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Development of a MHC-LC-LC-HRMS Method for Simultaneous Determination of Aflatoxin B_1, B_2, G_1, G_2 and Ochratoxin A in Snus

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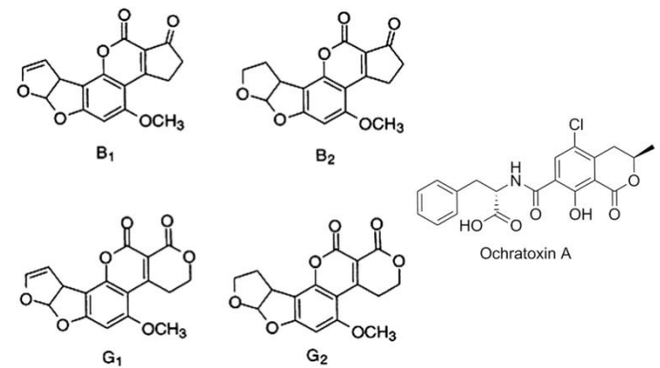
OUTLINE

1. Introduction
2. Method Optimization
3. Results & Discussion
4. Sample Analysis
5. Conclusions

INTRODUCTION

- Aflatoxins (AFs) and ochratoxin A (OTA), have been classified as carcinogenic or possibly carcinogenic to humans.
- The tobacco leaves could be contaminated by mycotoxins, thus the remained mycotoxins in snus may be ingested by humans.
- Swedish National Food Agency has regulated the total concentration of AFs (B_1, B_2, G_1, G_2) should not exceed 5.0 μg/kg in snus.
- Additionally, according to the data revealed by Swedish Match, OTA had been detected in snus.



INTRODUCTION

- AFs and OTA are typically identified by LC-MS/MS, while most methods employed the Immunoaffinity columns (IAC) technique to eliminate the interferences and reduce the matrix effect.
- But IAC technique is not suitable for multi-mycotoxins determine.
- It is necessary to establish a sensitive and feasible method to simultaneously determinate AFs and OTA in snus.
- This work reported the application of MHC-LC-LC-HRMS system for the multi-mycotoxins analysis.

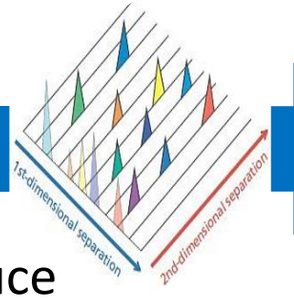
INTRODUCTION

- So, Why MHC-LC-LC system coupled to HRMS ?

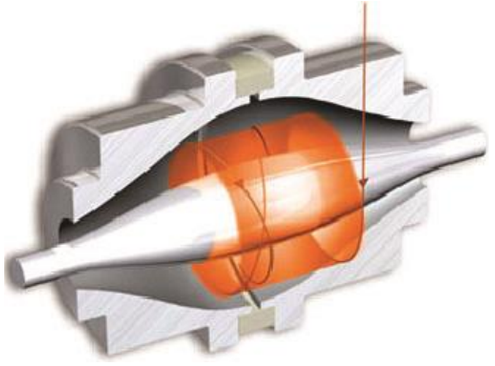
HRMS

- The hybrid high resolution mass spectrometry enables the measure of accurate mass to avoid false positives.

MHC-LC-LC



- The utilization of multiple heart-cutting LC-LC can reduce matrix effect and enhance method sensitivity.



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METHOD OPTIMIZATION

Basis for LC-LC system

01

Orthogonality of the separation mechanism

- ✓ 1st-LC C18 column
- ✓ 2nd-LC Pentafluorobenzene (PFP) column

02

Compatibility of the mobile phase

- ✓ 1st-LC ACN/H_2O
- ✓ 2nd-LC MeOH/H_2O

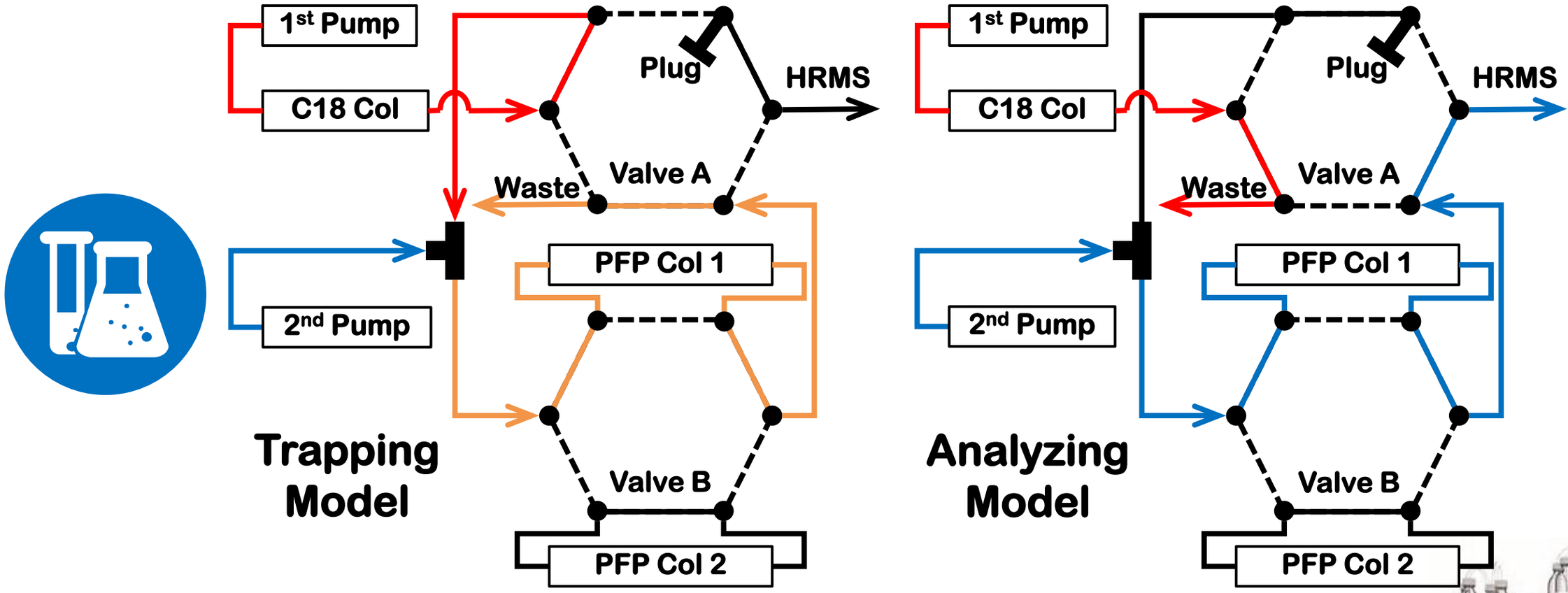
03

Modulating interface of LC-LC

- ✓ A set of 2-pos/6-port high pressure valves

METHOD OPTIMIZATION

Schematic of the MHC-LC-LC



1st-LC flow path — 2nd-LC flow path — mixed flow path

METHOD OPTIMIZATION

MHC-LC-LC conditions

Gradient for MHC-LC-LC

Time (min)	1st-LC B phase (%)	2nd-LC B phase (%)	Valve A Position	Valve B Position
0.0	10	100	To Waste	To Col 2
1.0	10	-	-	-
5.0	-	100	-	-
5.1	-	5	-	-
17.2	-	-	To 2nd-LC	To Col 1
20.4	-	-	To Waste	-
22.0	-	-	To 2nd-LC	To Col 2
23.5	-	-	To Waste	-
24.0	79	-	-	To Col 1
24.1	100	-	-	-
25.0	-	5	-	-
25.5	-	40	-	-
29.5	100	60	-	-
29.6	10	100	-	-
34.5	-	100	-	-
34.6	-	5	-	-
39.5	-	5	-	To Col 2
40.5	-	80	-	-
44.5	-	100	-	-

- 1st-LC: Waters ACQUITY UPLC HSS C18 column (2.1X100mm, 1.8µm); phase A is water, B is ACN/water/FA (950/49/1, v/v/v) contained 2mM NH_4FA; flow rate 0.3mL/min.
- 2nd-LC: Agilent Poroshell 120 PFP column (4.6x50mm, 2.7µm); phase A is water, B is MeOH/water/FA (950/49/1, v/v/v) contained 10 mM NH_4FA; flow rate 0.6mL/min.
- All the columns are held at 50 °C.
- Injection volume is 4 µL.

METHOD OPTIMIZATION
HRMS conditions

- ✓ Data were acquired under parallel reaction monitoring mode.
- ✓ Positive ion; Sheath gas pressure 35 psi; Auxiliary gas flow 3; Spray voltage 3.5 kV; Capillary temperature 300 ℃; Auxiliary gas heater temperature 350 ℃.
- ✓ A resolving power of 70,000 full width at half maximum (FWHM) atm/z 200 was used.

HRMS parameters for different analyte

Analyte	Formula	Precursor Ion (m/z)	Product Ion (m/z)	Mass Tolerance (ppm)	CE (eV)
AFG_2	$C_{17}H_{14}O_7$	331	313.0704	5	30
AFG_1	$C_{17}H_{12}O_7$	329	243.0653	5	30
AFB_2	$C_{17}H_{14}O_6$	315	259.0601	5	35
AFB_1	$C_{17}H_{12}O_6$	313	241.0496	5	40
d_3-AFB_1	$C_{17}H_9D_3O_6$	316	241.0496	5	40
OTA	$C_{20}H_{18}ClNO_6$	404	257.0208	5	30
d_5-OTA	$C_{20}H_{13}D_5ClNO_6$	409	257.0208	5	30

METHOD OPTIMIZATION

Sample pretreatment

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Liquid-Liquid Extraction

01 2g milled sample powder were weighed into a 50 mL centrifuge tube.

02 Add 10 mL de-ionized H_2O, and agitate for 1 min.

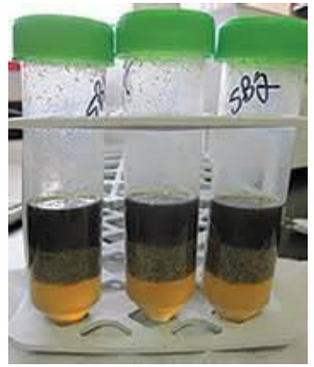
03 Add 10mL EA with 1% AA and 100 µL IS solution, and agitate for 30 min.

04 The organic phase was concentrated to dryness at 50 °C under nitrogen.

05 The residue was re-constituted with 0.5 mL MeOH/water (50/50, v/v) before analysis.

QuEChERS Extraction

Add 10mL ACN with 1% AA and 100 µL IS solution, and shake for 30 min.
Then add 4g $MgSO_4$, 1g NaCl, 1g trisodium citrate dehydrate, 0.5g disodium citrate sesquihydrate, and agitate for 2 min.



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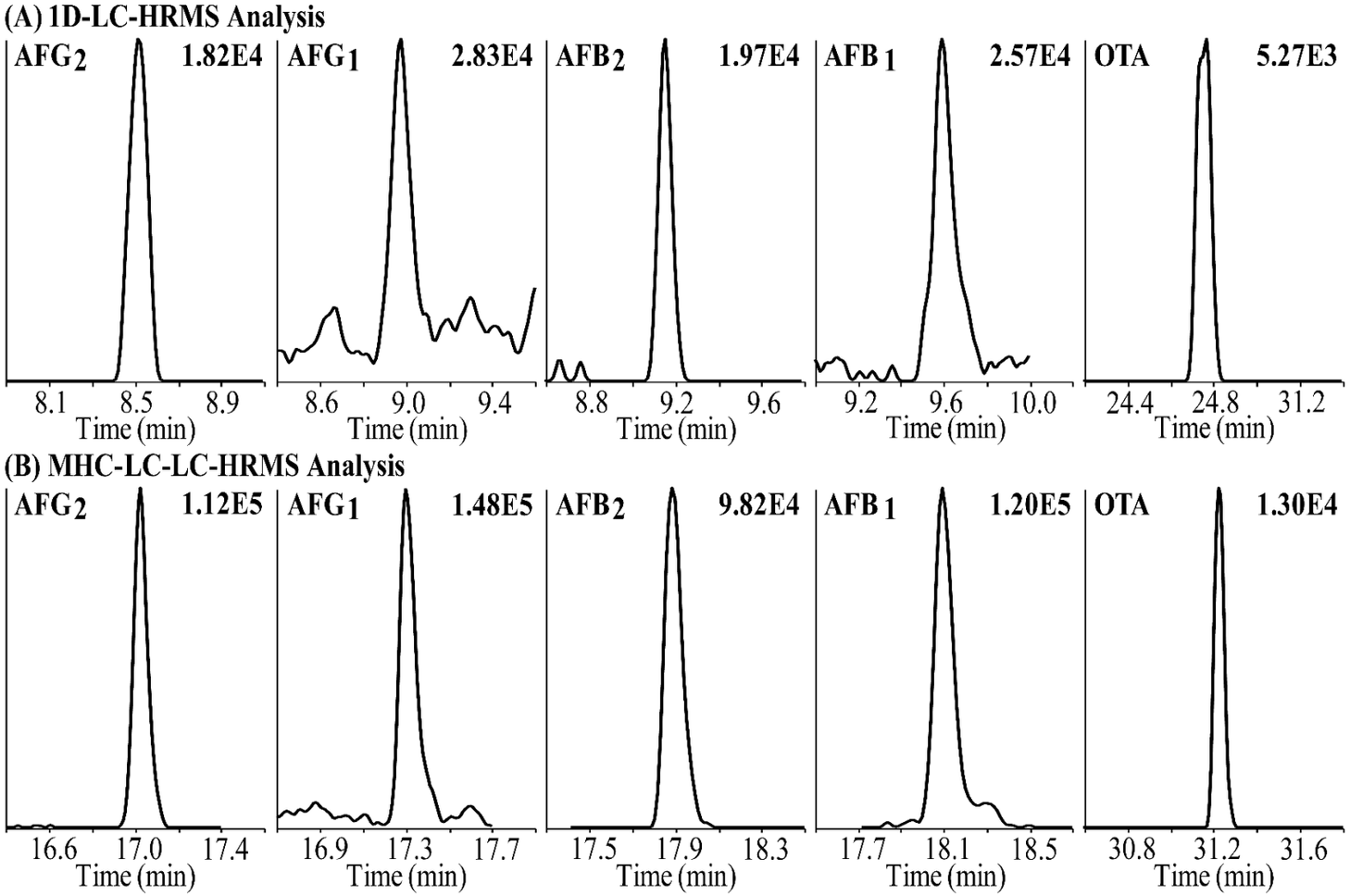
RESULT & DISCUSSION

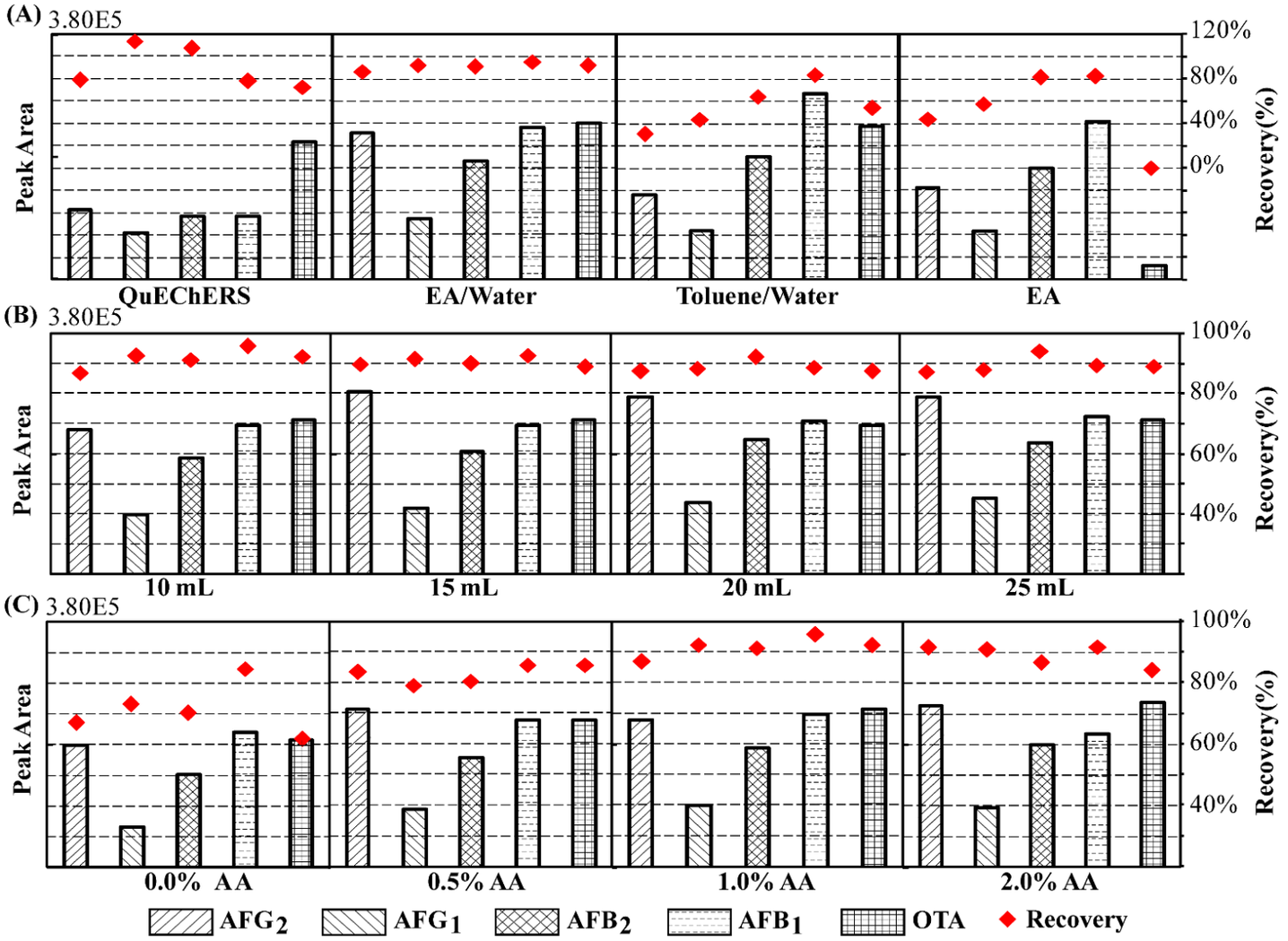
Comparison of LC-HRMS & MHC-LC-LC-HRMS



Chromatograms obtained from the CRP1 spiked with 1.0 µg/kg mycotoxins standards.

RESULT & DISCUSSION

Comparison of different pretreatment strategies

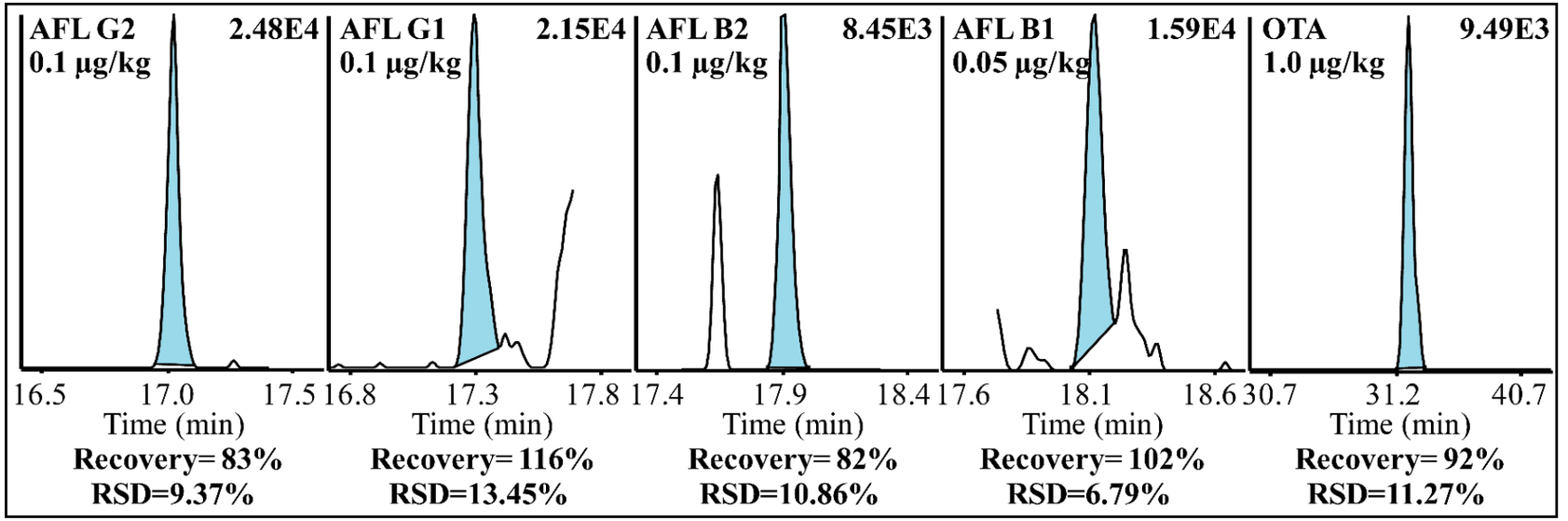


Comparison of (A) different extraction strategies; (B) different volumes of EA; (C) different concentration of AA on the responses and recoveries of AFs and OTA.

RESULT & DISCUSSION

LOQ & Recovery

LOQ



Analyte	R^2	Dynamic range (µg/kg)	Recovery (%) 1.0 µg/kg	2.0 µg/kg	5.0 µg/kg
AFG_2	0.9978	0.2-20	92.0	93.6	87.6
AFG_1	0.9965	0.2-20	88.4	90.4	91.6
AFB_2	0.9971	0.2-20	85.7	89.4	88.6
AFB_1	0.9993	0.2-20	103.2	92.3	85.4
OTA	0.9977	1.0-20	111.5	92.3	96.5

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SAMPLE ANALYSIS

Results of fifteen snus samples

Sample	Moisture (%)	Mycotoxins content in dry product (μg/kg)				
		AFG_2	AFG_1	AFB_2	AFB_1	OTA
sample 01	40.0%	--	--	--	--	2.81
sample 02	58.8%	--	--	--	--	1.37
sample 03	48.8%	--	--	--	--	1.56
sample 04	43.1%	--	--	--	--	2.79
sample 05	49.5%	--	--	--	--	4.31
sample 06	44.9%	0.13	--	--	--	2.53
sample 07	45.6%	--	--	--	--	3.45
sample 08	36.9%	--	--	--	--	3.55
sample 09	44.1%	--	--	--	--	1.78
sample 10	41.3%	--	--	--	--	1.86
sample 11	48.5%	--	--	--	--	3.63
sample 12	50.1%	--	--	--	--	3.06
sample 13	34.7%	--	--	--	--	1.74
sample 14	36.3%	--	--	--	--	1.76
sample 15	38.4%	--	--	--	--	1.01

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CONCLUSION

- A novel, accurate and sensitive method for the analysis of multi-mycotoxins in snus was proposed in this work
- With the advantage of LC-LC system, the chromatographic resolution was improved and the ion suppression was reduced, which led to higher method sensitivity.
- With the high confirming ability of HRMS, sample pretreatment procedures were simplified.
- A LLE approach utilizing EA with 1% AA/water (v/v, 50/50) solution was adopted to extract the AFs and OTA in snus.
- The developed method showed high sensitivities, good recoveries and repeatabilities for AFs and OTA.

Kitzbühel - Austria 2017
CORESTA SSPT

THANK YOU !