Development and validation of an UPLC-MS/MS multi-method for the quantitative analysis of important mycotoxins in rumen fluid

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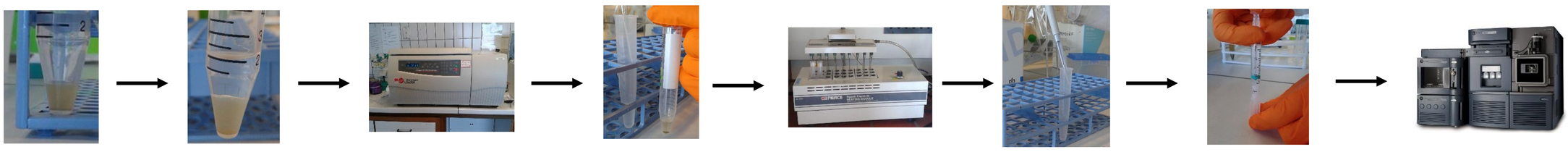
INTRODUCTION AND AIMS

In Belgium, the most important mycotoxins found in maize silage are deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEN), mycophenolic acid (MPA), roquefortine C (ROQ-C), citrinin (CIT) and enniatin B (ENN B)[1,2,3]. The detoxifying capacity of ruminal microbiota for the previously mentioned mycotoxins, especially in combinations, is not well known. To answer this question, a method to determine multiple mycotoxins in rumen fluid is needed. Therefore, a sensitive and specific analytical method for the quantitative determination of the mycotoxins DON, NIV, ZEN, MPA, ROQ-C, CIT and ENN B as well as their metabolites deepoxy-deoxynivalenol (DOM-1), α-zearalenol (α-ZOL), β -zearalenol (β-ZOL), zearalanone (ZAN), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL) in rumen fluid using UPLC-MS/MS was developed and validated.

MATERIALS AND METHODS

Sample extraction:

Rumen fluid (cattle/sheep) → 1) sample for extraction of CIT
→ 2) sample for extraction of other mycotoxins



sample + IS* (250 µl) → + 250 µl aqueous phase** + 1.5 ml organic phase** → mix + centrifuge → collect organic solvent → evaporate to dryness → dissolve in solvent* (200 µl) → filtrate → analyze on Xevo® TQ-S (UHPLC-MS/MS)

(*$^{13}C_{18}$ DON, $^{15}N_3$ ENN B, $^{13}C_{17}$ MPA, $^{13}C_{22}$ ROQ-C, $^{13}C_{18}$ ZEA & $^{13}C_{13}$ CIT are used as internal standard (IS); **other solvents for extraction CIT vs other mycotoxins)

Chromatography:

- HPLC instrument: Acquity UHPLC system (Waters, Zellik, Belgium)
- UPLC-column: HSS T3 column (2.1 x 50 mm, d.p.: 1.8 µm) in combination with a VanGuard pre-column (2.1 x 5 mm, d.p.: 1.8 µm) both from Waters (Zellik, Belgium)
- Aqueous and organic mobile phases with gradient elution program

MS/MS detection:

- MS instrument: Xevo® TQ-S triple quadrupool MS/MS (Waters, Zellik, Belgium)
- MS conditions: positive and negative electrospray ionization, multiple reaction monitoring mode (MRM)

→ Analysis Ⓐ (ESI -): CIT
→ Analysis Ⓑ (ESI +): NIV, DON, DOM-1, ENN B, MPA, ROQ-C
→ Analysis Ⓒ (ESI -): ZEN & metabolites

Validated according to European guidelines[4,5]

RESULTS

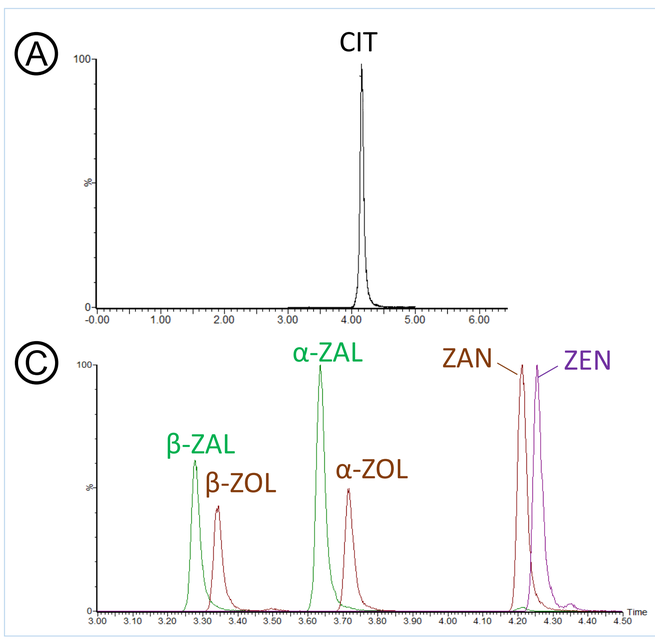


Figure 1: LC-MS/MS chromatograms of spiked rumen fluid (10 ng/mL)
Ⓐ CIT (ESI -)
Ⓑ NIV, DON, DOM-1, ENN B, ROQ-C & MPA (ESI +)
Ⓒ ZEN and metabolites (ESI -)

Table 1: Evaluation of validation parameters

	All mycotoxins, except ROQ-C	ROQ-C
Linearity	✓	✓
Accuracy & precision	✓	✓
Limit of quantification	0.05-0.6 ng/mL NIV: 36 ng/mL	0.1 ng/mL
Limit of detection	10-280 pg/mL NIV: 5.4 ng/mL	4 pg/mL
Specificity & selectivity	✓	✓
Carry-over	✓	✗

DISCUSSION AND CONCLUSIONS

The LC-MS/MS parameters resulted in good chromatographic properties (Figure 1). Furthermore, the method is validated according to European guidelines (Table 1). ROQ-C shows carry-over, but this can be countered by injecting the samples from low to high concentrations of ROQ-C. This analytical method can be used to determine the fate of multiple mycotoxins in rumen fluid on a specific and sensitive way. *In vitro* studies will be performed to determine the detoxifying capacity of ruminal microbiota when incubated with the mycotoxins DON, NIV, ZEN, MPA, ROQ-C, CIT and/or ENN B in different rumen conditions.

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