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Research Article

Nitrosamine Impurities in Active Pharmaceutical Ingredients: Formation Mechanisms, Analytical Profiling, Toxicological Risk Assessment, and Regulatory Control Strategies

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ABSTRACT

Nitrosamine impurities in active pharmaceutical ingredients (APIs) and finished medicines require careful control because some members of this chemical group are genotoxic and carcinogenic. They may be introduced through contaminated starting materials, reagents, solvents or shared equipment, or form when susceptible amines encounter nitrosating agents during synthesis, formulation or storage. Nitrosamine drug substance-related impurities (NDSRIs) are especially challenging because their structures and toxicological profiles may be specific to the parent drug. This narrative review discusses nitrosamine classes and formation pathways, analytical methods, toxicological assessment, regulatory expectations and risk-reduction measures. LC–MS/MS and GC–MS/MS support targeted measurement, whereas LC–HRMS can help investigate unexpected impurities. Performance must be established for each analyte and sample matrix. Assessment is also complicated by limited reference standards and toxicological data for some NDSRIs. A suitable control programme therefore combines product-specific risk assessment, validated testing, justified acceptable-intake limits and preventive controls throughout the product lifecycle. Better characterisation of NDSRIs, comparable reporting of analytical performance and further validation of predictive toxicology tools remain research priorities.

INTRODUCTION

An active pharmaceutical ingredient (API) is the drug substance that produces the intended pharmacological effect. Impurity profiling involves detecting,

identifying and characterising impurities and quantifying them where suitable methods are available; finding an impurity alone does not establish its toxicological significance¹⁻⁴. Pharmaceutical nitrosamines have

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drawn regulatory attention after their detection in several medicines led to investigations, recalls and other actions. Their hazards differ by compound, so the presence and level of each relevant nitrosamine must be evaluated rather than treating the group as uniformly toxic. Possible sources include raw materials, reagents, solvents, recovered materials, manufacturing reactions, excipients and reactions occurring during storage⁵⁻⁸.

Risk assessments commonly distinguish small-molecule nitrosamines, including N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), from nitrosamine drug substance-related impurities (NDSRIs), which are structurally associated with a particular API. Whether an NDSRI can form depends on the drug structure, available precursors and conditions during manufacture or storage. Consequently, the risk controls for one medicine should not automatically be applied to another^{7,8}.

A defensible control strategy goes beyond testing a finished batch. It considers manufacturing and formulation risks, uses analytical methods capable of distinguishing the target impurity from matrix components, evaluates toxicological evidence, establishes a justified acceptable intake (AI), and reduces opportunities for contamination or formation. Trace concentrations, matrix interference, limited reference standards and uncertain NDSRI toxicology can complicate these assessments^{6,7}.

This review examines the main pharmaceutical nitrosamine categories and their formation, compares analytical approaches, summarises toxicological and regulatory assessment, and discusses prevention and mitigation. It focuses on connecting root-cause assessment, confirmatory analysis and risk-based controls across the product lifecycle.

MATERIALS AND METHODS

This article is a narrative review, not a systematic review. The existing reference list was used as an initial source set; bibliographic details and regulatory documents were then checked through targeted searches of public bibliographic resources and official agency websites. Search topics covered pharmaceutical nitrosamines, NDSRIs, nitrosation chemistry, LC-MS/MS, LC-HRMS, GC-MS, the Carcinogenic

Potency Categorisation Approach (CPCA), acceptable intake and pharmaceutical risk management.

Priority was given to guidance from the US Food and Drug Administration (FDA), European Medicines Agency (EMA) and International Council for Harmonisation (ICH), alongside peer-reviewed reviews and primary studies on formation, analytical performance, toxicology and mitigation. Numerical method results are reported as examples from the cited studies, not as universal performance values. Regulatory limits and recommendations should be checked against the latest applicable guidance before submission.

The review did not use a predefined systematic-review protocol, comprehensive database searches, duplicate independent screening or a PRISMA flow diagram. It should therefore be interpreted as a narrative synthesis rather than an exhaustive systematic review.

RESULTS AND DISCUSSION

Classification and Sources

Pharmaceutical nitrosamine impurities can be grouped into two broad classes: small-molecule nitrosamines and NDSRIs. Regulatory documents and published studies describe this distinction^{7,8}.

Small-molecule nitrosamine impurities

Small-molecule nitrosamines generally have low molecular weights and lack a structural relationship to the API. They may enter the process through contaminated materials or arise from reagents, solvents and manufacturing chemistry.

NDMA:N-nitrosodimethylamine NDEA:N-nitrosodiethylamine NMBA:N-nitroso-N-methyl-4-aminobutyric acid NDIPA:N-nitrosodiisopropylamine NEIPA:N-nitrosoethylisopropylamine

Nitrosamine drug substance-related impurities (NDSRIs)

NDSRIs are structurally related to the API and are often associated with a particular drug substance. They may form when a susceptible amine group in the API or a related compound reacts with a nitrosating species during production, formulation or storage.



N-nitroso-varenicline, a varenicline-related nitrosamine.

N-nitroso-quinapril associated with quinapril

N-nitroso-orphenadrine, related to orphenadrine.

The likelihood of NDSRI formation depends on the API's molecular structure and whether the surrounding conditions support nitrosation.

Table 1 outlines key differences between the two categories.

Table 1: Comparison of Small-Molecule Nitrosamines and NDSRIs

Basis	Small-Molecule Nitrosamines	Nitrosamine drug substance-related impurities (NDSRIs)
Definition	Usually low-molecular-weight nitrosamines not structurally related to the API.	Nitrosamine impurities that are closely related in chemical structure to the active pharmaceutical ingredient or therapeutic compound.
Typical source	May arise from reagents, solvents, starting materials, intermediates, contamination, or process chemistry.	May form by nitrosation of a susceptible site in the API or a related substance.
Specificity	May occur across different products and manufacturing processes.	Often specific to a particular API and its formulation or process conditions.
Examples	NDMA, NDEA, NDIPA, NEIPA, and NMBA.	NDSRIs associated with specific drug substances; confirm the exact identity and nomenclature in the cited source.
Risk assessment	Use compound-specific toxicological data and the applicable authority's acceptable-intake recommendation.	May require structure-based assessment, read-across, or additional toxicological data when compound-specific evidence is limited.
Control focus	Identify and control contamination, nitrosating agents, amine sources, and process conditions.	Assess API structure, formulation/excipient compatibility, manufacturing conditions, and storage-related formation.

In general, small-molecule nitrosamines are not structurally linked to the API, whereas NDSRIs are. Both categories require evidence-based risk assessment and appropriate controls to protect patients.

Potential sources include starting materials, intermediates, reagents, solvents, process water, equipment, packaging and storage conditions ⁹. Contaminated inputs may introduce amines, nitrites or pre-existing nitrosamines. Under suitable conditions, sodium nitrite and certain solvents or reagents, including N,N-dimethylformamide (DMF), may contribute to nitrosamine formation ^{5,7}. Inadequately purified recovered solvents and shared equipment can also create

cross-contamination risks. In finished medicines, nitrite present in excipients or process water may react with susceptible drug substances ^{5,7}. Some packaging components, including nitrocellulose-containing lidding materials and printing inks, have also been identified as potential sources. Temperature, humidity and pH may influence product degradation and nitrosamine formation over time ^{5,7}. Risk assessments should therefore cover materials, process steps, packaging and stability conditions ^{5,7,9}.

Formation Mechanisms

Table 2 links common formation routes with contributing factors and practical points for risk reduction.

Table 2: Formation Pathways, Contributing Factors, and Risk-Reduction Focus

Stage/pathway	Potential reactants or source	Conditions/contributor	Risk-reduction focus
API synthesis	Susceptible secondary amines; tertiary amines may undergo nitrosative dealkylation; nitrite/nitrosating agents	Suitable pH, temperature, contact time, solvent and reaction sequence	Avoid/substitute nitrite where feasible; segregate reactive materials; optimize pH, temperature, addition order and quench
Formulation	Amine-containing API or related compounds plus nitrite impurities in excipients	Excipient nitrite level, moisture, pH, compatibility and processing	Qualify suppliers; assess lower-nitrite excipients where justified; perform compatibility and formulation risk studies
Storage/degradation	API/degradation products plus nitrosating species or precursors	Time, temperature, humidity/moisture, pH, packaging interaction	Assess stability and packaging; control moisture and storage conditions
Cross-contamination/materials	Pre-existing nitrosamines, amines or nitrites in solvents, reagents, water, recovered solvents or shared equipment	Material reuse, inadequate purification/cleaning, supplier variability	Supplier qualification, recovered-solvent controls, cleaning verification, material risk assessment
Packaging	Potentially reactive packaging components, inks or lidding materials	Product-contact or migration conditions over shelf life	Assess container-closure system using product-specific evidence and stability data

Nitrosation reactions involving nitrites, nitrosating agents and amines

Nitrosamines may form when susceptible amines are exposed to nitrite under suitable conditions, particularly in acidic environments. Acidification of nitrite produces nitrous acid (HNO_2), which can generate nitrosating species such as dinitrogen trioxide (N_2O_3) and the nitrosonium ion (NO^+).

Secondary amines can react with these species to produce N-nitrosamines⁵. Primary amines generally form other products rather than stable N-nitrosamines, while tertiary amines may undergo nitrosative dealkylation: loss of an alkyl group can generate a secondary amine that is subsequently nitrosated^{5,9}.

The reaction depends on pH, amine structure, nitrite concentration, temperature, contact time and solvent environment. In pharmaceutical production, risk can arise when susceptible amines and nitrosating agents come into contact through raw materials, reagents, solvents, recovered solvents, process water or specific process conditions^{5,9}.

Several process variables influence the extent of nitrosamine formation, particularly pH, temperature,

contact time and the choice of reagents and operating conditions.

Effects of pH, Temperature, Reaction Time, and Process Conditions

pH affects both the formation of reactive nitrosating species and the protonation state of amines. Under acidic conditions, nitrite can form nitrous acid and related reactive species. At very low pH, however, more amine may be protonated, reducing the availability of its unshared electron pair for reaction. The net effect therefore depends on the balance between nitrosating-species formation and the amount of reactive, unprotonated amine¹⁰.

Effect of Temperature

Temperature can alter the rate of nitrosation. When compatible amines and nitrosating agents are present, higher temperatures may increase formation rates. Tertiary amines may require additional reaction steps, such as dealkylation, before a nitrosamine is produced. Appropriate temperature control can help limit this risk^{5,10}.



Effect of Reaction Time

Longer contact between an amine and a nitrosating agent may increase nitrosamine formation when the reaction conditions favour nitrosation. For tertiary amines, additional time may be needed for dealkylation before nitrosation occurs. Hold times should therefore be assessed during process development and risk evaluation^{5,10}.

Effect of Manufacturing and Process Conditions

Reagent and solvent selection, reaction sequence, quenching and other process parameters can influence nitrosamine formation. Risk may increase when nitrite is added in acidic conditions in the presence of susceptible amines. Contaminated starting materials, recovered solvents, inadequate equipment cleaning and unsuitable process water are additional potential sources. Process design and material controls should address these factors together^{5,10}.

Formation During Synthesis, Formulation and Storage

During Synthesis

During API synthesis, secondary amines can react directly with nitrosating agents, while tertiary amines may first undergo nitrosative dealkylation. Starting materials, intermediates, reagents, solvents and reused materials may introduce precursors. The risk depends on the amines present and on reaction conditions, including acidity, temperature and reagent choice⁵.

During Formulation

Nitrosamines may also form in a finished dosage form when an amine-containing API or related impurity reacts with nitrite present in an excipient. The likelihood depends on excipient nitrite content, API chemistry and formulation conditions. Where supported by compatibility data, selecting lower-nitrite excipients can reduce this risk¹¹.

During Storage

Nitrosamine levels can rise during storage if the API degrades or reacts with nitrosating species in the formulation. Moisture and temperature may affect these reactions. Research on ranitidine formulations has reported temperature-dependent NDMA formation,

supporting the need to monitor relevant products under suitable stability conditions throughout shelf life⁶.

Analytical Methods for Detection

Analytical Approaches for Detecting Nitrosamine Impurities

Chromatography coupled with mass spectrometry is widely used to measure nitrosamines in APIs and finished medicines. Method selection depends on analyte properties, formulation matrix, required sensitivity and whether the task is targeted quantification or investigation of unknown compounds. LC-MS/MS, LC-HRMS and GC-MS/MS serve different purposes; conventional HPLC or UPLC with non-MS detection may not provide sufficient sensitivity for some trace-level measurements^{6,12,14}.

Liquid chromatography–tandem mass spectrometry (LC-MS/MS)

In LC-MS/MS, chromatography separates the target compounds and tandem mass spectrometry detects them through selected mass transitions. The technique can quantify known nitrosamines at trace levels, including compounds that are non-volatile or sensitive to heat. Reliability depends on sample preparation, chromatographic resolution, ionisation settings, matrix effects, suitable standards and validation. Multiple-reaction monitoring can improve selectivity when transitions are appropriately chosen^{6,12}.

Liquid Chromatography–High-Resolution Mass Spectrometry (LC-HRMS)

LC-HRMS provides accurate-mass measurements that support screening and structural investigation of suspected or unexpected impurities. Accurate mass alone may not prove a compound's identity; fragmentation patterns and, where available, comparison with a reference standard provide additional evidence. The FDA has published LC-HRMS methods for selected pharmaceutical formulations¹².

Gas chromatography–tandem mass spectrometry (GC-MS/MS)

GC-MS/MS is useful for volatile and some semi-volatile nitrosamines that remain stable under the analytical conditions. Gas chromatography separates



compounds according to volatility and interactions with the column, while tandem mass spectrometry adds selectivity. Headspace sampling can limit the introduction of non-volatile matrix components. LC-based methods may be preferable for analytes that are thermally unstable or poorly suited to gas chromatography⁶.

High-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC)

HPLC and UPLC separate analytes from the API and other formulation ingredients before detection. UPLC

commonly uses smaller stationary-phase particles, which can improve efficiency and shorten analysis time. UV or diode-array detection may be suitable when the analyte response and concentration are adequate, but these detectors may lack the sensitivity or selectivity required for some nitrosamines at trace levels. Coupling chromatography with mass spectrometry is often more appropriate for such applications¹².

Table 3 compares the typical uses and limitations of the main analytical platforms.

Table 3: Comparison of Analytical Methods

Method	Typical application	Strengths	Limitations
LC-MS/MS	Targeted quantification of known nitrosamines	High sensitivity and selectivity when transitions and sample preparation are suitable	Requires suitable standards and method development; matrix effects and interferences must be evaluated.
LC-HRMS	Screening and investigation of suspected or unexpected impurities	Accurate mass and fragmentation data support characterization	Data interpretation can be complex; confirmation and quantification may still require standards.
GC-MS/MS	Volatile or sufficiently thermally stable nitrosamines	Good chromatographic separation and selective detection	Not appropriate for every analyte; volatility, thermal stability, and matrix suitability matter.
HPLC/UPLC with non-MS detection	Separation and routine measurement where detector response is adequate	Robust chromatographic separation; UPLC may shorten run times	Conventional UV/diode-array detection may not reach the sensitivity required for trace nitrosamines.

These are general comparisons rather than guarantees of performance. Results depend on the target compound, sample matrix, instrument configuration and validation of the complete method^{6,12}.

Representative Method-Specific LOD and LOQ Data

Published limits of detection (LOD) and quantification (LOQ) vary with the compound, sample matrix and method. Table 4 gives one study-specific example.

Khorolskiy et al. reported an HPLC-MS/MS method using atmospheric-pressure chemical ionisation for model mixtures containing valsartan, losartan and irbesartan. The reported LOD was 0.2 ng/mL and LOQ was 0.4 ng/mL; these values apply to the conditions and matrices evaluated in that study and should not be assumed for other products¹⁵.

Table 4: Example of Method-Specific Analytical Performance

Study / method	Application	LOD	LOQ	Interpretation
Khorolskiy et al. ¹⁵	HPLC-MS/MS with APCI; model mixtures of valsartan, losartan, and irbesartan	0.2 ng/mL	0.4 ng/mL	Study-specific values; not directly transferable to other matrices or methods.



Method Validation of Nitrosamine Impurity Analysis

Validation demonstrates whether an analytical method is fit for its intended purpose in the presence of the API and other sample components. Studies of nitrosamine methods commonly examine accuracy, precision, recovery, matrix effects and robustness. The values below come from separate investigations and should be interpreted within each study's design and acceptance criteria ¹⁵⁻¹⁸.

Accuracy and Recovery

Accuracy describes agreement between a measured result and an accepted or expected value. Recovery is commonly assessed by adding a known amount of analyte to a sample and measuring the proportion recovered after preparation and analysis.

For a validated HPLC–MS/MS method covering NDMA, NDEA, NMBA and NEIPA in valsartan, losartan and irbesartan, reported recoveries met that study's 80–120% acceptance range for the compounds and levels tested. This supports performance under the investigated conditions, not automatic transfer to other matrices ¹⁵.

Precision

Precision describes how closely repeated measurements agree. It may be evaluated under repeatability conditions and through intermediate precision, for example by changing the day, analyst or column. Results are often expressed as relative standard deviation (%RSD); lower variability generally indicates better precision.

In an LC–APCI–MS/MS study of nitrosamines in sitagliptin, the reported intermediate-precision results met the study's criterion of less than 15% RSD. The assessment included changes in analyst, day and column of the same chemistry and brand ¹⁶.

Recovery

Recovery estimates how much of an added analyte the procedure measures. It should be checked at multiple

concentrations, including levels near the relevant specification or LOQ. In the cited sitagliptin study, recoveries ranged from 85.38% to 103.36%; results were more variable at the LOQ, showing why performance near the lower measurement limit needs particular attention ¹⁶.

Matrix Effects

Matrix effects occur when APIs, excipients or other sample constituents change the mass-spectrometric response, causing ion suppression or enhancement and potentially biasing the result even when chromatographic separation is adequate.

A study of nitrosamine measurement in rifampin and rifapentine evaluated matrix effects and mitigation options. These included improving sample preparation and chromatographic conditions and using appropriate internal standards. Matrix effects should be assessed for each relevant product matrix rather than assumed to be negligible ¹⁷.

Robustness

Robustness testing examines whether small, deliberate changes in analytical conditions affect method performance. Variables may include mobile-phase composition or pH, flow rate and column temperature.

In an LC–MS/MS study of a nitroso impurity in sitagliptin, investigators varied the flow rate from 0.36 to 0.44 mL/min and tested column temperatures of 27°C and 33°C. Reported recoveries for the unspiked samples at the studied concentrations were 97.73–104.17%. These findings illustrate the specific robustness checks performed in that method; they should not be treated as universal acceptance values ¹⁸.

Summary of Reported Validation Data

Table 5 summarises selected validation observations from separate studies; the entries do not describe one shared analytical method.



Table 5: Examples of Validation Findings Reported in the Literature

Validation aspect	Reported example	Source
Accuracy/recovery	85.38–103.36% across tested concentrations in the cited sitagliptin study	16
Precision	Reported precision met the study-defined criterion of less than 15% RSD	16
Matrix effects	Matrix-related interference required method optimization in rifampin/rifapentine analysis	17
Robustness	Deliberate changes in flow rate and column temperature were evaluated in a cited method	18

The values and observations in Table 5 come from different studies. Acceptance criteria should be justified for the method's intended purpose, relevant guidance and concentration range.

Challenges in Detecting Trace Nitrosamines and NDSRIs

1. Nitrosamines may occur at trace concentrations, so the method needs sufficiently low LOD and LOQ values. Accurate measurement may also be complicated by interactions between the API and excipients ¹².
2. Matrix components can suppress or enhance mass-spectrometric signals, creating biased concentration estimates if the effect is not evaluated and controlled ¹².
3. Because NDSRIs differ in structure and physico-chemical properties, an assay developed for one impurity may not be suitable for another. Compound-specific method development may be needed ⁶.
4. The absence of a suitable reference standard can limit identity confirmation, calibration, validation and reliable quantification of an NDSRI ⁶.

Toxicological Risk Assessment

Genotoxicity, Mutagenicity, and Carcinogenicity Concerns

Genotoxicity: Some nitrosamines undergo metabolic activation by cytochrome P450 enzymes, producing reactive intermediates that can bind to DNA and form DNA adducts. If such damage is not repaired correctly, it may contribute to carcinogenesis ¹⁹.

Mutagenicity: Experimental studies show that some nitrosamines can induce genetic mutations. The strength of this effect varies between compounds, so evidence should be evaluated for the individual substance ²⁰.

Carcinogenicity: Several nitrosamines produce tumours in experimental animals, but potency is not identical across the group. Risk from a pharmaceutical impurity depends on the compound and the level and duration of patient exposure ²¹.

Compound-Specific Acceptable Intake Limits and Lifetime Exposure

Acceptable intake (AI) is a compound-specific daily exposure limit derived to manage potential cancer risk. Because nitrosamines differ in potency, a single limit is not appropriate for every compound. When direct toxicological data are insufficient, agencies may consider structurally related compounds through read-across or use the Carcinogenic Potency Categorisation Approach (CPCA), where applicable ^{22–25}.

Structure-Based Approaches for Assessing Nitrosamine Impurities

When compound-specific toxicology is unavailable, structure-based methods can help estimate the potential carcinogenic potency of a nitrosamine. These methods are particularly relevant to NDSRIs, for which the parent API provides structural context. Common approaches include CPCA, structure–activity relationship (SAR) analysis and read-across from suitable analogues ²³.

The CPCA estimates the potency category of qualifying N-nitrosamines by examining features around the



N-nitroso group, including the number and arrangement of alpha hydrogens and structural features that may affect metabolic activation. It assigns one of five categories, each associated with a suggested AI. The approach is not suitable for every nitrosamine class; compounds such as N-nitrosamides and N-nitrosoureas may require other assessment methods ^{26,27}.

SAR analysis relates chemical structure to biological activity. Read-across estimates the toxicity of a substance using data from a sufficiently similar, well-characterised analogue. Either approach may inform an AI decision when the structural comparison and supporting toxicology are appropriate; uncertainty increases when the analogue is not closely matched ^{23,28}.

Role of Daily Dose, Treatment Duration, and Patient Exposure in Risk Assessment

Daily dose: Risk assessment uses the maximum daily amount of the medicine to estimate the impurity

concentration corresponding to a given AI. For a fixed AI, a higher daily dose means a lower allowable concentration in the medicine ²².

Treatment duration: Cumulative exposure depends on how long the medicine is taken. Lifetime-based limits commonly use a 70-year exposure assumption; adjustments for shorter treatment periods should only be applied where current regulatory guidance permits them ^{22,23}.

Risk-Assessment Calculation Example

For illustration, an AI of 26.5 ng/day and a maximum daily dose of 1000 mg/day give a concentration of 26.5 ng/day ÷ 1000 mg/day = 0.0265 ng/mg. This is equivalent to 0.0265 µg/g, 0.0265 ppm or 26.5 ppb. The example is hypothetical: the correct AI must be selected for the specific impurity using current applicable guidance ^{22,23}.

Table 6: Illustrative Acceptable-Intake Concentration Calculation

Parameter	Illustrative value	Calculation / interpretation
Illustrative acceptable intake (AI)	26.5 ng/day	Hypothetical example only; not a universal limit.
Maximum daily dose	1000 mg/day	Use the product-specific maximum daily dose.
Concentration calculation	AI ÷ maximum daily dose	26.5 ng/day ÷ 1000 mg/day = 0.0265 ng/mg.
Equivalent mass concentration	0.0265 ng/mg	Numerically equivalent to 0.0265 µg/g = 0.0265 ppm = 26.5 ppb.
Interpretation	Compare with a justified specification	Use the current compound-specific AI and applicable regulatory guidance.

Estimated daily exposure is calculated by multiplying the impurity concentration in the medicine by the maximum daily amount of that medicine. The estimated exposure is then compared with the applicable AI to determine whether further controls or regulatory action may be needed ²³.

Regulatory Guidelines

Risk assessment

Risk assessment is the starting point for controlling nitrosamines in APIs and finished medicines. Manufacturers should evaluate potential introduction or formation during synthesis, formulation, production and storage, including risks from starting materials,

reagents, solvents, excipients, water, process conditions and the API's structure. FDA and EMA guidance describes documented assessment and follow-up testing when a plausible risk is identified ^{7,28}.

Confirmatory Testing

Where a risk assessment identifies a credible possibility of contamination or formation, confirmatory testing should use a validated method with sensitivity appropriate to the relevant AI. FDA guidance discusses testing multiple batches—generally at least three unless a scientific justification supports an alternative—and comparison of results with the applicable compound-specific limit ^{7,23,29}.



Reporting Requirements

Verified nitrosamine findings and the associated corrective or preventive measures should be reported to the relevant authority when required by the applicable regulatory pathway. Results above the applicable limit, evidence of increased lifetime risk and newly identified impurities may trigger further reporting or application changes under FDA or EMA requirements. The exact obligations should be checked against current agency guidance ^{7,28}.

Specification Limits and Impurity Control

Specification limits should reflect the compound-specific AI, maximum daily dose, analytical capability and product-specific risk assessment. FDA guidance describes circumstances in which a separate specification may not be needed when confirmatory results are at or below 10% of the AI, provided the root cause is understood and effective manufacturing controls are established and verified. This is a conditional situation, not a general exemption from risk assessment or testing ⁷.

Corrective and Preventive Action

If levels exceed the applicable limit or the assessment identifies a meaningful formation risk, the manufacturer should investigate the cause and implement corrective and preventive actions. Options may include revising the synthesis route, controlling nitrite-containing excipients, strengthening supplier qualification, changing formulation or packaging, and adjusting process parameters. Follow-up testing should confirm that the actions are effective ^{7,28}.

ICH M7 (R2) Principles

ICH M7 (R2) sets out principles for identifying, assessing and controlling DNA-reactive mutagenic impurities to limit potential carcinogenic risk. It addresses hazard assessment, acceptable intakes, control strategies and lifecycle management. Nitrosamines may require more specific evaluation because their potency can differ substantially from that of other mutagenic impurities ³⁰.

Prevention, Control, and Mitigation

Table 7 summarises key control measures and the evidence that can be used to monitor their implementation across the product lifecycle.

Table 7: Prevention and Mitigation Strategies

Control point	Prevention / mitigation strategy	Evidence or monitoring
Raw materials and suppliers	Assess nitrite, amine and pre-existing nitrosamine risks; qualify suppliers; review specifications and certificates	Supplier documentation, targeted testing when risk justifies it, trend review
Process chemistry	Avoid unnecessary contact between nitrosating agents and susceptible amines; consider route/reagent substitution; optimize pH, temperature, addition order, hold time and quench	Process risk assessment, development studies, validated change control
Solvents, reagents and equipment	Control recovered solvents; verify purification; prevent cross-contamination through cleaning, maintenance and segregation	Incoming material controls, cleaning verification, recovery-process controls
Excipients and formulation	Assess excipient nitrite levels and API-excipient compatibility; choose lower-risk alternatives where justified	Excipient risk data, compatibility studies and confirmatory testing
Packaging and storage	Assess container-closure components; study moisture, temperature, pH and shelf-life effects	Stability studies, packaging assessment, batch trend monitoring
Analytical lifecycle control	Use fit-for-purpose validated methods and risk-based confirmatory testing	Method validation, batch testing as indicated, investigation and CAPA



Raw Material Selection and Control of Nitrite and Amine Sources

Materials used in API synthesis and finished-product manufacture should be assessed for nitrite, nitrosating agents, pre-existing nitrosamines and susceptible amines. Potential sources include excipients, water, starting materials and industrial process inputs. Supplier qualification, review of certificates of analysis and material specifications, and risk-based testing can help identify hazards. Testing and supplier controls should be strengthened when the assessment indicates a credible risk^{7,28}.

Process Changes to Reduce or Prevent Nitrosamine Formation

Manufacturing steps should be reviewed to identify conditions that favour nitrosamine formation. Possible changes include replacing nitrite-containing materials where feasible, altering the order of addition, controlling pH and temperature, limiting hold times under nitrosating conditions, or selecting alternative reagents and solvents. For NDSRIs, changes to the synthesis route or formulation may be needed to reduce contact between the API and nitrosating species. Each change should be validated to ensure impurity reduction without compromising API quality, stability or efficacy^{7,28}.

Prevention of Cross-Contamination and Control of Equipment, Solvents, Reagents, and Packaging

Cross-contamination controls include validated cleaning, equipment maintenance, segregation of materials and assessment of shared production equipment. Suspect solvents, recovered materials and reagents should be evaluated for nitrosamines and relevant precursors. Packaging components and the container-closure system should also be assessed when they could introduce contaminants or promote reactions during storage. Controls should follow a documented, product-specific risk assessment^{7,28}.

Stability Studies, Storage Conditions, and Ongoing Monitoring

Stability studies should determine whether nitrosamines form or increase during the proposed shelf life. Long-term and accelerated studies should be selected according to the product and risk assessment,

with additional investigations where needed. Temperature, humidity, moisture, pH and interactions among the API, excipients and packaging may affect formation. Manufacturers should review relevant batches and stability trends, investigate unexpected increases and update the risk assessment when processes or materials change^{7,28}.

Recent Advances and Research Gaps

Advances in High-Resolution Mass Spectrometry and Sensitive Analytical Methods

High-resolution mass spectrometry, particularly LC–HRMS, supports the investigation of nitrosamine impurities through accurate-mass measurements and structural information. MS/MS fragmentation can strengthen a proposed identification. Targeted techniques such as LC–MS/MS or GC–MS can then be used for quantification when the method is appropriate and validated. Platform choice depends on the impurity's properties, the formulation matrix, required sensitivity and reference-standard availability^{12–14}.

Approaches for Identifying Unknown Nitrosamines and NDSRIs

Untargeted HRMS screening can combine accurate mass, isotope-pattern assessment and MS/MS fragmentation to flag compounds that may not be included in a targeted method. Proposed structures can be examined using predicted fragmentation, plausible reaction pathways and comparison with a synthesised or purchased reference standard where available. Screening generates candidates; it does not by itself establish confirmed identity or validated quantitative results^{12,13}.

The API structure provides a starting point for anticipating possible NDSRIs. Investigators can map susceptible amine groups, consider plausible nitrosation routes and examine interactions with excipients and nitrosating agents during manufacture and storage. A suspected structure should be confirmed with appropriate analytical evidence and, where possible, a reference standard. A separate validated method is needed when reliable quantification is required^{12,13}.



Research Gaps and Analytical Challenges

Reference standards: Certified standards are not available for every newly identified nitrosamine or NDSRI, which can limit confirmation, calibration and validation. Toxicology: Carcinogenicity data are also missing for many NDSRIs. CPCA, read-across and other predictive methods can help prioritise assessment, but their uncertainty should be reported and empirical evidence sought when the risk or uncertainty is substantial.

Role of Artificial Intelligence and Machine Learning in Nitrosamine Research

AI and machine learning may help prioritise APIs for investigation by predicting susceptibility to nitrosation. Reiss et al. described two quantitative structure–activity relationship ((Q)SAR) approaches: a graph-neural-network model trained on 207 nitrogen-containing compounds and a separate expert-rule method. Both approaches reported approximately 80% precision on the study dataset³¹.

These models may help rank compounds for further risk assessment, but predicting a possible nitrosation site does not prove that an impurity forms under manufacturing conditions, establish its concentration or determine its carcinogenic potency. AI/ML should therefore support—not replace—experimental studies, validated analytical methods and toxicological assessment. Further evaluation should address dataset coverage, external validation, model transparency and performance across diverse API structures^{23,31}.

CONCLUSION

Control of pharmaceutical nitrosamines should be based on the specific medicine, combining knowledge of formation chemistry with suitable analytical testing, toxicological assessment and manufacturing controls. Small-molecule nitrosamines and NDSRIs may arise through different pathways, so assessment should cover the API structure, materials, process chemistry, excipients, packaging and storage. Validated LC–MS/MS and GC–MS/MS methods can quantify selected targets, while LC–HRMS can support investigation of unexpected impurities; method suitability must be demonstrated for each analyte and matrix.

Results should be compared with the relevant compound-specific AI and current regulatory guidance. Important limitations include gaps in NDSRI reference standards and toxicology, differences in analytical performance between methods and uncertainty in predictive models. Research should improve compound-specific evidence and reporting consistency, while manufacturers prioritise prevention throughout the product lifecycle rather than relying only on final-product testing.

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