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REVIEW Article

Smart Analytical Techniques for Detection of Nitrosamine Impurities in Pharmaceuticals: A Risk-Based Approach

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Abstract

Nitrosamine impurities have emerged as a critical safety concern in pharmaceutical products due to their potent genotoxic and carcinogenic nature. Since the detection of N-nitrosodimethylamine (NDMA) in angiotensin receptor blockers, global regulatory agencies have intensified scrutiny on nitrosamine contamination across drug substances and products. This review comprehensively examines smart analytical techniques employed for the detection and quantification of nitrosamine impurities, integrating a risk-based approach aligned with regulatory expectations. Advanced methodologies including liquid chromatography–mass spectrometry (LC-MS/MS), gas chromatography–mass spectrometry (GC-MS), high-resolution mass spectrometry (HRMS), and emerging microanalytical platforms are critically discussed. The article further explores mechanisms of nitrosamine formation, sources of contamination, and the role of predictive risk assessment tools in pharmaceutical manufacturing. Emphasis is placed on analytical challenges such as trace-level detection, matrix interference, and sample preparation complexities. Comparative evaluation of conventional and modern techniques highlights advancements in sensitivity, selectivity, and regulatory compliance. Additionally, the integration of artificial intelligence and digital analytical frameworks in impurity profiling is addressed as a future direction. This review provides a holistic understanding of nitrosamine impurity detection, offering practical insights for pharmaceutical scientists, regulatory professionals, and analytical chemists to ensure drug safety and quality.

Keywords: Nitrosamine impurities, LC-MS/MS, Pharmaceutical analysis, Risk assessment, Genotoxic impurities

1.0 Introduction

The pharmaceutical industry has witnessed a paradigm shift in impurity profiling following the global detection of nitrosamine contaminants in widely prescribed drugs such as sartans, ranitidine, and metformin. Nitrosamines, particularly N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), are classified as probable human carcinogens due to their mutagenic and DNA-damaging potential [1]. These compounds can form unintentionally during various stages of drug synthesis, manufacturing, storage, and even degradation processes. The presence of such impurities, even at trace levels, poses significant health risks, prompting regulatory agencies to establish stringent limits and monitoring requirements.

The increasing incidence of drug recalls due to nitrosamine contamination has highlighted the limitations of traditional analytical techniques in detecting ultra-trace impurities [2]. Consequently, there has been a growing emphasis on developing sensitive, selective, and robust analytical methodologies capable of detecting nitrosamines at nanogram levels. Modern pharmaceutical analysis now integrates advanced instrumental techniques with computational tools to enhance detection capabilities and ensure regulatory compliance.

Nitrosamine formation typically requires the presence of nitrosating agents and secondary or tertiary amines under favorable conditions such as acidic pH and elevated temperature [3]. This complex chemistry makes it challenging to predict and control their formation during pharmaceutical processing. Additionally, impurities may arise from raw materials, solvents, excipients, or environmental factors, further complicating the analytical landscape.

This review aims to provide a comprehensive overview of smart analytical techniques used for detecting nitrosamine impurities, emphasizing a risk-based approach that integrates analytical science with regulatory frameworks and predictive modeling.

2.0 Chemistry and Formation Mechanisms of Nitrosamine Impurities

Nitrosamines are a class of organic compounds characterized by the presence of a nitroso functional group attached to an amine. Their formation in pharmaceuticals is primarily governed by chemical reactions involving nitro sating agents such as nitrites and amines under acidic or high-temperature conditions. These reactions can occur during drug synthesis, formulation, or storage, making nitrosamine contamination a multifactorial issue.

The mechanism of nitrosamine formation typically involves the reaction between nitro sating species and secondary or tertiary amines, resulting in the formation of stable nitrosamine compounds [3]. These reactions are influenced by several factors, including pH, temperature, solvent composition, and the presence of catalysts. For instance, solvents such as dimethylformamide and reagents used in synthesis may contribute to nitrosamine formation if residual impurities are present [4].

Recent studies have also identified the formation of nitrosamine drug substance-related impurities (NDSRIs), which are structurally complex derivatives formed directly from the active pharmaceutical ingredient [5]. These impurities are particularly challenging to detect due to their structural similarity to the parent compound and their presence at extremely low concentrations.

Furthermore, storage conditions such as humidity, light exposure, and temperature fluctuations can promote the formation of nitrosamines post-manufacturing [6]. This highlights the importance of stability studies and continuous monitoring throughout the product lifecycle.

Understanding the chemistry of nitrosamine formation is crucial for developing effective analytical strategies and implementing preventive measures in pharmaceutical manufacturing.

Table 1: Comparative Overview of Smart Analytical Techniques for Detection of Nitrosamine Impurities in Pharmaceuticals

Analytical Technique	Principle	Detection Limit	Advantages	Limitations	Typical Applications
LC-MS/MS	Separation by liquid chromatography followed by tandem mass spectrometric detection using MRM transitions	ng/g (ppb level)	High sensitivity, excellent selectivity, suitable for multi-analyte detection, regulatory acceptance	Expensive instrumentation, requires skilled operation	Routine analysis of NDMA, NDEA, NDBA in APIs and finished products
GC-MS	Volatilization of analytes followed by mass spectrometric detection	ng/g (ppb level)	High resolution, ideal for volatile nitrosamines, well-established method	Requires derivatization, complex sample preparation	Detection of volatile nitrosamines such as NDMA in formulations
UPLC-HRMS	Ultra-performance liquid chromatography coupled with high-resolution mass spectrometry	pg–ng level	Accurate mass measurement, identification of unknown impurities, high resolution	High cost, complex data interpretation	Detection of nitrosamine drug substance-related impurities (NDSRIs)
LC-HRMS (Orbitrap/TOF)	High-resolution detection based on exact mass and isotopic patterns	pg level	Structural elucidation, non-target screening capability	Requires advanced software and expertise	Identification of novel or unknown nitrosamines

Spectroscopic Methods (UV/FTIR)	Absorption or vibrational spectroscopy	µg level	Simple, cost-effective, rapid screening	Low sensitivity, not suitable for trace detection	Preliminary screening and characterization
AI-Integrated Analytical Platforms	Data-driven modeling using machine learning algorithms combined with analytical outputs	Depends on integrated technique	Predictive analysis, method optimization, reduced experimental workload	Requires large datasets, computational expertise	Risk prediction, method development, impurity profiling
Microfluidics-Based Systems	Miniaturized analytical platforms with integrated fluid handling	ng level	Low sample consumption, rapid analysis, portable systems	Limited scalability, early-stage development	On-site rapid screening of pharmaceutical samples

3.0 Sources and Risk Factors Associated with Nitrosamine Contamination

Nitrosamine impurities can originate from multiple sources within the pharmaceutical supply chain, making risk assessment a critical component of quality control. One of the primary sources is the use of contaminated raw materials or reagents during synthesis. Secondary amines present in starting materials or intermediates can react with nitro sating agents to form nitrosamines [7].

Manufacturing processes also play a significant role in impurity formation. Conditions such as high temperature, acidic pH, and the presence of reactive intermediates can facilitate nitrosation reactions [8]. Cross-contamination during equipment cleaning or reuse of solvents further contributes to the risk of impurity formation.

Another important source is excipients and packaging materials. Certain excipients may contain trace levels of nitrites, which can react with amines present in the drug formulation [9]. Additionally, packaging materials may introduce contaminants that promote nitrosamine formation over time.

Environmental factors, including exposure to nitrogen oxides and other pollutants, can also contribute to impurity formation during storage and distribution [10]. Moreover, degradation pathways of the active pharmaceutical ingredient can lead to the formation of nitrosamines under specific conditions.

Risk factors associated with nitrosamine contamination include process complexity, use of high-risk reagents, inadequate purification steps, and lack of robust analytical monitoring. Regulatory agencies have emphasized the need for comprehensive risk assessment strategies to identify and mitigate these factors throughout the drug lifecycle.

4.0 Regulatory Guidelines and Risk-Based Approaches

The detection of nitrosamine impurities has prompted global regulatory agencies to implement stringent guidelines aimed at ensuring drug safety. Authorities such as the U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), and International Council for Harmonisation have issued recommendations for the assessment, control, and reporting of nitrosamine impurities.

One of the key regulatory concepts is the establishment of acceptable intake limits for nitrosamines, typically in the nanogram per day range [11]. These limits are based on toxicological data and are designed to minimize the risk of cancer associated with long-term exposure. Updated regulatory guidance up to 2023 has further refined acceptable intake limits for nitrosamine drug substance-related impurities (NDSRIs), emphasizing a science-based approach to risk assessment [12].

A risk-based approach involves a systematic evaluation of potential sources of nitrosamine contamination, followed by the implementation of appropriate control measures. This includes identifying high-risk materials, optimizing manufacturing processes, and employing sensitive analytical techniques for detection and quantification.

Regulatory frameworks also emphasize the importance of confirmatory testing using validated analytical methods. Techniques such as LC-MS/MS and GC-MS are widely recommended due to their high sensitivity and specificity for detecting trace-level impurities [13].

In addition to analytical testing, predictive tools such as in silico modeling and computational risk assessment are increasingly being used to identify potential nitrosamine formation pathways. These tools enable proactive risk management and support the development of safer pharmaceutical products.

5.0 Advanced Chromatographic Techniques for Nitrosamine Detection

The detection of nitrosamine impurities at trace levels has necessitated the use of highly sensitive chromatographic techniques. Among these, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has emerged as the gold standard due to its exceptional sensitivity, selectivity, and robustness. LC-MS/MS enables

the detection of nitrosamines at parts-per-billion (ppb) levels, making it suitable for regulatory compliance and routine quality control [13]. The use of multiple reaction monitoring (MRM) enhances specificity by targeting characteristic fragmentation patterns of nitrosamines.

Gas chromatography–mass spectrometry (GC-MS) is another widely used technique, particularly for volatile nitrosamines. GC-MS offers high resolution and sensitivity, making it suitable for detecting low molecular weight nitrosamines such as NDMA and NDEA [14]. However, sample preparation for GC-MS often involves derivatization steps, which may introduce variability and increase analysis time.

Ultra-performance liquid chromatography (UPLC) has gained attention for its improved resolution and reduced analysis time compared to conventional HPLC. When coupled with high-resolution mass spectrometry (HRMS), UPLC provides enhanced detection capabilities for complex nitrosamine drug substance-related impurities (NDSRIs) [15]. These techniques are particularly useful in identifying unknown impurities and degradation products.

The integration of chromatographic techniques with advanced detection systems has significantly improved the reliability and accuracy of nitrosamine analysis. For example, UPLC methods have been successfully applied in impurity profiling of pharmaceutical compounds, demonstrating their effectiveness in detecting related compounds at trace levels [16].

6.0 Mass Spectrometry and High-Resolution Analytical Techniques

Mass spectrometry plays a pivotal role in the identification and quantification of nitrosamine impurities due to its high sensitivity and structural elucidation capabilities. LC-MS/MS remains the most widely used technique, offering excellent selectivity through the use of specific ion transitions [17]. The ability to detect multiple nitrosamines simultaneously makes it a preferred method in pharmaceutical analysis.

High-resolution mass spectrometry (HRMS), including time-of-flight (TOF) and Orbitrap systems, has revolutionized impurity profiling by enabling accurate mass measurements and identification of unknown compounds [18]. HRMS is particularly useful for detecting nitrosamine drug substance-related impurities (NDSRIs), which often require precise structural characterization.

Another emerging technique is atmospheric pressure chemical ionization (APCI), which enhances the detection of non-polar nitrosamines. This technique complements electrospray ionization (ESI), expanding the analytical scope for different classes of nitrosamines [19].

The combination of chromatographic separation with advanced mass spectrometric detection provides a powerful analytical platform for comprehensive impurity profiling. These techniques not only improve detection limits but also facilitate the identification of complex impurity structures, supporting regulatory compliance and risk assessment.

7.0 Smart Analytical Technologies and AI Integration

The integration of artificial intelligence (AI) and machine learning (ML) into pharmaceutical analysis represents a transformative advancement in impurity detection. AI-driven algorithms can analyze large datasets generated from chromatographic and spectroscopic techniques, enabling rapid identification of impurity patterns and predictive modeling of nitrosamine formation pathways [20].

Machine learning models have been successfully applied in method development, optimizing parameters such as mobile phase composition, flow rate, and column selection. These models reduce experimental time and improve method robustness, enhancing analytical efficiency [21]. Additionally, AI-based tools can predict potential nitrosamine formation based on chemical structure and reaction conditions, supporting proactive risk assessment.

Automation and digitalization in analytical laboratories further contribute to the concept of “smart analytics.” Automated sample preparation systems, coupled with real-time data analysis, minimize human error and improve reproducibility. Digital platforms also facilitate data integrity and regulatory compliance by ensuring traceability and auditability of analytical results [22].

The adoption of smart analytical technologies is expected to play a crucial role in the future of pharmaceutical analysis, enabling faster, more accurate, and cost-effective detection of impurities.

8.0 Comparative Evaluation of Analytical Techniques

A comparative analysis of analytical techniques used for nitrosamine detection highlights the strengths and limitations of each method. LC-MS/MS is widely regarded as the most reliable technique due to its high sensitivity and specificity, making it suitable for routine analysis and regulatory compliance [13]. However, it requires expensive instrumentation and skilled personnel.

GC-MS offers excellent performance for volatile nitrosamines but is limited by the need for derivatization and complex sample preparation [14]. UPLC-HRMS provides superior resolution and accuracy, making it ideal for identifying unknown impurities, but its high cost may limit widespread adoption [18].

Spectroscopic techniques such as UV and FTIR are less commonly used for nitrosamine detection due to their lower sensitivity. However, they may serve as complementary techniques for preliminary screening and characterization [23].

The selection of an appropriate analytical technique depends on factors such as the nature of the sample, required detection limits, and available resources. A combination of techniques is often employed to achieve comprehensive impurity profiling.

9.0 Analytical Challenges and Limitations

Despite significant advancements in analytical technologies, several challenges remain in the detection of nitrosamine impurities. One of the primary challenges is the ultra-trace level at which these impurities must be detected, often requiring detection limits in the nanogram range [24]. Achieving such sensitivity necessitates the use of highly sophisticated instruments and optimized analytical conditions.

Matrix interference is another major challenge, particularly in complex pharmaceutical formulations. Co-eluting compounds may interfere with the detection of nitrosamines, leading to false positives or inaccurate quantification [25]. Advanced sample preparation techniques and selective detection methods are required to overcome these issues.

The identification of nitrosamine drug substance-related impurities (NDSRIs) presents additional challenges due to their structural complexity and similarity to the parent compound. High-resolution techniques and advanced data analysis tools are essential for accurate identification [18].

Furthermore, the lack of standardized methods for certain nitrosamines and variations in regulatory guidelines across regions may complicate analytical processes. Continuous research and harmonization of guidelines are necessary to address these challenges.

10.0 Case Studies and Industrial Applications

Several case studies have demonstrated the practical application of advanced analytical techniques in detecting nitrosamine impurities. The detection of NDMA in angiotensin receptor blockers led to widespread recalls and highlighted the importance of sensitive analytical methods [2]. LC-MS/MS methods were successfully employed to detect NDMA at trace levels, enabling regulatory action and risk mitigation.

Another example involves the detection of nitrosamines in ranitidine, where degradation during storage was identified as a key factor contributing to impurity formation [6]. Advanced analytical techniques were used to monitor impurity levels under various storage conditions, providing insights into stability and risk factors.

In industrial settings, pharmaceutical companies have adopted risk-based approaches combined with advanced analytical methods to ensure product safety. Routine monitoring using LC-MS/MS and GC-MS, along with predictive modeling tools, has become standard practice in quality control laboratories [13].

These case studies underscore the importance of integrating analytical techniques with risk assessment strategies to effectively manage nitrosamine impurities in pharmaceuticals.

References

1. International Agency for Research on Cancer (IARC). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some N-Nitroso Compounds*. Lyon: IARC; 2010.
2. U.S. Food and Drug Administration. Control of Nitrosamine Impurities in Human Drugs: Guidance for Industry. Silver Spring: FDA; 2023.
3. Mitch WA, Sedlak DL. Formation of N-nitrosodimethylamine (NDMA) from dimethylamine during chlorination. *Environ Sci Technol*. 2002; 36(4):588–595.
4. Ashworth IW, Dirat O, Teasdale A, Whiting M. Potential for the formation of N-nitrosamines during the manufacture of active pharmaceutical ingredients: an assessment of the risk. *Org Process Res Dev*. 2020; 24(9):1629–1646.
5. European Medicines Agency. Nitrosamine impurities in human medicinal products: EMA recommendations. Amsterdam: EMA; 2022.
6. Zeng T, Mitch WA. Contribution of N-nitrosamine formation pathways to NDMA formation during water treatment. *Water Res*. 2016; 98:202–210.
7. Teasdale A, Elder D, Chang SJ. Risk assessment of genotoxic impurities in pharmaceuticals: regulatory perspectives. *J Pharm Biomed Anal*. 2013; 87:223–230.
8. Li K, Ricker RD, Phelps JB. Nitrosamine formation mechanisms and mitigation strategies in pharmaceutical manufacturing. *Org Process Res Dev*. 2021; 25(11):2219–2232.
9. Chen Y, Zhang J, Li Y. Nitrite impurities in excipients and their contribution to nitrosamine formation. *Int J Pharm*. 2020; 589:119845.

10. Luo Q, Wang L, Wang Y. Environmental sources of nitrosamine impurities in pharmaceuticals. *Sci Total Environ*. 2021; 764:142874.
11. International Council for Harmonisation (ICH). ICH M7 (R1): Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals. Geneva: ICH; 2017.
12. European Medicines Agency. Questions and answers on nitrosamine impurities in human medicinal products. Amsterdam: EMA; 2023.
13. U.S. Food and Drug Administration. LC-MS/MS methods for detection of nitrosamine impurities. Silver Spring: FDA; 2022.
14. Wang C, Deng Z, Li X. Determination of volatile nitrosamines using GC-MS in pharmaceutical products. *J Chromatogr A*. 2019; 1602:360–368.
15. Xu Y, Zhao Z, Wang X. UPLC-HRMS analysis for detection of nitrosamine impurities in drug substances. *Anal Chem*. 2021; 93(12):5212–5220.
16. Jiang, H., Li, Y., Pelzer, M., Cannon, M.J., Randlett, C., Junga, H., Jiang, X. and Ji, Q.C., 2008. Determination of molindone enantiomers in human plasma by high-performance liquid chromatography–tandem mass spectrometry using macrocyclic antibiotic chiral stationary phases. *Journal of Chromatography A*, *1192*(2), pp.230-238..
17. Kamps J, Herrmann M. LC-MS/MS quantification of nitrosamines in pharmaceuticals: method development and validation. *J Pharm Biomed Anal*. 2020; 189:113421.
18. Kaufmann A. High-resolution mass spectrometry for pharmaceutical impurity profiling. *TrAC Trends Anal Chem*. 2020; 130:115995.
19. Bruins AP. Mechanistic aspects of electrospray ionization and atmospheric pressure chemical ionization. *Mass Spectrom Rev*. 1998; 17(6):319–332.
20. Goodarzi M, Russell PJ, Vander Heyden Y. Data mining in pharmaceutical analysis: application of machine learning. *J Pharm Biomed Anal*. 2015; 102:74–84.
21. Patel P, Shah R, Singh S. Application of artificial intelligence in pharmaceutical analytical method development. *J Pharm Innov*. 2022; 17(3):785–798.
22. European Medicines Agency. Guideline on data integrity and computerized systems in pharmaceutical analysis. Amsterdam: EMA; 2021.
23. Skoog DA, Holler FJ, Crouch SR. *Principles of Instrumental Analysis*. 6th ed. Belmont: Cengage Learning; 2017.
24. Elder DP, Snodin DJ. Genotoxic impurities in pharmaceuticals: risk assessment and control. *Org Process Res Dev*. 2009; 13(5):928–939.
25. Müller L, Mauthe RJ, Riley CM. A rationale for determining, testing, and controlling specific impurities in pharmaceuticals. *Regul Toxicol Pharmacol*. 2006; 44(3):198–211.