Screening Mycotoxins on Sorghum Grain via Superficially Porous Particle Columns

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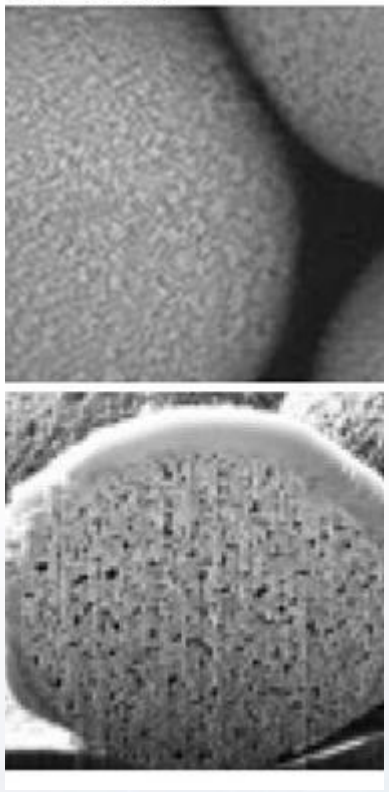
Overview

- Why SPP?
- High throughput screening
  - Speed, selectivity and sensitivity
- Qualitative results
- Summary/Conclusions

<10 minutes screening

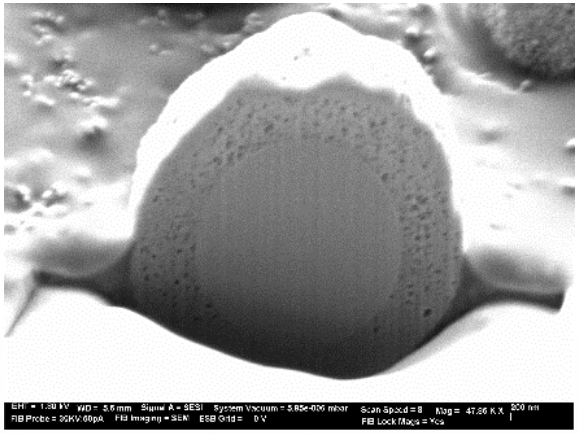
High resolution LC and MS are important

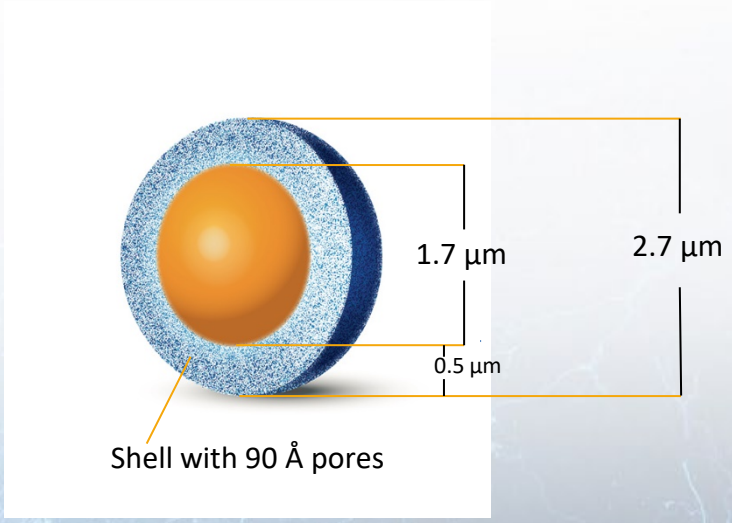
SPP Technology



Fully Porous Particle (FPP)

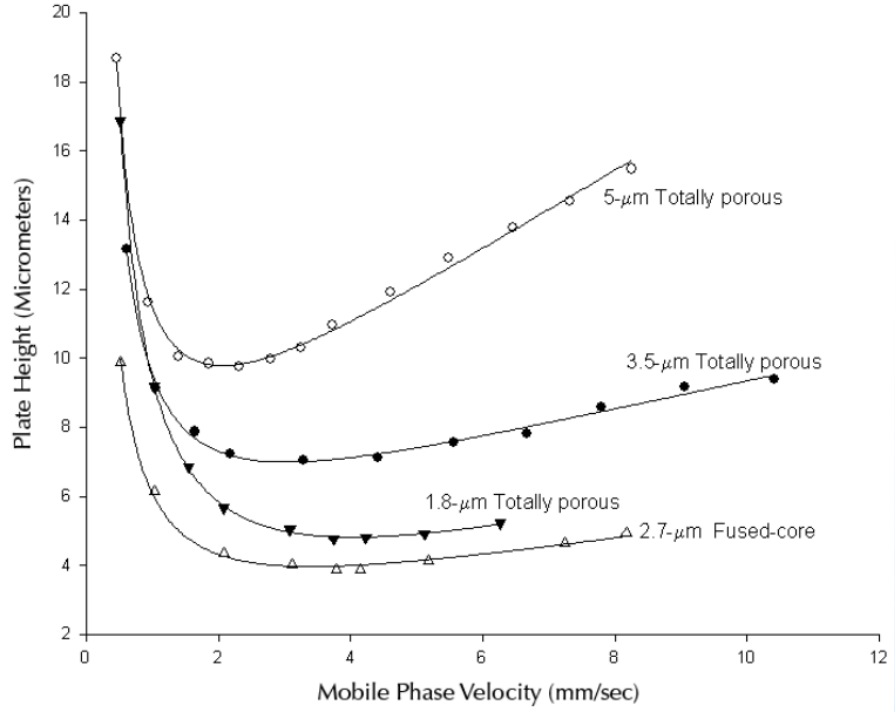
HALO 90 Å, 2.7 µm

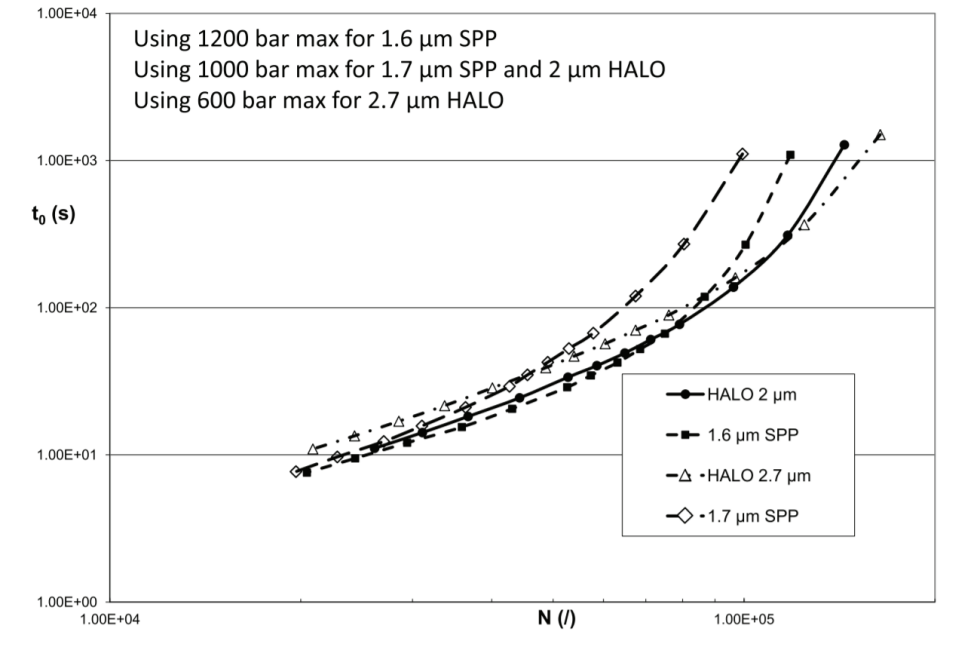




Superficially Porous Particle (SPP)

Column selection – SPEED & Performance





J.J. DeStefano, T.J. Langlois, & J.J. Kirkland, ***J. Chromato. Sci.***, 2008, 46(3), 254-260

Mycotoxins

- Secondary metabolites of fungi
  - Low molecular weight
  - Toxic in low concentrations
  - Chemically and thermally stable during food processing
  - Term first used in 1962 after 100,000 turkey chicks died after eating peanut meal contaminated with aflatoxins
- Major mycotoxins
  - Aflatoxins, citrinin, ergot alkaloids, fumonisins, ochratoxins, patulin, trichothecenes, and zearalenone










Sorghum - our grain of choice

- Drought tolerant
- 3 different lots were blinded
  - animal feed
  - human consumption

Cycle – how are we affected?

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Fungal infection

Cycle – how are we affected?

Fungal infection

MTOX Production

Cycle – how are we affected?

Fungal infection

MTOX Production

Primary mycotoxicosis

Secondary mycotoxicosis

Screening Methodology

Simple, fast and highly sensitive:

- Sample Preparation – UNADULTERATED
  - Pressure, liquid, temp
  - Small scale ‘ASE’ approach for sample preparation

Screening Methodology

Simple, fast and highly sensitive:

- Column separation
  - Superficially porous particle technology
  - PFP and C18 selectivities
  - **Column dimensions:** 2 µm d_p, 2.1 × 50 mm, 90Å

Temperature: 60 °C

Flow rate: 400 µL/min

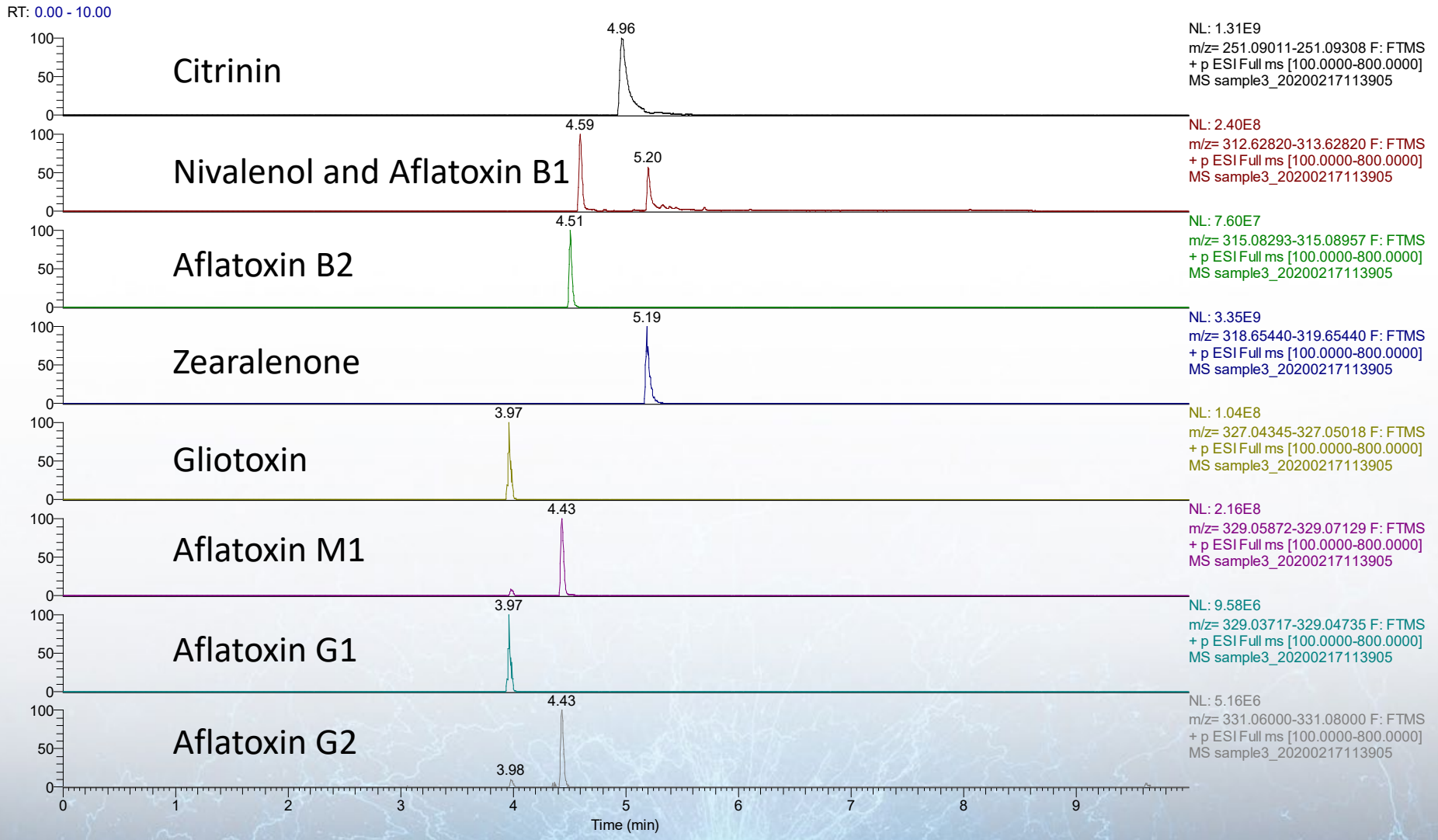
Injection volume: 0.5 µL

Mobile Phase A: 0.1% formic acid and 5 mM ammonium formate in water

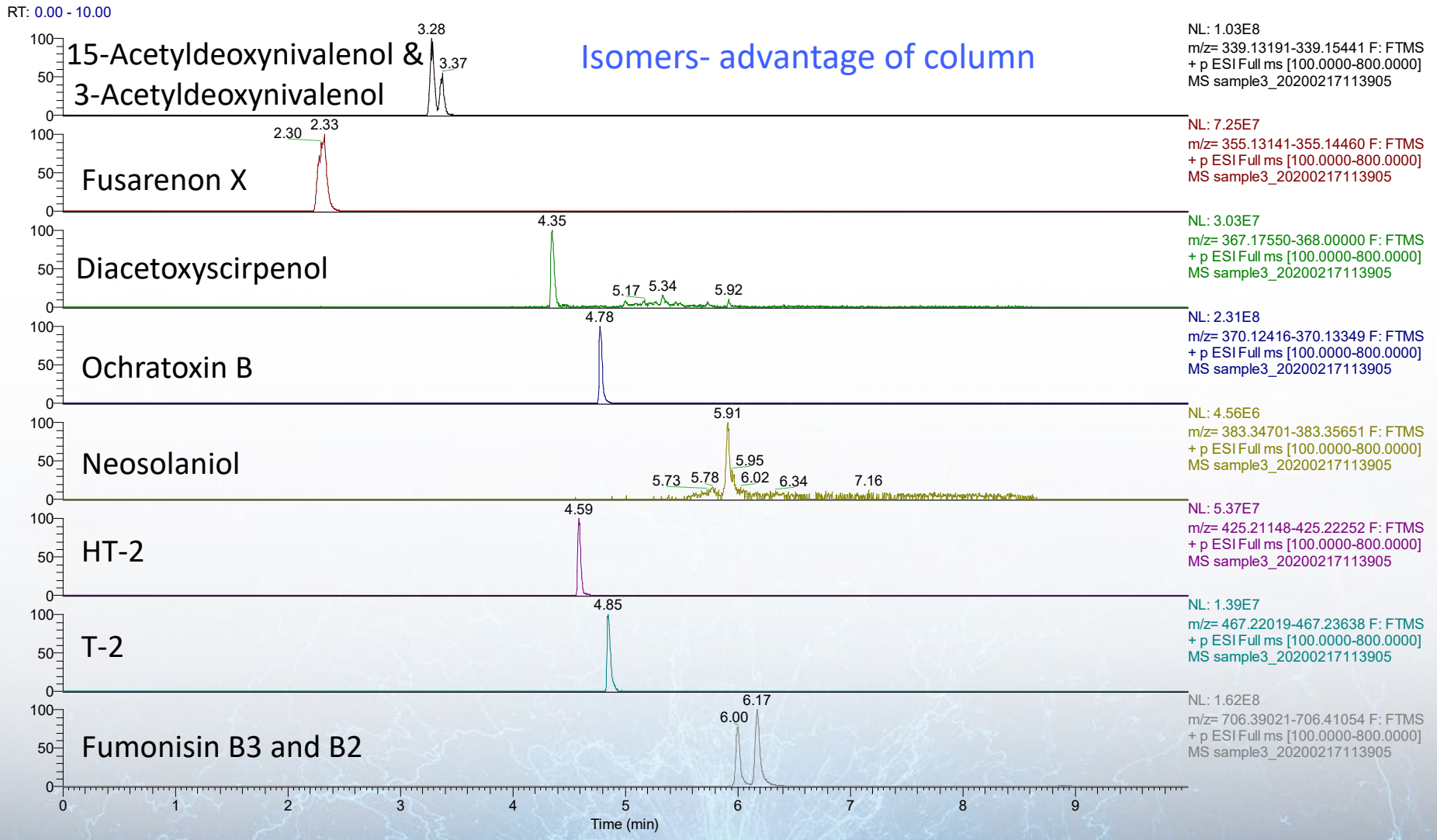
Mobile Phase B: : 0.1% formic acid and 5 mM ammonium formate in methanol

Gradient method

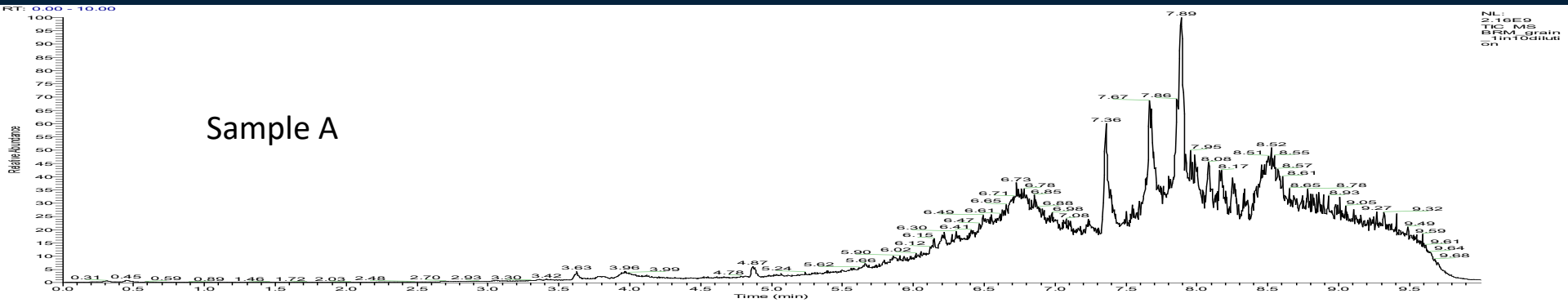
Standard separation - EIC

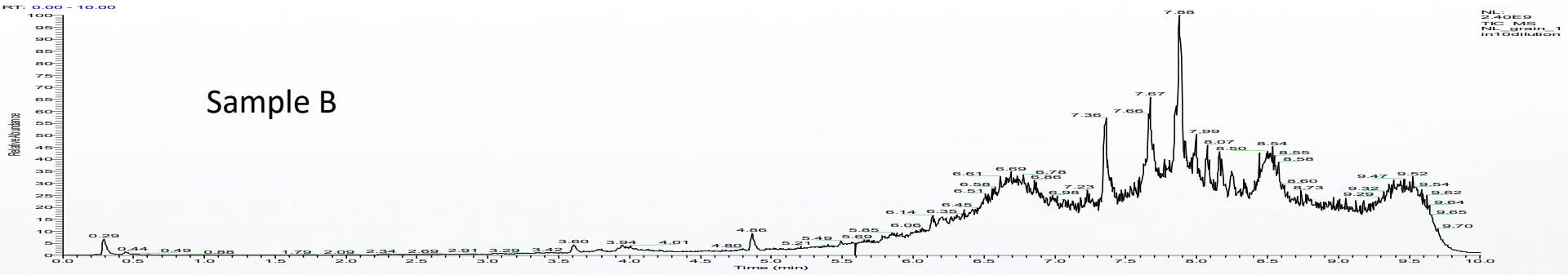


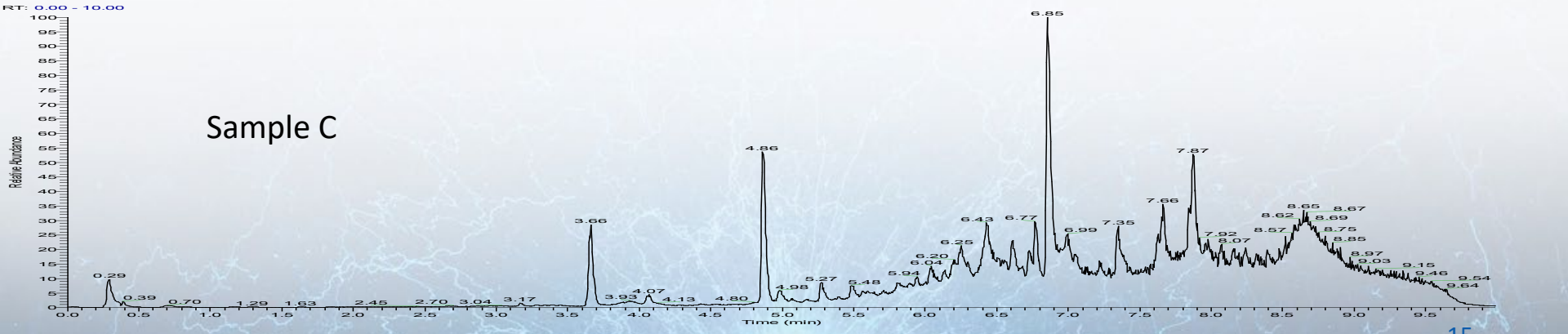
Standard separation EIC



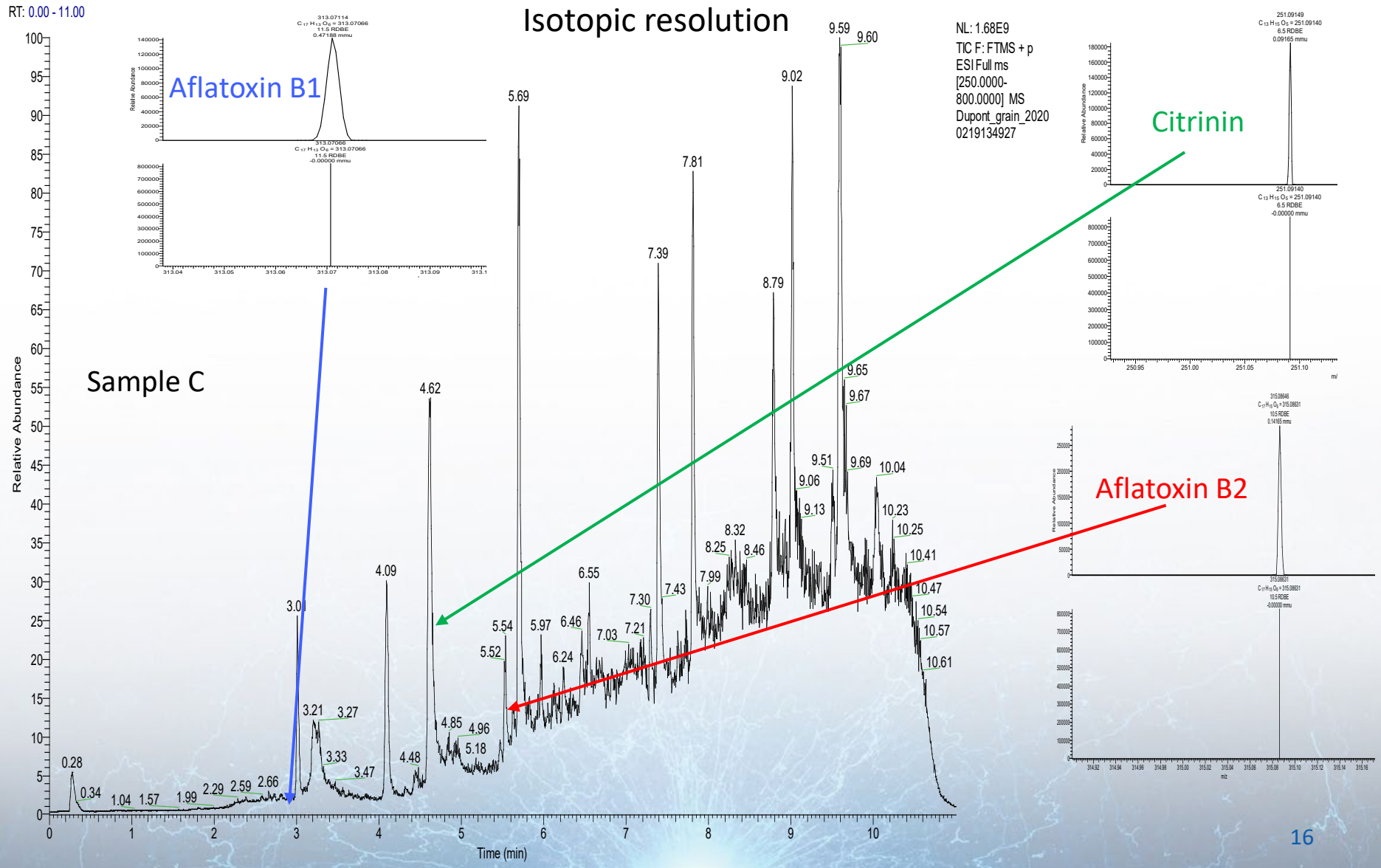
Screening 3 grains - TIC



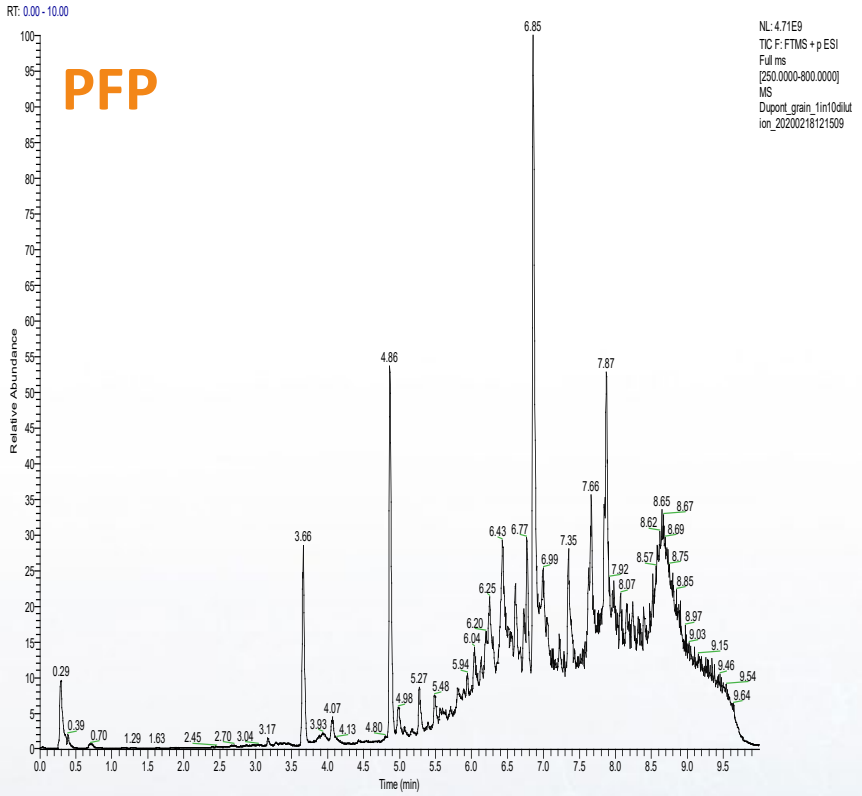


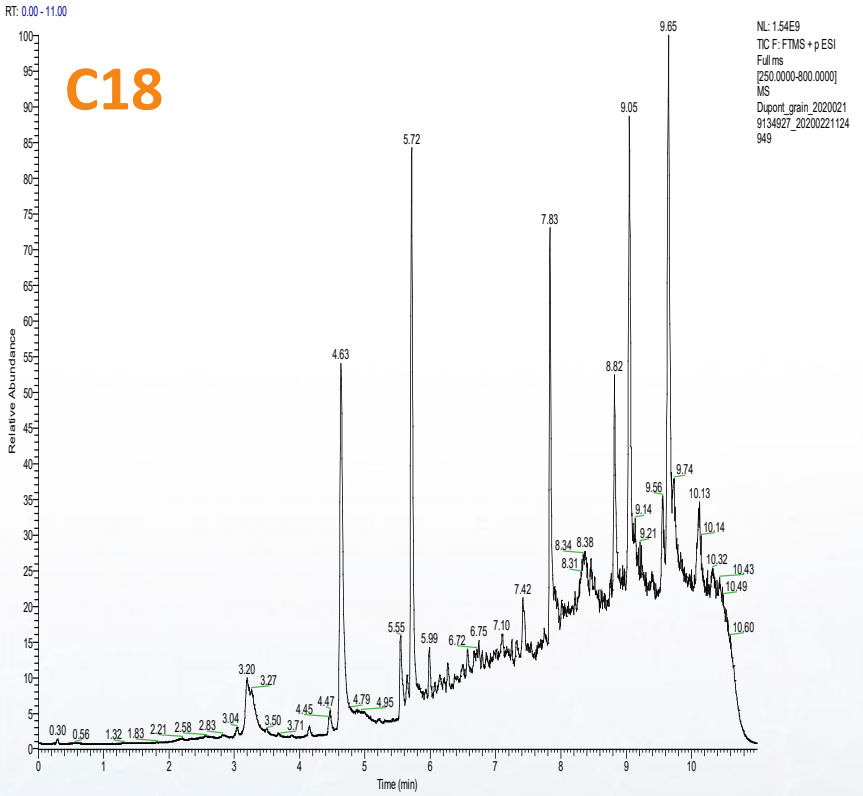


Identification: Exact Mass Confirmation



Selectivity Differences





Unique advantages with different retention behavior

- C18 is not a ‘universal’ column
- Mitigates difference in ion suppression/enhancement
- Matrix effects differ with PFP and C18

Mycotoxin screening

Compound	Formula	Precursor ion	Sample A	Sample B	Sample C
Citrinin	$C_{13}H_{14}O_5$	251.0860	Y	Y	N/D
Nivalenol	$C_{15}H_{20}O_7$	313.1235	N/D	Y	Y
Aflatoxin B1	$C_{17}H_{12}O_6$	313.0662	N/D	Y	Y
Aflatoxin B2	$C_{17}H_{14}O_6$	315.0820	N/D	Y	N/D
Zearalenone	$C_{18}H_{22}O_5$	319.1491	Y	Y	Y
Aflatoxin M1	$C_{17}H_{12}O_7$	329.0604	Y	N/D	Y
Aflatoxin G1	$C_{17}H_{12}O_7$	329.0601	N/D	Y	Y
Fumonisin B1	$C_{34}H_{59}NO_{15}$	722.3868	Y	N/D	N/D

Conclusion

- < 10 minutes screened mycotoxins in sorghum
  - High resolution and speed is critical
  - No universal separation column
    - » PFP and C18 selectivity differences

Acknowledgements

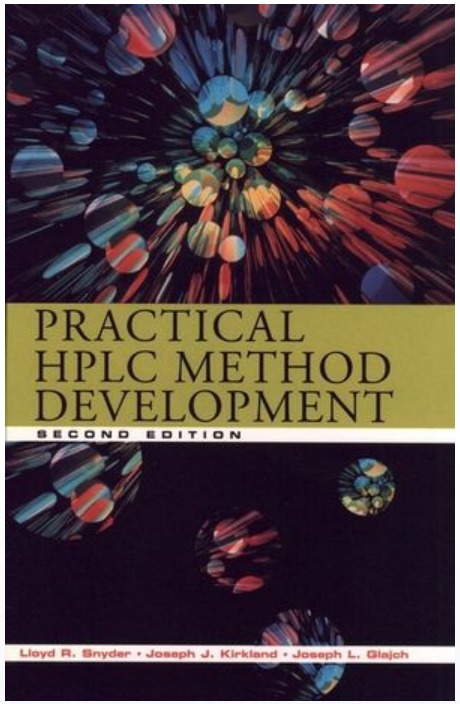
- USDA for the grains
- The team at AMT

EXTRA SLIDES

Superficially Porous Technology

Dr. Joseph Jack Kirkland

May 24, 1925 – October 30, 2016



Innovations

Zipax, 1969

Permaphase, 1972

Zorbax, 1972

Zorbax-Rx, 1988

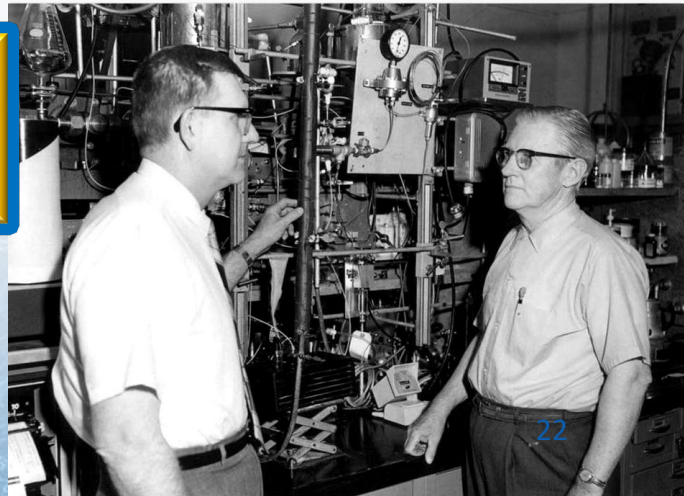
StableBond, 1989

Poroshell, 2000

HALO, 2006

150 peer reviewed publications
28 patents
8 books on HPLC
Educated > 5000 people

HPLC Pioneer
One of the founders of HPLC conference



Milestones in Fused-Core® History

1960 Superficially porous particles (SPPs) were first proposed by Golay

1969 Jack Kirkland (DuPont Company), inspired by Horvath, Preiss, and Lipsky's nucleotide separation using ~50 µm cores containing thin layer of anion exchange resin, develops Zipax®, a 30 µm particle of silica sol on glass beads

25 yrs

Kirkland continues to develop design advantages for SPP, but totally porous particles develop rapidly and dominate HPLC column market. However, demand for speed and resolution create need for particle innovation that delivers higher performance at lower pressures.

2006 **Kirkland, Langlois and DeStefano, at Advanced Materials Technology, Inc. were first to commercialize Fused-Core® silica particle smaller than 3 µm (HALO® 2.7 µm) to meet these demands**

2009 AMT continues to be a leader in development and commercialization of novel packing materials for both small and large molecules (**HALO® BioClass 160Å and 400Å and HALO® 90 Å 2µm**)

2014

2015 AMT celebrates its tenth year of innovative solutions for challenging separations

2017 **First 1000Å SPP product for large molecule analysis with development by Boyes and Kirkland**

Today **The innovation continues…..**

Columns: 4.6 x 50 mm,
FPP C18, 5 µm
FPP C18, 3.5 µm
FPP C18, 1.8 µm
HALO C18, 2.7 µm

Solute: naphthalene
Mobile phase: 60% ACN/40% water 24 °C