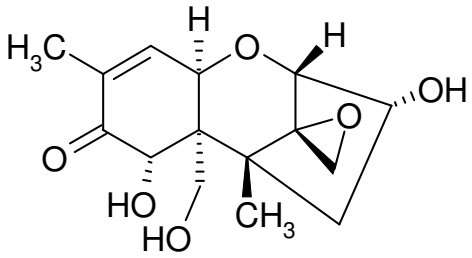
Analysis of deoxynivalenol in beer

Mycotoxins, toxic secondary metabolites of several fungal species, represent food safety issue of high concern. Deoxynivalenol (Figure 1), the most abundant trichothecene mycotoxin, can be found world-wide as a contaminant of wheat, barley, maize and other cereals.[1,2] The transmission of deoxynivalenol from barley into beer has been reported in several studies.[3,4] Therefore, its levels should be controlled.

Figure 1 Structure of deoxynivalenol, trichothecene B *Fusarium* toxin.



The AccuTOF-LC time-of-flight mass spectrometer equipped with a DART ion source and AutoDart HTC PAL autosampler, was used for examination of beer in this study. Donprep® immunoaffinity columns (R-Biopharm) was employed for selective isolation of target analyte from the sample. Briefly, 10 mL of beer with added internal standard ($^{13}C_{15}$-deoxynivalenol, 500 ng/ml) were passed through the cartridge, which was then washed with 5 mL of water. Deoxynivalenol was subsequently eluted with 4.5 mL of methanol. Calibration standards containing deoxynivalenol in the range from 100 to 1500 ng/mL and fixed amount of internal standard (500 ng/mL) were prepared for quantification.

Introduction of the sample ($n = 5$) into the gas beam was carried out automatically with the use of autosampler. Beer extract was placed in the sampling hole, Dip-it™ sampler stick was immersed into the sample and introduced in front of the DART ion source (Figure 2). After each sample analysis, PEG mixture solution was injected for mass drift compensation. TIC chromatogram of beer sample analysis is shown in Figure 3.

Figure 2 Sample introduction.

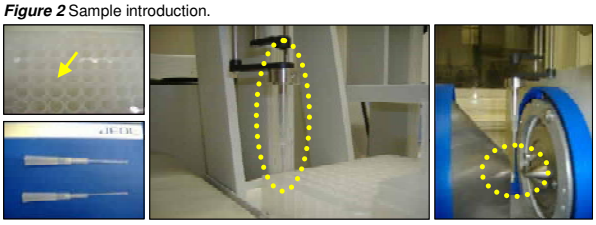
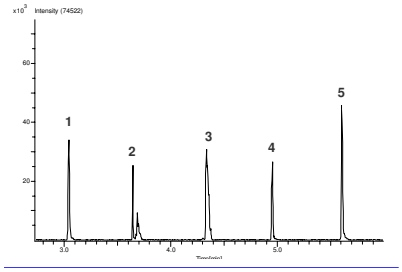


Figure 3 Repeated injections of beer sample.



To enhance negative ionization of target analytes, vial containing methylene chloride was placed beneath DART gun exit – MS orifice axis. After sample introduction, both deoxynivalenol and $^{13}C_{15}$-deoxynivalenol were immediately detected as $[M+Cl]^-$ (see Figure 4) under parameters setting shown in Table 1. Good mass accuracy was obtained (see Table 2).

Table 1 Optimized DART ion source parameters

Parameter	Setting
Polarity	negative
Helium flow rate	2.7 L/min
Discharge needle voltage	3000 V
Perforated electrode voltage	-150 V
Grid electrode voltage	-250 V
Gas beam temperature	300 °C

Figure 4 Positive DART spectrum: deoxynivalenol and internal standard in beer extract.

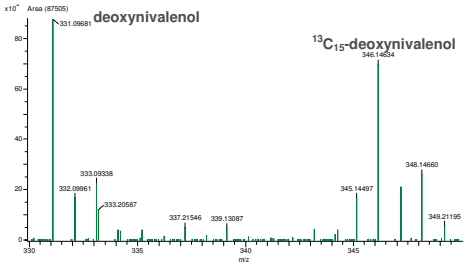
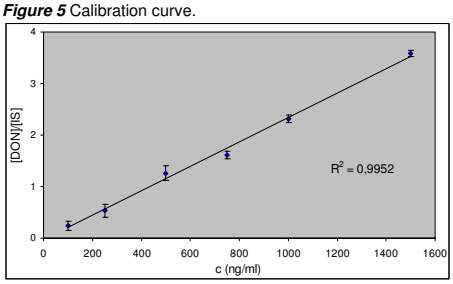


Table 2 Comparison of exact and measured masses.

Compound	Exact mass (mu)	Measured mass (mu)	Diference (mmu)	Elemental composition $[M+Cl]^-$
Deoxynivalenol	331.09484	331.09681	-1.97	$C_{15}H_{20}O_6Cl$
$^{13}C_{15}$-Deoxynivalenol	346.14516	346.14634	-1.18	$^{13}C_{15}H_{20}O_5Cl$

In Figure 5, calibration plot of deoxynivalenol is shown; analyte to internal standard ratio was linear in selected concentration range. Deoxynivalenol concentration determined with the use of DART-TOFMS in particular beer sample was 166 µg/L and repeatability of the method, estimated from five repetitive analyses, was 3%. In addition, accredited LC–MS/MS method was used for sample examination to confirm the trueness of results obtained by DART-TOFMS. The difference between deoxynivalenol obtained by respective methods was as low as 14 µg/mL.

Figure 5 Calibration curve.



In conclusion, AccuTOF-DART has been demonstrated as a suitable to screen for deoxynivalenol in beer samples purified by simple procedure employing immunoaffinity columns.

References

1. Hussein, H. S.; Brasel, J. M.: Toxicity, metabolism, and impact of mycotoxins on humans and animals, *Toxicology*, 167, 2001, 101-134.
2. Wiedenbörner, M.: Encyklopedia of food mycotoxins, Springer, Berlin.
3. Scott, P. M.: Mycotoxins transmitted into beer from contaminated grains during brewing, *Food Chemical Contaminants*, 79, 1996, 875-882.
4. Papadoulou-Bouraoui, A.; Vrabcheva, T.; Valzacchi, S.; Stroka, J.; Anklam, E.: Screening survey of deoxynivalenol in beer from the European matket by an enzyme-linked immunosorbent assay, *Food Additives and Contaminants,* 21, 2004, 607-617.