Talanta 191 (2019) 89–93

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Automated fluorimetric sensor for the determination of zearalenone mycotoxin in maize and cereals feedstuff

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ARTICLE INFO

Keywords:
Zearalenone
Mycotoxin
Cereal
Maize
Multicommutated flow injection analysis
Optosensor

ABSTRACT

Zearalenone (ZEA), a mycotoxin produced by several *Fusarium* molds, can be found in many cereals and related products. The toxicity of ZEA has been reported for both humans and animals. Therefore, many countries have adopted regulations in foods and feed materials to limit the exposure to this contaminant. In this paper, we propose a multicommutated flow-through optosensor to quantify ZEA in different cereal samples. ZEA was retained and pre-concentrated on C_{18} silica gel, and the use of the multicommutated flow manifold allowed the automated retention/desorption of ZEA on the solid microbeads by the use of appropriate carrier/eluting solutions, hence increasing the selectivity and sensitivity of the system. The native fluorescence of ZEA was recorded on the solid phase at $\lambda_{exc}/\lambda_{em}$ of 265/465 nm/nm. A QuEChERS procedure was used to carry out the extraction of ZEA from different cereal samples (feedstuff materials). Recovery studies were performed to assess the accuracy of the method, obtaining recovery yields between 93% and 107% in all the analyzed samples. LC-MS was employed as reference method. The quantitation limit of the proposed method was low enough to fulfill the maximum residue levels established by the Commission of the European Communities, thus demonstrating its potential use for the analysis of ZEA in feedstuffs.

1. Introduction

Mycotoxins are toxic secondary metabolites produced by different filamentous fungi, mainly members of the genera *Aspergillus*, *Fusarium*, and *Penicillium*. *Aspergillus* and *Penicillium* species frequently grow on foods and feeds during the storage, whereas *Fusarium* species often infect growing crops and produce mycotoxins before or immediately after harvesting [1]. It is estimated that approximately 25% of the world's crops are affected by mycotoxin contamination each year [2,3], which not only causes economic losses, but also high risks for human health. Hence, there are strict regulations to control mycotoxins levels in most of the countries worldwide. The most common mycotoxins found in foods and feeds are aflatoxins, ochratoxins, fumonisins, patulin, and zearalenone (ZEA).

ZEA (14,16-dihydroxy-3-methyl-3,4,5,6,7,8,9,10-octahydro-1H-2-benzoxacyclotetradecine-1,7-dione) (Fig. 1) is a *Fusarium* metabolite with potent estrogenic activity. It is biosynthesized by a variety of Fusarium fungi, including *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. equiseti*, *F. crookwellense* and *F. semitectum* [4], which are regular contaminants of cereal crops. ZEA is not only considered a mycotoxin, but also a phytoestrogen, a mycoestrogen, and a growth promotant [5].

ZEA is formed mainly after the harvesting of cereals due to inadequate hygiene practices and inadequate conservation of the cereals during transportation and storage. Among the different cereals that are contaminated with ZEA, maize and related products are the most commonly contaminated by this mycotoxin [6]. Considering the mean levels of ZEA in foods, the average daily intakes of this contaminant range between 0.8 and 29 ng kg^{-1} for adults and 6 − 55 ng kg^{-1} for small children [7]. Although the International Agency for Research on Cancer does not classify ZEA as potential carcinogenic to humans, several studies have suggested that further investigation is required concerning the potential effects of ZEA in the promotion of breast cancer [8,9]. Hence, due to the high consumption of cereals and cereals by-products by both human and animals, it is important to have accurate analytical methods for the routine control of this contaminant.

The most common analytical methods for the quantification of ZEA involve liquid chromatography with mass spectrometry detection (LC-MS) [10–14]. Moreover, the use of immunoassays with different approaches [15,16], as well as electrochemical methods [17,18] have been also reported. Regarding the use of luminescence methods, only a molecularly imprinted polymer-based fluorescence sensor has been developed [19]. We propose here an alternative flow-through luminescence optosensor for the determination of ZEA without using any derivatization step. With this purpose, we implemented solid-phase

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https://doi.org/10.1016/j.talanta.2018.08.049
Received 8 June 2018; Received in revised form 15 August 2018; Accepted 17 August 2018
Available online 18 August 2018
0039-9140/

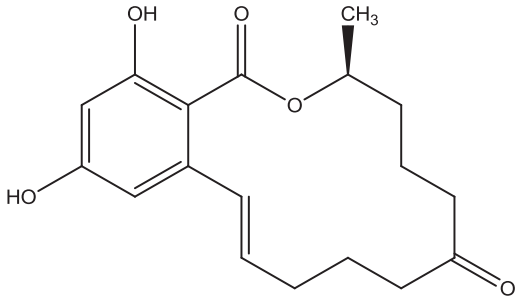

Fig. 1. Zearalenone structure.

spectroscopy (SPS) in a multicommutated system, making use of 3-way solenoid valves. The use of SPS provides an enhancement in the selectivity and sensitivity of the method by pre-concentrating the analyte in the detection zone [20]. In addition, the use of multicommutation allows the automation of the manifold, hence minimizing sample consumption and improving the sample throughput [21,22].

More data on occurrence of ZEA in feed materials are needed to improve exposure assessment for animals. The developed method has been satisfactorily applied to the analysis of ZEA in maize and maize-based feeds, fulfilling the maximum residue limit recommended by the European Union of 3 mg kg^{-1} [23].

2. Experimental

2.1. Reagents and solutions

ZEA (Sigma, Madrid, Spain) stock solution of 100 µg mL^{-1} was prepared in ethanol (EtOH) and was stored in the dark at −20 °C. Working solutions were prepared daily by dilution with deionized water.

Methanol (MeOH), ethanol (EtOH), acetonitrile, acetic acid, anhydrous sodium acetate, hydrochloric acid (HCl), aflatoxin B1, ochratoxin A, patulin, sodium hydroxide (NaOH), ammonium (NH_3), and ammonium chloride (NH_4Cl) were obtained from Panreac (Barcelona, Spain), whereas anhydrous magnesium sulphate ($MgSO_4$) was obtained from Sigma. Citrinin was purchased from Acros Organics (Thermo Fisher Scientific, Madrid, Spain).

Bakerbond C_{18} Prep LC Packing was purchased from Serviquimia (Tarragona, Spain) and Primary-Secondary Amine (PSA) (Supelclean PSA SPE bulk packing) was obtained from Supelco (Bellefonte, PA, USA). Sephadex-QAE A-25 and Sephadex-SP C-25 resins, both 40–120 µm average particle size, were bought from Sigma, whereas C_{18} bonded phase silica gel, 55–105 µm average particle size, was obtained from Waters (Milford, USA).

2.2. Instrumentation and apparatus

Luminescence measurements were made with a Cary-Eclipse Luminescence Spectrometer (Varian Inc., Mulgrave, Australia), controlled by a Cary-Eclipse (Varian) software. We constructed the flow system with a Gilson Minipuls-3 four-channel peristaltic pump (Villiers Le Bel, France), 161T031 NResearch three-way solenoid valves (Neptune Research, West Caldwell, NJ, USA), solvent-resistant pump tubing (Spetec, Erding, Germany), PTFE tubing of 0.8 mm internal diameter, and methacrylate connections. The three-way solenoid valves used in multicommutated system act as a switch between two different positions, “off” and “on”, remaining permanently connected two of the three valve ports. An electronic interface based on ULN 2803 integrate circuit (Motorola, Phoenix, AZ, USA) was used to generate the electric potential (12 V) and current (100 mA) needed by the solenoid valves, which were controlled by a software written in Java.

A Hellma flow cell 176.752-QS (Hellma, Mülheim, Germany) was used (25 µL of internal volume and a light path length of 1.5 mm). The cell was filled with C_{18} solid phase microbeads and blocked at the exit with glass wool to prevent the displacement of the particles.

A pH-meter Crison GLP21 (Crison Instruments, Barcelona, Spain), a Selecta Ultrasons ultrasonic bath (Barcelona, Spain) and a centrifuge Mixtasel-BL (Selecta, Barcelona, Spain) were also used.

The liquid chromatography system was an Agilent capillary HPLC 1100 (Agilent Technologies, Santa Clara, CA, USA), with a Kinetek C_{18} column (50 × 2.1 mm; 2.6 µm particle size) from Phenomenex (Madrid, Spain). The mobile phase consisted of water: formic acid (100:0.1, v-v) and acetonitrile. The gradient varied from 10% acetonitrile to 60% acetonitrile in 14 min, at a flow-rate of 0.2 mL min^{-1}. The chromatograph was connected to an ion trap mass spectrometer (Esquire 6000, Bruker Daltonics, Billerica, MA, USA) with an electrospray interface. Analyses were carried out in negative ion mode, in full scan (100–500 *m/z*) and MS/MS mode (317→175) for ZEA quantification. Five microliters of sample was inserted.

2.3. Sample treatment

We analyzed maize and samples of animal feed (rabbit and hamster) that contained maize. All samples were purchased at local markets. The QuEChERS extraction method [24] was designed for samples with a high percentage of humidity. Therefore, it has to be adapted for dry samples, such as the ones here analyzed.

Before performing the extraction procedure, 200 g of each sample were homogenized using a high-speed laboratory homogenizer. Then, 200 mL of deionized water were added and the mixture was homogenized again. For the extraction process, 15 g of the sample slurry were weighed in a 50 mL PTFE centrifuge tube and 15 mL of 1% (v: v) acetic acid in acetonitrile were added. The screw cap was closed and the tube was energetically shaken for 1 min by hand. Then, 1.5 g of anhydrous sodium acetate and 6 g of anhydrous $MgSO_4$ were added, repeating the stirring process for 1 min. Then, the tube was centrifuged at 3700 rpm for 3 min 5 mL of the supernatant (acetonitrile phase) were taken with a pipette and transferred to a 15 mL centrifuge tube. After the addition of 750 mg anhydrous $MgSO_4$, 250 mg PSA sorbent and 250 mg C_{18} sorbent, the tube was vigorously shaken for 30 s by hand and centrifuged again for 3 min. An appropriate volume of the obtained extract was further diluted with 0.2 M ammonium chloride/ammonium buffer, pH 8.6, before analysis.

2.4. General procedure

The flow manifold is shown in Fig. 2. In the initial state, all valves are switched off and the carrier, 50% MeOH, flows through the flow-through cell while the other solutions recycle to their containers. The sample (prepared in 0.04 mol L^{-1} NH_4Cl/NH_3, pH 8.6) is introduced by simultaneously switching valves V_1 and V_2 on for 30 s. The sample is carried towards the flow-through cell - filled with C_{18} silica gel - and ZEA is retained on the solid support, where its signal is recorded (265/465 nm/nm for excitation/emission wavelengths, respectively). ZEA is eluted by the carrier itself once the analytical signal has been recorded. However, when sample extracts are analyzed, an additional eluting solution is needed to completely regenerate the solid support (due to the absorption of some components of the samples). Hence, once every five samples, MeOH is inserted in the system by activating valves V_1 and V_3 for 30 s. Thus, the system is ready for the next sample insertion. All calibration standards and samples were analyzed in triplicate.

3. Results and discussion

3.1. Selection of the solid support

First of all, the level of the solid support in the flow cell needs to be carefully adjusted, so that the light beam is focused exactly on the