

# Investigation of mycotoxins in different beers with several clean-up techniques using the Mycotoxin Screening System

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## 1. Introduction

Mycotoxins are secondary metabolites, produced by fungi and were found to be toxic and carcinogenic [1]. Therefore, quantification in food and beverages is crucially important, in order to ensure the health of humans and animals. This poster presents simple and fast methods for sample preparation and analysis of several mycotoxins in different matrices using the Shimadzu "Mycotoxin Screening System". It is a package with ready-to-use methods and reports to screen mycotoxins for European maximum residue levels. This work focuses on determination of Aflatoxin B1, B2 (AFB1, AFB2), G1, G2 (AFG1, AFG2), Deoxynivalenol (DON), Nivalenol (NIV) and Ochratoxin A (OTA), as these mycotoxins can be produced by fungal infestation during or after harvest of grain and can therefore end up in processed products such as beer, brewed from malt.

All analytes of interest could be quantified with LOQs at or below the European maximum residue levels (EUMRL) (Table 1). Highly sensitive detection of all analytes was achieved by the combination of fluorescence and photodiode array (PDA) detectors.

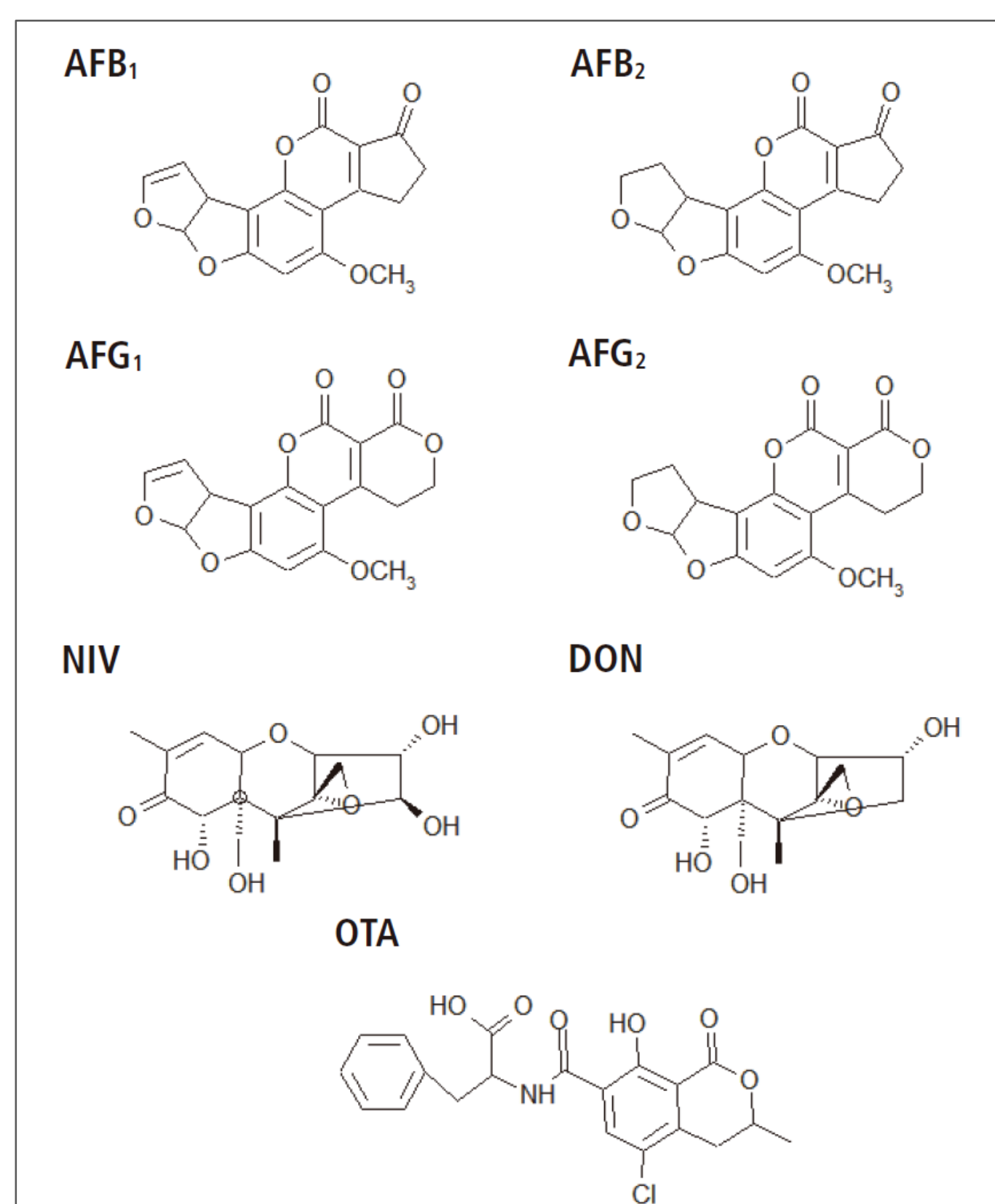


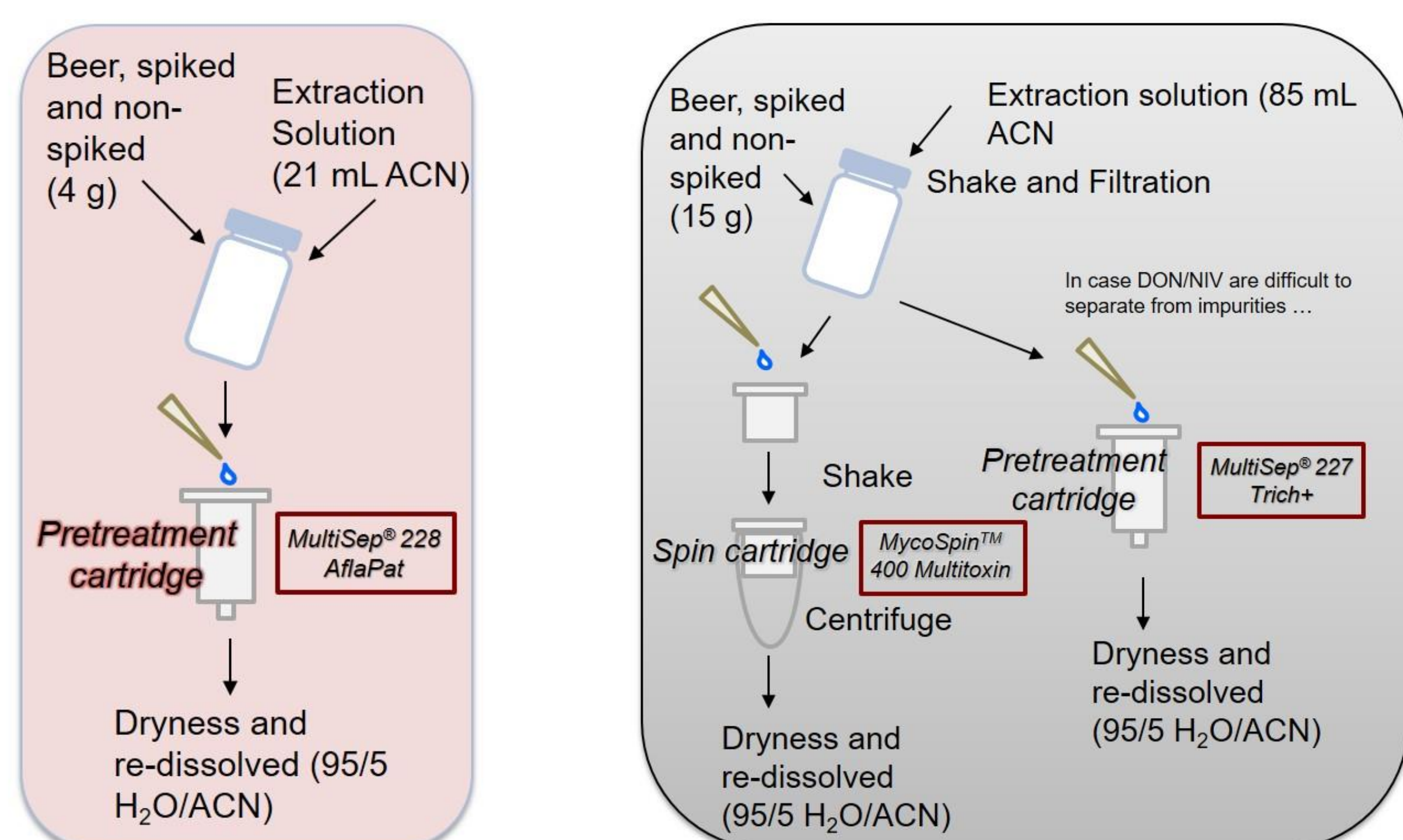
Figure 1: Structures of mycotoxins

Table 1: Different mycotoxin regulations in EU, US and Japan [2,3]

Target compound	Examples of targeted product	EU	US (Codex)	Japan
Aflatoxin B1,B2,G1,G2 (AFB1,B2,G1,G2)	Grain, Grain product (Flour etc.)	Total 4~15 µg/kg AFB1 2~12 µg/kg	Total 10~15 µg/kg	Total 10 µg/kg
Ochratoxin A (OTA)	Wheat, Wheat flour	2~10 µg/kg	5 µg/kg	Not regulated
Deoxynivalenol (DON)	Wheat, Wheat flour	500~1750 µg/kg	1000 µg/kg	1100 µg/kg (prov. std. value)
Nivalenol (NIV)	Wheat, Wheat flour		Not regulated	

## 2. Materials and Methods

### 2-1. Sample pretreatment with three different solid phase extraction cartridges



### 2-2. Analytical conditions

For analysis, a Shimadzu i-series system with integrated photo diode array (LC-2040C 3D) and additional fluorescence detector (RF-20AXs) was used (figure 2). The detailed analytical conditions used are shown in table 2.



Figure 2: Shimadzu Mycotoxin Screening System

Table 2: Analytical conditions for analysis of mycotoxins

System:	LC-2040C 3D (Shimadzu Corporation, Japan)
Column:	Shim-pack GIST C18 3.0 mm x 75 mm, 2 µm (Shimadzu Corporation, Japan)
Mobile Phase A:	20 mmol/L NaH <sub>2</sub> PO <sub>4</sub> in H <sub>2</sub> O, pH 2.5
Mobile Phase B:	Acetonitrile
Mobile Phase C:	Methanol
Gradient:	5 %B to 35 %B; 15 %C in 10 min
Injection volume:	10 µL
Oven Temp.	55 °C

## 3. Results

All mycotoxins were detected with fluorescence or PDA detection. For NIV and DON, the clean-up cartridge MultiSep 227 was used. Both were clearly identified in the spiked beer sample using PDA detection (figure 3).

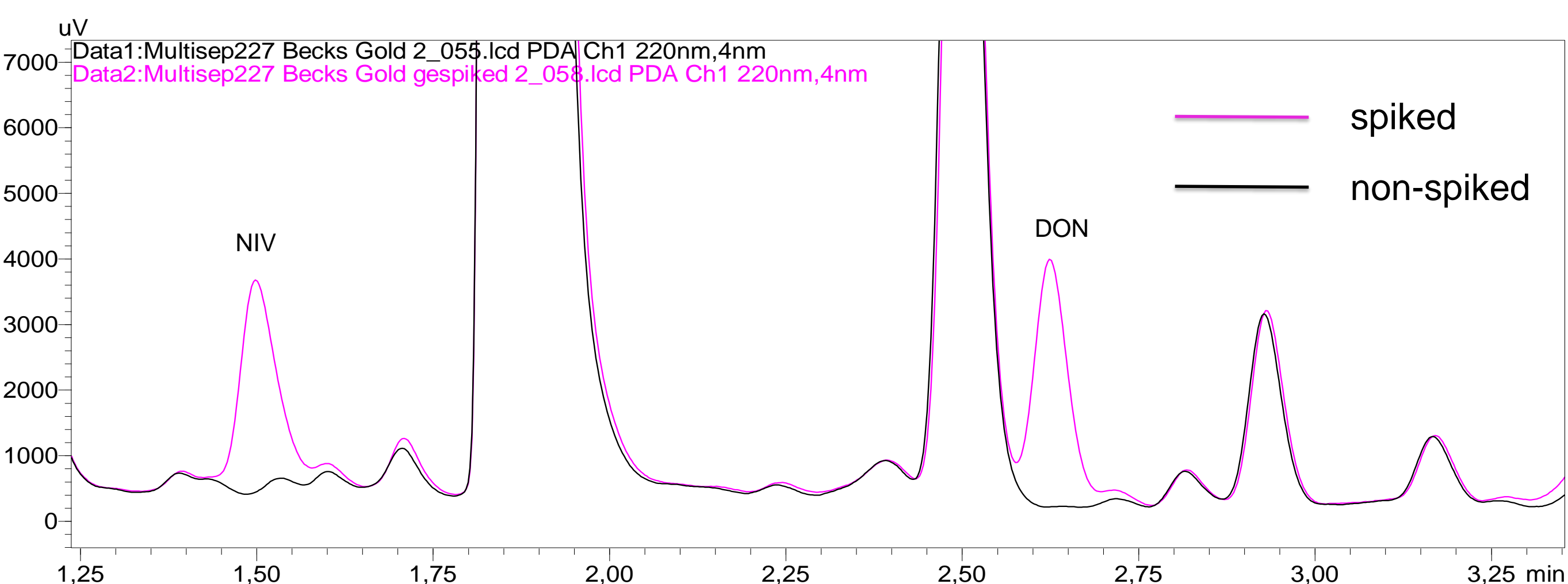


Figure 3: PDA Chromatogram of spiked and non-spiked beer sample

A chromatogram (fluorescence detection) of a spiked and a non-spiked beer sample can be found in figure 4. By comparison of the unaltered beer samples, a difference in the two batches was identified. The chromatogram obtained from batch 2 shows a slightly higher mycotoxin content than the sample of batch 1 (figure 5). However, the concentrations are still below the EU regulations and therefore acceptable. The clean-up cartridge MultiSep 228 was used.

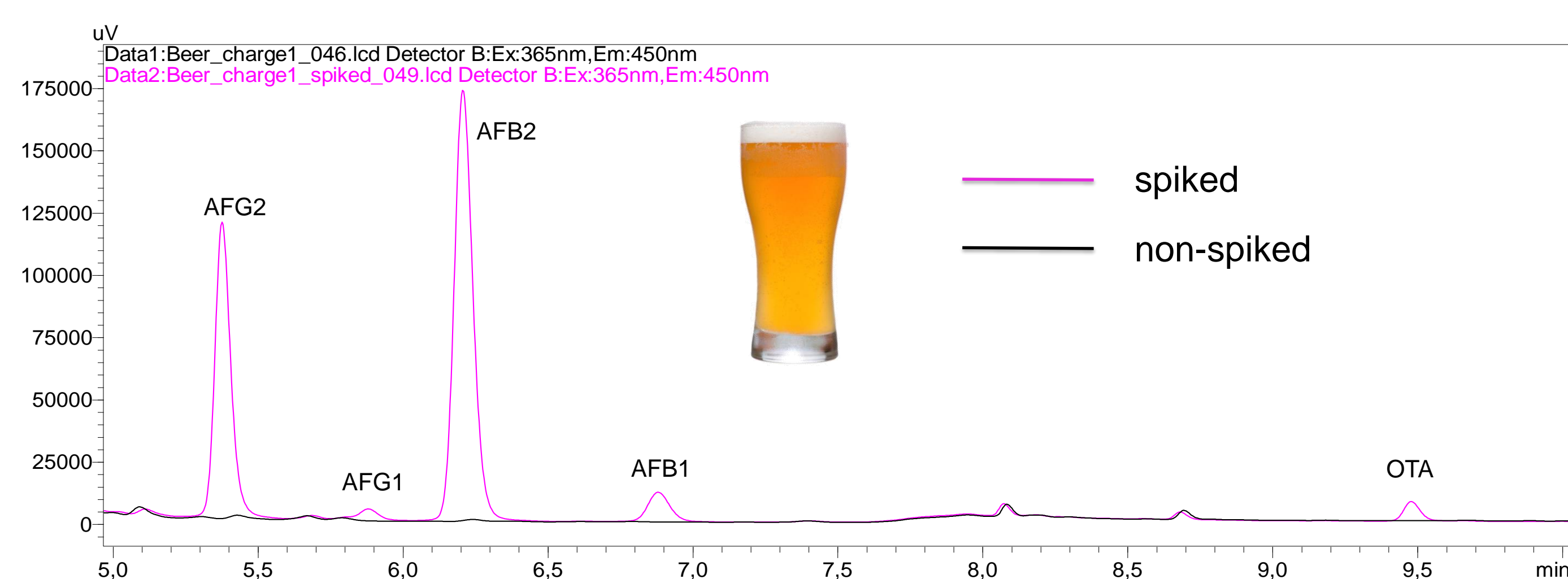


Figure 4: Chromatogram of the analysis of a spiked and a non-spiked beer sample

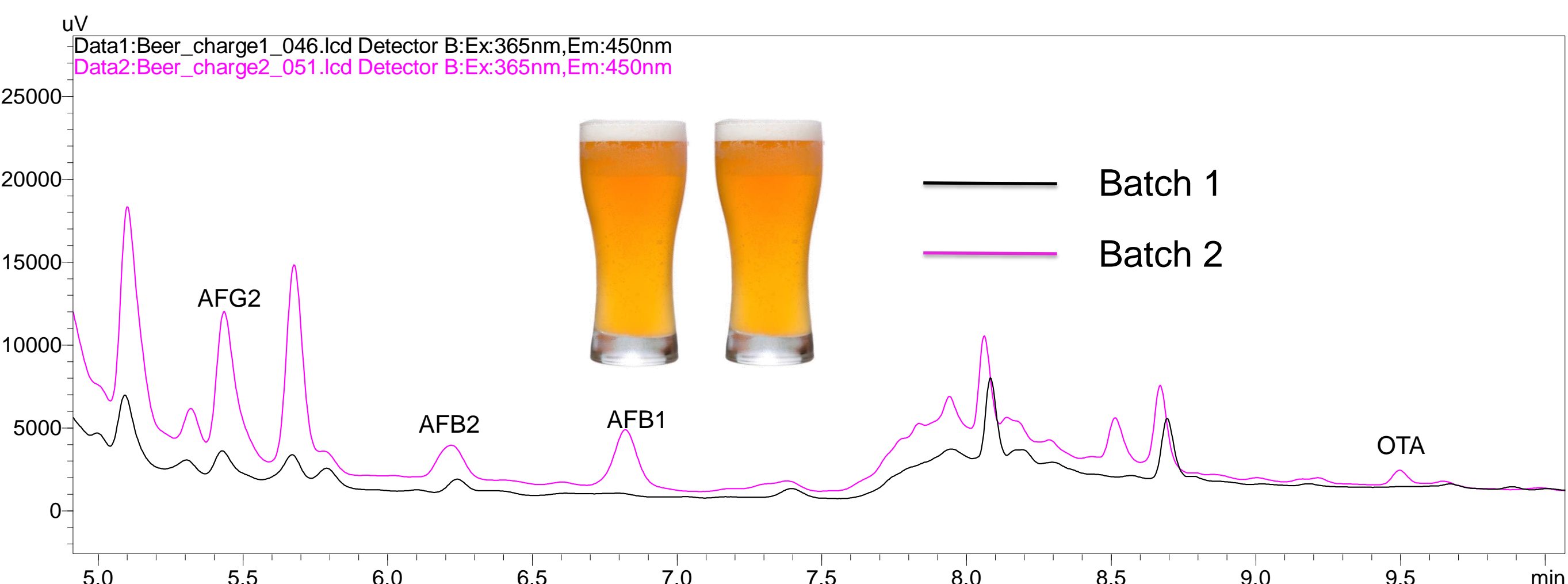


Figure 5: Comparison of samples of two different batches of the same beer brand

For quantification of the mycotoxins, calibration curves were produced in the range of 2.5 - 25 µg/L. These calibration curves all showed good linearity with R<sup>2</sup> values ≥ 0.97 (figure 6).

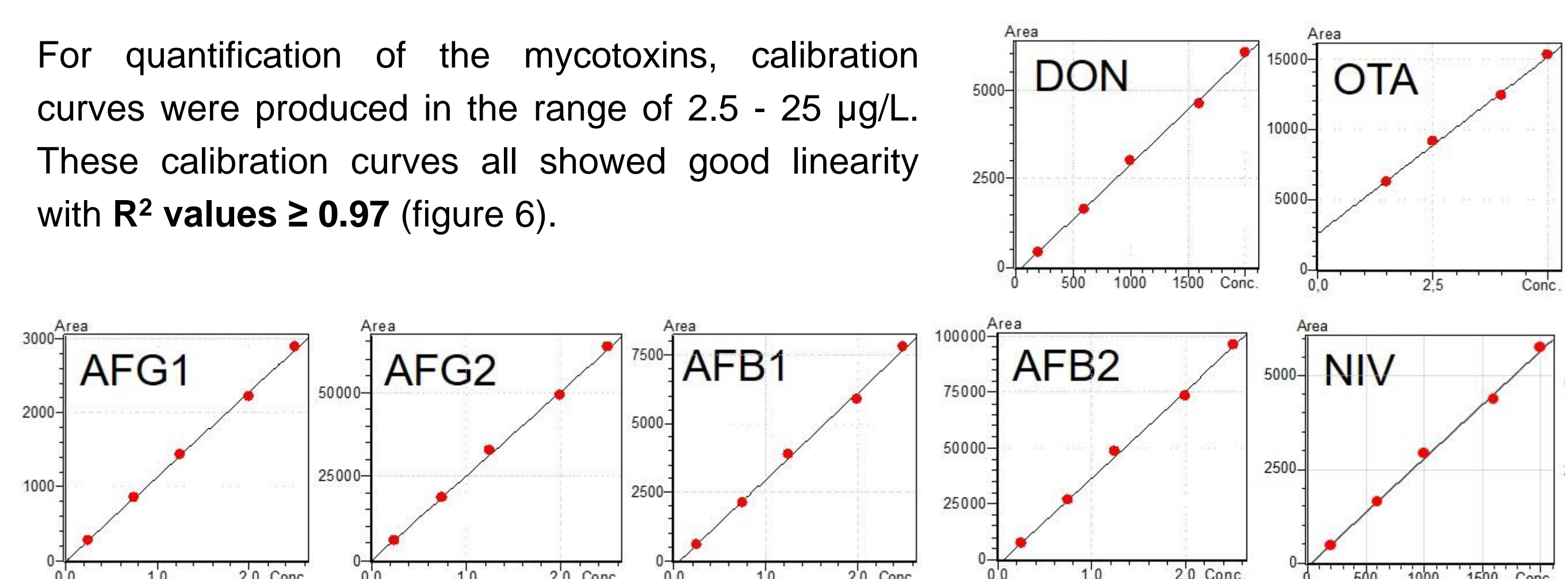


Figure 6: Calibration curves for mycotoxins of interest in the range of 2.5 – 25 µg/L

## 4. Conclusion

In this study, the applicability of the "Mycotoxin Screening System" from pretreatment to analysis and final reporting of analytical results for the simultaneous analysis of 7 mycotoxins in beer was shown. AFB1, AFB2, AFG1, AFG2, OTA, DON and NIV which are most commonly tested in malt products were extracted and analyzed in spiked and non-spiked beer samples. The sample preparation technique as well as the measurement were proven to be easy and fast. The beer samples analyzed contained mycotoxin concentrations below the EU regulations. The system offers a fast, safe and easy method for the analysis of mycotoxins in beverages such as beer.

### References

- [1] European Food Safety Authority (<http://www.efsa.europa.eu/de/topics/topic/aflatoxins>)
- [2] EU: Commission Regulation (EC) No 165/2010 of 26 December 2010 amending Regulation (EC) No 1881/2006. Setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins.
- [3] EU: Commission Regulation (EC) No 105/2010 of 5 February 2010 amending Regulation (EC) No 1881/2006. Setting maximum levels for certain contaminants in foodstuffs as regards ochratoxin A.