Determination of Aflatoxin contaminants in chrysanthemum by HPLC

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Abstract :A method was developed for the simultaneous determination of Afiatoxin (AFB_1、AFB_2、AFG_1、AFG_2) in chrysanthemum is developed by Liquid chromatograPhy with fluoreseence detection after immunoaffinity column clean一up and Pre一cloumn derivatization.four south medicine samples were extracted with methanol一water(70:30,v/v),The extracts were Purified by imunoaffinity columns and the toxins were separated by reversed phase HPLC, and quantified with fluorescenee detection after photoehemieal derivatization. The separation was perfored on a C_{18} column.Recoveries of the different medicines spiked with Aflatoxin, The average recoveries ranged from 81.0 %～94.3 % by addition of avermectin standards at three different concentration levels of 10 μg/kg) to chrysanthemum as matrixes. The sensitivity, accuracy and precisiothod were able to meet the requirements for pesticide residue analysis.

Introduction

chrysanthemum,which are rich in Chuzhou,Huangshan,Bozhou,Hangzhou of China. They are called the four most famous chrysanthemum. However, in the process of production, processing, storage, transport, due to the condition and technology simple, It is easy to mildew and produce aflatoxin.Aflatoxins (AFs) are secondary metabolites produced by Wlamentous fungi Aspergillus, particularly Xavus and parasiticus[1].

Aflatoxin contamination during storage is a major problem facing the Agriculture products industry in the world,Especially in the more humid parts. The carcinogenic, mutagenic and immuno-suppressive evects of aflatoxins on several animals have been fully documented[2],and AFB_1 has the strongest toxicity In four kinds of aflatoxins.Human liver cancer and levels of aflatoxins in the daily diet are strictly correlated as epidemiological studies show [3].

AFs do not decompose at the temperature of boiling water during the preparation of the drink[4]. South Korea KFDA (KFDA) issued the AFB_1 standard and test method of Chinese herbal medicine in the notice, requirements, cassia seed, peach kernel, licorice 9 Chinese herbal medicines of AFB_1 lower than 10 μg /kg. European Commission Regulations set limits for AFB_1 and total aflatoxins of 2 ug/kg and 4 ug/kg respectively in groundnuts,nuts, dried fruit and cereals since 1998[5]."China Pharmacopoeia" 2010 edition sets maximum limits also in spices (dried citrus peel. peach kernel and Scaphium scaphigerum (AFB 15 ug/kg,total AFs 10ug/kg).

Materials and methods

Instruments, reagents and materials

Waters 2695 HPLC (Water Corporation); High-speed Homogenizer; Nitrogen blow instrument(United States Organomation); Centrifuge (Japan-Hitachi); Vortex mixer (Germany IKA);Solid-phase extraction device (HP6019); Electronic balance (AUY220, Shimadzu Corporation).

Acetonitrile and dichloromethane was of analytical grade, and methanol was of analytical and HPLC grade. Water was distilled twice. The cartridges used for solid phase extraction was Aflatoxin immunoaffinity column(3 ml, 300ng). The concentration of AFs standards was high than 95 %. Stock solutions (1.0mg/ml) of the reference standards was dissolved in methanol and stored at -18℃.

Instrument conditions

The target was detected by high performance liquid chromatography (HPLC) with a fluorescence detector) (λ=360nm excitation,λ=435 nm emission). An Symmetry C_{18}(4.6×250mm, 5μm) chromatograph column was employed and was operated at 35℃.Optimum separation was achieved using Gradient elution. Mobile phase consisted of A: acetonitrile;B: water with 0.1 %(v/v) acetic acid. The gradient was set up as follows: 0～8.0 min, 20 %-30 %A; 8.0 min, 30 %A,takes ten minutes.The injection volume was 10 μL, and the flow-rate was 0.3 ml/min.

Preparation for analysis

Samples (5*0.01 g) was prepared in 250 mL cone flask with plug, added 5 g Sodium chloride and 150 ml methanol/water(70/30). After Homogeneous for 2 minutes, the sample extract was fltered. The immunoaffinity column was washed prior to use with 10 ml phosphatebuffer saline (pH 7.4) at flow rate of 3ml/min, then 15 ml of clear filtrate was diluted with 30 ml waters and applied to the conditioned column (6 ml

/min). After that, thecolumn was washed with 10 ml water (5 ml/min) twice and driedby passing air through it. Finally, bound aflatoxins were eluted slowly with 1.0 ml methanol and pushing air through the column to collect all elution liquid in glass test tube, and finally filtered through 0.22 μm filters. In the end,keep the supematant for analysis.

Accuracy of methods

Sample with no aflatoxins residue was analyzed following the same procedure as the above samples,at the same time, setting additional levels 10 ug/kg, Recovery and precision of the method were tested by standard addition method. The results of the recovery tests and statistical data are given in Table 1. It indicated that this method has a good accurate.

Table 1 Recovery of aflatoxins B1, B2, G1 and G2 from blanks samples spiked with known concentration of toxin and statistical data

		AFB_1	AFB_2	AFG_1	AFG_2
chrysanthemum	Recovery (%)	81.0	88.0	91.5	94.3
	Standard deviation (SD)	2.3	4.6	1.9	5.2
	Reproducibility (DSR) (%)	2.7	5.2	2.1	5.8

Results and discussion

All data were obtained without any interference in the analysis. Aflatoxins analysis on spices are not simple. because of the highly coloured contaminating materials
that are co-extracted with AFs. The 75% of chrysanthemum resulted contaminated
from four aflatoxins.and AFB_1 is high.

Conclusions

This study provides useful information about the risk of aflatoxins hazard and hopes to raise the consciousness among consumers, researchers, farmers and traders about
the importance to improve processing methods (planting,drying, transporting and storaging) and to establish a monitoring programme on medicine and the necessity to obtain more and more data on the distribution and contamination levels of AFs in south medicines.

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