Rapid and Simultaneously Screening and Quantitation of Mycotoxins in different Matrices using high resolution MSMS instrument

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ABSTRACT

Mycotoxins are known to harm the health of humans and animals. They take effect as either carcinogenic or cytotoxic and impair the immune system. We will present the possibility to analyse different mycotoxins at a comparable detection level to the European detection limits implemented into a high resolution Triple TOF screening/quantitation method. For our measurements an AB SCIEX Triple TOF® LC/MS/MS system was used. For each polarity a single run in positive and negative ion mode was applied here. We will demonstrate the using of a high resolution MSMS mycotoxin library for screening and identification of mycotoxins in different matrices.

INTRODUCTION

Mycotoxins are produced by several strains of fungi both in the field, during storage, mixing and delivery of grain and human and animal food. Mycotoxins are known to be toxic and harm humans and animals as they are carcinogenic or cytotoxic and impair the immune system. Mycotoxins fall into several major classes and those which can affect the health of humans or animals include the aflatoxins, ocratoxin A (OTA), fumonisins, zearalenone (ZON), tricothothecens and ergot alkaloids[1].

Regulations for mycotoxin contamination for some of the major classes have been set out in different countries. In the European Union the mycotoxin limits were harmonized in the regulation for contaminants in foodstuffs[2, 3] and amended by regulations in September 2007[4]. 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[5]. This has been due to the wide range of polarities 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 have been adopted as European bench mark methods in July 2010. Both methods are based on an immuno-affinity column cleanup of the sample followed by HPLC-fluorescence detection. However, there is a possibility that many different classes of mycotoxins could be present in the same sample of food or feed and not just ZON and AFB1.

In this work we show the ability to analyze mycotoxins at comparable detection level as well as implementing these compounds into a high resolution Triple TOF screening/quantitation method. For our measurements the AB SCIEX Triple TOF® 5600+ System were used in combination with an ultra fast HPLC System. Information of exact mass, retention time and Isotopic pattern of detected molecular ions was used for identification. Furthermore, high resolution MS/MS spectra searched against a LC/MS/MS library was used to confirm the identity of detected contaminants. LibraryView™ and MasterView™ software, new software tools provide the capability to build and modify the library and identify the compounds.

MATERIALS AND METHODS

Sample Preparation

Different types of milk samples were analyzed including bio sheep's milk drink, fresh bio sheep's milk, bio goat milk and also bovine milk from different suppliers. Proteins were precipitated by adding 200 µL of acetonitril to 100µl milk. After shaking for 15 minutes the samples were centrifuged, the supernatant dried and reconstituted in 100µl solvent (5% ACN, 95% water). 20 µL of the solution was directly injected on a chromatographic separation system.

HPLC Conditions

An Agilent 1290 Infinity system with a Kinetex XB-C18, 50 x 2.1mm 2.6µ Phenomenex column at set to 40° C was used. Gradient:

	Time [min]	Flow [µl/min]	% A	%B
A: H_2O with 5 mM NH_4Ac and 0.5 % AcOH	0	400	98	2
	2	400	98	2
B: MeOH with 5 mM NH_4Ac and 0.5 % AcOH	6	400	20	80
	6.2	400	1	99
	9.2	400	1	99
	9.4	400	98	2
	13	400	98	2

MS Conditions

AB SCIEX Triple TOF® 5600+Systeme with Duo-Source (Positive/Negative)
IDA Experiment (MS + MSMS in parallel)
Accumulation time TOF MS: 50 / 100 ms
Accumulation time TOF MSMS: 50ms (Unit Resolution)

Source parameter
GS1: 50.00
GS2: 60.00
CUR: 30.00
TEM: 500.00
ISVF: 5500.00 or -4500.00

Calibrant Delivery System (for automatic MS and MSMS calibration)

- TOF-MS (survey scan)
- IDA criteria (threshold, etc.)
- TOF-MS/MS
- TOF-MS/MS
- TOF-MS/MS

20 or 30 (!) dependent scans

Remember, the instrument can acquire scans at up to 100 Hz!

- TOF-MS/MS

RESULTS

The system has the power to acquire MS and MS/MS data on every peak present in a given sample, and those data may be processed in a targeted manner using MasterView™ Software then coupled to MultiQuant™ Software for targeted quantitation (not shown here), or processed in a non-targeted manner using MasterView™ Software, where samples can be qualitatively explored for any other chemical residue that might be presented beyond the lab's routine targeted list. This system and setup give labs more data on their samples in every injection.

Importance of MSMS Library Search

Figure 1 Mycotoxin Standard in a concentration of 10 ppb in water. As an example Aspinolide B identified only with MS and isotopes - but false MSMS library search.

Figure 2 Mycotoxin Standard in a concentration of 10 ppb in water. As another example Asperloxin identified with MS, isotopes and MSMS library search.

Figure 3 Mycotoxin Standard in a concentration of 10 ppb in water. As an example Paxiline (neg. ion mode) identified only with MS and isotopes - but false MSMS library search.

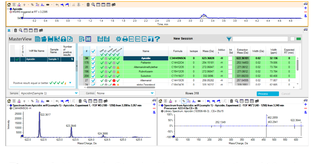

Figure 4. Mycotoxin Standard in a concentration of 10 ppb in water. As another example Apicidin (neg. ion mode) identified with MS,isotopes and MSMS library search.

Since structural isomer have same formula and exact mass, even some time they can separated by HPLC. It can makes false positive result with only exact mass and RT. Library search can make correct answer and reduce false positive results. Figure 5 shows an extract of the list used for HR MSMS library 350 mycotoxins and metabolites.

BoxA-01	FA_001	15-Acetyldeoxynivalenol	C17H22O7	88337-96-6
BoxA-02	FA_002	15-Hydroxyculmorin	C15H26O3	144447-99-4
BoxA-03	FA_003	15-Hydroxyculmorone	C15H24O3	144447-97-2
BoxA-04	FA_004	15-Monoacetoxyscirpenol	C17H24O6	2623-22-5
BoxA-05	FA_006	16-Keto-aspergillimide	C20H27N3O4	199784-50-4
⋮				
BoxA-67	FA_096	Citrinin	C13H14O5	518-75-2
BoxA-68	FA_097	Citromycetin	C14H10O7	478-60-4
BoxA-69	FA_098	Clonostachydiol	C14H20O6	147317-35-9
⋮				
BoxD-50	FA_442	Epiequisetin	C22H31NO4	255377-45-8
BoxD-51	FA_443	Fusapyrone	C34H54O9	156856-31-4
BoxD-52	FA_444	Infectopyrone	C14H16O5	590409-70-4

BoxC-59	FA_319	Verrucalogen	C27H33NO7	12771-72-1
BoxC-60	FA_320	Verticolorin A	C18H1007	6807-96-1
BoxC-61	FA_323	Viomellein	C30H24O11	55625-78-0
BoxC-62	FA_325	Viridicatin	C15H11NO2	129-24-8
BoxC-63	FA_326	Wortmannin	C23H24O8	19545-26-7
BoxC-64	FA_327	Xanthonegrin	C36H32O12	1685-91-2
BoxC-65	FA_328	Zearalenone	C18H22O5	17924-92-4
BoxC-66	FA_329	Zearalenone-14-glucoside	C24H32O10	105088-14-0
BoxC-67	FA_331	Deepoxy-deoxynivalenol	C15H20O5	88054-24-4
BoxC-68	FA_333	5-O-Methylsulochrin	C18H18O7	10056-14-1
BoxC-69	FA_334	6-Aminopenicillanic acid	C8H12N2O3S	551-16-6
BoxC-70	FA_336	Actinomycin D	C62H86N12O16	50-76-0
BoxC-71	FA_339	Amoxycillin	C16H19N3O5S	26787-78-0
BoxC-72	FA_340	Amphotericin B	C47H73NO17	1397-89-3
BoxC-73	FA_341	Arisomycin	C14H19NO4	22862-76-6
BoxC-74	FA_342	Ascomycin	C43H69NO12	11011-38-4
BoxC-75	FA_344	Bafilomycin A1	C35H58O9	88899-55-2

Figure 5. List of used mycotoxins and metabolites for the HR MSMS library

Targeted Analysis

Figure 6. Cow milk sample blank. Clearly observed blank cow milk contain mycotoxins

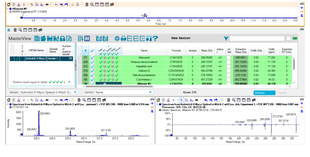

Figure 7. Cow milk sample spiked. List of the identified mycotoxins and metabolites.

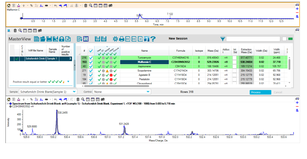

Figure 8. Sheep milk sample blank. Clearly observed blank sheep milk contain mycotoxins

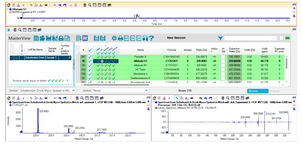

Figure 9. Sheep milk sample spiked. List of the identified mycotoxins and metabolites.

Figure 10. Cow goat sample blank. Clearly observed blank goat milk contain mycotoxins

Figure 11. Goat milk sample spiked. List of the identified mycotoxins and metabolites.

CONCLUSIONS

A simple and ease-of-use extraction method for milk was shown here to be sufficient. For a reliable identification of mycotoxins and the metabolites we could demonstrate that a MS spectrum only, plus the correct isotopic destribution (and also retention time – not shown here in this presentation) may not ne reliable enough. A high resolution MSMS library (HR MSMS DB) is necessary to correctly identify the individual mycotoxins and metabolites. As a result ABSCIEX will continue to build a comprehensive HR MSMS DB not only with mycotoxins and ist metabolites but also for pesticides, antibiotics, PPCP and other compounds in positive and negative ion mode.

REFERENCES

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TRADEMARKS/LICENSING