

ANALYSIS OF FOOD CONTAMINANTS

I. Definition and Classification of Food Contaminants

II. Chromatographic Techniques

III. Analysis of Specific Contaminants

- Pesticide Analysis
- Mycotoxins-Aflatoxins
- Immunoassays
- Food Additives

I. Definition and Classification

Contaminants are undesired nonnutrient substances that find their way into foods and food raw materials. They all have in common the potential for human toxicity, ranging from acute poisoning to accumulation diseases due to prolonged exposures. Their presence in the food chain is surely a risk factor for human health. Food contaminants are of very diverse character in their compositions, and may be classified as follows:

- A. Physical contaminants
- B. Biological contaminants
- c. Chemical Contaminants

A. Physical contaminants

- **Extraneous materials:** Any foreign matter (sand, soil, glass, rust etc.) associated with objectionable conditions of processing, storage or distribution
- **Filth:** Any matter contributed by animals such as rodents, insects, birds etc.
 - 1. **Light filth:** oleophilic and “floats”
 - 2. **Heavy filth:** separated by sedimentation

METHODS for Quantifying Physical Contaminants:

1. Microscopy
2. Physical Separations: on basis of differences in particle size(sieving), in density(floatation of insects and insect fragments)
3. Chemical Analyses of “indicators”: i.e. uric acid content measures insect or bird excrement.
4. Automated methods: ELISA based on detecting insect protein “myosin” in plant food samples.

DAL: Defect action levels: Current maximum levels of unavoidable defects that present no health hazard

B. Biological Contaminants:

Microorganisms that invade food commodities can be identified and quantified by making use of conventional microbiological analyses

C. Chemical Contaminants:

1. Heavy metals and radioactive metal contaminants (Environmental pollutants to be determined by either atomic absorption spectroscopy and/or measurement of radioactivity)

2. Toxins produced by microorganisms:

a-Bacterial toxins: Mycological detections followed by biological tests (brine shrimp larvae) or specific rapid toxin tests based on immunochemical methods.

b-Mycotoxins(toxic metabolites of moulds):
Mycotoxin contamination is at ppm levels.~mg/kg; mg/ton and is not homogenous throughout the food, which is an important problem for chemical analyses.

3. Chemicals used in agricultural practices

- a. **Pesticides:** Currently there are more than 300 Chemicals used to kill pests (insecticides-herbicides-fungicides)
- b. **Animal drugs** (hormones and antibiotics) for raising the productivity animals, increasing the ratio of lean meat to fatty meat, thus both the quantity and the quality of the meat being improved. These should be given much prior to slaughter, this period being called the "withdrawal time", which should cover sufficient time for all types of excretions of the chemical from the animal body.

4. Migration from packaging materials: monomers of the polymers used for food packaging (like vinyl monomer of PVC). In general, the polymers are inert and do not migrate, whereas monomers do. The vinyl monomer can be analyzed by GC using headspace technique after suspension in dimethylacetamide.

5. Food Additives: These chemicals which are intentionally added to foods during processing are made up of very diverse groups of chemical compounds, each requiring specific types of analysis.

II. Chromatographic Techniques

Analysis of almost all contaminants involve methods that make use of chromatographic separations. The first developer of this type of methods was Russian scientist, Tswelt, who partitioned plant pigments in 1903.

All types of chromatography involve two phases, one stationary and the other mobile, separations being based on the differences in the volatility, polarity, molecular size, electrical charge, solubilities and/or affinities of constituents in the respective two phases. There are five different techniques involved for these separations, the main two being the following:

1. Adsorption or displacement chromatography:

Here, the column is packed with an inert filling. First the sample solution (i.e. with solutes A+B in selected solvent) is given to the column, where components are adsorbed by noncovalent bonds-nonpolar in action. Then the fresh solvent (mobile phase) is given onto the column, which displaces some of the solutes of sample solution (A+B), leaving them free to migrate down the column. Separation of solute molecules occur by equilibration between adsorbed state and solution. This technique is effective primarily for separation of nonpolar compounds according to their functional groups and MW.

2. Partition chromatography:

Effective for fractionating mainly hydrophilic components of differing polarities. Here, the inert packing is coated with a film of high-boiling liquid that is strongly adsorbed by the particle surface, the adsorbent constituting the stationary phase. After giving first the fresh solvent as the mobile phase, and the sample solution, some more mobile phase (solvent) is passed through the column, redistributing the components of sample in the two phases, the mobile eluting solvent and the stationary adsorbent on the packing material. Higher homologues migrate before shorter molecules.

The other three chromatographic techniques are "size-exclusion", "affinity" and "ion-exchange" chromatography.

Rf value: A parameter that measures how far a substance moves with respect to how far the solvent moves at the same time.

Retention time, RT, is a similar concept in GC and HPLC.

Types of Chromatography

Stationary phase	Mobile phase	NAME	Physical principle
Solid	Gas	Gas-solid	Adsorbtion
Solid	Liquid	Thin layer column paper	Adsorbtion +partition
Liquid	Liquid	HPLC	partition
Liquid	Gas	Gas-liquid	partition

Parameters for selection of the stationary and mobile phases:

For Stationary Phase:

Adsorbivity (↓)

(Al silicate (Fuller's earth))

Mg silicate (Florisil)

Al oxides

Silica gel

MgO

Na₂CO₃

For Mobile Phase:Polarity↑

Petroleum ether (30-50°C)

CCl₄

Chlorinated Hydrocarbons

Ether

Acetone

Benzene

Alcohols

Water

Acids (organic-inorganic)

[Base+acid+water] mixtures

You can mix any of the solvents given under the eluotropic series till you reach the desired polarity in order to increase or decrease the R_f or RT (retention time) for the analyzed solute component.

Hints for Selection of Stationary and Mobile phases:

1. If you have a very polar solute molecule, it will be held more strongly on a hydrophilic adsorbent .

Therefore, use a weak adsorbent, but stronger elution solvent (mobile).

If the solute has weak affinity for adsorbent, use strong adsorbent(of high activity level*) and weak eluent .

*Activity Level of Stationary phase is also important.
Alumina(Al_2O_3) has 5 activity grades:
Grade I: 0% H_2O ; Grade II: 3% H_2O ; Grade III: 6% H_2O ;
Grade IV: 10% H_2O ; Grade V: 12% H_2O -most active.

Summary:

Normal Phase

- Stationary phase \rightarrow Polar
- Mobile phase \rightarrow Non-polar
- First to elute \rightarrow least polar components
- Last to elute: most polars

Reversed Phase

- Stationary phase \rightarrow Non-polar
- Mobile phase \rightarrow Polar
- First to elute \rightarrow most-non polars
- Last to elute \rightarrow least polars (non polar)

III. Analysis of Specific Contaminants:

EXAMPLE 1: Pesticide Analysis

First of all, check what types of pesticides are allowed and are used for your sample food commodity in respective food legislation. Then start with a multiresidue screening test type of analysis (i.e. Enzymatic acetylcholinesterase test for all organophosphorus pesticides) and for positive samples, conclude with official analytical method(either one of the multiresidue(MRM) or single residue(SRM) method) for the specific pesticide identified. Steps in analyses:

1. Sample preparation: Discarding inedibles and homogenization
2. Extraction from sample matrix: Organo chloro pesticides are non polar, thus require fat soluble solvents, but Organophosphates require more polar water soluble-solvents. For general screening purposes, you can use acetonitrile, which extracts both.

3. Isolation and clean-up:Removal of interfering co-extractives, preferably by column chromatography (partitioning) or liquid-liquid extraction in separatory funnel.

This can be done by transferring pesticides to petroleum ether and then purifying the pet-ether extract on a Fluorisisil column, to be followed by stepwise elution of chlorous and phosphorous pesticides with different solvent systems.

4. Separation:Components of purified extract are separated by differential partitioning between a mobile and a stationary phase(Chromotography)

5.Detection and Quantification : A physical parameter of separated components in mobile phase is measured as it passes through a detector, the measured signal being related to concentration

For TLC, Al oxide plates are used which are spotted with sample from microsyringe or capillary tubes. The development tank should be pre-saturated with the developing solvent, (i.e. acetone: heptane)

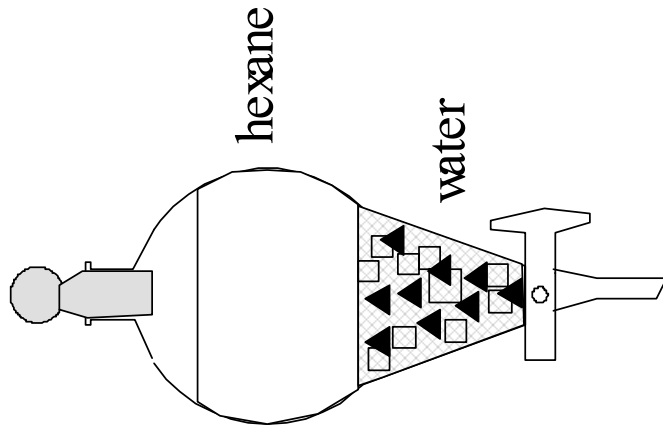
After development of the spotted plate, for visualisation of chlorinated pesticides, spray with AgNO_3 solution, then dry and observe under UV light. For visualization of organophosphates, spray with a dye solution (TBPP in acetone). Pesticides occur as bluish-purple spots against yellow background. Identification is done according to R_f values and quantification is by GC (Electron capture detector for chlorinated pesticides; AFD ionization for organophosphates). For % Recovery, internal standard is added to sample prior to extraction step.

EXAMPLE 2: Mycotoxins-Aflatoxins

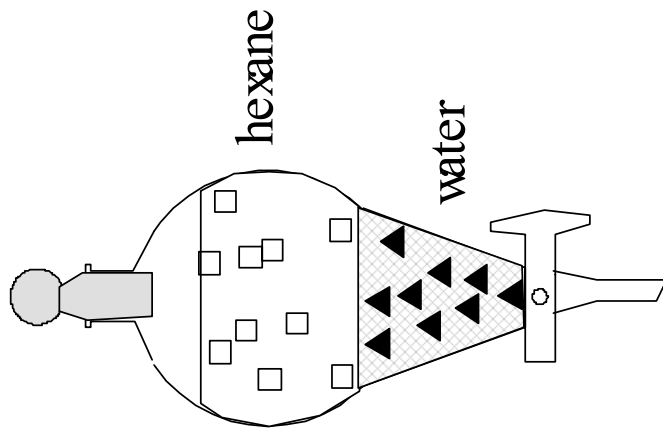
Different analytical methods have been developed for different mycotoxins and even for the same mycotoxin in different food types (matrix differentiation). A general procedure for aflatoxins is as follows:

- Following aqueous methanol extraction of aflatoxins from sample matrix, aflatoxins are partitioned to methylene chloride in a separatory funnel (liquid-liquid extraction) using these two immiscible solvent systems.
- Later, clean-up from interfering substances like pigments is realized with silica gel column chromatography.
- Then TLC silicagel plates are used for identifications of isolated toxins. Mobile phase is $[\text{CHCl}_3 : \text{methanol} : \text{acetone}]$. Visualization is done under UV light because aflatoxins B1 and B2 give blue fluorescence, whereas G1 and G2 give green fluorescence.

Before extraction

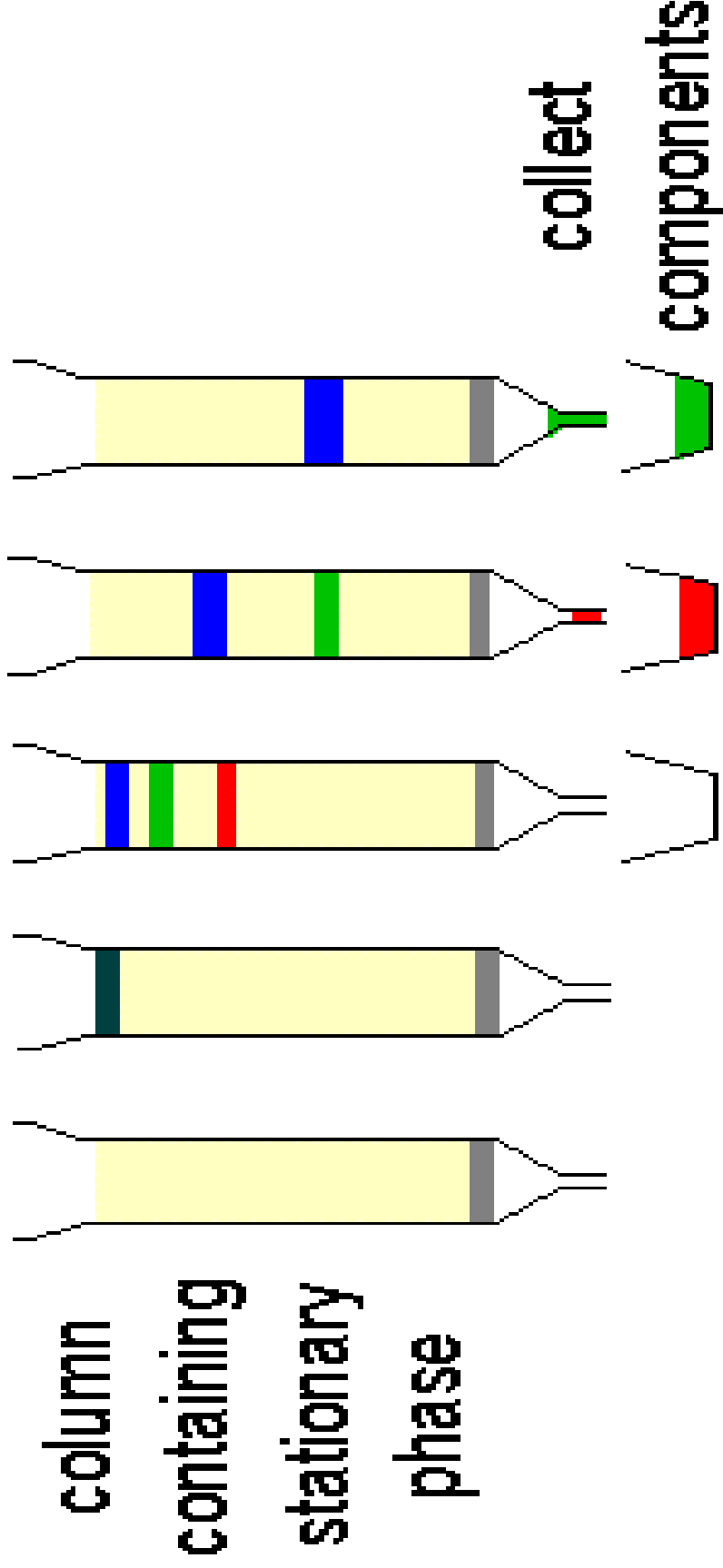


After extraction



load add

sample solvent



column
containing
stationary
phase

•For confirmation of identify of individual aflatoxins, two dimensional TLC development can be applied, where after development in first solvent, you rotate plate 90°C, and develop again in same solvent.

TLC methods are semi-quantitative because they are based on matching intensity of fluorescence of sample extract with those of aflatoxin standard solutions of different concentrations.

If a Densitometer is used, it can convert fluorescence intensity of spots (450nm) into peaks.

For optimum quantification, HPLC using fluorescence detector is required.

- Immunoassays

Antibodies are able to make a significant contribution to characterisation and quality assessment of agri-food materials. The unique properties of antibodies include a potential for extraordinary diversity of recognition, high affinity and specificity towards the target, and a stability and robustness well-suited to in vitro (and even 'in field') applications.

Immunoassays have been recognised for a number of properties:

- performance-specificity, sensitivity, precision, accuracy and reproducibility,
- high rates of sample through-put,
- dealing with analytes difficult to handle by alternative procedures,
- simplicity

Modern, non-isotopic methods such as the enzyme-linked immunosorbent assay (ELISA) have basic, comparatively cheap equipment requirements.

Many of the applications of immunoassays in the agri-food area have been related to food safety. Of the haptenic compounds, much interest has been devoted to the mycotoxins, the secondary metabolite mold products that can be found as contaminants in food and feed and which can have potent toxic bioactivities in animals and man.

Especially for the trichothecenes, and the fumosins, and many other similar low molecular weight materials, including pesticides, phycotoxins and veterinary drugs, immunochemical methods offer a very good analysis alternative.

Also, for specification of a sample of meat, immunoassay provides a rapid, simple and objective procedure that is difficult to reproduce with alternative methods of analysis.

EXAMPLE 3: Food Additives (ie:Colorants-Food dyes)

Food colorants are either water soluble [These being called (-N=N-)“azodyes” or “coal tar dyes”], or oil soluble like the natural carotenes. Their Qualitative analysis involve either dyeing with white defatted wool (to see if any added colorant is present or not) or for identification of individual dyes, paper or thin layer chromatography is performed. For this second purpose, the sample solution is spotted together with standard solutions of Orange S and Amaranth dyes.

After development in 4 different developing solvent systems, the sample spots observed in each are to be coded for their respective RF values obtained after developing in each of the respective solvent systems described in next slide. Coding is as follows:

The 4 following different solvent systems are used for the developments:

1. Trisodium citrate/H₂O/NH₄OH
2. T-butanol/propionic acid/water
3. T.S. citrate /hexane/H₂O
4. Methyl propanol/water/ethanol/NH₄OH

Each sample will have a four-lettered code(see next slide):

Example: ACDD means the dye in sample was A in solvent 1, C in solvent 2, and D in solvents 3 and 4

For Rf:

- A) The spots above orange S
- B) Same Rf with orange S, above A
- C) Below orange S, above Amaranth
- D) Same Rf with Amaranth, above B
- E) Below amaranth

Following proper identifications of the dye(s) in the food sample, the individual dye has to be extracted and studied for quantitative evaluations, using a visible spectrophotometer, by measuring its absorbance value at its peak value of the standard dyes's spectrum and comparing it to that of standard dye solutions on a calibration curve prepared for this purpose.