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FAST AND RELIABLE METHOD FOR DETECTION OF MYCOTOXIN METABOLITES IN PIG'S URINE AS POTENTIAL BIOMARKERS OF EXPOSURE

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• Introduction

Methods for the detection of mycotoxin metabolites in urine usually involve very sophisticated and expensive analytical equipment, such as mass spectrometers. However, it is possible to reliably detect and quantify these substances using more affordable equipment, making the possibility of performing analysis more accessible. The aim of this study was to evaluate the performance of the HPLC-FLD method for determination of aflatoxin and zearalenon metabolites in pig's urine. The method was evaluated for aflatoxin M1 (aflatoxin B1 metabolite), as well as zearalenone, α-zearalenol and β-zearalenol (zearalenone metabolites).

• Material and method

Urine samples were prepared after treatment with β-glucuronidase Helix pomatia type H-2 (Sigma-Aldrich, USA) and cleanup on immunoaffinity columns (RomerLabs, Austria). Mycotoxin standards used: AFM1 (BioPure, Austria), ZEA, α and β-ZOL (Sigma-Aldrich, USA).

HPLC analysis was carried out on a 1260 HPLC system using Hypersil ODS 150 x 4.6 mm, 5 µm column (Agilent Technologies, USA).

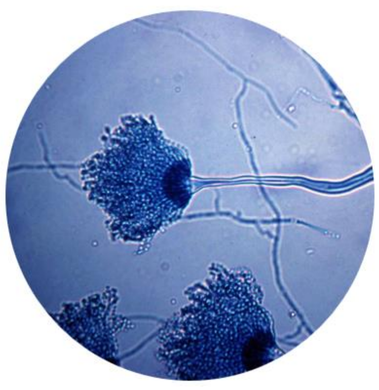
• Results and discussions

The method showed good specificity and selectivity, and good linearity (R2>0.995) for all metabolites. Limits of quantification were below 50 ng/ml for zearalenone metabolites, and below 1 ng/ml for aflatoxin M1. Average recovery rates ranged from 91.3 to 98.6%. Precision was calculated as relative standard deviation (RSD) of ten measurements, and it ranged from 4.9 to 8.7%. Reproducibility was evaluated after two sets of measurements on different days and operators, and the RSD was ranged from 7.7 to 11.2%.

Validation parameter	Aflatoxins	ZEA, α and β-ZOL
Accuracy	Recovery (N=6): R=98.6 %	Recovery (N=6): R=91.3 %
Repeatability	N=6; RSD= 4.9%	N=6; RSD= 8.7%
Interlaboratory reproducibility	N=10; RSD= 7.7%	N=10; RSD= 11.2%
Linearity	N=7; R^2=0.9995	N=7; R^2=0.9992
Detection limit	0.3 ng/ml	15.0 ng/ml
Quantification limit	1.0 ng/ml	50.0 ng/ml

• Conclusions

The results of method's performance evaluation showed that it can be used for the determination of aflatoxin B1 and zearalenone metabolites in pig's urine.

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