False Positive Detection of Aflatoxins in Herbal Preparations

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Abstract

Herbal preparations are tested for aflatoxins, using Thin Layer chromatographic (TLC) and High performance Liquid chromatographic (HPLC) detection methods.Such preparations however, are rich in various plant pigments as well as a wide range of other compounds, which can have florescence characteristics similar to those of aflatoxins. When one-directional TLC, shows positive results for aflatoxins, subsequent confirmation tests indicate that they are not really aflatoxins. However, by two-dimensional TLC followed confirmation those positive compounds would be detected more clearly; aflatoxin may not be detected below the level of 5 ppb using TLC. On the other hand, when HPLC analysis gives positive results for aflatoxins, confirmation is necessary using two-dimensional TLC. For instance, in this study, HPLC analysis indicated aflatoxin G_1 at 315 ppb and 227 ppb in two such herbo-mineral preparations, but two-dimensional TLC showed that they are not aflatoxins. It points to the fact that great care is necessary when TLC and HPLC methods are used to detect aflatoxins in herbal preparations. When high levels of contamination with aflatoxin are suspected in HPLC analysis, it is recommended to use two-dimensional TLC for the confirmation.

Introduction

Traditional herbal preparations as well as Ayurvedic medicinal formulations are well known to have curative properties against most vulnerable diseases. Many of them contain blends of herbs and in certain instances, they are combined with minerals too [1]. Presence of natural toxic compounds such as aflatoxins formed due to poor post harvest conditions and bad storage can lead to risk of food poisoning. Stringent regulations imposed by national and international regulatory authorities, require scrutinization of such preparations for quality assurance against toxic substances including aflatoxins [2].

Aflatoxins are secondary metabolites of fungi mainly *Aspergillus flavus* and *Aspergillus parasiticus* and they are known to be toxic to animals as well as humans [3].Herbs and spices should therefore be processed hygienically during the harvest and post harvest stages to minimize the risk of aflatoxin contamination. Herbs and spices contain large amount of organic compounds with curative properties on various ailments. Some of these compound also posses similar characteristics and same fluorescence properties as those of aflatoxins when separation is performed by TLC. Same phenomenon is experienced when they are analyzed by HPLC. Unless confirmation tests are performed, such compounds could be estimated erroneously as aflatoxins. Such false positive detection of aflatoxins in some instances; may give very high levels [4].

Confirmation of presence of aflatoxins therefore is an important step in aflatoxin analysis. Methodologies for aflatoxin analysis are well documented in AOAC. The successful validation of method with acceptable clean up followed by well established detection system are necessary for accurate reporting of results. A descriptive and sensitive method for the determination of aflatoxins by HPLC in various food matrices at a level of 2 ng/g for aflatoxin B_1 and 4 ng/g for total aflatoxins, has recently been successfully validated and it is presently in the process of being adopted as an official method (CEN and AOAC International) [5]. In order to obtain reliable results for convincing the consumers and producers of the confidence in testing methods, there is an urgent need for such internationally validated methods, which could serve as confirmatory methods.

During the survey on storage of selected species, a higher frequency of occurrence of *A. flavus* was observed in most spices samples stores in ambient temperature [6].

In the initial mycotoxin researches, TLC became a very common and popular technique to separate extracted

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