm



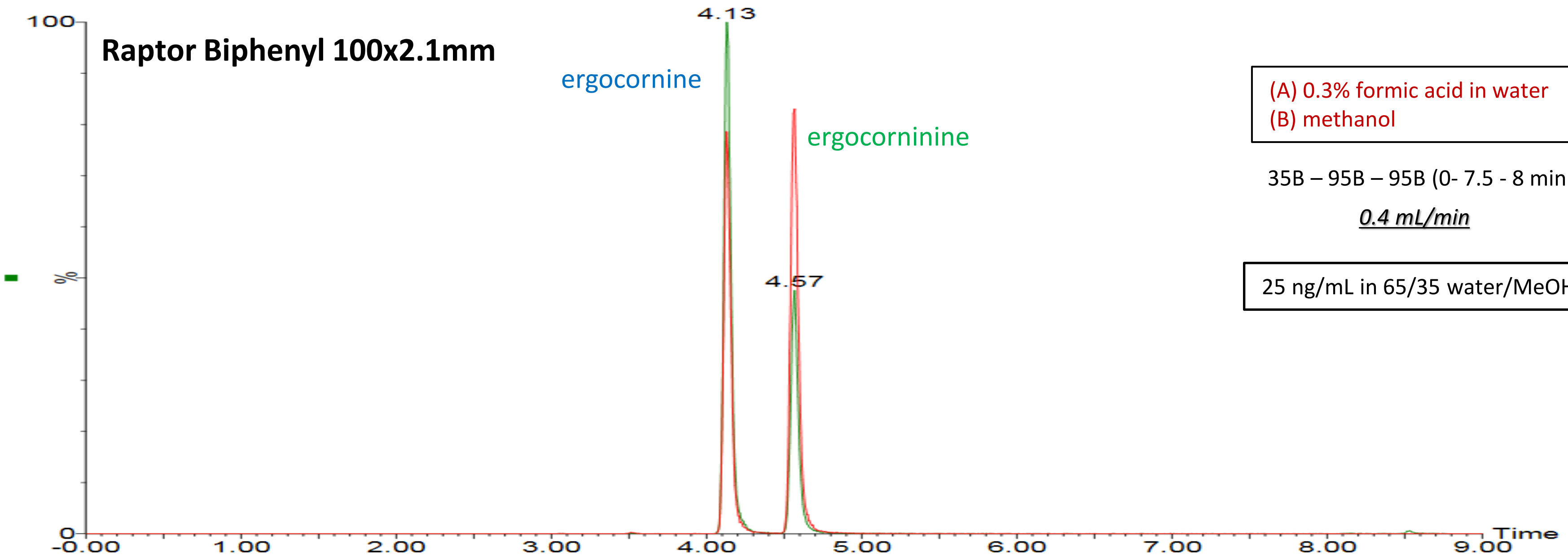
Raptor C18 50x2.1mm



LC Method Development

Biphenyl phase is the most suitable analytical column

- → The Tenuazonic Acid peak could only be integrated and quantified on the BiPh phase
- → Proper chromatographic separation of ergot epimers

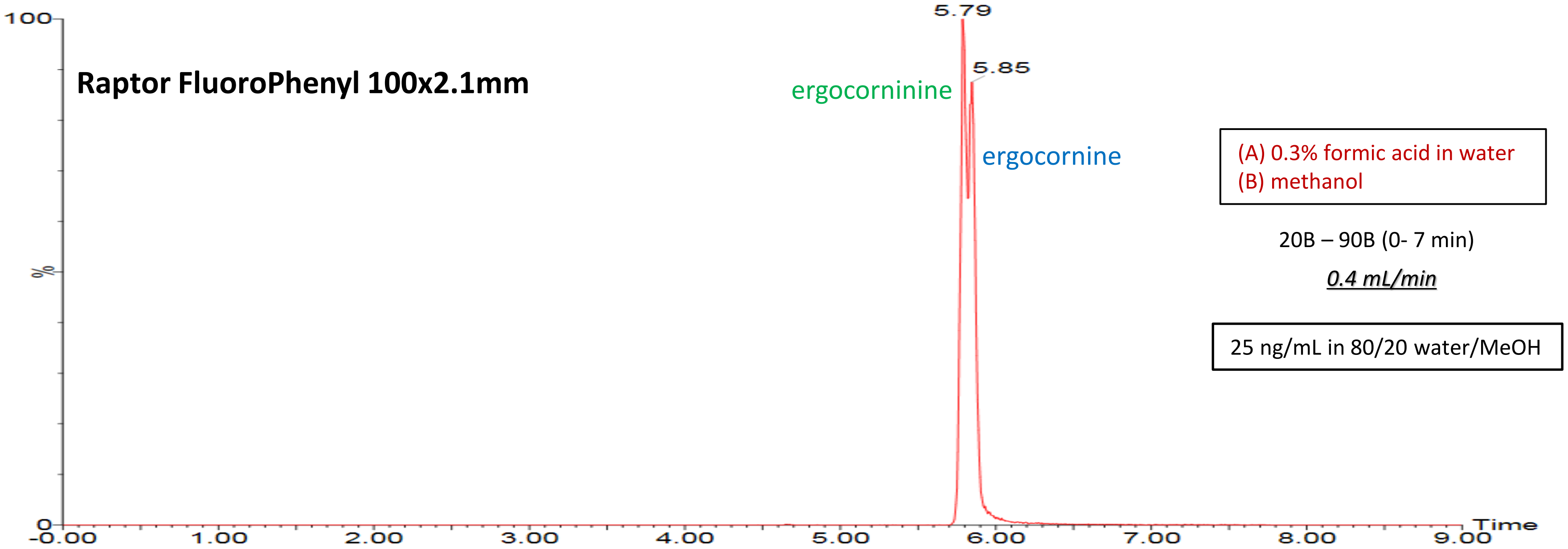


(A) 0.3% formic acid in water
(B) methanol

35B – 95B – 95B (0- 7.5 - 8 min)

0.4 mL/min

25 ng/mL in 65/35 water/MeOH



(A) 0.3% formic acid in water
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25 ng/mL in 80/20 water/MeOH

LC Method Development

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- → The Tenuazonic Acid peak could only be integrated and quantified on the BiPh phase
- → Proper chromatographic separation of ergot epimers

Aqueous formic acid/ methanol solution is the most satisfactory mobile phase coupled to a high column temperature for EA epimer separation

- → Addition of Ammonium acetate resulted in the loss of Fumonisin B signals
- → Aqueous acetid acid/ methanol mobile phase resulted in deteriorated TeA peak (but speparation of ergot epimers is greatly increased and detection sensitivity is incread for Alternarias
- → Formic acid/ammonium formate combination in mobile phase enabled separation of all EA epimers but resulted in reduced sensitivity and unstable detection of Alternaria toxin

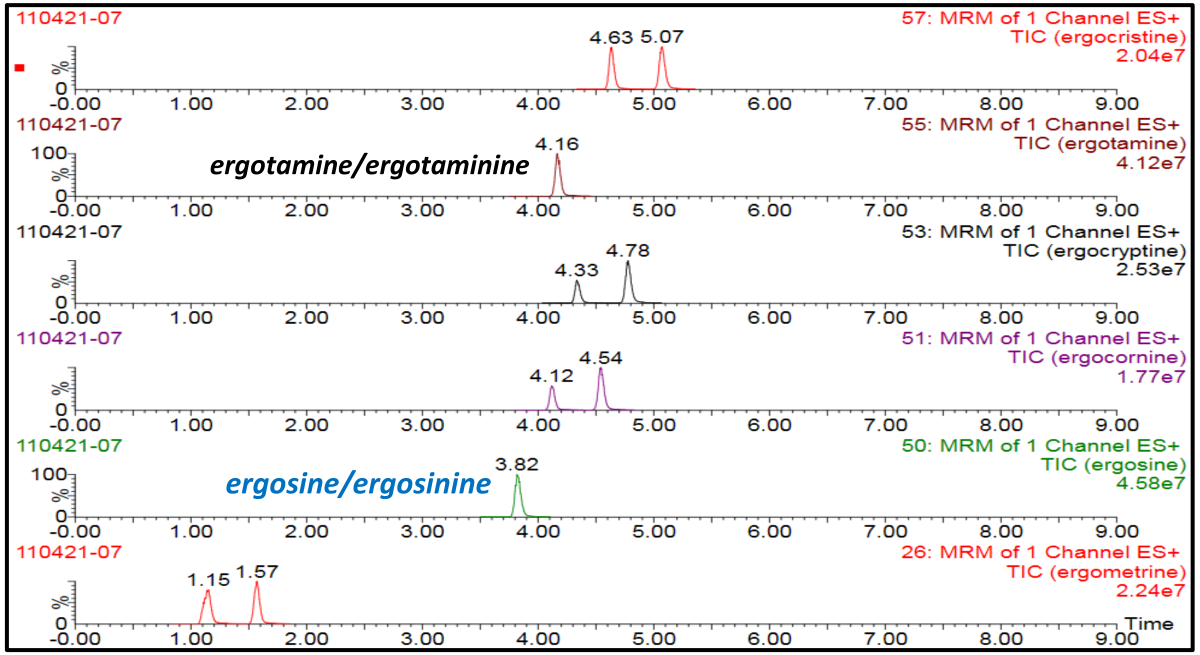
Raptor Biphenyl 100x2.1mm

(A) 0.3% formic acid in water
(B) methanol

35B – 95B – 95B (0- 7.5 - 8 min)

100B – 100B (8 - 10 min)

0.4 mL/min



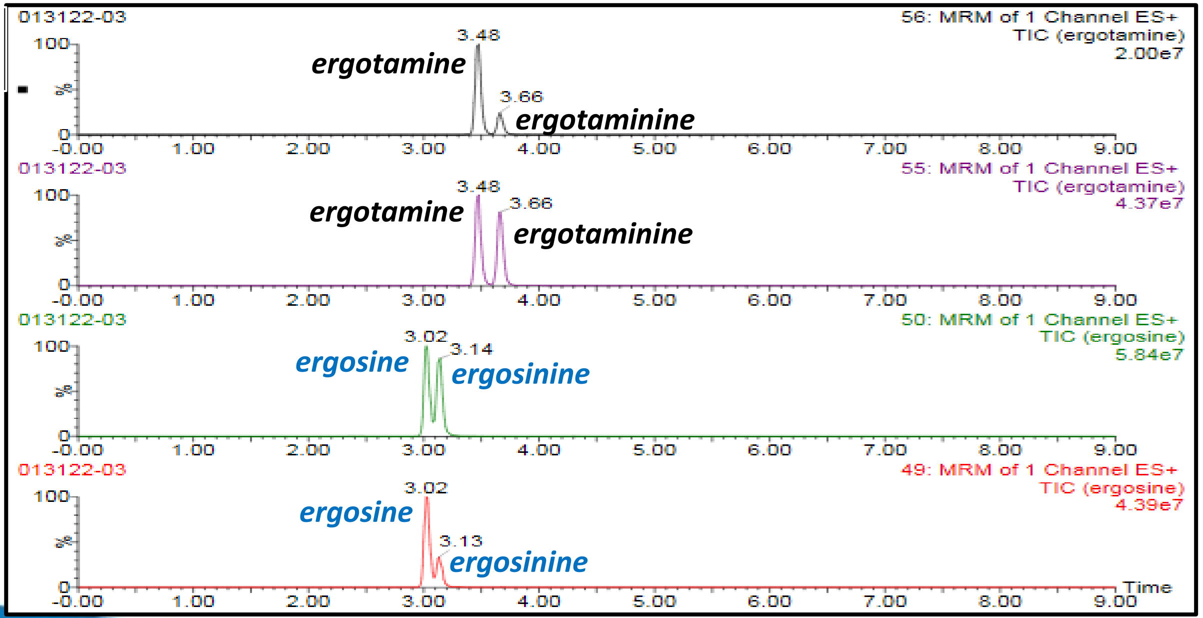
Column Temperature:

35°C

(A) 0.1% formic acid in water
(B) 0.1% formic acid in methanol

40B – 100B (0- 15 min)

0.4 mL/min



60°C

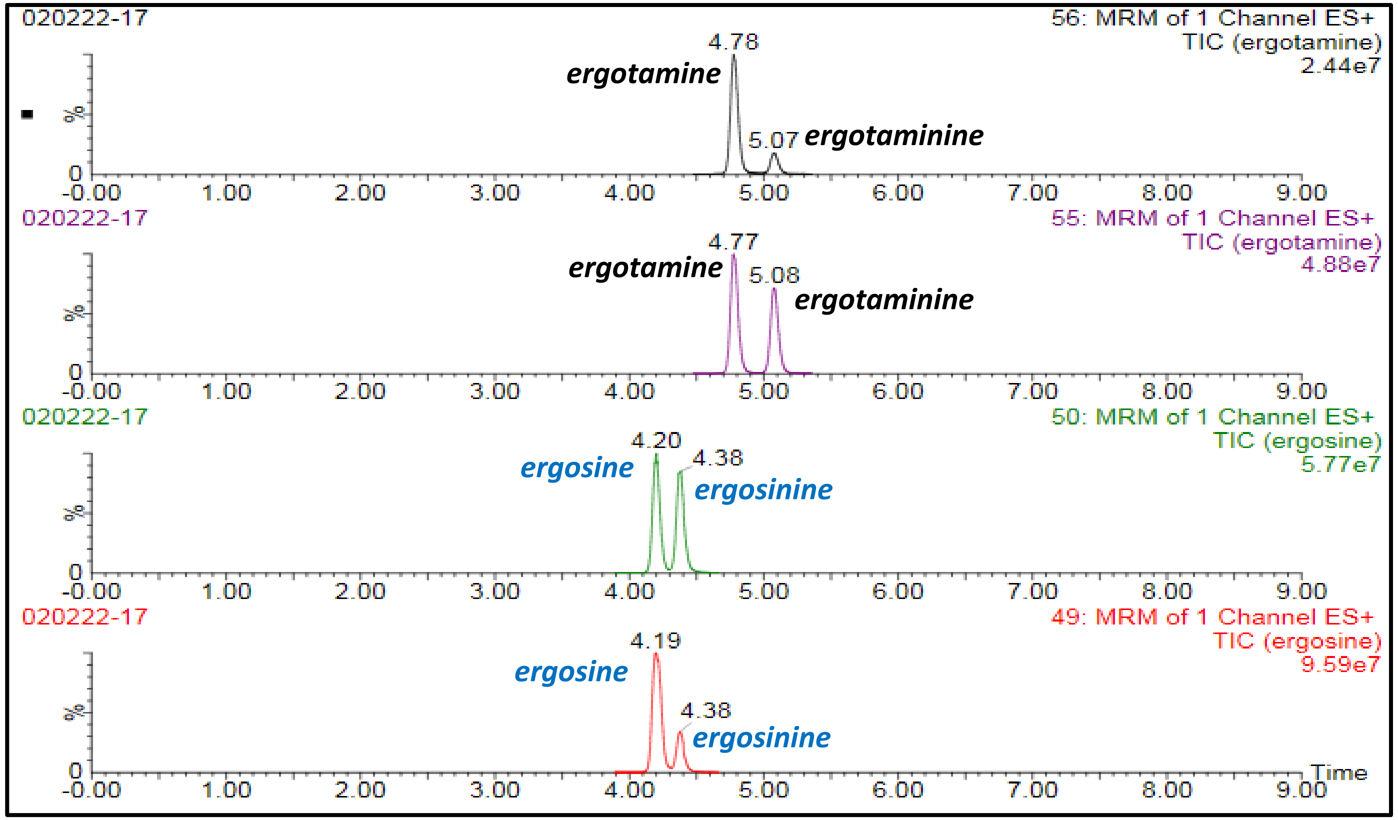
RESTEK Pure Chromatography

Waters Acquity – TQS

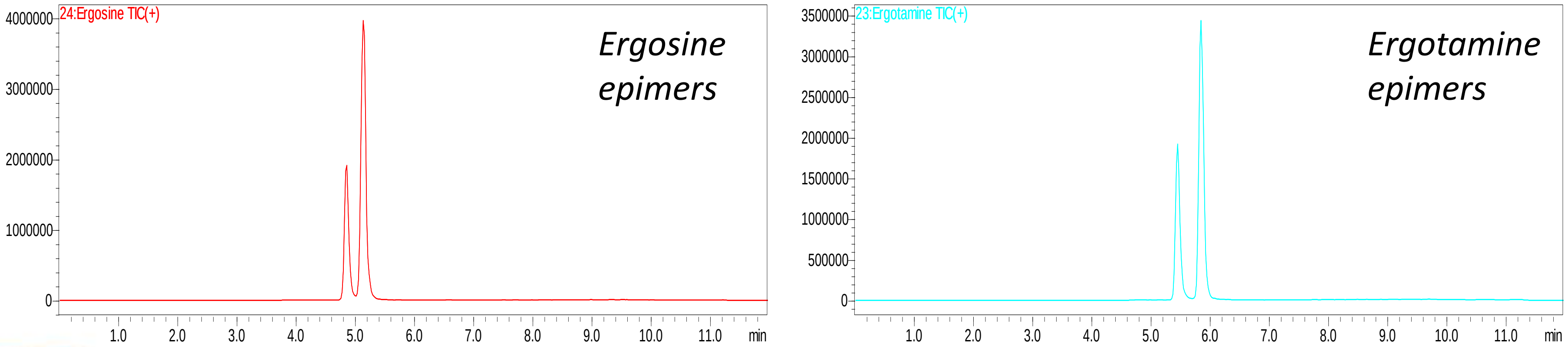
(A) 0.05% formic acid in water
(B) 0.05% formic acid in methanol

35B – 50B – 100B (0- 4.5 - 9 min)

0.4 mL/min   Column Temp. 60°C



Shimadzu Nexera X2 – 8060MS



LC Method Development

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Aqueous formic acid/ methanol solution is the most satisfactory mobile phase coupled to a high column temperature for EA epimer separation

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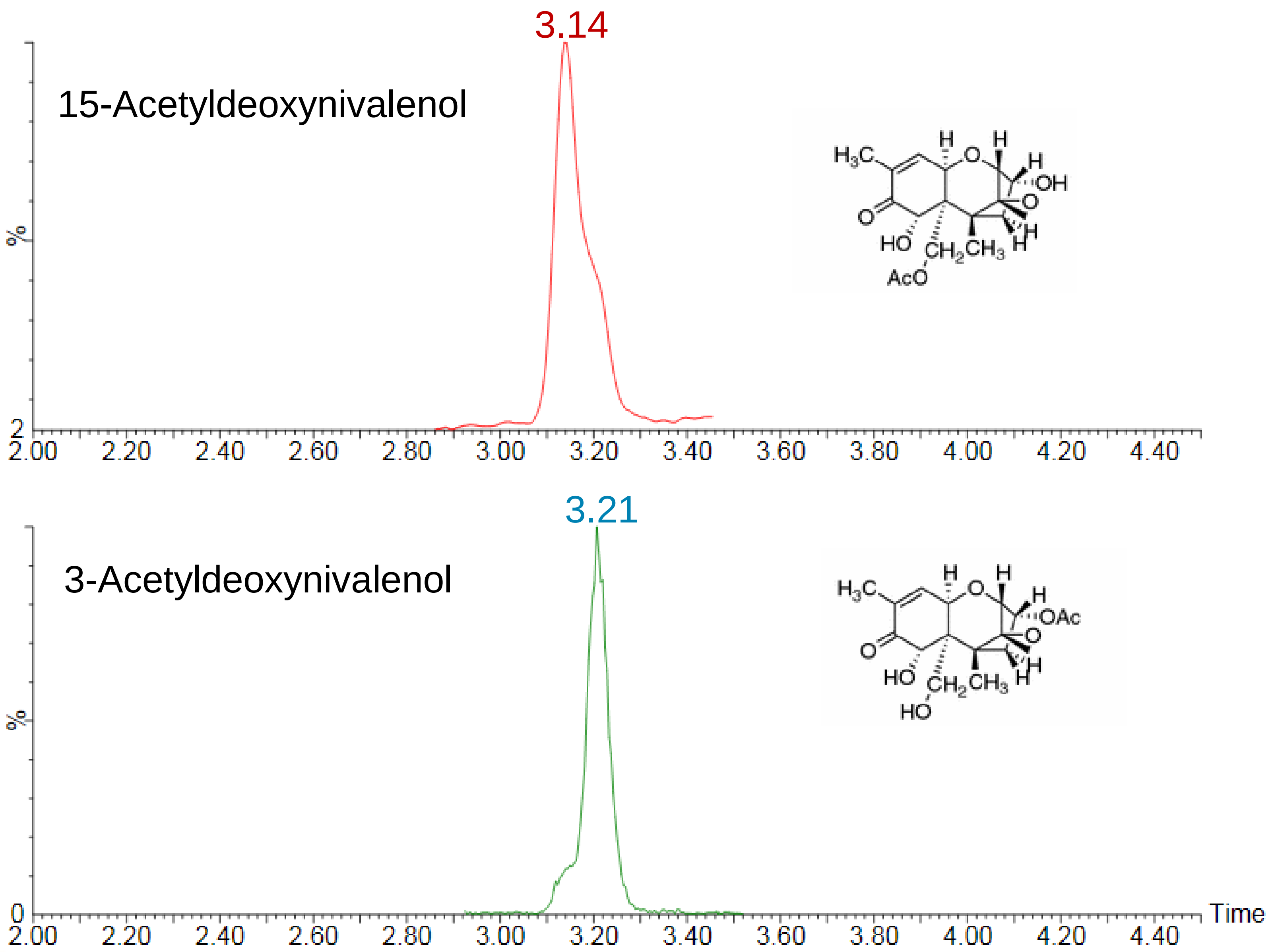
Aqueous methanol diluent (50/50 water/methanol) is neccesary for proper chromatografic performance of early eluting compounds and EAs

LC Method Development

Analytical Column	Raptor Biphenyl 2.7µm, 100 mm x 2.1 mm	
Guard Column	Raptor Biphenyl EXP Guard Colum Cartridge 2.7um, 5 mm x 2.1 mm	
Mobile Phase A	0.05% formic acid in water	
Mobile Phase B	0.05% formic acid in methanol	
Gradient	**Time (min)**	**%B**
	0.00	25
	5.00	50
	9.00	100
	9.01	25
	11.0	25
Flow Rate	0.4 mL/min	
Injection Volume	5 µL	
Column Temp.	60°C	
Ion Mode	Positive ESI	

Blended Flours
(50 µg/kg Fortification)


3.14
15-Acetyldeoxynivalenol
%
2
2.00 2.20 2.40 2.60 2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40
3.21
3-Acetyldeoxynivalenol
%
0
2.00 2.20 2.40 2.60 2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40
Time

Food Matrix & Sample Preparation

Four food products

- → Baby wheat cereal (baby food)
- → Peanut (High fat)
- → Tomato puree (high water content)
- → Blended Flour (high carbohydrate)

(white rice flour (75%), brown rice flour (5%), millet flour (5%), oat flour (5%), all-purpose wheat flour (5%), all-purpose gluten free flour (5%) **)**

Food Matrix & Sample Preparation

Sample preparation with single step extraction evaluated by matrix effect and recovery

- → The water/methanol extract had higher matrix effect than water/ACN extract
- → Lower matrix interference and higher recovery could be optained with higher content of ACN (80/20 ACN/water was chosen as extraction solution
- → Addition of formic acid was neccesary to obtain accetable recovery of Fumunisin Bs for solid samples at the expense of losing recovery of Citrinin
- → It´s not neccesary to use formic acid containing solution for the extraction of tomato puree to obtain acceptable recovery for both Fumonisin Bs and Citrinin

Food Matrix & Sample Preparation

Sample Extraction
with 0.5% formic acid
in 80/20 Acetonitrile/water

2 g sample (blank or fortified) + 16 mL of extraction solution

(5, 50, 200 µg/kg)

↓

Shaked on reciprocal shaker at 800rpm for 20 minutes

↓

Centrifugation at 4000rpm for 10 minutes

↓

Dry down 1 mL extract & reconstitute with 1 mL 50/50 water/methanol

Calibration Standards:
Reconstitute blank dry down with 1 mL of 50/50 water/methanol
containing 0.05, 0.10, 0.25, 0.50, 1.0, 2.5, 5.0, 10, 25, 37.5, 50 ng/mL
(0.4, 0.8, 2, 4, 8, 20, 40, 80, 200, 300, 400 µg/kg)

↓

Filtration with Thomson SINGLE StEP filter vials (0.2µm PTFE)

↓

Injection for the analysis

Method Accuracy & Precision

Matrix Matched Calibration

- Mitigatin incurred mycotoxins in food products
- No signal correction if the signals of incurred mycotoxins was ≤30% of the lowest concentrated standard (0.4µg/kg)
- The signals of incurred mycotoxins wer corrected by peak area subtraction of matrix matched standrad and fortified sample extract from the blank extract
- Consistent and suitable linearity of all analytes could be obtained with a 1/x weighted quadratic regression (or non-weighted quadratic regression for incurred mycotoxins)

r^2 > 0.997, deviations < 20%

Recovery & Precision

- 3 batches of analyses for each food matrix (n=9 for each fortified level)
- Except citrinin in solid samples, the average recovery was 72-112% and the %RSD was 0.5 – 12%
- Baby wheat cereal had a specific matrix signal blocking the detection of nivalenol

r^2 > 0.997, deviations < 20%

Average Recovery & Precision

	Average Recovery (RSD, %)											
	Baby Wheat Cereal			Peanut			Tomato Puree			Blended Flour		
Concentration, µg/kg	5	50	200	5	50	200	5	50	200	5	50	200
Aflatoxin B1	105 (4.8)	100 (3.0)	79.8 (2.6)	98.2 (6.4)	97.0 (5.2)	89.0 (5.7)	92.7 (3.8)	97.6 (5.2)	103 (3.0)	101 (2.8)	95.5 (1.3)	89.0 (1.5)
Aflatoxin B2	110 (1.4)	109 (2.8)	106 (2.3)	102 (5.8)	99.3 (4.7)	91.3 (2.9)	91.7 (4.2)	93.3 (0.9)	94.7 (0.4)	100 (2.3)	101 (0.9)	88.7 (1.3)
Aflatoxin G1	105 (6.1)	107 (1.7)	102 (2.1)	98.2 (4.2)	97.3 (3.2)	91.2 (4.1)	91.3 (1.9)	92.2 (3.6)	93.3 (2.5)	99.3 (1.7)	100 (1.6)	93.6 (2.2)
Aflatoxin G2	108 (3.0)	109 (1.3)	104 (2.2)	104 (5.3)	102 (3.8)	93.5 (1.9)	86.8 (8.3)	96.4 (2.5)	98.5 (2.5)	98.7 (3.1)	102 (2.6)	94.5 (2.0)
Ochratoxin A	109 (1.8)	108 (2.1)	94.5 (1.5)	102 (1.9)	101 (1.1)	97.7 (0.9)	90.9 (3.5)	93.8 (3.3)	101 (5.9)	98.1 (1.6)	98.2 (1.3)	82.8 (1.7)
3- + 15-Acetyldeoxynivalenol	104 (6.3)	108 (1.8)	104 (3.3)	101 (6.5)	95.9 (5.8)	91.0 (4.4)	91.9 (4.3)	98.1 (2.7)	95.0 (1.8)	98.4 (5.2)	101 (2.9)	100 (0.9)
Deoxynivalenol	112 (4.0)	102 (2.6)	95.7 (1.3)	98.1 (3.5)	93.7 (4.8)	88.2 (3.4)	-	90.3 (6.4)	94.5 (2.6)	102 (3.5)	97.5 (2.6)	96.9 (0.8)
Diacetoxyscirpenol	105 (4.0)	107 (1.5)	103 (1.2)	93.2 (4.3)	95.4 (3.9)	93.8 (5.0)	90.9 (3.8)	94.5 (4.7)	94.0 (1.9)	98.1 (6.3)	101 (3.1)	98.7 (1.8)
Fumonisin B1	94.3 (4.6)	94.0 (2.8)	92.3 (2.6)	87.2 (3.1)	88.2 (4.5)	87.8 (6.6)	91.8 (3.6)	91.5 (1.9)	91.9 (0.7)	100 (3.2)	99.6 (1.7)	96.1 (1.2)
Fumonisin B2	93.3 (4.1)	95.1 (4.8)	90.3 (2.9)	95.4 (4.7)	92.5 (2.3)	88.8 (3.9)	89.9 (4.1)	92.9 (2.3)	92.4 (0.8)	104 (2.7)	99.6 (1.4)	94.4 (1.6)
Fumonisin B3	91.8 (4.9)	94.6 (4.9)	91.6 (3.1)	90.6 (2.7)	90.1 (3.8)	87.7 (4.7)	91.1 (3.6)	93.1 (1.8)	91.9 (0.9)	104 (2.2)	99.9 (1.4)	95.9 (1.2)
Fusarenon-X	99.0 (3.9)	100 (2.9)	103 (2.8)	86.9 (7.0)	90.3 (11.0)	88.3 (10.1)	-	92.0 (6.8)	94.3 (1.9)	101 (3.8)	100 (3.7)	98.3 (1.6)
HT-2	110 (2.4)	111 (1.4)	108 (1.1)	100 (2.7)	100 (2.0)	94.3 (3.0)	96.8 (3.1)	96.1 (2.1)	99.0 (1.4)	101 (1.6)	103 (2.2)	98.3 (1.3)
Nivalenol	-	-	-	-	98.3 (6.2)	89.0 (3.6)	-	92.5 (4.5)	93.7 (5.0)	-	95.5 (4.7)	92.9 (2.3)
T-2	111 (2.1)	110 (1.8)	108 (2.8)	99.1 (2.7)	101 (1.7)	95.9 (2.1)	92.0 (6.3)	94.7 (1.3)	98.6 (1.5)	102 (1.3)	103 (1.3)	96.9 (1.3)
α-Zearalenol	100 (4.9)	102 (5.2)	90.1 (5.8)	89.2 (8.1)	93.6 (5.5)	94.7 (3.4)	97.7 (3.2)	88.9 (4.2)	90.0 (3.4)	96.9 (3.7)	99.0 (3.6)	95.0 (3.3)
Zearalenone	110 (6.7)	110 (3.0)	105 (3.7)	98.3 (7.3)	97.4 (2.8)	91.3 (1.5)	95.0 (4.5)	93.6 (2.2)	95.7 (2.0)	101 (3.8)	102 (2.1)	92.3 (1.4)
Citrinin	26.1 (9.2)	26.6 (3.1)	30.1 (3.8)	24.1 (8.7)	25.1 (1.9)	25.8 (3.5)	71.9 (4.7)	76.4 (1.6)	77.1 (1.7)	32.3 (3.5)	32.2 (6.3)	35.8 (4.5)
Patulin	106 (4.6)	95.6 (5.6)	89.2 (5.1)	88.8 (12.0)	83.6 (9.0)	86.0 (7.2)	-	98.9 (3.6)	103 (4.5)	93.6 (4.4)	86.1 (3.1)	92.2 (2.9)
Alternariol	108 (4.1)	108 (1.6)	104 (1.0)	94.2 (3.4)	95.4 (2.4)	96.2 (2.7)	89.3 (4.6)	91.8 (2.5)	91.4 (1.3)	98.4 (2.3)	101 (2.5)	96.3 (3.2)
Alternariol monomethylether	108 (4.1)	109 (2.2)	99.3 (2.7)	93.5 (3.3)	93.5 (3.7)	89.8 (2.4)	91.3 (6.6)	88.7 (5.1)	93.9 (3.9)	104 (2.9)	101 (1.7)	93.7 (1.9)
Altenuene	110 (2.1)	109 (2.1)	105 (2.1)	99.6 (2.0)	99.5 (1.2)	95.4 (1.2)	98.4 (3.4)	92.4 (2.1)	92.8 (1.8)	101 (2.9)	101 (3.1)	98.2 (0.5)
Tentoxin	111 (3.6)	109 (2.5)	103 (1.4)	104 (2.9)	101 (1.1)	95.3 (1.4)	92.5 (6.2)	94.2 (2.2)	95.8 (1.4)	104 (4.2)	105 (2.1)	98.2 (1.9)
Tenuazonic acid	-	85.8 (1.7)	87.4 (6.3)	92.5 (4.7)	91.0 (2.1)	88.5 (2.4)	-	89.3 (4.1)	88.5 (2.0)	-	92.5 (8.8)	90.0 (9.5)
Ergocornine	109 (1.5)	109 (1.4)	102 (1.3)	93.8 (3.5)	93.2 (4.4)	91.2 (3.3)	91.5 (3.0)	93.1 (1.9)	92.9 (0.6)	102 (2.5)	101 (1.9)	97.6 (1.7)
Ergocorninine	109 (3.0)	109 (2.0)	101 (1.9)	105 (3.0)	104 (2.4)	99.5 (3.1)	89.9 (3.8)	92.3 (2.2)	92.5 (3.1)	101 (2.5)	102 (2.6)	95.7 (2.4)
Ergocristine	108 (3.1)	108 (2.9)	101 (4.4)	92.1 (3.8)	91.7 (5.1)	92.0 (2.2)	91.3 (2.9)	94.2 (2.0)	94.3 (0.8)	101 (1.7)	99.8 (2.0)	96.7 (1.8)
Ergocristinine	106 (3.5)	105 (1.4)	101 (0.8)	102 (4.8)	104 (4.3)	102 (4.6)	91.6 (5.9)	94.4 (1.8)	95.6 (2.7)	102 (2.9)	102 (3.0)	99.3 (4.5)
Ergocryptine	107 (2.0)	109 (1.9)	104 (3.4)	95.0 (3.0)	94.7 (4.1)	92.1 (1.7)	90.1 (3.0)	93.5 (2.2)	93.2 (0.7)	99.5 (2.7)	99.9 (1.2)	97.4 (1.4)
Ergocryptinine	106 (1.7)	108 (2.0)	101 (1.1)	103 (5.3)	105 (4.0)	101 (4.2)	91.1 (4.3)	95.1 (1.5)	98.1 (1.6)	101 (2.0)	101 (1.8)	95.4 (1.9)
Ergometrine	92.8 (7.3)	90.0 (4.2)	88.3 (3.6)	101 (2.3)	96.2 (2.6)	86.7 (1.9)	90.7 (3.6)	88.9 (6.1)	87.6 (3.5)	101 (1.8)	99.7 (3.2)	95.3 (1.3)
Ergometrinine	101 (4.2)	99.1 (1.9)	94.3 (0.7)	93.2 (4.3)	95.5 (1.7)	89.1 (2.2)	90.6 (3.9)	90.1 (4.4)	89.7 (1.9)	100 (3.5)	98.5 (1.9)	91.1 (1.9)
Ergosine	108 (2.6)	106 (5.6)	101 (3.2)	90.8 (2.0)	91.8 (2.2)	89.2 (2.6)	91.7 (2.2)	90.4 (3.1)	90.3 (1.5)	99.9 (2.7)	99.1 (3.0)	98.2 (1.1)
Ergosinine	111 (1.8)	109 (0.9)	103 (1.1)	100 (1.1)	102 (2.0)	97.7 (2.2)	92.7 (1.4)	93.6 (2.5)	93.8 (0.9)	99.2 (2.8)	98.4 (2.8)	97.5 (1.0)
Ergotamine	109 (1.9)	108 (1.7)	102 (2.8)	91.0 (2.8)	92.6 (2.8)	89.8 (3.6)	91.1 (2.2)	90.6 (3.7)	90.7 (1.3)	101 (2.9)	100 (3.1)	96.4 (2.2)
Ergotaminine	109 (1.0)	109 (0.7)	101 (0.6)	98.2 (2.0)	101 (1.5)	96.6 (1.3)	93.6 (3.5)	94.7 (1.7)	94.5 (0.6)	101 (2.3)	99.7 (1.3)	97.1 (1.5)

Conclusions

The established workflow provides a unique solution for simultaneous determination of Alternaria toxins, ergot alkaloid epimers and other mycotoxins

Simple and fast workflow with a combination of convenient extraction procedure (no additional cleanup) and short-time chromatographic analysis

Workflow applied to multi-mycotoxin quantification in a wide variety of food products

For the vast majority of analytes, the limit of quantification is 0.4 µg/kg which is satisfactory to meet the regulatory or compliance level


THANK
YOU
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SHUKURIA
DANKSCHEEN
BÏYAN
SHUKRIA
TINGKI
YAQHANYELAY
TASHAKKUR ATU
SUKSAMA
EKHMET
GRAZIE
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