WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 10, 80-108. Review Article ISSN 2277– 7105

QbD APPROACH FOR DEVELOPMENT OF STABILITY INDICATING RP - HPLC METHOD AND IT'S VALIDATION: A REVIEW

Ajay Chaudhary[1*], Alka N. Choudhary[1] and Kamlesh K. Dutta[2]

[1]Quality Assurance Research Laboratory, Department of Pharmaceutical Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, India.

[2]Quest Pharmaceuticals Private Limited, Birganj, Nepal.

Article Received on 03 April 2018,
Revised on 24 April 2018,
Accepted on 15 May 2018,
DOI: 10.20959/wjpr201810-11522

***Corresponding Author**
Ajay Chaudhary
Quality Assurance Research Laboratory, Department of Pharmaceutical Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, India.

ABSTRACT

Untill a few years ago, the quality of drug products were mostly determined by purity rather than impurities. But the things has changed and the quality of drug products are now determined by not only purity but also the impurities/degradants present in them. Regulatory bodies are now more concerned on impurities along with purity because of the health concern. It is therefore necessary to develop scientifically sound method that is capable to separate, detect and quantify the drug - related degradants that can form on storage or manufacturing of the drug product and any drug – related impurities that may be introduced during synthesis of the drug. RP-HPLC is the most powerful and dominant analytical technique available in todays world for the purpose. Here in this review is presented a step-by-step QbD approach to for developing stability indicating RP-HPLC method and its validation. Method development, validation and its life cycle management must incorporate in it the concept of QbD and QRM.

KEYWORDS: Quality by Design, Stability Indicating, RP - HPLC, Method Development and Validation, Force Degradation, Gradient Scouting.

INTRODUCTION

Analytical method development and validation play important roles in the discovery, development and manufacturing of pharmaceutical products. [1] Method development and its validation should be an stepwise procedure (sequential approach) and concept of Quality by Design – QbD, Q8(R2) and Quality Risk Management – QRM, Q9 should be incorporated in

it. Both QbD and QRM are well proven approach in product and process development and validation and are similarly effective in the field of analytical method development and validation. [2-7] Quality by Design (QbD) is defined as

"A systematic approach to development thats begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management."[8]

Methods are commonly developed using a one-factor-at-a-time (OFAT) approach where one variable is changed sequentially until a suitable method is produced. This type of development may create an adequate method but provides a limited understanding of method capabilities and method robustness. A better understanding of the overall method capabilities and limitations in development phase can be achieved with QbD and hence ensures a greater chance of successful downstream method validation, transfer and routine use.[9] QbD approach for method development includes.[10-16]

- **Analytical Target Profile (ATP)**
  - Define what the method has to measure and to what level the measurement is required (i.e., performance level characteristics - such as precision, accuracy, working range, sensitivity - and the associated performance criteria).

- **Risk Assessment Process**
  - Define Critical Method Attributes, CMAs (e.g., Resolution, Peak Tailing, etc).
  - Define Critical Method Parameters, CMPs (e.g., pH, Flow Rate, Column Temperature, etc) which affect the CMAs.

- **Design of Experiment (DoE)**
  - Relationship between CMPs and CMAs is explored by varying multiple CMPs simultaneously to see its impact on CMAs (e.g., impact of flow rate and column temperature on peaks resolution).
  - Helps to avoid unnecessary experiments and speed up the optimization process.

- **Control Strategy**

❖ Determines the Design Space, the experimental region in which changes to method parameters will not significantly affect the results. Greater is the design space more the method is robust.

- **Lifecycle Management**

❖ Monitor method performance; update as needed as process and analytical technology evolves.

METHOD DEVELOPMENT

Among various analytical methods, HPLC (High Performance Liquid Chromatography) - dominant and most powerful analytical technique in todays modern pharmaceutical world, have been widely practiced for over five decades because of its wide applicability to innumerous analytes in very wide variety of matrices. But HPLC method development presents a significant bottleneck in analytical laboratories, as multiple CMAs, like column chemistry, eluents and its pH, separation temperature, etc need to be systematically investigated and optimized for targeted CMPs. To this constraint, latest automated method scouting techniques are available in todays world. This system combines automated multi-column, various solvent screening and intelligent run analysis software, which allow chromatographers to explore QbD principle more efficiently and develop method more rapidly saving valuable time and resources resulting in increased productivity compared to conventional manual approaches.[17]

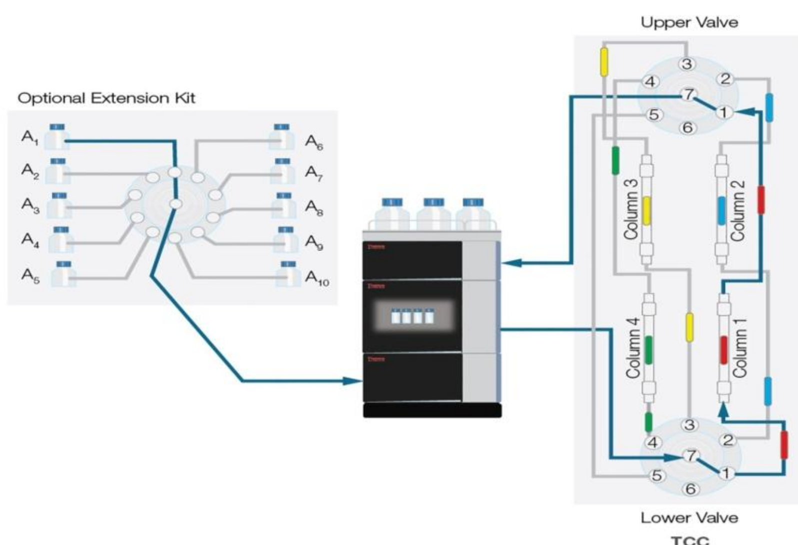

Figure 1: Latest automated method scouting technique for HPLC method development.[17]

There are two separation techniques available in HPLC methods,[18,19]

a. Isocratic Separation: Composition of mobile phase remain constant for the entire run

b. Gradient Separation: Composition of mobile phase changes with time during the run. It is further classified as, [20]

- **High Pressure Gradient**

❖ Mobile phase is mixed on the high pressure side of the pumps.

❖ Gradient delay volume is comparatively low.

- **Low Pressure Gradient**

❖ Mobile phase is mixed on the low pressure side of the pump.

❖ Gradient delay volume is comaparatively high.

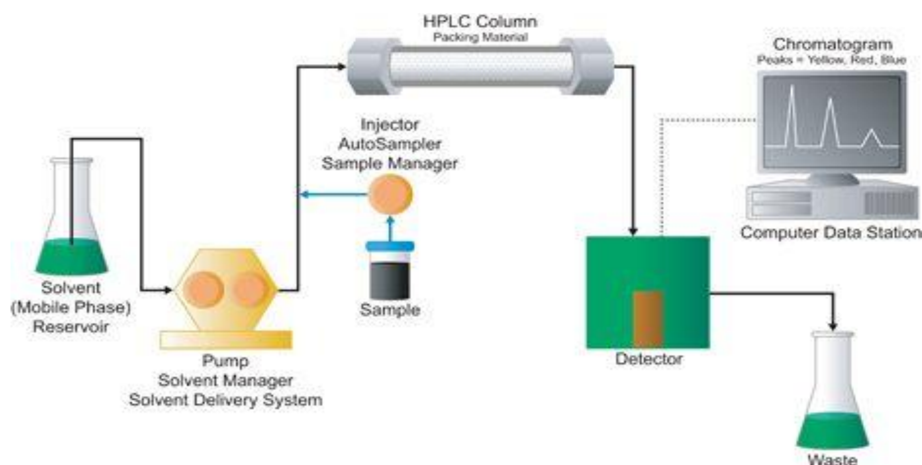

Figure 2: Isocratic Separation.[20]

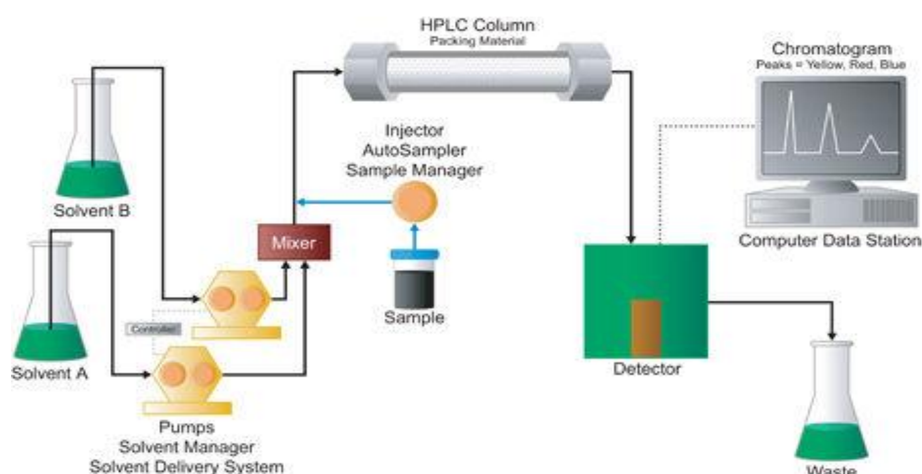

Figure 3: High Pressure Gradient Separation.[20]

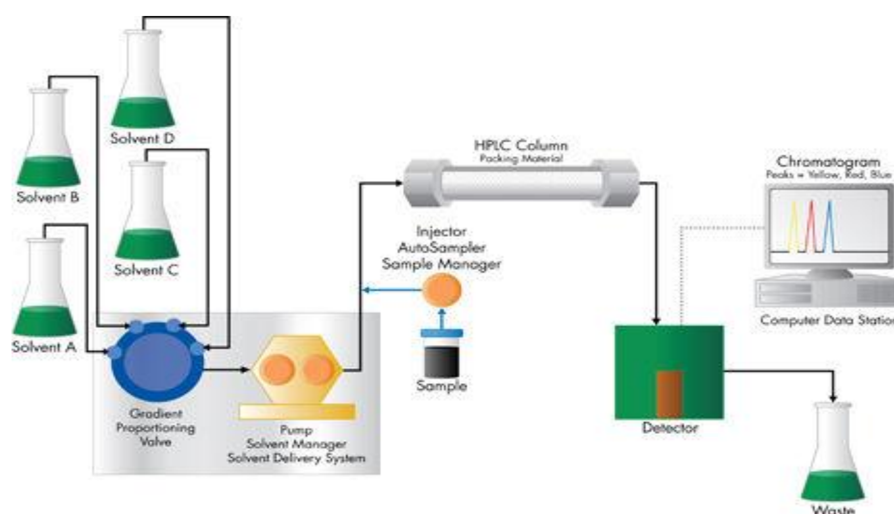

Figure 4: Low Pressure Gradient Separation.[20]

Whenever possible isocratic method is preferred but gradient method can be used for samples containing analytes with wide range of retentivities that would under isocratic condition provides chromatograms with capacity factor outside of the normally acceptance range of 0.5 – 15. Gradient HPLC is also a requirement for complex samples with large number of components like 20 – 30 because the maximum number of peaks that can be resolved with a given resolution is much higher in gradient HPLC than in isocratic HPLC. Gradient HPLC will also give greater sensitivity, particularly for analytes with longer retention times because of the more constant peak width, as for a given peak area, peak height is inversely proportional to peak width.[21]

Step-by-Step QbD approach to method development is detailed below,

1. Purpose of the Method

The purpose of the method to be developed should be clear that whether the method is to be employed for,[22,23]

- Identification tests,
- Limit tests for the control of impurities,
- Quantitative tests for impurities content or
- Quantitative tests of the active moiety.

Design of experiment(DoE) solely depends upon the purpose of the method.[4,5,22]

2. Target Measurement

- Determine the numbers of compounds present in the sample.
- Define the range of concentrations used to measure and the sample matrix it will be measured in.

3. Determine the CMAs

Table 1: CMAs for HPLC method development.[21,23-28]

S. No.	CMAs	General Requirement
1.	Resolution (R_S)	≥ 2 (For adequate separation of peaks)
2.	Run Time	5 – 10 Minutes (Desirable for speed analysis)
3.	Capacity Factor or Retension Factor (k)	0.5 – 20 (For optimum peak elution)
4.	Numbers of Theoretical Plates (N)	≥2000 (For higher sensitivity)
5.	Symmetry Factor or Tailing Factor(A_S)	≤2 (For adequate peak symmetry)
6.	Pump Pressure	<150 Bar (For prolonged life of hardware)
7.	Peak Purity (Purity Ratio)	Below Unity

4. Literature Survey

Before to start the experiments to develop method it is always suggested to consult the chromatographic literature to find out if anyone has already worked on the project and grab ideas that benefit in designing experiments, which certainly save one having to do a great deal of experimental works.[26,28]

5. Sample Information

It is preferable to have maximum information on sample and its related known impurities to make development work fast. Some of the Physical and chemical properties need to be investigated are;[26,29]

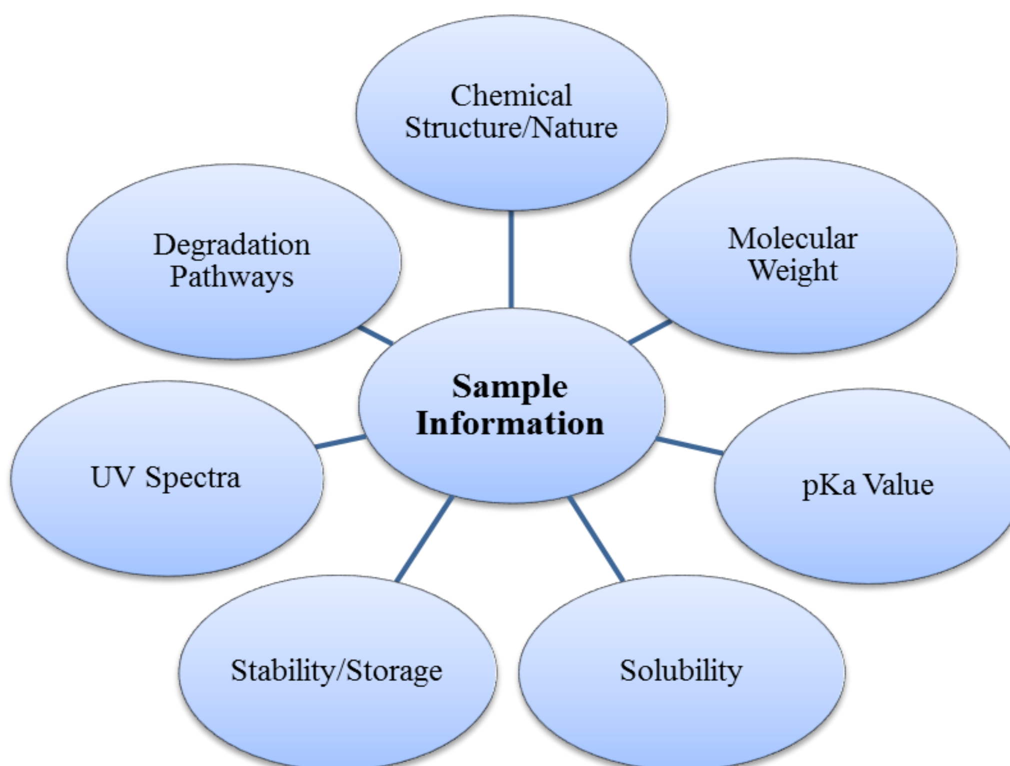

Figure 5: Sample Information.[17]

6. Sample Preparation

Select a sample preparation method that provides a clean sample.

- Diluent for the test preparation is selected based on solubility of the drug substance and known impurities. Analyte and impurities should not degrade in the selected diluent..
- Mobile phase as diluent is preferable for smooth baseline.[26]
- Sample may require sample pre-treatment to remove interferences and/or protect the column and equipment. As a part of sample pre-treatment; filter compatibility study is required.
- Is chemical derivatization required to assist detection sensitivity or selectivity?[21]

7. Selection of Mobile Phase[30]

- Selection of mobile phase is always done in combination with selection of column/stationary phase.
- Combination of Acetonitrile, Methanol, Tetrahydrofuran with water (or an aqueous buffer) give a sufficient range of polar and hydrogen bonding interactions with solute to separate a very large numbers of compounds in RP-HPLC.

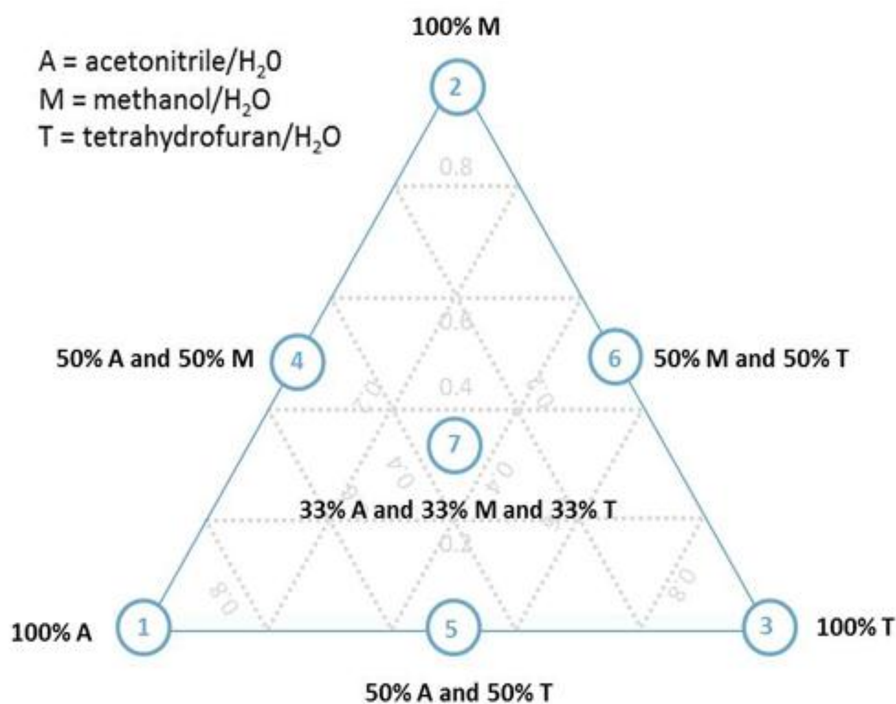

Figure 6: Preferable order of choice of solvent mixtures for HPLC method development.[31,32]

- Experiments shall be conducted using different aqueous buffers having varing strength and pH if optimum separation is not achieved with solvent combination (Acetonitrile, Methanol & Tetrahydrofuran with water) to obtain the required peak symmetry and separation. Care should be taken to keep the pH of the buffer between 2 – 8, as most of the column doesn't withstand the pH outside the range. [23,34,35]

Table 2: Properties of various commonly used HPLC solvents.[26,31,32,33]

Solvent	UV Cut – Off (nm)	Viscosity (mPa)	Polarity	Elution Strength ε°(C18)	Other Comments
Water	190	1.0	10.2	NA	NA
Acetonitrile	190	0.37	5.8	3.1	Low operation pressure
Methanol	205	0.60	5.1	1.0	NA
Tetrahydrofuran	215	0.55	4.0	3.7	Slowly oxidises and take longer time to equilibrate with stationary phase

Table 3: pH range of various commonly used buffers for HPLC method development.[31,32]

Buffer	pKa	pH Range	UV Cut - Off (nm)
Phosphate	2.1, 7.2, 12.3	1.0-3.1, 6.2-8.2, 11.3-13.3	<200
Citrate	3.1, 4.7, 6.4	2.1-4.1, 3.7-5.7, 5.4-7.4	230
Carbonate	6.1, 10.3	5.1-7.1, 9.3-11.3	<200
Formate	3.8	2.8-4.8	210 (10 mM)
Acetate	4.8	3.8-5.8	210 (10 mM)
Ammonia	9.3	8.3-10.3	210 (10 mM)
Borate	9.2	8.2-10.2	NA

- A rule of thumb known as **"2 pH Rule"** is very useful in predicting pH of the buffer with respect to the pKa value of analyte.

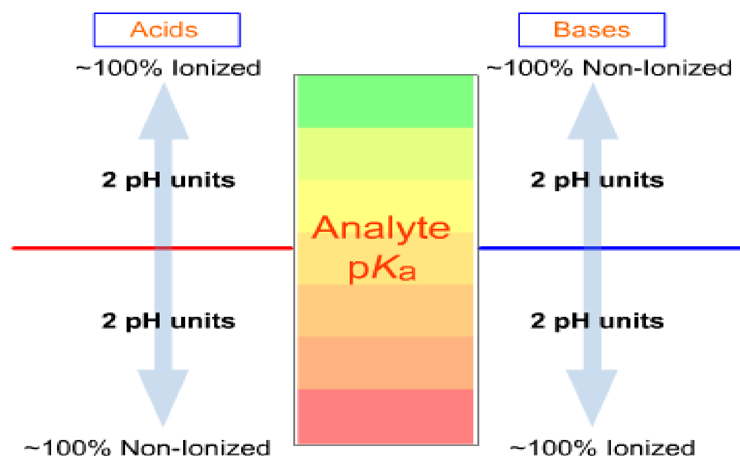

Figure 7: A rule of thumb – "2 pH Rule" for selection of pH of the buffer.[31,32]

- Acids show an increase in retention as pH is reduced, while bases show decrease in retention as pH is reduced.

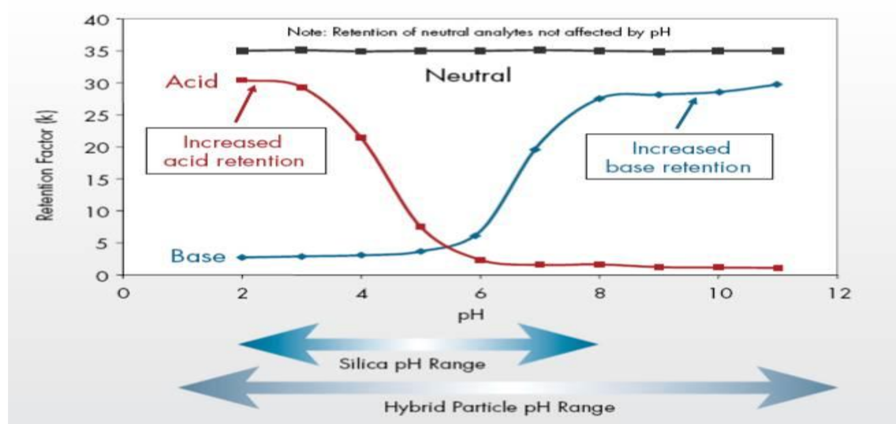

Figure 8: Retention of the analytes (Acids, Bases and Neutral Compounds) with respect to the pH of the buffer.[30]

- If acidic or basic analytes are present then ion suppression agent (for weak acids or bases) or ion paring agents (for strong acids or bases) may be required as additive in mobile phase for desired peak symmetry and separation.[21]

8. Selection of Column/Stationary Phase

Appropriate column selection is also a very important for method development. For reverse phase chromatography a wide variety of columns are available covering a wide range of polarity by cross linking the Si-OH group with alkyl chain like C4, C8, C18, Nitrile group(-CN), Phenyl group(-C6H6) and Amino group(-NH2). The most common stationary phase used in reverse phase chromatography is C18 as it can separate a wide range of compounds when the solvent is properly chosen.

- Length and diameter of the column
- Packing material chemistry
- Size of the particle
- Percentage Carbon Loading
- End Capping.

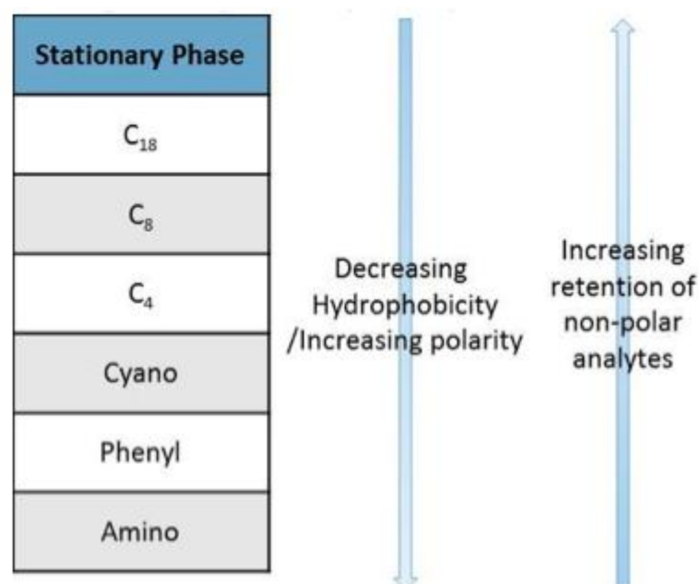

Figure 9: Polarity of various commonly used column for HPLC method development.[31,32]

Various parameters of stationary phase which are to be considered are, It is desirable to investigate separation on a range of same type of column to validate the effect of carbon load and end capping on peak symmetry.

9. Selection of Detector and Detection Wavelength

Following consideration must be given while selecting detector for the method,[21]

- Do the analytes have chromophores to enable UV detection?
- Will the sample require chemical derivatization to enhance detectability and/or improve the chromatography?
- What detection limits are necessary?
- Is more selective/sensitive detection required?

Different types of detectors are available such as [36-38]

- Ultraviolet(UV), most commonly used in pharmaceutical industry
- Photo Diode Array(PDA), is very well suited for method development
- Fluorescence
- Electrochemical
- Light Scattering
- Refractive Index(RI)
- Flame Ionization Detection(FID)
- Evaporative Light Scattering Detection(ELSD)

- Corona Aerosol Detection(CAD)
- Mass Spectrometric(MS)
- NMR, and others.

UV spectra of analytes must be taken and overlaid with each other, normalized the spectra due to different amounts present in the mixture and finally select a wavelength which is most common and give higher response for all analytes. For the greatest sensitivity λ_{max} that gives adequate response for all the analytes should be used.[36] UV wavelength below 200 nm should be avoided because detector noise increases in this region, while higher wavelength give greater sensitivity.[21]

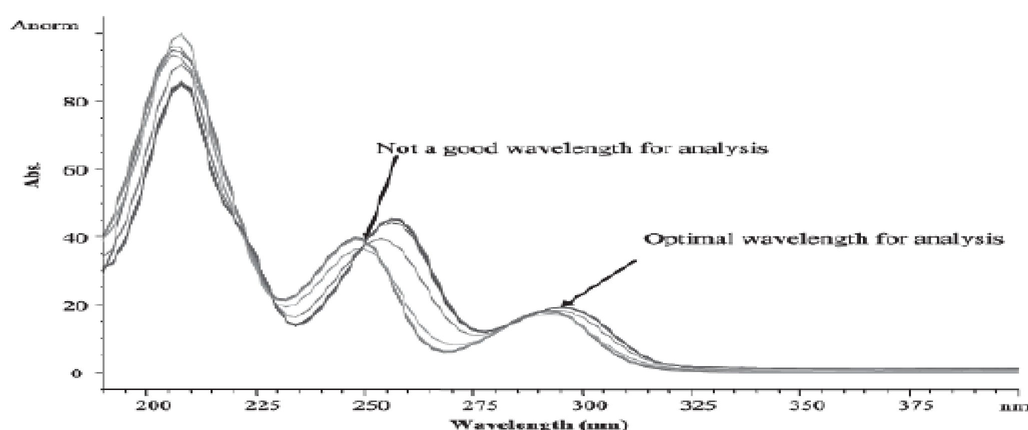

Figure 10: Overlay spectra for selection of detection wavelength.[36]

10. Selection of Solvent Delivery System[39]

Gradient scouting can be used as the fastest way to determine whether analytes is to be separated using isocratic solvent delivery or gradient solvent delivery system. In this technique, a broad gradient is run with selected mobile phase, stationary phase, and expected chromatographic conditions. Lets, T_g be the time over which the solvent composition is changed and T_e be the difference between the first and last desired peak eluted.

- If ratio of $T_e/T_g > 0.40$, gradient run is suggested.
- If ratio of $T_e/T_g < 0.25$, isocratic run is suggested.
- If ratio of T_e/T_g is between 0.25 – 0.40, either type of run may be possible.[19,39]

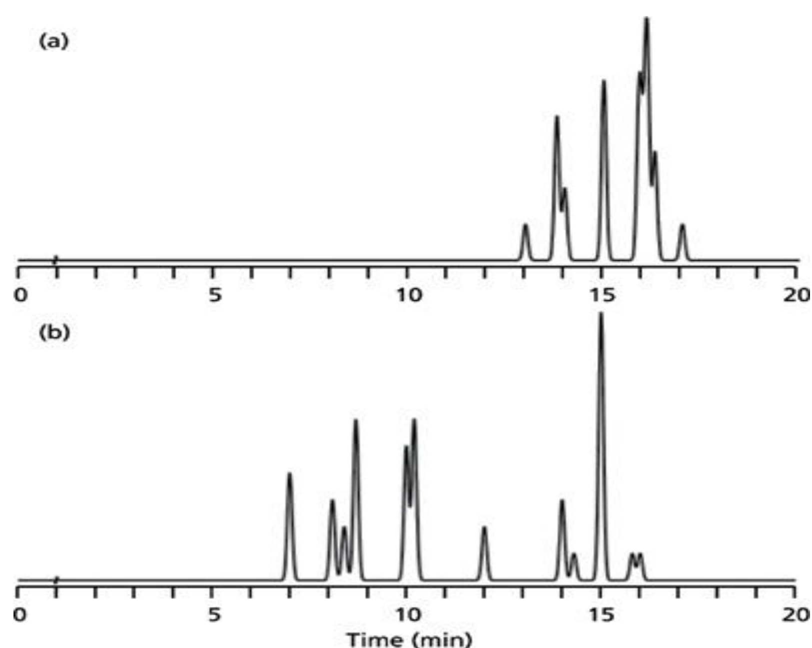

Figure 11: Simulated chromatograms for gradient scouting runs using a 150 mm × 4.6 mm column run at 2 mL/min with a 5–95% B gradient over 20 min.[39]

In this simulated condition (a), isocratic run may be possible with T_e/T_g of 0.2 and in simulated condition (b), gradient run is the possibility with T_e/T_g of 0.45.

Further, fine tuning and optimization of the method is required. If isocratic elution should be used, then good starting point is the solvent composition half-way between the first and the last peak. If gradient elution should be used, then priority for gradient type should be as follows,

Broad Gradient > Steeper Gradient > Segmented Gradient

11. Selection of Flow Rate

Selection of flow rate shall be based on,

- Retention time
- Column back pressure
- Separation of analytes
- Peak Symmetry
- Theoretical Plates
- Flow rate preferably shall not be more than 2.5ml/min.

12. Selection of Column Temperature

It is preferable to optimize the chromatographic condition with column temperature as ambient. However, if required to obtain desired peak symmetry and separation, the column temperature above ambient (30- 60°C) can be adopted. Column temperature affects the relative retention and peak symmetry of the analytes. Higher temperature allow for faster equilibration between mobile phase and stationary phase allowing for faster flow rate and thus shorter analysis time.[40,41]

13. Selection of Test Concentration and Injection Volume

- The test concentration is generally chosen based upon the response of the analytes and impurities at the selected detector wavelength.
- For a low concentration solution, injection volume could be increased but it is to be ensured that the selected injection volume for the column is not overloaded, resolution and peak symmetry are not compromised.

14. Forced Degradation Study[13,14,42-45]

These studies are undertaken to deliberately degrade the sample and evaluate the analytical method's ability to measure an active ingredient and its degradation products, without interference, by generating potential degradation products. These study comprises a series of chemical and physical stress tests. Typical test conditions are;

- **Heat/Thermal Stress**

Drugs are susceptible to degradation at higher temperature as the rate of reaction increases with increase in temperature (Arrhenius Equation). Thermal degradation is conducted normally at 40 – 80ºC. The most widely accepted temperature is 70ºC both at low and high humidity for 1 – 2 months. High temperature (>80ºC) may not produce predictive degradation pathway. Solid drug products should be exposed to both dry heat as well as wet heat, while liquid drug products should be exposed to only dry heat.

- **Photostability**

Photostability testing is conducted by exposing the drug to light of wavelength in the range of 300 – 800 nm and the most commonly recommended illumination is 1.2 – 6 million lux hours to cause the photolytic degradation.

- **Hydroltytic Stress**

Hydrolytic stress test is a common chemical degradation reaction of the analyte with water. Apart from water, hydrolysis reactions are normally performed over a wide range of pH by exposure of the sample to acidic or basic catalyzed stress conditions. Hydrochloric acid (0.1 – 1 M) for acid hydrolysis and sodium hydroxide or potassium hydroxide (0.1 – 1 M) for base hydrolysis are the most common and suggested as suitable reagents for hydrolysis. The hydrolytic stress testing normally is conducted at room temperature with or without co - solvent and if no degradation appears, continues under higher temperature of 50°C to 70°C. Stress testing normally should not exceed more than 7 days. The degraded sample is then neutralized before injection using suitable acid, base or buffer, to avoid further decomposition.

- **Oxidation**

Oxidative stress testing is one of the most conducted stress testings of drug degradation. When testing for oxidation, the common suggestion is to use hydrogen peroxide in the concentration range of 3% to 30%. But also other oxidizing agents can be used e.g. metal ions, oxygen, and radical initiators. It is suggested to conduct the degradation study initially at room temperature before opting for extreme conditions.

It is very crucial to know how much degradation is sufficient to provide adequate and reliable data. When the sample is overstressed this can lead to secondary degradation. Secondary degradation would not be formed in formal stability studies and would not support the purposeful stress testing. When stressing too little some degradation pathways may not be identified and when the samples are stressed too much it can result in unrealistic degradation. The extent of the stress applied in forced degradation studies should ensure formation of the desired amount (usually varies between 5 to 30%) of degradation. Not always forced degradation studies result in product degradation. The degradation experiments can be stopped if no degradation is observed after drug product has been exposed to a stress that exceeds accelerated stress conditions.

15. Peak Purity[46-48]

Peak purity analysis is an evaluation technique for detecting the presence of coeluting impurities with the analyte peak. Running a peak purity check prior to analytical quantitation helps to ensure accuracy. In the development of analytical methods, peak purity analysis can

reveal the presence of contamination during standardization and, by doing so, can prevent the subsequent generation of false analytical data.

For impurity detection with a single wavelength UV/visible detector, one must see a shoulder, valley or excessive tailing to suspect the presence of an impurity. The absence of these features on the chromatographic peak are not an assurance of peak purity. The impurity was not "seen" because the chromatographic resolution is too low, less than R=0.3, or the impurity concentration is quite low.

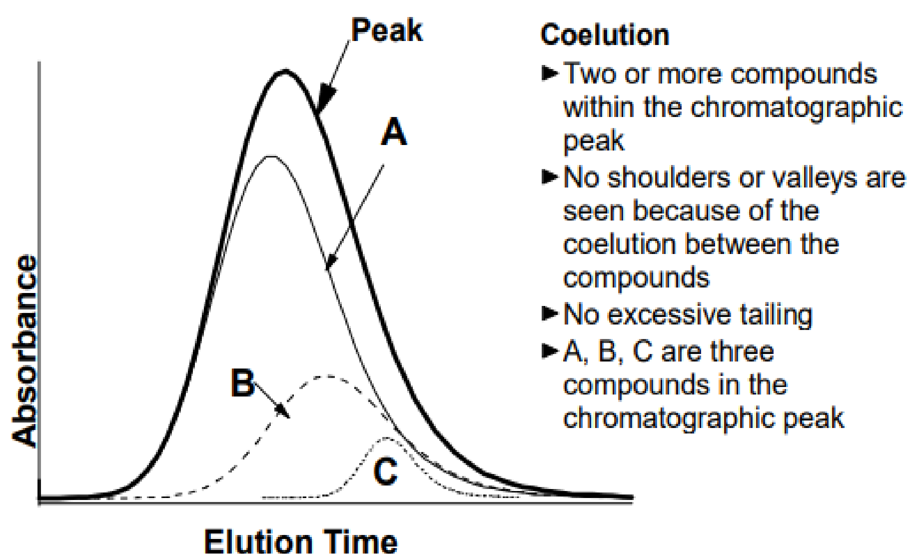

Figure 12: Coelution of three compounds A, B and C.[46]

A photodiode array detector can provide additional information using the acquisition of spectra to determine "peak purity". Various techniques employed for peak purity determination with PDA detector are;

- **Signal Overlay**

Based on the principle that two different compounds are unlikely to exhibit identical absorption over multiple wavelengths, the presence of an impurity is revealed by the deviation of the profiles. Peak profile acquired at several wavelengths is overlayed. Peaks free of impurities exhibit good overlap, but the presence of an impurity is indicated by a shift in the retention time maximum at different wavelengths.

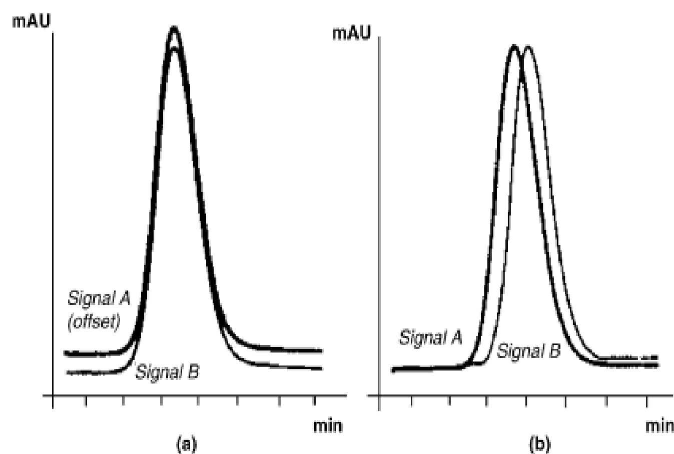

Figure 13: Normalized signals for (a) pure and (b) impure peaks.[47]

In addition to overlaying signals, their ratios ca be calculated and plotted. The resulting ratiograms are sensitive indicators of peak purity. Any significant distortion of the ideal rectangular form of the ratiogram indicates an impurity.

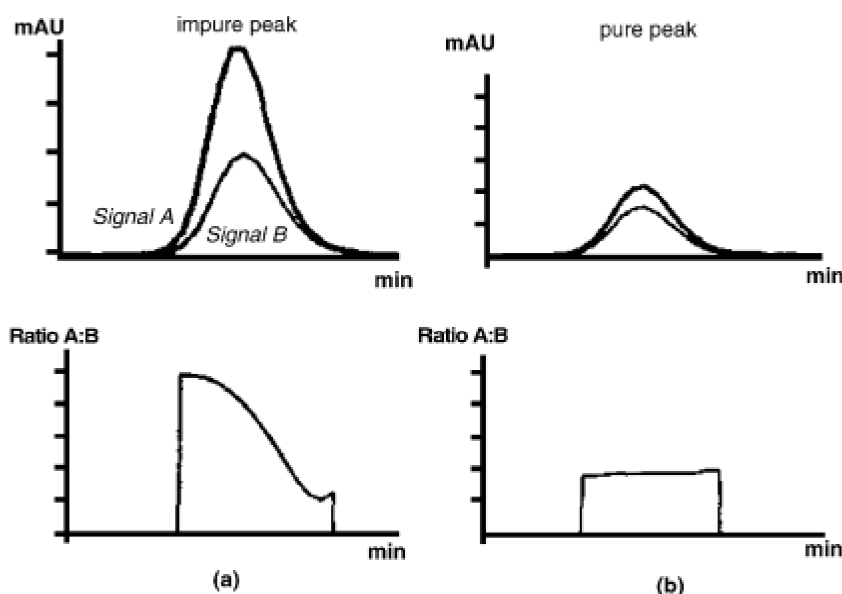

Figure 14: Ratiograms for (a) pure and (b) impure peaks.[47]

- **Spectral Overlay**

Comparing peak spectra is probably the most popular method to discover an impurity. If a peak is pure all UV-visible spectra acquired during the peak's elution or migration should be identical. The results obtained by comparison of these spectra against each other should be very close to a perfect 100% match. Significant deviations can be considered as an indication of impurity.

- **Similarity Factor**

It is a numerical value to characterize the degree of dissimilarity of the peak spectra, a so-called similarity factor, based on the match of the peak spectra to one another. At the extremes, a similarity factor of 0 indicates no match and 1000 indicates indentical spectra.

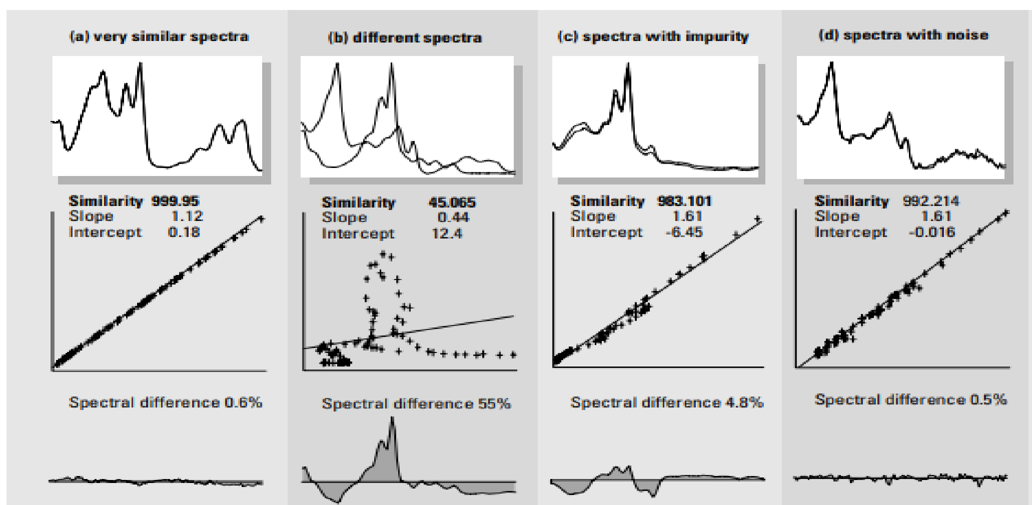

Figure 15: Graphical display of similarity factor for different pairs of normalized spectra.[48]

- **Similarity and Threshold Curve**

Similarity curves are plots of retention times versus similarity factors computed by comparing spectra across an eluted peak with one or more selected spectra. The threshold curve is a plot of retention time versus a similarity factor threshold, below which the presence of an impurity cannot be distinguished from spectral noise. If an impurity is present at a detectable concentration, the similarity curve will intersect the threshold curve.

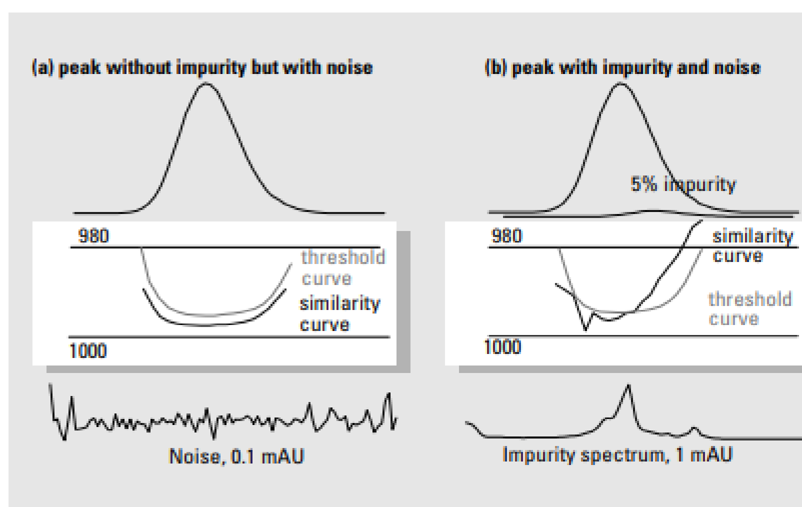

Figure 16: Effect of impurity and noise on similarity and threshold curves.[48]

- **Similarity/Threshold Ratio**

The ratio of the similarity and threshold curves is displayed as a single curve.

Ratio = (1000 – similarity)/ (1000 – threshold)

If the ratio is less than 1 the test for that spectrum passes, if it is greater than 1 then it fails.

- **Purity Ratio**

The purity value of each single spectrum is displayed as the logarithm of the difference from the threshold value. For a spectral pure peak the ratio values are below unity and for spectral impure peaks the values are above unity.

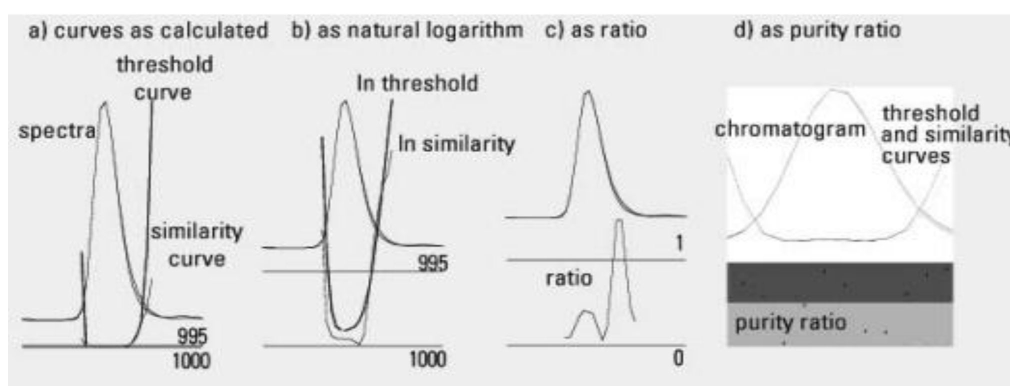

Figure 17: a) Threshold and Similarity Curves, b) ln Threshold and ln Similarity, c) Similarity/Threshold Ratio, d) Purity Ratio.[48]

METHOD VALIDATION[49-64]

Before transferring the developed analytical method to laboratory for routine implementation it is necessary to demonstrate that it is suitable for its intended purpose by performing analytical method validation.[49] Following validation characteristics are to be addressed during analytical method validation.

Table 4: Validation characteristics for analytical method validation as per ICH - Q2(R1).[19,65,66]

Validation Characteristics	Type of Analytical Procedure			
	Identification	Testing for Impurities		Assay
		Qauntitative	Limit	-Dissolution -Content/Potency
Accuracy	-	+	-	+
Precision	-	+	-	+
Specificity	+	+	+	+
Detection limit	-	-	+	-
Quantitation limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

1. System Suitability

System Suitability is to be demonstrated by injecting 6 replicate injections of the standard solution.

Acceptance Criteria

- System Suitability Parameters like Resolution,Tailing Factor, Number of Theoretical Plates(NTP) should comply for all analyte peaks.
- RSD ≤ 1%.

2. Accuracy

The accuracy of an analytical procedure expresses 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.

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations/3 replicates each of the total analytical procedure).[65]

Acceptance Criteria: Percent recovery of known amount added should be 95 - 105%.

3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels,

- **Repeatability:** Repeatability expresses the precision under the same operating conditions over a short interval of time. It is also termed as intra-assay precision.

  Repeatability should be assessed using:

a) A minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each); or

b) A minimum of 6 determinations at 100% of the test concentration.[65]

Acceptance Criteria: Individaul RSD ≤ 2%.

- **Intermediate Precision:** Intermediate precision expresses within-laboratories variations like different days, different analysts, different equipments, etc.

Acceptance Criteria: Individual and Overall RSD ≤ 2%

- **Reproducibility:** Reproducibility expresses the precision between laboratories.

Acceptance Criteria: Individual and Overall RSD ≤ 2%

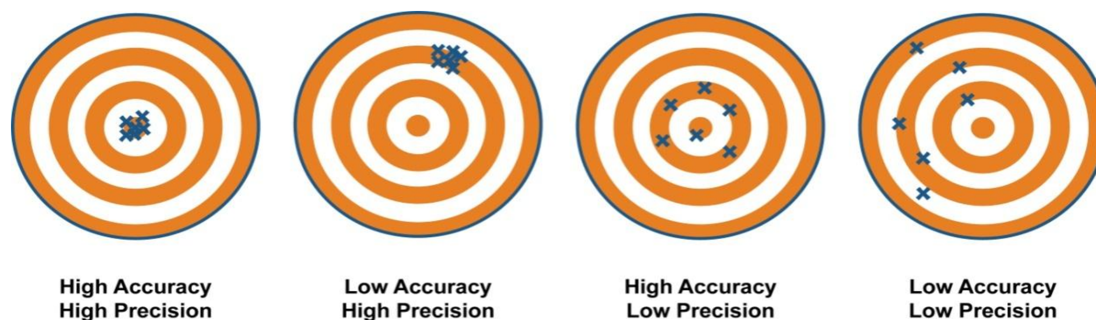

Figure 18: Relationship between accuracy and precision.[67]

4. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components like impurities, degradants, matrix, etc which may be expected to be present.

Acceptance Criteria:

- No interference from blank i.e. no peak in the blank should coelute (i.e. have same retention time) with any of the analyte peak.
- All analyte peaks should be well separated from its adjacent peak (i.e Resoluiton ≥2).
- All analyte peaks should pass the peak purity criteria to show that the analyte chromatographic peak is not attributable to more than one component.

5. Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value.

A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.[33,65]

Based on the Standard Deviation of the Response and the Slope of the calibration curve;

$LOD = 3.3\sigma/S$

Where σ = the standard deviation of the response

S = the slope of the calibration curve

6. Limit of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

A signal-to-noise ratio of 20 or 10:1 is generally considered acceptable for estimating the quantitation limit.[33,65]

Based on the Standard Deviation of the Response and the Slope of the calibration curve;

LOQ = 10σ/S

Where σ = the standard deviation of the response

S = the slope of the calibration curve

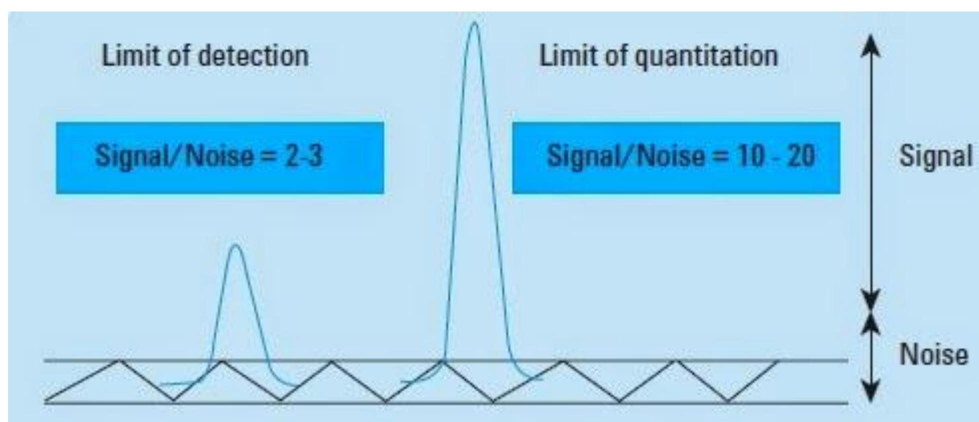

Figure 19: Acceptable S/N ratio for determination of LOD and LOQ.[68]

7. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. For establishment of linearity, a minimum of 5 concentrations is recommended.[65]

Acceptance Criteria: The regression line must have correlation coefficient ≥0.999.

8. Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

The following minimum specified ranges is acceptable,

Table 5: Specified range of various analytical parameter as per ICH - Q2(R1).[40,65]

For Assay	80 – 120 % of the test concentration
For Content Uniformity	70 – 130 % of the test concentration
For Dissolution	± 20 % over the specified range
For Determination of Impurity	From the reporting level to 120 % of the specification

9. Robustness

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

In case of HPLC, examples of typical variations may be,[65]

- Influence of variations of pH in a mobile phase (±0.2)
- Influence of variations in mobile phase composition (±2%)
- Different columns (different lots and/or suppliers)
- Temperature (±5°C)
- Flow rate (±10%)
- Detection wavelength (±5nm)

Acceptance Criteria: System Suitability Parameters like Resolution, Tailing Factor, Number of Theoretical Plates (NTP) should comply for all analyte peaks.

10. Stability of Sample Solution

Stability of samples is established to determine if special storage conditions are necessary, for instance, refrigeration. It is demonstrated by preparing one sample as per the method and analysing it initially and at different time intervals viz. 6 hrs, 12 hrs, 18 hrs and 24 hrs stored at normal laboratory condition. If the sample is not stable in normal laboratory condition, the stability study need to be performed in the cooling module at 4 – 8 °C.

Acceptance Criteria: RSD ≤ 1%

Statistical analysis of validation data shall be used to evaluate validation characteristics against predetermined acceptance criteria. All statistical procedures and parameters used in the analysis of the data should be based on sound principles and appropriate for the intended evaluation. Several statistical methods are useful for assessing validation characteristics, for example, percentage, mean, variance, standard deviation or coefficient of variation,

correlation coefficient, y-intercept and slope of the regression line, residual sum of squares, confidence interval, etc as appropriate.

Once an analytical procedure is successfully validated and implemented, the procedures (lifecycle management) should be followed during the life cycle of the product to continually assure that it remains fit for its intended purpose.[49]

Furthermore revalidation of analytical procedure may be necessary in the following circumstances,[69]

- Change in analytical procedure.
- Change in the route of synthesis of the drug substance.
- Change in manufacturing process or formulation.

The degree of revalidation depends upon nature of the change. Decision of revalidation of all or parts of the analytical procedure shall be based on risk based evaluation.

CONCLUSION

Analytical QbD offers the benefits of more through understanding of the method's capabilities and limitations, thus delivers more robust method in comparison to classical OFAT approach. In addition, analytical QbD could significantly reduce the efforts relating to post approval variation. Analytical QbD includes in sequence; defining ATPs, CMAs, CMPs, DoE, establishing design space & control strategies, and future method lifecycle management. Forced degradation study helps in validating stability indicating analytical procedure. Here drug substances or drug products are exposed to hydrolysis, heat, light and oxidizing agents to produce approximately 5% to 30% degradation of active substance. Peak purity analysis is very useful in chromatographic method development and validation, to confirm that all components have been chromatographically separated and thus ensure accuracy of the method.

ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatography

QbD: Quality By Design

QRM: Quality Risk Management

OFAT: One factor at a time

LOD: Limit of Detection

LOQ: Limit of Quantitation

UHPLC: Ultrahigh Pressure Liquid Chromatography

LC-MS: Liquid Chromatography-Mass Spectrometry

2D-LC: Two-Dimensional Liquid Chromatography

DOE: Design of Experiment

UV: Ultraviolet

PDA: Photo Diode Array

RI: Refractive Index

FID: Flame Ionization Detection

ELSD: Evaporative Light Scattering Detection

CAD: Corona Aerosol Detection

MS: Mass Spectrometric

NMR: Nuclear Magnetic Resonance

REFERENCES

1. Bhardwaj SK, Dwivedi K, Agarwal DD. A Review: HPLC Method Development and Validation. International Journal of Analytical and Bioanalytical Chemistry, 2015; 5(4): 76-81.
2. Bhatt DA, Rane ST. QbD Approach to Analytical RP-HPLC Method Development and its Validation. International Journal of Pharmacy and Pharmaceutical Sciences, 2011; 3(1): 179-187.
3. Gholve SB, Ajgunde RR, Bhusnure OG, Thonte SS. Analytical Method Development and Validation by QbD Approach – A Review. Pelagia Research Library, 2015; 6(8): 18-24.
4. Pande PP, Sayyad SF, Chavan MJ, Chaudhari SR. Quality by Design in Analytical Method Development and Validation. Journal of Environment and Life Sciences, 2017; 2(2): 39-45.
5. Deepa M, Reddy KR, Satyanarayan SV. A Review on Quality by Design Approach for Analytical Method Development. Journal of Pharmacy Research, 2017; 11(4): 272-277.
6. Thakor NS, Amrutkar SV. Implementing Quality by Design(QbD) in Chromatography. Austin Journal of Analytical and Pharmaceutical Chemistry, 2017; 4(1): 1-5.
7. Sumithra M, Ravichandiran V. Review on Quality by Design. International Journal of Frontiers in Science and Technology, 2014; 2(4): 47-56.

8. Pharmaceutical Development Q8(R2). International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (ICH), August 2009.
9. Summers M, Fountain KJ. A Quality by Design (QbD) Based Method Development for the Determination of Impurities in a Peroxide Degrade Sample of Ziprasidone. Waters Corporation, 2011.
10. Xue G, Harwood J, Cheng C, Fortin D, Wang J, Reid G, McBrien M, Vazhentsev A. Streamlined QbD HPLC Method Development with Automated Data Processing. Advanced Chemistry Development, Inc.
11. Tei A. Quality by Design (QbD) Solutions for Analytical Method Development – A Systematic Approach to Reducing Variability. Agilent Technologies.
12. Tang Y. Quality by Design Approaches to Analytical Methods – FDA Perspective. U.S. Food and Drug Administration, 2011.
13. Bossunia MTI, Urmi KF, Shaha CK. Quality-By-Design Approach to Stability Indicating RP-HPLC Analytical Method Development for Estimation of Canagliflozin API and It's Validation. Pharmaceutical Methods, 2017; 8(2): 92-101.
14. Rajkotwala AS, Sirajuddin S, Dedania ZR, Dedania R, Vijendraswamy SM. QbD Approach to Analytical Method Development and Validation of Piracetam by HPLC. World Journal of Pharmacy and Pharmaceutical Sciences, 2016; 5(5): 1771-1784.
15. Jadhav ML, Tambe SR. Implementation of QbD Approach to the Analytical Method Development and Validation for the Estimation of Propafenone Hydrochloride in Tablet Dosage Form. Chromatography Research International, 2013; 1-9.
16. Badhe N, Savkare A, Bagmar U. Quality By Design (QbD) Based Development and Validation of RP – HPLC Method for Amlodipine Besylate and It's Impurities from Bulk and Formulation. International Journal of Pharma and Bio Sciences, 2017; 8(3): 170-189.
17. Paul C, Fabel S, Squibb A. Automated Approach to HPLC Method Development. Thermo Fisher Scientific, 2017. Available from: URL: https://www.rdmag.com/article/2017/02/automated-approach-hplc-method-development.
18. Mishra K, Sing BK. A Review on Method Development and Validation by Using HPLC. International Journal for Pharmaceutical Research Scholars, 2016; 5(16): 71-81.
19. Rao G, Goyal A. An Overview on Analytical Method Development and Validation by Using HPLC. The Pharmaceutical and Chemical Journal, 2016; 3(2): 280-289.
20. Sirard T. Fundamentals of HPLC. Waters Corporation, 2012.

21. Sabir GA. HPLC Method Development and Validation for Pharmaceutical Analysis. Pharmaceutical Technology Europe, 2004; 16(3). Available from: URL: http://www.pharmtech.com/hplc-method-development-and-validation-pharmaceutical-analysis.
22. Thomas AL. Design of Experiments for Analytical Method Development and Validation. BioPharm International, 2014; 27(3). Available from: URL: http://www.biopharminternational.com/design-experiments-analytical-method-development-and-validation.
23. Patil PN. HPLC Method Development – A Review. Journal of Pharmaceutical Research and Education, 2017; 1(2): 243-260.
24. Shah BP, Jain S, Prajapati KK, Mansuri NY. Stability Indicating HPLC Method Development: A Review. International Journal of Pharmaceutical Sciences and Research, 2012; 3(9): 2978-2988.
25. Rao BV, Sowjanya GN, Ajitha A, Rao VUM. A Review on Stability Indicating HPLC Method Development. World Journal of Pharmacy and Pharmaceutical Sciences, 2015; 4(8): 405-423.
26. Reviewer Guidance: Validation of Chromatographic Methods. Center for Drug Evaluation and Research (CDER), November 1994.
27. Analytical Method Development and Validation. Available from: URL: http://shodhganga.inflibnet.ac.in/bitstream/10603/8513/9/09_chapter%202.pdf.
28. Ravisankar P, Navya N, Pravallika D, Sri ND. A Review on Step-by-Step Analytical Method Validation. IOSR Journal of Pharmacy, 2015; 5(10): 07-19.
29. Hema, Reddy GS. A Review on New Analytical Method Development and Validation by RP-HPLC. International Research Journal of Pharmaceutical and Biosciences, 2017; 4(3): 41-50.
30. HPLC Method Development. Available from: URL: http://quimica.udea.edu.co/~carlopez/cromatohplc/desarrollo-metodos.html.
31. Reversed Phase Chromatography. Available from: URL: http://www.chromacademy.com/lms/sco5/Theory_Of_HPLC_Reverse_Phase_Chromatography.pdf.
32. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development. John Wiley & Sons Inc; 2011.
33. Method Development and Validation of HPLC Method. Available from: URL: http://shodhganga.inflibnet.ac.in/bitstream/10603/22707/8/08_chapter%203.pdf.

34. Gupta V, Jain ADK, Gill NS, Gupta K. Development and Validation of HPLC Method – A Review. International Resdearch Journal of Pharmaceutical and Applied Sciences, 2012; 2(4): 17-25.
35. Sanap GS, Zarekar NS, Pawar SS. Review on Method Development and Validation. International Journal of Pharmaceutics and Drug Analysis, 2017; 5(5): 177-184.
36. Pathy K. How to Start Analytical Method Development. Available from: URL: https://www.myexperiment.org/files/807/download.
37. Phani RS, Prasad KRS, Mallu UR. Scientific Approach for RP-HPLC Method Development: Complete Review. International Journal of Science Innovations and Discoveries, 2012; 2(6): 218-228.
38. Thammana M. A Review on High Performance Liquid Chromatography(HPLC). Research and Reviews: Journal of Pharmaceutical Analysis, 2016; 5(2): 22-28.
39. John WB. Making the Most of a Gradient Scouting Run. LCGC Europe, 2013; 26(1): 18-22. Available from: URL: http://www.chromatographyonline.com/making-most-gradient-scouting-run?id=&sk=&date=&pageID=3#.
40. Saimalakondaiah D, Kumar VR, Reddy TRM, Ajitha A, Rao VUM. Stability Indicating HPLC Method Development and Validation. International Journal of Pharma Research and Review, 2014; 3(10): 46-57.
41. Tiwari P, Sing BK. HPLC: A Modern Approach of Development and Validation. World Journal of Pharmaceutical Research, 2016; 5(9): 1616-1631.
42. Charde MS, Welankiwar AS, Kumar J. Method Development by Liquid Chromatography with Validation. International Journal of Pharmaceutical Chemistry, 2014; 04(02): 57-61.
43. Von V, Janzen H, Susanowo A. Forced Degradation Studies – Comparision between ICH, EMA, FDA and WHO Guidelines and ANVISA's Resolution RDC 53/2015. Rheinischen-Friedrich-Wilhelms-University Bonn, 2016.
44. Iram F, Iram H, Iqbal A, Husain A. Forced Degradation Studies. Journal of Analytical & Pharmaceutical Research, 2016; 3(6): 2-5.
45. Rawat T, Pandey IP. Forced Degradation Studies for Drug Substances and Drug Product – Scientific and Regulatory Considerations. Journal of Pharmaceutical Sciences and Research, 2015; 7(5): 238-241.
46. Waters 996 Photodiode Detector: Peak Purity, What is Peak Purity Analysis? Performance Perspective, Waters Corporation, 1996.
47. Papadoyannis IN, Gika HG. Peak Purity Determination with a Diode Array Detector. Encyclopedia of Chromatography, 2003; 1-5.

48. Stahl M. Peak Purity Analysis in HPLC and CE using Diode – Array Technology. Agilent Technology, 2003.
49. Sood S, Bala R. Method Development and Validation Using HPLC Techniques – A Review. Journal of Drug Discovery and Therapeutics, 2014; 2(19): 23-29.
50. Patil R, Deshmukh T, Patil V, Khandelwal K. Review on Analytical Method Development and Validation. Research and Reviews: Journal of Pharmaceutical Analysis, 2014; 3(3): 1-10.
51. Sangeetha M, Reichal CR, Indulatha VN, Thirumoorthy N. Development and Validation of RP-HPLC Method: An Overview. Research and Reviews: Journal of Pharmaceutical Analysis, 2014; 3(4): 8-14.
52. Toomula N, Kumar A, Kumar S, Bheemidi VS. Development and Validation of Analytical Method for Pharmaceuticals. Journal of Analytical and Bioanalytical Techniques, 2011; 2(5): 1-4.
53. Sowjanya P, Subashini D, Rakesh S. A Review on Method Development and Validation by HPLC. Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences, 2015; 4(1): 42-50.
54. Pushpa LE, Sailaja B. Bioanalytical Method Development and Validation by HPLC: A Review. Journal of Medical and Pharmaceutical Innovation, 2014; 1(6S): 1-9.
55. Prathap B, Rao GHS, Jegannath S, Swathikrishna KV, Priyanka V. A Review – Analytical Method Development and Validation by RP-HPLC. Asian Journal of Research in Chemistry and Pharmaceutical Sciences, 2016; 4(2): 37-44.
56. Kirthi A, Shanmugam R, Prathyusha MS, Basha DJ. A Review on Bioanalytical Method Development and Validation by RP-HPLC. Journal of Global Trends in Pharmaceutical Sciences, 2014; 5(4): 2265-2271.
57. Murugan S, Elayaraja A, Niranjan BM, Chandrakala K, Prathap NK, Ramaiah P, Chathurya V. A Review on Method Development and Validation by Using HPLC. International Journal of Novel Trends in Pharmaceutical Sciences, 2013; 3(3): 78-81.
58. Srivastava RK, Kumar SS. An Updated Review: Analytical Method Validation. European Journal of Pharmaceutical and Medical Research, 2017; 4(09): 774-784.
59. Prathap B, Rao GHS, Devdass G, Dey A, Harikrishnan N. A Review on Stability Indicating HPLC Method Development. International Journal of Innovative Pharmaceutical Research, 2012; 3(3): 229-237.

60. Shah RR, Shah RS, Pawar RB, Gayakar PP. An Analytical Method Development of HPLC. International Journal of Institutional Pharmacy and Life Sciences, 2015; 5(5): 506-5013.
61. Pithiya GL, Patel NK, Patel AB, Vyas AJ, Patel A. A Review on Bioanalytical Method Development and Validation by LC-MS/MS. Journal of Pharmaceutical and Medicinal Chemistry, 2016; 2(1): 95-101.
62. Meduri MP, Agarwal P, Vimala G, Banu N. The Development and Validation Studies of RP-HPLC Method – A Review. World Journal of Pharmaceutical Sciences, 2016; 4(1): 85-92.
63. Kumar V, Bharadwaj R, Gupta G, Kumar S. An Overview on HPLC Method Development, Optimization and Validation Process for Drug Analysis. The Pharmaceutical and Chemical Journal, 2015; 2(2): 30-40.
64. Sanjay KD, Kumar DRH. Importance of RP-HPLC in Analytical Method Development: A Review. International Journal of Pharmaceutical Sciences and Research, 2012; 3(12).
65. Validation Of Analytical Procedures: Text and Methodology Q2(R1). International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (ICH), November 2005.
66. Chauhan A, Mittu B, Chauhan P. Analytical Method Development and Validation: A Concise Review. Analytical &Bioanalytical Techniques, 2015; 6(1): 1-5.
67. Uncertainty, Precision and Accuracy. Available from: URL: http://climatica.org.uk/climate-science-information/uncertainty.
68. Huber L. Validation of Analytical Methods, Germany: Agilent Technologies, 2010.
69. Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), July 2015.