PRACTICAL MANUAL
OF
ELE FST-351

Course :	ELE FST 351	**Credit:**	3(2+1)	**Semester-V**
Course title:	Food Safety and Standards (Elective)			

Syllabus

Theory

Food quality: physical, nutritional, microbial and sensory, quality control; Hazards in supply chain, biological, chemical and physical hazards, natural contaminants, allergens, Food adulteration, toxicities due to hazards, Food infection and intoxication, risk analysis, and detection and epidemiology of food borne pathogens. ISO Food Safety Management Systems.potential risks of food borne bioterrorism, bioterrorism protection measures, Personal hygiene and sanitary food handling.

Quality management and quality assurance: Total quality management, good manufacturing practices, good agricultural practices, good laboratory practices; ISO. HACCP: Principles, implementation; Plan documentation, types of records; Auditing: Surveillance, audit, mock audit, third party quality certifying audit, Certification, certification procedures, certifying bodies, accrediting bodies, international bodies.

Risk assessment and management during food preparation. Microbial standards of fresh and processed foods.

Concept of Quality management systems in India; Sampling procedures and plans; Food Safety and Standards Act, 2006, AGMARK, BIS, Global GAP, Global Food safety Initiative; BRC, SQF, SGS, Food Codex; Export import policy, Labeling issues. export documentation; and food safety.

Practical

Estimation of CFU of water, Estimation of TDS in water.Estimation of *Listeria* and *E. Coli/ Salmonella /Shigella/ Staphylococcus* from food samples.Estimation of fungal toxins from food samples.Heavy metal detection (lead),Estimation of any one commonly used pesticide,HACCP for food industries by taking few models,Study of national and international microbial quality standards,Visit to export oriented food processing industry,

Teaching Schedule

a) Theory

Lecture	Topics	Weightage (%)
1-4	Food quality: physical, nutritional, microbial and sensory, quality control	16
5-9	Hazards in supply chain, biological, chemical and physical hazards, natural contaminants, allergens, Food adulteration, toxicities due to hazards,	16
10-12	Food infection and intoxication, risk analysis, and detection and epidemiology of food borne pathogens.	8
13-15	ISO Food Safety Management Systems.potential risks of food borne bioterrorism, bioterrorism protection measures, Personal hygiene and sanitary food handling.	10
16-18	Quality management and quality assurance: Total quality management, good manufacturing practices, good agricultural practices, good laboratory practices; ISO. HACCP: Principles, implementation;	8

Lecture	Topics	Weightage (%)
19-21	Plan documentation, types of records; Auditing: Surveillance, audit, mock audit, third party quality certifying audit, Certification, certification procedures, certifying bodies, accrediting bodies, international bodies.	10
22-23	Risk assessment and management during food preparation. Microbial standards of fresh and processed foods.	8
24-25	Concept of Quality management systems in India; Sampling procedures and plans;	8
26-28	Food Safety and Standards Act, 2006; Domestic regulations; AGMARK, BIS, Global GAP	8
29-30	Global Food safety Initiative; BRC, SQF, SGS, Food Codex; Export import policy, Labeling issues. export documentation; and food safety.	8
	Total	**100**

Practical

Exercise	Topics
1&2	Estimation of CFU of water, Estimation of TDS in water.
3 &4	Estimation of *Listeria* and *E. Coli/ Salmonella /Shigella/ Staphylococcus* from food samples.
5 & 6	Estimation of fungal toxins from food samples.
7 & 8	Heavy metal detection (lead)
9 & 10	Estimation of any one commonly used pesticide
11 & 12	HACCP for food industries by taking few models of food industry
13 & 14	Study of national and international microbial quality standards
15 & 16	Visit to export oriented food processing industry
	Total

Suggested Reading:

1) Food Microbiology. W.C. Frazier and D.C. Westhoff, 4th Edn. Tata McGraw-Hill Publishing Company Limited, New Delhi.
2) Food Safety Handbook. Ronald H. Schmidt and Gary E. Rodrick. 2003. John Wiley & Sons, Inc., Hoboken. New Jersey, USA.
3) Food Safety and Food Quality. R.E. Hester and R.M. Harrison. 2001. Royal Society of Chemistry, Cambridge, UK.
4) The Safety of Foods (Sicherheit von Lebensmitteln). GrahamGraham, H. D**.** (Edit.) 2.
5) Auflage. AVI Publishing Co., Inc., Westport, Connecticut (USA)
6) Food Chemistry (New Edition).Owin R. Fenema
7) Handbook of Food Toxicology. S.S. Deshpande, CRC Press. 2002.
8) Food Hygiene and Sanitation. S. Roday,Tata McGraw-Hill Education
9) Food Microbiology. M.R. Adams and M.O. Moss

EXPERIMENT No-1

ESTIMATION OF TOTAL DISSOLVED SOLID IN WATER

AIM

To estimate total dissolved solids in the given water sample **.**

INTRODUCTION

The term total dissolved solids refer to materials that are completely dissolved in water. These solids are filterable in nature. It is defined as residue upon evaporation of filterable sample.

ENVIRONMENTAL SIGNIFICANCE

- Dissolved minerals, gases and organic constituents may produce aesthetically displeasing colour, taste and odour.
- Some dissolved organic chemicals may deplete the dissolved oxygen in the receiving waters and some may be inert to biological oxidation, yet others have been identified as carcinogens.
- Water with higher solids content often has a laxative and sometimes the reverse effect upon people whose bodies are not adjusted to them.
- High concentration of dissolved solids about 3000 mg/L may also produce distress in livestock.
- Estimation of total dissolved solids is useful to determine whether the water is suitable for drinking purpose, agriculture and industrial purpose.

PRINCIPLE

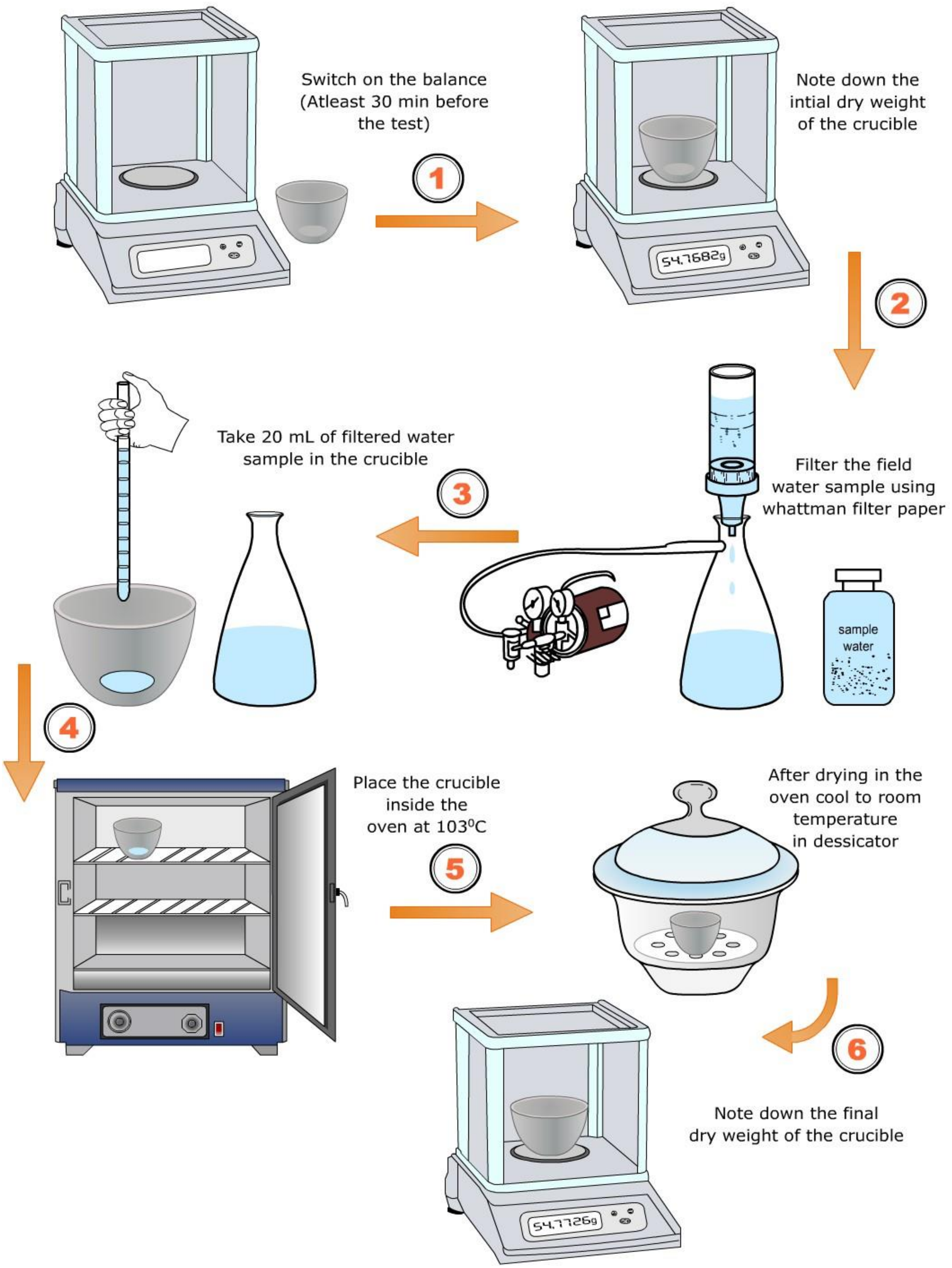
A well mixed sample is filtered through a standard glass fiber filter, and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 179- 181°C. The increase in dish weight represents the total dissolved solids.

1. MATERIALS REQUIRED

1.1 APPARATUS REQUIRED

1. Dish
2. Water sample
3. Oven
4. Desiccators
5. Analytical Balance
6. Graduated Cylinders
7. Pipette
8. Gooch Crucibles
9. Filter
10. Vacuum Pumps

PROCEDURE CHART



1. Switch on the balance (Atleast 30 min before the test)

Note down the intial dry weight of the crucible

2. Filter the field water sample using whattman filter paper

3. Take 20 mL of filtered water sample in the crucible

4. Place the crucible inside the oven at 103^0C

5. After drying in the oven cool to room temperature in dessicator

6. Note down the final dry weight of the crucible

1.2 SAMPLE HANDLING AND PRESERVATION

Preservation of sample is not practical. Because biological activity will continue after a sample has been taken, changes may occur during handling and storage.

Both the characteristics and the amount of solids may change.

To reduce this change in samples taken for solids determinations, keep all samples at 4^0 C.

Do not allow samples to freeze.

Analysis should begin as soon as possible.

1.2.1 PRECAUTIONS

- The following precautions should be observed while performing the experiment:
  - Water or Wastewater samples which contain high concentrations of calcium, chloride, magnesium or sulfate can rapidly absorb moisture from the air.
  - Such samples may need to be dried for a longer period of time, cooled under proper desiccation and weighed rapidly in order to achieve a reasonable constant weight.
  - We should be aware prolonged drying may result in loss of constituents, particularly nitrates and chlorides.
  - Volume of sample should be adjusted to have residue left after drying as 100 to 200mg. It is mainly to prevent large amount of residue in entrapping water during evaporation.
  - Samples with high concentrations or bicarbonate require additional drying at 180ºC to ensure that all of the bicarbonate is converted to carbonate.

1.3 PROCEDURE

TESTING OF SAMPLE FOR TOTAL DISSOLVED SOLIDS

To measure total dissolved solids, take a clean porcelain dish which has been washed and dried in a hot air oven at 180 C for one hour.

- Now weigh the empty evaporating dish in analytical balance. Let’s denote the weight measured as W1 = g.
- Mix sample well and pour into a funnel with filter paper.
- Filter approximately 80 -100 mL of sample.
- Using pipette transfer 75mL of unfiltered sample in the porcelain dish.
- Switch on the oven and allowed to reach 105°C. Check and regulate oven and furnace temperatures frequently to maintain the desired temperature range.
- Place it in the hot air oven and care should be taken to prevent splattering of sample during evaporation or boiling.
- Dry the sample to get constant mass. Drying for long duration usually 1 to 2 hours is done to eliminate necessity of checking for constant mass.
- Cool the container in a desiccator. Desiccators are designed to provide an environment of standard dryness. This is maintained by the desiccant found inside. Don't leave the lid off for prolonged periods or the desiccant will soon be exhausted. Keep desiccator cover greased with the appropriate type of lubricant in order to seal the desiccator and prevent moisture from entering the desiccator as the test glassware cools.
- We should weigh the dish as soon as it has cooled to avoid absorption of moisture due to its hygroscopic nature. Samples need to be measured accurately, weighed carefully, and dried and cooled completely.
- Note the weight with residue as W_2 =

CALCULATION

TABLE

Total Dissolved Solids

Description		Weight (g)
Weight of the clean porcelain evaporating dish (g)	W1	
Weight of the dish and the residue (g)	W2	
Weight of residue(g)	W	
Volume of the Sample (mL)	V	
Total Dissolved Solids (mg/L)	**TDS**	

Tabulation for Total Dissolved Solids (TDS):

Weight of the clean porcelain evaporating dish (g) W_1 =

Weight of the dish and the residue (g) W_2 =

Weight of residue (g) W =

The volume of the sample (mL) V =

DETERMINATION OF TOTAL DISSOLVED SOLIDS DATA SHEET

Date Tested :

Tested By :

Project Name :

Sample Number :

Sample Location :

Sample Description :

Description		**Weight (g)**
Weight of the clean porcelain evaporating dish (g)	W_1	
Weight of the dish and the residue (g)	W_2	
Weight of residue(g)	W	
Volume of the Sample (mL)	V	
Total Dissolved Solids (mg/L)	**TDS**	

Model CAlculAtion:

W_1 =
W_2 =
V =
Weight of residue (g) W = $W_2 - W_1$
=

Weight of residue in mg (To convert W (g) to W (mg), multiply W (g) with 1000)
W (mg) =

Multiply the weight of the dry solids (in mg) by 1,000 mL/L to convert the sAmple size from mL to L.

TOTAl Dissolved Solids (mg/L)

V = Volume of the sAmple (mL) (To convert mL to L, multiply by 1000)
= mg/50 mL = mg/mL
= mg/mL x 1,000 mL/L = mg/L

INFERENCE

Water can be classified by the amount of TDS per litre:

- fresh water < 1500 mg/L TDS
- brackish water 1500 to 5000 mg/L TDS
- saline water > 5000 mg/L TDS

The following charts give some common ranges for TSS results and possible removal efficiencies for various types of treatment.

Sample	**Common Ranges, mg/L**	
Influent	Weak < 150	400+ Strong
Primary Effluent	Weak <60	150+ Strong
Secondary Effluent	Good 10 -	60+ Bad
Tertiary Effluent	Less than 3	
Activated Sludge		
Mixed Liquor (MLSS)	1,000 - 5,000	
Return or waste sludge	2,000 - 12,000	
Digester Supernatent	3,000 - 10,000	
Sludge	20,000 - 60,000	

EXERCISE No- 2

Estimation of CFU of Water (COLIFORMS)

Equipment/ Materials-

1. Autoclave of sufficient size and number. Used for sterilization of media and for discarded plates / used media, etc (with calibrated thermometer and pressure gauge).
2. Balance sensitive to 0.1 g with 200 g load.
3. Colony Counter (Quebec or equivalent).
4. Dilution and media storage bottles. 120, 300, 600 and 1500 ml in capacity.
5. Durham's tubes
6. Glass test tubes 16 x150 mm. Rimless

7.Plastic caps for test tubes

8. Serological test tubes

9. Hot air ovens used for sterilization of glass and metal ware

10.Incubators. Microscope binocular with 900 x and higher magnification.

11.Microscopic slides and cover slips.

12.Non-adsorbant cotton.

13.Petri plate (glass or plastic)

14.Petri plate containers. (Stainless steel or aluminium, with covers) for hot air sterilization of glass petri plates.

15.Test tube racks and baskets to hold test tubes.

16 Laminar flow chamber

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Culture Media:

Lauryl Sulphate Tryptose (LST) broth tubes of single and double strength with inverted Durham tubes (10 ml quantities in tubes and 50 ml in bottles).

Procedure:

Dechlorination:

Samples collected in pre-sterilised bottles are mixed well. To every 100ml portion of chlorinated samples 0.1 ml of a 10% sterile solution of sodium thiosulphate is added.

Various combinations of sample volume are taken depending on the probable load of coliform in the sample. To 10 ml or 50 ml volume of double strength broths, equal volumes of sample is added. To 10ml of single strength broths 1 ml or 0.1 ml is added.

Tubes/bottles are incubated at 35°C for 24 and 48 hrs.

Interpretation

Record tubes showing gas production after 48 hrs. The MPN index per 100ml sample is determined using the following statistical tables.

Table : MPN index and 95% Confidence limits for various combinations of positive and negative results when five 10 ML portions are used.

No. of tubes giving positive reaction out of 5 of 10 ml each	MPN Index/100 ml	95% confidence Limits (Approximate)	
		Lower	Upper
0	< 2.2	0	6.0
1	2.2	0.1	12.6
2	5.1	0.5	19.2
3	9.2	1.6	29.4
4	16.0	3.3	52.9
5	> 16.0	8.0	Infinite

Table : MPN index and 95% Confidence limits for various combinations of positive and negative results when Ten 10 ML portions are used

No. of tubes giving positive reaction out of 10 of 10 ml each	MPN Index/100 ml	95% Confidence Limits (Approximate)	
		Lower	Upper
0	< 1.1	0	3.0
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12.0	4.3	27.1
8	16.1	5.9	36.8
9	23.0	8.1	59.5
10	> 23.0	13.5	Infinite

Expression of Results:

Coliforms = x MPN/250 ml or 100ml

Plating Technique:

Alternately take desired volume of water (250 ml or 100 ml) and pass it through a micropore filter of 0.2U. Take the filter disk and place on a well dried surface of LST Agar. Incubate at 35°C for 24 to 48 hrs. Count the typical colonies and express the result as given below.

Expression of Result:

Coliform count = x cfu/g

EXPERIMENT No-3

Detection of *Listeria monocytogenes* in Food sample

Equiupments:

1. Autoclave
2. Balance sensitive to 0.1 g with 200 g load.
3. Blenders with steel jar and lid / Stomacher.
4. Bunsen burners.
5. Colony Counter (Quebec or equivalent).
6. Dilution and media storage bottles. 120, 300, 600 and 1500 ml in capacity.
7. Durham's tubes
8. Glass test tubes 16 x150 mm. Rimless
10. Plastic caps for test tubes
11. Serological test tubes
12. Hot air ovens used for sterilization of glass and metal ware. They should have a thermostat range between 150-185^{o}C.
13. Howard Mold Counting Chamber
15. Haemocytometer.

16. Incubators.
17. Microscope binocular with 900 x and higher magnification.
18. .Microscopic slides and cover slips.
22. Non-adsorbant cotton.
23. Petri plate (glass or plastic)
24. Petri plate containers. (Stainless steel or aluminium, with covers) for hot air sterilization of glass petri plates.
25. Pipettes. (glass or plastic) Graduated, with 1, 5 and 10 ml total flow type / Automatic pipette with error < ±5 % with sterilisable or Autopipetor with Pre-sterilized plastic tips.
26. Pipette containers (Stainless steel plastic tip containers boxes)
27. pH meter. Electronic pH meter with accuracy of 0.1 pH unit shall be used.
28. Refrigerator and deep freezer.
29. Test tube racks and baskets to hold test tubes.
30. Thermometers.
31. Vortex – mixer.
32. Water bath for holding media at 44-46ºC.
33. Serological water bath
34. Laminar flow chamber

Culture media and reagents:

- Phosphate buffered peptone water
- Half Frazer broth
- Frazer broth
- Modified Oxford Agar
- PALCAM Agar
- Tryptone Soya Yeast Extract Agar
- Tryptone Soya Yeast Extract Broth
- Sheep Blood Agar
- Carbohydrate utilization broth (Rhamnose and Xylose)
- Motility Agar
- CAMP Medium and test organisms
- Hydrogen peroxide solution

Preparation of test sample:

Take 25 g of a well-mixed sample in stomacher bag and use 225 ml of Half Frazer broth. Stomach the sample for two minutes. Pour aseptically the contents in to a wide mouth bottle and incubate at 30°C for 24±2h (a black coloration may develop).

Take one ml of the above culture and transfer to 9ml of Frazer broth. Incubate the inoculated tube at 37°C for 48± 2h at 35-37°C.

From 24 h culture of Half Frazer broth and 48h Frazer broth streak out culture on Modified Oxford Agar and PALCAM agar so that well separated colonies are obtained.

Invert the plates and incubate at 35 or 37°C for 24 h and if required an additional 18 h if growth is slight or no colonies appear. Examine the plates for colonies presumed to be *L. monocytogenes*.

Appearance of colonies:

On M Ox agar the colonies are small (1mm) greyish surrounded by a black halo.

After 48 h the colonies turn darker with a possible green luster and are about 2 mm in diameter with black halos and sunken centres.

On PALCAM agar after 24 h the colonies appear1.5 to 2 mm in diameter greyish green or olive green some times with black centre and always surrounded by a black halo and depressed centre.

Confirmation of Listeria species:

Select five typical colonies from one plate of each medium. If presumed colonies are less than five on a plate, take all of them.

Streak the selected colonies from each plate on to the surface of a well dried TSYEA for obtaining well separated colonies. Invert the plates and incubate at 35°C or 37°C for 18 to 24 h or until the growth is satisfactory.

Typical colonies are 1 mm to 2 mm in diameter, convex, colorless and opaque with an entire edge. Carry out the following tests from colonies of a pure culture on the TSYEA.

Catalase reaction:

With the help of loop pick up an isolated colony and place it in H_2O_2 solution on a glass slide. Immediate production of gas bubbles indicates catalase positive reaction.

Gram staining:

Perform Gram staining on a colony Listeria are Gram positive slim short rods.

Motility Test:

Take colony from TSYEA plate and suspend it TSYE broth. Incubate at 25°C for 8 to 24 h until cloudy medium is observed. Take a drop of culture and place it on a glass slide. Cover the top with a cover slip and observe under a microscope. *Listeria* is seen as slim rods with a tumbling motility (cultures grown above 25°C fail to show this motion. Compare them with a known culture – *cocci* or large rods with rapid motility are not *Listeria.*

As an alternative stab motility agar tube with an isolated colony from TSYEA and incubate at 25°C for 48 h. typical umbrella like appearance around the stab indicate motility positive culture. If growth in not positive incubate up to five days and observe for the stab again.

Confirmation of *Listeria monocytogenes:*

Heamolysis test:

Take a colony from TSYEA and stab it on a well dried surface of sheep blood agar plate. Simultaneously stab positive (*L. monocytogenes*) and negative (*L. innocua*) control cultures. Inver the plates and incubate at 35°C or 37°C for 24±2 h. examine the plates.

L. monocytogenes show clear light zones of beta haemolysis. *L. innocua* does not show any haemolysis. Examine the plates in a bright light to compare test cultures with the controls.

Carbohydrate utilization:

Inoculate each of the carbohydrate utilization broths (rhamnose and xylose) with a culture from TSYE broth and incubate at 35 °C or 37v for upto 5 days. Appearance of yellow color indicates a positive reaction within24 to 48 h.

CAMP test

On a well dried surface of sheep blood agar streak each of the *Staphylococcus aureus* and *Rhodococcus equi* cultures in single lines and parallel to each other and diametrically opposite, a thin even innoculum is required.

Streak the test strain separated in a similar manner at right angles to these cultures as that the test strain and *S. aureus* and *R.equi* cultures do not touch but their closest are about 1 mm or 2 mm apart. Several test strains can be streaked on the same plate. Simultaneously streak control cultures of L *monocytogene*s, L innocua and *L. ivanovii.* Incubate plates at 35 to 37 ^{o}C for 18 to 24 h.

Observe plates against bright light. In *L. monocytogenes* case there is enhanced zone of beta haemolysis at the intersectiono of *S. aureus*.

EXPERIMENT No-4

Detection of *Salmonella species* in foods.

Equipment: Refer to exercise no 3. (Equipment, Materials and Glassware)

Culture Media:

- Lactose broth
- Trypticase Soy Broth
- Trypticase Soy Broth Containing Potassium Sulfite at a final concentration of 0.5%.
- Reconstituted Non-Fat Dry Milk
- 1% aqueous Brilliant Green Dye Solution.
- Selenite Cystine Broth
- Tetrathionate Broth
- Xylose Lysine Deoxycholate (XLD) Agar
- Hektoen Enteric Agar (HEA)
- Bismuth Sulphite Agar (BSA)
- Triple Sugar Iron (TSI) Agar
- Lysine Iron Agar (LIA)
- Urea Broth
- Phenol Red Dulcitol Broth
- Phenol Red Lactose Broth
- Tryptone Broth
- KCN Broth
- Malonate Broth
- Buffered Glucose (MR-VP) Medium
- Brain Heart Infusion (BHI) Broth
- Buffered Peptone Water

Procedure:

Preparation of sample and pre-enrichment

Aseptically open the sample container and weigh 25g sample into a sterile empty wide mouth container with screw cap or suitable closure.

Add 225ml of sterile lactose broth to the sample. Buffered peptone water, Trypticase soy broth, and nutrient broth can also be used for pre-enrichment. Make a uniform suspension by blending if necessary. Cap container and let stand at room temperature for 60 min. Instead of lactose broth the recommended pre-enrichment broth for the following food samples is as follows :

Nonfat dry milk and dry whole milk – Sterile distilled water. Add 0.45 ml of 1% aqueous briliant green dye before incubation.
Dried active yeast – Trypticase soy broth
Onion-garlic powder – Trypticase soy broth containing potassium sulfite at a final concentration of 0.5%
Milk Chocolate – Reconstituted non fat dry milk.
Shake and adjust pH (if necessary) to 6.8±0.2 with sterile 1N NaOH or 1N HCl.

Incubate at 35°C for 24±2 hours

Selective enrichment

Gently shake incubated sample mixture and transfer 1 ml to 10 ml of selenite cystine broth and an additional 1 ml to tetrathionate broth. Incubate 24±2 hours at 35^{o}C.

Selective media plating

Vortex – mix and streak 3 mm loopful of incubated selenite cystine broth on selective media plates of XLD, HEA and BSA. Repeat with 3mm loopful of incubated tetrathionate broth.

Incubate plates at 35^{o}C for 24±2 hours and 48±2 hours.

Observe plates for typical Salmonella colonies

On XLD (after 24h) - Pink colonies with or without black centres.

On HEA (after 24h) - Blue green to blue colonies with or without black centers.

On BSA (after 24 to 48h) – Brown, grey or black colonies sometimes with metallic sheen. Surrounding medium is usually brown at first, turning black with increasing incubation time.

Treatment of typical or suspicious colonies

Pick with needle typical or suspicious colonies (if present) from each XLD, HEA and BSA plates. Inoculate portion of each colony first into a TSI agar slant, streaking slant and stabbing butt and then do the same into an LIA slant.

Incubate TSI and LIA slants at 35°C for 24±2 hours and 48±2h respectively. Cap tubes loosely to prevent excessive H_2S production.

A Typical Salmonella reactions are :

	TSI	**LIA**
Slant	Alkaline (red)	Alkaline (Purple)
Butt	Acid (Yellow)	Alkaline (Purple)
H_2S production (blackening in butt)	+ or -	+

A culture is treated as presumptive postive if the reactions are typical on either or both TSI and LIA slants.

Biochemical tests

Using sterile needle inoculate a portion of the presumptive positive culture on TSI slant into the following broths. Incubate at 35°C for the specified period of days and read for Salmonella typical reactions.

Biochemical tests

Broth/ Media	Time of incubation	Results
Urea broth	24±2h	Negative (no change in yellow colour of medium)
Phenol red lactose broth	48±2h	*Negative for gas and/or acid reaction
Phenol red sucrose broth	48±2h	*Negative for gas and/or acid reaction
Phenol red dulcitol broth	48±2h	*Postive for gas and/or acid reaction
Tryptone broth	24±2h	Negative for indole test
KCN broth	48±2h	Negative (no turbidity)
Malonate broth	48±2h	*Negative (green colour unchanged)
MR-VP medium	48±2h	Negative for VP test but positive for MR test.

***(Note : Majority of *S. arizonae* are atypical for these reactions).**

Criteria for discarding Non-Salmonella Cutlures

Test(s) or Substrate(s)	Results
Urease test	Postive (purple-red)
Indole test	Positive (red)
Flagellar test (Polyvalent or spicer-Edwards	Negative (no agglutination)
Lysine decarboxylase test	Negative (yellow)
KCN broth	Positive (growth)
Phenol red lactose broth*	Positive (acid and/or gas)**
Lysine decarboxylase test	Negative (yellow)
Phenol red sucrose broth	Positive (acid and/or gas)**
Lysine decarboxylase test	Negative (yellow)
KCN broth	Positive (growth)
Voges-Proskauer test	Positive (red)
Methyl red test	Negative (yellow)

* Malonate broth positive cultures are tested further to determine if they are *Salmonella arizonae*

** Do not discard positive broth cultures if corresponding LI agar cultures give typical Salmonella reactions; test further to determine if they are Salmonella sp.

Serological Tests

To reduce number of presumptive positive cultures (TSI positive and urease negative) carried through biochemical identification tests, the following serological flagellar (H) screening test may be carried out.

Transfer 3mm loopful of culture into 5ml of BHI broth and incubate at 35ºC until visible growth occurs (About 4-6 hours).

Add about 2.5ml formalized physiological saline solution.

Test with Salmonella flagellar (H) antisera. Positive cultures show visible agglutination.

Further confirmation can be made by using Salmonella Polyvalent (O) antiserum.

Calculation:

NA

Expression of Result:

Salmonella = Present/Absent per 25 g

EXPERIMENT No- 5 & 6

ESTIMATION OF FUNGAL TOXIN (AFLATOXINS) IN CORN AND PEANUT POWDER / BUTTER - LIQUID CHROMATOGRAPHIC METHOD

Principle

Aflatoxins are extracted, purified, derivatised with trifluoroacetic acid and then separated by reverse phase liquid chromatography and detected by fluorescence. Method can measure 0.1 ng of aflatoxin B1, B2, G1, and G2. Detection limit is about 0.3 ng/gm.

Apparatus

(a) Liquid chromatograph – with Rheodyne septumless injector, Fluorichrom fluorescence detector, 7.54 and 7.60 excitation filters (360nm) 3-73 and 4-76 glass emission filters (440nm) fitted with flow cell integrator or recorder, 0.5 cm/min chart speed. Flow rate 1.0 mL/min. Set up detector preferably with tungsten source, using low lamp, high gain attenuation 20 or adjust range to give minimum half scale deflection with 1.25 ng aflatoxin B1 or G1. For optimum performance detector should be left on continuously.

(b) Column – 15 cm × 4.6 mm i.d. Supercosil LC -18 or equivalent

Note: - New LC columns or those that have been stored in methanol for extended periods require conditioning with concentrated standards in order to achieve optimum resolution and sensitivity to aflatoxin B1 and G1.

(c) Clean Up Column – 20 cm × 1cm i.d with Teflon stopcock and coarse frit bed support, detachable glass solvent reservoir with 24/40 fitting

(d) Adjustable autopipettes – 10-100 and100–200 µL with disposable tips

(e) Filter tube – glass 15 × 2.5 cm i.d with coarse frit bed support (glass wool not recommended)

Reagents

(f) Solvents – Glass distilled or HPLC grade: methanol, hexane, methylene chloride, benzene, acetone, acetonitrile. Anhydrous ethyl ether stored in metallic container (Glass bottled ether forms peroxides soon after opening which degrades aflatoxins)

(g) LC elution solvents – Water: acetonitrile: methanol (700:170:170). Adjust ratio of water to obtain baseline resolution of aflatoxin B2 and G2

(h) Silica gel for Column chromatography – Silica gel 60, (0.063-0.2 mm). activatd by drying at 100°C. Cool to room temperature. Weigh desired quantity (100gm) into glass stoppered container. Add one ml water in small increments, agitate silica gel between additions. Shake or tumble mechanically 4-6 hours. Let stand 16 hours

(i) Trifluoroacetic acid (TFA) – Assay by titration– equal to or more than 98.5%. Transfer 1-2mL TFA to a 1 dram vial with a Teflon lined cap. Keep in freezer when not in use. Discard if discoloration appears.

(j) Sodium sulphate, anhydrous. Sift out fines to obtain 20 – 40 mesh. Heat for 2-3 hours at 600°C to remove organic impurities.

(k) Aflatoxin standard solutions –

    (1) Aflatoxin stock solution – 10 µg/mL. Prepare individual stock solution in benzene-acetonitrile (98+2) and determine concentration of each by measuring UV absorption if desired.

(2) Working standard solutions - Use an autopipette (Pipetman) to transfer an appropriate quantity stock solution to each 4 dram vial to obtain the final concentrations of aflatoxins in each vial as indicated below.

Vial	B1and G1 ng	B2 and G2 ng
1	250	125
2	500	250
3	1000	500
4	2000	1000

Evaporate solutions to dryness under a gentle stream of nitrogen (drying may be facilitated by warming to 40°C). Using Eppendorf pipette add 200μL hexane and 50μL of trifluoroacetic acid to each vial, cap and vortex for 30 seconds. Let solutions stand 5 minutes, then add 10mL water: acetonitrile (9+1) and vortex for 30 seconds. Let layers separate for 5 -10 min or centrifuge at 1000 rpm for 30 seconds. Final concentration of aflatoxins shall be:

Vial	B1and G1ng/10.05 ml	B2and G2 ng/ 10.05 ml
1	0.25	0.125
2	0.5	0.25
3	1.0	0.50
4	2.0	1.0

Extraction and partition

Transfer 50gm prepared corn, or peanut powder or peanut butter to a one litre blender jar, add 200mL of methanol followed by 50mL of 0.1 M hydrochloric acid and blend for three minutes at high speed. Filter through 24 cm Whatman No 1 filter paper or equivalent. Filtrate may not be completely clear. Collect 50mL filtrate. Transfer to 250mL separatory funnel. Add 50mL 10% Sodium chloride solution, swirl, add 50mL hexane and shake gently for about 30 seconds. Let phases separate then drain lower aqueous layer into another 250mL separator funnel. Discard hexane layer. Add 25mL methylene chloride and shake moderately for 30 seconds. If emulsion occurs break up with clean pipette. Let phases separate then drain lower methylene chloride layer through coarse granular anhydrous sodium sulphate in glass filter tube. Collect elute in a 250mL beaker. Evaporate elute, on steam bath under a gentle stream of nitrogen, to 2 -3mL.

5.1 Column Chromatography

Make a slurry of 2gm silica gel with about 10mL ether–hexane (3+1) in a 30mL beaker. Pour slurry into a clean up column and wash beaker with additional 5mL ether–hexane solvent to effect complete transfer. Keep stop cock closed and let silica gel settle without tamping. Wash sides of column with 2-3mL ether–hexane using squeeze bottle. After gel settles, open stop cock and while column drains add about 1 cm anhydrous sodium sulphate. Transfer eluate collected after extraction to column. Wash beaker with about 2mL of methylene chloride and add wash to column. Do not use more than 5- 6mL methylene chloride to transfer eluate to column. With stop cock fully open, add 25mL benzene–acetic acid (9+1) and the 30mL ether–hexane (3+1) to column, draining each wash to top of sodium sulphate. Discard washes. Elute aflatoxin with 100mL methylene chloride – acetone (90+10) and collect elute in 250mL beaker. Evaporate elute on steam bath under a gentle stream of nitrogen to about 6mL. Quantitatively transfer to 3 dram vial.

Evaporate elute to dryness using a steam bath or an aluminium block under a gentle stream of nitrogen. Evaporate remaining 200µL just to dryness under a gentle stream of nitrogen by holding vial in palm of hand and slowly rotating vial.

Derivatisation

Add 200μL hexane to the residue obtained above. Then add 50μL of TFA using Eppendorf pipette, cap the vial and vortex vigorously for 30 seconds. This procedure must be followed closely to ensure consistent reaction yields. Let mixture stand 5 minutes. Using Eppendorf pipette add 1.950mL water-acetonitrile (9+1).Vortex vigorously for exactly 30 seconds and let layers separate 10 mm. Concentration is 10 gm/2mL aqueous acetonitrile.

[Note: Post column derivitization using Kobra Cell may also be used]

Determination

Using instrument parameters mentioned under apparatus successively inject 25μL of derivatised standard solution. Prepare standard curve to check linearity of responses. Inject 25μL of trfluoroacetic acid treated test solution (lower aqueous phase). If test peaks are outside the linear range, dilute aliquot of trfluoroacetic acid treated test solution to suitable volume with water –acetonitrile, remix on vortex mixer and inject another 25μL portion.

Calculate individual aflatoxin concentration as follows:

Use responses of standard containing 500 ng B1and G1 and 250 ng B2 and G2 for calculation

Aflatoxin ng/gm = $(P/ P') \times C \times (2 /10) \times 1000 \times D$

Where,

P and P' = peak areas or heights for test solution and standard per 25μL injection

C = concentration of individual aflatoxin in standard solution(0.5 or 0.25 μg/10 .05mL

D = dilution factor if 2mL test solution for injection is diluted

(Ref:- AOAC 17th edn, 2000 Official Method 990.33 Aflatoxins in Corn and Peanut Butter, Liquid Chromatographic Method)

EXERCISE No-7 & 8

DETERMINATION OF HEAVEY METAL (LEAD) IN FOOD

Principle :

The sample is ashed and the acid solution of ash is neutralized with ammonia in the presence of citrate. Several other interfering elements are complexed with cyanide and lead is isolated as lead dithizonate into $CHCl_3$.

The chloroform layer is shaken again with dilute nitric acid and chloroform layer is discarded. The aqueous phase is buffered to pH 9.5 to 10.0 and lead is re-extracted with dithizone in chloroform. The colour produced is read at 510 nm and is compared with known standard.

NOTE: It is suggested to go for Microwave digestion followed by AAS/ICP/ICP-MS. Microwave digestion will save chemical, time and sequential loss of target metal during digestion. Considering toxicity of the metals, highly precise and accurate method and equipment should be used for determination. The changes of cross contamination/false result are also very higher for lead. Hence proper QC and spike should be used during analysis

Reagents:

(a) Lead standard solution:

(i) Stock solution: (1 mg Pb/ml in 1% HNO3)

Dissolve 1.5985 g of pure Pb $(NO)_2$ crystal in 1% HNO_3 and dilute to 1000ml.

(ii) Working solution:

Prepare as needed by diluting stock solution suitably with 1% HNO_3 solution.

(b) Nitric acid:

(1%) Dilute 10 ml of fresh, colourless HNO3 (sp. Gr. 1.40) to 1000 ml with redistilled water. If acid is redistilled, boil off nitrous fumes before diluting.

(c) “Acid-aid” solution:

Dissolve 40 g Al $(NO_3)_3.9H_2O$ and 20 g Ca $(NO_3)_2.4H_2O$ in 100 ml water.

(d) Citric acid solution:

Concentrated lead free solution. Prepare the solution so that 1 ml of solution contains about 0.5 g of citric acid.

(e) Dithizone solution: (1 mg/ml in $CHCl_3$)

Dissolve 100 mg of purified dithizone in chloroform and dilute to 100 ml. Prepare dilute solutions as needed by diluting stock solution suitably with chloroform.

(f) Potassium cyanide solution: (10% w/v)

Dissolve 25 g of recrystallised, phosphate free KCN in water and dilute to 250 ml.

(g) Ammonia-cyanide mixture:

To 100 ml of 10% potassium cyanide solution in a 500 ml volumetric flask, add enough NH_4OH solution to introduce 19.1 g of NH_3 and dilute to volume with water.

(h) Washed filter paper:

Soak 9 cm quantitative papers overnight in 1% HNO_3. Wash with large volumes of water on buchner to remove acid and any traces of lead.

Preparation of sample:

Accurately weigh representative sample of 5 to 100 g, depending upon amount of sample available and expected lead content, into a clean silica dish. Dry wet sample on steam bath or in oven. If sample is difficult to ash (meats) or has low ash content (candies, gellies etc) add 2 to 5 ml of "Ash aid" solution, mix well and dry.

Char the sample carefully over burner. Do not let material ignite. Samples like milk, candies etc. may be charred without ignition by adding little at time to dish heated over burner or hot plate. Charring of sample is carried out by means of a soft flame (like that of Argand burner) to volatilise as much as possible of the organic matter. Oils and fats must be "smoked" away by heating at about 350ºC. MATERIALS BEING ASHED MUST NOT BE ALLOWED TO IGNITE DURING ANY PHASE OF ASHING.

When sample is dry and charred, place it in a temperature controlled muffle furnace and raise temperature SLOWLY without ignition. Cover floor of furnace with piece of asbestos board or SiO_2 plate so that sample receives most of its heat by radiation and not by conduction. Ash the sample at temperatures not exceeding 500ºC for about 8 to 10 hours. If ashing is not complete, remove dish from furnace, cool and moisten the char with 2 ml of ash aid solution. Dry contents of dish thoroughly and replace in furnace. If ashing is not complete after 30 minutes, remove dish, cool and cautiously add 2 to 3 ml HNO_3. Dry again and place in furnace and continue ashing until practically carbon-free ash is obtained.

When clean ash is obtained, cool, cover the dish with watch glass and cautiously add 15 to 20 ml conc. HCl. Rinse down watch glass with water and heat on steam bath. If clear solution is not obtained evaporate to dryness and repeat addition of HCl. Dilute the clear solution with water to a definite volume (filters solution if necessary, wash the insoluble material on filter successively with few ml of hot HCl, hot hydrochloric acid citric acid solution and hot 40% NH_4 OAC solution and make up to 100 ml).

PREPARE A SAMPLE BLANK SOLUTION using exactly the same amounts of reagents including water and exposing the sample blank in furnace or on steam bath for the same length of time and giving identical treatment as given in case of sample.

Isolation and Determination of Lead:

(i) Transfer suitable aliquot of ash solution of sample to a 250 ml separator and add citric acid reagent equivalent to 10 g of citric acid and mix well.

(ii) Make slightly alkaline to litmus with NH_4OH, keeping the solution cool, swirl gently and let stand 1 to 2 min. At this stage solution should be clear without any precipitate. (If precipitate forms, redissolve with HCl transfer solution to a stoppered conical flask and adjust to pH 3.0 to 3.4 (bromophenol blue) with NH_4OH. If enough Fe is present to colour solution strongly, make adjust with the help of spot plate. Dissolve any precipitate, if formed, by shaking and cooling.

If amount of lead expected is small, add 5 to 10 mg of pure $CuSO_4 . 5H_2O$ to solution. Pass in H_2S until solution is saturated (3 to 5 min), immediately filter with suction. Dissolve the residue of precipitated sulphides, without previous washing, with 5 ml of hot HNO_3 drawing solution through into original flask, wash with hot water. Stopper the flask, shake the HNO_3 and water washes well and boil the solution to remove H_2S. Transfer this solution to separator add citrate solution equivalent to 5 g of citric acid, make ammonical and proceed from step (iii) onwards.

(iii) To the clear solution obtained after step (ii), add 5 ml of 10% KCN solution (more may be necessary if large amount of Zn, Cu, Cd etc are present), shake and check the pH of the solution by adding a drop of thymol blue indicator solution (pH should preferably be 8.5 to 9.5 blue green to blue colour with thymol blue).

(iv) Immediately add 20 ml of dithizone solution (in this step usually solution of 8 mg dithizone / L of $CHCl_3$ is adequate), shake for 20 to 320 sec and let layers separate. Transfer the $CHCl_3$ layer to small separator containing 25 ml of 1% HNO_3.

(v) Repeat step (iv) and continue extractions till the $CHCl_3$ layer is distinctly green. Drain all the extracts into the small separator containing 1.0% HNO_3.

(vi)Shake well combined extracts in smaller separator well and drain green dithizone layer into another separator containing additional 25 ml portion of 1.0% HNO_3. Shake the contents of the separator, let layers separate and discard the organic layer.

(vii) Filter acid extracts, containing lead, in succession through small pledget of wet cotton inserted in stem of small funnel, into a 50 ml flask using second acid extract to rinse separator in which first extraction was made. Make up any slight deficiency in volume with 1.0% HNO_3 and mix.

(viii) Take suitable aliquot (or entire volume, if needed) of the 50 ml of 1.0% HNO_3 solution containing lead (obtained in step (vii) and enough 1.0% HNO_3 reagent to make total volume to 50 ml (add acid solution first and later lead extract).

(ix) Add 10 ml of ammonia-cyanide mixture and mix (pH is about 9.7).

(x) Immediately add appropriate volume of dithizone solution of suitable concentration, and shake for 1 min and let layers separate. The appropriate volume and concentration of dithizone solution can be chosen as per information provided in the table below:

Pb range (μg)	Concentration of dithizone in $CHCl_3$ (mg of dithizone/1 solution) soln.	Vol. of dithizone to be taken (ml)
0 to 10	8	5
0 to 50	10	20
0 to 200	20	25

(xi) Drain the lower dithizone layer into a clean and dry tube and read absorbance at 510 nm against a REAGENT BLANK by following all the steps from Step (i) to Step (xi) as was done for sample but substituting the aliquot of ash solution taken with same volume of 2N HCl. Carry out isolation and determination of lead content in SAMPLE BLANK SOLUTIONS in identical manner to that of SAMPLE SOLUTION.

Convert absorbance 'A' to μg of Pb from the calibration curve or preferably calculate with the help of the equation obtained by least squares method.

Subtract the lead content obtained for sample blank solution from that of sample solution to get actual lead content of sample solution and calculate the lead content of sample.

Preparation of Standard Curve:

Prepare working curve of required range (to be decided basing based on lead content of samples), starting with blank to final standard of range with four intermediate increments.

(1) Pipette appropriate amounts of lead solution into series of separators and add 1.0% HNO_3 solution so that total volume in each separator is always 50 ml. Add the required volume of acid solution first and then the proposed volume of standard solution.

(2) Add 10 ml of ammonia-cyanide mixture and mix (resultant pH will be 9.7).

(3) Immediately develop colour by shaking for 1 min with proper quantity of dithizone solution from the table. Let layers separate.

(4) Drain lower chloroform layer into a clean and dry tube. Read absorbance 'A' of each standard extract against the extract of zero lead content (i.e. reagent blank of standards). Plot absorbance 'A' against µg of Pb or calculate reference equation by method of least squares.

Standard lead and 1.0% HNO_3 solutions used in preparation of standard curve should be saturated with $CHCl_3$, before use, by shaking those solutions with clear $CHCl_3$ and discarding the organic layer.

EXPERIMENT No-9 and 10

Estimation of Dithiocarbamate (Pesticide) residues in sample by GC-MS(Gas chromatography- mass spectrometer)

Materials requirement for sample preparation

1. Fruits (grapes).
2. **Equipment:**
   - Screw capped bottle
   - Micro-Centrifuge: Rotor head with holding capacity: 2 mL, 10 -15 mL and 50 mL centrifugetubes
   - GC-MS/MS andLC-MS/MS
   - Electronic Balance: Capacities 3-5 kg & 0.01 to 100g
   - Waterbath

Procedure for sample preparation

a) Weigh 25 g sample in 250mL in screw capped bottle.

b) Add 75 mL of reaction mixture. [Weigh 30 g of Tin (II) Chloride and dissolve it in the 1000 mL of HCl]

c) Add 25 mL of isooctane and immediately close the bottle with screw cap.

d) Place the bottle in water bath at 80 (±5) °C.

e) Mix the content of the bottle by inversion after approximately 20 min.

f) Repeat step ‘e’ after every 20 min.

g) After the total reaction time of 60 min, remove the bottle from the water bath and mix the content of bottle.

h) Transfer the bottle in cool ice water bath to cool down the temperature quickly.

i) After cooling the reaction mixture to about 10-20°C, transfer 1.8 mL of isooctane layer in 2 mL micro centrifuge tube.

j) Centrifuge at 5000 rpm for 5 min at 10°C.

k) Transfer the supernatant (1 mL) to an auto sampler vial.

l) Inject in to GC-MS.

Flow chart for analysis of dithiocarbamate residues in sample

250 ml screw cap bottle

➡

Weigh 25 g of grape berry or homogenized sample

➡

Add 75 ml SnCl2 reaction mixture

➡

Add 25 ml of isooctane

➡

Keep on water bath at 80°C for 1 hour

➡

Intermediate of shaking after every 20 min.

➡

Keep bottle at room temperature or normal temperature of water

➡

Transfer the bottle in ice cold water.

➡

Two separate layers

➡

Take upper layer 1-2 ml

➡

Micro-centrifuge at 10 °C

➡

Transfer to GC vial in

GC-MS parameters

Sr. No. Parameter Specification	**Sr. No. Parameter Specification**	**Sr. No. Parameter Specification**
1.	Column	DB-5 ms (5 % diphenyl, 95 % dimethyl polysiloxane, 30 m x 0.25 mm ID, 0.1 µm film thickness) capillary column OR Rxi-624 30 m x 0.32 mm id, 1.8 µ film thickness having 6% cyanopropyl / 94% dimethyl polysiloxane
2.	Oven Temperature	40 °C for 5 min hold time and then ramp it from 40 °C to 200 °C with 5 min hold time.
3.	Injector program	Programmable Temperature Vaporization with Large Volume Injection volume:10 µl Injection Program: start temperature 40°C with 0.1 min hold, then ramp @10°C/s up to 80°C with 0.3 min hold, then ramp up to 110°C at rate of 10°C/s. For cleaning purpose of liner, the temperature was increased up to 290°C for 4 min. OR Splitless 2 µL at 200 °C
4.	Detector	EI - 70 eV Ion source temperature: 200°C Scan Range: In full scan mode, scanning range = 50-100 amu. Inselected ion monitoring mode, select the m/z 76 and m/z 78.

Preparation of Reaction Mixture:

a) Weigh 30 g of Tin (II) Chloride and dissolve it in the 1000 mL ofHCl.

b) Take a plastic can having capacity 2500-3000 mL.

c) Add 1000 mL water in it.

d) Then gradually add 1000 mL of Tin (II) chloride and dissolve inHCl with stirring. Finally a clear solution isobtained.

Standards

Carbon disulphide standard solution:

a) Take 20 mL bottle with screw cap (air tight) and tare the weight.

b) Fill the bottle with isooctane, put cap on the bottle and note down the weight in grams. This isthe weight of the solvent in gram. By using following formula convert weight in gram to mL.

Weight of the solvent in mL (A)

= (weight of the solvent in gram/ density of the solvent in g/mL)

c) Change the unit on the balance gram to milligram (mg)

d) Add pure CS_2 about 20 µL, cap the bottle and note down the weight in mg. This is the weightof the CS_2 standard.

Actual weight of the Standard (B) = (Weight of standard × Purity of standard)/100

Actual concentration of CS_2 stock std solution (C_1) = (Actual weight of the Std (B)/mL of the solvent (A))

e) Prepare the working standard of 100 µg/ mL (C_2) of 5 mL (V_2) in isooctane by using following formul

$C_1 V_1 = C_2 V_2$

$V_1 = C_2 V_2 / C_1$

Standard solution of Thiram:

a) Weigh accurately 10 mg of Thiram in closed standard bottle.

b) Note down the weight of the Thiram in the mg unit.

c) Check the purity of Thiram standard

d) Calculate the actual weight of the Thiram by using following formula

e) Actual weight of the Thiram Standard (x) = (Weight of standard × Purity of standard)/100

f) Cap the bottle with screw cap and tare it.

g) Change the unit of balance mg to g.

h) Add ethyl acetate in bottle and closed the bottle with screw cap.

i) Note down the weight of the ethyl acetate which has been taken in to the glass bottle in the gram.

j) Convert gram weight of the ethyl acetate in the mL weight. By using following formula.

Weight of the Solvent in mL (y) =

(Weight of the solvent in gram/ density of the ethyl acetate solvent in (g/mL))

k) Calculate the actual concentration of the standard (mother stock) by using following formula.

Actual concentration of thiram mother (stock) solution

(C_1) = (Actual weight of the Std (x)/ mL of the solvent (y))

Use isooctane for further dilution. Prepare working standard (C_2) 1µg/mL of 5 mL (V_2) in isooctane by using following formula. $C_1 V_1 = C_2 V_2$

l) Store mother stock solution at - 20°C.

m)Store the working standard solution at 0°C.

n) Perform further dilutions in isooctane.

o) 1 g of Thiram liberates 0.6333 g of CS_2

External calibration solvent standards for GC-MS

a) Freshly prepare fivepoints calibration standards at 0.01, 0.02, 0.05, 0.1 and 0.2µg/mL freshly every day from the working standard of 1µg/mL of CS_2.

b) Prepare matrix matched standard at the same concentrations by extracting fresh sample usingthe procedure and spiking the extract with appropriate volumesof working standards (1µg/mL)

Recovery sample preparation

For recovery experiment, spike calculated amount of thiram in the aqueous

layer, Immediatelyclose the bottle and follow the same extraction procedure.

For recovery level 1 ppm, spike quantity from working standard= 0.4 mL For recovery level 0.5 ppm spike quantity

EXERCISE No-11 & 12

STUDY OF NATIONAL AND INTERNATIONAL QUALITY STANDARDS

International standards and statutes:

- ✓ International Standards Organization (ISO)
- ✓ Codex Alimentaires Commission (CAC)
- ✓ World Health Organization (WHO)
- ✓ Food and Agriculture Organization (FAO)
- ✓ World Trade Organization (WTO)
- ✓ Food and Drugs Administration (FDA), USA
- ✓ International Commission on Microbiological Specifications for Foods (lCMSF)

Standards Organization (ISO):

The product sell based on the product quality as perceived by the customer is the major factor for sustained what makes a sales of a product. The method of quality control consisted largely of physical inspection of the end product against the product specification. However, technical specifications may not by themselves guarantee, that a customer's requirements will be met, if there happens to be any deficiency in the specifications or in the organizational system, to design and produce the products, or service. Consequently, this has led to the development of quality system standards and guidelines that complement relevant product, or service, requirements given in the technical specifications. It is the main criteria for a management system, which will improve a company's performance. It is a media for ensuring orderly and systematic maintenance and upkeep of system. It is applicable to all type of organizations, independent of product size and country and ensures consistent improvement of quality. The role of ISO series is given below.

The role of International Standards Organisation (ISO) series

ISO series Role of ISO

ISO 9000 – 1, 2, 3, 4 Quality management and quality assurance
ISO 9004 - 1 Quality management and quality system elements sub contractor
ISO 9004 – 2 Guidelines for services
ISO 9004 – 3 Guidelines for processed materials
ISO 9004 – 4 Guidelines for quality improvement
ISO 9004 – 5 Guidelines for project management
ISO 9004 – 6 Guidelines for quality plans
ISO 9004 – 7 Guidelines for configuration management
ISO 10011 – 1, 2, 3 Guidelines for auditing quality system
ISO 10012 – 1, 2 Quality assurance requirement for measuring equipment
ISO 10013 Guidelines for developing quality manual
ISO 10014 Guidelines for economic effect of quality
ISO 10015 Continuing education and training guidelines
ISO 22000 : 2005 Specifies requirements for a food safety management system where an organisation in food chain needs to demonstrate its ability to control food safety hazards in order to ensure that food is safe at the time of human consumption.

Salient features of International Standards Organization (ISO):

- Most important international standards setting nongovernmental organization
- World federation of 123 countries national standards bodies
- Develops consensus based international standards
- Develops standard through 200 technical committees split into 650 sub-committees and 200 working groups
- All of its standards are voluntary in nature
- 1SO-9000 series is the general quality certification standard
- Presently, India has about 5000 ISO-9000 companies

Codex Alimentarius Commission (CAC):

The term Codex Alimentarius is taken from Latin and means food code. The Codex Alimentarius Commission develops food standards, guidelines and related texts such as codes of practice under the Joint FAO / WHO Food Standards Programme. It is also called Codex (Hormonized International Standards due to involvement of both FAO and WHO. About 170 countries were member of the commission. The purpose of this program is to protect the health of consumers and to ensure fair practice in the food trade; to promote coordination of all food standards work undertaken by international governmental and non-governmental organizations; to determine priorities and initiate and guide the preparation of draft standards through and with the aid of appropriate organizations; to finalize standards and after acceptance by Governments, publish them in a Codex Alimentarius either regional or worldwide standards. It brings together all the interested parties viz. scientists, technical experts, governments, consumers and industry representatives to help develop standards for food manufacturing and trade.

These standards, guidelines and recommendations are recognized worldwide for their vital role in protecting the consumer and facilitating international trade. As Codex Alimentarius represent a consensus of food and trade experts from around the world, these standards are more and nl0re being used in international trade negotiations and also for setting of disputes by WTO. The Codex contract Point in India is the Directorate General of Health Services (DGHS) in the Ministry of Health; however, the Ministry of Food processing Industries is closely associated with the activities of Codex Alimentarius.

Salient Features of Codex Alimentarius:

- ✓ Protecting health of the consumers and ensuring fair trade practices
- ✓ Promoting coordination of all food standards work undertaken by international governmental and non-governmental organizations
- ✓ Determining priorities and initiating and guiding the preparation of draft standards.
- ✓ Finalizing standards
- ✓ Amending published standards
- ✓ Submission of a proposal for a standard
- ✓ A decision by the Commission or the executive committee
- ✓ Preparation of a proposed draft standard by subsidiary body
- ✓ Adoption of standard by the Commission
- ✓ Addition of Codex Standard in the Codex Alimentarius

Food and Drug Administration:

The Food and Drug Administration (FDA or USFDA) is an agency of the United States Department of Health and Human Services, one of the United States federal executive departments. The FDA is responsible for protecting and promoting public health through the regulation and supervision of food safety, tobacco products, dietary supplements, prescription and over-the-counter pharmaceutical drugs (medications), vaccines, biopharmaceuticals, blood transfusions, medical devices, electromagnetic radiation emitting devices (ERED), veterinary products, and cosmetics.

The FDA also enforces other laws liking sanitation requirements on interstate travel and control of disease on products ranging from certain household pets to sperm donation for assisted reproduction. The FDA has its headquarters at White Oak, Maryland. The agency also has 223 field offices and 13 laboratories located throughout the 50 states.

Organization: The FDA comprises several offices and centers.

- ✓ Office of the Commissioner
- ✓ Center for Biologics Evaluation and Research
- ✓ Center for Devices and Radiological Health (CDRH)
- ✓ Center for Drug Evaluation and Research (CDER)
- ✓ Center for Food Safety and Applied Nutrition
- ✓ Center for Tobacco Products
- ✓ Center for Veterinary Medicine
- ✓ National Center for Toxicological Research
- ✓ Office of Regulatory Affairs

National standards

- The Prevention of Food Adulteration Act, 1954
- The Fruit Products Order, 1955
- Essential Commodities Act, 1955.
- Agricultural Produce (Grading and Marking) Act (AGMARK), 1937
- Indian Standard Institute (ISI) : Bureau of Indian Standards (BIS) (ISI Mark), 1986
- The Meat Food Products Order, 1973
- The Vegetable Oil Products (Control) Order, 1947
- The Milk and Milk Products Order, 1992
- Food Safety and Standard Act, 2006

Prevention of Food Adulteration Act (1954) and Rules (1955): One of the early acts to be promulgated in food laws and standards was the Prevention of Food Adulteration Act of 1954, which has been in force since June I, 1955. The objective of this act was to ensure that food articles sold to the customers are pure and wholesome. This prevents fraud or deception and encourages fair trade practices. Amendments were made to plug loopholes in the act and to ensure stringent punishment for those indulging in malpractices.

The PFA Act lays down the guidelines for setting up standards for various food items like cereals and cereals products, pulses, ghee etc. All these processed items are expected to conform to these standards.

The Act prohibits the manufacture, sale and distribution of not only adulterated foods but also foods contaminated with toxicants and misbranded foods. A central committee for food standards has been constituted under the Act and has been charged with the function of advising the Central Government on matters relating to the Food Standards.

It is mandatory & comes under the Ministry of Health and Family Welfare to operate Central Committee for Food Standards (CCFS). The act provides minimum standards for all types and categories of food products. It extends to the whole of India. Adulteration also covers misbranding or mislabelling and anything else which may be injurious to consumers health. Not following of PFA Act lead to fine and imprisonment for a term of not less than 3 months to one year.

Salient features of the PFA Act:

- The act is mandatory
- PFA -1954 and 1955 protects consumers against impure, unsafe, and fraudulently labeled foods
- Applies to domestic and imported products and cover various aspects of food processing

Essential Commodities Act, 1955: This Act is administered by the Ministry of Consumer Affairs, Food and Public Distribution through the State/Union Territory. The aim of this Act is to regulate the manufacturer toward quality aspect, commerce and distribution of essential commodities like food. Number of orders has been listed in the provision of the act.

- distribution-food colour, preservatives, pesticide residues, packaging and lebelling, etc.
- All imported products must adhere to the rules specified in the Act and its regulations
- Focuses primarily on the establishment of regulatory standards for primary food products, which constitute bulk of the Indian diet
- According to the Act, an article of food shall be deemed to be adulterated if
- If the article sold by a vendor is not of the nature, substance or quality demanded by the purchaser and as it is represented to be.
- If it contains any other substance or processed as to affect injuriously the nature.
- If any inferior or cheaper substance has been substituted whollyor in part for the article.

Bureau of Indian Standards (BIS): BIS is the National Standards Organization established as Society in 1947 as Indian Standards Institution and subsequently made its statutory body as BIS under Bureau of Indian Standards Act 1986. It revoked Indian Standards Institutions (Certification Marks) Act 1952 but incorporated all its provisions. The Bureau is a body corporate and responsible for laying down policy guidelines for BIS. It comprises of members Representing Industry, Consumer Organizations, Scientific and Research Institutions, Professional/technical institutes, Central Ministries; State Government and Members of Parliament. The act is mandatory for milk powders, sweetened condensed milk, infant formula etc. This act generally covers hygienic conditions of manufacture, raw material quality and safety. It also ensures the quality to the consumers by certification.

Salient Features of the BIS:

· Standard Formulation,

· Certification :

  - ✓ Product Quality Management System, Eco Mark,
  - ✓ Environment Management System,
  - ✓ Hazard Analysis' and Critical Control Points,
  - ✓ Laboratory: Testing, Calibration and Management, Standards Promotion,
  - ✓ Affairs and Awareness and Training Programs.

Food Safety and Standards Act, 2006: An act to consolidate the laws relating to food and to establish the Food Safety and Standards Authority (FSSA) of India for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, import, to ensure availability of safe and wholesome food for human consumption and for matters connected therewith or incidental thereto. FSSA, 2006 bring the different pieces of legislation pertaining to food safety under one umbrella which will override the PFA, 1955 and various Quality Control Orders under Essential Commodities Act, 1955.

The aim is to better coordinate and integrate food safety controls across India to give highest level of health protection.

Salient Features of Food Safety and Standards Act:

- ✓ Enhanced food safety in the country should provide opportunities for additional value-added agri-food exports
- ✓ Food safety is no longer a national issue. It will become an increasingly important global issue. India can play an important role in enhancing food safety capacities of developing countries
- ✓ which in turn will bring additional benefits
- ✓ The food safety programs offer opportunities for human capital formation through food safety education and training

Agricultural Produce (Grading & Marketing) Act, 1937: It is popularly known as 'Agmark'. The AGMARK standard was set up by the Directorate of Marketing and Inspection of the Government of India by introducing an Agricultural Produce Act in 1937. The word 'AGMARK' seal ensures about quality and purity of the food products.

Salient Features of AGMARK Standards:

- ✓ Quality standards for agricultural commodities are framed based on their intrinsic quality.
- ✓ Food safety factors are being incorporated in the standards to compete in World trade.
- ✓ Standards are being harmonized with international standards to keeping in view the WTO requirements.
- ✓ Check is kept on the quality of certified products through 23 laboratories and 43 offices spread all over the country
- ✓ The grades incorporated are grades 1, 2, 3 and 4 or special, good, fair and ordinary. In this Act an article is said to be misgraded if does not meet any of the following points
- ✓ The article is not of the quality prescribed for the grade and designation with which it is marked.
- ✓ The composition of the article offered for grading is altered in any way after a sample has been drawn for analysis and determination of the grade designation of the article in accordance with the rules made under this Act.
- ✓ If the article is tampered with another material.
- ✓ Any false claim is made for the quality prescribed for its grade designation, upon the label or through advertisement or in any other manner.
- ✓ The actions which can be taken on misbranding are:
- ✓ Seize and detain any agricultural produce in relation to an offence
- ✓ under this Act or the rules.
- ✓ Penalty for un-authorized marking with grade designation mark
- ✓ Penalty for counterfeiting grade designation mark
- ✓ Penalty for selling misgraded articles
- ✓ Power to prescribe compulsory grade designations in respect of certain articles
- ✓ Eligibility for AGMARK Standards: Parties desirous to grade their commodities under AGMARK have to obtain Certificate of Authorization.
- ✓ For the purpose, they should have adequate infrastructure to process the commodity and access to an approved laboratory for the determination of quality and safety factors.