INTRODUCTION

Impurity profiling and degradation study of drug substances and drug products has attracted global interest because regulatory bodies have become more stringent in maintaining and controlling quality and purity of drugs. Also the regulatory requirements for impurity profiling and forced or stress degradation study have been extended to generic drugs and products in recent years. Due to the stringent environment laid down by regulatory authority, there is steady increase in product recalls from the market[1]. One of the reasons often cited is "due to the presence of impurities or degradation products (DPs) beyond the prescribed limits". In drug therapy two issues fundamentally important are efficacy and safety of drug. Two main factors determining the safety of drug therapy are:

1. Pharmacological toxicological profile of drug substances: the relationship of beneficial and adverse effects of the drug.
2. The impact of impurity present in drug substance. Analytical chemist play important role in monitoring and controlling impurities in drug substances.

As per ICH guideline Q3A impurity in a drug substance is "*any component of the drug substance that is not the chemical entity defined as the drug substance* (1)" and as per ICH guideline Q3B impurity in a drug product is "*any component of the drug product that is not the chemical entity defined as the drug substance or an excipient in the drug product* (2)"

Although exact definition for impurity profiling is not given in regulatory guidelines, Impurity profiling may be defined as "*the common name of analytical activities with the aim of detecting, identifying and/ or elucidating the structure and quantitatively determining organic, inorganic impurities and residual solvents in bulk drug and pharmaceutical formulations"* (3). Very broad definition of impurity is given in guideline and it can include DPs as impurity. DPs (4) are defined as "*a molecule resulting from a change in the drug substance brought about over time. For the purpose of stability testing of the products in this guidance, such changes could occur as a result of storage or processing for example oxidation, deamination, proteolysis and aggregation*"

[1] Available from: http://www.fda.gov/%20Safety/Recalls/EnforcementReports/default.htm

1.1 Classification of Impurities

As per ICH guidelines Q3A and Q3B Impurities can be classified as:

- Organic impurities

These can be formed during the manufacturing process or during storage of new drug substances. They can be identified/unidentified, volatile/nonvolatile. This includes: starting materials, intermediates, by-products, DPs, reagents, ligands and catalysts.

- Inorganic impurities

These can arise during manufacturing process; normally they are known and identified. This includes: reagents, ligands, catalysts, heavy metals, other residual metals, inorganic salts and other materials like filter aids, charcoal.

- Residual solvents

These are generally inorganic/organic liquids that are used during synthesis of drug substances as a vehicle for preparation of solution or suspension. Appropriate control of residual solvent is necessary since they have known toxicity. ICH Q3C (5) guideline provides the limits of residual solvent based on existing safety and toxicity data. These were classified in three categories:

Class 1 (The most toxic and/or environmentally hazardous): These are highly toxic in nature and are limited to 2–8 ppm, for environmentally hazardous chemical like trichloroethane the limit of 1500 ppm is applied. During manufacturing of pharmaceuticals Class 1 solvents should be avoided. But if their presence is unavoidable, the definite concentration limit is applied, regardless of the actual patient intake dose.

Class 2 (Considered a lesser risk): These should be limited in their usage. Two different approaches were described in guideline for setting limits of class 2 solvents. The first approach is used when PDE (permitted daily dose) can not be estimated; concentration limits are calculated on the basis of daily intake of theoretical product mass of 10g. The second approach is used when dose is known; the PDE and/or dose value can be used to determine the permissible concentration.

Class 3 (The lowest risk category): These have low toxic potential and are limited to 5000 ppm (0.5% w/w)

1.2 Identification and Qualification threshold of Impurities and DPs (1,2,6).

ICH guidelines Q3A and Q3B provide the reporting, identification and qualification threshold of impurities and DPs (table 1.1 and 1.2). The thresholds are slightly different for both as ICH treats DPs different than impurities, although DPs are impurities only.

Table 1.1: Thresholds for reporting impurities (6)

Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
Less or equal to 2 g/day	0.05%	0.10% or 1.0 mg/day (whichever is lower)	0.15% or 1.0 mg/day (whichever is lower)
>2 g/day	0.03%	0.05%	0.05%

Table 1.2: Threshold for reporting degradation products (6)

Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
≤1 mg		1.0% or 5 µg TDI (whichever is lower)	0.15% or 1.0 mg/day (whichever is lower)
1 mg–10 mg		0.5% or 20 µg TDI (whichever is lower)	0.05%
10 mg–100 mg			0.5% or 200 µg TDI whichever is lower
<10 mg			1.0% or 50 µg TDI whichever is lower
>10 mg–2 g		0.2% or 2 mg TDI whichever is lower	
>100 mg–2 g			0.2% or 3 mg TDI (whichever is lower)
≤1 g	0.1%		
>1 g	0.05%		
>2 g		0.1%	
>2 g			0.15%

1.3 Regulatory requirement

The regulatory requirements for the control and test of impurity and DPs in drug substances and products are summarized in table 1.3. Sections 3.2.S.3.2 and 3.2.P.5.5 of ICH CTD, M4Q (R1) (i.e. *Common Technical Document*) (7) clearly specify the requirement of characterization of impurities in new drug substances and DPs in new drug products. Multiple EMA guidelines also specify the same requirement e.g. CPMP/QWP/130/96 (i.e. *Chemistry of New Active Substances*) (8); EMEA/CHMP/CVMP/QWP/450653/2006 ((i.e. *Assessment of Quality of Medicinal Products Containing Existing or Known Active Substances*); CHMP/QWP/297/97 Rev 1 corr ((i.e. *Summary of Requirements for Active Substances in the Quality Part of the Dossier*) (9), etc. The latter has been adopted by TGA, Australia in revised form.

In sections 3.2.S.3 and 3.2.P.5.5 of USFDA ANDA checklist (FDA ANDA filing checklist, revision 9/2011) (10) provide the requirement of name, origin and structure of impurity/(s) in drug substance and drug product. Similarly in Sections S 3.2. and P 5.5, the Canadian Ministry of Health also specify the same requirement in the draft Quality (*Chemistry and Manufacturing*) Guidance: New Drug Submissions (NDSs) and Abbreviated New Drug Submissions (11).

In sections S 3.2 and P 5.5, the ACTD (i.e. *ASEAN Common Technical Dossier*) for registration of pharmaceuticals for human Use (12) requires information on the characterization of impurities. In section 3.2.S.3.2, WHO has also specified the clear requirement in its draft document QAS/10.373 (*Guideline on Submission of Documentation for a FPP, Generic Finished Pharmaceutical Product: Quality Part*) (13).

Table 1.3: International guidelines describing regulatory requirements for the control and test of impurities and DPs in drug substances and products for human use.

International agency	Guideline(s)	Issue date/date of coming into effect	Reference
ICH (USA, EU and Japan)	Q3A(R2): Impurities in New Drug Substances	25 October 2006	(1)
	Q3B(R2): Impurities in New Drug Products	2 June 2006	(2)
	Q3C(R5): Impurities: Guideline for Residual	4 February 2011	(5)

	Solvents		
	Q3D: Impurities: Guideline for Metal Impurities (final concept paper)	29 October 2009	(14)
	M7: Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk (final concept paper)	9 June 2010	(15)
	EMEA/CHMP/CVMP/QWP/450653/2006: Assessment of Quality of Medicinal Products Containing Existing/Known Active Substances	10 July 2007	(16)
	CPMP/QWP/1529/04: Control of Impurities of Pharmacopoeial Substances	22 April 2004	(17)
EMA (Europe)	CPMP/SWP/5199/02 and EMEA/CHMP/QWP/251344/2006: Guideline on the Limits of Genotoxic Impurities	28 June 2007	(18)
	CPMP/SWP/QWP/4446/00 corr.: Guidelines on Specification Limits for Residues of Metal Catalysts	January 2007	(19)
	EMA/CHMP/CVMP/QWP/199250/2009: Guideline on Setting Specifications for Related Impurities in Antibiotics (draft)	14 July 2010	(20)
	NDAs: Impurities in Drug Substances	February 2000	(21)
US FDA (USA)	Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (draft)	December 2008	(22)
	ANDAs: Impurities in Drug Products	November 2010	(23)
TGA (Canada)	Impurities in Existing Drug Substances and Products (draft)	6 September 2005	(24)
TPD (Australia)	Australian Regulatory Guidelines for Prescription Medicines; Appendix 18: Impurities in Active Pharmaceutical Ingredients and Finished Products	June 2004	(25)

1.4 Sources of Impurities

There are three important sources of impurities (6):

1.4.1 Synthesis Related Impurities

During synthetic process, impurities in new drug substance or new chemical entity (NCE) mainly originate from raw materials, intermediates, by products and solvents. The raw materials used in synthesis are generally manufactured to much lower purity level than a drug substance. Hence, they can contain a number of components that can affect the purity or may react with other chemicals used in synthesis of a drug substance. The impurities can also be produced by reaction of impurities present in solvent itself that is used in the synthesis, and may range from trace levels to significant quantity. Intermediates formed during synthesis are also not generally purified to higher level as in case of drug substance, hence can form impurity in final product.

Generally in pharmaceutical synthesis, the purity of final intermediate is controlled by performing regulatory purity/impurity testing. This typically involves residual solvents, which are not used in further downstream processing, in cases where it is conclusive that the moieties are not also DPs. The proper and rigorous analytical methodology should be adopted at this step, since this is the last major source of potential impurities. Base to salt/ acid to salt conversions could also generate new potential impurities. Moreover, thermally unstable compounds can also undergo decomposition if further processing involves heating.

1.4.2 Formulation Related Impurities

The excipients used to formulate drug product, can originate potential impurities. In addition during the process of formulation the drug is subjected to variety of conditions like heat, shear etc, that can attenuate undesirable reactions and form DPs. Hydrolysis or solvolysis generally takes place in solutions and suspensions that leads to degradation. These reactions can also occur in the dosage forms that are in solid state such as capsules and tablets, when water/ another solvent have been utilized for granulation. It provides ripe situation for hydrolysis and metal catalysis besides contributing its own impurities. Oxidation occurs for easily oxidizable materials if no precautions are taken. Similarly, photochemical reactions are feasible for light sensitive materials. Lyophilization and

vortex mixing are sometimes used during process of formulation that is considered high risk operations which can cause impurity formation.

1.4.3 Degradation Related Impurities

Number of impurities can be generated due to degradation of API and/or other interactions on storage. Therefore, it is important to conduct stability studies in order to predict, evaluate and ensure drug product safety(26). Stability studies include evaluation of API stability, pre-formulation studies in order to evaluate compatibility of API with the excipients to evaluate its stability in the formulation matrix, accelerated stability testing of the drug product, kinetic studies and determination of expiration date, stability studies of drug products in market. The stability studies were conducted under various exaggerated conditions of humidity, light and temperature that helps to determine which type of impurities can be generated by degradation reactions.

Kinetic Study

Mostly degradation reactions of pharmaceuticals are chemical in nature and occur at a finite rate. These reactions are influenced by conditions such as concentration of reactants, solvent, pH of the medium, temperature, presence of catalysts and radiation energy. The dependency of the reaction rate on concentration of reactant is described by order of that particular chemical reaction. Although some pharmaceuticals degrade by complicated mechanisms, mostly the degradation can be classified as zero, first or pseudo first order reaction.

In stability predictions, it is critical to understand the limitations of experimentally obtained heat of activation energy values. For example the heat of activation of analyte at pH value where two/ more degradation mechanisms are involved is not essentially constant with temperature. Therefore for different temperatures, it is necessary to find out heat of activation value for bimolecular rate constants that are involved in a pH rate profile, to predict degradation rates at all pH values (4).

1.4.4 Crystallization Related Impurities

If a substance has same elemental composition but can exist in different crystal packing arrangements, are called polymorphs and the phenomenon is called polymorphism. If the substances exist in different packing arrangements with different elemental composition,

the phenomenon is known as solvatomorphism. These two has profound effect on solid state properties. This requires the development and validation of assay methodology for the determination of phase composition. The nature of crystal structure can influence the conductivity, crystal shape and colour, density, dissolution rate, hygroscopicity, melting and sublimation property, rate of reaction, solubility, refractive index, surface tension and viscosity.

1.4.5 Stereochemistry Related Impurities

Stereoisomers can be considered impurity in drug substance, although ICH excludes stereo chemical impurities, pharmacopoeias consider them as ordinary impurity. The first set of guideline regarding this issue was issued by FDA in 1987 where the question of stereochemistry was approached directly on the manufacture of drug substance. The differences in pharmacological or toxicological profiles of stereoisomers suggest that it should be monitored carefully.

1.4.6 Genotoxic Impurities

Genotoxic compounds can be carcinogenic to humans due to their ability to induce chromosomal rearrangements and/or genetic mutations. The ICH M7 (15) guideline provides the limits for control of genotoxic impurities in pharmaceuticals to limit the risk of carcinogenicity.

1.5 Benefits of isolation and characterization of impurities and DPs

The identification or structural characterization of Impurities or DPs is beneficial in many ways:

(i) Provides understanding of source, origin and nature of impurities or DPs, that can be utilized for their control during drug synthesis and/or formulation development. There are many reported impurities or DPs that originate from totally unexpected sources. There was an example of recall of drug nelfinavir mesylate formulation from the market due to conversion of mesylate to genotoxic impurity, ethyl methane sulfonate formed by interaction of nelfinavir mesylate with residual ethanol (27). Another example is interaction of counterion maleate with amlodipine free base used to form the salt (28).

(ii) Impurities or DPs can be synthesized once structure has been elucidated, that leads to availability of a pure impurity standard. This can be used for (a) establishing quantitative validation parameters, like calibration curves, LOD, LOQ and response factor (b) spiking study (c) establishing safety in in-vitro and in-vivo qualification studies.

(iii) Side or adverse effects of drug substances and products can be explained on the basis of structure of impurities or DPs. Like polymeric DPs formed in aminopenicillins on storage were responsible for allergenic reactions attributed to these drugs.

(iv) Based on review of available literature or via computational toxicology assessment by using commercial software, genotoxic potential of particular impurities or DPs can be assessed.

(v) Correlating whether a specific impurities or DP is a significant metabolite.

(vi) Establishing drug degradation mechanisms and pathways.

(vii) To list impurities or DPs in compendial monographs for reference.

(viii) To create or add into library of spectral data of chemical compounds.

(x) Environmental mapping of drugs by focusing on their major impurities or DPs.

(xi) Addition into structure searchable drug degradation databases for e.g. Pharma D3 (29).

(xi) Sometimes understanding of chemical reactions that leads to formation of impurity or DPs, discloses some unusual chemistry that may even result in new chemical or drug leads.

1.6 Identification and Structure Elucidation of Impurities and DPs

1.6.1 Conventional Approach

The conventional approach of identification and structure elucidation of unknown impurities and DPs involves separation, impurity or degradant enrichment and isolation or synthesis which is followed by spectral analysis.

The separation are usually carried out by High Performance Liquid Chromatography (HPLC), Ultra High Performance Liquid Chromatography (UPLC/UHPLC), Thin Layer Liquid Chromatography (TLC), High Performance Thin Layer Liquid Chromatography (HPTLC), Hydrophilic Interaction Liquid Chromatography (HILIC), Gas Chromatography (GC), Capillary Electrophoresis (CE), Super Critical Fluid Chromatography (SFC) and/or

any other relevant separation technique. The detection is usually carried out by UV or other detectors like fluorescence, Evaporative Light Scattering Detector (ELSD), Chemiluminescent Nitrogen Detector (CLND), Corona CAD (C-CAD, Corona Charged Aerosol Detectors) have also been utilized advantageously in impurity profiling and degradation study. But usually UV/PDA detectors are employed, which also provides purity of each peak (30).

Simultaneous orthogonal techniques can also be used for ex. HPLC and CE (31); RPLC, MEKC, GLC and SFC (32); HILIC and LC (33); CE and CEC (34); SCF and LC (35). Due to differential selectivity these techniques ensure separation of large number of impurities and DPs. The presence of particular impurities and DPs can be checked by matching retention times of unknown analytes with their standards by developed method, usually done through spiking. Impurity or degradation peak that are above identification threshold (as specified in ICH guidelines Q3A and Q3B) are marked. For identification and structural elucidation of such impurity or DPs, it is usual practice to enrich and isolate them using appropriate tool (described in figure 1.1). For unknown DPs different stress conditions like acid, base, neutral hydrolysis, oxidation and light are used and optimized to obtain sufficient amount of desired DPs (36, 37). The structure of purified impurity or DPs is deduced from the spectral data. Some investigations at this stage may also include single crystal analysis of pure crystalline product (38). The prediction of the structure is then followed by actual synthesis of impurity or DPs, then followed by spectral matching by spiking to confirm the presence or absence of the identified and isolated compound (39).

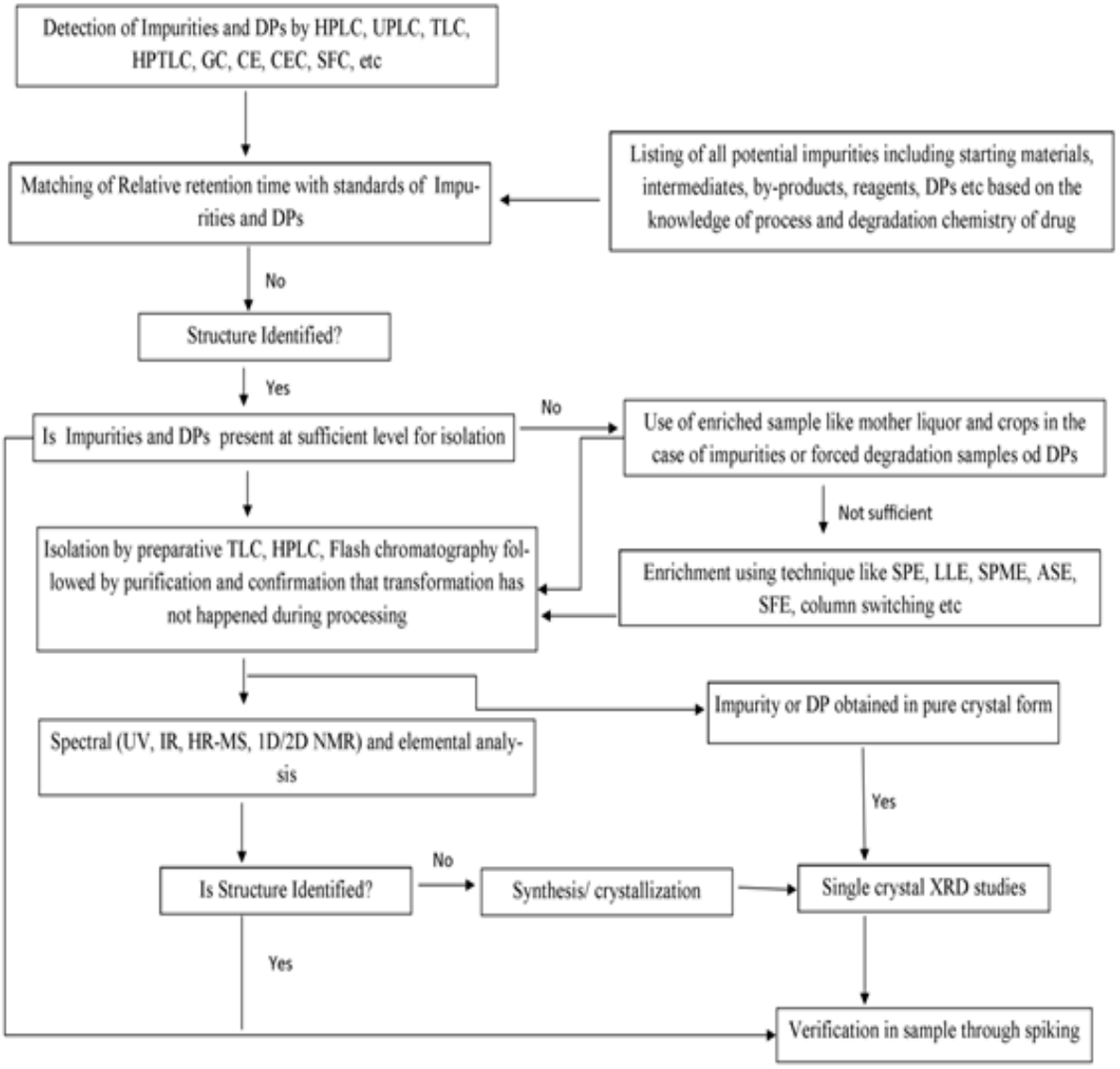

Figure 1.1: Conventional approach for the characterization of impurities and DPs

1.6.2 Hyphenated methods

There are few limitations of conventional approach, these are (40):

1. The process is time consuming and sometimes become complicated if several impurities and DPs have to be characterized in a single sample.

2. If the impurity or DPs formed are present in trace amount and can not be enriched, the process become more tedious.

3. If unstable impurity or DP is formed, or if there is possibility of secondary reaction during processing, isolation becomes difficult.

Due to these reasons, hyphenated techniques are preferred choice for the identification and characterization from last few years especially if impurity or DPs formed are in trace level. Mostly the available hyphenated instruments have LC, GC or CE on the front end connected to MS, NMR or IR on the detection side. These are LC-MS, GC-MS, CE-MS, LC-NMR, CE-NMR, LC-NMR/MS and LC-IR etc. Specially, LC-MS instruments are mostly used. Also combined LC-MS-NMR systems are available. Summary of hyphenated techniques that can be utilized for identification and characterization of impurities and DPs are illustrated in figure 1.2.

MS based hyphenated techniques

GC-MS

It was the first hyphenated technique introduced for determination of organic volatile impurities (41), and residual solvents (42) in a sample and used till today. However, the volatility and thermal stability of analytes is essential for GC-MS. Therefore few literatures exist on the use of GC-MS in the characterization of impurities and DPs.

LC-MS

The most popular hyphenated technique for characterization of impurities and DPs is LC-MS, as it has potential to give nearly clear structural information about unknown analyte. Although it was introduced much after GC-MS, several advancements and ranges of this instrument is available commercially. These are: LC-MS (Single Quad), LC-MS-MS (Triple Quad), LC-TOF, LC-MS-TOF (Q-TOF, Triple TOFTM), LC-MS-3DTRAP (MSn), LC-MS-2DTRAP (Q-TrapTM), LC-Hybrid Trap TOF Systems (LCMS-IT-TOF®), LC-OrbitrapTM, LC-FTICR (Fourier Transform Ion Cyclotron Resonance). These are either used alone or in combination to get desired information useful for structural characterization.

CE-MS

CE (Capillary electrophoresis) and CEC (capillary electro-chromatography) is important techniques for separation and identification of impurities and DPs. CEC is a hybrid technique that involves both high efficiency of CE and stationary and mobile phase selectivity of LC. Few literatures are available wherein CE/CEC have been hyphenated

with MS for characterization of impurities and DPs but are gradually gaining significance. The technique is usually restricted to separation of analytes.

SFC-MS

Small number of reports are available on the use of SFC-MS for characterization of impurities and DPs for pharmaceutical substances and products (3),(43). The technique has its advantage of saving LC solvents but it's bench-top instrument was not available commercially for analysis; recently it has been introduced in to the market.

NMR based hyphenated techniques

LC-NMR

In 1978 for the first time, the coupling of LC effluent to NMR was reported (44). To improve the instrument sensitivity and resolution, modern LC-NMR instruments are accompanied with multiple technological advancements, like microprobes, strong field magnets (above 500 MHz), and cryoprobe technology (45). SPE (Solid phase extraction) units are embedded in between LC and NMR to overcome the requirement of high volumes of expensive deuterated solvents in mobile phase. The LC effluent contains low sample concentrations, due to which ^{13}C detection is usually not possible. Also insufficient quantity of analyte did not allow acquisition of heteronuclear HSQC and HMBC spectra. Specific NMR pulse sequences are used to obtain clean spectra free from corresponding residual non-deuterated solvents (46). Usually supportive informations are gathered from LC-NMR for structural confirmation for the components separated on LC column. Several reports are available on the use of LC-NMR for structural characterization of impurities and DPs (47, 48). The useful LC-NMR could be collected for concentration of 0.06%, though most reported studies involves 0.5% and above. Isomers, that generate same mass and fragmentation pattern, for such compounds LC-NMR data is very useful to confirm their identity. Unlike LC-MS phosphate buffer is preferred choice for LC-NMR, because of the presence of multiple protons in volatile buffer like formate or acetate.

CE-NMR

If analytes are present in relatively small amounts, hyphenated CE-NMR provides similar advantages as LC-NMR with respect to separation, chemical identification, and structural information. Both continuous and stopped flow modes, similar to LC-NMR are used in

CE-NMR. The typical problem associated with CE-NMR is the shorter residence time of sample in NMR due to small sample volume output from CE that affects the detection sensitivity (49). Although, intensive innovative efforts have been made to improve this, only few publications reported the application of CE-NMR to identification and characterization of trace amount of impurities and DPs (50, 51).

LC-FTIR systems

Conventional FT-IR system requires 1–5 mg of sample hence recording becomes difficult when analytes are present or generated in trace quantities or cannot be isolated. LC-IR provides benefits in such cases and has been recently commercialized. Some limitations exist while recording IR spectrum of impurities or DPs at levels of 0.1% in LC-IR, these include (52, 53):

(i) On-line enrichment of analyte is essential.

(ii) Interference of mobile phase components.

(iii) It is difficult to apply chemometrics especially in case of gradient elution, since the background absorption is strongly influenced by the slight variation in mobile phase composition.

(iii) Complete removal/ elimination of the solvents are difficult.

(iv) Analytes should have low volatility than the mobile phase.

(v) Differential nature i.e. amorphous or crystalline; of analyte post deposition and also post solvent elimination.

Interface constitutes most critical component in LC-IR due to above cited reasons. It is available in two types- (i) flow cell (on-line) (ii) solvent elimination (semi on-line). On-line LC-IR have limited use and are restricted to major constituents only due to its poor detection limits, while semi on-line has comparatively better sensitivity and gives improved spectral data. Few literature reports are available on the use of LC-IR in characterization of impurities and DPs (54).

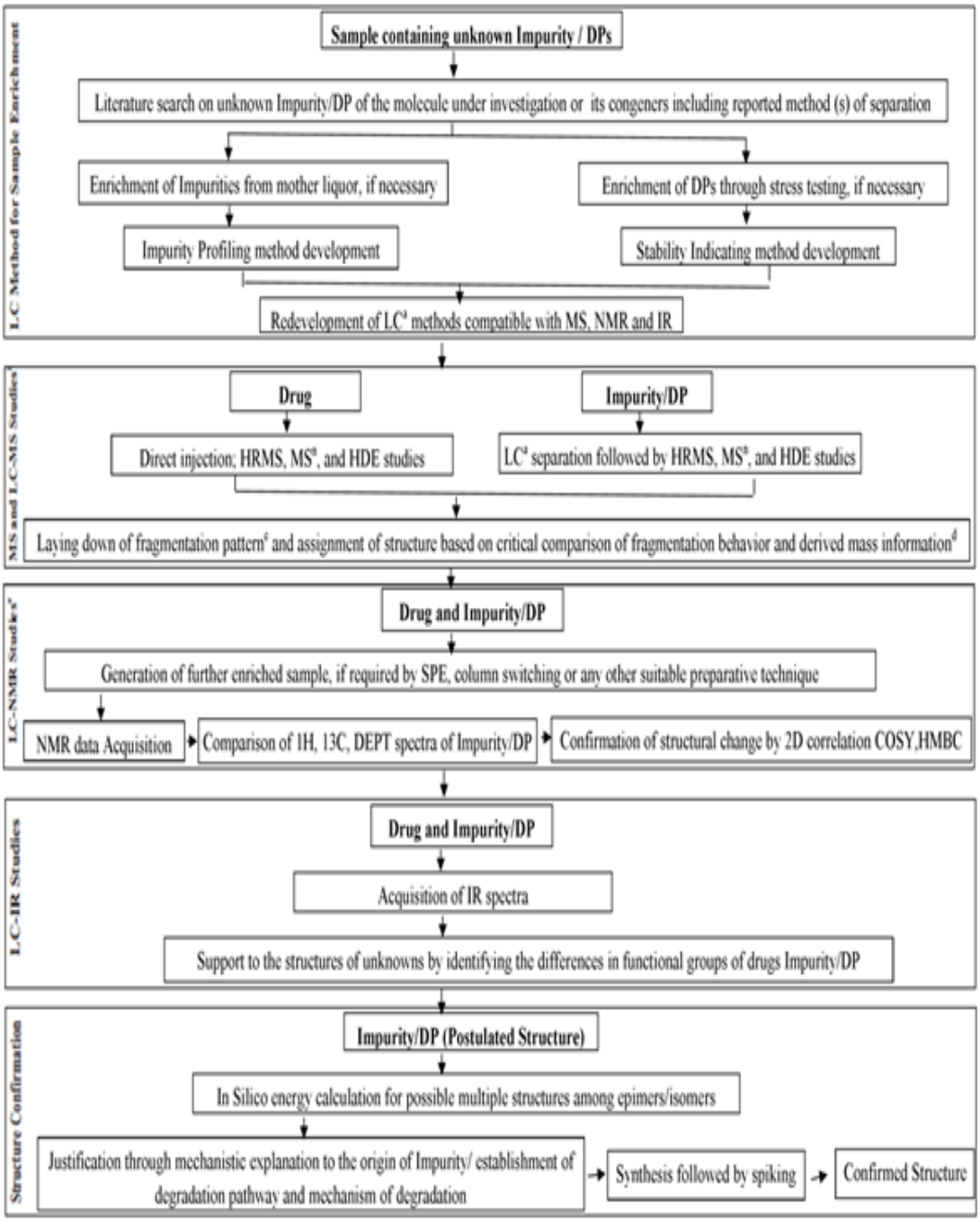

Figure 1.2: Comprehensive strategy for unequivocal structural characterization of Impurities/DPs using modern hyphenated techniques (11).

1.7 Stability Indicating Assay Method (SIAMs)

The main goal of SIAMs is the establishment of stability of drug substances and products by providing information about the conditions of stress testing. Several synonyms have been used in literature for stress testing theses are stress studies, forced degradation

studies, stress decomposition studies and forced decomposition studies. Although, in industry these studies have been in practice for a long time, but was mandated with the advent of ICH guidelines.

1.7.1 Regulatory status of stability indicating assay methods (SIAMs)

The ICH guideline Q1A (55) states the requirement of "a validated stability-indicating method" for testing of those features that change during storage and may influence the safety, efficacy and quality of drug substance and product. In order to establish inherent stability characteristics and to determine degradation pathways to support the applicability and suitability of the proposed analytical method, stress testing at 10 °C increments of temperature above the accelerated temperatures, photolytic, oxidative and extremes of pH conditions should be carried out on the drug substances. The ICH guideline Q3A (1) and Q3B (2) stresses on providing documented evidence regarding the validation of analytical procedures and its suitability for the identification, detection and quantitation of DPs. Also it is required that unique impurities of new drug substance are separated and do not interfere during analysis of specified and unspecified DPs. The ICH guideline Q6A (56) also states the requirement of stability indicating assays under universal tests or criteria for drug substances and drug products. In ICH guideline Q5C (57) same requirements are mentioned.

The exact definition of a SIAMs is not provided in any of the ICH guideline, however 1987's US-FDA (58) stability guideline and the 1998's (59) draft guideline defines SIAMs as *"validated quantitative analytical methods, that can detect the changes with time in the physical, chemical, and/or microbiological properties of the drug substance and drug product, that are specific so that the contents of active ingredient, DPs and other components of interest can be accurately measured without interference"*

Requirement of stability testing and SIAMs is also mentioned in European Committee for Proprietary Medicinal Products, World Health Organization (WHO), Canadian Therapeutic Products Directorate's guidelines (60, 61), United States Pharmacopoeia (USP) and ICH Q7A guideline (62) (*Good Manufacturing Practices for Active Pharmaceutical Ingredients*).

1.7.2 Steps involved in development of validated SIAMs

Regulatory documents states the requirements of development of validated SIAMs but information on the basic steps that has to be followed for the development of validated SIAMs is provided neither in pharmacopoeias nor in regulatory guidelines. However M. Bakshi and S. Singh (63) have described the practical steps that could be followed in the development of validated SIAMs and are as follows:

Step I- Critical study of the drug structure to determine the likely decomposition route.

Step II- Collection of necessary information on physicochemical properties of drug.

Step III- Stress decomposition or degradation studies.

Step IV- Preliminary separation studies on stressed or degraded samples.

Step V- Final method optimization and development.

Step VI- Identification and characterization of DPs and preparation of standards.

Step VII- Validation of SIAMs.

1.7.3 Degradation conditions and Sample Generation

Stress degradation is carried out to generate representative samples for developing SIAMs for drug substances and drug products. Figure 1.3 represents the general protocol of stress degradation.

Hydrolytic conditions

The most common degradation reaction that occurs over a wide range of pH is hydrolysis. Under acidic or basic condition the catalysis of ionizable functional group takes place. The structure and stability of particular molecule determines the selection of type and concentration of acids and bases to be utilized for stress degradation. Generally hydrochloric or sulfuric acid (0.1-1M) and sodium or potassium hydroxide (0.1-1M) is used for acid and base hydrolysis respectively. For poorly water soluble drugs co-solvents can be used based on structure of drug molecule. If no degradation takes place at room temperature elevated temperature can be utilized as per Arrhenius law.

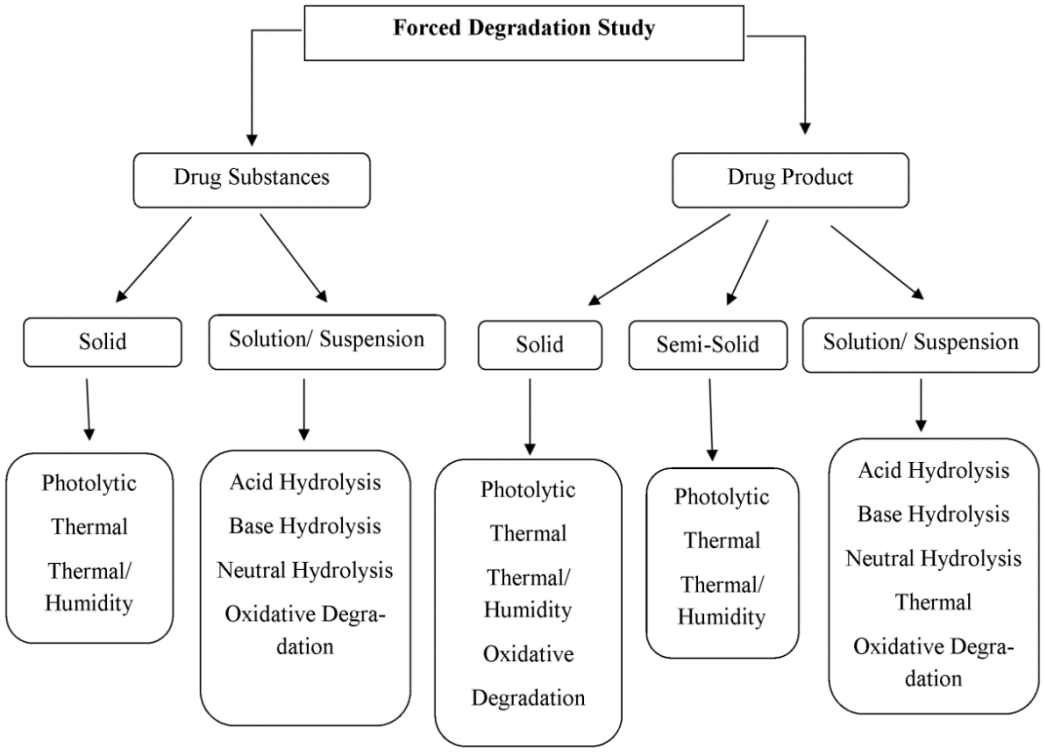

Figure 1.3: Flow chart describing various stress conditions used for degradation

Oxidative conditions

Hydrogen peroxide is most commonly used for oxidative stress degradation. Other oxidizing agents like oxygen, radical initiators (e.g., azobisisobutyronitrile, AIBN) and metal ions can also be used. The selection of type of oxidizing agent, its concentration and conditions depends on the nature and structure of drug substance. Usually oxidation involves formation of reactive anion or cataion by electron transfer mechanism. Functional groups like sulfides, phenols, amines undergo oxidation to form sulfones, sulfoxide, hydroxylamine and N-oxides (64). Also allylic carbon, benzylic carbon, tertiary carbon/ α-positions with respect to hetero-atom form hydroxide, hydro peroxides or ketone (36, 65) are susceptible to oxidation.

Photolytic conditions

Photo-stability testing is carried out by exposing the drug to UV or fluorescent light as per ICH Q1B guideline. Samples should be exposed to a minimum of 1.2 million l x h and 200 Wh/m^2 light with maximum recommended illumination of 6 million l x h (66). Free radical mechanism is involved in photo-degradation. Photosensitive functional groups are nitro-

aromatics, carbonyls, N-oxides, aryl chlorides, alkenes, weak C–H and O–H bonds polyenes, and sulfides (67).

Thermal conditions

Samples could be exposed to dry and wet heat conditions at higher temperatures for a shorter period (68). Effect of temperature on thermal degradation of a molecule is studied through the Arrhenius equation (69) $k = Ae^{-Ea/RT}$ where k= specific reaction rate, R = gas constant (1.987cal/deg mole), A = frequency factor, Ea = activation energy and T = absolute temperature.

1.8 QbD (Quality by Design) and DoE (Design of Experiments)

QbD has been considered as a fundamental pharmaceutical quality model by US Food and Drug Administration (US-FDA) in the development of pharmaceutical products and processes (70, 71) and stressed on critical significance of applying principles of QbD to ensure to build the product quality by design. ICH guidelines Q8 (R2) (*pharmaceutical development*) (72), Q9 (*quality risk assessment*) (73), and Q10 (*pharmaceutical quality system*) (74) also supports the QbD principles.

According to FDA, QbD is "*a systematic approach to development that begins with predefined objectives and emphasizes on product and process understanding and process control based on sound science and quality risk management*". QbD establishes the DS (design space) that determines process control and is defined as the "*multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality*". As per the definition, multivariate techniques should be used to characterize DS; hence for implementing QbD, DoE has emerged as a fundamental activity.

Analytical QbD, AQbD (75) is analogous to process QbD which is related to analytical method development and apply the similar principle. AQbD determines a MODR (method operable design region) defined as "*a multidimensional space based on the method factors and settings that provide suitable method performance*". Some key aspects of the AQbD are depicted in figure 1.4.

Analytical target profile (ATP)

A tool used for method development is ATP that describes the method requirements, expected to be measured. The aim of chromatographic method development is usually separation, identification and quantification of drug substance, impurity and/or DPs. ICH guidelines (76) describes accuracy, precision, robustness, ruggedness etc as method requirements. In order to set stringent method goals detailed information of compound properties should be collected like its pH, pka, solubility, UV chromophore, and stability.

Method design

To set various experimental conditions and for appropriate availability of material, method design is prepared. In this the reagents required are made available and regional and geographical conditions are taken into consideration. Feasibility and/or availability of instruments are checked and experimental design is prepared. In this use of various flowcharts and/or decision tree can be made for correct implementation.

Critical quality attributes (CQA)

The possible effect of factors on method development is studied. Proper understanding of the particular method will help in sorting CQA.

Risk assessment (RA)

It provides a link between input process variable and CQA. Tools utilized for RA are, Fishbone or Ishikawa or cause effect diagram, Failure mode effect analysis (FMEA), Cause and Effect (C and E) Analysis and Pareto analysis. To identify the effects of variables (like instrumental factors, raw materials, and environmental factors) on selected CQA, a fishbone diagram is constructed. Then to rank the variables FMEA or Cause and Effect (C and E) Analysis can be used to facilitate the identification of high risk variables/factors, based on risk, that is a combination of probability, severity, and delectability (77). Grouping of factors is done based on risk assessment: High-risk factors that should be stringently controlled; Potential noise factors and factors that can be experimentally explored to determine acceptable ranges.

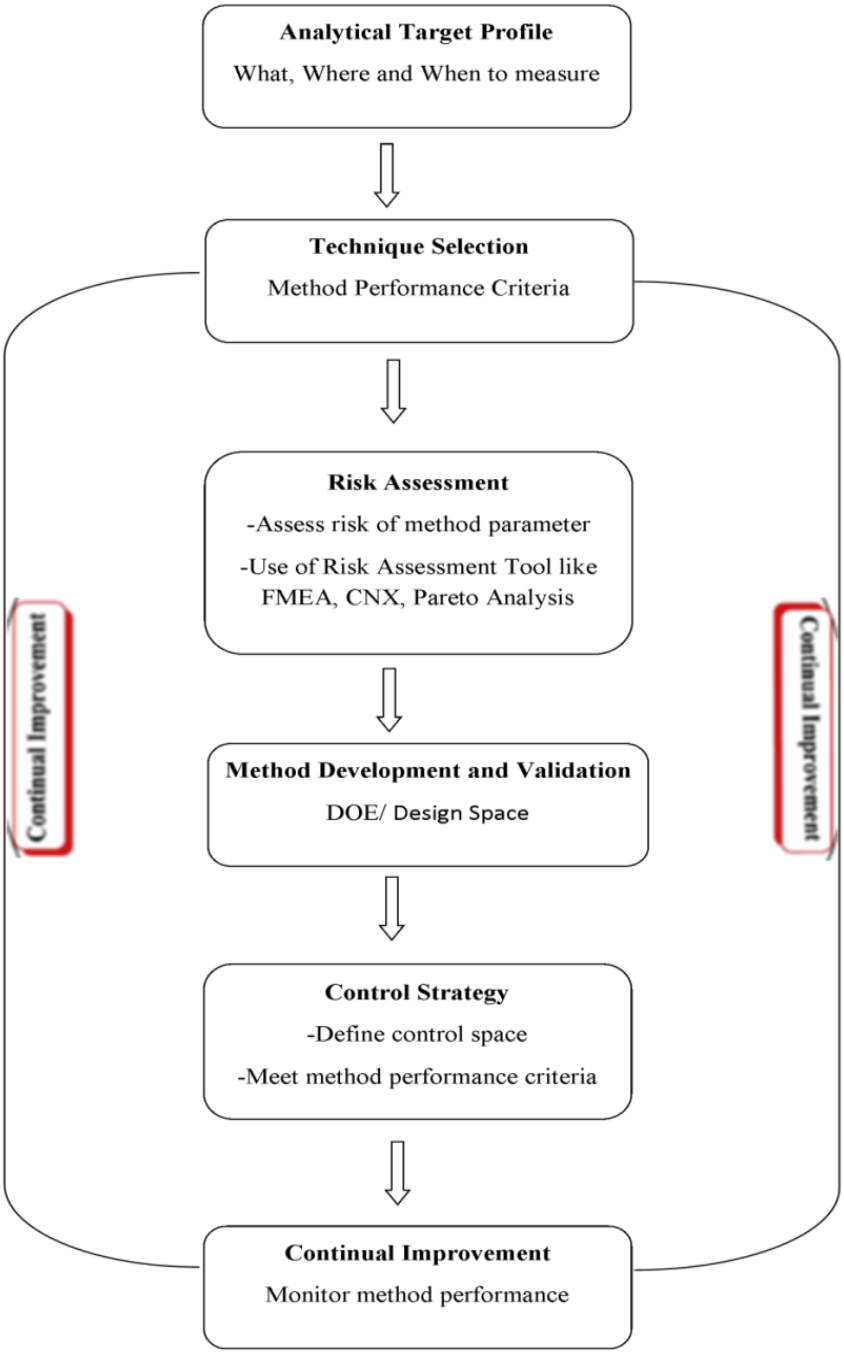

Figure 1.4: Analytical method development in QbD

Design of experiments (DoE)

After RA higher ranked variables can be subjected to screening studies by means of DoE (78). The objective of screening design is to reduce the number of experiments on a maximum number of variables to estimate the main effects, mostly with fractional two level and Plackett Burman designs (79). Screening design is applicable for both numeric

and categorical (buffer, column, solvent etc) variables. Then subsequent optimization by response surface methodology (RSM) is done for numeric variables to estimate the interaction and/or quadratic effects (80). Based on preliminary experiments and/or prior knowledge or RA, DoE screening can be eliminated enabling direct optimization by RSM (81, 82), these includes Full Factorial, Fractional Factorial, D-optimal, Doehlert designs Central Composite and Box–Behnken Designs (79).

Method verification

The chromatographic space evaluated by DoE is then validated usually in accordance with ICH Q2(R1) guidelines. Accuracy and precision study provides additional understanding of the method's uncertainty and confirms acceptability to the previously defined ATP.

Control strategy

Control strategy is required to confirm that the set method performs consistently and as intended and gives accurate results. Factors identified to have high risk have to be specially controlled. System suitability can also be checked and verified time to time.

Life cycle approach

It differs from traditional approach of method development because it includes continuous improvement of method performance based on design space that allows flexibility for Continuous improvement of analytical method, without prior regulatory approval. Justified changes can be done wherever required based on risk assessment and data collected from DoE that can be used as the repository of knowledge.

1.9 REFERENCES

1. Guideline ICH. Impurities in new drug substances Q3A (R2), in: International Conference on Harmonisation, IFPMA, Geneva (Switzerland), 2006.
2. Guideline ICH. Impurities in new drug products Q3B (R2), in: International Conference on Harmonisation, IFPMA, Geneva (Switzerland), 2006.
3. Görög S. Identification and determination of impurities in drugs: Elsevier, volume 4, 2000.
4. Ahuja S, Alsante KM. Handbook of isolation and characterization of impurities in pharmaceuticals: Academic press; volume 5, 2003.

5. Guideline ICH, Impurities Guideline for residual solvents Q3C (R5), in: International Conference on Harmonisation, IFPMA, Geneva (Switzerland), 2011.
6. Ahuja SS. Assuring quality of drugs by monitoring impurities. Advanced drug delivery reviews. 2007;59(1):3-11.
7. Guideline ICH. The common technical document for the registration of pharmaceuticals for human use: Quality – M4Q (R1), in: International Conference on Harmonisation, IFPMA, Geneva (Switzerland), 2002.
8. Guideline CPMP, Chemistry of new active substances (CPMP/QWP/130/96), Committee for Proprietary Medicinal Products, EMEA, London (UK), 2004.
9. Guideline CPMP, Summary of requirements for active substances in the quality part of the dossier (CHMP/QWP/297/97 Rev 1 corr. and EMEA/CVMP/1069/02), Committee for Proprietary Medicinal Products, EMEA, London (UK), 2005.
10. http://www.fda.gov/downloads/Drugs/Development Approval Process/How Drugs are Developed and Approved/Approval Applications/Abbreviated New Drug Application ANDA Generics/UCM151259.pdf (Accessed on: Mar-2014).
11. Guideline TPD, Draft Guidance for Industry, Quality (chemistry and manufacturing) Guidance: New Drug Submissions (NDSs) and Abbreviated New Drug Submissions (ANDSs), Therapeutic Products Directorate, Health Canada, Ontario (Canada), 2001.
12. http://www.moh.gov.bn/pharmacyservices/download/ASEAN%20Common %20Technical%20Document%20(ACTD).pdf (Accessed on: Mar-2014).
13. Guideline WHO, on submission of documentation for a multisource (generic) finished pharmaceutical product (FPP): Quality part (draft), Working document QAS/10.373 Rev.1, World Health Organisation, Geneva (Switzerland), 2011.
14. Guideline ICH, Impurities Guideline for metal impurities Q3D, in: International Conference on Harmonisation, IFPMA, Geneva (Switzerland), 2009.
15. Guideline ICH, Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk (final concept paper) M7, in: International Conference on Harmonisation, IFPMA, Geneva (Switzerland), 2010..
16. Guideline CPMP, Assessment of the Quality of Medicinal Products Containing Existing/Known Active Substances (EMEA/CHMP/CVMP/QWP/450653/2006), Committee for Proprietary Medicinal Products, EMEA, London (UK), 2007.
17. Guideline CPMP, Guideline on Control of Impurities of Pharmacopoeial Substances: Compliance with the European Pharmacopoeia General Monograph Substances for Pharmaceutical use and General Chapter Control of Impurities in Substances for Pharmacopoeial Use (CPMP/QWP/1529/04), Committee for Proprietary Medicinal Products, EMEA, London (UK), 2004.

18. Guideline CPMP, Guideline on the Limits of Genotoxic Impurities (CPMP/SWP/5199/02 and EMEA/CHMP/QWP/251344/2006), Committee for Proprietary Medicinal Products, EMEA, London (UK), 2007.
19. Guideline CPMP, Guideline on Setting Specifications for Related Impurities in Antibiotics (draft) (EMA/CHMP/CVMP/QWP/199250/2009), Committee for Proprietary Medicinal Products, EMA, London (UK), 2010.
20. Guideline CPMP, Guideline on Setting Specifications for Related Impurities in Antibiotics (draft) (EMA/CHMP/CVMP/QWP/199250/2009), Committee for Proprietary Medicinal Products, EMA, London (UK), 2010.
21. Guideline US FDA, NDAs Impurities in Drug Substances, Food and Drug Administration, Silver Spring, MD (USA), 2000.
22. Guideline US FDA, Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (draft), Food and Drug Administration, Silver Spring, MD (USA), 2008.
23. Guideline US FDA, ANDAs Impurities in Drug Products, Food and Drug Administration, Silver Spring, MD (USA), 2010.
24. Guideline TGA, Australian Regulatory Guidelines for Prescription Medicines, Appendix 18: Impurities in Active Pharmaceutical Ingredients and Finished Products, Therapeutic Goods Administration, Woden ACT (Australia), 2004.
25. Guideline TPD, Impurities in Existing Drug Substances and Products (draft), Therapeutic Products Directorate, Health Canada, Ontario (Canada), 2005.
26. Mollica JA, Ahuja S, Cohen J. Stability of pharmaceuticals. Journal of pharmaceutical sciences. 1978;67(4):443-65.
27. Pozniak A, Müller L, Salgo M, Jones JK, Larson P, Tweats D. Elevated ethyl methanesulfonate (EMS) in nelfinavir mesylate (Viracept®, Roche): overview. AIDS research and therapy. 2009;6(1):1.
28. Murakami T, Fukutsu N, Kondo J, Kawasaki T, Kusu F. Application of liquid chromatography-two-dimensional nuclear magnetic resonance spectroscopy using pre-concentration column trapping and liquid chromatography–mass spectrometry for the identification of degradation products in stressed commercial amlodipine maleate tablets. Journal of Chromatography A. 2008;1181(1):67-76.
29. Raillard SP, Bercu J, Baertschi SW, Riley CM. Prediction of drug degradation pathways leading to structural alerts for potential genotoxic impurities. Organic Process Research & Development. 2010;14(4):1015-20.

30. Görög S, Herényi B. Analysis of steroids: XXXVIII. The use of high-performance liquid chromatography with diode-array UV detection for estimating impurity profiles of steroid drugs. Journal of Chromatography A. 1987;400:177-86.
31. Saavedra L, Huidobro AL, García A, Cabanelas JC, González MG, Barbas C. CE as orthogonal technique to HPLC for alprazolam degradation product identification. Electrophoresis. 2006;27(12):2360-6.
32. Slonecker PJ, Li X, Ridgway TH, Dorsey JG. Informational orthogonality of two-dimensional chromatographic separations. Analytical chemistry. 1996;68(4):682-9.
33. Wang X, Li W, Rasmussen HT. Orthogonal method development using hydrophilic interaction chromatography and reversed-phase high-performance liquid chromatography for the determination of pharmaceuticals and impurities. Journal of Chromatography A. 2005;1083(1):58-62.
34. Reilly J, Saeed M. Capillary electrochromatography as an alternative separation technique to high-performance liquid chromatography and capillary zone electrophoresis for the determination of drug related impurities in Lilly compound LY300164. Journal of Chromatography A. 1998;829(1):175-86.
35. Wang Z, Zhang H, Liu O, Donovan B. Development of an orthogonal method for mometasone furoate impurity analysis using supercritical fluid chromatography. Journal of Chromatography A. 2011;1218(16):2311-9.
36. Boccardi G. Pharmaceutical Stress Testing Predicting Drug Degradation, NY:Taylor and Francis, first edition, 2005.
37. Singh S, Bakshi M. Stress test to determine inherent stability of drugs. Pharm Technol. 2000;4:1-14.
38. Brenna E, Frigoli S, Fronza G, Fuganti C, Malpezzi L. Isolation and characterisation of a phenolic impurity in a commercial sample of duloxetine. Journal of pharmaceutical and biomedical analysis. 2007;43(4):1573-5.
39. Görög S. The importance and the challenges of impurity profiling in modern pharmaceutical analysis. TrAC Trends in Analytical Chemistry. 2006;25(8):755-7.
40. Ferreirós N, Dresen S, Alonso RM, Weinmann W. Hydrolysis and transesterification reactions of candesartan cilexetil observed during the solid phase extraction procedure. Journal of Chromatography B. 2007;855(2):134-8.
41. Djozan D, Jouyban A, Norouzi J. Ultrasonic assisted SPME Coupled with GC and GC—MS using Pencil Lead as a Fiber for Monitoring the Organic Volatile Impurities of Ceftazidime. Journal of chromatographic science. 2008;46(8):680-5.
42. Pavón JLP, del Nogal Sánchez M, Laespada MEF, Pinto CG, Cordero BM. Analysis of class 1 residual solvents in pharmaceuticals using headspace-programmed temperature

vaporization-fast gas chromatography–mass spectrometry. Journal of Chromatography A. 2007;1141(1):123-30.

43. Brondz I, Ekeberg D, Bell DS, Annino AR, Hustad JA, Svendsen R, et al. Nature of the main contaminant in the drug primaquine diphosphate: SFC and SFC–MS methods of analysis. Journal of pharmaceutical and biomedical analysis. 2007;43(3):937-44.

44. Wanatabe N, Niki E. Direct-coupling of FT-NMR to high performance liquid chromatography. Proceedings of the Japan Academy Ser B: Physical and Biological Sciences. 1978;54(4):194-9.

45. Mukhopadhyay R. Liquid NMR probes: oh so many choices. Analytical chemistry. 2007;79(21):7959-63.

46. Neuhaus D, Ismail IM, Chung C-W. "FLIPSY"—a new solvent-suppression sequence for nonexchanging solutes offering improved integral accuracy relative to 1D NOESY. Journal of Magnetic Resonance, Series A. 1996;118(2):256-63.

47. Junwal M, Sahu A, Handa T, Shah RP, Singh S. ICH guidance in practice: degradation behaviour of oseltamivir phosphate under stress conditions. Journal of pharmaceutical and biomedical analysis. 2012;62:48-60.

48. Michalski R. Encyclopedia of Chromatography. 2010.

49. Jayawickrama DA, Sweedler JV. Hyphenation of capillary separations with nuclear magnetic resonance spectroscopy. Journal of Chromatography A. 2003;1000(1):819-40.

50. Wolters AM, Jayawickrama DA, Larive CK, Sweedler JV. Capillary isotachophoresis/NMR: extension to trace impurity analysis and improved instrumental coupling. Analytical chemistry. 2002;74(10):2306-13.

51. Eldridge SL, Almeida VK, Korir AK, Larive CK. Separation and analysis of trace degradants in a pharmaceutical formulation using on-line capillary isotachophoresis-NMR. Analytical chemistry. 2007;79(22):8446-53.

52. Somsen G, Gooijer C, Brinkman UT. Liquid chromatography–Fourier-transform infrared spectrometry. Journal of Chromatography A. 1999;856(1):213-42.

53. Somsen G, Gooijer C, Brinkman UT, Velthorst N, Visser T. Coupling of LC and FT-IR: Impurity profiling of testosterone undecanoate. Applied spectroscopy. 1992;46(10):1514-9.

54. Murakami T, Konno H, Fukutsu N, Onodera M, Kawasaki T, Kusu F. Identification of a degradation product in stressed tablets of olmesartan medoxomil by the complementary use of HPLC hyphenated techniques. Journal of pharmaceutical and biomedical analysis. 2008;47(3):553-9.

55. Guideline ICH, Stability Testing of New Drug Substances and Products Q1A (R2), in: International Conference on Harmonization, IFPMA, Geneva; 2003.

56. Guideline ICH, Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances Q6A, in International Conference on Harmonisation, IFPMA, Geneva; 1999.
57. Guideline ICH, Quality of biotechnological products: Stability testing of biotechnological/biological products Q5C: in International Conference on Harmonisation (ICH). Developments in biological standardization. 1995.
58. FDA, Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics. Food and Drug Administration, Rockville, MD, 1987.
59. FDA, Guidance for Industry: Stability Testing of Drug Substances and Drug Products (Draft guidance), Food and Drug Administration, Rockville, MD, 1998.
60. WHO, Guidelines for Stability Testing of Pharmaceutical Products Containing Well Established Drug Substances in Conventional Dosage Forms, in WHO Expert Committee on Specifications for Pharmaceutical Preparations. Technical Report Series 863, World Health Organization, Geneva, 1996, 65–79.
61. Guideline E. Stability testing: of existing active substances and related finished products. issued as CPMP. QWP/122/02, 2003.
62. Guideline ICH. Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients. Q7A, in International Conference on Harmonisation (ICH). Current Step. 2000.
63. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. Journal of pharmaceutical and biomedical analysis. 2002;28(6):1011-40.
64. Gupta A, Yadav JS, Rawat S, Gandhi M. Method development and hydrolytic degradation study of Doxofylline by RP-HPLC and LC-MS/MS. Asian Journal of Pharmaceutical Analysis. 2011;1(1):14-8.
65. Alsante KM, Hatajik TD, Lohr LL, Santafianos D, Sharp TR. Solving impurity/degradation problems: case studies. Separation Science and Technology. 2004;5:361-400.
66. Baertschi SW, Thatcher SR. Sample presentation for photostability studies: problems and solutions. Pharmaceutical Photostability and Stabilization Technology: Taylor & Francis New York; 2006. p. 445.
67. Ahuja S, Scypinski S. Handbook of modern pharmaceutical analysis: Academic press; 2010.
68. Alsante KM, Ando A, Brown R, Ensing J, Hatajik TD, Kong W, et al. The role of degradant profiling in active pharmaceutical ingredients and drug products. Advanced drug delivery reviews. 2007;59(1):29-37.

69. Qiu F, Norwood DL. Identification of pharmaceutical impurities. Journal of liquid chromatography & related technologies. 2007;30(5-7):877-935.
70. FDA. Pharmaceutical CGMPs for the 21st Century—a risk-based approach. Final report Rockville, MD. 2004.
71. FDA. PAT guidance for industry–a framework for innovative pharmaceutical development, manufacturing and quality assurance. US Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, Office of Regulatory Affairs, Rockville, MD. 2004.
72. Guideline ICH. Pharmaceutical Development Q8,(R2); US Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD. 2009.
73. GuidelineICH. Quality Risk Management Q9. in International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use. 2006.
74. Guideline ICH. Pharmaceutical Quality Systems Q10. International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use, 2008.
75. Vogt FG, Kord AS. Development of quality-by-design analytical methods. Journal of pharmaceutical sciences. 2011;100(3):797-812.
76. Borman P, Nethercote P, Chatfield M, Thompson D, Truman K. The application of quality by design to analytical methods. 2007.
77. Sangshetti JN, Deshpande M, Zaheer Z, Shinde DB, Arote R. Quality by design approach: Regulatory need. Arabian Journal of Chemistry. 2014.
78. Huang J, Kaul G, Cai C, Chatlapalli R, Hernandez-Abad P, Ghosh K, et al. Quality by design case study: an integrated multivariate approach to drug product and process development. International journal of pharmaceutics. 2009;382(1):23-32.
79. Hibbert DB. Experimental design in chromatography: a tutorial review. Journal of chromatography B. 2012;910:2-13.
80. McBrien M. Practical implications of quality by design to chromatographic method development. Chromatography Today. 2010;3:30-3.
81. Piepel G, Pasquini B, Cooley S, Heredia-Langner A, Orlandini S, Furlanetto S. Mixture-process variable approach to optimize a microemulsion electrokinetic chromatography method for the quality control of a nutraceutical based on coenzyme Q10. Talanta. 2012;97:73-82.
82. Molnár I, Monks K. From Csaba Horváth to quality by design: visualizing design space in selectivity exploration of HPLC separations. Chromatographia. 2011;73(1):5-14.