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Nitrosamine Impurities in Pharmaceutical Drug Substances and Drug Products: Risk Assessment, Analytical Validation, Regulatory Guidance, and Lifecycle Management

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Abstract

Few quality events in the modern pharmaceutical era have carried the regulatory and patient safety weight of the 2018 valsartan nitrosamine crisis. The discovery of N-Nitrosodimethylamine (NDMA) contamination in valsartan drug substance and the cascade of findings in ranitidine, metformin, losartan, and an expanding range of other drug classes that followed, established nitrosamine impurities as one of the most analytically demanding and regulatorily significant challenges facing the pharmaceutical industry. Several N-nitroso compounds are classified as probable or possible human carcinogens under the International Agency for Research on Cancer (IARC) and are now subject to rigorous regulatory control under the FDA Guidance Revision 2 (September 2024), ICH M7(R2), ICH Q2(R2), the EMA Nitrosamine Guidelines, and the WHO Technical Report. Detecting and quantifying nitrosamines reliably at acceptable intake (AI) limits, which can fall as low as 18 nanograms per day for the most potent compounds, demands a level of analytical sophistication that goes well beyond conventional pharmaceutical quality control practice. This review brings together the full landscape of what a pharmaceutical analytical scientist needs to know to build, validate, and sustain a credible nitrosamine impurity control program. The FDA three-step risk assessment framework risk assessment, confirmatory testing, and implementation of risk-mitigation measures are examined in detail, with worked numerical examples demonstrating how specification limits and required limits of quantitation are derived from AI limits and maximum daily dose data for real drug products.

The Carcinogenic Potency Categorization Approach (CPCA) for drug-substance-specific nitrosamines (NDSRIs) is explained through structural examples, showing precisely how the alpha-carbon hydrogen count, activating features, and deactivating features combine to determine a compound's potency category and its corresponding AI limit. Analytical method validation is covered across all ICH Q2(R2) parameters for GC-MS/MS, LC-MS/MS, and LC-HRMS platforms, with particular attention to the aspects that consistently prove most challenging in practice matrix effects and ion suppression in complex pharmaceutical matrices, isotope-labeled internal standard selection and performance, photostability of NDMA and related volatile nitrosamines, robustness evaluation using Design of Experiments approaches, and carryover control at trace-level concentrations. The ICH Q2(R2) and ICH Q14 lifecycle model encompassing Analytical Target Profile definition, Method Operable Design Region characterization, and Continued Method Performance Verification through statistical process control monitoring is discussed as the framework that transforms nitrosamine method management from a one-time compliance exercise into a continuous quality commitment. Case studies from the valsartan, ranitidine, metformin, and losartan recalls are integrated throughout to ground every technical discussion in the real events that shaped current regulatory expectations. This review serves as a technically rigorous and practically actionable reference for pharmaceutical analytical scientists, quality assurance professionals, and regulatory affairs practitioners engaged in nitrosamine impurity control across drug substance and drug product manufacturing.

Keywords

Nitrosamine Impurities, NDMA, NDEA, NDSRIs, ICH M7(R2), ICH Q2(R2), GC-MS/MS, LC-MS/MS, LC-HRMS, Analytical Validation, Risk Assessment, Mutagenic Impurities, CPCA, Acceptable Intake, Pharmaceutical Quality, Lifecycle Approach

1. Introduction

Nitrosamines are a class of organic compounds characterized by the nitroso functional group (–N–N=O), formed when secondary or tertiary amines react with nitrosating agents such as nitrous acid or nitrite salts under acidic conditions (**Figure 1**). Although nitrosamines occur naturally in processed foods, tobacco smoke, drinking water, and certain industrial environments, their presence in pharmaceutical drug products has generated profound regulatory and public health concern owing to their well-established carcinogenic and mutagenic properties. Many N-nitroso compounds induce tumors in multiple animal species at low doses, and several are classified as probable human carcinogens by the International Agency for Research on Cancer (IARC). As a result, their presence in chronically administered medicines, even at trace concentrations in the parts-per-billion range, represents a significant patient safety concern when cumulative lifetime exposure is

considered. Nitrosamine contamination in pharmaceuticals gained global prominence in 2018 with the detection of N-nitrosodimethylamine (NDMA) in Valsartan drug substance. This event triggered widespread product recalls, comprehensive regulatory investigations, and a fundamental reassessment of manufacturing risk assessment practices across the pharmaceutical industry. Subsequent findings of nitrosamine impurities in Ranitidine, Metformin, and an expanding range of other drug classes demonstrated that nitrosamine formation can occur through several mechanistically distinct pathways. These include changes in API synthetic routes, intrinsic API degradation under storage conditions, interactions between the API and excipient-derived nitrite, and contamination from recovered solvents and reagents. These findings also highlighted that no drug class or dosage form can be presumed exempt from risk without explicit evaluation. In response, the FDA, EMA, WHO, and ICH have collectively established a comprehensive regulatory framework governing nitrosamine risk assessment, confirmatory testing, acceptable intake (AI) limit derivation, and control strategy implementation. The most recent milestone is the FDA Guidance for Industry: Control of Nitrosamine Impurities in Human Drugs, Revision 2 (September 2024), which formally classifies nitrosamines into small-molecule nitrosamines and nitrosamine drug substance-related impurities (NDSRIs) (**Figure 2**), integrates the CPCA for AI limit derivation, and recognizes the ubiquitous nature of trace nitrite across the pharmaceutical supply chain. Central to compliance with this framework is the development and rigorous validation of ultra-sensitive analytical methods, primarily GC-MS/MS and LC-MS/MS, capable of detecting and quantifying nitrosamines at or below 30% of the AI-based specification limit in complex pharmaceutical matrices, a requirement that places exceptional technical demands on analytical laboratories [1]-[4].

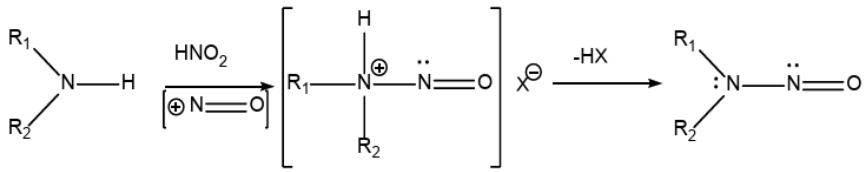


Figure 1. Representative reaction to form nitrosamines.

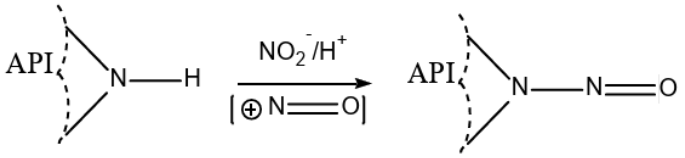


Figure 2. Representative reaction of NDSRI formation.

2. Scope of This Review: Inclusions and Exclusions

This review is scoped specifically for pharmaceutical analytical scientists, quality assurance professionals, and regulatory affairs practitioners involved in nitrosa-

mine impurity control programs. It covers the current regulatory frameworks, FDA Guidance Revision 2 (September 2024), EMA Guideline (EMA/409815/2020), FDA RAIL Guidance (August 2023), ICH M7(R2), ICH Q2(R2), ICH Q14, and USP <1469>, alongside the FDA three-step risk assessment framework with worked specification limit and LOQ calculations, the CPCA-based AI limit derivation for NDSRIs, development and full ICH Q2(R2) validation of GC-MS/MS, LC-MS/MS, and LC-HRMS methods, the lifecycle approach including ATP, MODR, and Continued Method Performance Verification (CMPV), and mechanistic case studies from the valsartan, ranitidine, metformin, and losartan recalls. The review does not address nitrosamines in foods, tobacco, drinking water, or occupational settings; in vivo carcinogenicity mechanisms beyond AI limit derivation; veterinary, cosmetic, or agrochemical products; biological or large-molecule drug products; detailed synthetic process chemistry; epidemiological patient outcome studies; or regulatory submission strategy. These boundaries reflect a deliberate prioritization of analytical and scientific depth over encyclopedic coverage, ensuring the review serves as a practically actionable technical reference for its intended audience.

3. Regulatory Framework Governing Nitrosamine Control

3.1. Global Regulatory Landscape

When nitrosamine contamination was identified in pharmaceutical products, regulatory agencies responded swiftly. The FDA recommended that manufacturers follow a three-step process: risk assessment, confirmatory testing, and mitigation, alongside established acceptable intake limits. The EMA similarly mandated reviews of APIs, excipients, manufacturing conditions, and packaging, with strict reporting timelines. Health Canada adopted a comparable risk-based approach and collaborated internationally to align expectations. Globally, the ICH M7 Guideline on mutagenic impurities has provided an overarching scientific framework for nitrosamine risk management [5].

3.2. Role of ICH Guidelines in Nitrosamine Risk Management

Three ICH guidelines underpin nitrosamine control. ICH M7 addresses mutagenic impurities, providing the toxicological framework for setting acceptable intake limits. ICH Q9 supplies quality risk management tools, hazard analysis, risk ranking, and failure mode analysis, to identify and prioritize nitrosamine formation risks across manufacturing steps. ICH Q10 embeds these controls into a lifecycle-wide pharmaceutical quality system, ensuring risks identified in development are monitored through commercial production. Together, they shift the focus from end-product testing alone to proactive process understanding and prevention [6] [7].

3.3. Acceptable Intake Limits for Nitrosamines

Because nitrosamines are potent mutagenic and carcinogenic compounds, even

trace amounts may present a meaningful risk to patients who take medicines chronically over many years. Regulatory authorities have therefore established acceptable intake (AI) limits, sometimes referred to as permitted daily exposures (PDEs) in other impurity contexts, that define the maximum amount of each nitrosamine a patient may be exposed to daily without incurring a clinically significant cancer risk over a 70-year lifetime. These limits are not arbitrary thresholds. They are derived from a toxicology-driven process in which regulatory scientists evaluate available animal carcinogenicity data, structural analogy with related compounds, and predictive models where direct experimental data are absent. The resulting AI values, almost universally expressed in nanograms per day, reflecting the extraordinarily low concentrations at which these compounds exert biological harm are then used to calculate product-specific specification limits by dividing the AI by the drug's maximum daily dose, as described in the FDA three-step framework.

The seven FDA-designated small-molecule nitrosamines, N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosomethylphenylamine (NMPA), N-nitrosodiisopropylamine (NDIPA), N-nitrosoisopropylethylamine (NIPEA), N-nitrosodibutylamine (NDBA), and N-nitroso-N-methyl-4-aminobutyric acid (NMBA), are all classified as members of the cohort of concern under ICH M7(R2) and are subject to compound-specific acceptable intake (AI) limits derived from rodent carcinogenicity data. Six of these compounds NDMA, NDEA, NMPA, NDIPA, NIPEA, and NDBA share a common AI of 26.5 ng/day, reflecting their comparable carcinogenic potency. NMBA carries a higher AI of 96 ng/day, consistent with its comparatively lower potency as determined from its tumor dose data. For nitrosamine drug substance-related impurities (NDSRIs) compounds structurally unique to each API and lacking their own carcinogenicity datasets the Carcinogenic Potency Categorization Approach (CPCA) provides a structure-based method for assigning provisional AI limits ranging from 18 ng/day at the highest potency tier (Category 1) down to 1500 ng/day at the lowest (Category 5), as illustrated in **Figures 3-5**. The structural basis for these CPCA categories is described in detail in Section 3.4.

An important practical principle underpins all AI limits: even where a measured nitrosamine level falls below the applicable threshold, manufacturers are expected to apply the principle of minimization, reducing impurity levels as far as is technically and practically feasible, rather than treating the AI limit as a target. This aligns with the proactive, prevention-first philosophy of ICH M7(R2), which positions risk-based design and process control above reliance on end-product testing. In practice, AI limits directly drive analytical method development by establishing the LOQ target the validated method must be capable of detecting and quantifying each nitrosamine at or below 30% of the AI-derived specification limit, ensuring that any result approaching the threshold is reliably distinguished from background noise. They also govern batch release specifications and provide the common numerical language through which regulatory agencies, manufacturers, and quality laboratories across different regions and products can communi-

cate and enforce a consistent standard of patient protection [8] [9].

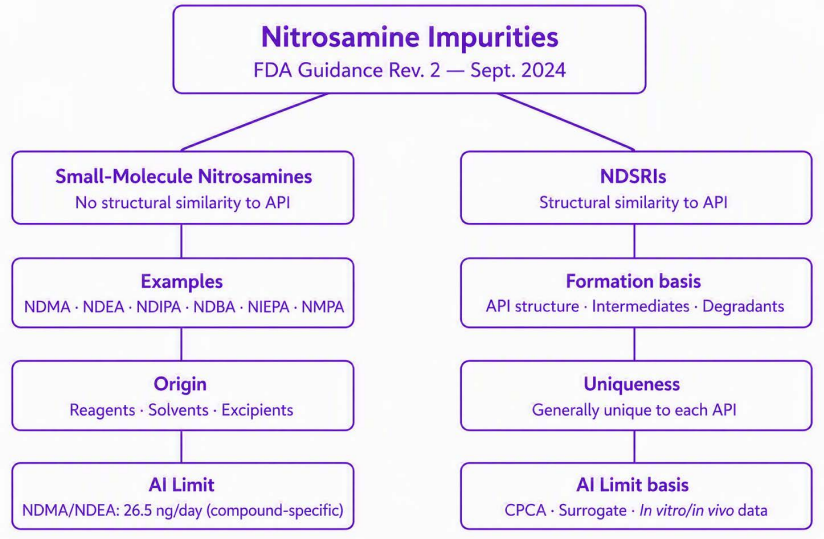


Figure 3. Types of nitrosamine impurities.

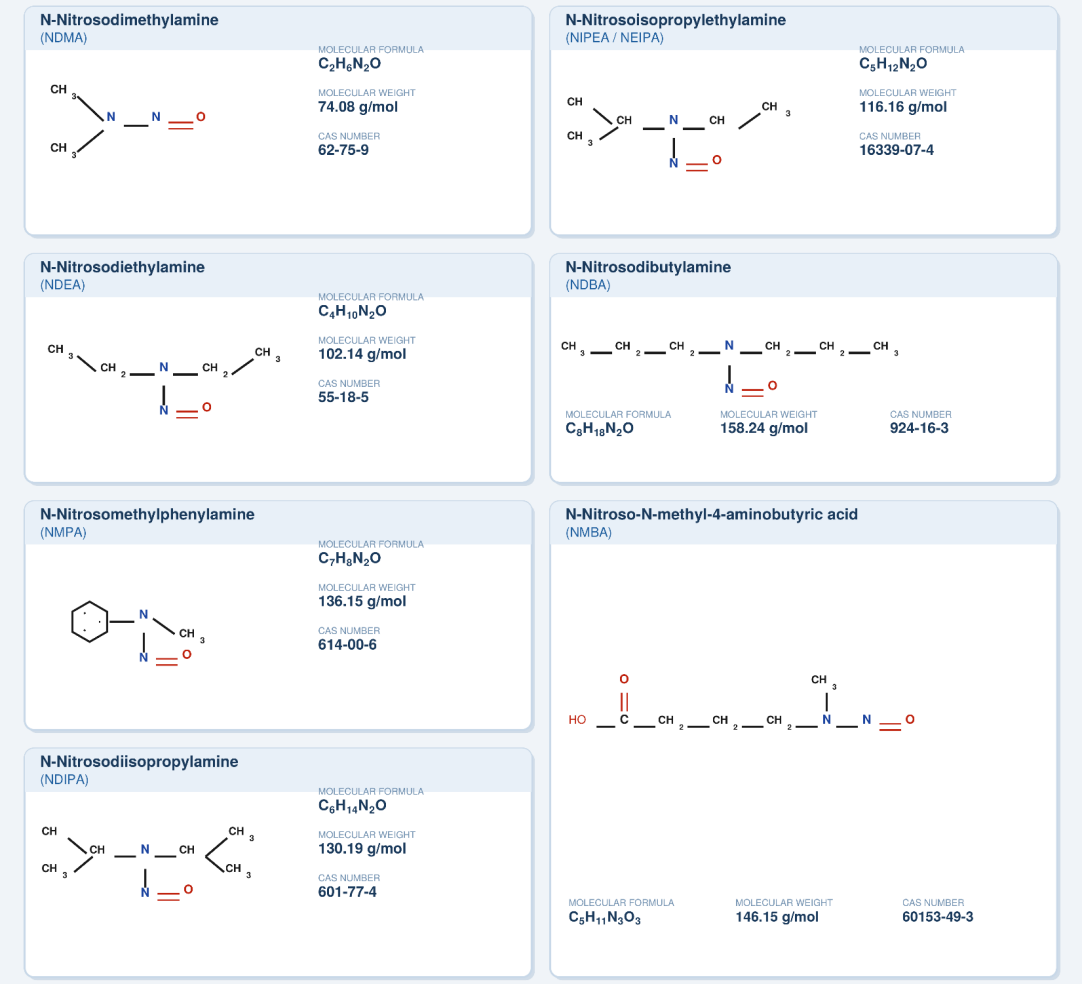


Figure 4. Chemical structures of potential small-molecule nitrosamine impurities in APIs and drug products.

Recommended AI Limits – Small-Molecule Nitrosamines (Compound-Specific Data)

Nitrosamine	AI Limit (ng/day)	Category
NDMA (N-Nitrosodimethylamine)	26.5	Cohort of Concern
NDEA (N-Nitrosodiethylamine)	26.5	Cohort of Concern
NMBA (N-Nitroso–N-methyl–4-aminobutyric acid)	96.0	Cohort of Concern
NDIPA / NDBA / NEIPA / NMPA	26.5	Cohort of Concern
NDSRIs (data-poor) – CPCA Category 1	18	Highest potency
NDSRIs — CPCA Categories 2–5	26.5 – 1500	Graduated by structure
Total nitrosamines (any) – aggregate	*< 26.5 – no further data*	*Rev. 2 key rule*

CPCA: Carcinogenic Potency Categorization Approach (RAIL Guidance + Rev. 2)

Category 1	Category 2	Category 3	Category 4	Category 5
18 ng/day	100 ng/day	400 ng/day	1,500 ng/day	1,500 ng/day
Very high potency	High potency	Moderate potency	Low potency	Very low potency

Author's Note: The NDMA acceptable intake (AI) values presented in this review article were intentionally based on an AI value of 26.5 ng/day to demonstrate a conservative risk-assessment scenario and to maintain consistency throughout the worked examples. The authors acknowledge that the current FDA-recommended AI for NDMA is 96 ng/day. Consequently, specification limits and corresponding LOQ requirements may differ when calculated using current FDA recommendations.

Figure 5. Acceptable Intake (AI) and CPCA framework.

3.4. Structural Determinants of Nitrosamine Carcinogenic Potency: The Role of Alpha-Carbon Hydrogen Availability

Not all nitrosamines are created equal. While every compound in this chemical class carries the same N–N=O functional group, the carcinogenic hazard that group actually poses to patients depends almost entirely on the structural environment of the carbon atoms immediately adjacent to the nitrogen bearing the nitroso substituent the alpha-carbons. It is this structural feature, rather than the nitroso group itself, that explains why one nitrosamine carries an AI limit of 18 ng/day while a closely related compound from the same chemical family is permitted at 1500 ng/day an eighty-fold difference in allowable patient exposure driven by molecular architecture alone. The mechanistic basis for this dependence is the primary metabolic activation pathway of nitrosamines. Cytochrome P450 enzymes principally CYP2E1 and CYP2A6 attack the alpha-carbon and abstract one of its hydrogen atoms in a reaction called alpha-hydroxylation. The resulting unstable alpha-hydroxy-nitrosamine intermediate rapidly decomposes to release a highly reactive alkyldiazonium ion, which alkylates DNA at the O^6 position of guanine, initiating the mutagenic events that underlie nitrosamine carcinogenicity. The critical structural requirement for this entire cascade is the presence of at least one hydrogen atom on the alpha-carbon because alpha-hydroxylation is by definition a hydrogen abstraction reaction, and a fully substituted alpha-carbon bearing no hydrogens cannot undergo it. A nitrosamine with quaternary alpha-carbons on both sides of the N-nitroso group is therefore metabolically inert through this pathway, however danger-

ous it might superficially appear, and is assigned directly to Potency Category 5 (AI 1500 ng/day) under the CPCA framework. Where alpha-hydrogens are present, the CPCA decision tree moves to quantify them specifically, how many hydrogens each alpha-carbon carries and how accessible those hydrogens are to the CYP450 active site. The EMA's alpha-carbon evaluation table codifies this into a numerical alpha-hydrogen score. A 0,2 pattern one quaternary alpha-carbon and one methylene alpha-carbon carrying two hydrogens gives a score of 3, reflecting that only one face of the molecule is metabolically productive. The same score of 3 applies to 1,2 and 1,3 patterns, where one alpha-carbon is a methine group carrying a single hydrogen alongside some degree of steric bulk, moderating CYP450 access. The most carcinogenically potent configurations are the 2,2 and 2,3 patterns both alpha-carbons are methylene or methyl groups, presenting multiple freely accessible C–H bonds on both sides of the N-nitroso group with minimal steric hindrance. These receive an alpha-hydrogen score of 1 and map to the most restrictive AI limits. This structural logic directly explains the potency of NDMA and NDEA: NDMA's two methyl alpha-carbons each carry three hydrogens in a completely unhindered geometry ideal for CYP450 hydroxylation; NDEA's two methylene alpha-carbons present two accessible hydrogens per side with comparable efficiency. NDIPA, by contrast, carries only a single hydrogen on each methine alpha-carbon, with bulky methyl substituents creating meaningful steric interference with CYP450 binding a structural difference that translates directly into a more moderate carcinogenic potency classification. The alpha-hydrogen score is not the complete picture. The CPCA framework adds activating feature scores accounting for cyclic alpha-carbon frameworks that constrain the molecule in a CYP450-favourable geometry, electron-withdrawing groups near the alpha-carbon that lower the C–H bond dissociation energy, and beta-carbon substitution patterns that open secondary metabolic activation pathways and subtracts deactivating feature scores for bulky substituents that sterically block enzyme access, electron-donating groups that strengthen the C–H bond, and structural elements that redirect metabolism away from the mutagenic alpha-hydroxylation pathway. The algebraic sum of these three contributions alpha-hydrogen score plus activating features minus deactivating features gives the compound's total CPCA potency score, which maps as follows: score ≤1 → Category 1 (AI 18 ng/day); score 2 → Category 2 (100 ng/day); score 3 → Category 3 (400 ng/day); score ≥4 → Category 4 (1500 ng/day); structural exclusion (no alpha-hydrogens or tertiary alpha-carbon) → Category 5 (1500 ng/day) (**Figures 6-8**). What gives this framework its practical power is that it requires nothing more than the two-dimensional molecular structure to operate. A pharmaceutical analyst or medicinal chemist encountering a novel NDSRI the N-nitroso derivative that forms when an API's secondary amine undergoes nitrosation under pharmaceutical manufacturing or storage conditions can work through the CPCA decision tree by inspection, count alpha-hydrogens, identify activating and deactivating features, and arrive at a provisional AI limit before any experimental carcinogenicity data are generated. This structural pre-assessment immediately defines the analytical challenge for the

method development scientist: the LOQ must be demonstrated at or below 30% of the specification limit derived from that provisional AI. It simultaneously guides the process chemist toward synthesis route modifications that might reduce or eliminate the vulnerable secondary amine, and the formulation scientist toward excipient and packaging choices that minimize the nitrite exposure needed to drive nitrosation in the first place. The CPCA is therefore not merely a regulatory classification exercise but a practically actionable framework that connects structural chemistry to analytical requirements, manufacturing controls, and ultimately patient safety all from a structural diagram and a scoring table.

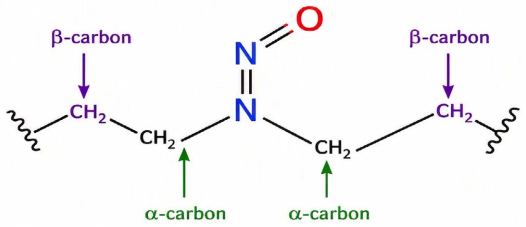


Figure 6. General nitrosamine structure showing α- and β-carbon locations.

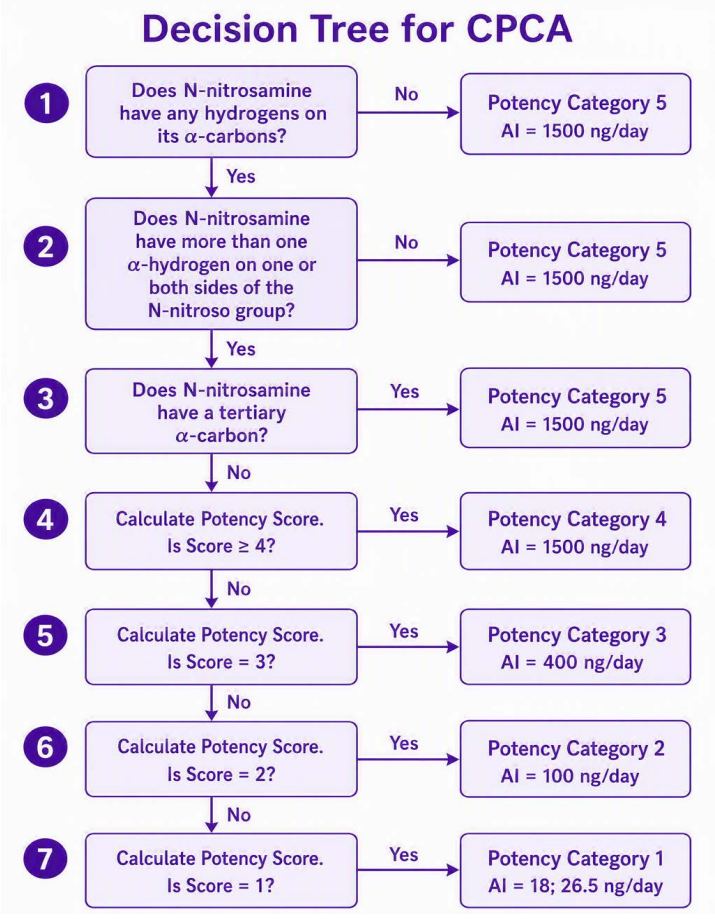
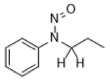
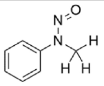


Figure 7. Decision tree for CPCA. EMA assigns Category 1 compounds an AI limit of 18 ng/day, whereas FDA currently applies 26.5 ng/day for Category 1 NDSRIs.

Count of Hydrogen Atoms on Each α-Carbon, Lowest First	Example	α-Hydrogen Score
0,2		3*
0,3		2
1,2		3
1,3		3
2,2		1
2,3		1

* A score of 3 applies when the methylene α-carbon is not part of an ethyl group. If the methylene α-carbon is part of an ethyl group, a score of 2 should be applied.

Figure 8. Evaluation of alpha carbon.

It is important to acknowledge that the CPCA is a provisional, structure-based risk assessment tool and carries inherent limitations that constrain its application as a definitive carcinogenicity determination. The framework was developed from a training set of nitrosamines with known rodent carcinogenicity data, and its predictive accuracy decreases for NDSRIs with structural features not well-represented in that training set particularly for compounds with complex heterocyclic frameworks, multiple stereocenters adjacent to the N-nitroso group, or unusual electronic environments that may modulate metabolic activation through pathways not captured by the alpha-carbon hydrogen scoring model. When an NDSRI is present at levels approaching or exceeding the CPCA-derived AI limit in a commercial drug product, or when the CPCA assignment places the compound in Category 1 or Category 2 where the AI limit is most restrictive, the provisional CPCA categorization should be reviewed for replacement by compound-specific carcinogenicity data either from a dedicated rodent bioassay of the NDSRI itself or from a structurally matched, scientifically justified surrogate nitrosamine for which TD50 data from guideline-compliant studies are available and peer-reviewed. Regulatory agencies including the FDA and EMA have both confirmed in their guidance documents that compound-specific data, where available and of sufficient quality, supersede the CPCA-derived provisional AI and should be used to set the definitive specification limit [10] [11].

3.5. Regulatory Requirements for Risk Evaluation and Confirmatory Testing

Once acceptable intake limits are established, manufacturers are recommended to follow a structured three-step approach. First, a thorough risk evaluation is con-

ducted across relevant product lifecycle elements, raw materials, excipients, API synthesis pathways, solvents, packaging, and equipment, to identify any potential sources of nitrosamine formation or contamination. Where risks are identified, confirmatory testing using sensitive analytical methods such as GC-MS, GC-MS/MS, and LC-MS/MS is performed on APIs, intermediates, and finished products to verify whether nitrosamines are present within acceptable limits. If detected, manufacturers should investigate root causes and implement appropriate corrective actions, such as process changes, raw material controls, or additional purification steps. All risk assessments, results, and corrective actions must be fully documented for regulatory traceability. Importantly, regulators emphasize that confirmatory testing complements rather than replaces proactive process design, with the overall goal being a continuous, science-driven cycle of identification, verification, and prevention to ensure patient safety (Figure 9).

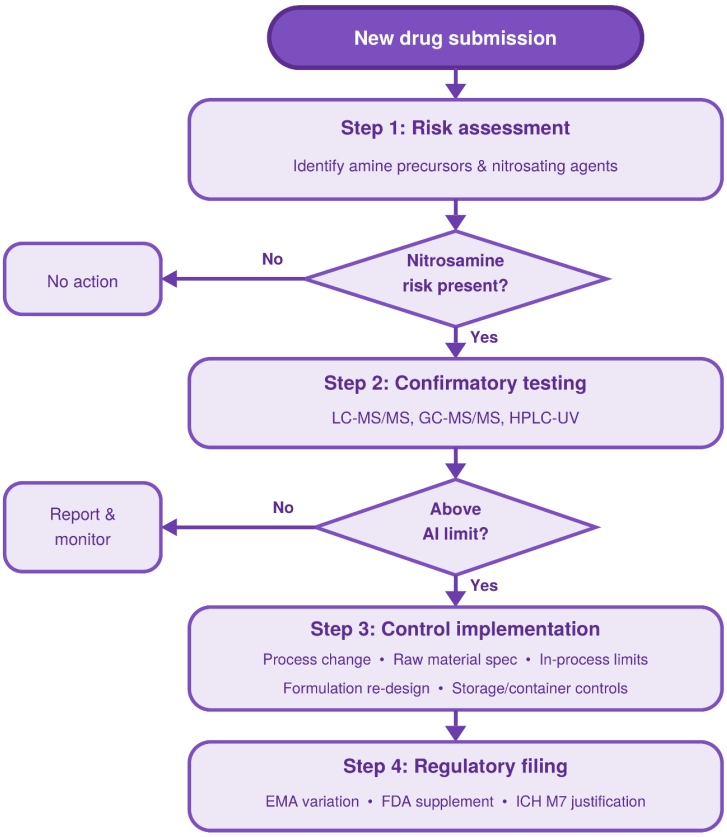


Figure 9. Risk based control framework for nitrosamine impurities.

3.6. Regulatory Divergences in Practice

While the FDA, EMA, Health Canada, and WHO are broadly aligned on the overarching risk-based philosophy for nitrosamine control, meaningful practical differences persist across these jurisdictions that analytical scientists and regulatory affairs professionals must navigate carefully. The most consequential divergence concerns the AI limit assigned to CPCA Category 1 compounds: the FDA's RAIL

Guidance (August 2023) sets Category 1 at 26.5 ng/day, consistent with FDA's class-specific limit based on NDEA, while the EMA's July 2023 decision tree assigns Category 1 a more stringent limit of 18 ng/day, derived from a more conservative carcinogenic potency estimate for the highest-risk structural tier. For a drug product submitted under both FDA and EMA jurisdictions, this 32% difference in AI limit translates directly into a 32% difference in the specification limit and therefore a correspondingly more demanding LOQ requirement under EMA assessment, which has practical implications for method sensitivity targets and instrument selection. Regarding combined nitrosamine exposure, the FDA Guidance Revision 2 explicitly addresses the aggregate daily exposure scenario and permits manufacturers to apply a total nitrosamine approach summing daily exposures from all individual nitrosamines present and comparing the sum to a reference threshold of 26.5 ng/day as an alternative to individual compound-by-compound specifications. The EMA has not formally adopted an equivalent aggregate approach in its published Q&A documentation as of July 2024, requiring instead that each identified nitrosamine meet its own individual AI-derived limit. Health Canada's Guidance for Industry (2020) aligns closely with the EMA framework on individual compound limits and does not yet incorporate the CPCA for NDSRIs as a formal acceptance basis, though it references ICH M7(R2) as the applicable toxicological guideline. WHO guidance, published through its Technical Report Series and updated prequalification expectations, follows the EMA AI limits for confirmatory testing of medicines included in prequalification programs, meaning that manufacturers supplying products to low- and middle-income country markets through international procurement mechanisms must meet the more conservative 18 ng/day Category 1 AI to satisfy WHO prequalification requirements. For confirmatory testing methodology, the FDA specifies in Revision 2 that both GC-MS/MS and LC-MS/MS are acceptable as primary confirmatory techniques with published validated methods, whereas the EMA's guidance additionally references Ph. Eur. general methods and explicitly permits confirmation by a second orthogonal technique, providing somewhat greater methodological flexibility for structurally complex NDSRIs that cannot be adequately resolved by a single chromatographic mode.

3.7. Challenges in Regulatory Implementation

Despite clear regulatory guidance, implementing nitrosamine controls in real-world manufacturing presents significant challenges. Nitrosamine formation pathways are complex and unpredictable, arising not only during API synthesis but also during formulation, storage, and interactions with excipients or packaging, therefore, even minor variations in raw materials or manufacturing conditions can affect impurity levels. Analytical detection is equally demanding, as acceptable intake limits in the nanogram-per-day range require ultra-trace sensitivity, specialized instrumentation, and rigorous method validation that not all facilities can readily achieve. Risk assessments themselves are resource-intensive, re-

quiring cross-functional expertise and thorough documentation, with significant process or supplier changes potentially triggering a reevaluation. Further complexity arises from global regulatory variability, as differences in guidance across the FDA, EMA, and Health Canada require careful navigation for companies operating in multiple markets. Finally, nitrosamine control is an ongoing lifecycle responsibility, not a one-time exercise, demanding continuous monitoring and periodic review as manufacturing conditions evolve. Successful compliance depends on strong cross-functional collaboration, advanced analytical capabilities, and a proactive quality system.

4. Sources and Mechanisms of Nitrosamine Formation in Pharmaceutical Manufacturing

Understanding how nitrosamines are formed is essential for preventing their occurrence in pharmaceutical products. Nitrosamines are not deliberately added to drugs; rather, they are unintended impurities that can arise from chemical reactions between amines and nitrosating agents under certain conditions. A comprehensive understanding of these sources and mechanisms is crucial for designing effective control strategies and analytical testing methods [9] (**Figure 10**).

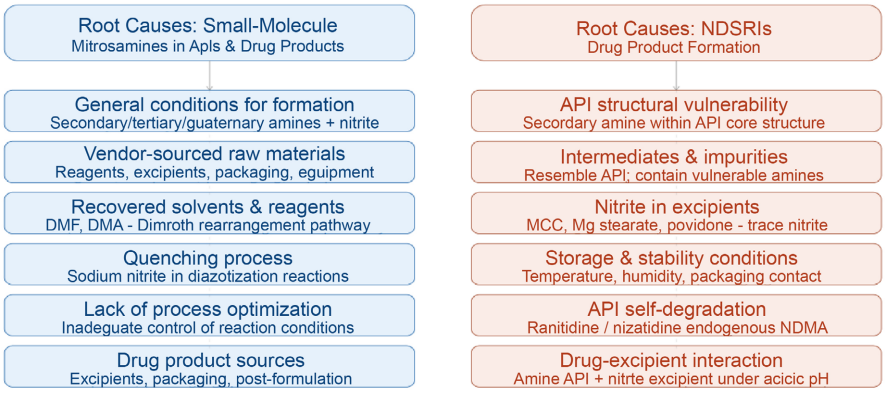


Figure 10. Root cause of nitrosamine formation.

4.1. Chemistry and Formation Mechanisms of Nitrosamines

Nitrosamines are typically formed when secondary or tertiary amines react with nitrosating agents such as nitrites (NO_2^-) in acidic or oxidizing environments. The general reaction involves the conversion of an amine group into a nitroso group (–N–N=O), which forms the nitrosamine structure. The likelihood of this reaction depends on several factors, including the type of amine, the presence of nitrosating agents, pH, temperature, and reaction time. Some nitrosamines, such as NDMA, are highly potent and can be generated even under mild conditions, making them particularly concerning in pharmaceuticals.

4.2. Sources in Active Pharmaceutical Ingredients (APIs)

Nitrosamine formation can occur at various stages of API synthesis. Reactions

that involve amines, nitrites, or certain solvents can inadvertently produce nitrosamines. For example, changes in reaction conditions, such as temperature or pH adjustments, can accelerate nitrosation. Contamination can also arise from residual solvents or reagents that contain trace nitrites, particularly if raw materials are not carefully controlled. The chemical complexity of API synthesis means that even small changes in reagents, catalysts, or manufacturing scale can alter the likelihood of nitrosamine formation.

4.3. Sources in Excipients and Raw Materials

Excipients, the non-active components of a drug product, can also contribute to nitrosamine formation. Some excipients contain amine groups or impurities that can react with nitrosating agents during drug product formulation or storage. Water, salts, or other additives may also contain trace nitrites, further increasing the risk. Raw material variability, which is common when sourcing from multiple suppliers, can also introduce unexpected nitrosating agents, highlighting the need for thorough raw material risk assessment and control.

4.4. Nitrosamine Formation during Drug Product Manufacturing

Nitrosamines can form during the actual formulation of drug products, particularly under conditions of heat, moisture, or acidic pH. Processes such as wet granulation, extrusion, or tablet compression can create localized environments that favor nitrosation reactions. Additionally, certain excipients may interact with APIs or environmental contaminants during processing, leading to nitrosamine formation even if the API itself is initially free of nitrosamine impurities.

4.5. Degradation Pathways and Storage Conditions

Beyond manufacturing, nitrosamines can also form during storage due to degradation reactions. Exposure to heat, light, moisture, or oxygen can induce chemical changes in the drug product or excipients, generating nitrosamines over time. Stability studies and accelerated aging experiments are therefore critical for understanding and mitigating these degradation pathways.

4.6. Packaging and Environmental Contributions

Packaging materials and the surrounding environment may also influence nitrosamine formation. For instance, metal catalysts from equipment surfaces, nitrite contamination in water or air, and interaction with certain plastics or container materials can contribute to nitrosation. Understanding these environmental factors is essential for designing control strategies that extend beyond the chemistry of the API and formulation.

In summary, nitrosamine formation in pharmaceutical products is a multifactorial problem, arising from a combination of chemical reactivity, raw material quality, manufacturing conditions, storage, and environmental factors. A thorough understanding of these sources and mechanisms is the foundation for effec-

tive risk assessment, control strategy development, and analytical testing. By identifying the specific points in the lifecycle where nitrosamines are most likely to form, manufacturers can implement targeted mitigation measures, ensuring patient safety and regulatory compliance (Figure 10).

5. Risk-Based Framework for Nitrosamine Assessment

Given the complex nature of nitrosamine formation in pharmaceutical products, a risk-based approach is essential for their effective identification, evaluation, and control. Rather than relying solely on end-product testing, modern regulatory guidance emphasizes proactive risk assessment throughout the drug lifecycle, combining scientific understanding of chemistry, manufacturing processes, and patient exposure to ensure safety (Figure 11).

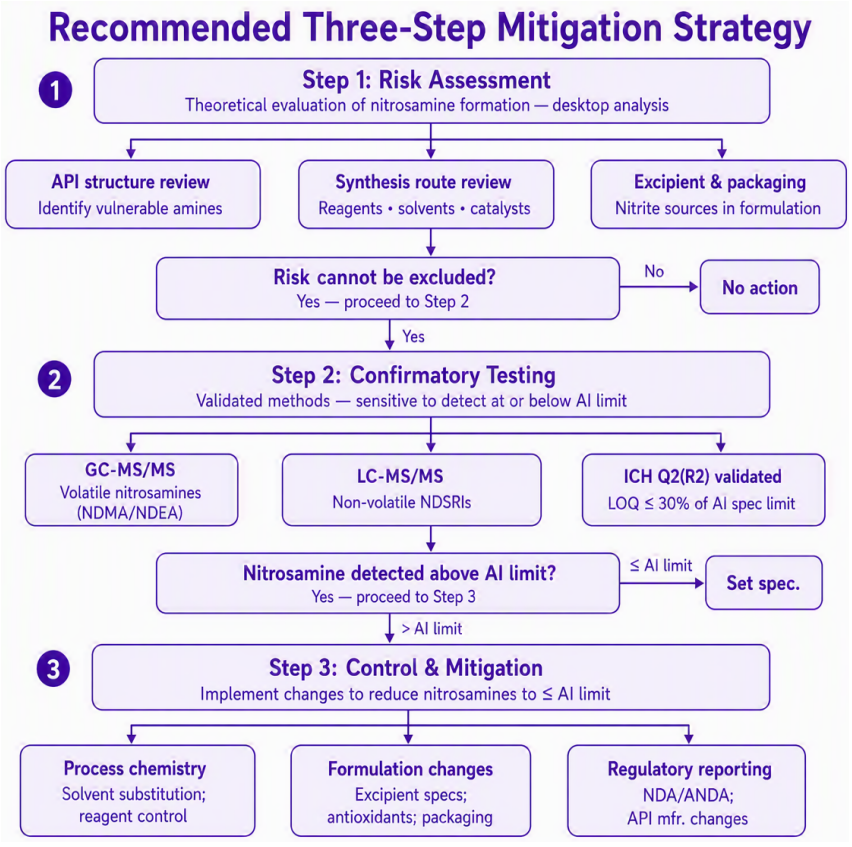


Figure 11. Recommended three step mitigation strategy.

5.1. Principles of Risk-Based Assessment

A risk-based framework begins with the identification of potential nitrosamine sources. This includes assessing the API synthesis pathway, excipients, solvents, manufacturing conditions, and storage or packaging environments. The goal is to systematically pinpoint steps where nitrosation reactions could occur. Once potential sources are identified, the likelihood and severity of nitrosamine formation are evaluated. Likelihood refers to how probable it is for a nitrosamine to form

under given conditions, while severity relates to the potential health impact if the impurity is present at a certain level. This approach is guided by ICH Q9(R1), which provides tools and methods such as risk ranking, failure mode and effects analysis (FMEA), and hazard analysis to systematically prioritize areas of concern. Using these tools, manufacturers can focus resources on the most critical steps in the production and formulation process, ensuring efficient use of testing and control measures.

5.2. Establishing Risk Mitigation Strategies

Once high-risk areas are identified, manufacturers design risk mitigation strategies to prevent or minimize nitrosamine formation. Mitigation can include modifying reaction conditions, replacing potentially reactive raw materials, implementing purification steps, or enhancing storage and packaging controls. Importantly, mitigation strategies should aim to prevent nitrosamine formation proactively, rather than merely relying on detection after the product is produced.

5.3. Role of Acceptable Intake Limits in Risk Assessment

Acceptable intake limits, discussed previously, provide a quantitative benchmark for evaluating risk. By comparing predicted or measured daily nitrosamine exposure against these limits, manufacturers can prioritize interventions and determine whether additional controls or testing are necessary. This ensures that risk assessment is both scientifically grounded and practically actionable.

5.4. Integration with Analytical Testing

Analytical testing serves as a verification tool within the risk-based framework. High-risk APIs, excipients, or formulations are subjected to confirmatory analysis using sensitive techniques such as LC-MS/MS or GC-MS/MS. The results of these tests are then used to refine risk assessments, validate mitigation strategies, and support regulatory submissions. Importantly, analytical testing is most effective when integrated with risk assessment rather than conducted in isolation.

5.5. Continuous Risk Management

Risk assessment for nitrosamines is not a one-time activity. Changes in raw materials, manufacturing processes, suppliers, or storage conditions can introduce new risks over time. A robust risk-based framework incorporates lifecycle monitoring and risk-based periodic reassessment, ensuring that mitigation strategies remain effective throughout the product lifecycle. In summary, a risk-based framework for nitrosamine assessment allows pharmaceutical manufacturers to identify potential sources, evaluate their impact, implement targeted mitigation strategies, and verify effectiveness through analytical testing. By combining scientific understanding, quality risk management, and regulatory guidance, this approach ensures patient safety while optimizing resources and maintaining compliance.

5.6. Common Challenges and Recommended Controls

Effective control of nitrosamine impurities across the pharmaceutical manufacturing lifecycle requires a coordinated set of technical, analytical, and organizational measures directed at every potential formation pathway. The common challenges and recommended controls are presented in Table 1.

Table 1. Common challenges and recommended controls of nitrosamine.

Challenge	Recommended Controls
Amines/nitrites in raw materials	Supplier declarations, incoming QC testing
Nitrosating agents in process	Process redesign, safer reagent substitution
Recycled solvents contamination	Dedicated recovery streams, impurity testing
Nitrite in utilities	Monitoring, filtration, RO systems
Shared equipment residues	Nitrosamine-specific cleaning validation
Excipient nitrite content	CoA review, low-nitrite grade selection
API degradation	Forced degradation studies, formulation adjustment
Storage-related formation	Optimized packaging, controlled humidity and temperature
Analytical detection limits	Validated analytical methods with LOQ below AI limits
Supplier variability	Quality agreements, supplier audits
Regulatory changes	Change control, rapid implementation plans
CMO gaps	Technical agreements, training, audits

6. Analytical Strategies for Nitrosamine Detection and Quantification

Accurate detection of nitrosamines is critical because these impurities can pose a significant health risk even at extremely low levels, often in the parts-per-billion (ppb) range. Analytical strategies must therefore be highly sensitive, specific, and reliable, and they must consider the complexity of pharmaceutical matrices, the potential for interference, and the risk of artifactual formation during analysis.

6.1. Analytical Challenges in Nitrosamine Determination

Detecting nitrosamines is not straightforward. Several challenges make this task complex.

1) Trace-Level Detection Requirements: Regulatory acceptable intake limits for nitrosamines are extremely low, often in the nanogram-per-day range. Analytical methods must therefore be capable of detecting and quantifying nitrosamines at ultra-trace levels.

2) Matrix Complexity: Pharmaceuticals often contain multiple active and inactive ingredients. Excipients, binders, and solvents can interfere with detection, requiring methods that can separate nitrosamines from these complex backgrounds.

3) Chemical Diversity of Nitrosamines: Different nitrosamines have different chemical properties, some are volatile, others non-volatile; some are polar, others non-polar. This diversity necessitates multiple analytical techniques to reliably detect all relevant nitrosamines.

4) Risk of Artifactual Formation: Nitrosamines can sometimes form during sample preparation if reactive amines encounter nitrites under conditions favorable to nitrosation or acidic conditions. Analytical methods must therefore minimize the risk of creating false positives.

6.2. Analytical Techniques for Nitrosamine Analysis

The selection of the appropriate analytical platform is among the most consequential decisions in developing a nitrosamine testing program. The physicochemical properties of the target nitrosamines, particularly volatility, thermal stability, and polarity together with the required sensitivity relative to the AI-based specification limit and the complexity of the drug product matrix collectively determine the optimal technique. No single method is universally appropriate for all nitrosamines across all drug product types.

6.2.1. Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) Headspace and Direct Injection

GC-MS/MS with headspace sampling, direct injection, or purge-and-trap sampling has historically been widely used for volatile and semi-volatile nitrosamines including NDMA, NDEA, NMEA, and NDBA. The technique offers excellent sensitivity (LOD typically 0.01 - 0.1 ng/mL), high chromatographic resolution, and robust performance for simple matrices. Electron ionization and chemical ionization (CI) mode using methanol as reagent gas improves selectivity for nitrosamines by generating characteristic $[M + H]^+$ and $[M + NO]^+$ ions. However, GC-MS/MS has significant limitations for thermally labile nitrosamines and for high-boiling Drug Substance Nitrosamine Impurities (DSNIs), which may not volatilize efficiently under headspace conditions or may decompose during gas-phase injection. Matrix-matched calibration and stable-isotope-labeled internal standards (e.g., NDMA-d_6, NDEA-d_{10}) are essential to compensate for matrix-dependent injection variability.

6.2.2. Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

LC-MS/MS has become the gold standard for non-volatile nitrosamines and complex DSNIs. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are the most widely used ionization modes, with positive-mode ESI offering superior sensitivity for most nitrosamines. Multiple reaction monitoring (MRM) acquisition with at least two diagnostic transitions (a quantifier and a qualifier) is required for regulatory compliance. Method development for LC-MS/MS requires careful attention to matrix effects, which can cause significant ion suppression or enhancement, particularly in tablet formulations with complex excipient backgrounds. Post-column infusion experiments and serial dilution ex-

periments are recommended to evaluate and correct for matrix effects. Stable isotope-labeled internal standards matched to each target nitrosamine are strongly recommended and are mandated by several regulatory agencies for confirmatory testing. Sample preparation strategies for solid dosage forms include aqueous extraction with ultrasonication, filtration, or centrifugation, protein precipitation for biological matrices, and solid-phase extraction (SPE) for enrichment of trace-level analytes. The use of mixed-mode SPE sorbents (e.g., Oasis WCX or MCX) has shown utility for achieving the clean extracts required at sub-nanogram-per-gram analyte concentrations.

6.2.3. Liquid Chromatography High-Resolution Mass Spectrometry (LC-HRMS)

Orbitrap and quadrupole-time-of-flight (QTOF) instruments operated in data-independent acquisition (DIA) or targeted MS^2 mode offer the capability to simultaneously screen for known nitrosamines and detect unknown or unexpected N-nitroso compounds based on accurate mass and isotope pattern matching. This is particularly valuable during initial risk assessment phases and for identifying novel DSNIs in new chemical entities. Full-scan HRMS data can be retrospectively mined for nitrosamine signals without the need for pre-specified MRM transitions, providing a significant advantage over triple-quadrupole instruments during investigational analyses. However, HRMS instruments are considerably more expensive, require specialized operator expertise, and their regulatory acceptance for routine QC testing while growing is not yet universal.

6.2.4. High Performance Liquid Chromatography Ultraviolet (HPLC-UV)

HPLC with ultraviolet detection (HPLC-UV) operates at sensitivity levels typically 5 to 50 ng/mL, equivalent to approximately 1 to 10 ppm in a standard pharmaceutical extract that are fundamentally insufficient for AI-limit compliance testing of any of the seven FDA-designated small-molecule nitrosamines or for any NDSRI assigned to CPCA Categories 1 through 3. Neither the FDA nor the EMA accepts HPLC-UV as a standalone method for nitrosamine specification testing or confirmatory analysis in regulatory submissions. Its appropriate role is strictly limited to high-level screening in early-stage process development and route scouting, where nitrosamine precursor concentrations may be orders of magnitude above the AI limit, and as a teaching or method scouting tool in laboratory training settings. Any wording in Section 4 or elsewhere in earlier drafts that placed HPLC-UV in a confirmatory testing context has been removed and replaced with the above standardized description.

6.3. Sample Preparation and Extraction Techniques

Proper sample preparation is essential to achieve accurate results. Techniques typically include solid-liquid extraction to isolate nitrosamines from solid formulations, filtration and dilution to reduce matrix interference, and derivatization for compounds that are poorly volatile or difficult to detect. The goal is to maximize

recovery of nitrosamines from the sample while avoiding loss or chemical alteration during preparation.

6.4. Avoiding Artifactual Nitrosamine Formation during Analysis

A critical consideration is preventing artifactual formation of nitrosamines during sample handling. Nitrosamines can form when reactive amines come into contact with nitrites under acidic conditions. To prevent this, analysts should avoid strong acids or nitrite contamination during extraction, use stabilizing agents, when necessary, minimize exposure to heat or light that could promote nitrosation, and validate that the analytical method does not generate nitrosamines artificially. Careful method design and validation ensure that detected nitrosamines reflect the true content in the sample, not a product of laboratory handling. Detecting nitrosamines is technically challenging due to their trace-level presence, chemical diversity, and complex sample matrices. Analytical strategies, including GC-MS, GC-MS/MS, LC-MS/MS, and LC-HRMS, provide highly sensitive and selective tools for quantification. However, successful analysis also depends on careful sample preparation and strategies to avoid artifactual formation. Together, these practices allow pharmaceutical manufacturers to reliably monitor nitrosamines, support risk-based control strategies, and ensure patient safety. The analytical techniques, principles, advantages, and limitations for nitrosamine analysis are presented in Table 2.

Table 2. Analytical techniques, principle, advantages and limitations of nitrosamine.

Technique	Principle and Ionization/ Detection Mode	Advantages	Limitations
GC-MS/MS (Headspace)	Volatile analytes partitioned into headspace vapor; GC separation followed by triple-quadrupole MS/MS detection in MRM mode. Electron Ionization (EI) or Chemical Ionization (CI)	1. Extremely high sensitivity (pg/mL range). 2. No sample extraction required reduced matrix effects. 3. Excellent for volatile nitrosamines (NDMA, NDEA). 4. Established in published FDA and EMA-associated methods. 5. ISTD deuterated standards commercially available. 6. Minimal sample preparation.	1. Restricted to volatile, thermally stable nitrosamines only. 2. Non-volatile DSNIs are generally not detectable. 3. Equilibration temperature must be tightly controlled. 4. Requires dedicated headspace autosampler. 5. NDMA can artefactually form from thermally labile matrices (e.g., ranitidine).
GC-MS/MS (Direct Injection)	Liquid sample or extract injected directly onto GC column; analytes separated by boiling point and polarity; MS/MS MRM detection Electron Ionization (EI) or Chemical Ionization (CI)	1. Broader analyte scope than headspace. 2. Suitable for semi-volatile nitrosamines. 3. High selectivity via MRM transitions. 4. Cost-effective relative to LC-MS/MS.	1. Risk of thermal degradation of labile analytes at injector port. 2. Matrix components co-injected requires clean-up step. 3. Not suitable for non-volatile DSNIs. 4. Solvent compatibility constraints.

Continued

LC-MS/MS Gold standard for non-volatile nitrosamines	Reversed-phase or HILIC liquid chromatographic separation followed by electrospray ionization (ESI) and triple-quadrupole MS/MS in MRM mode Electrospray Ionization (ESI+) or Atmospheric Pressure Chemical Ionization (APCI+)	1. Gold standard for non-volatile and complex nitrosamines 2. Extremely high sensitivity sub-pg/mL achievable 3. No thermal degradation risk 4. Suitable for all DSNIs regardless of volatility 5. Simultaneous multi-analyte quantitation in one run 6. Stable isotope-labeled ISTDs correct matrix effects 7. Broadly applicable across dosage form matrices	1. Significant matrix effects ion suppression/enhancement in complex matrices. 2. Expensive instrumentation and maintenance 3. Method development and optimization are technically demanding. 4. Requires validated ISTD for each analyte. 5. Mobile phase additive compatibility must be optimized for ESI. 6. Cross-contamination between highly sensitive injections.
LC-HRMS (Orbitrap/QTOF) For unknown nitrosamine identification	LC separation coupled to Orbitrap or quadrupole-time-of-flight (QTOF) instruments; full-scan accurate mass acquisition enabling targeted and untargeted detection Electrospray Ionization (ESI+/ESI−); Accurate mass resolution >50,000 FWHM.	1. Definitive structural confirmation via accurate mass (<5 ppm error). 2. Retrospective data mining unknown nitrosamines detectable without pre-specification. 3. High specificity distinguishes isobaric interferences impossible by unit-resolution MS/MS. 4. Simultaneous screening of all nitrosamines in a single injection. 5. Essential for NDSRI characterization and structural elucidation. 6. Supports CPCA-based risk assessment with confirmatory structural data.	1. Significantly higher instrument cost than triple-quadrupole platforms. 2. Complex data processing and bioinformatics expertise required. 3. Lower sensitivity in full-scan mode compared to MRM-based LC-MS/MS. 4. Not currently mandated for routine QC primarily investigational and development use. 5. Regulatory acceptance for routine specifications is still evolving. 6. Long analysis and data review times.
HPLC-UV/DAD "Early-stage process development screening only; not fit for AI-limit specification testing"	Reversed-phase HPLC separation with ultraviolet absorbance detection at 230 - 254 nm; nitrosamines detected based on chromophore absorption of N-nitroso group. N/A photometric detection.	1. Simple, widely available instrumentation in all QC laboratories. 2. Low cost of acquisition and operation. 3. No need for mass spectrometry expertise. 4. Useful for high-concentration screening in process chemistry. 5. Straightforward method development. 6. No ionization suppression concerns.	1. Insufficient sensitivity for AI-limit compliance testing (NDMA AI = 26.5 ng/day) 2. Poor selectivity co-eluting API impurities can cause false positives 3. Not capable of structural confirmation 4. Not accepted by FDA/EMA as a standalone method for nitrosamine specifications 5. LOD typically 5 - 50 ng/mL far above regulatory requirements for most nitrosamines 6. Cannot distinguish individual nitrosamines from other UV-absorbing species

6.5. Calculation of Specification Limits from AI Limits

The central calculation converts the regulatory AI limit expressed in nanograms per day into an analytical concentration limit in parts per million (μg/g) using the drug product's maximum daily dose (MDD). The FDA formula is:

$$\text{Specification Limit}(\text{ppm}) = \text{AI Limit}(\text{ng/day}) \div \text{MDD}(\text{mg/day}). \quad (1)$$

This formula derives from the dimensional analysis: ng/day ÷ mg/day = ng/mg = μg/g = ppm. The LOQ requirement is then calculated as 30% of the specification limit, per FDA guidance:

$$\text{Required LOQ}(\text{ppm}) \leq \text{Specification Limit} \times 0.30. \quad (2)$$

6.5.1. Example 1 NDMA in Valsartan 160 mg Tablet

NDMA AI limit: 26.5 ng/day. MDD of valsartan: 320 mg/day (160 mg twice daily per FDA-approved label). Specification limit = 26.5 ÷ 320 = 0.0828 ppm ≈ 0.083 ppm. Required LOQ ≤ 0.083 × 0.30 = 0.025 ppm. For a sample preparation concentration of 1 mg/mL (1 mg valsartan per 1 mL diluent), the NDMA concentration at the specification limit in the extract equals 0.083 ng/mL = 83 pg/mL, and the required LOQ corresponds to 25 pg/mL. This sub-100 pg/mL sensitivity requirement mandates GC-MS/MS in headspace mode or LC-MS/MS with isotope-labeled internal standards; conventional HPLC-UV cannot approach this sensitivity threshold.

6.5.2. Example 2 NMBA in Losartan 100 mg Tablet

NMBA AI limit: 96.0 ng/day. MDD of losartan: 100 mg/day. Specification limit = 96.0 ÷ 100 = 0.96 ppm. Required LOQ ≤ 0.96 × 0.30 = 0.288 ppm. For an extraction concentration of 2 mg/mL, the LOQ in the extract corresponds to 576 pg/mL, a less demanding sensitivity requirement reflecting NMBA's lower carcinogenic potency relative to NDMA.

6.5.3. Example 3 NDSRI Calculated via CPCA

For a novel NDSRI from a hypothetical API (MDD = 50 mg/day) with one alpha-hydrogen, no cyclic N-nitroso structure, and no strongly electron-withdrawing substituents, structural assessment assigns CPCA Category 2 (AI = 100 ng/day). Specification limit = 100 ÷ 50 = 2.0 ppm. Required LOQ ≤ 2.0 × 0.30 = 0.60 ppm. This limit is substantially more achievable than small-molecule nitrosamine limits, permitting method development without isotope-labeled ISTD correction in many cases, though ISTD use remains best practice.

6.5.4. Example 4 Multiple Nitrosamines: Total Exposure Assessment

FDA Revision 2 permits a total nitrosamine approach when multiple nitrosamines are present. For a drug product (MDD = 200 mg/day) containing NDMA at 0.05 ppm (daily exposure: 10.0 ng/day), NDEA at 0.03 ppm (6.0 ng/day), and NMBA at 0.10 ppm (20.0 ng/day), the total daily exposure is 36.0 ng/day. Since 36.0 ng/day exceeds the 26.5 ng/day threshold for the most potent nitrosamine present

(NDMA), process changes or alternative justification are required despite no individual nitrosamine exceeding its own AI limit. This rule prevents the accumulation of multiple nitrosamines at individually acceptable levels to a combined carcinogenic burden above the regulatory threshold.

The Worked Specification Limit and LOQ Calculations for Representative Drug Products presented in Table 3.

Table 3. Worked specification limit and LOQ calculations for representative drug products.

Drug/Nitrosamine	MDD (mg/day)	AI Limit (ng/day)	Specification Limit (ppm)	Required LOQ (ppm)	Extract Concentration at LOQ*
Valsartan/NDMA	320	26.5	0.083	0.025	25 pg/mL
Losartan/NMBA	100	96.0	0.960	0.288	576 pg/mL
Metformin/NDMA	2000	26.5	0.013	0.004	4 pg/mL
Hypothetical API/ NDSRI Category 2	50	100.0	2.000	0.600	600 pg/mL
Ranitidine/NDMA	300	26.5	0.088	0.026	26 pg/mL

*Extract concentration at LOQ was calculated assuming sample preparation at 1 mg/mL for valsartan, metformin, the hypothetical API, and ranitidine, and 2 mg/mL for losartan. Actual values should be optimized during method development.

7. Analytical Method Validation for Nitrosamine Determination

Nitrosamines are highly potent impurities that can pose health risks at extremely low levels, often in the parts-per-billion (ppb) or parts-per-trillion (ppt) range. Because of this, analytical methods must be carefully validated to ensure they are reliable, reproducible, and capable of detecting nitrosamines accurately. Validation provides confidence that the measured levels reflect the true content in the drug product and that decisions made based on these results, such as batch release or process changes, are scientifically sound. Regulatory agencies including the FDA, EMA, and Health Canada emphasize that analytical methods for nitrosamines must be fit for their intended purpose. This means that methods must detect and quantify nitrosamines at levels well below acceptable intake limits; be robust and reliable in complex sample matrices like tablets, capsules, or API powders; minimize risk of artifactual formation during sample handling and preparation; and be fully documented with validated parameters that support regulatory submissions and ongoing quality control. Validation is not simply a formal requirement; it is a safeguard to protect patients, ensure compliance, and support confidence in the manufacturing process [12]-[54].

7.1. Validation Parameters

A well-validated method must demonstrate performance across multiple dimensions. Each parameter provides unique assurance about accuracy, precision, and reliability.

7.1.1. System Suitability

System suitability tests ensure that the analytical instrument is working correctly before sample analysis begins. This may include confirmation of baseline stability of the detector, checking resolution and separation between nitrosamines and other peaks, verifying retention time consistency to ensure identification is accurate, and assessing signal sensitivity for trace detection. System suitability ensures that any problems with the instrument, such as contamination, drift, or hardware issues, are detected before analyzing critical samples, preventing unreliable results.

$$\%\text{RSD} = \frac{\text{Standard Deviation}}{\text{Average Peak Area}\left(n=6\right)} \times 100 \tag{3}$$

The number of replicate injections and acceptance criteria should be defined and justified for the analytical procedure.

7.1.2. Specificity and Selectivity

Specificity ensures the method measures only the target nitrosamine and no other chemicals, excipients, or degradation products. Selectivity is slightly broader: it confirms the method can distinguish nitrosamines from structurally similar compounds. For example: in a tablet formulation, multiple ingredients may have similar chemical properties, but a specific, selective method can identify NDMA (N-nitrosodimethylamine) without interference from other nitrogen-containing molecules. Without specificity and selectivity, measured results could be misleading, giving false positives or negatives.

7.1.3. Linearity and Calibration Range

Linearity confirms that the analytical response (signal) increases proportionally with nitrosamine concentration across the intended range. Calibration involves preparing standard solutions at multiple concentrations and plotting a response curve. A linear response ensures that the method can accurately interpolate concentrations between measured points. The calibration range must cover expected levels (at least 5 levels) in the sample, including ultra-trace amounts, to provide confidence across both low and high concentrations. A method that lacks linearity may underestimate or overestimate nitrosamine levels, potentially compromising safety.

$$y = mx + b\,. \tag{4}$$

where y = peak area, x = concentration, m = slope, b = y-intercept.

$$\text{Y bias} = \frac{\text{Y-intercept}}{\text{Peak Area at 100\%}} \times 100\,. \tag{5}$$

Any acceptance criterion for intercept bias should be scientifically justified and should not be treated as an ICH-mandated requirement.

7.1.4. Accuracy and Recovery

Accuracy measures how close the analytical result is to the true nitrosamine con-

centration. Recovery studies are commonly performed by spiking known amounts of nitrosamines into the sample matrix and measuring how much is recovered after preparation and analysis. High recovery (usually 90% - 110%) indicates that the method accurately reflects the true content. Recovery studies also account for matrix effects, which can cause signal suppression or enhancement in complex formulations. Accuracy is essential to trust the data used for regulatory compliance and to ensure patient safety.

$$\%\text{Recovery} = \frac{\text{Amount Found}\left(\frac{\mu g}{mL}\right)}{\text{Amound Added}\left(\frac{\mu g}{mL}\right)} \times 100. \tag{6}$$

7.1.5. Precision (Repeatability and Intermediate Precision)

Precision assesses the consistency of results under repeated measurements: Repeatability: Same analyst, instrument, and conditions over a short time period. Intermediate precision: Variation across different analysts, instruments, or days. High precision ensures that the method provides reliable, reproducible data, which is crucial for confirming that nitrosamine levels are consistently within safe limits.

$$\%\text{RSD} = \frac{\text{Standard Deviation}}{\text{Average of \%Impurity}\left(n=6\right)} \times 100. \tag{7}$$

7.1.6. Limit of Quantitation (LOQ)

LOQ is the lowest concentration that can be quantified with acceptable accuracy and precision. To determine the LOQ, linearity solutions are diluted near the expected quantitation limit and analyzed for signal-to-noise ratio (S/N), with six replicate injections performed to confirm method reliability at low concentrations. For QL, a ratio of at least 10:1 is considered acceptable. This QL will also be determined based on the standard deviation linear response and a slope.

$$\text{LOQ} = \frac{10\sigma}{S}. \tag{8}$$

where σ = the standard deviation of the response S = the slope of the calibration curve.

7.1.7. Limit of Detection (LOD)

LOD represents the lowest concentration that can be reliably detected, though not necessarily quantified. For nitrosamines, LOD must be well below regulatory thresholds because even trace amounts can be harmful. LOD ensures that no potentially dangerous nitrosamine goes undetected. LOD is similarly determined by diluting linearity solutions near the expected detection limit, evaluating S/N ratios, and performing three replicate injections. For DL, a ratio of at least 3:1 is considered acceptable. This DL will also be determined based on the standard deviation linear response and a slope.

$$\text{LOD} = \frac{3.3\sigma}{S}. \tag{9}$$

7.1.8. Robustness

Robustness measures the method's resilience to small, deliberate variations in experimental conditions: Minor changes in pH, flow rate, column temperature, and mobile phase composition should not significantly affect results. Robust methods provide confidence that the method will perform reliably in routine laboratory conditions, even when minor variations occur.

$$\%\text{Difference for Sample} = \frac{\text{Absolute}\left(\text{Initial Result} - \text{Each Robustness Result}\right)}{\text{Initial Result}} \times 100. \quad (10)$$

7.1.9. Solution and Sample Stability

Nitrosamines can form or degrade during sample handling. Stability studies confirm that: Nitrosamines remain unchanged during extraction, storage, and analysis. Sample solutions can be analyzed over a practical time window without affecting results. This prevents artificially inflated or decreased readings and ensures that the measured nitrosamine levels reflect the product as it exists in reality.

$$\%\text{Difference for Standard} = \frac{\text{Absolute}\left(\text{Initial Peak Area} - \text{Stability Point Peak Area}\right)}{\text{Initial Peak Area}} \times 100. \quad (11)$$

$$\%\text{Difference for Sample} = \frac{\text{Absolute}\left(\text{Initial Result} - \text{Stability Point Result}\right)}{\text{Initial Result}} \times 100. \quad (12)$$

The recommended Method Validation Parameters and Acceptance Criteria are presented in Table 4. The nitrosamine validation parameters are presented in Figure 12.

Table 4. Recommended method validation parameters and acceptance criteria.

Parameter	Recommended Acceptance Criteria
System Suitability	%RSD NMT 20%, USP Tailing between 0.8 to 1.8, Plate Count NLT 2000.
Specificity	No interference from matrix; resolution > 1.5 from closest peak
Linearity	$R^2 \geq 0.999$; y-intercept bias ≤ 2% of response at LOQ
LOD	S/N ≥ 3; typically, 10% - 30% of AI limit; Confirmed by peak shape assessment
LOQ	S/N ≥ 10; ≤30% of specification limit; precision ≤ 20% RSD
Precision	%RSD ≤ 10%
Intermediate Precision	%RSD ≤ 20%; across analysts, days, instruments
Accuracy	%Recovery 70% - 130% at LOQ; 80% - 120% at ≥ LOQ
Robustness	Evaluate pH ±0.2, flow rate ±0.1 mL/min, column temp ±5˚C
Matrix Effect/Ion Suppression	<±20% signal variation vs. neat standard
Stability	Deviation <±15% from nominal concentration

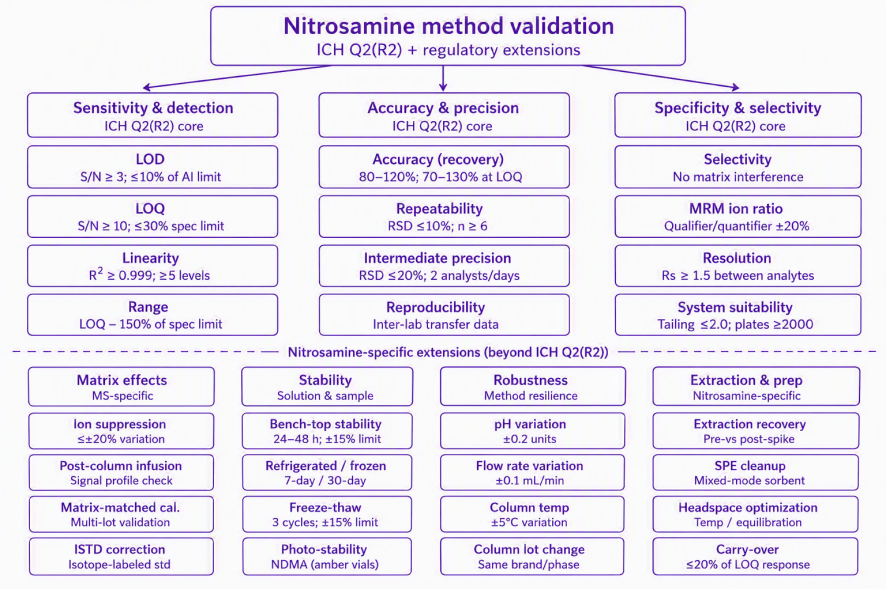


Figure 12. Nitrosamine method validation parameters.

8. Lifecycle Approach to Analytical Method Validation for Nitrosamine Impurity Testing

8.1. Overview

The adoption of ICH Q2(R2) in 2023 replaced the static, one-time validation paradigm of its predecessor with a continuous three-stage lifecycle model that begins at method conception and extends through the entire commercial life of the drug product. For nitrosamine analytical methods, this lifecycle framework carries particular regulatory weight because the impurity landscape continues to evolve new AI limits for previously uncharacterized NDSRIs are published on a rolling basis, novel nitrosamines are identified in drug classes previously considered low risk, and product reformulations undertaken to reduce nitrosamine burden require re-evaluation of method performance in modified matrices. A method validated against a fixed parameter checklist at a single point in time may be insufficient for a field characterized by this degree of regulatory and scientific dynamism. The lifecycle model addresses this by treating method validation not as a regulatory checkpoint but as part of an ongoing, scientifically managed analytical procedure lifecycle.

8.2. Stage 1 Analytical Target Profile and Method Operable Design Region

In the proposed framework used in this review, Stage 1 encompasses activities from the initial method concept through the characterization of the Method Operable Design Region (MODR). The defining deliverable of Stage 1 is the Analytical Target Profile (ATP), which must be established before any method development experiments are conducted and which serves as the performance contract against which all subsequent validation decisions are evaluated. For a nitrosamine

LC-MS/MS method, the ATP may specify the identity of each target analyte, the required LOQ expressed as a percentage of the AI-based specification limit (mandated at ≤30% per FDA Guidance Revision 2), the accuracy requirement at the LOQ (70% - 130%), the precision requirement expressed as percent RSD (≤20% at the LOQ), the acceptable range of ISTD-normalized matrix effects (0.85 - 1.15), and the ion ratio tolerance for each analyte (±20% of the reference value in neat standard). Design of Experiments approaches, typically Plackett-Burman screening designs followed by central composite or Box-Behnken response surface designs are used to map the MODR by characterizing the method's analytical response surface across the key variables identified during initial scouting, including mobile phase pH, organic modifier percentage, column temperature, flow rate, source temperature, and capillary voltage. The MODR defines the parameter space within which all ATP acceptance criteria are simultaneously satisfied, and its boundaries govern the operational flexibility of the method throughout its commercial lifecycle without requiring regulatory notification of minor adjustments that remain within the established design space.

8.3. Stage 2 Formal Qualification and Validation

Within the proposed framework, Stage 2 is the formal confirmation that the method meets its predefined acceptance criteria, under actual analytical conditions using the drug product matrix in which it will be deployed commercially. It corresponds most closely to the classical ICH Q2(R1) validation exercise but is explicitly framed as ATP verification rather than parameter compliance, permitting greater scientific flexibility in prioritizing validation resources proportionate to risk. For nitrosamine methods, this means that matrix effects, LOQ performance in the target matrix, and NDMA photostability receive elevated experimental attention relative to parameters that present lower analytical risk for the specific method-matrix combination.

8.4. Stage 3 Continued Method Performance Verification

Stage 3, Continued Method Performance Verification (CMPV), is the genuinely transformative element of the ICH Q2(R2) lifecycle model and the stage with the most direct implications for commercial nitrosamine QC operations. CMPV requires systematic, ongoing monitoring of method performance throughout the commercial life of the drug product, with the specific purpose of detecting performance changes before they cause incorrect analytical results. For a nitrosamine LC-MS/MS method, appropriate potential CMPV indicators include the ISTD peak area response per injection run, monitored on Shewhart X-bar statistical process control charts with illustrative warning limits at $\pm 2\sigma$ and action limits at $\pm 3\sigma$; the qualifier-to-quantifier ion ratio for each analyte in system suitability standards, tracked over time to detect changes in source conditions or column selectivity; analyte retention time relative to the ISTD, sensitive to mobile phase pH drift or column aging; and the ISTD-normalized matrix factor from matrix-

matched calibration standards prepared from successive drug product lots, detecting shifts in excipient background attributable to supplier or grade changes. The selected indicators, statistical methods, frequencies, and alert/action limits should be justified using method knowledge, historical performance, and risk.

8.5. Nitrosamine-Specific CMPV Example NDMA in Metformin Extended-Release Tablets

To illustrate CMPV in practice, consider a validated GC-MS/MS headspace method for NDMA in metformin extended-release 1000 mg tablets (MDD 2000 mg/day; AI-based specification limit 0.013 ppm; validated LOQ 0.004 ppm). Following commercial launch, the laboratory establishes CMPV monitoring of four indicators on every analytical sequence: (i) the deuterated NDMA-d_6 internal standard peak area per injection, baseline-established from the first 30 commercial runs and monitored with $\pm 2\sigma$ warning and $\pm 3\sigma$ action limits; (ii) the headspace equilibration temperature log, confirmed within ±2˚C of the validated nominal at each run; (iii) the S/N ratio at the LOQ from the lowest calibration standard, confirmed ≥10 at each run; and (iv) the matrix factor from a spiked drug product extract at 50% of the specification limit, confirmed within 0.85 - 1.15 after ISTD normalization. At month 14 of commercial manufacture, the ISTD peak area shows a progressive downward trend across five consecutive analytical sequences, crossing the -2σ warning limit on the fifth occasion. Root cause investigation reveals that the headspace vial septa have begun showing compression-related leakage at elevated temperatures, reducing headspace transfer efficiency. Corrective action such as replacement of the septum lot and re-qualification of the headspace system restores ISTD response to the established baseline within the control limits. The incident is documented in the laboratory deviation management system and reviewed at the next Annual Product Review alongside NDMA batch release trend data. This CMPV-driven intervention prevented continuation of a method performance degradation that, had it progressed, would have caused underestimation of NDMA levels in commercial batches at concentrations near the specification limit a patient safety outcome that end-product batch testing alone would not reliably have detected.

9. Case Studies of Nitrosamine Contamination

Understanding nitrosamine contamination in pharmaceutical drug products is greatly enhanced by examining high-profile real-world cases that have collectively reshaped regulatory expectations, analytical practice standards, and quality system requirements across the global industry. The cases of valsartan, ranitidine, and metformin are particularly instructive because each illustrates a mechanistically distinct pathway of nitrosamine formation process-derived contamination, intrinsic API instability, and formulation-dependent generation, respectively and because the regulatory and industry responses to each have progressively refined the risk assessment frameworks that now govern nitrosamine control under the FDA Guidance Revision 2 (September 2024) and ICH M7(R2).

9.1. Valsartan Process-Derived NDMA Contamination

The discovery of N-nitrosodimethylamine (NDMA) contamination in valsartan drug substance in 2018 constitutes the foundational event of the modern pharmaceutical nitrosamine crisis. Root cause investigations conducted by the FDA, EMA, and multiple independent laboratories established that the contamination originated from a manufacturing process change introduced several years earlier at certain API suppliers, specifically the use and recovery of dimethylformamide (DMF) as a process solvent in the tetrazole ring synthesis step. Recovered DMF contained trace dimethylamine impurities which, upon exposure to sodium nitrite used in the subsequent azide quench step under acidic conditions, underwent N-nitrosation via the Dimroth rearrangement to produce NDMA at concentrations of 50 - 200 ppm exceeding the established AI-based specification limit of 0.083 ppm by a factor of 600 - 2400. The public health impact was substantial: millions of patients across more than forty countries were potentially exposed to supra-limit NDMA levels for an extended period, necessitating one of the largest pharmaceutical recall events of the decade. The regulatory response was equally far-reaching, with the FDA and EMA issuing guidance mandating a three-step risk assessment, confirmatory testing, and mitigation strategy for all drug products and APIs. At the manufacturing level, the primary corrective actions included elimination of DMF and structurally related dimethylamide solvents from the synthetic route, introduction of in-process monitoring for nitrite and nitrosamine levels at critical synthetic steps, tightening of raw material specifications for sodium azide and sodium nitrite purity, and substantial strengthening of supplier qualification programs. The valsartan case established the paradigm that even well-established, long-marketed APIs must be proactively re-evaluated for nitrosamine risk following any process or supplier change, and that nitrosamine risk assessment must be embedded as a permanent component of the change control process [55] [56].

9.2. Ranitidine Endogenous NDMA Generation from API Instability

The ranitidine crisis, which unfolded between 2019 and 2020, illustrated a fundamentally different and in many respects more challenging mechanism of nitrosamine formation: endogenous generation of NDMA from the API itself rather than from process-derived contaminants or extraneous nitrosating agents. Ranitidine's inherent molecular architecture containing both a dimethylaminomethyl moiety and a nitroguanidino group renders the molecule susceptible to intramolecular rearrangement and degradation under thermal and oxidative conditions, releasing dimethylamine which subsequently undergoes N-nitrosation under physiological or storage-related conditions. Independent laboratory analysis demonstrated that NDMA levels in ranitidine drug products increased substantially under accelerated storage conditions at 37˚C and above, with some products generating NDMA equivalent to millions of nanograms per tablet orders of magnitude

above the AI limit of 26.5 ng/day. The FDA's April 2020 request for market withdrawal of all ranitidine products globally represented the first instance in the modern regulatory era of a drug being withdrawn primarily on the basis of intrinsic API chemical instability leading to nitrosamine generation, rather than manufacturing process contamination. The ranitidine case established two critical precedents that now inform the current regulatory framework: first, that API structural stability assessment must be considered an integral component of nitrosamine risk evaluation for any new or existing drug product; and second, that nitrosamines can continue to form after the drug product leaves the manufacturing facility during distribution and shelf storage, necessitating stability-indicating nitrosamine testing as part of the formal stability program rather than solely at the time of manufacture [57] [58].

9.3. Metformin Formulation-Driven NDMA Formation

Metformin, the world's most widely prescribed antidiabetic agent, presented a third mechanistic scenario: NDMA formation driven primarily by the interaction between the API and excipients within the formulation matrix, particularly in extended-release dosage forms with prolonged contact between drug substance and excipients during storage. Investigations revealed that metformin's chemical structure allows for release of trace dimethylamine as a degradation product, which then reacts with trace nitrite contributed by common excipients including microcrystalline cellulose, polyvinylpyrrolidone, and sodium starch glycolate to generate NDMA at levels exceeding the AI-based specification limit of 0.013 ppm in the highest-dose extended-release formulation (MDD 2000 mg/day). The extreme stringency of this limit 0.013 ppm, representing one of the most demanding nitrosamine specifications in the pharmaceutical industry arose from the exceptionally high maximum daily dose of metformin, which amplifies even trace nitrosamine concentrations into pharmacologically significant daily exposures. Several extended-release Metformin products were voluntarily recalled based on FDA and independent laboratory testing findings. The mitigation strategies investigated and implemented by manufacturers included excipient nitrite content specification and testing, antioxidant addition to the formulation (ascorbic acid, alpha-tocopherol) to scavenge nitrosating species, modified packaging using nitrogen purge and desiccant systems to minimize humidity-driven degradation during shelf life, and reformulation of certain extended-release matrix systems to reduce the API-excipient contact time and temperature during manufacturing. The metformin experience reinforced that nitrosamine risk assessment must be conducted at the formulation level not solely at the API synthesis level and that the combination of high drug load, reactive excipient impurities, and extended shelf life creates a uniquely demanding nitrosamine control environment [59] [60].

9.4. Lessons Learned and Industry Implications

Taken together, the valsartan, ranitidine, and metformin cases provide a compre-

hensive map of the mechanistic diversity of pharmaceutical nitrosamine contamination and a clear set of principles that now underpin industry best practice and regulatory expectation. Nitrosamine formation is inherently multi-factorial: it can originate from process chemistry decisions made years before the contamination is detected, from the intrinsic instability of the API molecule itself, or from apparently innocuous excipient-API interactions that only become significant under the right combination of temperature, humidity, pH, and time. This mechanistic diversity demands that risk assessment be genuinely comprehensive encompassing the complete synthetic route, all raw materials and reagents, the formulation composition and manufacturing process, excipient nitrite content, packaging configuration, and the entire anticipated shelf life under real and accelerated storage conditions. Analytical vigilance is inseparable from risk management. Each of these cases revealed a period during which contamination existed but was undetected, either because ultra-sensitive analytical methods had not been developed and validated for the specific matrix, because the formation pathway had not been anticipated in the risk assessment, or because routine quality control testing was not sensitive enough to detect nitrosamines at AI-relevant concentrations. The development and validation of methods capable of quantifying nitrosamines at or below 30% of the AI-based specification limit demanding sensitivity in the low parts-per-million to parts-per-billion range is therefore not merely a regulatory compliance obligation but a fundamental patient safety requirement. Finally, these cases collectively demonstrated that no drug class, dosage form, or manufacturing geography is inherently exempt from nitrosamine risk. From a small-molecule cardiovascular agent manufactured through a complex tetrazole synthesis to a simple antidiabetic tablet formulated with standard excipients, the potential for nitrosamine formation is present wherever vulnerable amines and nitrite sources coexist. The adoption of the FDA's three-step risk assessment framework, the application of the Carcinogenic Potency Categorization Approach for NDSRIs, and the implementation of the ICH Q2(R2) lifecycle model for ongoing analytical method performance verification collectively represent the industry's most mature response yet to a challenge that continues to evolve as new drug-substance-specific nitrosamines are identified and as the scientific understanding of nitrosamine formation mechanisms deepens.

10. Emerging Technologies and Future Perspectives

As the pharmaceutical industry continues to respond to nitrosamine contamination challenges, emerging technologies and innovative strategies are shaping the future of detection, risk mitigation, and regulatory compliance. These developments aim to enhance sensitivity, reliability, and proactive risk management, ensuring that nitrosamines are effectively controlled across the product lifecycle.

10.1. Advanced Analytical Techniques for Nitrosamine Detection

Traditional analytical methods such as GC-MS and LC-MS/MS have proven ef-

fective, but the field is advancing rapidly. High-resolution mass spectrometry (HRMS) offers high sensitivity and selectivity, allowing laboratories to detect ultra-trace nitrosamines even in complex matrices. Newer multi-analyte detection platforms can simultaneously detect multiple nitrosamines in a single run, saving time and resources while improving risk assessment. Miniaturized and automated sample preparation techniques, such as micro-extraction and automated solid-phase extraction, may reduce the risk of artifact formation and improve reproducibility. Coupled techniques combining GC or LC with HRMS provide structural confirmation, ensuring accurate identification of unknown nitrosamines. Definitive confirmation may additionally require an authentic reference standard and orthogonal analytical evidence. Together, these advances not only improve analytical sensitivity and accuracy but also support regulatory compliance by providing robust, reproducible, and scientifically defensible data.

10.2. Process Analytical Technology (PAT) Applications

Process Analytical Technology (PAT) is transforming how nitrosamines are controlled in real time. In-line or online monitoring may enable continuous surveillance of critical process parameters such as temperature, pH, and nitrite levels that can influence nitrosamine formation. Real-time sensors can identify the presence of amines or nitrites before they react to form nitrosamines, allowing for immediate intervention. Data from PAT systems can further be used to automatically adjust process conditions, reducing reliance on end-product testing alone. By integrating appropriately qualified PAT into manufacturing workflows, companies can proactively control nitrosamine risk and significantly reduce the likelihood of contamination and product recalls.

10.3. Predictive Risk Modeling and AI-Based Monitoring

Artificial intelligence and predictive modeling are becoming increasingly valuable tools in nitrosamine risk management. Predictive risk models leverage historical data, chemical knowledge, and process parameters to forecast where and when nitrosamines might form. Machine learning algorithms can analyze large datasets across multiple batches, identifying subtle trends that may indicate emerging risk before nitrosamines become analytically detectable. These models require adequate, representative data and experimental or analytical verification and should not replace confirmatory testing. Decision support systems further guide manufacturers in prioritizing testing, implementing controls, and optimizing processes. Collectively, these technologies move industry from a reactive posture to a predictive, science-based approach, enabling smarter resource allocation and more effective risk mitigation.

10.4. Continuous Manufacturing and Nitrosamine Risk Control

Continuous manufacturing presents potential opportunities for reducing nitrosamine formation. Continuous flow reactors maintain uniform temperature, pres-

sure, and pH, minimizing the conditions that favor nitrosation reactions. Smaller reaction volumes inherently limit opportunities for unwanted side reactions, and seamless integration with PAT and real-time analytics allows immediate process adjustments when conditions approach risky thresholds. Compared to traditional batch manufacturing, continuous processes enhance control, reduce variability, and improve overall product quality advantages that are especially significant for high-risk APIs. However, continuous manufacturing does not inherently eliminate nitrosamine risk, and its benefit must be demonstrated for the specific process chemistry.

10.5. Global Harmonization of Nitrosamine Regulations

A persistent challenge in nitrosamine control is regulatory variability, as different agencies maintain slightly different acceptable intake limits, testing requirements, and reporting expectations. Global harmonization efforts aim to align these guidelines, providing consistent frameworks for manufacturers operating across multiple regions. Harmonized regulations facilitate method standardization, risk assessment alignment, and efficient compliance, reducing duplication of effort while strengthening patient safety. Achieving this alignment will require sustained international collaboration among regulators, industry groups, and scientific communities to ensure clarity, consistency, and continued innovation in nitrosamine management. In summary, the future of nitrosamine control rests on a convergence of advanced analytical technologies, real-time process monitoring, predictive modeling, continuous manufacturing, and regulatory harmonization. By embracing these emerging capabilities, the pharmaceutical industry can move progressively from reactive detection to proactive prevention ultimately ensuring that patients receive safe, high-quality medicines in which nitrosamine impurities are prevented, minimized, and controlled within acceptable limits.

11. Conclusion

Nitrosamine impurities represent one of the most significant pharmaceutical quality and regulatory challenges of the current era. The evolution from the initial sartan recall crisis to the current state of comprehensive, validated, risk-based nitrosamine control programs reflects remarkable industry-wide progress within a compressed timeframe. However, significant work remains to close analytical sensitivity gaps for complex DSNIs, standardize global regulatory expectations, and establish long-term commercial monitoring frameworks. For pharmaceutical industry professionals, the key takeaways from this review are fourfold. First, a deep understanding of the chemistry of nitrosamine formation is indispensable for identifying and mitigating risks across the drug product lifecycle. Second, method validation for nitrosamine impurities demands exceptional rigor, particularly with respect to matrix effects, standard and sample-solution stability, and sensitivity at AI-limit concentrations. Third, risk assessment must be comprehensive and iterative, addressing synthesis, formulation, excipients, packaging, and stor-

age conditions as potential contributors to nitrosamine burden. Fourth, maintaining current awareness of rapidly evolving regulatory guidance is essential, as the scientific and regulatory consensus on nitrosamine risk management continues to develop. The ultimate goal, ensuring patient safety through the consistent delivery of drug products free from unacceptable levels of potent carcinogens, provides both the motivation and the framework for the ongoing collaborative effort among industry, regulators, and the scientific community to address this enduring pharmaceutical challenge.

Author Contributions

Sai Krishna Bompelliwar conceived the study, defined the review scope and structure, led the literature search and synthesis across all sections, drafted the manuscript in its entirety including the regulatory framework, risk assessment calculation methodology, analytical validation parameters, and lifecycle approach sections, and served as the corresponding author responsible for all revisions and submission. Ravi Teja Meduri contributed to the development of the nitrosamine formation mechanism content, the case study analyses for valsartan, ranitidine, metformin, and losartan, and provided critical review and editing of the analytical methods and validation sections. Rasheed Babu Shaik contributed to the compilation and verification of the reference list, the preparation of the chemical structure figures and flowchart diagrams, and reviewed the regulatory framework and acceptable intake limit sections for accuracy and completeness. Abhishek Kumar Mishra contributed to the CPCA structural determinants section, the alpha-carbon hydrogen scoring discussion, and provided technical review of the GC-MS/MS and LC-MS/MS method validation content. Mohit Chintakindi Bhasker contributed to the lifecycle approach section including the Analytical Target Profile, Method Operable Design Region, and Continued Method Performance Verification content, and assisted with the preparation and critical revision of the final manuscript. Naveen Kumar Mallampati supervised the study, reviewed, and edited the manuscript. All authors read and approved the final version of the manuscript for submission.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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