

15.0 ESTIMATION OF AFLATOXIN B₁ IN FEED

15.1 Estimation of Aflatoxin B₁ – HPLC method

Reagents

- Acetonitrile AR grade
- Distilled water
- Acetonitrile HPLC grade
- Water HPLC grade
- Nitrogen gas
- Aflatoxin B₁ standard from Sigma.

Apparatus

- Amber colored beaker
- Amber colored conical flask
- HPLC



Fig. 15.1 High performance liquid chromatography

Procedure

Extraction

- Weigh 25 g of ground sample into an Erlenmeyer flask or small blending jar.
- Add 100 ml of 80/20 acetonitrile/ water to the sample.
- Secure a lid tightly and shake for 1 hour or blend on high for minutes.
- Filter the extract into a sample jar and cover with a lid.

Purification process

- Place the purification column in a 15 x 125 mm test tube.
- Pipette 2 ml of extract and spiking solution into the top of the purification column.
- Push approximately 500 µl of solutions through the column.

HPLC conditions

- Mobile Phase : Combine 350 ml of water with 650 ml of Acetonitrile.
- Flow Rate : 0.5ml/min; Approximate Retention Time : 2.9
- Fluorescence Detector: Emission = 430 nm; Excitation = 369 nm

Preparation of standards

Take required quantity of standards (0, 0.25, 0.5, 1, 5, 10, 20, 50, 75, 100 and 200 ng) in clean vials and dry it under stream of nitrogen gas. Then add 150 ml of trifluoro acetic acid for derivatisation and after an interval of 10 minutes, add 850 ml of mobile phase to make it 1ml. Inject 20 µl of this solution in HPLC (Fig. 15.1) for standard peaks with areas.

HPLC injections

Take 100 ml extract from purified extraction and dry it under stream of nitrogen. Add 150 ml of trifluoro acetic acid and 850 ml of mobile phase to make 1 ml. Now it is ready for HPLC injection. Sample peak and area will be compared with standards.

Calculations

$$\text{Aflatoxin B}_1 (\mu\text{g/Kg}) = \frac{\text{Baf} \times \text{Vext}}{\text{Vb} \times \text{W} \times \text{Vf}/100}$$

Baf (ng)- Concentration of aflatoxin B₁ from calibration curved solution

Vex - Volume in which dried extraction dissolved

Vb - Volume injected in HPLC.

W - Weight of the sample.

Vf - Volume of filtrate taken in column.

100 - 100 ml of extraction solution.

Reference: AOAC (1997) 16th edition