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Navigating Advances and Challenges in Pharmaceutical Impurity Characterization and Quantification

ARCHITA TIWARI*, RAJESH KUMAR SHARMA[1] and BHUPENDRA SINGH[2]

[1]Teerthankar Mahaveer College of Pharmacy, Teerthankar Mahaveer University, Moradabad, India
*Professor, NGI College of Pharmacy, Neelkanth Group of Institutions, Meerut, India.

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ABSTRACT

Impurities are unavoidable foreign materials present in trace amounts in pharmaceuticals. Regulatory authorities like the FDA and EMA set guidelines to control impurity levels, ensuring drug safety and efficacy. Impurity profiling, which includes isolating, identifying, and characterizing impurities, is essential in maintaining these standards. The increasing complexity of drug formulations, due to advanced synthetic methods, novel excipients, and delivery systems like nanoparticles and liposomes, has led to more impurities. Analytical methods such as HPLC, GC, MS, NMR, FTIR, and hyphenated methods are used to detect and quantify impurities with extended accuracy and sensitivity. This review aims to explore both current and advanced methods for impurity detection, with a focus on recent advancements in analytical techniques and the complexity of formulations. It also examines the challenges of impurity detection, considering existing resources and newly updated regulatory guidelines for impurity control. This review aims to highlight the critical need for improved analytical techniques that minimize errors in impurity detection and enhance accuracy. Stringent regulatory guidelines and advanced analytical techniques like HPLC, MS, and NMR are vital for ensuring drug safety and quality. Continuous innovation in impurity analysis will be crucial to meet evolving standards.

Keywords: Impurity profiling, Hyphenated techniques, Complexities of drugs, Genotoxic impurities.

INTRODUCTION

Overview of impurity profile in Pharmaceuticals

Any component of a newly developed medicinal product that is not an essential component of the chemical entity is considered an impurity according to the International Council on Harmonisation. Tiny, undesirable chemicals that occur in the drug development process, such as API, solvent, or excipients, are known as impurities. In accordance with laws, ensure quality, efficacy, and safety of medications, and minimise dangers to patients and the environment, it is essential to detect trace contaminants. The medication's efficacy, quality, and safety may be compromised by even a minute quantity of this contaminant.[1]

The emphasis on quality control has significantly increased in recent years, with both the bulk drug and pharmaceutical industries facing the challenge of consistently delivering top-notch products. Ensuring the purity of active pharmaceutical ingredients involves multiple factors. Raw materials play a crucial role, as their quality directly impacts the final product. The manufacturing process, including methods like crystallization and purification, also influences API purity. Vigorous quality control measures are imperative at every step to maintain the desired quality and purity standards.[2, 3] Organic, inorganic, residual solvent, and elemental impurities are the four main types of contaminants, as illustrated in Figure 1. Various sources can contribute to the formation of pharmaceutical impurities during manufacture.[4] Packaging materials, such as containers, closures, and labels, can also contribute to impurities.[5] These impurities may leach into the drug product over time, especially if the packaging materials are not inert or if there are compatibility issues.[6] The incompatibility or instability in pharmaceutical formulations due to the interaction of ingredients in the formation of dosage forms, leading to the degradation of certain components. Thiamine is an example of this kind of impurity because, in the formulation of vitamin B complex, thiamine is degraded in the presence of nicotinamide. [7] Layers within pharmaceutical products produce no benefits to patients, so strict control over raw materials, equipment, and manufacturing conditions can reduce the introduction of impurities during production. Analytical performances like HPLC and MS are also used to identify and enumerate impurities at trace levels.[8] Impurity profiling is the cornerstone of pharmaceutical quality control; it assures ensures efficacy, safety, improves formulation, meets regulatory standards for impurity level in pharmaceuticals, and also helps identify the source of impurity.

The ICH Q3A guidelines provide a thorough explanation of how to conduct impurity profiling on both known and unknown contaminants in new pharmaceutical substances, while the ICH Q3B guidelines deal with contaminants that may have leached into the product during its degradation or interaction with additional ingredients.[9] Under investigation impurity profiling gives all possible types of impurities and also different kinds of impurities present in pharmaceutical formulation.[10].

The objective of this current review is to navigate through currently available and some advanced methods of impurity detection. This review highlights the recent advances in analytical methods for impurity detection, especially discussing the challenges of impurity detection with the available resources. To give an insight into the serious need for such an analytical technique or to improve analytical processes, which can minimize errors in impurity detection and improve the results. This review will give an in-depth insight to the researchers and the educators for the updated information about impurity detection.

Sources and Types of Impurities

Enantiomeric impurities

The scientific name for these mirrored copies is enantiomers. Due to these enantiomers, the drug results in different pharmacological activities. Some of them are active, less active, or remaining some causes adverse effects. Pharmaceutical companies are increasingly developing drugs that contain only the active enantiomer. This approach can improve drug efficacy and safety. However, the development and process for a single enantiomer drug can be a little costly and complex. [6, 11]

Since opposite enantiomers require particular chiral stationary phases or mobile phases, HPLC, which has been utilised to detect drug ingredient impurities, is incapable of doing chiral analysis without specialised equipment. For this complication, various unique methods including CE-UV[12], HPLC-CD[13], MEKC-UV[14], CZE-UV [15]and CZE-LIF[16], etc. helps in the separation of enantiomeric portion from chemical moiety.

Teratogenic impurities

Teratogenicity refers to the ability of a substance to cause birth defects by disrupting the normal development of an embryo or fetus. This is the side effect of many drugs like thalidomide. [17]Class 2 solvents like acetonitrile, N-dimethyl formamide are dangerous to health and sometime causes irreversible toxicities such as teratogenicity or neurotoxicity.[18-20]

Genotoxic Impurities

Regulatory body: Endocrine-related carcinogens are defined in the ICH recommendations. ICH S2 (R1) as “a general phrase that pertains to

any undesirable alteration in genetic material, taking into account the process by which the alteration is produced.[21] To rephrase, genotoxicity is defined as an effect that damages or destroys a cell's genetic material, specifically its DNA and RNA.[22] Because they can cause cancer in humans at extremely low concentrations, possible genotoxic impurities (PGIs) in medications are a major cause for concern. These impurities often arise from reactive functional groups used in the synthesis of pharmaceuticals. Genotoxic impurities (GTIs) in pharmaceuticals can indeed be both organic and inorganic in nature. Alkyl halides, aromatic amines, epoxides, and nitrosamines are some examples of organic impurities, while cadmium, lead, and chromium, etc. are related to inorganic impurities.[23-25] Because they can alkylate DNA bases on N-3 of adenine and N-7 of guanine, alkyl halides are regarded as possible genotoxic contaminants that are produced during the production of APIs through undesirable chemical reactions.[26-28]. Nitrosamines is category in which the amine group is linked with the nitroso group in their structures. They are of major concern because the ICH M7 guidelines classified them as class 1 impurities. [29, 30] The enzymes epoxide hydrolase and glutathione-S transferase, which are generated during metabolism, are involved in detoxification and the genotoxic activity of epoxides. Direct carcinogens include 2,4-diaminotoluene and 2,4-diaminoethylbenzene, as well as a small number of amines with nitro groups.[31-33] Much smaller concentrations of elemental contaminants like As (arsenic), Hg (mercury), Cd (cadmium), and Pb (lead) are hazardous. Lead has been linked to adult cardiovascular problems, elevated blood pressure, reduced cognitive development, and renal tumours. Lead consumption mostly affects the human brain.[34]

Nitrosamine Impurities

Nitrosamine impurities may get into the formulation through reagents, catalysts, solvents, or raw materials, etc. These impurities are known as carcinogenic and mutagenic because their small exposure can results cancer. This major concern attracts regulatory authorities' attention towards patients' safety. In this context, regulatory authorities release the limit of impurities and classify them under class 1 impurities as per ICH guidelines. Various batches of generic drugs and drug products, including sartans and angiotensin II blockers, were voluntarily recalled from the market by the FDA and EMA in July 2018 due to the presence of N-Nitrosodiethylamine (NDEA) and N-Nitrosodimethylamine (NDMA) impurities, which are said to be carcinogenic. By causing genetic mutations, these Nitrosamine impurities affect DNA by causing chromosome breaks, rearrangements, or covalent bindings.[35]

Focus on controlling genotoxic impurities in pharmaceutical products.

Definitive standards for medicinal substances and pharmaceutical products were published by ICH in the late 1990s. U.S. (USFDA), EU (EMA), and Japanese (PMDA) regulatory agencies have all accepted these standards. According to the ICH recommendations, a drug substance with a daily intake of up to 2 g is considered to have impurity levels of up to 0.15% (1500 ppm), or 1 mg. In most cases, no further study is necessary below this threshold. On the other hand, the product could be harmful if the threshold is crossed. In most cases, these limits are enough to detect typical contaminants that are caused by the procedure. Following recommendations from the USFDA and EMA, it was suggested that ICH limits might not be sufficient for genotoxic contaminants.[36] The possible health concerns posed by these contaminants necessitate more stringent control measures.

In December 2002, the Safety Working Party (SWP) of the European Committee for Proprietary Medicinal Products published a "Position Paper on the Limits of Genotoxic Impurities" to address gaps in ICH regulations.[37] Because of the increased risk of cancer, chromosomal damage, and genetic abnormalities that can result from genotoxic or carcinogenic contaminants, regulatory oversight of these substances has grown in recent years. Since the safety of patients is the main concern of regulatory authorities, the increased focus on genotoxicity in clinical development and drug licensing could cause delays.[36] Because of the increased risk of cancer, chromosomal damage, and genetic abnormalities that can result from genotoxic or carcinogenic contaminants, regulatory oversight of these substances has grown in recent years. Since the safety of patients is the main concern of regulatory authorities, the increased focus on genotoxicity in clinical development and drug licensing could cause delays.[38] Because there are many other organic chemicals present, although at

lesser amounts, this demands analytical equipment that is both very sensitive and selective. In addition, because genotoxic impurities can originate from a variety of sources and include a wide variety of functional groups, it is possible that a single analytical approach will not be adequate to detect them all. Consequently, it may be necessary to choose a combination of analytical procedures. In the past, methods such as gas chromatography (with FID detectors) and HPLC with UV/vis detectors were commonly employed to analyse these contaminants. Mass spectrometers have recently seen a surge in detector applications due to their superior sensitivity and selectivity. Because these contaminants are reactive, it is important to manage them while also protecting the analysers from damage during sample extraction, storage, and analysis.[39]

ICH guideline M7, which is titled "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk," lays out a system for assessing and controlling the dangers of toxic pharmaceutical contaminants. This guideline was created in 2013 to standardise the technical requirements for pharmaceutical registration worldwide. Its purpose is to keep products safe and effective while reducing the possibility of carcinogenic contaminants.

A risk assessment is conducted to determine the appropriate control techniques for genotoxic impurities, according to the ICH M7 guideline. These impurities are characterised according to their propensity to cause genetic mutations or chromosomal damage Table 1.

The safety of pharmaceuticals and their compliance with regulations depend on their prompt detection and control. These are chemical compounds with the potential to alter cellular genetic material, which in turn increases the risk of cancer. Guidelines on allowable levels for PGIs in pharmaceuticals are provided by several regulatory authorities with regard to genotoxic impurities. [40]

Importance of monitoring impurities in pharmaceutical products. Monitoring impurities in drugs is crucial to ensure their quality, efficacy, and safety. Impurities can affect stability, safety, and efficiency of pharmaceuticals, making their identification and control essential in drug development and manufacturing processes. Impurities in pharmaceuticals can originate from the synthesis of API itself, from raw materials like starting materials, solvents, catalysts, or excipients used in formulation, interaction between drug product and its packaging material, or environmental factors,

Table 1: Genotoxic impurities potential, risk assessment, and their control strategies

Class	Definition	Risk Assessment and Control Strategies
1	Known Carcinogens and Strongly Suspected Carcinogens	Maintain control at or below the permissible limit for each compound
2	Alerting Structure, DNA Reactivity, and No Carcinogenicity Data	Maintain control within acceptable ranges (standardised or modified TTC)
3	Alerting Structure, No DNA Reactivity, or Inadequate mutagenicity Data	Perform a bacterial mutagenicity test or keep control levels within permissible ranges (standardised or modified TTC): A class 5 substance is considered nonmutagenic if When class 2 is applied, mutagenicity is established
4	There is no indication of genotoxicity and no system for alerting	Extravagance as a nonmutagenic impurity
5	The absence of an alerting structure, insufficient data, or the presence of an alerting structure with enough evidence to prove that the substance is not carcinogenic	Extravagance as a nonmutagenic impurity

and may also arise from exposure to conditions during transportation that introduce impurities or cause degradation of pharmaceuticals.[41] For a pharmaceutical product to be completely safe for human consumption, even the tiniest amount of contaminants must be eliminated from the medication ingredient. Consequently, a crucial aspect of medication creation is the management of contaminants through quantitative or qualitative investigation.[42] An assortment of regulatory bodies have put out standards to deal with impurity control, including, but not limited to, [10, 43]

PMDA, ICH, The Canadian Drug and Health Agency,

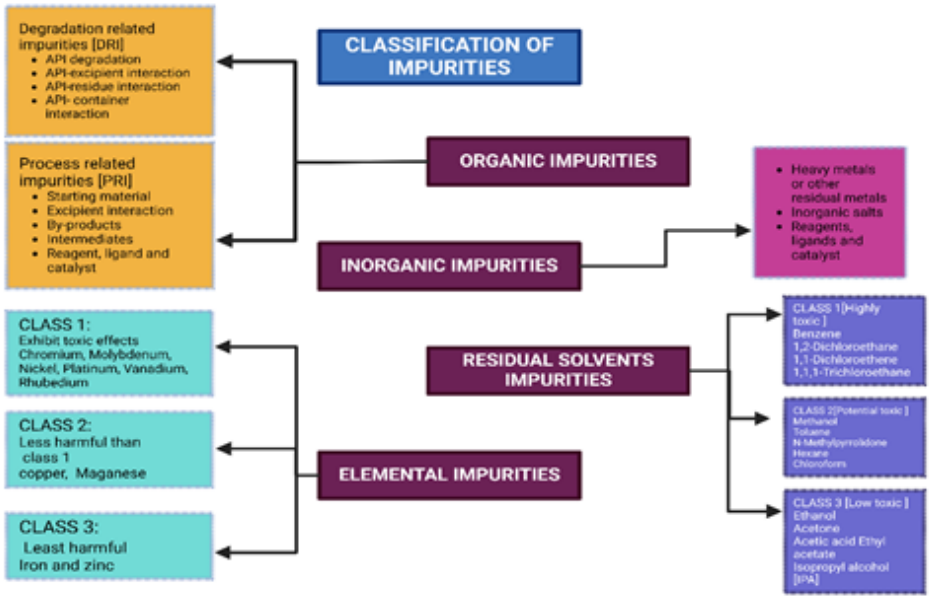

Fig. 1: Different forms of impurities originated from organic, inorganic, residual solvents, and elements

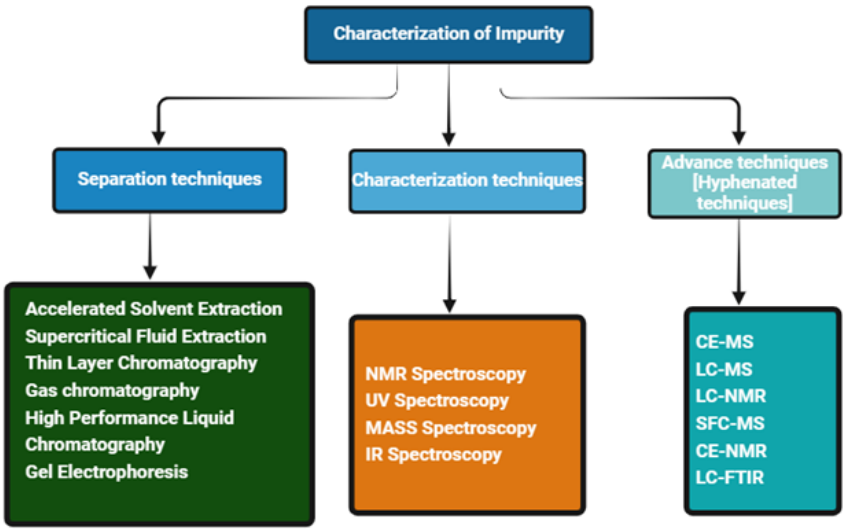

Fig. 2: Characterization approaches for analysis of impurities present in pharmaceutical drugs

Table:2 Analytical Methods Used for Detection of Genotoxic and Enantiomeric Impurities Present in Pharmaceuticals [Fig.3 and Fig. 4]

Type of Impurity	Drug	Impurity	Analytical Method	Optimized analytical conditions	References
Enantiomeric impurities	Magnesium-L-aspartate	D-aspartic acid	CZE-LIF	pH 7.0, 50 mM phosphate BGE, 18 mM (v/v) DMSO, 18 mM HP-βCD, 20 kV, 25 °C	50
	Alalevonadifloxacin	desfluoro analog levonadifloxacin N-Boc analog of ALA	LC–MS/MS	10 mM ammonium formate, C18 (250 x 4.6 mm I.D.) column, pH 2.1 [formic acid, acetonitrile], 295 nm,10 µL, 30°C	51
	Brivaracetam	(R, S)-brivaracetam, (S, S)-brivaracetam, (R, R)-brivaracetam	LC–MS	10 mM ammonium bicarbonate :acetonitrile, IG-U (100 × 3.0 mm; 1.6 µm 40°C, 215 nm	52
	Netarsudil (NTS), latanoprost (LTP), and benzalkonium chloride (BZC)	-	HPLC-DAD	phosphate and sodium pentane sulfonate (pH 3.0) and acetonitrile, Zorbax-SB Phenyl column, flow rate 1.5 mL/min, 254 nm	53
	Vildagliptin	R-vildagliptin	CZE-UV	20 mM SBE-βCD, 75 mM Tris-acetate BGE, pH 4.75, 25 kV, 200 nm, 15 °C	54
	Montelukast	R, R-trans-montelukast, S, R-cis-montelukast R, S-cis-montelukast	MEKC-UV	10 mM TM-βCD, 20 mM borate BGE, 10 mM SDS, 10 mM SBE-βCD, pH 9.0, 18 kV, 254 nm, 15 °C	55
	Sitafloxacin	R,R,S-sitafloxacin, R,S,R-sitafloxacin S,S,R-sitafloxacin	CZE-UV	15 mM d-phenylalanine, phosphate BGE, pH 4.5, 20 mM CuSO4, 20 mM βCD, 15 kV, 25 °C, 297 nm	56
	Lenalidomide	R-lenalidomide	CZE-UV	pH 6.5, 30 mM SBE-βCD, 30 mM phosphate BGE, 12 kV, 210 nm, 10 °C	57
	R-lansoprazole	S-lansoprazole	CZE-UV	20 mM βCD, 20 kV, 17 °C, 210 nm25 mM phosphate BGE, pH 7.0, 10 mM SBE-βCD,	58

	S-citalopram	R-citalopram	CZE-UV	pH 7.0, 3 mM CM-βCD, 25 mM phosphate BGE, 15 kV, 17.5 °C, 230 nm	59
	Linagliptin	S-linagliptin	CZE-UV	pH 6.10, 70 mM sodium acetate BGE, 4.7 mM CM-βCD, 28 kV, 25 °C, 200 nm	60
	R-solriamfetol	S-solriamfetol, R-phenylalaninol, S-phenylalaninol	CZE-UV	pH 4.5, 4 mM S-βCD, 45 mM Tris-acetate BGE, 19.5 kV, 21 °C, 200 nm	61
	Valsartan	-	HPLC-CD	ACN-water (0.01% acetic acid) (1:1), 233 nm	62
	Dextromethorphan	-	HPLC-CD	23 mM docusate sodium (7:3), 23 mM, ACN-ammonium nitrate, pH*3.4, 280nm	62
	Rivaroxaban	(R)-Rivaroxaban	HPLC	acetonitrile (ACN): water pH 4.5 (92:8 v/v),(150 × 4.6 mm; 5 µm) column, 0.35 mL/min, 250 nm, 40 °C	63
	Imeglimin	-	LC-ESI-MS/MS	Methanol and 10 mM ammonium acetate (95:5 v/v ratio),(100 × 4.6 mm, 3 µm), 0.5 ml/minutes	64
	Solriamfetol	R-solriamfetol	HPLC	0.15% diethylamine in methanol (MeOH), polysaccharide-based chiral columns, 2-propanol (IPA) or acetonitrile (ACN), 0.6 mL/min flow rate at 20 °C	65
	Mebeverine	-	LC-MS	acetonitrile: 10 mM ammonium acetate (85:15) 250 mm x 4.6 mm i.d, 5µm, 0.8 mL/min	66
Genotoxic impurities	Siponimod	alcohol and aldehyde impurity	LC-MS/MS	ammonium acetate (0.02M), pH 4.2, and methanol in 45:55 (v/v) at 0.5 mL/min flow rate, Waters C18 (150 × 4.6 mm; 5 µm) column,	67
	Febuxostat	-	GC-ECD	flow rate of 1.5 mL	68

			min−1,agitated at 250 rpm, Agilent J&W DB-624 (30 m × 0.32 mm, 1.8 µm film thickness),	
Ciprofloxacin	N-nitroso impurities	UPLC-ESI-MS/MS	acetonitrile, and methanol [475:500:25 v/v/v], Agilent Poroshell 120 Aq-C18 column (150 mm × 4.6 mm, 2.7 µm, 0.1% formic acid in a mixture of water, flow rate 0.5 mL/min.	69
Posaconazole	BSKZ155, BSKZ117 and BSKZ160	LC-MS/MS	0.1% formic acid aqueous solution and acetonitrile as mobile phase, YMC-Triart C18 column,	70
Doxofylline	methyl 4-methylbenzenesulfonate (PGI-1), (HPLC-UV) ethyl 4-methylbenzenesulfonate (PGI-2), 2- hydroxyethyl 4-methylbenzenesulfonate (PGI-3), and 2-(4-methylphenyl) sulfonyloxyethyl 4-methylbenzenesulfonate (PGI-4)		C18 column (250 mm × 4.6 mm, 5 µm), 20% acetonitrile, flow rate of 1.0 mL/min	71
Crisaborole	4-(4-(4-bromo-3-formylphenoxy)-3-formylphenoxy) benzonitrile and 4-(4-Bromo-3-formyl-phenoxy)-benzonitrile	UPLC-MS/MS	column (4.6 mm ×75mm, 3.5 µm), acetonitrile [0.1% trifluoroacetic acid]+ water [0.1% formic acid]	72
Crotamiton	Aromatic amine impurities	GC-FID	Column length 30 m, i.d. 0.53 mm, film thickness 1.0 µm, 40 °C	73
Ranitidine	NDMA impurity	LC-HRMS	Ace C18-AR, 3 µm 100 Å, 50 x 4.6 mm, 30 °C, 0.5 mL/min, 3 µL, 0.1% formic acid in water+ 0.1% formic acid in ACN, 3.5 kV	74

Metformin	N-nitroso-dimethylamine (NDMA)	(LC-HRMS)	X Select CSH C18 2.5 µm, 3.0 x 150 mm, 0.3 mL/min, 3 µL, 0.1% formic acid in water+ 0.1% formic acid in Methanol, 3.5 kV, 30 °C	75
valsartan	N-nitroso Valsartan and methyl N–((2β-(1H–tetrazol-5-yl)-[1,1β biphenyl]–4-yl)methyl)-N-nitroso-L-valinate	UPLC-MS/MS	C18 column (150 mm × 4.6 mm, 2.5 µm), ammonium acetate aqueous solution (0.01 mol/L), 0.5 mL/min,	76
Alogliptin	Pyridine, 3-aminopyridine, N, N-dimethylamine, and 4-dimethylaminopyridine	HPLC–MS	(250 mm × 3.9 mm, 3.5 µm) column, water-methanol (55:45, v/v), 0.5 mL/min	77
Imatinib Mesylate	N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-aminopyrimidine (IMA), N-(2-methyl-5-nitrophenyl)-4- (pyridin-3-yl) pyrimidin-2-amine (IMN)	LC–MS/MS	C18 (150×2.1 mm, 1.7 µm), 0.02 M ammonium formate buffer (pH 3.4) and acetonitrile (containing 0.05% formic acid), 0.4 mL min–1, 40 °C	78
Sitagliptin	7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4] triazolo [4,3-a] pyrazine (7-nitroso impurity)	(UPLC-MS/MS)	C18 column (100 mm × 4.6 mm,3.5 µm), 0.12% formic acid in water, with methanol, 0.6 mL/min, 40 °C	79
Valsartan	Nitrosodimethylamine, N-Nitrosodiethylamine, N-Nitrosoethylisopropylamine, N-Nitrosodiisopropylamine	GC-MS/MS	30 m x 0.25 mm, 0.5 µm, 70°C for 4 min, 3.0 mL/min, 70 eV in EI mode with 150°C “quadrupole temperature	80
Cinnarizine	Cinnamyl chloride and Benzhydryl chloride	LC-MS/MS	C18 column 150× 4.6 mm ,5 µm, 1.0 mL/min flow rate, 0.1% Trifluoroacetic acid in water and 100% acetonitrile,	81

Table 3: Regulatory guidelines and their current revisions

	Section	Guidelines	Year	Current Revisions
Q3	A[R2]	Give Directions, Identify, and Qualify Impurities in New Medicinal Substances	25 October 2006	The revisions aimed to clarify inconsistencies, revise the decision tree, harmonize with the ICH Q3B Guideline, and address editorial issues.
	B [R2]	Using chemical synthesis, create a new drug substance; then, identify and qualify any impurities in the product.	2 June 2006	The revision helped to clarify inconsistencies, align with updated scientific understanding, and ensure consistency with related guidelines, such as ICH Q3A. Ensure the guideline remains up-to-date and harmonized with related guidelines, maintaining high standards of quality in pharmaceuticals.
	C [R9]	Recommends Guidelines for an acceptable amount of Residual Solvents	24 January 2024	Revisions have been made to include new solvents and update PDE levels based on emerging toxicological information.
	D [R2]	Recommend Guidelines for an acceptable amount of Elemental Impurities	April 2022	Correction of Permitted Daily Exposures (PDEs) for Gold, Silver, and Nickel. Gold and Silver monographs have been updated. Cutaneous and transcutaneous routes for elemental impurity addition with restrictions.

The Australian Department of Health and Ageing Therapeutic Goods, EMA, USFDA.

Regulatory authorities are also focusing not only purity profile of the drug but also on impurity profiling. For the safety and efficiency of drug product different pharmacopoeias like IP, USP, BP, and JP are also revising their monographs for the drug substances every year by introducing the permissible limit of different types of impurities[10].

Use of advanced analytical techniques for impurity detection

It is possible to determine trace amounts and learn more about the structure of contaminants using a combination of analytical methods. Many variables, including the synthetic pathway, reaction conditions, starting material quality, reagents and solvents, purification stages, and storage conditions, determine kind and quantity of these contaminants. Commonly employed for impurity detection and structural elucidation are spectroscopic as well as microchemical methods, such as TLC and GC-MS.[44] and techniques like HPLC, GC, MS, and AAS are commonly employed to achieve such high sensitivity and accuracy in detecting trace or ultra-trace levels of impurities or substances present in pharmaceutical products. These methods are indeed critical for ensuring product safety and quality. There are different types of methods employed for the detection and identification of impurities currently in pharmaceutical formulations, as shown in Figure 2.[41] Separation methods 2. Hyphenated methods 3. Spectroscopic methods

As a result of recent advancements, LC-MS systems are now commonly utilised for complicated analysis of mixtures containing thermally labile and biologically essential compounds like domperidone. This is due to the mild nature of (APCI and APPI. The sensitivity and adaptability of APCI and APPI make them perfect for use in biochemical and pharmacological analysis. Several ionisation methods are employed in allethrin profiling to

Table 4: Purpose of Regulatory requirements and scientific/technical demands for impurity profiling

Aim	Regulatory requirements
Ensure Compliance with Standards	Maintain impurity levels within regulatory limits.
Address Regulatory Queries	Respond to specific questions or concerns raised by regulatory agencies regarding impurities.
Facilitate Cleaning Validation	Develop procedures to ensure equipment is adequately cleaned to prevent cross-contamination of impurities.
Ensure Batch Consistency	Ensure that each production batch maintains consistent impurity profiles.
Support Risk Assessment	Provide data to support risk assessments regarding impurity profiles.
Document and Report Impurities	Provide detailed documentation for regulatory submissions.
Develop Control Strategies	Implement measures to control and minimize impurities during manufacturing.
Assess Toxicological Impact	Evaluate the potential toxic effects of impurities on patient safety.
Identify and Quantify Impurities	Determine the type and amount of impurities present in APIs and DPs.
Technical Demands	
Investigate Degradation Products	Identify impurities that arise from the degradation of APIs or DPs over time.
Support Shelf-life Determination	Provide data to determine and justify the shelf-life of the drug product.
Investigate New Synthesis Routes	Explore how different synthesis routes impact impurity profiles and optimize accordingly.
Optimize Formulation Stability	Study how impurities affect the stability of the final drug product.
Enhance Process Understanding	Gain deeper insights into the manufacturing process to identify sources of impurities.
Improve Analytical Methods	Develop and refine analytical techniques for better detection and quantification of impurities.

Table 5: Qualification threshold for drug substance and drug product110

Maximum Daily Dose (MDD)	Identification Threshold	Reporting Threshold	Qualification Threshold
≤ 2 g/day	0.10% or 1.0 mg/day intake (whichever is lower)	0.05%	0.15% or 1.0 mg/day intake (whichever is lower)
> 2 g/day	0.05%	0.03%	0.05%

improve detection and identification capabilities; allethrin is a cyclopropane carboxylate ester. Combining the strengths of capillary electrophoresis with mass spectrometry, CE-MS allows for extremely precise separations and identifications.[45] ESI, APPI, and APCI are all ionisation processes that can be utilised by this procedure. By applying an electric field inside a small capillary, CE-MS enables the high-resolution separation of analytes according to their charge-to-size ratio. Other approaches that can reduce the time needed for impurity quantification are GC/MS/MS, GC/DAD/MS, GC/DAD/MS/NMR, SFC/MS, CE/MS, and HPLC/MS.[46] This separation is highly efficient and capable of resolving complex mixtures, including closely related compounds.[47-49] Table 2 details a few state-of-the-art analytical procedures utilised for detecting enantiomeric and genotoxic contaminants.

Challenges in impurity profiling

Identifying unknown impurities in pharmaceutical products

If unknown impurities are found in drug compounds or products over certain thresholds, it is essential to identify them to understand their chemical structures; this is an essential step in drug development. Improving synthetic processes and optimising formulations to minimise impurities can be achieved by conducting toxicological implications assessments and understanding the formation mechanisms of these impurities. This method is vital for these endeavours. [82] Because they may disturb the efficacy and safety of medication products, unknown contaminants pose a big problem for the pharmaceutical business. Detecting drug contaminants at quantities as low as 0.1% can be a technical nightmare.[83] Another obstacle to its identification and purification was the impurity's instability in acidic chromatographic conditions. Still, you can turn this acidic instability to your advantage by studying the impurity's deterioration, which will provide you with a wealth of knowledge about its structure.[84]

Challenges of impurity profiling using HPLC with UV/vis absorbance detection are significant and multifaceted, especially when dealing with analytes that lack suitable chromophores, possess high hydrophilicity, or exhibit ionic characteristics. Many substances do not have chromophores that absorb in the UV/visible range, making them difficult to detect with UV/vis absorbance detectors. Highly hydrophilic or ionic compounds often exhibit poor retention on conventional reversed-phase (RP) HPLC columns, leading to inadequate separation.[85] In pharmaceuticals, having enantiomers, formidable analytical challenges are faced during separation, as the D/L isomers have identical physicochemical properties except for the stereochemical configuration.[86] Drugs with enantiomers face difficulties in enantioseperations because of the choice of suitable chiral selectors and advanced understanding of underlying chiral recognition principles.[87] Absence of reference standard is another challenge in impurity profiling because reference standards are needed not only for API in dosage form, but also required for impurity.[88]

Ensuring consistency in impurity profiling across different batches of drugs

When any drug is manufactured, it is processed in discrete "batches" of materials. Because exact replication of production conditions is nearly impossible, variations in impurity content will occur among final products from the same source. These variations are categorized as inter-batch variation and intra-batch variation. Inter-batch variation refers to differences in impurity profiles between different batches of the same drug produced by the same manufacturer or laboratory. Which are caused by raw material differences, process conditions, equipment differences, and human factors.[89] Impurity standards play a critical role in ensuring consistency in impurity levels across different batches of pharmaceutical products, thereby maintaining product quality and safety. By adhering to these standards, manufacturers can ensure that impurity levels remain consistent across different batches, protecting patient health and meeting regulatory requirements.[90] Maintaining consistent impurity profiles across different batches

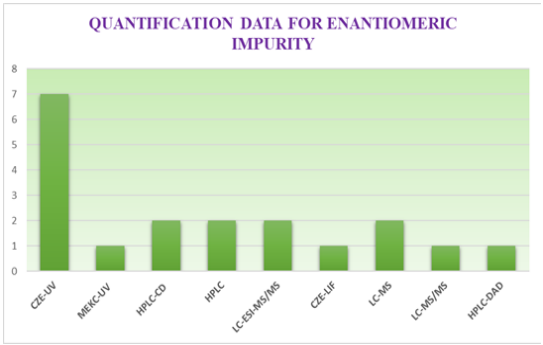

Fig. 3: Advanced Analytical methods used for enantiomeric impurities

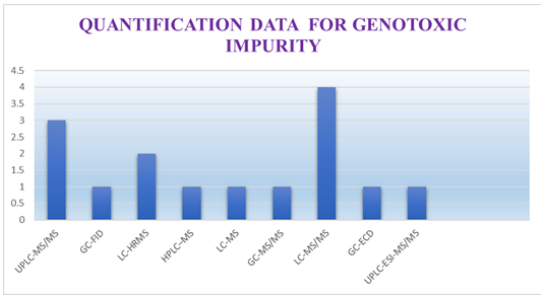

Fig. 4: Advanced Analytical methods used for genotoxic impurities

is essential for drug quality, safety, and efficacy. Through rigorous impurity profiling, manufacturers can identify and control impurities, ensuring that each batch meets the required standards and provides safe and effective treatment for patients.[91]

Addressing the impact of impurities on drug safety and efficacy

Ensuring the development of pharmaceutical items of high excellence is crucial for preserving the safety and effectiveness of drugs. Regulatory bodies and the manufacturing sector share this duty, as they are the ones responsible for establishing and enforcing rigorous quality standards and specifications. The clinical efficacy and safety of pharmaceutical drugs can be greatly exaggerated through a number of risk factors. These problems encompass issues with product quality, mistakes in pharmaceutical administration, and recognised side effects or adverse drug reactions (ADRs). [92] These problems encompass issues with product quality, mistakes in pharmaceutical administration, and recognised side effects or ADRs. The recall of topical medicines Tetracycline-ABC and Dibecline that occurred on June 14, 2017, in the US, as a result of Phillips Company, emphasises how important it is to follow correct processing procedures while making pharmaceuticals. An error in processing that could have serious consequences for patients led to this recall. [93]

Recent developments in the impurity profile of Pharmaceuticals

The increasing complexity of drug formulations is leading to more impurities

The deterioration of drugs is caused by their intrinsic properties. Drug degradation occurs in formulated dosage forms because of the drug's intrinsic properties. Depending on the inherent properties of the medicinal ingredient, the degradation process might be quite complex and involve either one or more chemical processes. Several factors can affect the degradation process. One of these is the formulation of the dosage form, which affects the product content. b. The procedure followed during the production of the dosage form. c. Environmental variables such as temperature, humidity, and light intensity during storage.

The pharmacological substance's physical form (e.g., amorphous or crystalline) might also influence the degradation process's characteristics. In order to minimise drug degradation and ensure stability and efficacy of the formed product, it is essential to understand the inherent features of the medicinal component and the functions of various excipients. [94] The drug substance or formulation proteins could degrade or be altered by even minute amounts of reactive contaminants in excipients. Because of their enormous size and intricate structure, proteins are especially vulnerable to changes brought about by reactive excipient contaminants. Despite their minute concentrations, these contaminants pose a threat to the active medication's stability and quality since they react with it and destabilise it as the product ages. Evaluating and understanding the potential implications of reactive impurities in the excipients used is crucial during medicinal product development.[95]

Herbal formulations have excellent therapeutic potential with fewer or no side effects, and they are also popular as traditional medicines that originate from plants. Tablets, capsules, powders, teas, and extracts from both fresh and dried plants are just a few of the various forms that phytoconstituents—active substances found in plants—are available in. Due to its accessibility and low cost, many communities depend on traditional medicine and natural remedies. Many problems with the effectiveness, standardisation, efficacy, and production processes of herbal formulations have arisen as a result of improper testing. In order to guarantee the safety of these items, results of research indicate that adequate quality inspections utilising innovative analytical techniques are necessary.[96] Microbiological and degrading contamination, which increases formulation contaminants, can easily compromise liquid dosage forms.

Teva Pharmaceuticals USA recalled 60 mL bottles of Fluocinonide Topical Solution USP, 0.05%, because of deterioration and contaminants that caused the product to be sub-potent. Even though pre-formulation experiments were successful, this issue shows that different dosage forms can affect the drug's stability.[97] The absorption of environmental moisture is a major concern for pharmaceutical and nutraceutical solids that are very hygroscopic. Chemical degradation and solid-state transitions are two mechanisms that might cause substantial

changes to their physicochemical properties due to their water-absorbing tendency. Hydrolysis, oxidation, photodegradation, and solid-state transitions such as polymorphic transformation, amorphous to crystalline transition, caking, and agglomeration all contribute to drug degradation, which in turn reduces the amount of active ingredients in the formulation and introduces impurities into the product. The therapeutic efficacy and longevity of these products may be negatively impacted by these alterations.[98]

Before or during the creation of pharmaceutical formulations, numerous investigations have been conducted to identify degradation products and the processes by which they degrade. The complexity of degradation studies can increase significantly when a combination product has dosage forms of more than one API. Degradation product may interfere in the formulation due to the interaction of API with solvent impurities, residual solvents, excipients, excipient impurities, salt, preservatives, excipient- preservative interaction, excipient degradation, extractables, leachable, and metal-ion catalyzed reaction, etc.[94]

In many instances, there are not enough reference standards available for all probable impurities. Identification and quantification of impurities become more difficult without reference standards. It is challenging to separate and quantify impurities or degradation products from analytes of interest due to coelution when they elute at a similar retention time as the analyte of interest.[99]

Regulatory requirements for impurity profiling

Impurity profiling, in addition to the purity profile, is essential to monitor a safer drug to reach the markets with high therapeutic effect, with no toxicity and without any side effects, because the presence of a minute amount of impurity results in low therapeutic effect, deterioration of the pharmaceutical drug product, and unstable product.[100] To establish a thorough framework for the assessment and management of contaminants in medicinal substances and pharmaceutical goods, numerous national and regional standards have been put out. These regulations ensure that pharmaceuticals are of high quality, effective, and safe standards. Table 3 shows that regulatory restrictions are crucial in ensuring the safety and quality of pharmaceutical products. Impurities in APIs or DPs can significantly impact the efficacy and safety of medications. As a result, regulatory bodies such as the FDA or EMA have established stringent standards for pharmaceutical impurity levels.[4]

For NDAs, ICH has produced the Q3B (R2) guideline, which provides recommendations for addressing drug product impurities.

However, it's noted that this guideline is also applicable to abbreviated ANDAs, providing a framework for assessing and controlling impurities. Regulatory agencies such as EMA, FDA, and ICH have published and revised regulations regarding genotoxic, mutagenic, and carcinogenic impurities. These guidelines, updated in 2006, 2008, and 2017, respectively, outline procedures for assessing, controlling, preventing, and reducing impurities in drug products. To provide a safe intake limit for unstudied substances with low carcinogenicity or other hazardous effects. Many marketing applications consider an exposure level of 1.5 µg per person per day (TTC) for each contaminant to be an acceptable qualification criterion.[101] Concerns about genotoxicity and carcinogenicity are typically not warranted for contaminants below this level. An important guideline for the management of residual solvents in APIs, excipients, and medicinal products is ICH Q3C. It provides information on how to identify and handle solvents that pose a risk of cancer and genetic damage in the pharmaceutical industry by classifying them according to these hazards. [83, 102]

Overview of guidelines from regulatory bodies

Regulatory agencies from various countries, such as the FDA in the USA, EMA, TGA in Australia, MHRA in the UK, and ICH, place a significant emphasis on impurity testing in APIs and pharmaceutical products globally. This stringent requirement aims to ensure the safety and efficacy of pharmaceutical products.[103] To aid in the preparation of marketing authorisation applications for human medicines, EMA offers scientific recommendations on contaminants in pharmaceuticals and pharmaceutical ingredients. In order to guarantee the safety and effectiveness of medicinal products, these standards make sure that any contaminants that may form during production, storage, or interactions with packaging materials are detected, measured, and managed.[104] In Q3A-Q3E, the ICH provides the standards for contaminants. The

most recent update to the ICH recommendations for contaminants is detailed in Table 4.

Importance of setting impurity limits in pharmaceutical products

The main goal of impurity profiling is indeed focused on the control approaches of chemical and physical changes in APIs and drug products throughout the drug development process. These control strategies are vital for ensuring both the safety and efficacy of drugs. Impurities can pose significant safety risks, including toxicity, carcinogenicity, and allergic reactions. By profiling impurities, pharmaceutical companies can identify and control these harmful substances, ensuring that only safe levels of impurities are present in the final product. By assessing how impurities may affect the physical and chemical stability of a drug, manufacturers can predict potential performance changes. This assessment is crucial for ensuring that the drug remains effective and safe over its shelf life.

Impurity profiling is not a one-time activity but a continuous process throughout the drug's lifecycle. It includes regular monitoring to detect any new impurities that may arise due to changes in manufacturing processes or environmental factors.105 Impurities in APIs have the potential to greatly impact the bioavailability, safety, and effectiveness of these substances.

Regulatory bodies like the US FDA and ICH have established strict guidelines and limits for allowable levels of impurities in APIs to guarantee the highest quality of pharmaceutical products.106 Indeed, the ICH recommendations give a wealth of information regarding the various pharmaceutical impurity categories and the permitted levels for each. In the ICH guideline, you can find further details on the different kinds of impurities and the maximum allowable levels in pharmaceutical drug products and drug substances. The aforementioned document's Sections Q3A, Q3B, Q3C, and Q3D are concerned with chemical substances, as well as the requirements, testing protocols, and approval standards for novel medicinal drugs and pharmaceutical goods.36, Q6B, M7, and S9 all pertain to biotechnological and biological product specifications, testing procedures, and acceptance criteria, intending to minimise carcinogenic risk from DNA-reactive (mutagenic) impurities in pharmaceuticals[76]. Drug safety and efficacy are dependent on pharmaceutical contaminants being effectively reduced and controlled. The goal of this procedure is to reduce patient risk of adverse health effects by setting impurity limits based on safety considerations. Commonly, these restrictions are based on maximum daily exposure levels. The TTC, Acceptable Intake (AI), Permissible Daily Exposure (PDE), and Staged TTC are important techniques in this field [107].

Impurities in drug materials can significantly impact the safety and efficacy of pharmaceuticals. Physiologically highly active or toxic impurities have the potential to contribute to the drug's side-effect profile, potentially making it inconsistent and unpredictable. This variability in the impurity profile, which can arise from differences in synthesis routes and other manufacturing factors, poses a risk to drug safety and patient health[108].

The ICH Q3A/B guidelines primarily focus on quality aspects, setting limits on impurities based on their potential impact on product quality. On the other hand, ICH Q3C emphasizes safety considerations, establishing limits for specified and unspecified impurities to ensure patient safety [Table 5] [83, 109] .To determine if a particular impurity or a specific impurity profile is biologically safe at defined concentrations, it is necessary to qualify the impurity by collecting and analysing relevant data. When an impurity satisfies at least one of these criteria, we say that it is qualified. The levels and proposed acceptance criteria are within the range of what is found in human drug products approved by the FDA. Being a significant metabolite of the drug substance, they are also supported by scientific literature. Additionally, they have not been found to exceed the levels evaluated in comparative in vitro genotoxicity studies.

The ICH Q3A(R2) guidelines for medicinal substances and the ICH Q3B(R2) guidelines for medicinal products, as shown in Table 5, provide the qualification requirements. These cutoffs are derived from the highest recommended daily dosage of the medicinal compound or product.[44]

Establishment of safety-based impurity limits in medicinal products is crucial for ensuring their safety and effectiveness. These limits are

designed to control the presence of impurities, which could otherwise pose significant health risks to patients.[111]

Challenges in meeting regulatory requirements for impurity profiling

Pharmaceutical companies aiming to register their products globally face significant challenges due to varying regulatory requirements across various regions. In the context of pharmaceutical manufacturing, controlling elemental impurities such as lead is crucial to ensure the safety and efficacy of drug products. Lead, often used as a catalyst in drug synthesis, is classified as a human toxicant under the ICH Q3D guideline, which sets a permissible daily exposure (PDE) limit of 0.5 parts per million (ppm). However, different countries may adopt varying standards for allowable impurity levels. The case studies explore these challenges by comparing the registration process in two hypothetical regions:

Region 1 (Country A) and Region 2 (Country B). [112] Under ICH Q3D guidelines, lead is a class-1 elemental impurity, which is regarded as highly toxic. As specified in the guideline, 0.5 ppm is the maximum allowable PDE for lead. The lead concentration was controlled within 0.5 ppm by Country A based on ICH safety specifications. Country B's decision to tighten the specification limit for lead to 0.1 ppm, following its regional regulatory body's suggestion, is based on the rationale that lead offers no therapeutic benefits and poses significant health risks. In order to tighten the lead specification limit to 0.1 ppm by country B, as advised by its regional regulatory body, has necessitated the addition of a new step has been added in the API manufacturing process to fully scavenge lead. This added step, while crucial for ensuring compliance with the new safety standards, has led to increased manufacturing costs and extended production times.[112]

CONCLUSION

In conclusion, impurity profiling is a critical aspect of pharmaceutical development and manufacturing. Ensuring that impurities are monitored, controlled, and within regulatory limits is essential for producing safe, effective, and high-quality pharmaceutical products. The improved analytical technologies have tremendously enhanced the ability to identify impurities at very low concentrations. High-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), nuclear magnetic resonance (NMR) and a number of hyphenated methods (LC-MS/MS, GC-MS/MS, etc)[113] have greatly increased sensitivity, specificity, and accuracy of analysis of impurities. To regulate the amount of impurities in pharmaceutical products, regulatory agencies in the world, including the International Council for harmonisation (ICH), U.S. Food and Drug Administration (FDA), and European Medicines Agency (EMA), have put precise guidelines including ICH Q3A, Q3B, Q3C, Q3D, and ICH M7. These guidelines offer a standardized system of identifying and qualifying contaminants and risk evaluation using toxicological thresholds and exposure limits. In spite of the great improvements, there are still a number of challenges in the profiling of impurities. These are the identification of unknown impurities, absence of reference standards, co-elution in chromatographic methods and difference in impurity profile among manufacturing batches. Moreover, the growing frequency of the genotoxic and nitrosamine impurities has increased the necessity of more sensitive analytical techniques and the effective risk assessment strategies[30, 50]. The constant observation of impurities, as well as process optimization and high-quality control measures, is thus necessary to reduce possible risks of pharmaceutical impurities.

The further development of impurity profiling will be oriented on introducing high-resolution analytical methods, automation, data analysis through artificial intelligence, and green analytical chemistry methods. The introduction of Quality by Design (QbD) principles and sophisticated process analytical technologies (PAT) can also help to improve the capacity of predicting, monitoring, and controlling impurities in the pharmaceutical production[114]. All these developments will enhance quality assurance systems in the pharmaceutical industry and promote production of safer, effective, and high-quality medicines in the healthcare sector of the world.

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Authors' Contributions

Bhupendra Singh: Conceptualization, edited the final version of the manuscript; Archita Tiwari: Data collection, write the original version of the manuscript. Rajesh Sharma: Supervised the complete manuscript.

Conflict of Interest

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