Nitrosamines Analysis with LC/MS-MS

Ensuring Safety and Quality in Pharmaceutical Manufacturing

Waters™

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Introduction

Since 2018, global regulatory bodies have required pharmaceutical manufacturers to control and strive to mitigate the presence of N-nitrosamines (nitrosamines) in their products through a combined approach of risk assessment and analytical testing where needed. Waters analytical and laboratory automated technologies, comprehensive end to end analytical workflows, and scientific expertise, combine to support streamlined analytical solutions for nitrosamines analysis.

In this book you will gain a comprehensive understanding of nitrosamine analysis:

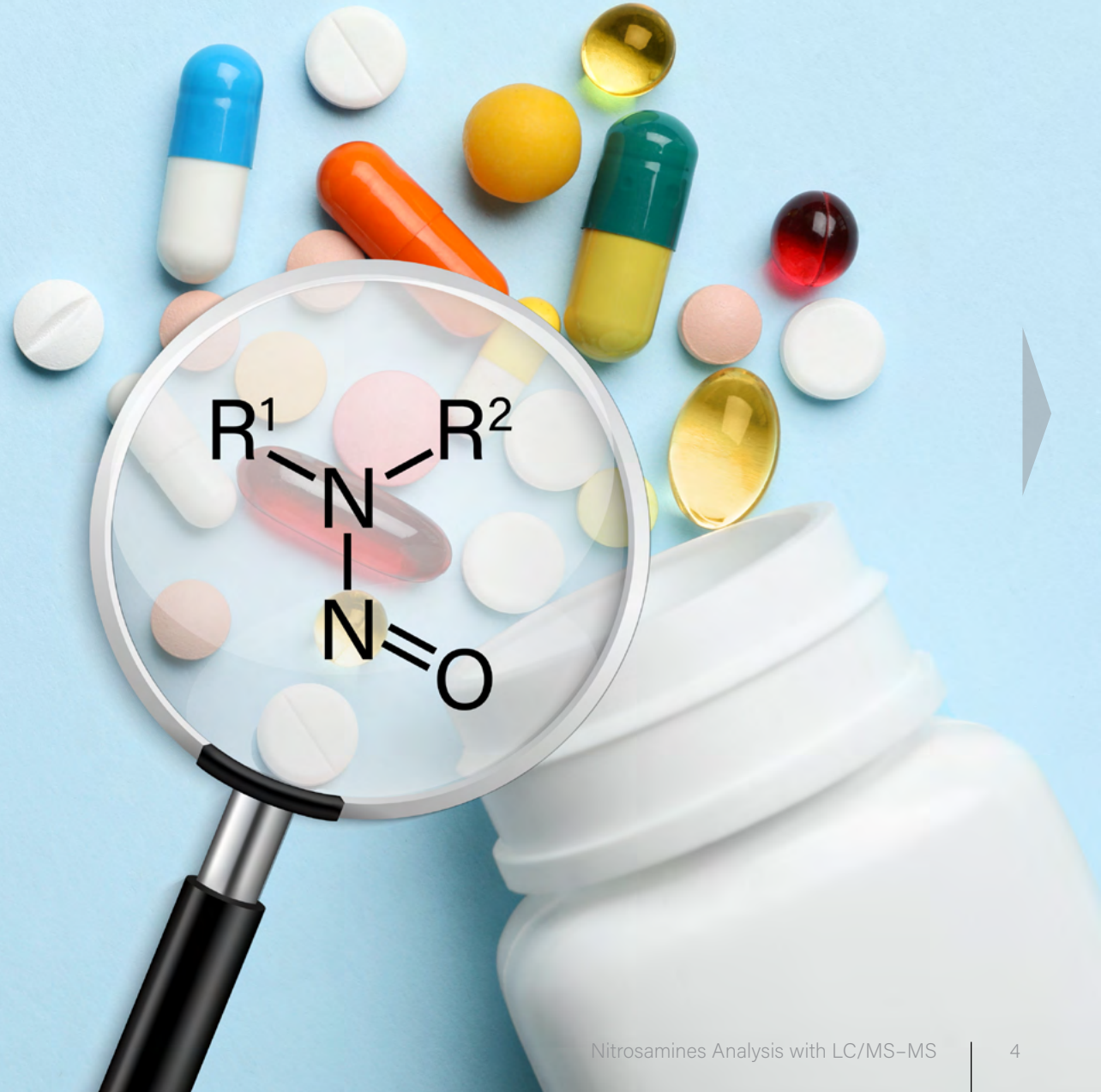
- History
- Formation
- Method development
- Waters Solutions
- Nitrosamines and Beyond

Trust in Waters to provide scalable application procedures and technologies to help you adapt quickly to challenges brought in by new regulations, new opportunities and competitive pressures. By partnering with us you gain access to unmatched levels of application support and market leading service which all aim to have your labs running effectively and consistently day–in–day–out.

Controlling Nitrosamines

N–nitrosamines, are a potentially mutagenic class of impurities that may pose a risk of cancer when individuals are exposed to them, above acceptable levels, for extended periods. In recent years, nitrosamines have been detected in various widely marketed medicines, examples include varenicline, metformin, ranitidine, and the sartan class of medicines, which has led to voluntary product recalls from the market.

Maintaining the safe production of critical medicine is a global priority for the pharmaceutical industry. To mitigate the risk of nitrosamines, regulatory bodies and health authorities are emphasizing the importance of identifying, monitoring, and controlling nitrosamine impurities in active pharmaceutical ingredients (APIs) and other raw materials. Analytical testing plays a vital role in controlling nitrosamines in both marketed medicines and drugs in development.



The Formation of Nitrosamines

Nitrosamines can form when an amine source and nitrosating agent react under specific conditions of temperature and pH. The API synthesis and drug product manufacturing processes may introduce risk of nitrosamine formation via starting materials, reagents, solvents, catalysts, intermediates, excipients and raw materials and storage packaging and conditions. Initially, industry focus was the formation of low–mass nitrosamines such as NDMA and NMBA however the prevalence for nitrosamines formation expanded significantly with the detection of Nitrosamine Drug Substance Related Impurities (NDSRIs), which are structurally similar to the API and can form in numerous marketed medicines. As regulations for nitrosamine control evolve towards a pragmatic approach, there is a need for analytical workflows that support both flexibility and sensitivity and meet a wide range of regulatory thresholds.

Method Development for Nitrosamine Analysis

When conducting a nitrosamines risk assessment, it is crucial to develop highly sensitive and specific analytical methods that are capable of quantifying at or below regulatory-approved thresholds. Whilst GC and other detection techniques are employed, LC/MS-MS (Liquid Chromatography with Tandem Mass Spectrometry) emerges as the gold standard solution for quantification of all types of nitrosamines. This is due to the robust sensitivity and selectively afforded by MS/MS and the accessible instrumentation solutions that are well suited for method development and routine analysis in a regulated environment. As with any trace analysis, the development of methods for the quantification of nitrosamines can present diverse challenges.

Andrew the pipetting robot

OneLab design & execute

Sample Preparation for Nitrosamine Analysis

Comprehensive sample preparation is often necessary when extracting low levels of nitrosamines from a high concentrated API or the matrix component of a drug product. Effective sample preparation has a significant impact on the final performance of the quantitative assay. Since nitrosamines encompass a wide range of compounds with varying physicochemical properties, methods are usually specific to the particular assay, therefore straightforward methods with simplified workflows are favored. However, in cases where greater selectivity is required due to challenging matrix of API or API interference, approaches like solid–phase extraction can be beneficial. Automated sample preparation techniques can further enhance reproducibility, reduce external contamination, and improve assay precision, thereby enhancing inter–assay and inter–laboratory reproducibility, particularly for low–level impurities.

As demand for impurity testing increases, the Andrew+™ Platform will support productivity and successful method transfer between laboratories in a regulated environment is facilitated by the traceability and security afforded by compliance ready OneLab™ Software.

Optimized Chromatographic Separation

Selecting the appropriate chromatography and column chemistry is critical when developing a method for analyzing nitrosamines in drug substances or products. It is essential to establish resolution between the large API peak and trace nitrosamines. By selectively directing the API peak to waste and redirecting the well–resolved nitrosamines to the mass spectrometer (MS) inline, issues such as suppression and source contamination can be minimized. It's important to note that different substances, drug products, and formulations may require tailored approaches to achieve optimized separation. Although there is no one-size-fits-all solution, Waters has a range of separation technologies to meet your specific testing and analytical needs.

Furthermore, our ACQUITY™ Premier UPLC™ and Premier Chromatography products, featuring MaxPeak™ High Performance Surfaces, support a risk–mitigation approach to LC–MS analysis, improving separation and minimizing the risk of missing challenging metal sensitive analytes.

Optimized MS Sensitivity for Impurities Analysis

To meet regulatory requirements for detecting low levels of nitrosamines in APIs or drug products, tandem quadrupole mass spectrometry is the preferred method for quantification. With the sensitivity and selectivity afforded by tandem MS, this accessible technique also offers flexible ionization options that can be tailored to the specific nitrosamines being measured. For simple LC/MS analysis, low–mass nitrosamines are often best detected using atmospheric pressure chemical ionization (APCI), while electrospray ionization (ESI) is better suited for analyzing complex nitrosamines or NDSRIs.

To ensure high sensitivity and selectivity, multiple reaction monitoring (MRM) is recommended for nitrosamine measurement. It is important to note that different published methods may use different transitions for a given impurity, so each laboratory must validate its analytical methodologies to within specific regulatory compliance requirements, before submitting data to regulatory authorities.

Regulatory Compliance

REGULATORY GUIDELINES FOR ANALYTICAL METHOD DEVELOPMENT

The control of nitrosamines is subject to evolving regulatory requirements in the pharmaceutical industry. Regulatory agencies demand a comprehensive understanding of a drug product's impurity profile, which includes quantitation of impurities present at low levels in sample matrices. This necessitates the development and validation of sensitive analytical methods that adhere to specific regulatory guidance.

Several key regulatory guidelines provide direction for the management of impurities in pharmaceuticals. These include the ICH (International Council for Harmonization) guidelines which support approaches to adhering with global regulatory requirements and harmonized guidance throughout the industry.

Specific to nitrosamines, ICH M7 provides guidance on the assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk, which encompasses nitrosamine analysis.

Relevant chapters within the United States Pharmacopeia (USP) serve as valuable resources for impurity guidance. For example, USP <1469> specifically addresses nitrosamines impurity analysis.

By staying up-to-date with the latest guidelines and harmonized procedures, laboratories can ensure the safety and quality of pharmaceutical products, reinforcing their position as reliable partners in the industry.

Waters Comprehensive Solutions for Nitrosamines Quantification

Sensitivity beyond today's regulatory thresholds and future proofing for evolving requirement in trace genotoxic impurities quantification.

Our comprehensive solutions for impurity analysis with LC–MS/MS is designed to assist laboratories in meeting stringent regulatory requirements for all, small to complex, nitrosamines. By offering state–of–the–art LC–MS/MS instrumentation, optimized sample preparation workflows, and validated analytical methods, Waters empowers laboratories to develop methods to achieve the sensitivity, selectivity, and accuracy necessary to quantify all nitrosamines at or below regulatory-approved thresholds. From the quantification of higher threshold nitrosamines (NDSRI) with the Xevo Cronos, to the flexibility across a range of small and complex NDSRI with the Xevo TQ-S micro, you can depend on the Xevo Tandem Family. For ultimate sensitivity in trace impurity quantification, the Xevo TQ Absolute enables laboratories to grow their ultimate nitrosamine analytical testing capability across a range of threshold requirements, with a future proofing analytical performance that prepares you for today's and tomorrow's emerging needs. Our integrated solutions support method development and testing in a regulated environment with our compliance ready software platform, MassLynx™ Security, while supporting regulatory submission and audit across the globe.

Fit for Purpose or Future Proofing?

Fit for routine testing requirements.

ACQUITY PREMIER/ XEVO TQ-CRONOS

- Dependable performance
- Fit for purpose sensitivity
- Higher threshold nitrosamines (e.g. some NDSRI's)

Dependable sensitivity and versatility across impurity thresholds..

ACQUITY PREMIER/ XEVO TQ-MICRO

- Dependable and versatile performance
- Sensitivity beyond regulatory requirements
- Flexibility across NDSRI and small nitrosamines

Future proofing performance with absolute sensitivity.

ACQUITY PREMIER/ XEVO TQ-ABSOLUTE

- Dependable, versatile, and future proofed performance
- Sensitivity beyond lowest threshold regulatory requirements for all nitrosamines
- Flexibility for limited sample load and sample preparation

Nitrosamines and Beyond

The evolving nitrosamines concern and their potential impact on drug safety has raised questions about the future for mutagenic and genotoxic impurity control in Pharmaceuticals. What will the next challenge be?

Can laboratories meet today's testing requirements and also prepare for future genotoxic and mutagenic impurities testing capabilities that continue to support strict quality standards?

Regulatory requirements for nitrosamine control have continued to evolve and expand, therefore increasing the versatility and sensitivity required for analytical testing. To support an effective and efficient strategy for nitrosamine control, and stay ahead of future regulatory changes, it is essential to implement high-performance technologies and flexible analytical workflows that support assays and exceed today's regulatory thresholds. Keeping up with advancements in technology and staying up-to-date with regulatory changes will allow laboratories to prepare for future requirements and support the ongoing supply of safe and effective pharmaceuticals.

Conclusion

The analysis of nitrosamines with LC/MS-MS plays a crucial role in pharmaceutical manufacturing. By developing robust methods, optimizing sensitivity, implementing effective sample preparation techniques, and staying ahead of regulatory requirements, laboratories can position themselves as trusted partners in the control of nitrosamines. With a future-proofed laboratory environment, you can meet current challenges and confidently navigate the evolving landscape of highly regulated mutagenic and genotoxic impurities analysis; ultimately ensuring the safety and quality of pharmaceutical products for patients worldwide.

At Waters, our global team of scientists support your next genotoxic impurity analysis challenge, from training to ongoing application specific expertise and services.

Learn about Waters Analytical solution for trace-level Mutagenic and genotoxic impurity analysis by visiting our website at waters.com/nitrosamines.

waters.com/nitrosamines

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