Chapter I - General Introduction
Focus on impurities and degradation products

Over the last one and a half decade a sea change has been occurred in the definition of ‘quality’ of pharmaceuticals by the introduction of a series of International Conference on Harmonisation guidelines\(^1\)–\(^4\).

Due to this the Focus has been shifted from ‘purity’ to ‘impurities’ (IMPs) in drug substances and their ‘degradation products’ (DPs) in finished pharmaceutical products. There are select examples which are having IMPs and DPs induce side effects in human beings. DP of tetracycline, i.e., epianhydrotetracycline, causes Fanconi’s Syndrome leading to renal failure\(^5\). The Polymeric DPs in aminopenicillins cause allergenicity and not the drugs themselves\(^6\)\(^,\)\(^7\). The most recent case is the presence of oversulfated chondroitin sulfate in heparin samples that caused allergenic reactions and over 100 deaths\(^8\).

A subset of IMPs and DPs even carry for genotoxicity, which pose an additional safety where attributed potential concern, because these molecules can cause genetic mutations, chromosomal breaks, and/or chromosomal rearrangements, leading to significant risk for carcinogenicity or other toxic effects\(^9\). Due to these reasons, intense attention has been paid by regulatory agencies world-wide and accordingly multiple guidelines have been issued for the control and testing of IMPs and DPs. Even the pharmacopoeial authorities have recently taken key policy decisions to implement possible principles and terminology of the revised ICH Q3 guidelines already published in new monographs\(^10\). Further decision has been taken to include related substances tests and list of IMPs/DPs in all the monographs, even updating of related substances test in old monographs also. For example replacement of TLC by HPLC and addition of impurity in lists where ever needed.

Today the industry is forced to invest heavily in trained manpower and buying sophisticated instruments, and also acquiring reference standards of IMPs/DPs from compendial agencies and commercial sources for the purpose of their characterization and control. All major companies now have specialized impurity and stability profiling groups. However, despite their best efforts, there are failures of products complying with stringent limits of IMPs and DPs lay down by regulatory agencies.
This is resulting in recalls of large quantities of pharmaceutical products from the market. A few examples of recall notifications issued by US FDA\textsuperscript{[11]} are listed in Table: 1.1. Similar recalls have been affected by agencies of other countries\textsuperscript{[12, 13]}.

### Table: 1.1

Typical recalls of finished pharmaceutical products by the US FDA due to the presence of IMPs/DPs (September 2011–March 2012).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Product</th>
<th>Volume</th>
<th>Declared reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adagen(Pegademasebovine) injection, 250 units/ml, 1.5ml single-dose vials, 4 vials/carton</td>
<td>249 cartons</td>
<td>IMPs/DPs: During routine stability testing, levels of IMPs were out of specification.</td>
</tr>
<tr>
<td>2</td>
<td>Azelastin hydrochloride ophthalmic solution, 0.05%(sterile), 6 ml bottles</td>
<td>155,363 bottles</td>
<td>IMPs/DPs: Total IMP results were out of specification During the analysis of 18 months controlled stability samples.</td>
</tr>
<tr>
<td>3</td>
<td>Brimonidine Tartrate ophthalmic solution 0.2% (Sterile),(sterile),15 ml bottles</td>
<td>18137 bottles</td>
<td>IMPs/DPs: 9 months stability sample does not comply specification.</td>
</tr>
<tr>
<td>4</td>
<td>Budeprion XL (Buproprion HCl ER Tablets), 300 mg</td>
<td>7718 tubes</td>
<td>IMPs/DPs: Recall is being carried out due to the potential that lots may not meet IMP specification over product shelf life.</td>
</tr>
<tr>
<td>5</td>
<td>Cyclopirox Gel 0.77% 45 g tube</td>
<td>24664 tubes</td>
<td>IMPs/DPs: Two lots did not conform to product specifications for an unspecified IMP at the 9 month stability test station.</td>
</tr>
<tr>
<td></td>
<td>Product</td>
<td>Quantity</td>
<td>IMPs/DPs:</td>
</tr>
<tr>
<td>---</td>
<td>----------------------------------------------</td>
<td>----------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>6</td>
<td>Diflorasone Diacetate Cream USP 0.05%</td>
<td>27557</td>
<td>Out-of-specification results, 0.05% were obtained for known and total DPs.</td>
</tr>
<tr>
<td></td>
<td>(a) 15 g tube (b) 30 g tube (c) 60 g tube</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Fludeoxyglucose F18 injections 20–300mCi/ml, multi-dose vials</td>
<td>8 doses</td>
<td>IMPs/DPs: Product contains residual by-products of the manufacturing process above allowances</td>
</tr>
<tr>
<td>8</td>
<td>Hydroxyzine Hydrochloride Oral Solution, USP, 10 mg/5 ml, 120 ml &amp; Pint(473 ml) bottles</td>
<td>30740</td>
<td>IMPs/DPs: Out of Specification results for an individual IMP at the 12 month room temperature time point.</td>
</tr>
<tr>
<td>9</td>
<td>Leflunomide Tablets, 10 mg, 30-count Bottle</td>
<td>32325</td>
<td>IMPs/DPs: One lot of this product does not meet IMP specifications.</td>
</tr>
<tr>
<td>10</td>
<td>Migergot (Ergotamine Tartrate and Caffeine) Rectal Suppositories USP</td>
<td>10968</td>
<td>IMPs/DPs: Product was out of specification for a known DP, ergotaminine.</td>
</tr>
<tr>
<td>11</td>
<td>Pediatric Atropine Sulfate Injection, USP, 0.05 mg/ml; Single Dose</td>
<td>37100</td>
<td>IMPs/DPs: Out of specification result for total IMPs at the 8 months Stability testing interval.</td>
</tr>
<tr>
<td>12</td>
<td>Prednisolone Sodium Phosphate Oral Solution, 15 mg (base)/5 ml</td>
<td>24980</td>
<td>IMPs/DPs: Out of specification for related compounds at the 12-month room temperature stability time point.</td>
</tr>
</tbody>
</table>
13 Ropinirole Hydrochloride Tablets, 0.25 mg, 0.5 mg, 1 mg And 2 mg, 100-count tablets per bottle 1,103,81 IMPs/DPs: Ropinirole Hydrochloride Tablets may not meet the specification for a known IMP over the product shelf life.

14 Topiramate 25 mg & 200 mg tablets 82653 IMPs/DPs: High out of specification results for IMP.

15 TYLENOL Gelcaps ® Cold Multi-Symptom Nighttime/Rapid Release 2,372,47 IMPs/DPs: The level of chlorpheniramine ammonioacetate (CPAA) was Slightly higher Than expected Some TYLENOL® in Cold Multi-Symptom Night time Rapid Release Gelcaps.

Types of impurities and degradation products

Impurity profile is the description of identified and unidentified impurities present in new drug substances. Impurities can be described as shown in Table:1.2. Impurities have been named differently or classified as per the ICH\textsuperscript{14} as follows:

a) Common names
- By-products
- Degradation products
- Interaction products
- Intermediates
- Penultimate intermediates
- Related products
- Transformation products
Table: 1.2 Description of impurity types and their sources

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Impurity type</th>
<th>Impurity source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Process-related drug substance</td>
<td>- Organic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Starting material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Intermediate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- By-product</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Impurity in starting material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Inorganic</td>
</tr>
<tr>
<td>2</td>
<td>Process-related drug product</td>
<td>- Reagents, catalysts, etc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Organic</td>
</tr>
<tr>
<td>3</td>
<td>Degradation drug substance or drug</td>
<td>- Degradation products</td>
</tr>
<tr>
<td></td>
<td>product</td>
<td>- Excipient interaction</td>
</tr>
</tbody>
</table>

b) United State Pharmacopoeia
The United States Pharmacopoeia (USP) classifies impurities in various sections
- Impurities in Official Articles
- Ordinary Impurities
- Organic Volatile Impurities
c) ICH Terminology
According to ICH guidelines, impurities in the drug substance produced by chemical synthesis can broadly be classified into the following three categories;
- Organic Impurities (Process and Drug related)
- Inorganic Impurities
- Residual Solvents
Organic impurities may arise during the manufacturing process and or storage of the drug substances may be identified or unidentified, volatile or non-volatile, and may include
- Starting materials or intermediates
- By-products
- Degradation products
ICH limits of impurities

With respect to listing of IMPs/DPs in registration dossiers for drug substances and drug products, respectively, the various types are ‘specified’, ‘unspecified’, ‘identified’ and/or ‘unidentified’. Evidently, IMPs for which structural characterization has been achieved are the ones considered as ‘Identified’. Majority of the International regulatory Guidelines require that any IMP or DP at a level greater than ICH identification threshold should be identified. The prescribed identification thresholds in ICH guidelines\[^{1, 2}\] are listed Table:1.3.

**Table: 1.3** Dose-dependent thresholds for the identification of IMPs and DPs in drug substances and drug products, respectively\[^{1, 2}\].

<table>
<thead>
<tr>
<th>Maximum daily dose</th>
<th>Identification threshold for IMPs in Drug substance</th>
<th>Maximum daily dose</th>
<th>Identification threshold for DPs in drug product</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2 g</td>
<td>0.1% or 1 mg per day intake, whichever is lower</td>
<td>&lt;1 mg</td>
<td>1.0% or 5 µg per day intake, whichever is lower</td>
</tr>
<tr>
<td>&gt;2g</td>
<td>0.05%</td>
<td>1 mg–10 mg</td>
<td>0.5% or 20 µg per day intake, whichever is lower</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;10 mg–2 g</td>
<td>0.2% or 2 mg per day intake, whichever is lower</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;2 g</td>
<td>0.10%</td>
</tr>
</tbody>
</table>

**Stability indicating analytical method development**

In order to accurate detection and to quantify IMPs and DPs, a stability indicating analytical method is needed. Ideally, such a method should resolve all IMPs and DPs from the parent and from each other. As outlined by the International Conference on Harmonization (ICH)\[^{4}\], the process for establishing a stability indicating method involves stress testing: “Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help establish the
degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used”.

The impurities can be identified predominantly by following methods:

- Spectroscopic method
- Separation method
- Isolation method
- Characterization method

The regularly used method for separation of impurities and degradation products are Capillary electrophoresis (CE), Chiral Separations, Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), TLC, HPTLC, and HPLC. Currently these are used in the pharmaceutical industry, the choice is overwhelmingly gradient reversed phase high-performance liquid chromatography (RP-HPLC) with UV detection.

Analytical separations are moving rapidly toward higher resolution, improved peak capacities, and multi-dimensional approaches. Utilization of HPLC columns with smaller and smaller particles, leading to higher and higher backpressures, is resulting in dramatically improved analysis time and improved resolution. Increasing the column temperature can significantly decrease backpressure, speed up analyte diffusivity, and can lead to significantly reduced analysis times.

HPLC versus UPLC

High performance liquid chromatography (HPLC) has proven to be the predominant technology used in laboratories worldwide during the past 30-plus years. One of the primary drivers for the growth of this technique has been the evolution of packing materials used for effective separations. The underlying principles of this evolution are governed by the van Deemter equation, with which any student of chromatography is intimately familiar\footnote{15}. The van Deemter equation is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP, or column efficiency). And, since particle size is one of the variables, a van Deemter curve can be used to investigate chromatographic performance.

For many years, researchers have looked at “fast LC” as a way to speed up analyses\footnote{16,17}. The “need for speed” has been driven by the sheer numbers of samples
in some laboratories particularly in drug discovery and the availability of affordable, easy to use mass spectrometers. Smaller columns and faster flow rates (amongst other parameters) have been used. Elevated temperature, having the dual advantages of lowering viscosity, and increasing mass transfer by increasing the diffusivity of the analytes, has also been investigated\textsuperscript{[18]}. However, using conventional particle sizes and pressures, limitations are soon reached and compromises must be made, sacrificing resolution for time.

However, as the particle size decreases to less than 2.5 mm, not only is there a significant gain in efficiency; but the efficiency doesn’t diminish at increased flow rates or linear velocities. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC.

Pumps in conventional HPLC systems reach a pressure of max. 400 bars. Pumps in UPLC systems can reach pressures of 1000 bar and more. This allows the use of smaller particles (< 2.0 mm) and still produce acceptable flow rates (up to 5 ml/min). The use of smaller particles allows to

- Obtain better resolution (separation efficiency) or
- Perform faster chromatography or a combination of both or
- Increase sensitivity, due to sharper (narrower) and higher peaks

**Chemistry of small particles**

Smaller particles provide not only increased efficiency, but also the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and speed. Efficiency is the primary separation parameter behind UPLC since it relies on the same selectivity and retentivity as HPLC. In the fundamental resolution (Rs) equation: resolution is proportional to the square root of N.

\[
Rs = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k}{k + 1} \right)_{\text{Physical}} \left( \frac{k}{k + 1} \right)_{\text{Chemical}}
\]
But since N is inversely proportional to particle size (dp): as the particle size is lowered by a factor of three, for example, 5 µm (HPLC scale) to 1.7 µm (UPLC-scale), N is increased by three and resolution by the square root of three or 1.7. N is also inversely proportional to the square of the peak width:

\[ N \propto \frac{1}{W^2} \]

This illustrates that the narrower the peaks are, the easier they are to separate from each other. Also, peak height is inversely proportional to the peak width:

\[ \text{Height} \propto \frac{1}{W} \]

So as the particle size decreases to increase N and subsequently Rs, an increase in sensitivity is obtained, since narrower peaks are taller peaks. Narrower peaks also mean more peak capacity per unit time in gradient separations, desirable for many applications, e.g. peptide maps. Still another equation comes into play when migrating toward smaller particles:

\[ \text{FR}_{\text{opt}} \propto \frac{1}{dp} \]

This relationship also is revealed from the van Deemter plot. As particle size decreases, the optimum flow F opt to reach maximum N increases. But since back pressure is proportional to flow rate, smaller particle sizes require much higher operating pressures, and a system properly designed to capitalize on the efficiency gains; a system that can both reliably deliver the requisite pressures and that can maintain the separation efficiency of the small particles with tightly managed volumes. Efficiency is proportional to column length and inversely proportional to the particle size:

\[ N \propto \frac{L}{dp} \]

Therefore, the column can be shortened by the same factor as the particle size without loss of resolution. Using a flow rate three times higher due to the smaller particles and shortening the column by one third (again due to the smaller particles),
the separation is completed in 1/9 the time while maintaining resolution. Although high efficiency, nonporous 1.5-µm particles are commercially available, they suffer from poor loading capacity and retention due to low surface area. Silica-based particles have good mechanical strength but can suffer from a number of disadvantages, which include a limited pH range and tailing of basic analytes. Polymeric columns can overcome pH limitations, but they have their own issues including low efficiencies, limited loading capacities and poor mechanical strength.

Packing a 1.7µm particle in reproducible and rugged columns was also a challenge that needed to be overcome, however. A smoother interior surface for the column hardware, and re-designing the end frits to retain the small particles and resist clogging were necessary. Packed bed uniformity is also critical, especially if shorter columns are to maintain resolution while accomplishing the goal of faster separations\textsuperscript{[19]}

**Advantages\textsuperscript{[20]}**

- Better resolution (separation efficiency)
- Faster chromatography
- Better sensitivity (sharper and higher peaks)
- Less solvents
- Withstand high back pressure system

**Disadvantage\textsuperscript{[20]}**

- Higher price of instruments, spare parts and columns
- Also detector and data collection system (CDS) may not cope with sharper peaks (data acquisition rate).
- So far only binary pump systems (not ternary or quaternary). This may make method transfer not straightforward.
- Number of stationary phases still limited (improves quickly)

**Potential areas of use\textsuperscript{[20]}**

- Analysis of complex mixtures (e.g. impurity profiles, formulation inerts)
- At-line analysis in manufacturing (analysis at the vessel)
- Analysis of large amounts of samples
- For LC/MS to get better spectra (improved signal to noise)
**A Quality by Design approach to analytical methods**

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. Rather, a systematic screening approach that evaluates a number of stationary phases, pH ranges and organic modifiers provides a more thorough approach to method development. A quality by Design (QbD) approach to method development uses statistical design of experiments (DoE) to develop a robust method ‘design space’. The design space defines the experimental region in which changes to method parameters will not significantly affect the results.

A potential QbD approach to analytical methods can be exemplified as follows:

**Step 1: Define method intent**\(^{21-25}\)

The goal of LC method development have to be clearly defined, as pharmaceutical QbD is a systematic, scientific, risk based, holistic and proactive approach that begins with predefined objectives and emphasizes product and process understanding and control. The ultimate goal of the analytical method is to separate and quantify the impurities as well as main compound.

**Step 2: Perform experimental designs**

A systemic experimental design is needed to assist with obtaining in-depth method understanding and performing optimization. Here an efficient and comprehensive experimental design based on systemic scouting of key components of the RP-LC (mobile phase composition, mobile phase delivery system, and column, pH, and flow rate and column temperature) is presented. The QbD forms a chromatographic database that will assist with method understanding, optimization and selection. In addition to this, it can be used to evaluate and implement change of the method and it should be needed in the future, for example should the chromatographic column used no longer be commercially available, or an impurity is no longer relevant.
Initially an experimental design comprised of a standard set of 2 columns, 3 pH values and 4 mobile phases (2 compositions, 2 delivery systems) developed, after this a set of 3 column temperatures, 3 flow rates was developed to further optimize the peak symmetric properties. This led to a total of 30 (2 column x 3 pH x 4 mobile phases, + 3 column temperatures + 24 x 3 flow rates) chromatographic conditions. In addition, it enabled the creation of a database that describes the relationship of the compound retention and possible RP-LC conditions.

**Steps 3: Evaluate experimental results and select final method conditions.**

These method conditions were evaluated using the three tiered approach. At the first level, the conditions were evaluated for peaks symmetry, peak fronting and peak tailing. At the second level, condition was evaluated for the better separation of impurities and drug substances.

**Step 4: Perform risk assessment with robustness and ruggedness evaluation.**

As the final method is selected against method attributes, it is highly likely that the selected method is reliable and will remain operational over the lifetime of product. Therefore, the evaluation of method robustness and ruggedness to be carried out as the fourth step method development is mainly for the method verification and finalization. A risk-based approach based on the QbD principles set out in ICH Q8 and Q9 was applied to the evaluation of method robustness and ruggedness. Structured methodologies for risk assessment, such as Cube plots or 3D surface plots can be implemented to identify the potential risk of the method due to a small change of method parameters or under a variety of conditions such as different laboratories, analysts, instruments, reagents, days etc.

A) Robustness

To establish the robustness of test method and to demonstrate its reliability for minor changes in chromatographic condition

B) Ruggedness

The ruggedness of analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, different instruments, different lots of reagents, different assay, temperatures, different days, different analysts, etc.
Step 5: Define analytical method performance control strategy

As a result of robustness and ruggedness studies, the overall method gives us an understanding about method performance under various conditions can be improved. The analytical method performance control strategy along with appropriate system suitability criteria can be defined to manage risk and ensure the method delivers and the desirable method attributes. If the risk is high and is hard to manage, it is an opportunity for the analyst to go back to the database described in step 2 to find a more appropriate method and to go through the procedure as described to ensure method robustness and ruggedness.

OFAT versus Factorial design

The traditional approach would be to use a one-factor-at-a-time (OFAT) optimization where each factor or parameter to be optimized is systematically varied while the other factors are fixed at a specific value.

Factorial designs are a powerful, underutilized set of tools for the analytical scientist. Factorial designs consist of a series of experiments in which factors are varied simultaneously, rather than one at a time. The most basic factorial design examines each factor at two separate levels. Known as a two-level factorial design, the number of experiments required can be determined by the formula \( n = 2^x \) where \( n \) represents the number of experiments and \( x \) is the number of factors being studied. Therefore, a two-level factorial design for optimizing three factors would require a total of 23 or eight experiments. In a factorial design, every possible combination of factors is represented (Fig: 1.1).

![Figure 1.1: OFAT vs. Factorial Cube design](image-url)
Factorial designs are often denoted in a matrix format known as the design matrix. Table 1.4 shows that the standard design matrix for a two-level factorial design for three factors. In the matrix, the levels are represented with the standard notation of -1 and 1. By common convention, the -1 level represents the lower level for a factor whereas 1 represents the higher level. The design matrix provides a guide for the analyst to identify the conditions for each experiment. All the analyst has to do now is to perform the series of experiments with the factors and levels according to the specifications in the design matrix. It is also important for the analyst to run the experiments in random order to eliminate any experimental bias.

**Table 1.4** Two level Factorial design matrix for three factors

<table>
<thead>
<tr>
<th>Experiment No</th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>4</td>
<td>-1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>7</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Once the experiments have been conducted and the response for each experimental run has been recorded, the question of how to analyse the data arises. Using several simple statistical tools, the analytical scientist can easily identify the combination of factors which produces the best results. Most statistical software packages such as Minitab, SAS, SPSS, Design Expert or even Microsoft Excel have the tools required to analyse data from a factorial design. Analysis of variance (ANOVA) is often used to determine the effect size for each of the factors as well as a measure to identify the factors that have a statistically significant effect on response. However, for determining the optimal levels for each factor, the most useful tools are graphical plots of the data, such as contour plot, 3D surface plot and cube plot are used.
Contour plot

The contour plot (Fig: 1.2) is a two-dimensional representation of the response across the select factors. The full range of two factors at a time can be displayed. If there are more than two factors the 2D surface can be thought of a slice through the factor space.

Fig: 1.2 Typical Contour plot.

3D Surface

The 3D Surface plot (Fig: 1.3) is a projection of the contour plot giving shape to the color. Except for zoom functions, the 3D surface has all the same options as the contour plot plus the ability to rotate the plot.

Fig: 1.3 Typical 3D Surface plot
Cube Plot

Cube plots are useful for representing the effects of three factors at a time. They show the predicted values from the coded model for the combinations of the –1 and +1 levels of any three factors that you select. (Fig: 1.4)

Fig: 1.4 Typical cube plot.

Method validation of related substances

“Methods validation is the process of demonstrating that analytical procedures are suitable for their intended use” (ICH Topic Q2B, March 1995)

"Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical application -”

Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories. The type of validation programme required depends entirely on the particular method and its proposed applications.

Typical analytical parameters used in validation include:

- Specificity
- Precision
- Accuracy
- Linearity
- Range
- Robustness
- Limit of detection
- Limit of quantification

Specificity

Ability to assess unequivocally the analyte in the presence of components which may be expected to be present (impurities, degradants, matrix).
Aspects:
- Identification
- Purity tests
- Assay (Content/potency)

Precision

Closeness of agreement (‘scatter’) between a series of measurements obtained from multiple sampling of the same homogeneous sample.
Aspects
- Repeatability
- Intermediate precision
- Reproducibility

Accuracy

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.

Linearity

Ability (within a specified range) to obtain test results which are directly proportional to the concentration of analyte in the sample
Aspects:
- Test across the range (at least 5 concentrations)
- Evaluate linearity by visual inspection of the plot and by statistical techniques
- Calculate corr. coefficient, y-intercept, slope and res. sum of squares
Range

Interval between upper and lower concentration of the analyte in the sample for which it has been demonstrated that the procedure has a suitable level of precision, accuracy and linearity.

Aspects:
- Defined from linearity study
- Depends on the application of the method (assay, dissolution, content uniformity)

Robustness

Measure of the capacity of a method to remain unaffected by small variations in method parameters.

Aspects
- To be considered during development
- To be used for establishment of system suitability criteria
- Include testing of stability of solutions
- To be tested by introducing small variations in method parameters

Detection limit

Lowest amount of an analyte in a sample which can be detected but not necessarily quantitated.

Method
- Based on visual evaluation
- Based on signal-to-noise ratio (3:1)
- Based on st.dev. (SD) of response and slope (DL=3.3xSD/S)
- Report results and method of choice

Quantification limit

Lowest amount of an analyte in a sample which can be quantitatively determined with a suitable precision and accuracy.

Method
- Based on visual evaluation
- Based on signal-to-noise ratio (10:1)
- Based on st.dev. (SD) of response and slope (DL=10xSD/S)
- Report results and method of choice
Relative Response Factor (RRF)

Relative Response Factor (RRF) is an analytical parameter used in chromatographic procedures to control impurities/degradants in drug substance and drug product. RRF is used to correct the difference in detector response of impurities with analyte peak. RRF is established by slope method with linear range of solutions. Different Pharmacopoeias refer the term RRF differently.

As per United States Pharmacopoeia (USP) The Relative Response Factor, is the ratio of the responses of equal amounts of the impurities and the drug substance[27]. The USP refers RRF as a Correction factor or Response factor or Relative response factor.

As per European Pharmacopoeia (Ph.Eur) the Relative detector response factor, commonly referred as Response Factor, expresses the sensitivity of a detector for a given substance relative to a standard substance. The correction factor is reciprocal of the response factor[28]. Ph.Eur refers RRF is a Correction factor or Response factor.

As per British Pharmacopoeia (BP) the Response Factor is a relative term, being the response of equal weights of one substance relative to that of another in the conditions described in the test[29]. BP refers RRF as Response factor.

Establishment of RRF is required to avoid the stability issues with standards, to reduce the cost on preparation of Impurity Standards, to reduce Maintenance of Impurity Standards, due to the lack of donation of Impurity Standards, difficulty in synthesis and isolation of Impurity Standards, for convenience and time saving. Relative Response factor (RRF) is used in different stages: Phase 1 to Phase 4 studies, in drug purity tests, Mass balance tests, in limit tests, in stability indicating methods etc.
Role of Mass Balance during method development:

Mass balance correlates the measured loss of a parent drug to the measured increase in the amount of degradation products. It is a good quality control check on analytical methods to show that all degradation products are adequately detected and do not interfere with quantization of the parent drug (i.e., stability-indicating methods). Regulatory agencies use mass balance to assess the appropriateness of the analytical method as a stability-indicating method and determine whether all degradants have been accounted for [30].

In mass balance calculations, the loss of parent drug or the amount of drug remaining is determined from a sample assay, and the increase in degradation products is determined by a related substances method. The fundamental approach for determining mass balance is to quantitative the decomposition peaks using degradation methods and then reconcile the measured loss in the parent drug with the amount of degradation products. If the loss in potency can be reasonably accounted for by the amount of degradants measured, then mass balance is achieved.

The assessment of degradation in pharmaceutical products involves two aspects of analytical measurement. Firstly, a specific or selective analytical method must be available for accurate assay of parent drug compound, in order to measure any loss. Second, methodology should be in place for quantification of the degradation products formed. Ideally, when degradation occurs, the measured amount of parent drug lost should correlate well with the measured increase in degradation products. This correlation is referred to as “mass balance” [31].

More recently, the International Conference on Harmonization (ICH) has provided definition of “mass balance; material balance” as follows: The process of adding together the assay value and levels of degradation products to see how closely these add up to 100% of initial value, with due consideration of the margin of analytical precision.
Mass balance is also useful in method validation\cite{32}. In order to demonstrate that analytical methods are stability-indicating, unstressed and stressed materials are often compared. Any increase in degradation product that correlates well with loss of parent drug, aids in demonstrating that the methods can accurately assess degradation. Mass balance is also important in understanding alternative degradation pathways\cite{33}. For example, consider a situation where both acid catalyzed and oxidative degradation produces a substantial loss of parent compound in stress-testing studies. If good mass balance is achieved for the acid-catalyzed degradation, but not for the oxidative degradation, further work to better understand the oxidative degradation pathway(s) is warranted. It may be that the poor mass balance in the latter case results from important oxidative products that are unaccounted for or from structures, which need to be more fully elucidated to understand response factor differences. Mass balance is an important consideration in assessing degradation pathways of pharmaceutical products. Often, response factor differences between degradation products and the parent compound are responsible for mass balance problems. RRFs should, therefore, be incorporated, when possible, in the quantification of degraded samples.

Mass balance in pharmaceutical analysis is very important for several reasons. By demonstrating the degardative losses of parent drug correlate well with the measured increase in degradation products unaccounted for. Conversely, if one observes, for example, a 20% loss of parent drug but only measures a 5% increase in degradation products, it is likely that additional degradation products formed are not accurately determined by the given method. Because unknown degradation products could potentially be toxic or otherwise compromise the safety of drug, it is important to have methods that detect all major degradation products. Thus, safety is the major reason for the study of mass balance.
Aim and Scope of work:

Aim:

Analytical methods used for the impurities analysis of active pharmaceutical ingredients (API) and finished pharmaceutical product by HPLC having limitations such as longer run time, longer re-equilibration time and usage of maximum solvent. So author planned to develop the analytical methods with shorter run time.

Methods are commonly developed using 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. So author decided to work on systematic screening approach to development and optimization of method to determine the drug in the presence of its organic non-volatile impurities in active pharmaceutical ingredients and finished pharmaceutical products.

Objective:

The author focused on systematic screening of various factor such as number of stationary phases, pH ranges, column temperature and organic modifiers, which provides a more thorough approach to method development with in short span of time using UPLC.

A Quality by Design (QbD) is a statistical approach to design of experiments (DoE) and to develop a robust method ‘design space’. The design space defines the experimental region where in which changes occur in method parameters will not significantly affect the results.

The author has selected Omeprazole and Domperidone active pharmaceutical ingredients, two non-pharmacopeial drug substance i.e Sparfloxacin and Dxlansoprazole and one finished pharmaceutical product (Amlodipine Besylate and Benazepril HCl Capsule) for determination of related substances and other drugs with combination of four sartans (Candesartan, Irbesartan, Telmisartan, Valsartan) in active pharmaceutical ingredients.
Scope:

When analytical method performed on UPLC gives better resolution (separation efficiency), faster chromatography (shorter run time), better sensitivity (sharper and higher peaks), less solvents, less injection volume and withstand high back pressure.

Software driven method optimization affords considerable time savings for the scientist and the use of QbD can produce a significantly more robust and quality submission to regulatory authorities.

A QbD based method development provides better understanding of the overall method capabilities and limitations in development ensures a greater chance of successful method validation, transfer and routine use.
REFERENCES

28. European Pharmacopoeia 7.0, Section 2.2.46 Chromatographic Separation Techniques, 2010.