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Development and validation of an LC-MS/MS method for the determination of DON, 3ADON and 15ADON in chicken plasma

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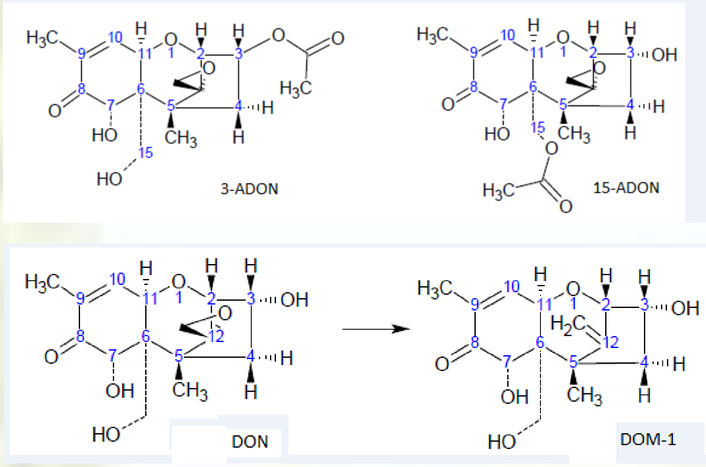
INTRODUCTION

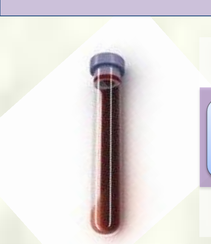
The research towards the toxicity and occurrence of deoxynivalenol (and mycotoxins in general) has made significant progress. As the information and insight on native mycotoxins increases, the focus of this research domain tends to shift towards the gathering of occurrence and toxicity data on masked mycotoxins. These are mycotoxin conjugates or derivatives that are undetectable by conventional methods due to changes in their structural formation and physicochemical properties. For this study we focused on two important and frequently occurring masked forms of deoxynivalenol, 3-acetyldeoxynivalenol (3ADON) and 15-acetyldeoxynivalenol (15ADON), both precursors in the fungal biosynthesis of deoxynivalenol (DON). Conversion of these masked mycotoxins back to their native form by *in vivo* hydrolysis cannot be excluded. Consequently, this would imply an underestimation of the degree of contamination upon analysis. Deepoxy-deoxynivalenol (DOM-1) is the main *in vivo* metabolite, with highly reduced toxicity due to the removal of the epoxide function.



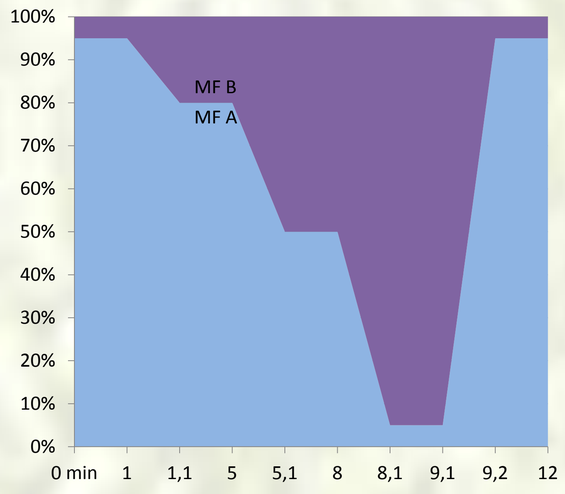
OBJECTIVES

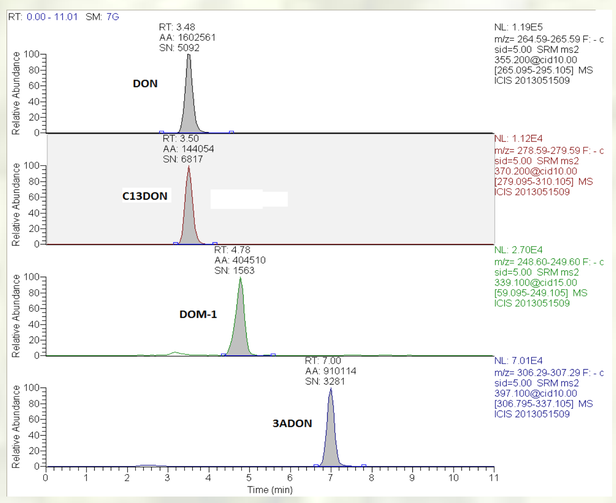
Determination of the toxicokinetic parameters and degree of *in vivo* hydrolysis of 3ADON and 15ADON in broiler chickens. For this, an LC-MS/MS method needed to be developed and validated for the above mentioned compounds in chicken plasma. For sample clean-up, three methods were tested, a liquid-liquid extraction, a solid-phase extraction and a deproteinization by means of organic solvents. In the following steps of the development, critical factors (e.g. precipitation solvent, temperatures, etc ..) were examined by linear regression with the use of Plackett-Burman designs in order to determine their statistical significance.

METHOD DEVELOPMENT AND VALIDATION



Centrifuge chicken blood sample to collect plasma → 250 µl chicken plasma + 750 µl ACN + C_{13}DON (IS) → Centrifuge 10' at 8517g and 4°C → Isolate supernatant and dry at 70 °C under N_2 → Reconstitute in 200 µl UPLC water and vortex 15" → Filter (0.22 µm) and analyse





MS/MS instrument: TSQ Quantum Ultra triple quadrupole mass spectrometer
HPLC instrument: Surveyor autosampler Plus and MS Pump Plus
Column: Hypersil Gold (50 mm x 2.1 mm i.d., d.p.: 1.9 mm)
MFa: UPLC water + 0.1% acetic acid
MFb: UPLC grade methanol + 0.1 % acetic acid
Column temperature: 60°C
Injection volume: 10 µl
Flow rate: 0.5 ml/min
Run Time: 12 minutes
Detection: MRM-mode

Linearity: three calibration curves (3 x n=7) in plasma up to 200 ng/ml
LOQ: lowest concentration (n=6) with acceptable results for accuracy and precision
LOD: calculated from S/N values from the LOQ samples (n=6)
Precision: determined at three levels (LOQ, 50 and 100 ng/ml), values presented in table are for LOQ, values are listed as relative standard deviation, within day (n=6), between day (n=3x3)
Accuracy: determined at three levels (LOQ, 50 and 100 ng/ml), values presented in table are for LOQ (n=6)
Specificity: results not shown, tested by injection of blank sample, no interfering peaks
Carry-over: results not shown, tested by injecting a 50/50 (v/v) mixture of both mobile phases after a 200 ng/ml spiked plasma sample, none of the compounds showed carry-over
Matrix effect: results not shown, ion suppression was observed in plasma, demonstrating the need of matrix matched calibration curves

	Linearity R^2 & GOF	LOQ (ng/ml)	LOD (ng/ml)	Accuracy	Within-day precision	Between-day precision
DON	0.9975 13.2%	1	0.04	-5.4%	28.7%	17.6%
3ADON	0.9989 4.9%	1	0.13	-28.4%	22.6%	41.2%
15ADON	0.9986 3.1%	2	0.70	2.0%	6.0%	19.2%
DOM-1	0.9987 7.2%	2	0.51	5.5%	14.3%	32.8%

CONCLUSION

- Solid-phase extraction as well as liquid-liquid extraction gave comparable results to a simple deproteinization by means of acetonitrile.
- The latter method was further developed and validated.
- C_{13}DON was well suited as internal standard for all the compounds included in the LC-MS/MS method.
- The method will be used to determine toxicokinetic parameters (AUC, C_{max}, t_{max}, bioavailability, …) of the compounds in broiler chickens.

MYTOX

With thanks to Ghent University for the funding (BOF-project)
Presented at the 35th MYCOTOXIN WORKSHOP
Ghent, Belgium 22 – 24 May 2013

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