

Supplementary Materials: Presence of 19 Mycotoxins in Human Plasma in a Region of Northern Spain

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Table S1. Examples of the obtained calibration curves.

DOM-1			AFG2			AFM1			AFG1		
ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)
6.0	6.40	5.66	0.8	0.90	12.91	0.8	0.91	13.22	0.3	0.32	7.18
12.0	10.84	15.51	1.6	1.44	9.72	1.6	1.39	12.85	0.6	0.59	1.61
24.0	22.91	6.77	3.2	3.19	0.30	3.2	3.09	3.30	1.2	1.23	2.09
36.0	35.92	0.23	4.8	4.61	3.89	4.8	4.96	3.24	1.8	1.76	2.23
48.0	49.28	2.67	6.4	6.81	6.34	6.4	7.00	9.34	2.4	2.52	5.08
60.0	64.44	7.39	8.0	7.58	5.29	8.0	7.74	3.29	3.0	2.89	3.63
120.0	119.22	0.65	16.0	15.44	3.47	16.0	15.38	3.87	6.0	5.94	0.92
180.0	176.99	1.67	24.0	24.82	3.43	24.0	24.33	1.39	9.0	9.05	0.50
y = 83.44x – 63.85 R ² = 0.9981 Weight: 1/x			y = 626.99x – 27.40 R ² = 0.9969 Weight: 1/x			y = 410.34x – 15.98 R ² = 0.9978 Weight: None			y = 86.15x – 73.82 R ² = 0.9995 Weight: 1/x		

B-C: back-calculated; RE: relative error.

Table S1. (Continued). Examples of the obtained calibration curves.

AFB2			AFB1			HT-2			OTB		
ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)
0.3	0.36	18.79	0.2	0.23	14.47	6.0	6.26	4.28	1.0	1.17	17.21
0.6	0.58	3.39	0.4	0.37	8.64	12.0	10.73	10.61	2.0	1.94	3.06
1.2	1.21	0.93	0.8	0.74	7.63	24.0	24.03	0.10	4.0	4.24	6.11
1.8	1.83	1.91	1.2	1.15	4.33	36.0	37.14	3.16	6.0	5.99	0.20
2.4	2.50	4.07	1.6	1.73	8.07	48.0	49.95	4.06	8.0	8.21	2.59
3.0	2.88	3.86	2.0	1.96	1.91	60.0	58.65	2.26	10.0	9.73	2.68
6.0	5.80	3.36	4.0	3.94	1.45	120.0	118.44	1.30	20.0	19.15	4.27
9.0	9.14	1.53	6.0	6.09	1.43	180.0	180.81	0.45	30.0	30.57	1.90
y = 1433.4x – 90.03 R ² = 0.9986 Weight: None			y = 2844.47x – 136.89 R ² = 0.9974 Weight: 1/x			y = 52.85x – 16.61 R ² = 0.9995 Weight: None			y = 747.54x – 241.03 R ² = 0.9982 Weight: None		

B-C: back-calculated; RE: relative error.

Table S1. (Continued). Examples of the obtained calibration curves.

T-2			ZEA			OTA-d ₅			STER		
ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)
1.0	1.15	15.07	4.0	4.35	8.72	2.0	2.05	2.70	1.0	1.06	6.43
2.0	1.86	6.84	8.0	7.10	11.25	4.0	4.16	3.99	2.0	1.79	10.39
4.0	4.18	4.59	16.0	-	*	8.0	8.28	3.55	4.0	4.08	2.08
6.0	6.09	1.53	24.0	25.26	5.23	12.0	11.74	2.13	6.0	6.13	2.12
8.0	8.13	1.66	32.0	32.31	0.97	16.0	16.36	2.35	8.0	8.47	5.89
10.0	9.81	1.86	40.0	39.22	1.94	20.0	19.80	0.98	10.0	10.11	1.15
20.0	19.33	3.36	80.0	76.31	4.61	40.0	38.84	2.90	20.0	18.61	6.96
30.0	30.44	1.45	120.0	123.45	2.87	60.0	60.74	1.23	30.0	30.74	2.47
y = 3576x – 935.47 R ² = 0.9989 Weight: None			y = 185.95x – 115.93 R ² = 0.9977 Weight: 1/x			y = 236.91x – 85.24 R ² = 0.9992 Weight: None			y = 737.25x – 72.43 R ² = 0.9960 Weight: None		

B-C: back-calculated; RE: relative error*; not included.

Table S1. (Continued). Examples of the obtained calibration curves.

NIV			DON			FUS-X			NEO		
ng/mL	B-C (ng/mL)	ng/mL	ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)
20.4	20.52	6.0	8.7	7.77	10.90	8.7	9.91	13.65	0.8	0.88	10.00
40.8	36.27	12.0	17.4	15.20	12.82	17.4	18.85	8.08	1.6	1.52	4.80
81.6	82.39	24.0	34.9	37.03	6.16	34.9	33.77	3.20	3.2	3.13	2.34
122.4	125.52	36.0	52.3	51.64	1.29	52.3	53.50	2.26	4.8	4.98	3.84
163.2	183.19	48.0	69.8	72.18	3.46	69.8	74.23	6.41	6.4	6.56	2.50
204.0	195.47	60.0	87.2	90.09	3.31	87.2	85.78	1.63	8.0	7.22	9.74
408.0	383.88	120.0	174.4	168.36	3.47	174.4	160.07	8.22	16.0	15.24	4.73
612.0	625.17	180.0	261.6	264.05	0.94	261.6	270.22	3.29	24.0	25.26	5.26
y = 4.64x – 18.09 R ² = 0.9957 Weight: None			y = 24.5x + 18.42 R ² = 0.9987 Weight: None			y = 16.19x – 31.98 R ² = 0.9943 Weight: None			y = 181.00x – 16.54 R ² = 0.9952 Weight: 1/x		

B-C: back-calculated; RE: relative error.

Table S1. (Continued). Examples of the obtained calibration curves.

3-ADON			15-ADON			DAS		
ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)	ng/mL	B-C (ng/mL)	RE (%)
1.8	2.08	19.04	2.7	2.77	1.92	0.7	0.84	19.35
3.5	2.98	14.78	5.4	5.41	0.52	1.4	1.23	11.84
7.0	7.15	2.18	10.9	10.64	2.20	2.8	2.62	6.59
10.5	10.62	1.16	16.3	16.71	2.37	4.2	4.23	0.80
14.0	15.08	7.70	21.8	23.15	6.38	5.6	5.54	1.13
17.5	17.35	0.86	27.2	27.83	2.33	7.0	6.97	0.36
35.0	32.81	6.26	54.4	49.70	8.64	14.0	13.36	4.55
52.5	53.70	2.29	81.6	84.11	3.07	21.0	21.91	4.32
$y = 64.22x - 40.31$			$y = 77.18x - 39.69$			$y = 381.06x - 37.92$		
$R^2 = 0.9963$			$R^2 = 0.9941$			$R^2 = 0.9965$		
Weight: None			Weight: None			Weight: 1/x		

B-C: back-calculated; RE: relative error.

Table S2. Revalidation parameters after enzymatic treatment. Precision and accuracy at three concentration levels (LOQ, 6xLOQ and 30xLOQ). Calculated as RSD or RE (%).

Mycotoxin	Precision (%RSD)						Accuracy (%RE)					
	Within-run (n=3)			Between-run (n=9)			Within-run (n=3)			Between-run (n=9)		
	L	M	H	L	M	H	L	M	H	L	M	H
DOM-1	1.96	4.61	11.82	5.04	7.37	6.34	6.89	3.45	6.53	8.80	2.15	4.96
AFG2	6.53	5.31	2.61	7.86	9.83	8.02	1.25	5.90	6.14	7.36	5.02	1.83
AFM-1	5.23	4.20	3.14	8.39	6.98	5.12	3.33	6.11	4.69	6.67	2.43	3.73
AFG1	3.45	7.73	4.05	8.18	9.40	7.26	3.33	0.74	7.89	8.89	5.00	6.93
AFB2	6.37	5.10	1.89	5.20	6.02	8.53	8.89	3.89	8.07	11.48	2.16	3.99
AFB1	5.56	5.12	3.14	6.16	7.64	8.56	10.00	1.67	8.83	14.44	4.63	7.83
HT-2	10.76	4.45	0.83	7.01	12.02	6.87	11.83	5.87	0.94	14.89	3.27	5.93
OTB	3.33	3.32	1.08	4.54	10.05	4.11	4.00	5.78	4.37	7.22	4.54	5.75
T-2	9.67	4.80	5.94	12.08	11.47	13.06	5.00	4.61	9.38	0.44	6.41	3.95
ZEA	6.26	4.68	4.70	11.61	6.97	8.05	1.25	1.21	9.31	6.31	6.18	7.76
OTA-d ₅	5.38	6.66	1.76	12.21	11.49	3.93	10.67	3.94	1.54	5.61	6.74	1.78
STER	7.21	7.58	3.90	8.22	14.17	13.20	0.00	5.06	10.37	0.44	0.96	0.29
NIV	12.56	8.62	4.08	14.28	9.82	13.14	4.28	3.52	11.84	0.58	7.72	3.55
DON	7.99	3.02	2.21	10.39	5.08	14.28	11.92	3.02	10.32	9.68	1.04	0.80
FUS-X	11.04	5.17	9.10	11.68	7.41	13.21	8.31	5.51	10.98	10.16	3.69	5.88
NEO	9.80	2.55	2.46	15.77	5.82	10.09	11.25	0.14	7.94	4.22	5.09	2.79
3-ADON	2.91	1.20	1.44	4.71	7.65	9.04	10.19	5.27	3.06	7.85	3.02	3.10
15-ADON	3.84	6.97	2.55	9.36	6.17	6.74	2.35	0.61	3.12	0.05	5.60	3.21
DAS	6.21	3.53	3.67	6.62	12.89	7.86	3.81	4.37	3.51	4.11	4.39	2.91

RSD: relative standard deviation; RE: relative error; L: low level; M: medium level; H: high level.

Table S3. Revalidation parameters after enzymatic treatment. Recovery and matrix effect (%RSD).

	DOM-1	AFG2	AFM-1	AFG1	AFB2	AFB1	HT-2	OTB	T-2	ZEA
Recovery	81.17	82.59	89.88	78.77	85.14	93.11	101.06	96.32	103.05	90.43
	83.31	93.15	88.16	88.16	81.58	86.36	90.20	89.78	87.39	83.02
	94.71	79.23	77.88	76.75	76.27	74.36	78.95	74.55	77.19	68.83
Mean	86.40	84.99	85.31	81.22	81.00	84.61	90.07	86.89	89.21	80.76
RSD (%)	8.43	8.55	7.60	7.50	5.51	11.22	12.27	14.60	14.60	13.59
Matrix effect	71.77	90.95	115.31	88.59	105.34	86.89	104.15	100.03	106.01	89.59
	72.83	92.04	95.24	82.97	100.87	88.63	103.66	103.50	103.37	99.81
	68.89	93.12	101.21	84.42	94.76	88.52	104.60	106.34	107.91	101.36
Mean	71.16	92.04	103.92	85.32	100.32	88.02	104.14	103.29	105.76	96.92
RSD (%)	2.86	1.18	9.92	3.42	5.29	1.11	0.45	3.06	2.16	6.60

RSD: relative standard deviation.

Table S3. (Cont.). Revalidation parameters after enzymatic treatment. Recovery and matrix effect (%RSD).

	OTA-d ₅	STER	NIV	DON	FUS-X	NEO	3-ADON	15-ADON	DAS
Recovery	89.39	90.84	79.30	92.56	97.47	85.43	90.45	92.58	93.47
	87.85	74.42	76.53	94.66	94.95	82.45	84.85	90.08	96.01
	70.23	56.50	65.39	78.96	68.25	78.37	81.63	76.07	73.87
Mean	82.49	73.92	73.74	88.73	86.89	82.08	85.64	86.24	87.79
RSD (%)	12.91	23.23	9.99	9.61	18.64	4.32	5.22	10.32	13.80
Matrix effect	99.26	66.58	92.23	91.98	86.31	94.26	103.53	89.56	104.18
	100.81	72.69	92.52	79.94	83.70	86.22	87.31	86.18	99.12
	102.67	83.41	94.25	91.49	102.85	101.05	94.94	99.36	111.89
Mean	100.91	74.23	93.00	87.81	90.95	93.84	95.26	91.70	105.06
RSD (%)	1.69	11.48	1.17	7.76	11.42	7.91	8.52	7.46	6.12

RSD: relative standard deviation.

Table S4. OTA reanalysis in plasma samples.

Women Samples	OTA (ng/mL)			Men Samples	OTA (ng/mL)		
	First analysis	Reanalysis	RE (%)		First analysis	Reanalysis	RE (%)
47	45.7	42.3	7.7	46	< LOQ	< LOQ	0.0
58	< LOQ	2.4	18.2	57	< LOQ	< LOQ	0.0
68	2.4	< LOQ	17.4	67	< LOQ	< LOQ	0.0
76	< LOQ	< LOQ	0.0	77	2.2	< LOQ	11.3
91	< LOQ	< LOQ	0.0	92	2.2	< LOQ	7.7
101	4.8	4.0	17.2	100	2.7	< LOQ	30.1
107	2.1	< LOQ	2.5	105	< LOQ	< LOQ	0.0
120	2.8	< LOQ	32.6	113	3.4	< LOQ	52.1
215	< LOQ	< LOQ	0.0	250	< LOQ	2.1	6.8
218	< LOQ	< LOQ	0.0	288	< LOQ	< LOQ	0.0
240	10.1	8.6	15.6	333	< LOQ	< LOQ	0.0
300	3.1	2.7	12.4	360	4.0	< LOQ	67.1
335	< LOQ	< LOQ	0.0	390	4.4	< LOQ	75.0
380	4.2	< LOQ	71.2	425	2.6	< LOQ	24.6
400	5.0	2.5	64.7	445	2.2	< LOQ	9.5
422	3.0	< LOQ	40.3	470	2.4	< LOQ	19.8
446	2.9	< LOQ	37.4	500	2.1	< LOQ	4.9
475	< LOQ	2.2	8.2	510	2.2	< LOQ	8.6
496	2.3	< LOQ	13.5	530	5.8	3.8	41.7
518	2.3	< LOQ	14.4				
522	3.5	2.5	33.4				

RE: relative error; LOQ: limit of quantification (included in the statistical study as 2 ng/mL).