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Impurity profiling in pharmaceuticals and its regulatory requirements for ensuring product quality and safety

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Abstract:

Impurities whether organic, inorganic, or residual are unavoidable components that may arise during the synthesis, formulation, or storage of active pharmaceutical ingredients (APIs) and finished products. Even trace levels of these unwanted substances can significantly influence a drug's efficacy, stability, and patient safety. Therefore, impurity profiling has become an integral part of modern pharmaceutical quality assessment, focusing on the identification, quantification, and control of impurities to ensure product reliability and regulatory compliance. Regulatory authorities across the globe, including the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), the United States Food and Drug Administration (USFDA), and the European Medicines Agency (EMA), have established specific guidelines that define impurity thresholds, analytical validation requirements, and reporting standards. These frameworks help pharmaceutical manufacturers maintain consistent product quality and mitigate toxicological risks. The present review provides an overview of the regulatory aspects governing impurity profiling, outlines current global guidelines, and discusses the analytical and toxicological considerations essential for developing safe and compliant pharmaceutical products.

Keywords: **dossier, drug product, impurity, impurity profiling, quality.**

Classification numbers: **2.2, 3.3, 3.5**

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1. Introduction

Impurity profiling is a critical component of pharmaceutical quality assurance, directly influencing drug safety, efficacy, and regulatory compliance. Impurities may arise during synthesis, formulation, storage, or packaging, and even trace levels can alter pharmacological response or introduce toxicological risk. Therefore, systematic identification, quantification, and control of impurities have become essential throughout the product lifecycle. With increasing analytical sensitivity, previously undetected trace contaminants are now routinely identified, shifting impurity control from a reactive quality check to a proactive risk management strategy [1, 2].

Global regulatory expectations for impurity control have evolved significantly over the past two decades. The International Council for Harmonisation (ICH) established foundational frameworks through: ICH Q3A (R2) - Impurities in New Drug Substances, ICH Q3B (R2) - Impurities in New Drug Products, ICH Q3C (R8) - Residual Solvents, ICH M7 (R2) - Assessment and Control of DNA Reactive (Mutagenic) Impurities.

These guidelines define reporting, identification, and qualification thresholds, toxicological assessment strategies, and acceptable intake limits. More recently, regulatory authorities such as USFDA and EMA have intensified scrutiny of high-risk impurities, particularly genotoxic impurities and nitrosamines, leading to product recalls and strengthened post-marketing surveillance [3-7].

Recent years have seen an increase in regulatory measures, product recalls, warning letters, and compliance reviews, reflecting not only the expansion of regulatory control but also the growing challenge of achieving unified international standards. Regulatory guidelines usually set limits, actions, and documentation requirements, which in practice are implemented in relation to institutional capacity, analytical frameworks, and risk-management policies. This gap between regulatory expectations and operational capabilities has become a problematic area in pharmaceutical governance. The available literature has mainly been dedicated to describing regulatory frameworks or summarising analytical procedures. However, comparative analyses of regulatory thresholds across various jurisdictions, the analytical constraints involved, and industry behavioural responses to compliance deviations remain limited. In addition, the current dynamic background of risk-based regulation, data-integrity demands, and global harmonisation initiatives calls for a more methodical and analytical discussion rather than a purely descriptive one. Moreover, the rapid development of analytical tools has enhanced detection capabilities, paving the way for identifying impurities and deviations at trace levels that were not previously noticed. Although this shift supports patient safety, it creates regulatory issues related to acceptable limits, toxicological relevance, and proportional response strategies. There is no widely

accepted global enforcement practice, which further increases the compliance burden, especially for multinational manufacturers that operate in different regulatory contexts [3-5].

Despite the availability of multiple review articles on impurity profiling, most existing literature primarily provides descriptive summaries of analytical techniques or reproduces regulatory provisions without comparative or critical evaluation. There remains a lack of structured analysis comparing impurity thresholds across jurisdictions, evaluating the regulatory decision logic behind reporting, identification, and qualification limits, and assessing how analytical sensitivity influences regulatory risk assessment. Furthermore, few reviews integrate real-world regulatory case studies, such as nitrosamine recalls, azido impurity findings, or cross-contamination events, with a discussion of the associated analytical and compliance implications. Fig. 1 depicts the lifecycle of impurity formation. This review uniquely integrates impurity lifecycle analysis with current ICH regulatory frameworks, including Q3A, Q3B, Q3C, Q3D, and M7. It provides a practical, application-oriented approach supported by root-cause analysis and real-case threshold examples, enhancing its relevance for pharmaceutical quality and regulatory science.

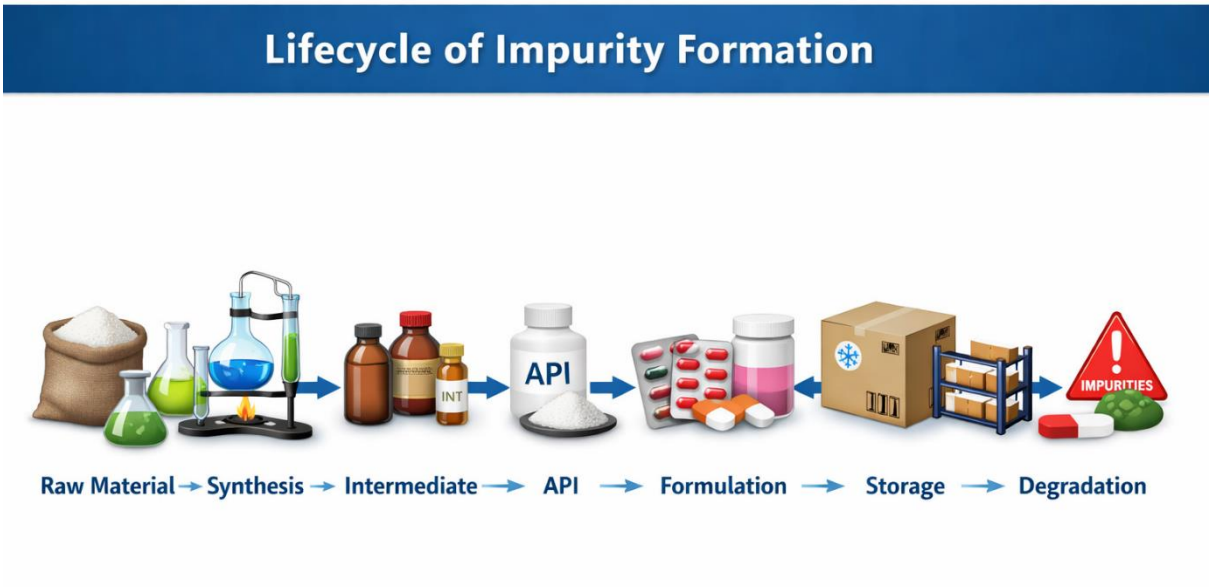

Fig. 1. Schematic of the impurity formation lifecycle.

Objectives of the review: The primary objective of this review is to provide a comprehensive overview of current knowledge related to impurity profiling in pharmaceuticals and its regulatory requirements. The review aims to summarise the available literature, analyse recent advances, and highlight key findings reported in previously published studies. In addition, the review seeks to identify existing research gaps and potential directions for future investigation. By integrating findings from

multiple studies, this work intends to provide a clear understanding of scientific developments and their potential applications.

2. Methodology

The present review was conducted through a comprehensive survey of the scientific literature available in major academic databases, including PubMed, ScienceDirect, Scopus, SpringerLink, and Google Scholar. Relevant articles published in peer-reviewed journals were identified using combinations of keywords related to the subject of the review. The selection of the literature was based on its relevance to the topic, scientific quality, and contribution to the understanding of the underlying mechanisms, applications, or developments within the field. Both original research articles and previously published review papers were considered to ensure broad coverage of the available information. The collected literature was carefully analysed and synthesised to provide a structured interpretation of current scientific knowledge. The review emphasises key findings, emerging trends, and potential challenges reported in the literature.

3. Regulatory and scientific overview of impurities

3.1. Impurity

Impurities are defined as any component present in a drug substance or drug product other than the intended active pharmaceutical ingredient (API) or excipients. These may arise from raw materials, manufacturing processes, degradation during storage, or interactions with excipients and packaging materials. Even at trace levels, certain impurities may affect product quality, stability, and patient safety, making their identification and control a critical aspect of pharmaceutical development [8-13].

According to ICH guidelines, impurities in pharmaceutical products are broadly classified into three major categories: organic impurities, inorganic impurities, and residual solvents, as described in ICH Q3A (R2) and Q3B (R2). Organic impurities include process-related impurities and drug-related degradation products. Inorganic impurities originate mainly from reagents, catalysts, elemental impurities, and inorganic salts used during synthesis. Residual solvents are volatile organic chemicals remaining from manufacturing processes and are regulated under ICH Q3C (R8).

In addition to these traditional categories, genotoxic impurities (GTIs), including nitrosamine impurities, have gained significant regulatory attention in recent years because of their potential carcinogenic risk. These impurities are addressed under ICH M7 and require stringent risk assessment, control strategies, and ultra-sensitive analytical detection. Impurities can be classified into three types:

A. Product-related impurities originating from chemical or biosynthetic processes.

B. Impurities developed as a result of spontaneous drug degradation during storage or when exposed to severe temperatures.

C. Substances that appear before the final product yet may remain as impurities.

Selective methods should be used for the detection and measurement of impurities above 0.1%. Spectroscopic methods are used to ascertain the possible structures of impurities, which may then be synthesised to provide conclusive evidence of their structures. Therefore, in order to alter the reaction conditions and reduce the quantity of contaminants to an acceptable level, it is necessary to understand the structure of these contaminants in the bulk medicament. The isolation, identification, and quantification of impurities are helpful in producing a pure chemical with reduced toxicity and safer pharmacological treatment. Pharmacological chemicals may be controlled and validated through the quantitative assessment of these pollutants [6].

3.2. Lifecycle of impurity

The lifecycle of impurities in pharmaceutical products is influenced by multiple interconnected factors. These include raw material quality, synthetic pathways, processing conditions, storage environment, packaging interactions, and manufacturing controls. To systematically represent these contributing factors, an Ishikawa (fishbone) diagram is presented (Fig. 2). This diagram highlights the root causes of impurity formation across the product lifecycle and aids in identifying critical control points for impurity mitigation.

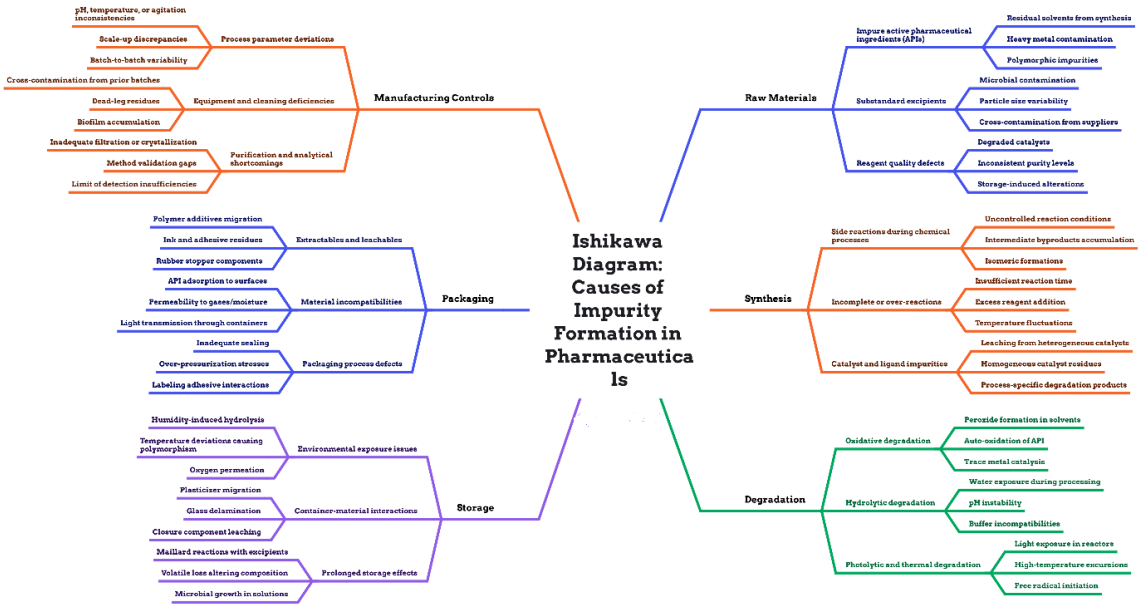

Fig. 2. An Ishikawa diagram of the lifecycle of impurity formation and root cause analysis.

3.3. Classification of impurities

Classification of impurities as per ICH Q3A/Q3B is as follows: Organic impurities (process and drug related); inorganic impurities (reagents, ligands, catalysts); residual solvents (volatile solvents) [7, 14-18].

3.3.1. Organic impurities

These types of impurities arise mainly during the manufacturing process and/or during storage of the drug substance.

These include the following impurities:

Starting materials or intermediates: If each stage of a multi-step synthesis is not executed with the utmost care, every API will contain impurities originating from the starting materials or intermediates. Even if solvents are used to wash the final product, there is still a possibility that some unreacted starting material may remain unless the manufacturers take extra precautions to avoid contamination [8, 19-21].

Degradation products: Impurities are created as the final products of bulk medication manufacture degrade. Synthesis, storage, dosage-form formulation, and ageing all contribute to the formation of degradation products. Impurities from breakdown products are often seen in antibiotics such as penicillin and cephalosporin [21-23].

By-products: Obtaining a single final product with a perfect yield is rather rare in synthetic organic chemistry. Potential by-products are always present. This is because they may be produced by a wide range of unintended events involving chemical reagents, catalysts, starting materials, or intermediates, including incomplete reactions, overreactions, isomerisation, dimerisation, rearrangement, and undesired side reactions. One possible by-product of bulk paracetamol manufacture is diacetylated paracetamol [23, 24].

3.3.2. Inorganic impurities

The procedures used to manufacture bulk medicines may also result in inorganic contaminants. Commonly recognised examples include the following [25].

Reagents, ligands, and catalysts: While the presence of these contaminants is very unlikely, it may pose a problem in some processes if manufacturers do not exercise sufficient care during manufacturing [26].

Elemental impurities (ICH Q3D): Elemental impurities arise from sources such as catalysts, raw materials, manufacturing equipment, and container-closure systems. According to ICH Q3D, these impurities are classified based on their toxicity and route of administration into Class 1 (high toxicity), Classes 2A and 2B (moderate toxicity),

and Class 3 (low toxicity). The guideline establishes Permitted Daily Exposure (PDE) limits for each element, considering oral, parenteral, and inhalation routes. Risk assessment-based approaches are recommended to evaluate the presence of elemental impurities, focusing on identifying sources, estimating exposure, and implementing appropriate control strategies. Compliance with ICH Q3D requires manufacturers to adopt a systematic risk-based approach, including supplier qualification, process optimisation, and routine monitoring, to ensure patient safety [27].

Other materials: Activated carbon is often used in conjunction with filters or filtering aids such as centrifuge bags during the manufacture of medicines in large quantities. It is critical to routinely check bulk drugs for black particles and fibres in order to avoid such contamination [28, 29].

3.4. Sources of impurities

The principal impurity types and their corresponding sources are summarised in Table 1. Specifically:

i. Beginning materials, by-products, intermediates, degradation products, reagents, ligands, catalysts, and organic impurities (drug- and process-related) may be volatile or non-volatile, identified or unknown.

ii. Reagents, ligands, catalysts, elemental impurities, inorganic salts, and other materials such as filter aids and charcoal are examples of inorganic contaminants.

iii. Liquids used to make solutions or suspensions during drug production may be inorganic or organic and may therefore contain miscellaneous impurities. Polymorphic forms, including enantiomeric impurities, are common [30].

Table 1. Types and their sources of impurity profiling [28, 29]**.**

No.	Impurity type	Impurity source
1	Process-related drug substance	Organic starting material intermediate by-products impurity in starting material
2	Process-related drug products	Organic or inorganic reagents, catalysts
3	Degradation of drug substance or drug product	Organic degradation products
4	Degradation drug product	Organic excipient interaction

3.5. Genotoxic and nitrosamine impurities

Genotoxic impurities are chemical entities capable of interacting with DNA and potentially inducing mutations, which may lead to carcinogenesis. Because of their high toxicological concern, regulatory agencies require their presence to be controlled at extremely low levels. Regulatory frameworks such as ICH M7 recommend a risk-based approach for the assessment and control of such impurities. This includes hazard identification using (Q)SAR tools, establishing acceptable intake limits, and

implementing control strategies such as process modification, impurity purge assessment, and analytical monitoring [31-33].

In addition to nitrosamines, other structurally alerting impurities, such as azido and hydrazine derivatives, require careful evaluation because of their genotoxic potential. Recent regulatory guidance emphasises the need for continuous monitoring and lifecycle management to ensure that impurity levels remain within acceptable limits [34].

Recent years have highlighted the regulatory significance of specific chemical impurities through multiple drug recalls worldwide. Notable examples include nitrosamine impurities such as N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), which led to recalls of ranitidine, valsartan, metformin, and related products. In addition, azido impurities detected in sartans and genotoxic sulfonate esters formed during alkylation reactions have raised serious safety concerns. Cross-contamination events, such as the detection of cetirizine-related impurities in ranitidine products, further underscore the complexity of impurity control in shared manufacturing facilities [35-38].

The detection and quantification of these impurities at trace levels have relied on advanced analytical techniques including LC-MS/MS, GC-MS, headspace GC for residual solvents, and NMR spectroscopy for structural confirmation. High-resolution mass spectrometry has been particularly valuable for non-targeted impurity screening and root-cause investigations. These real-world cases highlight the critical role of sensitive analytical tools and robust regulatory oversight in ensuring patient safety [8-13, 39, 40]. Table 2 describes selected nitrosamine-related impurities and actions by regulatory authorities.

Table 2. Selected nitrosamine-related recalls and regulatory actions [8-13, 39, 40]**.**

Drug/product	Impurity detected	Regulatory action	Key root cause
Valsartan	NDMA	Global recall (2018)	Solvent process modification
Ranitidine	NDMA	Market withdrawal	Degradation during storage
Metformin	NDMA	Batch recalls	Contaminated raw materials
Sartans (multiple)	NDEA	Import alerts	Nitrite contamination

3.6. Threshold of toxicological concern (TTC)

ICH M7 introduces the TTC concept, which establishes a generic acceptable intake limit for mutagenic impurities when compound-specific carcinogenicity data are unavailable. The default TTC value for lifetime exposure is 1.5 µg/day, corresponding to an estimated theoretical cancer risk of 1 in 100,000 individuals. Acceptable intake

limits may vary based on treatment duration and patient population. This risk-based framework enables scientifically justified impurity control while avoiding unnecessary toxicological studies [8-10, 39, 40].

3.7. Analytical method for identification of impurity

Since crude estimates of impurities are usually performed against the drug substance itself, which may be inaccurate, isolating and characterising impurities is essential for reliable monitoring. For these estimations, it is often assumed that the detector response will be the same because the impurities are structurally related to the target material. A variety of methods are useful for separating and identifying impurities.

3.7.1. Techniques for separation and characterisation of impurities

Through the integration of chromatographic, spectroscopic, and hyphenated methods, a comprehensive framework is developed for assessing the impurity profile of bulk pharmaceutical substances. Various chromatographic and spectroscopic methods are illustrated with numerous examples. These methods include thin-layer chromatography (TLC), gas chromatography (GC), analytical and preparative high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), high-performance thin-layer chromatography (HPTLC), mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), ultraviolet spectroscopy, and many more. The ease and rapidity of analysis made possible by hyphenated methods such as GC-MS, LC-MS/MS, LC-NMR, and LC-MS have made them very valuable [41-43].

High-performance liquid-chromatography (HPLC): HPLC is a commonly used chromatographic method for identifying and separating contaminants. Drug compounds are identified using retention time and direct comparison with existing standards. An appropriate column, an optimised system, and a suitable mobile-phase system are necessary for impurity isolation and identification using HPLC. The preparative mode of HPLC requires the use of specialised equipment. Among the several analytical methods for impurity separation, HPLC has received the most attention [44].

Gas chromatography (GC): Quantitation using GC is crucial. In terms of resolution, selectivity, and ease of quantitation, it can deliver what is required. The main restriction is that the sample must be inert or must undergo derivatisation to become volatile. Organic volatile contaminants benefit greatly from this method [45].

UV-Visible spectroscopy: UV-Vis spectroscopy is a modest but useful tool for identifying and understanding the structure of drug impurities without using chromatography. This method is useful only for impurities that absorb in the ultraviolet range above 200 nm. UV-Visible analysis is an effective method for identifying pure pharmaceutical substances. Chromophores are organic compounds that absorb light at a specific wavelength, which is proportional to the sample concentration [45, 46].

FT-IR spectroscopy: Infrared spectroscopy is a method for analysing contaminants that is not often employed. If the chemical structure of the impurities is known and they are detected above certain percentage limits in the material, FT-IR spectrometry may be used to determine whether they are present in raw pharmaceutical compounds. It has been reported that FT-IR spectroscopy may be used to examine impurities in statins such as atorvastatin and simvastatin [45, 46].

Capillary electrophoresis (CE): CE is a viable method for situations in which high resolution is needed but only small sample volumes are available. The main difficulty is the low level of repeatability [34, 35].

3.7.2. Modern trends in impurity profiling

Recent advances in pharmaceutical analysis have significantly enhanced impurity detection capabilities. High-resolution mass spectrometric techniques such as liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) and quadrupole time-of-flight mass spectrometry (QTOF-MS) have become powerful tools for impurity identification and structural elucidation. These methods offer superior mass accuracy, enabling the detection of unknown and low-level impurities.

Non-targeted impurity profiling has emerged as an important strategy, particularly during early development and stability studies, in which unknown degradation products may be present. When combined with chemometric and data-driven approaches, non-targeted analysis allows comprehensive impurity mapping without prior structural assumptions. These modern techniques support proactive impurity risk management and align with Quality by Design principles [45, 46]. Table 3 shows the comparative analysis of various analytical techniques and their efficiency in impurity profiling.

Table 3. Comparative summary of analytical techniques used in impurity profiling [13, 25, 35-38]**.**

Technique	Sensitivity	Applicability	Advantages	Limitations
HPLC	Moderate	Routine impurity quantification	Wide availability and reproducibility	Limited structural information
GC	High	Volatile impurities, residual solvents	High resolution, sensitivity	Requires volatility or derivatisation
LC-MS/MS	Very high	Trace and genotoxic impurities	Excellent sensitivity and selectivity	High cost, skilled operation
LC-HRMS/ QTOF-MS	Extremely high	Unknown and non-targeted impurities	Accurate mass, structural elucidation	Complex data interpretation
FT-IR	Low to moderate	Functional group identification	Rapid and non-destructive analysis	Limited sensitivity
NMR	Moderate	Structural confirmation	Definitive structural data	Requires higher impurity levels

4. Remedies to prevent impurities in pharmaceutical products

Impurity prevention cannot rely solely on end-product testing but requires integration of process control, validated analytical monitoring, and lifecycle risk assessment. In practice, implementation gaps arise due to differences in equipment capability, solvent recovery practices, water quality systems, and cleaning validation robustness. Regulatory inspections frequently identify deficiencies in cross-contamination control, residual solvent management, and impurity trending documentation. Therefore, impurity control must be embedded within process validation, change management, and quality risk management systems rather than treated as an isolated analytical responsibility [39, 47].

4.1. ICH guidelines for impurity profiling

Regulatory authorities are now giving this issue serious attention. A number of guidelines regarding residual solvents and contaminants in pharmacological substances and products have been released by the International Conference on Harmonisation.

A. Q3A (R2) Impurities in New Drug Substances.

B. Q3B (R2) Impurities in New Drug Products.

C. Q3C (R5) Impurities: Guidelines for Residual Solvent.

D. US-FDA “NDAs - Impurities in New Drug Substances”.

E. US-FDA “ANDAs - Impurities in New Drug Substances”.

According to the ICH guidelines for contaminants in new pharmaceutical goods, it is not required to detect pollutants below the 0.1% level unless the likely contaminants are expected to be generally hazardous or potent. Table 4 provides the impurity limits for pharmacological substances.

Table 4. Threshold limits of impurities as per regulatory guidelines [8, 9, 39, 40, 47]**.**

Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
≤2 g/day	0.05%	0.10% or 10 mg per day intake (whichever is lower)	0.15% or 10 mg per day intake (whichever is lower)
>2 g/day	0.03%	0.05%	0.005%

4.1.1. Q3A (R2) Impurities in New Drug Substances

The purpose of this document is to lay out the rules for submitting registration applications regarding the amount and type of contaminants in newly synthesised medicinal compounds that have not been registered in any member state or region

before. Novel pharmaceutical substances still in the early stages of clinical trials are not subject to this guideline. Herbal products, crude animal or plant products, fermentation products, radiopharmaceuticals, peptides, oligonucleotides, and biological or biotechnological pharmacological compounds are not covered by this guideline. Two approaches are used to address impurities in new pharmaceutical compounds. Topics covered in the chemistry section include analytical processes in brief, impurity listing in specifications, report generation, and impurity identification and categorisation. In the safety section, the process for identifying and evaluating contaminants that were either absent or present at significantly lower concentrations in the clinical and safety study batches of a novel medicinal compound is described in depth [39].

4.1.2. Q3B (R2) Impurities in New Drug Products

This document should be used as a guide for registration applications; it will help in understanding impurities in freshly produced pharmaceutical products created from chemically synthesised novel pharmacological compounds that have not been registered in a specific region or member state. This guideline supplements ICH Q3A(R2), 'Impurities in New Drug Substances', and should also be read together with the ICH Q3C guideline, 'Residual Solvents', where applicable [48-51].

The only contaminants addressed by this standard are those that occur as a result of drug breakdown, interaction with an excipient, or the container-closure system; these are referred to as 'degradation products' in this standard. Impurities in a new medicinal product should not be disclosed or tracked if they are not degradation products. The excipients of the new pharmaceutical product and any pollutants that may have leached or been removed from the container-closure system are not taken into account in this guideline. Furthermore, this guideline does not apply to innovative medicinal products used in clinical studies [48-51].

The following types of products are not covered in this guideline: Medicinal herbs, peptides, oligonucleotides, radiopharmaceuticals, fermentation by-products and semi-synthetic derivatives, herbal remedies, and unprocessed animal or plant substances are not covered by this guideline. Also excluded from this document are extraneous contaminants that should not occur in new drug products and are more appropriately addressed as good manufacturing practice issues, as well as polymorphic forms and enantiomeric impurities [40].

4.1.3. Q3C(R8) Impurities: Guideline for Residual Solvents

This proposal aims to determine what amounts of residual solvents in medications are acceptable in order to ensure patient safety. Recommendations for safer solvent alternatives and toxicologically acceptable amounts of various residual solvents are detailed in the publication. Organic volatile chemicals that remain after pharmaceutical

manufacturing processes are called residual solvents when medicinal components or excipients are made or used in the pharmaceutical industry, as well as when medicinal products are manufactured. Solvents are still used in some capacity in realistic manufacturing processes. Maximising yield while controlling properties such as crystal shape, purity, and solubility is crucial when synthesising pharmaceutical components. The correct solvent for the job is essential. Consequently, the solvent may at times play a crucial role in the synthesis process.

Therefore, the solvent may at times be pivotal to the synthesis procedure. The use of solvents as excipients or solvates is outside the scope of this regulation. However, it is important to evaluate and justify the presence of solvents in these commodities. Any residual solvents in pharmaceutical components, excipients, or finished pharmaceutical products are addressed in this standard. For this reason, it is important to carry out residual-solvent testing whenever it is known that a manufacturing or purification process generates these solvents. Solvent testing is required only for compounds that are either made or employed in the pharmaceutical industry, whether they are excipients, components, or final products. The residual solvent levels in the drug product can be calculated using a cumulative approach, which takes into account the levels in the materials used to make it. However, manufacturers still have the option to test the product directly. There is no requirement to test the medicinal product for residual solvents if the calculation produces a level that is equal to or lower than the one indicated in this guideline. However, if it is higher than what is suggested, it is important to test the medicinal product to see whether the formulation process reduced the solvent level to a safe level. It is also important to check whether any solvents were used to make the medication [8, 39, 40].

Classification of residual solvents is as follows:

Class 1 solvents: solvents to be avoided. These are known carcinogens, substances with a high probability of causing cancer in humans, and potential environmental hazards.

Class 2 solvents: solvents to be limited. These are substances that may cause neurotoxicity or teratogenicity but do not cause cancer in animals, although they may cause other types of irreversible toxicity. These solvents are also considered to have additional significant toxicities that may be reversible.

Class 3 solvents: potentially low-toxic solvents that pose little threat to human health; no exposure limit is necessary. The daily PDE for Class 3 solvents is 50 mg or higher [8].

4.1.4. US-FDA NDAs - Impurities in New Drug Substances

The identification, qualification, and reporting of impurities in non-new drug substances may be found in Q3A, 'Impurities in New Drug Substances', which applicants are advised to study according to this guidance (Table 5). Although Q3A was created by the International Conference on Harmonisation (ICH) to outline what information should be included in a new drug application (NDA) regarding impurities in chemically synthesised new drug substances, the Agency considers that the guidelines given there regarding the identification, qualification, and reporting of impurities should also be taken into account when assessing impurities in such substances [9]. Impurities in chemically synthesised therapeutic compounds should be reported, identified, and qualified in the following ways, according to new guidelines in this guidance on chemistry, manufacturing, and controls (CMC) information such as original abbreviated new drug applications (ANDAs), drug master files (DMFs), including type II DMFs, and ANDA supplements for changes in the synthesis or processing of a drug substance. The guidance also provides recommendations for establishing acceptance criteria for impurities in drug substances. Drug substances such as biological or biotechnological products, peptides, oligonucleotides, radiopharmaceuticals, fermentation products, semisynthetic products derived from fermentation products, and herbal products are not covered in this guidance [10].

Table 5. Comparative regulatory thresholds (ICH vs USFDA).

Parameter	ICH Q3A/Q3B	USFDA NDA guidance	Practical industry challenge
Reporting threshold	0.05-0.1%	Aligned with ICH	Detection sensitivity differences
Identification threshold	0.10%	Case-dependent	Structural elucidation cost
Qualification threshold	0.15%	Toxicology-based	Need for additional studies
Genotoxic impurities	ICH M7 (TTC-based)	Mandatory risk assessment	Ultra-trace detection requirement

4.2. Practical application of ICH guidelines

The implementation of ICH Q3A (drug substances), Q3B (drug products), and Q3C (residual solvents) can be illustrated using a representative oral drug product. According to these guidelines, impurities are controlled based on reporting, identification, and qualification thresholds, which depend on the maximum daily dose. For example, for a drug with a daily dose ≤2 g/day, the reporting threshold is typically 0.05%, the identification threshold is 0.10%, and the qualification threshold is 0.15%. Impurities exceeding these thresholds must be reported, structurally identified, and toxicologically qualified. For residual solvents (ICH Q3C), solvents are categorised into

Class 1 (to be avoided), Class 2 (to be limited), and Class 3 (low toxic potential), with specified concentration limits. Manufacturers must ensure that solvent levels remain within permissible limits using validated analytical methods. This framework ensures a consistent and scientifically justified approach to impurity control, supporting regulatory compliance and patient safety [37].

4.3. Future perspectives

Although significant progress has been made in establishing regulatory frameworks and analytical standards for impurity profiling, several challenges and opportunities remain for future research and industry practice. One key area for advancement lies in the development of more sensitive, rapid, and high-resolution analytical platforms capable of detecting ultra-trace-level impurities with minimal sample preparation. The integration of hyphenated techniques (such as LC-MS/MS, GC-MS, QTOF-MS, and NMR) with data-driven chemometric tools has the potential to enhance structural elucidation and impurity trend analysis. Another emerging direction is the implementation of Quality by Design (QbD) and real-time release testing (RTRT) to proactively control impurities throughout the product lifecycle rather than relying solely on end-product testing. The increasing use of continuous manufacturing also necessitates updated regulatory guidance for dynamic impurity-control strategies [25, 38, 52-54].

Furthermore, toxicological risk assessment models for novel, unidentified, or metabolite-related impurities require refinement to better predict long-term patient safety. The adoption of computational toxicology and artificial intelligence (AI) may support more reliable prediction of impurity hazards and reduce dependence on animal testing. Lastly, there is a need for global harmonisation of impurity reporting standards, as variations between regulatory regions can complicate dossier preparation and delay market approval. Strengthening collaborative regulatory frameworks will enhance consistency in drug quality evaluation and facilitate smoother international pharmaceutical distribution [48, 49, 53-56].

Future advancements in impurity profiling are expected to be driven by the integration of intelligent analytical systems and digital technologies. Automated, AI-assisted workflows combining high-resolution mass spectrometry and NMR data are likely to support rapid impurity detection and tentative structural assignment with reduced analyst intervention. In parallel, advanced process analytical technology tools, such as Raman and near-infrared spectroscopy coupled with chemometric models, may enable real-time monitoring of impurity formation during synthesis and manufacturing [48-51, 56, 57].

These developments will support a shift from retrospective impurity testing to predictive and preventive quality control, particularly in continuous manufacturing environments. However, their successful implementation will require appropriate regulatory frameworks and validation strategies [48-51, 56-59].

Beyond regulatory and analytical considerations, impurity profiling has important implications for clinical and pharmaceutical practice. The identification and control of impurities play a critical role in ensuring the safety, efficacy, and stability of pharmaceutical products used in patient care. Undetected or poorly characterised impurities may contribute to unexpected adverse reactions, reduced therapeutic efficacy, or long-term toxicity. Therefore, advanced impurity-detection strategies can assist clinicians and regulatory authorities in assessing potential safety risks associated with drug formulations. In clinical settings, improved impurity characterisation supports pharmacovigilance activities and helps healthcare professionals better understand drug-related adverse effects. Furthermore, the integration of advanced analytical technologies with pharmaceutical manufacturing processes may enable early detection of impurity formation during drug development, thereby facilitating safer formulation design and more reliable therapeutic outcomes. In addition, collaborative efforts between pharmaceutical scientists, clinicians, and regulatory agencies are essential to translate impurity-profiling research into practical applications. Future research should focus on linking impurity-characterisation data with clinical safety outcomes, which may help refine risk-assessment models and guide regulatory policies. Such interdisciplinary approaches will strengthen the bridge between pharmaceutical analysis and clinical practice, ultimately contributing to improved drug quality and enhanced patient safety [60-66].

5. Conclusions

Impurity profiling has evolved from a quality-control exercise into a multidisciplinary regulatory-science domain integrating analytical chemistry, toxicology, risk management, and compliance strategy. While ICH guidelines provide a harmonised framework, practical implementation remains influenced by analytical capability, manufacturing complexity, and regional regulatory interpretation. The increasing detection of ultra-trace impurities, particularly genotoxic and nitrosamine species, underscores the need for high-resolution analytical platforms and proactive lifecycle control. From an industrial perspective, proactive impurity control through quality by design, advanced HRMS platforms, AI-supported risk prediction, and lifecycle management is essential for sustainable compliance. Regulatory alignment and science-based flexibility will remain critical to balancing innovation with patient safety.

CRediT author statement

Moin Shaikh: Writing original draft; Kranti Satpute: Critical revision; Shoaeb Mohammad Syed: Conceptualisation & Methodology.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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