Simultaneous Determination of 17 Mycotoxins by LC-MS/MS Using a Novel Polyaromatic HPLC Column

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INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by several species of fungi on agricultural commodities in the field and during storage. To date more than 300 mycotoxins, possessing varying degrees of toxicity, have been identified in agricultural commodities. Mycotoxins are chemically stable and are not always destroyed during food processing and heat treatment. Thus, monitoring of these compounds in food is an important health, agricultural production, food processing and trade concern.

The analysis of mycotoxins is challenging due to the large number of compounds to be detected and the wide physicochemical properties they possess. In addition, the food commodities tested are typically complex in nature and may simultaneously contain several mycotoxins at low concentrations. Sample preparation approaches used for mycotoxin analysis, including solid-phase extraction (SPE), immunoaffinity chromatography and QuEChERS, are complicated by the different polarity and solubility of the mycotoxins. Due to the limited sample cleanup that can be incorporated into a method, sample extracts may still contain a large amount of matrix components that can negatively affect the detection system.

LC-MS/MS has become the detection system of choice for mycotoxin analysis due to its good sensitivity, selectivity and ability to detect a wide range of compounds. However, challenges still remain, including the retention of the very polar trichothecenes and the reduction of matrix effects. This poster details a LC-MS/MS method for the simultaneous analysis of 17 mycotoxins. The compounds included in this method represent a wide range of mycotoxins, including type A- and B-trichothecenes, aflatoxins, ochratoxin A, zearalenone and fumonisins. HPLC separation is conducted on a Selectra® DA column, which contains a novel polyaromatic stationary phase that is capable of greater retention of polar compounds compared to a standard C18 column.

METHOD DEVELOPMENT

- A variety of mobile phase conditions were evaluated:
  - Solvent – MeOH and MeCN.
  - Additive – formic acid, ammonium acetate, ammonium hydroxide and ammonium bicarbonate.
- MeOH gave better peak shape and increased MS response for trichothecenes compared to MeCN.
- Formic acid gave poor peak signal for some trichothecenes.
- Ammonium acetate gave poor peak shape for deoxynivalenol and fusarenon X.
- Ammonium hydroxide gave overall good peak shape and sensitivity. However, at high pH the fumonisins are ionized and too polar to be retained on the column. Acidifying the sample extract helps to overcome this problem, but has a negative effect on other analytes.
- Ammonium bicarbonate gave good peak shape and sensitivity for all analytes, including satisfactory retention of the fumonisins.
- α- and β-zearalanol were successfully baseline resolved using the Selectra® DA column.
- The method can detect mycotoxins at ≤10 ng/mL.

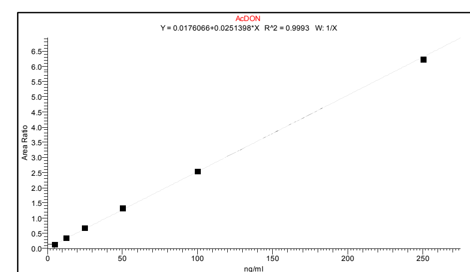
ANALYSIS

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000 LC system
HPLC column	UCT Selectra® DA, 100 × 2.1 mm, 3 µm (p/n: SLDA100ID21-3UM)
Guard column	UCT Selectra® DA, 10 × 2.1 mm, 3 µm, (p/n: SLDAGDC21-3UM)
Guard column holder	p/n: SLDGRDHLDR
Mobile phase A	H_2O + 10 mM ammonium bicarbonate
Mobile phase B	Methanol + 10 mM ammonium bicarbonate
Flow rate	300 µL/min
Column temp.	30°C
Run time	20 min (including a 5 min equilibration step)
Sample composition	methanol: water (50:50, v/v)
Injection volume	10 µL
Autosampler temp.	10°C
Wash solvent	methanol: water (50:50, v/v)
Divert valve	Mobile phase was sent to waste from 0-3.5 and 15-20 min to reduce ion source contamination.

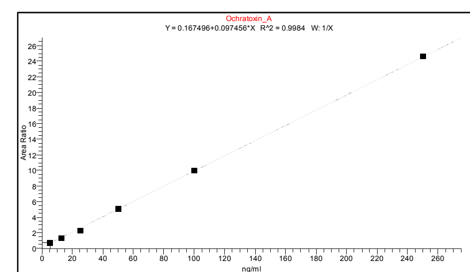
LC Gradient:

Time (min)	% A	% B
0.0	95	5
4.0	40	60
7.0	40	60
9.0	0	100
15.0	0	100
15.2	95	5
20.0	95	5

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™ tandem mass spectrometer
Ionization mode	ESI^+ and ESI^-
Spray voltage	4500 V (ESI^+) / 3500 V (ESI^-)
Vaporizer temperature	450°C
Capillary temperature	240°C
Sheath gas pressure	60 arbitrary units
Auxiliary gas pressure	55 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	0 V
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas	Argon
Collision gas pressure	1.7 mTorr
Acquisition method	EZ method (scheduled SRM)
Cycle time	1 sec

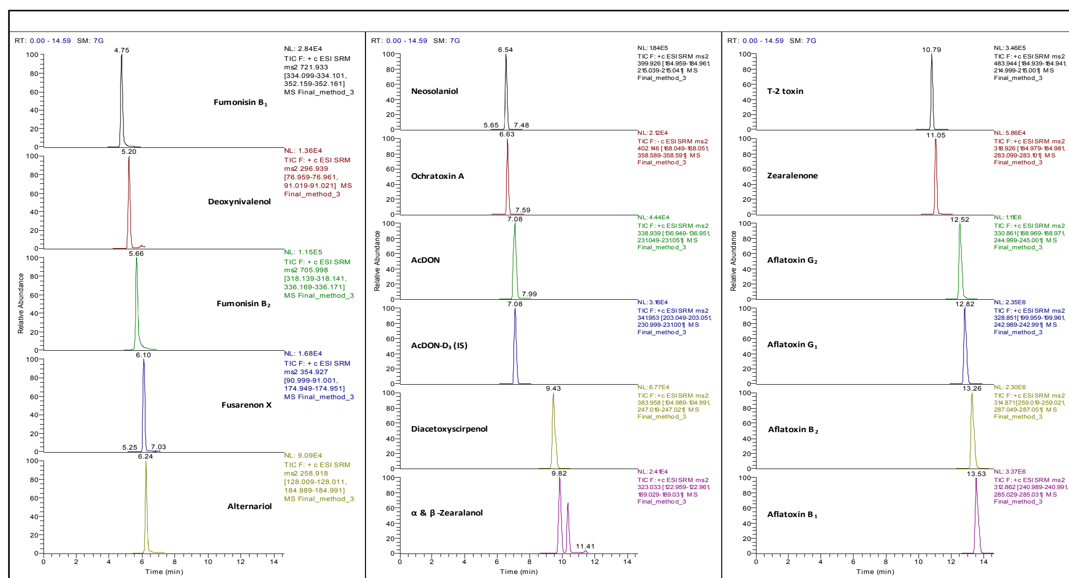


Calibration curve example (ESI^+)



Calibration curve example (ESI^-)

CHROMATOGRAM



CONCLUSIONS

An LC-MS/MS method has been successfully developed for the simultaneous determination of 17 mycotoxins. Separation of the mycotoxins, including α- and β-zearalanol, was achieved within 15 min on a Selectra® DA HPLC column. Ammonium bicarbonate was found to be the best mobile phase additive to incorporate into the method. The use of LC-MS/MS detection provides sufficient selectivity and sensitivity for the identification and quantification of the mycotoxins. The use of rapid polarity switching allows all target analytes to be detected in a single run. Future work will focus on the development of an accompanying SPE sample preparation procedure for the isolation and purification of mycotoxins from food samples prior to analysis using the LC-MS/MS method outlined in this poster.

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