Method 24.2

Analysis of Aflatoxins B_1, B_2, G_1, and G_2 by HPLC

Purpose: To determine the levels of aflatoxins in spices and herbs by HPLC.

A. Apparatus:

1. Blender jars.
2. 150 mL beakers.
3. Glass funnels.
4. 10 mL pipets.
5. 50 mL graduated cylinders with ground glass stoppers.
6. 10-100 μL adjustable air displacement pipet.
7. 10 mL volumetric flask.
8. Parafilm.
9. Aflatest Pump Stand.
10. Hewlett-Packard HPLC 1050 or equivalent.
11. Fluorescence Detector.
12. Kobra Cell.
13. Timer.

B. Reagents:

1. Supleco Aflatoxin Mix Kit-M, (Cat.No.4-6304).
2. HPLC grade Methanol.
3. 80% Methanol/20% DI Water (200 mL of DI water is added to 800 mL of HPLC grade Methanol and mixed thoroughly.).

4. 20% Tween/80% DI Water (100 mL of Tween-20 is added to a 500 mL volumetric flask and brought to volume with DI water.).

5. DI Water.

6. 1% Acetic Acid in DI water (1 mL of ACS grade Acetic Acid is added to a 100 mL volumetic flask and brought to volume with DI water.).

7. Aflatest-P columns.

8. Sodium Chloride - ACS grade.

9. Fluted filter paper.

10. Glass fiber filter paper.

C. Preparation of Sample:

1. Weigh 25 g of sample into a blender jar.

2. Weigh 5 g of salt into blender jar.

3. Add 100 mL of 80% methanol/20% water mix.

4. Cap blender jar and seal with parafilm.

5. Blend at high speed for 1 minute.

6. Filter blender contents through a fluted filter into a 150 mL beaker.

7. Pipet 10 mL of filtrate into 50 mL graduated cylinder.

8. If the sample is nutmeg, oregano, or black pepper, add 40 mL of 20% Tween-20 solution to the graduated cylinder. If the sample is not one of the products mentioned, add 40 mL of DI water to the cylinder. Mix.

9. Filter the contents of the graduated cylinder through a glass fiber filter into a 150 mL beaker. This filtrate will be used for the aflatest column.

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D. Procedure:

1. Standard Preparation:
   a. Allow Supelco Aflatoxin Mix Kit-M standard to come to room temperature.
   b. Put 5 mL of methanol into a 10 mL volumetric flask.
   c. Add 25 uL of undiluted Supelco standard to the methanol. Mix well.
   d. Bring to volume with 1% Acetic Acid in DI water solution.

2. Immuno-Affinity Column Clean-Up:
   a. Attach an Aflatest-P column to the pump stand.
   b. Pipet 10 mL of filtrate on the column and allow to absorb on column.
   c. Once all the sample has passed through the column, rinse the column with 10 mL of DI water. Repeat DI water rinse.
   d. Place an HPLC vial under the tip of the column and add 1 mL of methanol to the column.
   f. Collect all the methanol eluent in the HPLC vial and cap. The sample is now ready for injection into the HPLC.

3. Chromatographic Conditions:
   a. Mobile Phase - 63% DI water with 0.1 mg/L KBr and 0.02% Nitric Acid/22% Methanol/ 15% Acetonitrile.
   b. Flow Rate - 1 mL/minute.
   c. Column - Supelcosil LC-18, 25cm x 4.6 mm ID (cat.no. 5-8298).
   d. Injection Volume - 50 uL.
   e. Detection - Excitation 365 nm, Emmission 464 nm, Filter 408 nm.
   f. Kobra Cell at 100 u-amps.

E. Calculations:

1. Calculation of response factor.

$$RF = \frac{\text{Conc. of Std.}}{\text{Area of Std.}}$$

Conc. of Std. for B_1 and G_1 = 2.5 ppb
Conc. of Std. for B_2 and G_2 = 0.75 ppb

2, Calculation of aflatoxin content in sample.

$$\text{Aflatoxin (ppb)} = \frac{\text{Area of Sample x RF x 50}}{\text{Sample Weight}}$$

F. Statistics:

TBD

G. NOTES:

N/A

H. REFERENCES:

1. Official Methods of AOAC International (1996) 990.33 (49.2.17); for corn and peanut butter.

2. Aflatest Manual.

3. Hewlett Packard HPLC manual.

4. J. Leek Associates, Inc.