GENERAL LABORATORY MANUAL

ON

FOOD, WATER &DRUGS ANALYSIS

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This manual covers methods of analysis of following products:

- **Food products (oils, package food, drink, grains etc)**
- **Water (both package and unpackaged water**
- **Drugs**

This manual was compiled from approved standard regulatory bodies' manuals. This includes:

- **National agency for food and drugs administration and control (NAFDAC)**
- **World health organization (WHO)**
- **Food Safety and Standards Regulations, India.**
- **United state pharmacopeia standard operating procedure**
- **British pharmacopeia standard operating procedure**

Table of content

CHAPTER ONE

REAGENTS PREPARATION

Standard solution is a solution whose concentration is known. Standard solutions are mostly prepared from primary solution. Preparation of standard solution for analysis/practical has been a hot case to most chemistry teachers, undergraduate students, and some analysts. Hence, this chapter will help a lot in understanding how to go about preparing standard solution from the stock.

Preparation of standard solution has to do with either solid substances (salt) or liquid substances (mostly acid).

When dealing with solid substances (salts) weighing of the calculated amount of the solid substance is very necessary, while when dealing with liquid substances, measuring of the calculated volume of the liquid is very paramount.

A. PREPARATION OF STANDARD SOLUTION FROM LIQUID SUBSTANCE

When dealing with liquid substances, the following information about the stock liquid is very important and must be noted:

1. Specific density of the liquid in g/cm^3
2. Specific gravity of the stock liquid
3. %purity by mass of the stock liquid
4. Molar mass of the stock liquid

The above information are obtained from the label of the stock liquid usually provided by the manufacturer.

When the above information is available, the preparation can be done in either of the two ways:

1. Determining the molarity of the stock liquid substance (acid) first, followed by applying dilution principle
2. calculating the volume of stock (acid) require for the preparation of a required volume of standard solution.

Below is a table showing the specification of some common acids.

Specification	HNO_3	HCl	H_2SO_4
% by mass	70	36	98
Density g/cm^3	0.994	0.425	1.760
Specific gravity	1.42	1.18	1.80
Molar mass	63.00	36.50	98.00

1. DETERMINE THE MOLARITY OF THE STOCK FOLLOW BY DILUTION PRINCIPLE

The general formula for calculating the molarity of a stock acid is given as

$$C_S = \frac{10pd}{m.m}$$

Where Cs = molarity of stock liquid
P = %by mass of the liquid
d = density in g/cm3 or specific gravity
m.m = molar mass of the liquid

While dilution principle formula is given as:

C1V1= C2V2

Where C1 = conc. of the stock solution
V1 = volume of stock solution
C2 = desired conc. to be prepared
V2 = desired volume of the standard solution

Example: How can you prepared 500ml, 0.031M of HCl whose %by mass = 36, specify gravity = 1.18 and m.mass = 36.5g/mole?

Solution
Firstly determine the molarity of the stock solution by using the formula

$$C_S = \frac{10pd}{m.m}$$

Cs =?, P=35, d=1.18 and m.mass = 36.5g/mole

$$C_S = \frac{10 \times 35 \times 1.18}{36.5}$$

$$= 11.64 mol/dm^3$$

: . Molarity of the stock acid = 11.64mol/dm^3

The molarity of the stock acid is now known to be 11.64mol/dm3. Therefore, the volume of the stock acid required to prepare 500cm3 of 0.031M must be calculated using dilution formula.

$C_1V_1 = C_2V_2$

Where C_1 = 11.64M, V1=? C_2 = desire conc. = 0.031M

V_2 = desire volume = 500cm3

11.64M x V_1 = 0.031M x 500cm3

$$V_1 = \frac{0.031M x 500cm^3}{11.64M}$$

= 1.336cm3

:. $V_1 \approx$ 1.3cm3

Prepare 500 cm^3 of 0.031M HCl, transfer 1.3cm3 of the stock acid to 30cm3 portion of distilled water in a beaker. Transfer the resulting solution into 500cm3 volumetric flasks and fill to mark with distilled water, cork and shake very well. You now have 500cm^3 of 0.031M HCl solution.

Example

How can you prepare 200cm^3 of 1.2M HNO_3 acid? Given that, the specific gravity = 1.426, % by mass = 70 and m.mass = 63g/mole

Solution

$$C_S = \frac{10pd}{m.m}$$

Where C_s =?, P = 70, d = 1.41 and m.mass = 63g/mole

$$C_S = \frac{10 \; x \; 70 \; x \; 1.42}{63}$$

= 15.8M

:. C_s = 15.8M

Dilution principle

$C_1V_1 = C_2V_2$

$C_1 = 15.8M$, $V_1 = ?$, $C_2 = 1.2M$, $V_2 = 200cm^3$

15.8M x V = 1.2M x 200cm3

$$V_1 = \frac{1.2M x 200cm^3}{15.8M}$$

:. V1 = 15.2cm3

Therefore, $15.2cm^3$ of conc. HNO_3 should be measured and transfer into $30cm^3$ of distilled water. Transfer the resulting solution into $200cm^3$ volumetric flasks and fill to mark with distilled water.

1. CALCULATING THE VOLUME OF STOCK REQUIRED FOR THE PREPARATION OF REQUIRED VOLUME OF STANDARD SOLUTION.

Calculating the volume of the stock acid required can be done using the formula below.

$$Cs = \frac{C_r V_r m.mass}{10pd}$$

Where Cs = volume of stock solution
Cr = required conc.
Vr = required volume of standard solution
P = % by mass of the acid
d = specific gravity of acid
m.mass = molar mass of acid

Example

How can you prepare $500cm^3$ of 0.23M H_2SO_4? When the acid has the following specifications; m.mass 98g/mole, %by mass = 98% and specific gravity ≈ 1.80

Solution

Volume of stock acid required to prepare $500cm^3$ of 0.23M H_2SO_4 can be calculated using the formula.

$$V_s = \frac{C_r V_r \, m.m}{10pd}$$

$$Vs = \frac{0.23 \times 500 \times 98}{96}$$

$Vs = 0.0064dm^3$

$1dm^3 \approx 1000cm^3$

$0.0064dm3 = xcm^3$

$$x = \frac{0.0064dm^3 x 1000cm^3}{1dm^3}$$

$Vs = 6.4cm^3$

Therefore, measure $6.4cm^3$ of stock (acid) into a beaker containing $30cm^3$ of distilled water. Transfer the resulting solution into $500cm^3$ volumetric flasks and fill to mark with distilled water.

In some cases, you will be asked to prepare a standard solution in which its concentration is expressed in g/dm^3 from a liquid substance. In such cases, the given conc. in g/dm^3 must first be converted to mol/dm^3, followed by calculation of volume of stock solution required for the preparation using normal formula.

Example
How can you prepare $300cm^3$ $18.2g/dm^3$ of HNO_3?
(m.mass =63g/mole)

Solution
It is not convenient to weigh liquid substances; therefore, conversion of unit of expression from g/dm^3 to mol/dm^3 is necessary.

$$\text{Conc. in mol/dm}^3 = \frac{\text{conc. in g/dm}^3}{\text{m.mass}}$$

$$\text{Conc. in mol/dm}^3 = \frac{18.2g/dm^3}{63g/mole}$$

$= 0.2889mol/dm3 \approx 0.29mol/dm3$

:. 300cm^3 of 18.2g/dm^3 of HNO_3 is equivalent to300cm^3 0.29mol/dm^{3}of HNO_3. Now, how to prepare 300cm^3 of 0.29mol/dm^3.

$$V_s = \frac{C_r V_r \, m.m}{10pd}$$

$$where\ V_r = 300cm^3 = 0.3dm^3$$

$$V_s = \frac{0.29M x 0.3dm^3 x 63g/mole}{10x70x1.41}$$

$$Vs = 0.0056dm^3 \approx 5.6cm^3$$

Therefore, measure 5.6cm^3 of conc. HNO_3 into 30cm^3 of distilled water in a beaker. Transfer the resulting solution into 300cm^3 volumetric flasks and fill to mark with distilled water, now you have 300cm^3 18.2g/dm^3 of HNO_3 solution.

2. PREPARATION OF STANDARD SOLUTION FROM SOLID SUBSTANCES

When preparing standard solution from solid substances, weighing is one of the things that must be done carefully. After calculating the amount of solid required for the preparation of the solution, the next thing is weighing of the solid. The solid weighed is transferred into a small portion of distilled water in a beaker. All traces of the solid must be washed into the beaker and stirred to dissolve completely. The resulting solution should then be transferred to a specific volume of volumetric flask and filled to mark with distilled water.

The amount of the solid substance in gram that is required for the preparation of a standard solution can be calculated by the formula.

$$\text{Amount in gram} = \frac{\text{conc. in mol/dm}^3 x \text{ volume(cm}^3) \ x \ \text{m.mass}}{1000}$$

Where : conc. in mol/dm3 = desire conc. of standard solution
m.mass = molar mass of the solid

Example
How can you prepare 250cm3 of 0.014M NaOH Solution? (m.mass = 40g/mole)

Solution

$$\text{Amount of NaOH in gram} = \frac{\text{conc. in mol/dm}^3 x\ \text{volume(cm}^3) \ x\ \text{m.mass}}{1000}$$

$$= \frac{0.014\text{mol/dm}^3 x\ 250\text{cm}^3 \ x\ 40\text{g/mole}}{1000\text{cm}^3}$$

:. Amount of solid = 0.14g

NaOH is delinquent and extract weight of 0.1g is usually added to the calculated weight (0.14g + 0.1 = 0.24g)

:. Weigh 0.24g of NaOH and dissolve in $50cm^3$ of distilled water in a beaker, stir the solution until it dissolves completely. Transfer the resulting solution to $200cm^3$ volumetric flask and fill to mark with distilled water. Now you have $200cm^3$ of 0.014M NaOH solution.

Example 2
How can you prepare $1000cm^3$ of 0.18M oxalic acid ($H_2C_2O_4$). (molar mass = 90g/mole)

Solution

$$\text{Amount of oxalic required in } \boldsymbol{g} = \frac{0.18x1000x90}{1000}$$

= 16.2g

Weigh 16.2g of oxalic acid and dissolve in $100cm^3$of distilled water. Transfer the resulting solution into $1000cm^3$volumetric flasks and fill to mark with distilled water.

PREPARATION OF STANDARD SOLUTION IN WHICH IT'S CONCENTRATION IS EXPRESSED IN TERM OF %

Most times, some standard solutions required for analytical work, their concentrations are expressed in term of %w/v or %v/v

Example

How can you prepare $400cm^3$ of 30%(v/v) H_2SO_4 solution?

Solution

30%(v/v) means 30% of conc. H_2SO_4 and 70% of distilled water.
i.e $30cm^3$ H_2SO_4 contained in $100cm^3$ of the solution
xcm3 H_2SO_4 will be contained in $400cm^3$ solution.

$$x = \frac{30cm^3 x\ 400cm^3}{100cm^3}$$

$$= 120cm^3$$

Therefore, to prepare $400cm^3$ of 30%(v/v) H_2SO_4 solution, measure $120cm^3$of conc. H_2SO_4 into $280cm^3$ of distilled water. Now you have $400cm^3$ of 30%(v/v) H_2SO_4 solution.

DILUTION PRINCIPLE

During analysis, you may require a solution of lesser concentration to the available prepared solution, and it becomes necessary for you to use the available prepared solution. The available solution which has a higher concentration than your required solution can be diluted to desire concentration by adding more water to achieve the desire concentration, principle of dilution must be applied using formula below.

C1V1 = C2V2

Example

Describe how you dilute accurately a solution of 0.10mol/dm3ethanoic acid to $250cm^3$ $0.01mol/dm^3$.

Solution

In this question, you are expected to calculate the required volume of the solution to be diluted.

C1V1 = C2V2

Where: C1 = 0.10mol/dm^3, V1 = ?, C2 = 0.01mol/dm3, V2 = 250cm^3

0.10mol/dm^3 x V1 = 0.01mol/dm^3x 250cm^3

$$V_1 = \frac{0.01\text{mol/dm}^3 \, x \, 250\text{cm}^3}{0.10\text{mol/dm}^3}$$

V1= 25cm3

:. To prepare 250cm^3 0.01mol/dm^3 of ethanoic acid from 0.10mol/dm^3. Transfer 25cm^3 of 0.10M to 250cm^3 volumetric flask and fill to mark with distilled water.

Now you have 250cm^3 0.01M ethanoic acid solution.

Example 2

What volume of distilled water will be required to dilute 20cm^3 of NaOH solution whose concentration is 0.2M to 0.001M?

Solution

C1V1 = C2V2

Where C1 = 0.2M, V1 = 20cm^3, C2 = 0.001M, V2 = ?

0.2Mx 20cm^3 = 0.001M x V1

$$V_2 = \frac{0.2\text{M} \, x \, 20\text{cm}^3}{0.001\text{M}}$$

= 4000cm^3

This means, the final volume of the solution should be 4000cm^3. Now, 4000cm^3 minus 20cm^3 of the solution to be diluted (4000cm^3 – 20cm^3 = 3980cm3)

:. Therefore, 3980cm^3 of distilled water is required for the dilution.

PREPARATION OF 100ml OF 1% PHENOLPHTHALEIN INDICATOR

1% means 1g in 100ml.

Therefore, weight 1g of powder phenolphthalein indicator into 100ml volumetric flask, and dissolve with small portion of 95% ethanol and fill to mark with 95% ethanol.

PREPARATION OF FEHLING'S I SOLUTION (500ML).

44.2990g of CuSO4.5H20 per l000ml.

Xg of $CuSO_4.5H_2O$ per 500ml

$$X = \frac{44.2990g \times 500ml}{l000rnl}$$

$$= 22.1495g$$

Weight 22.1495g of $CuSO_4.5H_20$ into 500ml Volumetric flask and dissolve with small portion of distilled water. Fill to mark with distilled water.

CHAPTER TWO

FOOD ANALYSIS

MOISTURE CONTENT ANALYSIS

Title: Determination of moisture content analysis

Principle: Heating of sample in an oven at 105 ± 2^0C and the loss in weight of sample is calculated as the moisture content. This method is applicable to all food and non-food substances e.g flour, pasta, chinchin, salt, grains, cake, cement, yoghurt seasonings etc.

Equipment: Analytical weighing balance electric oven: desiccators, Porcelain crucible and metal tong

PROCEDURE

- Dry a clean weighing crucible in an oven at 105 ± 2^0C for 15 minutes
- Cool in desiccators and then weigh (w_1)
- Introduce 3-5g of sample in weighing crucible and take the weight (w_2)
- Place crucible in water bath to evaporate the water completely. (This applicable liquid substance such as yoghurt, ice cream etc).
- Place the weighing crucible in an oven set at 105 ± 2^0C for 3 hours
- Return the weighing crucible to the desiccators using crucible tong and allow to cool for 15-30 minutes
- Weigh the crucible containing the sample (w_3)

Expression of Result

% moisture content = $\frac{w_2 - w_3}{w_2 - w_1} \times \frac{100}{1}$

where, w_1 = weight of the empty crucible

w_2 = weight of empty crucible + sample before drying

w_3 = weight of the crucible + sample after drying

PROTEIN ANALYSIS

Title: Determination % protein content in a given sample.

Scope: This method applies for crude protein content determination for all solid food products. This method is also known semi-micro kjeldal method.

principle: Protein compounds are digested by the addition of sulphuric acid and selen catalyst to ammonium sulphate. By addition of sodium hydroxide, the solution is then distilled to produce ammonium and it is trapped in a boric acid solution hich results in ammonium borate. Ammonium borate solution is quantitatively titrated by a standard acid solution.

Apparatus: Kjeldal digestion flask, conical flash, burette, volumetric flask, measuring cylinder, analytical balance,

Reagents: concentrated H_2so_4, 50% a solution of sodium hydroxide, screened methyl red indicator, 5% sodium thiosulphate, 2% boric acid, kjeldal catalyst tablets

PROCEDURE

1. Weigh about 2g of the sample and transfer with the paper into a kjeldal flask.
2. Add 25ml concentrated H_2SO_4 using 10ml pipette

3. Add 2 table of selenium catalyst tablet.
4. Heat the flask gently in the digestion unit (fume cupboard) at 30^0C .
5. Swirl the flask occasionally to wash down charred particles from the sides of the flask.
6. When the initial vigorous reaction has died down increase the heat and continue digestion unto the liquid turns clear and free from black or brown colour. Allow to cool.
7. Dilute content with about 200ml of distilled water and connect the distillation apparatus.
8. Measure 50ml of 2% boric acid into a conical flask.
9. Add 80ml of 40% sodium hydroxide
10. Add seven drops of screened methyl red indicator and swirl
11. Place it on the receiver so that the end of the delivery tube dips just below the level of the boric acid and set the apparatus at 60^0C.
12. Run in about 85ml of sodium hydroxide into the distillation flask.
13. Add 50ml distilled water, boil vigorously until about 250ml have distilled over.
14. Titrate with standard 0.02M HCl until a neutral grey end point is obtained

CALCULATION

$$\% \text{ protein content} = \frac{(\text{ml acid}) \times (\text{N acid}) \times (0.014\ \text{N}) \times (100)}{\text{Weight of sample}}$$

ASH CONTENT ANALYSIS

Title: Determination of % ash content in a given sample

INTRODUCTION: Ash is defined as the organic residue remaining after water and other volatile materials have been removed by heat. It helps to check for adulteration, it helps to depict the mineral content of the food substance.

AIM: To determine of % ash content in a given sample

PRINCIPLE: Ashing of sample in a furnace at 600^0C for certain period of time and the ash content is calculated by weight difference between crucible and ash left in crucible after drying

EQUIPMENT: Porcelain crucible, spatula, analytical balance, muffle furnace.

PROCEDURE

1. Weigh an empty crucible which has been previously dried and cooled in a deccicator and weigh (w_1)
2. Weigh accurately 3-5g of samples (w_2)
3. Transfer the crucible (w_2) to a muffle furnace which has been maintained at 600^0C. Ash the sample until constant weight is reached.
4. Remove the crucible and place in a desiccator provided with efficient desiccator.
5. Allow the dish to cool and take the weight (w_3)

CALCULATION

$$\% \text{ ash content} = \frac{w_3 - w_1}{w_2 - w_1} \times \frac{100}{1}$$

Where: w_1 = weight of empty crucible

w_2 = weight of empty crucible + sample before drying

w_3 = weight of empty crucible + sample after drying.

FAT CONTENT ANALYSIS

Title: Determination of percent fat content present in a given sample (Sohxlet method).

Principle: Extracting fat material with organic solvent, the extracted fat compared to the sample weight is expressed as the fat content. (This is applicable in all solid food substances e.g meat, groundnut, seeds, noodles, grains, flours, milk powder etc)

Equipment: Sohxlet extractor, weighing balance, desiccators, electric oven, a pair of tongs.

PROCEDURE

1. Into an empty filter paper, weigh 3-5g of the blended sample and wrap carefully.
2. Into an empty 250ml quick fit flat bottom flask, add a pinch of antibumping granules and weigh
3. Set up the experiment in a soxhlet apparatus by adding 200ml of petroleum ether and set the temperature knob at 25^0C
4. Leave the experiment to stand for 4hrs and at the end extraction process remove the sample using a crucible tong.
5. Allow the petroleum ether to reflux twice before the covering of petroleum ether.
6. Remove sample and place in oven for 3hrs at 105^0C and send to the desiccators to cool for 30mins and weigh sample immediately

CALCULATION

$$\% \text{ fat content} = \frac{w_2 - w_3}{w_2 - w_1}$$

Where w1 = weight of empty flask + antibumping granules

w_2 = weight of flask + antibumping granules + sample before extraction

w_3 = weight of flask + antibumping granules + sample after extraction.

TOTAL CARBOHYDRATE TEST

This is obtained by calculation, after the percentage moisture, fat, protein and ash content has been determine then, total carbohydrate can be determines using this formula:

% Total carbohydrate = 100 – (%moisture + %fat + %protein + %ash)

ESTIMATION OF REDUCING SUGAR IN DRINKS/FOOD

INTRODUCTION: In fruits, both reducing and non-reducing sugars are present in varying amount. Reducing sugars are those hexose ($C_6H1_2O_6$) sugars, which can reduce compounds such as alkaline (ammoniacal) silver nitrate solution, cupric salt solution etc., because they themselves are oxidized. Hexose sugars are divided into 2 main groups, which are aldo-hexose and keto-hexose. Aldo-hexose or aldose contains aldehyde group and keto-hexose or ketose contains ketone group. Aldehyde are strong reducing agents. Hexose sugars which contain aldhyde groups eg. glucose, galactose, mannose etc., are reducing sugars. Ketones are however, more resistant to oxidation than aldehydes, because it involves the breaking of a relatively stable C-C bond. Hence, they do not ordinarily reduce alkaline silver

nitrate or cupric salt solution. But those fructose contains ketone, it is able to reduce readily as easily oxidizable CO-CH_2OH group is present in it and it acts as reducing sugar. Non-reucing sugar eg., sucrose is a disaccharide and cannot reduce alkaline silver nitrate or cupric acid solution. (This method is applicable to all soft drink.

PRINCIPLE: When sugars are extracted and titrated, the reducing sugars only take part in the reaction in making reduction, but the non-reducing sugars that are present in it, do not take part in reduction and remains as such. Accordingly, only the reducing sugars are estimated by titration.

REAGENT:

- Fehling Solution – 5ml Fehling's A + 5ml Fehling's B + 20 ml Water
- 45 % Lead acetate ($C_2H_3O_{22}Pb$, 3H2O): 45g of Lead Acetate in 100 ml water
- 22 % Oxalic Acid: 22g of Oxalic Acid in 100 ml water

PROCEDURE:

- Take 25 g of sample (filtered juice) and heat for 3 minutes, till it turns to a curd like appearance.
- Add 2ml of 45% Lead Acetate and wait for 2 minutes.
- Add 22% Oxalic acid to the sample to remove the excess Lead acetate.
- Wait till a yellowish tint appears and add NaOH until the bubble retains in the sample to neutralize the solution.
- Make up to 250 ml and titrate against hot Fehling's solution. Add Methylene Blue at the end point and heat.

- End point of the reaction is a green colour appearance. On addition of methylene blue and heating red colour appears.

$$\text{Total Reducing sugar} = 0.05 \times \frac{250}{T.V} \times \frac{100}{\text{wt. of sample}}$$

Where : T.V = Titre value

0.05 = Normality of Fehling's solution

Wt. = weight of sample

250 = dilution volume.

TEST FOR TOTAL SUGAR IN DRINKS/FOOD

AIM: To estimate total non-reducing sugar in drinks.

PRINCIPLE: The non-reducing sugars which are not titritable are first hydrolyzed to reducing sugars. Thus after hydrolysis, the non-reducing sugars are converted to reducing sugars while the reducing sugars that are already present in the sample remain unchanged. Accordingly, all the sugars that are present after hydrolysis remain as reducing sugars. This is conveniently termed as total sugars.

REAGENTS

- Fehlings Solution – 5ml Fehlings - A + 5ml Fehlings - B + 20 ml Water
- 45 % Lead acetate ($C_2H3O_{22}Pb.\ 3H_2O$): 45g of Lead Acetate in 100 ml water
- 22 % Oxalic Acid: 22g of Oxalic Acid in 100 ml water
- NaOH drops to neutralize

PROCEDURE

- Procedure is same for reducing sugar the volume made up to 250 ml.
- Out of the 250ml sample solution, take 50 ml and add 5 gm citric acid.
- Heat the sample and make up to 250 ml with water.
- Titrate against the Fehling's solution.
- End point of the reaction is a green colour appearance. On addition of methylene blue and heating brick red colour appears.

$$\text{Total sugar} = 0.05 \times \frac{250}{T.V} \times \frac{100}{\text{wt. of sample}}$$

Where : T.V = Titre value

0.05 = Normality of Fehling's solution

Wt. = weight of sample

250 = dilution volume.

The non-reducing sugars present in the sample may be determined from the values of the total and the reducing sugars as follows.

Percentage of non-reducing sugars = [Percentage of (Total sugars)- (Reducing sugars)] x 0.95

QUANTITATIVE TEST OF BENZOIC ACID IN DRINKS (YOGHURT)

INTRODUCTION:

Benzoic acid is a food preservative commonly used in sugary soft drinks and yoghurt. Benzoic acid and its salts (sodium benzoate) have inhibitory effect on the growth of yeast, a major cause of food spoilage. Benzoic acid is not a suitable preservative for fruit juice because it reacts with ascorbic acid to produce benzene, a potential carcinogenic compound.

AIM: To determined the concentration of benzoic acid in drinks (Yoghurt and yoghurt-like products).

PRINCIPLE: The benzoic in the drink is extracted using organic solvent. The extracted benzoic is quantified by neutralization reaction.

MATERIALS/REAGENTS:

- 50rnl measuring cylinder
- 50ml Burette
- 250m1 volumetric flask
- 100ml Separation funnel
- Litmus papers
- Water bath
- 0.005M NaOH
- 10% NaOH.
- 25% HCl
- Chloroform
- Phenolphthalein indicator

PROCEDURE:

- Measure 25ml of the sample into 250ml volumetric flask
- Add 50ml of saturated NaCl solution
- Neutralize the solution in the flask by adding drops of 10% NaOH
- Test the solution with litmus paper to confirms in the solution been neutralize

- Fill the flask to mark with more saturated NaCl solution and keep if for 2hrs
- After 2hrs, filter the solution.
- Measure 100ml of the filtrate into separating funnel
- Add 1ml of 25% HCl and test the solution for acidity using litmus paper
- Add 20ml of chloroform into the solution in separating funnel. Shake very well and allow the chloroform layer to separate from water.
- Gently open the tap of the funnel and collect the aqueous layer into a beaker while chloroform layer into a clean conical flask (Extraction).
- Return the aqueous solution into the separating funnel and Repeat the extraction process using 30, 30 and 20ml of chloroform. Collect the entire chloroform layer into one conical flask.
- Place the chloroform solution in a water bath to evaporate to dryness.
- Add 30ml of ethanol to the residues left after complete evaporation.
- Add three drops of indicator.
- Titrate against 0.01N NaOH in the burette.

CALCULATION

$$\textbf{Conc. Of Benzoic acid (ppm)} = \frac{\textbf{Titre x 0.0061x10}^{6}}{\textbf{Volume of sample}}$$

CAC/NAFDAC Standard: $\leq$ 300ppm.

DETERMINATION OF ACIDITY AND PROTEIN CONTENT BY TITRATION METHOD

INTRODUCTION: Acidity is the degree of hydrogen ion concentration present in the sample while acidity in respect to this analysis is the measured the amount of lactic acid present in the sample. More also, the amount of amino acid present in the sample depicts the proteins and quality of the product.

AIM: To determine the degree of acidity and protein content in yoghurt and yoghurt- like products.

PRINCIPLE: The principle is base on standardization of the sample using standard alkaline. The volume of standard alkaline required to neutralized the sample is used to determined the acidity of the sample. Formylaldehycle is added to after neutralization to bind lo amino group of the amino acid, making the solution more acidic. The volume of base required to neutralize the effect of carboxyl group of amino acid depict the protein content of the product.

MATFRIALS/REAGENT : 50ml burette, retort-stand, conical flask, measuring cylinder,, dropper, 0.1M NaOH, phenolphthalein indicator, and Formylaldehyde

METHODOLOGY

1. Transfer 10ml of the sample into a conical flask
2. Add 3 drops of indicator
3. Fill the burette with 0.1M NaOH and adjust to zero mark
4. The burette was filled with O.1M NaOH.
5. Titrate until permanent faint pink colour is form. Note the volume (first titre value).
6. Add 2ml of formylaldehyde to the solution in the conical flask and shake very well

7. Continue the titration until permanent faint pink colour is form. Record the volume (second titre value).

CALCULATION

%Acidity = First titre value X 0.09008 X 100.

%Protein = Second titre value – (0.1) X 1.95.

CAC/NAFDAC standard: (Acidity: ≤4%)

DETERMINATION OF IODINE CONTENT IN SALT (NaCl)

AIM: Todetermine the amount of iodine in a table salt.

Apparatus:

- Analytical balance
- 250ml conical flask
- Burette (25ml)
- Spatula

Reagent:

- Distilled water
- Potassium iodide
- Sodium thiosulphate – 0.005N
- Starch indicator (1%)
- Phosphoric acid (H_3po4) – 85% H_3PO_4

Procedure:

- Weigh accurately 25g of sample into 250ml of conical flask
- Add 100ml of distilled water to dissolve the sample
- Add 2ml of 85% H_3po_4 solution
- Add 2ml of 1% starch solution
- Add 0.5grams of KI crystals
- Titrate the mixture with 0.005N $Na_2S_2O_3$ solution.

EXPRESSION OF RESULT:

Iodine content (ppm) $= \frac{V \times N \times 35.67}{W} \times 100$

Where,

V = ml of $Na_2S_2O_3$ for sample titration

N = Normality of $Na_2S_2O_3$

35.67 = Equivalent mass of KIO_3

W = Weight of sample taken.

QUANTITATIVE DETERMINATION OF METAL ELEMETS FROM FOOD PRODUCT

INTRODUCTION: Metal elements are elements that readily loose electrons to forms positive ions, this include: zinc, magnesium, potassium, sodium, scandium, mercury, copper, cobalt, nickel, calcium, lead etc. Most of these metals have detrimental health effects particularly heavy metals hence, the need to check their concentration in food products.

AIM: To check concentration of metal elements in food products

PRINCIPLE: When metal ions are introduce into a flame during flame test analysis, each metal ions has its own characteristic flame colour, although some metal ions flame colour are said to be same, this is not actually true but, it is because the distinction of the flame colour is not visible to human eye. For example, Idium ion and lead ion are said to give blue colour flame. Chloride salts are the most volatile salts. The sample is to be diluted in dil. HCl, this helps to convert metal ion in the sample to metal chloride salts, which are very volatile; so that on heating the chloride salt, the metal ions can be made available easily for burning. The AAS burnt the metals element and measure the intensity of the flame colour produce by the metal ion.

MATERIALS/REAGENT:

- Porcelain crucible
- Spatula
- Analytical balance
- Muffle furnace
- Atomic Absorption Spectrophometer (AAS)
- 0.1N HCl

PROCEDURE:

- Weigh accurately 5g of samples into crucible
- Transfer the crucible to a muffle furnace which has been maintained at 600^0C. Ash the sample until constant weight is reached.
- Remove the crucible and allowed to cool
- Add 20ml of 0.1N HCl solution into the ash obtained from complete combustion
- Filter the solution using filter paper (Dilute the filtrate if necessary)
- Analyze the filtrate above using AAS base on the manufacturer operating procedure using ethylene gas. (Note: select the wavelength depending on the metal element to be analyzed).

CALCULATION/EXPRESSION OF RESULT

After AAS analysis, the result will be display on the screen.

$$\textbf{Conc. Of metal (mg/kg)} = \frac{\textbf{AAS Result (mg/L) x v. sample x D.F}}{\textbf{Weight of sample}}$$

Where: V. sample = volume of the sample (liquids sample) or volume of dil. HCl used for the dilution of the ash.

D. F = Dilution factor

See how to calculate D.F

For example, you dissolve your ash with 50ml dilute HCl, then you transfer 10ml of the solution and dilute to 50ml with distilled water.

Dilution factor = 50ml/100rn1 X 50ml/l0rnl

D.F = 2.5

DETERMINATION OF NaCl CONTENT IN COOKING SALT BY MOHR METHOD

AIM: To determine the % salt (Nacl) content in a salt sample

APPARATUS/REAGENTS:

- Analytical balance
- 250ml conical flask
- Burette (50ml)
- Indicator bottle.
- Distilled water
- Silver nitrate solution (0.1N)
- Potassium chromate solution (5%)

PROCEDURE:

- Weigh accurately 0.1 – 0.5grams of sample into the conical flask
- Add 100ml of distilled water into the sample to dissolve it
- Add 5 drop of 5% K_2CrO_4 indicator solution
- Titrate the solution with 0.1N $AgNO_3$ solution
- Discontinue the titration and take your end point reading when a reddish orange precipitate is beginning to form.

EXPRESSION OF RESULTS:

$$\% \text{ Nacl} = \frac{V \times N \times 58.45}{W} \times 100$$

Where,
V = Titre value
N = Normality of $AgNO_3$
W = Weight of sample
58.45 = Equivalent mass Nacl

DETERMINTION OF SALT (NaCl) CONTENT IN FOOD SUBTANCES

INTRODUCTION: salt is one of the ingredients used in most food particularly process food, such as bread, cake, noodles, pasta, chin chin, snacks, cookies seasonings, etc. The salt may be added to a product, to add taste to the product or to serve as a preservative.

AIM: To determine the percentage of salt in food substances

MATERIAL/REAGENT:

- Porcelain crucible
- Spatula
- Analytical balance
- Muffle furnace
- Burette (50ml)
- Silver nitrate solution (0.1N)
- Potassium chromate solution (5%)

PRINCIPLE: The organic component of the product is allowed to burn completely into carbon dioxide and water majorly. The inorganic component (NaCl) remains undisruptive. The ash after complete combustion contains the inorganic salt which can be quantified by titametric method.

PROCEDURE:

- Weigh accurately 5g of samples into crucible
- Transfer the crucible to a muffle furnace which has been maintained at 600^0C. Ash the sample until constant weight is reached.
- Remove the crucible and allowed to cool
- Add 100ml of distilled water into the ash obtained from complete combustion
- Filter the solution using filter paper
- Add 5 drop of 5% K_2Cro_4 indicator solution to the filtrate

- Titrate the solution with 0.1N $AgNO_3$ solution
- Discontinue the titration and take your end point reading when a reddish orange precipitate is beginning to form.

EXPRESSION OF RESULTS:

$$\% \text{ Nacl} = \frac{V \times N \times 58.45}{W} \times 100$$

Where,
V = Titre value
N = Normality of $AgNO_3$
W = Weight of sample
58.45 = Equivalent mass Nacl

QUANTITATIVE DETERMINATION OF MELAMINE IN FOOD SUNSTANCES

INTRODUCTION: Melamine is an organic base with the chemical formula $C_3H_6N_6$. Melamine has been discovered to have caused serious health effect to animal that were feed with food contaminated with melamine. More also melamine is known to be carcinogenic. Looking at the adverse health effect, some manufacturers still add it to infant formula (milk powder, yoghurt etc) to deceive the government regulatory body, that their product contain high protein whereas, it is melamine; since the estimation of protein content is base on the nitrogen content and melamine has six nitrogen atoms.

AIM: To determine the concentration of melamine in food (Dairy products)

PRINCIPLE:
Melamine assay is a direct competitive enzyme-linked immunosorbent assay (ELISA). Melamine is allowed to compete with enzyme- conjugated antibody for binding sites during the first incubation period. The micro well are then wash with distilled water. After the wash a substrate is added to the well and a blue color is develops. The intensity of the color is inversely proportion to the concentration of the melamine in the sample or standard. The absorbance is read using **ELISA MACHINE.**

MATERIAL/REAGENTS/EQUIPMENT

Milk powder Antibody coated micro well, measuring cylinder, melamine standard 100ppb and 500ppb), substrate solution, stop solution, sample, vortex mixer, weighing balance, centrifuge.

PROCEDURE:

- Weight 5g or 5ml of the sample into centrifuge tube
- For solid add 25ml of 70:30 methanol/water and
- Place centrifuge tube to its position in the centrifuge machine and centrifuged for 20mins at 3000rpm (Extraction)
- Start the machine to shake the flask at 250rpm for three minutes (Extraction)
- Remove the tube from the centrifuge and allow to settled for 3minutes
- Gently decant the upper layer of the solution in the tube into a clean beaker
- Filter the decanted solution above
- Concentrate the filtrate by heating on nitrogen heating block
- Transfer 200µl of conjugate enzyme solution into 5 dilution wells each using micro pipette
- Transfer 100 µl standard of melamine of different concentration (100ppb, 200ppb etc) into four first dilution wells respectively while to the fifth well transfer 100µl of concentrated filtrate.
- Mixed the solution in the wells very well by pipetting up and down 3 times using multifunctional micro pipette
- Immediately transfer the solution from dilution wells to antibody coated micro wells.
- Incubate at room temperature for 15mins
- Discard the solution from antibody coated micro wells and rinse the wells very well with distilled water
- Transfer 100µl of substrate solution into each of the antibody coated wells and incubate at room temperature for 5mins (blue colour developed)
- After 5mins add 100µl stop solution to each well in order to stop the reaction
- Place the well strip into ELISA reader machine and take absorbance of each solution from the well.
- Plot a standard curve of absorbance against concentration of the standard
- From the standard curve used the absorbance of solution from well 5 (sample) to extractpolate the concentration of melamine in the sample
  Note: The concentration can also be determine using High Performance Liquid Chromatography (HPLC))

WHO STANDARD: ≤200ppb

QUANTITATIVE TEST FOR TOTAL AFLATOXINS IN FOOD SUBTANCES BY ELISA METHOD

INTRODUCTION:
Mycotoxin are secondary metabolite of fungi(asperginus species), afiatoxin is one of the mycotoxin found in farms products such as cereals, legumes, flour, smoking food substances etc. food like cereals fish are contaminated with this aflatoxin either from the farm or during storage. Aflatoxin is known to be harmful and carcinogenic. Complete elimination of this toxin is not possible hence; there is need for quantitative determination to make sure they are present in minor quantity.

AIM: To determine the level of total aflatoxins (B1, B2, G1 and G2) in food substance

PRINCIPLE: The principle is base on antigen antibody reaction. The toxins are specific with enzymes coated on the surface of the micro well thereby compete with substrate. The principle of ELISA machine is base on Beer-lambert law

MATERIALS/EQUIPMENT:
Aflatoxin test kit, micropipette, weighing balance, hot block and ELISA machine, orbital shaker

PROCEDURE:

- Blend the sample in fine powder
- Weight 5g of the sample into a conical flask
- Add 25ml of 70:30 methanol/water and cover the mouth of the flask
- Place the conical flask into orbital shaker holder
- Start the machine to shake the flask at 250rpm for three minutes (Extraction)
- Remove the flask from the shaker and allow to settled for 3minutes
- Gently decant the upper layer of the solution in the flask into a clean beaker
- Filter the decanted solution above
- Concentrate the filtrate by heating on nitrogen heating block
- Transfer 200µl of conjugate enzyme solution into 5 dilution wells each using micro pipette
- Transfer 100 µl standard of aflactoxin of different concentration (0.1, 0.2, 0.3, 0.4) into four first dilution wells respectively while to the fifth well transfer 100µl of concentrated filtrate.

- Mixed the solution in the wells very well by pipetting up and down 3 times using multifunctional micro pipette
- Immediately transfer the solution from dilution wells to antibody coated micro wells.
- Incubate at room temperature for 15mins
- Discard the solution from antibody coated micro wells and rinse the wells very well with distilled water
- Transfer 100µl of substrate solution into each of the antibody coated wells and incubate at room temperature for 5mins (blue colour developed)
- After 5mins add 100µl stop solution to each well in order to stop the reaction
- Place the well strip into ELISA reader machine and take absorbance of each solution from the well.
- Plot a standard curve of absorbance against concentration of the standard
- From the standard curve used the absorbance of solution from well 5 (sample) to extractpolate the concentration of total aflactoxin in the sample
  Note: The concentration can also be determine using High Performance Liquid Chromatography (HPLC))
  NAFDAC STANDARD: for ready to eat food = $\leq$ 4µg/kg or 4ppm
  Others food = $\leq$ 5µg/kg or 5ppm

QUALITATIVE TEST FOR POTASSIUM BROMATE

INTRODUCTION: Bread is an important staple food of many countries of the world especially in Nigeria. Manufacturer preferred fortified their bread flour with potassium bromate for selfish interest. Potassium bromate act as effective maturing agent to the bread, but it effect is detrimental to human health.

AIM: To check whether potassium brornate was used for the preparation of bread, cake, dough nut, snack etc.

PRINCIPLE:

The principle for determination of the bromate in bread, snack, dough nut etc. is base on redox reaction. In electrochemical series bromine is highly electronegativity than iodine, therefore if bromine is present in the bread it will displaced iodide ion and oxidized it to iodine molecule which will then react with the starch indicator to produce blue black colour.

MATERIALS/REAGENTS

- Crucible
- Droppers,
- 10% w/v potassium iodide
- 1:7 HC1 with water Starch indicator.

METIIODOLOGY:

- Place small quantity of the sample into a crucible
- Add some quantity of starch indicator solution, to soak the sample completely
- Add equal volume of 10% KI and 1:7 HCl solution using dropper
- Mixed very well and observed any colour change after 5 minutes.

Sample the contain potassium bromated will turn to blue black.

CHAPTER THREE

ANALYSIS OF OIL

DETERMINATION OF MOISTURE CONTENT OF OIL

AIM : To determine the moisture content of RBDPO/Olein

Apparatus:

- Oven (electric oven) set at 105^{o}c
- Desiccator
- Beaker
- Analytical balance
- Crucible
- Tong

PROCEDURE:

- Dry a clean beaker in an oven at 105$_{0}$C for 15minutes
- Cool the beaker in the desiccator
- Weigh empty beaker and record as W1
- Add about 5 -10grams of sample and record as W2
- Place the beaker with the sample into the oven at 105^{o}c for 3hours
- Remove the beaker from oven after 3hours
- Cool in the desiccator for 30minutes
- Weigh the beaker and oil after cooling and record as W3

EXPRESSION OF RESULTS:

Moisture content = $\frac{w2 - w1}{W3 - w1} \times 100$

Where,

W1 = weight of empty beaker

W2 = weight of empty + sample before oven dried

W3 = weight of beaker + sample after oven dried.

DETERMINATION OF FREE FATTY ACID

Aim: To determine free fatty acid in oil /fat.

Apparatus/Reagents:

- Analytical balance
- Digital burette/straight burette
- Conical flask/beaker
- Hot plate
- Isopropyl alcohol
- Standardized potassium hydroxide (KOH)
- 1% phenolphthalein indicator

Procedure:

- Accurately weigh 5grams of sample into a clean dry beaker / conical flask
- Add 25ml of neutralized isopropyl alcohol into the sample
- Swirl to dissolve the sample
- When the oil/fat is hard, heat the sample over a hot plate 40-50^{o}c
- Titrate with a known concentration of KOH until pink colour appeared which last for about 30seconds
- Stop titration and record the volume of KOH solution required when the mixture turn pink.

Note : Isopropyl neutralization: Take 100ml of isopropyl into a conical flask, add 0.5ml of 1% phenolphthalein indicator

Add few drops of 0.05NKOH to neutralize until faint purple colour appeared.

EXPRESSION OF RESULT:

Free fatty acid as palmitic acid

$$\%FFA = \frac{T.V \times N \times 25.6}{W}$$

Where,

TV = Volume of KOH used for titration

N = Normality of KOH solution

W = Weight of oil/fat sample.

DETERMINATION OF ACID VALUE IN OILS/FATS

INTRODUCTION: The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat or oil. The acid value test gives us the idea on how to make a good choice for oil to be used for soap production. Oil with high acid value will in turn have high saponification value hence suitable for soap production.
It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of oil glycerides. The value is a measure of the amount of fatty acids which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature and/or lypolytic enzyme lipase. The value is also expressed as per cent of free fatty acids calculated as oleic acid.
AIM: To determine acid value in oils

PRINCIPLE: The acid value is determined by directly titrating the oil/fat in an alcoholic medium against standard potassium hydroxide/sodium hydroxide solution

REAGENT:

- 95% ethanol
- Phenolphthalein indicator solution or Alkali blue 6B for dark colour oils.
- 0.1N KOH or 0.5N NaOH solution.

PROCEDURE:
Weight 1-2g of melted oil sample into 250ml round bottom flask
Measure 200ml of 95% ethanol into a beaker
Add three drops of phenolphthalein indicator and neutralize the ethanol by adding 0.1N KOH drop wise until permanent pink colour is form.
Pour the neutralize ethanol into the flask containing the sample and attach it to reflux set-up.
Heat the solution to boil for about 1hr 30mins.
Fill the burette with 0.1 KOH and adjust to zero.
After 1hr 30mins remove from reflux set-up and titrate until permanent pink colour.

CALCULATION

$$\%\text{Acid value} = \frac{56.1 \times N \times T.V}{wt}$$

where : N = Normality of the standard solution (KOH)
T.V= Titre value
Wt. = weight of the sample
56.1 = conversion factor.

PEROXIDE VALUE TEST

INTRODUCTION: Peroxide value is a measure substances in a sample expressed in terms of milli-equivalent of peroxide per 100g with sample which oxidizes potassium iodide (This method is applicable to all kinds of oil. The peroxide value depicts the level of freshness of oil. Under normal conditions these peroxides can breakdown into secondary oxidation products, usually containing carbonyl groups. Changes in the peroxide value can often be used to monitor the potential shelf-life of oil or products.

AIM: To check the peroxide value of oil

PRINCIPLE: Treatment of a test portion in a mixture of acetic acid and chloroform solution in the presence of potassium iodide followed by titration of free iodide with sodium thiosulphate solution.

Apparatus: 250ml Erlenmeyer flask, burette, pipette
REAGENT: Acetic acid, saturated potassium iodide solution, sodium thiosulphate, starch indicator and distilled water

PROCEDURE

1. Weigh 2-5g of sample into 250ml Erlenmeyer flask.
2. Add 30ml acetic acid (chloroform solution) and swirl the flask until the sample is completely dissolved in solution
3. Add 1ml of saturated potassium iodide solution and swirl the solution for 1mins
4. Add 30ml distilled water and store in a dark cupboard for 2-3mins
5. Titrate with 0.01N sodium thiosulphate solution ($Na_2S_20_3$) solution. Titrate gradually and with constant and vigorous shaking until the yellow colour has almost disappeared.

6. Add 1ml of starch indicator solution, continue to titrate shaking the flask until all the iodine from chloroform layers has been liberated.
7. Titrate the sodium thiosulphate solution drop wise until the blue colour has disappears.

EXPRESSION OF RESULT

$$\text{Peroxide value (PV)} = \frac{V \times N \times 1000}{\text{Weight of sample}}$$

Where: V = volume of $Na_2S_20_3$ solution

N = normality of 0.01N $Na_2S_20_3$ solution

W = weight of samples (g)

DETERMINATION OF IODINE VALUE IN OILS/FATS

INTRODUCTION:

The iodine value of an oil/fat is the number of grams of iodine absorbed by 100g of the oil/fat, when determined by using Wijs solution. The iodine value is a measure of the amount of unsaturation (number of double bonds) in a fat. The iodine value tells us the nutritional value of oils in question. Oils with high iodine value are highly nutritive for consumption and have shorter shelve life due to present of double bonds.

PRINCIPLE: The oil/fat sample taken in carbon-tetrachloride is treated with a known excess of iodine monochloride solution in glacial acetic (Wijs solution). The excess of iodine monochloride is treated with potassium iodide and the liberated iodine estimated by titration with sodium thiosulfate.

REAGENTS:

- Carbon tetrachloride
- 15% Potassium iodide (free from potassium iodate)
- 1% Starch solution
- 0.1N sodium thiosulphate
- Distilled water

PROCEDURE:

- Weigh 0.1-0.3g of melted oil sample into 500ml volumetric flask
- Add 15ml of carbon tetrachloride (CCl_4)
- Add 25ml of Wij's solution
- Corked the mouth of the flask and keep in a dark cupboard for 1hr 30mins
- Fill the burette with 0.1N sodium thiosulpate and adjust to zero mark.
- After 1hr 30mins remove the solution from dark and add 20ml of 15% potassium iodide
- Add 150ml of distilled water
- Titrate the solution in the flask against solution in the burette until the solution turns yellow
- Add 1ml of starch solution and continue with the titration until the blue black colour disappear
- Carry out the same procedure above for blank except there will be no sample in flask.

  NOTE: this analysis must be carryout in fume cupboard

CALCULATION

$$\textbf{Iodine value} = \frac{12.69 \times (B - S) \times N}{Wt}$$

Where: B = Titre value for the blank
S = Titre value for the sample
W = weight in g of the sample
12.69 = conversion factor

QUALITATIVE TEST FOR MINERAL OIL

INTRODUCTION: Mineral oils are produced from refined hydrocarbons obtained from crude oil by distillation. Some time oil manufacturer add this mineral oil for selfish interest.

PRINCIPLE

The presence of mineral oil is indicated by the development of turbidity when hot distilled water is added to a freshly made alcoholic solution of the soap formed by the oil.

REAGENT:

0.5 N Alcoholic potassium hydroxide solution

PROCEDURE

- Take 25 ml of the alcoholic KOH solution in a conical flask and add 1 ml of the sample of oil to be tested.
- Boil on a water bath using an air or water cooled condenser till the solution becomes clear and no oily drops are found on the sides of the flask.
- Take out the flask from the water-bath and transfer the contents to a wide mouthed warm test tube
- Carefully add 25 ml of boiling distilled water along the sides of the test tube.
- Keep on shaking the tube lightly from side to side during the addition.
    - The turbidity indicates presence of mineral oil, the depth of turbidity depends on the percentage of mineral oil present.

DETERMINATION OF COLOUR IN OIL

INTRODUCTION: There are many visual signs in the development of degradation of the oil, the most obvious of which are darkening in colour and rancid flavour (Khalil, 1979). Colour has become important parameter when you are try to check quality of oil. Adulterated oil gives different colour result in Tintometer. This has become an important parameter to check in the laboratory to ascertain adulterated status of every oil.

PRINCIPLE:

The method determines the colour of oils by comparison with Lovibond glasses of known colour characteristics. The colour is expressed as the sum total of the

yellow and red slides used to match the colour of the oil in a cell of the specified size in the Lovibond Tintometer.

Apparatus:

- Lovibond Tintometer
- Glass cells (cell size 0.25 inch, 0.5 inch. 1.0 inch, 5.25 inch or 1.0 cm, 2.0 cm, 5.0 cm as required)

PROCEDURE:

- Melt the sample if it is not already liquid and filter the oil through a filter paper to remove any impurities and traces of moisture. Make sure sample is absolutely clear and free from turbidity.
- Clean the glass cell of desired size with carbon tetrachloride and allow it to dry.
- Fill it with the oil and place the cell in position in the tintometer.
- Match the colour with sliding red, yellow and blue colours. Report the colour of the oil in terms of Lovibond units as follows :- Colour reading = (a Y + 5 b R) or (a Y + 10 b R) in (* cell) Where, a = sum total of the various yellow slides (Y) used, b = sum total of the various red (R) slides used Y + 5R is the mode of expressing the colour of light coloured oils; and Y + 10 R is for the dark-coloured oils.

KREIS TEST FOR RANCIDITY OF OIL

INTRODUCTION: Rancidity is the spoilage of oil, and this occur when there is present of high concentration of free fatty acid in oil which oxidized in the present of oxygen to produce peroxide which make oil rancid. This is carryout to check freshness status of oil

AIM: To qualitatively determine the rancidity of an oil sample.

PRINCIPLE:

The principle is based on the oxidation at a double bond of free fatty acid to produce epihydrinaldehyde fatty acid. The epihydrinaldehyde reacts with phloroglucinol to produce red to violet colour.

REAGENTS:

- Concentrated HCl
- 1% phioroglucinol solution.

PROCEDURE:

- Transfer 10ml of the oil sample into a stopped boiling tube
- Add 10 ml of 0.1% phioroglucinol solution
- Add 10ml of conc. HCl and cover the tube with stopper
- Shake vigorously for 40second and allow standing for 20mins

EXPRESION OF RESULT

Appearance of red-violet colour indicates rancid oil while appearance of yellow colour indicates good oil.

DETERMINATION OF SCPECIFIC GRAVITY OF OIL

INTRODUCTION: Specific gravity is the ratio of the density of a substance to the density of a given reference material. The reference material mostly is water. Specific gravity is used to depict purity of liquids. Each pure liquid has it standard reference value; any significant deviation in analysis result of any substances from standard reference value indicate present of impurities in that substance.
AIM: To check the purity of oil

MATERIALS:

- Specific gravity bottle
- Analytical weighing balance
- Pure distilled water.

PROCEDURE:

- Dry a clean density bottle and its stopper in oven for 15mins and cooled in dessicator
- Take the weight of the bottle with its stopper (w1)
- Fill the bottle with distilled water and fixed the stopper
- Clean all the overflow water with cotton wool and take the weight (w2)
- Pour the water out and return the bottle with its stopper to oven. Allowed to stay in oven for 45mins; remove and cool in dessicaor

- Fill the bottle with sample and fixed its stopper, and clean the entire overflow sample with cotton wool
- Take the weight (w3)

CALCULATION

$$\text{SPECIFIC GRAVITY} = \frac{w3 - w1}{W2 - w1}$$

DETERMINATION OF SAPONIFICATION VALUE

INTRODUCTION: The saponification value is the number of mg of potassium hydroxide required to saponify 1 gram of oil/fat. The saponification value is an index of mean molecular weight of the fatty acids of glycerides comprising a fat. Lower the saponification value, larger the molecular weight of fatty acids in the glycerides and vice-versa.

AIM: To determine the saponification value of oil/fat

PRINCIPLE: The oil sample is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric acid.

MATERIALS/REAGENTS:

- 250 ml capacity conical flask with ground glass joints (or round bottom flask)
- 1 m long air condenser, or reflux condenser (65 cm minimum in length) to fit the flask
- Hot water bath or electric hot plate fitted with thermostat
- 0.5N Alcoholic potassium hydroxide solution
- 1% Phenolphthalein indicator solution
- 0.5N hydrochloric acid

PROCEDURE:

- Melt the sample if it is not already liquid and filter through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample is completely dry.
- Mix the sample thoroughly and weigh about 1.5 to 2.0 g of dry sample into a 250 ml Erlenmeyer flask. Pipette 200ml of the alcoholic potassium hydroxide solution into the flask. Conduct a blank determination along with the sample.
- Connect the sample flasks and the blank flask with air condensers, keep on the water bath, and boil gently but steadily until saponification is complete, as indicated by absence of any oily matter and appearance of clear solution. Clarity may be achieved within one hour of boiling.
- After the flask and condenser have cooled somewhat wash down the inside of the condenser with about 10 ml of hot ethyl alcohol neutral to phenolphthalein
- Add 1ml of 1% phenolphthalein indicator.
- Fill the burette with 0.5N HCl and adjust to zero mark
- Titrate the excess potassium hydroxide with 0.5N hydrochloric acid, using about 1.0 ml

CALCULATION

$$\text{Saponification Value} = \frac{56.1 \times (B-S) \times N}{W}$$

Where, B = Volume in ml of standard hydrochloric acid required for the blank.
S = Volume in ml of standard hydrochloric acid required for the sample
N = Normality of the standard hydrochloric acid and
W = Weight in gm of the oil/fat taken for the test.

DETERMINATION OF UNSAPONIFIABLE SUBSTANCES

INTRODUCTION: The unsaponifiable matter is defined as the substances soluble in oil which after saponification are insoluble in water but soluble in the solvent used for the determination. It is used to depict amount of substance like mineral oil, organic foreign matter, vitamins etc in the oil.

AIM: To check the saponinfication value of oild/fat

PRINCIPLE: The unsaponifiable matter is defined as the substances soluble in oil which after saponification are insoluble in water but soluble in the solvent used for the determination. It includes lipids of natural origin such as sterols, higher aliphatic alcohols, pigments, vitamins and hydrocarbons as well as any foreign organic matter non volatile at 100°C e.g (mineral oil) which may be present. Light Petroleum or diethyl ether is used as a solvent but in most cases results will differ according to the solvent selected and generally the use of diethyl ether will give a higher result

MATERIALS/REAGENTS:

- Flat bottom flask or conical flask with a ground glass joint, 250 ml capacity.
- Air condenser 1 metre long to fit the flask
- Separating funnel, 500 ml capacity
- Weighing balance
- 0.1N alcoholic potassium hydroxide solution
- 95% Ethyl alcohol
- 1% Phenolphthlein indicator solution
- Petroleum ether (40-60 ºC)
- 10% Aqueous ehtanol:
- 0.02N sodium hydroxide solution
- Acetone
- Anhydrous sodium sulphate.

PROCEDURE:

- Weigh accurately 5g of well mixed oil / fat sample into a 250ml conical flask.
- Add 50ml of alcoholic potassium hydroxide solution.
- Boil the content under reflux air condenser for one hour or until the saponification is complete (complete saponification gives a homogeneous and transparent medium).

- Take care to avoid loss of ethyl alcohol during the saponification.
- Wash the condenser with about 10 ml of ethyl alcohol.
- Transfer the saponified mixture while still warm to a separating funnel,
- wash the saponification flask first with some ethyl alcohol and then with cold water, using a total of 50 ml of water to rinse the flask, Cool to 20 to 25ºC.
- Add to the flask 50 ml of petroleum ether, shake vigorously, and allow the layers to separate. Transfer the lower soap layer into another separating funnel and repeat the ether extraction for another 3 times using 50 ml portions of petroleum ether. Some oils high in unsaponifiable matter, e.g., marine oils, may require more than three extractions to completely remove unsaponifiable matter.
- Wash the combined ether extract three times with 25 ml portions of aqueous alcohol followed by washing with 25 ml portions of distilled water to ensure ether extract is free of alkali (washing are no longer alkaline to phenolpthalen).
- Transfer ether solution to 250 ml beaker, rinse separator with ether, add rinsing to main solution.
- Evaporate to about 5ml and transfer quantitatively using several portions of ether to 50ml Erlenmeyer flask previously dried and weighed.
- Evaporate ether. When all ether has been removed add 2-3 ml acetone and while heating on steam or water bath completely remove solvent under a gentle air. To remove last traces of ether, dry at 100°C for 30 minutes till constant weight is obtained
- Calculate the weight of the residues by initial weight of the empty flask from the weight after complete dryness
- Dissolve residue in 50 ml of warm ethanol which has been neutralized to a phenolpthalien end point.
- Titrate with 0.02N NaOH.

CALCULATION

Weight in g of the free fatty acids in the extract as oleic acid = 0.282 x V x N

Where, V = Volume in ml of standard sodium hydroxide solution

N = Normality of standard sodium hydroxide solution

$$\%\text{Unsaponifiable matter} = \frac{100 \times (A-B)}{W}$$

Where, A = Weight in g of the residue

B = Weight in g of the free fatty acids in the extract

W = Weight in g of the sample

STABILITY TEST

AIM: Determination of stability on a given oil sample

INTRODUCTION: Stability test determined how stable the oil is, particular oil used for industrial purpose. Unstable oil can lead to production of products with short shelves life.

PROCEDURE

1. Pour substantial amount of oil sample into deep fryer.
2. Allow to heat to a temperature of 150^0C.
3. Fry ten samples (either dough nut, cake etc) for 2mins each.
4. After the entire process scoop out about 100ml of sample using a beaker
5. Analyze for Free Fatty Acid (see free fatty acid procedure), for both the initial oil and after fried oil
6. The difference in these values is the stability value of the oil.
   Good oils should have stability value $\leq$ 0.0014%

CHAPTER FOUR
ANALYSIS OF FRUIT JUICE

TOTAL ACIDITY TEST IN FRUIT JUICE BY TITRIMETRIC METHOD

INTRODUCTION: Acids are important constituents in fruits as together with sugars, they determine quality and taste of the fruits. Maturity of many fruits for their harvest is also judged from their level of acids along with sugars, or the soluble solids.

PRINCIPLE: The total acidity of a fruit could be determined by titrating a known amount of aqueous extract of it against an alkali solution of known concentration. It is expressed as equivalence of any organic acid, eg. Citric, malic etc.

REAGENT/ APPARATUS:
Sodium hydroxide solution: Make 50 ml 0.1N NaOH by dissolving 0.2 gm of NaOH in 50ml water
Phenophthalein ($C_{20}H_{14}O_4$) indicator: Approx. 0.5 % in 80 % ethanol

PROCEDURE:

- Take 25 ml sample add 100 ml water and heat IN water bath for 10 minutes.
- Make up the sample to 250 ml with water.
- Take 10 ml from the solution above (2) into conical flask.
- Add few drops of phenolphthalein indicator and shake very well.
- Fill the burette with 0.1N NaOH after washing and rinsing.
- **Titration the solution from the burette against the solution in the conical** flask until persistent pink colour forms that at least last for few second seconds.

CALCULATION

$$\text{Total acidity} = T.V \times N \times 0.064 \times \frac{50}{50} \times \frac{100}{\text{wt. of sample}}$$

Where : T.V = Titre value
N = Normality of NaOH
0.064 = conversion factor
Wt. = weight of sample
50/50 = dilution.

CHAPTER FOUR

ANALYSIS OF FRUIT JUICE

TEST FOR pH OF FRUIT JUICE

INTRODUCTION
pH simply refer to the degree of alkalinity or acidity of a solution. Fruit contain acid such as ascorbic acid, citric acid as such pH value of fruit juice fall on the acidic side in the pH scale. Fruit juice produce from unripe fruit will always give very low pH due to high concentration of acid.

AIM: To check the level of acidity of drinks.

MATERIALS:
- pH meter
- beaker.

PROCEDURE:
- Switch on the pH meter and allow for equilibration
- Pour some quantity of the juice into the beaker
- Insert the pH meter electrode into the juice in the beaker and allow for equilibration
- Take the reading display on the screen as the pH value of the solution.

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- Add few drops of phenolphthalein indicator and shake very well.
- Fill the burette with 0.1N NaOH after washing and rinsing.
- Titration the solution from the burette against the solution in the conical flask until persistent pink colour forms that at least last for few second seconds.

CALCULATION

$$\text{Total acidity} = T.V \times N \times 0.064 \times \frac{50}{50} \times \frac{100}{\text{wt. of sample}}$$

Where : T.V = Titre value
N = Normality of NaOH
0.064 = conversion factor
Wt. = weight of sample
50/50 = dilution.

QUANTITATIVE TEST FOR ASCORBIC ACID OR VITAMIN C IN FRUIT JUICE BY TITRIMETRIC METHOD

INTRODUCTION: Ascorbic acid or Vitamin C Vitamin C or ascorbic acid is an enediol isomer of 2-keto-L-gluconolactone with a configuration similar to that of L-glucose. Oxidation of ascorbic acid gives rise to dehydroascorbic acid and both forms are physiologically active

.

AIM: To determine the ascorbic acid content in fruit juice drink (fruit juice content)

PRINCIPLE: Titrimetric estimation of vitamin C is conventionally done using 2, 6-dichlorophenol indophenol dye solution. This dye is blue in alkaline solution and red in acidic solution. Ascorbic acid reduces the dye to a colorless form. Reaction is quantitative and specific for ascorbic acid at pH 1.0-3.5.

REAGENTS/APPARATUS:

- 4 % Oxalic acid : 40 gm of oxalic acid dissolved in 1000ml of distilled water (W/V)
- DCPIP Dye solution: Dissolve 0.250 g of sodium salt of 2,6-dicholorophenol indophenols in about 500ml of water containing 0.210 g of $NaHCO_3$ and dilute to 1 liter of water. Store the solution in refrigerator and standardize it with freshly prepared standard solution of ascorbic acid every time just before use
- Standard ascorbic acid ($C_6H_8O_6$): 0.01 % ascorbic acid is dissolved in oxalic acid
- All titration apparatus.

PROCEDURE

- Fill the burette with DCPIP dye solution and adjust to zero mark
- Take 5g of fruit sample (filtered juice) and make up to 100 ml with 4% oxalic acid.
- Take 5ml sample from the 100 ml above and add 10 ml 4% oxalic acid and titrate against the dye 2, 6- dicholorophenol indophenols in the burette until pink colour is form that last for 15seconds.

Procedure for standardization of DCPID dye solution

- Fill the burette with DCPID dye solution and adjust to zero mark
- Take 5ml of standard ascorbic acid solution into conical flask,
- Add 10ml of 4% oxalic acid
- Mixed well and titrated against DCPIP dye solution in the burette.

Titre value range: between 0.1 -1 ml.

CALCULATION

$$\text{Concentration of ascorbic acid in mg/100g of sample} = \frac{0.5\text{mg}}{\text{T.V2}} \times \frac{\text{T.V1}}{5\text{ml}} \times \frac{100\text{ml}}{\text{wt. of sample}} \times 100$$

Where: 0.5mg = concentration of standard ascorbic acid solution
T.V1 = Titre value with sample
T.V2 = Titre value with standard ascorbic solution
Wt. = Weight of the sample.
5ml = volume of standard ascorbic solution
100ml = Dilution volume of the sample.

Or

Concentration of ascorbic in mg/100g sample = 60.6 X Titre value

When standardization is not done.

ESTIMATION OF REDUCING SUGAR IN FRUIT JUICE

INTRODUCTION: In fruits, both reducing and non-reducing sugars are present in varying amount. Reducing sugars are those hexose ($C_6H1_2O_6$) sugars, which can reduce compounds such as alkaline (ammoniacal) silver nitrate solution, cupric salt solution etc., because they themselves are oxidized. Hexose sugars are divided into 2 main groups, which are aldo-hexose and keto-hexose. Aldo-hexose or aldose contains aldehyde group and keto-hexose or ketose contains ketone group. Aldehyde are strong reducing agents. Hexose sugars which contain aldhyde groups eg. glucose, galactose, mannose etc., are reducing sugars. Ketones are however, more resistant to oxidation than aldehydes, because it involves the breaking of a relatively stable C-C bond. Hence, they do not ordinarily reduce alkaline silver nitrate or cupric salt solution. But those fructose contains ketone, it is able to reduce readily as easily oxidizable CO-CH_2OH group is present in it and it acts as reducing sugar. Non-reucing sugar eg., sucrose is a disaccharide and cannot reduce alkaline silver nitrate or cupric acid solution. (This method is applicable to all soft drink.

PRINCIPLE: When sugars are extracted and titrated, the reducing sugars only take part in the reaction in making reduction, but the non-reducing sugars that are present in it, do not take part in reduction and remains as such. Accordingly, only the reducing sugars are estimated by titration.

REAGENT:

- Fehling Solution – 5ml Fehling's A + 5ml Fehling's B + 20 ml Water
- 45 % Lead acetate ($C_2H_3O_{22}Pb$, 3H2O): 45g of Lead Acetate in 100 ml water
- 22 % Oxalic Acid: 22g of Oxalic Acid in 100 ml water

PROCEDURE:

- Take 25 g of sample (filtered juice) and heat for 3 minutes, till it turns to a curd like appearance.
- Add 2ml of 45% Lead Acetate and wait for 2 minutes.
- Add 22% Oxalic acid to the sample to remove the excess Lead acetate.
- Wait till a yellowish tint appears and add NaOH until the bubble retains in the sample to neutralize the solution.
- Make up to 250 ml and titrate against hot Fehling's solution. Add Methylene Blue at the end point and heat.
- End point of the reaction is a green colour appearance. On addition of methylene blue and heating red colour appears.

$$\text{Total Reducing sugar} = 0.05 \times \frac{250}{T.V} \times \frac{100}{\text{wt. of sample}}$$

Where : T.V = Titre value

0.05 = Normality of Fehling's solution

Wt. = weight of sample

250 = dilution volume.

TEST FOR TOTAL SUGAR IN FRUIT JUICE

AIM: To estimate total non-reducing sugar in drinks.

PRINCIPLE: The non-reducing sugars which are not titratable are first hydrolyzed to reducing sugars. Thus after hydrolysis, the non-reducing sugars are converted to reducing sugars while the reducing sugars that are already present in the sample remain unchanged. Accordingly, all the sugars that are present after hydrolysis remain as reducing sugars. This is conveniently termed as total sugars.

REAGENTS

- Fehlings Solution – 5ml Fehlings - A + 5ml Fehlings - B + 20 ml Water
- 45 % Lead acetate ($C_2H3O_{22}Pb.\ 3H_2O$): 45g of Lead Acetate in 100 ml water
- 22 % Oxalic Acid: 22g of Oxalic Acid in 100 ml water
- NaOH drops to neutralize

PROCEDURE

- Procedure is same for reducing sugar the volume made up to 250 ml.
- Out of the 250ml sample solution, take 50 ml and add 5 gm citric acid.
- Heat the sample and make up to 250 ml with water.
- Titrate against the Fehling's solution.
- End point of the reaction is a green colour appearance. On addition of methylene blue and heating brick red colour appears.

$$\text{Total sugar} = 0.05 \times \frac{250}{T.V} \times \frac{100}{\text{wt. of sample}}$$

Where : T.V = Titre value

0.05 = Normality of Fehling's solution

Wt. = weight of sample

250 = dilution volume.

The non-reducing sugars present in the sample may be determined from the values of the total and the reducing sugars as follows.

Percentage of non-reducing sugars = [Percentage of (Total sugars)- (Reducing sugars)] x 0.95

DETERMINATION OF TOTAL SOLIDS IN FRUIT JUICE

PRINCIPLE: Heating of sample in an oven at 105 ± 2^0C and additional weight to the empty beaker is calculated as the percentage total solid

EQUIPMENT: Analytical weighing balance electric oven: desiccators, Porcelain crucible and metal tong

PROCEDURE

- Dry a clean beaker in an oven at 105± 2^0C for 15 minutes
- Cool in desiccators and then weigh (w_1)
- Introduce 10g of sample into the beaker (w_2)
- Place beaker in water bath to evaporate the water completely. (This applicable liquid substance such as yoghurt, ice cream etc).
- Place the weighing beaker with the residue in an oven set at 105± 2^0C for 3 hours

- Return the weighing beaker to the desiccators using crucible tong and allow to cool for 15-30 minutes
- Weigh the beaker containing the residue (w_3)

CALCULATION

$$\% \text{ Total solid} = \frac{W3 - W1}{W2 - W1} \times 100$$

Where: w_1 = weight of the empty beaker

w_2 = weight of empty beaker + sample before drying

w_3 = weight of the beaker + residue after drying

Reference: Joy P. P. 2013. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala Tel.: 0485-2260832, 9446010905;

ESTIMATION OF TOTAL SOLUBLE SOLIDS IN FRUIT JUICE

INTRODUCTION

Total soluble solids (TSS) of a given sample of fruit juice represent the various chemical substances present in it in soluble form. It indicates a measure of sugars present in the sample. The amount of TSS present in the juice of a fruit is also considered to be a reliable index in judging its maturity. In accordance with, the harvest-maturity of many fruits is assessed in considering the TSS of their juices. (This method is applicable to all drinks)

AIM: To estimate total soluble solid in juice.

PRINCIPLE: Total soluble solid of a given fruit juice sample is determined in a quicker way with the help of a Refractometer, which is also known as hand or pocket Refractometer. The instrument works on the principle of refractive index of the sample and gives the refractive index as o Brix

REQUIREMENT:

- A hand- Refractomete
- A dropper or a glass-rod
- Blotting pape
- Absorbent cotton
- Rectified spirit
- Distilled water

PROCEDURE:

- The lid, that is, covering plate of the Refractometer which rests over the prism-plate and is attached with it at the base end with a hinge is unfolded backward. By doing so, both the lid and the prism-plate are exposed.
- The lid and the prism-plate are then carefully and scrupulously washed with jet of clean water to ensure that they have no stain on their surfaces.
- Water adhered on the prism-plate and the lid as well as the surrounding parts of them, if any is completely wiped off with blotting paper or absorbent cotton.
- The lid of the prism-plate is then washed with distilled water and the water adhered on them is blotted out. The cleaning is best done by rubbing the lid and the prism-plate gently and carefully with absorbent cotton, soaked with rectified spirit.

- Then, with the help of a previously cleaned dropper or a glass-rod, a drop of distilled water is carefully dropped on the surface of the prism-plate. The lid is folded forward and placed over the prism-plate to cover it. At this position, the lid and the prism-plate are firmly held together with fingers to avoid unfolding of the lid.
- The Refractometer is held to point towards light. The eyepiece of it is brought close to any eye of the observer who should look into the eye-piece to have a view of the image of the scale. The scale-focusing knob should be conveniently rotated to adjust it at such a position where the scale is most clearly visible. The shaded part would be seen to intersect the unshaded part at zero position of the scale which indicates no reading with respect to distilled water. If it's not there then the reading should brought to zero by rotating the scale-calibrating screw.
- The lid is then folded backward. The distilled water that remains adhered over the lid and the prism-plate is completely blotted out and these are dried in air for a few minutes.
- A clear sample of fruit juice, TSS of which is to be determined is taken in the dropper, or a drop may be taken with the glass-rod. A drop of juice is, then carefully placed on the prism plate.
- Reading of the juice sample as oBrix is obtained and amount of TSS is expressed accordingly. *Reference:* ***Joy P. P. 2013. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala Tel.: 0485-2260832, 9446010905 ;***

CHAPTER FIVE

ANALYSIS OF FLOUR

MOISTURE CONTENT ANALYSIS

Title: Determination of moisture content analysis

Principle: Heating of sample in an oven at 105 ± 2^0C and the loss in weight of sample is calculated as the moisture content. This method is applicable to all food and non-food substances e.g flour, pasta, chinchin, salt, grains, cake, cement, yoghurt seasonings etc.

Equipment: Analytical weighing balance electric oven: desiccators, Porcelain crucible and metal tong

PROCEDURE

- Dry a clean weighing crucible in an oven at 105 ± 2^0C for 15 minutes
- Cool in desiccators and then weigh (w_1)
- Introduce 3-5g of sample in weighing crucible and take the weight (w_2)
- Place the weighing crucible in an oven set at 105 ± 2^0C for 3 hours
- Return the weighing crucible to the desiccators using crucible tong and allow to cool for 15-30 minutes
- Weigh the crucible containing the sample (w_3)

Expression of Result

$$\% \text{ moisture content} = \frac{w_2 - w_3}{w_2 - w_1} \times \frac{100}{1}$$

where, w_1 = weight of the empty crucible

w_2 = weight of empty crucible + sample before drying

w_3 = weight of the crucible + sample after drying

PROTEIN ANALYSIS

Title: Determination % protein content in a given sample.

Scope: This method applies for crude protein content determination for all solid food products. This method is also known semi-micro kjeldal method.

principle: Protein compounds are digested by the addition of sulphuric acid and selen catalyst to ammonium sulphate. By addition of sodium hydroxide, the solution is then distilled to produce ammonium and it is trapped in a boric acid solution hich results in ammonium borate. Ammonium borate solution is quantitatively titrated by a standard acid solution.

Apparatus: Kjeldal digestion flask, conical flash, burette, volumetric flask, measuring cylinder, analytical balance,

Reagents: concentrated H_2so_4, 50% a solution of sodium hydroxide, screened methyl red indicator, 5% sodium thiosulphate, 2% boric acid, kjeldal catalyst tablets

PROCEDURE

15. Weigh about 2g of the sample and transfer with the paper into a kjeldal flask.
16. Add 25ml concentrated H_2SO_4 using 10ml pipette
17. Add 2 table of selenium catalyst tablet.
18. Heat the flask gently in the digestion unit (fume cupboard) at 30^0C .
19. Swirl the flask occasionally to wash down charred particles from the sides of the flask.

20. When the initial vigorous reaction has died down increase the heat and continue digestion unto the liquid turns clear and free from black or brown colour. Allow to cool.
21. Dilute content with about 200ml of distilled water and connect the distillation apparatus.
22. Measure 50ml of 2% boric acid into a conical flask.
23. Add 80ml of 40% sodium hydroxide
24. Add seven drops of screened methyl red indicator and swirl
25. Place it on the receiver so that the end of the delivery tube dips just below the level of the boric acid and set the apparatus at 60^0C.
26. Run in about 85ml of sodium hydroxide into the distillation flask.
27. Add 50ml distilled water; boil vigorously until about 250ml have distilled over.
28. Titrate with standard 0.02M HCl until a neutral grey end point is obtained

CALCULATION

$$\% \text{ protein content} = \frac{(\text{ml acid}) \times (\text{N acid}) \times (0.014\ \text{N}) \times (100)}{\text{Weight of sample}}$$

ASH CONTENT ANALYSIS

Title: Determination of % ash content in a given sample

INTRODUCTION: Ash is defined as the organic residue remaining after water and other volatile materials have been removed by heat. It helps to check for adulteration, it helps to depict the mineral content of the food substance.

AIM: To determine of % ash content in a given sample

PRINCIPLE: Ashing of sample in a furnace at 600^0C for certain period of time and the ash content is calculated by weight difference between crucible and ash left in crucible after drying

EQUIPMENT: Porcelain crucible, spatula, analytical balance, muffle furnace.

PROCEDURE

6. Weigh an empty crucible which has been previously dried and cooled in a deccicator and weigh (w_1)
7. Weigh accurately 3-5g of samples (w_2)
8. Transfer the crucible (w_2) to a muffle furnace which has been maintained at 600^0C. Ash the sample until constant weight is reached.
9. Remove the crucible and place in a desiccator provided with efficient desiccator.
10. Allow the dish to cool and take the weight (w_3)

CALCULATION

$$\% \text{ ash content} = \frac{w_3 - w_1}{w_2 - w_1} \times \frac{100}{1}$$

Where: w_1 = weight of empty crucible

w_2 = weight of empty crucible + sample before drying

w_3 = weight of empty crucible + sample after drying.

Reference: Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality: Version 2

GLUTEN ANALYSIS

INTRODUCTION: Gluten is protein component of flour which helps in enhancing the firmness of an elasticity of dough

AIM: To determination percentage gluten content in wheat flour .

PROCEDURE

1. Weigh 25g of flour in an empty filter paper
2. Pour the flour into a bow
3. Add 12ml of distilled water and mix until it becomes homogeneous and work into a dough
4. Soak in water at cool temperature in 1hr
5. Wash the dough over a running tap water until soluble matter and starch are completely removed
6. Spread the gluten in pre-weigh filter paper
7. Dry the filter paper and sample the oven at 105^0C for 3hrs.
8. Transfer to the desiccators for cooling and take the final weight.

CALCULATION

$$\% \text{ gluten content} = \frac{w_3 - w_1}{w_2 - w_1} \times \frac{100}{1}$$

where: w_1 = weight of empty filter paper

w_2 = weight of empty filter + sample before drying

w_3 = weight of empty filter + sample after drying

DETERMINATION OF FALLING NUMBER IN FLOUR (Falling number test)

INTRODUCTION: The level of enzyme activity measured by the falling number test affects product quality. Yeast in bread dough, for example, requires sugars to develop properly and therefore needs some level of enzyme activity in the dough. Too much enzyme activity, however, means that too much sugar and too little starch are present. Since starch provides the supporting structure of bread, too much activity results in sticky dough during processing and poor texture in the finished product. If the falling number is too high, there is no problem because enzymes can be added. Falling number results are recorded as an index of enzyme activity in a wheat or flour sample and the results are expressed in time as seconds. A high falling number (for example, above 300 seconds) indicates minimal enzyme activity and sound quality flour. A low falling number (for example, below 250 seconds) indicates substantial enzyme activity and damaged flour.

REAGENT/MATERIALS: Falling number tube or boiling tube, measuring cylinder, water bath, thermometer and stirrer.

PROCEDURE:

1. weight 7g of flour sample and add 25ml of distilled water in a glass falling number tube or boiling tube with a stirrer and shaken to form a slurry.
2. Heat the slurry a boiling water bath at 100 degrees Celsius and stirred constantly, the starch gelatinizes and forms a thick paste.
3. Gently drop a stirrer from the mouth of the tube
4. The time it takes the stirrer to drop through the paste is recorded as the falling number value. ***Reference:*** ***Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality: Version 2***

SEDIMENTATION TEST

INTRODUCTION: The sedimentation test provides information on the protein quantity and the quality flour samples. Positive correlations were observed between sedimentation volume and gluten strength or loaf volume attributes. The sedimentation test is used as a screening tool in wheat breeding as well as in milling applications. Sedimentation values can be in the range of 20 or less for low-protein flours with weak gluten to as high as 70 or more for high-protein wheat with strong gluten.

AIM: To determine the sedimentation volume of flours.

REAGENT/MATERIALS: Lactic acid, weighing balance Graduated cylinder with stopper, stop watch,

PRINCIPLE: During the sedimentation test gluten proteins present in the flour swells and precipitate as a sediment.

PROCEDURE:

1. A weight 3.2g of flour place in 100ml glass-stoppered graduated cylinder.
2. Add 50ml of distilled water and stirred for 5minutes
3. Add 20ml of lactic acid solution into the cylinder and mixed for 5 minutes.
4. Stop the mixing and keep the cylinder in an upright position for 5 minutes.
5. The cylinder is removed from the mixer and kept in upright position for 5 minutes.
6. Check and record the volume of the flour that sediment after 5 minutes in upright position

ACIDITY IN FLOUR (Alcohol Extract)

PRINCIPLE: The sample is shaken with ethanol and the extract titrated with standard alkali.

REAGENTS

1. Ethanol, 90%, v/v, neutralized to phenolphthalein.
2. Phenolphthalein, 1% in neutral ethanol.
3. 0.05 N Sodium hydroxide solution.

PROCEDURE:

Weigh 5.0 g of sample into a conical flask, add 50 ml of 90% ethanol, stopper the flask, shake and leave to stand overnight.

Decant the extract through a filter, wash the residue with 20 ml of 90% v/v neutral ethanol and decant through the same filter.

Titrate the combined filtrate with 0.05 N sodium hydroxide solutions to the phenolphthalein end-point.

CALCULATION

$$\% \text{ acidity (as sulphuric acid)} = \frac{\text{titre}}{1000} \times .05 \times \frac{100}{\text{Wt of sample}}$$

INTERPRETATION: Recently milled flours have alcohol extract acidity of 0.03 - 0.04%. ***Reference:*** ***Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality: Version 2***

ESTIMATION OF IRON IN FLOUR

PRINCIPLE: Iron is often added as a nutrient to enriched flours, usually as ferrous sulphate, ferric ammonium citrate or iron powder. This method involves ashing the flour to remove organic material, reducing ferric ion to ferrous with sulphur dioxide and reacting with o-phenanthroline. The resultant red compound is determined spectrophotometrically.

APPARATUS

1. Muffle furnace.
2. Steam bath.
3. Hot plate.
4. Pipettes and volumetric flasks.
5. Spectrophotometer (visible range).

REAGENTS:

1. Glycerol-alcohol mixture (1+1).
2. Nitric acid, concentrated.
3. Hydrochloric acid, 5 N
4. Hydrochloric acid, dilute (1 ml acid to 100 ml with water).
5. Sulphur dioxide solution, 2%. Dissolve 3.25g sodium meta - bisulfite in 100 ml water.
6. Sodium acetate solution, 2 N.
7. Congo red indicator paper
8. o-Phenanthroline solution, 0.25% in water.
9. Ferrous ammonium sulphate hexahydrate standard - dissolve 0.7024 g in water , add 2 drops HC1 and dilute to 1 litre. Dilute 50 ml of this to 1 litre (1 ml = 0.005 mg Fe).

PROCEDURE

- Weigh an amount of flour containing up to 0.4 mg iron into a silica ashing dish. (Note that iron is often added to flour at 1.6 mg/10 0 g).
- Add 10 ml of the glycerol-alcohol mixture.
- Ash overnight at 600°C (after initial heating with an infrared lamp or at the mouth of the open muffle). Cool and add 1 ml concentrated nitric acid. Evaporate and re-ash 1 hour.
- Cool and add 5 m l 5 N HC1. Heat on a steam bath 15 minute s and filter through hardened filter paper into a 100 ml volumetri c flask.
- Add 3 ml dilute HC1 to the silica dish, bringing to boil on a hot plate and filter using the same filter and flask as above. Repeat this process four more times. Then wash the dish and filter with hot water to make the filtrate flask to the mark.
- Mix and pipette 10 ml into a 25 ml volumetric flask. Add 1 ml 2% sulphur dioxide solution. Add 2 N sodium acetate solution by burette until the solution changes
- red paper from blue to pink. Next, add 2 ml 0.25% o-phenanthroline solution and make to the mark. Let stand overnight to develop the red colour.
- Read the absorbance in a 4 cm cell at 520 nm versus a blank prepared the same way as the sample. Prepare a standard curve by pipetting 0, 2, 5, 7 and 10 ml of the dilute standard solution (0.005 mg Fe/ml) into five 25 ml volumetric flasks. Add reagents as with the sample and read the absorbances.
- Plot the absorbance versus the mg iron.

CALCULATION

Find the mg iron corresponding to the sample absorbance using the standard curve.

$$\text{Conc. Of iron (mg)} = \frac{\text{corresponding mg from standard curve X 1000}}{\text{Weight of sample}}$$

CHAPTER SIX

WATER ANALYSIS

INTRODUCTION:

Water generally is very important to the body or in short water is life because life will never exist in the absent of water. Water is one of the six classes of nutrient that the body needs to maintain good health. at the same time this water the body need to maintain good health can serves as a means of diseases transferred if the water is poorly processed and packaged. Therefore analysis for water quality and purity is necessary since water is one of six classes of nutrient the body need. Water laboratory is charge with the responsibility of testing the water quality and purity, before a water sample is recommended satisfactory it passed all the analytical parameters and microbiology analysis.

PHYSICAL TEST

Basically there are four parameters which are used in water analysis, they are:

1. Sensory parameters (it involves the use of sense organs,), this include:

- Appearance (good/pure water for human consumption should be colourles to human eyes).
- Odour (good/pure water for human consumption should be odourless to human nose).
- Taste (good/pure water for human consumption should be tasteless to human tongue). .

2. Physical parameters; this include:

- pH; (this is done using pH meter (acceptable range 6.85 — 8.50)
- Total dissolve solid (TDS)
- Total insoluble particles
- Turbidity test

3. Chemical parameters; this involves chemical reaction; this includes:

- Test for free dissolved carbon dioxide
- Total alkalinity test
- Sulphate content test
- Nitrite test
- Hardness test

- Test for chloride
- Potassium test
- Test for heavy metals

pH TEST

INTRODUCTION: The pH for refers to the degree of acidity/alkalinity of a solution.

AIM: To check the pH of water

EQUIPMENT: pH meter.

PROCEDURE:

- Pour the solution of the syrup/injection into beaker
- Calibrate the pH meter using buffer
- Deep the pH meter electrode into the solution in the beaker
- Note the reading on the pH meter screen

DETERMINATION OF TOTAL INSOLUBLE SOLIDS (TIS) IN WATER BY GALVIMETRIC METHOD

INTRODUCTION:
A total insoluble particle is the measured of all insoluble substance present in the sample. This also, helps in determining the purity of water.

AIM: To determine the quantity of total dissolve solids in water samples.

PRINCIPLE: The sample is filtered and the filtrate evaporated in a tarred dish on steam bath. The residue after evaporation is dried to constant mass at 103-105°C.

MATERIALS/REAGENTS:
Evaporation dish, measuring cylinder, filter paper funnel water bath, oven, analytical balance

PROCEDURE:

- Dry clean filter in the oven for 15mins at 103-105oC
- Cool in desssicator and take the weight (W1)
- Filter 100ml of water sample through the pre-weighing filter paper
- Transfer the filter paper together with the residues to oven set temperature of 103-105oC. Allowed to stay in oven for 1hr 30mins
- Remove from oven and allowed cool in dessicator. Take the weight (W2)
  NOTE: the value of W1 and W2 must be in milligram

CALCULATION

$$\textbf{Total insoluble particles in mg/L} = \frac{\textbf{1000 x (W2-W1)}}{\textbf{Vol. of sample}}$$

DETERMINATION OF TOTAL DISSOLVES SOLIDS (TDS) IN WATER BY GALVIMETRIC METHOD.

INTRODUCTION:

TDS is the measured of all constituents dissolved in water. The principal inorganic inions dissolve in water include carbon, chlorides sulfate and nitrates while cations include sodium, potassium, calcium and magnesium. The quantity of the total dissolve solids indicates the level of water purity.

AIM: To determine the quantity of total dissolve solids in water samples.

PRINCIPLE: The sample is filtered and the filtrate evaporated in a tarred dish on steam bath. The residue after evaporation is dried to constant mass at 103-105°C.

MATERIALS:

- Evaporation dish
- Measuring cylinder
- Filter paper
- Funnel
- Water bath
- Oven
- Analytical balance

PROCEDURE:

- Dry clean evaporation dish in the oven for 15mins at 103-105oC
- Cool in desssicator and take the weight (W1)
- Filter 100ml of water sample
- Transfer the filtrate into evaporation dish and place in water bath to evaporate to dryness
- Take the evaporation dish to oven set temperature of 103-105oC. Allowed to stay in oven for 1hr 30mins
- Remove from oven and allowed cool in dessicator. Take the weight (W2)
  NOTE: the value of W1 and W2 must be in milligram

CALCULATION

$$\textbf{TDS in mg/L} = \frac{\textbf{1000 x (W2-W1)}}{\textbf{Vol. of sample}}$$

NAFDAC/WHO STANDARD:≤200mg/L

TURBIDITY TEST (DIGITAL TURBID METER)

INTRODUCTION:
Turbidity is the interference of light passage through water by small suspended particles such as, silt extracted from soil and humus and mineral matters. This test is very important during water treatment. The amount of coagulant to be added depends on the value of turbidity test.

AIM: To check the cleanliness of water.

PRINCIPLE: The light from a light source in the turbid
meter passes through the sample, and thentensity light scattered is measured by a p hotoelectric cell and converted to electrical energy which is proportional to the turbidity of the sample and the turbidity is
read directly on the meter and reported in NTU

EQUIPMENT/REAGENT:

- Turbid meter
- Hydrozinium sulphate
- Hexamethylene tetramine

PROCEDURE: standardized the meter
with a prepared stock solution of hydrazinium sulphate and
hexamethylene tetramine
Pour the water sample to the sampler and press start button on the machine
The machine will directly display the turbidity value of the water
WHO standard ≤5NTU

DETERMINATION OF TOTAL ALKALINITY TEST IN WATER

INTRODUCTION: Total alkalinity is test with aim of determining the quantity of alkaline molecules present in the sample. The higher the quantity of alkaline the higher the pH and lower the quantity of alkaline the lower the pH and the more acidic is the sample.

AIM: To quantify total alkalinity in water samples.

PRINCIPLE:
The principle of the analysis is base on neutralization reaction. The sample is titrated against standard acid, the amount of the acid require to neutralized the solution is used for the determination of total alkalinity.

MATERIALS/REAGENT:
Burette, conical flask, measuring cylinder, methyl orange indicator, 0.02M H_2SO_4

PROCEDURE:
Fill the burette with 0.02M and adjust to zero mark
Transfer 100ml of the water sample into conical flask
Add 3 drops of methyl orange indicator
Titrate the solution against the solution in the burette until pink colour end point.(

CALCULATION
Total Alkalinity (mg/L) = Titre value x 10
NAFDAC STANDARD: ≤100mg/L
Reference: Natural Mineral Water- Food Safety & Standard Regulations - 2011 and IS 13428:2005 Reaff.

QUANTITATIVE TEST FOR CHLORINE BY TITRATION METHOD

INTRODUCTION: Chlorine is formed when gas dissolved in water, chlorine is also used during purification of water because of its ability to kill microorganism. Chlorine is also dietary mineral needed by the body for good health. Chloride play very important roles in the body such as resting membrane potential and maintain proper cell volume, it react with hydrogen to form HCl which aid digestion, it is also involve in transportation of CO_2 from the tissues to the lungs etc. although chloride is important to the body but high concentration has adverse effect to the body hence, determining the quantity present in water became necessary. (Nelson *et al* 2008). This method prescribes the determination of chloride and it is suitable for use in relatively clear waters when 0.15 to 10mg of chloride is present

PRINCIPLE: In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of the silver nitrate titration of chloride. Silver chloride is precipitated before red silver chromate is formed. Bromide, iodide and cyanide register equivalent chloride concentrations. Sulphite, thiosulphate and sulphide ions interfere but can be removed by treatment with hydrogen peroxide. Orthophosphates in excess of 25mg/L interfere. Iron in excess of 10mg/L interferes by masking the end point.

MATERIALS/REAGENTS:

- 5% Potassium chromate indicator solution
- 0.1N silver nitrate solution

Special reagents for removal of interferences

- 1.25g/L Aluminum hydroxide
- 1% Phenolphthalein indicator solution
- 1N Sodium hydroxide
- 1N Sulphuric acid
- 30% Hydrogen peroxide

PROCEDURE:

(a) Clean water sample without interferences

- Measure 100ml of water into conical flak
- Add 1ml of 5% potassium chromate indicator
- Fill the burette with 0.1N silver nitrate solution
- Titrate the solution from the burette against solution in the conical flask until reddish brown precipitate is form.
-

(b) Turbid water with interferences:

- Use 100ml sample or a suitable portion diluted to 100ml. If the sample is highly colored, add 3ml of aluminum hydroxide suspension, mix, let settle and filter
- If sulphide, sulphite or thiosulphate is present, add 1ml of hydrogen peroxide and stir for 1 minute.
- Adjust sample pH to 7-10 with sulphuric acid or sodium hydroxide if it is not in the range.
- Add 1ml of potassium chromate indicator solution.
- Titrate with standard silver nitrate solution to a pinkish yellow end point. Standardize silver nitrate solution and establish reagent blank value by titration method.

$$\textbf{Conc. of chlorine in mg/L} = \frac{(V_1 - V_2) \times N \times 35450}{V_3}$$

Where:

V_1 = Volume in ml of silver nitrate used by the sample
V_2 = Volume in ml of Silver nitrate used in the blank titration
V_3= volume in ml of sample taken for titration
N= Normality of silver nitrate solution
35450 = Conversion factors

NAFDAC/WHO STANDARD: < 0.2mg/L

Reference: Natural Mineral Water- Food Safety & Standard Regulations - 2011 and IS 13428:2005 Reaff.

QUANTITATIVE ANALYSIS FOR TOTAL CARBONDIOXIDE (CO_2) IN PACKAGE WATER

INTRODUCTION:

The amount of CO_2 in water depicts the number of microorganism present in the water sample. CO_2 is a by-product of microorganism metabolic activities.

AIM: To determine the quantity of total CO_2 in package water sample.

PRINCIPLE:

The principle is base on neutralization reaction. The carbon dioxides present in sample react with NaOH, at the neutral point the colour of the indicator change which indicate end point.

MATERIALS/REAGENTS:
1ml pipette filler test tube, phenolphthalein indicator and reagent 1% NAOH

PROCEDURE:

- Measure 20ml of the sample into test tube
- Add 2 drops of phenolphthalein indicator
- Fill 1ml pipette with 1% NOH and adjust to zero mark
- Gradually dispense the solution from the pipette drop wise into the solution in the test tube until pink colour end point.
- Check the volume of 1% NaOH used by counting the number of divisions from the pipette

CALCULATION

Concentration of CO2 in mg/L = Numbers of division *X* 4mg/L
NAFDAC STANDARD: ≤50mg/L

QUALITATIVE AND QUANTITATIVE TEST FOR NITRITE IN WATER SAMPLE

INTRODUCTION:
In water nitrogen is present in different forms namely Nitrate, Nitrite Ammonia & organic Nitrogen. All these forms are biochemically interconvertible and are component of nitrogen cycle. Nitrite is form when nitrogen in water reacts with oxygen to from nitrite. This nitrogen or nitrogen compound are introduce into the water when nitrogen fertilizer are wash from farm into the source of water used for packaging. Nitrogen also indicates contamination from industrial waste.

AIM: To determine the present of nitrite and its quantity in water samples.

PRINCIPLE:
Glacial acetic acid donates H^+ which react with the nitrite to form nitric acid. Nitration reaction occurs between the nitric acid and sulfalinic acid to formed diazoniurn salt. This product reacts with napthalarnine to form azodye which have a pink colour. The colour form help in quantifying the quantity of nitrite in the sample.

PROCEDURE:

- Measure 50ml of water sample into conical flask
- Add 1ml of nitrite reagent
- Add 1ml of nitrite reagent allowed for 1mins
- Fill cuvatte with the solution above
- Take the absorbance at 234nm using smart spectrophotometer
- The smart spectrophotometer will display the concentration in mg/L

QUANTITATIVE TEST FOR POTASSIUM BY SPECTROPHOMETRIC METHOD

INTRODUCTION:

Potassium occurs in drinking water as a consequence of adding potassium permanganate as an oxidant during water treatment. Increase in exposure to potassium could result to significant health effects in people with kidney diseases or other conditions like diabetes, hypertension and adrenal insufficiency. Hence the need for this test.

AIM: To determine the quantity of sulphate and potassium in water sample.

PRINCIPLEPotassium present in the sample reacts with tetraphenylborate in the present of NaOH to produce a compound that absorbed light at wavelength 273nm..

MATERIALS/REAGENTS:

smart spectrophotorneter, analytical balance, 1M NaOH, pure tetraphenylborate (solid)

PROCEDURE:

- Switch the smart spectrophotometer and allowed to equilibrate to 273nm
- Pour 10ml of the water sample into cuvette scan as blank using smart spectrophotometer
- Removed the blank and add 5mg of tetraphenylborate, follow by 3 drops of 1M NaOH and allowed to stand for 5mins
- Scan the solution in the cuvette at 273nm using smart spectrophotometer
- The concentration of the potassium will be display directly from the screen.
  NAFDAC STANDARD: ≤12mg/L
  Reference: Nafdac water analysis manual.

QUANTITATIVE TEST FOR SULPHATE SPECTROPHOMETRIC METHOD

INTRODUCTION:
Sulphate can be found in almost all natural water but, the concentration of the sulphate give information about the source of the water either close to sulphate ore or contamination with industrial waste. High concentration of sulphate in drinking water has laxative effect when combine with calcium and magnesium to form hard water in the body

AIM: To determine the quantity of sulphate and potassium in water sample.

PRINCIPLE:
The sulphate present in the sample react barium chloride which present in the reagent to form barium sulphate which absorb light maximally at 235nm.

MATERIALS/REAGENTS:
smart spectrophotorneter 2% $BaCl_2$,

PROCEDURE:

- Switch the smart spectrophotometer and allowed to equilibrate to 235nm
- Pour 10ml of the water sample into cuvette scan as blank using smart spectrophotometer
- Removed the blank and pour it out
- Transfer 10ml of the water sample to the cuvette. Add 1ml of 2% $BaCl_2$ solution and allowed to stand for 5mins
- Scan the solution in the cuvette at 235nm using smart spectrophotometer
- The concentration of the sulphate will be display directly from the screen.
  NAFDAC STANDARD: $\leq$200mg/L
  Reference: Nafdac water analysis manual

HARDNESS TEST (EDTA Titrimetric Method)

INTRODUCTION: Hard water is the water that does not form lather easily with soap due mainly to the presence of Ca++ and Mg++.

PRINCIPLE:
These ions form a chelated soluble complex in the presence of EDTA. Eriochrome Black indicator is used, and as the EDTA is added, the solution will turn from wine red to blue at the endpoint. The sharpness of the endpoint is pH dependent (pH 10.0 ± 0.1). To minimize chance of $CaCO_3$ a precipitation, titration should take no more than 5 minutes. The indicator functions best at room temperature.

REAGENTS

- **Buffer solution**: Dissolve 1.179g EDTA disodium salt and 0.780 g $MgSO_4 \cdot 7H_2O$ in 50ml distilled water. Add this solution to 16.9 9 NH_4Cl and 143ml conc. NH_4OH with mixing and dilute to 250ml with distilled water. Store in stoppered plastic or resistant-glass container.
- **Eriochrome Black Indicator**: Mix together 0.5 9 dye and 4.5 9g hydroxylamine hydrochloride. Dissolve this mixture in 100ml of 95% ethyl or isopropyl alcohol.
- **0.010M EDTA** Titrant,

PROCUDURE:

- Measure a 50ml sample (or a 25ml aliquot diluted to 50ml) into a 125ml Erlenmeyer flask.
- Add 1ml buffer; usually 1ml buffer will be sufficient to give pH 10.0 - 10.1.
- Add 1-2 drops of indicator
- Fill burette with 0.010M EDTA solution
- Titrate slowly, stirring continuously, until the last reddish tinge disappears from the solution (adding the last few drops at 3-5 sec. intervals).
- 1ml of 0.010 M EDTA is equivalent to 1mg of $CaCO_3$

CALCULATION

$$\text{Hardness in mg/L} = \frac{A \times B \times 1000}{\text{Volume of sample}}$$

A = mt titration for sample where
B = mg $CaCO_3$ equivalent to 1ml EDTA titrant

WHO /NAFDAC STANDARD: ≤50mg/L

CHAPTER SEVEN

DRUGS ANALYSIS

Drugs analysis includes:
(A) PHYSICAL TEST (ANALYSIS)
(B) ASSAY (CHEMICAL ANALYSIS)
(C) MICROBIAL ANALYSIS

PHYSICAL TEST OF DRUGS

The physical test of drug refers to those parameters that do involve chemical reactions, they include the following parameter:

- The uniformity test for tablets/capsules
- The disintegration time test for of tablets/capsules
- The pH test, for syrups/injection
- The hardness test for tablets
- The dissolution test tablets/capsules

UNIFORMITY TESR FOR TABLETS/CAPSULES DRUGS

INTRODUCTION: The tablets/capsules drugs need to be in uniform because; the doctors prescribe every drug base on the concentration of the active content in each tablet. The tablets are said to be uniform if the weight of each of the tablet fall in between the upper and lower limit weight of at least 10 tablets/capsules

AIM: This is done to confirm the uniformity of tablets.

EQUIPMENT: Analytical weighing balance

PROCEDURE:

- Take the weight of each of the tablet (at least 10 tablets), using analytical weighing balance
- Calculate the average weight of the tablet (the sum of weight of tablets divided by the number of the tablets).

- Calculate the percentage deviation of the tablets, this can be done using the formulae below:

1. For drugs that contain 250mg and above of the active content per tablet: Used; **5/100 *X* average weight of the tablets.**

2. For drugs that contain below 250mg to 80mg of the active content per tablet use; 7/**100 *X* average weight of the tablets**.

3. For drugs that contain below 80mg of the active content per tablet: use; **10/1 00 X average weight of the tablets**

- Calculate the upper limit and the lower limit. This can be done using the

Formula:

i. Upper limit = average weight + % deviation

ii. Lower limit = average weight % deviation

If the upper limit value fall below the highest weigh of the tablets, then, the tablets is consider to be in uniform. But if the upper limit value, fall above the highest weight of the tablet; the tablets or capsules are consider being non-uniform.

HARDNESS TEST FOR TABLETS DRUG

INTRODUCTION: This test is carrying out in all tablets drugs using harness tester machine. This is to analyses the hardness of tablet. Normally, tablets drugs should not be too soft or too hard. Hardness test give information on how the active content are been bind together to the binder, and how freely the active content can be release in the body when such drugs are taken.

PRINCIPLE: The principle of the machine is base on the force the machine applied to break the tablet. The unit of measurement is "Newton"

EQUIPMENT/MATERIALS: Hardness tester machine and ruler

PROCEDURE:
This is done using hardness tester machine.

- Measure the diameter of the tablet to be analyze using a meter rule
- Switch on the harness machine
- Click on action and highlight measurement, a dialog box will open, to
- Select new, a dialog box will open contain the following information to he filled; number of tablets to be analyze, diameter of tablet, oblong (i.e shape of the tablet either round or rectangular)
- Filled the information and place the tablets in the sample inserting space, starting from the inserting mark
- Click ok

The hardness of the tablet will be display on the screen.
NAFDAC STANDARD: ≤200N

DISINTEGRATION TIME TEST FOR TABLETS/CAPSULES

INTRODUCTION: The disintegration tester is a machine that works just as how the stomach works. The time taken for the tablet to disintegrate completely in the disintegrating tester is equivalent to the time it will take for the tablet to dissolve completely in human system when taken in the body.

AIM: To check the readiness of tablets and capsules to dissolves completely in human body.
EQUIPMENT/MATERIALS: Disintegration tester machine, 1000ml beaker

PROCEDURE:

- Switch on the machine
- Adjust the temperature to $37^{O}C$, that is normal body temperature
- Fill the beaker with 800ml of distilled water
- Insert beaker into the disintegration tube
- Place the tablet into the disintegrating tube and press the start button
- The disintegration will be display on the screen.
- Note the time taken for the tablet to disintegrate completely in the tube.

NAFDAC STANDARD
The disintegration time for uncoated tablets drugs should not be more than 1 5mins.
The disintegration time for coated tablets drugs should not be more than 60mins
The disintegration time for capsule drugs should not be more than 30mins

pH TEST FOR SYRUPS/INJECTION

INTRODUCTION: The pH for refers to the degree of acidity/alkalinity of a solution. The syrups/injections are expected not to be too acidic or too alkaline because that can also alter the pH of the body when such syrups/injections are taken into the body.

AIM: To check the pH of drugs

EQUIPMENT: pH meter.

PROCEDURE:

- Pour the solution of the syrup/injection into beaker
- Calibrate the pH meter using buffer
- Deep the pH meter electrode into the solution in the beaker
- Note the reading on the pH meter screen

DRUGS ASSAY

Drug assay is a qualitative or quantitative analysis of active ingredient present in drugs. The procedure for assaying every active ingredient of a drug is determined by the SOP (Standard Operating Procedure) which could be majorly BP (British Pharmacopoeia) or US P (United State Pharmacopoeia).

The following points are to be consider when carryout assay for drugs

1. **CALCULATION OF WEIGHT EQUIVALENT (ACTUAL WEIGHT)**

For example the average weight of tablets is 0.334g. The manufacturer declared that each tablet contain 50mg of the active. The SOP said, weight equivalent of 17mg into 50m1 volumetric flask.

The actual weight:

50mg = 0.334g

17mg = Xg

:. Actual weight = l7mg/50mg X 0.334g

= 0.11356g

Therefore, weight 0.11356g into 50m1 volumetric flask and dissolve as the instruction given from the SOP.

2. WEIGHT OF THE SAMPLE TAKEN.

It is possible that one cannot weight exactly what is calculated as the actual weight, but one can weight a closer weight. The weight of the sample taken should be

3. DILLUTION FACTOR

For example, the SOP instructed you to weight equivalent of 25mg into 50ml volumetric flask, dissolved with methanol and filter, dilute 1ml of the resulting filtrate to 25ml using methanol.

Dilution factor = 50rn1/100rn1 X 25ml/lrnl

= 12.5

4. PROCEDURE FOR ASSAY

This is base on the SOP. The analysis could be done either by chromatography method, Spectrophotometer method, titration method or extraction method.

DISSOLUTION TEST/ ASSAY OF PREGNISOLONE TABLET

INTRODUCTION: Dissolution test give information on the amount of the active content that will be absorbed into the blood stream for immediate action at a given time when such drugs are taking into the body. Normally, drugs should readily dissolve in the body to release the active content so that they can be biologically available for absorbed into the blood stream for immediate effect.

AIM: To determine dissolution percentage of prednisolone tablets using Dissolution tester apparatus

MATERIAL/APPARATUS/REAGENTS/EQUIPMENT:
20ml and 50ml volumetric flask, 2ml pipette, beaker, dissolution tester, standard Prednisolone, Uv-spectrophometer and prednisolone tablets

.

PROCEDURE:
The procedure is base on BP SOP.

- Transfer 900ml of distilled water in each of the dissolution tube
- Adjust the temperature to normal physiological body temperature (37^oC)
- Set the time for dissolution to 45mins
- Drop one tablet of the drug to each of the dissolution tube.
- Subject the machine into action by pressing start button
- After 45mins, fetch portion of the solutions from each tube and filter
- Keep the filtrate for drug assay.
- Check the manufacturer claims e.g each tablet contain 5mg of Prednisolone (Note: if the tablet dissolve completely, the concentration of solution from the dissolution tube should be 0.00555mg/ml.

PROCEDURE FOR ASSAY

- Take the absorbance of the filtrate from each tube using UV-spectrophometer at 256nm. Use distilled water as blank
- Calculate the average of the absorbance.

Procedure for preparation of standard:

- Weigh 10ml of pure standard prednisolone into 20ml volumetric flask which contain at least 10ml of methanol
- Shake very well and fill to mark with methanol
- From 20ml of the solution above, transfer 2.5ml into 200ml volumetric flask
- Fill the flask to mark with distilled water (this also give concentration of 0.0055mg/ml)
- Take the absorbance at 265nm using UV-spectrophometer

CALCULATION

$$\%\text{Dissolved} = \frac{\text{Ab.S} \times \text{Conc. Of Std} \times \text{Vd}}{\text{Ab.St} \times \text{Mc}} \times 100$$

Where: Ab.S = Average absorbance of filtrate from dissolution tube
Conc. Of Std = Conc. Of standard (0.0055mg/ml)
Vd = volume of distilled water in the dissolution tube (900ml)
Ab.St = Absorbance of standard
Mc = manufacturer claims (e.g each tablet contain 5mg of prednisolone

NAFDAC acceptable range: ≥70

Reference: British pharmacopeia standard operating procedure manual.

ASSAY OF PARACETAMOL

INTRODUCTION: Paracetamol is one of the active ingredients in pain reliever drugs

AIM: To check concentration of paracetamol active in pain reliever drug

PRINCIPLE: Sodium nitropruside reagent react with paracetamol in the basic solution to forms a products with colour O-nitrosamine which absorb UV-light maximally at 700nm wavelength.

MATERIALS/REAGENT:

10% sodium nitropruside reagent: mixed 10% potassium cyanide and 5% sodium hydroxide
Volumetric flask
UV-spectrophometer

PROCEDURE:

Weight equivalent of 15mg of the sample into 50ml volumetric flask
Add 30ml of sodium nitropruside reagent and shake very well
Fill the flask to mark with distilled water, shake very well and filter immediately
Keep the filtrate for 30mins at 37^{o}C (used water bath to maintain the temperature
Measure the absorbance of the filtrate at 700nm against sodium nitropruside reagent as the blank. (molar absorptivity = 3.4x10L/molcm)

CALCULATION/EXPRESSION OF RESULT

$$\text{Conc. of active ingredient (mg)} = \frac{A.Bs}{M.AB} \times D.F \times \frac{A.W}{W.T} \times 1000$$

Where : A.Bs = Absorbance
D.F = Dilution factor (see how to calculate in page)
A.W = Average weight of the capsules
M.AB = Molar absorptivity
W.T = weight of sample taken

ANALYSIS OF CIPROFLOXACIN BY SPECTROPHOMETRY METHOD (USP SOP)

INTRODUCTION: Ciproflocacin is an antibiotics commonly used for the treatment of various bacterial infections. This includes; joint infections, intra abdominal infections, typhoid fever, urinary tract infections etc.

MATERIALS/REAGENTS:

PROCEDURE

- Weight equivalent of 100mg of the sample into 100ml volumetric flask
- Add 5ml of 0.1N HCl
- Add 60ml of distilled water and shake vigorously for 20mins
- Fill the flask to mark with distilled water
- Filter the solution and transfer 10ml of filtrate into 50ml volumetric flask
- Add buffer solution to maintain pH of 2.5
- Fill to mark with solution of iron III
- Take absorbance of the resulting solution against solution of iron III as blank at 430nm (M. absorptivity = 4480/Mcm)

CALCULATION

Conc. Of ciprofloxacin (mg/Tab.) = absorbance x D.F x Av.w of tabs. x 1000
M.Ab. w.t

Where: D.F = Dilution factor (5)
Av.w of tabs = average weight of at least 10 tablets
M.Ab = Molar absorptivity
W.t = weight of the sample used.

Reference: United state pharmacopeia standard operating procedure

ASSAY OF DICHLOFENAC POTASSIUM (CAPSULE)

NAME OFACTIVE CONTENT: Dichlofenac potassium
MANUFA CTURER CLAIM: Each tablet contain 25mg of the active content
SOP: BP (British Pharmacopoeia, range: 24mg-25mg

AIM: To confirm the concentration of the active content as declared by the manufacturer.

MATERIALS/EQUIPMENT: 50m1 volumetric flask, filter paper, funnel, beaker, 2m1 pipette methanol and UV spectrophotometer

PROCEDURE:

- Weigh equivalent of 5mg of the sample into 50ml volumetric flask containing some quantity of methanol
- Shake very well and fill to mark with methanol
- Filter the resulting solution
- Transfer 1ml from the filtrate above into 25ml volumetric flask and dilute to mark with methanol
- Take the absorbance of the solution at 277nm using methanol as blank

Molar Absorptivity of Dichlofenac potassium= 357.14

CALCULATION

$$\text{Conc. of active ingredient (mg)} = \frac{\text{A.Bs}}{\text{M.AB}} \times \text{D.F} \times \frac{\text{A.W}}{\text{W .T}} \times 1000$$

Where : A.Bs = Absorbance
D.F = Dilution factor (see how to calculate in page)
A.W = Average weight of the capsules
M.AB = Molar absorptivity
W.T = weight of sample taken

Reference: British pharmacopeia standard operating procedure manual

ANAL YSIS ON HYDROGEN PEROXIDE SYRUP

NA ME OFA CTIVE CONTENT: Hydrogen peroxide
MANUFA CTURER CLAIM: the solution contain 6% w/v hydrogen peroxide
SOP: USP
SOP TOLERANCE RANGE: 6-7%

AIM: To confirm the concentration of the active content "hydrogen peroxide".

REAGENTS/APPARATUS: Drug sample (hydrogen peroxide syrup), l0ml pipette, 150m1 measuring cylinder, Dropping pipette, 50m1 burette, conical flask, 1M H_2SO_4 and 0.02M $KMnO_4$

PROCEDURE:

- Measure 10ml of the sample into 100ml volumetric flask and fill to mark with distilled water
- From 100ml of the solution above transfer 10ml into a conical flask
- Add 20ml of 1M H_2SO_4
- Fill the burette with 0.02M $KMnO_4$ and adjust to zero mark
- Titrate against the solution in the conical falsk until permanent faint pink colour is form. Note the volume of 0.02M $KMnO_4$. 1ml of is equivalent to 1.701mg of hydrogen peroxide.

CALCULATION

$$\textbf{\% Active ingredient/100ml = Titre value x equivalent of active/ml x } \frac{\textbf{10}}{\textbf{100}}$$

ANAL YSIS ON ECZEMA SKIN CREAM SAMPLE

NAME OFACTIVE: Betamethasone

SOP: BP (British Pharmacopoeia)

METHOD: HPLC (high Performance Liquid Chromatography.

AIM: To determine the concentration of the active content "betamethasone" in eczema skin cream.

MATERIAL/APPARATUS/REAGENTS/EQUIPMENT:
Eczema skin cream, cotton, reference standard of betamethasone, separating funnel, measuring cylinder, conical flask, funnel, 50m1 volumetric flask, 96%, 75% ethanol, hot hexane, analytical weight balance and HPLC machine

PROCEDURE:
Preparation of mobile phase:

- Measure 290rn1 of absolute ethanol into 600rn1 reagent bottle
- Add 210ml of distilled water, that gives 500rn1 of 58% ethanol.
- Sonicate the solvent for 20mins at 25°C to remove bubles.

Preparation of reference standard:

- Weight 2mg of the reference standard "betamethasone" into 50ml volumetric flask
- Add 20ml of the mobile phase solution and shake very well
- Fill to mark with the same solution. This means the concentration of betamethasone in the prepared solution is 0.04mg/rnl.

Preparation of sample:

- weight equivalent of 2mg of the sample into 100ml hot hexane and shake for 2rnins, allowed to cool,
- Pour the solution into separation funnel and add 20ml of 96% ethanol, shake very well and allowed to stand for 3mins
- Open the tap and collect the ethanol layer through absorbent cotton wool previously soaked in 75% ethano l(extraction)
- Repeat the extraction of the hexane mixture with 2 X l0ml ethanol (57%).
- Dilute the ethanol layers collected to 50ml with 75% ethanol.
- Fill the mobile phase bottles with mobile phase solution

- Fill sample valve with the dilulated solution after extraction and place in auto-sampler of the machine
- Fill another valve with reference standard solution and place in auto-sampler of the machine
- Carryout chromatography process base on HPLC Manufacturer procedure using the following Chromatography conditions:

✓ . Use a stainless steel column (10cm x 5mm) packed with acetylsilyl silica gel for

✓ chromatography (5µl)

✓ . Use isocratic elution and the mobile phase described below

✓ . Use a flow rate of 2m1 per minute

✓ . Use a column temperature of 60°C

✓ . Use detection wavelength of 238nm

✓ . Injection 20µl of each solution

CALCULATION/EXPRESION OF RESULT

After chromatography process the result will be display on the screen as chromatogram.
The chromatogram contains peak, retention time and total area of the peak.

For example, Chromatogram of 0.04mg standard betamethason shows the total area of the peak to be 13530855cm^2 while the sample total area of the peak show to be 2594270cm^2

Now, the concentration of the sample can be calculated as:
0.04mg of standard = 13530855cm^2
Xmg of sample = 2594270cm^2

$$Xmg = \frac{0.04mg \times 2594270cm^2}{13530855cm^2}$$

:. Conc. Of sample (mg) = 0.0077mg

Reference: British pharmacopeia standard operating procedure manual

ASSAY OF WORMZEL TABLETS BY TITRATION METHOD

NAME OFA CTJVE CONTENT: Albendazole
SOP: USP (United State Pharmacopoeia,)
AIM: To confirm manufacturer claimed

MATERIAL/APPARATUS/REAGENT/EQUIPMENT:
Wormzel tablets, 50ml burette, beaker, funnel, 150m1 conical flask, glacial acetic acid, acetic anhydride, 0.0 1M perchioric acid, crystal violet indicator.

PROCEDURE:

- Weight equivalent of 100mg into conical flask
- Add 40ml of glacial acetic acid
- Add 100ml of acetic anhydride
- Add two drops of crystal violet indicator
- Fill the burette with 0.1M Perchloric acid
- Titrate the solution in the burette against solution in the conical flask until green colour appears.

CALCULATION

1ml of 0.1M perchloric acid used is equivalent to 26.5mg /tablet of alberdazole.

$$\text{Acceptable percentage} = \frac{\text{calculate conc. From titration}}{\text{Manufacturer claims}} \times 100$$

NAFDAC ACCEPTABLE RANGE: 90% - 110%
Reference:United State Pharmacopoeia standard operating procedure

STERILITY TEST

INTRODUCTION: Sterility testing is defined as the testing which confirms that products are free from present of viable microorganism. Sterility test is very important for medical devices (surgical equipment), pharmaceutical (tablets/capsules, injection, eye drops), tissues material (bandage, tissues paper) and others materials that claims to be sterile or free from viable microorganism. This test can be done either using British pharmacopeia (BP) or united state pharmacopeia (USP) method.
AIM: To check sterility of sterile products

MEDIA: Fluid thioglycollate medium or Soya-bean casein digest medium

PRINCIPLE/PROCEDURE:

Procedure for suitability test: suitability test is carryout to ascertain in the you are to carryout sterility test antimicrobial or not. There are two main methods

1.Membrane filtration method: After transferring the content of the container or containers to be tested to the membrane add an inoculum of a small number of viable microorganisms (not more than 100 CFU) to the final portion of sterile diluent used to rinse the filter.

- **2. Direct inoculation method:** After transferring the contents of the container or containers to be tested to the culture medium add an inoculum of a small number of viable microorganisms (not more than 100 CFU) to the medium. In both cases use the same microorganisms as those described above under Growth promotion test of aerobes, anaerobes and fungi. Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 5 days. If clearly visible growth of microorganisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without further modification. If clearly visible growth is not obtained in the presence of the product to be tested, visually comparable to that in the control vessels without product, the product possesses antimicrobial activity that has not been satisfactorily eliminated under the conditions of the test.
- Modify the conditions in order to eliminate the antimicrobial activity and repeat the method suitability test.

- The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined. (Appropriate negative controls must be included)
- The technique of membrane filtration is used whenever the nature of the product permits, that is, for filterable aqueous preparations, for alcoholic or oily preparations and for preparations miscible with or soluble in aqueous or oily solvents provided these solvents do not have an antimicrobial effect in the conditions of the test.
- **Membrane filtration:** Use membrane filters having a nominal pore size not greater than 0.45 µm whose effectiveness to retain microorganisms has been established. Cellulose nitrate filters, for example, are used for aqueous, oily and weakly alcoholic solutions and cellulose acetate filters, for example, for strongly alcoholic solutions. Specially adapted filters may be needed for

certain products, e.g. for antibiotics. The technique described below assumes that membranes about 50 mm in diameter will be used. If filters of a different diameter are used the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and membrane are sterilized by appropriate means. The apparatus is designed so that the solution to be examined can be introduced and filtered under aseptic conditions; it permits the aseptic removal of the membrane for transfer to the medium or it is suitable for carrying out the incubation after adding the medium to the apparatus itself.

- **Aqueous solutions**: If appropriate, transfer a small quantity of a suitable, sterile diluents such as a 1 g/l neutral solution of meat or casein peptone pH 6.9 to 7.3 onto the membrane in the apparatus and filter. The diluents may contain suitable neutralizing substances and/or appropriate inactivating substances for example in the case of antibiotics.
- Transfer the contents of the container or containers to be tested to the membrane or membranes, if necessary after diluting to the volume used in the method suitability test with the chosen sterile diluents but in any case using not less than the quantities of the product to be examined.
- Filter immediately. If the product has antimicrobial properties, wash the membrane not less than three times by filtering through it each time the volume of the chosen sterile diluent used in the method suitability test. Do not exceed a washing cycle of five times 100 ml per filter, even if during method suitability it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity.
- Transfer the whole membrane to the culture medium or cut it aseptically into two equal parts and transfer one half to each of two suitable media. Use the same volume of each medium as in the method suitability test or transfer the medium onto the membrane in the apparatus.
- Incubate the media for not less than 14 days

Direct inoculation of the culture medium:

- Transfer the quantity of the preparation to be examined directly into the culture medium so that the volume of the product is not more than 10% of the volume of the medium, unless otherwise prescribed. If the product to be examined has antimicrobial activity, carry out suitability test, after neutralizing this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent

dilution. Where appropriate the concentrated medium may be added directly to the product in its container.

- Oily liquids products: Use media to which have been added a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability of the test, for example polysorbate 80 at a concentration of 10 g/l.
- Ointments and creams. Prepare by diluting to about 1 in 10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluents such as peptone (1 g/l) TS1.
- Transfer the diluted product to a medium not containing an emulsifying agent. Incubate the inoculated media for not less than 14 days.
- Observe the cultures several times during the incubation period. Shake cultures containing oily products gently each day. However when fluid thioglycollate medium is used for the detection of anaerobic microorganisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

OBSERVATION/EXPRESSION OF RESULTS

- At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination 14 days after the beginning of incubation
- Transfer portions (each not less than 1 ml) of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days.
- If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined.

POTENCY TEST FOR ANTIBIOTIC DRUGS

POTENCY TEST FOR ANTIBACTERIA DRUG

INTRODUCTION:

The general relationship between an antibiotic and an infectious organism is one of antibiosis. This word refers to an association of two organisms in which one is harmed or killed by the other. It is believed that antibiotics interfere with the surface of bacteria cells, causing a change in their ability to reproduce. Testing the action of an antibiotic in the laboratory shows how effective a particular drugs will be when used for treatment against particular bacterial infection. This test is commonly used in hospital to screen drugs, to determine the drugs suitable for a particular ailment.

AIM: To determine the effectiveness of antimicrobial drugs

3.5.3.3 PRINCIPLE:

Nutrient Agar (NA) is a non selective medium that support the growth of broad spectrum of bacteria. The microorganisms are inoculated into the medium and the antibiotic is place at the center of the plate, the antimicrobial activities of the antibiotic is observed as clear zone due to inhibition of the growth of the organism.

MATERIALS/MEDIA/EQUIPMENT:

- Antibiotic drugs
- Sterilized plates
- Swab stick
- Nutrient Agar (NA)
- 70% ethanol
- Incubator
- Autoclave
- Pure isolate of bacterial
- Top loading weight balance, incubator and
- UV-safety cabinet.

PROCEDURE:

- Prepare the nutrient agar base on manufacturer procedure
- Sterilize the medium by autoclaving using autoclave
- Remove the medium and allowed to cooled to 44-50^{o}C
- Sterilize the testing environment with 70% ethanol
- Aseptically pour substantial quantity of the agar into petri dish and allowed to solidify
- Deep the swab stick into the solution of bacteria isolates and inoculates into the plate by streaking all over the surface
- Place at least three antibiotic tablets at different position at the top of the solidify agar on the plate
- Incubate at 37^{o}C for 24hrs
- After 24hrs check the zone of inhibition on each tablet
- ***Note: If the drugs is not a broad spectrum antibacterial, use pure isolate of the corresponding bacterial in which the drug is meant for***

OBSERVATION/EXPRESSION OF RESULT

- Large and very clean zone of inhibition indicate highly effective antibiotic
- Small and unclear zone of inhibition indicate less effective antibiotic
- Absent of zone of inhibition indicate ineffective antibiotic

POTENCY TEST FOR ANTIFUNGAL DRUGS

INTRODUCTION: Antifungal drugs are used to treat fungal infections, which most commonly affect the skin, hair and nails. Some of the examples of antifungal drugs include; clotrimazole, econazole, ketoconazole, amphotericin etc.

AIM: To determine the effectiveness of antifungal drugs.

PRINCIPLE:

Saborous dextrose agar (SDA) is a non selective medium that support the growth of broad spectrum of fungi. Antifungal drugs work by either killing the fugal cells; for example, by affecting a substance in the cell walls, causing the contents of the fungal cells to leak out and the cells die or preventing the fungal cells growing and reproducing. The ability of the drug to kill or prevent growth/reproduction can be seen in its zone of inhibition

MATERIALS/MEDIA/EQUIPMENT:

- Antibiotic drugs
- Sterilized plates
- Swab stick
- Saborous Dextrose Agar (SDA).
- 70% ethanol
- Incubator
- Autoclave
- Pure isolate of fungi
- Top loading weight balance, incubator and
- UV-safety cabinet.

PROCEDURE:

- Prepare the nutrient agar base on manufacturer procedure
- Sterilize the medium by autoclaving using autoclave
- Remove the medium and allowed to cooled to 44-50^{o}C
- Sterilize the testing environment with 70% ethanol
- Aseptically pour substantial quantity of the agar into petri dish and allowed to solidify
- Deep the swab stick into the solution of fungi isolates and inoculates into the plate by streaking all over the surface
- Place at least three antifungal tablets (or scurf the cream using wire loop) at different position at the top of the solidify agar on the plate
- Incubate at 37^{o}C for 24hrs, 48hrs and 72hrs
- After each 24hrs check the zone of inhibition on each tablet

  Note: If the drug is not a broad spectrum antifungal drugs, use pure isolate of the corresponding fungus in which the drug is meant for

OBSERVATION/EXPRESSION OF RESULT

- Large and very clean zone of inhibition indicate highly effective Antifungal drug
- Small and unclear zone of inhibition indicate less effective Antifungal drug
- Absent of zone of inhibition indicate ineffective Antifungal drug

ANALYSIS OF HERBAL DRUGS

INTRODUCTION: In herbal drugs, secondary metabolites of plants are the key drivers of the pharmacological actions. Secondary metabolites are products of subsidiary pathways in plants. Secondary metabolites in traditional medicine play important role in alleviating several aliments.
Secondary metabolites are classified according to their chemical structure.
The major classes include:

1. Flavonoids
2. Saponins
3. Tannins
4. Alkaloids
5. Glycoside

The analysis of herbal drugs includes the following parameters:

- Phytochemical screening
- Potency test
- Quantitative test for secondary metabolites

PROCEDURE/EXPRESSION OF RESULT FOR PHYTOCHEMICAL SCREENING

1. QUALITATIVE TEST FOR FLAVONOIDS

- Take 2ml of the sample test tube
- Add 5ml of dilute ammonia
- Add 1ml of concentrated sulphuric acid

EXPRESSION OF RESULT

Appearance of yellow colouration which disappears on standing indicates present of flavonoids.

2. QUALITATIVE TEST FOR SAPONNINS

- Take 5ml of the sample into test tube
- Shake very well till persistence froth is observed
- Add 3 drops of olive oil and shake

EXPRESSION OF RESULT

Formation of emulsion indicates present of saponins

3. QUALITATIVE TEST FOR TANNINS

- Take 5ml of the sample into test tube
- Heat to boil and filter
- Add few drops of 0.1% ferric chloride (Ayoola *et al* 2008)

EXPRESSION OF RESULT

Formation of brownish green or blue-black indicates present of tannins

4. QUALITATIVE TEST FOR ALKALOIDS

- Take 5ml into test tube and dilute to 10ml with acid alcohol
- Boil and filter
- Add 2ml of dilute ammonia to the filtrate
- Add 5ml of chloroform
- Shake and gently decant the chloroform layer into another test tube
- Add 10ml of acetic acid
- Add Mayer's reagent (Trease *et al,*. 1989)

EXPRESSION OF RESULT

Formation of creamy colouration indicates present of alkaloids

5. QUALITATIVE TEST FOR GLYCOSIDES

- Take 5ml of the sample into test tube
- Add 2ml of glacial acetic acid containing few drops of ferric chloride solution
- Add 1ml of concentrated sulphuric acid (Trease *et al,*. 1989)

EXPRESSION OF RESULT

Formation of brown ring at the interface indicates present of glycosides.

POTENCY TEST (ANTIMICROBIAL ACTIVITITY) OF HERBAL DRUGS

MATERIALS/MEDIA/EQUIPMENT:

- Antibiotic drugs
- Sterilized plates
- Swab stick
- Nutrient Agar (NA)
- Incubator
- Autoclave
- Pure isolate of bacteria and fungi
- Top loading weight balance, incubator and

PROCEDURE:

- Prepare the nutrient agar and saborous dextrose agar base on manufacturer procedure
- ppour substantial quantity of the agar each to separate to petri dish and allowed to solidify
- Deep the swab stick into the solution of bacteria isolates and inoculates into the plate containing nutrient agar by streaking all over the surface
- Deep the swab stick into the solution of fungal isolates and inoculates into the plate containing saborous dextrose agar by streaking all over the surface
- Make three wells in each of the solidify agar in the plates
- Place at least o.5ml of the herbal solution (liquids) or small quantity of the herbal powder (solids) into the wells.
- Incubate at $37^{o}C$ for 24hrs, 48hrs and 72hrs
- After each 24hrs check the zone of inhibition on each well

OBSERVATION/EXPRESSION OF RESULT

- Large and very clean zone of inhibition in nutrient agar plate indicate high effective broad spectrum antibacterial herbal drugs
- Small and unclear zone of inhibition in nutrient agar plate indicate less effective broad spectrum antibacterial herbal drugs
- Absent of zone of inhibition in nutrient agar plate indicate high ineffective broad spectrum antibacterial herbal drugs
- Large and very clean zone of inhibition in saborous dextrose agar plate indicate high effective broad spectrum antifungal herbal drugs

- Small and unclear zone of inhibition in saborous dextrose agar plate indicate less effective broad spectrum antifungal herbal drugs
- Absent of zone of inhibition saborous dextrose agar plate indicate high ineffective broad spectrum antifungal herbal drugs

Note: If the herbal drugs is not a broad spectrum antibacterial or antifungal, use pure isolate of the corresponding bacteria or fungus in which the drug is meant for

CHAPTER EIGHT
MICROBIAL ANALYSIS

TEST FOR E. COLI AND COLIFORM

INTRODUCTION: Coliform bacteria are members of Enterobacteriacae family that express β-Dgalactosidase activity and E.coli are members of coliform group that express β-Dgalactosidase and β-D-glucuronidase activity. . More precisely, present of coliforms in a sample is an vidence of recent human fecal contamination of that particular sample or product.

PRINCIPLE:
(For water sample or liquid food substance). A test portion of water sample is passed through a membrane filter, which is then placed for incubation on Chromogenic coliform agar plate. All presumptive coliform bacteria grow as pink to red colonies which can be confirmed by negative oxidase test. Due to β-D-galactosidase and β-D-glucuronidase activity E.coli bacteria appear as Dark blue to violet in color. This method is not applicable to β-D-glucuronidase negative E.coli strains like E.coli O157.

MATERIALS/MEDIUM:
- Petri dish (plate)
- Chromogenic coliform Agar (CCA)

PROCEDURE:
- Prepare the agar base on manufacturer procedure and sterilize by autoclaving using autoclave
- Allow the medium to cooled to 44°C–47°C
- Pour substantial amount to sterilize petri three petri dish
- Disinfect the surface of the bottle/pouch/cups containing sample with 70% ethanol. Thoroughly mix the sample by vigorous shaking to achieve uniform distribution
- Filter the sample (required volume) through a sterile membrane filter (0.45µm pore size) and place the filter in first two plate CCA plate (third plate is control) and Incubate overnight at 36±2°C for 18- 24hr.
- For solid products, allowed the agar in the plate to solidify and swab on the products then aseptically strike on the surface of the solidified agar in the plate and incubate overnight at 36±2°C for 18- 24hr.

EXPRESSION OF RESULT

- Count all pink to red colonies as presumptive coliform. Confirmatory test on all presumptive colonies can be carryout by negative oxidase test
- Count all dark blue to violet colonies as presumptive E.coli.

TEST FOE SALMONELLA SPECIES

INTRODUCTION: Salmonella are Gram negative, oxidase negative, non-spore forming, rod shaped bacteria which is able to ferment glucose and capable of facultative anaerobic growth.

AIM: To test for the present of salmonella species in water and food products. Salmonella species are the major pathogens that causes typhoid fever in human being hence, this test necessary on every consumable substances

MATERIAL/ MEDIA:

- Autoclave
- Petri dish (plate)
- Buffered peptone water
- Rappaport-Vassiliadis (RVS) broth
- Brilliant green/phenol red lactose agar (BGA)
- Xylose lysine deoxycholate agar (XLD)

PRINCIPLE: Detection of Salmonella is based on pre-enrichment, selective enrichment followed by isolation on selective media. Presumptive Salmonella colonies are confirmed by biochemical and serological tests. Pre-enrichment broth is necessary to enable injured cells to grow. Further selective enrichment is done to increase the proportion of Salmonella in relation to background flora. Selective media are used for further isolation and preliminary confirmation.

PROCEDURE:

- Pre-enrichment: Aseptically clean the surface of bottle/pouch/cups containing sample with 70% ethanol and filter 250ml (or as specified) of water sample through a membrane filter of 0.45µm pore size using sterile membrane filtration assembly. Place the filter disk in 50 mL buffered peptone water and incubate at 36±2°C for 16-20 hrs.

- Selective enrichment: Transfer 0.1ml of the pre-enrichment culture to 10mL or 1mL to 100 mL of malachite green/magnesium chloride (Modified RVS broth) and incubate at 42±0.5°C for 18-24hrs.
- Confirmation on Selective agar media: After incubation streak a loopful from RVS broth on selective medium i.e. BGA, XLD and BSA (Optional). Incubate the plates at 36±2°C for 24 hr (48 hrs for BSA).
- In order to detect slow growing Salmonella, re-inoculate BGA, XLD and BSA (optional) after continued incubation of RVS broth for further 24 hrs.

NOTE: ***whenever you are testing liquid transfer some milliliter of the sample as inoculums while for solid product; swab on the products and strike on the medium.***

OBSERVATION/RESULT EXPRESSION

Salmonella's Colonies on BGA are red or slightly pink-white and opaque with red surrounding.

Salmonella's Colonies on XLD agar are colorless but appear red usually with a black centre.

On BSA medium black *Salmonella's* colonies appear surrounded by a metallic sheen.

Salmonella's H2S negative strains appear on XLD agar as pink with a darker pink center.

Lactose positive *Salmonella* strains grow on XLD agar as yellow with or without blackening (***Reference: Natural Mineral Water- Food Safety & Standard Regulations - 2011 and IS 13428:2005 Reaff.)***

TEST FOR STAPHYLOCOCCUS AUREUS IN WATER/FOOD SUBSTANCES

INTRODUCTION: Staphylococcus aureus:Aerobic, gram positive cocci in cluster show coagulase activity. Produce golden yellow colored colonies on Nutrient agar and Blood agar and shiny black colonies on Baired-Parker medium.

PRINCIPLE:

Baird Parker agar is used for the isolation and differentiation of coagulase-positive Staphylococci. Staphylococci can reduce tellurite to telluride, which results in grey to black coloration of the colonies. With the addition of egg yolk, the medium becomes yellow, slightly opaque. A clear halo develops around colonies from coagulase positive Staphylococcusaureus. Grey-black colonies and a halo on this medium are presumed to be indicative of coagulase positive staphylococci.

MATERIALS/ MEDIA:

- Swab stick/wire loop
- Incubator
- Autoclave
- Cooked Salt Meat medium
- Baired-Parker agar
- Blood agar

PROCEDURE:

- Disinfect the surface of the bottle/pouch/cups containing sample with 70% ethanol. Thoroughly mix the sample by vigorous shaking to achieve uniform distribution
- Filter the sample (requisite volume) through a sterile membrane filter (0.45µm pore size) and place the filter in 50mL of Cooked Salt Meat medium. Incubate overnight at 37°C (Liquids sample). Swab on the sample and Streak on the medium in plates using swab stick (for solids sample)
- After incubation streak a loopful from Cooked Salt Meat medium on Baired Parker agar for at least 30 hrs at 37°C and overnight on Blood agar at 37°C.

OBSERVATION/EXPRESSION OF RESULT:

- S. aureus show shiny black colonies with or without grey-white margins on Baired-Parker agar
- S. aureus show golden yellow colonies on Blood agar.

TEST FOR PSEUDOMONAS AERUGINOSA

INTRODUCTION: *Pseudomonas aeruginosa* a Gram negative, non-sporing rod is oxidase and catalase positive. When grown on asparagine and ethanol, it produces water soluble fluorescent pigment.

PRINCIPLE: Detection of *Pseudomonas aeruginosa* is based on selective enrichment followed by isolation on confirmation media. Asparagine proline broth is used as selective medium for cultivation of P. aeruginosa. Milk Agar is used for selective isolation of Pseudomonas *aeruginosa*. Strains of *Pseudomonas aeruginosa* are identified by their pigment i.e. pyocyanin production. Pseudomonas aeruginosa hydrolyzes casein and produces a yellow to green diffusible pigment on Milk agar.

MATERIALS/MEDIA:

- Pseudomonas asparagine proline broth
- Milk agar with cetrimide
- Petri dish
- 10ml bottles
- Autoclave
- Incubator

PROCEDURE:

- Aseptically clean the surface of bottle/pouch/cups containing sample with 70% ethanol and filter 250mL (or as specified) of water sample through a membrane filter of 0.45µm pore size using sterile membrane filtration assembly and place the filter in 50mL of concentrated Asparagine proline broth (only for liquids).
- Incubate at 37±1°C for 48 hrs.
- After incubation examine the medium showing either growth or fluorescence under U.V. light (360±20 nm)
- Subculture a loopful on Milk agar plate and incubate for 24h at 42±0.5°C

OBSERVATION

- On Asparagine proline broth; Production of Pyocyanine (Blue/green) pigment indicate present of *Pseudomonas aeruginosa*

On Milk agar with cetrimide; Pseudomonas aeruginosa hydrolyzes casein and produces a yellow to green diffusible pigment. ***Reference: Natural Mineral Water- Food Safety & Standard Regulations - 2011 and IS 13428:2005 Reaff.***

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AEROBIC MICROBIAL COUNT TEST IN WATER AND FOOD

INTRODUCTION: Plate count agar (PCA) is a general purpose growth medium commonly used to assess "total" or “viable” bacterial growth of a water sample or food substances. The number of microorganisms per milliliter of sample is calculated from the number of colonies obtained on PCA plate from selected dilution.

AIM: To detect the microbial load in the sample.

PRINCIPLE:
Plate count agar (PCA) is a general purpose growth medium commonly used to assess "total" or “viable” bacterial growth of a water sample. The number of microorganisms per milliliter of sample is calculated from the number of colonies obtained on PCA plate from selected dilution. Poured plates are prepared using a specified culture medium and a specified quantity of the sample. The plates are aerobically incubated at two different temperatures i.e. 37°C for 24 hr and 20 – 22°C for 72 hr

MEDIA:
Plate count Agar (PCA)

PROCEDURE:

- Prepared the medium base of manufacturer procedure and autoclave it.
- Disinfect the surface of the bottle/pouch/cups containing sample with 70% ethanol. Thoroughly mix the sample by vigorous shaking to achieve uniform distribution.
- Aseptically inoculate 1ml of the water sample using sterile pipette (swab on the products) into sterile petri plates in duplicate in two sets. The petri plates should be labeled with the sample number, date and any other desired information.
- Pour into each plate 15–18ml of the molten sterilized PCA media (cooled to 44°C–47°C). Avoid pouring of molten medium directly onto the inoculum. Mix the media and inoculum by swirling gently clockwise and anti-clockwise so as to obtain homogenous distribution of inoculum in the medium.

- Allow to cool and solidify. In case, where in sample microorganism having spreading colonies is expected, add 4ml of overlay medium onto the surface of solidified plates.
- After complete solidification, invert the prepared plates and incubate one set at 370C for 24 hr and other set at 20 – 22°C for 72 hr.
- After specified incubation period count all colonies including pinpoint colonies. Spreading colonies shall be considered as single colony. If less than one quarter of dish is overgrown by spreading, count the colonies on the unaffected part of the dish and calculate corresponding number in the entire dish. If more than one quarter is overgrown by spreading colonies discard the plate.

CALCULATION AND EXPRESSION OF RESULT

Plates having microbial count between 10 and 300cfu:

$$N = \frac{\text{ColoniesPlate1} + \text{ColoniesPlate2}}{2}$$

If microbial load is from 3 to 1 then reporting of results shall be: "Microorganisms are present, but, less than 4 per ml"

When the test sample/plates contain no colonies then reporting of results shall be: "Less than 1 cfu/ml".

TEST FOR YEASTS AND MOULDS IN WATER/FOOD SUNSTANCES

INTRODUCTION: Yeast & Moulds Microorganism are capable of forming colonies in a selective medium (Chloroamphenicol Glucose Yeast Extract Agar medium) at 25°C. Yeast and moulds are the major causes of food spoilage.
AIM: To detect present of yeast and mould in food sample/water sample

PRINCIPLE:
Chloramphenicol Yeast Glucose Agar is a selective medium recommended for isolation and enumeration of Yeast & Moulds. The medium contains yeast extract, which provides nitrogenous nutrient and vitamin B complex. Dextrose is the energy source. Chloramphenicol a thermostable antibiotic, suppresses the bacterial flora. Aerobic incubation of plates is done at $25^0C \pm 1^0C$ and count is taken on 3rd, 4th or 5th day.

MATERIALS/MEDIA:

- Chloroamphenicol Glucose Yeast Extract Agar (CGYEA)
- Swab stick/wire loop
- Incubator
- Autoclave

PROCEDURE:

- Prepare the media base on the manufacturer procedure
- Aseptically clean the surface of bottle/pouch/cups containing sample with 70% ethanol and filter 250mL (or as specified) of sample through a membrane filter of 0.45µm pore size using sterile membrane filtration assembly (liquid sample). Swab on the sample and Streak on the medium in plates using swab stick (for solids sample)
- Place the filter (Streak) on CGYEA media and incubate at 25±1°C
- Observe the plates for colonies of Yeast & Moulds on 3rd, 4th and 5th days of incubation.

OBSERVATION/EXPRESSION OF RESULT

- Yeast colonies are smooth, moist and elevated.

Mould colonies are easily recognized by the profuse growth of hyphae. ***Reference: Natural Mineral Water- Food Safety & Standard Regulations - 2011 and IS 13428:2005 Reaff.***

TEST FOR VIBRIO CHOLERAE IN FOOD/WATER

INTRODUCTION: *Vibrio cholera* a Gram negative bacterium is a curve shaped rod. It is catalase and oxidase positive and ferment glucose without gas production. *V. cholera* occurs naturally in the plankton of fresh, brackish and salt water. Vibro cholerae a causative agent of cholera, one of the fast killing diseases hence, this test becomes very necessary to every consumable substance.

AIM: To test for the present of Vibrio cholera in food/water.

PRINCIPLE:

Bile Salt agar and Thiosulphate Citrate Bile salt sucrose agar (TCBS) is used selectively for the isolation, identification and enumeration of *Vibrio cholerae*. Vibrio species grow in the presence of relatively high levels of bile salts which inhibit the growth of grampositive microorganisms. TCBS has a very high pH (8.5-

9.5) which suppresses growth of intestinal flora other than Vibrio sp. *V. cholerae* ferment sucrose, which results in a pH shift and production of yellow colonies.

MATERIALS/MEDIA:

- Alkaline peptone water
- Thiosulphate-Citrate-Bile salts-Sucrose agar (TCBS)
- Bile salt agar (BSA)
- Swab stick/wire loop
- Incubator
- Autoclave

PROCEDURE:

- Disinfect the surface of the bottle/pouch/cups containing sample with 70% ethanol. Thoroughly mix the sample by vigorous shaking to achieve uniform distribution.
- Filter the sample (requisite volume) through a sterile membrane filter (0.45µm pore size) and place the filter in 50 mL of alkaline peptone water. (Swab on the sample and inoculate into 50ml of alkaline peptone water) Incubate at 37°C overnight.
- After incubation streak a loopful on TCBS and BSA medium plates and incubation at 37°C overnight.

OBSERVATION/EXPRESSION OF RESULT

- Suspicious colonies of V. cholera on TCBS appear as opaque yellow colored with entire round margins.

Cholera colonies show a distinctive appearance on BSA medium. ***Reference: Natural Mineral Water- Food Safety & Standard Regulations - 2011 and IS 13428:2005 Reaf.***

References

- *Natural Mineral Water- Food Safety & Standard Regulations - 2011 and IS 13428:2005 Reaff.*
- *Joy P. P. 2013. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam, Kerala Tel.: 0485-2260832, 9446010905;*
- *Wheat and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality: Version 2.*
- *Nafdac laboratory standard operating procedures manual.*
- *WHO food safety manual.*
- *Food Safety and Standards Regulations, India.*
- *United state pharmacopeia standard operating procedure.*
- *British pharmacopeia standard operating procedure.*