Guidance Document

General Calculations in Indian Pharmacopoeia

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Introduction

Pharmacopoeial calculations are indispensable part of almost every monograph procedure. Each monograph procedure uses various test methods, experimental designs and corresponding formulae to calculate results. Following are examples of general calculations applied in IP:

1. Chemical Analysis

(i) Molarity

In IP, as a practice all concentrations of solutions are expressed in the terms of molarity. Molarity (M) is defined as the number of moles of solute per litre of solution. The solute is defined as the substance being dissolved, while the solvent is the substance where the solute is dissolved (usually water).

$$M = \frac{1000 \times W}{V \times \text{Mol wt.}}$$

Where, M is molarity; V is volume in ml; W is weight in g

Illustration: *Preparation of 500ml 0.1M NaOH solution (Mol.wt. of NaOH = 40)*

$$0.1 = \frac{1000 \times W}{500 \times 40}$$

$$W = 2.0\ g$$

To prepare 0.1M NaOH solution, dissolve 2.0 g of sodium hydroxide pellets in 250 ml distilled water and make up the volume to 500 ml.

(ii) Normality

Normality (N) is defined as the number of equivalent weight of solute per litre of solution. Equivalent weight is molecular mass of substance divided by its number of replaceable H^+ ions or OH^- ions or valency or number of electrons.

$$N = \frac{1000 \times W}{V \times \text{Eq. wt.}}$$

Where, N is normality; V is volume in ml; W is weight in g

$$\text{Equivalent wt. (E)} = \frac{\text{Mol. wt. of substance}}{n}$$

Where, n is number of replaceable H^+ ions (for acids) or number of replaceable OH^- ions (for bases)

Illustrations:

a) *Preparation of 1000 ml 1N H_2SO_4 solution (Mol. wt. of H_2SO_4 = 98)*
Number of replaceable H^+ ions per mole = 2 (as $H_2SO_4 = 2H^+ + SO_4^{2-}$) and therefore E= 98/2 =49

$$1 = \frac{1000 \times W}{1000 \times 49}$$

$$W = 49.0\ g \text{ (or } 26.6 \text{ ml as sulphuric acid has about } 1.84 \text{ g weight per ml)}$$

1N sulphuric acid solution is prepared by dissolving 49.0 g (26.6 ml) in 250 ml distilled water and making up the volume to 1000 ml.

b) *Preparation of 500 ml 0.1N NaOH solution (Mol. wt. of NaOH = 40)*
Number of replaceable OH^- ions per mole = 1 (as $NaOH = Na^+ + OH^-$) and therefore E= 40/1 =40

$$0.1 = \frac{1000 \times W}{500 \times 40}$$

$$W = 2.0\ g$$

0.1N sodium hydroxide solution is prepared by dissolving 2.0 g in 250 ml distilled water and making up the volume to 500 ml.

(iii) ppm

ppm is an abbreviation of parts per million, equivalent to 10^{-6} and used to describe very low concentrations. One ppm is equivalent to 1 milligram of substance per litre of solvent (mg/L) or 1 milligram of substance per kilogram of solid or solvent (mg/Kg).

ppm shows that the value is multiplied with one million (10^6) and % shows that the value is multiplied with one hundred (10^2). So in order to convert ppm into %, divide the value by 10000. On the contrary, in order to convert % into ppm, multiply the value by 10000 (e.g. 1 ppm = 0.0001%, 10 ppm = 0.001% and so on)

Illustration 1: Making up 1000 ppm solution

a) From the Pure Metal

Example: Make a 1000 ppm standard of sodium (Na) using sodium metal.

Weigh accurately 1.0 g of metal, dissolve in 1:1 concentrated nitric or hydrochloric acid, and make up volume to 1000 ml with deionised water.

b) From a Salt of the Metal

Example: Make a 1000 ppm standard of Na using sodium chloride.

Molecular weight of sodium chloride (NaCl) = 58.44

Atomic weight of sodium (Na) = 23

1 g Na in relation to molecular weight of sodium chloride (NaCl) = 58.44/23 = 2.542 g

Hence, weigh accurately 2.542 g NaCl, dissolve and make up volume to 1000 ml to make a 1000 ppm Na standard solution.

c) From an Acidic Radical of the Salt

Example: Make a 1000 ppm phosphate standard using potassium dihydrogen phosphate salt (KH_2PO_4).

Molecular weight of potassium dihydrogen phosphate (KH_2PO_4) = 136.09

Molecular weight of phosphate (PO_4) radical = 95

1 g PO_4 radical in relation to molecular weight of potassium dihydrogen phosphate (KH_2PO_4) = 136.09/95 = 1.432 g

Hence, weigh accurately 1.432 g KH_2PO_4 and dissolve up to 1000 ml volume to make a 1000 ppm PO_4 standard.

Illustration 2: Dilution from stock solution

Dilution Formula: $C_1V_1 = C_2V_2$

C_1 = available stock solution concentration

V_1 = volume of stock solution required for dilution

C_2 = required final concentration

V_2 = required final volume

Example: Prepare 100 ml of 100 ppm sodium solution from 50 ml of 1000 ppm stock solution of sodium (Na) standard using water as diluent.

C_1 = 1000 ppm sodium (Na) standard solution; V_1 = ? ml; C_2 = 100 ppm; V_2 = 100 ml

$V_1 = (C_2 \times V_2) / C_1$

= (100 ppm x 100 ml) / 1000 ppm

= 10 ml

Therefore pipette out 10.0 ml of the 1000 ppm sodium standard stock solution, dilute up to 100 ml with water to get solution of 100 ppm.

2. Titrimetric Analysis

Titration is a method of volumetric analysis. A reagent, called the titrant is prepared as a standard solution. A known concentration and volume of titrant reacts with a solution

of analyte to determine concentration. The volume of titrant reacted is called titration volume. There are many types of titrations. The most common types of qualitative titrations are acid-base titrations, redox titrations, precipitation titrations and complexometric titrations.

Illustration: Assay of Albendazole (Acid-Base Titration)

Assay: *Dissolve 0.5 g in 80 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration. Factor: 1 ml of 0.1M perchloric acid is equivalent to 0.02653 g of $C_{12}H_{15}N_3O_2S$ (Albendazole)*

To proceed with assay, first 0.1M perchloric acid ($HClO_4$) is to be standardised as per IP.

Standardisation of 0.1M perchloric acid ($HClO_4$)

Weigh accurately about 0.35 g of potassium hydrogen phthalate ($C_8H_5KO_4$), previously powdered lightly and dried at 120° for 2 hours and dissolve it in 50 ml of anhydrous glacial acetic acid. Add 0.1 ml of crystal violet solution and titrate with the perchloric acid solution until the violet colour changes to emerald-green. Perform a blank determination and make any necessary correction.

Acceptance Criteria: *The volumetric solutions should not differ from the prescribed strength by more than 10 per cent and the molarity should be determined with a precision of 0.5 per cent.*

Factor : 1 ml of 0.1M perchloric acid is equivalent to 0.02042 g of $C_8H_5KO_4$
Diluent : Anhydrous glacial acetic acid
Indicator : Crystal violet solution
Blank : Diluent + Indicator

Excel calculation format for Molarity determination

	SET 1	***SET 2***	***SET 3***
Purity of potassium hydrogen phthalate	*99.96*		
Theoretical molarity	*0.1*		
Weight of potassium hydrogen phthalate (g)	*0.3505*	*0.3508*	*0.3502*
Blank (ml)	*0.2*	*0.2*	*0.2*
Volume consumed (ml)	*17.2*	*17.2*	*17.1*
Factor	*0.02042*		
Calculated molarity	*0.101*	*0.101*	*0.101*
Relative standard deviation	*0.0*		
Molarity (Average)	*0.101*		

$$\text{Molarity} = \frac{\text{Wt. of potassium hydrogen pthalate} \times \text{Theoretical molarity} \times \text{Purity of potassium hydrogen pthalate}}{(\text{Volume consumed} - \text{Blank}) \times \text{Factor} \times 100}$$

$$\text{Molarity (Set-1)} = \frac{0.3505 \times 0.1 \times 99.96}{(17.2 - 0.2) \times 0.02042 \times 100}$$

$$= 0.101$$

Similarly calculate for Set-2 and Set-3. Final molarity is the average all three sets.
The standardised Perchloric acid is used for assay of Albendazole.

Albendazole Assay Calculation

Excel calculation format for Assay by titration

	SET 1	SET 2
Theoretical Molarity	0.1	
Calculated Molarity	0.101	
Weight of sample(g)	0.5009	0.5006
Blank (ml)	0.2	0.2
Volume consumed (ml)	18.8	18.8
Factor	0.02653	
Assay% w/w(on as is basis)	99.49	99.48
Loss on Drying (LOD) %	0.12	
Assay (%w/w)(on dried basis)	99.62	99.68
Mean	99.65	

Calculation Formula:

$$\text{Assay (\%w/w) (on as is basis)} = \frac{\text{(Volume consumed – Blank) x Calculated molarity x Factor x 100}}{\text{Weight of sample x Theoretical molarity}}$$

$$\text{Assay (\%w/w) (SET-1)} = \frac{(18.8 - 0.2) \times 0.101 \times 0.02653 \times 100}{0.5009 \times 0.1}$$

$$= 99.50$$

$$\text{Assay (\%w/w) (as dried/anhydrous basis)} = \frac{\text{Assay \%w/w (on as is basis) x 100}}{\text{(100 – Water content/Loss on drying)}}$$

$$= \frac{99.50 \times 100}{(100 - 0.12)}$$

$$= 99.62$$

3. Water Determination by Karl Fischer Titration

Karl Fischer (KF) titration is a titration method for measuring water content in all types of substances. Standardisation of KF Reagent is performed by using disodium tartrate. The water equivalence factor F, in mg of water per ml of reagent, is given by the formula:

$$F = \frac{W \times 2\ (18.02)}{V \times 230.08}$$

or

$$F = \frac{W \times 0.1566}{V}$$

Where, 36.04 is two times the molecular weight of water and 230.08 is the molecular weight of sodium tartrate dihydrate. W is the weight in mg of sodium tartrate dihydrate and V is the volume in ml of the reagent consumed in the titration.

Illustration: Cefaclor (IP 2018)

Water (2.3.43). *3.0 to 6.5 per cent, determined on 0.2 g.*

Excel calculation format for Water Determination

	SET 1
KF Reagent Factor (mg/ml)	5.01
Weight of sample (g)	0.2016
Volume of KF reagent consumed (ml)	1.65
Water Content (% w/w)	4.10

Calculation Formula:

$$\text{Water content (\%w/w)} = \frac{\text{Volume of KF reagent consumed by test x Factor x 100}}{\text{Weight of sample x 1000}}$$

$$= \frac{1.65 \times 5.01 \times 100}{0.2016 \times 1000}$$

$$= 4.10$$

4. Loss on Drying

Loss on drying is the loss of weight expressed as percent w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions.

Illustration: Levosalbutamol Sulphate (IP 2018)

Loss on Drying (2.4.19). *Not more than 2.0 per cent, determined on 1.0 g at 105°.*

Excel calculation format for LOD

	SET 1
Weight of empty bottle (W1) (g)	*53.42917*
Weight of bottle + Weight of sample before drying (W2) (g)	*54.42919*
Weight of sample (g)	*1.00002*
Weight of bottle + Weight of sample after drying) (W3) (g)	*54.42010*
Loss on Drying (LOD) (%w/w)	*0.91*

Calculation Formula:

$$LOD\ (\%w/w) = \frac{(W2 - W3) \times 100}{(W2 - W1)}$$

$$= \frac{(54.42919 - 54.42010) \times 100}{(54.42919 - 53.42917)}$$

$$= \frac{0.00909 \times 100}{1.00002}$$

$$= 0.91$$

Note: Drying and ignition to constant weight. Two consecutive weighing after the drying or igniting operation shall not differ by more than 0.5 mg.

5. Sulphated Ash

The sulfated ash test utilizes a procedure to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulfuric acid. The test is usually used for determining the content of inorganic impurities in an organic substance.

Illustration: Levosalbutamol Sulphate (IP 2018)

Sulphated Ash (2.3.18). *Not more than 0.1 per cent.*

Excel calculation format for Sulphated Ash

	SET 1
Weight of empty crucible (W1) (g)	*27.17329*
Weight of crucible + Test weight before ignition (W2) (g)	*28.17364*
Test weight (g)	*1.00035*
Weight of crucible + Weight of sample after ignition (W3) (g)	*27.17374*
Sulphated Ash (%w/w)	*0.05*

Calculation Formula:

$$Sulphated\ ash\ (\%w/w) = \frac{(W3 - W1) \times 100}{(W2 - W1)}$$

$$= \frac{(27.17374 - 27.17329) \times 100}{(28.17364 - 27.17329)}$$

$$= \frac{0.00045 \times 100}{1.00035}$$

$$= 0.05$$

Note: Drying and ignition to constant weight. Two consecutive weighing after the drying or igniting operation shall not differ by more than 0.5 mg.

6. Specific Optical Rotation (SOR)

Optical rotation, 'α' is the property shown by certain substances of rotating the plane of polarisation of polarised light. Such substances are said to be optically active. This property is characteristic of some crystals and of many pharmaceutical liquids or solutions of solids.

For liquids

$$\text{Specific Optical Rotation } [\alpha]_D^{25} = \alpha / ld^{25}$$

For solids

$$\text{Specific Optical Rotation } [\alpha]_D^{25} = 100\alpha / lc$$

Where,

α = corrected observed rotation, in degrees, at 25º

D = D line of sodium light (l = 589.3 nm)

l = length of polarimeter tube in dm

d^{25} = specific gravity of liquid or solution at 25º

c = concentration of solution in %w/v

Illustration: Levosalbutamol Sulphate (IP 2018)

Specific optical rotation (2.4.22): *-30º to -40º, determined on 1.0 per cent w/v solution.*

Excel calculation format for SOR

	SET 1
Test Weight (g)	*1.00032*
Test dilution (ml)	*100*
Test concentration (%w/v)	*1.00032*
Observed angle of rotation (°)	*-0.35*
Loss on Drying (%w/w)	*0.93*
Specific Optical Rotation (°) (on as is basis)	*-34.99*
Specific Optical Rotation (°) (on dried basis)	*-35.32*

Calculation Formula:

$$\text{SOR (°) (on as is basis)} = \frac{\text{Observed angle of rotation} \times 100}{l\ (dm) \times \text{Test conc. (\%w/v)}}$$

$$= \frac{-0.35 \times 100}{1 \times 1.00032}$$

$$= -34.99$$

$$\text{SOR (°) (on dried basis)} = \frac{\text{SOR (on as is basis)} \times 100}{(100 - \text{LOD/Water content})}$$

$$= \frac{34.99 \times 100}{(100 - 0.93)}$$

$$= -35.32$$

7. Ultra-Violet Spectrophotometer

Illustration: Triamcinolone (IP 2018)

Assay: *Dissolve 25 mg in sufficient ethanol (95 percent) to produce 100.0 ml and mix. Dilute 2.0 ml to 50.0 ml with ethanol (95 percent) and measure the absorbance of the resulting solution at the maximum at about 238 nm* ***(2.4.7)****.*

Calculate the content of $C_{21}H_{27}FO_6$ taking 389 as the specific absorbance at 238 nm

Calculations

a) Using Specific Absorbance

Excel calculation format for Assay by UV using specific absorbance

Specific Absorbance	*389*
	SET 1
Test weight (g)	*0.02517*
Test dilution (ml)	*0.02517 g/100 ml→2 ml/50 ml*
Test absorbance	*0.390*
Assay (%w/w) (on as is basis)	*99.58*
Water content/LOD (%w/w)	*0.37*
Assay (% w/w) (on dried /anhydrous basis)	*99.95*

Calculation Formula:

$$\text{Assay (\%w/w) (as is basis)} = \frac{\text{Test absorbance}}{\text{Specific absorbance}} \times \frac{1}{100} \times \frac{\text{Test dilution}}{\text{Test wt. (g)}} \times 100$$

$$= \frac{0.390}{389} \times \frac{1}{100} \times \frac{100 \times 50}{0.02517 \times 2} \times 100$$

$$= 99.58$$

$$\text{Assay (\%w/w) (on dried/anhydrous basis)} = \frac{\text{Assay \% w/w (on as is basis)}}{(100 - \text{Water content/LOD})} \times 100$$

$$= \frac{99.58}{(100 - 0.37)} \times 100$$

$$= 99.95$$

b) Using Standard Substance

Note: Standard and test solutions are prepared for similar concentrations.

Excel calculation format for Assay by UV using standard

	SET 1
Purity of Standard	*99.8*
Standard Weight (g)	*0.02500*
Standard dilution (ml)	*0.02500 g/100 ml →2 ml/50 ml*
Test weight (g)	*0.02517*
Test dilution (ml)	*0.02517 g/100 ml→2 ml/50 ml*
Standard Absorbance	*0.389*
Test Absorbance	*0.390*
Assay(%w/w) (on as is basis)	*99.38*
Water content/LOD (%w/w)	*0.37*
Assay (%w/w) (on dried /anhydrous basis)	*99.75*

Calculation Formula:

$$\text{Assay (\%w/w) (as is basis)} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \frac{\text{Standard wt.(g)}}{\text{Standard dilution}} \times \frac{\text{Test dilution}}{\text{Test wt. (g)}} \times \text{Purity of Std.}$$

$$= \frac{0.390}{0.389} \times \frac{0.02500 \times 2}{100 \times 50} \times \frac{100 \times 50}{0.02517 \times 2} \times 99.8$$

$$= 99.38$$

$$\text{Assay (\%w/w) (on dried/anhydrous basis)} = \frac{\text{Assay \% w/w (on as is basis)}}{(100 - \text{Water content/LOD})} \times 100$$

$$= \frac{99.38}{(100 - 0.37)} \times 100$$

$$= 99.75$$

8. High Performance Liquid Chromatography (HPLC)

Assay by HPLC involves estimation of the content of active substance present in sample with respect to standard of known purity.

Proposed sequence arrangement in assay:

a) Blank
b) Standard preparation (5 injections)
c) Sample preparation (2 injections)
d) Standard preparation (bracketing)

Note: System suitability parameters such as asymmetry, theoretical plates, RSD, resolution or any other parameter shall comply as per monograph.

a) Calculation of Assay for API

Illustration: Ethionamide (IP 2018)

Ethionamide contains not less than 98.5 per cent and not more than 101.0 per cent of ethionamide ($C_8H_{10}N_2S$) calculated on dried basis.

Assay. Determine by liquid chromatography (2.4.14)

Reference Solution Preparation (Standard): Dissolve 50 mg of the ethionamide RS in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Test Solution Preparation: Dissolve 50 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Calculation of assay for active pharmaceutical ingredient (API).

Excel calculation format for API Assay by HPLC

Standard	*Ethionamide*
Purity of Standard	*99.50*
Standard weight (g)	*0.05055*
Dilution	*0.05055 g/100 ml→5 ml/50 ml*
No of injection	***Area***
1	*2953606*
2	*2921057*
3	*2920293*
4	*2936718*
5	*2928947*
Mean	*2932124*
Std. dev.	*13740*
RSD	*0.47*

Test (Ethionamide API)	
Test weight(g)	*0.05075*
Dilution	*0.05075 g/100 ml→5 ml/50 ml*
No of injection	***Area***
1	*2929104*
2	*2929463*
Mean	*2929284*
Assay (mg) as is basis	*99.01*
LOD (%w/w)	*0.12*
Assay (%w/w) on dried basis	*99.13*

Calculation Formula:

$$\text{Assay (\%w/w) (on as is basis)} = \frac{\text{Test area x Standard wt. x Test dilution x Purity of standard}}{\text{Standard area x Standard dilution x Test wt.}}$$

$$= \frac{2929284 \times 0.05055 \times 5 \times 100 \times 99.50}{2932124 \times 100 \times 50 \times 0.05075 \times 5}$$

$$= 99.01$$

$$\text{Assay (\% w/w) (on dried/anhydrous basis)} = \frac{\text{Assay \% w/w (on as is basis)}}{(100 - \text{Water content/LOD})} \times 100$$

$$= \frac{99.01}{(100 - 0.12)} \times 100$$

$$= 99.13$$

b) Calculation of Assay for Tablets/Capsules

Illustration: Ethionamide Tablets

Ethionamide tablets contain not less than 95.0 per cent and not more than 105.0 per cent of Ethionamide.

Assay. Determine by liquid chromatography (2.4.14)

Reference Solution Preparation (Standard): Dissolve 50 mg of the Ethionamide RS in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Test Solution Preparation: Weigh and powder 20 tablets. Weigh a quantity of the powder containing 50 mg of Ethionamide in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Excel calculation format for tablet/capsule assay by HPLC

Standard Ethionamide	
Potency of Standard	*99.50*
Standard weight (g)	*0.05055*
Dilution	*0.05055 g/100 ml → 5 ml/50 ml*
No of injection	***Area***
1	*2953606*
2	*2921057*
3	*2920293*
4	*2936718*
5	*2928947*
Mean	*2932124*
Std. dev.	*13740*
RSD	*0.47*

Test (Ethionamide Tablets 250 mg)	
Label Claim (mg)	250
Weight of sample (g)	0.05875
Average weight (g)	0.295
Dilution	0.05875 g/100 ml → 5 ml/50 ml
No of injection	**Area**
1	2901134
2	2897463
Mean	2899299
Assay (mg)	249.73
Assay (%) of label claim	99.89

Calculation Formula:

$$\text{Assay (mg/Tab) (on as is basis)} = \frac{\text{Test area x Standard wt. x Test dilution x Purity of standard x Average wt.}}{\text{Standard area x Standard dilution x Test wt. x 100}}$$

$$= \frac{2899299 \times 0.05055 \times 5 \times 100 \times 50 \times 99.50 \times 0.295 \times 1000}{2932124 \times 100 \times 50 \times 0.05875 \times 5 \times 100}$$

$$= 249.73$$

$$\text{Assay label claim (\%) (on as is basis)} = \frac{\text{Assay (mg) (on as is basis)}}{\text{Label claim of formulation (mg)}} \times 100$$

$$= \frac{249.73}{250} \times 100$$

$$= 99.89$$

c) Calculation of Assay for Suspension/Syrup

Illustration: Trimethoprim and Sulphamethoxazole Oral Suspension (IP 2018)

Trimethoprim and Sulphamethoxazole Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of Trimethoprim, $C_{14}H_{18}N_4O_3$, and Sulphamethoxazole, $C_{10}H_{11}N_3O_3S$.

Assay. Determine by Liquid Chromatography (2.4.14)

Test Solution: Dilute a volume of oral suspension containing 80 mg of sulphamethoxazole with 30 ml of methanol and sonicate for 10 minutes and dilute to 50.0 ml with methanol and filter. Dilute a volume of filtrate to obtain a solution containing 0.016 %w/v of sulphamethoxazole with the mobile phase.

Reference Solution: Dilute 5.0 ml of a solution containing 0.032 %w/v of trimethoprim RS and 0.16 %w/v of sulphamethoxazole RS in methanol to 50.0 ml with mobile phase.

Excel calculation format for suspension/ syrup assay by HPLC

Standard		
	Trimethoprim	Sulphamethoxazole
Potency of Standard	99.70	99.60
Standard weight(g)	0.03248	0.08026
Dilution	0.03248 g/100 ml → 5 ml/50 ml	0.08026 g/50 ml → 5 ml/50 ml
No of injection	**Area**	**Area**
1	738407	10200012
2	738399	10195088
3	736941	10202988
4	735672	10191089
5	740302	10191110
Mean	737944	10196057
Std. dev.	1743	5333
RSD	0.24	0.05

Test (Cotrimoxazole Suspension)		
Co-Trimoxazole 40 mg/200 mg per 5 ml Paediatric Suspension		
	Trimethoprim	*Sulphamethoxazole*
Label Claim (mg/5 ml)	*40*	*200*
mg/ml as per label claim	*8*	*40*
Weight of sample (g)	*2.06590*	
Weight per ml (g/ml)	*1.03295*	
Dilution	*2.06590 g/50 ml → 5 ml/50 ml*	
No of injection	***Area***	***Area***
1	*717177*	*10161098*
2	*719000*	*10133215*
Mean	*718089*	*10147157*
Assay (mg/ml)	*7.88*	*39.78*
Assay (mg/5 ml)	*39.40*	*198.90*
Assay (%) of label claim	*98.50*	*99.45*

Calculation Formula:

$$\text{Assay (mg/ml) (on as is basis)} = \frac{\text{Test area} \times \text{Standard wt.} \times \text{Test dilution} \times \text{Purity of standard} \times W \times \text{Average wt.}}{\text{Standard area} \times \text{Standard dilution} \times \text{Test wt.} \times 100}$$

Where W = weight (g) per ml

$$\text{Trimethoprim (mg/ml) (on as is basis)} = \frac{718089 \times 0.03248 \times 5 \times 50 \times 50 \times 99.70 \times 1.03295 \times 1000}{737944 \times 100 \times 50 \times 2.06590 \times 5 \times 100}$$

$$= 7.88$$

$$\text{Trimethoprim (mg/5 ml) (on as is basis)} = 7.88 \times 5 = 39.40$$

$$\text{Assay label claim (\%)(on as is basis)} = \frac{\text{Assay (mg/5 ml) (on as is basis)}}{\text{Label claim of formulation (mg/5 ml)}} \times 100$$

$$= \frac{39.40}{40} \times 100$$

$$= 98.50$$

$$\text{Sulphamethoxazole (mg/ml) (on as is basis)} = \frac{10147157 \times 0.08026 \times 5 \times 50 \times 50 \times 99.60 \times 1.03295 \times 1000}{10196057 \times 50 \times 50 \times 2.06590 \times 5 \times 100}$$

$$= 39.78$$

$$\text{Sulphamethoxazole (mg/5 ml) (on as is basis)} = 39.78 \times 5 = 198.90$$

$$\text{Assay label claim (\%)(on as is basis)} = \frac{\text{Assay (mg/5 ml) (on as is basis)}}{\text{Label claim of formulation (mg/5 ml)}} \times 100$$

$$= \frac{198.90}{200} \times 100$$

$$= 99.45$$

9. Dissolution

Dissolution testing is used to provide drug release information for both quality control purposes and drug development.

Preparation of solutions (according to monograph):

a) Blank preparation

b) Reference solutions/ working standard: working standard in diluents/mobile phase (concentration of working standard in ppm should be similar with concentration of test in ppm)

c) Test solution: 1 tablet/capsule in 250 ml/500 ml/900 ml of dissolution medium

Calculation for Tablet/Capsule

a) Evaluation using UV-Visible Spectrophotometer

Illustration: Ethionamide Tablets (IP 2018)

Dissolution (2.5.2)

Apparatus No.2,
Medium. 900 ml of 0.1M hydrochloric acid
Speed and Time: 100 rpm and 45 minutes
Withdraw a suitable volume of the medium, filter and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 274 nm (2.7.4). Calculate the content of $C_8H_{10}N_2S$ from the absorbance of a solution of known concentration of ethionamide RS.
D. Not less than 75 per cent of the stated amount of $C_8H_{10}N_2S$

Excel calculation format for dissolution (UV)

Standard	*Ethionamide*		
Purity of Standard	*99.50*		
Standard weight (g)	*0.02782*		
Dilution preparation	*0.02782 g/100 ml→2 ml/50 ml (11.13 ppm)*		
Standard Absorbance	*0.462*		
Test	*Ethionamide Tablets 250 mg*		
Label claim (mg)	*250*		
Dilution	*1Tablet/900 ml →2 ml/50 ml (11.11 ppm)*		
No. of Tablet	*Absorbance*	*Content (mg/tab)*	*Content%*
Tablet-1	*0.457*	*246.43*	*98.57*
Tablet-2	*0.453*	*244.27*	*97.71*
Tablet-3	*0.448*	*241.58*	*96.63*
Tablet-4	*0.460*	*248.05*	*99.22*
Tablet-5	*0.458*	*246.97*	*98.79*
Tablet-6	*0.453*	*244.27*	*97.71*
Max Dissolution (%)	*99.22*		
Min Dissolution (%)	*96.63*		
Mean Dissolution (%)	*98.11*		

Calculation Formula:

$$\text{Content (mg/Tab)} = \frac{\text{Test absorbance x Standard wt. x Volume of dissolution medium x Dilution x Purity of standard x 1000}}{\text{Standard absorbance x Standard dilution x 1 Tablet/Capsule x 100}}$$

$$\text{Content (mg/Tab)} = \frac{0.457 \times 0.02782 \times 2 \times 900 \times 50 \times 99.50 \times 1000}{0.462 \times 100 \times 50\ 1 \times 2 \times 100}$$

$$= 246.43$$

$$\text{Content (\%)} = \frac{\text{Content (mg/Tab) x 100}}{\text{Label claim (mg)}}$$

$$= \frac{246.43 \times 100}{250}$$

$$= 98.57$$

b) Evaluation using HPLC

If evaluation is to be performed using HPLC, proceed as per assay calculation by HPLC using the following calculation formula:

$$\text{Content (mg/Tab)} = \frac{\text{Test area x Standard wt.x Volume of dissolution medium x Dilution x Purity of standard x 1000}}{\text{Standard area x Standard dilution x 1 Tablet/Capsule x 100}}$$

$$\text{Content (\%)} = \frac{\text{Content (mg/Tab) x 100}}{\text{Label claim (mg)}}$$

c) Calculation for Controlled Release Tablet/Capsule

Evaluation using HPLC

Illustration: Norethisterone Acetate Controlled Release Tablet

Dissolution Apparatus: Paddle

Medium. 900 ml of 0.3%w/v solution of sodium lauryl sulphate

Speed: 50 rpm

Temperature: 36.5° to 37.5°

Time interval (n): 4^{th} hour, 8^{th} hour, 12^{th} hour and 16^{th} hour.

Standard Preparation: Weigh accurately about 75 mg of Norethisterone acetate standard in 100 ml volumetric flask, dissolve in methanol and makeup the volume with methanol. Dilute 2 ml of the resulting solution to 100 ml with dissolution medium, filter with 0.45 µm nylon filter.

Sample preparation: At specified time interval (4,8,12,16 hours) withdraw 10 ml of aliquot from a zone midway between surface of dissolution medium and top of rotating blade not less than 1cm from the vessel wall and filter with 0.45 µm nylon filter. Replace aliquot drawn with 10 ml of fresh dissolution medium to make up the dissolution volume to 900 ml.

Evaluation is performed using HPLC

Acceptance Criteria:

4^{th} hour 10-40%

8^{th} hour 30-60%

12^{th} hour 50-80%

16^{th} hour Not less than 70% of the labelled amount

Calculation

Drug release $D_{(n)}$ in % at time point n

$$= \frac{\text{Test area x Standard wt. x Volume of dissolution medium x Dilution x Purity of standard x 1000 x 100}}{\text{Standard area x Standard dilution x 1 Tablet/Capsule x 100 x Label claim}}$$

Calculation for correction factors

*% Content present in sample volume after 8 hour C1= (D1/900)*10*

*% Content present in sample volume after 12 hour C2= (D2/900)*10*

*% Content present in sample volume after 16 hour C3= (D3/900)*10*

Calculation of corrected results

Calculate cumulative % content release by applying correction factor for the amount of sample withdrawn as shown below.

For 4 hour = D1 (no correction factor required)

For 8 hour = D2 + C1

For 12 hour = D3 + C1+ C2

For 16 hour = D4 + C1+ C2 +C3

**Similarly, if there are more time intervals for sample withdrawal, calculate correction factors at respective interval and keep adding to %content value along with previously calculated correction factors.*

No correction factor is to be added at the first sample withdrawal time interval.

Excel calculation format for dissolution (HPLC) at end of 4 hours

Standard Norethisterone Acetate	
Purity of Standard	*99.11*
Standard weight (g)	*0.07510*
Dilution	*0.07510 g/100 ml → 2 ml/100 ml*
No of injection	***Area***
1	*862189*
2	*864458*
3	*861171*
4	*890038*
5	*863136*
Mean	*868198*
Std. dev.	*12269*
RSD	*1.41*

Test	*Norethisterone Acetate Controlled Release Tablet*	
Label claim (mg)	*15*	
Dilution	*1 Tablet/900 ml*	
Time Interval	*At end of 4 hours*	
No. of Tablet	*Area (mAU)*	*% Content (D1)*
Tablet-1	*156174*	*16.07*
Tablet-2	*176213*	*18.13*
Tablet-3	*236372*	*24.32*
Tablet-4	*155945*	*16.04*
Tablet-5	*182967*	*18.82*
Tablet-6	*267936*	*27.56*
Content (Max)		*27.56*
Content (Min)		*16.04*
Content (Avg)		*20.16*

At end of 4 hours

Content (mg/tab) for Tablet-1

$$D1 = \frac{156174 \times 0.07510 \times 2 \times 900 \times 99.11 \times 1000 \times 100}{868198 \times 100 \times 100 \times 1 \times 100 \times 15}$$

$$= 16.07$$

Excel calculation format for dissolution (HPLC) at end of 8 hours

Test	*Norethisterone Acetate Controlled Release Tablet*			
Label claim (mg)	*15*			
Dilution	*1 Tablet/900 ml*			
Time Interval	*At end of 8 hours*			
No. of Tablet	*Area(mAU)*	*CF 4 hours C1=(D1/900)x10*	*% Content (D2)*	*%Cumulative content (D2+C1)*
Tablet-1	*345396*	*0.18*	*35.53*	*35.71*
Tablet-2	*375841*	*0.20*	*38.67*	*38.87*
Tablet-3	*491364*	*0.27*	*50.55*	*50.82*
Tablet-4	*358491*	*0.18*	*36.88*	*37.06*
Tablet-5	*376751*	*0.21*	*38.76*	*38.97*
Tablet-6	*539816*	*0.31*	*55.53*	*55.84*
Content (Max)			*55.53*	*55.84*
Content (Min)			*35.53*	*35.71*
Content (Avg)			*42.65*	*42.88*

At end of 8 hours

% Content for Tablet-1

$$D2 = \frac{345396 \times 0.07510 \times 2 \times 900 \times 99.11 \times 1000 \times 100}{968198 \times 100 \times 100 \times 1 \times 100 \times 15}$$

$$= 35.53$$

Correction Factor-C1 $= \frac{16.07 \times 10}{900} = 0.18$

%Cumulative content (D2+C1) = 35.53 + 0.18 = 35.71

Excel calculation format for dissolution (HPLC) at end of 12 hours

Test	*Norethisterone Acetate Controlled Release Tablet*				
Label claim (mg)	*15*				
Dilution	*1 Tablet/900 ml*				
Time Interval	*At end of 12 hours*				
No. of Tablet	*Area (mAU)*	*CF 4 hours C1=(D1/900)x10*	*CF 8 hours C2=(D2/900)x10*	*% Content (D3)*	*%Cumulative content (D3+C1+C2)*
Tablet-1	*765980*	*0.18*	*0.39*	*78.80*	*79.37*
Tablet-2	*772611*	*0.20*	*0.43*	*79.48*	*80.11*
Tablet-3	*767577*	*0.27*	*0.56*	*78.97*	*79.80*
Tablet-4	*762834*	*0.18*	*0.41*	*78.48*	*79.07*
Tablet-5	*775233*	*0.21*	*0.43*	*79.75*	*80.39*
Tablet-6	*772950*	*0.31*	*0.62*	*79.52*	*80.45*
Content (Max)				*79.75*	*80.45*
Content (Min)				*78.48*	*79.07*
Content (Avg)				*79.17*	*79.87*

At end of 12 hours

% Content for Tablet-1

$$D3 = \frac{765980 \times 0.07510 \times 2 \times 900 \times 99.11 \times 1000 \times 100}{968198 \times 100 \times 100 \times 1 \times 100 \times 15}$$

$$= 78.80$$

Correction Factor-C2 $= \frac{35.53 \times 10}{900} = 0.39$

%Cumulative content (D3+C1+C2) = 78.80 + 0.18 + 0.39 = 79.37

Excel calculation format for dissolution (HPLC) at end of 16 hours

Test	*Norethisterone Acetate Controlled Release Tablet*					
Label claim (mg)	*15*					
Dilution	*1 Tablet/900 ml*					
Time Interval	*At end of 16 hours*					
No. of Tablet	*Area (mAU)*	*CF 4 hours (C1) =(D1/900)x10*	*CF 8 hours (C2) =(D2/900)x10*	*CF 12hours (C3) =(D3/900)x10*	*% Content (D4)*	*%Cumulative content (D4+C1+C2+C3)*
Tablet-1	*715965*	*0.18*	*0.39*	*0.88*	*73.66*	*75.11*
Tablet-2	*754563*	*0.20*	*0.43*	*0.88*	*77.63*	*79.14*
Tablet-3	*867352*	*0.27*	*0.56*	*0.88*	*89.23*	*90.94*
Tablet-4	*726037*	*0.18*	*0.41*	*0.87*	*74.69*	*76.15*
Tablet-5	*931514*	*0.21*	*0.43*	*0.89*	*95.83*	*97.36*
Tablet-6	*917159*	*0.31*	*0.62*	*0.88*	*94.35*	*96.16*
Content (Max)					*95.83*	*97.36*
Content (Min)					*73.66*	*75.11*
Content (Avg)					*84.23*	*85.81*

At end of 16 hours

% Content for Tablet-1

$$D4 = \frac{715965 \times 0.07510 \times 2 \times 900 \times 99.11 \times 1000 \times 100}{868198 \times 100 \times 100 \times 1 \times 100 \times 15}$$

$$= 73.66$$

$$\text{Correction Factor-C2} = \frac{78.80 \times 10}{900} = 0.88$$

%Cumulative content (D4+C1+C2+C3)= 73.66 + 0.18 + 0.39 + 0.88 = 75.11

10. Uniformity of Content by HPLC

The test for uniformity of content of single dose preparations is based on the assay of the individual contents of active substance(s) of a number of single-dose units to determine whether the individual contents are within limits set with reference to the average content of the sample. This test is applicable to tablets/capsules containing 10 mg or less than 10 mg or less than 10 per cent w/w of active ingredient. This test is not applicable to tablets/capsules containing multivitamins and trace elements.

Acceptance Limits: Uniformity of Content of Single-Dose Preparations IP 2018(2.5.4).

Preparation of solutions (according to monograph):

a) Blank preparation

b) Reference Solutions/ Working standard: working standard in diluents/mobile phase

c) Test solution: 1tablet/capsule in diluents

Sequence arrangement:

(i) Blank
(ii) Reference solution/Standard preparation (5 injections)
(iii) Sample preparation 1
(iv) Sample preparation 2
(v) Sample preparation 3
(vi) Sample preparation 4
(vii) Sample preparation 5
(viii) Sample preparation 6
(ix) Sample preparation 7
(x) Sample preparation 8
(xi) Sample preparation 9
(xii) Sample preparation 10
(xiii) Reference solution (bracketing)

System suitability observation such as Asymmetry, Theoretical plates, and RSD or as per method/monograph.

Calculation for uniformity of content in tablet/capsule (Formulations)

$$\text{Content (mg/Tab)} = \frac{\text{Test area} \times \text{Standard wt.} \times \text{Dilution} \times \text{Purity of standard} \times 1000}{\text{Standard area} \times \text{Standard dilution} \times 1\ \text{Tablet/Capsule} \times 100}$$

$$\text{Content (\%)} = \frac{\text{Content (mg/Tab)} \times 100}{\text{Average content (mg/Tab)}}$$

Calculation for tablet/capsule (Formulations) with API used as salt

$$\text{Content (mg/Tab)} = \frac{\text{Test area} \times \text{Standard wt.} \times \text{Dilution} \times \text{Purity of standard} \times 1000 \times \text{Factor}}{\text{Standard area} \times \text{Standard dilution} \times 1\ \text{Tablet/Capsule} \times 100}$$

$$\text{Content (\%)} = \frac{\text{Content (mg/Tab)} \times 100}{\text{Average content (mg/Tab)}}$$

$$\text{Factor (F)} = \frac{\text{Mol. Wt. of API}}{\text{Mol. Wt. of API (salt form)}}$$

Illustration: Primaquine Tablets

Primaquine Phosphate tablets

Statement of content: Primaquine Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of Primaquine $C_{15}H_{21}N_3O$.

Usual strength. 7.5 mg, 15 mg.

(13 mg of Primaquine Phosphate is approximately equivalent to 7.5 mg of primaquine).

Determine by liquid chromatography (2.4.14), as described in the Assay, using the following solutions.

Test solution. Powder one tablet, dissolve in 20.0 ml of the mobile phase with the aid of ultrasound for 3 minutes and dilute to 50.0 ml with the mobile phase and filter.

Reference solution. Dissolve a weighed quantity of primaquine phosphate RS in the mobile phase and dilute with the mobile phase to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Inject the reference solution and the test solution. Calculate the content of $C_{15}H_{21}N_3O$ in the tablet.

Excel calculation format for Uniformity of content

Standard Primaquine phosphate	
Purity of Standard	*99.50*
Standard weight (g)	*0.02661*
Dilution	*0.02661 g/100 ml*
No of injection	***Area***
1	*88.643*
2	*87.815*
3	*87.902*
4	*87.103*
5	*85.780*
Mean	*87.449*
Std. dev.	*1.08*
RSD	*1.24*

Test	*Primaquine Phosphate eq. to primaquine*		
Label claim (mg)	*7.5*		
Dilution	*1 Tablet/50 ml*		
No. of Tablet	*Area*	*Content (mg/tab)*	*Content%*
Tablet-1	*80.362*	*6.93*	*94.29*
Tablet-2	*84.869*	*7.32*	*99.59*
Tablet-3	*87.334*	*7.53*	*102.45*
Tablet-4	*84.333*	*7.27*	*98.91*
Tablet-5	*92.258*	*7.95*	*108.16*
Tablet-6	*84.255*	*7.26*	*98.78*
Tablet-7	*90.691*	*7.82*	*106.39*
Tablet-8	*81.814*	*7.05*	*95.92*
Tablet-9	*82.698*	*7.13*	*97.01*
Tablet-10	*83.597*	*7.21*	*98.10*
AverageContent (mg/tab)	*85.221*	*7.35*	*99.96*
Minimum		*6.93*	*94.29*
Maximum		*7.95*	*108.16*

$$\text{Content (mg/Tab)} = \frac{\text{Test area x Standard wt. x Dilution x Purity of standard x 1000 x Factor}}{\text{Standard area x Standard dilution x 1 Tablet/Capsule x 100}}$$

$$\text{Factor (F)} = \frac{\text{Mol. Wt. of Primaquine}}{\text{Mol. Wt. of Primaquine phosphate salt}}$$

Tablet-1 = $\frac{80.362 \times 0.02661 \times 50 \times 99.50 \times 1000 \times 259.347}{87.449 \times 100 \times 1 \times 455.340}$

= 6.93

Content (%) = $\frac{\text{Content (mg/Tab)} \times 100}{\text{Average content (mg/Tab)}}$

= $\frac{6.93 \times 100}{7.35}$

= 94.29

11. Residual Solvents by Gas Chromatography

Residual solvents are defined as organic volatile impurities that may remain in active pharmaceutical substances, excipients or medicinal products after processing. Depending upon the safety data and their risk to the human health, these solvents are classified as class I, class II and class III solvents.

Acceptance Limits: Residual Solvents (5.4)

Illustration: Residual Solvent Determination (Methanol, Acetonitrile, Dichloromethane)

Instrument: *The Gas Chromatograph equipped with flame-ionization detector and headspace sampler.*

Reagents: *Methanol, Acetonitrile, Dichloromethane, N, N-Dimethylformamide.*

Preparation of vial for blank

Pipette 5.0 ml of N,N-Dimethylformamide to a 20 ml headspace vial and close the vial immediately using suitable septa and screw/crimp cap.

Preparation of standard solution

Weigh accurately about 0.240 g of Methanol, 0.033 g of Acetonitrile, 0.048 g of Dichloromethaneto a 100 ml volumetric flask containing about 25 ml of N, N-Dimethylformamide and make up the volume with N, N-Dimethylformamide. Pipette 5.0 ml of this solution to a 100 ml volumetric flask and dilute to volume with N, N-Dimethylformamide.

(The final concentration of standard solution containing all the above solvents is in accordance with Class 2 solvents limit as mentioned in IP 2018 which is 3000 ppm of Methanol, 410 ppm of Acetonitrile, 600 ppm of Dichloromethane with respect to amount of test sample taken for residual solvent determination.)

Preparation of vial for standard

Pipette 5.0 ml of the standard solution to a 20 ml headspace vial and close the vial immediately using suitable septa and screw/crimp cap.

Preparation of vial for test sample

Transfer about 0.2 g of test sample, accurately weighed, to a 20 ml head space vial, add 5.0 ml of N,N-Dimethylformamide and close the vial immediately using PTFE septa and screw cap.

Preparation of system suitability solution

Use standard preparation as system suitability solution.

Evaluation of blank

Place the vial of blank and record the chromatogram. No interfering peak should be observed at the retention time of the analytes. If any interference peak observed in the blank, the area of peak should be subtracted from the area of corresponding peak observed in standard and sample preparation.

Procedure

Place two vials of blank and record the chromatograms.

Place six vials of standard and record the chromatograms.

Place two vials of test sample and record the chromatograms.

Place one vial of bracketing standard and record the chromatogram.

Elution order: Retention time to be confirmed from chromatogram of standard solution.

Note the area counts of the solvent peaks from the test chromatograms for calculation of residual solvents.

System suitability criteria:

The relative standard deviation of areas obtained for each solvent from six injections of standard preparation should not be more than 15.0%. [Note: RSD can be set at a value lower than 15% also]

The relative standard deviation of areas obtained for each solvent from six injections of standard preparation and bracketing standard should not be more than 15.0%. [Note: RSD can be set at a value lower than 15% also]
Note: Bracketing standard should be injected at the end of each new sample or end of sequence or after injecting ten injections of test preparations, whichever is earlier.

Calculate the content of each solvent by using the following formula:

$$\text{Solvent (ppm)} = \frac{\text{Test solvent area x Standard solvent wt. x Test Dilution x Purity of standard solvent x 10000}}{\text{Standard solvent area x Standard solvent dilution x Test wt.}}$$

The solvent content in sample is the average of the results obtained from the two headspace vials. Calculate the content of solvent for each sample preparation and report the average.

Solvent	Methanol	Acetonitrile (ACN)	Dichloromethane (DCM)
wt (g)	0.23811	0.03484	0.04730
Dilution	Weight taken/100 ml→ 5 ml/100 ml		
Purity of Standard	99.90	99.88	99.96
Std. Inj.	Area	Area	Area
1	3494	586	398
2	3591	569	393
3	3480	584	357
4	3502	557	346
5	3598	602	372
6	3586	576	349
AVG	3542	579	369
STDEV	55	15	22
% RSD	1.56	2.67	6.06

Test Sample	Test-1			Test-2		
Test sample weight (mg)	0.20171			0.20164		
Dilution	020171 g→ 5 ml			020164 g→ 5 ml		
Solvents	Methanol	ACN	DCM	Methanol	ACN	DCM
Area(mAU)	2150	200	195	2121	218	210
Residual solvent present (ppm)	1790	149	310	1766	162	333
Average Methanol content (ppm)	1788					
Average Acetonitrile content (ppm)	156					
Average Dichloromethane content (ppm)	321					

$$\text{Test-1 (Methanol) (ppm)} = \frac{2150 \times 0.23811 \times 5 \times 5 \times 99.90 \times 10000}{3542 \times 100 \times 100 \times 0.20171} = 1790$$

$$\text{Test-1 (Acetonitrile) (ppm)} = \frac{200 \times 0.03484 \times 5 \times 5 \times 99.88 \times 10000}{579 \times 100 \times 100 \times 0.20171} = 149$$

$$\text{Test-1 (Dichloromethane) (ppm)} = \frac{195 \times 0.04730 \times 5 \times 5 \times 99.96 \times 10000}{369 \times 100 \times 100 \times 0.20171} = 310$$

Similarly calculations are performed for Test-2. Average of both tests is taken.
Test-2 (Methanol) (ppm) = 1766
Test-2 (Acetonitrile) (ppm) = 162
Test-2 (Dichloromethane) (ppm) = 333
Average (Methanol) (ppm) = 1788
Average (Acetonitrile) (ppm) = 156
Average (Dichloromethane) (ppm) = 321

12. Related Substances

Test for related substances given in monographs covers manufacturing impurities (intermediates and by-products) and/or degradation products. In chromatographic determinations in the absence of a reference substance it is usual practice to limit the levels of impurities by the simple test of comparision of the unknown peak with a peak obtained with a dilute solution of the substance under examination. Acceptance criteria is set taking into account the qualification of the degradation products, accelerated and long term stability data, the expected expiry period and the recommended storage conditions for drug products.

Where for any reasons, data on quantification and qualification of impurities is not available, a workable criterion for acceptance could be find in general chapter of the Indian Pharmacopoeia.

To understand the how impurity limits are calculated considering test and standard/reference concentrations, refer following illustration.

Illustration: Anastrazole

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of acetonitrile and 50 volumes of water.

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system

- *a stainless steel column 25 cm x 4.6 mm, packed with phenyl group (5 µm),*
- *mobile phase: a mixture of 65 volumes of water, 35 volumes of acetonitrile, and 0.5 volume of ortho-phosphoric acid, adjust pH to 3.0 with 1 M sodium hydroxide,*
- *flow rate: 1 ml per minute,*
- *spectrophotometer set at 220 nm,*
- *injection volume: 20 µl*

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 percent). Ignore any peak with an area less than 0.1 times the area of the peak in the chromatogram obtained with the reference solution (0.1 per cent).

In the above Anastrazole illustration, the test concentration is 0.05% w/v (500 ppm), Reference solution concentration is 0.0005%w/v (5 ppm).

Calculations determining limit of Impurities

$$\% \text{ Impurity} = \frac{\text{Standard concentration} \times 100 \times \text{Factor (times the area of peak)}}{\text{Test concentration}}$$

Note: Same formula can be used to calculate factor(times the area) when it is not given in the monograph and only impurity limit is given.

Calculation for Determining 0.5 per cent ImpurityLimit (for Any Secondary Peak)

'In the chromatogram obtained with the test solution, area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent)'.

$$= \frac{0.0005 \times 100 \times 0.5 \text{ (times the area of principal peak)}}{0.05}$$

$$= 0.5\%$$

Calculation for Determining 1.0 per cent ImpurityLimit (Sum of Areas of All the Secondary Peaks)

'The sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 percent).'

$$= \frac{0.0005 \times 100 \times 1.0 \text{ (times the area of principal peak)}}{0.05}$$

$$= 1.0\%$$

Note: factor (times the area) is calculated by same formula used to calculate per cent impurity.

Calculation for Determining 0.1 per cent Impurity Limit

'Ignore any peak with an area less than 0.1 times the area of the peak in the chromatogram obtained with the reference solution (0.1 per cent).'

$$= \frac{0.0005 \times 100 \times 0.1 \text{ (times the area of principal peak)}}{0.05}$$

$$= 0.1\%$$

General Methods for Determination of Related Substances/Impurities (by HPLC)

(i) Using Different Concentrations of Test as Reference Solution

Illustration: *Example of this is found in the drug substance monograph of Telmisartan-Related Substances, IP-2018, page-3319.*

(ii) Using Different Concentrations of Test as Reference Solution and Relative Retention Time

Illustration: *Example of this is found in the drug substance monograph of Clozapine-Related Substances, IP-2018, page-1685.*

(iii) Using Different Concentrations of Test as Reference Solution, Relative Retention Time (RRT) and Correction Factor (CF) or Relative Response Factor (RRF)

Illustration 1: *Example of this is found in the drug substance monograph of Trimetazidine Hydrochloride - Related Substances, IP-2018, page-3437-3438.*

Illustration2: *Example of this is found in the drug dosage monograph of Mesalazine Prolonged-release Tablets -Related Substances IP-2018, page-2539.*

(iv) Using Different Concentrations of Reference Standard (RS) as Reference Solution, Relative Retention Time (RRT) and Correction Factor (CF) or Relative Response Factor (RRF)

Illustration: *Example of this is found in the drug dosage monograph of Terazosin Tablets -Related Substances IP-2018, page-3334-3335.*

Response Factor

Response factor (or relative response factor) expresses the sensitivity of a detector for a given substance relative to a standard substance i.e. the ratio of the detector response of the impurity to the detector response of the test substance at the same concentration.

Correction Factor

The correction factor given in monograph is the reciprocal value of the response factor and the area of the impurity peak in the chromatogram obtained with the test solution must be multiplied by this corrector factor.

Note: As per IP if correction factor is given in monograph it should be multiplied with the area (place it in numerator). If relative response factor (RRF) is given in monograph, divide area with RRF (place it in denominator).

(v) Using Impurity Reference Standard

Preparation of solutions:

- Blank preparation: according to monograph
- System suitability solution: In combination of specified impurity and working standard; or Impurity mixture in diluents according to monograph.
- Reference solution: According to monograph using impurity standard or API standard.
- Test solution: API or formulation in diluent/mobile phase according to monograph.

Sequence arrangement:

Note: Following this sequence is not mandatory, *in house* procedure may be used.

- Blank
- System suitability solution
- Reference solution/Standard preparation (5 injections)
- sample preparation 1
- sample preparation 2
- Standard preparation (bracketing)/ System suitability solution

System suitability observation such as Asymmetry, Theoretical plates, Resolution, and RSD shall be as per monograph.

For Active Pharmaceutical Ingredient

$$\text{Known Impurity (\%)} = \frac{\text{Area of known impurity in test solution} \times \text{Conc. of impurity standard} \times \text{Purity of impurity standard} \times CF}{\text{Area of known impurity standard} \times \text{Conc. of test solution}}$$

CF= Correction Factor (if given in monograph/method)

Illustration1: *This calculation formula can be used in the drug substance monograph of Gliclazide – Gliclazide Impurity-B.*

Illustration2: *This calculation formula can be used in the drug dosage monograph of Clotrimazole Cream – For Determination of 2-Cholrotritanol (Clotrimazole Impurity-A).*

$$\text{Unknown Impurity (\%)} = \frac{\text{Area of unknown impurity in test solution} \times \text{Conc. of API standard} \times \text{Purity of API standard}}{\text{Area of API standard} \times \text{Conc. of test solution}}$$

Note: Diluted API standard can be used to determine unknown impurities

Total Impurities = Summation of all the known and unknown impurities.

For Tablet/Capsule

$$\text{Impurity (\%)} = \frac{\text{Area of known impurity in test} \times \text{Conc. of standard (Imp/API)} \times \text{Purity of standard (Imp/API)} \times \text{Avg. wt.}}{\text{Area of standard (Imp/API)} \times \text{Conc. of test} \times \text{Label claim}}$$

Where Imp = Impurity

13. Bacterial Endotoxin Test (BET)

(i) Maximum Valid Dilution (MVD)

MVD is the maximum allowable dilution of a sample at which the endotoxin limit can be determined. MVD is calculated by following general formula:

$$\text{MVD} = \frac{\text{Endotoxin limit} \times \text{Concentration of the sample solution*}}{\lambda}$$

Where, λ is the labelled sensitivity of the lysate (EU/ml)

*Concentration of the sample solution is expressed as mg/ml in case the endotoxin limit is specified by weight (EU/mg), or as Units/ml in case the endotoxin limit is specified by unit (EU/unit), or as 1.0 ml/ml in case the endotoxin limit is specified by volume (EU/ml).

(ii) Endotoxin Limit

Endotoxin limit for a parenteral products define on the basis of dose (effect of endotoxins are related to the amount of endotoxin in the product dose administrated to a patient). As dose varies from product to product, the endotoxin limit for a product is calculated from the following expression:

Endotoxin Limit = K/M

Where,

K is the threshold pyrogenic dose of endotoxin per kg of body mass. The value of K is the 5.0 EU per kg for parenteral preparations except those administrated intrathecally, and is 0.2 EU per kg for preparations intended to be administrated intrathecally, and

M is the maximum dose administered to an adult (taken as 70 kg for this purpose). If a product is infused or injected to a patient at frequent intervals over an extended time, M is based on maximum total dose administered in a one hour period.

Illustration: *If an injection having maximum dose of 300 mg is administrated intramuscularly to an adult (taken as 70 kg for this purpose), then the endotoxin limit of this injection can be calculated as given below.*

$$\text{Endotoxin Limit} = \frac{5.0\ \text{EU/kg} \times 70\ \text{kg}}{300\ \text{mg}} = 1.16\ \text{EU/mg}$$

Note: *For radiopharmaceutical products not administrated intrathecally, the endotoxin limit is calculated as 175/V, where V is the maximum recommended dose in ml. For intrathecally administrated radiopharmaceuticals, the endotoxin limit is obtained by the formula 14/V. For formulations (anticancer products) administrated on a per square meter of body surface, the formula is K/M, where K= 2.5 EU per kg and M is the (maximum dose/m^2/hour/×1.80 m^2)/ 70 kg.*

For medical devices the endotoxin limit is calculated as K×N/V. Where, K is amount of endotoxin per device i.e. endotoxin limit NMT 20 EU/medical devices and NMT 2.15 EU per medical device for those medical devices in contact with the cerebrospinal fluid, N is equal to the number of devices tested and V is equal to the total volume of the extract or rinse medical devices.

(iii) BET by Gel Clot Method

Illustration: *If an injection has the concentration of 100 mg/ml and Endotoxin limit is 0.20 EU/mg. Lysate to be used in the test has the labelled sensitivity of 0.03 EU/ml. Then MVD will be calculated as given below:*

$$MVD = \frac{0.2\ \text{EU/mg} \times 100\ \text{mg/ml}}{0.03\ \text{EU/ml}} = 666.66 \approx 667$$

$$\frac{MVD}{4} = \frac{667}{4} = 166.75 \approx 167$$

Sample Dilution

0.03 ml sample + 4.98 ml of LRW ⟶ 1:167 (MVD/4)

Control Standard Endotoxin (CSE) Dilution

If potency of CSE after reconstitution is 20 EU/ml.

0.1 ml of CSE (20 EU/ml) + 1.9 ml of LRW ⟶ 1 EU/ml

0.12 ml of CSE (1 EU/ml) + 0.88 ml of LRW ⟶ 0.12EU/ml (4λ)

Test Procedure

Test	LRW	Product	CSE	Lysate
NWC	100 µl	-	-	100 µl
PWC	50 µl	-	50 µl (4λ)	100 µl
NPC	50 µl	50 µl (MVD/4)	-	100 µl
PPC	-	50 µl (MVD/4)	50 µl (4λ)	100 µl

Observation

Test	1st Tube	2nd Tube	Dilution of Sample Tested
NWC	-	-	N.A
PWC	+	+	N.A
NPC	-	-	MVD/2
PPC	+	+	MVD/2

-Denotes no Gel Clot Formation; + Denotes Gel Clot Formation

Results: Less than 0.20 EU/mg

(iv) BET by KTA Method

Control Standard Endotoxin (CSE) Dilution

If potency of CSE after reconstitution is 50 EU/ml.

0.1 ml of CSE (50 EU/ml) + 0.9 ml of LRW ⟶ 5 EU/ml

0.1 ml of CSE (5 EU/ml) + 0.9 ml of LRW ⟶ 0.5 EU/ml

0.1 ml of CSE (0.5 EU/ml) + 0.9 ml of LRW ⟶ 0.05 EU/ml (λ)

Lysate sensitivity is the lowest CSE concentration of the standard curve expressed in EU/ml.

Illustration: *If an injection has the concentration of 100 mg/ml and Endotoxin limit is 0.20 EU/mg. Then MVD will be calculated as given below:*

$$MVD = \frac{0.2\ EU/mg \times 100\ mg/ml}{0.05\ EU/ml}$$

$$= 400$$

$$\frac{MVD}{8} = \frac{400}{8} = 50$$

Sample Dilution

0.05 ml sample + 2.45 ml of LRW ⟶ 1:50 (MVD/8)

Test Procedure

Test	LRW	Product	CSE	Lysate
Std.1 (5 EU/ml)	-	-	100 µl	100 µl
Std.2 (0.5 EU/ml)	-	-	100 µl	100 µl
Std.3 (0.05 EU/ml)	-	-	100 µl	100 µl
NWC	100 µl	-	-	100 µl
NPC	-	100 µl (1:50)	-	100 µl
PPC	-	100 µl (1:50)	10 µl (5 EU/ml)	100 µl

Results: Endotoxin Content in Sample <0.0250 EU/mg

Spike Recovery 0.5465 EU/ml (99%)

These results are generated by automated software for KTA method.

14. Antibiotic Assay Calculation

(i) Cup-Plate Method

Illustration

Sample Name : Erythromycin
Test Organism Used : *Kocuria rhizophila* ATCC-9341
Assumed Potency of Ref. Std. : 974 units/mg (*as is* basis)
Weight of Ref. Std. : 10.4 mg
Weight of Sample : 10.6 mg

Standard Dilution $= \frac{10.4 \times 974}{10} = 1012.96$ units/ml or ≈ 1013 units/ml

10.4 mg of Ref. Std. + 1.0 ml methanol + 9.0 ml phosphate buffer → 1013 units/ml
1.0 ml of 1013 units/ml + 9.13 ml phosphate buffer pH 8.0 → 100 units/ml
1.0 ml of 100 units/ml + 9.0 ml phosphate buffer pH 8.0 → 10 units/ml
4.0 ml of 10 units/ml + 6.0 ml phosphate buffer pH 8.0 → **4 units/ml (S_H)**
1.0 ml of 10 units/ml + 9.0 ml phosphate buffer pH 8.0 → **1 units/ml (S_L)**

Sample Dilution $= \frac{10.6 \times 974}{10} = 1032.44$ units/ml or ≈ 1032 units/ml

10.6 mg Sample + 1.0 ml methanol + 9.0 ml phosphate buffer → 1032 units/ml
1.0 ml of 1032 units/ml + 9.32 ml phosphate buffer pH 8.0 → 100 units/ml
1.0 ml of 100 units/ml + 9.0 ml phosphate buffer pH 8.0 → 10 units/ml
4.0 ml of 10 units/ml + 6.0 ml phosphate buffer pH 8.0 → **4 units/ml (T_H)**
1.0 ml of 10 units/ml + 9.0 ml phosphate buffer pH 8.0 → **1 units/ml (T_L)**

Observations

Plate	Sample Zone Diameter (mm)		Standard Zone Diameter (mm)	
	T_H	T_L	S_H	S_L
1	25.3	21.3	25.4	21.3
2	25.0	21.2	25.3	21.2
3	25.1	21.2	25.1	21.4
4	25.2	21.1	25.4	21.5
Average	25.15	21.2	25.3	21.35

Calculation

% potency = antilog (2.0 ± a log I)
Where; I = ratio of dilution (4:1 = 4)

$$a = \frac{(T_H+T_L) - (S_H+S_L)}{(T_H-T_L) + (S_H-S_L)}$$

$$a = \frac{(25.15+21.2) - (25.3+21.35)}{(25.15-21.2) + (25.3-21.35)} = \frac{46.35 - 46.65}{3.95 + 3.95} = -\frac{0.3}{7.9} = -0.0380$$

log I = 4 = 0.6021

% of potency = antilog (2.0 − 0.0380×0.6021)
= antilog 1.9771 = 94.86

Potency of sample = $\frac{94.86 \times 974}{100}$ = 923.94 units/mg (*as is* basis)

Water content in sample = 3.42%

Potency of sample (*on anhydrous* basis) = $\frac{923.94 \times 100}{100-3.42}$ = 956.66 units/mg (*on anhydrous* basis)

(ii) Turbidimetric Method

Illustration: Amikacin sulphate (IP 2018)

Amikacin Sulphate having a molar ratio of Amikacin to H_2SO_4 of 1:2 contains the equivalent of not less than 674 µg and not more than 786 µg of $C_{22}H_{43}N_5O_{13}$ per mg, calculated on the dried basis. Amikacin Sulphate having a molar ratio of Amikacin to H_2SO_4 of 1:1.8 contains the equivalent of not less than 691 µg and not more than 806 µg of $C_{22}H_{43}N_5O_{13}$ per mg, calculated on the dried basis.

Assay: Determine by the microbiological assay of antibiotics, method B (2.2.10), and express the result in µg of amikacin, $C_{22}H_{43}N_5O_{13,}$ per mg.

Sample Name : Amikacin sulphate

Test Organism Used : *Staphylococcus aureus* ATCC-29737(0.1 ml per 100 ml medium)

Assumed Potency of Ref. Std. : 690.76 µg/mg (*as is* basis)

Product Analysis

Prepare five different concentrations of standard solution for preparing the standard curve by diluting the stock solution of the Standard Preparation of the antibiotic (as per Table 3 given in IP 2018 Vol. I Page 52) and increasing stepwise in the ratio 5:4. Select the median concentration (as per Table 3 given in IP 2018 Vol. I Page 52) and dilute the solution of the substance being examined (unknown) to obtain approximately this concentration.

Standard Stock Solution					**Test Dilution**		
Assay Method	Prior Drying	Initial Solvent	Final Stock Concentration	Use Before (number of days)	Final Diluent	Median Dose µg or Units per ml	Incubation Temp(°)
B	No	Water	1 mg	14	Water	10 µg	32-35

Reference Standard Dilution $= \frac{14.48 \text{ mg} \times 690.76 \text{ µg/mg}}{10 \text{ ml}} = 1000.22$ µg/ml or ≈ 1000 µg/ml

Weight of Reference Standard = 14.48mg ⟶ 10 ml in water ⟶ Stock Solution (1000 µg/ml)
1 ml of stock solution diluted up to 10 ml ⟶ Solution 1 (100 µg/ml)
0.64 ml of solution 1 diluted up to 10 ml ⟶ Std. a (6.4 µg/ml)
0.8 ml of solution 1 diluted up to 10 ml ⟶ Std. b (8.0 µg/ml)
1.0 ml of solution 1 diluted up to 10 ml ⟶ Std. c (10.0 µg/ml)
1.25 ml of solution 1 diluted up to 10 ml ⟶ Std. d (12.5 µg/ml)
1.56 ml of solution 1 diluted up to 10 ml ⟶ Std. e (15.6 µg/ml)

Sample Dilution $= \frac{14.48 \text{ mg} \times 690.76 \text{ µg/mg}}{10 \text{ ml}} = 1000.22$ µg/ml or ≈ 1000 µg/ml

Weight of Sample = 14.48 mg ⟶ 10 ml in water ⟶ Sample Stock Solution (1000 µg/ml)
1.0 ml of Sample Stock Solution diluted up to 10 ml ⟶ 100 µg/ml
1.0 ml of 100 µg/ml diluted up to 10 ml ⟶ Test dilution* (10 µg/ml)
*concentration of sample same as Std. c

Place 1 ml of each concentration of the standard solution and of the sample solution in each of tubes in duplicate or triplicate. To each tube add 9 ml of nutrient medium previously seeded with the appropriate test organism and shake vigorously.

At the same time prepare three control tubes, one containing the inoculated culture medium (culture control), another identical with it but treated immediately with 0.5 ml of dilute *formaldehyde solution* (blank) and a third containing uninoculated culture medium. Place all the tubes in an incubator or water bath and maintained them at specified temperature for three to four hours. After incubation add 0.5 ml of dilute formaldehyde solution to each tube and shake vigorously. Measure the growth of test organism by determining the absorbance at 530nm against the blank.

Observations

Concentration of Reference Standard/ Sample	Log Value of Reference Standard Concentration	Absorbance at 530 nm			Average of Absorbance
		I	II	III	
Std. a (6.4 µg/ml)	0.8061	0.1437	0.1432	0.1433	0.1434
Std. b (8.0 µg/ml)	0.9030	0.1205	0.1200	0.1201	0.1202
Std.c (10.0 µg/ml)	1.0	0.0987	0.0989	0.0994	0.0990
Std. d (12.5µg/ml)	1.0969	0.0688	0.0689	0.0693	0.0690
Std. e (15.6 µg/ml)	1.1931	0.0468	0.0467	0.0472	0.0469
Sample	-	0.0990	0.0985	0.0989	0.0988

ESTIMATION OF POTENCY: Construct the best straight response line through the points by means of following expressions:

$$L = \frac{3a + 2b + c - e}{5} \qquad H = \frac{3e + 2d + c - a}{5}$$

$$L = \frac{(3 \times 0.1434) + (2 \times 0.1202) + 0.0990 - 0.0469}{5} \qquad H = \frac{(3 \times 0.0469) + (2 \times 0.0690) + 0.0990 - 0.1434}{5}$$

$$L = \frac{0.4302 + 0.2404 + 0.0990 - 0.0469}{5} \qquad H = \frac{0.1407 + 0.1380 + 0.0990 - 0.1434}{5}$$

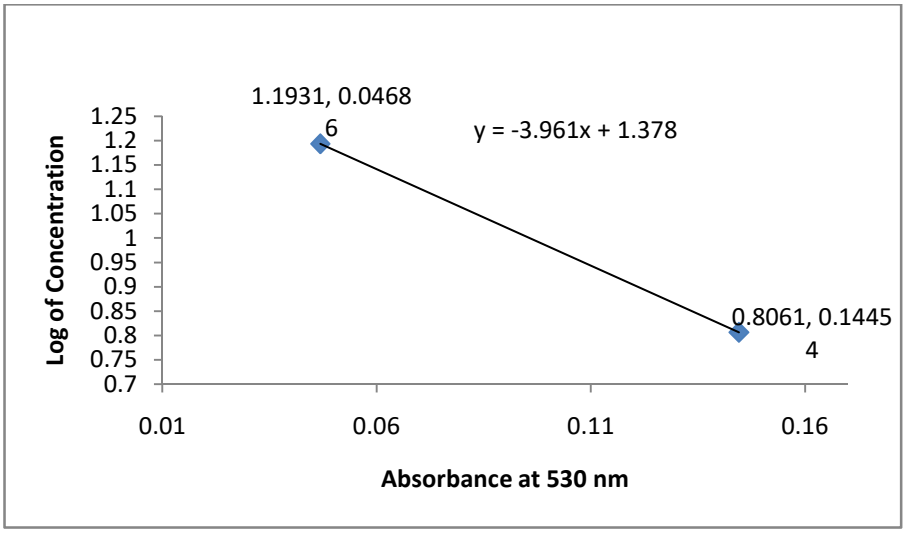
L=0.14454 H=0.04686

Where, L = the calculated absorbance for the lowest concentration of the standard response line.

H = the calculated absorbance for the highest concentration of the standard response line.

a, b, c, d, e = average absorbance values for each concentration of the standard response line lowest to highest respectively.

However, Average the absorbances for the sample and read the antibiotic concentration from the standard response line.



Concentration of Sample	Absorbance (x)	Obtained Log concentration of Test from the graph	Antilog concentration of Test
10 mcg/ml	0.0988	y = -3.961x + 1.378 y = 0.9866	9.70

RESULTS

Potency of sample = Antilog concentration of Test × Dilution factor

$= \frac{9.70}{10.0} \times \frac{14.48}{10.0} \times \frac{10.0}{14.48} \times 690.76$

= 670.04µg of amikacin per mg(on as is basis)

If, LOD = 3.88%w/w

Potency of sample (on dried basis)$= \frac{670.04 \times 100}{(100\text{-}3.88)}$ = 697.09µg of amikacin per mg(on dried basis)

(Limit-Amikacin sulphate has molar ratio of Amikacin to H_2SO_4 of 1:2, NLT 674 & NMT 786 µg of amikacin per mg, on dried basis)