

Determination of Mycotoxins in Tobacco and Smokeless Tobacco Products by Liquid Chromatography Tandem Mass Spectrometry

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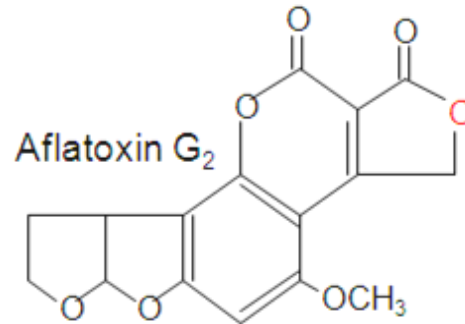
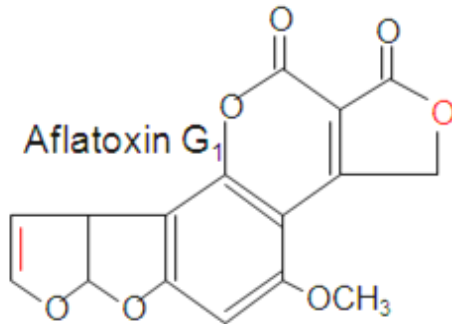
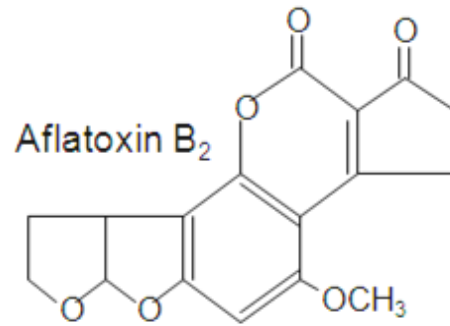
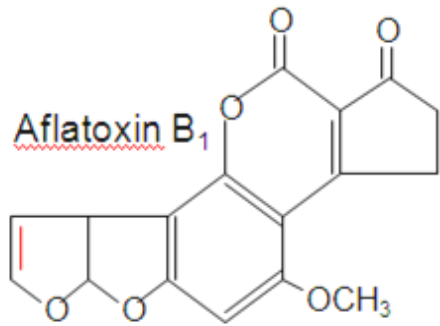
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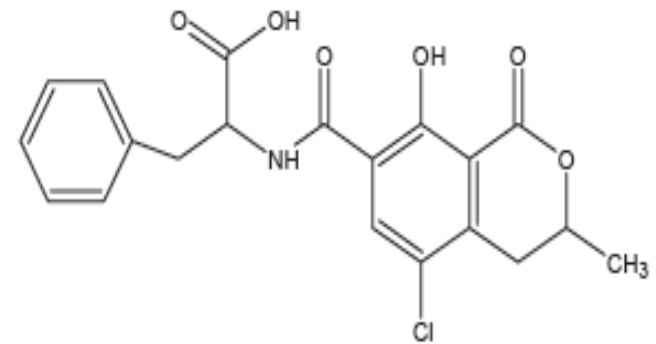
Why Mycotoxins?

- Aflatoxins B1, B2, G1, G2 (Group 1 by IARC, Vol. 82 in 2002; Vol. 100 in 2012) – “carcinogenic to humans”
<http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-23.pdf> (accessed on September 11, 2013)
- Ochratoxin A (Group 2B by IARC, Vol. 56 in 1993) – “possibly carcinogenic to humans”
<http://monographs.iarc.fr/ENG/Monographs/vol56/volume56.pdf> (accessed on September 11, 2013)
- Regulated Limit in food (by EC No1881 in 2006): low ppb (ng/g)
- Smokeless Tobacco (EU by ESTOC in 2009): 5ppb sum of Aflatoxins (B1, B2, G1, G2)
- Aflatoxin B1 in Tobacco (HPHC List, FDA, USA, 2012)

Mycotoxins



Ochratoxin A



Highly soluble in methanol and polar organic solvents
Sensitive to Heat and UV Light (emitting fluorescence)

Sample Preparation

2g tobacco sample

Spike Internal Standard (IS)

Extract in 20mL methanol-water solution (8:2 in v/v)

Shake 30min and then stand for 10min

Syringe filter to a collection tube

Direct LC-MS/MS

Dilute with PBS buffer and then pass through SPE for clean up



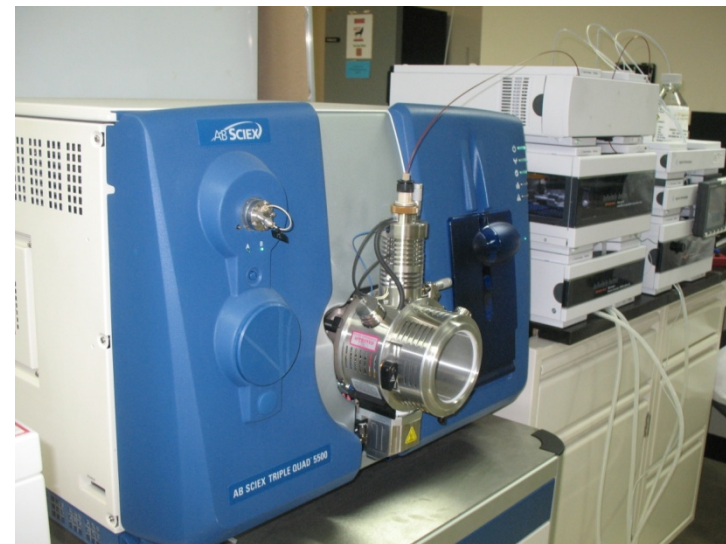
Instrumentation- LC-MS/MS

Agilent 1200 HPLC - AB/MDS Sciex API5500 Mass

Agilent ZORBAX Eclipse XDB-C18 column

Mobile phase: A, water /0.1% formic acid

B: methanol /0.1% formic acid /5mM ammonium formate



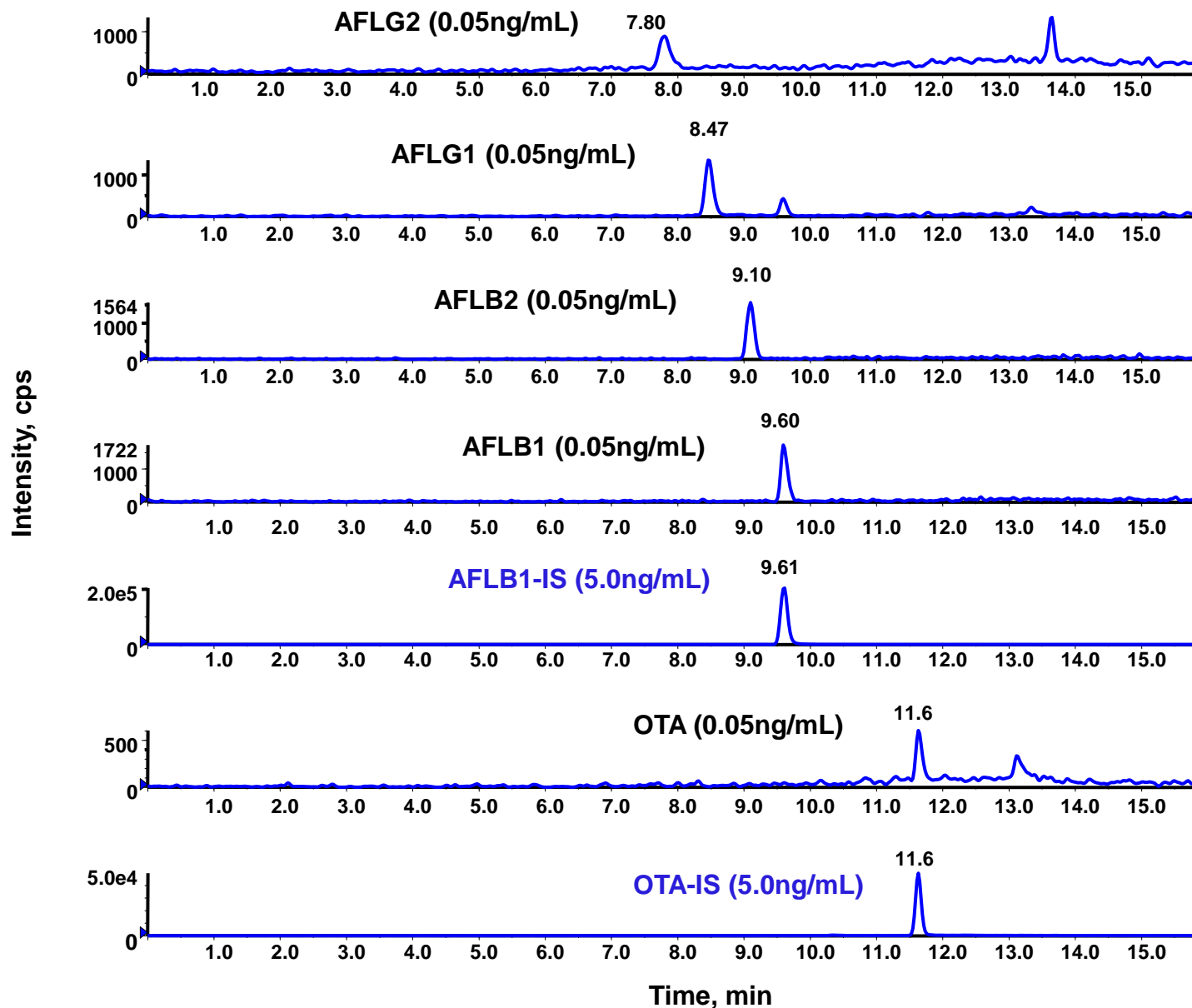
Gradient steps	Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (µl/min)
Column equilibration	2.0	70	30	200
1	7.0	0	100	200
2	11.5	0	100	200
3	12.0	70	30	200
4	16.0	70	30	200

Mass Detection Parameters

Ion Source (Positive ESI, IonSpray at 4500V; Temperature at 600 °C)

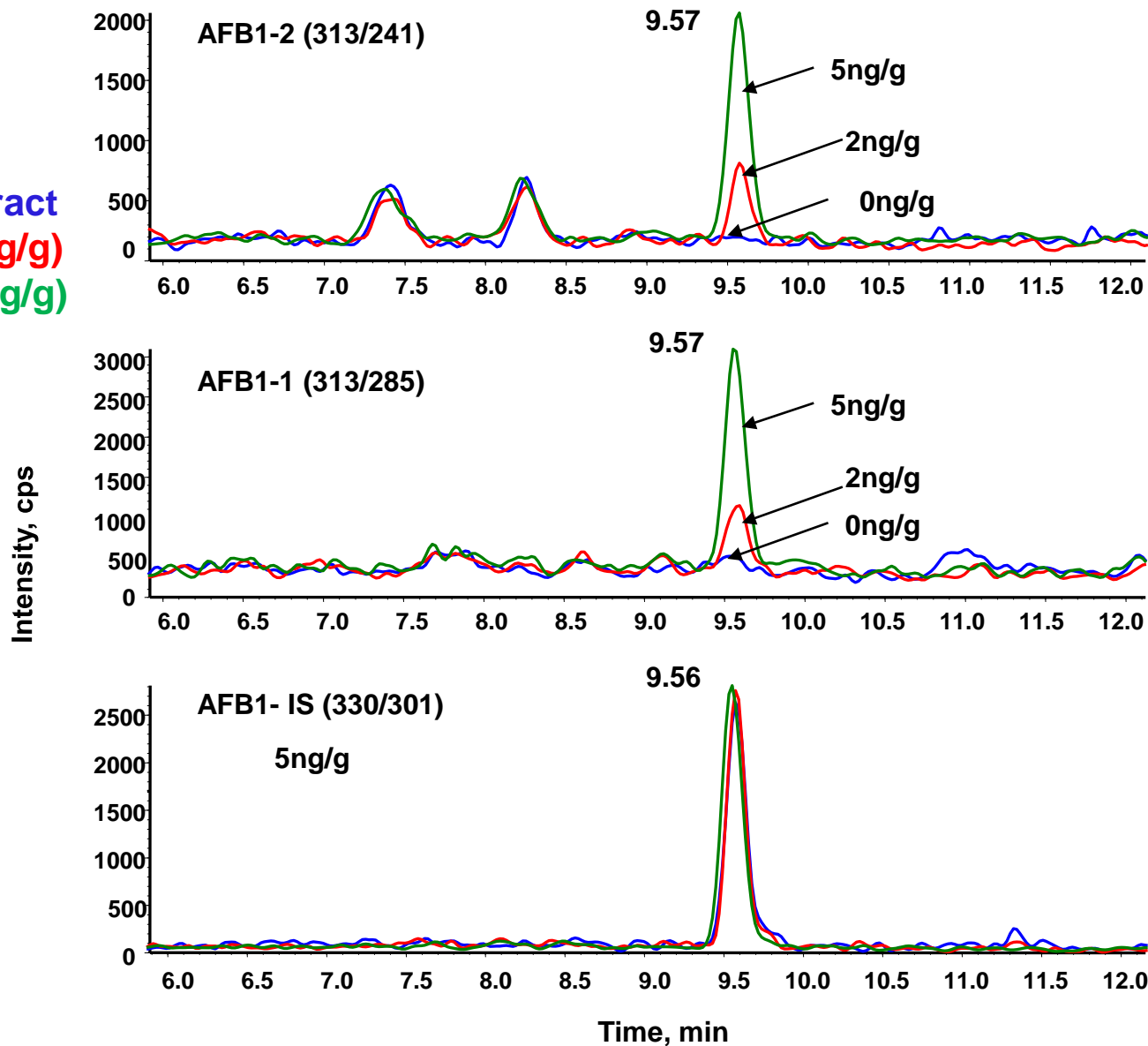
Analyte	Retention Time (min)	Precursor (m/z)	Product (m/z)	CE (eV)
Aflatoxin G2	7.7	331.1	313.1	36.0
			245.1	38.0
			285.1	36.0
Aflatoxin G1	8.4	329.3	243.0	40.0
			311.3	32.0
			283.0	38.0
Aflatoxin B2	9.1	315.1	259.1	40.0
			287.3	38.0
			271.1	40.0
Aflatoxin B1	9.6	313.1	285.1	34.0
			241.1	45.0
			269.1	40.0
U- [¹³ C ₁₇] Aflatoxin B1	9.6	330.2	301.2	34.0
			284.2	42.0
Ochratoxin A	11.6	404.1	239.1	40.0
			221.1	45.0
			102.1	75.0
U- [¹³ C ₂₀] Ochratoxin A	11.6	424.1	250.1	40.0
			232.1	50.0

Instrument Sensitivity – A Low Standard



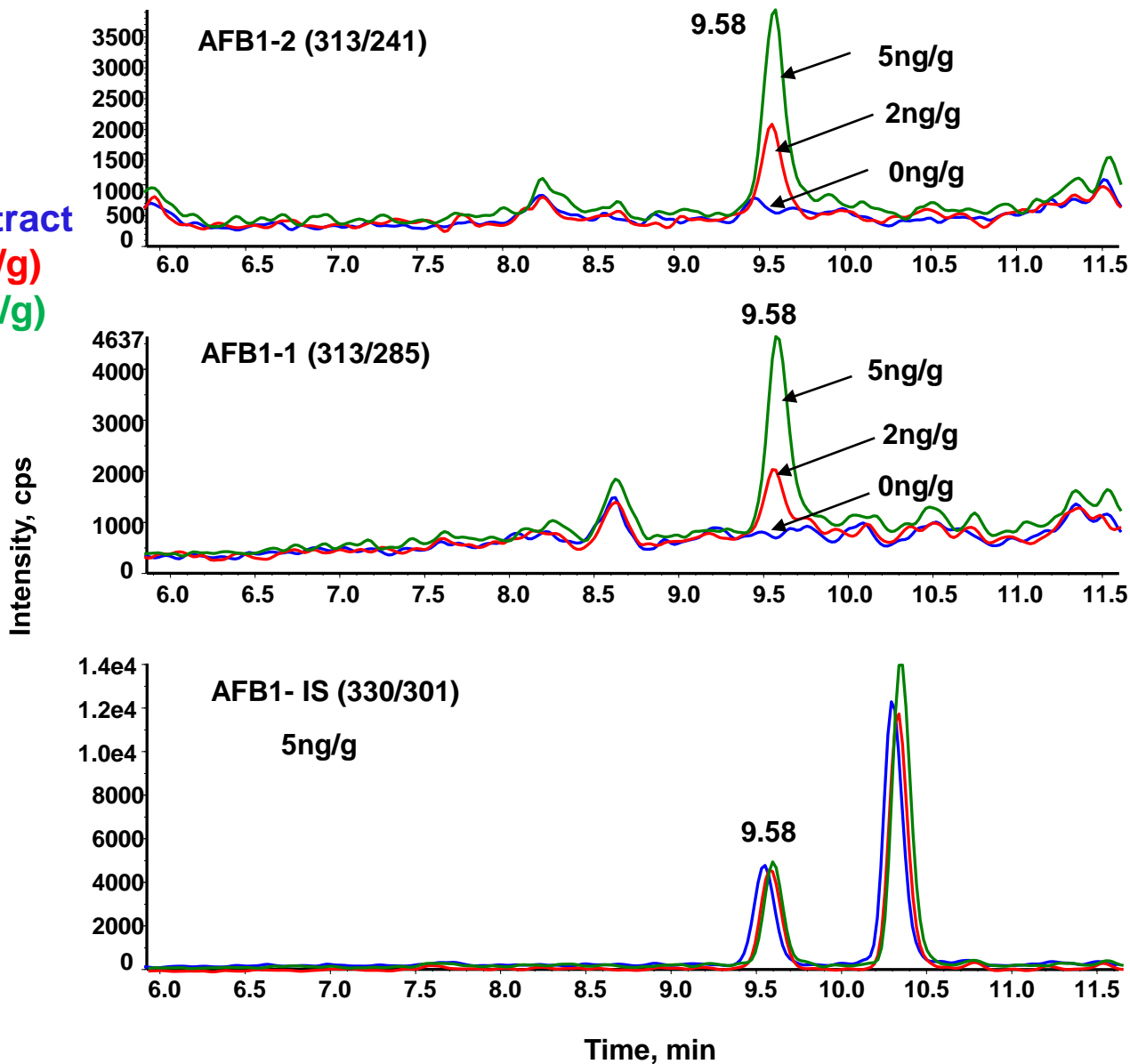
Direct LC-MS/MS Analysis - KY 3R4F

Blue – Cigarette filler extract
Red – Spiked Sample (2ng/g)
Green – Spiked sample (5ng/g)



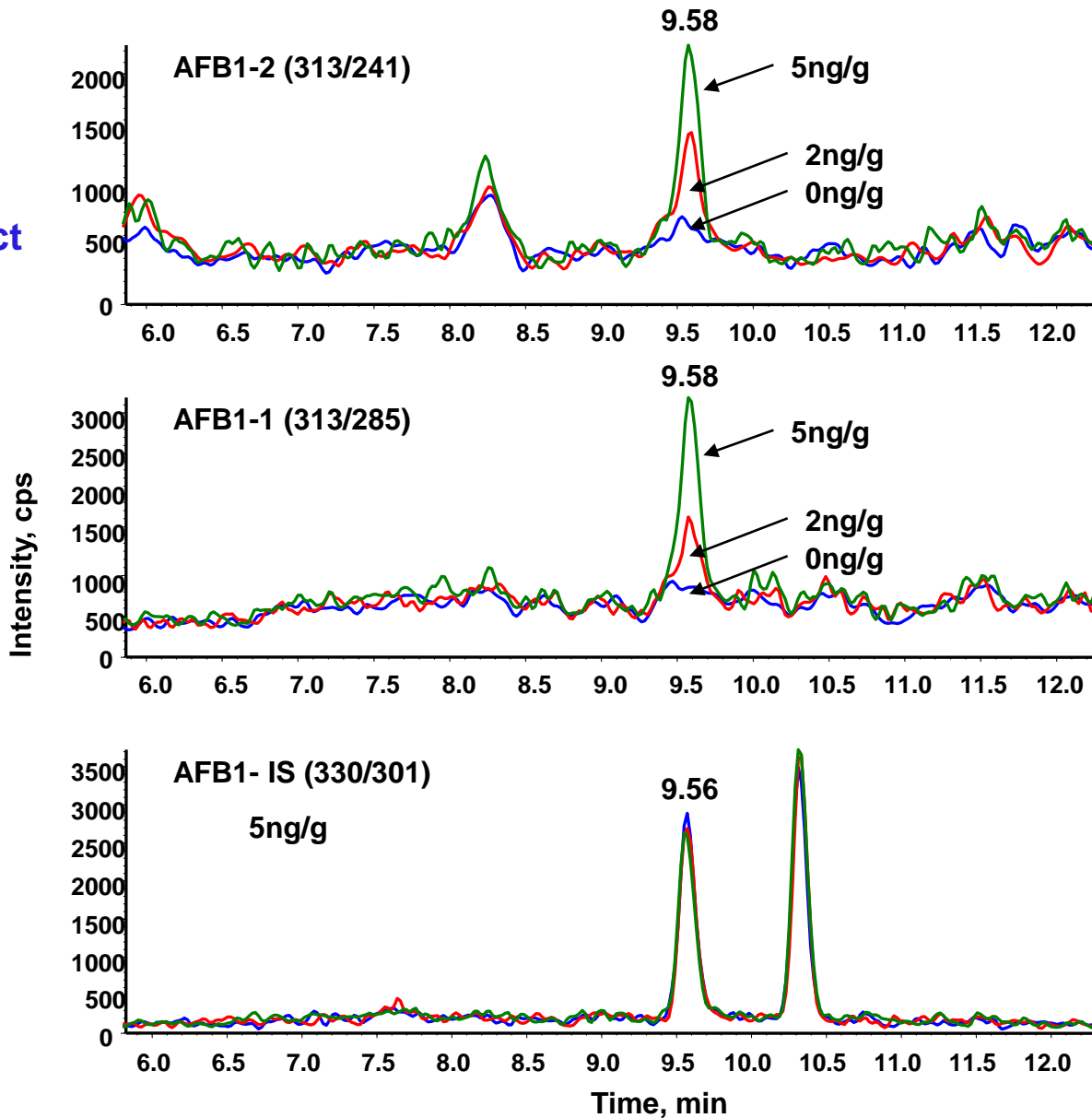
Direct LC-MS/MS Analysis - CORESTA CRP-2

Blue – Ground tobacco extract
Red – Spiked Sample (2ng/g)
Green – Spiked sample (5ng/g)



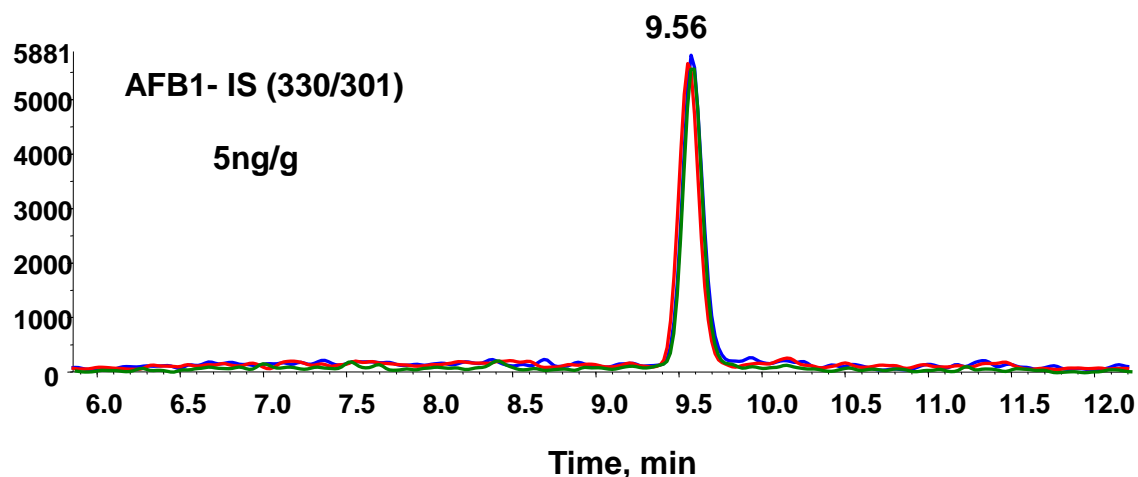
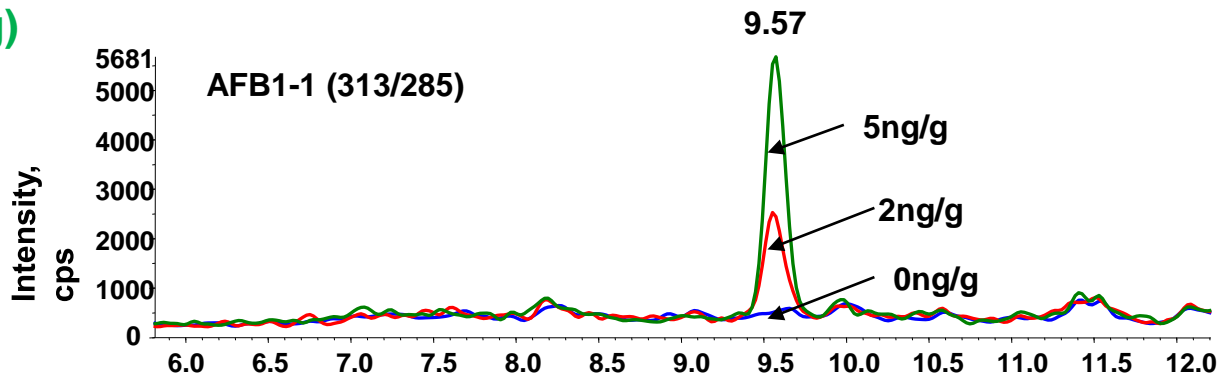
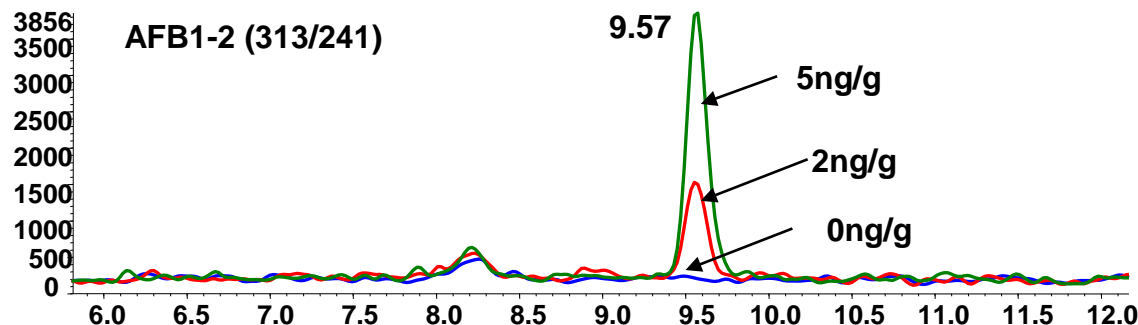
Direct LC-MS/MS Analysis - CORESTA CRP-3

Blue – Ground tobacco extract
Red – Spiked Sample (2ng/g)
Green – Spiked sample (5ng/g)



Direct LC-MS/MS Analysis - CORESTA CRP-4

Blue – Ground tobacco extract
Red – Spiked Sample (2ng/g)
Green – Spiked sample (5ng/g)



Method Validation: Direct LC-MS/MS



Linearity

Linear range (ng/mL):	0.025 ~ 100
Correlation coefficient (R^2):	>0.999

Precision

Lowest standard (0.025ng/mL, n=10),	RSD (%) < 15%
LFM (spiked at 2 & 5ng/g level, n= 3),	RSD (%) < 15%



Recovery (Accuracy)

Laboratory Reagent Blank (LRB) (ng/mL):	0
Laboratory Fortified Blank (LFB: 0.5, 5ng) (%):	93 -105
Laboratory Fortified Matrix (LFM: 1, 2, 5ng) (%):	80 - 120



Limit of Detection (LOD)

Method LOD (S/N =2) (ng/g):	0.5 (AfIB1)
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Limit of Quantification (LOQ)

Method LOQ (S/N =3) (ng/g):	2.0 (AfIB1),
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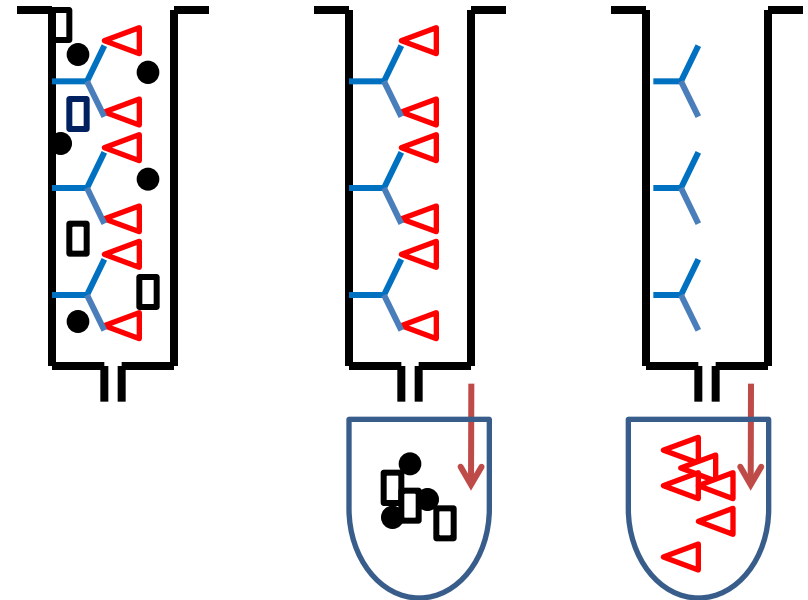
Immunoaffinity SPE - LC-MS/MS

Immunoaffinity SPE

- Antibody-Antigen Specific interaction
- Selective and accurate
- Complex sample matrices

SPE procedures

- Load sample: 6mL sample + 40mL 0.01M PBS (0.05% Tween 20)
- Wash: 4 x 3mL water
- Elute analyte: 2mL methanol
- Dilute sample: 0.1% formic acid in water
- Analyze sample: LC-MS/MS



Antibody



Analyte-Antigen



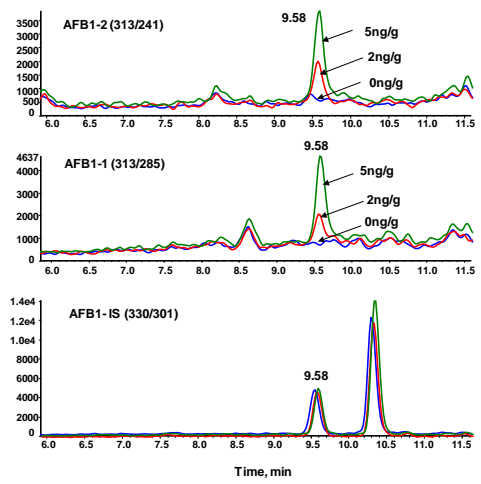
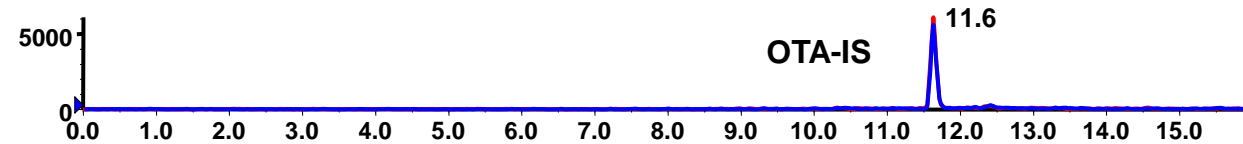
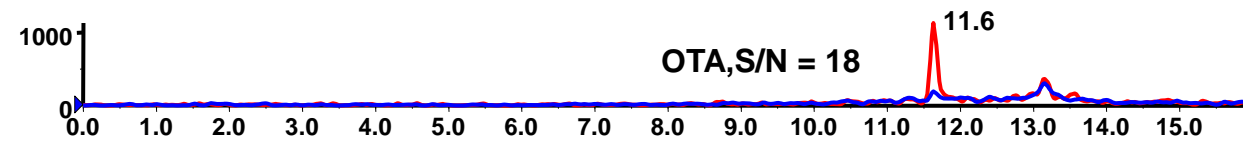
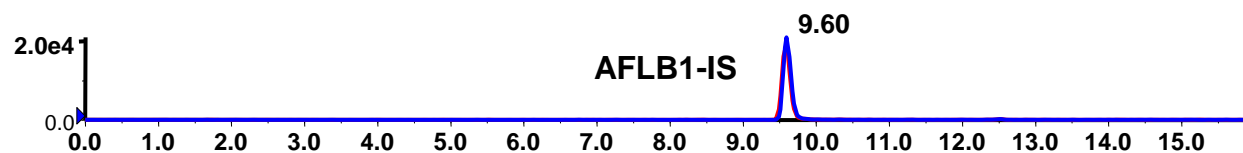
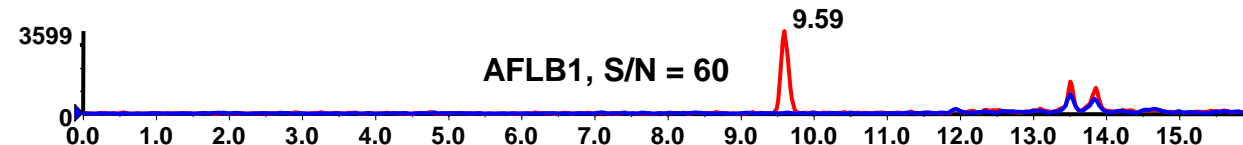
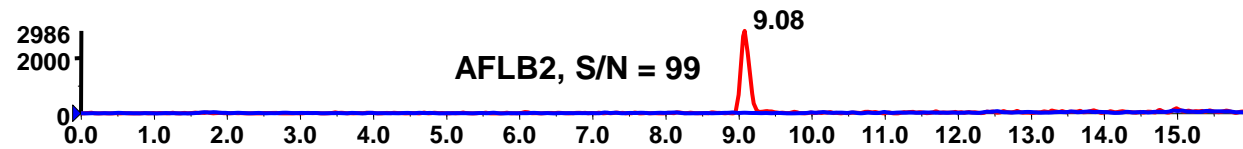
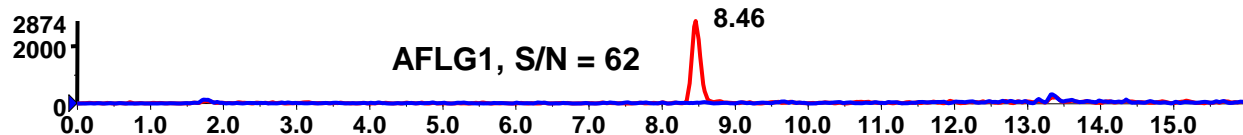
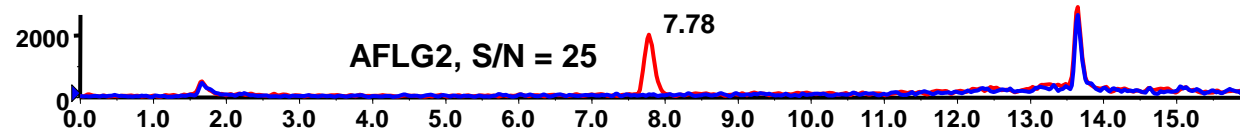
Interfering Components

Immunoaffinity SPE-LC-MS/MS for CRP-2

Blue – CRP-2 Extract

Red – Spiked Sample
(1.0 ng/g)

Intensity, cps



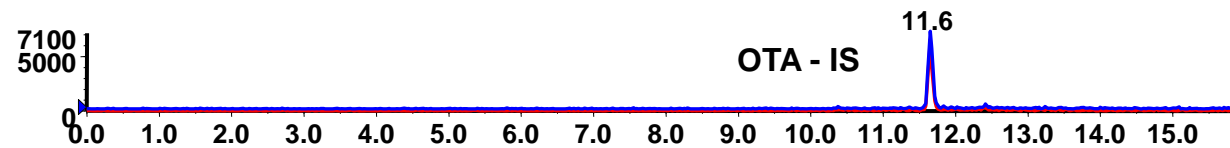
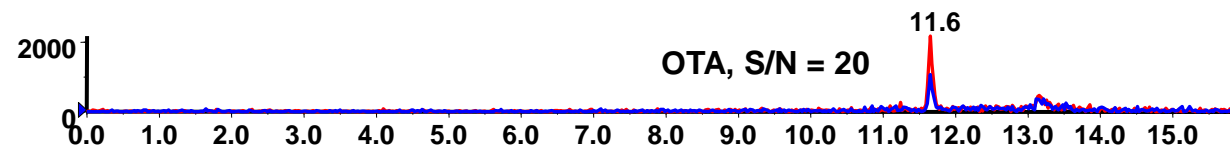
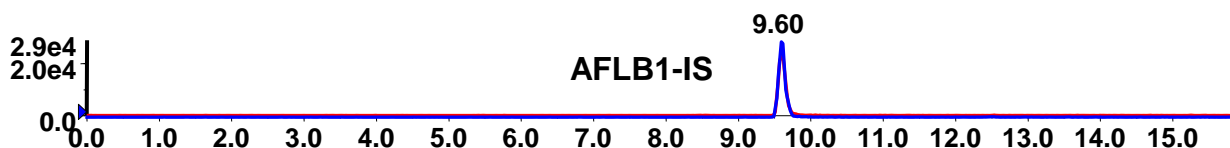
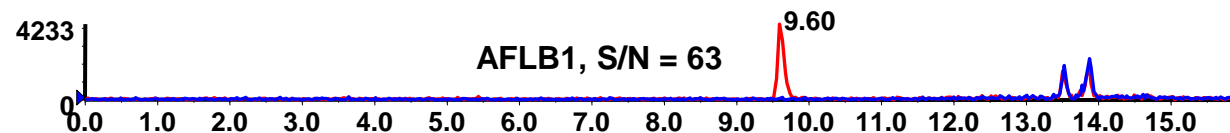
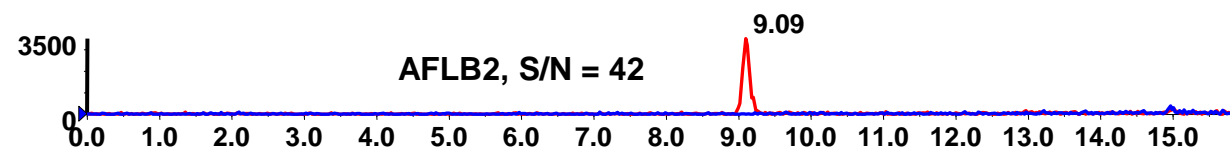
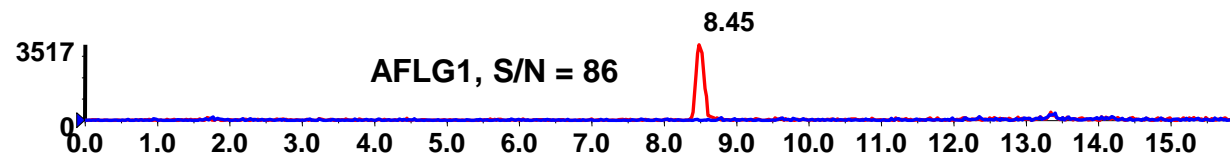
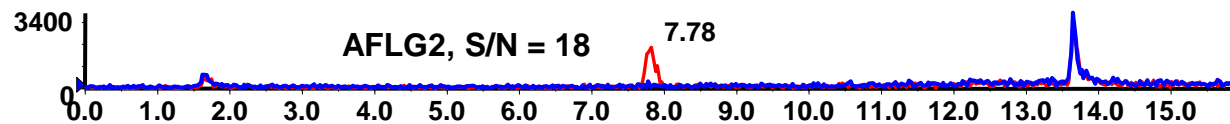
Time, min

Immunoaffinity SPE-LC-MS/MS for CRP-3

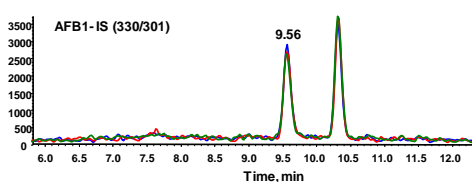
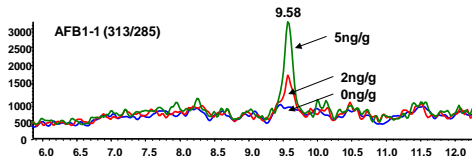
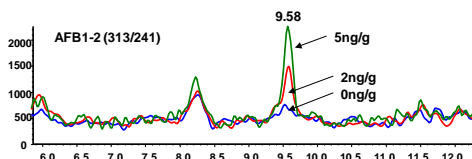
Blue – CRP-3 Extract

Red – Spiked Sample
(1.0 ng/g)

Intensity, cps



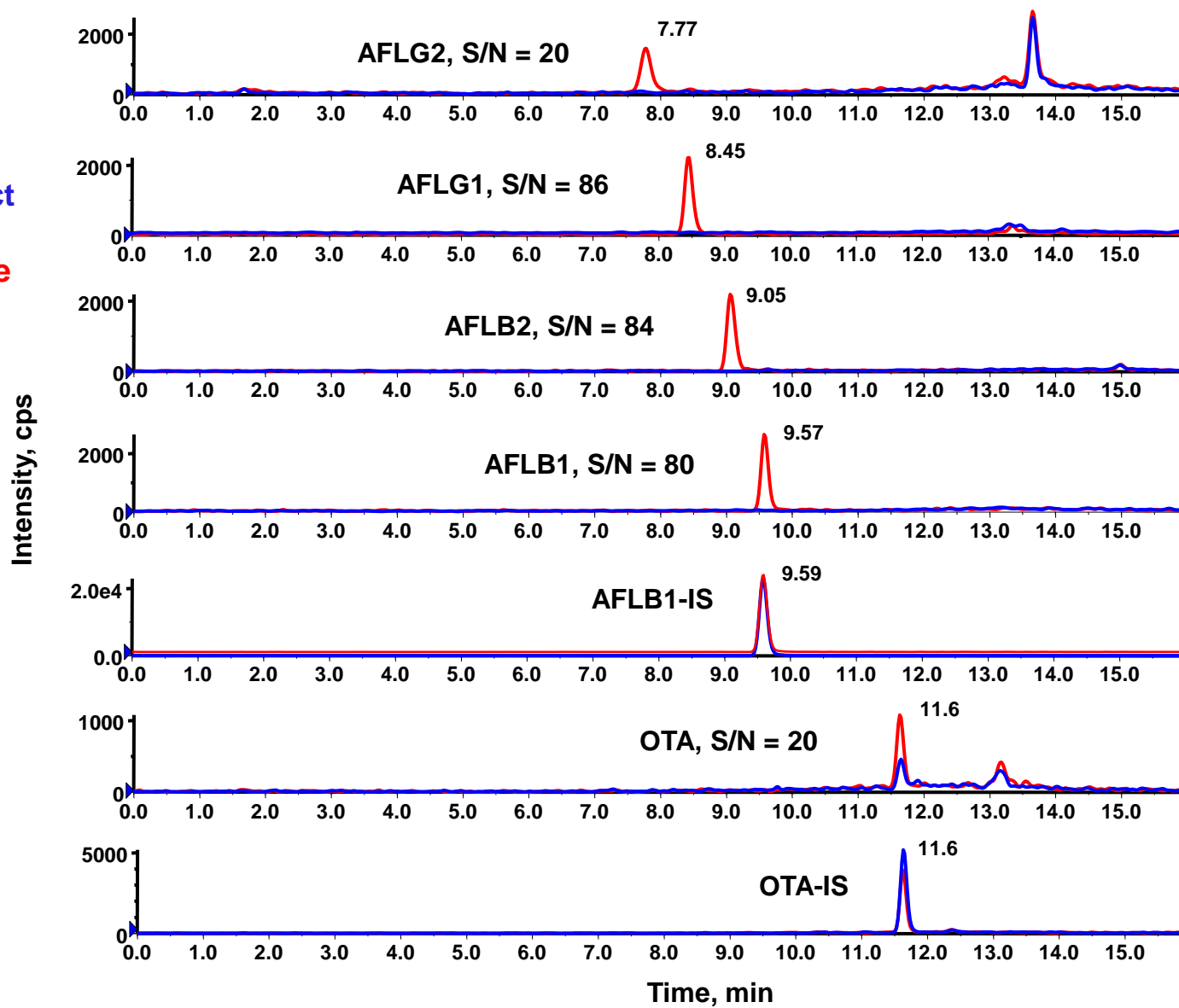
Time, min



Immunoaffinity SPE-LC-MS/MS for CRP-4

Blue – CRP-4 Extract

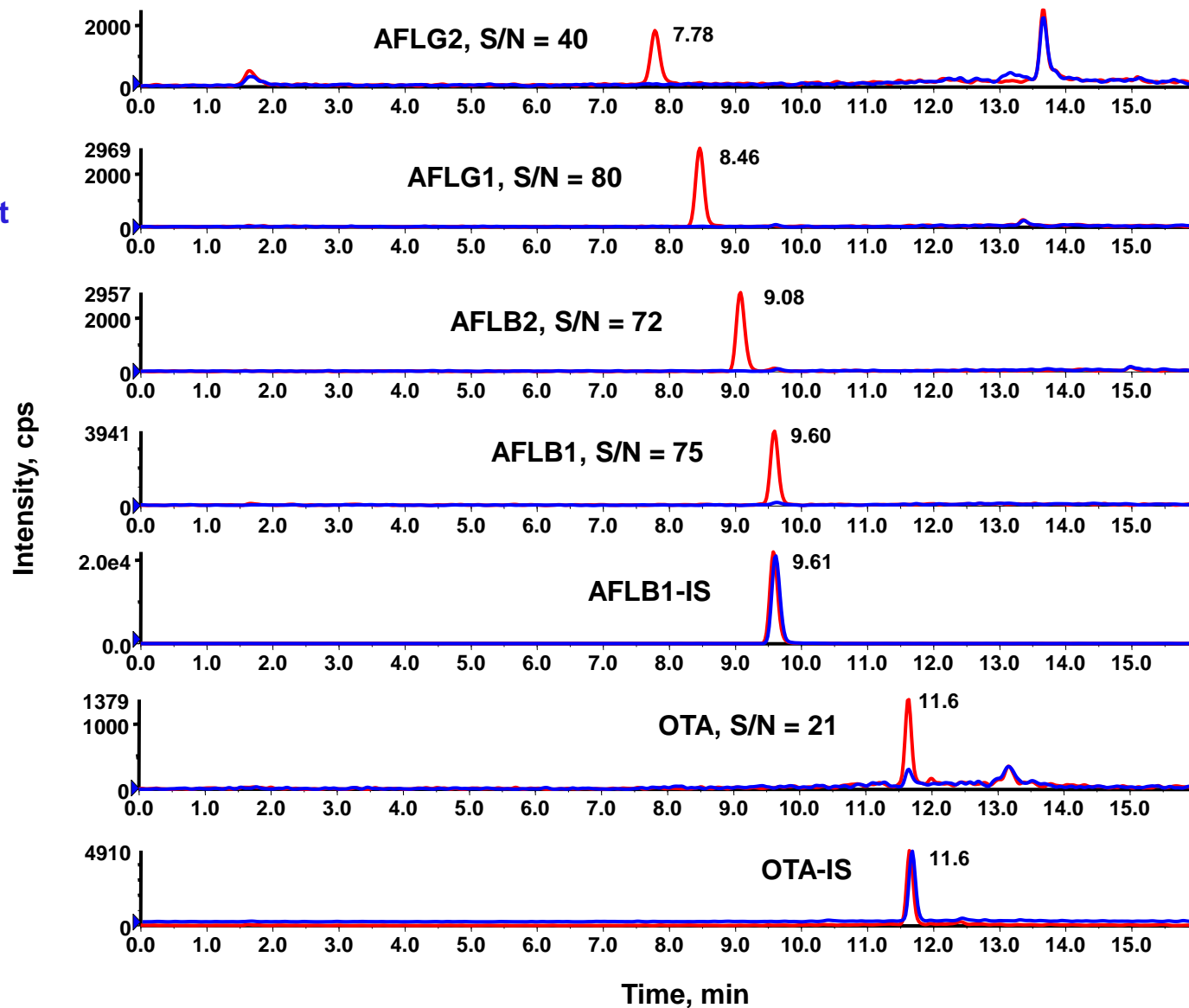
Red – Spiked Sample
(1.0 ng/g)



Immunoaffinity SPE-LC-MS/MS for 3R4F

Blue – KY 3R4F
Cigarette Filler Extract

Red – Spiked Sample
(1.0 ng/g)



Method Validation: SPE-LC-MS/MS



Linearity

Linear range (ng/mL): 0.025 ~ 100
 Correlation coefficient (R^2): >0.999

Precision

Lowest standard (0.025ng/mL, n=10), RSD (%) < 15%
 LFM (spiked at 1, 2, 5ng/g level, n= 3), RSD (%) < 15%



Recovery (Accuracy)

Laboratory Reagent Blank (LRB) (ng/g): 0
 Laboratory Fortified Blank (LFB: 0.5, 2ng/g) (%): 95 -102
 Laboratory Fortified Matrix (LFM:1, 2, 5ng/g) (%): 86 - 115



Limit of Detection (LOD)

Method LOD (S/N =3) (ng/g): 0.057 (AflB1), 0.076 (AflB2),
 0.077 (AflG1), 0.072 (AflG2), and
 0.099 (OTA)



Limit of Quantification (LOQ)

Method LOQ (S/N =10) (ng/g): 0.191 (AflB1), 0.253 (AflB2),
 0.255 (AflG1), 0.239 (AflG2), and
 0.328 (OTA)

Summary

- A highly selective and sensitive method was developed for the analysis of Mycotoxins in tobacco products by coupling immunoaffinity SPE to LC-MS/MS.
- A simple and fast screening LC-MS/MS method was also developed for Aflatoxin B1 analysis in tobacco products without the need for sample clean up and concentration.
- Sample matrix effects and variations in analytical procedures were minimized using isotope-labelled internal standard and sample clean up procedures.

Acknowledgements

- Labstat Sample Preparation Technicians and Analytical Team (Mr. Bor Cha and Mr. Seydina Lo)
- Your attention

