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Micro-bioaffinity nano-liquid chromatography mass spectrometry of mycotoxins

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Introduction

Functional binding assays with antibodies, transport proteins and molecular receptors are important tools in the future detection of known and unknown chemical contaminants in food.

However, mass spectrometric identification of both known and unknown bioactive compounds in positive (non-compliant) samples will be crucial. Therefore, bioaffinity-based isolations and detections, based on the same bioreagents, are being developed in this project. Since biomaterials (e.g. IgGs, receptors) are costly, microscale isolations are combined with nano-LC-Q-TOF-MS. The detection of ochratoxins, a group of the most abundant food-contaminating mycotoxins in the world, with monoclonal antibodies (Mabs) on superparamagnetic colour-encoded microspheres is used as a first model.

Luminex-based screening

A flow cytometric immunoassay (Luminex FM3D) was developed using functionalised superparamagnetic microspheres coated with Mabs directed against ochratoxin A (OTA) in combination with a fluorescent label (OTA-phycoerythrin) and an easy extraction method for wheat and cereal samples (1 g + 10 mL buffer). Results obtained for OTA and a non-chlorinated analogue (ochratoxin B (OTB)) with this easy and quick inhibition screening assay with “blank” and spiked wheat samples are shown in Fig. 1.

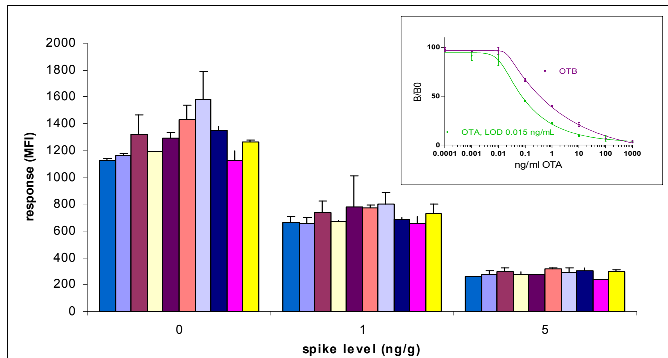

Figure 1. Average screening results (n=2) of 10 “blank” wheat samples (<0.6 ng/g as found by LC-MS), the same samples spiked at 1 and 5 ng/ng and the average dose-response curves of OTA (n=3) and OTB (n=2) in wheat extract (insert). Mab cross-reactivity to OTB is 12.5%.

Nano-LC-MS-based identification

Following the screening, a microscale bioaffinity-based isolation method was developed (Fig. 2), using the same sample extract and Mab-coated superparamagnetic microspheres, for confirmation and identification purposes with nano-LC-Q-TOF-MS. With this method, a naturally incurred cereal sample was analysed and next to OTA, OTB was also detected (see Fig. 3).

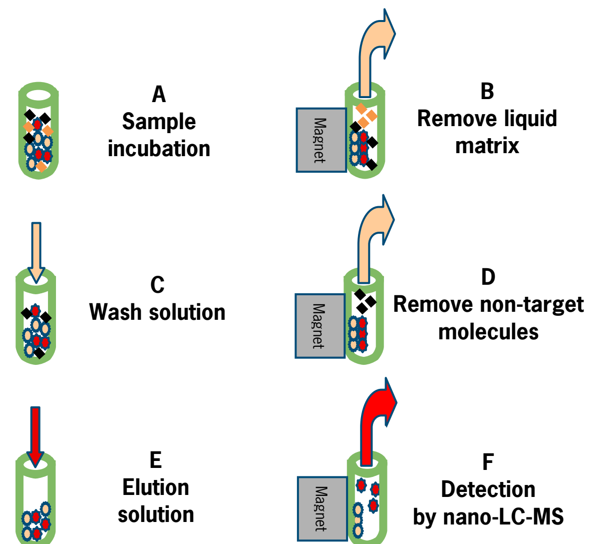

Figure 2. Bioaffinity-based isolation using Mab-coated superparamagnetic microspheres. = superparamagnetic microspheres, = target molecules, = sample compounds, = impurities

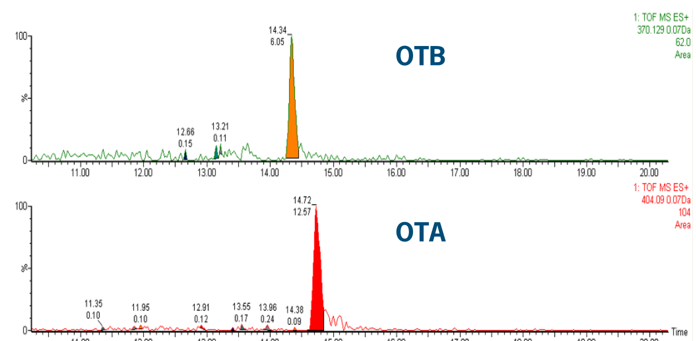

Figure 3. Nano-LC-Q-TOF-MS chromatogram of a naturally incurred cereal sample after the bioaffinity clean-up.

Conclusions

Using the same bioreagents in screening and in the isolation prior to identification, OTA was detected at relevant levels (<MRL of 3 ng/g and 5 ng/g) in wheat by Luminex (FM3D) and identified by LC-MS. Moreover, the presence of the analogue OTB in a naturally contaminated cereal sample was identified by means of the bioaffinity-based sample treatment and nano-LC-Q-TOF-MS.

Using couplings of quick and inexpensive screening assays to bioaffinity mass spectrometry, new efficient tools will become available for the detection, confirmation and identification of known and especially unknown food contaminants.

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Acknowledgement

This research was (partly) financed by the Dutch Ministry of Agriculture, Nature and Food Quality