Advancing Nitrosamine Detection in Pharmaceutical Systems

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Addressing the Growing Nitrosamine Challenge

N-nitrosamines are a class of potentially carcinogenic compounds that have posed significant challenges for the pharmaceutical industry, leading to product recalls and heightened regulatory scrutiny of commercialized drugs and those in development. These impurities can form through complex mechanisms involving nitrosating agents and amine-containing compounds during manufacturing, storage, or packaging. Accurate and sensitive quantification of nitrosamines is critical because regulators have set limits for these impurities at extremely low levels. The limits often range in the ng/day range, meaning that even minor inaccuracies can lead to incorrect risk assessments or regulatory non-compliance. Stringent quantification with high sensitivity is often required down to parts per billion (ppb) or parts per trillion (ppt) ranges. Advanced techniques are required for detection and quantification with a strong linearity over a wide concentration range. To address this challenge, Aptar Active Material Science invested in analytical capabilities that provide the scientific foundation needed to evaluate impurities, understand formation mechanisms, and support data-driven risk management strategies.

This paper presents a validated LC/QTOF/MS analytical approach designed to accurately quantify three key nitrosamines as identified in the paper published by Moser et al.[1] By combining high-sensitivity LC/QTOF/MS quantification with N-Sorb℠ nitrosamine mitigation technology, Aptar delivers both detection and mitigation solutions, enabling pharmaceutical partners not only to measure nitrosamine impurities at trace levels, but to actively reduce them and verify mitigation effectiveness with high confidence. This integrated capability enables detection and quantifiable verification of impurity reduction, ensuring that mitigation strategies are supported by high-confidence analytical evidence. The validated analytical framework serves as a critical tool within Aptar Active Material Science's laboratories to generate high-confidence, quantitative evidence of the efficacy of our proprietary N-Sorb℠ technology as a robust solution for nitrosamine mitigation.

Sensitive Detection as a Foundation for Risk Management

Nitrosamines originate from reactions between nitrosating agents and amines, with precursors often arising from excipients, solvents, or environmental exposure. Even when mitigation strategies are implemented, analytical verification remains critical to confirm acceptable impurity levels and ensure product safety. Sensitive and reliable analytical methods are foundational to effective nitrosamine risk management. An LC/QTOF/MS system combines high-performance liquid chromatography (LC) with quadrupole time of flight mass spectrometry (QTOF MS) to separate, detect, and accurately measure analytes.

IDEA IN BRIEF

THE PROBLEM

Nitrosamine contamination continues to challenge pharmaceutical manufacturers, driving increased regulatory scrutiny and product risk. Addressing this issue requires more than detection; it requires an integrated approach combining ultra-sensitive analytics with proven mitigation technologies.

THE CHALLENGE

A holistic approach to nitrosamine mitigation is needed — one that integrates analytical capabilities that enable trace-level quantification and robust calibration methodologies to ensure high-confidence data across wide concentration ranges, while also mitigating nitrosamine formation and accumulation.

THE SOLUTION

Aptar Active Material Science combines analytical precision with material science innovation to deliver measurable nitrosamine risk reduction. This empowers pharmaceutical partners to confidently meet regulatory requirements while protecting product quality and ensuring patient safety.

In operation, a sample is loaded and injected into the LC system where compounds are first chromatographically separated, then ionized and filtered by the quadrupole. The compounds then enter the time of flight analyzer, where their mass to charge ratios are determined with high resolution and mass accuracy. This configuration enables both qualitative identification and precise quantitative analysis at trace levels, making it well suited for complex applications such as nitrosamine detection.

While LC/QTOF/MS instrumentation is widely recognized for its analytical power, Aptar's differentiation lies in the integration of this capability within applied pharmaceutical and packaging systems. This enables evaluation of nitrosamine formation and mitigation in realistic product environments, bridging the gap between laboratory detection and real-world risk management.

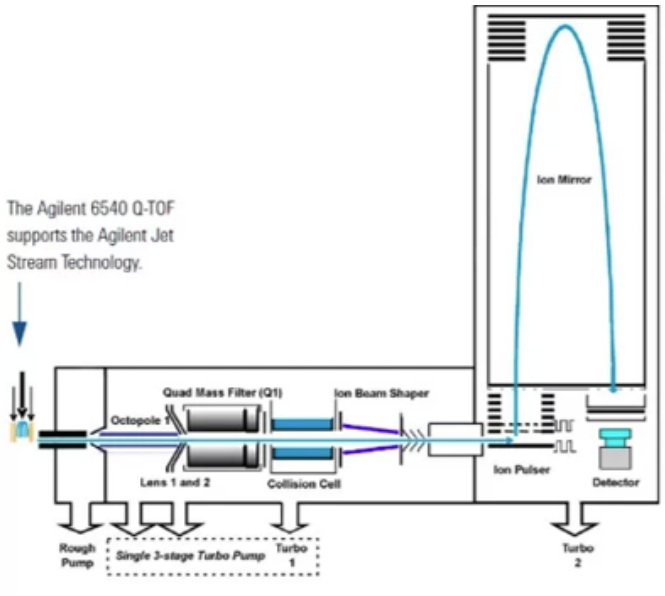

Figure 1: Diagram of Agilent 6540 UHD QTOF MS[2]

Adapted from Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS Systems Concepts Guide (p. 30), by Agilent Technologies, Inc., 2017.

Building a High-Sensitivity LC/QTOF/MS Method

The analytical method was developed using an Agilent 1290 UHPLC system coupled to an Agilent 6546 LC/QTOF/MS (Figure 1). The APCI ionization source was used and operated in positive ion mode. A kinetex 2.6 um XB-C18 100A column and two solvent solutions were used for chromatography purposes, 0.2% formic acid in water and methanol. The analytes of interest included N-nitrosodimethylamine (NDMA), 1-nitroso-4-phenylpiperidine, and 1-nitroso-4-phenylpiperidin-4-ol. Calibration curves were generated using mixed standard solutions across multiple concentration levels, with weighted regression applied to improve accuracy at low concentrations as necessary.

The method was developed and refined in alignment with industry standards, including assessment of linearity, sensitivity, and reproducibility across relevant concentration ranges. Particular attention was given to low-level quantification accuracy to ensure suitability for regulatory-driven nitrosamine limits. In addition, the method is designed to support evaluation of drug product and packaging interactions, where trace impurity formation and adsorption mechanisms are critical to overall risk mitigation strategies.

Analytical Performance Validation

The method demonstrated strong sensitivity and linearity across all analytes. Quantifier and qualifiers were identified and used to determine limits of detection and quantitation. Limits of detection were achieved at 50 ppb for NDMA and 5 ppb for the phenylpiperidine-related nitrosamines. Corresponding limits of quantitation were 100 ppb and 10 ppb, respectively. Calibration curves demonstrated strong linearity across the evaluated concentration ranges, supported by multi-point calibration and appropriate regression modeling to ensure accuracy at low concentration levels, which is critical to nitrosamine risk assessment. Reproducibility was confirmed through multiple injections and statistical evaluation of signal response, demonstrating consistent and reliable analytical performance. The use of weighted regression modeling supports improved accuracy, reinforcing the method's suitability for stringent nitrosamine regulatory thresholds.

Collectively, these performance characteristics establish a reliable analytical foundation for both baseline impurity assessment and subsequent mitigation studies, where accurate quantification at low concentrations is essential for detecting meaningful changes in nitrosamine levels.

The calibration curve for NDMA demonstrates a consistent and reliable analytical response across the tested concentration range, supporting accurate quantification at trace levels (Figure 2). It demonstrates consistency starting at 50 ppb, supporting its use for both baseline quantification and post-mitigation verification studies.

The analytical method shows strong performance for phenylpiperidine-related nitrosamines, with stable response characteristics across low and mid concentration ranges. The resulting calibration curve for 1-nitroso-4-phenylpiperidine, as shown in Figure 3, demonstrates linear consistency from 5-5000 ppb, supporting its use for both baseline quantification and post-mitigation verification studies.

1-nitroso-phenylpiperidin-4-ol's calibration curve demonstrates consistency up to 10,000 ppb, supporting its use for both baseline quantification and post-mitigation verification studies (Figure 4).

Table 1 shows the overall LOD and LOQ that was achieved for each corresponding analyte under the specified method.

Analyte	LOD (ppb)	LOQ (ppb)
NDMA	50	100
1-nitroso-4-phenylpiperdine	5	10
1-nitroso-4 phenylpiperdin-4-ol	5	10

Table 1: Established LOD/LOQ for Nitrosamine Analytes

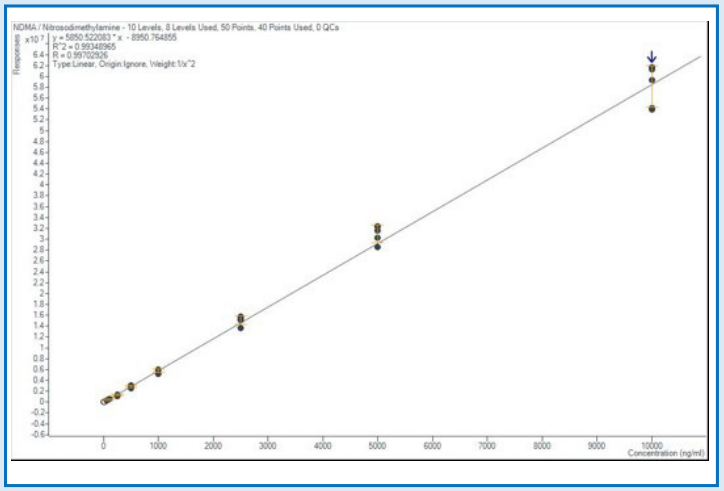

Figure 2: NDMA Calibration Curve

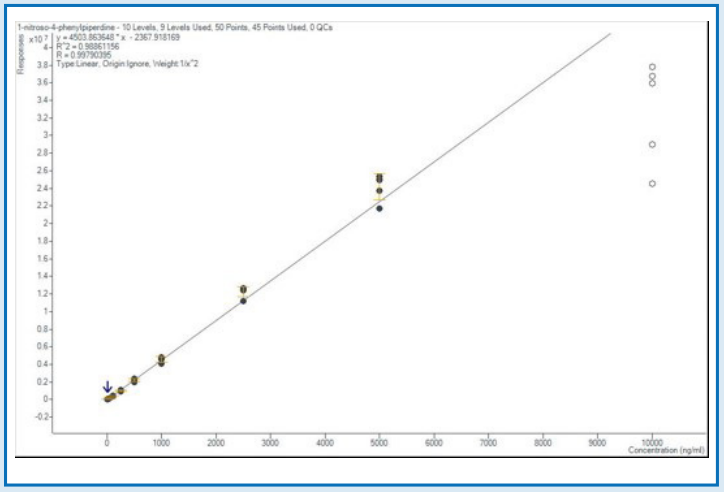

Figure 3: 1-Nitroso-4-Phenylpiperdine Calibration Curve

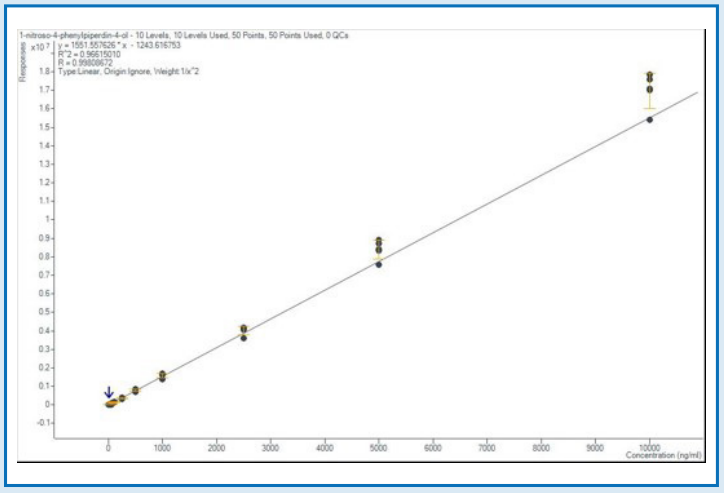

Figure 4: 1-Nitroso-4-Phenylpiperdin-4-ol Calibration Curve

Analytical Verification of Mitigation Performance

The true value of sensitive analytical capability lies in its ability to validate mitigation strategies with quantitative confidence. Within Aptar Active Material Science, this LC/QTOF/MS method is applied directly to evaluate the performance of N-Sorb℠ technology, a proprietary active material engineered to prevent nitrosamine formation and reduce volatile nitrosamine impurities post-formation.

Using this approach, baseline nitrosamine concentrations can be established and directly compared to levels observed following N-Sorb℠ technology integration, enabling clear, data-driven assessment of impurity reduction and providing measurable evidence of mitigation performance under relevant conditions.

The sensitivity of the method ensures that even small reductions in nitrosamine concentration can be accurately detected, which is critical given the extremely low regulatory thresholds. This capability supports generation of high-quality data suitable for strategic decision-making processes, transforming nitrosamine control from a reactive testing requirement to a proactive, engineered solution. This enables generation of comparative data sets that clearly demonstrate N-Sorb℠ performance, supporting both internal development decisions and external regulatory justification.

By combining advanced analytics with active material science, Aptar delivers a closed-loop solution—detect, mitigate, and verify—supporting pharmaceutical partners across development, scale-up, and commercial lifecycle management. These analytical capabilities provide the level of confidence required to support critical decisions regarding nitrosamine control strategies, including mitigation effectiveness and regulatory compliance.

Integrating Detection, Mitigation, and Verification

Nitrosamine contamination remains a significant challenge requiring both effective mitigation strategies and precise analytical verification. The validated LC/QTOF/MS method presented here delivers the sensitivity, accuracy, and robustness required for modern nitrosamine risk management. When combined with Aptar Active Material Science's N-Sorb℠ technology, this capability offers a fully integrated approach to detection, mitigation, and verification, empowering pharmaceutical companies to move beyond detection toward predictive and preventive impurity control strategies.

References

1. Moser, J., Ashworth, I.W., Harris, L., Hillier, M.C., Nanda, K.K., & Scrivens, G. (2023). N-nitrosamine formation in pharmaceutical solid drug products: Experimental observations. Journal of Pharmaceutical Sciences, 112(5), 1255-1267. https://doi.org/10.1016/j.xphs.2023.01.027
2. Agilent Technologies, Inc., (2017). *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS system concepts guide* (Publication No. G3335-90231). Agilent Technologies. Agilent Concepts Guide PDF (https://www.agilent.com/cs/library/usermanuals/public/G3335-90231_TOF_Q-TOF_Concepts.pdf)

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