

Simultaneous Analysis of 14 Mycotoxins and 163 Pesticides in Crude Extracts of Grains by LC-MS/MS

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Overview

Multi-component methods for the detection of different compound classes, such as mycotoxins or pesticides, have been established and are widely used to analyze a broad range of food or feed. However, there is a continuing demand to test for a larger number of compounds in shorter times. The development of a combined method for different compound classes can help to meet those new challenges. In this paper we present a fast, robust, and reliable method, which has been validated for the detection of 14 mycotoxins and 163 pesticides in the matrix grain. The LC-MS/MS method using the Scheduled Multiple Reaction Monitoring (Scheduled MRM™ algorithm) detects all mycotoxins with Limits of Quantitation (LOQ) between 1 µg/kg and 10 µg/kg. The LOQ for pesticides were found to be 10 µg/kg and less. All LOQ meet the requirements of the EU.

Introduction

Pesticides and mycotoxins are known to harm the health of humans and animals. Many of these compounds are known either as carcinogenic, cytotoxic, or ecotoxic. Therefore, different countries have set regulations on pesticides and mycotoxins. For example, in the EU, maximum residue levels of pesticides in or on certain products are regulated by EC/396/2005 and the amended regulation EC/839/2008 and, in Japan, by the Japanese Positive List Syoku-An No.0124001 January 14, 2005 and amendments May 26, 2006. Mycotoxin limits are harmonized in the regulation for contaminants in foodstuffs EC 1881/2006 and the amended regulation EC 1126/2007 in the EU.¹⁻⁶

Regulations on food and environmental analysis require the analysis of contaminants using confirmatory techniques, such as GC-MS and LC-MS/MS. More than 1000 pesticides are used worldwide and, along with their metabolites and degradation products, are present in food and the environment. Thus, there is a demand for powerful and rapid analytical methods that can detect very low concentrations of pesticides in mycotoxins in a variety of sample matrices.



Over the last years, LC-MS/MS replaced traditional GC and LC methods for the screening of pesticides and mycotoxins because of its ability to analyze a wider range of compounds in a single analysis and the unmatched selectivity and sensitivity of Multiple Reaction Monitoring (MRM).

Traditionally, mycotoxins and pesticides require different sample preparation. A simplified extraction procedure was established to analyze the two compound classes simultaneously in one sample, without additional cleanup steps by SPE or immunoaffinity columns. This new simplified sample preparation in combination with high resolution LC, and sensitive MRM detection allows detecting pesticides and mycotoxins faster and less labor-intensive and time-saving.

Experimental

Sample Preparation

10g of grain sample was extracted using a mixture acetonitrile/water. The extract was filtered and diluted with water + 5 mM ammonium acetate to optimize LC peak shape.⁷

LC

A Shimadzu Prominence LC system with an Agilent ZORBAX Eclipse XDB C18, 100x4.6 mm, 1.8 µm column at 40°C with a gradient of eluent A water/methanol (80/20) + 5 mM ammonium

acetate and eluent B water/methanol (10/90) + 5 mM ammonium acetate was used at a flow rate of 500 μ L/min. The injection volume was set to 100 μ L.

MS/MS

A SCIEX API 4000™ LC-MS/MS System with Turbo V™ source and Electrospray Ionization (ESI) probe was used. A number of 14 mycotoxins and 163 pesticides were detected using 2 MRM transitions per compound to allow quantitation and identification based on the ratio of quantifier and qualifier transitions as defined by regulation 2002/657/EC. The *Scheduled* MRM™ algorithm was used for best accuracy and reproducibility (Figure 1). Every sample was injected twice in positive and negative polarity.

Results and Discussion

A method for quantitation and identification of 9 fusarium toxins: Nivalenol (NIV), Deoxynivalenol (DON), Fusarenon X (FUS X), 3-Acetyldeoxynivalenol (3-AcDON), 15-Acetyldeoxynivalenol (15-AcDON), Diacetoxyscirpenol (DAS), HT-2 toxin, T-2 toxin, Zearalenon (ZON), and Ochratoxin A (OTA) was developed (Figure 1). This method was extended to also detect aflatoxins B1, B2, G1, and G2 (Figure 2). The complete method was validated for the analysis of wheat, barley, corn, and oat samples (Table 1).⁷⁻⁸

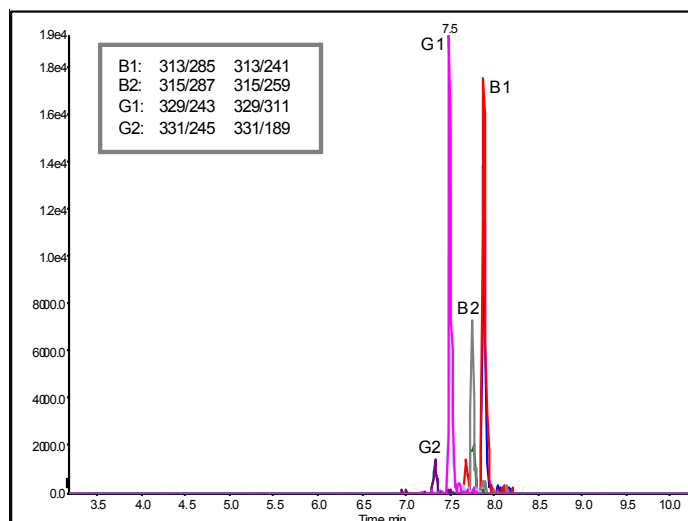


Figure 2. Detection of aflatoxins by LC-MS/MS

Table 1. LOQ and linear range of detected mycotoxins

Mycotoxin	LOQ (μ g/kg)	Linear Range (μ g/kg)	EU MRL #
3-AcDON	10	400	(1)
15-AcDON	10	150	(1)
DON	10	10000	1750* 1250** (2)
FUS X	10	2000	(1)
DAS	10	400	(1)
NIV	10	4000	(1)
OTA	1	>10	5***
HT-2	5	200	(2)
T-2	5	1000	(2)
ZON	5	80	100*** (2)
Aflatoxin B1	1	>20	2
Aflatoxins	1	>20	$\Sigma=4$

Footnotes to Table 1:

EC 1881/2006 and the amended EC 1126/2007

* Unprocessed durum wheat and oats

** Unprocessed cereals other than durum wheat and oats

*** Unprocessed cereals

(1) Due to co-occurrences and as "generally low" considered levels no MRL was estimated

(2) Appropriateness of setting a maximum level should be considered by 1 July 2008

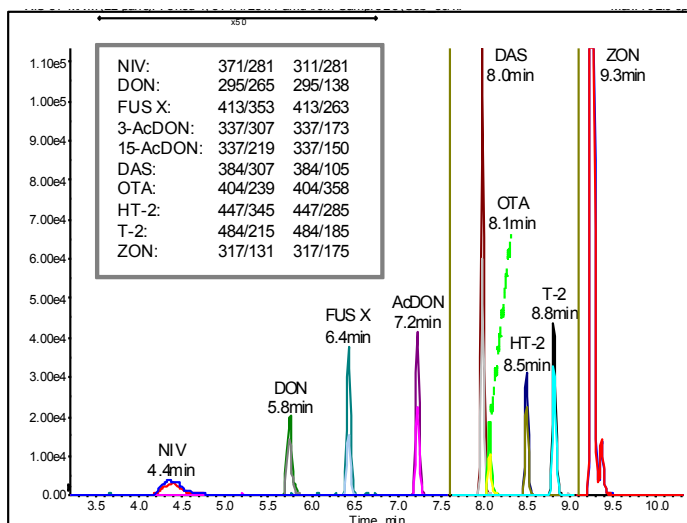


Figure 1. Detection of fusarium toxins and Ochratoxin A by LC-MS/MS

The developed method was recently updated to also quantify and identify 163 pesticides (Figure 3). The use of the *Scheduled* MRM™ algorithm allows the monitoring of such a large panel of analytes without sacrificing sensitivity and reproducibility. The method was validated in different grain matrices. Limits of

Quantitation (LOQ) of all mycotoxins were found between 1 µg/kg and 10 µg/kg. Pesticides were quantified at 10 µg/kg and less. All LOQ meet the requirements of the EU. Positive findings in two selected grain samples are shown in Figure 4.

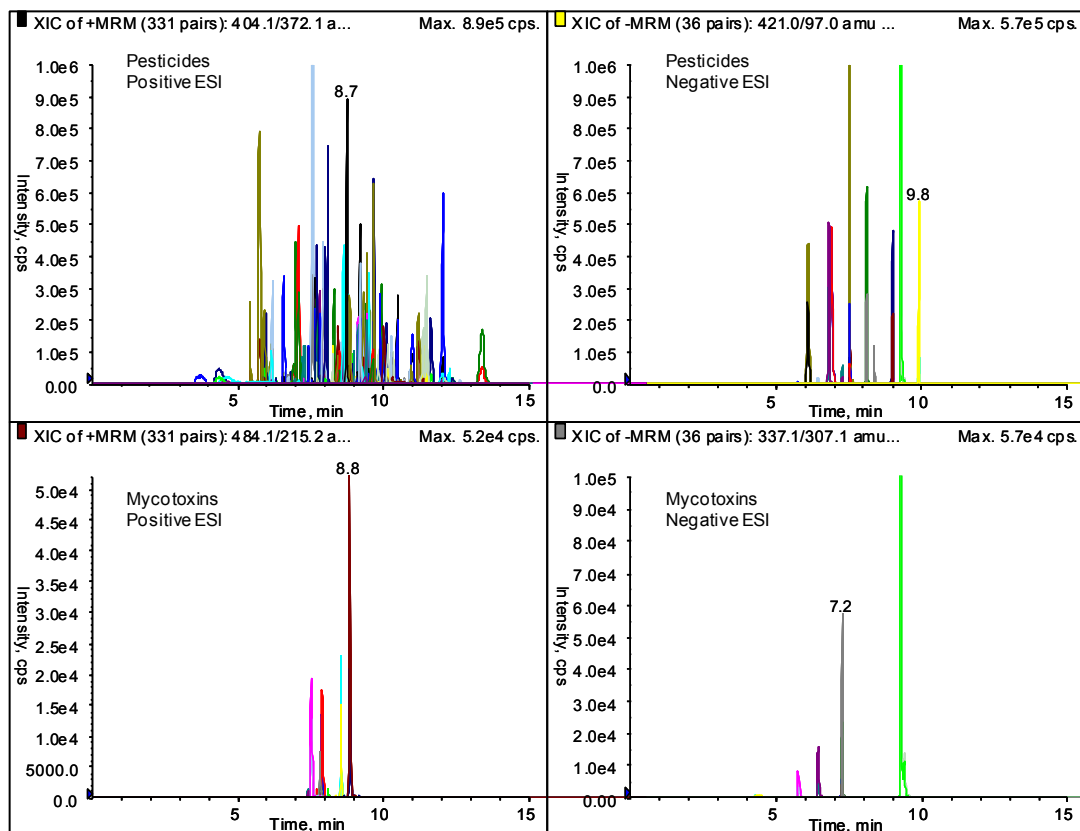


Figure 3. Detection of 14 mycotoxins and 163 pesticides using LC-MS/MS in two injections (positive and negative polarity) using the *Scheduled* MRM™ algorithm for best sensitivity and reproducibility

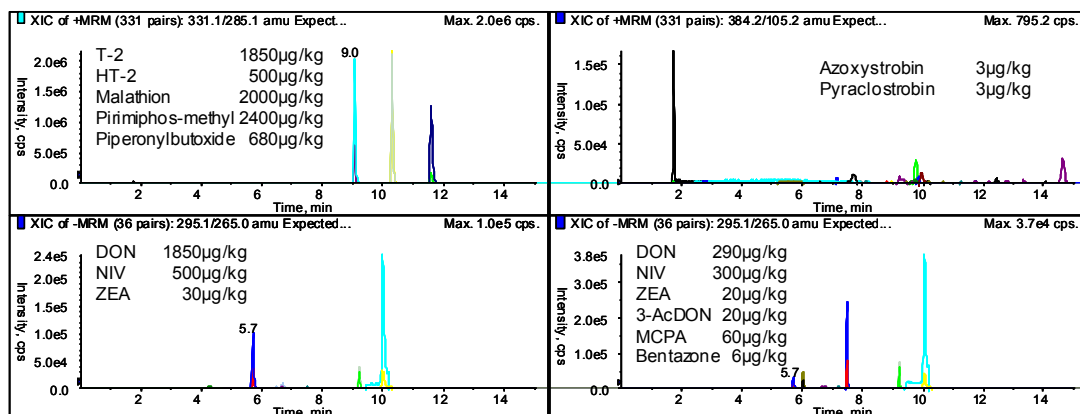


Figure 4. Detection of mycotoxins and pesticides in a durum wheat sample (left) and a barley sample (right)

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Summary

A fast, robust, and reliable method, for the detection 14 mycotoxins and 163 pesticides in the matrix grain was developed and validated. A generic extraction procedure followed by a dilution step was used to cover the large panel of analytes. High resolution LC was combined with high sensitivity detection using a SCIEX API 4000™ LC-MS/MS System. Multiple Reaction Monitoring (MRM) was used because of its high selectivity and sensitivity. With the *Scheduled* MRM™ algorithm activated for accuracy and reproducibility.

The method was validated in different grain matrices. Limits of Quantitation (LOQ) of all mycotoxins were found between 1 µg/kg and 10 µg/kg. Pesticides were quantified at 10 µg/kg and less. All LOQ meet the requirements of the EU.

References

- 1 D. Elbert et al.: presentation at AOAC conference (2008) in Dallas
- 2 A. Voller et al.: presentation at AOAC conference (2009) in Philadelphia