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ZEARALENONE AND ITS MAJOR METABOLITES – A SIMPLE ISOCRATIC METHOD

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SUMMARY

The Food and Agricultural Organization of the United Nations estimated that 25 % of the global food are contaminated with mycotoxins [1]. Zearalenone (ZON) is a non-polar mycotoxin and a common contaminant in cereal grain used for animal and human food. It exerts an estrogenic activity that disrupts endocrine function in animals and possibly humans. The major metabolites of ZON are α- and β- zearalenol. All three components can be determined with a robust and simple isocratic method.

INTRODUCTION

Mycotoxins are secondary metabolites produced by mould fungus. The mycotoxin ZON, which is an intermediate catabolic product of filamentous fungi of the genus Fusarium, can be determined on almost all type of cereal. Although the overall toxicity is low, animal testing unraveled teratogenic, hepatotoxic, immunotoxic, genotoxic and cancerogenic effects [2, 3]. ZON furthermore influences the tumor progression of hormonal sensitive tissues as it shows estrogen-like characteristics. α- and β- zearalenol are the main metabolites of ZON and mainly formed in the liver but also to a lesser extent in the intestines during first-pass metabolism [4, 5]. A relatively low proportion of β-zearalenol is formed from zearalenone compared to α-zearalenol in human [4]. α-zearalenol is about 3- to 4-fold more potent as an estrogen relative to zearalenone.

RESULTS

All samples and standards were provided from the Leibniz-Zentrum für Agrarlandschaftsforschung (ZALF) e.V. In a range from about 2 ng up to 20 ng (absolute) calibrations of all three mycotoxins were determined using six different volumes of the standard ZALF 1. **Tab. 1** gives a short summary of the retention times of the substances and achieved correlation coefficients of calibration. **Fig. 1** shows the separation of the components in the standard ZALF 1. All peaks are baseline separated. The sample ZALF 2 is made of extracted grain of wheat which were inoculated with a fungus of the genus Fusarium. For the determination of zearalenol the sample ZALF 2 was used as provided. For the determination of zearalenone a dilution was necessary. **Fig. 2** shows the measurement of the sample ZALF 2 with and without dilution. With quantification based on the determined calibration curves a concentration of 2.80 ng/µL for β-zearalenol, 0.29 ng/µL for α-zearalenol and 390 ng/µL for zearalenone were calculated.

Tab. 1 Retention times and correlation coefficients of β-Zearalenol, α-Zearalenol and Zearalenone calibration

Peak	Substance	Retention time	Correlation coefficient
1	β-Zearalenol	7.573 min	0.99985
2	α-Zearalenol	11.160 min	0.99997
3	Zearalenone	12.331 min	0.99997

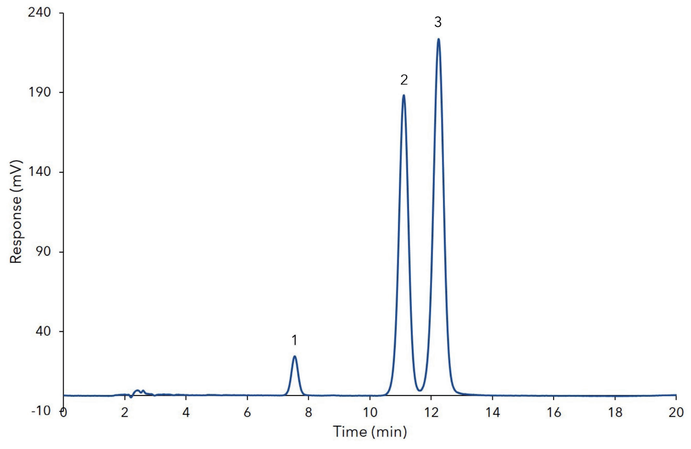

Fig. 1 Standard ZALF 1: 1 - β-Zearalenol, 2 - α-Zearalenol, 3 - Zearalenone

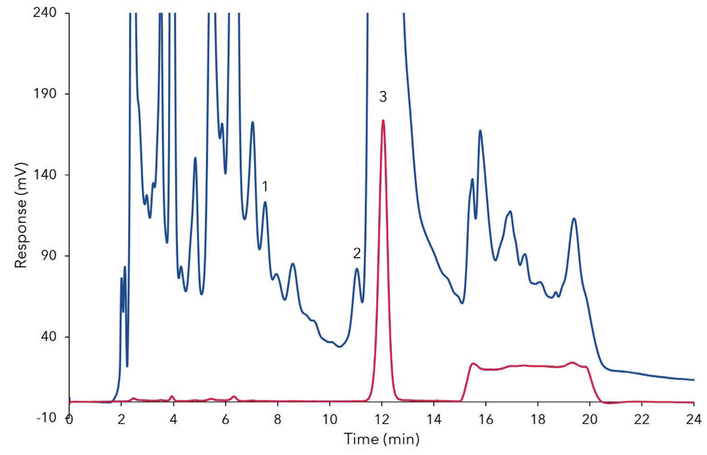

Fig. 2 Sample ZALF 2, blue - without dilution, red - 1:50 dilution, 1 - β-Zearalenol, 2 - α-Zearalenol, 3 - Zearalenone

MATERIALS AND METHOD

An AZURA Analytical HPLC Plus system was used for this application. The system consisted of an isocratic AZURA P 6.1L pump, an AZURA AS 6.1L autosampler, an AZURA CT 2.1 column thermostat and a RF 20 Axs fluorescence detector in combination with CBM 20 A under the Chromeleon™ software. The isocratic method [6] was applied for 25 minutes at a flow rate of 0.65 mL/min with a mixture of methanol and 3 mM phosphoric acid in a ratio 65:35 (v/v). The column temperature was set to 25 °C. The substances were measured with an excitation at 274 nm and emission at 456 nm. The used column in a dimension 150 x 4 mm ID with precolumn was filled with LiChrospher 100-5 RP 18 silica.

CONCLUSION

It was possible to identify and quantify ZON and its metabolites with the described isocratic method. Using a fluorescence detector for enhanced sensitivity allows measurements of small amounts of mycotoxins even in a complex sample matrix. This application can be used for quality control to make sure the set limit values according to Commission Regulation (EC) No 1881/2006 [7] will be maintained.

REFERENCES

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Additional information:

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ADDITIONAL MATERIALS AND METHODS

Tab. A1 Method parameters

Eluent A	Methanol:3 mM phosphoric acid 65:35 (v/v)		
Eluent B	Methanol		
Gradient	Volume [mL]	% A	% B
	0.0	100	0
	13.0	100	0
	13.5	65	35
	18.0	65	35
	18.5	100	0
	25.0	100	0
Flow rate	0.65 mL/min	System pressure	ca. 120 bar
Column temperature	25 °C	Run time	25 min
Injection volume	2-20 µL	Injection mode	Partial loop
Detection wavelength	Ex 274 nm / Em 456 nm	Data rate	100 Hz
		Time constant	0.01 s

Tab. A2 System configuration & data

Instrument	**Description**	**Article No.**
Pump	AZURA P6.1L, isocratic	APH30EA
Autosampler	AZURA AS 6.1L	AAA00AA
Detector	RF 20Axs with CBM-20A	A59201
Thermostat	AZURA CT 2.1	A05852
Column	LiChrospher 100-5 RP 18, Vertex Plus 150 x 4 mm ID with precolumn	15WE189LSJ
Software	Chromeleon 7.2