The Quantitation of Mycotoxins in Baby Food using a Simple Extraction and LC-MS/MS with Fast Polarity Switching and MRM Scheduling

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INTRODUCTION

Mycotoxins are produced by several strains of fungi both in the field, during storage, mixing and delivery of grain, human and animal food. Mycotoxins are known to be toxic and to cause disease in humans and animals as well as to cause carcinogenic or otherwise cytotoxic and impair the immune system. Mycotoxins fall into several major classes with those which can affect the health of humans or animals include the aflatoxins, ochratoxins, Fusarium toxins, including fumonisin, zearalenone (ZON), trichothecenes, and ergot alkaloids.

Regulations for mycotoxin contamination for some of the major classes have been set in different countries. In the European Union the mycotoxin limits were harmonized in the regulation for contaminants in foods(12) and amended by regulations in September 2007(13) and amended by regulations in September 2007. Traditionally mycotoxin analyses have been carried out using multiple methods, each method just suitable for one single mycotoxin or a group of chemically similar compounds e.g. aflatoxins. This has been due to the wide range of properties and physical properties of these compounds. These single mycotoxin methods include two new analytical methods for measuring aflatoxin B1 (AFB1) and ZON in baby food which were adopted as European benchmark methods in July 2010.(14)

Both methods are based on an immuno-affinity column cleanup of the sample followed by HPLC with fluorescence detection. However, it is possible that many different classes of mycotoxins could be present in the same sample of food. Therefore and not just AFB1 and ZON.

In this work we show the ability to analyze AFB1 and ZON at comparable detection levels, to the benchmark methods, as well as implementing these two mycotoxins into an LC-MS/MS screening method. For these measurements, the AB SCIEX Triplet Quad™ 5500 system was used. In one single LC-MS/MS run of 13 minutes 17 compounds were detected, 12 of them in the positive ionization mode and 5 of them in the negative ionization mode. The crude extracts of different foods were diluted and injected without any extensive sample clean up or concentration steps. Detection limits of AFB1 and ZON were found to be better than 20% of the use of any internal standards. The method itself incorporates fast polarity switching using the Scheduled MRM™ algorithm, unlike previous work(15) and extends on the previous number of toxins detected.

EXPERIMENTAL

Sample Preparation

A very simplified sample preparation was used, which has been presented previously.(16) 2 g of homogenized sample and extraction with acetonitrile/water (8:ml, 80:20)

Roller mixed for 20 minutes

Centrifuged 10 minutes 3500 rpm

Filtered using Phenomenex PHENEX 15 mm RC Membrane 0.45 um

Filterate diluted 1:4

HPLC

- Column: Phenomenex, Kinex™, 2.6 μm XB-C18, 50 x 2.1 mm
- Column Oven Temperature: 40°C
- Eluents A: H2O + 5 mM amnonium acetate + 0.5 % acetic acid
- B: CH3CN + 5 mM ammonium acetate + 0.5 % acetic acid
- Flow: 450 μL/min
- Gradient: 0 -2 min 2% (B), 5 min increase to 80%, 0.2 min increase to 98% and hold for 5 minutes

MS/MS

An AB SCIEX Triplet Quad™ 5500 system equipped with Turbo™ source and ESI probe was used. The following parameters were kept constant during a single LC-MS/MS run of 13 minutes 17 compounds were detected; 12 of which in the positive ionization mode and 5 of them in the negative ionization mode. The crude extracts of different foods were diluted and injected without any extensive sample clean up or concentration steps. Detection limits of AFB1 and ZON were found to be lower than 20% of the use of any internal standards. The method itself incorporates fast polarity switching using the Scheduled MRM™ algorithm, unlike previous work(15) and extends on the previous number of toxins detected.

RESULTS

In previous work we used chromatographic separation and a fixed switch between the negative and positive polarity experiments. Here we fixed up the single looped experiment combining both negative and positive polarity and combined it with the Scheduled MRM™ algorithm (Figure 1). The presented method has been tested on several cereal based samples and has been shown to be robust enough to detect these toxins below the required limits and met European legislation.

The simple solvent extraction followed by dilution and the use of small particle size LC columns has meant that the method is fast and simple to apply. The use of polarity switching with Scheduled MRM™ acquisition has enabled shorter run times with an improvement in sensitivity, while extending the target list of compounds.

SUMMARY

The presented method has been tested on several cereal based samples and has been shown to be robust enough to detect these toxins below the required limits and met European legislation.

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REFERENCES

6. EN 15850:2010 and EN 15851:2010

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