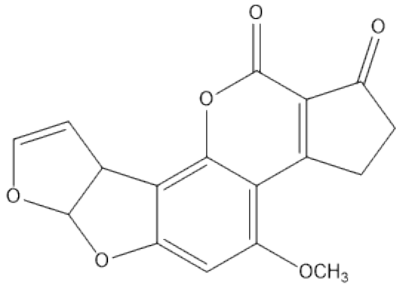
Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information (ESI)

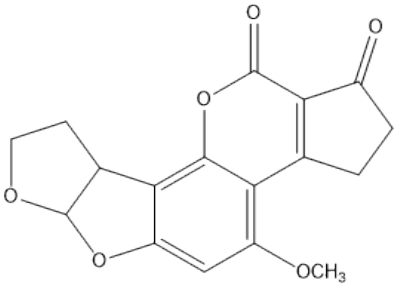
Miniaturized vortex assisted – dispersive molecularly imprinted polymer micro-solid phase extraction and HPLC-MS/MS for assessing trace aflatoxins in cultured fish

G.D.Thilini Madurangika Jayasinghe, Raquel Domínguez-González, Pilar Bermejo-Barrera, Antonio Moreda-Piñeiro

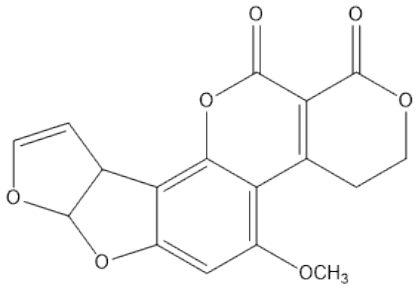
Table S1. Chemical structures of aflatoxins (B1, B2, G1, and G2) and dummy template (5,7-dimethoxycoumarin, DMC)



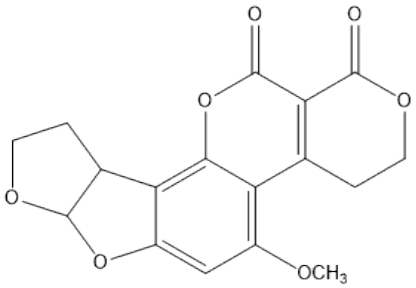
Aflatoxin B1



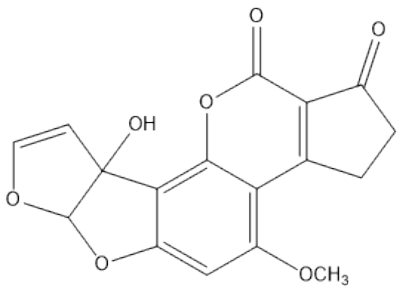
Aflatoxin B2



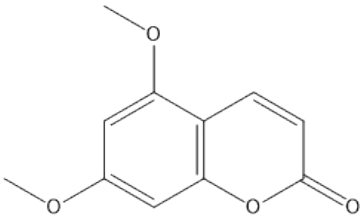
Aflatoxin G1



Aflatoxin G2



Aflatoxin M1



DMC

Table S2: Instrument parameters for FIA-MS/MS

MS/MS						
	Precursor ion (m/z)	Product ion (m/z)	DP(V)[a]	EP(V)[a]	CE(V)[a]	CXP(V)[a]
Vitamin D	385.400	367.300	40.110	10.150	15.000	5.000
Vitamin A	269.300	95.100	40.000	10.000	20.500	5.000
β Carotene	537.300	295.100	86.000	9.000	45.100	20.000
Lysine	147.200	84.100	84.700	10.000	21.000	10.000
Methionine	150.100	104.000	6.320	8.500	18.000	3.000
Cysteine	241.100	152.000	20.000	10.650	19.000	12.000
Glycine	76.100	30.100	6.120	8.000	19.500	14.000
FIA						
Injection volume		20 µL				
Flow rate		60 µL min^{-1}				
Carrier		0.1 % formic acid in methanol				
Elution time		3.0 min				

(a) DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential

(b) Electron spray operation conditions are: Ion spray voltage (IS), 5500 kV; Ion source temperature, 300 °C; nebulizer gas and curtain gas (N_2), 40 psi; collision gas (N_2), high

Table S3. ANOVA outputs when comparing slopes of calibration and standard addition

AFB1					
Source	Sum of squares	Degree of freedom	Mean square	F ratio	p-value
Between groups	96123	1	96123	28.70	0.0003
Within groups	33490	10	3349		
Total (Corr.)	129613	11			
AFB2					
Source	Sum of squares	Degree of freedom	Mean square	F ratio	p-value
Between groups	10800	1	10800	34.56	0.0002
Within groups	3125	10	312.5		
Total (Corr.)	13925	11			
AFG1					
Source	Sum of squares	Degree of freedom	Mean square	F ratio	p-value
Between groups	410700	1	410700	238.43	0.0000
Within groups	17225	10	1722.5		
Total (Corr.)	427925	11			
AFG2					
Source	Sum of squares	Degree of freedom	Mean square	F ratio	p-value
Between groups	2028	1	2028	19.13	0.0014
Within groups	1060	10	106		
Total (Corr.)	3088	11			
AFM1					
Source	Sum of squares	Degree of freedom	Mean square	F ratio	p-value
Between groups	79707	1	79707	972.04	0.0000
Within groups	820	10	82.0		
Total (Corr.)	80527	11			

Table S4. ANOVA outputs when comparing D-μ-SPE procedures by using several loading sample volumes (pre-concentration factors of 15, 30 and 50)

AFB1					
Source	Sum of squares	Degree of freedom	Mean square	F ratio	p-value
Between groups	0.1266	2	0.0633	4.74	0.0583
Within groups	0.0802	6	0.0133667		
Total (Corr.)	0.2068	8			

Table S5: AFs concentrations in ERM-BE376 (compound feeding stuff) certified reference material

Compound	Certified value (μg kg^{-1})	Calculated value (μg kg^{-1})	Recovery %
AFB1	12.9±1.8	13.0±0.7	101
AFB2	0.68±0.10	<LOQ	_
AFG1	5.2±0.8	5.6±0.4	107

Figure S1: Scanning electron microscope (SEM) images for MIP (a) and NIP (b)

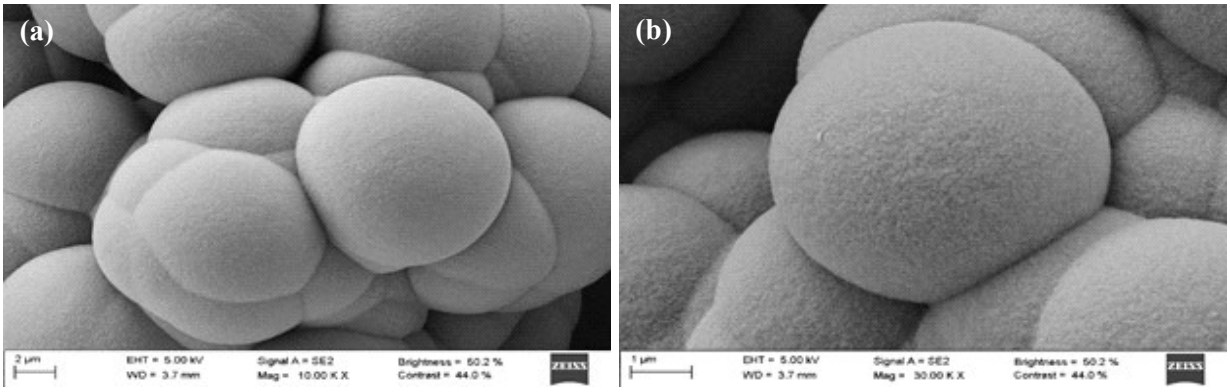


Figure S2: Fourier Transform Infrared Spectroscopy (FT-IR) Images of MIP after template removal (a) and NIP (b)

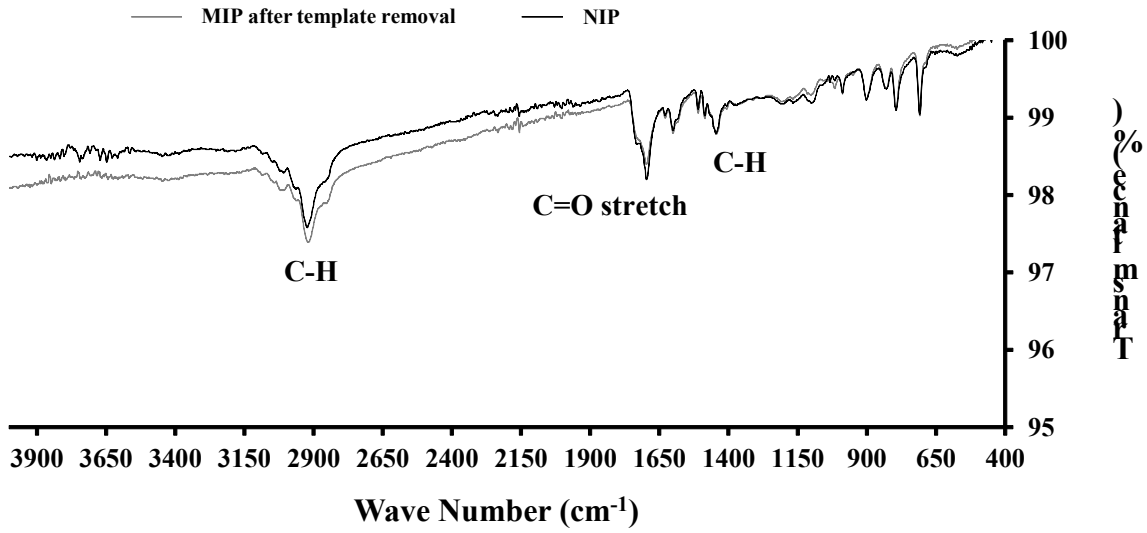


Figure S3. Effect of the number of loading/elution cycles on the analytical recovery of each D-µ-SPE (AFB1 concentration of 6.67 µg L^{-1}).