1. Introduction

Drug substances (DS) process development and Drug product (DP) formulation development are two major areas of the drug development process. Impurities/degradants can be generated in either of the processes, from DS degradation or DS-Excipient interaction. These Impurities either non-genotoxic or genotoxic in nature. Regardless, they are regulated by food and drug Administrator (FDA)/ International conferences on Harmonization (ICH) guidelines. Routine impurity analysis in pharmaceuticals requires identification at levels of 0.05 percent to 0.2 percent depending on the daily dose. However, genotoxic impurities can be much harder to detect due to their presence at low ppm levels. This review concentrates on the regulations and analytical technologies used to detect and quantitate impurities (genotoxic and non-genotoxic) in pharmaceuticals[1]

Genotoxicity: This symposium focuses on the management of genotoxic impurities in the synthesis of pharmaceuticals. Recent developments in both Europe and United States require sponsors of new drug applications to develop processes to control the risks of potential genotoxic impurities. Genotoxic impurities represent a special case relative to the International Conference on Harmonisation Q3A/Q3B guidances, because genotoxicity tests used to qualify the drug substance may not be sufficient to demonstrate safety of a potentially genotoxic impurity. The default risk management approach for a genotoxic impurity is the threshold of toxicological concern unless a more specific risk characterization is appropriate. The symposium includes descriptions of industry examples where impurities are introduced and managed in the synthesis of a pharmaceutical. It includes recent regulatory developments such as the “staged threshold of toxicological concern” when administration is of short duration (eg, during clinical trials). [2]

Cancer: Cancer is a complex genetic disease that is caused primarily by environmental factors. The cancer-causing agents (carcinogens) can be present in food and water, in the air, and in chemicals and sunlight that people are exposed to. Since epithelial cells cover the skin, line the respiratory and alimentary tracts, and metabolize ingested carcinogens, it is not surprising that over 90% of cancers occur in epithelia.
The causes of serious ill-health in the world are changing. Infection as a major cause is giving way to non communicable diseases such as cardiovascular disease and cancer. In 1996 there were 10 million new cancer cases worldwide and six million deaths attributed to cancer. In 2020 there are predicted to be 20 million new cases and 12 million deaths. Part of the reason for this is that life expectancy is steadily rising and most cancers are more common in an ageing population. More significantly, a globalization of unhealthy lifestyles, particularly cigarette smoking and the adoption of many features of the modern Western diet (high fat, low fibre content) will increase cancer incidence.

The management of patients with cancer is costly, but there is the daunting prospect that 70% of tomorrow’s patients are likely to live in countries that between them have only 5% of global resources. Huge steps in improving the prognosis of patients with cancer are almost immediately achievable with present-day technology and sufficient financial resource, and all essentially relate to early detection. Cancer does not develop overnight, instead often evolving over many years with detectable premalignant lesions presaging the development of full-blown malignancy. Malignant tumours not only invade surrounding tissue, but are able to colonize other, often vital, organs, a process known as metastasis. Widespread metastatic disease is usually a harbinger of imminent patient death. Thus, immediate referral to the oncologist after detection of any suspicious lump or symptom is paramount; in many parts of the world with poor health education patients present with very advanced disease. In the same vein, cancer screening programmes are designed to detect not only early asymptomatic malignant tumours but also premalignant lesions. Even in the richer countries, such programmes are a significant financial burden, and the more cost-effective programmes target the higher-risk groups denoted by age (e.g. cervical screening, mammography, colonoscopy) or occupation (e.g. blood in the urine of dye workers for bladder cancer).[3]

**Chronic myelogenous leukemia** Chronic myeloid leukaemia (CML) is a malignant, myeloproliferative disorder of haemopoietic stem cells. It arises from a stem cell acquiring a specific translocation t(9;22) which results in the formation of a hybrid oncogene, BCR-ABL. Selecting the most appropriate therapy for a patient with CML remains difficult. Currently, stem cell transplantation is generally accepted as offering the best prospect of a cure. However, advances in the study of tyrosine kinase inhibitors and immunological treatments may direct the future of CML treatment.[4].
**Gastrointestinal stromal tumor** A gastrointestinal stromal tumor (GIST) is one of the most common mesenchymal tumors of the gastrointestinal tract (1-3% of all gastrointestinal malignancies). They are typically defined as tumors whose behavior is driven by mutations in the Kit gene or PDGFRA gene, and may or may not stain positively for Kit.\(^5\)

**Kidney cancer** Renal cell carcinoma (RCC, also known as hypernephroma) is a kidney cancer that originates in the lining of the proximal convoluted tubule, the very small tubes in the kidney that filter the blood and remove waste products. RCC is the most common type of kidney cancer in adults, responsible for approximately 80% of cases. It is also known to be the most lethal of all the genitourinary tumors. Initial treatment is most commonly a radical or partial nephrectomy and remains the mainstay of curative treatment. Where the tumour is confined to the renal parenchyma, the 5-year survival rate is 60-70%, but this is lowered considerably where metastases have spread. It is resistant to radiation therapy and chemotherapy, although some cases respond to immunotherapy. Targeted cancer therapies such as sunitinib, temsirolimus, bevacizumab, interferon-alpha, and possibly sorafenib have improved the outlook for RCC (progression-free survival), although they have not yet demonstrated improved survival.\(^6\)

**Liver cancer** Hepatocellular carcinoma (HCC, also called malignant hepatoma) is a primary malignancy (cancer) of the liver. Most cases of HCC are secondary to either a viral hepatitide infection (hepatitis B or C) or cirrhosis (alcoholism being the most common cause of hepatic cirrhosis).\(^6\) In countries where hepatitis is not endemic, most malignant cancers in the liver are not primary HCC but metastasis (spread) of cancer from elsewhere in the body, e.g., the colon. Treatment options of HCC and prognosis are dependent on many factors but especially on tumor size and staging. Tumor grade is also important. High-grade tumors will have a poor prognosis, while low-grade tumors may go unnoticed for many years, as is the case in many other organs, such as the breast, where a ductal carcinoma in situ (or a lobular carcinoma in situ) may be present without any clinical signs and without correlate on routine imaging tests, although in some occasions it may be detected on more specialized imaging studies like MR mammography. The usual outcome is poor, because only 10 - 20% of hepatocellular carcinomas can be removed completely using surgery. If the cancer cannot be completely removed, the disease is usually deadly within 3 to 6 months.\(^6\)
**Impurity Profiling** There is an increasing interest in impurities present in API’s. Recently, not only purity profile but also impurity profiling has become essential as per various regulatory requirements. In the pharmaceutical world, an impurity is considered as any other organic material, beside the drug substance, or ingredient, arise out of synthesis or unwanted chemicals that remains with API’s. The impurity may be developed either during formulation, or upon aging of both API’s and formulated API’s in medicines. A good illustration of this definition may be identification of impurities in API’s like 1-(1,2,3,5,6,7 hexahydro-s-indacen-4yl)-3-4[-1-hydroxy-1methyl-ethyl]-furan-2sulphonyl urea using Multi disciplinary approach[7]. The presences of these unwanted chemicals, even in small amount, may influence the efficacy and safety of the pharmaceutical products. Impurity profiling (i.e. the identity as well as the quantity of impurity in the pharmaceuticals), is now gaining critical attention from regulatory authorities. The different pharmacopoeias, such as the British pharmacopoeias (BP), United state pharmacopoeias (USP), and Indian pharmacopoeias (IP) are slowly incorporating limits to allowable levels of impurities present in the API’s or formulations.

The international conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human Use (ICH) has also published guidelines for validation of method for analyzing impurities in new drug substances, products, residual solvents and microbiological impurities[8-11]

A number of articles[12-14] have stated guidelines and designed approaches for isolation and identification of process-related impurities and degradation products, using Mass spectrometry (MS), Nuclear Magnetic Resonance (NMR), High Performance Liquid Chromatography (HPLC), Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS), and Tandem Mass Spectrometry for pharmaceutical substances.

**Introduction to High-Performance Liquid Chromatography (HPLC)**

High performance liquid chromatography is the fasted growing analytical technique for the analysis of drugs. It simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. The rapid growth of HPLC has been facilitated b the development of reliable, moderately priced instruments and efficient columns.
Introduction to Ultra-Performance Liquid Chromatography (UPLC)

Acquity Ultra performance system takes advantage of technological strides made in particle chemistry performance, system optimization, detector design, and data processing and control. When taken together, these achievements have created a step-function improvement in chromatographic performance. Defined as UPLC, this new category of analytical separation science retains the practicality and principles of HPLC while increasing the overall interlaced attributes of speed, sensitivity, and resolution.

Normal phase chromatography

Retention by interaction of the stationary phase’s polar hydrocarbon chain with non-polar parts of the sample molecules.

Reverse-phase chromatography

Retention by interaction of the stationary phase’s non-polar hydrocarbon chain with polar parts of the sample molecules.