Determination of Aflatoxins in Groundnut Meals by High Performance Liquid Chromatography

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Bestimmung von Aflatoxin in Erdnußschrot durch Hochleistungsfliessigchromatographie


Summary. A method for determination of aflatoxins in groundnut meals by high performance liquid chromatography (HPLC) was investigated. After extraction and purification according to the AOAC “CB” method, an extract is injected on a Corasil II column and eluted by n-hexane/chloroform/ethanol (50+49,7+0,3), Detection is performed with an UV detector. The developed method makes it possible to determine aflatoxins B₁, B₂, G₁, and G₂. The detection limit for aflatoxin B₁ is 50 p.p.b. using a 254-nm UV detector, or less with more effective detectors (absorption at 350—360 nm).

Introduction

Systematic inspection of commercial samples of imported groundnut feed products, mainly from India, carried out in this laboratory, has proved that aflatoxin B₁ was present in all 3100 samples analysed in concentrations from 20 to 8500 p.p.b. The annual averages were as follows (in p.p.b.): 1970 — 350, 1971 — 290, 1972 — 320, 1973 — 770, 1974 — 420. The large range of concentration indicates the need to inspect the largest number of samples possible. Therefore, it is necessary to have a rapid and accurate method of analysis available.
Thin-layer chromatography, used in the majority of methods developed, has well-known disadvantages, such as difficulties in standardisation of resolution, moderate precision [1]. High performance liquid chromatography (HPLC) is free from these disadvantages. Until now, a few papers on the possibilities of application of this new analytical technique to aflatoxin analysis have appeared [2–7]. Recently, Seitz [7] has studied separations of aflatoxins on HPLC columns packed with different adsorbents.

The following report presents a procedure for the determination of aflatoxins in groundnut meals by HPLC on a Corasil II column at a pressure of 10—30 atm. The results for commercial samples of groundnut meals were compared with those previously obtained by TLC.

Experimental

Apparatus

The measurements were carried out on a liquid chromatograph built in the Institute of Chemical Engineering and Measurement Techniques, Technical University in Gdańsk. Operative pressures were: 10–30 atm. Stainless-steel columns 0.2 cm bore and 50 cm long, packed with Corasil II 37–50 µ (Waters Associates, Inc., Mass., USA). Detector UV, monochromatic, 254 nm with 0.01 A full-scale-sensitivity.

Reagents

a) Quantitative standard solution of aflatoxin B₆ in chloroform. Determination of concentration was carried out by measurement of absorbance of benzene-acetonitrile solution at max. absorption (347 nm) by the AOAC method [8]. After the determination, the solvent was evaporated and residue was redissolved in chloroform.

b) A mixture of aflatoxins B₁, B₂, G₁, and G₃ for separation studies was prepared by dissolving the aflatoxins standards in chloroform. The standards were from Makor Chemicals, Jerusalem, Israel (aflatoxin B₁) and from Carl Roth, Karlsruhe, GFR (aflatoxins B₂, G₁, G₃).

c) Chloroform, p.a., containing 0.6% of ethanol as stabilising agent.

d) n-Hexane, chemically pure, from Reachim, Moscow, USSR.

Analytical Procedure

Sample Extraction. 20 g of groundnut meal is mixed with 10 ml of water, 10 g of diatomaceous earth and 100 ml of chloroform. Extraction is carried out by 30 min on a wrist-action shaker. The extract is filtered through anhydrous sodium sulfate and 50 ml of extract is evaporated nearly to dryness in a rotary evaporator.

Extract Purification. Purification is carried out on a column packed with silica gel (0.05—0.2 mm). The column is prepared according to the AOAC Method 1 ("CB" method) [8]. The extract residue is transferred from the evaporator on to the top of the column, and elution is carried out with 150 ml of n-hexane, 150 ml of ethyl ether and 150 ml of 3% methanol in chloroform. The methanol — chloroform eluate is collected and evaporated to dryness in a rotary evaporator and then in an inert gas stream in a 1-ml-phaial.

High Performance Liquid Chromatography. The residue in the phial is dissolved in 100—500 µl of chloroform. About 5 µl of extract, accurately measured, is injected on to the column. The concentration and amount of extract to be injected is adjusted to give 40—80% of chart response. The mobile phase is n-hexane/chloroform/ethanol (50 + 49.7 + 0.3). The optimal flow-rate is about 1 ml/min. The elution volume for aflatoxin B₁ is about 8 ml, and varies for solvents of different purities. Results are calculated from peak areas by integrator, or by other convenient method, after calibration with quantitative standard solutions.