Review Article

A review on impurity profiling, degradation studies and bioanalytical methods on anti-diabetic drugs

Abstracts

According to ICH Q3A(R), the impurity in new drug substance is “any component of new drug substance that is not the chemical entity defined as new drug substance”. As Per ICH Q3B(R), the impurity in new drug product is “any component of drug product that is not the drug substance andexcipientsinthedrugproduct.”The forced degradation studies are used to facilitated the development of analytical methodology, to achieve a better understanding of drug substance and drug product stability, and to determine degradation pathways and degradation products. This study will help to get most stableformulation.The bioanalytical method development and validation is an essential part in drug discovery and development.There is need to develop and validate bioanalytical methods, as sponsors have to submit clinical pharmacology, bioavailability, bioequivalence, pharmacokinetic evaluation along with non-human pharmacology and toxicology studies and preclinical studies to regulatory authorities.

There are number of spectroscopic methods includes Ultraviolet spectroscopy, Mass spectroscopy, Nuclear magnetic resonance spectroscopy and Chromatographic methods includes HPLC,HPTLC,GC,UPLC as well as hyphenated techniques like LC-MS, LC/ESI-MS, LC-NMR-MS used for identification and characterization of impurities in API and drug products forced degradation study to obtain stability data and bioanalytical methods.

The uniqueness of this reviewis that it describesthe detail information and background explainingimpurities, forced degradation and bioanalytical method development and validation as well as all literature available regarding development and validation of all said methods for drugsand the drug products used to treat type 2 diabetes.

Keywords- Antidiabetic drugs, Impurity Profiling, Force degradation study, Analytical methods, Regulatory guidelines.

1. Introduction

According to ICH Q3A(R), theimpurity in new drug substance is "any component of new drug substance that is not the chemical entity defined as new drug substance". As Per ICH Q3B(R), the impurity in new drug product is "any component of drug product that is not the drug substance and excipients in the drug product."The impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredient (API), or develop throughout formulation development or upon aging of APIs and formulated API to medicine. The presence of these unwantedchemicaleveninsmallamountsmayinfluencetheefficacyandsafetyofpharmaceutical products.[1]

There is an ever-increasing interest in impurities present in APIs recently, not only purity profile butalsoimpurityprofilehasbecomeessentialaspervariousregulatoryrequirements.[2] Impurity is initial quality issue in pharmaceutical product that would compromise the efficacy of drug product. Hence any impurity present in drug product should be absolutely understood each qualitatively and quantitatively, and qualify, if necessary, through toxicological assessment. So, identification,isolation,qualificationsofimpuritiesareanimportantpartofdrugdevelopmentan

d regulatory assessment. The pharmaceutical impurities are unavoidable as a result of no chemical reaction has 100% selectivity and chemical compound is 100% pure. Ever so, it is possible to reduce impurities via synthetic improvement and appreciate preformulation /formulation studies.[3]

Common terms of impurities: [1,2,3]

There are various terms associated with impurities are as follow,

1. Intermediate
2. Penultimate intermediate
3. By-products
4. Transformation products
5. Interaction products
6. Related products
7. Degradation products

1. **Intermediate -** The compounds produced during synthesis of the desired material are called intermediates, especially when they have been isolated and characterized.
2. **Penultimate intermediate -** This is the last compound in the synthesis chain prior to the production of the final desired compound.
3. **By-product -** The compound produced in the reaction other than the required intermediates. They can occur through a variety of side reactions, such as overreaction, incomplete reaction, demonization and rearrangement, unwanted reactions between starting materials or intermediates with chemical reagents or catalysts.
4. **Transformation products -** This is a relatively nondescript term that relates to theorized and non-theorized products that may be produced in the reaction, which can include

synthetic derivatives of by-products. Transformation products are very similar to by-products.

5. **Interaction products** -These products formed either intentionally or unintentionally interaction between various chemicals involved. Two types of interaction products that can be commonly encountered are drug substance–excipient interactions and drug substance container/closure interaction.
6. **Related products** - These are chemically similar to drug substance and may exhibit potentially similar biological activity.
7. **Degradation products** - They are formed by the decomposition of active ingredient or other material of interest by the effect of external factors like heat, light and moisture.

Now a day ICH, EMA and USFDA have claimed the importance of impurities in the analysis of drug substances. Alternatively going for determination of purity, the identification and quantification of impurities in formulation & bulks are characterized. This is the best approach to designate the Identity, Quality, Safety, Efficacy, Purity, Strength and Stability of APIs and pharmaceutical formulations. It has become a mandatory requirement in various pharmacopoeias (BP, EP, USP) to include impurities present in drugs in specified amount.[4]

1.1 Classification of Impurities

The impurities present in new drug substance can be divided in to chemistry and safety aspects. According to chemistry aspect the impurities are identified, classified and analytical methods are developed to set their specifications.The safety aspects explain the qualification of impurities which are not performed at the time of clinical trials and their threshold limit are also determined. According to ICH guidelines the impurities are classified as Organic, Inorganic, and Solvent. This ICH guidance classifies impurities in three classes as Organic, Inorganic and

Solvent. Every class and aspects should be clearly reported related to each impurity for new drug.[5]Refer fig.1.

According to ICH Q3A(R2) guidelines, impurities classified as[6]

1. **Organic impurities** can be identified or unidentified, volatile or nonvolatile. They can arise from manufacturing process or storage of new drugsubstances.
    - Startingmaterials
    - Byproducts
    - Intermediates
    - Degradationproducts
    - Reagents, ligands and catalysts
2. **Inorganic impurities** can result from manufacturing process. They are normally known and identified.
    - Reagents, ligands and catalysts
    - Heavy metals and other residualSolvents
    - Inorganicsalts
    - Other materials (e.g, filter aid andcharcoal)
3. **ResidualSolvents**arethevolatileorganicchemicalsusedduringthemanufacturingprocessor generated during drug production. They are categorized into three classes with their limits in pharmaceutical products set by ICH guidelines Q3C.The class 1 solvents includes Benzene, Carbon tetrachloride,1,1-dichloroethane,1,2-dichloroethylene and 1,1,1-trichloroethnane should be avoided. The class 2 solvents such as methanol, toluene, pyridine, N, N-dimethylformamide and acetonitrile have permitted daily exposure limits (PDEs). The class 3 solvents such as acetone, isopropyl alcohol, butanol, ethanol and

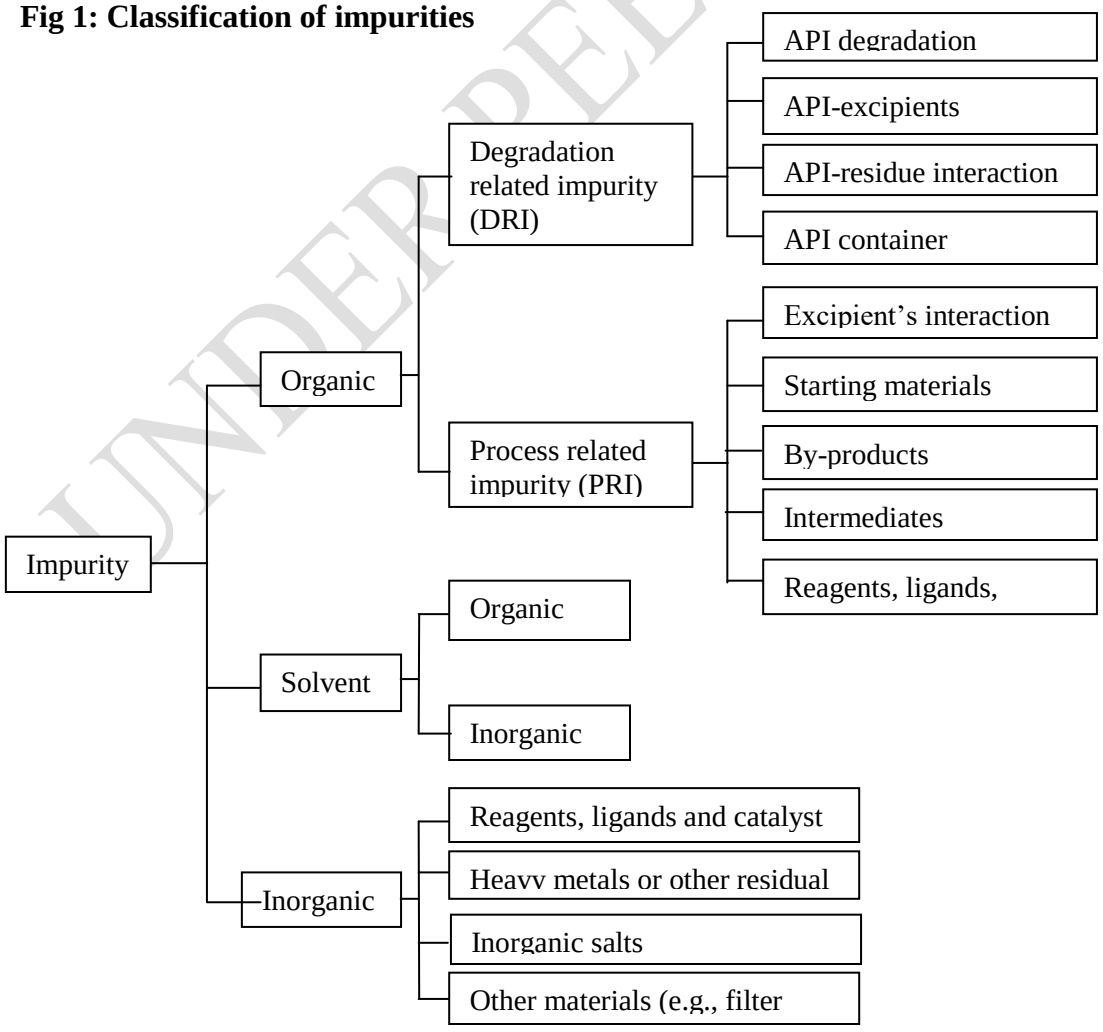
ethyl acetate should be limited by GMP or other quality-basedrequirements.[7]

ICH limits for selected list of common organic solvents found as volatile impurities.[8]

Chart 1: list of common organic solvents

Volatile Organic Impurity	Limits(ppm)	PDE (mg/day)
Acetonitrile	410	4.1
1,4-dioxane	380	3.8
Chloroform	60	0.6
Methylene chloride	600	6.0
Pyridine	200	2.0
1,1,2-tricholroethane	80	0.8

Fig 1: Classification of impurities



1.2 Elemental Impurity

The elemental impurities in drug and drug product such as As, Cd,Cu,Pb,Hg,V and Pt may arisefrom several sources; they may be added intentionally in synthesis, or may be present ascontaminant,e.g., through interaction with manufacturing equipment, containers and closures. They do not provide therapeutic benefits to the patients; their level should be controlled within acceptable limits in drug product i.e., Permitted Daily Exposure [P.D.E] for each element of toxicological concern and application of risk approach to control elemental impurities in drug product.[9]

They are classified from ICH Q3D and USP;

Class 1:Cd, Pb, As, Hg

Class 2A:Co, V, Ni

Class 2B: Ag, Au, Tl, Pd, Pt, Ir, Os, Rh, Ru

Class 3:Sb, Ba, Li, Cr, Cu, Sn, Ni

Class 4:B, Fe, Zn, K, Ca, Na, Mn, Mg, W, Al

According to USP impurities classified as;

1. Impurities in officialarticles
2. Ordinaryimpurities
3. Organic volatileimpurities

1.3 Sources of Impurities

1.Crystallization related impurities

The polymorphism is the term used to denote crystal systems where a substance can exists in numerous crystal packing arrangement, all of which has same elemental composition. It is also possible to possess crystal system where the substance exists in numerouscrystalpackingarrangement,eachofwhichhasadifferentelementalcomposition;this phenomenon is known assolvatomorphism.[10]

2.Stereochemistry related impurities

It is of paramount importance to seems for stereochemistry related compounds i.e. those compounds that have similar chemical structure but different spatial orientation These compounds will be considered impurities within theAPI.[10]

3.Impurities arising during storage

A variety of impurities can originate from during storage condition or shipment of drug products. The impurities can come from glass,rubberstoppers and plastic packaging materials. Metal oxide like NaO2, SiO2, CaO2, MgO are the main components leached fromglass.[11]

4.Mutual interaction amongst ingredients

Most vitamins are extremely labile and because of ageing they generate problems of instability in many dosage forms, particularly in liquid dosage forms. A vitamin on degradation doesn't give toxic impurities; on the opposite hand, thepotency of active ingredients lowers pharmacopeialspecifications.[11]

5.Residualsolvents

Theseareorganicvolatilechemicalsusedduringmanufacturingprocessor generating during the production. They have toxic or environmentally hazardousproperties; their complete removal can

be very difficult. Gas chromatography is employed for detection of residual solvents because they're mostly volatile in nature.[12]

6.SyntheticIntermediatesandbyproducts

The impuritiesinapharmaceuticalcompoundoranew chemical entity originate mainly during the synthetic process from raw materials, solvents, intermediateandbyproducts.Therawmaterialsarenormally used tomanufactureddrugsubstance that having minor purity.Similarly,solventsutilizedinthesynthesisarelikelytocontain variety of impurities which will change range from trace levels to significant amounts that may react with various chemicals utilized in the synthesis to produceimpurities.[13]

7.Formulation related impurities

Number of impurities in a drug product can arise out of inert ingredients used to formulate a drug substance. In the process of formulation, a drug substance is subjectedtoavarietyofconditionsthatmayresultinitsdegradationorotherdeleteriousreaction.

Solutions and suspensions are potentially susceptible to degradation due to hydrolysis. The water utilized in the formulation cannot only contribute its own impurities; it may also provide a ripe situation for hydrolysis and catalysis. Similar reactions are possible in other solvents that may be used.[14]

8.Functional group related impurities

Ester hydrolysis can be seen in a few drugs *viz* aspirin, benzocaine, cefotaxime, ethyl paraben, and cefpodoxime proxetil. Oxidative degradation ofdrugs that have hydroxyl group directly bonded to an aromatic ring (*viz* phenol derivatives like catecholamine and morphine) some drugs like hydrocortisone; methotrexate, and, conjugated dienes (*viz* vitamin A and unsaturated free fatty acids), heterocyclic aromatic rings, nitroso and nitrite derivatives, and

aldehydes (especially flavone rings) are all susceptible to oxidative degradation.[11,15]

9.Degradation related impurities

The Impurities can also be formed by degradation of the end productduringmanufacturingofthebulkdrugs.Thedegradationofpenicillinandcephalosporin's are well-known examples of degradation products. The presence of a β-lactam ring, likewise a α- amino group in the C6/C7 side chain plays a critical role in theirdegradation.[14]

10.Method related impurities

The diclofenac sodium in parenteral formulation containing an impurity;1-2,6dichlorophenylindole.Indiclofenacsodiumindolinonederivativescouldalsobe formed due to condition of autoclave (i.e., 123±20ºC) as it induced intramolecularreaction.[14]

1.4 Regulatory guidelines onImpurities[10,14,16]

There are several regulatory guidelines for impurities are given below:

1) ICHGuidelines

Q1A(R) – Stability testing of new drug substances and products.

Q3A- Impurities in new drug substances.

Q3B- Impurities in new drug products.

Q3C-Guidelines for residual solvents.

Q3D- Guidelines for elemental impurities

2) US-FDA Guidelines

NDAs- Impurities in new drug substances

ANDAs - Impurities in new drug substances

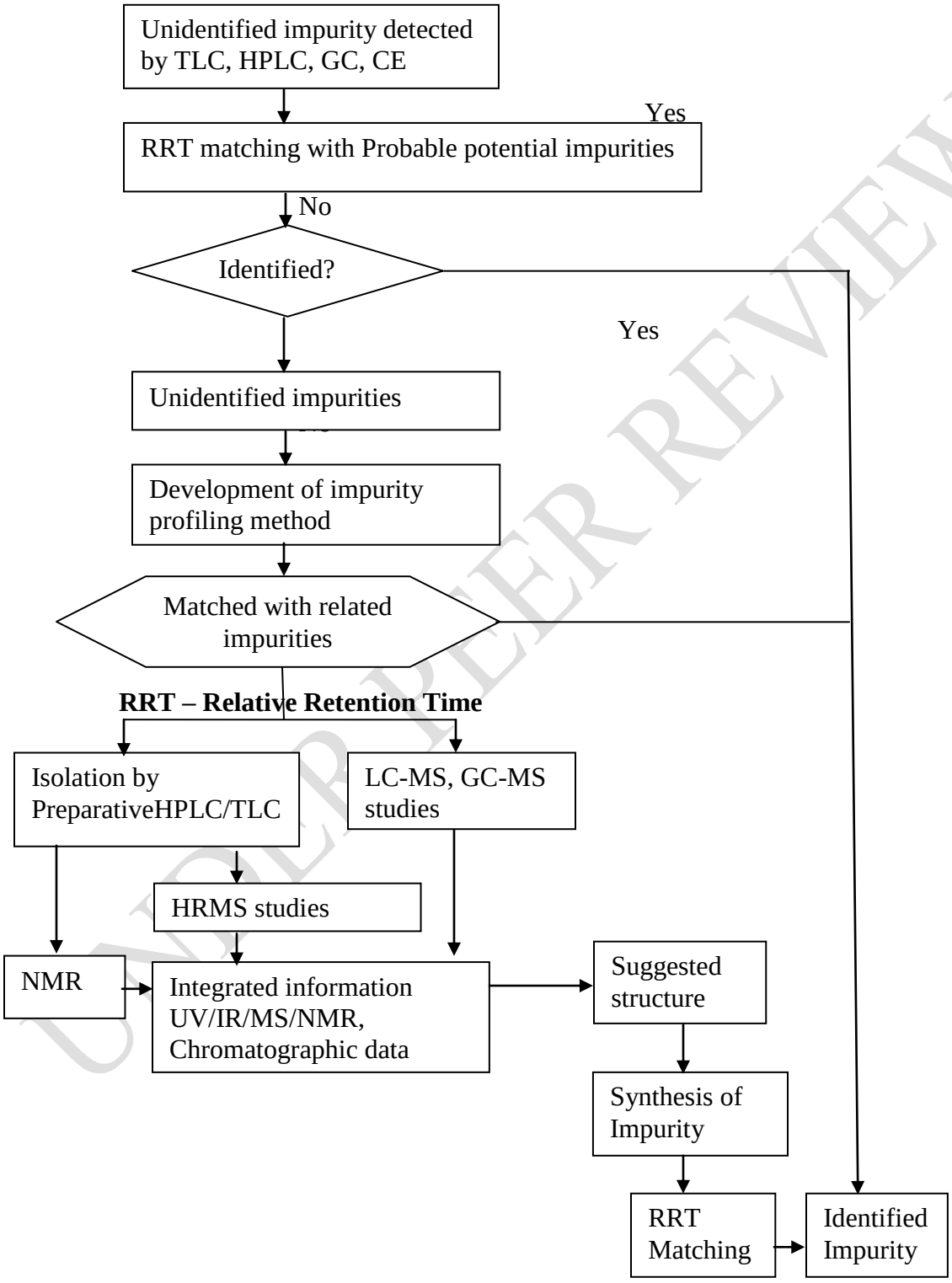
3) Australian regulatory guidelines for prescription medicine, Therapeutic Governance Authority(TGA),Australia

1.5 Impurity Profiling[17,18]

The impurity profiling is common name of analytical activities, the aim of which is the detection, identification, structure elucidation and quantitative determination of organic and inorganic impurities and also residual solvents in bulk drug and pharmaceutical formulation. In case of all phases of development, production and stability testing of drug product, the impurity profiling of API is a critical quality control variable.Itshouldcontainthedetailsofimpuritiesboth qualitatively and quantitatively. It describes route of investigating impurity related to with API.

Schematic representation of scheme for impurity profiling of drugs is shown in Fig2.

Fig2: Schematic representation of scheme for impurity profiling of drugs



2. Force degradation study[19]

The forced degradation studies are used to facilitated the development of analytical methodology, to achieve a better understanding of drug substance and drug product stability, and to determine degradation pathways and degradation products. This study will help to get most stableformulation. The drug substance and drug product stability are a critical parameter which may influence purity, potency and safety. There may be risk in patient's safety by formation of toxic degradation products due to changes in drug stability. Therefore, how much degradation is sufficient has been a question for pharmaceutical scientist. Degradation of drug substances between 5% and 20% has been accepted as reasonable for validation of chromatographic assays.As opinion of some pharmaceutical scientists, acceptable stability limit of 90% of label claim is usual and 10% degradation is ideal for use in analytical validation for small drug molecule. Forced degradation study Chart is shown in Fig 3.

2.1 Hydrolytic condition

Hydrolytic study under acidic and basic condition involves catalysis of ionizable functional groups present in the molecule. Hydrochloric acid or sulfuric acids (0.1–1 M) for acid hydrolysis and sodium hydroxide or potassium hydroxide (0.1–1 M) for base hydrolysis are suggested as suitable reagents for hydrolysis.

2.2 Oxidation condition

Oxidation conditions Hydrogen peroxide is widely used for oxidation of drug substances in forced degradation studies but other oxidizing agents such as metal ions, oxygen, and radical initiators (e.g., azobisisobutyronitrile, AIBN) can also be used.

2.3 Photolytic condition

Photo stability studies are carried out toproduce primary degradants of drug substance by exposure to UV or fluorescent conditions. Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 1.2 million lx h and 200 W h/m2 light.

2.4 Thermal condition

Thermal degradation (e.g., dry heat and wet heat) studies are carried out at exhausting conditions than approved ICH Q1A accelerated testing conditions.Solid-state drug substances and drug products samples should be exposed to dry and wet heat, while liquid drug products should be exposed to dry heat.

The International Conference on Harmonization addresses the questions regarding to stability as follows:[20-24]

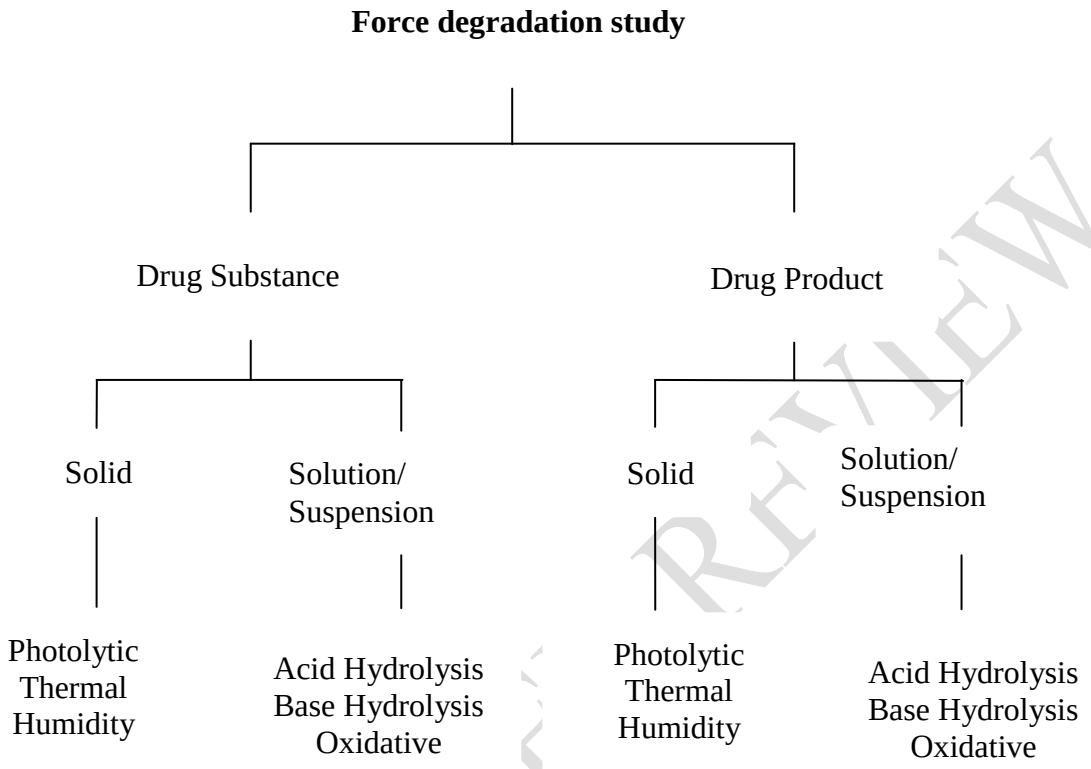
Q1A(R2) Stability testing of new drug substances and products.

Q1B Photostability testing of new drug substances andproducts.

Q1C Stability testing for new dosage form

Q1DBracketing and Matrixing designs for stability testing of new drugsubstances anddrug products

Fig 3. Force degradation study



3. Analytical methods for identification and characterization of Impurity

A) Reference standard method: The main purpose of this method is to quantify and to control reference standards which is used in the process of development and control of new drug. we knowthatthereferencestandardsallowtounderstandthefundamentalinformationforevaluation and observing the performance of bulk drug, by products, impurities, degradationproducts, excipients, raw materials, intermediates.[25]

B) Spectroscopic methods

a) Ultraviolet- visible

TheUV-VIS spectroscopy is based on the absorption of visible and ultraviolet (UV) radiation in the wavelength range of 200-800 nm. UV at singlewavelength doesn't give sufficient information. To confirm greater selectivity and to induce maximum information about molecule diode array detectors are used nowadays.[25]

b) Infrared(IR)

The sample is subjected to electromagnetic radiation which is within the range of 600 cm^{-1} and 4000 cm^{-1}. This radiation effects the bonds present in the molecule and then it stretches or causes bending in molecule due to absorption of energy of a specific wavelength. The wavelength at which they're absorbed gives us information about different types of bonds which might be used for knowing the structure of samples. They are mostly use to characterize solid and semisolid. It provides though little complex but significant or unique fingerprint ofany molecule which can be used for analysis of samples and thus determining the impurities present.[26]

c) Ramanspectroscopy

Itisaspectroscopictechniqueusedtostudyvibrational,rotational,and otherlowfrequencymodesinasystem.ItreliesontheinelasticscatteringortheRamanscattering of the monochromatic light usually from a laser, in the visible, near infrared, or near ultraviolet range. The laser light interacts with the photons or other excitations in the system, leading to the energy of the laser photons being shifted up or down. The shift in energy gives information about the phonon modes in the system.[27]

D) Mass spectrometry (MS)

The technique, where charged species (ions) are separated and detected according to their mass to charge ratio (m/z) is known as Mass spectrometry (MS). The MS isspecifichighlyselectiveandsensitivemethodsformolecularanalysisprovideinsightsto the structure of the analyte. It is also used for monitoring, characterizing and quantification of drugrelatedsubstances inAPI.Ifsinglemethodfailstoprovidenecessaryselectivity,couplingof thistechniquewithGC,HPLC,andLCleadtoincreasesthepowerofthetechniquewhencomplex samples are to beanalyze.[28]

E) Nuclear magnetic resonance (NMR)

TheNMR plays a vital role in identifying low level impurities in bulk drug materials with or without chromatographic isolation. Structural elucidation of impurities in drug materials mostly involve 1H and 13C experiments, the information obtained from these experiments is sufficient to ascertain the structure of the unknown impurity in the drug material. The introduction of NMR with on-line coupling to HPLC reduces the need for preparative isolation of impurities.[28]

F) Separationmethods

a) Capillary electrophoresis (CE)

The high separation efficiencies compared to other chromatographictechniquesisachievedbyCEfordeterminationofdrug-relatedimpurities.When HPLCtechniquesfailtoadequatelymeasureimpuritiesCEcanbeemployed,especiallyinthecase of very polar compounds. The CE is very useful for the separation of closely related compounds,such as Diastereomers and Enantiomers. Various modes of electrophoresis

methods have been developed in combination with chromatography which are as follows[14,29]

- Capillary zoneelectrophoresis.
- Capillary gelelectrophoresis.
- Micellar electrokinetic capillarychromatography.
- Capillaryelectrochromatography.
- Capillary isoelectricfocusing.
- Capillaryisotachotachophoresis.

b) Gaschromatography(GC)

TheGCtechniqueinvolvesvaporizationofthesampleandsubsequent injectionintothegaschromatographiccolumn.Thesampleispassedthroughthecolumnbymeans of gas flow. The solvent used is an inert gas and the stationary phase is a liquid film coated on a supportoffusedsilicaorapackedsorbent.Thesampleinvapourformmoves throughthecolumn byadsorptionandpartitionphenomenon.Thecomponentswithinthesamplemixtureareseparated by means of their individual affinity to involve in the adsorption and desorption processes. The separated components are eluted from the column and detected by a suitabledetector.[14]

c) High pressure liquid chromatography (HPLC)

HPLC is especially used for identifying, quantifying and purifying the impurities and each component of a substance. This method establishes itself as a critical method in the field of Pharmaceutical Analysis for both qualitative and Quantitative analysis. The USFDA has made a special attention and directed all the pharmaceutical countries of its state to ensure the quality of its product by using HPLC before selling to global market. The HPLC is used to elucidate structuresand quantitativelydetermine impurities and degradation products in bulk

drug substances and pharmaceuticalformulations.[11]

d) Thin layer chromatography (TLC)

TLC is the technique used for the identification of various components up to trace amounts. This technique has been used for developing stability-indicating analytical method. Its disadvantages are variability, non-quantitive most easy, simple, and simultaneous determination is possible. It can be used as a quantitative technique, in conjunction with densitometric detection i.e., high performance thin layer chromatography (HPTLC) for compounds which are difficult to analyze by other chromatographic method because of the absence of chromophore. TLC is very much used during initial degradation and stress studies to study the number of degradation products formed. HPTLC is more sensitive and faster compare to conventional TLC technique.[26]

e) Supercritical fluid chromatography (SFC)

The SFC is considered a normal phase technique because it utilizes the relatively nonpolar, "liquid" carbon dioxide as the bulk of the mobilephase thatisusedfortheanalysisandpurificationoflowtomoderate molecularweight,thermallylabile molecules. It is often used for the separation of chiral compounds. SFC primarily uses supercritical CO2 as eluent. This compound has an acceptable critical pressure (73.8 bar) and its critical temperature is close to ambient conditions (31.18^0C).[27]

G) Isolation methods

These methods are mandatory to separate impurities for their structural identification. Generally chromatographic and non-chromatographic methods are utilized for isolation of impurities before characterization. A list of methods that may use for isolation of impurities are given below.

a) Solid phase extraction methods

b) Liquid- Liquid extractionmethods

c) Accelerated solvent extractionmethods

d) Columnchromatography

e) FlashChromatography[30]

H) Hyphenatedmethods

Thismethodisreceivinggreateverattentionasutilizedforsolving various analytical problems. As it combines separation methods with spectroscopic methods like attachment of Mass spectroscopy with gas chromatography or HPLC, it is being used for both quantitative and qualitative analysis of unknown compounds in complex products. These methods are necessary tool in identifying minor components like impurities, degradation products, metabolites in various matrices.[30]

There are several hyphenated techniques used are given below;

a) Gas Chromatography- Mass spectrometry (GC-MS)

b) Liquid Chromatography- Mass spectrometry (LC-MS)

c) Liquid chromatography- diode array- Mass spectrometry (LC-DAD-MS)

d) Liquid chromatography- Nuclear magnetic resonance (LC-NMR)

e) Liquid chromatography- mass spectrometry- mass spectrometry (LC-MS-MS)

f) High performance liquid chromatography- DAD- Mass spectrometry (HPLC-DAD-MS)

g) High performance liquid chromatography- DAD- Nuclear magnetic resonance- mass spectrometry (HPLC-DAD-NMR-MS)

An

antidiabeticagentcomprisesachemicallyandpharmacologicallyheterogeneousgroupofdrugs.

The target in treating diabetes mellitus is to prevent excessive rises in blood glucose throughout each successive 24 hours period. In type 1 diabetes, where there is absent or little endogenous beta-cell function, insulin treatment is crucial topreventdiabeticketoacidosis,andtheaimisthattheprecisereplacementofinsulininthefasting state and after meals. In type 2 diabetes, a choice of treatment, including insulin is obtainable.

Thesecomprisesdrugthatincreaseinsulinsecretion(s u l f o n y l u r e a suchasglibenclamide,gli pizide,gliclazide,andthemeglitinide-likedrugssuchasrepaglinideandnateglinide), drugsthat improve insulin sensitivity (biguanides. metformin and the thiazolidinedione such as rosiglitazone, pioglitazone and troglitazone), and drug that reduce carbohydrate absorption(acarbose).Intype2diabetes,

choiceoftherapydependsonseveralfactors(pregnancyandpresence

ofoftenusedtoachievebettercontrolthanwhenoneagentisalone(e.g.,insulinplussulfonylurea,sul fonylureaplusmetformin). Withineachcategoryofagent,choiceiscommonlyassociatedwith pharmacokinetics consideration.[31]

4. Regulatory requirements for pharmaceutical impurity identification

According ICH, FDA and USP guidelines impurities classified in three categories as, organic impurities, inorganic impurities, and residual solvents and these impurities can be form from variety of sources, as given in Fig 1. The control of organic impurities in new drug substance is based on the Maximum Daily Dose and Total Daily Intake (TDI) of the impurities. Table 1. (Provides the ICH threshold for control of organic impurities in new drug substance). Depending on whether the MDD higher or lower than 2g, organic impurities in new drug substance at (or greater than) 0.05% or 0.1% require identification.

The control of organic impurities in new drug products are outlined in Table 2. Based on MDD, the identification thresholds for organic impurities in new drug products are divided into four groups to give more consideration to low dose drug products. For most new drug products,the MDD is between 10mg – 2g/day,therefore, any impurities at 0.2% or greater would have to be identified.[32]

Table 1: Reporting, Identification, Qualification thresholds for impurities in new drug substance according to ICH Q3A(R2).[33]

Maximum daily dose	**Reporting Threshold**	**Identification Threshold**	**Qualification Threshold**
≤ 2g/day	0.05%	0.10% or 1.0mg per day intake(whichever is lower)	0.15% or 1.0mg perday intake (whichever is lower)
>2g/day	0.03%	0.05%	0.05%

Table 2: Reporting, Identification, Qualification threshold for Degradation Products in New DrugProducts according to ICH Q3B(R2).[34]

ReportingThresholds	
Maximum Daily Dose	Threshold
≤ 1g	0.1%
> 1g	0.05%
Identification Thresholds	
Maximum Daily Dose	Threshold

< 1mg	1.0% or 5µg TDI, whichever is lower
1mg – 10mg	0.5% or 20µg TDI, whichever is lower
> 10mg – 2 g	0.2% or 2 mg TDI,whichever is lower
> 2 g	0.10%
Qualification Thresholds	
Maximum Daily Dose	Threshold
< 10 mg	1.0% or 50µg TDI, whichever is lower
10mg – 100mg	0.5% or 200µg TDI, whichever is lower
> 100mg – 2 g	0.2% or 3mg TDI,whichever is lower
> 2 g	0.15%

5. Bioanalytical Method development and validation

The bioanalytical method development and validation is an essential part in drug discovery and development.There is need to develop and validate bioanalytical methods, as sponsors have to submit clinical pharmacology, bioavailability, bioequivalence, pharmacokinetic evaluation along with non-human pharmacology and toxicology studies and preclinical studies to regulatory authorities. The bioanalytical methods are developed in biological matrices such as blood, serum, plasma or urine.[35]The bioanalysis process means analysis of drugs, metabolites and biomarkers in biological samples, and it involves various steps from the sample collection to sample analysis and data reporting. sample preparation it is very important in bioanalysis. Robust and stable sample preparation method should be applied to produced reliable results. The interferences from sample matrix are remove and improve analytical system performance are the role of sample preparation. Sample preparation is intensive and time consuming. The sample analysis and

detection are conducted. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is method of choice in bioanalytical laboratories for separation and detection due to high selectivity and high sensitivity of the LC-MS/MS technique. The information known about the analyte chemical structure and their properties is important before the start of bioanalytical work. It has important role in drugdevelopment. During the drug development bioanalysis is an essential part in pharmacokinetic, pharmacodynamic studies and toxicological evaluation. Additionally, bioanalytical method validation is a essential for the quantitative determination of various types of analytes in biological matrices. The bioanalysis procedure includes sample preparation, analysis, calibration and dataevaluation and reporting. A good sample preparation and a hyphenated instrumentation arerequired in modern bioanalysis. In pharmaceutical research companies the process of drugdiscovery and development of comprehensive bioanalytical methods is very important. In addition, the method validation has an important role in bioanalysis to ensure the quality of the performed method. Bioanalytical method validation is very important for supporting of new drug applications or biologics license applications. [36,37]

1. **Bioanalytical method development and validation**
2. Before the bioanalytical method development study of analyte chemical structure, pKa value, solubility properties, stability and adsorption properties are carried out.
3. Bioanalytical method developed and validated can be divided into:
4. 1 Preparation of sample
5. 2 Bioanalytical method development and establishment of assay procedure and
6. 3. Application of validated bioanalytical method to the analysis of drug [36]

5.1 Bioanalysis

Bioanalysis means the identification and quantification of analytes in biological samples (blood, plasma, serum, saliva, urine, feces, skin, hair, organ tissue). Bioanalysis is not only detection of small molecules eg. drugs and metabolites but also identify large molecules eg. proteins and peptides. Bioanalysis is well established to support drug discovery and drug development. Bioanalysis has an important role to perform the, pharmacokinetic (PK), pharmacodynamics (PD)and toxicokinetic (TK) studies of new drugs. It also established in clinical, preclinical and forensic toxicology laboratories. Thus, bioanalysis is an important in many researches such as the development of new drugs, forensic analysis, doping control and diagnostic of many diseases.[36,38]

5.2 Extraction technique in drug bioanalysis

Preparation of sample is aprimary step, regularly used sample preparation methods is protein precipitation (PPT), Liquid-liquid extraction (LLE), solid phase extraction (SPE).

Following are the commonly used sample preparationtechniques [36]

Protein precipitation (PPT)

Protein precipitation is fast and simple extraction technique applied for both hydrophilic and hydrophobic compounds. Precipitation is usually induced by addition of a miscible organic solvent (Methanol, acetonitrile, or acetone), salt (aluminium chloride), metal ions (zinc sulphate) or by changing the sample pH to change the nature of the solution (acids such as trichloroacetic, perchloric, metaphosphoric). In some cases, extraction of some drugs and metabolites the protein precipitation method can be followed by LLE or SPE in order to achieve higher efficiency.[36]

Liquid-liquid extraction (LLE)

LLE has been widely used for the preparation of aqueous and biological samples (eg. Plasma,urine) also for extraction of acidic and basic drug from biological samples. Liquid-Liquid

Extraction technique is not suitable for extraction analytes with different polarity from same sample.[36]

Solid phase extraction (SPE)

The SPE it has high efficiency, cost-effective, high reproducibility, and easy to operate. It is used for separating and concentrating of trace analytes in biological samples.[36]

Types of SPE techniques

1. Reversed phase- solid phase extraction

This is less selective compared to normal phase or ion-exchange SPE, Methanol, acetonitrile ormixed buffer/solvent are used as elution sample in RP-SPE. The Sorbent used bonded silica (C4, C8, C18 and Ph, with 40 μm particle size and 60Å pore size) and polymer sorbent as polystyrene can be used. This technique used in biological fluids and environmental pollutants in water[36]

2.Normal phase-solid phase extraction

Typical sorbents in NP-SPE are silica with polar functional groups (Si-CN, Si-NH2, Si-Diol and pure silica). The retention mechanism in this technique is based on hydrogen bonding between analytes and sorbent. [36]

4. Ion exchange-solid phase extraction

It is most selective method in SEE, based on acidic drugs can be isolated with quaternary amine bonded silica or Si-NH2 as anion exchange, for basic drugs strong cation exchange, Si-SCX and week cation exchange, Si-WCX can be used for isolating the cationic analytes.[36]

5.3 Separation and detection instrumentation

Liquid chromatography-UV (LC-UV)

High performance liquid chromatography (HPLC) is commonly used technique in bioanalysis. The main detector used in HPLC is UV-visible detector, due to wide range of selectivity of HPLC column it is applied for separation of drug and many metabolites indifferent matrices. [36]

Liquid chromatography-Tandem mass spectrometry (LC-MS/MS)

Liquid chromatography-Tandem mass spectroscopy is having high selectivity as an important tool in drug discovery. it has advantages to reduced analysis time.[36]

Ultra-performance liquid chromatography-Tandem mass spectrometry (UPLC-MS-MS)

As compared to LC, UPLC has many advantages such as higher resolution, high peak capacity, improved sensitivity and high speed of analysis, and reduced the ion suppression. The UPLCis usedfor smaller particles (<2.5 μm) and higher flow rates.[36]

Supercritical fluid chromatography-Tandem mass spectrometry (SFC-MS/MS)

As **compared** to HPLC, SFC has some advantage like rapid separation without using hazardous organic solvents.The diffusion rate of solute in supercritical fluid is ten times greater than organic solvents in LC. This technique has higher flow rate and higher sample capacity for determination of different drugs and metabolites in biological fluids. [36]

5.4 Validation parameters[39]

1. **Linearity**

   The ability of the method to obtained test result that are directly proportional to the concentration of analyte in the sample. The linearity of the method must be determinedregardless of the drug development phase.

2. **Selectivity(specificity)**

The ability to assess unequivocally the analyte in the presence of expected component, which may consist of excipient, degradant etc. For high performance liquid chromatography (HPLC) identification test, peak purity evaluation should be used to assess the homogeneity of the peak corresponding to the analyte of interest.

3. Calibration model

The selection of an appropriate calibration model is necessary for reliable quantificationof components. This can be done by sample analyzing and plotting of response verses corresponding concentration

4. Precision and repeatability

Repeatability means closeness of agreement of a series of measurements under the same operating conditions over a short interval of time.can be evaluated by performing a minimum of six replicate of a single sample solution prepared at the 100% test concentration. Intermediate precision performed within-laboratory variations such as different analyte, different days, and different equipment's. Repeatability also termed intra-assay precision and within day precision.

5. Intermediate Precision

Intermediate precision expresses within-laboratories variations: different analytes, different days, different equipment's, etc. Intermediate precision is also called between-day, between-run, or inter-assay precision.

6. Reproducibility

Reproducibility expresses the precision between laboratories, it is usually applied to standardization of methodology. Reproducibility studied a supposed method used in different laboratories.[39,40]

7. Limit of detection

According to ICH, limit of detection (LOD) means the lowest concentration of an analyte in a sample which is can be detected but not quantified as an exact value.

8. Limit of quantification

The limit of quantification (LOQ) is the lowest amount ofconcentration of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. [39,41]

***9.* Ruggedness (Robustness)**

Ruggedness is an measure for the susceptibility of a method to small changes that might occur during the analysis like small changes of (mobile phase composition, temperature, pH values etc). it can be very helpful during the method development/prevalidation phase.

Stability

The chemical stability of an analyte in matrix under specific conditions for given time ofintervals. The stability of the analyte during the analytical procedure is a prerequisite for reliable quantification.

Long term stability

Inprocess stability

6.Classification of Oral Antidiabetic drugs [42]

1. Enhance InsulinSecretion

A) KATP channelblockers

a) Sulfonylurea – Tolbutamide, Glibenclamide, Glipizide, Gliclazide,Glimepiride

b) Meglitide/Phenylamine analogues – Repaglinide,Nateglinide

B) Dipeptidyl peptidase -4 (DPP-4)inhibitors - Sitagliptin, Vildagliptin, Saxagliptin, Alogliptin,Linagliptin, Teneligliptin

2. **Overcome InsulinResistance**

a) Biguanide (AMPk activator) – Metformin

b) Thiazolidinedione (PPARyactivator) – Pioglitazone,Troglitazone

3. **Miscellaneous drugs**

a) α – Glucosidase inhibitors- Acarbose,Miglitol,Voglibose

b) Amylin analogue -Pramlintide

c) Dopamine D2 agonist –Bromocriptine

d) Sod-glucose cotransport-2 (SGPT-2) inhibitors – Dapagliflozin,Canagliflozine, Empagliflozin.

7. Category wise analytical perspectives of antidiabeticdrugs

Variousdrugsfromdifferentclassof Antidiabeticdrugswerestudiedforstudyrelatedtoimpurity profiling and force degradation. The study was based on following analyticalperspectives.

- **Column–**Columnisoneofthemostimportantpartofchromatographictechniqueswhere separation of analyte is performed. Column dimensions, chemistry of column, nature of stationaryphasefilledincolumn,particlesizeofstationaryphaseareimportantparameters for separation of different components from a mixture. C18 are widely used columnwhile other C8, Phenyl, Amino, Carbohydrate ES,Cyano, Silica, HILIC column used wherever they are suitable. Fig4.
- **Type of elution –** Both Isocratic and Gradient elutionare widely used. Fig5.
- **Matrix –**Maximum work is carried out on Active pharmaceutical ingredients followedby Tablets are used for impurity profiling and force degradation study.

Fig6.

- **Categories -** Anti-diabetic drugs (Oral hypoglycemic agents) categories as Dipeptidyl peptidase -4inhibitors, K-ATP channel blockers, Sulfonylureas, Alpha-Glucosidase inhibitors, Sodium glucose co-transport-2 inhibitors, Biguanide and Thiazolidinedione. Impurity profiling and forced degradation study are carried out on drugs belongs to these categories. Fig 7.
- **Chromatographic techniques –**HPLC is widely used technique while UPLC, HILIC, HPTLC are also used for the separation of mixtures. Fig8.
- **Detectors -** PDA detector are widely used as compared to UV and MS. Fig9.

Fig 4. Different column used for of Impurity and Forced degradation profiling of Antidiabetic drugs.

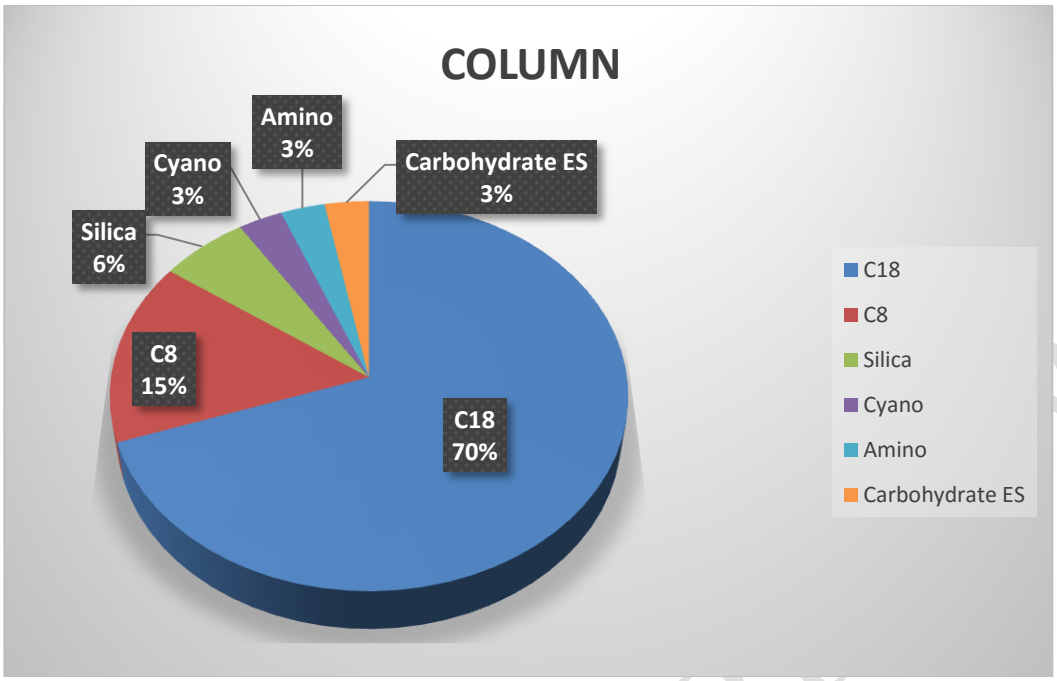


Fig 5. Types of Elution performed in analysis for Impurity degradation profiling of Antidiabetic drugs.

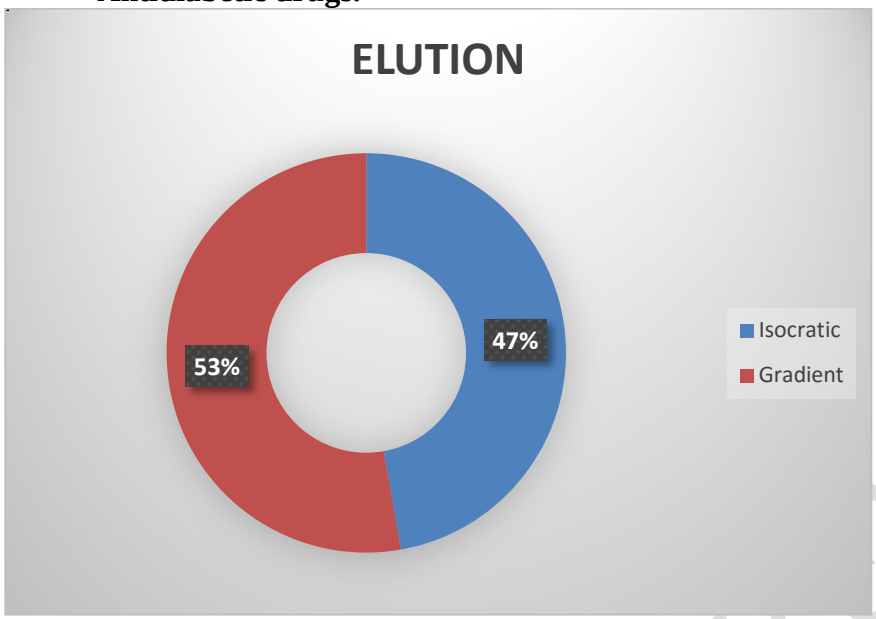


Fig 6. Different matrix used for Impurity and Forced degradationprofiling of Antidiabetic drug

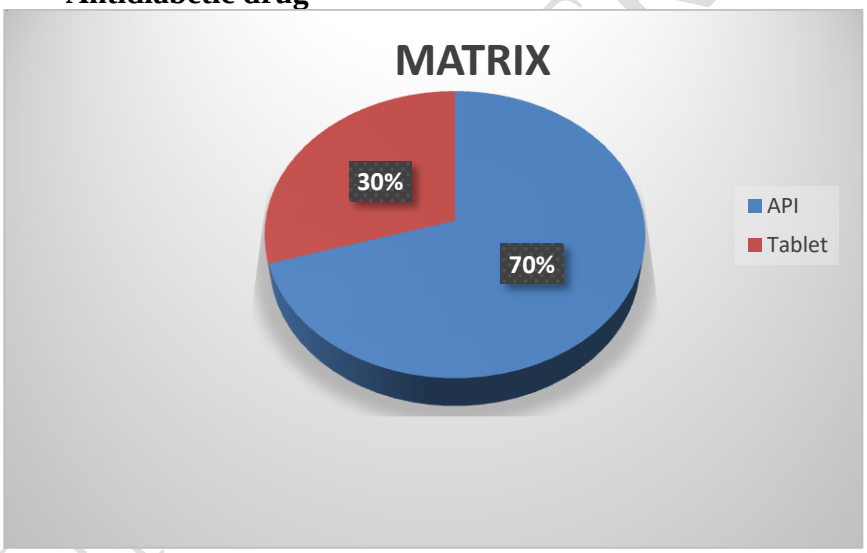


Fig 7. Categories of Antidiabetic drugs on which impurity profiling and forced degradation are studied.

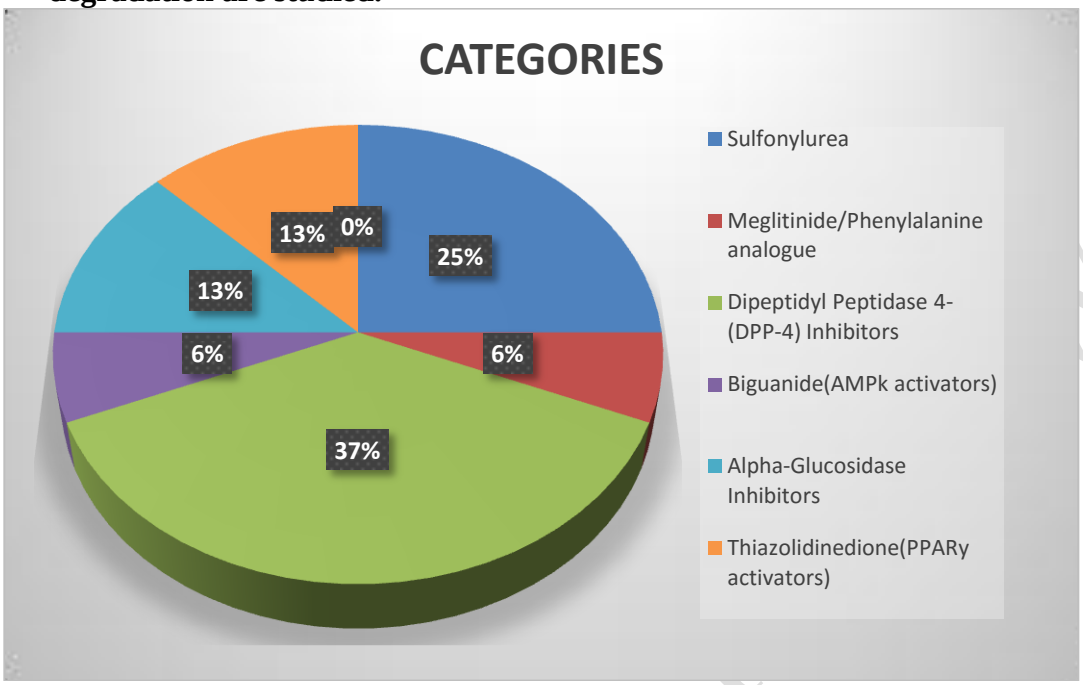


Fig 8. Different Chromatographic Technique used for Impurity and Forced degradation profiling of Antidiabeticdrugs

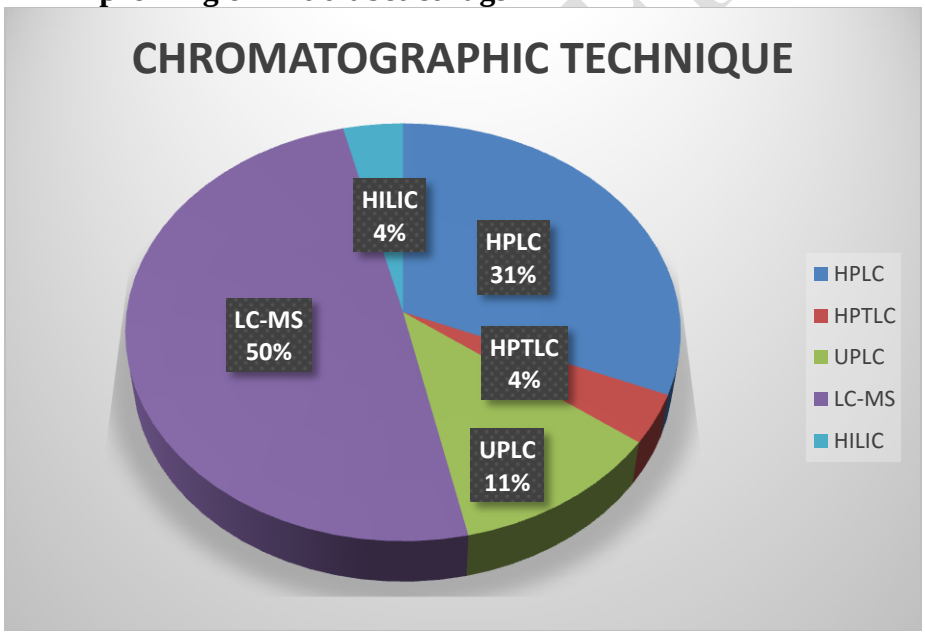
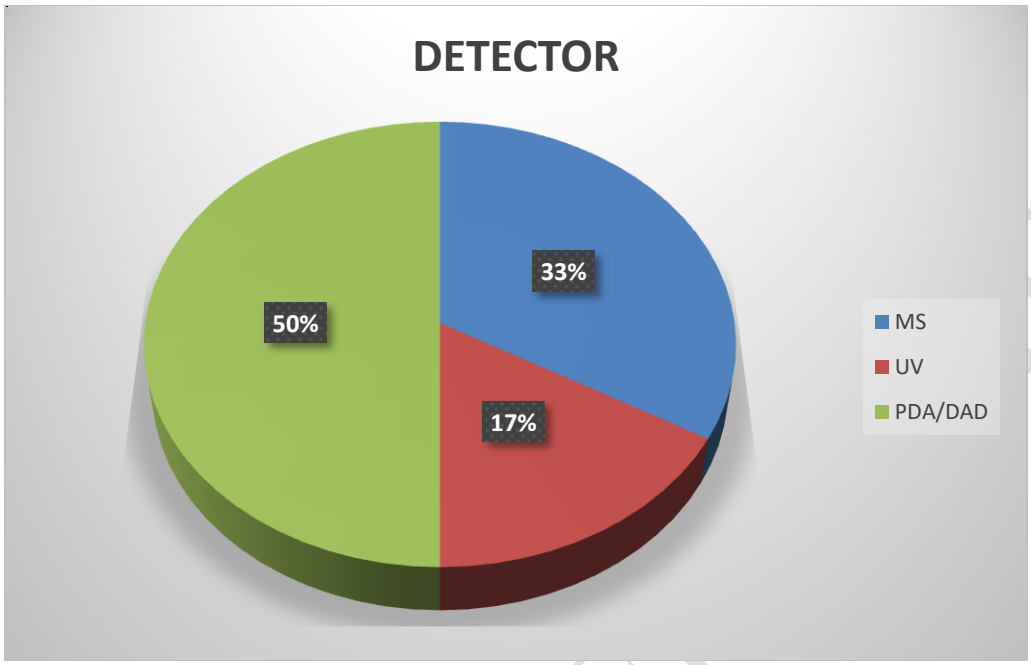


Fig 9. Different detectors used for Impurity and Forced degradation profiling of Antidiabetic drugs.



Conclusion

This review article provides information regarding analytical methods used for identification and characterization of impurities and degradation products of Antidiabetic drugs. Theanalyticaltechniquesemployedforimpurityanalysiswere HPLC,UPLC,LC-MS,LC-NMR, HPTLC, and HILIC. Among all these analytical techniques the most widely used technique is HPLC and LC-MS. This review consequently focuses on basic aspects of impurities in drug substance and drug products, forced degradation and bioanalytical methods which will be helpful to researchers engaged in said areasfor the analysis of antidiabetic drugs. Although different regulatory bodies have provided individual guidelines describing identities and permissible limits ofall said methods.

Abbreviations

API - Active PharmaceuticalIngredient

BEH - bridged ethylene hybrid;

CE - capillary electrophoresis

DP's- degradation products;

ESI/MS - electrospray ionization mass spectrometry;

FT-IR - Fourier transform infrared;

GC–MS - gas chromatography – mass spectrometry;

GTIs - genotoxic impurities;

HILIC - Hydrophilic Interaction liquid chromatography;

HPLC - High performance liquid chromatography;

HPLC/ESI-MS - High-performance liquid chromatography/electrospray ionization mass spectrometry;

HPTLC - High performance thin layer chromatography;

ICH - International Conference on Harmonization;

LC/MS/MS - liquid chromatography–tandem mass spectrometry;

LC–ESI-MS- liquid chromatography–electro spray ionization mass spectroscopy;

LC-ESI/MS/MS- liquid chromatography-electrospray ionization tandem mass spectrometry;

LC–ESI-QT/MS/MS liquid chromatography–tandem mass spectrometry using electrospray ionization source and Q-trap mass analyzer;

LC–MS - liquid chromatography–mass spectrometry;

MeOH - methanol;

MS - mass spectrometry;

NDA - New Drug Application;

PDA- Photodiode array;

PRIs- process related impurities;

QTOF- Quadrupole-time-of-flight

SIAM - Stability indicating assay method

SFC - supercritical fluid chromatography;

TEA-Triethylamine;

TFA -Trifluoroacetic acid

UPLC -Ultra-Performance liquid chromatography.

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