Simultaneous determination of deoxynivalenol, zearalenone and their metabolites in bovine and porcine urine

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Zearalenone (ZEN) and deoxynivalenol (DON) are mycotoxins, which are formed by various *Fusarium* species on cereals. They might have negative effects on animal health and performance when toxicologically relevant diet concentrations are exceeded. These mycotoxins and their degradation products can potentially be used as biomarkers in urine to assess the exposure of humans and animals. However, faster, cheaper and more practical multi-toxin methods with lower detection limits are needed. Therefore, the aim of this study was to develop and validate a method for ZEN, DON and their metabolites alpha-zearalenol, beta-zearalenol, zearalanone, alpha-zearalanol, beta-zearalanol and de-epoxy-DON in bovine and porcine urine, which combines an economic sample preparation and the selectivity and sensitivity of LC-MS/MS.
Samples were incubated with β-glucuronidase over night before cleaning up with solid phase extraction (Oasis HLB, Waters). ZEN, DON and their metabolites were eluted with methanol and evaporated to dryness. After resuspension in methanol/water (70/30 v/v) sample analysis was performed on an Agilent 1200 series HPLC system using a Pursuit^{TM} XRs Ultra 2.8 (Varian) column coupled with a 4000 QTrap (Applied Biosystems) LC-MS/MS system with negative electrospray ionization (ESI). The developed and validated method showed recovery rates ranging from 26-63%. By using ^{13}C-labelled and deuterated internal standards (alpha-ZEL-d_4, beta-ZEL-d_4, alpha-ZAL-d_4, beta-ZAL-d_4, $^{13}C_{18}$-ZEN und $^{13}C_{15}$-DON) the recovery was corrected up to 76-131%.
The applicability was confirmed with urine samples obtained from different feeding trials with practically relevant ZEN and DON concentrations. Furthermore, it could be shown that the developed method is suitable as a multi-biomarker method to assess animal exposure to these mycotoxins.

High Speed Countercurrent Chromatography for analysis of Chlorophylls *a/b* ratio and their derivates in plants

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Chlorophylls play a central role in the life processes of plants and for these main reasons the biochemistry of chlorophyll catabolism in senescent leaves and fruits has been broadly studied previously. The higher susceptibility to pheophytinization of chlorophyll *a* compared to that of chlorophyll *b* has been well reported in the literature and hampers the exact measurement and quantification of Chlorophylls *a* and *b* and their derivates. [1]

The isolation and quantification of Chlorophylls *a* and *b* and their derivates by HSCCC was successfully used for different plants such as spinach and grass as well as banana fruit. CCC is especially suited for large-scale fractionation of complex extracts. The separation is based on partitioning between two immiscible solvent pairs, and up to 50.000 partitioning steps per hour can be obtained. Irreversible adsorptions and sample loss can be neglected. [2]

The chlorophylls *a/b* ratio of the different plants and fruits change meaningfully and it is clearly reflected in the chromatograms obtained by the application of the preparative HSCCC where the elution mode used in the separation of compounds was head to tail, the flow rate of the mobile phase was 3.5 mL/min and the wavelength 445 nm.[3]

On-line high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry was applied to the identification of the fractions by means of the mass spectra in positive (+) mode. The solvent elution program for HPLC was A: TBME/MeOH/H_2O 4/92/4 (v/v/v) B: TBME/MeOH/H_2O (90/6/4), using a RP-18 column (Prontosil C_{18} 250 x 2mm), flow rate of 0.8 mL/min. Gas was nitrogen with a flow rate of 7.0 mL/min to 350 °C, temperature of APCI: 400 °C. ESI-MS parameter: capillary, 4500V, cap, 2800V.

With the present results it may be possible to elicit the physiological and biochemical behavior of chlorophylls in plants and fruits during the degradation of the pigments and consequently to detect problems during the senescence stage.

References:

[1] M. I. Minguez-Mosquera and J. Garrido-Fernández, *J. Agric. Food Chem.* **1989**, 37, 1-7. [2] P. Winterhalter, *Trends Food Sci. Technol.* **2007**, 18, 507-513. [3] F. das Neves Costa and G. Guimarães Leitão, *J. Sep.Sci.* **2010**, 33, 336-347.

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