BIOMARKERS FOR EXPOSURE OF MYCOTOXINS IN PIGS AND BROILER CHICKENS

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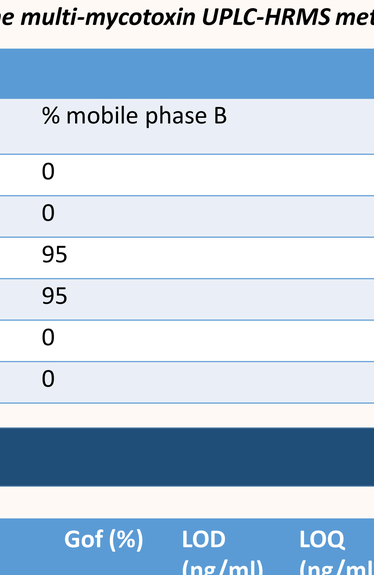
INTRODUCTION AND AIMS

Poultry and pigs are highly exposed to mycotoxins due to their cereal based diet. Acute and chronic exposure to mycotoxin contaminated feed can cause deleterious effects on the performance and wellbeing of the animals and leads to economic losses. Therefore it is of great importance to assess mycotoxin exposure in livestock and to correlate this with the health status of the flock. Biomarkers for exposure of the animal need to be established as feed analysis has some drawbacks, for instance, the feed may already be consumed, 'hot spots' with inhomogenous mycotoxin levels, and no clear dose-response correlation. Typical biomarkers for exposure are parent compounds and their phase I and II metabolites, which can be detected in biological matrices, such as plasma and excreta. The first objective of this PhD research is to develop an ultra-high performance liquid chromatography coupled to HR-MS (type Synapt G2-Si HDMS) screening method to determine the mycotoxins aflatoxins, deoxynivalenol, T-2 toxin, zearalenone and enniatins and their major phase I and II metabolites in plasma, urine and faeces of pigs and plasma and excreta of poultry.

EXPERIMENTAL

Plasma sample preparation

20 mycotoxin analytical standards (eg aflatoxin B1, deoxynivalenol, T2 toxin, zearalenone ,enniatins and their major phase I metabolites) are added to plasma of pigs.



Evaporate under nitrogen stream (45°C+/-5°C)

250 µl plasma → Supernatant → Dry residue → LC HRMS

+10 µL of 100 ng/mL mix C^{13}-OTA/AFB1/T2/ZEA/DON
Vortexing
+ 750 µL acetonitrile
Vortexing
Centrifugation (8517g, 10min, 4°C)

Reconstitution in 250 µL 80/20 ACN/H_2O
Vortex-mixing
Nylon-filter (0.20 µm)

Chromatography

- *UPLC instrument*: Acquity I class ultra-high performance liquid chromatograph (Waters, Zellik, Belgium)
- *UPLC-column*: Acquity UPLC BEH C18 column (50 mm × 2.1 mm i.d., dp: 1.7 µm) in combination with a guard column of the same type (5 mm × 2.1 mm i.d., dp: 1.7 µm), both from Waters (Zellik, Belgium).
- Mobile phases and gradient elution program are summarized in table 1A and 1B.

Table 1A: Overview of the mobile phases used for the multi-mycotoxin UPLC-HRMS method

	Mobile phase A	Mobile phase B
ESI -	0.1% acetic acid in water	0.1 % acetic acid in acetonitril
ESI +	0.1% FA , 10mMNH_4AC water/methanol (98/2)	0.1% FA , 10mMNH_4AC methanol/water (98/2)

Table 1B: Overview of the LC gradient program used for the multi-mycotoxin UPLC-HRMS method

GRADIENT	Mobile phase	
Time (min)	% mobile phase A	% mobile phase B
Initial	100	0
0.5	100	0
5.5	5	95
7	5	95
7.50	100	0
10	100	0

High Resolution mass spectrometry

- MS instrument: HR-MS type Synapt G2-Si HDMS (Waters, Zellik, Belgium)
- MS conditions: positive and negative electrospray ionization.

RESULTS

Mycotoxin	m/z value	Retention time (min)	Detection mode
Deoxynivalenol	295.1182	1.58	ESI -
3-acetyl-DON	337.1287	2.16	ESI-
15-acetyl-DON	337.1287	2.16	ESI-
Zearalenone	317.1389	3.55	ESI-
α-zearalenol	319.1545	3.25	ESI-
β-zearalenol	319.1545	3.04	ESI-
α-zearalanol	321.1702	3.20	ESI-
β-zearalanol	321.1702	3.00	ESI-
Zearalanone	319.1545	3.53	ESI-
Enniatin A	682.4643	5.64	ESI+
Enniatin B	640.4173	5.36	ESI+
Enniatin A1	668.4486	5.55	ESI+
Enniatin B1	654.433	5.46	ESI+
Beauvericine	784.4173	5.49	ESI+
Alternariol	259.0606	3.77	ESI+
Alternariol MethylEther	273.0763	4.53	ESI+
Aflatoxin B1	313.0712	3.36	ESI+
Ochratoxin	404.0901	4.36	ESI+
T2-toxin	467.2281	4.24	ESI+
HT-2 toxin	425.2175	3.95	ESI+

Table 2: Mycotoxins, m/z value , retention time and detection mode (negative or positive ionisation mode, ESI)

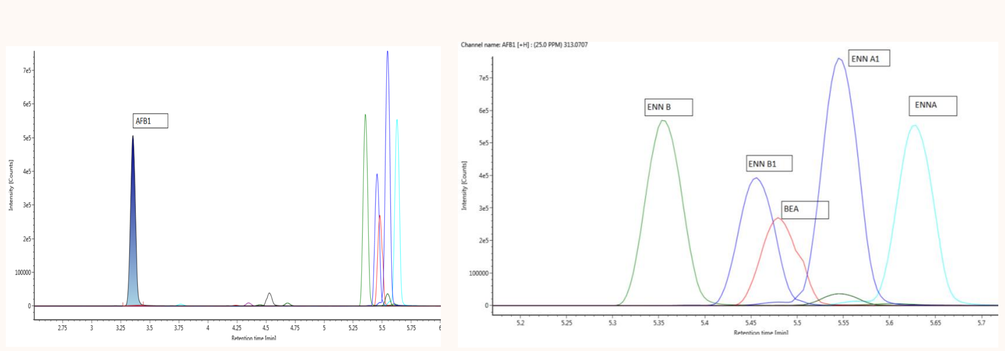


Figure 1. Chromatogram for the separation of the enniatins (ENN A, B, A1, B1), beauvericine (BEA) and aflatoxin B1 in ESI+

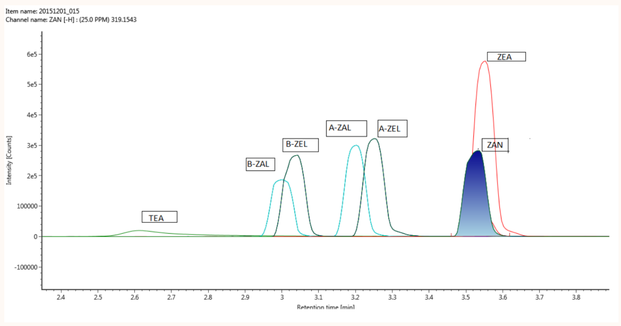


Figure 2. Chromatogram for the separation of zearalenone (ZEA) and its phase I metabolites : α-zearalanol (α-ZAL),β-zearalanol (β-ZAL),α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), zearalanone (ZAN) in ESI-

Toxin	R	Gof (%)	LOD (ng/ml)	LOQ (ng/ml)
AFB1	0.999	8	0.46	5
ZEA	0.999	8.29	0.18	5
15/3-ADON	0.996	13.01	2.41	10
T2	0.999	13.27	0.40	5
ENN B	0.998	11.42	0.85	1
OTA	0.993	13.13	1.66	5
AME	0.998	16.72	0.38	5
Acceptance criteria: R≥0.99; Gof≤20%; LOD: S/N=3				

Table 3: Evaluation of linearity (correlation coefficient (r) and goodness-of-fit coefficient (Gof), limit of quantitation (LOQ), limit of detection (LOD)

CONCLUSION

The chromatography resulted in the succesful separation of 20 mycotoxins (example in fig 1 and 2). Furthermore, it was shown that this method covers a wide concentration range (5-300 ng/ml) with a good correlation coefficient (R) and goodness-of-fit coefficient (Gof) . The results of the within- and between-run precision and accurracy are in compliance with the relevant specifications.