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Pharmaceutical Sciences **Review Article……!!!**

ANALYTICAL DEVELOPMENT LABORATORY FORMULATION (ADL-F): INTERVIEW QUESTIONARY FOR RESEARCH SCIENTIST POSITION

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ABSTRACT

Current Article Explain some important questions which asked frequently in the interview for Regulatory Affairs department, ADL formulation department, Quality control department, ADL API department for the selection of various posts like research associate, junior scientist, senior scientist, research scientist, chemist, quality control officer, Quality Assurance officer etc.

1. **Explain Current Pharmacopoeia:**

Pharmacopoeia	Volume	Published Date	Started Date
USP	USP 42 NF 47	1 February 2019	1 May 2019
USP	USP 43 NF 48	1 February 2020 1 Nov 2019	1 May 2020
BP	BP 2020	1 August 2019	1 January 2020
IP	8 TH EDITION IP 2018 ADDENDUM 2019		
EP	10 th edition	July 2019	1 Jan 2020

2. **What are the ICH guidelines: International Council on hormonization**

1. Quality Guidelines	2. Efficacy Guidelines
3. Safety Guidelines	4. Multidisciplinary Guidelines

ICH Quality Guidelines:

1. Q1A(R2) Stability Testing of New Drug Substances and Products.
2. Q1B Stability Testing : Photostability Testing of New Drug Substances and Products.
3. Q1C Stability Testing for New Dosage Forms.
4. Q1D Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products
5. Q1E Evaluation of Stability Data.
6. Q1F Stability Data Package for Registration Applications in Climatic Zones III and IV
7. Q2(R1) Validation of Analytical Procedures: Text and Methodology
8. Q2(R2)/Q14 EWG Analytical Procedure Development and Revision of Q2 (R1) Analytical Validation
9. Q3A(R2) Impurities in New Drug Substances
10. Q3B(R2) Impurities in New Drug Products
11. Q3C(R6) Impurities: Guideline for Residual Solvents
12. Q3C(R7) Maintenance of the Guideline for Residual Solvents.
13. Q3C(R8) Maintenance EWG Maintenance of the Guideline for Residual Solvents
14. Q3D Guideline for Elemental Impurities
15. Q3D training Implementation of Guideline for Elemental Impurities

16. Q3D(R1) Maintenance EWG Guideline for Elemental Impurities

17. Q3D(R2) Maintenance EWG Revision of Q3D(R1) for cutaneous and transdermal products

18. Q4 Pharmacopoeias

19. Q4A Pharmacopoeial Harmonisation

20. Q4B Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions

21. Q4B Annex 1(R1) Residue on Ignition/Sulphated Ash General Chapter

22. Q4B Annex 10(R1) Polyacrylamide Gel Electrophoresis General Chapter

23. Q4B Annex 11 Capillary Electrophoresis General Chapter

24. Q4B Annex 12 Analytical Sieving General Chapter

25. Q4B Annex 13 Bulk Density and Tapped Density of Powders General Chapter

26. Q4B Annex 14 Bacterial Endotoxins Test General Chapter

27. Q4B Annex 2(R1) Test for Extractable Volume of Parenteral Preparations General Chapter.

28. Q4B Annex 3(R1) Test for Particulate Contamination: Sub-Visible Particles General Chapter.

29. Q4B Annex 4A(R1) Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests General Chapter.

30. Q4B Annex 4B(R1) Microbiological Examination of Non-Sterile Products: Tests for Specified Micro-Organisms General Chapter.

31. Q4B Annex 4C(R1) Microbiological Examination of Non-Sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use General Chapter.

32. Q4B Annex 5(R1) Disintegration Test General Chapter

33. Q4B Annex 6 Uniformity of Dosage Units General Chapter

34. Q4B Annex 7(R2) Dissolution Test General Chapter

35. Q4B Annex 8(R1) Sterility Test General Chapter

36. Q4B Annex 9(R1) Tablet Friability General Chapter

37. Q5A(R1) Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

38. Q5B Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products

39. Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products.

40. Q5D Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/ Biological Products

41. Q5E Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process.

42. Q6A Specifications : Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances

43. Q6B Specifications : Test Procedures and Acceptance Criteria for Biotechnological/Biological Products

44. Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients

45. Q7 Q&As Questions and Answers: Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients

46. Q8 Pharmaceutical Development

47. Q8 & Q9/10 Q&As (R4) Q8/Q9/Q10 – Implementation

48. Q8(R2) Pharmaceutical Development

49. Q9 Quality Risk Management

50. Q9 & Q8/10 Q&As (R4) Q8/Q9/Q10 – Implementation

51. Q10 Pharmaceutical Quality System

52. Q10 & Q8/9 Q&As (R4) Q8/Q9/Q10 – Implementation

53. Q11 Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities)

54. Q11 IWG Questions & Answers: Selection and Justification of Starting Materials for the Manufacture of Drug Substances

55. Q11 Q&As Questions & Answers: Selection and Justification of Starting Materials for the Manufacture of Drug Substances

56. Q12 EWG Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management.

57. Q13 EWG Continuous Manufacturing of Drug Substances and Drug Products

3. HPLC Principle:

When a mixture of components are introduced into column, various chemical and/or physical interactions takes place between sample molecules and the particles of the column packing. They travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent, travels slower. The component which has less affinity towards the adsorbent, travels faster. Since no two components have same affinity towards the stationary phase, the components are separated.

4. UV Principle:

When a beam of monochromatic light passes through a homogeneous absorbing medium, the rate of decrease in intensity of light is directly proportional to the concentration and the path length of the absorbing medium.

5. Dissolution and Disintegration Difference:

Disintegration:

Breaks down into tiny granules, molecules or particles.

Applied on solid substances.

Can proceed dissolution in braking substances of low solubility.

Dissolution:

Disolves substances in different solvents.

Applied on solids, gaseous, liquid.

Happens after substances with low solubility are broen down But can happens in the absence of disintegration.

6. BCS Classification:

Class 1: High solubility and High Permeability (Propranolol)

Class 2: Low solubility and High Permeability (Naproxen)

Class 3: High solubility and Low Permeability (Ranitidine, Atenolol)

Class 4: Low solubility and Low Permeability (Furosemide, Hydrochlorthiazide)

7. Discriminatory Dissolution Media:

An in vitro discriminatory test would be the test to reflect differences in physical characteristics of the test products (formulation/manufacturing) with no direct or definite consequences in vivo. Such tests may be conducted using any of the experimental conditions necessary concerning apparatuses (paddle/basket, Erlenmeyer flask with magnetic stir etc.) and media (organic or aqueous solvents having any pH), etc. In this respect, disintegration test may be considered as a discriminating test, if formulation/manufacturing differences be linked to disintegrating time.

An in vivo discriminatory test or a bio-relevant test, on the other hand, would be a test which would relate differences in formulation/manufacturing of products to corresponding differences in vivo such as bioavailability/bioequivalence characteristics. For an in vivo discriminatory test, an essential requirement is that the test must be conducted using physiologically relevant experimental conditions.

8. Dissolution apparatus Types:

Apparatus 1 (basket apparatus)

Apparatus 2 (paddle apparatus)

Apparatus 3 (reciprocating cylinder)

Apparatus 4 (flow through cell)

Apparatus 5 (paddle over disk)

Apparatus 6 (rotating cylinder)

Apparatus 7 (reciprocating holder)

9. What is F1 and F2 related to dissolution development study:

The difference factor (f1): It is defined by FDA calculate the % difference between 2 curves at each time point and is a measurement of relative error between 2 curves.

Acceptance Criteria: 0-15

The similarity factor (f2): It is defined by FDA is logarithmic reciprocal square root transformation of sum of squared error and is a measurement of the similarity in the percentage (%) dissolution between two curves.

Acceptance Criteria: 50-100

10. How to calculate Unknown Impurity:

$$\% \text{ Known Impurity} = \frac{\text{Area of known Impurity}}{\text{Total area of sample}} \text{X } 100\text{X RF}$$

$$\% \text{ unknown Impurity} = \frac{\text{Area of unknown Impurity}}{\text{Total area of sample}} \text{X } 100\text{X } 1$$

% Total Impurity = % of Known impurity + % of Unknown Impurity.

11. How to calculate Dissolution/Assay:

Assay Calculation:

$$\% \text{ of assay} = \frac{\text{Area of test}}{\text{Area of Std}} \text{X } \frac{\text{Wt of Std}}{\text{Std dilution}} \text{ X } \frac{\text{Sample Dilution}}{\text{Wt of test}} \text{X} \frac{\text{Avg weight}}{\text{L.C.}} \text{X Potency X Factor}$$

$$\text{Mg/Tab} = \frac{\% \text{ of assay}}{100} \text{ X L.C.}$$

Potency: As such.

Factor: Molecular weight of Standard to molecular weight of test.

L.C.: Label Claim.

Dissolution Calculation:

$$\% \text{ of Diss}^{n} = \frac{\text{Area of test}}{\text{Area of Std}} \times \frac{\text{Wt of Std}}{\text{Std dilution}} \times \frac{\text{Disso volume}}{1} \times \frac{\text{Potency}}{\text{L.C.}} \times \text{Factor}$$

12. Assay on dried basis and Assay on Anhydrous Basis:

$$\% \text{ of assay on dried basis} = \frac{\% \text{ of assay as is basis}}{(100 - \%\text{Water})} \times 100$$

13. What is RRF related to impurity:

$$\text{Response factor} = \frac{\text{Area of API/Impurity}}{\text{Amount/concentration}}$$

If $\text{RRF} = \frac{\text{Response factor of Impurity}}{\text{Response Factor of API}}$ used in denominator.

If $\text{RRF} = \frac{\text{Response factor of API}}{\text{Response Factor of Impurity}}$ used in numerator.

14. Mass Balance :

$$\% \text{ mass balance} = \frac{(\% \text{ Assay} + \% \text{ Total impurity}) \text{ of treated sample}}{(\% \text{ Assay} + \% \text{ Total impurity}) \text{ of Control sample}} \times 100$$

15. What is mean by Stability Indicating Method :

Stability Indicating Method (SIM) is defined as a validated analytical procedure that accurate and precisely measure active ingredients (drug substance or drug product) free from process impurities, excipients and degradation products.

16. Explain Validation and its Parameters:

Validation:

Establishment a documented evidence which provides a high degree of assurance that a specified process will consistently produce a product meeting its predetermined specifications and quality attributes.

System Precision: It gives the degree of reproducibility of analytical procedure for given method parameters.

Method Precision: It is an analytical method which gives the degree agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample.

Intermediate Precision: Reproducibility expressed within inter laboratory variation, such as different days, different analyst and different instrument.

Linearity: the ability of a method to produce results that is directly or by a well-defined mathematical transformation proportional to concentration of the analyte in sample within a given range.

Range: The interval between the upper and lower levels of the analyte that have been shown to be determined with precision, accuracy and linearity.

Accuracy: the accuracy of an analytical procedure express the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Limit of detection: The lowest concentration of an analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Limit of Quantification: The lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under stated experimental conditions. It is characteristic of quantitative determinations of low levels for compounds in sample mixtures, such as impurities in drug substance and degradation products.

Robustness: The robustness of an analytical procedure is mesure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Solution Stability: It is time interval within which the standard and sample solution is observed to give reliable and reproducible results without degradation products under normal laboratory condition.

Filter study: A composition of filtered to unfiltered solution in method validation to determine whether the filter being used retains any active compounds or contributes unknown compounds to analysis.

Response Factor: A response factor is a ratio of the response of an analyte divided by its concentration.

Relative response factor: RRF of an analyte is the ratio of its response factor to the response factor of reference compound under same operation content.

17. How to Develop Method for Assay:

Step 1 For Assay method development Literature Study from following area

1. Pharmacopoeias.
2. Literature databases.
3. Pharmaceutical news.
4. Internal or external guidelines.
5. Electronic books & journals.
6. DMF from drug substance supplier.
7. Originator information available from data base for drug product.
8. Source of some analytical method for API and finished products are pharmacopoeia (USP-NF, British pharmacopoeia, European pharmacopoeia).
9. The most important external guidelines are published by US Food and Drug agency(FDA),European medicines evaluation agency (EMEA), the international council for harmonization of technical requirements for pharmaceuticals for human use(ICH).

Step 2 Conduct literature survey and collect all information available targeting the following area for information

1. **Solubility profile -** which includes the solubility of drug substance in different solvents and at different pH conditions which is useful for selecting the diluents for sample preparation , dissolution medium (if in case of non availability of dissolution medium in pharmacopeia and office of generic drug (OGD) and selecting mobile phase.
2. Analytical profile – Physico- chemical and spectroscopic properties. Search for the impurities and degradation profile of the drug substance and their formation pathways.
3. Stability profile – Search for reported stability of the drug substance / drug product with respect to storage condition.
4. Additional physical tests beyond scope of a monograph which also needed to be developed for any dosage forms are

i) Water content (crystal properties / particle size / stability)

ii) Particle size (bioavailability / content uniformity / solubility / stability)

iii)Crystal properties and polymorphism (solubility / bioavailability / stability).

Step 3. The approach for method development for finished dosage forms shall be as follows:

For the product with pharmacopeial monograph is available.

i) Check the feasibility of pharmacopeial method → if pharmacopeial method is suitable → use pharmacopeial method.

ii) Check the feasibility of pharmacopeial method → if pharmacopeial method is not suitable → develop in-house method → demonstrate equivalency and superiority of method / check the suitability of developed method→

For the product with pharmacopeial monograph is not available.

i. Check the feasibility of pharmacopeial method → if pharmacopeial method is not available →develop in house method→ refer DMF, literature and in house method → check suitability of developed method→ perform partial validation for method challenges.

* Suitability shall be demonstrated by performing verification for selected parameter.

Step 4. Method goals are determined by different by factors

a. Specification parameter and limits**.**

b. Level of detection, sensitivity

c. Minimum resolution between components

d. Availability of standard materials Number of samples.

Step 5 Selection of method type influenced

e. Sample characteristics and sample preparation procedure**.**

f. Detection methodology

g. Speed of separation

h. Chromatographic run time

i. Critical parameter viz, matrix interruption non-destructive or destructive type of detection , compatibility etc.

Step 6. Optimization of method:

Preparation of representative sample:

As it was mentioned earlier that chromatographer has to collect all possible data about the sample components. When chemical properties, stability data and standards (i.e. degradation product and process related impurities) are available, spiked sample test with all related substance at limit level concentration can be used as representative sample for method development. If the chemical properties data, stability data and available standard do not cover the crucial input data, some experiments have to be done to prepare the representative sample. In that step it is recommended to test solubility and stability in different solvent and different pH. After that stress sample has to be prepared in following media.

i) Acidic media with concentration of HCL between 0.01 and 5.0M

ii) Basic media with concentration of NaOH between 0.01 and 5.0M

iii) Oxidative media with concentration of H_2O_2 between 0.03 and 30%

iv) Influence of temperature (powder and solution) from 40 to 100 ° C

v) Influence of light (powder and solution)with wavelength from 300 to 800 nm and powder 250 W / m^2

vi) Target level of degradation is from 5 to 20% of the sample.

Mass balance of the degraded sample:

It is very important to establish the mass balance for analytical method developed for related substance. It is a good quality to check a analytical methods to show that all degradation product are adequately detected and do not interfere with quantitation of the parent drug substance. Mass balance in pharmaceutical analysis is very important for several reasons. As unknown degradation products couid potentially be toxic or otherwise compromise the safety of drug . it is important to have methods to detect all major degradation products. While developing stability indicating method for degradants , we have to ensure whether the method is exactly qualifying all possible degradants or not hence mass balance is useful element for that. After each degradation study,

Total % drug = % of drug remained + % of known degradants + %of unknown degradants

$$\% \text{ mass balance} = \frac{(\% \text{ Assay} + \% \text{ Total impurity}) \text{ of treated sample}}{(\% \text{ Assay} + \% \text{ Total impurity}) \text{ of Control sample}} \times 100$$

For forced degradation study :

Mass balance correlates the measured loss of drug (API) to the measures increased in degradation products. In mass balance calculation the amount of drug (remaining in stressed sample) is determined from an assay by HPLC method, and increase degradation product is determined by related substance by HPLC method.

Factors which play important role in calculating mass balance are as given below :

Importance of relative response factor(RRF)

For example , if the RRF of an impurity (RRF should establishe while developing analytical method) is 0.6 and if amount of degradants formed as per area calculation is 3.5 then after applying the RRF the exact amount of impurity formed will be as given below

3.5*1/0.65 = 5.83 %

Importance of molecular weight (MW)

Suppose for example, MW of main drug is 151 and MW of degradant formed is 195. If the percent

degradation formed as per percent area is 3.5 , now exact amount of impurity formed is

3.5*151/195 =2.71%

Suppose for example MW of main drug is 151 and MW of dedardant found as per % area is 3.5,now the exact amount of impurity formed is

3.5*151/125=4.23%

The ultimate goal is that the analytical method should achieve 100 % mass balance. However the applicable limit for the mass balance will be as follows

If observed degradation is ≥ 10.0 % then mass balance shall be 90.0%

If observed degradation is < 10.0 % then mass balance shall be 95.0%

Sometime 100% mass balance may not be achieved because of following reason

- The degradant formed may not be eluted in developed method(extend runtime and modify method condition)
- Degradation products formed are volatile in nature.
- Degradation products formed have different wavelength and maxima than the parent drug resulting into significantly different response factor.
- Precipitation of drug substance during sample preparation
- The response may be zero at method wavelength (select appropriate wavelength or develop separate method for the particular degradant may be UV inactive (identify the impurity by different techniques like refractive index (RI), Evaporative light scattering detector(ELSD),Liquid chromatography mass spectroscopy (IMS) etc
- If mass balance not achieved different experiments shall be performed as follows
  a) perform forced degradation study on different concentration degradation reagent (e.g. 0.1N,0.5N,5.0N etc)
  b) Perform forced degradation study on different temperature .

Note: if mass balance is not achieved possible reason shall mention with appropriate justification. proper justification should be given for not achieving mass balance.

Choice of separation technique:

The choice of separation methods depends upon the sample characteristics. There are several criteria to the separation technique. according to the molecular weight samples are divided into two major groups.

1. MW > 2000 are classified as large molecules that are usually separated by size exclusion chromatography (SEC) and ion exchange chromatography (IC) or by reverse phase chromatography (RPC) with wide-pore particles.

2. MW < 2000 are classified as small molecules that are divided into two group

   a)Water soluble with polar character

   b)Soluble with organic solvent with non polar character both group of small molecules have similar list of separation mode (Reverse phase chromatography (RPC) , Normal phase chromatography (NPC), hydrophilic interaction chromatography(HILIC) ,Hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IC) with different experimental condition. method development often begins with RPC, Ion-pair RPC(is a type of RPC with modification – ion pair reagent in the mobile phase) and NPC are the second choice . Other options related to less frequent samples..

According to sample characteristics chromatography techniques are classified as follows:

i. RPC is the first choice for the neutral or non-ionized molecules and sample that dissolve in mixture of water and organic components.
ii. Ion pair RPC is a good choice for ionic or ionized molecules in most cases bases or cations.
iii. NPC is an alternative mode , but the first choice for lipophilic molecules or mixtures of isomers and samples are not soluble well in mixtures of waters and organic components.
iv. IEC is used for separation of inorganic ions, proteins , nucleic acids and other molecules that are sensitive to the ion-exchange mechanism.
v. HILIC and HIC modes are less universal that are used for special separation.

Optimization of mobile phase :

The primary objective in selection and optimization of mobile phase is to achieve the optimum separation of all impurities and degradants from each other and from analyte (Drug substance) peak. The selection of mobile phase is always done in combination with selection of column (stationary phase).the following are the points which shall be taken into consideration while selecting and optimizing mobile phase.

Buffer and strength :

selection of the appropriate buffer system with the pH at a point where the analyte has a charge or it is neutral – the choice depends on its chromatographic mode you wish to employ latex. It is strongly recommended to avoid pH where the samples have their pKa value these is the region where components have two different appearance viz as charged and uncharged molecules with different chromatographic behavior.

Buffer and its strength play an important role in deciding the peak symmetries and separations. Various types of buffer can be employed to achieve the required separations.

The following are most commonly used buffer for chromatographic separation:

Buffer *MS compatible buffer	pKa (25°C)	Useful pH Range
TFA*	< 2	1.5 - 2.5
Phosphate	2.1	1.1 – 3.1
Citrate*	3.1	2.1 – 4.1
Formate	3.8	2.8 – 4.8
Citrate*	4.7	3.7 – 5.7
Carbonate*	4.8	3.8 – 5.8
Citrate*	5.4	4.4 – 6.4
Carbonate*	6.4	5.4 – 7.4
Phosphate*	7.2	6.2 – 8.2
Tris	8.2	7.2 – 9.2
Ammonia	9.2	8.2 – 10.2
Borate	9.2	8.2 -10.2
Glycine	9.8	8.8 – 10.8
Carbonate	10.3	9.3 – 11.3
DEA	10.5	9.5 – 11.5
TEA	11.0	10.0 – 12.0
Phospahte	12.3	11.3 – 13.3

Concentration of Buffer.

Selectivity is affected by buffer concentration (strongly in some chromatographic modes and less in other modes). It is important to use the buffers with suitable strength to cope up for injection load on the column otherwise peak tailing may arise during chromatography. The buffer concentration should be in suitable range to hold the pH at the required level, otherwise as a consequence the method will not be robust. Solubility of buffer will be issue when the solution is mixed with an organic solvent , epically when gradient elution is used carefully check the solubility to avoid the precipitation . Cut off values has to be check before the buffer is used(the cut-off is set for the wavelength where the absorbance for the 10 mm path length of the solvents particular absorbance unit relative to the water.

pH of Buffer

pH plays an important role to achieve the chromatography separation as it controls the illusion properties by controlling the ionization characteristic .depending upon the pKa the retention of drug molecule changes example a sideshow and increasing retention time as ph is reduced , while beast show

decreasing retention time excrements shall be conducted using piers having different ph to obtain the required separation order silica based faces are not stable to ph seven or eight this is due to the fact that the siloxane linkages are cleaved below pH 2.0 ,while at pH values above 7.5 silica may dissolve. Modern materials are modified or made by hybrid techniques to improve pH stability up to pH 12.0.Additional factor is observed when HPLC columns packed with stationary phase that have significant silanol activity are used .The consequence is peak tailing in reverse phase Hplc mode because of iron exchange interactions that takes place between a positively charged component (nitrogen containing compounds)and a negatively charged silanol on the surface of silica stationary phase particles. The usual corrective action is to lower the ph of the mobile phase typically below pH 3.0 where the silanols or not ionized(pKa of the silanol is 3.5).This actions can change the selectivity and resolution of separation. Another possibility is to increase the buffer strength of mobile phase or addition of triethylamine(TEA) to the mobile phase. TEA acts as competing base reducing the interaction of the analyte with the silanols.

Mobile phase composition

In reverse phase chromatography the separation is mainly controlled by the hydrophobic interaction between drug molecules and the alkyl chains on the column packing material. Most chromatographic separation can be achieved by choosing the optimum mobile phase composition. This is due to the fact that fairly large amount of selectivity can be achieved by achieved by qualitative and quantitative composition of aqueous and organic portions. Many chromatographic parameters are controlled and influenced by the organic component of the mobile phase. Important factors are UV cut-off value, viscosity, miscibility and solubility, volatility, elution strength and selectivity impact.

Solvent	Cut-off value	Solvent	Cut-off value
Acetic acid 260	260	N,N-Dimethylacetamide	268
Acetone	330	N,N-Dimethylformamide	270
Acetonitrile	190	Dimethyl sulfoxide	265
Benzene	280	1,4-Dioxane	215
2-Butanol	260	Ethanol	210
Butyl acetate	254	Ethyl acetate	255
Carbon disulfide	380	Ethylene glycol dimethyl ether	240
Carbon tetrachloride	265	Ethylene glycol monoethyl ether	210

1-Chlorobutane	220	Ethylene glycol monomethyl ether	210
Chloroform	245	Glycerol	207
Cyclohexane	210	Dichloromethane	235
1,2-Dichloroethane	226	Diethyl ether	218
N,N-Dimethylformamide	270	Ethanol	210
Dimethyl sulfoxide	265	Ethyl acetate	255

Solvent	Cut-off value	Solvent	Cut-off value
1,4-Dioxane	215	Ethylene glycol dimethyl ether	240
Ethylene glycol monoethyl ether	210	Hexadecane	200
Ethylene glycol monomethyl ether	210	Hexane	210
Glycerol	207	Methanol	210
Heptane	197	Methylcyclohexane	210
Methyl ethyl ketone	330	Nitromethane	380
Methyl isobutyl ketone	335	Pentane	210
2-Methyl-1-propanol	230	Pentyl acetate	212
N-Methyl-2-pyrrolidone	285	1-Propanol	210
Pyridine	330	Toluene	286
Tetrachloroethylene	290	1,1,2-Trichloro-1,2,2 trifluoroethane	231
Tetrahydrofuran	220	2,2,4-Trimethylpentane	215
Water	191	m-Xylene	290
o-Xylene	290	p-Xylene	290

Most widely used solvents in reverse phase chromatography are Methanol and Acetonitrile, Tetrahydrofuran is also used but to a lesser extent. Experiments shall be conducted with mobile phase to check for the best separation between the impurities A drug solution having at possible knows impurities can be used for checking the extent of separation with different mobile phase ratios. Alternatively solutions of stressed drug substances can be used to check for the separation.

Selection of column :

The following are the parameters of a chromatographic column which are to be considered while choosing a column for separation of impurities and degradants.

I) Length and diameter of the column

Ii) packing material

Iii)Shape of the particles

Iv)Size of particles

V)% carbon loading , pore volume

Vi) Surface area

Vii) End capping

Column plays a most important role to achieve the chromatographic separations .Most chromatographic separations are achieved due to wide variety of column available and due to flexibility to change and control each of the above parameters. Columns with silica as a packing material is widely used in normal phase chromatography , where the eluent (mobile phase) is n non polar consisting of various organic solvents like n-hexane , ethyl acetate , methylene chloride etc . For reverse phase chromatography , a wide variety of columns are available covering a wide range of polarity by cross linking the Si-OH groups with alkyl chains C6, C8, C18 and nitrile groups (CN), Phenyl groups (-C6H5) and amino groups (- NH2) Silica based columns with different cross linking in the increasing order of polarity are as follows ------Non-polar ---- moderately polar----polar---C_{18}<C_8<C_6 < phenyl columns with different mobile phase to achieve best separations in chromatography . A column which gives separation of all impurities and degradants from each other and from drug substance peak and which is rugged for variation in mobile phase shall be detected. The ruggedness of the method can be confirmed by using columns of different brands having same column dimensions or different brands having same column dimensions or different lots of same brand.

Selection of solvent delivery system

Chromatographic separation with single eluent (isocratic elution) i.e. All the constituemts is mixed and pumped together as single eluent , is always preferable. However gradient elution is a powerful tool in achieving separation between closely eluting compounds or having widely differing in polarities. The important feature of the gradient elution which makes it a powerful tool, is that the polarity and ionic strength of the mobile phase can be varied (can be increased or decreased) during the run. Conduct experiments using different mobile phase combinations and different gradient programs to achieve separation of all impurities and degradants from each other and from Drug substance peak. In general while running a gradient, two mobile phase having different compositions will be kept in different

channels. The two mobile phase are then introduced into the column by different ways.

By low – pressure gradient – i.e. mobile phase are mixed at the predetermined ratio and pumped using a single pump.

By High – pressure gradient – i.e. mobile phase are pumped at different flow rates so as to achieve the required ratios and then mixed in chamber before introducing into the Column.

While optimization the separation of impurities, it is to be detected whether to optimize for low pressure gradient or for high pressure gradient. Low pressure gradients can be adopted when not more than 80% organic phase is to pumped. High pressure gradient is preferred when more than 80% of the organic phase is to be pumped.

While optimizing the gradient program especially using low viscous solvents like Acetonitrile and phosphate buffer, it is recommended to mix about 10% aqueous portion preferable the same buffer in mobile phase to avoid pumping problems.

While optimizing the gradient program it is important to monitor the following

Pressure gap is to be monitored so as to ensure that the overall system pressure will not cross 300 bar at any point during the run.

Flow rate is to be physically checked by collecting the output from the detector during the run at different time intervals, especially when the gradient is running with the high organic phase composition so as to ensure that there were no pumping problems during the runs when mobile phase of different compositions are pumped.

Compatibility of buffer with organic solvents should be checked physically adding the ratios. Also it is important to optimize the program after each run and before going to next injection. The programme for initialization shall be optimized such that there should not be no carry over to the next run and system get stabilize with initial composition before the next injection. Check the ruggedness of the gradient program up to ± 0.2 mL flow rate.

Select the gradient program which is rugged for organic phase variation and flow variation.

Selection of flow rate

Flow rate shall be selected on the basis of following data :

i) Retention times

ii) Column back pressure

iii) Separation of impurities

iv) peak symmetries

Check the ruggedness of the method by varying the flow rate by ± 0.2 mL from the selected flow rate. Select the flow rate which gives least retention time, good peak symmetries least back pressure and

better separation of impurities form each other and from drug substance peak.

Selection of column temperature :

Always it is preferable to optimize the chromatographic conditions with column temperature as ambient. However if the peak symmetry could not be achieved by any combination of column and mobile phase. Then the column temperature above the ambient can be adopted. The increase in the column temperature generally will result in reduction in peak asymmetry and peak reactions. When found necessary , the column temperature between 30°C and 80°C shall be adopted. If a column temperature of above 80°C is found to be necessary, packing materials, which can withstand to that temperature shall be chosen.

Selection of detector wavelength:

Selection of detectors wavelength is critical step in finalization of the analytical method for impurities and degradants. Inject the impurity and drug substance standard solutions into the chromatographic system with photo diode array detector and collect the spectra. Also conduct the forced degradation studies and collect the UV spectra for all major degradation products. Overlay spectra for all the components and select the spectra which is most common and gives higher response for all compounds.

Selection of diluent for test preparation:

Diluent of test preparation is selected initially based on solubility of the drug substance and known impurities. However finalization of the diluent depends upon its extraction efficiency, peak symmetries and resolution of impurities and diluent blank injection interference.

Inject the diluent blank and test solution spiked with known impurities into the chromatographic system and establish the non interference of the blank in estimation and the effect of diluent resolution of impurities and peak symmetries.

Select the diluent in which all the known impurities/ degradants and the drug substances/products are soluble, in which the extraction is complete due to which there is no blank interference, in which peak symmetries and resolution between impurities found to be satisfactory.

Selection of test concentration injection volume:

The test concentration is generally chosen based upon the response drug substance peak and impurities at the selected wavelength.

However the test concentration shall be finalized keeping in the mind the specification level of the impurities and their acceptance LOQ's. Ensure that signal to noise ratio(S/N) > 10 using both Diode Array detector (DAD) and variable wavelength detector (VWD).

Generally an injection volume of 5, 10 to 20 µl is recommended for estimation of impurities. However if the UV response found to be poor, then the test concentration can be kept high and injection volume

can be increased. But it is to be insured that at the selected injection volume the column is not overloaded, resolution between individual impurities and peak symmetries are not compromised.

After test concentration and diluent are finalized, prepare the solution on the bench top and observe for any precipitation or turbidity after 24 hours. The solution should not show any turbidity or precipitation.

Establishment of stability of test preparation:

Prepare the test solution spiked with known impurities and programme for the stability of solution on auto injector at least 12 hours. As far as possible select a stability solution on auto injector at least 12 hours. If the solution is found to be unstable by its nature, the incorporate the stability of the solution in test method. Auto sampler temperature can be set between 4°C to 20°C if test sample is not stable at room temperature.

Establishment of system suitability:

System suitability parameter (Tailing factor, theoretical plates, resolution, capacity factor etc) is to be selected based on criticality of separation, while doing the robustness and ruggedness studies. In that case, resolution factor for closely eluting compounds is selected as system suitability requirements.

If the separation of impurities from each other and from Drug substance peak is found to be satisfactory, there is no need keep resolution factor as a system suitability parameter. In such a case, system suitability requirement is asymmetry factor and theoretical plates of diluted standard peak.

Establishment of specificity of test method:

Conduct specificity study to check interference if any by injecting blank solution, placebo solution, individual component of placebo, individual impurity and spike solution of impurity and active.

Conduct force degradation study by stressing Drug product, Placebo and Drug substance using wet method (e.g. acid, base, peroxide and water) and dry method (e.g. thermal and photolysis).

If the purity of the peak is found to be satisfactory as per individual software requirements, then the method can be considered as stability indicating method can be considered as stability indicating for estimating of impurities and degradants.

Establishment of response factor for impurities:

After finalizing the test method, establish the response factor of all known impurities with respect to Drug substance and the procedure as follows:

Prepare a series of solutions of impurities and drug substance in the concentration ranging about from 50% to 150% of specification limit and inject into the HPLC system. Plot a linearity graph for impurities and drug substance and determine the slope for the same.

$$\text{Response factor (RF) of impurity} = \frac{\text{Slope of API}}{\text{Slope of impurity}}$$

*RF needs to be used in multiplication.

$$\text{Relative response factor(RRF) of impurity} = \frac{1}{\text{RF}}$$

*RRF needs to be used in division.

18. Explain Cleaning Validation Calculations and Acceptance Criteria:

a. Based on health based data:

1. Permitted daily exposure ICH :

$$\textbf{PDE} = \frac{\textbf{NOAEL x Weight Adjustment}}{\textbf{F1 x F2 x F3 x F4 x F5}}$$

NOAEL: No observed Adverse Effect Level.

F1: A factor (values between 2 and 12) to account for extrapolation between species

F2: A factor of 10 to account for variability between individuals

F3: A factor 10 to account for repeat-dose toxicity studies of short duration, less than 4-weeks.

F4: A factor (1-10) that may be applied in cases of severe toxicity, e.g. non-genotoxic carcinogenicity, neurotoxicity or teratogenicity

F5: A variable factor that may be applied if the no-effect level was not established. When only an LOEL is available, a factor of up to 10 could be used depending on the severity of the toxicity.

2. Allowable daily exposure ICH (mg/day):

$$\textbf{ADE} = \frac{\textbf{NOAEL x Weight Adjustment}}{\textbf{UFc x MF x PK}}$$

NOAEL: No observed Adverse Effect Level (mg/kg/day).

UFc: Composite Uncertainty Factor: combination of factors which reflects the inter- individual variability, interspecies differences, sub-chronic-to-chronic extrapolation, LOEL-to-NOEL extrapolation, database completeness.

MF: Modifying Factor: a factor to address uncertainties not covered by the other factors

PK: Pharmacokinetic Adjustments**.**

$$\textbf{MACO} = \frac{ADE/PDE_{previous} \times MBS_{Next}}{TDD_{Next}}$$

MACO = Maximum Allowable Carryover: acceptable transferred amount from the previous product into your next product (mg).

TDD_{Next} : Standard Therapeutic Daily Dose for the next product (mg/day)

MBS_{Next} : Minimum batch size for the next product(s) (where MACO can end up) (mg).

b. Based on therapeutic Daily Dose:

$$\textbf{MACO} = \frac{TDD_{previous} \times MBS_{Next}}{SF \times TDD_{Next}}$$

MACO : Maximum Allowance Carryover: acceptable transferred amount from the previous product into your next product (mg)

TDDprevious : Standard Therapeutic Daily Dose of the investigated product (in the same dosage from as TDDnext) (mg/day)

TDDnext: Standard Therapeutic Daily Dose for the next product (mg/day)

MBSnext: Minimum batch size for the next product(s) (where MACO can end up (mg)

SF: Safety factor (normally 1000 is used in calculations based on TDD).

c. Based on LD_{50}:

$$\textbf{NOAEL} = \frac{\textbf{LD}_{50} \textbf{ x BW}}{\textbf{2000}}$$

$$\textbf{MACO} = \frac{\textbf{NOAEL}_{previous} \textbf{ X MBS}_{Next}}{\textbf{SF}_{Next} \textbf{ X TDD}_{Next}}$$

MACO: Maximum Allowance Carryover: acceptable transferred amount from the previous product into your next product (mg)

NOEL previous No Observed Effect Level (mg/day)

LD50 Lethal Dose 50 in mg/kg animal. The identification of the animal (mouse, rat etc.) and the way of entry (IV, oral etc.) is important (mg/kg)

BW Is the weight of an average adult (e.g. 70 kg) (kg)

2000 2000 is an empirical constant

TDDnext Standard Therapeutic Daily Dose for the next product (mg/day)

MBSnext Minimum batch size for the next product (s) (where MACO can end up)

SFnext Safety factor

The safety factor (SF) varies depending on the route of administration (see below). Generally a factor of 200 is employed when manufacturing APIs to be administered in oral dosage forms.

Safety factors:

Topicals 10 – 100

Oral products 100 – 1000

Parenterals 1000 – 10 000.

d. Based on 10 PPM:

Calculations:

$$\textbf{Target value}\ [\mu g/dm2] = \frac{\textbf{MACO}\ [\mu g]}{\textbf{Total surface}\ [dm2]}$$

Summary:

1. **Based on therapeutic Daily Dose**
2. **Based on 10 PPM**
3. **Based on health based data(PDE/ADE)**
4. **Based on LD $_{50}$**

   From above 4 method whichever is very stringent or lowest ppm concentration is selected for its limit of cleaning method.

19. Assay Validation Acceptance Criteria:

Sr. No.	Test	Assay
1	Specificity	**1.** There should be no interference due to blank, placebo, any impurity, degradation product with component of interest. **2.** The minimum resolution between main peak and any peak if elutes near the main peak should not be less than 1.5. **3.** Peak purity criteria= purity angle < purity threshold.
2	System Precision	RSD NMT 2%.
3	Method Precision	RSD NMT 2%. Assay for all individual values should be within specification limit. (95-105 if any)
4	Intermediate Precision	RSD not more than 2%.

		Assay for all individual values should be within specification limit. Absolute difference NMT 2.0
5	Accuracy	Individual Recovery and Mean recovery 98-102% with RSD NMT 2% for assay of sample.
6	Linearity	Correlation coefficient value not less than 0.995 Slope of the regression line, Y-Intercept, Residual Sum of the square to be reported
7	Range	The % RSD at higher level and Lower Level NMT 2.0
8	Robustness Folw Wavelength Temperature	System Suitability results should meet the test method requirements for all remaining robustness parameter.
9	Solution stability (Carousal, Bench top)	The % similarity factor between the standard under stability and initial standard between 98.0 to 102.0. The % similarity factor between the sample under stability and initial samaple between 98.0 to 102.0. Deviation nmt **±2%** If any extra peak of impurities observed in chromatographic method than that of initial, consider that the solution in not stable.
10	Filter Study	The % similarity factor between filtered standard solution and unfiltered standard should between 98.0 to 102.0. The % similarity factor between filtered test solution and centrifuged should between 98.0 to 102.0.
11	System suitability	Resolution should not be less than 3.0 %RSD of Standard NMT 2.0

REFRENCES:

1. ICH Guidelines
2. USP pharmacopoeia
3. IP pharmacopoeia
4. WHO guidelines