

Mycotoxin Biomarkers in Pigs—Current State of Knowledge and Analytics

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Table S1. Summary of recent LC-MS/MS methods for determination of mycotoxin biomarkers in pig urine, faeces and serum

Matrix	Sample preparation	Creatinine adjustment	Enzymatic hydrolysis (enzyme)	Analytes	LOD [ng/mL]	LOQ [ng/mL]	Recovery [%]	Matrix effect (SSE) [%]	Analytical column	Mobile phase	LC-MS	Reference
urine	RP-18 SPE (Phenomenex)	-	glucuronidase/arylsulfatase from <i>Helix pomatia</i>	ZEN	0.1	0.5	94-105	-	RP-18 column	10% ACN, 45% MeOH, 45% H ₂ O (v/v/v)	PE Sciex API 365 (APCI)	[1]
				α -ZEL	0.2	0.5						
				β -ZEL	0.2	0.5						
				α -ZAL	0.5	1						
	SPE columns	-	type H-2 from <i>Helix pomatia</i>	β -ZAL	0.5	1	76-118	-	-	-	1200 series HPLC system (Agilent Technologies) coupled to a 4000 QTrap (Applied Biosystems)	[2]
				ZEN	0.03	0.10						
				α -ZEL	0.07	0.24						
				β -ZEL	0.16	0.52						
	Oasis TM HLB columns (Waters)	-	type H-2 from <i>Helix pomatia</i>	ZAN	0.09	0.30	64-100	-	-	MeOH:H ₂ O, 20:80, v/v)	QTrap MS/MS system (Applied Biosystems, Foster City, CA, USA), equipped with an ESI interface, an 1100 series micro-LC system	[3]
				α -ZAL	0.11	0.35						
				β -ZAL	0.13	0.44						
				DON	0.11	0.38						
	Mycobin1 immunoaffinity (Vicam) column (IAC) (Vicam) and an OASIS HLB solid phase extraction (SPE) column (Waters) connected in tandem	-	β -glucuronidase/sulfatase type H-2 from <i>Helix pomatia</i>	DOM-1	0.04	0.15	94-103 (R _E) 56-114 (R _A)	12 18 33 152 44 14 29 42	-	-	1290 Infinity series UHPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a 4000 QTrap mass spectrometer	[4]
				DON	0.18	0.6						
				DOM-1	0.36	1.21						
				AFM ₁	0.01	0.03						
				FB ₁	0.02	0.06						
				ZEN	0.02	0.07						
				α -ZEL	0.04	0.13						
				β -ZEL	0.04	0.15						
	D&S	-	-	OTA	0.006	0.02						
				DON	0.9	8						
				DON-3-Glc	1.3	2.1						
				DON-3-GlcA	9.0	37.3						
	D&S	-	-	DOM-1	1.4	3.7						
				DON	0.9	8						
				DON-3-Glc	1.3	2.1						
				DON-3-GlcA	9.0	37.3						

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urine	D&S	urine diluted to the same creatinine concentration (0.2 mM)	-	DON DON-GlcAc DON-3-S DOM-1 ZEN ZEN-14-GlcAc α -ZEL α -ZEL-14-GlcAc	-	-	84-115	-	Kinetex Biphenyl column (150×3 mm, 2.6- μ m particle size; Phenomene x, Aschaffenburg, Germany)	water/acetic acid and methanol/acetic acid (each 99.9/0.1, v/v)	Agilent 1290 series UHPLC system coupled to a Sciex 6500 QTrap mass spectrometer	[6]
				α -ZEL β -ZEL ZEN ZEN-14-Glc ZEN-16-Glc ZEN-14-S α -ZEL-GlcAc β -ZEL-GlcAc ZEN-14-GlcAc	0.11 0.16 0.15 0.07 0.18 0.02 0.73 0.42 0.30	0.38 0.54 0.49 0.23 0.60 0.08 2.4 1.4 1.0	84-113 (R _A)	-	Kinetex Biphenyl column (150 mm × 3 mm, 2.6 μ m, Phenomene x, Aschaffenburg, Germany)	water/acetic acid (99.9/0.1, v/v), and ACN/acetic acid (99.9/0.1, v/v)	Agilent 1290 series UHPLC system coupled to a Sciex 6500 QTrap mass spectrometer	[7]
				T-2 HT-2 T-2 triol	0.3 0.6 2	1 2 5	74.3-102.4	79.3-93.8	Zorbax XDB-C18 column (150 mm × 2.1 mm, 3.5 μ m; Agilent, USA)	5.0 mM ammonium acetate in water (A) and acetonitrile (B)	1290 Infinity series UHPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a 4000 QTrap mass spectrometer	[8]
Matrix	Sample preparation	Creatinine adjustment	Enzymatic hydrolysis (enzyme)	Analytes	LOD [ng/mL]	LOQ [ng/mL]	Recovery [%]	Matrix effect (SSE) [%]	Analytical column	Mobile phase	LC-MS	Reference
urine	LLE EtOAc	-	-	ZEN	-	1 for all analytes	56.2 (T-2)-212.8 (AOH)	90.6	Waters	10 mM ammonium formate,	Agilent 1290 series UHPLC system coupled	[9]
				α -ZEL	-	except:	(R _E)	84.8	Acquity			
				α -ZAL	-			85.8	HSS T3			[10]

β-ZAL	DOM-1	85.1	column (100	0.3% formic	to a Sciex 6500
β-ZEL	(4 ng/mL)	85.5	mm × 2.1	acid in	QTrap mass
ZAN	and	90.6	mm i.d., dp:	water and	spectrometer
TeA	T2G	52.4	1.8 µm)	10 mM	
AOH	(2 ng/mL)	11.1		ammonium	
AME		73.6		formate,	
DON		79.5		0.3% formic	
DOM-1		5.2		acid in	
3/15-		72.6		methanol	
AcDON		90.5			
T-2		100.4			
HT-2		107.6			
T2-G		72.4			
AFB ₁		79.3			
AFM ₁		106.3			
OTA		81.3			
ENNA		80.2			
ENNA ₁		83.7			
ENNB		82.8			
ENNB ₁		89.5			
BEA					

Matrix	Sample preparation	Creatinine adjustment	Enzymatic hydrolysis (enzyme)	Analytes	LOD [ng/mL]	LOQ [ng/mL]	Recovery [%]	Matrix effect (SSE) [%]	Analytical column	Mobile phase	LC-MS	Reference
urine	LLE 1. step : 0.1% formic acid- ACN + 0.8 g of sodium chloride. 2. step : upper supernatant + 500 mg of anhydrous magnesium sulfate, 50 mg of C18, 50 mg of PSA, and 50 mg of aluminia A	-	-	AFB ₁		0.05	80.8 - 114.3	signal suppressi on for the majority of compound s	BEH RP18 chromatogra phic column (Acquity UPLC, 100 mm × 2.1 mm; 1.7 µm; Waters, USA)	0.1% formic acid aqueous solution and 0.1% formic acid- methanol	Waters Acquity ultra-performance liquid chromatography (UPLC) system coupled to a Micromass Quatro Micro triple quadrupole mass spectrometer (Waters, Milford, MA, USA)	[11]
				AFB ₂		0.05						
				AFG ₁		0.05						
				AFG ₂		0.05						
				AFM ₁		0.05						
				AFM ₂		0.05						
				STC		0.05						
				T-2		0.05						
				LYS		0.05						
				MET		0.05						
				RC		0.05						
				DAS		0.05						
				DON	-	0.25						
				3-AcDON		0.5						
				15-AcDON		0.5						
				NEO		0.25						
				WOR		0.25						
				VER		0.25						
				HT-2		0.25						
				ZEN		0.25						
				α-ZEL		0.5						
				β-ZEL		0.5						
				ZAN		0.5						
				α-ZAL		0.5						
				β-ZAL		0.5						
	LLE 1. step: EtOAc/FA (99/1, v/v) + MgSO ₄ (2 M) 2. step: remaining aqueous phase+ ACN/FA (99/1, v/v).	-	-	DON	1	3.3	70-100	4	Symmetry C18, 5 µm, 2.1×150 mm (Waters, Milford, MA, USA)	water, 0.3% formic acid, 5mM ammonium format and methanol, 0.3% formic acid, 5mM ammonium formate	Waters Acquity ultra-performance liquid chromatography (UPLC) system coupled to a Micromass Quatro Micro triple quadrupole mass spectrometer (Waters, Milford, MA, USA)	[12]
				AFB ₁	0.1	0.33		5				
				AFM ₁	0.1	0.33		5				
				T-2	0.04	0.13		32				
				HT-2	0.3	3.3		17				
				NEO	1	0.17		4				
				FB ₁	0.05	0.07		57				
				OTA	0.02	0.8		46				
				OTα	0.25	1		4				
				ZEN	0.4	1.3		20				
				α-ZEL	0.3	1		23				
				β-ZEL	0.3	1		16				

Matrix	Sample preparation	Creatinine adjustment	Enzymatic hydrolysis (enzyme)	Analytes	LOD [ng/mL]	LOQ [ng/mL]	Recovery [%]	Matrix effect (SSE) [%]	Analytical column	Mobile phase	LC-MS	Reference
	extraction MeOH/water (50/50, v/v, 3/2/2 mL x3)	-	-	DON	3.4	11.2	85-95 (RE) 52-59 (RA)	63	Zorbax RRHD Eclipse XDB-C18 column	Water and MeOH/water (97/3, v/v), both containing 0.01% acetic acid and 5 mM ammonium acetate	1290 Infinity series UHPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a 4000 QTrap mass spectrometer	[5]
				DON-3-Glc	2.6	8.7		62	Agilent Technologies, Waldbronn, Germany)			
				DOM-1	3.2	10.8		59	Agilent Technologies, Waldbronn, Germany)			
faeces	extraction ACN/water (50/50, v/v) x3	-	-	α -ZEL	.0.08	0.28	103-139 (RE)	47	Kinetex Biphenyl column (150 mm × 3 mm, 2.6 μ m,	water/acetic acid (99.9/0.1, v/v), and ACN/acetic acid (99.9/0.1, v/v)	1290 Infinity series UHPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a 6500 QTrap mass spectrometer (Sciex, Foster City, CA, USA)	[7]
				β -ZEL				75	Phenomenex, Aschaffenburg, Germany)			
				ZEN				77	Phenomenex, Aschaffenburg, Germany)			
				ZEN-14-Glc	0.10	0.33	49 - 124 (RA)	66	Phenomenex, Aschaffenburg, Germany)			
				ZEN-16-Glc	0.06	0.19		75	Phenomenex, Aschaffenburg, Germany)			
				ZEN-14-S	0.73	2.4		104	Phenomenex, Aschaffenburg, Germany)			
	extraction (EtAc) x2 + SPE columns			T-2	0.3	1	74.3-102.4 (RE)	79.3-	Zorbax XDB-C18 column (150 mm × 2.1 mm, 3.5 μ m; Agilent, USA)	5.0 mM ammonium acetate in water and acetonitrile	1290 Infinity series UHPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a 4000 QTrap mass spectrometer	[8]
				HT-2	0.6	2		93.8				
				T-2 triol	2	5						

Matrix	Sample preparation	Creatinine adjustment	Enzymatic hydrolysis (enzyme)	Analytes	LOD [ng/mL]	LOQ [ng/mL]	Recovery [%]	Matrix effect (SSE) [%]	Analytical column	Mobile phase	LC-MS	Reference
faeces	1.LLE MeOH/ethyl acetate/formic acid (75/24/1; v/v) for OTA, TeA, AME and AOH. 2. the other mycotoxins a LLE with acetone and a solid phase extraction with a HybridSPE-phospholipid column	-	-	ZEN	-	5	41.2 - 266.1 (R _E)	35.4	Waters Acquity HSS T3 column (100 mm × 2.1 mm i.d., dp: 1.8 µm)	10 mM ammonium formate, 0.3% formic acid in water and 10 mM ammonium formate, 0.3% formic acid in methanol	Agilent 1290 series UHPLC system coupled to a Sciex 6500 QTrap mass spectrometer	[9] [10]
				α-ZEL		1		22.4				
				α-ZAL		5		18.5				
				β-ZAL		1		30.5				
				β-ZEL		1		40.3				
				ZAN		1		30.3				
				TeA		1		12.4				
				AOH		5		13.5				
				AME		1		22.7				
				DON		5		27.5				
				DOM-1		5		23.7				
				3/15-AcDON		1		16.7				
				T-2		1		82.4				
				HT-2		5		62.8				
				T2-G		5		66.4				
				AFB ₁		1		35.0				
				AFM ₁		1		35.3				
				OTA		1		68.1				
				ENNA		1		78.6				
				ENNA ₁		1		304.5				
				ENNB		1		61.9				
				ENNB ₁		1		72.8				
				BEA		1		64.3				
serum	SPE columns	Oasis™ HLB (Waters)	β-glucuronidase type H-2 from Helix pomatia	ZEN	0.03	0.08	85-117		100× 2 mm Pursuit XRs Ultra 2.8 µm C18 column (Agilent Technologies)	water and MeOH/ACN (70/30 v/v)	1200 series HPLC system (Agilent Technologies) coupled to a 4000 QTrap (Applied Biosystems)	[2] [13]
				α-ZEL	0.24	0.78						
				β-ZEL	0.71	2.37						
				ZAN	0.13	0.43						
				α-ZAL	0.16	0.52						
				β-ZAL	0.28	0.94						
				DON	0.14	0.45						
				DOM-1	0.23	0.76						

Matrix	Sample preparation	Creatinine adjustment	Enzymatic hydrolysis (enzyme)	Analytes	LOD [ng/mL]	LOQ [ng/mL]	Recovery [%]	Matrix effect (SSE) [%]	Analytical column	Mobile phase	LC-MS	Reference
serum	LLE ACN	-	-	T-2	0.3	1	74.3- 102.4	79.3-93.8	Zorbax XDB-C18 column (150 mm × 2.1 mm, 3.5 µm; Agilent, USA)	5.0 mM ammonium acetate in water and acetonitrile	1290 Infinity series UHPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a 4000 QTrap mass spectrometer	[8]
				HT-2	0.6	2						
				T-2 triol	2	5						
				DON	0.40	10	77.57(OTA) - 109.8 (ZAN)		Hypersil Gold column (50 mm × 2.1 mm i.d., dp: 1.9 µm) ThermoFisher Scientific	0.1% acetic acid in water and methanol	LC ThermoFisher Scientific (Breda, The Netherlands) TSQ® Quantum Ultra triple quadrupole mass spectrometer ThermoFisher Scientific	[14]
				DOM-1	0.22	5						
				ZEN	0.52	5						
				ZAN	0.05	5						
				α-ZEL	0.02	5						
				β-ZEL	0.02	5						
				α-ZAL	0.02	5						
				β-ZAL	0.02	5						
				OTA	0.02	5						
				T-2	0.05	2						
				HT-2	0.15	5						
				FB ₁	0.01	1						
				AFB ₁	0.05	2						
			β-Glucuronidase Type HP-2 from Helix pomatia	ZEN	0.003	0.2-5	83.5 - 86.0 (R _E) 82.4-96.1 (R _A)	97-112	Hypersil Gold column (50 mm × 2.1 mm i.d., dp: 1.9 µm) ThermoFisher Scientific	0.01% acetic acid in water, and acetonitrile	LC ThermoFisher Scientific (Breda, The Netherlands) TSQ® Quantum Ultra triple quadrupole mass spectrometer ThermoFisher Scientific	[15]
				α-ZEL	0.012							
				β-ZEL	0.020							
				α-ZAL	0.020							
				β-ZAL	0.020							
				ZAN	0.004							
				ZAN	0.040							

Matrix	Sample preparation	Creatinine adjustment	Enzymatic hydrolysis (enzyme)	Analytes	LOD [ng/mL]	LOQ [ng/mL]	Recovery [%]	Matrix effect (SSE) [%]	Analytical column	Mobile phase	LC-MS	Reference
serum	LLE ACN	-	-	BEA	0.010	0.2	81.94 (BEA) – 94.29 (ENNB)	90.6	Hypersil Gold column (50 mm × 2.1 mm i.d., dp: 1.9 µm)	0.1% glacial acetic acid in water and ACN	LC TSQ® Quantum Ultra triple quadrupole mass spectrometer ThermoFisher Scientific	[16]
				ENNA	0.001	0.1		111				
				ENNA _i	0.003	0.1		81.5				
				ENNB	0.004	0.2		91.6				
				ENNB _i	0.008	0.2	(R _E)	92.3				
				DON	0.01	0.1	28.2 (15-AcDON)- 88.3 (DON)	81.5	Hyper-sil Gold (50 × 2.1 mm i.d., 1.9 µm) column	water +0.1% glacial acetic acid and methanol +0.1% glacial acetic acid	Acquity UPLC system Xevo® TQ-S mass spectrometer (Waters, Zellik, Belgium)	[17]
				DOM-1	0.07	0.5		97.0				
				3-AcDON	0.01	1.0		80.3				
				15-AcDON	0.2	1.0		44.4				[18]
	LLE ACN with 0.1% formic acid	-	-	ZEN	0.001 - 1.68	1	72.9 (TEA)- 132.2 (ENNA) (R _E)	86.2	Waters Acquity HSS T3 column (100 mm × 2.1 mm i.d., dp: 1.8 µm)	10 mM ammonium formate, 0.3% formic acid in water and 10 mM ammonium formate, 0.3% formic acid in methanol	Agilent 1290 series UHPLC system coupled to a Sciex 6500 QTrap mass spectrometer	[9] [10]
				α-ZEL		1		74.7				
				α-ZAL		1		73.0				
				β-ZAL		1		74.4				
				β-ZEL		1		79.1				
				ZAN		1		86.5				
				TeA		1		96.3				
				AOH		1		114.9				
				AME		1		90.1				
				DON		1		40.4				
				DOM-1		1		42.6				
				3/15-AcDON		1		93.5				
				T-2		1		83.3				
				HT-2		1		61.7				
				T2-G		2		71.5				
				AFB _i		1		87.4				
				AFM _i		1		50.1				
				OTA		1		101.3				
				ENNA		1		62.1				
				ENNA _i		1		66.8				
				ENNB		1		50.9				
				ENNB _i		1		45.5				
				BEA		1		64.3				

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