PEER REVIEWED

Key Challenges in API Characterisation and its Impacts

The cost of bringing a new drug to the market has soared to over $2 billion (Mullin, 2014). This results from drug development activities that are long, arduous, and costly, with complex modality and a high degree of uncertainty. This is partly due to insufficient knowledge regarding the mechanism of disease, process understanding, and keeping up with regulatory pressure to deliver drugs that are safe, efficacious, and of high quality.

This paradigm shift in the industry to build quality from the ground up has resulted in scientists investing a great deal of their effort in the early phase of drug development activities. These include understanding the ADME (absorption, distribution, metabolism, and excretion) profile of an active pharmaceutical ingredient (API; how can it be delivered and what does the body do with it?), toxicology and safety assessment (is it safe for human consumption?) and pharmaceutics (is the chemistry viable, scalable, and does it allow characterisation of APIs and controls for related compounds?). The focus of this article will be on some of the key challenges and their impact on the characterisation of APIs.

Understanding the physical and chemical properties of an API and its interaction with the excipient in its container closure system will determine the success or failure of a drug product. Some of the physical attributes of an API that can impact stability, efficacy, and release are: particle size, polymorphism, and solubility.

Particle Size

Most drugs are administered in a solid dosage form. Properties such as the particle size distribution (PSD) of an API will impact not only flowability and mixing properties but also dissolution rate, bio-availability, and content uniformity. APIs are typically micronised during formulation studies and may undergo physical stress during granulation, milling, blending, and drying. The particle size can be determined using microscopy, sieving, dynamic light scattering (DLS), etc. However, these analytical technologies may have limitations in analysis in complex matrices with various excipient or multi-APIs, as it may be hard to individually assess them. More recently, vibrational spectroscopy techniques such as infrared (IR), near-infrared (NIR), and Raman have been implemented into workstreams to characterise functional groups and perform chemical imaging. This enables the determination of the concentration and distribution of ingredients within a formulation and API particle size and distribution within the excipient.

Polymorphism

One of the key objectives in early drug development is to identify a stable form of delivery. This will ensure the API and its excipients do not alter their physical properties and prevent the drug from delivering the desired therapeutic benefit to its patients. One of the biggest challenges in pharmaceutical development is the identification of polymorphs. Polymorphism is the ability of a molecule to exist in more than one crystalline form. This may potentially impact the solubility of the API, which in turn can affect the bio-availability of the drug. The mechanisms by which polymorphism typically occurs are *packing polymorphism and conformational polymorphism.* The fundamental difference between the mechanisms is that, in packing polymorphism, molecules share the same conformation but are packed differently in 3D space. By contrast, in conformational polymorphism, the same molecule exists in different conformations in the crystalline state. Various analytical techniques can be used to determine the forms of polymorph that an API transforms into. The physical form can be determined using X-ray diffraction, IR, solid-state NMR, differential scanning calorimeters (DSC), and microscopy. Agglomeration and attritions can occur during drying, which may affect the quality of the drug product. Although particle size information is helpful, it is hard to ascertain the effect in real time. NIR and Raman spectroscopy can be deployed *in situ* or inline, which offers the opportunity to monitor critical drying parameters during the evaporation of solvents.

Solubility

Solubility is the property of a compound to dissolve in a given medium to result in a homogeneous mixture. A drug molecule with poor solubility carries a higher risk of failure, as it will be hard to achieve a desired concentration in the systemic circulation, resulting in poor therapeutic response. Factors responsible for affecting the solubility of a drug are lipophilicity, hydrogen bonding, crystal energy, and ionizability. Techniques to enhance solubility include *physical modification* such as particle size reduction, solid dispersion, modification via the hot-melt method, or solvent evaporation, among other methods. *Chemical modification* can be achieved by change of pH, buffers, derivatisation, salt formation, or surfactants. The impact of poor solubility may lead to compound precipitation, reduced target concentration, and low bio-availability. As a result, this may delay formulation development and miss key milestones. Most importantly, the burden is on the patient, who may need to take a higher dosage to get the desired therapeutic response.

Content Uniformity

Content uniformity is a critical step in guaranteeing the quality of the final solid dosage form. This is to ensure that the right concentration of API is delivered to the target site, therefore the API distribution in a tablet should be homogenous. It is a significant

challenge for formulation development scientists to ensure homogeneous blending of excipients with either low dose or potent APIs. The central aim is to accurately represent the ratios of the individual components within the formulation. Typically, content uniformity is assessed by using high performance liquid chromatography (HPLC), as it provides specificity, selectivity, and sensitivity. Although the method is accurate and reproducible, sample preparation can be time-consuming. Techniques such as NIR and Raman spectroscopy have been successfully used in the process workstream to improve efficiency by performing real-time monitoring of the blending process without the need for any sample preparation. Although vibrational spectroscopy offers information rich data, these techniques should not be used as the only analytical technique, but as a complementary approach to analysis.

Impurities in an API can significantly affect stability, efficacy, and patient safety. Impurities present in the API need to be identified to ensure that they are not mutagenic or toxic. The analysis of these low-level unknown impurities and degradants can be challenging. Regulatory bodies around the world are emphasising the purity requirements and calling for the identification of impurities in an API. Unknown impurities that are generated during the process, degradation products formed during stability studies, or impurities generated during forced degradation studies are of paramount importance. These help in the understanding of degradation mechanisms and the prediction of potential pathways for impurities. This understanding helps in optimising production conditions as a molecule moves from clinical studies to commercialisation.

Toxic Impurities

According to ICH M7 guidelines, mutagenic impurities (MTIs) are residual reagents, intermediates, degradation products, or by-products generated during production much like other impurities (ICH Expert Working Group, 2014). Their distinguishing feature is the ability to damage DNA. Reactive reagents and key intermediates often form the building blocks of a complex molecule. These typically require changes to the production process, such as the addition of a purging step or a change to the chemical production. Regulatory agencies routinely perform paper-based mutagenic risk assessment of synthetic routes and may request proof of the absence of all identified potential mutagenic impurities in the API. These toxic impurities need to be monitored and controlled, typically at the sub-ppm level. For this, it is necessary to have instrumentation that is both sensitive and specific (fit for purpose).

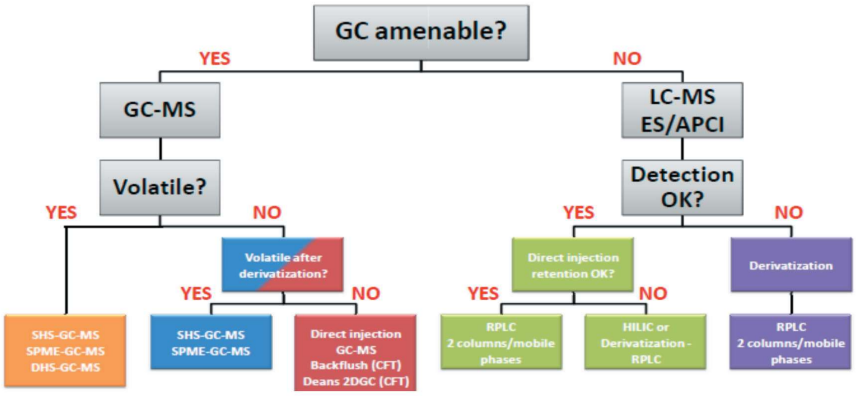


Figure 1: (Method selection flow chart for mutagenic impurity analysis for APIs)

A key challenge in identifying, detecting, and monitoring these potential mutagenic impurities is the disparate nature of these motifs. Gas chromatography (GC) relies on the volatility of the analyte, which can be modified via derivatisation. For detection, flame ionisation is probably the most widely used method, but its limitation is sensitivity. This can be overcome with a mass spectrometer (MS), or an electron capture detector (ECD), which is suitable for electronegative moieties. An LC system equipped with UV and MS detection provides selectivity, specificity, and sensitivity, especially when dealing with non-volatile and non-chromophoric impurities. Controlling heavy metals in the API is key, especially for inhalation and parenteral products, as they require a stringent control for class 1 and 2 (A and B) metals at sub-ppm levels (ICH Expert Working Group, 2014). Hence, hyphenated analytical techniques increase the sensitivity, selectivity, and specificity of a method and minimise the risk of reporting false negative results. In addition to these challenges, the sensitivity of these instruments is greatly impacted by the sample matrix. This can be overcome by selective filtration, solid phase extraction (SPE), liquid extraction, solid phase microextraction (SPME) or by QuEChERS.

Degradation Products

Degradation products are unwanted chemicals that are generated during manufacturing, transportation, or storage. These could potentially affect the efficacy and stability of the drug product, affecting the safety for the patient. In recent years, the pharmaceutical industry has developed a better understanding of extractable and leachable (E&L) species and how they are generated (David B. Lewis, 2011). Are they toxic in nature, are their control strategies, and what is their impact on patient safety? This has resulted in increased scrutiny by regulators who are concerned with migration of materials such as antioxidants, polymers, metals, inks, lubricants etc. into the drug product and their interaction with delivery and container closure systems.

Leachables that migrate into the drug product need to be identified and monitored over the shelf life of the drug product. Two factors affecting the detection limits are the route of administration and the degree of contact with packaging material. Products such as aerosols and injectables have the highest associated risk, while tablets and capsules have the lowest risk. E&L compounds can be broadly classified into non-volatiles (LC/MS is ideally suited for this type of application),

semi-volatiles and volatiles (GC/MS), and ICP-MS for elemental analysis.

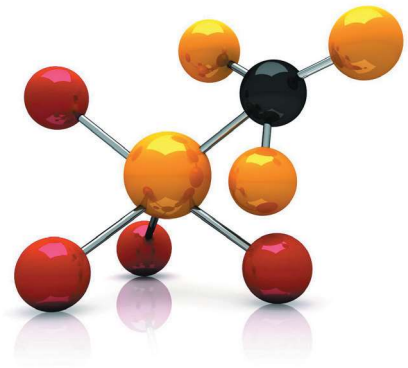
Isolation and Purification

The purity of an API depends on several factors, such as starting material, reaction kinetics (especially during scale-up), and the crystallisation process.

The ICH Q3A/Q3B guidelines for impurities in new drug substances/products states that degradation products observed during manufacturing and stability studies should be identified when present at a level greater than the *identification threshold* (ICH Expert Working Group, 2006). This is typically >0.10 % for a maximum daily dose of ≤2.0 g or >0.05 % for a maximum daily dose >2 g.

When impurities in API are known, they are often identified by their retention time and with spectral matching (UV and/or MS). However, if an unknown impurity is formed during manufacturing, isolation and purification are required if it is above the identification threshold limit. Such impurities can result from a change in supplier for a key intermediate or degradation products generated during stability studies.

Purification is a critical step in the process of drug manufacturing as it removes unwanted process-related impurities, which will ultimately affect the efficacy of the drug product. Usually, recrystallisation of the API will purge the impurities down to the desired level with a potential loss in yield. Occasionally, recrystallisation may not be an ideal option, as the risk of sample loss outweighs the benefit of purification and, in such cases, preparative HPLC is better suited. This technique requires loading of the sample on a preparative HPLC column, and fractions are collected based on retention time or mass-based fraction collection. If the impurity level is above 0.10 %, analytical scale purification system enables collection of micro to milligram quantities of impurities, which allows identification using MS and NMR. However, if the impurity level is above 0.15 %, it requires qualification of the impurity, requiring up to a few grams. Hence, it is important to optimise the



chromatography conditions such as flow rate, sample concentration, mobile phase, and selectivity on an analytical scale before scale-up is performed. This will help minimise excess solvent usage and maximise efficient method transfer.

Conclusion

To achieve greater production efficiencies and higher economies of scale, API manufacturers around the world are moving towards more agile and flexible modes of production. To manufacture APIs of high quality and to comply with global regulatory requirements, it is critical to perform the appropriate risk assessment earlier in the process. This enables insight into troubleshooting undesirable side products and to obtain a better understanding of factors that affect the efficacy, safety, and quality of a drug product.

REFERENCES

1. Mullin, R. (2014, November 20). Tufts Study Finds Big Rise In Cost Of Drug Development. Retrieved from Chemical & Engineering News: https://cen.acs.org/articles/92/web/2014/11/Tufts-Study-Finds-Big-Rise.html
2. ICH Expert Working Group. (2014, December 16). Guideline for Elemental Impurities, Q3D. ICH Harmonised Guideline. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Retrieved from www.ich.org: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3D/Q3D_Step_4.pdf
3. ICH Expert Working Group. (2014, March 31). Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk M7(R1). ICH HARMONISED GUIDELINE. Retrieved from http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_R1_Addendum_Step_4_2017_0331.pdf
4. David, B., Lewis, P. (2011). Current FDA Perspective on Leachable Impurities in Parenteral and Ophthalmic Drug products. AAPS Workshop on Pharmaceutical Stability – Scientific and Regulatory Considerations for Global Drug Development and Commercialization. Washington DC: Office of New Drug Quality Assessment, FDA.
5. ICH Expert Working Group. (2006, Oct 25). ICH Harmonised Tripartite Guideline; Impurities in New Drug Substances Q3A(R2). ICH Haromonised Guideline. Iternational Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Retrieved from www.ich.org: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3A_R2/Step4/Q3A_R2__Guideline.pdf

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