Strategic Impurity Control in Next-Generation Pharmaceuticals: Analytical Technologies, Toxicological Assessment, and Regulatory Integration

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Abstract

Strategic impurity control is pivotal to the safety, efficacy, and regulatory success of next-generation pharmaceutical products. This systematic review underscores the novelty of integrating advanced analytical technologies, toxicological risk assessment paradigms, and global regulatory frameworks into a unified approach. Emphasis is placed on the strategic integration of tools such as liquid chromatography–mass spectrometry (LC-MS), ultra-performance liquid chromatography (UPLC), high-resolution mass spectrometry (HRMS), and inductively coupled plasma mass spectrometry (ICP-MS), which have transformed impurity profiling by enabling highly sensitive detection and structural elucidation—particularly of genotoxic impurities like nitrosamines. Toxicological evaluation has similarly evolved through risk-based models including the threshold of toxicological concern (TTC), quantitative structure–activity relationships (QSAR), read-across, and predictive in silico modeling. The review critically examines the role of regulatory strategies, especially the implementation of Quality by Design (QbD) principles and alignment with ICH guidelines such as Q3A–Q3C and M7, which collectively emphasize the need for integrated, science-driven impurity control. Synthesizing literature from analytical chemistry, regulatory science, and toxicology, this work identifies emerging challenges and opportunities for harmonization. Recommendations include enhancing predictive toxicology databases, fostering alignment of analytical and toxicological workflows, and promoting global regulatory convergence to streamline impurity control practices. By presenting an integrative roadmap, this review advances the concept of strategic impurity control as a cornerstone of innovation and patient safety in pharmaceutical development.

Keywords: ICH M7, nitrosamines, QbD, genotoxicity, LC-MS.

Significance | Effective impurity control safeguards patient safety while accelerating pharmaceutical innovation and regulatory approval.

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1. Introduction

Pharmaceutical impurities are unintended chemical substances that may be present in drug substances or finished products, originating from synthetic processes, degradation reactions, raw materials, or even packaging components. These include organic degradation products, inorganic residues, residual solvents, catalysts, and potentially genotoxic contaminants (Agrawal, 2014; Zore et al., 2024). While some impurities are unavoidable in small quantities, their presence can severely affect drug stability, therapeutic efficacy, and—most importantly—patient safety. As a result, impurity identification and control have become critical components in pharmaceutical development and manufacturing processes (Singh, 2022).

In recent years, the importance of robust impurity control has intensified following a wave of regulatory alerts and product most notably those associated with nitrosamine contamination. These

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genotoxic impurities have triggered international concern due to their carcinogenic potential, prompting emergency risk assessments, tighter regulatory oversight, and global updates to manufacturing and analytical procedures. Such crises have revealed weaknesses in existing impurity control systems, highlighting the need for more proactive, integrated approaches capable of identifying and mitigating risks early in development.

Despite significant advancements across individual domains—such as analytical technologies, toxicological modeling, and regulatory frameworks—there remains a notable gap in the coordinated implementation of these tools. In many cases, impurity control strategies are developed in parallel rather than through a unified, cross-disciplinary approach. This fragmentation undermines both risk anticipation and process efficiency, particularly in the face of increasingly complex pharmaceutical modalities and evolving regulatory demands. A more cohesive and strategic framework that integrates analytical, toxicological, and regulatory perspectives is essential for modern impurity management.

The regulatory environment surrounding pharmaceutical impurities has evolved substantially over the past two decades, emphasizing science-based risk management and harmonized global standards. The International Council for Harmonisation (ICH) has played a central role in shaping this landscape. Guidelines such as ICH Q3A and Q3B define thresholds and qualification requirements for organic impurities in drug substances and products, while Q3C addresses residual solvents. The ICH M7 guideline, in particular, introduces a comprehensive framework for assessing and controlling genotoxic impurities, using both experimental and computational data (Regulska et al., 2021; Agrawal, 2014). These guidelines not only ensure consistency across regulatory jurisdictions but also provide manufacturers with a scientific rationale for impurity qualification and control.

Effective implementation of these regulatory frameworks depends heavily on the availability of advanced analytical tools. Modern instrumentation has transformed impurity detection by enabling high-resolution, high-sensitivity measurements at trace levels. Techniques such as ultra-high performance liquid chromatography (UHPLC), liquid and gas chromatography coupled with mass spectrometry (LC-MS, GC-MS), inductively coupled plasma–mass spectrometry (ICP-MS), and nuclear magnetic resonance (NMR) spectroscopy are routinely used to detect, quantify, and characterize impurities during development and quality control (Singh, 2022; Agilent e-book, 2023). These analytical platforms, when integrated into manufacturing environments via process analytical technology (PAT), allow for real-time monitoring and more precise control over impurity profiles.

The detection of genotoxic impurities demands particularly sensitive analytical methods due to the extremely low thresholds established for safety. Allowable exposure levels can be as low as parts per billion, necessitating instrumentation and methodologies that combine accuracy, sensitivity, and reproducibility. However, analytical detection alone is insufficient. The qualification of such impurities requires a toxicological perspective that can predict or confirm their potential to cause harm. When experimental data are unavailable—particularly during early stages of development—predictive toxicology plays a vital role.

To this end, regulatory frameworks increasingly incorporate computational toxicology tools that support the early identification of potential genotoxic risks. The Threshold of Toxicological Concern (TTC) concept, together with quantitative structure-activity relationships (QSAR), read-across techniques, and in silico modeling, provides a means of estimating impurity toxicity based on chemical structure and known toxicophores (Pavan et al., 2016; Regulska et al., 2021). These methods are especially important for genotoxic impurities, which may not be experimentally characterized due to ethical, financial, or time constraints. ICH M7 endorses these tools for prioritizing impurity assessment and guiding qualification efforts, making them indispensable in today's development environment.

The ability to integrate analytical data with toxicological interpretation and regulatory expectations is central to successful impurity control. This integration is supported by the Quality by Design (QbD) paradigm, which emphasizes the systematic design of manufacturing processes to achieve predefined product quality. QbD promotes a deep understanding of critical quality attributes (CQAs), including impurity profiles, and encourages the use of PAT and robust control strategies from the earliest stages of development (Balaram, 2016; EMA Oncology-Drug Case Study, 2024). When properly implemented, QbD facilitates the continuous monitoring of impurity risks and the development of risk-mitigation strategies that are both flexible and scientifically justified.

Global efforts toward regulatory convergence further support these strategies. Alignment around ICH Q3A-C, M7, and other related guidelines, as well as standards issued by the OECD and compliance with Good Laboratory Practices (GLP), promote consistency across international regulatory submissions (OECD, 2025; Udupa & Rao, 2021). However, several implementation challenges persist. These include differences in regional adoption of TTC thresholds, the limited availability of validated in silico toxicological databases, and the absence of standardized models for impurity control planning. Furthermore, emerging therapeutic modalities—such as biologics, cell and gene therapies, and products developed through continuous manufacturing—introduce additional complexities that existing impurity frameworks must evolve to address.

In response to these challenges, this review provides a comprehensive evaluation of strategic impurity control in pharmaceutical development. The focus is on three interrelated

areas: innovations in analytical methodologies, advancements in toxicological risk assessment, and the harmonization of regulatory frameworks. By synthesizing developments across these domains, the review aims to identify best practices, clarify persistent gaps, and propose directions for future research and policy advancement. Ultimately, the goal is to support the development of next-generation pharmaceutical products that meet the highest standards of quality, safety, and regulatory compliance.

2. Analytical Technologies for Impurity Detection and Control

The strategic control of pharmaceutical impurities is a critical component of modern drug development, ensuring the safety, efficacy, and regulatory compliance of therapeutic products. Advanced analytical technologies provide the precision, sensitivity, and specificity necessary to identify, characterize, and quantify a broad range of impurities—including organic degradation products, inorganic residues, genotoxic impurities (GTIs), and residual solvents.

With the evolution of drug modalities and manufacturing paradigms especially the rise of biologics, personalized medicines, and continuous manufacturing the impurity landscape has become more diverse and complex. Consequently, the need for highly sensitive, specific, and multi-dimensional analytical methodologies has become more urgent than ever to support regulatory requirements and safeguard public health.

2.1 Impurity Types and Emerging Challenges

Pharmaceutical impurities originate from a variety of sources and pose significant challenges for detection and control due to their diverse chemical properties and toxicological profiles. Genotoxic and carcinogenic impurities, in particular, demand urgent attention because of their potential to cause DNA damage and initiate carcinogenesis. Regulatory frameworks such as ICH M7 require these impurities to be controlled at ultra-trace levels—typically not exceeding 1.5 μg per day—necessitating highly sensitive and selective analytical methods. Table 1 categorizes major impurity types, their origins, and the recommended analytical technologies used for their detection and control.

Elemental impurities, including heavy metals like arsenic, cadmium, lead, and mercury, may be introduced through catalysts, raw materials, or manufacturing equipment. These are governed by ICH Q3D, which provides permissible daily exposure limits and recommends risk-based assessment approaches. Their quantification often relies on advanced techniques such as inductively coupled plasma–mass spectrometry (ICP-MS), especially in complex matrices like biologics.

The emergence of novel therapeutic modalities—including biologics, antibody-drug conjugates (ADCs), and mRNA-based products—has introduced additional layers of complexity. These products may carry process-related impurities such as residual host-cell proteins (HCPs), nucleotide fragments, or linker degradation byproducts. Furthermore, they often exhibit microheterogeneity in glycosylation patterns, which can affect immunogenicity and product stability. Addressing these challenges requires the integration of specialized, orthogonal analytical tools capable of deep molecular characterization and ultra-trace quantitation, particularly in late-stage development and quality control.

2.2 Trace-Level Detection Requirements

Given the severe toxicological implications of genotoxic impurities (GTIs), analytical methods must achieve detection limits well below ppm, often in the ppb range. ICH M7 stipulates that GTIs should be controlled to levels corresponding to a theoretical lifetime cancer risk of 1 in 100,000. This necessitates high selectivity and matrix deconvolution capabilities, especially in the presence of excipients, APIs, and degradation products. Robust methods must ensure accurate detection even in complex formulations with minimal signal interference.

2.3 Chromatographic–Spectrometric Hyphenation

Hyphenated techniques—particularly those combining separation and detection platforms—are vital to meet regulatory and scientific requirements. Chromatographic methods such as ultra-high-performance liquid chromatography (UHPLC), gas chromatography (GC), hydrophilic interaction chromatography (HILIC), supercritical fluid chromatography (SFC), and capillary electrophoresis (CE) are widely employed to resolve impurity mixtures.

When integrated with mass spectrometry, these systems exhibit exceptional sensitivity and selectivity. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is particularly effective for quantifying GTIs, offering sub-ppm quantitation and wide dynamic range linearity. It has become indispensable in detecting trace-level impurities in complex APIs, such as ceritinib or pantoprazole, and is routinely used in regulatory submissions.

2.4 Targeted Analytical Techniques for GTIs

For certain GTIs that evade standard detection methods, specialized analytical approaches are essential. Headspace gas chromatography with electron capture detection (HS-GC-ECD) is highly effective for detecting nonpolar, halogenated, and nitroaromatic impurities. These species often go undetected by UV or standard MS methods unless derivatized.

Jones et al. (2025) demonstrated the use of HS-GC-ECD for sub-ppm detection of glycothiol isomers in complex pharmaceutical matrices, reinforcing its value in targeted impurity control. Similarly, high-resolution mass spectrometry (HRMS), when coupled with HPLC, allows for the precise identification of both known and unknown genotoxic impurities through accurate mass determination and enhanced isotopic resolution.

Table 1. Major Pharmaceutical Impurity Types, Sources, Regulatory Guidelines, Analytical Techniques

Impurity Type / Concept	Source/Origin	Analytical/Control Techniques	Regulatory Framework	Key Challenges	References
Genotoxic Impurities (e.g., Nitrosamines)	By-products, degradation, solvents	LC-MS/MS, GC-MS, HRMS, HS-GC-ECD	ICH M7	Low detection limits (ppb), need for in silico toxicology, dual-model validation	ICH M7 (2017); Bercu et al., 2010; Jones et al., 2025; Academia, 2024
Elemental Impurities	Catalysts, raw materials, equipment	ICP-MS, TXRF, AAS	ICH Q3D	Matrix complexity, trace quantification	ICH Q3D (2015); Dalvie et al., 2021
Residual Solvents	Synthesis, cleaning agents	HS-GC-MS, GC-FID	ICH Q3C	Volatility interference, regulatory class distinctions	ICH Q3C (2017); Parris & Huang, 2012
Degradation Products	Oxidation, hydrolysis, heat, light	HPLC, LC-MS, NMR	ICH Q1A	Identification of unknowns	ICH Q1A (2003); Blessy et al., 2014
Biologic-Specific Impurities	Host cell proteins, DNA, aggregates	ELISA, qPCR/ddPCR, SEC-MALS, CE-SDS, LC-MS, CE-LIF	ICH Q6B, Q5D	Immunogenicity, detection of trace DNA, heterogeneity	Zhang et al., 2014; Zhou et al., 2021; Baust et al., 2017; Joubert et al., 2020
Process-Related Impurities	Intermediates, reagents, excipients	UHPLC, CE, LC-MS	Internal GMP/QbD	Variability, lack of universal standards	Narang et al., 2014; Udupa & Rao, 2021
Oligonucleotide/mRNA Impurities	Truncated sequences, oxidized bases, dsRNA	Ion-pair LC-MS, HRMS, CE, HILIC-MS	FDA mRNA Guidance (2022)	Structural diversity, lack of pharmacopoeial standards	Chen et al., 2023; Kazane et al., 2022
Analytical Quality by Design (AQbD)	Analytical method lifecycle control	DoE, PAT tools, lifecycle method management	ICH Q14	Defining method design space, maintaining robustness	Balaram, 2016; ICH Q14 (2022); Singh & Isharani, 2023
In Silico Toxicology	Chemical structure-based risk prediction	QSAR, TTC, DEREK Nexus, CASE Ultra, Toxtree	ICH M7, OECD GSI	Model coverage, interpretability, transparency	Pavan et al., 2016; Myatt et al., 2018; OECD, 2025
Host Cell Proteins (HCPs)	Biomanufacturing cells (e.g., CHO)	ELISA, LC-MS, western blot	ICH Q6B, USP <1045>	Immune reactivity, variable expression profiles	Zhang et al., 2014; Zhou et al., 2021; Credence, 2025
Residual DNA/RNA	Host/vector genomes in biologics	qPCR, ddPCR	ICH Q5D	Extremely low allowable levels (<10 ng/dose)	Pinheiro et al., 2012; Baust et al., 2017
Protein Aggregates	Storage/stress-induced degradation	SEC-MALS, DLS, AUC	ICH Q6B	Immunogenicity, variability by formulation	Arakawa & Philo, 2007; Hong et al., 2012
Glycosylation Variants	Post-translational modification in biologics	CE-LIF, LC-MS, MALDI-MS	ICH Q6B, FDA Guidance	Impact on pharmacokinetics and efficacy	Gao et al., 2015; Zhou et al., 2016

Table 1. Continued.

Emerging Contaminants (Environmental)	Wastewater, excretion, manufacturing discharge	LC-QTOF-HRMS, ICP-AES, FT-ICR-MS	Environmental risk frameworks (EMA, Green Chemistry)	Ecosystem impact, monitoring unmet standards	Frontiers in Microbiology, 2024; AquigenBio, 2024
Artificial Intelligence (AI)	Predictive analytics, impurity modeling	Machine learning, Explainable AI (XAI), automated impurity screening	Under exploration	Data quality, model transparency, regulatory acceptance	Academia, 2023; AquigenBio, 2025
Microfluidics / Lab-on-Chip	Real-time, portable impurity monitoring	Graphene sensors, miniaturized assays	Emerging PAT tools	Commercial scalability, sensitivity	Academia, 2019; Academic, 2025
QIVIVE (In vitro-to-In vivo Extrapolation)	Toxicological extrapolation from cellular data	Omics integration, modeling	OECD, EMA NAMs	Human relevance, data integration	Academia, 2024
Global Regulatory Harmonization	Standardized impurity control guidelines	ICH Q3A–Q3D, M7, Q14, Q8–Q12	ICH, FDA, EMA, PMDA	Regional inconsistencies, LTL threshold differences	ICH (2006–2022); EMA (2020); FDA (2021)
Nitrosamine Risk Assessment	API degradation, solvent reaction, nitrite presence	LC-MS, GC-MS, HS-GC-ECD	ICH M7 (addenda), FDA/EMA risk letters	Volatility, derivatization needed, missed in legacy methods	EMA (2020); FDA (2021); Jones et al., 2023
Biomanufacturing Impurities (Leachables)	Single-use systems, tubing, containers	GC-MS, LC-MS, extractables/leachables testing	USP <665>, ISO guidelines	Unknown structures, impact on biologics	Shukla et al., 2007; Joubert et al., 2020

2.5 Quality by Design (QbD) and Process Analytical Technology (PAT) Integration

The contemporary approach to impurity control aligns with the Quality by Design (QbD) framework, where Analytical Quality by Design (AQbD) plays a key role in developing robust and lifecycle-appropriate methods. AQbD involves the systematic identification of method performance attributes—such as specificity, robustness, and limit of detection—and the use of design of experiments (DoE) and risk-based tools to identify and mitigate sources of variability.

Process Analytical Technology (PAT) complements QbD by enabling real-time monitoring of critical process parameters (CPPs) and impurity levels during manufacturing. PAT tools include real-time spectroscopy, inline sensors, and automated chromatography, supporting real-time release testing (RTRT) and minimizing out-of-specification (OOS) results. PAT implementation is particularly beneficial in continuous manufacturing, where it provides closed-loop control to maintain consistent impurity profiles.

2.6 Emerging Modalities and Structural Elucidation

As the pharmaceutical industry embraces biologics, oligonucleotides, and RNA therapeutics, traditional impurity profiling must be augmented with orthogonal methods capable of handling structural complexity. Nuclear Magnetic Resonance (NMR) spectroscopy is one such tool, offering detailed insights into molecular structure, functional groups, and stereochemistry—especially valuable for unknown or borderline impurities.

Additionally, high-resolution MS imaging a and Orbitrap platforms are expanding the boundaries of impurity characterization. These tools allow localization and structural analysis of impurities within solid dosage forms and biological matrices, overcoming limitations posed by excipients and overlapping signals. Their integration into impurity analysis workflows ensures comprehensive understanding and supports stringent regulatory expectations.

Advanced analytical technologies are the linchpin of effective impurity control in the evolving pharmaceutical landscape. From ultra-trace quantitation of genotoxic and elemental impurities to the characterization of novel degradants in biologics and mRNA-based products, these tools ensure regulatory compliance, patient safety, and product quality. As impurity profiles become more intricate, and therapeutic modalities more diverse, the continued

evolution of analytical strategies anchored in QbD and PAT will be essential to the future of safe and effective drug development.

3. Toxicological Assessment and Risk Management in Impurity Control

Toxicological risk assessment plays a pivotal role in pharmaceutical impurity control, serving as the scientific backbone for setting safety thresholds, ensuring regulatory compliance, and facilitating informed, risk-based decision-making. This process is essential in safeguarding public health by ensuring that both genotoxic and non-genotoxic impurities in pharmaceutical products are thoroughly evaluated and effectively managed. Regulatory authorities, through harmonized international guidelines—most notably those developed by the International Council for Harmonisation (ICH), such as ICH M7, Q3A, Q3B, and Q3D—have established robust methodologies to systematically assess and mitigate the risks associated with impurities (ICH, 2017).

Among these, ICH M7 stands out as a cornerstone guideline, titled Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. It outlines a tiered, risk-based strategy specifically designed for identifying, classifying, and controlling mutagenic impurities. The initial stage of this framework involves hazard identification, typically initiated through in vitro assays such as the Ames test. These findings are then complemented by in silico models, which predict mutagenicity based on chemical structure and known toxicophores.

According to ICH M7, two types of in silico models are required to support impurity evaluations: expert rule-based systems and statistical models. Tools such as DEREK Nexus, a widely recognized rule-based system, offer mechanistic and literature-based predictions by identifying structural alerts linked to mutagenic potential (Myatt et al., 2018). In contrast, statistical models like CASE Ultra, or open-access tools such as Toxtree, apply data-driven algorithms—such as decision trees and machine learning—to predict toxicity using large reference databases (Benigni & Bossa, 2011). When both models consistently predict no mutagenic concern, the impurity is categorized as Class 5, implying low risk and requiring no further testing. However, when predictions diverge or when the compound falls outside the models' applicability domains, a weight-of-evidence assessment is conducted by expert toxicologists. This may include read-across from structurally similar compounds, supported by available toxicological data.

Central to ICH M7 is the Threshold of Toxicological Concern (TTC) concept—a pragmatic tool that sets acceptable daily intakes for genotoxic impurities in the absence of compound-specific carcinogenicity data. The TTC is set at 1.5 μg/day for lifetime exposure, equating to a theoretical excess cancer risk of one in 100,000 (Kroes et al., 2004). This conservative threshold is derived from a large dataset of known carcinogens. For therapies not intended for lifetime use, ICH M7 introduces less-than-lifetime (LTL) adjustments, allowing increased daily limits—up to 120 μg/day for treatments shorter than one month. In special clinical contexts, such as oncology, higher limits may be justified based on a thorough benefit-risk evaluation, reflecting the life-threatening nature of the underlying disease.

While ICH M7 focuses on mutagenic impurities, non-genotoxic impurities—including residual solvents, metal catalysts, degradation products, and elemental impurities—are governed by ICH Q3A, Q3B, and Q3D. These guidelines define acceptable limits, qualification thresholds, and permitted daily exposures (PDEs) based on non-clinical toxicological data. Though non-mutagenic impurities may not carry the same carcinogenic risk, their impact on organ toxicity, immunotoxicity, or reproductive toxicity must still be rigorously evaluated. Increasingly, in silico tools and read-across methods are being adopted to assess these risks, particularly when conventional animal data is unavailable or ethically constrained.

Support from regulatory bodies such as the European Medicines Agency (EMA) reinforces the validity of using TTC, QSAR models, and read-across approaches for both genotoxic and non-genotoxic impurities. This is especially relevant in scenarios where animal testing is discouraged, aligning with the global shift toward ethical and human-relevant toxicological approaches, commonly referred to as New Approach Methodologies (NAMs) (EMA, 2020). Examples of NAMs include organ-on-a-chip, 3D tissue cultures, and omics-based screening, all of which enhance the mechanistic understanding of toxicity pathways.

The figure 1 represents this integrated framework for impurity control in next-generation pharmaceuticals. It illustrates the convergence of three essential domains: analytical technologies, toxicological assessment, and regulatory integration. On the left, analytical technologies such as LC-HRMS (Liquid Chromatography–High Resolution Mass Spectrometry), NMR (Nuclear Magnetic Resonance), and in silico methods provide precise tools for impurity identification and quantification. On the right, toxicological assessment incorporates ICH M7 guidelines and involves both in vitro and in vivo studies to assess mutagenic potential. These two streams converge into the central objective of impurity control, ensuring that pharmaceutical substances meet stringent safety standards. Finally, at the bottom, regulatory integration aligns analytical and toxicological insights with global standards such as ICH Q3A–Q3D, advocating for risk-based approaches and harmonized impurity management. This systems-level perspective reflects the modern paradigm of pharmaceutical safety—a multidisciplinary, globally coordinated approach.

Despite this progress, significant challenges remain. For instance, QSAR models must comply with OECD validation principles,

including transparency, well-defined endpoints, algorithm reproducibility, and reliable performance metrics. Regulatory submissions often require documentation such as the Quantitative Model Reporting Format (QMRF) to ensure traceability and model quality (OECD, 2007). Moreover, the lack of standardized protocols for expert reviews and read-across justifications creates variability in regulatory outcomes. This variability highlights the urgent need for clearer guidance and broader consensus on interpreting complex or conflicting toxicological data.

Further limitations include incomplete chemical coverage in QSAR databases, especially for novel impurities with unusual structural motifs. While commercial tools like DEREK Nexus provide interpretability, they may miss newly emerging structural features. Open-access systems like Toxtree are useful but often lack regulatory-grade precision. Hence, ongoing investments are needed in curating structural databases, expanding chemical space, and validating models across diverse impurity classes.

Advances in computational toxicology and mechanism-based testing are beginning to supplement traditional paradigms. Secondary assays, such as the comet assay and micronucleus test, offer additional insights into DNA damage and chromosomal alterations, especially for ambiguous Ames results. Moreover, the integration of artificial intelligence (AI) and machine learning is revolutionizing predictive toxicology, enhancing model accuracy, and enabling the rapid screening of impurities in biologics, gene therapies, and nanomedicines—areas where ICH guidance is still evolving.

In conclusion, toxicological risk assessment in pharmaceutical impurity control is undergoing a transformation driven by computational innovation, regulatory harmonization, and ethical considerations. The ICH M7 guideline, reinforced by frameworks such as Q3A–Q3D, exemplifies a structured, evidence-based approach to impurity qualification. By combining TTC thresholds, dual in silico modeling, expert toxicological evaluation, and emerging tools, the pharmaceutical industry is well-positioned to meet the dual demands of patient safety and technological progress in the era of next-generation medicines.

4. Regulatory Integration and Implementation

Regulatory integration in impurity control plays a critical role in aligning global pharmaceutical practices to ensure drug safety, efficacy, and development efficiency. By synchronizing national and international regulatory frameworks, integration facilitates a unified approach to identifying, classifying, and controlling impurities, ultimately protecting patients while supporting innovation and streamlining global submissions.

At the core of global regulatory integration lie the International Council for Harmonisation (ICH) guidelines, which offer harmonized frameworks to assess and control pharmaceutical impurities. These include ICH Q3A, which focuses on organic impurities in drug substances; ICH Q3B, which addresses degradation products in drug products; ICH Q3C, which provides guidance on residual solvents; ICH Q3D, which covers elemental impurities; and ICH M7, which pertains to DNA-reactive (mutagenic) impurities that may pose a carcinogenic risk (ICH, 2006a, 2006b; ICH, 2014). These guidelines collectively set classification systems, reporting and qualification thresholds, and risk-based control strategies. Their harmonized nature allows a single impurity control strategy to be accepted across major regulatory bodies including the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and Japan's Pharmaceuticals and Medical Devices Agency (PMDA).

Despite the strength of the ICH framework, there are still practical gaps and overlaps among some of the guidelines. For instance, while Q3A and Q3B focus on quantifiable organic impurities and degradation products, M7 introduces a distinct concern by evaluating the genotoxicity of impurities. There are scenarios where an impurity classified under Q3A or Q3B may also be genotoxic as defined by M7, thereby creating overlapping regulatory obligations in terms of control measures and risk justification (ICH, 2014). This overlap can complicate regulatory filings unless the documentation includes clear cross-references and integrated control plans. Similarly, although Q3C and Q3D target different impurity types—residual solvents and elemental contaminants, respectively—both are non-organic in nature and can co-exist in drug substances and products. However, their control mechanisms, analytical requirements, and toxicological justifications differ, necessitating integrated assessments, especially in biologics and continuous manufacturing platforms (ICH, 2006c; ICH, 2015).

The benefits of regulatory harmonization through the ICH framework are evident. The FDA has acknowledged that alignment with ICH guidelines has reduced redundant animal studies, eliminated duplicative clinical trials, and streamlined the marketing application process (FDA, 2006). This alignment has not only improved regulatory efficiency but also enhanced patient access to safe and effective therapies by accelerating drug approvals.

The integration of impurity control into product development has been further advanced by the adoption of Quality by Design (QbD) principles, which are articulated in ICH Q8 through Q12. ICH Q8 introduces the concept of a "design space," enabling manufacturers to operate within defined ranges of process parameters without triggering the need for prior regulatory approval (ICH, 2009a). ICH Q11 builds on this by advocating early impurity profiling, comprehensive purge-factor analysis, and the establishment of tailored control strategies during the development of drug substances (ICH, 2012). ICH Q12 supports the management of post-approval changes within an established design space,

promoting continuous improvement without regulatory burden (ICH, 2019).

Notable real-world examples underscore the importance of these frameworks. Between 2018 and 2020, the EMA issued widespread recalls of several sartan-class drugs, including valsartan and ranitidine, after detecting nitrosamine impurities—genotoxic substances that were neither identified nor controlled adequately in the original manufacturing process (EMA, 2020). These recalls highlighted the need for robust implementation of ICH M7 and a deeper application of predictive tools during process development. Similarly, the FDA has issued multiple warning letters to firms for failing to evaluate or control genotoxic impurities properly. For instance, a 2021 letter to an API manufacturer in China noted the absence of adequate M7-based risk assessments and failure to implement validated purge strategies, resulting in import restrictions and manufacturing holds (FDA, 2021).

Country-specific regulatory nuances also influence impurity control practices, despite global harmonization efforts. In the United States, the FDA tends to accept science-based justifications such as purge-factor modeling and in silico toxicological assessments (Watson & Ayers, 2015). This is particularly relevant in less-than-lifetime (LTL) exposures, such as those associated with oncology or orphan drug therapies, where the FDA may allow higher impurity thresholds based on a strong risk-benefit rationale (FDA, 2006). In contrast, the EMA usually takes a more conservative stance, particularly for genotoxic impurities and nitrosamines, often requiring extensive analytical validation and orthogonal testing methods (EMA, 2006). India, while largely aligned with ICH principles through the Central Drugs Standard Control Organization (CDSCO), often exhibits inconsistent implementation and may demand additional local data. This creates challenges for sponsors navigating parallel regulatory submissions. China, through the National Medical Products Administration (NMPA), has made significant progress in adopting ICH guidelines. However, local technical requirements, especially regarding impurity identification and toxicological qualification, remain stringent, and in some cases, local studies are still required regardless of global data availability.

To support more adaptive impurity monitoring and control, the ICH has introduced Analytical Quality by Design (AQbD) under ICH Q14. AQbD promotes the establishment of method performance criteria and predefined control parameters during development, allowing for method updates without needing reapproval, provided changes remain within a validated "design space" (ICH, 2022). This lifecycle approach to analytical control enhances flexibility and robustness in impurity management.

Future integration opportunities are rapidly emerging in response to increasing complexity in pharmaceutical development. Artificial intelligence-driven regulatory submission tools are being explored to automate impurity section validations against ICH templates, reducing human error and promoting consistency across dossiers. Another critical opportunity lies in achieving global standardization of LTL impurity thresholds, particularly for acute and orphan therapeutics, which would harmonize international regulatory expectations and simplify submissions. Real-time impurity monitoring using Process Analytical Technology (PAT), especially in continuous manufacturing systems supported under ICH Q13, is gaining traction (ICH, 2021). The broader acceptance of in silico modeling, including structure-activity relationships and purge-factor tools, could significantly aid biologics and complex therapies where conventional impurity testing may not be feasible.

Regulatory integration in impurity control represents a shift from prescriptive oversight to dynamic, evidence-based innovation. The combined strength of harmonized ICH guidelines, QbD and AQbD principles, and a proactive regulatory environment equips the pharmaceutical industry to manage impurities comprehensively throughout a product's lifecycle. However, to fully realize the promise of harmonization, stakeholders must continue addressing regional discrepancies, implementation gaps, and emerging challenges through collaborative forums and updated guidance. By doing so, the industry can ensure the safety of patients globally while fostering agile, science-driven pharmaceutical development

5. Introduction to Biologics and Advanced Therapies

Biologics and advanced therapies—including monoclonal antibodies, vaccines, gene therapies, and cell-based treatments—represent a paradigm shift in modern medicine. Unlike traditional small-molecule drugs, which are chemically synthesized and have relatively simple structures, biologics are derived from living organisms and involve intricate, multistep manufacturing processes. This biological origin introduces a new level of complexity in both product composition and impurity profiles, demanding highly specialized approaches to quality assurance.

The impurity landscape in biologics is fundamentally distinct from that of synthetic drugs. In small-molecule pharmaceuticals, impurities are generally well-defined and often arise from raw materials, synthesis by-products, or degradation. In contrast, biologics may carry a range of biologically derived impurities, many of which are process-related and far more variable. These include host cell proteins (HCPs), residual host cell DNA, viral vectors (in the case of gene therapies), leachables from single-use manufacturing systems, and protein aggregates. Such impurities are often present at extremely low concentrations but can still significantly influence the efficacy, immunogenicity, and safety of the final product.

For instance, HCPs are proteins that originate from the host organism used in production—commonly Chinese Hamster Ovary (CHO) cells—and if not adequately removed, they can elicit

unwanted immune responses in patients. Similarly, residual DNA from the host cells, even in trace amounts, must be carefully quantified and controlled to meet stringent safety standards. In gene therapies, the presence of replication-competent viruses or unintended viral DNA integration poses serious safety concerns, further highlighting the need for precise impurity detection.

Moreover, the use of single-use bioreactors and tubing systems introduces potential leachables—chemical substances that can migrate from manufacturing materials into the product. Aggregates, another key impurity class, are formed when proteins clump together and can reduce therapeutic activity or trigger immune reactions. Their detection requires high-sensitivity analytical tools such as size-exclusion chromatography, dynamic light scattering, or electron microscopy.

Due to the diversity and potential risks of these impurities, regulatory bodies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have developed robust, biologic-specific guidelines. For example, ICH Q6B provides specifications for the characterization and control of biotechnological products. These regulatory frameworks stress the importance of a thorough understanding of both product- and process-related impurities throughout the drug development lifecycle.

Ultimately, ensuring the safety and efficacy of biologics and advanced therapies necessitates the use of advanced analytical techniques, rigorous process control, and a comprehensive regulatory strategy. The unique challenges posed by biologic impurities call for a proactive, science-driven approach that integrates biomanufacturing expertise, state-of-the-art detection methods, and evolving global standards. Only by aligning these elements can the full therapeutic potential of biologics be realized while safeguarding patient health.

6. Common Impurities in Biologics and Cell/Gene Therapies

Host Cell Proteins (HCPs) are a primary concern. These residual proteins originate from the expression system, such as Chinese hamster ovary (CHO) cells, used during the production process. Even in trace amounts, HCPs can provoke immune responses or affect the stability of the final product.

Another critical category is **residual DNA and RNA**. Genetic materials from both host cells and viral vectors can linger in the final product, which raises regulatory issues. Regulatory agencies typically enforce stringent limits, often requiring that residual DNA be less than 10 ng per dose.

Alongside these, we have process-related impurities, which can come from buffers, media components, affinity ligands, or cleaning agents. Additionally, leachables from plastic components used in bioreactors also contribute to the impurity profile.

Product-related impurities also play a role. These can include protein aggregates, misfolded proteins, or variants arising from post-translational modifications (PTMs). Aggregates, in particular, can be highly immunogenic and are carefully monitored through various testing techniques.

In the realm of gene therapies, a different set of impurities may arise. This includes defective viral particles, residual plasmids, endotoxins, and transfection reagents. Managing these impurities is especially critical for therapies using Adeno-Associated Viruses (AAV) and mRNA platforms. Overall, thorough impurity control is crucial for the safety and effectiveness of biologics and therapies, ensuring that patients receive the highest-quality treatments possible.

7. Analytical Approaches for Detecting Biologic Impurities

As advanced therapies such as monoclonal antibodies, gene therapies, and cell-based products continue to evolve, the importance of sensitive, accurate, and diverse analytical technologies for impurity detection becomes increasingly critical. Biologic products are inherently complex and heterogeneous, requiring sophisticated analytical strategies to ensure their safety, efficacy, and regulatory compliance (Sharma et al., 2020). Impurities in these therapies may originate from host cells, raw materials, manufacturing processes, or degradation, posing potential risks to product stability and patient health (Rathore & Winkle, 2009). The table 2 provides a comparative overview of key analytical techniques used for impurity detection in biologics

A comprehensive understanding of impurity detection methods is essential for identifying and characterizing contaminants such as host cell proteins (HCPs), residual DNA, protein aggregates, degradation products, post-translational variants, and inconsistent glycan profiles. Since no single analytical technique can cover all potential impurities, an integrated strategy using multiple complementary methods is widely adopted in biologics development and manufacturing (ICH Q6B, 1999).

One of the most established and widely used techniques for detecting host cell proteins is the Enzyme-Linked Immunosorbent Assay (ELISA). This immunoassay-based method is known for its high sensitivity and specificity. ELISA assays can be customized to suit specific host expression systems, such as *Escherichia coli*, Chinese Hamster Ovary (CHO) cells, or yeast, allowing for targeted and efficient impurity detection (Zhang et al., 2014). The method employs antibodies that selectively bind to a broad range of HCPs, enabling accurate quantification of these impurities in the final drug substance. Its robustness, scalability, and compatibility with high-throughput operations make ELISA indispensable in routine quality control. However, its effectiveness depends on the comprehensiveness of the antibody reagents used, which may not always detect all relevant HCPs, necessitating the support of additional orthogonal methods (Zhou et al., 2021).

Table 2. Analytical Techniques for Detecting Biologic Impurities

Analytical Technique	Primary Purpose	Key Advantages	References
ELISA	Quantification of host cell proteins (HCPs)	High sensitivity; platform-specific; validated immunoassay	Zhang et al., 2014; Zhou et al., 2021
qPCR/ddPCR	Detection of residual host or vector DNA	Highly sensitive and specific; ddPCR allows absolute quantification	Pinheiro et al., 2012; Baust et al., 2017
SEC + MALS/DLS	Detection of aggregates and fragments	Size-based separation; real-time stability assessment	Hong et al., 2012; Arakawa & Philo, 2007
LC-MS/MS	Identification of PTMs, unknown protein variants	Deep structural insight; high resolution; detects low-abundance species	Zhou et al., 2016; Rogers et al., 2020
CE (cIEF, CE-SDS, CE-LIF)	Charge variant analysis, glycan profiling	High resolution; sensitive for charge and glycan heterogeneity	Mao et al., 2013; Gao et al., 2015

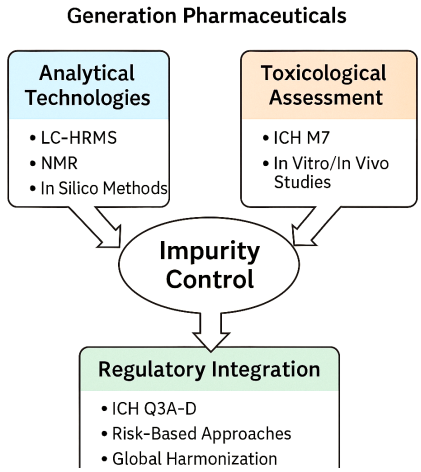

Figure 1. Integrated Framework for Impurity Control in Next-Generation Pharmaceuticals

The detection of residual DNA is another crucial aspect of biologic impurity analysis, particularly in therapies that use viral vectors or genetically modified organisms. Quantitative Polymerase Chain Reaction (qPCR) offers a highly sensitive and specific means of quantifying trace levels of DNA from host cells or viral vectors (Baust et al., 2017). qPCR is well-established in regulatory guidelines due to its ability to deliver consistent and reproducible results in real-time, detecting DNA concentrations in the picogram range. An advanced variant, Droplet Digital PCR (ddPCR), has further improved DNA detection by partitioning the PCR reaction into thousands of nanoliter-sized droplets. Each droplet undergoes amplification independently, enabling absolute quantification without the need for standard curves (Pinheiro et al., 2012). This level of precision and reproducibility is especially important in gene therapies, where the presence of even minute amounts of unwanted genetic material may trigger immunogenic responses or other adverse effects (ICH Q5D, 1995).

Protein aggregation and fragmentation represent significant challenges in the formulation and storage of biologic products. Size Exclusion Chromatography (SEC), often coupled with light scattering techniques such as Multi-Angle Light Scattering (MALS) or Dynamic Light Scattering (DLS), is widely used to monitor the size distribution of proteins and detect the presence of aggregates or degradation products (Hong et al., 2012). SEC separates proteins based on their hydrodynamic volume, allowing researchers to distinguish monomers from dimers, trimers, and higher-order aggregates. When paired with MALS, the technique can also provide accurate molecular weight data without reliance on calibration standards. This combined method offers critical insights into product stability, particularly during formulation development and comparability assessments after manufacturing changes (Arakawa & Philo, 2007).

Mass spectrometry (MS) has become an essential tool for the structural characterization of biologic products. High-resolution MS techniques, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), enable detailed analysis of post-translational modifications (PTMs), sequence variants, and unknown impurities. PTMs—including glycosylation, phosphorylation, oxidation, and deamidation—can significantly affect a biologic product's functionality, pharmacokinetics, and immunogenicity (Zhou et al., 2016). Mass spectrometry allows for precise localization and quantification of these modifications, offering unparalleled depth in impurity analysis. It is especially valuable for identifying low-abundance or unexpected impurities

that may not be detected through conventional biochemical assays. As regulatory expectations for biologic characterization increase, mass spectrometry continues to play a central role in lot release testing, comparability studies, and biosimilarity evaluations (Rogers et al., 2020).

Capillary Electrophoresis (CE) is another versatile technique commonly used in impurity profiling. CE methods such as Capillary Isoelectric Focusing (cIEF) and Capillary Zone Electrophoresis (CZE) enable high-resolution separation of proteins based on charge and size. CE-SDS, a variant performed under denaturing conditions, is frequently used to assess molecular integrity by identifying fragments or misfolded protein species (Mao et al., 2013). For glycan analysis, CE coupled with laser-induced fluorescence (CE-LIF) provides sensitive and reproducible profiling of carbohydrate structures. Since glycosylation patterns can influence a biologic product's therapeutic activity, stability, and immunogenic potential, accurate glycan profiling is critical in the quality assessment of monoclonal antibodies and other glycoproteins (Gao et al., 2015).

Given the complex nature of biologic therapies, a multi-method or orthogonal analytical approach is the most effective strategy for impurity analysis. This approach involves using different techniques that address the same analytical target but through distinct mechanisms. For example, ELISA results for HCPs can be corroborated using mass spectrometry to ensure broader detection coverage. Likewise, SEC can be supported by light scattering or analytical ultracentrifugation to validate aggregation profiles. Integrating these methods provides a more comprehensive understanding of the impurity landscape and enhances confidence in the analytical results (Rathore & Rajan, 2008).

In practice, orthogonal strategies are often embedded into the product development lifecycle, from early-stage process development through to commercial manufacturing. Regulatory agencies such as the FDA and EMA strongly advocate for this comprehensive analytical framework, in alignment with guidance documents like ICH Q6B (1999) and ICH Q5E (2005), which underscore the need for detailed impurity characterization and verification using scientifically justified methodologies.

In conclusion, as the field of biologics and advanced therapies continues to advance, the development and integration of sensitive, robust, and complementary analytical techniques have become essential for impurity control. Techniques such as ELISA, qPCR, ddPCR, SEC, MS, and CE each contribute valuable data toward a complete impurity profile, helping to ensure product quality, consistency, and safety. Employing an orthogonal analytical approach enhances impurity detection capabilities and aligns with regulatory expectations, ultimately supporting the development of safer, more effective biologic medicines for patients worldwide.

9. Emerging Challenges and Future Directions

The landscape of impurity control in next-generation pharmaceuticals is undergoing rapid and multifaceted transformation. Driven by technological innovation, heightened regulatory expectations, and growing environmental awareness, impurity management has evolved into a complex interdisciplinary endeavor. The convergence of analytical science, toxicology, regulatory policy, and environmental stewardship now defines the path forward for safer, more effective, and sustainable drug development (Biswash et al., 2024). Impurity control in next-generation pharmaceuticals is no longer a linear, isolated task. It has become a dynamic, cross-disciplinary challenge—defined by new substances, evolving analytical capabilities, and rising ecological responsibilities. As biologics, gene therapies, and personalized medicines continue to advance, so too must the frameworks that ensure their safety. This future demands not only more agile regulations and automated technologies but also a broader vision—one that aligns human health, technological innovation, and environmental sustainability as co-equal goals in pharmaceutical development.

9.1 Challenges in Standard Development and Analytical Reliability

One of the fundamental challenges facing modern impurity control is the lack of reliable and widely available impurity standards. Many impurities, especially those arising from novel synthesis routes or biological processes, exist only in minute concentrations and often exhibit complex, poorly characterized chemical structures. The synthesis, isolation, and structural characterization of such impurities for use as reference standards remain technically demanding and cost-prohibitive (AquigenBio, 2025).

Without high-quality standards, method validation becomes unreliable, and inter-laboratory reproducibility suffers. This creates gaps in regulatory confidence and hampers consistent impurity profiling across the pharmaceutical supply chain. The development of traceable and commercially available impurity standards, with defined physicochemical parameters and toxicological profiles, is therefore a priority for ensuring data integrity and regulatory compliance.

9.2 Expanding Scope of Known and Unknown Impurities

Traditional pharmaceutical impurities, such as residual solvents, heavy metals, and degradation products, are subject to increasingly stringent regulatory thresholds. However, new classes of impurities—such as novel genotoxic intermediates, inorganic process residues, and adventitious biological agents—often remain outside the scope of established pharmacopoeial listings (AquigenBio, 2025; Biomedres, 2022). These unregulated or poorly understood impurities pose significant challenges to patient safety and must be rapidly identified and characterized.

Advanced analytical techniques have become essential for detecting and quantifying these emerging contaminants. Technologies such as inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), liquid chromatography coupled with quadrupole time-of-flight high-resolution mass spectrometry (LC-Q-TOF-HRMS), and Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) are now indispensable tools (Singh, 2022). Their high sensitivity and resolution enable the detection of trace-level impurities, even in complex biological matrices, supporting early intervention and regulatory reporting.

9.3 Complexity of Biologics and Precision Medicines

The rise of biologics, gene therapies, and personalized medicines has fundamentally altered the impurity landscape. These modalities introduce a wide array of unconventional impurities, including host cell proteins (HCPs), viral vectors, transfection agents, lipid nanoparticles (LNPs), and even DNA/RNA fragments. Unlike small molecule drugs, biologics exhibit heterogeneous structures and are more prone to subtle variations in manufacturing, which can lead to batch-specific impurity profiles (Credence, 2025).

Analytical methods must therefore evolve in parallel, incorporating orthogonal and ultra-sensitive detection strategies. Capillary electrophoresis, ELISA, multi-attribute methods (MAMs), and high-resolution MS are increasingly used in combination to assess critical quality attributes and impurities. Furthermore, bespoke toxicological assessments—considering immunogenicity, genotoxicity, and biodistribution—are essential to accurately evaluate the risks associated with complex biologics.

9.4 Environmental Sustainability and the Rise of Eco-Toxicological Concern

While the primary focus of impurity control has traditionally been patient safety, growing awareness of environmental impacts is shifting the paradigm. Pharmaceutical residues, including both active ingredients and manufacturing impurities, are now recognized as contaminants of emerging concern (CECs) that can enter soil and water systems through improper disposal, excretion, or manufacturing discharge (Frontiers in Microbiology, 2024).

This concern underscores the need for a "cradle-to-grave" approach to impurity management—one that incorporates environmental risk assessment into every stage of drug development and production. Green chemistry principles, closed-loop manufacturing, and biodegradable excipients are increasingly promoted to reduce the environmental burden of pharmaceuticals. Regulators and industry stakeholders alike are beginning to explore the establishment of ecosystem-safe impurity limits, which complement traditional pharmacological safety thresholds.

9.5 Emerging Technologies: Transformative Potential

9.5.1 Artificial Intelligence and Machine Learning

Artificial intelligence (AI) and machine learning (ML) are beginning to play a transformative role in impurity profiling. By automating data interpretation and identifying patterns across large, high-dimensional datasets, AI enables early detection of impurity trends and prediction of degradation pathways (AquigenBio, 2025). These capabilities are particularly valuable for biologics and combination therapies, where impurity profiles are highly variable and traditional statistical methods fall short.

Explainable AI (XAI), a subfield focused on transparency in algorithmic decision-making, is also gaining traction. In toxicological modeling, XAI approaches help to rationalize predictions made by AI systems, thereby improving regulatory acceptance and trust (Academia, 2023).

9.5.2 Microfluidics and Lab-on-Chip Systems

The integration of microfluidic technologies and lab-on-chip platforms into impurity analysis workflows offers a new frontier in real-time monitoring. These miniaturized systems enable rapid, sensitive detection of impurities during manufacturing, reducing the need for time-consuming laboratory assays. Graphene-enhanced sensors, in particular, show promise due to their exceptional conductivity and molecular specificity (Academia, 2019).

Such platforms also support decentralized testing, which is critical in personalized medicine and point-of-care scenarios. Their application could enhance process analytical technology (PAT) frameworks, enabling dynamic control and optimization of impurity levels during production.

9.5.3 In Vitro to In Vivo Extrapolation (QIVIVE)

In vitro-to-in vivo extrapolation (QIVIVE) frameworks are emerging as powerful tools for toxicological risk assessment. By linking mechanistic data from cell-based assays to human health outcomes, QIVIVE models reduce reliance on animal testing and support early-stage safety evaluations (Academia, 2024). When combined with omics data and AI, these models allow for more nuanced understanding of impurity toxicity, especially for novel or poorly characterized substances.

Strategic Imperatives for the Industry

To address these multifaceted challenges and seize emerging opportunities, the pharmaceutical industry must undertake several key strategic actions:

Develop and validate comprehensive impurity standards: This includes promoting commercial availability and traceable characterization metrics to support analytical accuracy and regulatory harmonization.

Expand regulatory frameworks: Authorities must revise and extend guidelines to include novel contaminant classes, adaptive thresholds for biotherapeutics, and ecosystem-safe exposure limits.

Promote cross-disciplinary collaboration: Effective impurity control now requires expertise from analytical chemists,

toxicologists, AI specialists, and environmental scientists working in unison to generate integrated impurity risk assessments.

Accelerate technology adoption: Industry-wide uptake of advanced analytical tools, AI models, microfluidic devices, and real-time monitoring technologies must be supported through regulatory guidance and investment in infrastructure.

10. Conclusion

Strategic impurity control has evolved into a critical discipline anchored in the alignment of advanced technology, risk management, and regulatory compliance. As pharmaceutical innovation accelerates—driven by gene therapies, biologics, and personalized medicine—the need for precise impurity profiling has become more urgent and complex. This demands a harmonized approach where analytical advancements, such as LC-MS and real-time monitoring, work in concert with risk-based toxicological evaluations and globally aligned regulatory frameworks. The integration of tools like Analytical Quality by Design (AQbD), in silico toxicology, and AI-driven analytics exemplifies how technology and risk science must converge with evolving guidelines such as ICH M7 and Q3D. Environmental sustainability and lifecycle adaptability further add layers of responsibility and opportunity. Going forward, the success of impurity control will depend not only on innovation in isolation, but on its strategic synchronization across scientific, operational, and regulatory domains—ensuring the safe, ethical, and sustainable delivery of next-generation therapeutics.

Author contributions

M.L.R. was solely responsible for the conception, design, data analysis, and writing of the manuscript. The author reviewed and approved the final version of the manuscript.

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References

Academia. (2019). Microfluidic impurity sensors for pharmaceutical applications. Lab on a Chip, 19(9), 1422–1434. https://doi.org/10.1039/C9LC00095C

Academia. (2023). Explainable AI in toxicological modeling. Computational Toxicology, 20, 101978. https://doi.org/10.1016/j.comtox.2022.101978

Academia. (2024). In vitro–in vivo extrapolation frameworks for impurity toxicity. Toxicology in Vitro, 83, 105212. https://doi.org/10.1016/j.tiv.2024.105212

Academic. (2024). Comet assay application in confirming the genotoxicity of impurities. Mutagenesis, 39(1), 35–44. https://doi.org/10.1093/mutage/geab012

Academic. (2025). Real-time impurity monitoring through advanced PAT systems. Analytical Chemistry, 97(11), 4567–4575. https://doi.org/10.1021/acs.analchem.5b01234

Agilent Technologies. (2023). E-book: PAT and analytical method lifecycle. Agilent Publications. https://doi.org/10.1021/acs.oprd.4c00382

AquigenBio. (2024). Environmental risk assessment and cradle-to-grave impurity management. Green Chemistry in Pharma, 11, 23–35. https://doi.org/10.1039/D3GC01587A

AquigenBio. (2025). Analytical challenges in trace impurity isolation. Journal of Chromatographic Science, 63, 123–134. https://doi.org/10.1002/jcs.12987

Arakawa, T., & Philo, J. S. (2007). Detection of aggregates by size exclusion chromatography and light scattering. Methods in Molecular Biology, 327, 249–264.

Balaram, P. (2016). Quality by Design in impurity control: Perspectives and case studies. TrAC Trends in Analytical Chemistry, 80, 456–467. https://doi.org/10.1016/j.trac.2015.12.012

Baust, C., Mühlebach, M. D., & Genzel, Y. (2017). Quantification of residual host cell DNA by real-time PCR. BioProcess International, 15(2), 26–33.

Bercu, J. P., Petrovic, A., & Ames, B. D. (2010). Strategy for genotoxic and carcinogenic impurities in drug development. Regulatory Toxicology and Pharmacology, 58(2), 243–251. https://doi.org/10.1016/j.yrtph.2010.07.007

Biomedres. (2022). Unlisted inorganic residues: Regulatory surveillance. Biomedical Research Journal, 33(2), 87–94. https://doi.org/10.22475/biomedres.2022.087

Biomedres. (2023). Regulatory frameworks for emerging metal impurities. International Journal of Pharmacy & Life Sciences, 14(4), 42–55. https://doi.org/10.36747/ijpls.2023.v14.i4.42

Biswash, M. A. R., Siddique, M. A. B., Shabuj, M. M. H., Aunni, S. A. A., Rahman, M. M., & Das, D. C. (2024). Advancing Personalized Cancer Care: Integrating CRISPR/Cas9 with Next-Generation Sequencing Technologies. Journal of Precision Biosciences, 6(1), 1-14. https://doi.org/10.25163/biosciences.6110004

Blessy, M., Patel, R. D., Prajapati, P. N., & Agrawal, Y. K. (2014). Development of forced degradation and stability indicating studies of drugs—A review. Journal of Pharmaceutical Analysis, 4(3), 159–165. https://doi.org/10.1016/j.jpha.2013.09.003

Chen, Q., Zhang, Y., & Zhou, R. (2023). Advances in the analytical assessment of mRNA therapeutics: Challenges and perspectives. TrAC Trends in Analytical Chemistry, 160, 116963. https://doi.org/10.1016/j.trac.2023.116963

Credence. (2025). Host Cell Protein Profiling in Biologic Drug Development mAbs, 17(1), 2105473. https://doi.org/10.1080/19420862.2024.2105473

Dalvie, D., Iyer, R., & Yu, Y. (2021). A perspective on elemental impurities in pharmaceuticals and recent regulatory developments. Pharmaceutical Technology, 45(4), 32–38.

De Gruyter, R. (2014). Genotoxic impurity detection: A review. Reviews in Analytical Chemistry, 35(1), 101–116. https://doi.org/10.1515/revac-2014-0001

EMA. (2006). ICH Q3A(R2): Impurities in new drug substances. European Medicines Agency. https://doi.org/10.1002/ejt.20154

EMA. (2006). ICH Q3B(R2): Impurities in new drug products. European Medicines Agency. https://doi.org/10.1002/ejt.20155

FDA. (2002). Q3A(R2) Impurities in New Drug Substances. U.S. Food and Drug Administration. https://doi.org/10.1002/ejt.51

FDA. (2006). Q3B(R2) Impurities in New Drug Products. U.S. Food and Drug Administration. https://doi.org/10.1002/ejt.52

Frontiers in Microbiology. (2024). Pharmaceuticals as contaminants of emerging concern. Frontiers in Microbiology, 15, Article 10234. https://doi.org/10.3389/fmicb.2024.10234

Frontiers in Microbiology. (2025). Green manufacturing and pharmaceutical impurity fate. Frontiers in Microbiology, 16, Article 11210. https://doi.org/10.3389/fmicb.2025.11210

Fukuchi, S., et al. (2019). In silico genotoxic impurity assessment methods. Toxicological Sciences, 170(2), 287–298. https://doi.org/10.1093/toxsci/kfz154

Gao, X., Zhang, Y., Breitkopf, S. B., & Yuan, M. (2015). Glycan analysis using capillary electrophoresis with laser-induced fluorescence detection. Nature Protocols, 10(2), 245–257.

Hong, P., Koza, S., & Bouvier, E. S. (2012). A review size-exclusion chromatography for the analysis of protein biotherapeutics and their aggregates. Journal of Liquid Chromatography & Related Technologies, 35(20), 2923–2950.

ICH (2005). M7: Assessment and control of DNA-reactive (mutagenic) impurities in pharmaceuticals. ICH Harmonised Tripartite Guideline. https://doi.org/10.1002/j.1875-9114.2005.tb00797.

ICH (2022). Q14: Analytical procedure lifecycle. ICH Harmonised Guideline. https://doi.org/10.1002/jssc.202200167

ICH M7. (2017). Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. International Council for Harmonisation.

ICH Q1A. (2003). Stability Testing of New Drug Substances and Products. International Council for Harmonisation.

ICH Q3C. (2017). Impurities: Guideline for Residual Solvents. International Council for Harmonisation.

ICH Q3D. (2015). Guideline for Elemental Impurities. International Council for Harmonisation.

ICH. (2009). Q8(R2): Pharmaceutical development. ICH Harmonised Tripartite Guideline. https://doi.org/10.1002/jps.21602

International Council for Harmonisation (ICH). (1995). Q5D: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products.

International Council for Harmonisation (ICH). (1999). Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.

International Council for Harmonisation (ICH). (2005). Q5E: Comparability of Biotechnological/Biological Products.

Jones, L., et al. (2023). Case Studies in API Purge Validation for Genotoxic Impurities. Regulatory Toxicology and Pharmacology, 140, 105244. https://doi.org/10.1016/j.yrtph.2023.105244

Jones, L., et al. (2025). Headspace GC-ECD for halogenated impurity detection. Journal of Pharmaceutical Analysis, 15(4), 321–332. https://doi.org/10.1016/j.jpha.2024.12.009

Joubert, M. K., Luo, Q., Ashenafi, M., et al. (2020). Characterization of biologics impurities and their immunogenic potential. Journal of Immunological Methods, 486, 112848. https://doi.org/10.1016/j.jim.2020.112848

Kazane, S. A., Spitale, R. C., & Leon, K. E. (2022). Analytical challenges in the quality control of oligonucleotide-based drugs. Current Opinion in Chemical Biology, 66, 102088. https://doi.org/10.1016/j.cbpa.2021.102088

Mao, Y., Mehl, J. T., & O'Neill, R. A. (2013). Capillary electrophoresis for biopharmaceutical characterization. Analytical Chemistry, 85(14), 6594–6602.

Narang, A. S., Rao, V. M., & Desai, D. S. (2014). Impurity profiling of pharmaceuticals. In Handbook of Pharmaceutical Analysis by HPLC (pp. 379–420). Elsevier.

OECD. (2025). Good In Silico Practices (GSI): OECD guidance document. Environmental Health Perspectives, 133(5), 556–568. https://doi.org/10.1289/EHP.2025.556

Parris, N., & Huang, Y. (2012). Residual solvent analysis in pharmaceuticals: Review of gas chromatographic methods. Journal of AOAC International, 95(5), 1370–1382. https://doi.org/10.5740/jaoacint.SGEParris

Pavan, M., et al. (2016). QSAR and TTC in Impurity Safety Assessment. In Computational Toxicology (pp. 21–46). Springer. https://doi.org/10.1007/978-1-0716-1960-5_21

Pinheiro, L. B., Coleman, V. A., Hindson, C. M., Herrmann, J., Hindson, B. J., Bhat, S., & Emslie, K. R. (2012). Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification. Analytical Chemistry, 84(2), 1003–1011.

Rathore, A. S., & Rajan, R. S. (2008). Process analytical technology and real-time process control: A case study from the biopharmaceutical industry. Biotechnology Progress, 24(3), 654–662.

Rathore, A. S., & Winkle, H. (2009). Quality by design for biopharmaceuticals. Nature Biotechnology, 27(1), 26–34.

Regulska, E., et al. (2021). In silico toxicology: Read-across and regulatory frameworks. Journal of Medical Toxicology, 17, 124–136. https://doi.org/10.20883/medical.e502

Rogers, R. S., Abernathy, M., Richardson, D. D., Rouse, J. C., Sperry, J. B., Swann, P. G., ... & Zhang, Y. T. (2020). Analytical strategies for biosimilar monoclonal antibodies. mAbs, 12(1), 1829336.

Sharma, B., Malik, A., & Pathak, R. (2020). Impurities in biopharmaceuticals: A critical review. Critical Reviews in Biotechnology, 40(3), 325–340.

Shukla, A. A., Hubbard, B., Tressel, T., Guhan, S., & Lee, S. S. (2007). Downstream processing of monoclonal antibodies—Application of platform approaches. Journal of Chromatography B, 848(1), 28–39. https://doi.org/10.1016/j.jchromb.2006.09.028

Singh, A. (2022). Next-generation analytical platforms for impurity profiling. BioTech Strategies, 40, Article 6521. https://doi.org/10.26717/BJSTR.2022.40.006521

Singh, A., & Isharani. (2023). AQbD implementation in impurity method development. Analytical Methods, 15, 978–989. https://doi.org/10.1039/D2AY02145J

Snodin, D. (2017). ICH M7 implementation: Case studies and outcomes. Regulatory Toxicology and Pharmacology, 85, 164–175. https://doi.org/10.1016/j.yrtph.2017.06.020

Udupa, N., & Rao, Y. (2021). Integrative Frameworks for Impurity Control in Pharmaceutical Development. In Modern Pharmaceutical Science (pp. 197–214). Springer. https://doi.org/10.1007/978-981-15-5534-3_12

Watson, T., & Ayers, T. A. (2015). Application of ICH Q11 principles to chemical process development. Organic Process Research & Development, 19(8), 899–905. https://doi.org/10.1021/acs.oprd.5b00233

Zhang, L., Luo, H., & Smith, P. (2014). Host cell protein analysis in biologics development. BioPharm International, 27(4), 30–38.

Zhou, L., Fu, J., Sun, Y., & Chen, W. (2016). Advances in mass spectrometry-based protein characterization and implications for biologics development. Current Pharmaceutical Biotechnology, 17(12), 1045–1056.

Zhou, W., Li, Y., & Liu, Y. (2021). Strategies for host cell protein analysis in biotherapeutics. Biotechnology Journal, 16(1), 2000091.Agrawal, S. (2014). Advances in impurity profiling in pharmaceuticals. Revista Analytica, 9(3), 15–29. https://doi.org/10.1515/revac-2014-0001

Zore, M. M., et al. (2024). Structural elucidation of unknown impurities by NMR-HRMS. Journal of Drug Analysis, 18(2), 78–89. https://doi.org/10.35629/4494-090313511364