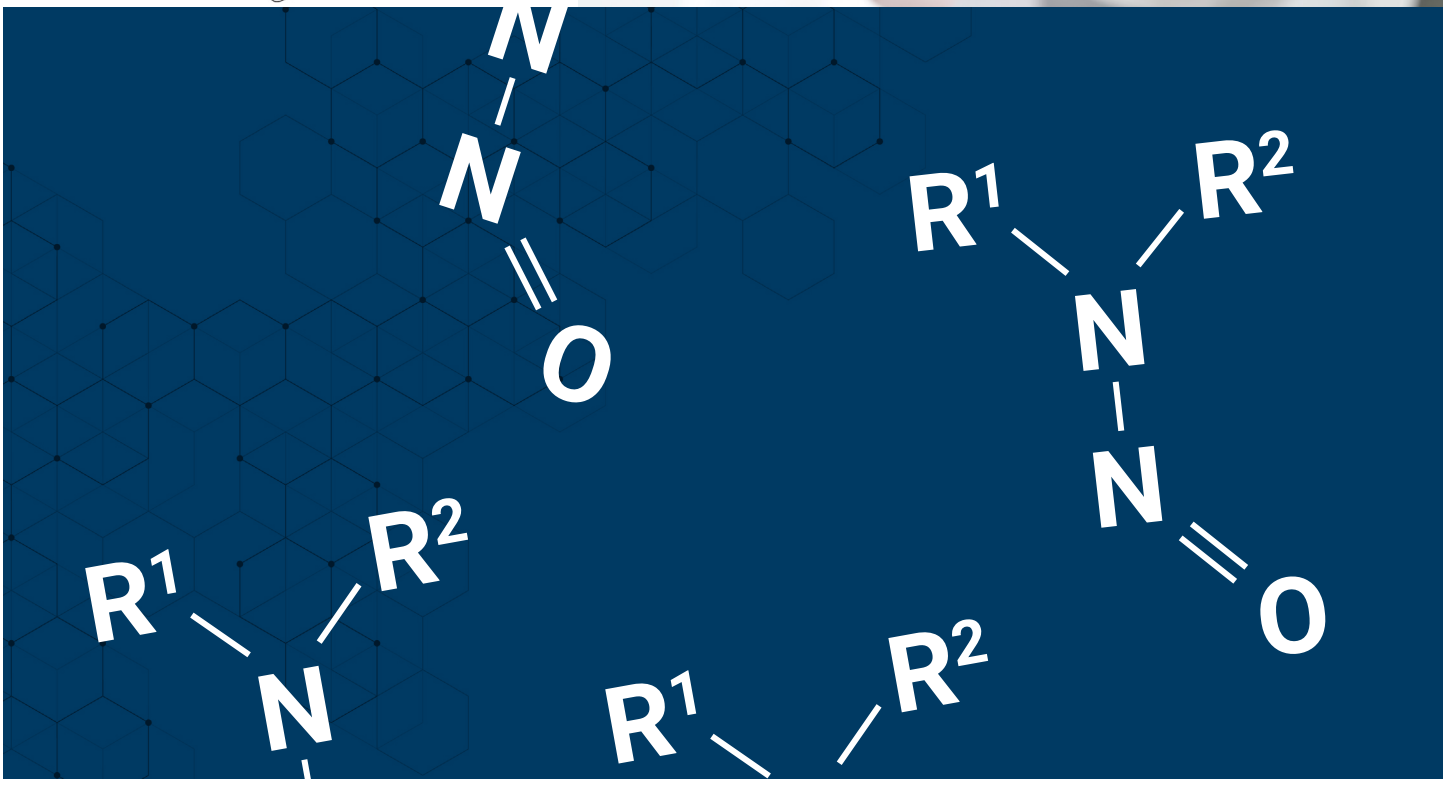
Agilent CrossLab
From Insight to Outcome

Nitrosamines Analysis in Pharmaceuticals

Using Triple Quadrupole LC/MS/MS and Quadrupole Time of Flight LC/MS
Consumables workflow ordering guide



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Mutagenic impurities in APIs and drug products pose a significant risk to health and safety—even in small quantities—and thus are a major concern for drug makers. Mutagenic impurities can damage DNA, leading to mutations and potentially cancer. Efforts to address and control the presence of trace levels of mutagenic impurities is of special concern to global regulators. As a result, the U.S. FDA and other regulatory agencies have taken steps to address the issue of mutagenic impurities in pharmaceuticals[1]. Detection and quantification of these trace nitrosamines in APIs and drug products can be challenging, necessitating the use of advanced and sensitive tools to meet regulatory requirements.

The list of APIs and drug products for nitrosamine determination has expanded beyond angiotensin II receptor blocker (ARB) Sartan drugs and include metformin, an oral diabetes drug and histamine-2 receptor antagonists such as ranitidine. This is evidenced by the recent recalls of metformin by various regulatory bodies like the U.S. Food & Drug Administration (FDA), European Directorate for the Quality of Medicines (EDQM), and Health Sciences Authority (HSA) due to the presence of N-nitroso-dimethylamine (NDMA). These impurities: (N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodiisopropylamine (NDIPA), N-nitrosoethylisopropylamine (NEIPA) and N-nitrosodibutylamine (NDBA) are classified as probable human carcinogens and are believed to have been introduced into the finished products due to chemical reactions that occur during the API manufacturing process.

These impurities can be detected using either a single quadrupole GC/MS (GC/MSD), or triple quadrupole GC/MS/MS (GC/TQ)(*1*), a triple quadrupole LC/MS/MS (LC/TQ), or quadrupole time of flight LC/MS (LC/Q-TOF)(*2-7*) . LC/MS/MS-based methods are generally very specific and highly sensitive. For this reason, these have served as the basis for development of methods to detect and quantify nitrosamine impurities in drug substance and drug products such as metformin, valsartan, losartan and irbesartan.

Figure 1. From left to right: 1260 Infinity II LC System, 6470B Triple Quadrupole LC/MS, 6550 iFunnel Q-TOF LC/MS, and Ultivo Triple Quadruple LC/MS.

[1]*U.S. FDA: www.fda.gov/media/131868/download Council of Europe: www.edqm.eu/en/news/omcls-release-three-methods-determination-ndma-sartans Health Canada: healthycanadians.gc.ca/recall-alert-rappel-avis/hc-sc/2020/72963a-eng.php Taiwan FDA: www.fda.gov.tw/ENG/siteList.aspx?sid=10360*

Column Choices

The pentafluorophenyl (PFP) ligand on the **InfinityLab Poroshell 120 PFP column** used in Method 2 (Table 1) (*8*) provides an orthogonal separation mechanism with C18 chemistries. PFP phases can separate analytes based on small differences in structure, substitution, and steric access to polar moieties. The resulting selectivity for positional isomers, halogenated compounds, and polar analytes is particularly useful when analyzing complex mixtures. Since NDIPA and NDPA are positional isomers, the InfinityLab Poroshell 120 PFP is the ideal recommended column for this separation for *ease of use*.

The **InfinityLab Poroshell HPH-C18 column**, which has also been used for this analysis in Method 1 (Table 1) (*8*) incorporates hybrid particle technology which improves particle *ruggedness at extended pH*, enabling long lifetimes and fewer column changes. When using this column, however, a robust method development process is critical to ensure that the method is long lasting, stable and reliable. Because the retention and selectivity of ionizable compounds can change significantly with varying pH, it is becoming standard practice to employ low-, medium-, and high-pH analyses during method development. In addition to optimizing the gradient conditions, in order to achieve separation between the positional isomers NDIPA and NDPA, the instrument MS/MS parameters also need to be optimized to maximize sensitivity.

LC configuration and parameters

Table 1. UHPLC configuration and settings. For method details, see Reference 8.

Parameter	Value	
	Method 1	**Method 2**
Instruments	Agilent 1290 Infinity II high-speed pump (G7120A) Agilent 1290 Infinity II multisampler (G7167B) Agilent 1290 Infinity II multicolumn thermostat (G7116B) Agilent 1260 Infinity diode array detector (G1315C)	Agilent 1290 Infinity II high-speed pump (G7120A) Agilent 1290 Infinity II multisampler (G7167B) Agilent 1290 Infinity II multicolumn thermostat (G7116B) Agilent 1260 Infinity diode array detector (G1315C)
Needle Wash	Methanol: water (80:20)	Methanol: water (80:20)
Sample Diluent	Water: methanol (95:5)	Methanol
Multisampler Temp.	10 °C	10 °C
Injection Volume	20 µL	5 µL
Analytical Column	Agilent InfinityLab Poroshell HPH-C18, 4.6 × 150 mm, 2.7 µm (p/n 693975-702(T))	Agilent InfinityLab Poroshell 120 PFP, 3.0 x 150 mm, 2.7 µm (p/n 693975-308)
Column Temp.	40 °C	40 °C
Mobile Phase A	0.1% formic acid in water	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in methanol	0.1% formic acid in methanol
Flow Rate	0.5 mL/min	0.5 mL/min
Gradient	**Time (min) / % A / % B / Flow (mL/min)** 0 / 95 / 5 / 0.5 2 / 95 / 5 / 0.5 7 / 40 / 60 / 0.5 10 / 25 / 75 / 0.5 11 / 10 / 90 / 0.5 16.5 / 10 / 90 / 0.5 16.6 / 95 / 5 / 0.5 20.0 / 95 / 5 / 0.5	**Time (min) / % A / % B / Flow (mL/min)** 0 / 95 / 5 / 0.5 3 / 95 / 5 / 0.5 14 / 40 / 60 / 0.5 17 / 10 / 90 / 0.5 19 / 10 / 90 / 0.5 19.1 / 95 / 5 / 0.5 22 / 95 / 5 / 0.5
Stop Time	20 minutes	22 minutes
UV Wavelength	230 nm	230 nm

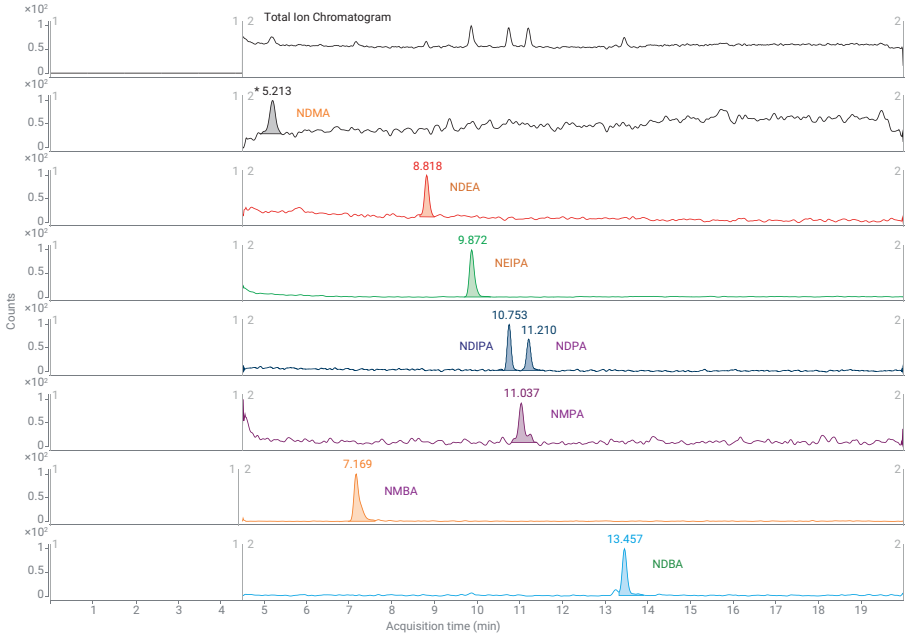

Figure 2. Representative MRM chromatogram of all the nitrosamine impurities at 0.5 ng/mL using Method 1 (For method details see Reference 8).

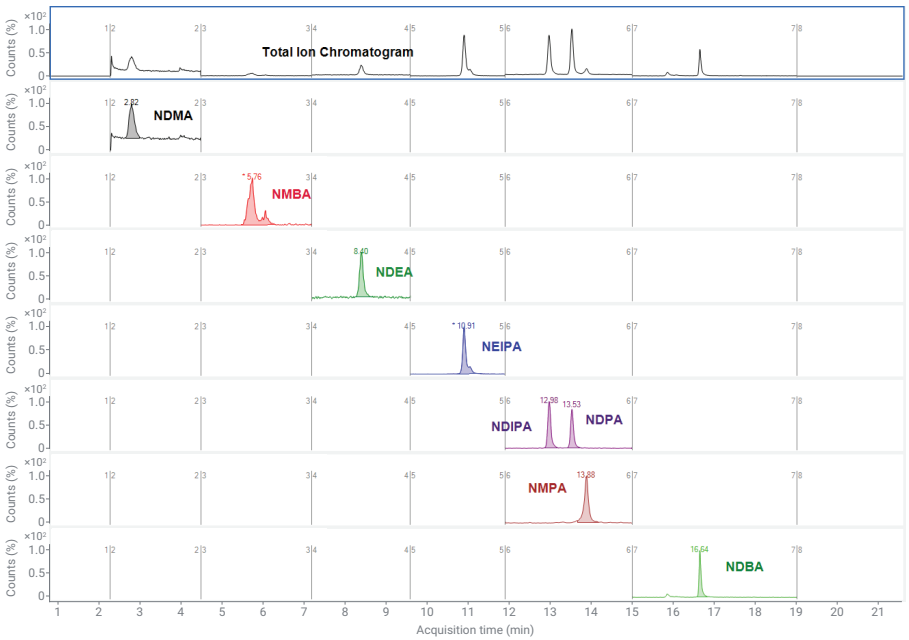

Figure 3. Representative MRM chromatogram of all eight nitrosamine impurities at 3 ng/mL using Method 2 (For method details see Reference 8).

Easy Selection and Ordering Information

This guide provides recommendations for Agilent products used in this analysis, so you can find what you're looking for quickly. Click the MyList* links in the header below to add items to your "Favorite Products" list at the Agilent online store. Then, enter the quantities for the products you need. Your list will remain under "Favorite Products" for your use with future orders.

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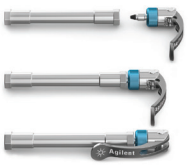
Product Description	Part Number
Nitrosamine Standards and Solvents	
Nitrosamine standards (in methylene chloride)	US-113N-1
InfinityLab Ultrapure LCMS MeOH	5191-4497
InfinityLab Ultrapure LCMS water	5191-4498
Formic acid, 5 mL	G2453-85060
Nitrosamine - Sample Preparation	
15 mL Centrifuge tubes and caps, 50/pk	5610-2039
Captiva Econofilter, PVDF membrane, 13 mm diameter, 0.2 µm pore size, 1000/pk	5190-5261
Captiva Premium Syringe Filter PES membrane, 15 mm diameter, 0.2 µm pore size, 100/pk (LCMS certified)	5190-5096[1]
Captiva Premium Syringe Filter Nylon membrane 15mm diameter, 0.2 µm pore size, 100/pk (HPLC certified)	5190-5088[2]
Captiva Disposable Syringe, 5 mL, 100/pk	9301-6476
Captiva Disposable Syringe, 10 mL, 100/pk	9301-6474
Nitrosamine - LC Column	
InfinityLab Poroshell 120 PFP, 3.0 x 150 mm, 2.7 µm (recommended)	693975-308
InfinityLab Poroshell 120 PFP guard column, 3.0 mm, 2.7 µm (recommended)	823750-915
InfinityLab Poroshell HPH-C18, 4.6 × 150 mm, 2.7 µm	693975-702
InfinityLab Poroshell HPH-C18, 4.6 mm, 2.7 µm, UHPLC guard, 3/pk	820750-922
Nitrosamine - LC Supplies	
InfinityLab Quick Connect assembly, 0.12 x 105 mm, for column inlet connection on UHPLC	5067-5957
InfinityLab Quick Connect assembly, 0.17 x 105 mm, for column inlet connection on UHPLC	5067-6166
InfinityLab Quick Turn fitting, for column outlet	5067-5966
Quick Turn capillary 0.12 x 280 mm, for connection from column to detector	5500-1191
Kit of stay safe waste cap GL45 with 4 ports and waste can 6 L	5043-1221
Charcoal filter with time strip for waste container	5043-1193
InfinityLab Stay Safe cap, starter kit	5043-1222
Stainless steel solvent inlet filter, 10 µm pore size	01018-60025
InfinityLab solvent filtration assembly includes glass funnel, 250 mL, membrane holder glass base, glass flask, 1 L, and aluminum clamp	5191-6776[3]
Regenerated cellulose membrane 47 mm, 0.20 µm 100/pk	5191-4340[3]



Nitrosamines-Vials and Caps	
Vial, screw top, amber, write-on spot, certified, 2 mL, 100/pk. Vial size: 12 x 32 mm (12 mm cap)	5182-0716
Cap, screw, green, preslit PTFE/silicone, 100/pk. Cap size: 12 mm	5183-2077
Vial insert, 250 µL, deactivated glass with polymer feet, 100/pk 5	5181-8872
Nitrosamines-MS Supplies	
APCI Needle Replacement Kit	G1946-68704
APCI Needle	G1960-20030
Capillary, Fast Switching, 0.6 mm	G1960-80060

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1. Having similar performance to PVDF, this membrane has low protein binding and is ideal for protein analysis. Available in a smaller pack size (100/pk) and is LC/MS certified.

2. Ideal for general filtration needs. Should not be used for protein analysis. Available in a smaller pack size (100/pk) and is HPLC certified.

3. Solvent filtration assembly and associated filter membranes are not recommended for use with InfinityLab Ultrapure LC/MS solvents.

References

1. Nitrosamines analysis in pharmaceuticals using single quadrupole GC/MS and triple quadrupole GC/MS/MS: Consumables workflow ordering guide, Agilent publication (5994-2979EN)
2. Nitrosamine Impurities Application Guide – Confidently detect and quantify mutagenic impurities in APIs and Drug Products (5994-2393EN)
3. Determination of a Genotoxic NDMA Impurity Using the High-Resolution Agilent 6546 LC/Q-TOF in Ranitidine Drug Substance and Drug Products (5994-1626EN)
4. Simultaneous Determination of Eight Nitrosamine Impurities in Metformin Using the Agilent 6470 Triple Quadrupole LC/MS (5994-2286EN)
5. Determination of Nitrosamine Impurities Using the Ultivo Triple Quadrupole LC/MS (5994-1383EN)
6. Determination of NDMA Impurity in Ranitidine Using the Agilent 6470 Triple Quadrupole LC/MS (5994-1668EN)
7. Determination of Nitrosamine Impurities Using the High-Resolution Agilent 6546 LC/Q-TOF (5994-1372EN)
8. Simultaneous Determination of Eight Nitrosamine Impurities in Metformin Extended-Release Tablets Using the Agilent 6470 Triple Quadrupole LC/MS (5994-2533EN)

Agilent Chemical Standards

Looking for high quality standards for additional applications? Search our catalog of 5000 chemical standards to find the best standard to use in your method, application or workflow. Can't find the right catalog standard? Our website also has an automated custom standard quoting tool to help you develop the standard that fits your needs.

Agilent LC Selector Tool

Are you looking for an LC column best suited for your applications? Use Agilent's LC column selector tool to select the optimal LC column.

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