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Multi-detection of mycotoxins by membrane based flow-through immunoassay

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ABSTRACT

A membrane-based flow-through immunoassay for rapid non-instrumental multi-detection of ochratoxin A (OTA), zearalenone (ZEN) and fumonisin B1 (FB1) in cereal grains and silage was developed. The test is based on a direct competitive enzyme immunoassay performed on a membrane. Alkaline phosphatase was used as enzyme label to decrease matrix interference. The analytical method involved a fast extraction procedure (extraction with a mixture of methanol, water and acetic acid 79:20:1, v/v) followed by a multi-detection. The immunotest allows the visual estimation of the presence of three mycotoxins in less than 15 min. The cut-off limit of this test have been set at a value not higher than half of EU legislative limits (2.5, 50 and 1000 μg kg^{-1} for OTA, ZEN and FB1 correspondingly in wheat; 2.5, 100 and 1000 μg kg^{-1} in maize; 25, 125 and 2500 μg kg^{-1} in silage). Evaluation and validation studies have been performed using spiked and naturally contaminated samples and results were compared with LC–MS/MS. Also the possibility of analyte detection in wheat at two concentration levels by the immunotest was demonstrated with OTA and ZEN as an example. In spite of the semiquantitative-qualitative character of the test, the results demonstrate that the test provides a very good estimation of the mycotoxins concentration in wheat, maize and silage. This test format could be adapted to the multi-detection of other contaminants in food and feed.

1. Introduction

Mycotoxins are natural toxins which are produced by moulds of various species growing on agricultural commodities in the field or after harvest - during storage and transportation. They have different chemical structures and could contaminate commodities during all stages of their cycle. As mycotoxins are potentially hazardous for humans and domestic animals some surveys are performed to predict mycotoxin occurrence and restrict they distribution and consumption, but till now the only way to control mycotoxins' presence or absence is analysis of food and feed samples for mycotoxin content. Methods for mycotoxin analysis can be divided into two groups: screening methods with enzyme-linked immunosorbent assay (ELISA) as the most popular one and chromatography-based confirmatory methods (Köppen et al., 2010). Simultaneous presence of several mycotoxins and necessity of their control demand development of methods for multidetection. Among chromatography-based methods liquid chromatography tandem mass spectrometry (LC–MS/MS) dispose of the widest possibilities for multiple mycotoxin assay (Ediage, DiMavungu, Monbaliu, Van Peteghem, & De Saeger, 2011; Van Pamel, Verbeken, Vlaemynck, De Boever, & Daeseleire, 2011). Biosensors present great opportunities for multi-assay in the area of screening methods (Ricciardi et al., 2013; Wang et al., 2012; Wu et al., 2012) while rapid tests are cost-effective alternatives for on-site screening or for developed countries. Lateral flow membrane-based multi-assays were developed for multiple mycotoxins detection (Huang et al., 2012; Won-Bo, Dzantiev, Eremin, & Chung, 2009). Several tests are commercially available, but their wide distribution is limited by sensitivity. Gel based flow-through multi-assay allows achievement of good sensitivity and minimization of matrix influence, but comparing to the membrane tests they are more labour-intensive (Basova et al., 2010). Membrane-based flow-through immunoassay tests are simpler to develop and reach good sensitivity compared to lateral flow membrane-based tests and more easy to operate compared to gel-based tests (Goryacheva, Lenain, & De Saeger, 2013). So they could be an optimal solution for use in developing countries with high probability of mycotoxin (multiple mycotoxins) contamination.

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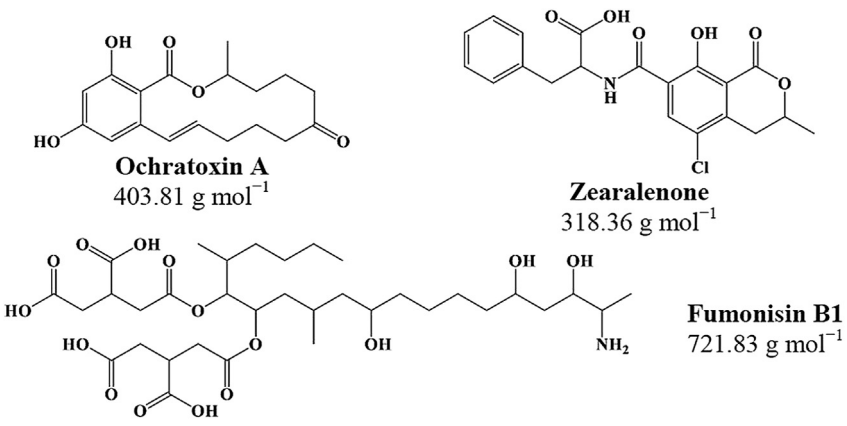

Fig. 1. Chemical structures and molecular weights of investigated mycotoxins.

In flow-through tests, specific antibodies are immobilized onto a membrane, creating a reaction zone for all subsequent reaction steps. In the end, a specific colour development on the membrane, which is dependent on the presence/absence of the analyte is evaluated. Membrane-based flow-through enzyme immunotests show many advantages to the conventional analytical techniques since many samples can be processed simultaneously and often direct determination of low concentration levels is possible with a high specificity. Moreover the tests do not require skilled personnel nor large volumes of sample. An enzyme, namely horse radish peroxidase (HRP), is usually used as label in the flow-through membrane-based immunotests. Application of colloidal gold (Pal, Acharya, Saha, Roy, & Dhar, 2005; Saha, Acharya, Roy, Shrestha, & Dhar, 2007) and colloidal dyes (Xiang, Tianping, & Zhigang, 2003) was investigated but did not become widespread because of low sensitivity. Numerous flow-through membrane tests were suggested for determination of mycotoxins, such as sporidesmin A (Collin, Schneider, Briggs, & Towers, 1998), ochratoxin A (OTA) (De Saeger, Sibanda, Desmet, & Van Peteghem, 2002; Saha, Acharya, & Dhar, 2006), aflatoxin B_1 (Pal & Dhar, 2004), T-2 toxin (De Saeger et al., 2002; Pal, Acharya, Saha, & Dhar, 2004; Sibanda, De Saeger, Van Peteghem, Grabarkiewicz-Szczesna, & Tomczak, 2000); fumonisins (Paepens et al., 2004; Schneider, Usleber, & Martlbauer, 1995). Commercial products are also available (R-Biopharm, Europroxima).

Several approaches to increase assay sensitivity were developed through homogeneously spotting of antibodies (Saha et al., 2006) and signal amplification (Bhattacharya, Bhattacharya, & Dhar, 1999; Pal et al., 2004; Pal & Dhar, 2004) suggested a device capable of performing simultaneous immunoassays on a bunch of samples for one analyte. Most of papers are devoted to individual analyte detection. Only several attempts were made for multi-detection. Schneider et al. (2004) developed the 8-well immunofiltration test device for simultaneous toxin detection. However, antibodies were immobilized on separate wells and detection of each mycotoxin was carried out individually. Insecticides endosulfan and carbaryl can be simultaneously detected using a membrane with two test lines and one control line (Zhang, Zhang, & Wang, 2006). A membrane-based immunoassay has been developed for simultaneous estimation of aflatoxin B1 and OTA in chili samples (Saha et al., 2007); the assay procedure included additional wetting and drying steps. Recently, the membrane-based format was successively used to screen OTA, aflatoxin B1, deoxynivalenol, and zearalenone (ZEN) in maize, peanuts, peanut cake and cassava flour (Ediage, DiMavungu, Goryacheva, Van Peteghem, & De Saeger, 2012); aflatoxin B1, ZEN, deoxynivalenol, OTA, and fumonisin B1 (FB1), in cereal samples (He et al., 2012).

The development of multitests for simultaneous detection of several mycotoxins, that are more economical in respect of time, work and materials, is an actual task. Flow-through tests provide more potential for multi-assay compared to lateral flow strips since the analyte solution simultaneously contacts with the separate immunoreagent spots. Therefore the effect of other test zones and non-specific interaction is expected to be less.

In this study OTA, ZEN and FB1 were chosen as model analytes for multitest development (Fig. 1). These mycotoxins occur widely in cereals and feed and are produced by different fungal spices. OTA has nephrotoxic, carcinogenic, genotoxic, immunosuppressive and teratogenic properties. ZEN is a nonsteroidal oestrogenic mycotoxin frequently implicated in reproductive disorders of farm animals and occasionally in hyperestrogenic syndromes in human. FB1 causes severe animal diseases like leukoencephalomalacia in horses, pulmonary oedema in pigs and liver cancer in rats (Weidenbörner, 2007). The main purpose was to show the possibility of membrane-based flow-through immunoassay for on-site simultaneous detection of several analytes with an incorporated internal negative control. The paper describes the development of a flow-through membrane-based enzyme immunoassay for simultaneous detection of OTA, ZEN and FB1 in wheat, maize and silage at levels two times lower than the maximum EU permitted level (Com.Rec., 2006; Com. Reg., 2006). This cut-off limit was chosen to increase the reliability of mycotoxin detection and to avoid false-negative results. A negative control was included to simplify the results evaluation and to assure functionality. Moreover the visual detection of the mycotoxins at two concentration levels is suggested based on spots covered with different amounts of antibodies.

2. Materials and methods

2.1. Reagents and materials

OTA, ZEN, FB1, rabbit anti-alkaline phosphatase (anti-AP) antibodies and casein from bovine milk (technical grade) were purchased from Sigma Chemical Co. (Bornem, Belgium). Rabbit anti-mouse immunoglobulins (IgG) (protein concentration 2.5 g L^{-1}) were supplied by Dako (Heverlee, Belgium). Monoclonal antibodies (Ab) against FB1 and OTA were prepared by the Institute for Animal Sciences, Agricultural Biotechnology Center, Gödöllö, Hungary. The anti-FB1 Ab was an IgG1 (protein content, 1 g L^{-1}) with kappa light chains with cross-reactivities for FB1, FB2, and FB3 100, 91.8, and 209%, respectively. The anti-OTA Ab was an IgG1 with kappa light chains with a 9.3% cross-reaction with ochratoxin B, but none at all with ochratoxin α, coumarin, 4-hydroxy-coumarin, and D,