Analysis of Aflatoxin using LC with Post-Column Photochemical Derivatization

The PHRED is applicable to determination of aflatoxins in test extracts of corn and peanuts when using AOAC Method 991.31, Method 999.07, or Method 970.45 with LC. While no significant difference exists for the PHRED in comparison to other post column methods with peanuts, a slightly high bias is obtained with corn when compared with the iodine or Kobra cell possibly due to higher recovery.

Caution

Mycotoxins are toxic substances. Perform manipulations in a hood wherever possible, taking particular precautions, such as using a glove box, when toxins are in dry form because of their electrostatic nature and tendency to disperse. Swab any accidental spills and all glassware and waste materials with 5% NaOCl bleach. Use UV glasses if there is exposure to any direct or reflected UV light from the light source. *(See D. Notes 1 & 3)*

A. Principle

Post-column derivatization of aflatoxins can increase detectability and /or selectivity of responses for the HPLC detector. By performing the derivatization photochemically, the derivative structures B2a and G2a are apparently obtained which provide the enhanced signals for the B1 and G1 aflatoxins without effect on the B2 and G2 aflatoxins.

B. Materials

Apparatus (See D. Note 1)

Equipment noted is not restrictive: equivalent systems can be substituted.

1. SP 8700 XR pump, SP 4200 computing integrator, SP 8780 auto sampler and SP WINNER software (Spectra-Physics Analytical San Jose, CA.) with Kratos FS 970 LC fluorometer set to provide 365nm excitation and 435nm emission.
2. Column, ACE C18, 150mm by 4.6mm with 5μm particle size.
3. "PHRED" photochemical reactor with low-pressure mercury lamp and knitted reactor coils, preferably KRC 25-25 with a 25m coil 0.25mm i.d. (VICAM, # G8501).
4. Silanized vials, 4ml, amber with Teflon lined screw caps.
5. Class A volumetric pipette, 2ml.
6. Replacement plastic grooved ferrules, VICAM # G8510.

Reagents

1. Degassed mobile phase: 40% methanol in 60% water (v/v), or a suitable mixture of methanol, acetonitrile and water that results in baseline separation of the aflatoxins. *(See D. Note 2)*
2. Injection solvent should be the same as that used in mobile phase.
3. Aflatoxin standards: Supelco Inc., Bellefonte, PA, or other suppliers.

Fluorescence detector conditions

Detector parameters should be used which are applicable for the available equipment in accordance with the manufacturer's recommendations. (Conditions found optimum in one lab for their fluorescence detector: Excitation @ 365nm; Emission @ 435nm; Filter Mode, RC Response setting of slow; Digital Filter, 3s or 5s; Gain, 10 or above; Attenuation, 1 or as needed.)

Standards

To establish a standard curve, prepare five concentrations of a mixed aflatoxin standard containing from 0.1 to 1.0ng of toxin per 20μl of injection solvent. These individual extracts should be evaporated to dryness under nitrogen in silanized vials. Subsequently, reconstitute each of the extracts with 2.0ml of injection solvent and stir with a vortex mixer for at least 2 minutes.

C. Analysis

Sample extracts, which are prepared by any of the officially recognized aflatoxin extraction methods of the AOAC using post-column derivatization such as 991.31 and 999.07, or the non-LC method 970.45, should be dried to a film in silanized vials and stored frozen at -20 C until ready for evaluation.

1. Reconstitute sample extracts with 2.0ml of injection solvent and stir with a vortex mixer for at least 2 minutes.

2. Utilizing a 1ml/min. flow rate of the mobile phase and 20µl injections of standards and extracts, begin with the use of a fresh preparation of aflatoxin standards and confirm that the equipment operation is providing the expected standard values for aflatoxins B1, B2, G1 and G2. Adjust flow rate if necessary to effect best separation.
3. Inject 20µl of each sample extract, recording both peak height and area data. If concentration is found to be below 5ng/g, a re-injection of 100µl is recommended which will increase sensitivity by a factor of 5.
4. Calculate concentrations using the equation in 991.31, 999.07 or derived from
   Aflatoxin ng/g = A x (T/I) x (1/W)
   A = ng of aflatoxin as eluate injected.
   T = Final sample eluate volume (µl).
   I = Volume eluate injected into LC (µl).
   W = Mass (g) of commodity represented by final extract.

The use of at least a three-point calibration curve is preferable but a single point calibration can be used if the response has previously been shown to be linear and injections of the standard have been made throughout the run to demonstrate the detector response is within acceptable limits. For example, the 0.5ng/20µl may be used for the calibration after the curve is verified. The useful life of the ultraviolet bulb is approximately 3000 hours, or until a consistent decrease of response (i.e. 10%) is noticed by a change of the B1/B2 peak area ratio for the aflatoxin standard.

D. Notes

1. Evaluation should be made of any leakage of UV light from source equipment and if detected, provide shielding or protective glasses during use.
   To prevent leakage of the knitted reactor coil do not over tighten connection. If leakage occurs, disconnect power to the photochemical reactor before inspecting unit.
2. Unless otherwise specified, use only analytical grade reagents.
   One of the solvent systems indicated for the photochemical system, permits the elimination of acetonitrile. This can be advantageous for some laboratories who wish to eliminate its use. Any combination of methanol, water and acetonitrile can be used so long as the mobile phase provides baseline separation of the aflatoxins.
3. Aflatoxins must be handled with extreme caution as noted in caution statement above since they are known to be carcinogens. Utilize hypochlorite bleach as a cleaning agent when cleaning glassware and disposing of waste materials.