SUMMARY

Impurities in pharmaceutical drug substances are the unwanted compounds that remain with the active pharmaceutical ingredients (APIs) or develop during the formulation or upon aging of both API and formulated API’s to medicines. The presence of these unwanted substances even in small amounts will influence the efficacy and safety of pharmaceutical products. So, it has become absolutely necessary to ascertain and examine critically their physical characteristics, chemical equivalence, chemical impurities and their prescribed limits and degradation products. Impurity profiling is the common name of a group of analytical activities, the aim of which is the detection, identification, structural elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations.

Impurity profiling is now receiving critical attention from regulatory authorities. Different pharmacopoeias, such as the British pharmacopoeia (BP) and the United States pharmacopoeia (USP) are slowly incorporating limits to allowable levels of impurities present in the API’s or formulations. Various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in API. Qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research.

International Conference on Harmonisation (ICH) has published guidelines on impurities in new drug substances, products and residual solvents. There is a good significant demand for the impurity-reference standards along with the API reference standards from both regulatory authorities and pharmaceutical companies. These ICH guidelines suggest a designed approach and guidance for isolating and identifying process-related impurities and degradation products using Chromatographic and Spectrometric techniques, either alone or in combination with other techniques. These guidelines define what investigations and documentation should be made in determining the impurities and degradation products seen in stability studies at recommended storage conditions.
In the present study, the author made an attempt to detect, identify and quantitatively determine the impurities along with API based on above mentioned guidelines. Structural elucidation of the impurities was done with the help Mass and NMR techniques.

**Chapter-I** begins with the significance of drug analysis and classification of impurities. It also discusses the need for the determination and characterization of process related impurities, as well as degradation impurities in API. The contents in brief were also discussed related to that of HPLC in particular to its use in pharmaceutical analysis followed by several method development and validation procedures for the methods as per ICH quality guidelines.

**Chapter-II** starts with an introduction of the drug, its chemical structure, mechanism of action and literature on various methods reported for Tolvaptan (TPN) drug substance. This chapter mainly deals with the method development, characterization and validation of process impurities and also subjected to forced degradation studies. Literature review reveals the information for the existence of various methods like UV and HPLC for determination of TPN in bulk and pharmaceutical formulations. There were no reports for the determination of TPN drug substance along with its process impurities. An accurate and sensitive reverse phased liquid chromatographic method (RP-HPLC) was developed for the determination of three process impurities in TPN drug substance. The chromatographic conditions were optimized by employing a Zodiac C18 (150 mm × 4.6 mm, 5.0 µm) column with flow rate of 1.0 mL min⁻¹ at 30°C and detector wavelength of 215nm. The mobile phase-A consists of 0.01M potassium dihydrogen ortho phosphate (pH 3.0 with trifluoroacetic acid) and mobile phase-B consists of Acetonitrile: water (90:10) with a gradient programme (Time/M-B): 0/40, 10/50, 28/66, 32/80, 35/40, 40/40. All impurities were well resolved from TPN main peak with proper baseline separation. Structural confirmation and characterization of these three process impurities was carried out by using proton, carbon magnetic resonance spectroscopy (PMR, CMR) and mass spectrometry (MS). Based on the spectroscopic studies three unknown process impurities were characterized as (4-Amino-2-methylphenyl)(7-chloro-5-hydroxy-2,3,4,5-tetrahydro-1H-1-benzo[b]azepin-1-yl)methanone (TPNRC01) 7-Chloro-1-[2-methyl-4-[(2-methylbenzoyl)amino]benzoyl]-5-oxo-2,3,4,5-tetrahydro-1H-1-
benzazepine (TPNRC02) and 4-[(5-Hydroxy-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl)carbonyl]-3-methylphenyl-2-methylbenzamide (TPNRC03). The newly developed method was validated according to ICH guidelines considering three process impurities to demonstrate specificity, precision, linearity and accuracy of the method. The Limit of detection for the three process impurities was 0.0005, 0.0008 and 0.0005 mg/mL and the limit of quantitation detection was 0.0015, 0.0016 and 0.0016 mg/mL respectively. The newly developed method was found to be highly efficient, selective, sensitive and accurate. Stability studies have also been performed for TPN under different stress conditions such as acid, base, oxidative, thermal and photolytic. Two new degradants were identified in acid degradation process, whose mass numbers were identified with the help of Mass spectrometric analysis.

Chapter-III starts by giving a brief description about the structure, chemical nature, mechanism of action and literature on various methods reported for Cidofovir dihydrate (CDV) drug substance. Literature survey reveals the existence of methods related to pharmacokinetics and pharmacodynamics. So far, no method was reported for the quantitative determination and characterization of process impurities. This chapter mainly deals with two parts. Part-A is the quantitative determination and characterization of process impurities with development of a sensitive and selective reversed phase liquid chromatographic method for the determination of two process impurities in Cidofovir (CDV) drug substance. Two newly identified process impurities were determined by High performance liquid chromatography using Inertsil- C8 column (250mm × 4.6 mm× 5.0 μm) with flow rate of 1.0 mL min⁻¹ at 40°C. The wavelength of detection was 275nm. Mobile phase-A consists of 0.001M Tetra butyl ammonium dihydrogen phosphate and disodium hydrogen orthophosphat(1:1) in water and mobile phase-B consists of acetonitrile with a gradient programme (Time/M-B) 0/5, 15/5, 18/20, 40/20, 45/5, 50/5 (v/v). Structural confirmation and characterization of these impurities were unambiguously carried out by using Nuclear magnetic resonance spectroscopy (NMR) and Mass spectrometry (MS). Based on the spectroscopic data unknown impurities were characterized as {((4-amino-1-((s)-2, 3-dihydroxypropyl) pyrimidin-2(1h)-one}) (Impurity-1), [(s)-2-(4-benzoyleamino-2-oxo-2h-pyrimidin-1-yl)-1-hydroxymethyl-ethoxy methyl]-phosphonicacid (Impurity-2). The newly developed method was validated according
to ICH guidelines considering two process impurities to demonstrate specificity, precision, linearity and accuracy of the method.

Part-B, is the quantitative determination of the Enantiomeric impurity of CDV. The structure of CDV itself shows that, it is an optically active compound with a chiral centre (R-isomer), which is considered to be enantiomeric impurity (Impurity-3). Hence, the author felt necessary to develop a suitable RP-HPLC method for the determination of enantiomeric impurity. The method was established using achiral column in presence of chiral mobile phase additives (CMPA). Hypersil-ODS (100x4.6mm, 5µ) column and the mobile phase consists of L-phenylalanine and copper sulphate penta hydrate were used to serve the purpose. The newly developed method was validated according to ICH guidelines considering the impurity to demonstrate specificity, precision, linearity and accuracy of the method. The newly developed method was found to be highly efficient, selective and sensitive. This method is for intended use in routine pharmaceutical analysis as it is cost effective.

Chapter-IV begins by introducing the structure and chemical nature of Ketobemidone Hydrochloride (KBH) drug substance. In the synthesis of Ketobemidone Hydrochloride (KBH), 3-Methoxy benzyl chloride (3-MBC) is a key starting material and 2, 2-dichloro-N-methyldiethanolamine (DNMDA) is an important reagent. The literature search identified these two as potential genotoxic compounds. As there were no reports in literature for the determination of the above impurities, the author felt necessary to develop a suitable method to quantify the impurities at threshold level as per ICH and EMEA guidelines. Hence, a specific and simple solvent extraction procedure has been adopted in the present study followed by Gas chromatographic method to determine the impurities. Since one of the genotoxic impurities of KBH i.e., DNMDA exists in the form of HCl salt and due to its immiscible nature in many of the organic solvents, solvent extraction procedure is preferred. Hence, the compound was neutralized with 3M Ammonia and subsequently extracted into a suitable solvent Toluene which can facilitate smooth analysis with good response at threshold level. Good resolution between the impurities was achieved with DB-624 (30 meters length, 0.53mm id, and 3µm film thickness) column using Nitrogen as carrier gas and FID as detector. This method was validated as per International Conference on Harmonization (ICH) guidelines and is able to
quantitate DNMDA and 3-MBC at 4.0 ppm level. The method is linear in the range of 50 to 150% of estimated permitted levels (4 ppm). The QL for DNMDA and 3-MBC were found about 1.5 ppm and 0.5 ppm respectively. The DL for DNMDA and 3-MBC were found about 0.5 ppm and 0.2 ppm respectively. The % RSD obtained for DNMDA and 3-MBC were 1.7 and 1.5 respectively. The results obtained indicated that the method was capable of quantifying DNMDA and 3-MBC in KetobimedoneHCl.

Chapter-V begins with the introduction giving a brief account of chemical name, structure, mechanism of action and literature of Pazopanib Hydrochloride (PBH). Literature survey reveals the existence of methods related to pharmacokinetics and pharmacodynamics. No methods were reported for the the quantitative determination and structural confirmation of process impurities of PBH drug substance. Hence it was felt necessary for the author to determine an RP-HPLC method for the determination of process related impurities along with PBH drug substance. The study of the method exposed the presence of two new process related compounds which were determined by HPLC using Zorbax RP18 column,(50 mm × 4.6 mm, 3.5 µm) with flow rate of 0.8 mL min⁻¹ at 40°C. The detected wavelength is 215nm. Mobile phase-A consists of 0.015M potassium dihydrogen ortho phosphate (pH 3.0 with ortho phosphoric acid) and mobile phase-B consists of Buffer: Methanol (16:84) with a gradient programme (Time/ %M-B) 0/30, 5/30, 30/80, 32/30, 45/30. Structural confirmation and characterization of these compounds were unambiguously carried out by using Nuclear magnetic resonance spectroscopy (NMR) and the mass numbers were confirmed by mass spectrometry (MS). Based on the spectroscopic data the unknown impurities were characterized as 5-amino-2-methyl benzene sulphonamide [PBHRC01] and N-(2-chlorophyrimidin-4-yl) N-2,3-trimethylyl-2H-indazol-6-amine [PBHRC02]. The newly developed method was validated according to ICH guidelines considering two impurities to demonstrate specificity, precision, linearity and accuracy of the method. The limits of detection of the two new impurities were about 0.0061 and 0.0062 mg mL⁻¹ and the limit of quantitation were about 0.024 and 0.021 mg mL⁻¹ respectively. The newly developed method was found to be highly efficient, selective and sensitive.
Chapter-VI starts by giving introduction, which includes the structure, chemical nature and mechanism of action and the significance for the simultaneous estimation of multi-component formulations. Multi-component formulations proved to be effective on functioning, due to greater patient acceptability, increased potency, fewer side effects and quicker relief. The development of these multi-component pharmaceuticals brought a revolution. These pharmaceuticals would serve their intent only if they are free from impurities and are administered in an appropriate amount. Hence, in the present study the author made an attempt to establish a stability indicating method for the simultaneous estimation of Ambroxol and Cefadroxil in bulk as well as in its tablets. Literature studies revealed that there were a few HPLC, HPTLC and UV methods available for the determination of Ambroxol and Cefadroxil in combined form. In the present study, the author developed a reverse phase high performance liquid chromatographic method for the determination of Ambroxol and Cefadroxil in bulk drug and pharmaceutical dosage form. The developed method was validated as per ICH guidelines. Separation and quantification were achieved on an Phenomenex-C18 (5μm, 150 x 4.6 mm) column using PDA detector. The mobile phase was 0.1% orthophthalaldehyde: Methanol (50:50), at a flow rate of 1 ml/min and injection volume was 10μL. Detection was carried out at a wavelength of 249 nm. The method was validated as per ICH guidelines. Degradation studies were performed for Ambroxol and Cefadroxil under different stress conditions which include acidic, basic, oxidative, thermal and photolytic. No significant degradants were observed under different stress conditions. Good linear relationship in the concentration range of 12-36μg/mL for Ambroxol with correlation coefficient of 0.999 and 60-180μg/ mL for Cefadroxil with correlation coefficient of 0.999. The LOD and LOQ for Ambroxol were found to be 0.0680mg/ml, 0.2268mg/ml respectively. The LOD and LOQ for Cefadroxil were found to be 0.244mg/ml, 0.813mg/ml respectively. The precision and accuracy were less than 2 % RSD for both analytes. The stressed sample chromatograms demonstrate the specificity of the proposed method for the determination of target analytes in presence of degradants.
PUBLICATIONS
