

Article

Study on Molecularly Imprinted Polymers Obtained Sonochemically for the Determination of Aflatoxins in Food

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Table S1. Comparative table summarizing the literature works related to MIPs-SPE for AFs using HPLC-MS as detection method.

Target	Mono- mer	Dummy plate	tem-	Method/Time of synthesis	Selectivity	LOD	Real sample	REF
Aflatoxins B1, B2, G1, and G2	MAA	DMC (5,7- ycoumarin)	dimethox-	precipitation polymerization/26 h.	- β Carotein -Carboxymethyl cellulose - carrageenan (kappa and lambda) -Agar-agar -Vit A -Vit D	AFB1LOD=0.42 μ g /kg AFB2LOD=0.88 μ g /kg AFG1LOD=1.1 μ g /kg AFG2LOD=1.2 μ g /kg	Fish feed	[1]
Aflatoxins B1, B2, G1, and ,M1	APTES and TEOS	DMC (5,7- ycoumarin)	dimethox-	-Sol-gel method/ 16 h.	Ochratoxin, patulin, Zearalenon, Fumonisin B1, Couma- rin, Bisphenol-A, Chlorpyrifos, Cypermethrin, diazinon, dichlorvos, and malathion, with a concentration of 10 ng mL ⁻¹ and glucose, lactose, fructose, sucrose, vitamin C, cysteine, serine, histidine, and urea with a concentration of 2 μ g mL ⁻¹	AFB1LOD=0.021 ng /mL AFB2LOD=0.024 ng /mL AFG1LOD=0.023 ng /ml AFM1LOD= 0.026 ng /mL	Rice; Wheat Flour and Milk.	[2]
Aflatoxins B1, B2 and G1.	MAA	ethyl 3- coumarincarbox- ylate		- Atom transfer rad- ical precipitation polymerization (ATRPP)/8 h.	Ochratoxin and Zearalenon.	AFB1LOD= 0.05ng /mL AFB2LOD= 0.05 ng /mL AFG1LOD= 0.07 ng /ml	spiked corn	[3]
Aflatoxins B1, B2 G1, G2 and M1.	MAA	5,7-Dimethoxycoumarin (DMC)		Bulk polymeriza- tion/24h	-	AFB1LOD=0.9 ng /Kg AFB2LOD=0.7 ng /Kg AFG1LOD=1.0 ng /Kg AFG2LOD=1.7 ng /Kg AFM1LOD= 0.3 ng /Kg	Baby food	[4]
Aflatoxins B1, B2, G1, and G2	Acryla- mide	Quercetin		precipitation polymerization/7 h.	Zearalenone, coumarin and isoxepac.	AFB1LOD=0.09ng/mL AFB2LOD=0.13ng/mL AFG1LOD=0.10ng /ml AFG2LOD=0.06ng/mL	Wheat, Soybean, rice and corn.	[5]
Aflatoxins B1, B2, G1, and G2	Methac- rylamide	Naphtoic acid		Free radical polymeriza- tion/5min	Citrinin, patulin, ochratoxin A and ochratoxin B.	AFB1LOD=0.005ng/mL AFB2LOD=0.027ng/mL AFG1LOD=0.007ng/ml AFG2LOD=0.009ng/mL	Ginger, Echiancea pur- purea, Ginseng, Hyper- icum, Red elm, Saffron Mango, Red rice, Pars- ley, Red fruits, Grape- fruit, Magnolia, Tilia Cordata, Root Salsopariglia Hop, Ver- bene Officinalis, Galega Officinalis.	This work

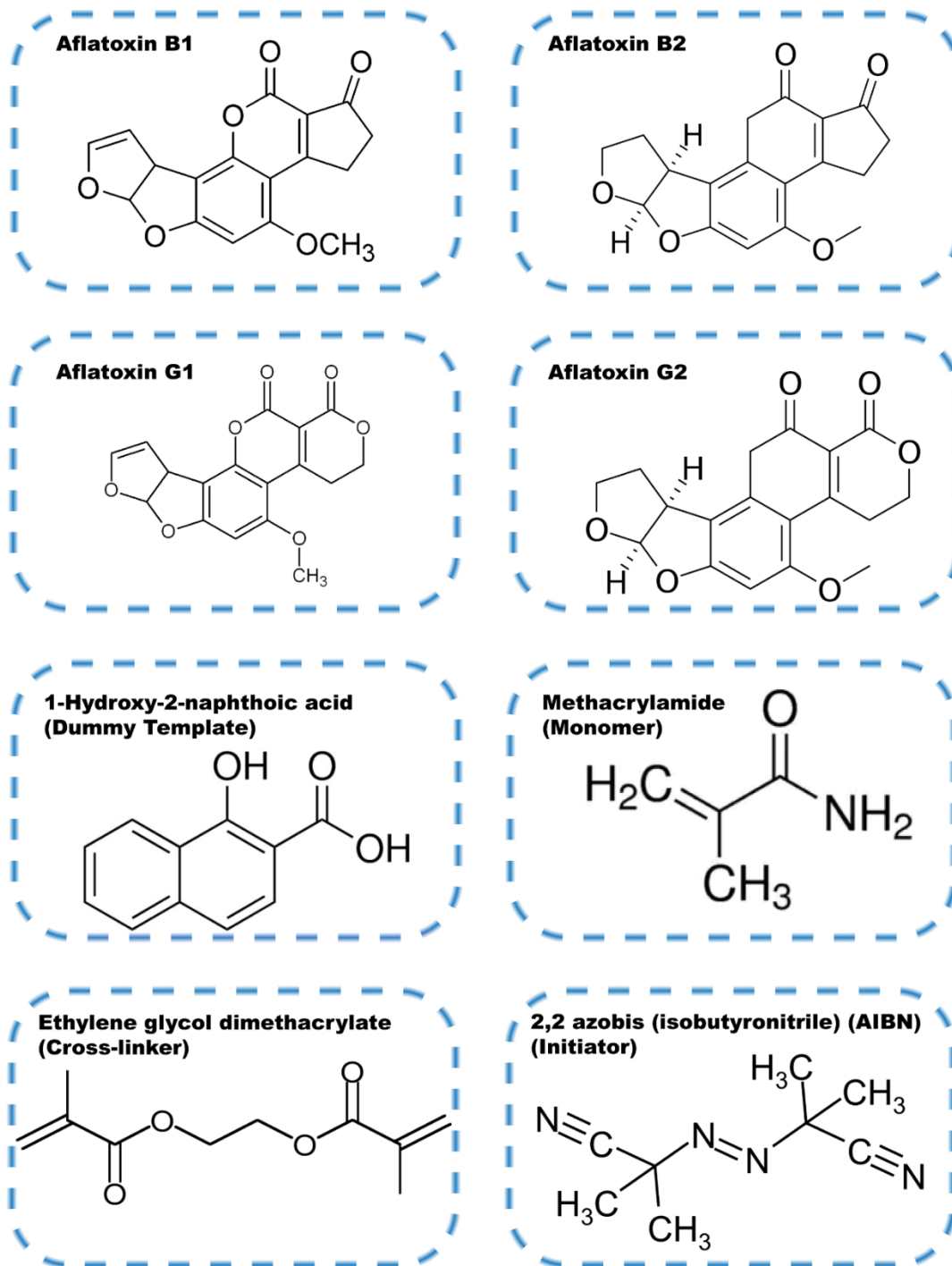


Figure S1. Structures of aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, 1-hydroxy-2-naphthoic acid, methacrylamide (MMA), ethylene glycol dimethacrylate (EGDMA) and 2,2 azobis (isobutyronitrile) (AIBN).

Table S2. Data obtained for the MIPs-SPE procedure optimization; the values are expressed as % of AFs adsorbed (A and B) and released from the MIP. In red are reported the chosen parameters. 5 ppb of AFs were employed for all the tests performed.

Amount of Polymer				
	AFB1 (%)	AFB2 (%)	AFG1 (%)	AFG2 (%)
2 mg	50	55	60	57
5 mg	85	85	90	90
10 mg	85	85	90	90
20 mg	85	85	90	90
Washing				
water	8	10	7	5
(80:20) ACN:H ₂ O	70	70	48	50
0.5%ACN H₂O	4	2	2	3
5% ACN H ₂ O	40	31	39	42
1% ACN H ₂ O	25	20	17	27
Elution				
MeOH	63	64	65	64
2% acetic acid in MeOH	85	85	90	90

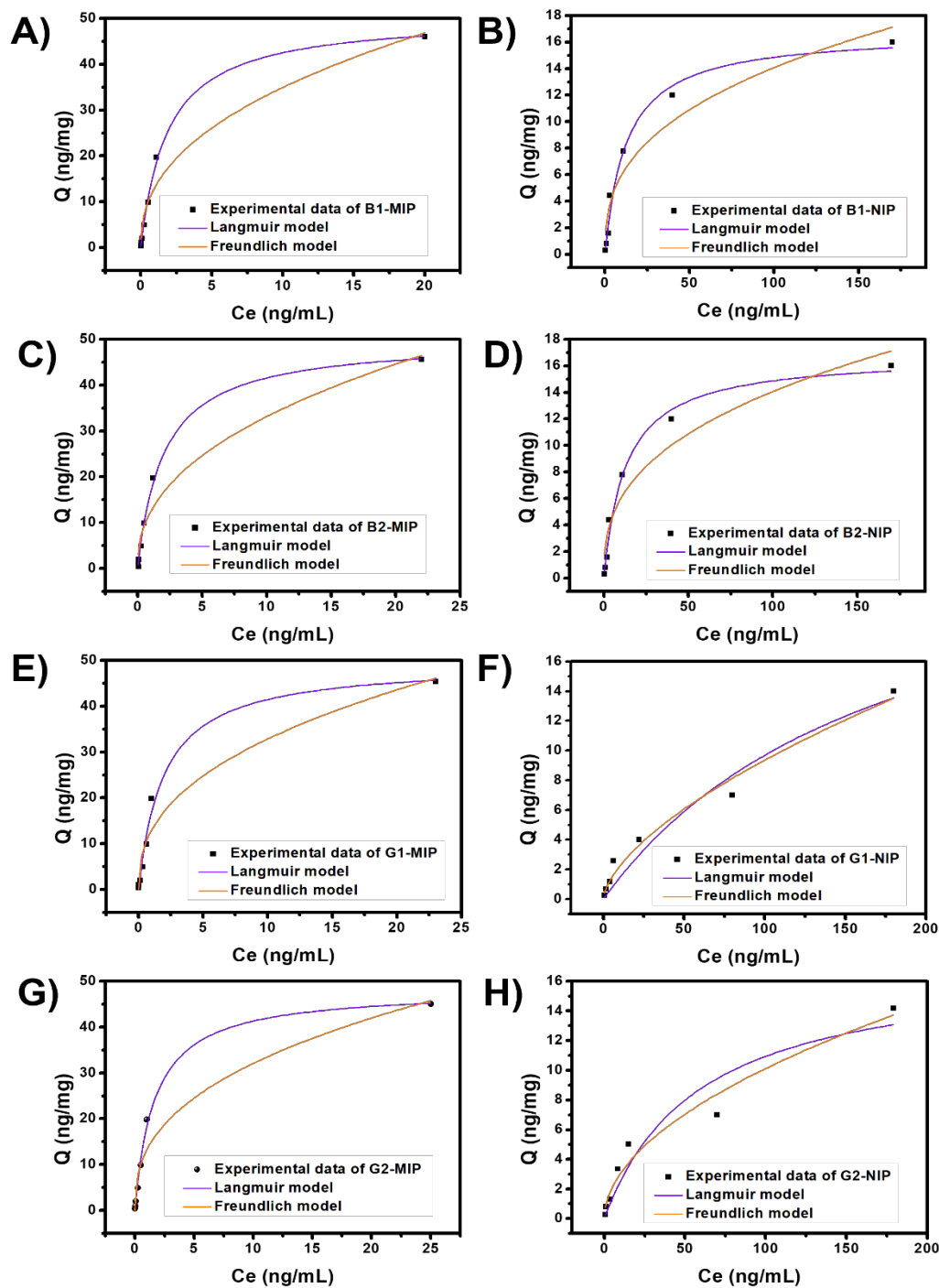


Figure S2. Adsorption isotherms models for the adsorption of AFBs onto MIP and NIP. A, C, E, and G represent the experimental data of adsorption by MIP at different concentrations at room temperature and the corresponding adsorption isotherms models including Langmuir and Freundlich models for AFB1, AFB2, AFG1, AFG2, respectively. B, D, F, and H represent the experimental data of adsorption by NIP at different concentrations at room temperature and the corresponding adsorption isotherms models including Langmuir and Freundlich models for AFB1, AFB2, AFG1, AFG2, respectively.

Table S3. Lower limit of quantification (LLOQ), lower limit of detection L(LOD), calibration curve equation and determination coefficient obtained in analytical procedure validation.

Analyte	LLOQ (ng mL ⁻¹)	LLOD (ng mL ⁻¹)	Calibration Curve	R ²
AFG1	0.02	0.005	y = 5696x - 1021	0.996
AFG2	0.09	0.027	y = 1683x - 3697	0.999
AFB1	0.02	0.007	y = 2875x - 6827	0.999
AFB2	0.03	0.009	y = 1889x - 1519	0.997

Table S4. Accuracy of the result obtained in the validation reported as BIAS percentage (%).

	Accuracy (BIAS %)											
	AFG1			AFG2			AFB1			AFB2		
	0.2 µg/kg	5µg/kg	10 µg/kg	0.2 µg/kg	5 µg/kg	10 µg/kg	0.2 µg/kg	5 µg/kg	10 µg/kg	0.2 µg/kg	5 µg/kg	10 µg/kg
Ginger	+4%	+3%	+3%	+4%	+3%	+3%	+4%	+4%	+4%	+3.0%	+3%	+4%
Echiancea pur- purea	+3%	+3%	+4%	+3%	+3%	+4%	+4%	+3%	+4%	+4%	+3%	+5%
Ginseng	+4%	+3%	+5%	+3%	+3%	6%	+4%	+4%	+2%	+4%	+3%	+4%
Hypericum	+4%	+5%	+5%	+3%	+4%	+6%	+4%	+3%	+3%	+4%	+4%	+5%
Red elm	+4%	+5%	+5%	+4%	+2%	+3%	+4%	+4%	+3%	+4%	+2%	+4%
Saffron	+4%	+3%	+4%	+4%	+4%	+3%	+4%	+2%	+5%	+4%	+4%	+5%
Mango	+4%	+4%	+5%	+4%	+3%	+5%	+4%	+3%	+4%	+4%	+3%	+4%
Red rice	+4%	+5%	+4%	+4%	+3%	+4%	+4%	+3%	+5%	+4%	+4%	+5%
Parsley	+4%	+4%	+4%	+4%	+5%	+3%	+4%	+5%	+5%	+4%	+5%	+5%
Red fruits	+3%	+3%	+5%	+4%	+5%	+4%	+4%	+3%	+5%	+4%	+5%	+5%
Grapefruit	+4%	+5%	+5%	+3%	+3%	+5%	+4%	+3%	+4%	+2%	+3%	+5%
Magnolia	+3%	+5%	+5%	+3%	+3%	+6%	+4%	+4%	+5%	+4%	+5%	+5%
Tilia Cordata	+4%	+4%	+5%	+4%	+4%	+6%	+4%	+4%	+5%	+4%	+5%	+4%
Root	+3%	+5%	+4%	+3%	+5%	+4%	+4%	+4%	+3%	+3%	+5%	+5%
Salsopariglia												
Hop	+3%	+4%	+4%	+4%	+5%	+6%	+5%	+3%	+5%	+3%	+5%	+3%
Verbene Offic- inalis	+3%	+5%	+5%	+3%	+5%	+6%	+4%	+4%	+5%	+5%	+4%	+5%
Galega Offici- nalis	+4%	+4%	+5%	+3%	+4%	+4%	+4%	+4%	+5%	+5%	+4%	+5%

Table S5. Intra-day and inter-day precision reported in percentage (%).

	Precision (RSD %)																							
	AFG1						AFG2						AFB1						AFB2					
	Intra-day			Inter-day			Intra-day			Inter-day			Intra-day			Inter-day			Intra-day			Inter-day		
	0.2 µg/ kg	5 µg/ kg	10 µg/ kg	0.2 µg/ kg	5 µg/ kg	10 µg/ kg	0.2 µg/ kg	5 µg/ k	10 µg/ kg	0.2 µg/ kg	5 µg/ kg	10 µg/ kg	0.2 µg/ kg	5 µg/ kg	10 µg/ kg	0.2 µg/ kg	5 µg/ kg	10 µg/ kg	0.2 µg/ kg	5 µg/ kg	10 µg/ kg	0.2 µg/ kg	5 µg/ kg	10 µg/ kg
Ginger	2%	3%	2%	2%	4%	4%	4%	3%	3%	4%	4%	4%	4%	3%	4%	4%	4%	4%	4%	2%	4%	3%	4%	3%
Echian cea pur- purea	3%	3%	3%	3%	3%	2%	2%	3%	4%	2%	2%	4%	3%	2%	3%	4%	3%	4%	5%	3%	4%	4%	4%	4%
Gin- seng	2%	3%	3%	3%	3%	4%	3%	3%	4%	3%	4%	2%	4%	1%	4%	4%	2%	3%	4%	3%	4%	3%	3%	3%
Hy- peri- cum	3%	3%	4%	2%	4%	4%	3%	4%	2%	3%	3%	3%	5%	4%	5%	2%	3%	4%	5%	3%	2%	4%	4%	4%
Red elm	3%	3%	4%	4%	4%	4%	2%	2%	3%	2%	4%	3%	4%	2%	4%	3%	3%	5%	5%	4%	3%	2%	3%	3%
Saf- fron	2%	3%	2%	3%	4%	3%	4%	4%	3%	4%	2%	2%	4%	4%	4%	3%	2%	4%	4%	2%	3%	3%	3%	4%
Mango	3%	4%	4%	3%	4%	3%	4%	3%	2%	4%	3%	4%	4%	3%	4%	2%	4%	5%	2%	2%	2%	3%	3%	4%
Red rice	4%	5%	5%	3%	3%	3%	4%	3%	4%	3%	3%	3%	2%	4%	3%	5%	3%	5%	3%	4%	4%	4%	3%	4%
Pars- ley	7%	4%	4%	2%	7%	5%	5%	7%	3%	3%	5%	4%	3%	5%	4%	5%	5%	2%	3%	3%	2%	4%	5%	4%
Red fruits	4%	3%	5%	2%	4%	3%	4%	4%	4%	2%	3%	5%	3%	5%	3%	4%	5%	3%	2%	4%	3%	2%	4%	5%
Grape- fruit	5%	4%	5%	3%	4%	3%	4%	3%	5%	3%	3%	4%	2%	3%	3%	3%	4%	3%	4%	3%	3%	3%	4%	5%
Mag- nolia	5%	5%	5%	3%	3%	3%	2%	3%	4%	3%	4%	2%	4%	4%	2%	4%	4%	2%	3%	4%	2%	3%	4%	3%

Tilia	5%	4%	5%	2%	3%	6%	4%	4%	4%	2%	4%	3%	4%	4%	4%	5%	4%	2%	4%	4%	3%	3%	5%	4%
Cor-																								
data																								
Root	4%	5%	5%	4%	5%	4%	3%	4%	4%	4%	4%	3%	3%	5%	2%	4%	5%	3%	5%	5%	3%	5%	4%	4%
Salsop																								
ariglia																								
Hop	5%	4%	5%	4%	5%	4%	2%	4%	5%	5%	3%	2%	3%	5%	3%	5%	4%	3%	4%	5%	2%	5%	5%	4%
Ver-	5%	5%	4%	4%	5%	3%	3%	5%	4%	4%	4%	4%	5%	4%	3%	5%	4%	2%	5%	5%	5%	4%	4%	4%
bene																								
Offici-																								
nalis																								
Galega	5%	4%	5%	4%	4%	3%	3%	4%	4%	4%	4%	4%	5%	4%	2%	4%	5%	5%	4%	5%	4%	5%	5%	4%
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Table S6. The isotherm equations

Model	Equation	Parameters
Langmuir	$Q_e = \frac{Q_{max}K_L C_e}{1 + C_e}$	k_L (mL/ng) is the Langmuir constant; Q_{max} (ng/mg) represents the maximum adsorption capacity obtained with Langmuir model.
Freundlich	$Q_e = K_F C_e^{1/n}$	k_F (ng/mg) and n_F are the Freundlich model constants.

Table S7. MS/MS parameters. Multi-reaction monitoring (MRM) transitions: Q1 MASS = precursor ion mass (amu); Q3 MASS = product ion mass (amu). TIME = dwell time. DP (V) = declustering potential (amu). EP (V) = entrance potential. CE (V) = collision energy. CXP (V) = cell exit potential.

Analytes	Q1	DP	EP	DT	Q3	CE	CXP
MRM (+)							
Aflatoxin G1	329.1	110	10	50	242.9	36	9
					310.9	31	10
Aflatoxin G2	331.1	110	8	50	313.1	35	18
					245.1	43	14
Aflatoxin B1	313.1	107	7	50	285.0	32	11
					241.0	50	9
Aflatoxin B2	315.1	100	9	50	287.1	36	9
					258.8	40	11
Ochratoxin A	404.3	81	9	50	358.1	21	15
					239.1	32	9
Aflatoxin M1	329.2	96	7	50	273	35	10
					258.7	33	12
Citrinina	251.0	55	13	50	233.0	24	9
					205.0	36	7
Ochratoxin B	370.2	50	7	25	205.0	23	12
					324.0	20	12
MRM (-)							
Patulina I	153.0	-50	-12	50	109.0	-13	-7
					81.0	-17	-5

IAC Procedure

The AFs extraction via IAC was performed as follow: 25 g of sample were extracted by adding 5g of NaCl and 100 mL of MeOH:H₂O 80:20 v/v; the extraction was performed shaking at high speed with a magnet for 2 min; then the sample was centrifuged at 4000 g for 10 min.

A 4 mL aliquot was diluted with 36 mL of PBS solution and then filtered; 20 mL of filtered sample was loaded on IAC at flow rate of 2 mL min⁻¹; the washing step was performed with 20 mL of H₂O at flow rate of 5 mL min⁻¹; finally the elution of the target analytes was achieved with 1 mL of MeOH and subsequently 1 mL of H₂O; 100 µL of elution was analyzed by HPLC-MS/MS (see section 2.6).

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