Detection of mycotoxins in foods and feeds

The analytical methodology for the detection of contamination in foods and feeds involves a sequence of five steps :

1- Sample preparation

2- Extraction

3- Clean- up

4- Quantification

1- Sampling :- The sampling stage is one of the most critical steps for a precise analytical procedure . This is even more important in the case of mycotoxins is very heterogeneous . The sampling probe should be long enough to reach the bottom of container when possible . Attempts should be made to use a sampling rate similar to the (200 g) per (200 Kg) mentioned above .

2-Extraction :- Determination of Mycotoxins from any food or feed requires a step for the solubilization of the Mycotoxins with an appropriate solvent . In most of the cases this is done with an immiscible solvent (i. e. , a solid – liquid or a liquid – liquid extraction step) however in some cases with liquid foods it is possible to omit the liquid – liquid extraction step . When the food sample is a solid matrix the solid – liquid is usually carried out in a blender over a defined period of time .

The commonly solvent used in extraction of Mycotoxins in food and feed are :

1- Water

2- Chloroform

3- Methanol

4- Hexane

5- Methyl ether ▪

6- Ethanol ▪

7- Another ▪

3- <u>Clean –up</u> : The clean –up of the analytical procedure involves the separation of the myctoxins from other Co-extracted compounds .The design of this stage is highly dependent on the sub sequent quantification stage . Qualitative assay will require less or NO clean –up , while quantitative ones will probably require more extensive clean – up . The specificity and sensitivity of the quantification will determine the required extent of the clean –up . Highly sensitive and specific immunoassay may not need a clean-up stage , (e. g. enzyme liked immune sorbent assay "ELISA") , where as method of TLC will require extensive clean- up . ▪

The available clean –up procedures ▪

include the following: ▪

1- SPE = Solid Phase Extraction . ▪

2- IAC = Immuno Affinity Colum . ▪

3- Chromotography Colum . ▪

4- **<u>Quantification</u>** : - After clean-up and before final measurement (quantification) the Mycotoxins must be free of all inter ferences . Separation of the Mycotoxins is usually achieved by a chromatographic method either : ▪

1- Thin Layer Chromatographic (TLC) . ▪

2- Gas Chromatographic (GC) . ▪

3- Liquid Chromatographic (LC) . ▪

Lec-8- **Zearalenone**

Is an estrognic secondary metabolites mycotoxins produced by several Fusarium species , which are commonly infect various cereal crops including ; maize , barley , oats , wheat , rice . sorghum and bananas .

Production of zearalenone in crops

Infection of the plant with fusarium ⟶ growth of fusarium ⟶ production of zearalenone .

Important Characteristics :

1 -Zearalenone is a whit crystalline solid . it exhibits blue-green fluorescence when examined by wavelength UV light (360 nm) .

2 - LD50 of this mycotoxins is 4000 mg/kg .

3 - Characterized chemically as a phenolic acid lactone and has a molecular weight of 318.36 .it is only slightly soluble in water and is quite stabe .

4 – Estrogen receptors are the main target .

5 – Zearalenone produced by many certain strains of various species of the genus Fusarium .incloding :

a - *F. culmorum .*

b - F. equiseti.

C - F. graminearum.

d – F. moniliforme.

6 - Have many type of metabolites isomers include : α (zearalenol , zearalenol) , β (zearalenol, zearalenol) differs in the binding affinity to estrogen recptors .

Metabolism and toxic effect of zearalenone :

1 – Following oral administration ,zearalenone is rapidly absorbed .

2 - The principal site of zearalenone metabolism appears in the liver .

3 -This mycotoxins is converted to different isomers of zearalenol (α and β) as well as zearalenol .

4 - zearlenone and its metabolites can undergo further conjugation reactions to produce glucuronide conjugates (in liver) .

5 -in human , pigs and rabbits the excretion tends to predominate through urinary system .

6 -The ability of zearalenone and its structurally related metabolites to exert a toxic effect lies in their ability to assume a molecular conformation similar to that Estrogen hormone so that they have the ability to bind to estrogen receptors in number of in vitro tissue system , including uterus, mammary gland , brain , bone and liver .

7 - the binding of mycotoxins to estrogen receptors leading to alterations in reproductive tract and a variety of symptoms including decreased fertility , embryo lethal resorptions and reduced size .

8 –This mycotoxin also has been found to induce chromosomal aberrations , sister chromatid exchange .

9 – May cause hepatocellular adenomas and pituitary adenomas and others .

Determination of Zearlenone by TLC teqnique

1- Make straight light line on the TLC plate is just (1.5) cm from the base plate .

2-Take 15 micro liters with micropipette from standard zearalenone toxin and transferit and placed on the line at a distance 2 cm from the ledge .

3- Put 15 micro of extract sample with a distance of 2 cm from the standard toxin drop .

4- Leave stains to dry and then placed in a tank of dichloromethane- acetone mixture 90:10 volume / volume and observe until the arrival of the solution to a distance of aboute 2 cm for 5 cm .

5- Exit the TLC plate for air drying for 5 minutes .

6- Examine the TLC plate under UV light with wavelength 360 nm to detect the presence of zearalenone toxin by corresponding RF , color of standard toxin and RF , of sample toxin .