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IMPURITY PROFILING AND DRUG CHARACTERIZATION: BACKDROP AND APPROACH

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Abstract:

Impurities are always an 'essential evil', that will however be present with the drug products with but with the dawn of more safety based limits for controlling the related impure substances (ICH Q3A-D and M7) it can be relied that such controlling authorities will better comprehend the course of action and consent to an appropriate tolerable limits to possibility (commercial and patient needs).Impurity produced either through formulation or in the lead ageing of both API's and formulated products in medicines. These unwanted chemicals, present even in small amount, may influence the efficacy and safety of the pharmaceutical products. Any substance/ unwanted chemical that is present in the active ingredient or drug substance which affects the purity of the material is not always inferior in quality. From the point of view of its applicability, the drug substance is compromised in terms of purity even if it contains another material with superior pharmacological or toxicological properties. Highly sophisticated instrumentation, viz MS attached to a Gas Chromatography or HPLC, LC-MS and other hyphenated and double hyphenated techniques are foreseeable tools in the identification of minor components (drugs, impurities, degradation products, metabolites) in various matrices.

Keywords: *Analytical Method Validation, Forced degradation studies, ICH Guidelines Impurity profiling, Hyphenated techniques.*

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INTRODUCTION:

Pharmaceutical Impurity Analysis: Overview

As acknowledged world wide the need of impurity profiling and drug characterization, the assortment of various authoritarian powers be it either USFDA [1]. Indian Pharmacopoeia [2], British Pharmacopoeia [3], European Pharmacopoeia [4], Canadian Drug and Health Agency, or ICH every regulation is emphasizing on not only the purity necessities but also the identification of impurities in the active pharmaceuticals ingredients (APIs). For critical conditions, drugs with reasonable risk for adverse effects are generally accepted. Exemplar, some life-saving cancer chemotherapies are known human carcinogens [5]. However, if one is suffering from a life-threatening tumour, a 5% risk of a secondary, treatment-related tumour is generally considered acceptable. Perhaps, this cannot be comparable for impurities found in drug substances and products as impurities bring the only risk and have no therapeutic efficacy [6].

Whether plant-based viz morphine, taxols (anticancer) digoxin (cardiac glycoside) or chemically synthesised the drug is seldom present unaccompanied. It is a complex mixture which contains two different types of key components:

1. Natural components – that are not completely removed during the extraction process.
2. By- products- generate during the manufacturing of the drug and are related to the manufacturing process (Process related impurities) [7]

International Community for Harmonization (ICH) Impurities can be classified as three types:

- Organic impurities (process and drug related)
- Inorganic impurities
- Residual solvents

As stated by ICH (International Community for Harmonization) the definition of Impurity profiling follows "A description of the identified and unidentified impurities, present in a new drug substance". The definition can be simplified in a layman's outlook that impurity profiling is a general name for a procedure involving all the analytical activities including the detection, identification, structural and quantitative determination of organic, inorganic as well as residual solvents in pharmaceutical dosage forms [8,9].

Sources of Impurities in Pharmaceuticals

The foregoing discussion makes it unambiguous that impurities can begin from numerous sources; such as; a) Crystallization-related impurities, b)Stereochemistry-related impurities, c) Residual solvents, d) Synthetic intermediates and by-products, e) Formulation & Method related impurities, f) Impurities arising during storage, g) Mutual interaction amongst ingredients, h) Functional group-related impurities, i) Degradation related impurities[8]

a) Crystallization-related impurities

Polymorphism and solvatomorphism have laid a strong significance to the pharmaceutical industries as it has been well accepted that the structural nature of a given compound can exert a profound effect on the physical properties of that molecule. Polymorphism elaborated as the crystal system where a solid material can exist in more than one crystal forms, having same elemental composition. In contrary, Solvatomorphism is a phenomenon where a substance has different crystal packing arrangement with different elemental composition [10].

The increasing advent of the molecular modelling tools has made it quite easy to determine the crystal structure of the compound and simultaneously the crystal structure of impurity and the solvent interacted. Both impurity and the solvent influence the intermolecular interaction that not only hampers the crystallization process but also remains as impurity leading to poor substantial quality of the drug. Because of the requirement of high-purity crystalline products, an understanding of the effects of solvents on purification is also necessary [11].

b) Stereochemistry-related impurities

Stereoisomeric compounds are the compounds having same chemical formula but different spatial arrangement that can become an impurity for other isomeric forms of the drug that has a therapeutic effect. Resolving a single Enantiomeric form of the chiral compound will show a greater therapeutic index, better pharmacological action and minimal adverse reactions.

However, the pharmacokinetic profile of levofloxacin (S-isomeric form) and ofloxacin (R-isomeric form) are comparable, suggesting the lack of advantages of single-isomer in this regard. The prominent single isomer drugs, which are being marketed, include levofloxacin (S-ofloxacin), levalbuterol (R-albuterol), and esomeprazole (S- omeprazole) [12].

c) Residual solvents

Residual solvents are organic volatile chemicals used during the manufacturing process or generated during the production. A number of organic solvents used in the synthesis of pharmaceutical products have toxic or environmentally hazardous properties, and their

complete removal can be very difficult. In addition, the final purification step in most pharmaceutical drug substance processes involves a crystallization step which can lead to the entrapment of a finite amount of solvent which can act as a residual impurity or can cause potential degradation of the drug. Residual solvent levels are controlled by the ICH, USP, and EP. Depending on the possible risk to human health, residual solvents are divided into three classes [12]. Table 1.

Table 1: Classification of solvents

Class	**Solvent/Limits**	**Specifications (ICH Q3C guidelines)**
Class I	Benzene (2ppm), Carbon Tetrachloride(4 ppm), Methylene Chloride (600 ppm) Methanol(3000ppm) Pyridine (200 ppm) Toluene (890 ppm)	More than the stated limit should be avoided
Class II	N, N dimethylformamide (880ppm), Acetonitrile (410 ppm)	More than the stated limit should be avoided
Class III	Acetic acid, Ethanol, Acetone	Permitted a daily exposure of 50 mg or less per day

A few examples of common organic solvents which are found as volatile impurities and have their limits set by ICH guidelines are depicted in Table 2. As stated above Class III solvents, such as acetic acid, acetone, isopropyl alcohol, butanol, ethanol, and ethyl acetate should be limited by GMP or other quality-based requirements.

ICH limits for a selected list of common organic solvents found as volatile impurities.

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

USP <467> 2009 General Chapter contains a more comprehensive method for residual solvent analysis that is similar to the ICH guidelines developed in 1997 where a limit test is prescribed for class 1 and class 2 solvents while class 2C solvents are usually determined by non-headspace methods due to their higher boiling point. The limits of detection (LOD) recommended for class 3 solvents are up to 5000 ppm. When the levels of residual solvents exceed USP or ICH limits, quantization is required [1,2].

d) Synthetic intermediates and by-products

A new chemical entity (NCE) that could be regarded as an impurity in pharmaceuticals originate during the process of synthetic preparations from raw materials, intermediates or as by-products of the reaction that has not been completely eliminated during the purification step. To cite some examples impurity profiling of ecstasy tablets by GC-MS [14], and MDMA samples, produced impurities in intermediates via reductive amination route [15]. The multi-step synthesis for example; in paracetamol bulk, there is a limit test for p-aminophenol, which could be a starting material for one manufacturer or be an intermediate for the others. In synthetic organic chemistry, getting a single end product with 100% yield is very rare; there is always a chance of having by-products. In the case of paracetamol bulk, diacetylated paracetamol may be formed as a by-product.

e) Formulation and method related impurities

During the formulation of a drug product different excipient used creates impurities. Also, the drug substances undergo a variety of formulation conditions in the process of manufacture that can lead to degradation of substance and other adverse reactions. Suspension, emulsion and solutions are

most likely to degradation because of hydrolysis and solvolysis. Such formulations are also prone to instability conditions like phase separation, creaming, creaming etc [16]. Fluocinonide Topical Solution USP, 0.05%, in 60-mL bottles, was withdrawn in the United States because of degradation/impurities leading to sub- potency [17]. As discussed, liquid dosage like suspension, emulsion, solutions are susceptible to both degradation and microbiological contamination. Incidentally, water content, compatibility of anions and cations, pH, mutual interactions of components, and the primary container are crucial factors.

The growth of bacteria, fungi, and yeast in a humid and warm environment results in instability of an oral liquid product for safe human consumption. Microbial contamination may occur during the shelf life and subsequent consumer use of a multiple-dose product, either due to inappropriate use of certain preservatives in the preparations or because of the leaching of primary containers [18].

A method relating to the process of manufacture leads to the development of impurity. An impurity, 1-(2, 6-dichlorophenyl) indolin-2-one is formed in the production of a parenteral dosage form of Diclofenac sodium, if it is terminally sterilized by autoclave [19]. The conditions of the autoclave method (i.e., 123 + 2°C) enforce the intramolecular cyclic reaction of Diclofenac sodium that forms an indolinone derivative and sodium hydroxide. The formation of this impurity has been found to depend on initial pH of the formulation.

f) Impurities arising during storage

A number of impurities can originate during storage or shipment of drug products. It is essential to carry out stability studies to forecast, evaluate, and ensure drug product safety [6].

Leachable or Extractables- can come from glass, rubber stoppers, and plastic packaging materials. Metal oxides such as NaO2, SiO2, CaO, MgO, are the major components leached/extracted from glass [20]. Generally, most synthetic materials contain leachable oligomers/monomers, vulcanizing agents, accelerators, plasticizers, and antioxidants [21]. Some examples of leachable/extractable\ from synthetic materials include styrene from polystyrene [22] diethylhexylphalate (DEHP, plasticizer in PVC) [23] dioctyltin isooctylmercaptoacetate (stabilizer for PVC) [24] zinc stearate (stabilizer in PVC and polypropylene) [25] , 2-mercaptobenzothiazole (accelerator in rubber stopper) [26] and furfural from rayon [27]. These impurities are needed to be analyzed by using different analytical methods.

g) Mutual interaction amongst the components

Generally, vitamins are extremely labile and due to ageing they generate problems of instability in many dosage forms, particularly liquid dosage forms.
A vitamin on degradation does not give toxic impurities; on the other hand, the potency of active ingredients lowers Pharmacopoeial specifications.

The presence of nicotinamide in a formulation containing four vitamins (nicotinamide, pyridoxine, riboflavin, and thiamine) causes the degradation of thiamine to a sub-standard level within a year shelf life of vitamin B-complex injections due to the mutual interactions of ingredients [28]. Similarly, the marketed samples of vitamin B-complex injections were found to have a pH range of 2.8 - 4.0. A custom-made formulation with simple distilled-water and a typical formulated vehicle including disodium edetate and benzyl alcohol were evaluated, and similar mutual interactions causing degradation were found.

h) Functional group-related typical degradation

Hydrolysis is the frequent observable fact for ester type of drugs where esters get hydrolysed easily to carboxylic acid and alcohol, especially in liquid dosage forms *like* benzylpenicillin, oxazepam and lincomycin. Ester hydrolysis can be can be seen in a few drugs *viz* aspirin, benzocaine, cefotaxime, ethyl paraben [28], and cefpodoxime proxetil[29]. Oxidative degradation of drugs that have hydroxyl group directly bonded to an aromatic ring (*viz* phenol derivatives such as catecholamines 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 flavon rings) are all vulnerable to oxidative degradation. Literature reveals that in mazipredone, the hydrolytic and oxidative degradation pathway in 0.1molL-1 HCl and NaOH at 800° C [30].
All pharmaceutical products are exposed to light while either at the time of manufacture as solid or solution, or packaged, or when being stored in pharmacy shops or hospitals for use by patients where it has a large probability of undergoing photolytic cleavage.

For instance, Ergometrine [31] nifedipine [32], nitroprusside, riboflavin and phenothiazines are prone to photo-oxidation. Photochemical energy creates free radicals, which can propagate chain reactions in a susceptible compound. Fluroquinolone from the

category of antibiotics are also susceptible to photo degradation [33]. In ciprofloxacin eye drop preparation (0.3%), sunlight induces photolytic cleavage reaction producing ethylene diamine derivative of ciprofloxacin [34]. Decarboxylation of some dissolved carboxylic acids, such as p-aminosalicylic acid; shows the loss of carbon dioxide from the carboxyl group when heated. Such an example of decarboxylation is the photoreaction of rufloxacin [35].

The reactive species in most of the drugs consist of;
• Water- that can hydrolyze some drugs or affect the dosage form efficacy
• Small Electrophiles- like hydroxyl, aldehyde and carboxylic acid derivatives
• Peroxides are reactive species that can oxidize some drugs
• Metals-which can catalyze oxidation of drugs and the degradation pathway
• Leachable

i) Degradation related Impurities

Impurities can also be formed by degradation of the end product during manufacturing of the bulk drugs. The degradation of penicillin and cephalosporins are well-known examples of degradation products. The presence of a β-lactam ring, as well as that of a α-amino group in the C6/C7 side chain, plays a critical role in their degradation. Another example that may be quoted is, the degradation of ibuprofen (IBP) to 2-(4-formylphenyl) propionic acid (FPPA), 2-(4-isobutylphenyl) propionic acid (IBP), 2-(4-methylphenyl) propionic acid (MPPA), 2-(4-ethylphenyl) propionic acid (EPPA), 4-isobutylacetophenone (4-IBAP), 2-(4-n-propylphenyl) propionic acid (PPPA) and 2-(4-n-butylphenyl) propionic acid (BPPA), which are reported to be well known impurities in IBP [36]. The degradation products of diclofenac-Na and clotrimazole [37], paclitaxel [38] are also reported.

Guidelines for the Control of Pharmaceutical Impurities

ICH Guidelines

ICH Q3A covers drug substances and ICH Q3B covers Drug Products. Impurities in new drug substances are addressed from two perspectives:

- Chemical aspects include classification and identification of impurities, report generation, listing of impurities in specifications, and a brief discussion of analytical procedures
- Safety aspects for toxicity and clinical studies of the substance those were not present or present in substantially lower quantity.

ICH guidelines do not cover the following drug substances: Biological, peptides and oligonucleotides, radiopharmaceuticals, herbal drugs, fermented products, plant or animal origin crude products.
According to ICH guidelines, impurities in the drug substance produced by chemical synthesis can broadly be classified into following three categories;
- Organic Impurities (Process and Drug-related)
- Inorganic Impurities
- Residual Solvents

Organic impurities may arise during the manufacturing process and or storage of the drug substance may be identified or unidentified, volatile or non-volatile, and may include;
- Starting materials or intermediates
- By-products
- Degradation products
-Reagent, ligand and catalyst

Inorganic impurities can result from the manufacturing process. They are normally known and identified and include:
-Reagents, ligands and catalysts
-Heavy metals or other residual metals
-Inorganic Salts
-Other materials (e.g., filter aids, charcoal) [39,40].

Solvents are inorganic or organic liquids used as vehicles for the preparation of solutions or suspensions in the synthesis of a new drug substance. Since these are generally of known toxicity, the selection of appropriate controls is easily accomplished (see ICH Q3C on Residual Solvents).

Impurities will be present API's unless; a proper care is taken in every step involved throughout the process [41].

2. Rationale for reporting ICH limits for impurities

According to ICH guidelines on impurities in new drug products, identification of impurities below 0.1% level is not considered to be necessary, unless potential impurities are expected to be unusually potent or toxic. According to ICH, the maximum daily dose qualification threshold to be considered is as follows; < 2g/day 0.1 % or 1 mg per day intake (whichever is lower) >2g/day 0.05% [42].

ICH Thresholds for Degradation related Products in New Drug Products

Table 3: Reporting Thresholds

Maximum Daily Dose	Threshold
≤ 1g	0.1%
> ≤ 1g	0.05%

Table 4: Identification Thresholds

Maximum Daily Dose	Threshold
< 1 mg ; whichever is lower	1.0% or 5 µg TDI
1 mg-10 mg; whichever is lower	0.5% or 20 µg TDI
> 10 mg- 2g; whichever is lower	0.2% or 2mg TDI
> 2 g	0.10%

Table 5: Qualification Threshold

Maximum Daily Dose	Threshold
< 10 mg ; whichever is lower	1.0% or 50 µg TDI
10 mg-100mg ; whichever is lower	0.5% or 200 µg TDI
> 100 mg-2g ; whichever is lower	0.2% or 3 mg TDI
> 2g	0.15%

Table 6: Selected Publications for Control of Impurities

Key Topics	Title
Guidelines for the control of impurities	International Conference on Harmonization (ICH) Q3A (R2) Impurities in New Drug Substances, 25 October 2006
	ICH Q3B (R2) Impurities in New Drug Substances, 2 June 2006
	US-FDA guidelines "NDAs -Impurities in New Drug Substances"
	US-FDA guidelines "ANDAs – Impurities in New Drug Substances" Australian regulatory guideline for prescription medicines, Therapeutic Governance Authority (TGA), Australia
Specific guidelines for the control of genotoxic impurities	Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended approaches; US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER); Silver Spring, MD, USA, December 2008
	EMA/CHMP/SWP/431994/2007 Rev. 3, Questions and answers on the guideline on the limits of genotoxic impurities, adopted September 23, 2010
	Guideline on the Limits of Genotoxic Impurities, CPMP/SWP/5199/02, EMEA/CHMP/QWP/2513442006; Committee for Medicinal products (CHMP), European Medicines Agency (EMEA); London 28 June 2006
	Pharmeuropa, Vol 20, No. 3, July 2008, Potential Genotoxic Impurities and European Pharmacopoeia monographs on Substances for Human Use
	ICH M7 Guideline (in preparation) for control of Mutagenic genotoxic impurities.
Guidelines relevant to analytical methods for the control of genotoxic impurities	ICH Guidance for Industry: Pharmaceutical Development Q8, (R2); US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER); Aug, 2009, http://www.fda.gov/RegulatoryInformation/Guidances/ucm128028.htm
	ICH Guidelines, Q9: Quality Risk Management Q9; US Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, Nov, 2005, http://www.fda.gov/RegulatoryInformation/Guidances/ucm128050.htm
	ICH S2A: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, April 1996 ICH S2B: A Standard Battery for Genotoxicity Testing of Pharmaceuticals, July 1997
	ICH S2 (R1): DRAFT Consensus Guideline (Expected to combine and replace ICH S2A and S2B): Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, March 6, 2008

Guidelines for the control of elemental impurities	Elemental impurities – Limits (Pharm. Forum, 2011), 37 (3), Chapter -232 Elemental impurities – Procedures (Pharm. Forum, 2011), 37(3), Chapter -233
Guidelines for the control of residual solvents	ICHQ3C, International Conference on Harmonization, Impurities Guidelines for Residual Solvents. Federal Register, 62 (247), 1997, 67377
	International Conference on Harmonization, ICH Q3C (R3) Impurities: Guideline for Residual solvents, November 2005
	ICH Topic Q3C (R4) Impurities: Guideline for Residual Solvents, European Medicines Agency, 2010
	USP Method 467, US. Pharmacopeia, updated June 2007, USP 32 – NF 18

NOTE: This list is a limited selection of key, recent regulatory publications. For complete, current regulatory information and the latest updates, please check the websites of the various regulatory authorities.

Analytical Techniques empowering Impurity profiling

To troubleshoot the impurities present in drug an analytical tool having high sensitivity and specificity is the best helping hand for a pharmaceutical scientist to better detect, identify and characterise the impurities. Chief analytical techniques for impurity include spectroscopy, chromatography or combination of both. Based on the types of impurity and information needed an appropriate analytical technique is selected. During the process of analytical method development and impurity profiling of drugs, researchers come across several complex analytical problems that can't be resolved with a single technique. Analytical techniques such as LC-MS, LC-MS-MS, LC-UV, GC-MS and CE-MS provide the needed information that can overcome these challenges in an efficient manner. Hence, these play an important role in impurity profiling of pharmaceuticals from identification to structure elucidation of unknown compounds. Following table no 7 summarizes of some of the techniques used in impurity analysis [43,44].

Table 7: Impurity analysis techniques

Organic impurities	FTIR, Preparative LC, LC/UV, LC/MS (SQ, Q-TOF, and QQQ), CE, SFC, and NMR
Inorganic/elemental impurities	ICP-OES and ICP-MS
Residual solvents	GC and GC/MS

Extraction techniques: Various extraction techniques have gained much development in last few years and are very helpful in isolation of impurities which is generally bypassed during the instrumental analysis techniques. As stated in the above guidelines the impurities and its degradation products should be according to the limits and elucidation is needed if it exceeds the level greater than0.1%. Latest Analytical techniques are helpful in providing structure determination (e.g. High field NMR, LC-MS-MS, GC-MS etc).Software tools for the prophecy of shore up these studies. Few of these techniques have been elaborated as follows:

a. Solid-Phase Extraction Methods

Solid phase extraction (SPE) is a progressively more useful sample preparation technique. When compared with liquid-liquid extraction, drawbacks such as incomplete phase separation, less quantitative recoveries, specific expensive and breakable glass wares and wastage of large quantity of organic solvent is avoided.SPE is less time consuming and less solvent is needed. It is more efficient and automated.SPE is used very frequently to prepare liquid samples and extract semi-volatile or non-volatile analytes, and can also be used with solids that are pre-extracted into solvents. SPE products are admirable for sample extraction, concentration, and cleanup. They are available in a wide variety of chemistries, adsorbents, and sizes.

b. Liquid –Liquid Extraction Methods

Liquid-liquid extraction is partitioning technique that separates compounds into two immiscible liquid one organic and other inorganic on the basis of their relative solubility. It is an extraction of a compound from a liquid phase into another liquid phase. Liquid-liquid extraction is a commonly performed technique in laboratories with help of a separating funnel.

c. Accelerated Solvent Extraction Methods

Accelerated Solvent Extraction (ASE) is uses higher temperature and pressures for extracting solid and semisolid sample matter with common solvent.ASE systems are ASE 150 system and the fully automated ASE 350. It is a fast method that reduces the extraction time hours to minutes using Dionium™ components when compared to techniques such as Soxhlet and sonication, ASE generates results in a little of the time. Filtration and clean up of solid samples can be achieved as part of the solvent extraction process in a single step. ASE is less expensive than other techniques and reduces solvent usage to 90%.

d. Supercritical Fluid Extraction

SFE is a process that is primarily used for the separation of components of extract from the matrix using a supercritical fluid as the solvent. The matrix used is mostly solid but can also be liquid. It is a type of preparative method used for preparing samples for analysis. It has been also widely used for separation of unwanted material /impurities from a substance. The extensively used Supercritical fluid is carbon dioxide along with some co solvent ethanol or methanol at temperature 31°C and pressure of 72 bars.

e. Supercritical Fluid Chromatography (SFC)

SFC, which uses supercritical CO_2 as mobile phase, is another orthogonal technique that can be used for impurity detection because it offers HPLC-level sensitivity with reduced organic solvent usage. SFC also offers the advantage of chiral impurity analysis enabling the determination of Enantiomeric excess at very low impurity levels. The reported studies suggest the determination of Salbutamol sulphate impurities using Achiral supercritical fluid chromatography [45].

f. Flash Chromatography

It is the widely used technique in modern research. A flash chromatography, simply defined as a rapid form of preparative column chromatography or medium pressure column chromatography. Previously, the air pressure was used to the flow of the solvent, therefore decrease in time to purify sample and nowadays pre-packed column with automated pumps are developed. The glass columns have been replaced with pre-packed plastic cartridges and computer linked with detectors and fractions collectors.

Isolation Methods

a. High-PerformanceLiquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is a chromatographic technique that is widely used in the field of analytical chemistry and biochemistry. HPLC is mainly used for identifying, quantifying and purifying the impurities and the 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. 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. HPLC helps in the structure elucidation and quantitative determination of impurities and degradation products in bulk drug materials and pharmaceutical formulations [46,47].

Table 8: HPLC Method for Separation of Impurities.

S.No.	Drug	Solvent\mobile phase	Impurities	References
1.	Salicylic acid and Betamethasone dipropionate	Methanesulfonicacid: CAN (0.05%)	Salicylic acid related 7-betamethasone dipropionate	48
2.	Almotriptan malate	Sodium phospate buffer : ACN (80:20)	3 impurities	49
3.	Alogliptin benzoate	Gradient system A-0.1%perchloric acid (ph adjusted 3.0 with triethethylamine B-acetonitrile	9 impurities	50
4.	Atomoxetine hydrochloride	Ortho-phosphoric acid, octanesulfonic acid n-propanol	Phenyl methylaminopropanol and mandelic acid	51
5.	Atorvastatin calcium	ACN : CH_3COOH_4 BUFFER gradient	Diastereomer-atorvastatin (DSAT) desfluoro-atorvastatin (DFAT)	52
6.	Bendazac lysine	Acetonitrile: aqueous buffer (1.0ml glacial acetic acid in 1000 ml water) (47:53)	2 impurities 2-(1,5-dibenzyl-1*H*-indazol-3-yloxy)acetic acid.	53

7.	Clopidogrel	Eluent A:ACN: potassium phosphate buffer (20:80): Eluent B:ACN: potassium phosphate buffer (pH2.3: 10mM) (80:20):	5-[1-(2-chlorophenyl)-2-methoxy-2- oxoethyl]-6,7-dihydrothieno[3.2-c]pyridine-5-ium	54
8.	Efavirenz	Water acetonitrile and methanol	5 impurities	55
9.	Eplerenone	ammonium acetate adjusted to pH 4.5, methanol and acetonitrile	2 impurities	56
10.	Ezetimibe	A-Orthophosphoric acid In water B- acetonitrile and water (50:50)	2 impurities	57
11.	Nevirapine	20:80 (v/v) acetonitrile-25mM $NH_4H_2PO_4$ (pH 5.0),	4 impurities	58
12.	Norgestrel	Gradient system A-0.1%formic acid B-acetonitrile with0.1%formic acid	(3,17α-diethinyl-13-ethyl-3,5-gonadiene-17-ol, 17α-ethinyl-13-ethyl-4-gonene-17-ol,	59
13.	Omlesartan	Phosphate buffer :acetonitril	Omlesartan acid	60
14.	Paclitaxal	H_2O: ACN (52:48)	10 DeacetylbaccatinIII,baccatinI II 10-deacet-yl-7-xylosyltaxol C,photo-degradient taxol C,ceph-alomannine,10-deacetyl-7-epitaxol, 7-epitaxol	61
15.	Phenazopyridine	H_2O:ACN (25:75)	3-phenyl-1-5-phenylazo-pyridine-2-6-diamin	62
16.	Retigabine	Acetonitrile and water	2 impurities	63
17.	Rizatriptan	Ammonium di hydrogen orthophosphosphate 20 mM + 2ml TEA pH 2 ACN;Gradient	Rizatriptan -1-2-dimer and Rizatriptan -2-2-dimer	64
18.	Ropinirole	ACN: Sodium heptane sulphonate 5mM, pH 2	4-[2 Dipropyl(aminoethyl)] 1 H indole-2,3 dione	65
19.	Sumatriptan	Acetonitrile and methanol	4 impurities	66
20.	Temozolomide	Gradient System: A-water with 5% Acetic acid B- Acetonitrile	3 impurities	67
21.	Trans-resveratrol	A-Sodium di hydrogen orthophosphate dehydrate in water B- Acetonitrile	5 impurities	68
22.	Trimethoprim	Gradient elution A triethylamine (0.25%) and formic acid (1.1%) in water (pH 5.8) elution B- Acetonitrile	2 impurities	69
23.	Verenicillin	Ammonium acetate buffer: Methanol	4,6,7,8,9,10 hexahydro 1H 6,10 methanopyrazino[2,3-h]3- benzazepine2,3 dione	70

b. Fourier Transform Infrared Spectroscopy (FTIR)

One of the most promising approaches for identification and determination of an impurity or a degradation product is FTIR as it provides a composite fingerprint that corresponds to a particular compound. The functional group region of an organic compound determines its FTIR spectrum. The technique helps to identify the structure and measure the concentration of the compound under investigation. Any change in the FTIR spectrum of a known compound can be correlated to the impurity or the degradant in that compound.

The FTIR packs a powerful combination of precision and compliance, making one of the best Analytical Techniques for routine analysis in pharmaceutical

laboratories. Measuring contaminants, such as ethylene glycol and diethylene glycol in glycerol, is quick and easy with FTIR because its accessory reduces the tedious process of finding the right path length and optimum measurement conditions. In addition, soft ware's of FTIR make it easy to meet regulatory requirement by alerting users when the impurity level is outside specification range, while proprietary liquid analysis technology simplifies sampling and reduces the risk of user error [72]. There are reported papers that included impurities analysed by FTIR in statins: Atorvastatin and Sinvastatin [73].

c. UV Visible Spectroscopy:

The most commonly use technique in pharmaceutical analysis is the UV Vis Spectroscopy that measures the amount of UV visible radiation that is absorbed by the compound. The UV Spectroscopy is a simple, rapid and specific technique that can be used for very small amount of sample [74].

Table 9: UV-Visible Spectroscopy Method.

S. No.	Drug	Mobile phase/solvent	Impurities	References
1.	Amphotericin B	Dimethyl sulfoxide and methanol	tetraenes	3
2.	Atropine sulphate	Methanol	Apo atropine	3
3.	Dextrose	Water	5-hydroxyl methyl furfural	2
4.	Mercaptopurine	Dimethyl sulphoxide and 0.1 M HCl	Hypoxanthine	1
5.	Norgestrel	Ethanol	3,17 α-diethinyl-13-ethyl-3,5-gonadiene-17-ol	59

d. Preparative Liquid Chromatography

Impurities or the degradants present in drugs are in a very low amount that hampers the identification because detailed analysis can be done after isolation of impurities. This becomes a major challenge in pharmaceutical labs. Preparative LC is such a technique that helps in easy isolation of the impurity in sufficient quantity for structural identification using FTIR, NMR, LC-MS or GC-MS.

e. Liquid Chromatography and Ultraviolet Spectrometry (LC/UV)

A number of impurity analysis methods found in pharmaceutical quality control (QC) laboratories use high-performance liquid chromatography (HPLC) coupled with UV detection (HPLC/UV methods). UV spectrometry helps identify impurity or degradant in drug substances based on absorption maxima. This technique is one of the most important and versatile analytical methods available for impurity profiling today due to its high selectivity (i.e., ability to quantitatively determine a number of the individual components present in a sample using a single analytical procedure), especially for quantitative routine analysis where standards are accessible. Newer, stationary phase systems are existing which operate in several modes, such as ion pairing, increased hydrophobic interactions, and variable pH, allowing a variety of samples to be analyzed altogether based upon their unique properties. The chances of errors are reduced and there is a possibility of analysis of all impurities that complies with the higher resolution of LC/UV.

f. Liquid Chromatography and Mass Spectroscopy (LC/MS)

LC/MS is a potent analytical tool that is regularly used in pharmaceutical development identifies product impurities and degradants. The detection limit of a few hundred ppm is easily achievable; hence it is possible to identify all the impurities present at concentrations greater than 0.1 %. An additional robustness and ruggedness are present with MS-based techniques as compared to UV as they have high specificity and sensitivity. Highly sensitive Time of flight (TOF) mass spectrophotometer has higher resolution and accuracy that enables the unequivocal identification of unknown trace impurity, creating a path for genotoxic impurity analysis. On the other hand single quadruple MS helps in confirmation of known impurities and preliminary structure assessment of unknown impurity.MS-based methods are often selected for the impurity profiling of APIs during process development. Triple-quadruple LC/MS/MS systems have become a standard platform for the quantitative analysis of organic impurities in pharmaceutical analytical laboratories [76].

Table 10: Hyphenated Techniques in Impurity Profiling of Drugs.

S. No.	Drug	Method	Mobile phase	Impurity	References
1.	Bendazac lysine	LC/MS/MS	Acetonitrile: aqueous buffer (1.0ml glacial acetic acid in 1000 ml water) (47:53)	2-(1,5-dibenzyl-1*H*-indazol-3-yloxy)acetic acid.	53
2.	Deferasirox	HPLC-UV	Water-methanol	Deferasirox A and B	77
3.	Dup 941	LC-UV-Diode array	Acetonitrile: water: trifluoro acetic acid	PC, SL, LS	78
4.	Salicylaldehyde-isonicotinoly-hydrazone	HPLC-DAD and MS/MS	Phosphate buffer methanol (60:40)	2-hydroxy-acetophen-one, isonicotinoylhydrazone, 2-hydroxy-propiophen-oneisonicotinoylhydrazone	79
5.	Capreomicin	LC-MS	CAN and formic acid	20-N-delysine-20-N-glutamine, 20N-delysin-36-N-lysine	80
6.	Lumefantine	HPLC-DAD/UV-ESI/MS/MS	H_2O:CAN and formic acid	Desbenzylketo derivative	3
7.	Meglumine	LC/MS	A-0.1% formic acid in water B-0.1% Formic acid in 90:10 Methanol acetonitrile	2 impurities	81
8.	Pholcodine	LC-ESI-MS	Conc. Ammonium solution and ACN	Pholcodine A,B,C	82
9.	d-allethrin	GC-FID/MS	Helium	Crysolectone, allethrolone, chrysanthemic acid	83
10	Saxagliptin	LC-ESI-MS/MS	A-aq. Ammonium formate solution B-methanol	7 impurities	84
11	Ritonavir	LC-MS/MS	HPLC –water : methanol : Acetonitrile (40:20:40) MS-nitrogen gas	8 impurities	85
12	Toremifene	MS^2/TOF and LC-MS/TOF	HPLC-methanol and water (85:15) MS-nitrogen gas	6 degradation impurities	86

Capillary Electrophoresis (CE)

High separation efficiencies compared to other chromatographic techniques is achieved by CE for determination of drug-related impurities. When HPLC techniques fail to adequately measure impurities CE can be employed, especially in the case of very polar compounds.

A detection limit of 0.1 % is widely accepted as a minimum requirement for a related impurities determination method and this can be achieved using CE. Adding up, CE is very useful for the separation of closely related compounds, such as Diastereomers and Enantiomers. An example of the value of CE in impurity analysis can be demonstrated using heparin (a polymeric anticoagulant) as an example. In this case, standard chromatography failed to distinguish drug lots associated with adverse events while CE was easily able to identify an unknown impurity As a result, the use of CE helped to solve this analytical challenge [87,88].

Table 11: Capillary Electrophoresis.

S.No.	Drug	Impurity	References
1.	Alcuronium	Diallylcaracurine (DAC), Monomeric allyl-Wieland- gumlich-aldehyde (WAG)	89
2.	Cefotaxime	6 impurities	90
3.	Cephradine	cephalexine	91
4.	Meclophenoxate	N,N-dimethyl ethanolamine	92
5.	Minocycline	4-epiminocyline, 6-deoxy- 6-demethyltetracycline, 7-didemethylminocycline, 7-monodemethyminocycline. 9-minocycline	93

Nuclear Magnetic Resonance (NMR)

NMR is potent analytical with wide applicability as it enables the study of compounds in both solid and solution phase and provides specific information about bonding and stereochemistry within a molecule, which is particularly important in the structural characterization of drug characterization of drug impurities and degradant present in very less quantity. The best advantage that lies with NMR is that it does not destroys the sample which makes it a valuable tool for characterization of impurities and degradants present in traces. It is also helpful in providing a quantitative output that is a vital division of impurity profiling [94-96].

Mass Spectrometry (MS)

Inductively-Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Inductively-Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) is one of the methods of MS that is useful for detecting metallic and non-metallic impurities at a very concentration of 10 [15] (part per quadrillion, ppq). The ionization of the sample is done by inductively couple plasma and then by the use of MS the ions are separated and quantified. ICP-MS is one of the best techniques that are very powerful and sensitive to deliver a consistently good trace-level examination of all 16 elements whose limits are defined in USP (232). The low detection limits of ICP-MS ensure that all regulated elements in drug substances or drug products can easily be determined using the new method, at or below regulated levels, and even when large sample dilutions are required. ICP-MS can also be used in combination with a variety of separation techniques, such as HPLC, GC, and CE, providing several options for separation (or speciation) of the different chemical forms of the elements, and depending upon the nature of the sample. ICP-MS achieves low detection limits for almost all elements, including those found in the more extensive analyte list proposed in the ICH Q3D, such as Au and Tl.

ICP-OES

ICP-OES provides parts per billion (ppb) detection limits for most regulated elements in pharmaceutical products, easily meeting the specified limits in cases where direct sample analysis or small dilution factors are appropriate. It also provides extended dynamic range, robust plasma, and one-step measurement of major, minor, and trace elements. Therefore, ICP-OES addresses the needs of a wide range of users, including those seeking a cost-effective solution for the direct analysis of elemental impurities in bulk raw materials and pharmaceutical products.

The Draft for elemental impurities (USP233) states the need of an instrument based method for determination of elemental impurities that is achieved by either ICP-MS or ICP-OES. With both methods, sample analysis can be accomplished in three ways: directly (unsolvated), following sample preparation

by solubilisation in an aqueous or organic solvent, or after acid digestion using a closed-vessel microwave system [97,98].

Gas Chromatography (GC)

Gas-liquid chromatography (GLC) also known as gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without any decomposition. The only difference over here is Gas used as a mobile phase instead of liquid. In many situations, GC is helpful in identification of a compound. As such in preparative chromatography, GC can be a promising technique to obtain a pure compound from a mixture.

In combination with flame ionization detection (FID), GC is the paradigm alternative for the analysis of volatile organic impurities that is mainly residual solvents. As stated by ICH Q3C guidelines the GC with headspace method plays a major role and globally recognized for residual solvent analysis in Q C analysis. Hence the use of FID detector helps to identify and quantitate the amount of residual solvent present. The recent technology is an adjunct of GC-MS that has been very successful in confirming and identifying the impurities [99,100].

Table 12: Gas chromatography,

S. No.	Drug	Mobile phase/Solvent	Impurity	References
1.	Cloxacillin	cyclohexane	N, N dimethyl aniline	3
2.	Doxorubicin hydrochloride	Dioxane	Acetone and ethanol	2
3.	Fluorescence sodium	Methanol	Dimethyl form amide	2
4.	Methamphetamine	n-Hexane and phosphate buffer	1,2-dimethyl-3-phenylaziridine,ephedrine, methyl ephedrine, N-formylmethamphetamine,N-acetylephedrine,N,O-diacetylephedrine, methametamine dimmer	101

CONCLUSION:

Impurity profiling is not an absolute term as impurities will be present in all drug substances and drug products, i.e. nothing is 100% pure if one looks in enough depth. When a quality of a product is protected, it is ultimately the consumers who will be on the beneficiary side. Hence keeping in mind patient needs and societal benefit, the role of impurity and degradant identification and control to the optimum limits is very imperative. The manufacturing units should have a prime agenda of identifying impurities and establishing the complete impurity profile of the drug that includes its toxicity and safety limits, LOD, LOQ of several organic and inorganic impurities, usually accompany with bulk drugs and finished products. The process of impurity profiling starts from the manufacture of pure Active pharmaceutical ingredient till the formulation of a dosage form and its storage. This has been accepted worldwide and there are standards and specification of control and isolation of impurity in guidelines by various regulatory authorities and manufacturers. Method validation for identification and characterization of impurity is the best approach and can be adopted for the evaluation of impurity. It has been rightly said that change is the only constant hence it's needed that one ought to go for innovating methods and techniques to identify and isolate impurities for safe and effective drug products.

The present review provides an insight into the current development of analytical techniques to investigate and quantify impurities in drug substances and drug products providing discussion of progress particular within the field of chromatography to ensure separation of and quantification of those related impurities by the advanced hyphenated and double hyphenated techniques.

FUTURE PROSPECTS:

Various regulatory authorities have stated the guidelines that govern the limits of impurities present to a safe and effective level nevertheless the need of having a proper standards or specification for control of impurities that need to be included in monograph of each drug is still an indispensable area for the Pharmaceutical Researchers.

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