CASE STUDY 73263

Overcoming the challenges of nitrosamine impurities in drugs

What pharmaceutical QA/QC laboratories need to know: Advanced GC-MS capabilities for cGMP nitrosamine testing

Why we need to analyze nitrosamine impurities in drugs

Unacceptable levels of nitrosamine impurities were first reported in June 2018 when Valsartan, an angiotensin II receptor blocker containing a tetrazole group, was recalled due to the presence of N-nitrosodimethylamine (NDMA) contamination. Further nitrosamine impurities were subsequently detected in other medications belonging to a group of sartans, including: N-nitrosodiethylamine (NDEA), N-nitrosodiisopropylamine (NDIPA), N-nitrosoethylisopropylamine (NEIPA) and N-nitroso-N-methyl-4-aminobutyric acid (NMBA).[1] Nitrosamines are considered a matter of concern as the ICH M7 (R1)[2] Guideline classifies them as Class 1 impurities or mutagenic carcinogens and they are categorized as probable carcinogens by the International Agency for Cancer Research (IARC).[3] There are many possible sources of impurities in pharmaceutical substances; nitrites or secondary or tertiary amines can be present as unintentional contaminants of raw materials, reagents and solvents used during the production processes and they can result in the formation of nitrosamine impurities by reaction with a nitrosating agent (e.g., sodium nitrite ($NaNO_2$).[1] Regulatory agencies all over the world have allowed a period of two years for manufacturers to review and make changes to their manufacturing processes to minimize nitrosamine impurities to the extent practically possible. During this transition period, interim limits are being applied to products based upon maximum daily intake.[4] Manufacturers of sartans with a tetrazole ring have to implement a control strategy for N-nitrosamines and from April 2021, the batches of active pharmaceutical ingredient or drug substance they produce must not contain quantifiable levels (corresponding to less than 0.03 ppm) of the two principal N-nitrosamine impurities: NDMA and NDEA.

Challenges of NDMA and other nitrosamine analysis in pharmaceuticals

The challenges of nitrosamine analysis in pharmaceuticals relate to sensitivity, selectivity, and compliance all in the light of obtaining reliable and timely results; high sensitivity must be achieved to fulfill the regulation requirements, and selectivity is also critical to avoid false positive non-compliant results.

ThermoFisher SCIENTIFIC

"A challenge with headspace GC-based testing is carryover of residual organic solvents. We found that the TriPlus 500 headspace sampler fully addresses the carryover problem due to directly connecting to the column. TriPlus RSH autosampler is also a great option as it allows a quick switching between headspace and liquid injection modes, significantly increasing our throughput"

– Dr. Dujuan Lu (SGS, USA)

The U.S. Food and Drug Administration (FDA) has published several analytical methods that may be considered when determining nitrosamine content in active pharmaceutical ingredient (API) or finished pharmaceutical product (FPP).[5] These methods include both liquid chromatography (LC) and gas chromatography (GC) coupled with mass spectrometry (MS) or high resolution accurate mass (HRAM) mass spectrometry. LC-MS methods have been developed to cover a wider range of analytes which are not amenable by GC-MS methods. In particular, GC-MS methods cannot directly detect N-nitroso-N-methyl-4-aminobutyric acid (NMBA) and so sample derivatization is required, increasing sample preparation time and efforts. Moreover, LC-MS methods offer a suitable solution when ranitidine is tested for N-nitrosamine impurities. However, the FDA reported that possible degradation effects can occur when ranitidine is stored or analyzed at high temperatures resulting in subsequent formation of NDMA.[6]

Thermo Fisher Scientific supports pharmaceutical testing laboratories with comprehensive chromatographic solutions including LC-MS/MS, LC-HRAM, GC-MS, GC-MS/MS and high resolution accurate mass Orbitrap GC-MS, for targeted and untargeted analysis of nitrosamine impurities, in compliance with FDA guidelines. This guide will focus on GC-MS solutions and the LC-MS information can be found on the dedicated Nitrosamine resource page.

Analysis of nitrosamines using GC-MS coupled to headspace sampling

Thermo Scientific™ ISQ™ 7000 GC-MS system with Thermo Scientific™ TriPlus™ 500 Headspace Autosampler

Key features

- Headspace sampling with no sample preparation and low chemical background
- Headspace autosampler with up to 240 vial capability for increased productivity
- Thermo Scientific™ Extractabrite™ ion source with Thermo Scientific™ NeverVent™ technology for quick preventive maintenance without venting the MS ensuring maximum uptime
- Timed-SIM method set up for optimized selectivity and sensitivity

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) for instrument control, method development, quantitative analysis and reporting with a full suite of compliance-ready features to ensure data security, data integrity, and 21 CFR Part 11 compliance for operation in environments governed by Current Good Manufacturing Practice (cGMP) regulations.

For the determination of the most volatile nitrosamine impurities, headspace sampling technique is preferred as no sample preparation is required. One of the advantages of using this technique is that it removes the complexity of the matrix while improving the selectivity for the compounds of interest and reducing the risk of false positive results. Thermo Scientific TriPlus 500 HS autosampler coupled with Thermo Scientific™ ISQ™ 7000 single quadrupole GC-MS system achieves the required sensitivity with calculated LOD (S/N>3) of 0.004 ppm for NDMA and 0.009 ppm for NDEA and LOQ (S/N>10) = 0.015 ppm for NDMA and 0.030 ppm for NDEA for the detection of nitrosamines at trace levels exceeding the FDA nitrosamine method requirements (LOD = 0.005 ppm for NDMA and 0.020 ppm for NDEA and LOD = 0.100 ppm for NDMA and 0.050 ppm for NDEA).[5]

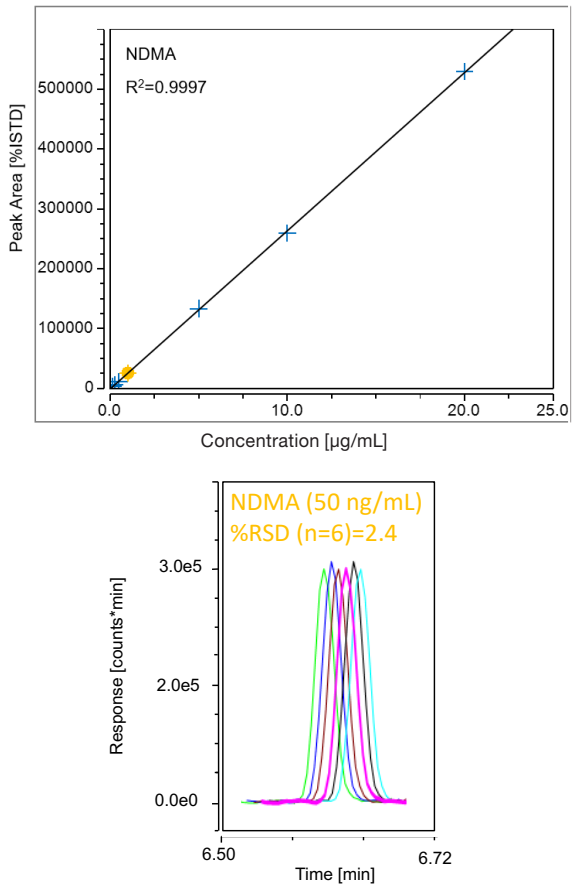


Figure 1. Examples of linearity for NDMA ranging from 0.03 to 20 µg/mL and repeatability at 50 ng/mL in solvent standard

Determination of nitrosamines using triple quadrupole GC-MS/MS coupled to direct liquid injection

Thermo Scientific™ TSQ™ 9000 GC-MS/MS system with Thermo Scientific™ AS™ 1310 Autosampler and Thermo Scientific TriPlus 500 Headspace Autosampler

Key features

- AS 1310 autosampler with up to 155 vials for increased sample throughput
- ExtractaBrite ion source with NeverVent technology for quick preventive maintenance without the need for venting the MS ensuring maximum uptime
- Auto-SRM and Time-SRM for fast method set up
- SRM selectivity reduces matrix interferences and enables low detection limits
- Chromeleon CDS for instrument control, method development, quantitative analysis and reporting in compliance-ready, enterprise, cGMP environments.

The published FDA methods include both liquid and gas chromatography coupled to mass spectrometry to ensure high selectivity and low limits of detection. Single or triple quadrupole mass analyzers provide acceptable selectivity to separate the analytes from the chemical background by the use of single ion monitoring (SIM) or selected reaction monitoring (SRM). Direct liquid injection coupled with triple quadrupole is the method of choice for determination of nitrosamines. Thermo Scientific™ TSQ™ 9000 triple

quadrupole GC-MS/MS system coupled with Thermo Scientific™ AS™ 1310 Autosampler delivers high selectivity for detection and accurate quantitation of NDMA, NDEA, NEIPA, NDIPA and NDBA with linearity and sensitivity, exceeding the FDA requirements.[5]

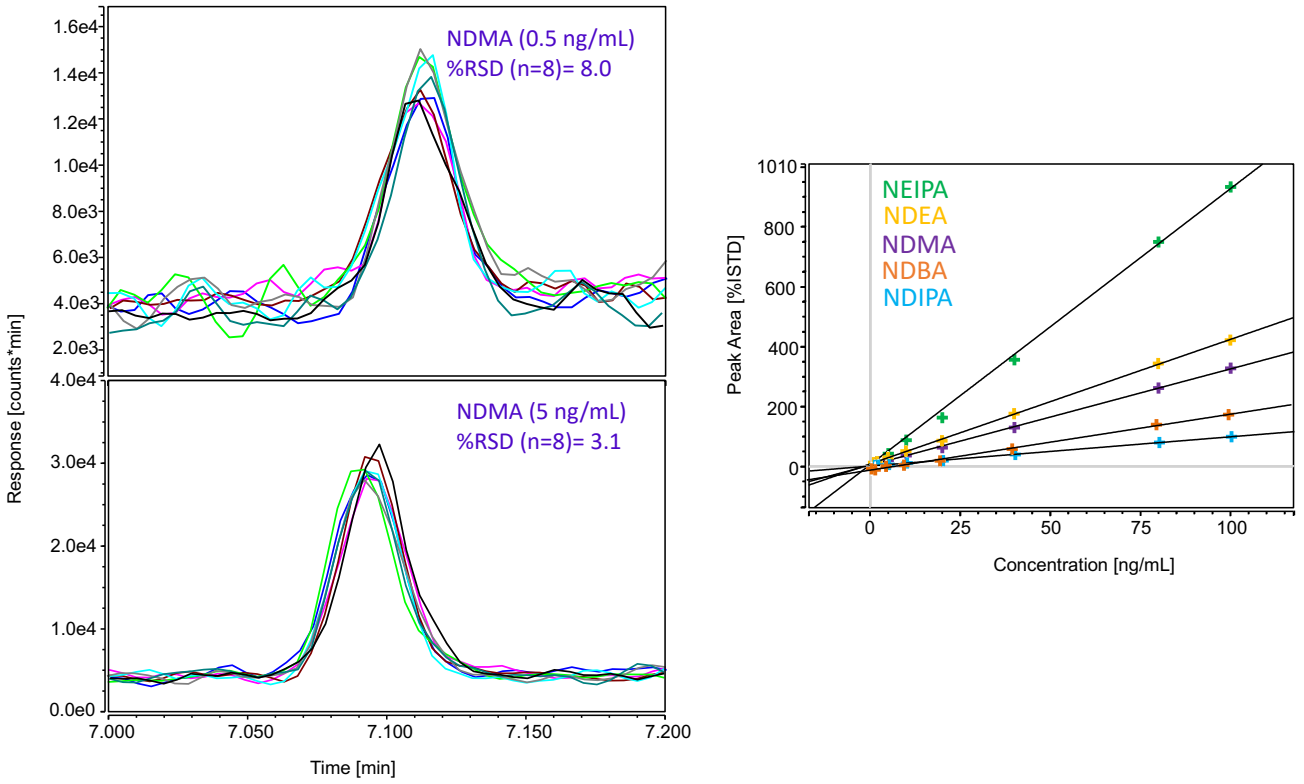


Figure 2. Examples of repeatability for NDMA at 0.5 ng/mL and 5.0 ng/mL in solvent standard and linearity for NDMA, NDEA, NEIPA, NDIPA and NDBA (1–100 ng/mL)

Table 1. Calculated limit of detection (LOD) and limit of quantification (LOQ), correlation coefficient (R^2) and average calibration factors (AvCF %RSD) obtained from eight calibration levels (1–100 ng/mL)

Nitrosoamine Impurity	Retention Time (min)	FDA LOD Requirement (ppm)		Calculated LOD (S/N>3) (ppm)	FDA LOQ Requirement (ppm)		Calculated LOQ (S/N>10) (ppm)	Coefficient of Correlation (R^2)	AvCF %RSD
		API	FPP		API	FPP			
NDMA	7.11	0.005	0.008	0.0002	0.008	0.013	0.0005	0.9997	3.0
NDEA	7.69	0.001	0.002	0.0002	0.005	0.008	0.0005	0.9999	2.0
NEIPA	7.95	0.001	0.002	0.0005	0.005	0.008	0.001	0.9997	3.3
NDIPA	8.14	0.001	0.002	0.0002	0.005	0.008	0.0005	0.9998	2.6
NDBA	9.65	0.01	0.016	0.0005	0.025	0.04	0.01	0.9988	5.1

"Dealing with nitrosamines impurities in pharmaceutical industry is not a challenge if you have HS-GC-MS/MS TSQ technology. Selectivity and sensitivity to quantitate target volatile nitrosamines at low levels are the key to assure compliance now and for the future."

– Dr. Siva Lakshmi (Laurus Labs, India)

Anticipating future regulatory requirements with increased sensitivity from advanced electron ionization (AEI) ion source

Key features

- AEI ion source provides improved sensitivity with a more efficient ionization and improved robustness

As regulations are evolving quickly and limits become stringent, TriPlus 500 HS autosampler coupled with TSQ 9000 triple quadrupole GC-MS/MS system equipped with AEI technology provides the sensitivity performance to meet the current regulation and to future-proof against any reduction in limits of detection. The innovative design of the AEI ion source provides a highly efficient ionization of analytes and a more tightly focused ion beam, lowering the detection limits to < 0.5 ng/mL (S/N > 3).

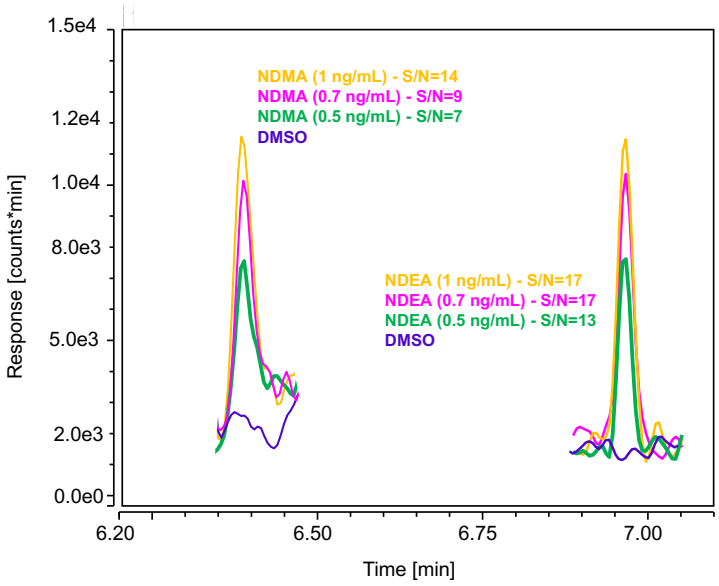


Figure 3. Peak-to-peak S/N for NDMA and NDEA spiked at 0.5, 0.7 and 1 ng/mL

Improved analytical precision with the TriPlus 500 HS Autosampler

Key features

- Precise pneumatic control, short and inert sample path for highly reliable analyte transfer and reproducible results

When analyzing nitrosamines it is essential that results are consistent. If the analysis produces variable results it could cause uncertainty, which in turn could lead to the reanalysis of samples or even worse a false negative. The highly efficient pneumatic control and the short and inert sample path of the TriPlus 500 HS Autosampler ensure reliable and reproducible analyte injection and transfer, offering outstanding repeatability and precision in routine analysis at ultra-trace concentrations with peak area %RSD < 7 % at 0.7 ng/mL.

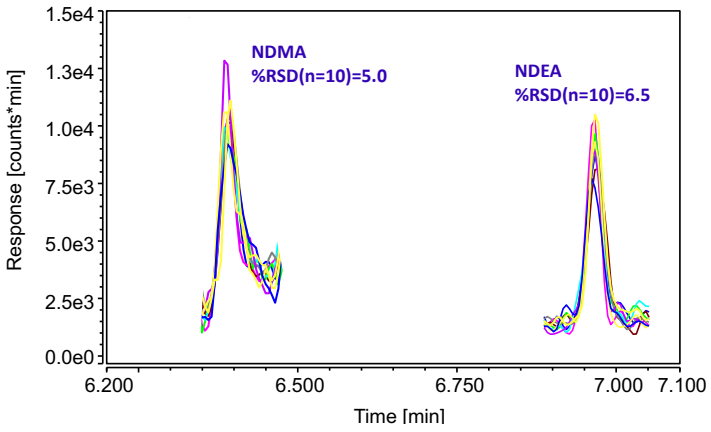


Figure 4. Overlaid injections (n=10) of NDMA (*m/z*=74>42) and NDEA (*m/z*=102>85) in solvent standard at 0.7 ng/mL (n=10)

"The Q Exactive GC mass spectrometer coupled with headspace sampler helps us a lot with confident identification of volatile organic compounds (VOCs) due to its excellent mass accuracy down to sub-1 ppm level."

– Dr. Dujuan Lu (SGS, USA)

Screening and quantification of genotoxic impurities in pharmaceuticals using Orbitrap GC-MS technology

The Thermo Scientific™ Exactive™ GC-MS system using Orbitrap™ technology offers the advantage of full-scan (FS) operation with higher mass resolving power than single or triple quadrupoles and therefore, providing high levels of selectivity and quantitative performance. This enables not only target compounds to be confidently detected, but also widen the scope and identify additional compounds. Combined SIM and FS data acquisition at high resolution and high mass accuracy allow for targeted analysis of nitrosamine impurities with compliance to FDA method validation requirements,[5] as well as untargeted screening of contaminants with retrospective analysis capability.

Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS system with Thermo Scientific™ TriPlus™ RSH Autosampler

Orbitrap GC-MS coupled to direct liquid injection

Key features

- High resolving power and sub-1-ppm accurate mass for improved selectivity, lower limits of detection and confident compound identification
- Combined SIM and FS acquisition for targeted analysis of known impurities and untargeted screening for a broader and deeper understanding of samples
- Sample handling flexibility with TriPlus RSH autosampler offering liquid, syringe-based HS and dynamic headspace (ITEX-DHS) for further sensitivity
- ExtractaBrite ion source with NeverVent technology for quick preventive maintenance without venting the MS ensuring maximum uptime
- Chromeleon CDS for instrument control, method development, quantitative analysis and reporting in compliance-ready, enterprise, cGMP environments.

Orbitrap technology ensures high selectivity with very low background noise for the detection of ultra-trace level impurities with high confidence in compound identification. Impurities are identified based on the retention time, accurate mass information (±5 ppm mass error) and the characteristic fragment ion. Moreover, the elemental composition of the quantification ions can be used to check the isotopic pattern fit (measured versus theoretical). The Exactive GC-MS coupled with TriPlus RSH autosampler for liquid injection delivers excellent sensitivity with linearity ranging from 0.1 to 50 ng/mL and average

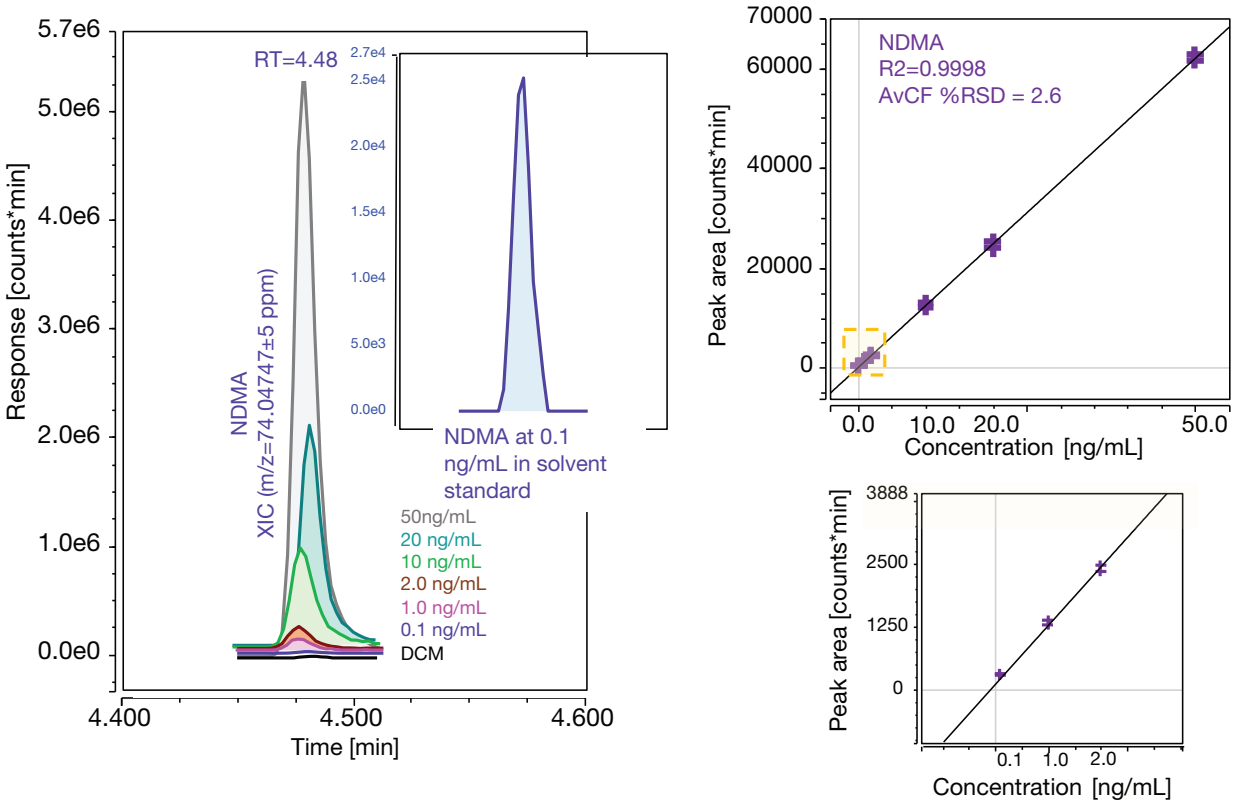


Figure 5. Linearity for NDMA (*m/z*=74.04747 ± 5 ppm) ranging from 0.1 to 50 ng/mL with R^2 = 0.9998 and AvCF %RSD=2.6

calibration factor %RSD=2.6.

Thermo Scientific Q Exactive GC Orbitrap GC-MS/MS system with Thermo Scientific TriPlus 500 Headspace Autosampler

Orbitrap GC-MS coupled to headspace sampling

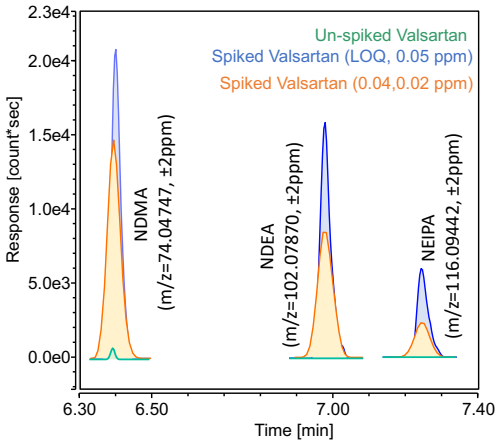
Key features

- High resolving power and sub-1 ppm accurate mass for improved selectivity and confident compound identification
- Headspace sampling for easier sample preparation and lower chemical noise
- Headspace autosampler with up to 240 vial capability for increased productivity
- ExtractaBrite ion source with NeverVent technology for quick preventive maintenance without venting the MS ensuring maximum uptime
- Chromeleon CDS for instrument control, method development, quantitative analysis and reporting in compliance-ready, enterprise, cGMP environments.

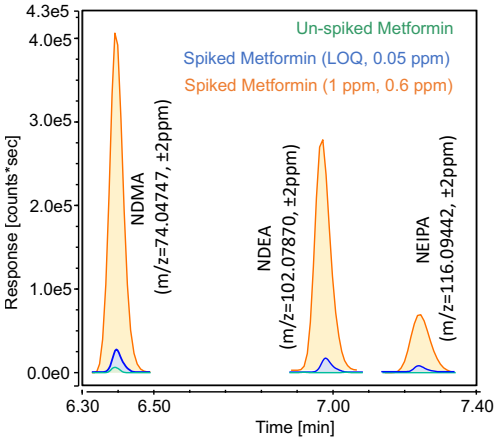
The high selectivity and accurate mass (sub-1 ppm) of the Orbitrap technology add confidence in compound identification and ensure reliable quantitative performance irrespectively of the sample concentration. Quantitative analysis of un-spiked and spiked Valsartan and Metformin samples showed calculated amounts within ±15% of the spiked concentration with % recovery ranging from 80–120% and consistent sub-1-ppm mass accuracy.

Untargeted screening of impurities in pharmaceutical substances

Untargeted screening and retrospective analysis can be carried out broadening the scope of the analysis. FS data are acquired at 60,000 resolution (Full Width at Half Maxima measured at *m/z* 200) (1), deconvoluted with Thermo Scientific™ TraceFinder™ CDS and chromatographic peaks are putatively identified based on spectral library match against NIST 17 nominal mass library (2). The elemental composition and the mass accuracy information are used to confirm the molecular ion (3). Moreover, the isotopic patter match (measured vs theoretical) adds confidence in compound identification (4). Benzene and other residual solvent impurities could be detected in the Metformin sample spiked with some Class 1 and Class 2A residual solvents at 1/5 the concentration limits (Appendix 2) reported in the USP <467> method.[7]



Compound	Retention Time (min)	Valsartan Amount (ppm) Spiked	Valsartan Amount (ppm) Measured	Recovery (%)	Mass Accuracy
NDMA	6.40	-	0.0008	-	-0.1
		0.04	0.036	90	0.6
		0.05	0.044	88	0.4
NDEA	6.98	-	-	-	-
		0.04	0.034	85	0.2
		0.05	0.052	104	0.7
NEIPA	7.24	-	-	-	-
		0.02	0.022	110	0.6
		0.05	0.048	96	0.4



Compound	Retention Time (min)	Metformin Amount (ppm) Spiked	Metformin Amount (ppm) Measured	Recovery (%)	Mass Accuracy
NDMA	6.40	-	0.01	-	0.4
		0.05	0.056	112	0.7
		1.0	1.05	105	0.5
NDEA	6.98	-	-	-	-
		0.05	0.056	112	0.4
		1.0	0.99	99	0.1
NEIPA	7.24	-	-	-	-
		0.05	0.054	108	0.7
		0.60	0.65	109	0.3

Figure 6. Quantitative performance assessed for un-spiked and spiked Valsartan and Metformin samples with calculated concentrations within 15% the spiked amount, recoveries ranging from 80% to 120% and mass accuracy consistently < 1 ppm. Samples spiked at the FDA required LOQ (0.05 ppm), below the LOQ (0.04 and 0.02 ppm) and above the LOQ (1.0 and 0.6 ppm)

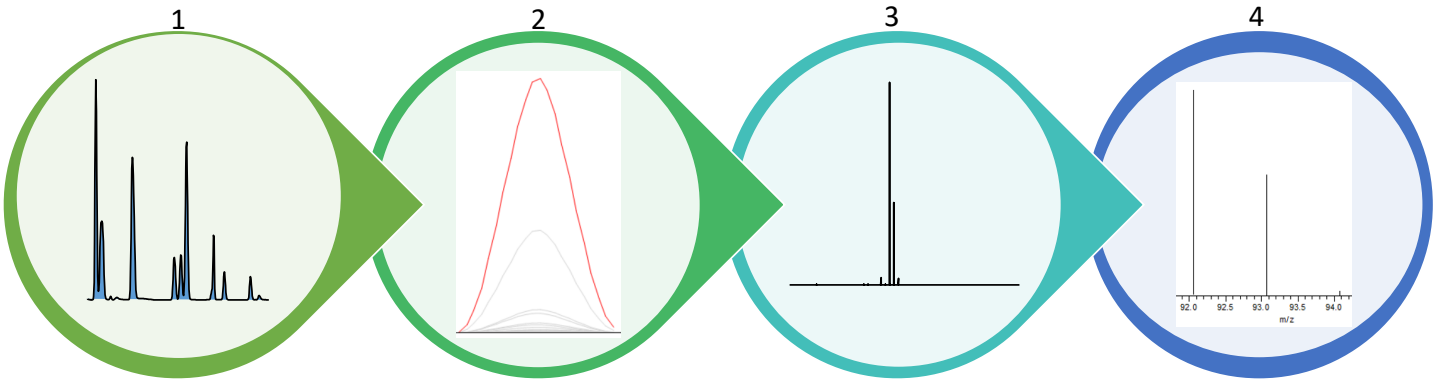
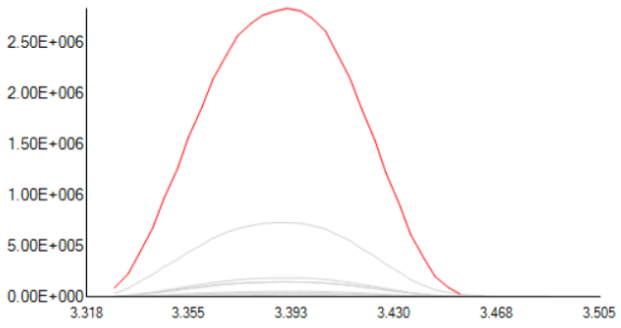


Figure 7. Workflow for unknown screening of volatile impurities. FS data are acquired at 60,000 resolution (FWHM at *m/z* 200) (1), chromatographic peaks are deconvoluted and putatively identified based on spectral library match against NIST 17 nominal mass library (2). The elemental composition and the mass accuracy information are used to confirm the molecular ion (3). Moreover, the isotopic pattern match (measured vs theoretical) adds confidence in compound identification (4)

A

Compound	Retention Time (min)	Reference *m/z*	Theoretical *m/z*	Total Score	Mass Accuracy (ppm)
1,1-Dichloroethene	2.76	95.95286	95.95281	98	0.50
Benzene	3.39	78.04639	78.04640	99	0.13
cis-1,2-Dichloroethene	3.74	95.95282	95.95281	98	0.18
Toluene	4.17	92.06202	92.06205	97	-0.32
1,4-Dioxane	4.32	88.05189	88.05188	95	0.11
Ethylbenzene	4.88	106.0777	106.07770	97	0.30
p-Xylene	4.94	106.0776	106.07770	97	-0.44
m-Xylene	5.00	106.0776	106.07770	97	-0.58
o-Xylene	5.37	106.0778	106.07770	97	0.91
Chlorobenzene	5.62	112.00752	112.00743	96	0.79

B



Component Name	Acquired *m/z*	Fragment ID	Theoretical *m/z*	Mass Error (ppm)
Benzene	78.04639	C6H6	78.0464	0.179381
Benzene	79.04982	C(12)5 C(13)1 H6	79.04976	0.812146
Benzene	78.04194	C(12)5 C(13)1 H5	78.04193	0.105072
Benzene	77.03854	C6H5	77.03858	0.415376
Benzene	77.03412	C(12)5 C(13)1 H4	77.03411	0.171353
Benzene	76.03072	C6H4	76.03075	0.35512
Benzene	75.02304	C6H3	75.02293	1.532862
Benzene	74.01508	C6H2	74.0151	0.243194
Benzene	73.00724	C6H	73.00728	0.493102

C

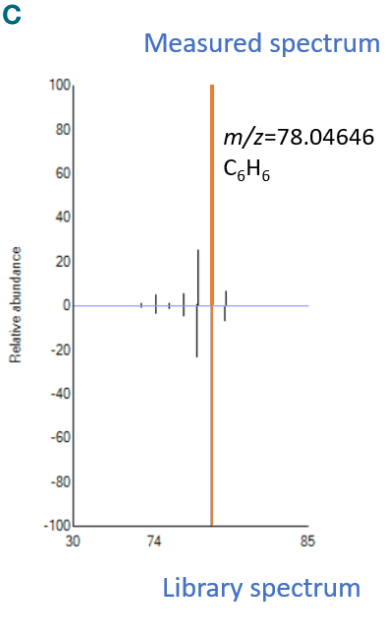


Figure 8. Example of deconvolution result table listing some of the compounds spiked in Metformin sample (A), deconvolution results with annotated fragments for peak eluting at RT=3.93 min and putatively identified as benzene based on NIST 17 library match, SI = 931 (B), spectrum comparison (measured vs library) (C)

Summary

The low levels at which the nitrosamine impurities could be present in pharmaceutical products pose challenges for analytical laboratories. The limits imposed by the current regulations are strict and could potentially be lowered in the future to ensure patients safety. Thermo Fisher Scientific offers a complete analytical solution for nitrosamine testing using LC-MS and GC-MS to cover a large range of pharmaceutical product types.

Highly versatile sample introduction configurations with liquid injection, static headspace (valve and loop and syringe based) and dynamic headspace (ITEX-DHS) coupled to single quadrupole, triple quadrupole and Orbitrap mass spectrometers are available to meet the current regulatory requirements and to address the future analytical needs.

Single quadrupole ISQ 7000 GC-MS and triple quadrupole TSQ 9000 GC-MS/MS systems are compliance-ready with FDA regulation, meeting or often exceeding the method requirements and providing the sensitivity, selectivity and precision needed for reliable quantitative analysis of these impurities.

It is critically important that the manufacturers of pharmaceutical products are assessing their products for any condition that might potentially lead to nitrosamine formation. Although quantification of known compounds, such as nitrosamines and precursors is essential, screening for unknown precursor chemicals that may contribute to the formation of nitrosamines is equally important. For this, Orbitrap GC-MS system combines the quantitative and qualitative strengths in one single platform allowing for targeted and untargeted screening of impurities and a deeper characterization of samples.

The high resolving power, consistent sub-1 ppm mass accuracy and the wide dynamic range of the GC-Orbitrap technology allows for fast and confident detection and identification of volatile impurities in pharmaceutical products regardless of concentration or complexity of the matrix.

Compliance-ready Chromeleon CDS software offers fully-integrated instrument control for streamlined workflows. This software delivers superior data integrity and security tools, networking capabilities, instrument control, automation, and data processing in compliance to the 21 Code of Federal Regulations (CFR) part 11.

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4. 20 August 2019 EMA/351053/2019 rev 1: Temporary interim limits for NMBA, DIPNA and EIPNA impurities in sartan blood pressure medicines.
5. Combined N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC/MS-Headspace, 28/01/2019, https://www.fda.gov/media/117843/download

   Combined Direct Injection N-Nitrosodimethylamine (NDMA) and NNitrosodiethylamine (NDEA) Impurity Assay by GC/MS, 11/12/2018, https://www.fda.gov/media/117807/download

   Combined Direct Injection N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), and N-Nitrosodibutylamine (NDBA), Impurity Assay by GC-MS/MS , 19/04/2019, https://www.fda.gov/media/123409/download

   Combined Headspace N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), and N-Nitrosodiisopropylamine (NDIPA) Impurity Assay by GC-MS/MS, 29/04/2019, https://www.fda.gov/media/124025/download

   Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs, 21/05/2019, https://www.fda.gov/media/125478/download

   Development and validation of a RapidFire-MS/MS method for screening of nitrosamine carcinogen impurities N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), NNitrosodibutylamine (NDBA) and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA) in ARB drugs, 24/07/2019, https://www.fda.gov/media/125477/download

   Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of NDMA in Metformin Drug Substance and Drug Product, 04/02/2020, https://www.fda.gov/media/134914/download
6. Important Information about NDMA impurities in ranitidine products, https://www.fda.gov/drugs/drug-safety-and-availability/questions-and-answers-ndma-impurities-ranitidine-commonly-known-zantac
7. USP <467> Organic Volatile impurities, Chemical Tests, United States Pharmacopeia, Interim Revision Announcement Official November 1, 2019; Official December 1, 2020

Appendix 1

GC-MS, GC-MS/MS and GC-Orbitrap analytical methods

TriPlus 500 HS Autosampler Parameters	
Incubation Temperature (°C)	150
Incubation Time (min)	15
Vial Shaking	Fast
Vial Pressurization Mode	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen)	130
Vial Pressure Equilibration Time (min)	1
Loop Size (mL)	1
Loop/Sample Path Temperature (°C)	180
Loop Filling Pressure (kPa)	70
Loop Equilibration Time (min)	1
Needle Purge Flow Level	2
Injection Mode	Standard
Injection Time (min)	1

Trace 1310 GC Parameters	
Inlet Module and Mode	SSL, split
Split Ratio	5:1
Septum Purge Flow (mL/min)	5, constant
Carrier Gas, Flow (mL/min)	He, 1.0
Oven Temperature Program	
Temperature (°C)	45
Hold Time (min)	1
Rate (ºC/min)	15
Temperature 2 (°C)	180
Rate (ºC/min)	20
Temperature 3 (°C)	250
Hold Time (min)	1
Column	
Trace GOLD TG-WAXMS	30 m, 0.25 mm, 0.25 µm (P/N 26088-1420)

ISQ 7000 GC-MS Parameters	
Transfer Line Temperature (°C)	250
Ionization Type	EI
Ion Source	ExtractaBrite
Ion Source Temperature (°C)	300
Electron Energy (eV)	70
Aquisition Mode	t-SIM

ISQ 7000 GC-MS Parameters for t-SIM		
Compound	**Retention Time (min)**	**SIM Ion (*m/z*)**
NDMA	6.08	74
NDMA	6.08	42
NDEA	6.80	102
NDEA	6.80	57
NDEA	6.80	42

Method parameters: Thermo Scientific ISQ 7000 GC-MS system coupled with Thermo Scientific TriPlus 500 Headspace Autosampler

Appendix 1

Liquid Injection-GC-MS/MS

AI/AS 1310 Autosampler Parameters	
Injection Volume (µL)	2
Draw Speed	Slow
Fill Stroke	5
Air Volume (µL)	1
Sample Depth	Bottom
Pre-Injection Delay Time (s)	2
Post-Injection Delay Time (s)	3
Pre-Injection Washing Cycles	5
Post-Injection Washing Cycles	5
Sample Wash Cycles	2

Trace 1310 GC Parameters	
Inlet Module and Mode	SSL, splitless
Inlet Temperature (°C)	240
Splitless Time (min)	1
Split Ratio	25:1
Septum Purge Flow (mL/min)	5, constant
Carrier Gas, Flow (mL/min)	He, 1.0
Oven Temperature Program	
Temperature (°C)	40
Hold Time (min)	0.5
Rate (ºC/min)	20
Temperature 2 (°C)	200
Rate (ºC/min)	60
Temperature 3 (°C)	240
Hold Time (min)	3
Column	
TR-WAX	30 m, 0.25 mm, 1.0 µm (P/N 260X296P)

TSQ 9000 GC-MS/MS Parameters	
Transfer Line Temperature (°C)	240
Ionization Type	EI
Ion Source	Extractabrite
Ion Source Temperature (°C)	230
Electron Energy (eV)	70
Aquisition Mode	RM
Detector Gain Multplier	3
Emission Current (µA)	50

TSQ 9000 GC-MS/MS Parameters for SRM

Compound	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Start (min)	End (min)	Collision Energy (eV)
NDMA	74	42	6.12	8.12	35
NDMA	74	44	6.12	8.12	10
NDMA-d6	80	50	6.12	8.10	14
NDEA	102	44	6.68	8.70	25
NDEA	102	56	6.68	8.70	35
NDEA	102	85	6.68	8.70	5
NEIPA	116	70	6.98	8.98	35
NEIPA	116	99	6.98	8.98	10
NDIPA	130	42	7.16	9.16	20
NDIPA	130	88	7.16	9.16	15
NDBA	158	99	8.66	10.66	25
NDBA	158	116	8.66	10.66	10

Method parameters: Thermo Scientific TSQ 9000 GC-MS/MS system coupled with Thermo Scientific AS 1310 Autosampler

Appendix 1

HS-GC-MS/MS (AEI)

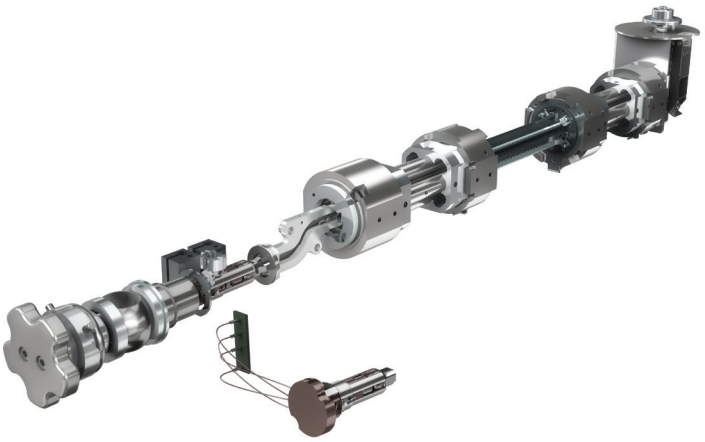
TriPlus 500 HS Autosampler Parameters	
Incubation Temperature (°C)	120
Incubation Time (min)	15
Vial Shaking	Fast
Vial Pressurization Mode	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen)	115
Vial Pressure Equilibration Time (min)	1
Loop Size (mL)	1
Loop/Sample Path Temperature (°C)	180
Loop Filling Pressure (kPa)	52
Loop Equilibration Time (min)	1
Needle Purge Flow Level	2
Injection Mode	Standard
Injection Time (min)	1

Trace 1310 GC Parameters	
Inlet Module and Mode	SSL, split
Split Ratio	5:1
Septum Purge Flow (mL/min)	5, constant
Carrier Gas, Flow (mL/min)	He, 1.0
Oven Temperature Program	
Temperature (°C)	40
Hold Time (min)	0.5
Rate (ºC/min)	20
Temperature 2 (°C)	160
Rate (ºC/min)	10
Temperature 3 (°C)	220
Hold Time (min)	1
Column	
Trace GOLD TG-WAXMS B	30 m, 0.25 mm, 0.5 µm (P/N 26086-2230)

TSQ 9000 GC-MS/MS Parameters	
Transfer Line Temperature (°C)	220
Ionization Type	EI
Ion Source	AEI
Ion Source Temperature (°C)	250
Electron Energy (eV)	70
Aquisition Mode	SRM
Detector Gain Multplier	10
Emission Current (µA)	100

TSQ 9000 GC-MS/MS Parameters for SRM

Compound	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Start (min)	End (min)	Collision Energy (eV)
NDMA	74	42	4.90	7.90	15
NDMA	74	44	4.90	7.90	5
NDMA-d6	80	50	4.88	7.88	5
NDMA-d6	80	46	4.88	7.88	15
NDEA	102	44	5.48	8.46	10
NDEA	102	85	5.48	8.46	5
NEIPA	116	70	5.74	8.74	35
NEIPA	116	99	5.74	8.74	10



Thermo Scientific TSQ 9000 GC-MS/MS system equipped with AEI ion source

Appendix 1

Liquid Injection-Orbitrap-GC-MS

TriPlus RSH Autosampler Parameters	
Injection Volume (µL)	2
Air Volume (µL)	1
Plunger Strokes	7
Filling Volume (µL)	3
Pre-Injection Delay Time (s)	0
Post-Injection Delay Time (s)	0
Sample Pullup Speed (µL/s)	0.4
Delay After Plunger Strokes (s)	1
Viscosity Delay (s)	1
Pre-Injection Washing Cycles	0
Sample Rinse Volume(µL)	1
Post-Injection Washing Cycles	4
Washing Volume (µL)	3

Trace 1310 GC Parameters	
Inlet Module and Mode	SSL, splitless w/ Surge
Inlet Temperature (°C)	250
Splitless Time (min)	1
Split Flow (mL/min)	80
Surge Pressure (kPa)	385
Surge Duration (min)	1
Septum Purge Flow (mL/min)	5, constant
Carrier Gas, Flow (mL/min)	He, 1.5
Oven Temperature Program	
Temperature (°C)	35
Hold Time (min)	3
Rate (°C/min)	120
Temperature 2 (°C)	270
Hold Time (min)	1
Column	
TraceGOLD TG-1701MS	30.0 m, 0.25 mm, 0.5 µm (P/N 26090-2230)

Exactive GC-MS Parameters for EI	
Transfer Line Temperature (°C)	260
Ionization Type	EI
Ion Source Temperature (°C)	230
Electron Energy (eV)	70
Aquisition Mode	Full Scan
Mass Range (Da)	50–400
Resolving Power (FWHM at *m/z* 200)	60,000
AGC Target	$1e^6$
Lockmass, Column Bleed	207.03235

Exactive GC-MS Parameters for SIM	
Transfer Line Temperature (°C)	260
Ionization Type	EI
Ion Source Temperature (°C)	230
Electron Energy (eV)	70
Aquisition Mode	SIM
Mass Range (Da)	50–400
Resolving Power (FWHM at *m/z* 200)	30,000
AGC Target	$5e^4$
MSX Counts	3
Isolation Window (*m/z*)	6

Exactive GC-MS Inclusion List for SIM

Compound	Mass (*m/z*)	Start (min)	End (min)	MSX ID
NDMA-d6	80.08514	3.80	4.9	1
NDMA	74.04747	3.80	4.9	2

Method parameters: Thermo Scientific Exactive GC-MS system coupled with Thermo Scientific TriPlus RSH Autosampler

Appendix 1
HS-Orbitrap-GC-MS

TriPlus 500 HS Autosampler Parameters	
Incubation Temperature (°C)	120
Incubation Time (min)	15
Vial Shaking	Fast
Vial Pressurization Mode	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen)	115
Vial Pressure Equilibration Time (min)	1
Loop Size (mL)	1
Loop/Sample Path Temperature (°C)	180
Loop Filling Pressure (kPa)	52
Loop Equilibration Time (min)	1
Needle Purge Flow Level	2
Injection Mode	Standard
Injection Time (min)	1

Trace 1310 GC Parameters	
Inlet Module and Mode	SSL, split
Split Ratio	5:1
Septum Purge Flow (mL/min)	5, constant
Carrier Gas, Flow (mL/min)	He, 1.0
Oven Temperature Program	
Temperature (°C)	40
Hold Time (min)	0.5
Rate (°C/min)	20
Temperature 2 (°C)	160
Rate (°C/min)	10
Temperature 3 (°C)	220
Hold Time (min)	1
Column	
Trace GOLD TG-WAXMS B	30.0 m, 0.25 mm, 0.5 µm (P/N 26086-2230)

Exactive GC-MS Parameters for EI	
Transfer Line Temperature (°C)	220
Ionization Type	EI
Ion Source Temperature (°C)	250
Electron Energy (eV)	70
Aquisition Mode	Full Scan
Mass Range (Da)	40–300
Resolving Power (FWHM at *m/z* 200)	60,000
AGC Target	$1e^6$
Lockmass, Column Bleed	207.03235

Exactive GC-MS Parameters for SIM	
Transfer Line Temperature (°C)	220
Ionization Type	EI
Ion Source Temperature (°C)	250
Electron Energy (eV)	70
Aquisition Mode	Full Scan
Mass Range (Da)	70–300
Resolving Power (FWHM at *m/z* 200)	30,000
AGC Target	$5e^4$
MSX Counts	4
Isolation Window (*m/z*)	20

Exactive GC-MS Inclusion List for SIM

Compound	Mass (*m/z*)	Start (min)	End (min)	MSX ID
NDMA	74.04801	6.00	6.65	1
NDEA	102.07931	6.70	7.20	2
NEIPA	116.09496	7.00	7.40	3

Method parameters: Thermo Scientific Q Exactive GC-MS/MS system coupled with Thermo Scientific TriPlus 500 Headspace Autosampler

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Appendix 2

Compound Name	Concentration Limit (ppm)
Class 1	
1,1-Dichloroethene	8
1,1,1-Trichloroethane	1500
Benzene	2
Carbon Tetrachloride	4
1,2-Dichloroethane	5

Compound Name	Concentration Limit (ppm)
Class 2 A	
Methanol	3000
Acetonitrile	410
Dichloromethane	600
trans 1,2-Dichloroethene	1870
cis 1,2-Dichloroethene	1870
Tetrahydrofuran	720
Cyclohexane	3880
Methycyclohexane	1180
1,4-Dioxane	380
Toluene	890
Chlorobenzene	360
Ehylbenzene	2170
m-Xylene	2170
p-Xylene	2170
o-Xylene	2170

USP <467> concentration limits in ppm for Class 1 and Class 2A residual solvents

Find out more at
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