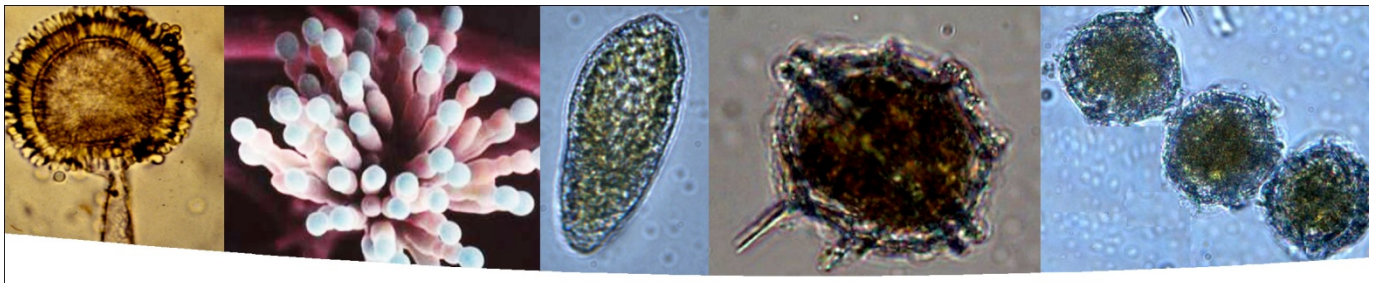

PROCEEDINGS

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P-14 COMPARISON OF PRECOLUMN AND POSTCOLUMN DERIVATIZATION SYSTEMS FOR THE LIQUID CROMATOGRAPHIC DETERMINATION OF AFLATOXINS IN PEANUTS

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Background: The monitoring of aflatoxins in food commodities as a consequence of the regulations established by many countries depends on the availability of adequate analytical methods. The most widely used method for the determination of aflatoxins in food and feed uses HPLC coupled with fluorescence detection. However, since aflatoxin B_1 and aflatoxin G_1 present less natural fluorescence, in order to improve the signals during analysis, various pre or postcolumn derivatization methods are used for signal enhancement.

Aim: To compare the method recovery when precolumn and postcolumn derivatization systems are used in the determination of aflatoxins in peanuts by liquid cromatography with fluorescence detection.

Materials and Methods: 25g samples of raw shelled peanuts and free of aflatoxins contamination were spiked with a pool of aflatoxin standards (2.02µg/Kg for B_1 and G_1 aflatoxins and 1.02µg/Kg for B_2 and G_2, 6.13 µg/kg total aflatoxins). The recovery tests were performed in triplicate.
The extraction and cleanup of the extract, using immunoaffinity column (Aflatest, Vicam Somerville, MA, USA), were done according to the AOAC Official Method 991.31 (AOAC, 2005a).
The precolumn derivatization was based on the AOAC Official Method 994.08 (AOAC, 2005b). A solution of trifluoroacetic acid, acetic acid and water 20:10:70 (v/v/v) was prepared to catalyze the conversion of aflatoxin B_1 and G_1 in B_{2a} and G_{2a}, respectively. The mixture of the extract and the derivatizing solution was heated to 65°C for 9 minutes to complete the derivatization reaction. Chromatographic conditions: mobile phase was methanol : acetonitrile : water in a gradient elution mode, starting composition 10:10:80 v/v/v, reaching 15:25:60 v/v/v in 3 minutes at a flow rate of 1.2 mL/minute; the C_{18} column, 150 mm x 4.6 mm, 5µm (XTerra® Waters) was maintained at 40˚C; the fluorescence detector operated at 364 nm excitation and 440 nm emission; 10 µL of the derivatized extract were injected.
Postcolumn derivatization was carried out as described in AOAC Official Method 999.07 (AOAC, 2005c), using an electrochemical cell (Kobra cell® - Rhône Diagnostics Ltd, Glasgow, UK). Chromatographic parameters: the mobile phase consisted of methanol : acetonitrile : water (for each 1L mobile phase 120 mg of potassium bromide and 350 µL of 4M nitric acid were added) in a gradient elution mode, starting composition at 20:20:60 v/v/v reaching 20:30:50 v/v/v in 3 minutes at a flow rate of 1.2 mL/minute; a C_{18} column, 250 mm x 4.6 mm, 5µm (XBridge® Waters) was kept at 40˚C; the fluorescence detector operated at 364 nm excitation and 440 nm emission; 30µL of the extract were injected.

The aflatoxins quantification was carried out based on a calibration curve established by the external standard method with seven concentrations within the working range of 0.0004 to 0.0203 µg/mL, for B_1 and G_1 aflatoxins, and 0.0002 to 0.0103µg/mL, for B_2 and G_2 aflatoxins.

Results and Discussion: The recovery rates for the precolumn derivatization method (95%, 88%, 89%, and 59% for B_1, B_2, G_1, and G_2, respectively) were within the acceptable recovery range (70 to 110%) indicated by the European Union Regulation n° 401, 2006, for concentrations between 1 and 10 µg/Kg of total aflatoxins, except for aflatoxin G_2, that shows inherent difficulty in recovering when it is purified by immunoaffinity columns. High recovery rates (90%, 85%, 100%, and 69% for B_1, B_2, G_1, and G_2, respectively) were obtained for the postcolumn derivatization.
Recovery is an important validation parameter of analytical methods, since both derivatization modes showed recovery values within the acceptable range, the viability of each method can be considered. The procedure for precolumn derivatization with trifluoroacetic acid promotes the aflatoxin detection and quantification at much lower cost when compared to the postcolumn method, since it is not necessary to purchase additional equipment. However, the analyst's cost must be considered. Also, the time required for the sample derivatization is an addicional step in the analysis, resulting in further human exposure to a solution composed of toxic substances such as acetic acid and trifluoroacetic acid. The postcolumn derivatization of aflatoxins, using the Kobra Cell®, occurs rapidly at room temperature. Moreover, it is not necessary to prepare the derivatizing agent daily and the maintenance is simple and easy. The derivatization reaction becomes part of the chromatographic run. The automation of the derivatization step increases the repeatability of results (results not shown).

Conclusion: Both derivatization methods have shown good recovery rates in the determination of aflatoxins in peanuts.

References:

1. AOAC Official Method 991.31, 2005a: aflatoxins in corn, raw peanuts and peanut butter: immunoaffinity column (aflatest) method. AOAC International, p.49.2.18.
2. AOAC Official Method 994.08, 2005b: aflatoxins in corn, almonds, Brazil nuts, peanuts, and pistachio nuts. AOAC International, p.49.2.19A.
3. AOAC Official Method 999.07: aflatoxin B_1 and total aflatoxins in peanut butter, pistachio paste, fig paste, and paprika powder. AOAC International, p.49.2.29.
4. Commission Regulation (EC) n° 401/2006 of 23 February 2006. Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union, L. 70, p. 12-34 (9.3.2006).