

Analysis of Aflatoxins by ChroZen TQ LC/MS

- TQ LC/MS Application



Abstract

Aflatoxins are the most common mycotoxins and are produced by *Aspergillus* species as secondary metabolites in crops such as maize (corn), peanuts, cottonseed, and tree nuts. They are potent carcinogens that are strictly regulated in most countries.

According to Food and Drug Administration (FDA), the allowed limit of aflatoxins is ≤ 20 ppb for food and ≤ 300 ppb for animal feed. The European Commission (EU) has set the allowed limit of $\leq 2-8$ ppb for aflatoxin B1 and $\leq 4-15$ ppb for four aflatoxins (B1, B2, G1 and G2) which is much lower than FDA's level.

In many aflatoxin analysis, HPLC with fluorescence detection is widely used and purification steps and derivatization are needed for high sensitivity. However, our ChroZen Triple Quadrupole LC/MS can efficiently perform the analysis of aflatoxin B1, B2, G1 and G2 without derivatization and solid phase extraction (SPE).

Instruments and Software

Item	Description	Part No.
ChroZen TQ LC/MS System	ChroZen LC-TQ	CRZLCTQAY60
	1) Heater Electrospray (H-ESI) Source	
	2) Atmospheric Pressure Chemical Ionization (APCI) Source	
	3) Foreline Pump x 2set	
	4) PC, 24-inch Monitor	
ChroZen UHPLC System	5) Workstation Software	
	UHPLC Solvent Tray	5451011010
	ChroZen UHPLC Pump	9431011010
	ChroZen UHPLC Column Compartment	5431011010
ACC	ChroZen UHPLC Autosampler (Cool, Heat)	9451011011
	UHPLC Performance Kit	9361011150
	ESI Needle	3944450A
	APCI Needle	3944365
	Hamilton 500ul	81265
	ChroZen TQ Installation Kit	394440790
	Programmable Syringe Pumps for MS Infusion	NE-1000
	Autosampler vial 100/pk	5182-0715
Autosampler cap, Septa 100/pk	5182-0717	

Table 1. ChroZen TQ LC/MS system configuration

Reagents and Standards

Aflatoxin standard solution



Fig 1. ChroZen TQ LC/MS

Optimized MRM Transitions

Aflatoxin	RT (min)	Precursor Ion [M+H] ⁺	Product Ion (m/z)	Collision Energy (eV)
Aflatoxin B1	2.304	313	241/285	35/21
Aflatoxin B2	2.219	315	259/287	29/19
Aflatoxin G1	2.158	329	200/243	37/26
Aflatoxin G2	2.079	331	245/275	22/27

Table 2. Optimized aflatoxin MRM transitions

LC Conditions and MS Conditions

LC conditions			
Mobile Phase	A: 5mM AF + 0.1% FA in Water B: 5mM AF + 0.1% FA in ACN		
Flow Rate	0.4ml/min		
Injection Vol.	5.0 µL		
Column	2.1mm I.D. x 50mm, 2.7 µm		
Column Temperature	40°C		
Pump Gradient	Time(min)	A (%)	B (%)
	0	90	10
	0.5	90	10
	1	30	70
	3	5	95
	3.5	5	95
	3.6	90	10
	5	90	10
MS conditions			
Source Type	HESI		
Spray Voltage	4500V (Positive)		
Cone Gas Temperature	350°C		
Cone Gas Flow Rate	15 units		
Heated Probe Temperature	450°C		
Probe Gas Flow Rate	50 units		
Nebulizer Gas Flow Rate	50 units		
Active Exhaust	On		

Table 3. LC conditions and MS conditions

Result

1) Total Ion Chromatogram

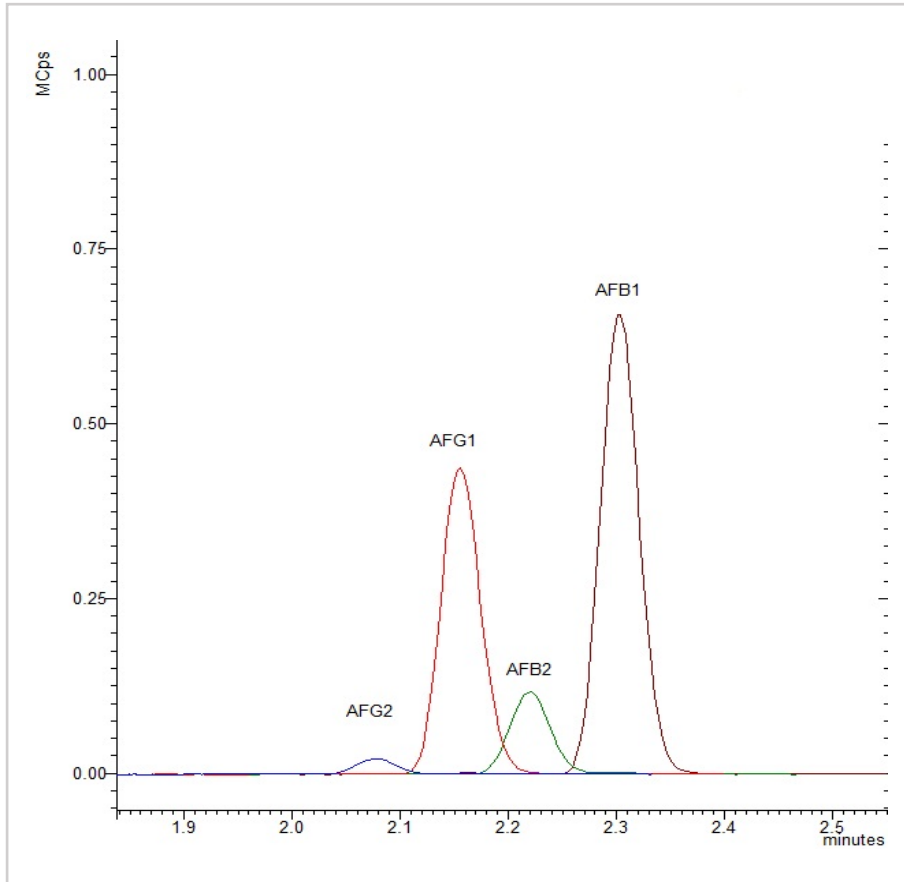


Fig 2. TIC of 10 µg/L AFB1 and AFG1, 3 µg/L AFB2 and AFG2 standards

2) Extracted Ion Chromatogram

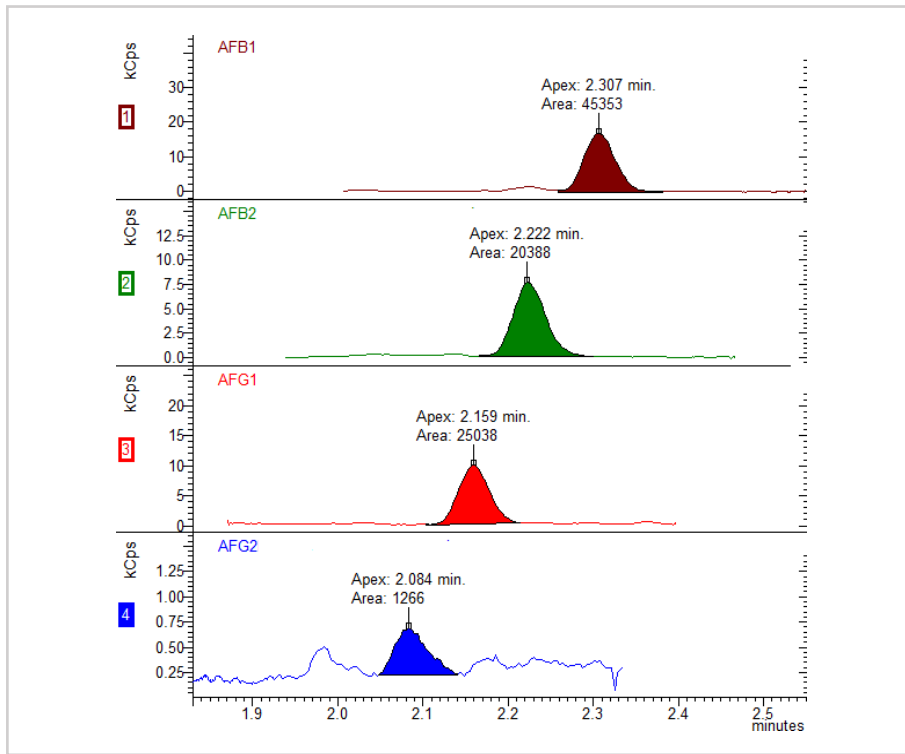


Fig 3. EIC of 1 µg/kg AFB1 and AFG1, 0.3 µg/kg AFB2 and AFG2 samples

3) Chromatogram Overlay

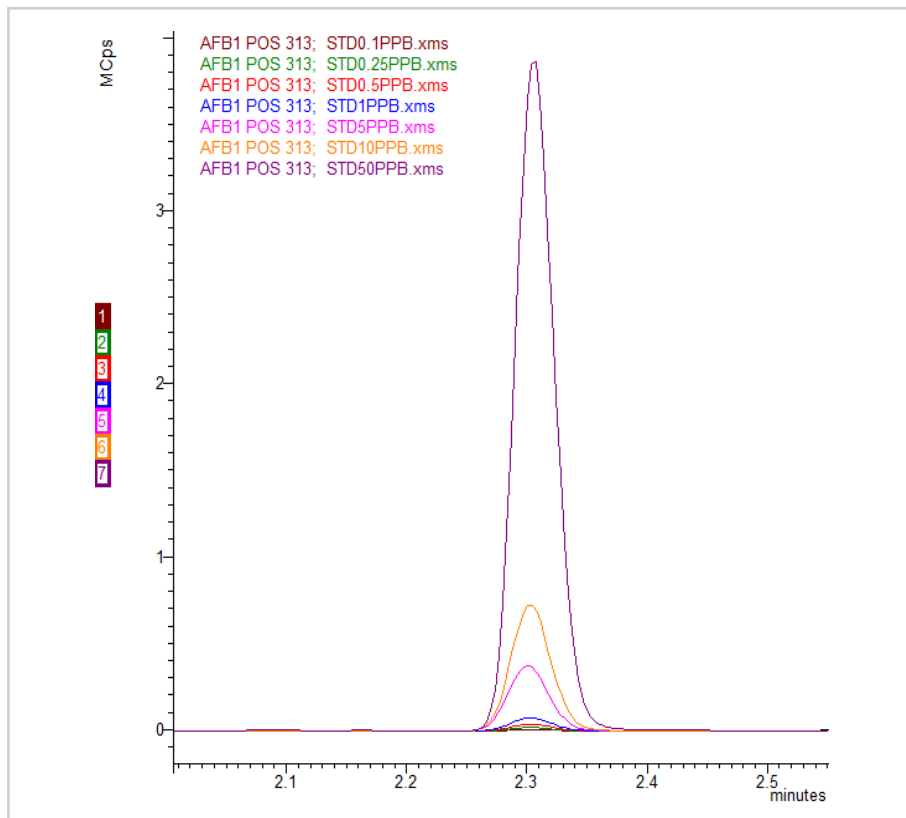


Fig 4. Overlay of 0.1 - 50 ppb AFB1

4) Calibration Curves

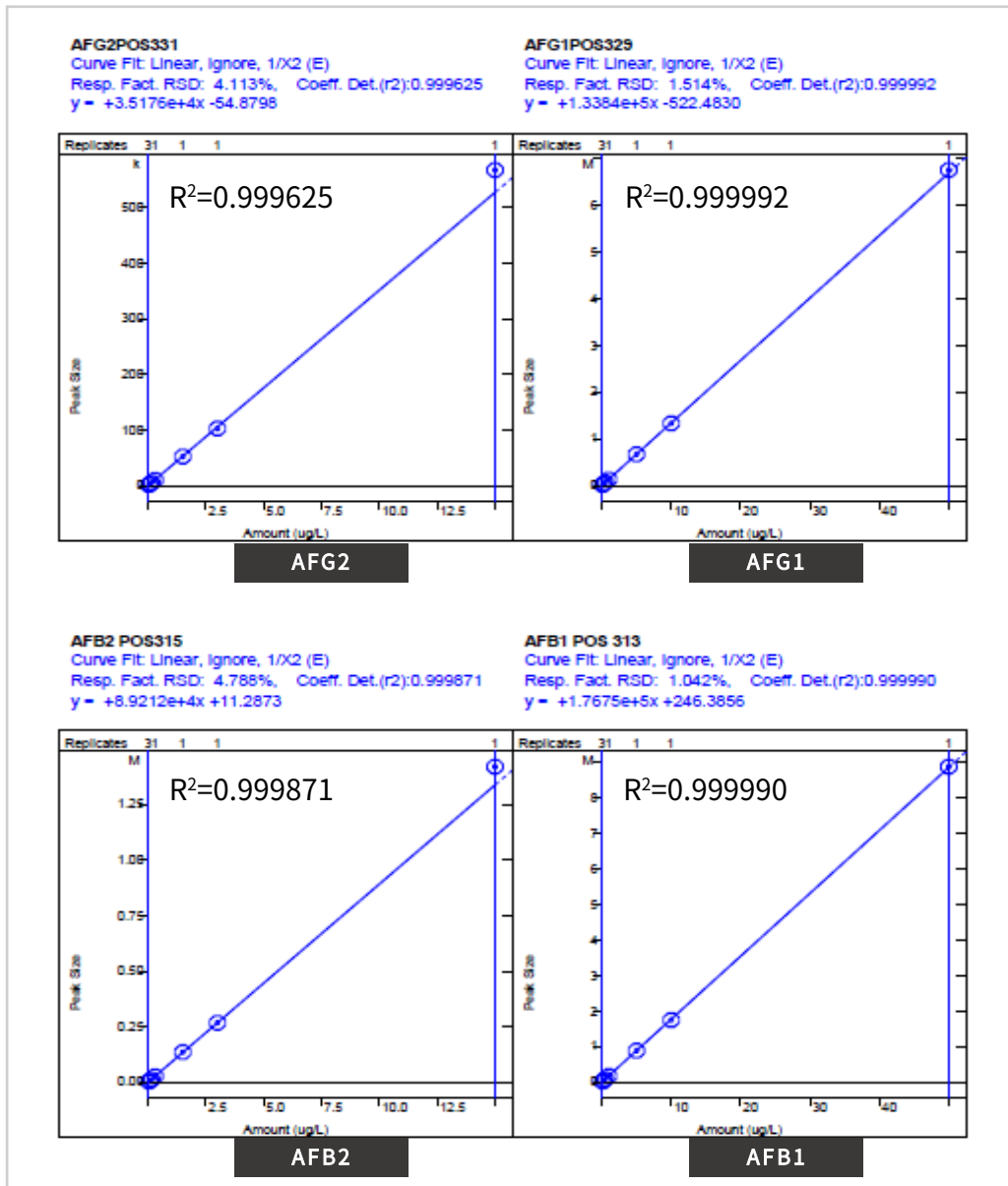


Fig 5. Calibration curves of 0.1 – 50 ppb aflatoxin solvent standards

Conclusion

This study determines the efficient detection and quantitation of aflatoxin B1, B2, G1 and G2 using ChroZen TQ LC/MS without the need of derivatization or solid phase extraction (SPE).

The results show excellent calibration linearity for 4 aflatoxins as the correlation coefficients (R^2) greater than 0.999 at the standard curve range of 0.1 ~ 50 ppb.

The limit of detection (LOD) was 0.0016, 0.0029, 0.0017 and 0.0063 ppb for B1, B2, G1 and G2 (S/N=3) and the limit of Quantitation (LOQ) was 0.0053, 0.0097, 0.0058 and 0.0208 (S/N=10) by seven replicate injections of 0.25 ppb standard solutions.

References

- FDA Guidance Documents, Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed; CPG 683.100 Animal Feeds, CPG 555.400 Foods
- Commission Regulation(EU) No 165/2010. Amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins.



60, Anyangcheondong-ro, Dongan-gu,
Anyang-si, Gyeonggi-do, 14042, Republic
of Korea

TEL: +82-31-428-8700

FAX: +82-31-428-8787

E-mail: export@youngincm.com

Homepage: www.youngincm.com