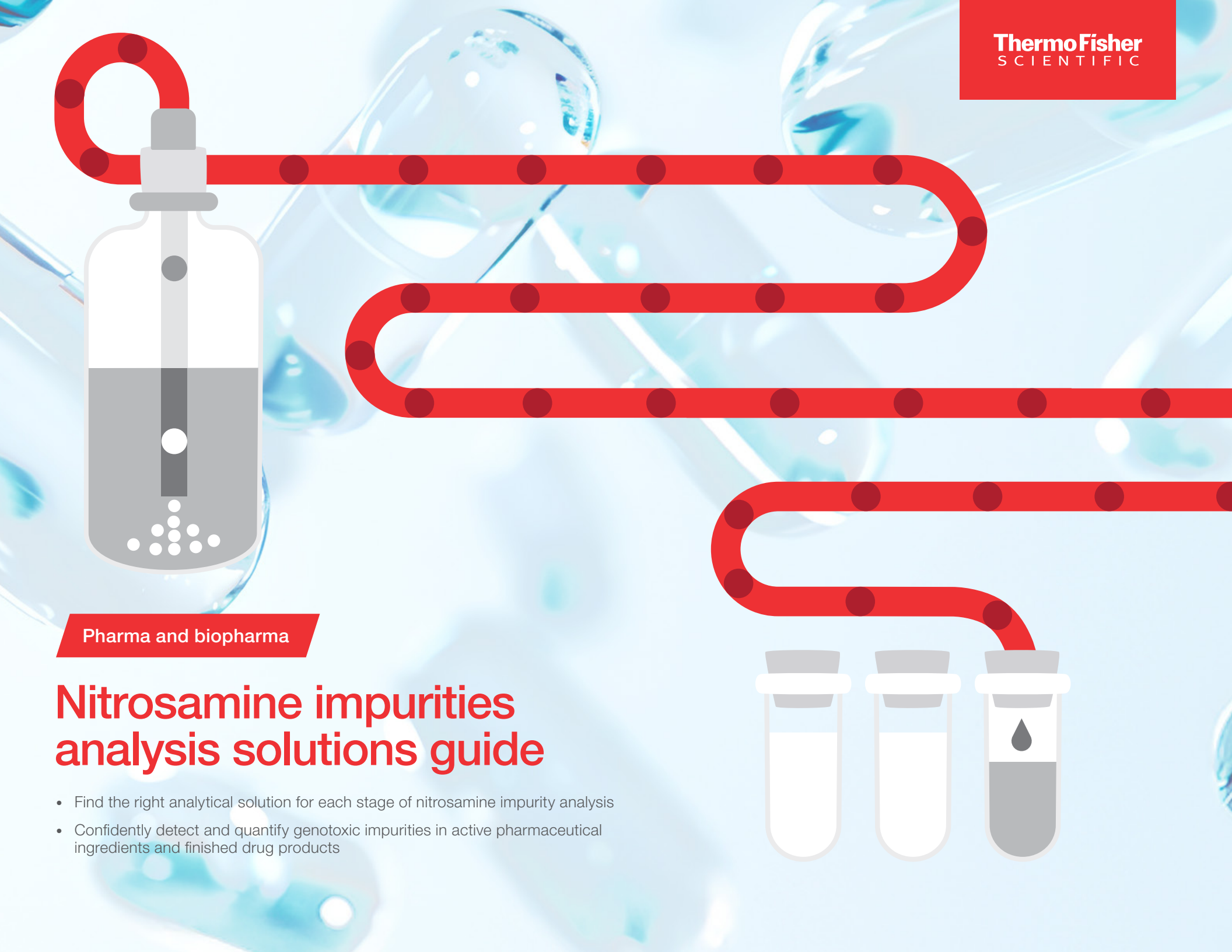


Pharma and biopharma

Nitrosamine impurities analysis solutions guide

- Find the right analytical solution for each stage of nitrosamine impurity analysis
- Confidently detect and quantify genotoxic impurities in active pharmaceutical ingredients and finished drug products

Contents

Advancing confidence in nitrosamine analysis: Solutions for a critical challenge

Since the discovery of elevated levels of small molecule nitrosamines in certain pharmaceuticals in July 2018, the pharmaceutical industry has faced growing pressure to identify, quantify, and control these class 1 genotoxic impurities. As a trusted partner in pharmaceutical analysis and quality control, Thermo Fisher Scientific delivers robust analytical solutions designed to help you meet evolving regulatory demands with confidence.

Whether you're working to:

- Monitor nitrosamines classified as known mutagenic carcinogens under IARC and ICH M7(R1) guidelines
- Detect and quantify Nitrosamine Drug Substance Related Impurities (NDSRIs)
- Assess and mitigate nitrosation risk by understanding nitrite levels in excipients
- Minimize the risk of costly drug recalls
- Stay ahead of complex global regulatory requirements

Thermo Fisher Scientific offers the expertise, technology, and support to keep your workflow both compliant and efficient. We can help navigate the path forward in nitrosamine analysis with greater precision and peace of mind.

Reliable results start with the right tools for nitrosamine analysis

Accurate nitrosamine impurity analysis demands robust, highly sensitive analytical techniques to ensure confidence in every result, especially when regulatory scrutiny is high.

Thermo Scientific™ technologies offer a proven, end-to-end solution for both exploratory and routine workflows, helping you meet global regulatory requirements with precision and efficiency.

Our comprehensive portfolio supports every step of the analytical process, empowering scientists to detect and quantify nitrosamines with clarity and consistency.

Key technologies include:

- Liquid, gas, and ion chromatography for reliable and reproducible separation
- High-resolution accurate-mass spectrometry (HRAM) to minimize false positives and deliver ultimate confidence
- Fast, sensitive tandem mass spectrometry (MS/MS) ideal for high-throughput routine analysis
- Automated sample extraction for streamlined workflows and minimized contamination risk
- Integrated compliance-ready software for unified data handling across all techniques

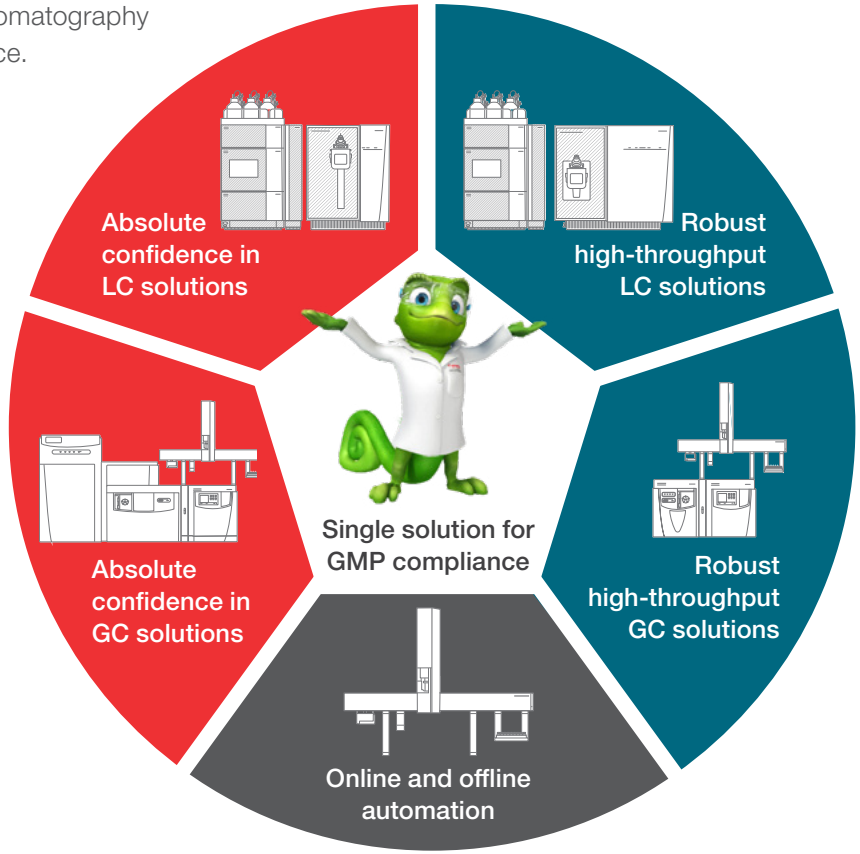
With Thermo Fisher Scientific as a collaborator, you can be confident in your results and your regulatory readiness.

Compliance for all analytical solutions

No matter which solution fits your needs, Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) is going to be there to assure confidence in your compliance.

Powerful, compliance-ready single solution platform for:

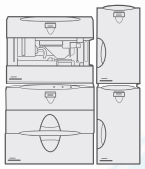
- Instrument control
- Data analysis and reporting
- In a secure, audit-trailed space
- LC-HRAM
- LC-MS/MS
- GC-HRAM
- GC-MS/MS
- IC
- Automation
- Fit within existing laboratory infrastructure of chromatographic systems



Get expert insights delivered, sign up for our scientific updates

Enabling analytical tools for each stage of nitrosamine impurity analysis

Ingredient and product monitoring

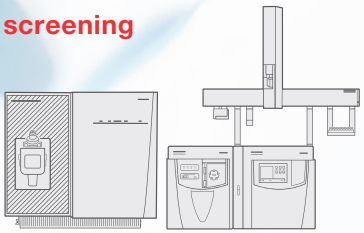


- Monitor nitrite and amine levels during manufacturing process and storage
- Risk monitoring

Highly confident detection and characterization

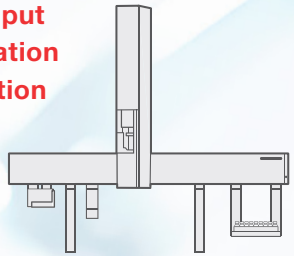
- High resolution accurate mass (HRAM) detection
- Impurity assessment and exploratory analysis
- Confident confirmation
- Routine assay capable

Routine screening



- Sensitive, high-throughput assays
- Complementary analysis
- LC and GC-MS/MS

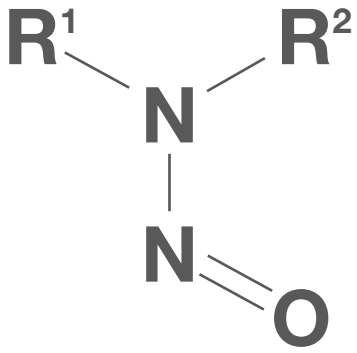
High-throughput assay automation and optimization

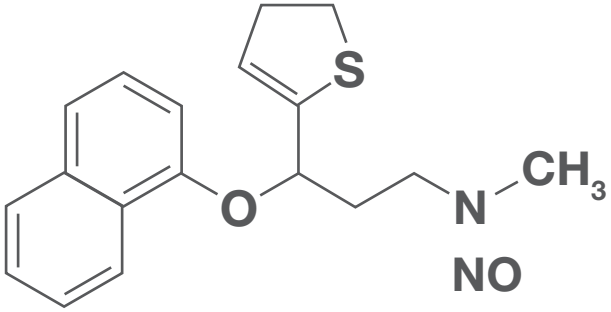


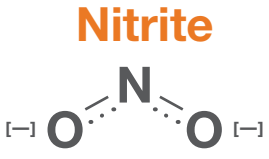
- To enable fast and reproducible
- Less hands-on
- Efficient monitoring

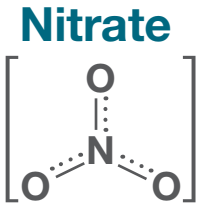
GMP compliance-ready software

Focus areas for nitrosamine analysis









N-nitrosamine impurity

- Small *N*-nitrosamines impurities belonging to a cohort of concern of ICH M7
- Individual and total *N*-nitrosamines content in drug substance (API) and drug product must be reported and meet acceptable limits

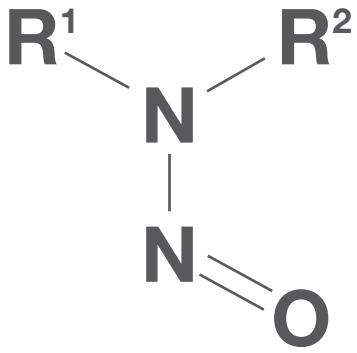
NDSRI

- Nitrosamine Drug Substance Related Impurity
- The API itself is being nitrosated to form *N*-nitrosamine
- Additional testing is needed to understand *N*-nitrosamine formation and routine monitoring

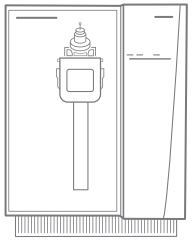
Precursor monitoring

- Batch-to-batch differences of nitrite concentration can increase the likelihood of nitrosamine formation
- Nitrite levels should be limited in drug substances and drug product to avoid rising impurity levels

Small *N*-nitrosamine impurity solutions



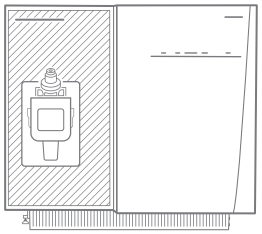
Nitrosamines are small molecular weight chemical substances that are probable human carcinogens, and their presence in medicines is considered unacceptable by regulators. Following risk assessment for their potential presence in a drug substance, routine batch monitoring maybe required.



LC-HRAM MS

Thermo Scientific™ Orbitrap Exploris™ 120 Mass Spectrometer

Gold standard in selectivity

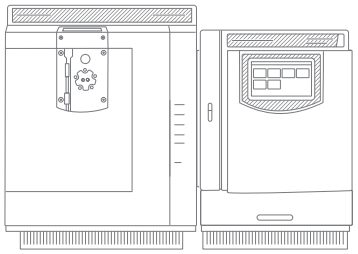


LC-MS/MS

Thermo Scientific™ TSQ Altis™ Plus Triple Quadrupole Mass Spectrometer

Thermo Scientific™ TSQ Quantis™ Plus Triple Quadrople Mass Spectrometer

Productivity and universal applicability

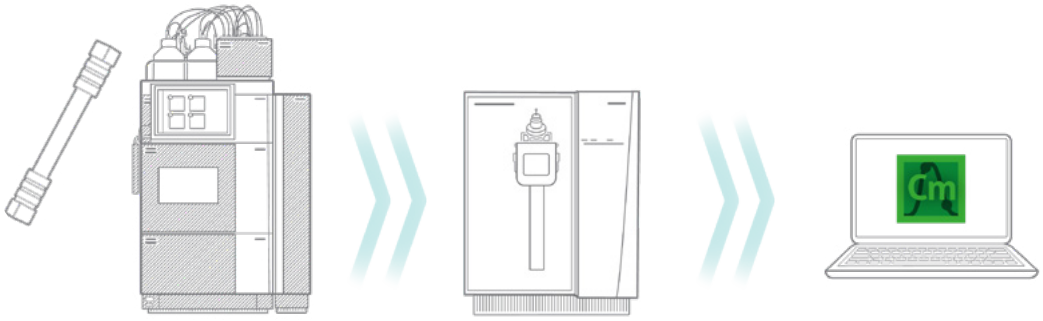


GC-MS

Thermo Scientific™ Orbitrap Exploris™ GC Mass Spectrometer

Ultimate chromatographic resolution and sensitivity

LC-HRAM MS solution for absolute mass confirmation



Robust separation, high load ability

Confident mass detection

Fit-for-purpose software

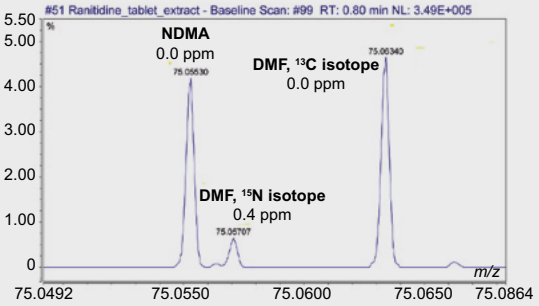
- Stable chromatography over extended period and hundreds of injections
- Confidence in analytical results allowing exceptional screening or confirmatory analysis
- Ease-of-use and compliance-ready software

The Thermo Scientific Orbitrap Exploris 120 mass spectrometer system provides high resolution accurate mass assuring absolute identification and quantitation of nitrosamines.

Featured Application note

HRAM LC-MS method for the determination of nitrosamine impurities in drugs

- Chromatographic co-elution of NDMA and DMF may cause over-estimation (false positive) if mass resolution is not sufficient
- The mass difference between NDMA and DMF ^{15}N isotope is only 21 ppm, 0.002 amu difference—requires minimum 45K resolution and 3 ppm mass accuracy



Mass spectrum of ranitidine drug tablet when co-elution of NDMA and DMF happens, with a resolution setting of 120,000 confident and accurate identification of isotope masses possible and allows for accurate quantitation.

Learn more about our LC-HRAM MS solution

Products and resources

Click on each listing for more information

Products

Robust separation, high load ability

Impurity separation with (U)HPLC platforms

- Thermo Scientific™ Acclaim™ 120
- Thermo Scientific™ Vanquish™ Flex UHPLC System
- Thermo Scientific™ Vanquish™ Core HPLC System

Confident mass detection

Mass detection

- Thermo Scientific™ Orbitrap Exploris™ 120 Mass Spectrometer

Fit-for-purpose software

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software

Interested in optimizing your LC-HRAM workflows? Start the conversation with a specialist today.

Resources

Literature

- Application note: HRAM LC-MS method for the determination of nitrosamine impurities in drugs
- Application note: High-resolution accurate-mass liquid chromatography methodology
- Article: How to comply with FDA imposed nitrosamine impurity testing
- Brochure: Vanquish Core
- Brochure: Orbitrap Exploris 120
- Brochure: Chromeleon CDS software
- Brochure: Consumables

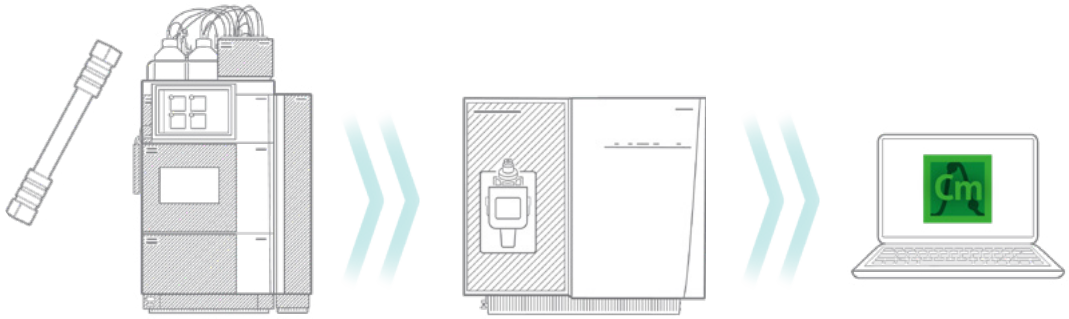
Web tools

- Vanquish selection tool
- Nitrosamine impurity analysis
- Orbitrap LC-MS
- HPLC and UHPLC platforms

Webinars

- Nitrosamine analysis: A leading CDMO's perspective
- Virtual seminar: Nitrosamine analysis

LC-MS/MS solution for your targeted monitoring needs



Robust separation, high load ability

Robust and sensitive mass detection

Fit-for-purpose software

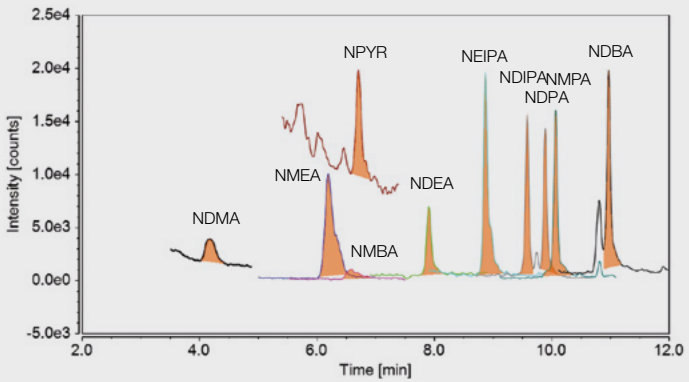
- Stable chromatography over extended period and hundreds of injections
- Great for screening or confirmatory analysis
- Ease-of-use and compliance-ready software

The Thermo Scientific Triple Quadrupole systems assure absolute confidence in results for any targeted quantitation needs.

Featured Application note

Highly sensitive and robust LC-MS/MS solution for quantitation of nitrosamine impurities in metformin drug products

- Thermo Scientific™ Vanquish™ Horizon UHPLC, Thermo Scientific™ Hypersil GOLD™ phenyl column coupled to a TSQ Quantis Chromeleon CDS
- LOQ 5 ppb using APCI and 10 ppb using HESI
- Excellent reproducibility for over 1,000 sample injections



XIC of nitrosamine quantifier ions in a 20 ppb spiked sample. APCI data are shown.

Learn more about our LC-MS/MS solution

Products and resources

Click on each listing for more information

Products

Robust separation, high load ability

Impurity separation with (U)HPLC platforms

- Thermo Scientific™ Acclaim™ 120
- Thermo Scientific™ Vanquish™ Flex UHPLC System
- Thermo Scientific™ Vanquish™ Core HPLC System

Robust and sensitive mass detection

Mass detection

- Thermo Scientific™ TSQ Altis™ Plus Triple Quadrupole Mass Spectrometer
- Thermo Scientific™ TSQ Quantis™ Plus Triple Quadrupole Mass Spectrometer

Fit-for-purpose software

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software

Would you like to learn more? Contact a LC-MS/MS specialist now.

Resources

Literature

- Application note: Highly sensitive and robust LC-MS/MS solution for quantitation of nitrosamine impurities in metformin drug products
- Application note: TSQ Quantis application note
- Article: How to comply with FDA imposed nitrosamine impurity testing
- Article: Nitrosamine, a CDMO perspective
- Brochure: Chromeleon CDS software
- Brochure: TSQ Quantis
- Brochure: Consumables
- Brochure: Vanquish Core

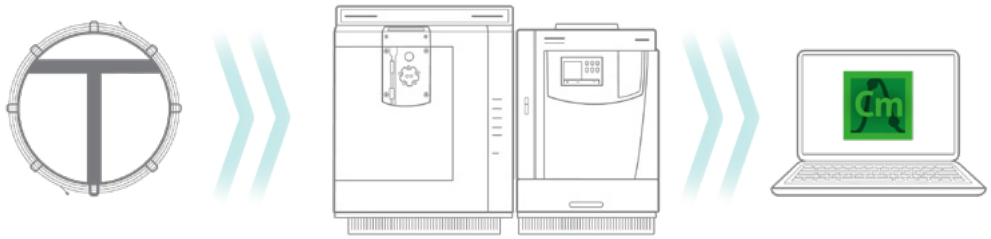
Web tools

- Nitrosamine impurity analysis
- TSQ LC-MS/MS portfolio
- HPLC and UHPLC platforms

Webinars

- Nitrosamine analysis; a leading CDMO's perspective
- Virtual seminar: Nitrosamine analysis

GC-HRAM MS solution for nitrosamine analysis gives ultimate sensitivity and confidence



Robust separation, high load ability

Confident mass detection

Fit-for-purpose software

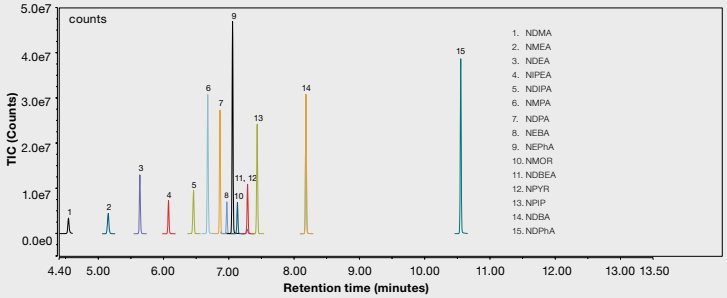
- Ultimate sensitivity
- Eliminate false positive results
- Ease-of-use and compliance-ready software

The Thermo Scientific Orbitrap Exploris GC-MS system provides high resolution accurate mass confirmation while beating LOQ requirements.

Featured Application note

A validated method for the rapid determination of fifteen nitrosamines in metformin drug substance

- Rapid separation of 15 nitrosamines in <12 minutes
- 10× <FDA regulatory limits of 30 ppb (ng/g) <2 ppb
- Excellent robustness over 2 weeks continual analysis



XIC of nitrosamine quantifier ions in a 50 ppb spiked sample.

Learn more about our GC-HRAM MS solution

Products and resources

Click on each listing for more information

Products

Robust separation, high load ability

Impurity separation with GC platforms

- Thermo Scientific™ TraceGOLD TG-1701MS GC Column

Confident mass detection

Mass detection

- Thermo Scientific™ Orbitrap Exploris™ GC Mass Spectrometer

Fit-for-purpose software

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software

Would you like to learn more? Contact a GC-HRAM specialist now.

Resources

Literature

- Applicaton note: GC Exploris HRAM validation of 15 nitrosamines in metformin drug substance
- Article: How to comply with FDA imposed nitrosamine impurity testing
- Case study: GC solutions for nitrosamines
- Brochure: Orbitrap Exploris GC
- Brochure: Chromeleon CDS software
- Catalogs: Consumables

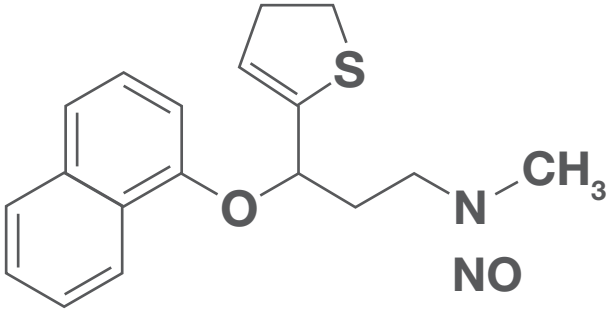
Web tools

- Orbitrap GC-MS 3D demo

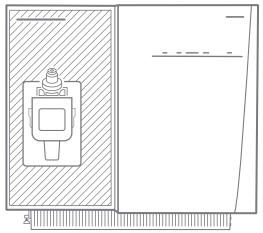
Webinars

- Analytical challenges and the effective management of nitrosamines: The evolving landscape of NDSRI analysis
- Virtual seminar: Nitrosamine analysis

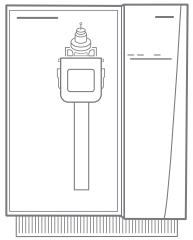
Solutions for Nitrosamine Drug Substance Related Impurity



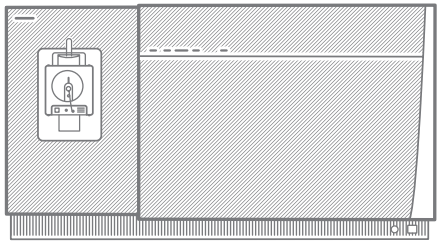
Nitrosamine Drug Substance Related Impurity (NDSRI) represent the next challenge in this story. Active pharmaceutical ingredients (APIs) containing a secondary (or even tertiary amine) are at risk of nitrosation, and as such need to be monitored for levels. To date the FDA has established a list of over 200 drugs on the market, and provided guidance of the acceptable intact (AI).



LC-MS/MS

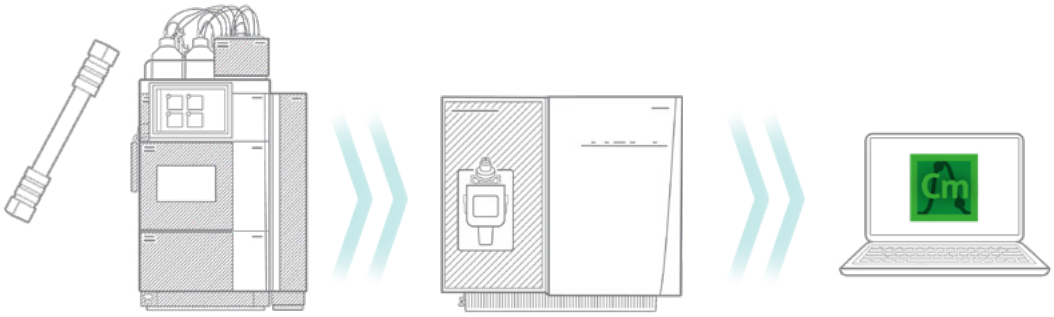


LC-HRAM



SQUAD
Simultaneous **QUA**ntitation and **D**iscovery

Routine quantitation of NDSRI by LC-MS/MS



Robust separation, high load ability

Robust and sensitive mass detection

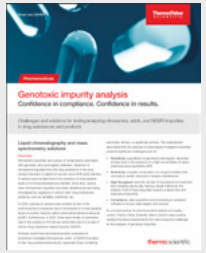
Fit-for-purpose software

- Stable chromatography over extended period and hundreds of injections
- Great for screening or confirmatory analysis
- Ease-of-use and compliance-ready software

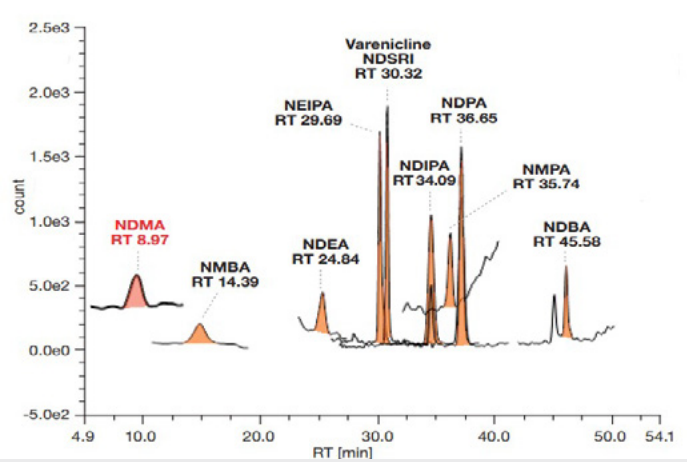
The Thermo Scientific Triple Quadrupole systems assure absolute confidence in results for any targeted quantitation needs.

Featured Application Note

Challenges and solutions for testing/analyzing nitrosamine, azido, and NDSRI impurities in drug substances and products



- Vanquish Horizon UHPLC, Acclaim 120 C18 column coupled to a TSQ Quantis plus with Chromeleon CDS software
- Nitrosamine impurities and NDSRI in same method



SRM chromatograms of eight NSM impurities and N-nitroso-varenicline neat standard at 1 ng/mL level.

Advantage of Orbitrap technology for NDSRI quantitation

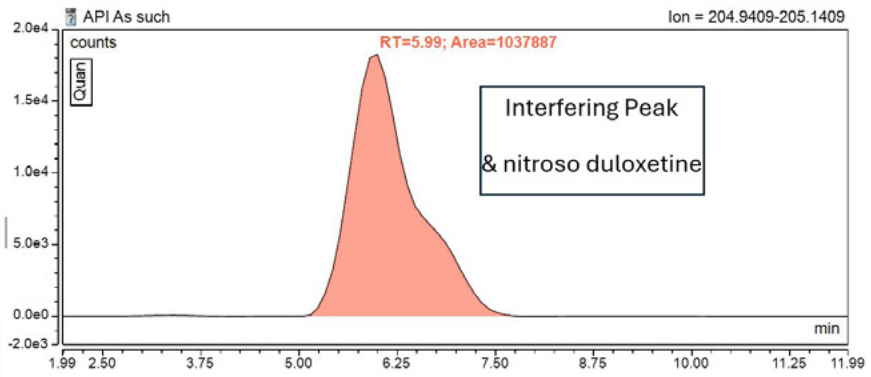
High resolution accurate mass enables the separation of ions with very close m/z values, effectively resolving interfering peaks that would otherwise overlap in unit resolution mass spectrometry.

Greater confidence in drug safety and efficacy: This technology ensures that the drug on market is safe and maintains its efficacy. Additionally, it helps to avoid unnecessary financial impacts due to false positive results.

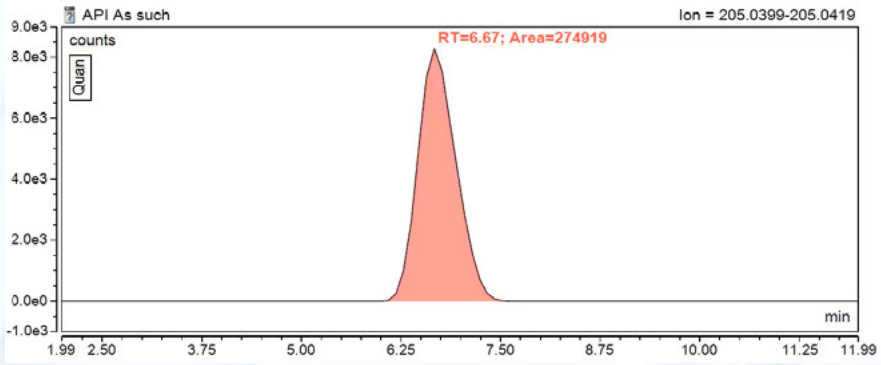
Efficiency in analysis: Extensive method develop times, and long analysis durations. Implementing Orbitrap technology reduces extensive method develop times, and long analysis durations by complementing chromatographic separation with the high resolution and mass accuracy of Orbitrap acquisition.

Ultimate confidence—eliminate false positive results with HRAM. Read our case study.

Analysis of *N*-nitroso duloxetine by quadrupole MS/MS

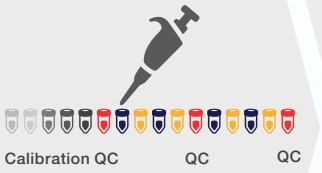


Analysis of *N*-nitroso duloxetine by Orbitrap™ HRAM



Simultaneous Quantitation and Discovery (SQUAD)

This approach focuses on confidently annotating and accurately **quantifying a targeted set of compounds** using authentic standards and calibration curves. **Simultaneously**, it performs **untargeted analysis to identify novel impurities**, all within a single injection.



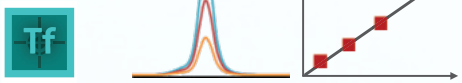
Sample preparation

Data acquisition
Single injection

Targeted analysis

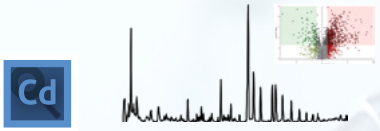
Confident identification

Accurate quantitation



Untargeted analysis

Differential analysis

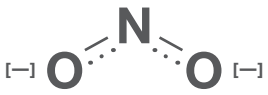


Unknowns' annotation

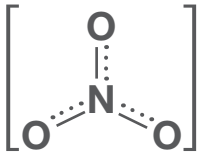
Stay up-to-date

Solutions for nitrite and nitrate detection

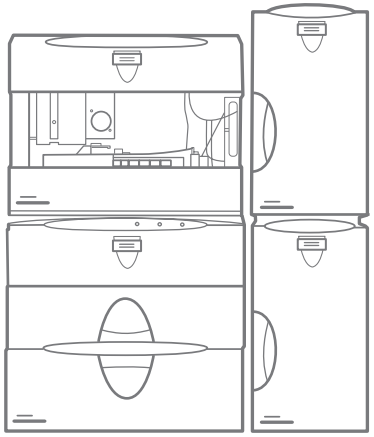
Nitrite



Nitrate

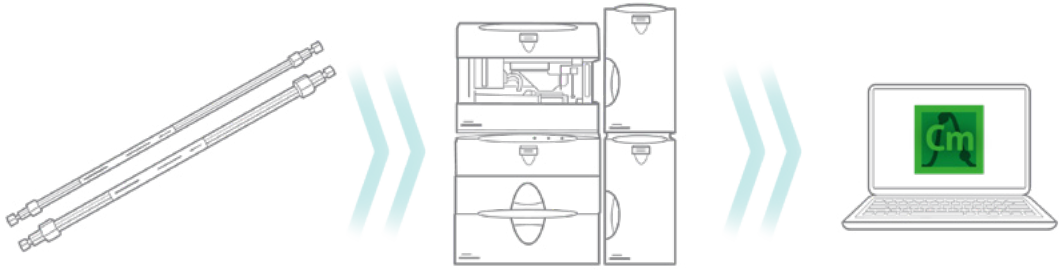


Nitrite, a known precursor in *N*-nitrosamine formation, can react with secondary or tertiary amines present during API synthesis or in the drug formulation process. Accurately measuring nitrite and nitrate levels is critical for drug manufacturers. Similarly, excipient manufacturers must monitor nitrite content to ensure compliance with quality standards.



IC-MS
Thermo Scientific™ Dionex™ ICS-6000 HPIC™ System
Reliable and sensitive detection of ions such as nitrite and nitrate

IC solution for nitrite analysis needs with confident targeted approach



High capacity columns

Selective separations

Fit-for-purpose software

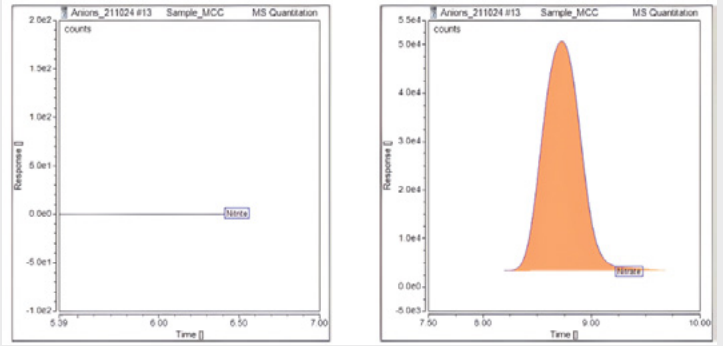
- Little or no sample preparation is required
- Thermo Scientific™ Dionex™ Reagent-Free™ (RFIC™) ion chromatography system with electrolytically generated KOH eluent
- Multi-drug product workflow

IC can be coupled with many detectors, here coupling to single quad mass spectrometry gives added levels of selectivity and sensitivity.

Featured Application note

Analysis of nitrite and nitrate from microcrystalline cellulose by IC-MS

- Quantitation from 1 to 150 ppb
- The method is accurate and precise
- Trace levels of nitrite and nitrate in the same run



SIM chromatogram of nitrite (left) and nitrate (right) in the tested microcrystalline cellulose sample.

Learn more about our IC solution

Products and resources

Click on each listing for more information

Products

High capacity columns

Impurity separation with (U)HPLC platforms

- Thermo Scientific™ Dionex IonPac™ AS19-4µm Column

Selective separation

Ion chromatography

- Thermo Scientific™ Dionex™ ICS-6000 HPIC™ System

Fit-for-purpose software

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software

Would you like to learn more? Contact a IC specialist now.

Resources

Literature

- Application note: Determination of nitrite in pharmaceuticals
- Brochure: Chromeleon CDS software
- Brochure: Consumables

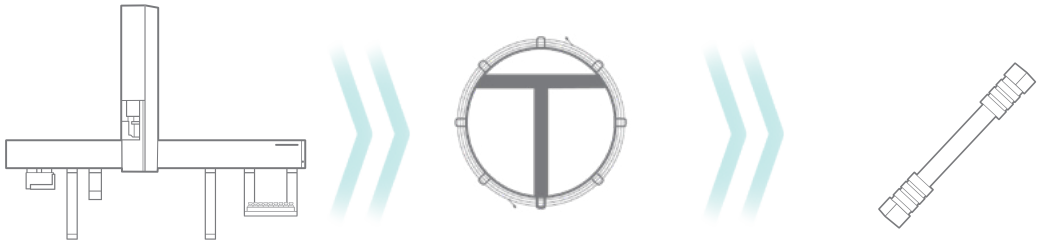
Web tools

- IC home page
- Nitrosamine impurity analysis

Webinars

- Nitrosamine analysis; a leading CDMO's perspective
- Virtual Seminar: Nitrosamine analysis

Automated sample preparation for LC and GC



TriPlus RSH performs sample preparation

Samples can be immediately run on GC-MS method

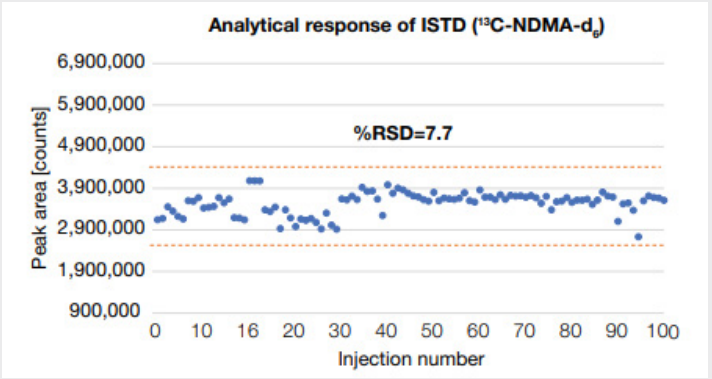
Or solvent exchanged (automated) for LC-MS method

- Sample prep automated from start to finish
- Thermo Scientific™ TriPlus™ RSH controlled by compliant ready Chromeleon CDS software as part of the analytical method
- Method can be adapted to end in GC-MS or LC-MS analysis

Featured Application note

An automated sample preparation workflow for the analysis of nitrosamines in metformin

- Unattended operation and improved sample throughput
- Reduce the risk of contamination by handling
- Samples can be run on LC or GC analysis



ISTD (13C-NDMA-d6) peak area % RSD obtained analyzing extracts (n=100) over a period of several weeks.

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Example proven solutions to help address regulatory needs

Requirement	Example approach	API	Separation	Detection	Download
Fit-for-purpose, reliable quantitative screening	Highly sensitive and robust LC-MS/MS solution for quantitation of nitrosamine impurities in metformin drug products	Metformin	LC	Triple quad MS	
	Determination of genotoxic nitrosamines in Valsartan	Valsartan	GC	Triple quad MS	
Future proof your lab with absolute confidence and quantitation	A validated method for the rapid determination of fifteen nitrosamines in metformin drug substance	Metformin	GC	High resolution MS	
	HRAM LC-MS method for the determination of nitrosamine impurities in drugs	Ranitidine	LC	High resolution MS	
	HRAM LC-MS methodology for the determination and quantitation of nitrosamine impurities in drug products	Ranitidine Valsartan	LC	High resolution MS	
	Overcoming the challenges of nitrosamine impurities in drugs: What Pharmaceutical QA/QC laboratories need to know	Metformin Valsartan	GC	High resolution MS Triple quad MS	
Ingredient and product monitoring	Determination of dimethylamine and nitrite in pharmaceuticals by ion chromatography to assess the likelihood of nitrosamine formation	Multiple	IC	UV / CD	

Stay up-to-date with latest releases on this topic.

Need more information? Contact a specialist

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