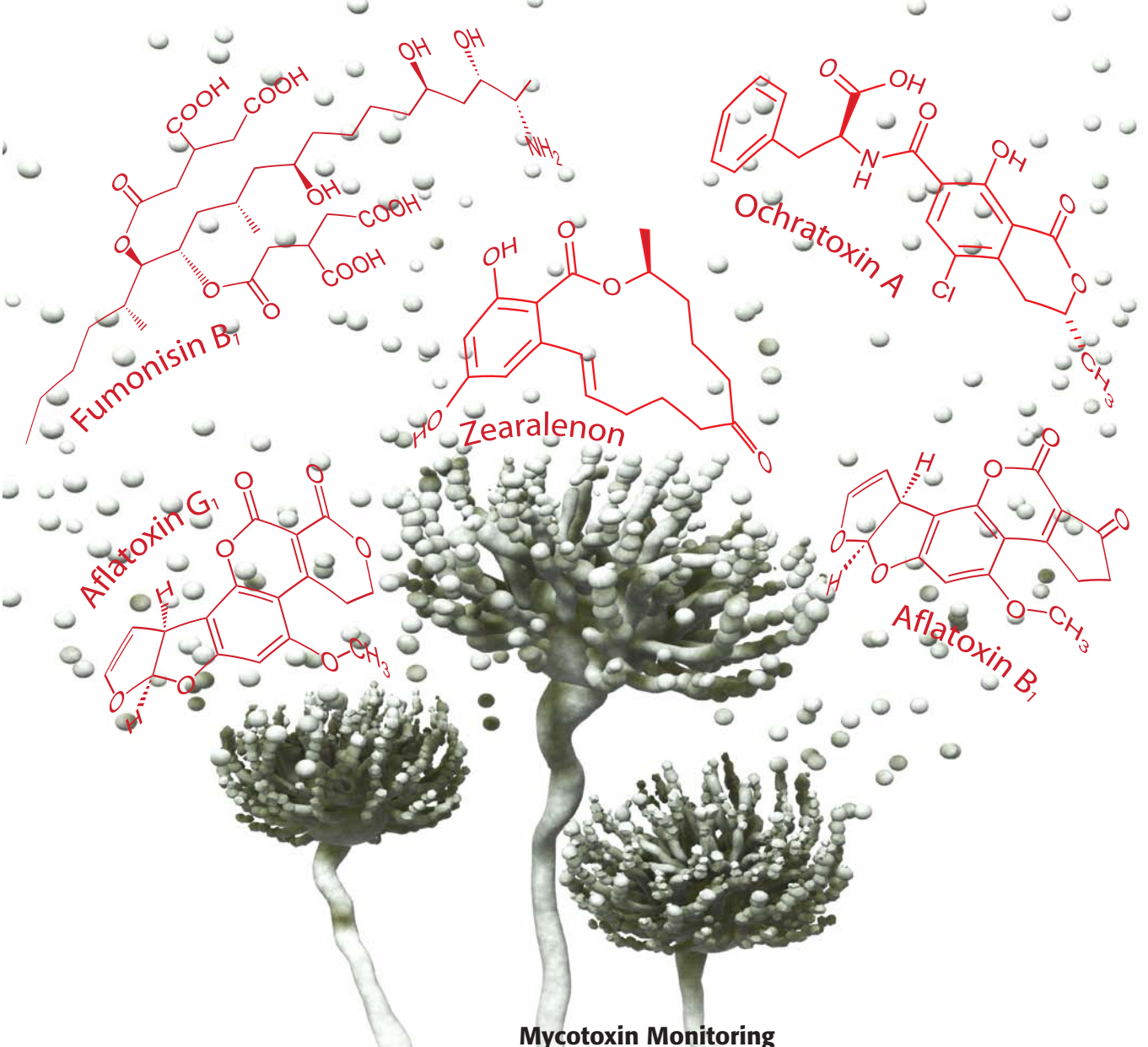

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Mycotoxin Monitoring

Nine steps forward

Laboratories are constantly under pressure to improve productivity – many times without the addition of human resources. To support this demand, more and more scientists are turning to automation of their time consuming and costly manual sample preparation procedures. This article describes how scientists at a contract laboratory specializing in food and environmental analysis developed and implemented an automated SPE-HPLC-MS/MS multi-method to determine nine mycotoxins in one run without relying on immunoaffinity cartridges or human intervention.

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Their Latin names seem almost regal, and those who know their powers are aware that the molds Aspergillus parasiticus and Aspergillus flavus should be treated with the utmost respect. They should be admired only from a safe distance, with proper protection, and never ingested. Luckily, the visual and olfactory appearance of molds is not appealing to humans and we are genetically programmed to avoid them, but they are not always visible to the naked eye, nor do they necessarily have an odor. Among the metabolites of these molds are potent toxins known as mycotoxins. Apart from being acutely toxic they have carcinogenic, genotoxic and endocrine disrupting traits [1].

Only the dose makes the poison

While Paracelsus obviously was right for most poisons, much more detailed toxicological knowledge at a mo-

lecular level is available to today's scientist. The dose considered toxic for some mycotoxins would have surprised the old master, since for these it is safe to assume that any measurable amount could be unsafe. Whoever processes grain, fruit, spices and other agricultural products should know that is not a question of whether these contain molds, but rather how much they contain. Molds are ubiquitous and cannot be eliminated, only controlled. Accordingly, depending on their individual toxicity, European Union (EU) lawmakers have established maximum levels of mycotoxins in food and feed with a special view to protecting the youngest humans [2]:

Aflatoxin B_1: 8,0 µg/kg (Peanuts), 0,1 µg/kg (Baby food); Sum total of aflatoxins B_1, B_2, G_1 and G_2: 15 µg/kg (Peanuts), 4,0 µg/kg (Grain)

Ochratoxin A: 10 µg/kg (Coffee, raisins), 0,5 µg/kg (Baby food)

Zearalenon: 200 µg/kg (Corn/Maize), 20 µg/kg (Baby food)

T-2-/HT-2-Toxin: not yet established

Fumonisin B_1: 2000 µg/kg (Corn/Maize), 200 µg/kg (Baby food)

Challenges in standard mycotoxin analysis

When maximum levels for toxins are established, this implies that adequate analysis methods and technologies are available with which these levels can be accurately determined and our food quality monitored. Standard methods prescribe the determination of mycotoxins in food and feed in separate groups of analytes using HPLC and fluorescence detection with a clean-up process based on Solid Phase Extraction (SPE) using immunoaffinity cartridges. For some, namely aflatoxins and fumonisin, a derivatization must be performed [3]. An HPLC-MS/MS method is specifically required only for T-2 and HT-2. Standard methods used for the determination of mycotoxins are of course effective in that they produce correct results, but they generally offer significant potential for optimization in terms of productivity. In addition, the use of immunoaffinity cartridges is quite expensive and several different cartridges and clean-up steps are required for each compound group making the total analysis very time consuming.

Realizing the optimization potential

Upon closer inspection, it seemed feasible to dramatically increase the efficiency and productivity of our mycotoxin analysis. Several steps of the process were analyzed and the optimization potential determined. Among other things, it became clear that a major bottle neck was the need for multiple SPE process steps for different groups of mycotoxins. If these could be combined into one SPE step based on a standard sorbent cartridge, the need for expensive immunoaffinity cartridges could be eliminated

Image: TeLA GmbH

The mycotoxin multi-method for the determination of nine mycotoxins was successfully automated on an LC-MS/MS system from Agilent Technologies in combination with a GERSTEL MultiPurpose Sampler (MPS Dual Head version). The solvent gradient used was (A: 5 mM formic acid, B: Acetonitrile; Flow: 0.2 mL/min, 50 °C). The stationary phase used was a C18 reversed phase (RP) material. Mycotoxins were detected in positive ESI Mode.

and the time spent per sample could be reduced significantly, of course provided the quality of results were comparable or could even be improved. A further positive point was that all steps could be automated using a standard sampler

Most Unwanted

Aflatoxins

Aflatoxins are formed as secondary metabolites by different Aspergillus species, including Aspergillus flavus and Aspergillus parasiticus. These are mainly found in nuts and spices. Aflatoxins are highly toxic and carcinogenic; the most critical Aflatoxins are the types B_1, B_2, G_1 and G_2. Aflatoxin B1 is the one most frequently found in food. Aflatoxins are thermally stable and are therefore not destroyed by cooking.

Ochratoxins

Next to the Aflatoxins, Ochratoxin A is counted among the most dangerous mycotoxins. Ochratoxin A is known to cause kidney damage and animal experiments have shown it to be carcinogenic. This mycotoxin is often found in coffee, cereals, beer and dried fruits. As is the case for Aflatoxins, Ochratoxin A is thermally stable and is not even destroyed during coffee roasting.

Fusarium toxins

Fusarium toxins are produced by various Fusarium species among others and are frequently found in cereals. Among these are Zearalenon, T-2 Toxin and HT-2 Toxin. Their acute toxicity is considered low.

Fumonisins

Fumonisins are strongly polar mycotoxins formed by the molds Fusarium verticillioides and Fusarium proliferatum, the most potent of these toxins is Fumonisin B1. Fumonisins are found everywhere in the world. They are formed especially on corn/maize, the amount formed on grains or in foods depends mainly on environmental factors and on storage conditions. Fumonisins are water soluble and are not deactivated by most food preparation processes.

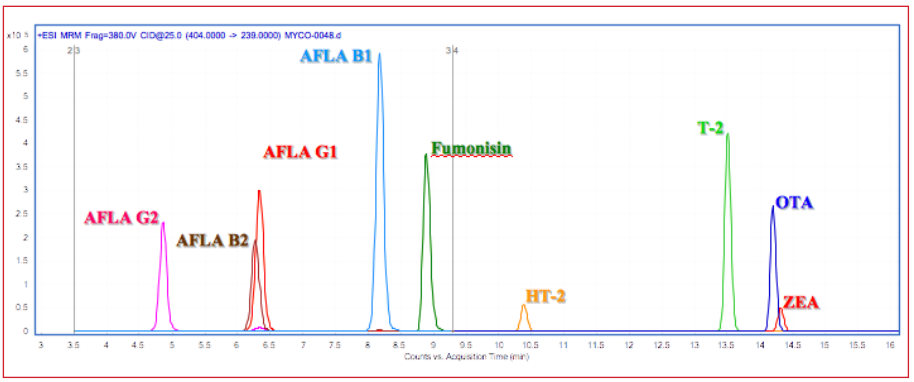

Image: TeLA GmbH

Chromatogram of a standard mixture of the mycotoxins Aflatoxin B_1, B_2, G_1 and G_2, Ochratoxin A, Zearalenon, T-2- and HT-2 Toxin as well as Fumonisin B_1.

Automated Sample Preparation makes the difference

for sample preparation and introduction to the LC-MS/MS system, reducing the laboratory workload. Finally, using MS/MS technology instead of fluorescence detection would improve both the specificity and the sensitivity of the analysis. Following a great number of carefully calibrated experiments, the mycotoxin multi-method was established for the determination of the mycotoxins Aflatoxin B_1, B_2, G_1 and G_2, Ochratoxin A (OTA), Zearalenon (ZEA), T-2- and HT-2 Toxin and Fumonisin B_1. The system used for the analysis consisted of an Agilent 1290 HPLC with 6495 Triple Quadrupol Mass Spectrometer from Agilent Technologies in combination with a GERSTEL MultiPurpose Sampler (MPS) in Dual Head configuration equipped with two towers. This version enables different types of process steps to be processed efficiently without the need to change tools.

Following the method development work, the automated sample preparation process was implemented as follows: The SPE cartridge was conditioned using methanol and water and a 7 mL sample was added to the cartridge. The packed bed is washed with 4 mL of water and dried with nitrogen gas. The analyte elution is performed using 1.5 mL acetic acid ester. The vial with the eluate is transferred to the MultiPosition Evaporation Station (*m*VAP) and the eluate evaporated to dryness. The residue is taken up in 500 µL of the mobile phase and injected to the separation column. In summary, the goal of developing a fully automated multi-method for the determination of mycotoxins was reached and the method implemented for the following compounds: Aflatoxin B_1, B_2, G_1 and G_2, Ochratoxin A, Zearalenone, T-2 and HT 2 Toxin as well as Fumonisin B1. The simplified analysis process with unified sample preparation steps for all mycotoxins lead to significant time savings compared

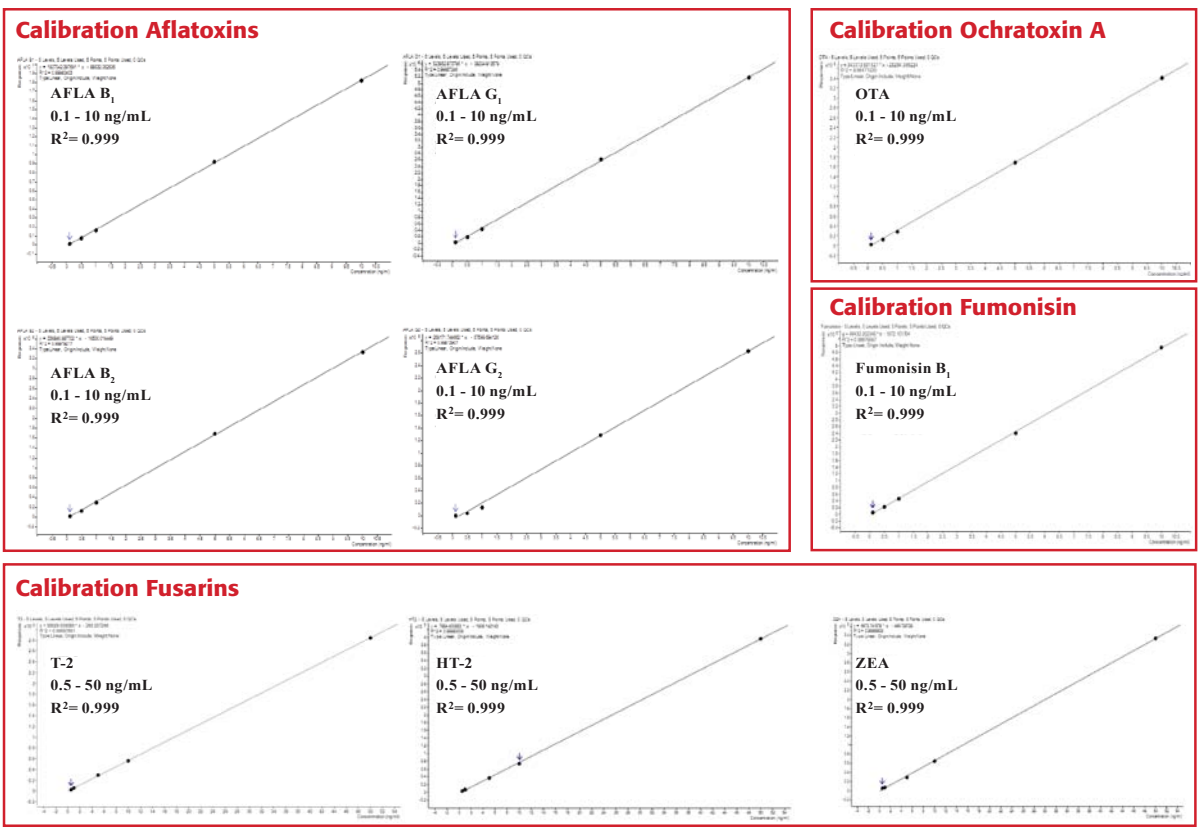

Image: TeLA GmbH

Excellent linearity over a wide range of concentrations for all compounds.

Mycotoxin	Level [µg/kg]	Recovery [%]	RSD [%]
Fumonisin	2	79	5.4
T-2	2	105	6.1
HT-2	2	103	6.5
OTA	2	88	4.3
Aflatoxin G_2	2	94	5.7
Aflatoxin G_1	2	91	4.8
Aflatoxin B_2	2	95	4.5
Aflatoxin B_1	2	89	3.9
ZEA	2	108	5.9

Validation data for the mycotoxin Multi-method for the determination of nine mycotoxins.

with the traditional manual sample preparation process. By using parallel processing of sample preparation steps and chromatography analysis (PrepAhead function) the analysis time was reduced to 52 minutes for the first sample and 30 minutes for all subsequent samples.

Results more than satisfactory

Using a solid phase extraction for sample clean-up instead of performing costly clean-up steps with immunoaffinity cartridges enabled us to reduce the cost per analysis significantly. Furthermore, fluorescence detection was replaced by much more sensitive and selective MS/MS detection, leading to lower limits of determination (0.1-0.3 µg/kg). Statistical values were good, average recoveries ranged from 94.6 percent and upwards and Relative Standard Deviations averaged 5.2 percent. Calibration curves for all mycotoxins showed excellent linearity from 0.1 to 10 ng/µL (Aflatoxins, Ochratoxin A and Fumonisin) and 0.5 to 50 ng/µL respectively (T-2, HT-2 and Zearalenone). Our routine analysis implementation showed that the Mycotoxin Multi-Method was rugged and reliable.

References

[1] Fast and reliable answers regarding aflatoxins in foods, GERSTEL Solutions Magazine No. 7, pp. 18-21 www.gerstel.com/pdf/solution_07_18_21.pdf

[2] COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

[3] Improving MS Detection of Aflatoxins using Automated Solid Phase Extraction and Derivatization coupled with an LC/MS System, GERSTEL AppNote 6/2007

GERSTEL NEWS

Thermal Desorption Unit – TDU 2

The new GERSTEL Thermal Desorption Unit (TDU 2) is the heart of the most flexible and powerful sample introduction platform available for your GC/MS.

The TDU 2 is a fourth generation Thermal Desorption instrument from GERSTEL, building on more than 20 years of experience. The TDU 2 offers the highest performance, new low split pneumatics for improved LODs, built-in alignment support for easy liner change, and automation performed by the MPS robotic offering high precision delivery of samples, Barcode reading capability and up to 240 TDU tube sample capacity in standard configurations.

The TDU 2 enables ultra-trace analysis of a wide range of samples. Mounted directly on top of the CIS inlet without the need for a transfer line, the TDU provides a completely inert sample path for best possible analyte recovery.

The GERSTEL MultiPurpose Sampler (MPS/MPS robotic) is extremely versatile. The system performs automated spiking of adsorbent tubes with liquid standards as well as multiple sample introduction techniques such as Liquid, HS, SPME plus the following seven:

- Thermal Desorption of sorbent packed tubes
- Direct Thermal desorption/extraction of solids
- Thermal Extraction of liquids in µ-vials
- Dynamic Headspace – DHS and DHS Large (1 L)
- Hot injection and trapping (HIT) for HS
- Stir Bar Sorptive Extraction (SBSE)
- Pyrolysis using the GERSTEL PYRO

It is extremely easy to add – or switch between – sample introduction techniques. All techniques are fully controlled by GERSTEL's powerful, yet easy to use MAESTRO software. The complete integration of MAESTRO into Agilent's GC/MS software allows set up through dropdown menus and combined storage and reporting of all method parameters.

Image: GERSTEL / Wolfram Schroll