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Detection of Aflatoxins- A Critical Review

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ABSTRACT *Aflatoxins are metabolic products produced by toxigenic strains of Aspergillus species. They are mutagenic and carcinogenic substances. Assesment of exposure to these toxic metabolites is of prime importance both in environmental and food commodities as well as in vivo in human samples. There are several electrochemical and immunochemical methods of detection of aflatoxins. Also, some newer techniques have been developed in recent years. This review is an attempt in this direction to discuss the methods of aflatoxin detection so that potential and actual exposure to aflatoxins can be determined.*

KEYWORDS : Aflatoxins, Aspergillus, Detection, Chromatography

1. Introduction

Aflatoxins are naturally occurring mycotoxins that are produced primarily by some strains of *Aspergillus flavus* and by most strains of *A. parasiticus*, and also some other species like *A. nomius, A. ochraceoroseus, A. bombycis, A. pseudotamari* (Bennet & Klich, 2003). The four major aflatoxins are B_1, B_2, G_1, and G_2.Many other aflatoxins (e.g., M1, M2 P_1. Q_1, B_{2a}, and G_{2a}) have been described which are the biotransformation products of the major metabolites (Heathcote & Hibbert 1978).

Aflatoxin B_1 (AFB1) is the most potent natural carcinogen known and is usually the major aflatoxin produced by toxigenic *Aspergillus*.

2.Methods for aflatoxin detection

Several methods for detection and quantification of AFs have been devised. The principal immunochemical based assay is the enzyme linked immunosorbent assay (ELISA). Other methods are based on electrochemical and optical principles like: chromatographic technique, UV-absorption, fluorescence, spectrometry, and immunochemical assays. However, these methods are more demanding as they need equipped laboratories, trained personnel, harmful solvents and are time consuming. Novel methods have been developed like biosensors, electrokinetics, electrochemical transduction, amperometric detection, and adsorptive stripping voltammetry.

2.1. Electrochemical methods

This technique uses electricity and electrochemical immunosensors. It employs two electrodes, one of which (called as measuring electrode) is coated with specific antibodies which will retain aflatoxins in the sample, whereas the other electrode is ususally made of a combination of Ag / AgCl.

2.2.Chromatography:

The most common techniques of chromatography are Gas chromatography (GC), liquid chromatography (LC), High performance liquid chromatography (HPLC) and thin layer chromatography (TLC). They may be followed by fluorescence detections stage (Cavaliere et al, 2006). These techniques are sensitive, but they frequently require lengthy sample pretreatment and high quality equipment (Sapsford et al. 2006, Vosough et al. 2010).

2.2.1.Thin-Layer Chromatography:

Thin layer chromatography (TLC) is one of the most widely used separation techniques for aflatoxins. It is the method of choice for identification and quantification of aflatoxins at levels of even 1 ng/g (Stroka & Anklarn, 2000). TLC is based on the separation of compounds by how far they migrate on a specific matrix with a specific solvent. The distance that a compound will travel is a unique identifier for specific compounds, and a retention factor (Rf) has been determined for most mycotoxins.

2.2.2.Liquid Chromatography:

Liquid chromatography (LC) is similar to TLC. Liquid chromatography methods in the field of aflatoxin detection include normal-phase LC (NPLC), reversed-phase LC (RPLC) with pre-column derivatization (BCD), RPLC followed by postcolumn derivatization (PCD), and RPLC with electrochemical detection.

2.2.3.High performance liquid chromatography (HPLC):

This is similar to TLC owing to same type of sampling and extraction methods. However, it is much rapid and accurate as compared to TLC. Both normal-phase and reverse-phase HPLC separations can be used for aflatoxin analyses.

2.3. Immunochemical Methods:

These highly specific tests are based on the affinities of the monoclonal or polyclonal antibodies for aflatoxins.

The three types of immunochemical methods are:

2.3.1. Radioimmunoassay (RIA): It is a very sensitive technique used to measure antigens. Although the RIA technique is extremely sensitive and specific, it requires special precautions, since radioactive substances are employed. It is similar to ELISA,except the fact that here antigen-antibody reaction is measured using radioactive signal rather than colorimetric method.

2.3.2.Enzyme-linked immunosorbent assay (ELISA): ELISAs are usually performed in 96-well plates. ELISA is a five-step procedure: 1) Coat the microtiter plate wells with antigen (if antibody is to be detected and vice versa); 2) block all unbound sites to prevent false positive results; 3) add primary antibody to the wells; 4) add secondary antibody conjugated to an enzyme 5) substrate will combine with the enzyme to produce a colored reaction, thus indicating a positive reaction. Direct competitive type of ELISA is commonly used in mycotoxin analysis

2.3.3.Immunoaffinity column assay (ICA): It contains anti-mycotoxin antibody that is immobilized onto a solid support.The sample extract is applied to an IAC. The mycotoxin binds to the antibody and water is passed through the column to remove any impurities. By passing a solvent such as methanol through the column, the captured mycotoxin is removed from the antibody and thus eluted from the column. The mycotoxin elute is developed by the addition of a chemical substance.

2.4.Other methods

2.4.1.Fluorescence

All the aflatoxins have a maximum absorption around 360 nm (Akbas & Ozdemir, 2006). 'B' and 'G' of the aflatoxins refer to its blue (425nm) and green-blue (450nm) fluorescence colours produced by these compounds under Ultra Violet (UV) light.

2.4.2.Ultra violet absorption

The aflatoxins have a maximum absorption around 360 nm with a molar absorptivity of about 20,000 cm^2 /mol nm (Akbas & Ozdemir, 2006). Even though aflatoxins could be detected by UV absorption, the sensitivity of such systems is not sufficient to detect these compounds at the parts per billion (ppb) levels required for food analyses (AlcaideMolin et al. 2009). Hence, fluorescence (FL) techniques have

become popular for AFs detection. UV absorption technique can also be combined with HPLC. HPLC-UV systems still are less sensitive than HPLC-FL systems, especially at low AF levels (Herzallah, 2009).

2.4.3.Spectrometry:Ion mobility spectrometry

It is used in the characterization of chemicals on the basis of speed acquired by the gas-phase ions in an electric field. To detect aflatoxins in a test sample, this is evaporated and mixed with a carrier gas and entered into the Ion Mobility Spectrometer (IMS) where the mixture is ionized and passed through an electric field gradient, where ions of different substances will travel at different speeds.

2.4.4. Rapid enzymatic method: AFB determination is based on acetylcholinesterase (AChE) inhibition, and the AChE residual activity is determined using the colorimetric method (Ellman's method) (Moscone et al 2011).

2.4.5. Permeation chromatography- high performance liquid chromatography-fluorescence detection (GPC-HPLC-FLD): A newer technique called GPC-HPLC-FLD has been developed for determination of benzo(*a*)pyrene and aflatoxins (B1, B2, G1, G2) in vegetable oil. Sample is extracted with ethyl acetate/cyclohexane and cleaned with the GPC. The separation of target compounds is performed on a Extend C_{18} column with methanol and ammonium acetate solution as mobile phase with gradient elution at a flow rate of 1.0 mL min^{-1}(Wang et al. 2014).

3.Assessment of human exposure to aflatoxins

Presence of aflatoxin-albumin or aflatoxin-DNA adduct in human body are the surrogates for genotoxicity in people.

AFB requires bioactivation to a reactive metabolite mainly exo-AFB1 8,9-epoxide, which if not detoxified, binds to double-helix DNA to form AFB1-N7-guanine adduct or, following hydrolysis to the AFB1-dihydrodiol, with proteins such as albumin (Baertschi et al. 1988). AF-albumin can accumulate after chronic exposure (Wild et al. 1996). The only AFB1 adduct structurally identified to date in enzymatically digested plasma albumin is AFB1-lysine (Wild et al. 1990, Guengerich et al. 2002). This is measured by ELISA,HPLC with fluorescence detection and by isotope dilution mass spectrometry (IDMS) Wild et al. 1990 , McCoy et al. 2005). Relatively inexpensive equipments and good performance of the ELISA has made it the method of choice.

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