Chapter 1

Introduction

And

Review of Literatures
1.1 Introduction

Impurities have a huge financial impact on the cost of a drug substance / product as practical yield, physico-chemical characteristics and Chromatographic purity are important factors involved in API and FP.

Any material- desired or undesired, that affects the purity of a drug substance or product is considered as an impurity. Impurities gets incorporated in the drug pathway usually from the starting material(s), intermediate (s) (whRG1 may be reactive / non-reactive or one whRG1 can probably undergo dimerisation), penultimate and pre-penultimate intermediates, by-product(s), transformation product(s), interaction product(s), related substance(s), degradation product(s), solvent(s), catalyst(s), tautomers and also stereoisomers whRG1 can also be well-defined under the subject of Chiral impurities.

Impurities might form due to rearrangements in the moiety or in-situ reactions wherein the reagents and by-products are not separated and can participate in incomplete reactions, over-reactions, isomerisations and undesired during reaction

Reaction of reagent and chemicals in the synthetic pathway, intentionally or unintentionally leads to interaction impurities such as API and expcipient interaction(s)

Impurity that has structural similarity to the drug substance, may exhibit similar biological activity. However, it does not ensure similar biological activity.

Impurities may also be formed due to the decomposition. It also includes impurities whRG1 enter the drug pathway during storage, formulation and also from aging. Impurities can also be from extraneous contaminants.

If one enantiomer is the active ingredient, the other isomer can be termed as impurity. The Polymorhic form of a drug substance can also add to its impurity profile, thereby reducing the drug quality and efficacy.
1.2 Classification of Impurities

Any impurity/ Impurities can be classified into the following characteristics:

1. Organic Impurities
2. Inorganic impurities
3. Residual solvents
4. Chiral impurities
5. Impurities arising due to Polymorphic forms.
6. Genotoxic Impurities based on the structural alerts and whRG1 can also be any of the above listed class of impurities.

Organic impurities can enter the drug synthesis pathway during the manufacturing process and/or storage and packaging of the new drug substance. They may be identified / unidentified, specified/unspecified, volatile / non-volatile, and also include:

1. Starting materials.
2. Reaction by-products.
3. Reaction Intermediates (whRG1 may be reactive species).
5. Reagents, ligands and catalysts.

Inorganic impurities, also, arise from the manufacturing process. They are usually known and identified. Inorganic impurities include:

1. Reagents and catalysts (Phase transfer catalysts, Ligands and Metal chelates).
2. Heavy metals and/or other residual metals.
3. Inorganic salts.
4. Other materials (e.g. Packaging materials, Storage containers, filter aids, charcoal)

Solvents are inorganic or organic liquids that are used as vehicles for preparing solutions or suspensions both in the synthesis of new drug substances as well as in the formulation of the drug product. Since database for the toxicity profiling of these solvents is available, the residue of these solvents in the drug substance can be aptly controlled.

Chiral impurities may occur owing to the presence of chiral centre(s) in the reaction intermediates which introduce chirality in the final moiety. These impurities may exhibit differential ADME and PK-PD pattern. These impurities should be appreciably controlled, so that the desired efficacy is achieved.

Genotoxic impurities in drug substance(s) are substances that are known to be potentially mutagenic or carcinogenic, especially those capable of causing genetic mutation. Genotoxic impurities can cause -

- Potential damage to DNA
- Genetic Mutations
- Chromosomal breaks
- Chromosomal rearrangements.

Genotoxic Impurities can be classified into the following categories:

- Organic impurities Eg. Hydrazine, Nitroso compound, Aromatic Nitro compounds, etc.
- Inorganic impurities Eg. Arsenic, Palladium, Platinum, etc.
- Residual solvents E.g. Pyridine, Benzene, Methyl chloride, etc. (1,2,3)

**RATIONAL E FOR DEVELOPMENT OF RELATED SUBSTANCES METHODS**

**1.3 IMPURITY PROFILE STUDY IN PHARMACEUTICAL DOSAGE FORMS**

Globally, impurity profiling (i.e., characterization i.e., qualitative and quantitative determination of impurities associated with drug substances or drug products) is looked upon as an indispensable and much-needed part of quality requirements. Regulatory authorities like United States Food and Drug Authority (USFDA) (4), European Directorate of Quality Medicine (5)
(EDQM), Therapeutic Goods administration\(^6\), World Health Organization (WHO)\(^7\) and other health agencies\(^8\text{-}^{11}\) have also emphasized on the purity requirements by identifying and quantifying the impurities in the API and Formulation. A vital component of the overall quality of a pharmaceutical product is the control of impurities, as their presence, even in traces, may affect the safety and efficacy of the drug product. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (RG1) guidelines\(^12\text{-}^{17}\) are developed by the joint efforts of regulators and industry representatives from the European Union, Japan and the United States. These guidelines ensure that different regions have requirements that are consistent for the data to be submitted to the respective regulatory agencies. As per RG1 Q3A (R2)\(^1\) and RG1 Q3B (R2)\(^2\) guidelines, unknown impurities associated with bulk drug and dosage form or the final drug product, greater than the identification threshold should be identified and quantified. The impurity profiles for pharmaceutical products require a basis for well reasoned and rational argument that seeks to limit both the number and amount of impurities on the grounds of safety. The efficacy, safety and the dosage of drug product determines the limit for these impurities in the drug product. The daily dose of active substance administered is different for different products. Though ignored often, it is obvious that this will have an influence on the amount of impurity administered when impurities are controlled on a percentage basis or parts per unit basis\(^18\text{-}^{19}\). Therefore, impurity profiling for any pharmaceutical drug product is a critical part of product development when necessitated and triggered the development of the modern chromatographic methods for the qualitative and quantitative determination of impurities associated with any drug product.

The toxicological assessor of a regulatory agency cannot necessarily reassure on safety, by simply limiting an impurity to a lower percentage level. Some drugs are administered orally at doses of \(>\text{30 g/day}\), whereby even \(0.01\%\) of an uncharacterized impurity gives the patient a potential exposure of \(>\text{3 mg daily}\). On the other hand, the analyst may not struggle to achieve lower limit (e.g. \(0.1\%\)) for detection, quantification, validation and reporting, provided the daily dose for humans is in \(\mu\text{g}\) or lower mg range. Roughly, the limit for 1 mg daily oral intake of an uncharacterized / poorly characterized impurity will possibly satisfy the safety assessment for regulatory purposes\(^{13\text{-}14}\).
It is, therefore, very essential from the safety point of view, to know the impurity profile (both qualitative and quantitative), so as to judge that an impurity will / will not pose any serious concern or to have an acceptable risk factor for treating critical ailments for particular patients for whRG1 no other therapy can be administered.

An impurity profile is generally defined by the chromatographic method(s) used to evaluate impurities in the drug substance(s) / product(s). An ideal impurity profile includes chromatograms for all the specified and unspecified impurities, enantiomers, conjugates and associated species. During formulation, application of energy to a mixture of chemicals increases the potential of reaction, thereby enhancing the chances of degradation, conjugation; adduct formation, dimerization and polymerization, changes in polymorphic form or even racemization. In fact, the energy should be greater than the activation energy of the modification, whRG1 is seldom accomplished in routine production. However, increased stress in the process can also lead to formation of impurities. These include high shear areas in the blending process, grinding, granulation, drying and tableting. Each drug substance(s) and excipient(s) has its own impurity profile and the potential to exhibit reactions and interactions.

The generic representation in Fig.2 illustrates the various types of impurities that can potentially arise during the production of a dosage form.
As per RG1 guidelines, an impurity in a new drug product is defined as any component of the new drug product that is not the drug substance or an excipient in the drug product. Impurities are usually assumed to be inferior to the related API (active pharmaceutical ingredient), since they may exhibit differential pharmacological activity.
United States Pharmacopeia (USP) deals with impurities in several of its sections, viz.,

(a) Impurities in official articles (b) Ordinary impurities (c) Organic volatile impurities

This also includes inorganic impurities, organic impurities, and impurities with biochemical origin, isomeric impurities and polymeric components.

According to the RG1 guidelines, impurities produced during chemical synthesis of the drug substance(s).

(a) **Process impurity e.g Organic impurities**

(b) **Metal impurities e.g Inorganic impurities**

(c) **Volatile impurities e.g Residual solvents**

![Flow sheet diagram for classification of impurities](image)

The word “impurities” comprises of both process impurities and degradation products. Process impurities, whRG1 are indicatively due to the process, could be either starting material,
impurities of starting materials, reagents, or products of side reactions. Degradation products are formed by the interactions of the drug substance and related compounds under certain specific conditions to which the active drug substance is susceptible to breakdown.

**Some analytical method for Imatinib Mesylate is given below.**

In this study HPLC method was developed for quantification of genotoxic impurity in Imatinib mesylate and method was validated as per RG1. No pharmacopeial method is available for chromatographic purity of Imatinib mesylate by HPLC method, only few literature are available for quantification of Imatinib mesylate in dosage form. High performance liquid chromatographic method has reported for Imatinib. The column used in this method was C18 column (waters bondapack) 15cm x 3.9mm, 5µ. Heptane sulphonic acid (Ion pair reagent) with potassium phosphate buffer is used as Buffer: RR2 (42:58). Wavelength selected was 237 nm. The method is linear form 300ppm to 800ppm with respect sample concentration. %RSD for intra- and inter-day RV5 studies was 1.7 and 2.6. RV6 ranged between 96.2 and 101.4. (20)

Another quantitative HPLC method has also been developed for detection and quantification of Imatinib mesylate in rat serum. This method demonstrated high sensitivity with lower limit of quantification of 25 ng/ml and the stability of Imatinib mesylate in serum was also studied (21).

Another developmental study has also been reported of a HPLC method with UV detection, whRG1 is also well optimized and validated for determining Imatinib mesylate concentration in biological matrices of mouse viz., brain, spleen, liver, kidney and plasma. Further optimization of the method revealed the significance of accurate pH values for sample processing and analytical conditions. The method is simple, quick and sensitive and has also been successfully employed for the quantification of accumulated Imatinib in the tissues after multiple doses of oral administration to mice. Imatinib also had appreciable tissue penetration in all tissues / organs except brain, where the procedure of multiple dose administration did not produce any accumulation (22).

Another Reverse-phase HPLC method has also been reported for the simultaneous determination of Imatinib mesylate and the related impurities in Glivec capsules. The method was also
validated statistically for its selectivity, linearity, RV5, RV6 and robustness. Due to its speed and RV6, the method may be employed for quality control analysis.\(^{(23)}\)

In this study of Imatinib mesylate indicated that, Imatinib is used for treatment of chronic myelogenous leukemia or gastrointestinal stromal tumors. Liquid chromatographic-electrospray ionization mass spectrometric (LC-MS) method was developed for quantification of Imatinib mesylate and its main metabolite in plasma. The HPLC column used for method development was Luna C18 (5 µm, 50 x 4.6 mm), Mobile phase used for 0.1% formic acid and RR2. Deuterated Imatinib was used as Internal Standard. Lower limit of quantification was 30ng/ml linearity covered from 30- 10000ng/ml. They have also proven that the suitability of the method for Imatinib for quantifying the concentrations of Imatinib and CGP 74588 in the plasma of a patient who was given 200 mg dose of Imatinib orally.\(^{(24)}\)

PATIENTS AND METHODS:
In a Phase I conducted on 64 adult patients with Philadelphia chromosome-positive leukemias. In PK parameters, plasma concentration-time curves derived for Imatinib. After one month treatment WBC defined for PD response, A model for maximum inhibition-effect was used to describe the relationship between reduction in WBC and exposure parameters of the drug.\(^{(25)}\)

A study was also conducted on Relative Response Factor by changing the High Performance Liquid Chromatography (HPLC) method conditions like different HPLC columns, Flow rate, pH, Temperature, Buffer concentration, Detector wavelength, Detectors (Ultraviolet & Photo Diode Array Detectors) and various Solvent grades and the variations in established RRF were observed. The authors have also studied the impact on RRF by changing the Robustness parameters and HPLC columns and all the results were compared. The comparison study revealed that any slight variation in method conditions affects the established RRF values.\(^{(26)}\)

In another reported study, a simple, precise and accurate method was developed for the test of Related substances of Imatinib mesylate for both Drug substances and drug products. The column used was Acquity UPLC BEH (50 mm x 2.1mm, 1.7 µm) C18 Column with column oven temperature 30°C and flow rate 0.3 mL/minute. Gradient method was set. Wavelength
used for detection was 237 nm. Good resolution were obsevred between eight related compound. The correlation coefficients for all the peak were found to be >0.999 whRG1 indicate that linearity of the method is precise. RS2 for RV5 and intermideate RV5 is found to be less that 5.0%. RV6 of all the eight peak were found to be well withint the limit of acceptance criteria. (27)

In another study, different polymorphic form (α and β) of Imatinib mesylate was studied.. The grinding and thermal behavior of Imatinib mesylate was studied by differential scanning calorimetry and X-ray powder diffraction. Molecules in the both form showed considerable conformational differences as a result of dissimilar intramolecular interactions, whRG1 stabilizes their molecular conformations. Hydrogen bonding and π–π interactions is normally useful Dimer chain arrangement. Mesylate ion is useful for the connection between dimmer ion , it determine the chain of dimmers. The neighboring chains are linked by very weak interactions: C-H….π interaction(s) in form α and π–π interaction(s) in form β. At ambient temperature, a thermal disorder was observed in the mesylate ion in form α, whRG1 was reportedly removed at low temperatures (−123°C). The β Form was found to be more stable at room temperature. (28)

Some reported analytical methods for Sorafenib tosylate is given below

The recoveries of sorafenib under the provided method conditions were found to be between 98.12 % and 100.50 %. This indicated that the commonly used excipients in the pharmaceutical formulations did not interfere in the proposed method. This method had been considered very useful for determination of Sorafenib in Pharmaceutical dosage forms. The observations of RS2, whRG1 was less than 2.0 for both intra- and inter-day variation measurements, also indicated a very high degree of RV5. This linearity range of this method is also proved to be in the range 20-120 μg /mL; whereby, this method can be applied for quantifying low levels of Sorafenib in pharmaceutical dosage forms (29).

The electrochemical reduction behavior and determination of sorafenib has also been studied by differential pulse polarography (DPP) at dropping mercury electrode (DME). In this method, the reduction of the carbonyl group to the saturated compound in a four electron process and the reduction mechanism has been proposed and discussed (30). The method was also employed in
routine practice for monitoring the plasma concentrations of Sorafenib in cancer patients. Large inter-individual variability and higher exposure in patients experiencing severe toxicity, supports the need for the therapeutic drug monitoring to ensure optimal exposure to Sorafenib \(^{(31)}\).

Another HPTLC method for the estimation of Sorafenib tosylate and its formulation has been reported. This method has also been validated as per RG1 guidelines. This method can also be used to determine the purity of the drug available from possible sources by detection of the related impurities \(^{(32)}\).

In this study a sensitive, rugged method was reported using liquid chromatography mass spectrometry for sorafenib Tosylate. Proteín precipitation was used for sample preparation using 0.1ml of plasma nd 0.5ml Acetonitrile. The analysis was performed using IS ((\((2)H(3)(15)N\) sorafenib) with waters xterra c18 column (150 mm x 2.1 mm, 3.5 \(\mu\)m) analytical column, using organic modifier: Buffer 0.01M ammonium acetate (65:35, v/v) containing 0.1% formic acid as the mobile phase and a isocratic flow at 0.2 mL / minute for 6 minutes. Analytes were monitored using using LCMS technique in positive mode. Linearity for human plasma were covered form 7.3ng/ml to 7260ng/ml with coefficient of determination value > 0.96. The values for both inter and intra day RV5 and RV6 were well within the generally accepted criteria for the analytical methods (<15%). \(^{(33)}\)

Reportedly, a rapid and highly LCMS method for assay was reported for Sorafenib in human plasma. Proteín precipitation was used for sample preparation using 0.1ml of RR1whRG1 contain IS (IS) ([2H3, 15N] standard Sorafenib and 50\(\mu\)l of plasma solution. Good separation achived when using a Waters Symmetry Shield RP8 (2.1mm x 50 mm, 3.5 \(\mu\)m) column at RT using an isocratic elution pattern with RR1/ 0.1% Formic acid in water in the ratio 65:35 (v/v) at a flow rate of 0.25 mL/ minute. The detection was performed using an electrospray ionization in the positive ion Multiple Reaction Monitoring (MRM) mode and by monitoring the ion transitions from m/z values 464.9 \(\rightarrow\) 252.0 (Sorafenib) and m/z values 469.0\(\rightarrow\)259.0 Internal Standard). \(^{(34)}\)

As reported, in this Open - label, Single - centred, Phase I Dose - Escalation study the drug safety, PK (pharmacokinetics) and drug efficacy of Sorafenib, a multi-kinase inhibitor along with
Irinotecan (a cytotoxic agent) as a combination therapy was further investigated in patients bearing advanced refractory solid tumours. Patients were administered Irinotecan 125 mg/m² and Sorafenib twice a day in dosages of 100, 200 and 400 mg (bid) (cohorts 1-3) in the Initial Dose - Escalation phase, whereas, the patients suffering from the condition of colorectal cancer (CRC) were administered fixed - dose of Irinotecan 140 mg and Sorafenib 400 mg bid (cohort 4) in the extended phase.\(^{(35)}\)

A simple UV-Spectrophotometric Method, validated for all the analytical parameters such as Specificity, RL1, RL2, Range and Linearity, RV5 – System, Method and Spiked along with the RV6, Ruggedness as well as the Sensitivity is also reported for the determination of sorafenib in both bulk drug and pharmaceutical formulations in an analytical developmental study. As reported, the method also showed excellent Linearity over the range 2 – 10 ppm with the equation for Regression: \(y = 0.079x - 0.0081\) and a RS1 value of \(r^2 = 0.999\). Recoveries were also consistent in the range of 97 – 99% with the RSD as low as 5% at the UV detection wavelength of 265 nm. The RL1 (RV2) was found to be very low (0.028 ppm) deeming the method to be very sensitive, whereas the RL2 (Limit of Quantitation) was found to be 0.085 ppm. This method being validated and highly sensitive was employed for the drug content analysis from the formulated drug preparations (tablets). Moreover, the method being rugged was also utilized for the pH metric solubility analysis of the pharmaceutical drug substance as well as the formulated drug product.\(^{(36)}\)

This study was also reportedly the first report for the solubilization of Sorafenib (SFB), a water insoluble drug, by polyamidoamine (PAMAM) dendrimers and (-) CycRL1extrin (CD). The study also reported that whole generations (G4 and G5) of PAMAM dendrimers were used. The aqueous solubility of Sorafenib was also measured in the presence of dendrimers and (-) CycRL1extrin at 30°C and at pH - 4, 7.4 and 10 using the Higuchi Rotating Bottle method. Further, the amount of solubilized SFB was also measured by a HPLC-UV method. FTIR and UV-Vis spectroscopy were also used to further confirm the complexation. From the phase solubility studies, it was also found that the PAMAM dendrimers increased the solubility of Sorafenib at pH 4. The maximum solubilizing effect was from G4 PAMAM dendrimers at pH - 4 whRG1 was up to 36 folds. (-) CD did not / slightly increased the solubility of Sorafenib.\(^{(37)}\)
References:

7. The National Health Surveillance Agency (ANVISA), Brazil.


