15.3 Estimation of aflatoxin B_1 - TLC method

Principle

Methods of aflatoxin analysis are mainly based on their solubility characteristics in different solvents as well as on their characteristic fluorescent properties. The solubility of aflatoxins in organic solvents like chloroform, methanol, ethanol, acetone, acetonitrile, benzene etc., helps in their quantitative extraction from the various samples. Their insolubility in diethyl ether, petroleum ether and hexane helps in separating them from certain interfering pigments and fats. Their characteristic fluorescence and absorption in the long wavelength range have visible and UV light aids their detection and estimation.

Thin layer chromatography

Thin layer chromatography (TLC), also known as flat bed chromatography or planar chromatography is one of the most widely used separation techniques in aflatoxin analysis. Since, 1990 it has been considered the AOAC official method and the method of choice to identify and quantitative aflatoxins at levels as low as 1 ng/g. The TLC method is also used to verify findings by newer, more rapid techniques.

Reagents

- Petroleum ether (40-60ºC)
- Chloroform
- Anhydrous sodium sulphate
- Silica gel
- Diethyl ether
- Hexane
- Methanol
- Benzene
- Acetone
- Nitrogen gas
- Aflatoxin B_1 standard

Apparatus

- Amber colored beaker
- Amber colored conical flask
- Rotary vacuum evaporator
- Mechanical shaker
- Chromatography column (300 x 22)

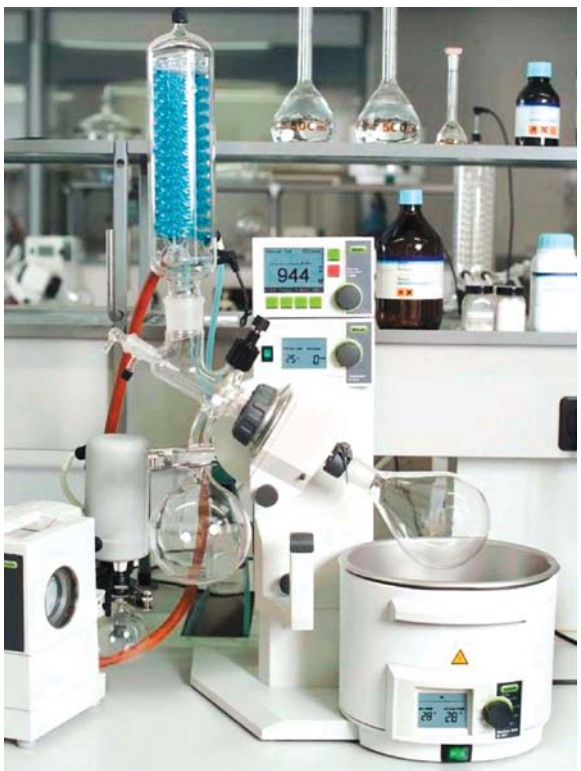

Fig. 15.3 Rotary vacuum evaporator

Procedures

Extraction

Weigh accurately 50 g of the well mixed sample in a 500 ml glass stoppered conical flask. Add 150 ml of petroleum ether, mix and keep overnight. Filter out the ether using an ordinary filter paper. To defatted sample in the flask, add 250 ml of chloroform mix and place in the mechanical shaker for 30 min. Filter the contents through an ordinary filter paper followed through a bed of anhydrous sodium sulphate.

Column chromatography

Prepare a chromatographic silica gel column (300 mm x 22 mm i.d.) using glass wool, anhydrous sodium sulphate (5 g), slurry of activated (at 110 ± 1 ºC for 1 h) silica gel (10 g in almost an equal amount of chloroform) and anhydrous sodium sulphate (7.5 g), in the

order mentioned. Pour 50 ml of the prepared extract on the column and elute it. Then pour 150 ml of diethyl ether: hexane mixture (3:1, v/v). Elute the aflatoxin from the column using 150 ml of chloroform: methanol mixture (97:3, v/v) and collect the elute in a 250 ml round bottom flask.

Concentrate the extract in a rotary vacuum evaporator (Fig. 15.3) and then dry the concentrated extract on a water bath under a gentle stream of inert (nitrogen) gas.

Preparation of activated TLC plate

Prepare TLC plates of silica gel G (8-10 g with about 20 ml of water on a plate of 20 x 20 cm size) using an applicator (0.40 mm thickness). Air-dry the plates overnight. Activate the plates in oven at 110 ± 1ºC for 1 h. Dilute the dried extract to a fixed volume (e.g. 200 µl) with benzene: acetonitrile mixture (98:2, v/v). Apply 5 or 10 µl of the diluted extract on the marked TLC plates (3 cm above the bottom edge of the plate) along with standard aflatoxin B_1 solution representing 2.5, 5.0, 7.5 and 10 µg concentrations. Run the plates to about 15 cm height in a solvent system (chloroform: acetone, 90:10 v/v). Remove the plates and air dry. Observe the plates under UV light at 360 nm in a UV chromatography view cabinet. Compare the intensities of the fluorescent of the spots produced by the sample with those produced by the standard for quantifying the aflatoxin content in the sample.

$$\text{Aflatoxin } B_1 \text{ content } (\mu g/kg) = \frac{C \times V_1 \times V_3}{M \times V_2}$$

Where,

C - Concentration of aflatoxin B_1 from calibration curved solution (µg/ml)

V_1 - Final volume of extract

V_2 - Volume of extract applied on the TLC plate.

V_3 - Volume of standard B_1 solution applied on the TLC plate whose fluorescence intensity is similar to that of sample (µg)

M - Weight of the sample.

Reference: IS 1992b.