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Analysis of Fumonisin Mycotoxins by LC/MS

Application Brief

Agilent 1100 Series LC/MSD
Foods, Environmental

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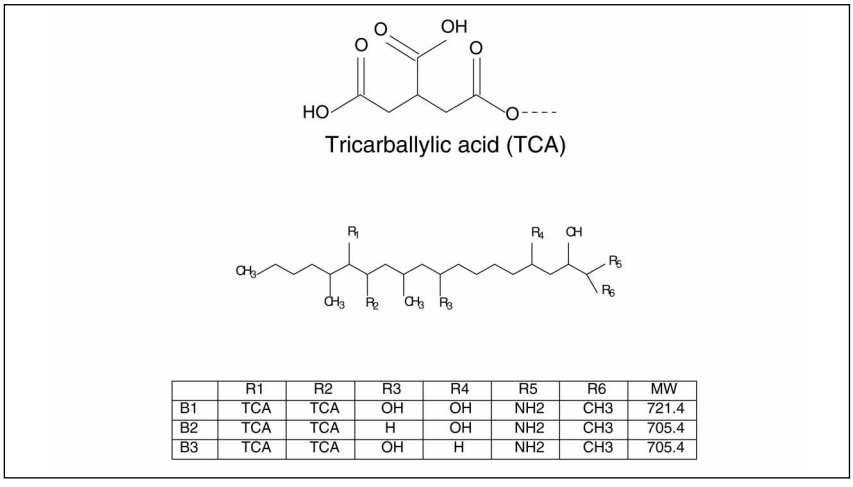
Introduction

The fungus *Fusarium*, which is known to infest corn and corn products, produces a group of mycotoxins called fumonisins. The toxicities of the most abundant fumonisins, $B_{1\text{-}3}$, have been extensively studied, and a variety of species-specific toxicities have been published. These compounds may be carcinogenic to humans. Fumonisins are characterized by a 19-carbon aminopolyhydroxyalkyl chain that is diesterified with propane-1-2, 3- tricarboxylic acid. Analogues $B_{1\text{-}3}$ show a difference in the number and position of the hydroxyl groups (Figure 1). Fumonisins B_2 and B_3 have the same molecular weight.

Most analytical methods exclude the detection of one or more of the known fumonisins. Traditional HPLC analysis requires the derivatization of the amino group. In this paper, we show that the Agilent 1100 Series LC/MSD can detect fumonisins without derivatization.

Experimental

The system comprised of an Agilent 1100 Series binary pump, vacuum degasser, autosampler, thermostated column compartment, diode-array detector (DAD), and LC/MSD. The LC/MSD used electrospray ionization (ESI). Complete system control and data evaluation were done on the Agilent ChemStation for the LC/MSD.



	R1	R2	R3	R4	R5	R6	MW
B1	TCA	TCA	OH	OH	NH2	CH3	721.4
B2	TCA	TCA	H	OH	NH2	CH3	705.4
B3	TCA	TCA	OH	H	NH2	CH3	705.4

Figure 1. Structure of fumonisins.

Results and Discussion

The fumonisin analogues were analyzed in scan mode at a high concentration (25 ng) to determine the molecular ion and confirming fragments. The initial conditions showed the molecular ion $[M+H]^+$, but no significant fragment ions. Collision induced dissociation (CID) was used to generate more fragments for structural confirmation. Fumonisin B_2 and B_3, indistinguishable by their spectra, were easily separated chromatographically (Figure 2).

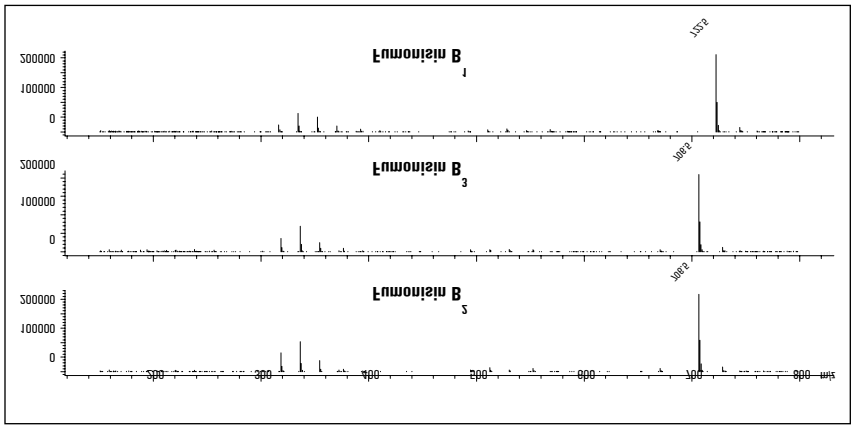


Figure 2. Mass spectra for fumonisin analogues.

The total ion chromatogram (TIC) shows very good sensitivity at 25 ng (Figure 3). To further improve sensitivity, the standards were run in the selected ion monitoring (SIM) mode.

Chromatographic Conditions

Column:	150 x 2.1 mm Zorbax Eclipse XDB, C18, 5 μm
Mobile phase:	A = 5 mM ammonium acetate in water, pH 3 B = acetonitrile
Gradient:	Start with 33% B at 8 min 60% B at 9 min 33% B
Flow rate:	250 μl/min
Injection vol:	5 μl
Column temp:	40°C
Diode-array detector:	Signal 220, 4 nm; reference 550,100 nm

MS Conditions

Source:	ESI
Ion mode:	Positive
Vcap:	4000 V
Nebulizer:	30 psig
Drying gas flow:	10 l/min
Drying gas temp:	350°C
Scan range:	120-820 amu
Step size:	0.1
Peak width:	0.15 min
Time filter:	On
Fragmentor:	Variable 230 V (100-680) 100 V (680-800)

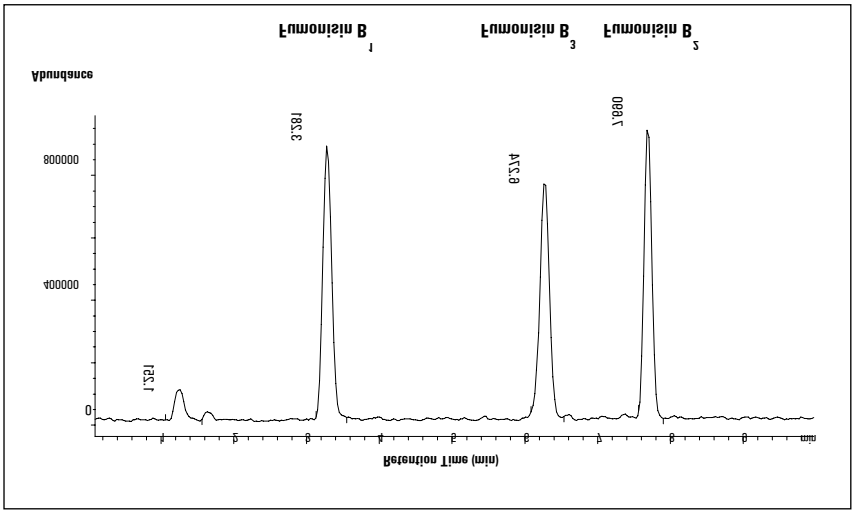


Figure 3. Chromatographic separation of fumonisin analogues at 25 ng.

Figure 4 shows the extracted ion chromatograms for 250 pg of fumonisins in a corn extract. The mass spectra showing the molecular and fragment ions provide high-confidence identification and quantification.

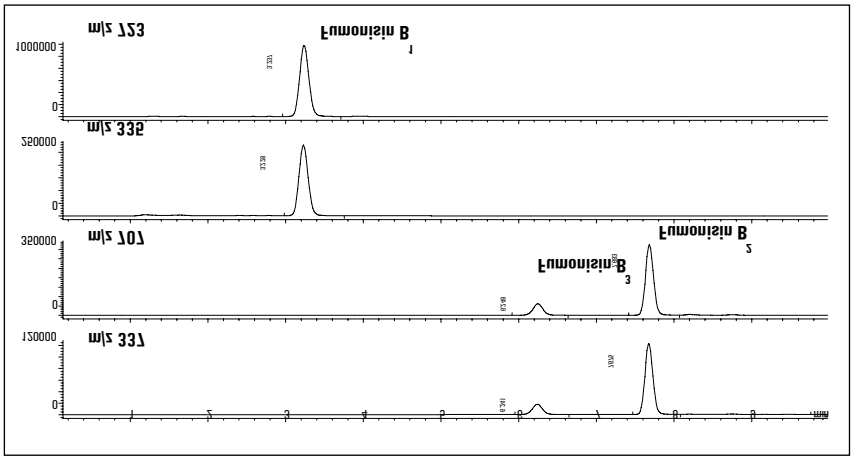


Figure 4. SIM of molecular and fragment ions for fumonisins in spiked corn extract.

Chromatographic Conditions

Column:	150 x 2.1 mm Zorbax Eclipse XDB, C18, 5 μm
Mobile phase:	A = 5 mM ammonium acetate in water, pH 3 B = acetonitrile
Gradient:	Start with 33% B at 8 min 60% B at 9 min 33% B
Flow rate:	250 μl/min
Injection vol:	5 μl
Column temp:	40°C
Diode-array detector:	Signal 220, 4 nm; Reference 550,100 nm

MS Conditions

Source:	ESI
Ion mode:	Positive
Vcap:	4000 V
Nebulizer:	30 psig
Drying gas flow:	10 l/min
Drying gas temp:	350°C
SIM ions:	at 0 min, 334.4, 352.4, 370.4, 722.5 at 5 min, 336.4, 354.4, 706.5
Step size:	0.1
Peak width:	0.15 min
Time filter:	On
Fragmentor:	Variable 230 V (334.5, 352.4, 370.4) 100 V (706.5, 722.5)

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Conclusion

The Agilent 1100 Series LC/MSD is capable of detecting fumonisins at low levels without derivatization. Mass spectrometry allows specific and sensitive detection in complex matrices such as corn extract.

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