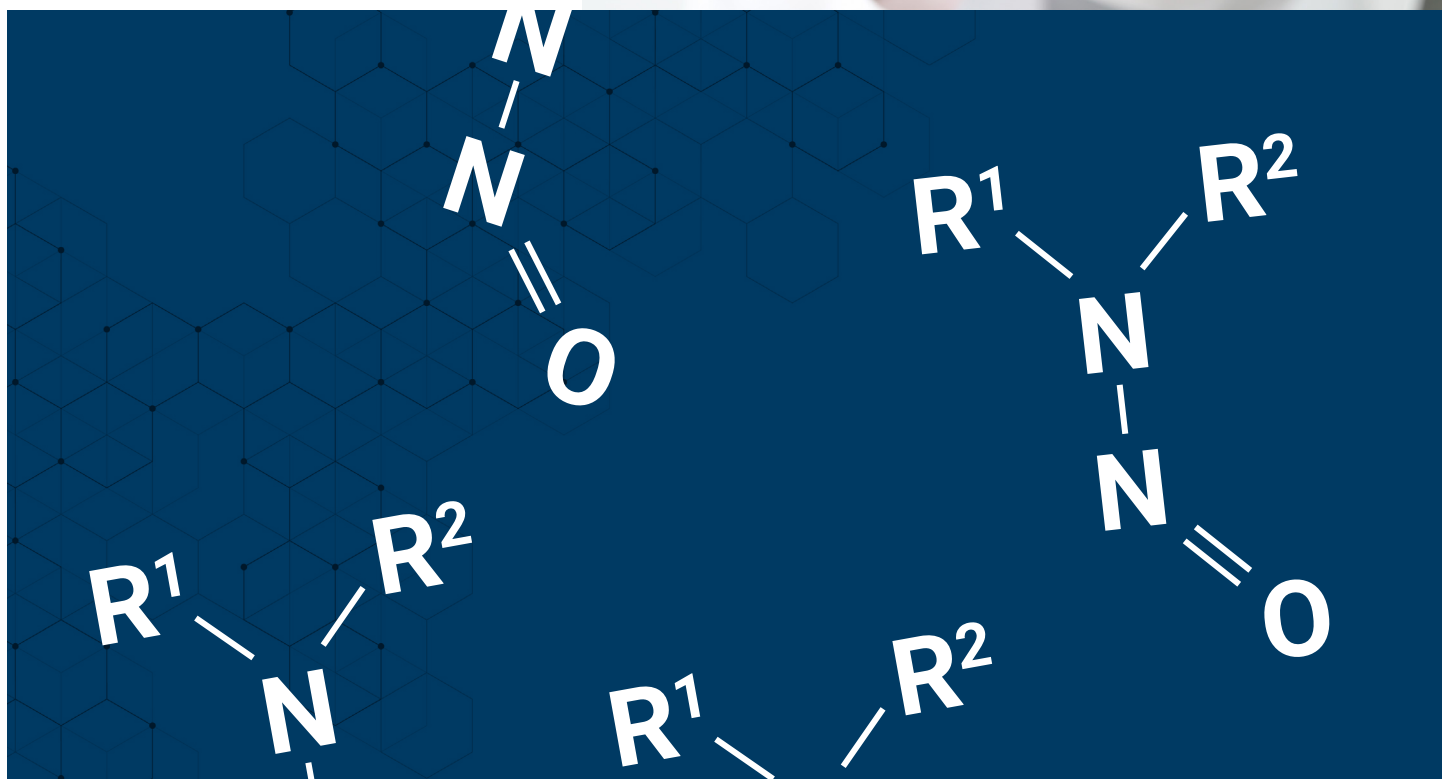
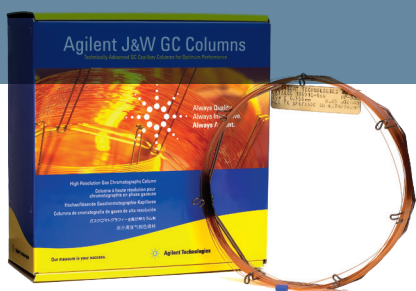


# Nitrosamines Analysis in Pharmaceuticals

Using Single Quadrupole GC/MS or Triple Quadrupole GC/MS  
Consumables workflow ordering guide



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<sup>1</sup>. 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.

Sartan drugs are angiotensin II receptor blockers (ARBs), used to treat high blood pressure and congestive heart failure (1). Metformin is an oral diabetes drug used to control blood sugar levels. Ranitidine products treat heartburn and acid reflux. All have recently been recalled by the FDA due to the presence of high levels of nitrosamine impurities:

- N-nitrosodimethylamine (NDMA)
- N-nitrosodiethylamine (NDEA)
- N-nitrosodiisopropylamine (NDIPA)
- N-nitrosoethylisopropylamine (NEIPA)
- N-nitrosodibutylamine (NDBA)

These impurities are classified as probable human carcinogens, may be introduced into the finished products through the manufacturing process.

Nitrosamine impurities can be detected using either single quadrupole GC/MS (GC/SQ), triple quadrupole GC/MS/MS (GC/TQ) or triple quadrupole LC/MS/MS (LC/TQ) or Quadrupole Time of Flight LC/MS (LC/Q-TOF). (2).

There are three GC/MS methods that follow United States Food and Drug Administration (US FDA) guidance, two use a headspace method and the third uses liquid injection. The methods differ in sensitivity and the number of impurities being analyzed. This guide provides recommendations for Agilent products based on these methods so you can find what you're looking for quickly.



The Agilent 8890 GC/7693 LS/ 7010B GC/TQ



The Agilent 8890 GC/7697A HSS/5977B GC/MSD

## FDA Method 1: GC/MS headspace method to detect NDMA and NDEA

This method (3) detects the presence of two impurities, NDMA and NDEA, using GC/MS headspace analysis.

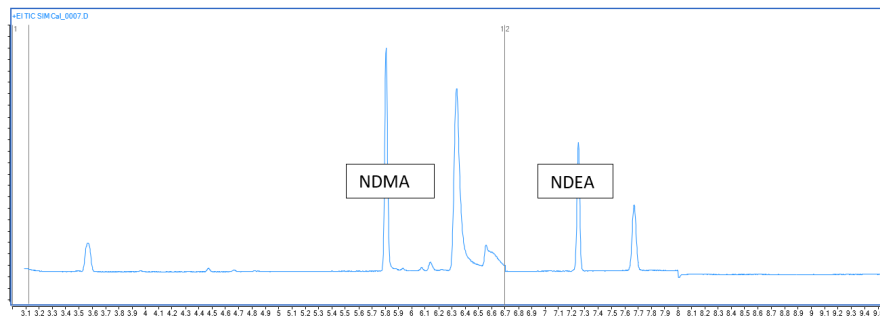


Figure 1. Generated chromatogram in select ion mode of a 1 µg standard mix of NDMA and NDEA in NMP. NDMA retention time is 5.80 minutes, and NDEA is 7.25 minutes.

### Method Parameters

The Agilent 8890/5977 GC/MSD equipped with a 7697A headspace sampler is recommended for this analysis (4). Method parameters are shown below.

Agilent 7697A Headspace Sampler Conditions	
Oven Temperature	130 °C
Loop Temperature	180 °C
Transfer Line Temperature	185 °C
Vial Equilibration	15 minutes
Injection Duration	1 minute
Vial Size (mL)	20 mL
Vial Shaking	Level 5
Fill Mode	Default
Fill Pressure	15 psi
Loop Fill Mode	Default

Agilent 8890 GC Parameters	
Inlet (Split/Splitless)	Helium
Temperature	220 °C
Mode	Split
Split Ratio	5:1
Inlet Pressure (Initial)	7.33 psi
Oven Type	240 V-Fast oven
Equilibration Time	1 minute
Oven Program	40 °C for 0.5 minutes, 20 °C/min to 160 °C, 10 °C/min to 240 °C, Hold 2 minutes Total cycle time: 16.5 minutes
Column	Agilent J&W DB-1701, 30 m × 0.25 mm, 1.0 µm (part number 122-0733)
Mode	Constant flow
Flow	1 mL/min

Table 1. The limits of detection (LOD) and limits of quantitation (LOQ) for FDA method 1.

Impurity	LOD (ppm)	LOQ (ppm)
NDMA	0.005	0.10
NDEA	0.02	0.05

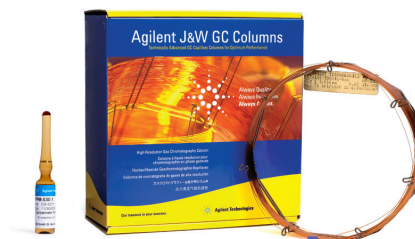
Agilent 5977 GC/MSD Conditions	
Source Type	Extractor
Source Temperature	230 °C
Mass Filter Mode	Selection ion monitoring (SIM)
NDMA <i>m/z</i>	74.00
NDEA <i>m/z</i>	102
Lens Diameter	6 mm
Quad Temperature	150 °C
NDMA Dwell	150
NDEA Dwell	150

## FDA Method 1 Ordering Information

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	
Nitrosamine standards	<a href="#">US-113N-1</a>
Nitrosamine - GC Column	
DB-1701 30 m x 0.25 mm, 1.0 µm	<a href="#">122-0733</a>
<b>Nitrosamines- GC Inlet Liners</b>	
Inlet liner, Ultra Inert <sup>1</sup> , splitless, straight, 2 mm id	<a href="#">5190-6168</a>
Inlet liner, Ultra Inert, split, low pressure drop, glass wool	<a href="#">5190-2295</a>
<b>Nitrosamines-Inlet Supplies</b>	
Inlet septa, Advanced green, non-stick, 11 mm, 50/pk	<a href="#">5183-4759</a>
Inlet septa, Advanced green, non-stick, 11 mm, 100/pk	<a href="#">5183-4759-100</a>
Ultra Inert <sup>1</sup> Gold seal, with washer, 1/pk	<a href="#">5190-6144</a>
Ultra Inert Gold seal, with washer, 10/pk	<a href="#">5190-6145</a>
Self-Tightening Column Nut, collared, inlet	<a href="#">G3440-81011</a>
Self-Tightening Column Nut, collared, MSD	<a href="#">G3440-81013</a>
Replacement collar for Self-Tightening Column Nut	<a href="#">G3440-81012</a>
15%Graphite/85% Vespel Ferrules, 0.4 mm i.d., 10/pk	<a href="#">5181-3323</a>
20x magnifier loop	<a href="#">430-1020</a>
<b>Nitrosamines-Vials and caps</b>	
Vial, headspace, certified, crimp, clear, flat bottom, 20 mL, 100/pk	<a href="#">5182-0837</a>
Cap, crimp, headspace, aluminum, PTFE/silicone septa, 20 mm, 100/pk	<a href="#">5183-4477</a>
<b>Gas Filters</b>	
Gas Clean Carrier Gas Kit for 7890	<a href="#">CP17988</a>
Gas Clean Carrier Gas Kit for 8890 and 8860	<a href="#">CP179880</a>
Gas Clean carrier gas purifier replacement cartridge	<a href="#">CP17973</a>
<b>Nitrosamines-MS Supplies</b>	
EI Filament (for 7000A/B/C/D, 5977B Inert Plus, 5977A Extractor, Inert or Stainless steel and 5975 systems)	<a href="#">G7005-60061</a>
HES Filament for 7010 Triple Quadrupole GC/MS	<a href="#">G7002-60001</a>
Drawout plate, 6mm for 5973/75, 5977	<a href="#">G3163-20530</a>
Drawout plate, 6mm, extractor source	<a href="#">G3870-20448</a>



<sup>1</sup> Ultra Inert supplies provide excellent surface inertness through the entire flow path, prevent analyte catalytic breakdown, response loss and peak shape distortion and thus support reliable qualitative and quantitative analysis for sensitive analysis. GC column inertness is critical as columns contribute toward the largest surface area within the flow path.

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## FDA Method 2: GC/MS headspace method to detect NDMA, NDEA, NEIPA and NDIPA

Method 2 (5) is a later version of the FDA method and uses a single quadrupole GC/MS with headspace-based injection to detect four impurities. The method was validated using an Agilent 7890B GC System with an Agilent 5977A MSD and an Agilent 7697A Headspace Sampler.

Table 2. The limits of detection (LOD) and limits of quantitation (LOQ) for this method.

Impurity	Drug Substance LOQ (ppm)	Drug Substance LOD (ppm)	Drug Product LOQ (ppm)	Drug Product LOD (ppm)
NDMA	0.05	0.01	0.05	0.01
NDEA	0.05	0.01	0.05	0.01
NEIPA	0.05	0.025	0.05	0.025
NDIPA	0.05	0.025	0.05	0.025

### Method parameters

HS Sampler Parameters	
Oven Temperature:	120 °C
Loop Temperature:	125 °C
Transfer Line Temperature:	130 °C
Vial Equilibration Time:	15 min
Injection Time:	1.0 min
Vial Size:	20 mL
Vial Shaking:	Level 9 (250 shakes/min)
Fill Pressure:	15 psi
Loop Size:	1 mL

MS Parameters	
MS Source Temperature:	230 °C
Quad Temperature:	150 °C
Acquisition Type:	SIM
Gain Factor	5
Solvent Delay:	6.0 min.

<b>Group 1 (NDMA &amp; NDMA-d6)</b>
Group Start Time : 6min
Number of Ions : 4 (NDMA: 74.0 Dwell 60, 42.1 Dwell 60), (NDMA-d6: 80.1 Dwell 60, 46.1 Dwell 60)
<b>Group 2 (NDEA &amp; NDEA-d4)</b>
Group Start Time : 7min
Number of Ions : 4 (NDEA: 102.1 Dwell 60, 57.0 Dwell 60), (NDEA-d4: 106.1 Dwell 60, 61.1 Dwell 60)
<b>Group 3 (NDIPA &amp; NEIPA)</b>
Group Start Time : 7.52min
Number of Ions : 4 (NDIPA: 130.0 Dwell 60, 43.0 Dwell 60), (NEIPA: 116.0 Dwell 60, 56.0 Dwell 60)

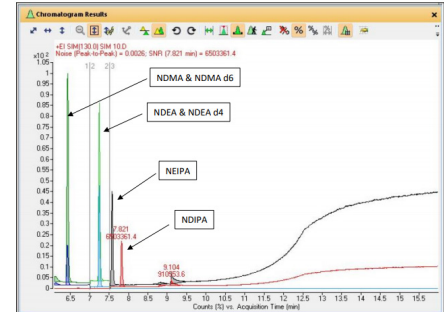


Figure 2. Chromatogram: 0.25 µg NDMA, NDEA, NDIPA, NEIPA Working Standard and IStd NDMA d6 and NDEA d4.

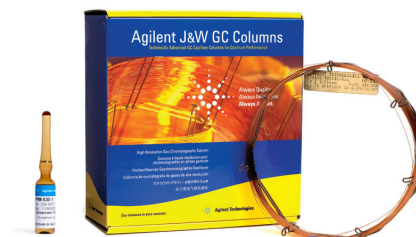
GC/MS - HS Parameters	
Instrument:	Agilent 7890B GC with Agilent 5977A MSD and Agilent 7697A HS sampler
Column:	DB-WAX, 30 m x 0.25 mm, 0.5 µm (PN: 122-7033), or equivalent
Inlet Temperature:	220 °C
Column Flow:	1 mL/min
Split Ratio	5:1
Oven Program:	70 °C for 4 min.; 20 °C/min to 240 °C, Hold for 3.5 min.
GC Run Time	16 min.
GC Cycle Time:	24 min.

## FDA Method 2 Ordering Information

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Product Description	Part Number
<b>Nitrosamine Standards</b>	
Nitrosamine standards	<a href="#">US-113N-1</a>
<b>Nitrosamine - GC Column</b>	
DB-WAX 30 m x 0.25 mm, 0.5 µm	<a href="#">122-7033</a>
DB-WAX Ultra Inert <sup>1</sup> 30 m x 0.25 mm, 0.5 µm	<a href="#">122-7033UI</a>
<b>Nitrosamines- GC Inlet Liners</b>	
Inlet liner, Ultra Inert <sup>1</sup> , splitless, straight, 2 mm id	<a href="#">5190-6168</a>
Inlet liner, Ultra Inert <sup>1</sup> , split, low pressure drop, glass wool	<a href="#">5190-2295</a>
<b>Nitrosamines-Inlet Supplies</b>	
Inlet septa, Advanced green, non-stick, 11 mm, 50/pk	<a href="#">5183-4759</a>
Inlet septa, Advanced green, non-stick, 11 mm, 100/pk	<a href="#">5183-4759-100</a>
Ultra Inert <sup>1</sup> Gold seal, with washer, 1/pk	<a href="#">5190-6144</a>
Ultra Inert <sup>1</sup> Gold seal, with washer, 10/pk	<a href="#">5190-6145</a>
Self-Tightening Column Nut, collared, inlet	<a href="#">G3440-81011</a>
Self-Tightening Column Nut, collared, MSD	<a href="#">G3440-81013</a>
Replacement collar for Self-Tightening Column Nut	<a href="#">G3440-81012</a>
15% Graphite/85% Vespel Ferrules, 0.4 mm i.d., 10/pk	<a href="#">5181-3323</a>
20x magnifier loop	<a href="#">430-1020</a>
<b>Nitrosamines-Vials and caps</b>	
Vial, headspace, certified, crimp, clear, flat bottom, 20 mL, 100/pk	<a href="#">5182-0837</a>
Cap, crimp, headspace, aluminum, PTFE/silicone septa, 20 mm, 100/pk	<a href="#">5183-4477</a>
<b>Gas Filters</b>	
Gas Clean Carrier Gas Kit for 7890	<a href="#">CP17988</a>
Gas Clean Carrier Gas Kit for 8890 and 8860	<a href="#">CP179880</a>
Gas Clean carrier gas purifier replacement cartridge	<a href="#">CP17973</a>
<b>Nitrosamines-MS Supplies</b>	
EI Filament (for 7000A/B/C/D, 5977B Inert Plus, 5977A Extractor, Inert or Stainless steel and 5975 systems)	<a href="#">G7005-60061</a>
HES Filament for 7010 Triple Quadrupole GC/MS	<a href="#">G7002-60001</a>
Drawout plate, 6 mm for 5973/75, 5977	<a href="#">G3163-20530</a>
Drawout plate, 6 mm, extractor source	<a href="#">G3870-20448</a>



<sup>1</sup>See footnote under ordering information table on page 4.

\* See footnote at the bottom of page 4.

## FDA Method 3: GC/TQ method utilizing liquid injection to simultaneously quantify NDMA, NDEA, NEIPA, NDIPA and NDBA

This method (6) is also a later version of the first FDA method. It is a liquid injection based method using triple quadrupole GC/TQ and analyzes for five impurities. Single quadrupole MS (methods 1 & 2) often gives ambiguous results and is less sensitive. GC/TQ methods are more sensitive and give better specificity. A headspace transfer line accessory allows both headspace and liquid injection capability on the same inlet, without changing the configuration.

Drug substance LOD/LOQ calculations for this method were based on 500 mg of Valsartan API. Increasing the amount weighed out and extracted will lower the reported LOQ. Drug product LOD/LOQ calculations were based on one tablet containing 30 mg of Valsartan API.

Table 3. The limits of detection (LOD) and limits of quantitation (LOQ) for the FDA method 3.

Impurity	Drug Substance LOQ (ppm)	Drug Substance LOD (ppm)	Drug Product LOQ (ppm)	Drug Product LOD (ppm)
NDMA	0.008	0.005	0.013	0.008
NDEA	0.005	0.001	0.008	0.002
NEIPA	0.005	0.001	0.008	0.002
NDIPA	0.005	0.001	0.008	0.002
NDBA	0.025	0.010	0.040	0.016

Agilent offers a comprehensive solution (7) for the determination and estimation of five nitrosamine impurities (NDMA, NDEA, NEIPA, NDIPA, and NDBA) in metformin (8) and sartan (9) drug products and drug substances at trace levels using an Agilent 7890B or 8890 GC coupled to an Agilent 7010B triple quadrupole GC/MS system. An Agilent 7010B triple quadrupole GC/MS, equipped with a high efficiency source, can achieve LOQs that are 2–20 times lower than those required by current regulations.

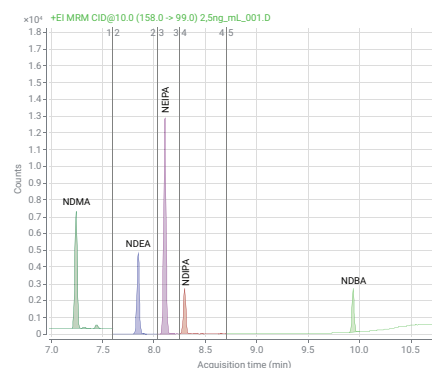


Figure 3. Extracted MRM chromatogram (quant transition) of lowest calibration standard at 2.5 ng/mL mix of five impurities in dichloromethane (Agilent 7890B GC).

### Method parameters

GC Parameters	Value
MMI Injection Mode	Pulsed splitless: 12.285 psi until 0.5 min
Inlet Temperature	250 °C
Oven Temperature Program	40 °C (0.5 min) 20 °C/min to 200 °C (0 min) 60 °C/min to 250 °C (3 min)
Total Run Time	12.33 min
MS Transfer Line Temperature	250 °C
Injection Volume	2 µL
Carrier Gas	Helium, 1 mL/min

MS Parameters	Value	
Mode	Electron ionization, 40 eV	
Source Temperature	250 °C	
Quadrupole Temperature	Q1 and Q2 = 150 °C	
<b>MRM Mode Conditions</b>		
MS1 Resolution	All compounds Unit	
MS2 Resolution	All compounds Unit	
Collision Gas Flow	Nitrogen at 1.5 mL/min,	
Quenching Gas Flow	Helium at 4 mL/min	
Detector Gain	1	
Quant./Qual. Transitions (FDA method)	Start time: 6.5 min	NDMA 74 → 44, CE 15, dwell 150 ms 74 → 42, CE 20, dwell 50 ms NDMA:C13-d <sub>6</sub> 82 → 48, CE 20, dwell 100 ms
	Start time: 7.60 min	NDEA 102 → 85, CE 10 V, dwell 150 ms 102 → 56, CE 18 V, dwell 150 ms
	Start time: 8.03 min	NEIPA 116 → 99, CE 10 V, dwell 150 ms 71 → 56, CE 10 V, dwell 150 ms
	Start time: 8.25 min	NDIPA 130 → 88, CE 10 V, dwell 150 ms 130 → 42, CE 10 V, dwell 150 ms
	Start time: 8.70 min	NDBA 158 → 99, CE 10 V, dwell 150 ms 84 → 56, CE 22 V, dwell 150 ms

## FDA Method 3 Ordering Information

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Product Description	Part Number
<b>Nitrosamine Standards</b>	
Nitrosamine standards	<a href="#">US-113N-1</a>
<b>Nitrosamine - Sample prep</b>	
Captiva Premium Syringe Filter Nylon membrane 15 mm diameter, 0.45 µm pore size, 100/pk (HPLC certified)	<a href="#">5190-5091</a>
Captiva Disposable Syringe, 5 mL, 100/pk	<a href="#">9301-6476</a>
<b>Nitrosamine - GC Column</b>	
VF-WAXms 30 m x 0.25 mm, 1.0 µm	<a href="#">CP9206</a>
<b>Nitrosamines- GC Inlet Liners</b>	
Single taper splitless Inlet liner 900 µL with deactivated wool	<a href="#">5062-3587</a>
Single taper, splitless, Inlet liner 900 µL with glass wool Ultra Inert <sup>1</sup> (1/pk)	<a href="#">5190-2293</a>
Single taper, splitless, Inlet liner 900 µL with glass wool Ultra Inert <sup>1</sup> (5/pk)	<a href="#">5190-3163</a>
Single taper, splitless, Inlet liner 900 µL with glass wool Ultra Inert <sup>1</sup> (25/pk)	<a href="#">5190-3167</a>
Single taper, splitless, Inlet liner 900 µL with glass wool Ultra Inert <sup>1</sup> (100/pk)	<a href="#">5190-3171</a>
Inlet liner O-ring, non-stick fluorocarbon, certified, 100/pk	<a href="#">5190-2269</a>
<b>Nitrosamines-Inlet Supplies</b>	
Inlet septa, Advanced green, non-stick, 11 mm, 50/pk	<a href="#">5183-4759</a>
Inlet septa, Advanced green, non-stick, 11 mm, 100/pk	<a href="#">5183-4759-100</a>
Ultra Inert <sup>1</sup> Gold seal, with washer, 1/pk	<a href="#">5190-6144</a>
Ultra Inert <sup>1</sup> Gold seal, with washer, 10/pk	<a href="#">5190-6145</a>
Self-Tightening Column Nut, collared, inlet	<a href="#">G3440-81011</a>
Self-Tightening Column Nut, collared, MSD	<a href="#">G3440-81013</a>
Replacement collar for Self-Tightening Column Nut	<a href="#">G3440-81012</a>
15% Graphite/85% Vespel Ferrules, 0.4 mm i.d., 10/pk	<a href="#">5181-3323</a>
5 µL ALS syringe, fixed needle, 23-26s/42/cone	<a href="#">5181-1273</a>
5 µL ALS syringe, fixed needle, 23-26s/42/cone 6/pk	<a href="#">5181-8810</a>
10 µL ALS syringe, fixed needle, 23-26s/42/cone	<a href="#">5181-1267</a>
10 µL ALS syringe, fixed needle, 23-26s/42/cone 6/pk	<a href="#">5181-3360</a>
<b>Nitrosamines-Vials and caps</b>	
MS analyzed vial kit, 2 mL clear screw top, write-on spots, blue caps, PTFE/silicone septa, 100/pk	<a href="#">5190-2278</a>
Vial, screw top, amber, write-on spot, certified, 2 mL, 100/pk	<a href="#">5182-0716</a>
Vial, screw top, amber, write-on spot, deactivated (silanized), certified, 2 mL, 100/pk	<a href="#">5183-2072</a>
Screw cap, blue PTFE/red silicone septa, 100/pk	<a href="#">5182-0717</a>
Screw cap, blue PTFE/silicone/PTFE septa, 100/pk	<a href="#">5182-0723</a>
Vial insert, 100 µL, 500/pk Insert size: 5.0 x 30 mm	<a href="#">9301-1387</a>



<sup>1</sup>See footnote under ordering information table on page 4.

\* See footnote at the bottom of page 4.



<b>Gas Filters</b>	
Gas Clean Carrier Gas Kit for 7890	<a href="#">CP17988</a>
Gas Clean Carrier Gas Kit for 8890 and 8860	<a href="#">CP179880</a>
Gas Clean carrier gas purifier replacement cartridge	<a href="#">CP17973</a>
<b>Nitrosamines-MS Supplies</b>	
El Filament (for 7000A/B/C/D, 5977B Inert Plus, 5977A Extractor, Inert or Stainless steel and 5975 systems)	<a href="#">G7005-60061</a>
HES Filament for 7010 Triple Quadrupole GC/MS	<a href="#">G7002-60001</a>
Drawout plate, 6 mm for 5973/75, 5977	<a href="#">G3163-20530</a>
Drawout plate, 6 mm, extractor source	<a href="#">G3870-20448</a>



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8. Quantification of Nitrosamine Impurities in Metformin Using Agilent GC/MS/MS Instrumentation, Agilent publication ([5994-2419EN](#))
9. Analysis of Five Nitrosamine Impurities in Drug Products and Drug Substances Using Agilent GC/MS/MS Instrumentation, Agilent publication ([5994-1821EN](#))

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