Nitrosamine Impurity Analysis in Pharmaceuticals

Application Guide

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Analytical Products

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In July 2018, regulatory authorities for medicines were informed about the occurrence of the nitrosamine impurity N-nitrosodimethylamine (NDMA) in valsartan-based products. The active pharmaceutical ingredient (API) valsartan is an angiotensin-II-receptor blocker. It is used to treat hypertension, heart failure, and heart attack. It belongs to a group of structurally related compounds known as sartans. Later, other nitrosamine impurities such as N-nitrosodiethylamine (NDEA), N-nitrosodiisopropylamine (NDIPA), N-nitrosoethylisopropylamine (NEIPA), and N-nitrosodibutylamine (NDBA) were found to be present in other medicines belonging to the sartan family.

Due to the carcinogenic nature of some nitrosamine impurities, drug manufacturers have made efforts to reduce and/or eliminate the levels of these compounds in their products. Different test methods were developed, utilizing both HPLC and GC. As of 2021, analytical methods had been published by both the US Food and Drug Administration (FDA), European Medicines Agency (EMA), as well as other regulatory bodies. In addition, USP published chapter <1469> on nitrosamine impurities, which became official December 1, 2021.

Nitrosamines have also been found in some formulations of these prescription and over the counter medicines:

ranitidine, nizatidine: histamine-2 blockers used to treat gastrointestinal ailments such as heartburn

metformin: used to treat type II diabetes

rifampin & rifapentine: used to treat some bacterial infections; most notably tuberculosis

Testing using these published methods is based on chromatographic separation, by HPLC or GC, and mass spectral detection. The target compound list varies by method, with some focusing solely on NDMA in specific formulations such as ranitidine. Others, such as those found in USP chapter <1469> cover more nitrosamine impurities in a wider array of formulations. All methods require high sensitivity and selectivity to be able to detect very low levels of these compounds and to distinguish them from other matrix components.

Regardless of the method used in your laboratory for nitrosamine testing, we can provide you with products to support every part of the workflow. See the illustrations on the following pages for details and links to more information about recommended products.

Explore our full portfolio of nitrosamine reference materials

Nitrosamine Analysis – HPLC Workflow

Sample Prep

- Millex® Syringe Filters
- Autosampler & other vials
- Pipettes & pipette tips
- Solvents for Chromatography

Standardization & Calibration

- Nitrosamine impurity standards

HPLC Analysis

- HPLC Columns
- HPLC and LC-MS Solvents

HPLC Columns per method

- US FDA published methods:
  - Ascentis® Express AQ-C18
  - Ascentis® Express Biphenyl
- Taiwan FDA published method
  - Ascentis® Express AQ-C18
- EP 2.5.42
  - Ascentis® Express C18
- USP <1469>
  - L1: Ascentis® Express AQ-C18
  - L1: Ascentis® Express C18
  - L3: Ascentis® Express F5

See the end of this guide for a list of specific supporting products.

Nitrosamine Analysis – GC Workflow

Sample Prep

- Millex® Syringe Filters
- Autosampler & other vials
- Pipettes & pipette tips
- SupraSolv® solvents for GC

Standardization & Calibration

- Nitrosamine impurity standards

GC Analysis

- GC Columns & Accessories

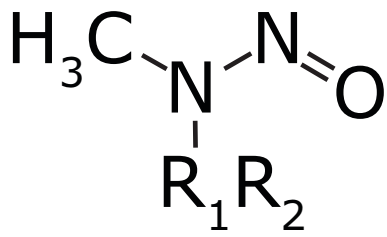
GC Columns per method

- US FDA & Taiwan FDA published methods: SUPELCOWAX 10
- Health Canada published method: SPB-624
- EP 2.5.42: SPB-624
- USP <1469>: SUPELCOWAX 10

See the end of this guide for a list of specific supporting products.

Reference Materials for Nitrosamine Analysis

To aid in the accurate analysis of these compounds, we offer multiple nitrosamine impurities from USP and EP, as well as certified reference materials (CRMs), produced and tested in our ISO 17034 and ISO/IEC 17025 accredited facilities. Many of these CRMs are offered as pharmaceutical secondary standards, which can be used to replace the time-consuming process of preparing and qualifying in-house working standards.



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SigmaAldrich.com/nitrosamines

What are Pharmaceutical Secondary CRMs?

Pharmaceutical Secondary Certified Reference Materials (CRMs) provide a time-saving, convenient and cost-effective alternative to making in-house working standards.

Important Features of Secondary CRMs:

- **Traceability** to United States Pharmacopeia (USP) and (if available) also to European Pharmacopoeia (EP) and British Pharmacopoeia (BP)
- **Certified purity value** according to ISO 17034 and ISO/IEC 17025 utilizing mass balance and/or qNMR approaches
- **Comprehensive certificate** according to ISO Guide 31

Our Secondary Standards are Certified Reference Materials (CRMs) manufactured under the scope of ISO 17034 and tested in an ISO/IEC 17025 accredited laboratory. Comprehensive Certificates of Analysis demonstrate traceability to USP, EP and/or BP primary standards (where available). In addition, an independent certified purity value is provided. This allows these Secondary CRMs to be used as reference materials for quantitative purposes in a variety of applications.

The US Food and Drug Administration (FDA) and EP both recognize the use of secondary standards or working standards which are established with reference to the corresponding primary standard (references available on request).

applications

Determination of N-Nitrosamines in Valsartan

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Introduction

In July 2018 regulatory authorities for medicines have been informed about the occurrence of a nitrosamine impurity (in particular N-nitrosodimethylamine, NDMA, **Figure 1**) in Valsartan products.[1] The active pharmaceutical ingredient (API) Valsartan is an angiotensin-II-receptor blocker for the treatment of hypertension, heart failure and heart attack, when the patient is intolerant to ACE inhibitor therapy.[2] Valsartan is part of a group of structurally related compounds known as sartans, which have a tetrazole group (a ring with four nitrogen and one carbon) in common. Later other nitrosamine impurities such as N-nitrosodiethylamine (NDEA), N-nitrosodiisopropylamine (NDIPA), N-nitrosoethylisopropylamine (NEIPA) and N-nitrosodibutylamine (NDBA) have been discovered in other medicines belonging to the sartan family, but also in ranitidine drugs. Therefore, a worldwide recall was issued on pharmaceutical products that are using Valsartan bulk drug substances, which lead to an interim shortage of Valsartan drugs.

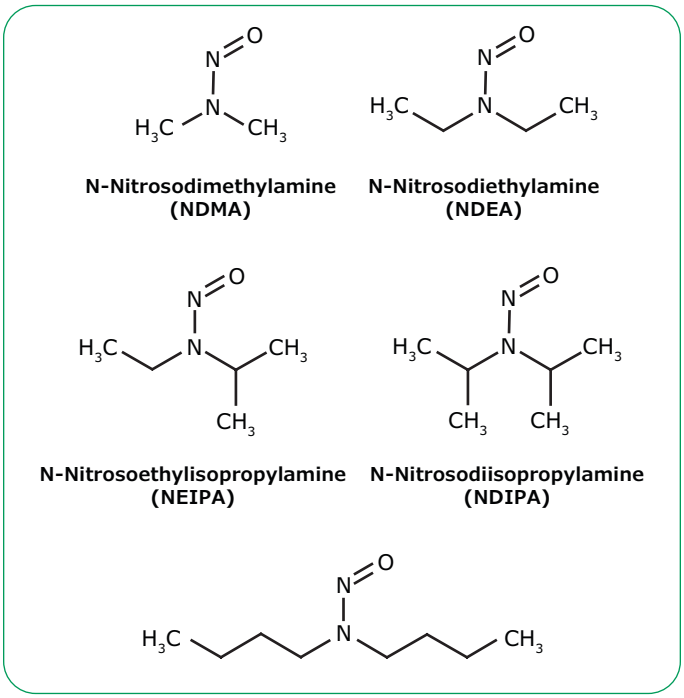

Figure 1. Molecular structure of the N-nitrosamines investigated in this study

N-nitrosamines contain a nitroso functional group (NO). According to the International Agency for Research on Cancer (IARC) from the World Health Organization (WHO) the majority of nitrosamines are carcinogenic and genotoxic in animals and probable human carcinogens. Investigations by the authorities and the API manufacturers determined that the nitrosamines are formed from reaction of secondary or tertiary amines and nitrite under acidic conditions in the manufacturing process. In the case of sartans, the formation of the tetrazole ring requires sodium nitrite which can then result in the formation of N-nitrosamines. Or these compounds may derive from contaminated solvents, reagents, or manufacturing equipment.[3] An interim limit for nitrosamines was set by the European Pharmacopoeia (Ph. Eur.) Commission to be less than 1 ppm in APIs, but the limit was reduced to 30 ppb at end of 2020.[4]

These limits require the use of highly sensitive and highly selective analytical techniques. Most methods are either done by GC-MS or by HPLC-MS.[5-7] Another challenge is the variety of nitrosamines, APIs, and formulations, which require specifically tailored methods for the impurity testing.

This work presents a procedure for determination of 5 nitrosamine impurities (NDMA, NDEA, NEIPA, NDIPA and NDBA) in a Valsartan drug product at trace level by GC-MS/MS in EI MRM mode according to US FDA guidelines. For the method development one of the methods suggested by the Office of Testing and Research (OTR) of the FDA was used as base.[8] Method validation was conducted according to the requirements of USP.[9]

Experimental

The GC-MS/MS method used liquid injection to cover a broad range of nitrosamines. Compared to the OTR method a wax column with a thinner film thickness (0.5 µm instead of 1 µm) was chosen, but this deviation complies with USP general chapter <621> on chromatography.[10] The chromatographic conditions as well as the MS/MS conditions are shown in **Tables 1-3**.

Table 1: Chromatographic conditions

Experimental Conditions	
Column:	SUPELCOWAX 10, 30 m x 0.25 mm I.D., 0.5 µm (**24284**)
Detector:	MS/MS
Inj. temp.:	250 ° C
Oven:	40 °C (0.5min), 20 °C /min to 200 °C, 60 °C /min to 250 °C (3 min)
Carrier gas:	helium, 1.0 mL/min
Liner:	4 mm single taper liner with glass wool
Injection:	2 µL – Splitless pulsed injection
Sample Diluent:	Dichloromethane
Sample Preparation:	Using a pill cutter, each tablet was quartered, and the pieces were placed in 15 mL centrifuge tube followed by 5 mL of dichloromethane. Sample was vortexed for 1 min and placed in centrifuge and centrifuged at 4000 rpm for 2.5 min. Using a disposable pipet, approximately 2 mL of dichloromethane layer was transferred to a 5 mL syringe fitted with a 0.45µm PVDF filter. Approximately 0.5 mL of sample was filtered into a 2 mL vial and capped.
Standard Solution:	2.5 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, 40 ng/mL, 80 ng/mL and 100 ng/mL each prepared in dichloromethane (NDMA/NDEA/NEIPA/NDIPA/NDBA)

Table 2: MS/MS conditions

MS/MS Conditions	
Tuning	auto-tuning
Acquisition	MRM (EI mode)
Collision Gas	nitrogen @ 1.5 mL/min
Quench Gas	helium @ 4.0 mL/min
Solvent Delay	7 min
MS source temperature	230 °C
Quad Temperature	150 °C
Electron Energy	70 eV
Dwell Time	50 ms

Table 3: MRMs for the five different nitrosamines

Peak	Compound	Transition	Retention Time
1	NDMA MRM 1	74->42	6.95
	NDMA MRM 2	74->44	6.95
2	NDEA MRM 1	102->85	7.53
	NDEA MRM 2	102->56	7.53
3	NEIPA MRM 1	116->99	7.78
	NEIPA MRM 2	71->56	7.79
4	NDIPA MRM 1	130->42	7.97
	NDIPA MRM 2	130->88	7.98
5	NDBA MRM 1	158->99	9.5
	NDBA MRM 2	84->56	9.49

The five nitrosamines were sufficiently separated in less than ten minutes and the target peaks were well resolved from the solvent and matrix species (**Figure 2**). The retention times are a little bit shorter compared with the OTR method, which is likely due to the lower film thickness.

Low limits of detection were achieved by multiple reaction monitoring (MRM) for two transitions. Two examples are shown in **Figure 3** by the chromatograms of NDEA and NDIPA at the lowest concentration of 2.5 ppb.

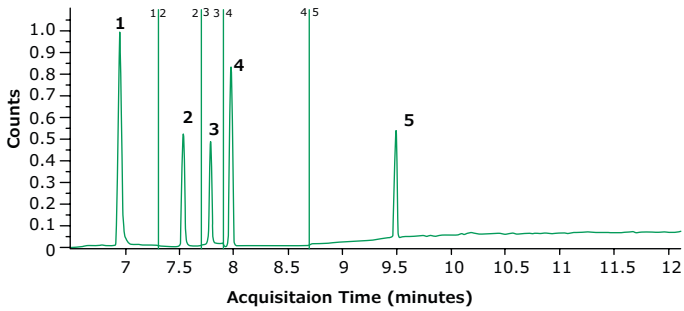

Figure 2. Exemplary chromatogram of the system suitability solution with a concentration of 40 ng/mL. For peak labeling please see **Table 3**.

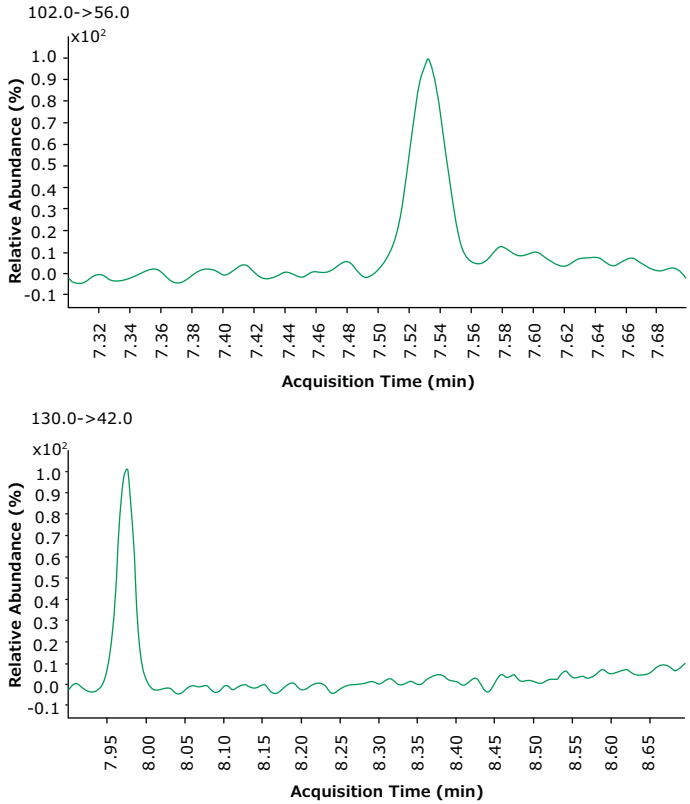

Figure 3. Chromatograms of NDEA (top) and NDIPA (bottom) at the lowest concentration of 2.5 ppb.

Method Suitability

The validated FDA-OTR method requires that the % RSD for six replicate injections of the 40 ng/mL standard is ≤ 5 %. Using our method, the % RSD for six consecutive injections of the 40 ng/mL standard was less than 5 for all the impurities at both MRMs as shown in **Table 4**.

Table 4: Precision of six consecutive injections of the 40 ng/mL nitrosamine standard.

Compound	% RSD for MRM1	% RSD for MRM2
NDMA	1.8	1.3
NDEA	1.1	1.1
NEIPA	4.2	1.5
NDIPA	0.9	2.2
NDBA	4.3	3.0

Furthermore, the correlation coefficient (R^2) of the linear calibration curves should be ≥ 0.998. This was exceeded for all five nitrosamines at both MRMs (**Table 5**).

Table 5: Correlation coefficient (R^2) of the nitrosamines at both MRMs

Impurity	MRM 1	MRM 2
NDMA	0.9994	0.9995
NDEA	0.9991	0.9995
NEIPA	0.9995	0.9995
NDIPA	0.9996	0.9994
NDBA	0.9983	0.9981

Application on Valsartan Drug Product

A commercial Valsartan product purchased in a local pharmacy was spiked with nitrosamine impurities to a concentration of 10 ppb (40 ppb for NDBA) in the drug product. The recovery rates of the nitrosamines were in the range from 94.5 to 105.7% respectively. (**Table 6**).

Table 6: Recovery rates of nitrosamines in spiked drug product

Impurity	Recovery of 10 ppb in drug product
NDMA	99%
NDEA	103.50%
NEIPA	94.50%
NDIPA	103.90%
NDBA	105.70%

In the OTR method, limits of quantification (LOQs) for the determination of the nitrosamines in Valsartan products were given in the range of 8 – 40 ppb. Our method achieves similar LOQs in a Valsartan drug product (**Table 7**). The LOQs were calculated from the calibration curves based on a signal/noise (S/N) ratio of 10 for each of the compounds and have been validated by standard addition experiments to Valsartan tablets. Limits of detections (LOD) were calculated using a signal/noise (S/N) ratio of 3.

Table 7: LOQs in drug product for OTR method and our method.

Impurity	FDA LOQ in drug product [ppb]	LOQ in drug product obtained in this study [ppb]
NDMA	13	3
NDEA	8	5
NEIPA	8	3
NDIPA	8	5
NDBA	40	32

Summary

The determination of nitrosamine impurities can be easily achieved by GC-MS/MS in MRM mode using the SUPELCOWAX column based on the suggested method by OTR-FDA. All nitrosamines were well separated from each other as well as from solvent and matrix peaks. All system suitability requirements were met. The method was successfully applied for the analysis of a Valsartan drug product, that was spiked with nitrosamine impurities.

References

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9. USP General Chapter <1225> Validation of Compendial Procedures, USP43-NF38
10. USP General Chapter <621> Chromatography USP43-NF38

Featured Products

Description	Cat. No.
GC Column	
SUPELCOWAX 10 GC Capillary Column 30 m × 0.25 mm, df 0.50 µm	**24284**
Solvents	
Dichloromethane for gas chromatography MS SupraSolv®	**1.00668**
Reference Materials	
N-Nitrosodimethylamine (NDMA), Certified Reference Material 5000 µg/mL in methanol	**CRM40059**
N-Nitrosodiethylamine (NDEA), Certified Reference Material 5000 µg/mL in methanol	**40334**
N-Nitrosodi-n-butylamine (NDBA), Analytical Standard (NDBA)	**442685**
N-Nitroso-ethyl-isopropylamine, European Pharmacopoeia (EP) Reference Standard (NEIPA/ NIPEA)	**Y0002262**
N-Nitroso-diisopropylamine (NDIPA), European Pharmacopoeia (EP) Reference Standard	**Y0002263**
Accessories	
Millex® HV Durapore(PVDF) 0.45µm syringe filter	**SLHV033NS**
Inlet Liner, Split/Splitless Type, Single Taper FocusLiner™ Design (wool packed), pkg of 5 ea (also available as pkg of 1 ea (2879901-U) and pkg of 25 ea (2879925-U))	**2879905-U**
Supelco® Helium Purifier SS fittings, 1/4 in. Swagelok (nuts and ferrules included)	**27601-U**
Supelco® Helium Purifier SS fittings, 1/8 in. Swagelok (nuts and ferrules included)	**27600-U**

Description	Cat. No.
OMI® Tube Holder for use with OMI-2 purifier tubes	**23921**
OMI®-2 Purifier Tube	**23906**
Super Clean (Base-Plate Design) Gas Purifier triple trap (hydrocarbon, moisture, oxygen)	**SU861026**
Super Clean (Base-Plate Design) Kit carrier gas kit (includes SU861026 + SU861011)	**28878-U**

Supporting Products

Description	Cat. No
N-Nitrosodimethylamine (NDMA) Pharmaceutical secondary standard; Certified reference material	**PHR2407**
N-Nitrosodiethylamine (NDEA) Pharmaceutical secondary standard; Certified reference material	**PHR2408**
N-Nitrosodibutylamine (NDBA) Pharmaceutical secondary standard; Certified reference material	**PHR3608**
N-Nitrosoethylisopropylamine (NEIPA/NIPEA) Pharmaceutical secondary standard; Certified reference material	**PHR3609**
N-Nitrosodiisopropylamine (NDIPA) Pharmaceutical secondary standard; Certified reference material	**PHR3607**

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Nitrosamine Testing using LC-MS Methods from the USP General Chapter <1469>

Tim Mueller[1] and Patrik Appelblad[2]*

[1]Site Management-Analytical
[2]Advanced Analytical

Introduction

Nitrosamines are unwanted side products in many substances and are suspected to be toxic and carcinogenic. In pharmaceutical raw material and finished drug products, nitrosamines may also form as side-products from synthesis, during storage, from packaging, etc. Demand for nitrosamine analysis has rapidly increased worldwide. The list of nitrosamine impurities resulting from drug substances produced using specific synthetic routes has grown after extensive assessments of these protocols.

The United States Pharmacopeia (USP) published in December 2021 new procedures in response to the unexpected detection of nitrosamines, such as N-nitrosodimethylamine (NDMA), **Figure 1**, in certain active pharmaceutical ingredients (API) and corresponding final formulations.1 The new USP chapter <1469> provides recommendations regarding the creation of controls of nitrosamine levels to ensure their elimination or reduction, and analytical method performance characteristics for nitrosamine testing procedures using both GC-MS (procedures 2 and 4) and LC-MS (procedures 1 and 3).

This paper focuses on the LC-MS based test procedures (procedure 1 and 3) for quantitative analysis of known nitrosamine impurities in drugs and pharmaceutical raw materials using liquid chromatography and mass spectrometric detection. Even though both methods were evaluated, final run conditions and data from procedure 3 will be presented. System suitability criteria for procedure 1 could not be met with the instrumentation available, as will be described.

Procedure 1 designates the use of a high-resolution mass spectrometer (HRMS) and can be used for the quantitation of NDMA, NDEA (nitrosodiethylamine), NDBA (nitrosodibutylamine), NDIPA (N-nitrosodiisopropylamine), NEIPA (N-nitrosoethylisopropylamine), NMBA (N-nitrosomethylaminobutyric Acid), and NMPA (N-nitrosomethylphenylamine) in selected sartans (valsartan, irbesartan, and losartan potassium). Procedure 3 uses MS/MS and can be used for the quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA in selected sartans (valsartan, losartan potassium, olmesartan medoxomil, candesartan cilexetil, and telmisartan).

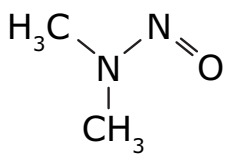

Figure 1. Chemical Structure of N-Nitrosodimethylamine (NDMA).

Experimental Conditions

Standard and Sample Preparation – Procedure 3

Standards Preparation:	
Internal Standard Solution:	10 µg/mL each of NDMA-d6 and NMBA-d3, 1 µg/mL each of NDEA-d10 and NDBA-d18 in water
Nitrosamine Standards Stock Solution Mixture:	Prepare a mixture of 200 ng/mL each of N-nitrosodimethylamine (NDMA), N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), N-nitrosodibutylamine (NDBA), and N-nitrosomethylaminobutyric acid (NMBA) by mixing appropriate volumes of the respective USP Reference Standards and dilute with water.
Ndea Standard Stock Solution:	Prepare a solution of 132 ng/mL of N-nitrosodiethylamine (NDEA) by diluting USP N-Nitrosodiethylamine RS with water.
Standard Solutions:	Depending on the targeted nitrosamine concentration in the sample, prepare a set of 5 consecutive linearity solutions as described in Table 1 from the nitrosamine standards stock solution mixture and NDEA standard stock solution by mixing specified volumes of each solution as indicated.
Sample Preparation:	
Sample Preparation	1. Transfer 80 mg of the drug substance into a 2-mL lidded centrifuge tube. 2. Add 1188 µL of diluent and 12 µL of the Internal standard solution. 3. Vortex at 2500 rpm for 20 min (except for losartan potassium, which should be vortexed NMT 5 min). 4. Centrifuge at about 10,000 rpm for 10 min. 5. Filter into a vial using a PTFE filter of 0.45-µm pore size.
Valsartan Samples	The valsartan sample solution was prepared by dissolving about 80 mg of ground valsartan tablets in 1188 µL of diluent (1% formic acid in water) + 12 µL internal standard. "Valsartan" in Table 8 means drug product (tablet/tablet powder).
Losartan Samples	The Losartan sample solution was prepared by dissolving about 80 mg of ground valsartan tablets in 1188 µL of diluent (1% formic acid in water) + 12 µL internal standard. "Losartan" in Table 10 corresponds to losartan tablets or ground losartan tablets

Table 1. Preparation of the nitrosamine standard solutions (dilution protocol) for procedure 3

Linearity Solution #	Concentration Level	Concentration of NDMA, NMBA, NDBA, NEIPA, NDIPA/NDEA (ng/mL)	Content of NDMA, NMBA, NDBA, NEIPA, NDIPA/ NDEA (ppb)	Nitrosamine Standard Stock Solution Mixture (µL)	NDEA Standard Stock Solution (µL)	Water (µL)	Internal Standard (µL)	Total Volume (µL)
1	L1	1.33/0.66	19.95/10	8	6	1174	12	1200
2	L2	2/0.88	30/13.5	12	8	1168	12	1200
3	L3	5/3.3	75/49.5	30	30	1128	12	1200
4	L4	7.5/4.95	112.5/74.25	45	45	1098	12	1200
5	L5	10/6.6	150/99	60	60	1068	12	1200
6	L6	15/9.9	225/148.5	90	90	1008	12	1200
7	L7	30/19.8	450/297	180	180	828	12	1200
8	L8	60/39.6	900/594	360	360	468	12	1200
9	L9	90/59.4	1350/891	540	540	108	12	1200

Table 2. Experimental conditions for procedure 3

HPLC Parameters	
Column:	Ascentis® Express C18, 2.7 µm HPLC column 150 mm x 3.0 mm I.D.
Mobile Phase:	[A] 0.1% Formic acid in H_2O; [B] 0.1% Formic acid in methanol
Gradient:	

Time (min)	% A	%B
0.0	97	3
1.5	97	3
4.0	50	50
7.0	25	75
8.1	15	85
9.2	5	95
12.0	5	95
12.1	97	3
15.0	97	33

Flow Rate:	0.5 mL/min
Column Temperature:	60 °C
Autosampler Temperature:	18 °C
Detection:	MRM, APCI (+), see **Table 3 & 4**
Injection Volume:	20 µL
Samples:	Standards & valsartan/losartan solutions as indicated

Table 3. MS instrument parameters used for quantitative purposes for procedure 3

MS Detector Parameters	
Instrument:	Agilent 6495C Triple Quadrupole MS
Source:	APCI
Detection:	Instrumental parameters as shown in this table. Further detailed detector settings can be found in the procedure 3, USP chapter<1469>
Source Parameter	
Gas Temperature:	290 °C
Drying Gas Flow:	11 L/min
Nebulizer Pressure:	25 psi
Vaporizer Temperature:	350 °C
Capillary Voltage:	3000 V
Corona Current:	4 µA
Fragmentor:	166 V
Ion Polarity:	positive

Table 4. MRM Transitions for nitrosamine impurities for procedure 3

Nitrosamine	Polarity	MRM Transitions (m/z)			
		MRM-1 (Quantitation)		MRM-2	
NDMA	Positive	75.0	43.0	75.0	44.1
NDMA-D_6	Positive	81.2	46.0	81.2	64.1
NDEA	Positive	103.1	75.1	103.1	47.1
NDEA-D_{10}	Positive	113.2	34.2	113.2	49.1
NMBA	Positive	147.1	44.1	147.1	117.1
NMBA-D_3	Positive	150.1	47.1	150.1	120.2
NDBA	Positive	159.2	41.1	159.2	29.1
NDBA-D_{18}	Positive	177.3	66.2	177.3	46.2
NEIPA*	Positive	117.1	75.1	117.1	47.2
NDIPA*	Positive	131.2	89.1	131.1	47.1

*NDEA-D_{10} was used as internal standard for NEIPA and NDIPA

Results and Discussion

Evaluation of Procedure 1

The USP procedure 1 method describes the use of liquid chromatography and high-resolution mass spectrometric detection (LC-HRMS), but the given experimental conditions[1] appear not generic enough to allow implementation and validation on any given HRMS platform. Our laboratory experienced sensitivity issues with an ultra-modern HRMS detector (Agilent 6546 Q-TOF), and to verify the results different HPLC columns, solvents, and reagents were tested. Chromatographic system suitability criteria could be met, but it was not possible to meet system suitability for overall identification and sensitivity.

Comparable signal intensities were attained at 1 µg/mL, 100 ng/mL, and 50 ng/mL for NDEA, NEIPA, NDIPA, NDBA, and NMPA (ESI positive mode) with a Supelco® L43 column (Ascentis® Express F5), see **Figure 2** and two other manufacturers L43 columns (not shown). NDMA impurity was not detected with ANY column at all concentration levels, and NMBA was not detected with any column in (-)-ESI mode. The comparison of different L43 columns (pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer) showed similar overall behaviour with procedure 1 using the LC-HRMS instrument, but it was not possible to meet the system suitability for identification and sensitivity. More recently, there has been further

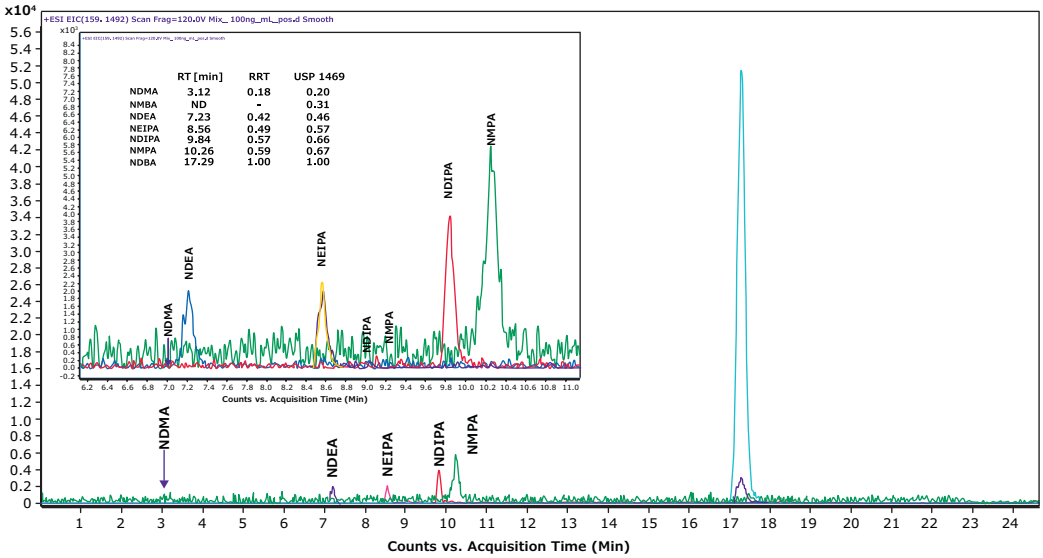

Figure 2. Chromatogram of a 100 ng/mL nitrosamine mixture analyzed with a Supelco® L43 column (Ascentis® Express F5), ESI (+) mode.

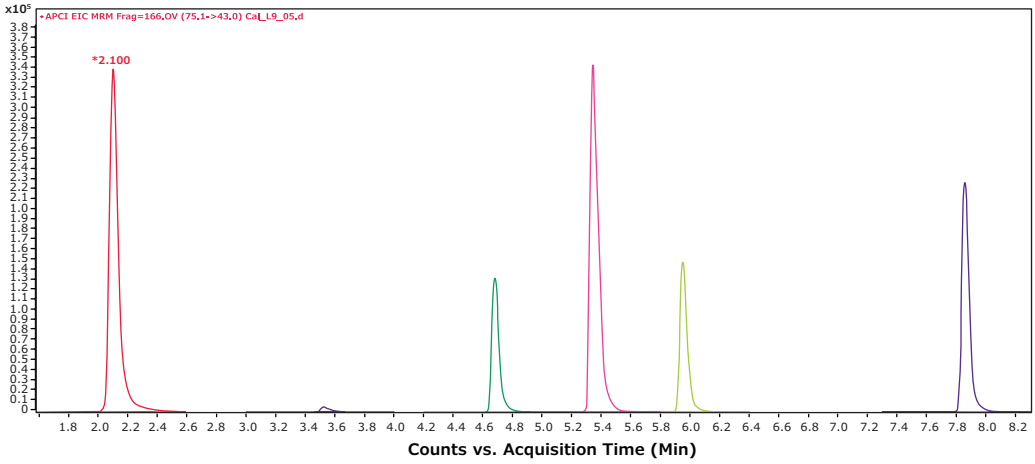

Figure 3. MRM Chromatogram (no scaling) of a 90 ng/mL nitrosamine standard solution using an Ascentis® Express C18, 2.7 µm HPLC column for procedure 3.

clarification posted on the USP Pharmacopeial Forum (USP-PF) regarding procedure 1. It mentions that analyses were performed and validated with an Orbitrap Fusion™ Lumos™ Tribrid™ brand of mass spectrometer. Since this type of instrumentation was not available in our laboratory, further validation of procedure 1 was not pursued.

Evaluation of Procedure 3

This method describes the use of liquid chromatography and tandem-mass spectrometric detection (LC-MS/MS), for the quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA in selected sartans (valsartan, losartan potassium, olmesartan medoxomil, candesartan cilexetil, and telmisartan). The procedure listed two system suitability criteria: 1. Correlation coefficient: NLT 0.99 and 2. y-Intercept: Not more than (NMT) 25% of the response of the medium concentration solution used in standard curve generation. Going forward, analytical data will be presented from the work establishing a validated analytical procedure 3 using a 150 x 3.0 mm Ascentis® Express C18 column (USP L1 packing) with 2.7 µm particles. An example of a nitrosamine impurity standard on this column is shown in **Figure 3**.

The method linearity was determined over nine calibration levels after optimizing the instrumental set-up. Triplicate injections were made of each linearity solution. The USP Chapter <1469> has defined two system suitability requirements for procedure 3. The correlation coefficient should not be less than (NLT) 0.99 and the y-intercept for each calibration graph should not be more than (NMT) 25% of the response of the medium concentration solution used in standard curve generation. As shown in **Table 5** both of these requirements were met.

Table 5. The method system suitability requirements for procedure 3

Analyte	Correlation coefficient	y-Intercept (max. y-Intercept)
NDMA	0.9960	0.001851 (< 0.031125)
NMBA	0.9932	0.000409 (< 0.022675)
NDEA	0.9986	0.000167 (< 0.018575)
NEIPA	0.9952	0.001380 (< 0.0939)
NDIPA	0.9959	0.000144 (< 0.032175)
NDBA	0.9936	0.019615 (< 0.2201)

The method precision (**Table 6**) and accuracy (**Table 7**) were determined using data from ten injections of calibration levels 1, 5 and 9 (L1, L5 and L9). Accuracies of the level 1, 5 and 9 solutions were calculated using the 9-point calibration curves described in **Table 1**.

Table 6. Procedure 3 method precision determined from calibration level 1, 5, and 9 (n=10 at each level)

Analyte	Concentration (ng/mL)	Average Response	Relative Standard Deviation (%)
NDMA	1.33	21687	1.7
	10.00	168086	2.3
	90.00	1513884	0.7
NMBA	1.33	398	5.6
	10.00	3093	7.6
	90.00	27009	2.7
NDEA	0.66	4443	2.5
	6.60	46576	2.4
	59.40	455404	1.1
NEIPA	1.33	18033	1.6
	10.00	148983	2.9
	90.00	1395374	0.9
NDIPA	1.33	6470	1.6
	10.00	54903	3.8
	90.00	523857	0.8
NDBA	1.33	12255	4.0
	10.00	92155	6.2
	90.00	827071	3.3

Table 7. Procedure 3 method accuracy determined for level 1, 5, and 9 using the calibration curve of each corresponding analyte (n=10 at each level)

Analyte	Concentration (ng/mL)	Average Accuracy (%)	Relative Standard Deviation (%)
NDMA	1.33	100.85	0.6
	10.00	97.90	1.1
	90.00	100.03	0.5
NMBA	1.33	109.93	7.0
	10.00	95.65	5.3
	90.00	98.66	3.7
NDEA	0.66	106.56	2.4
	6.60	96.04	1.0
	59.40	103.51	0.9
NEIPA	1.33	105.93	1.4
	10.00	97.86	2.4
	90.00	100.38	1.1
NDIPA	1.33	106.34	1.4
	10.00	96.80	3.6
	90.00	100.55	1.0
NDBA	1.33	95.64	2.2
	10.00	100.86	3.7
	90.00	98.44	2.2

The method's limit of detection (LOD) and limit of quantification (LOQ) were determined by spiking 3.3 ng/mL (2.2 ng/mL for NDEA) into a valsartan/losartan sample solution and using the signal-to-noise (S/N) ratio for the calculation. The limit of detection is defined as a signal-to-noise S/N ratio of 3, whilst the limit of quantification is defined as a S/N ratio of 10. The S/N ratio was calculated by the instrument software, and where the S/N ratio for each peak is established automatically using the peak height and a defined region of noise. Resulting limits for the measured samples are shown in **Tables 8-11**

Table 8. The method limit of detection (LOD), and limit of quantification (LOQ), in a valsartan sample solution

NDMA		NBMA		NDEA		NEIPA		NDIPA		NDBA	
LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)
12	41	103	343	19	63	19	63	7	22	63	211

Table 9. The method limit of detection (LOD), and limit of quantification (LOQ), for analyte content in valsartan

NDMA		NBMA		NDEA		NEIPA		NDIPA		NDBA	
LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)
0.18	0.62	1.55	5.15	0.28	0.94	0.28	0.95	0.10	0.33	0.95	3.16

Table 10. The method limit of detection (LOD), and limit of quantification (LOQ), in losartan sample solution

NDMA		NBMA		NDEA		NEIPA		NDIPA		NDBA	
LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)
12	41	96	320	25	85	13	43	8	27	474	1580

Table 11. The method limit of detection (LOD), and limit of quantification (LOQ), for analyte content in losartan potassium

NDMA		NBMA		NDEA		NEIPA		NDIPA		NDBA	
LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)
0.18	0.62	1.44	4.80	0.38	1.27	0.19	0.64	0.12	0.41	7.11	23.70

Table 12. Procedure 3 method - method specificity

Analyte	Average Retention time (min)	Relative Retention time (RRT)	Relative Retention time (RRT) per USP 1469
NDMA	2.100	0.27	0.20
NMBA	3.528	0.45	0.31
NDEA	4.681	0.60	0.46
NEIPA	5.345	0.68	0.57
NDIPA	5.951	0.76	0.66
NDBA	7.859	1.00	1.00

Table 13. Procedure 3 method - analyte recovery

Analyte	Spiked concen-tration* (ng/mL)	NDMA (%)	NMBA (%)	NDEA (%)	NDEIPA (%)	NDIPA (%)
Valsartan	3.3 / 2.2	96.70	105.00	106.83	93.52	96.84
	16.6 / 11	94.72	95.50	102.50	80.52	82.79
	33.3 / 22.2	99.27	99.32	108.09	84.06	85.54
Losartan potassium	3.3 / 2.2	100.50	97.58	82.45	87.67	94.31
	16.6 / 11	103.25	97.19	109.20	92.29	96.04
	33.3 / 22.2	105.94	95.30	115.22	97.54	103.74

*for all analytes / NDEA

The method specificity was determined by monitoring the analytes retention time, and their relative retention to the retention of NDBA for a series of injections of nitrosamine standard solutions (n=40) and is shown in **Table 12**.

The analyte recovery was determined in one valsartan batch and in one losartan potassium batch **(Table 13)**. The drug substance batches were spiked with all analytes over three concentration levels as triplicates during the sample preparation procedure. The prepared sample solutions were measured and evaluated against an external calibration curve to calculate the individual analyte concentration. The ratio of internal standard signal versus analyte signal was determined in the sample solution and in the solutions of the (external) calibration row, i.e., signal NDMA-D6 / signal NDMA. Then the signal ratios were used to calculate the concentration in the sample solution against the calibration solutions.

During the determination of the analyte recovery, a systematic issue with the determination of the spike recovery for NDBA was observed (data not shown), as the found concentrations of this analyte were always too high (recoveries > 130% and thus excluded).

The analysis of possible reasons for this issue showed that coelution with one or several unknown substances occurs during the elution of NDBA.

Conclusion

This paper shows intriguing findings from work with USP Chapter <1469>, Procedure 1, and a successful implementation of Procedure 3 meeting all system suitability requirements.

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1. United States Pharmacopeia. 2022. General Chapter, <1469> Nitrosamine Impurities. USP-NF. Rockville, MD: United States Pharmacopeia. DOI: https://doi.org/10.31003/USPNF_M15715_02_01

Featured Products

Description	Cat. No.
HPLC	
Ascentis® Express F5, 2.7 µm HPLC column 100 mm x 4.6 mm I.D.	**53590-U**
Methanol hypergrade for LC-MS LiChrosolv®	**1.06035**
Water for chromatography (LC-MS Grade) LiChrosolv®	**1.15333**
Formic acid for LC-MS LiChropur™	**00940**
Reference Materials and Accessories	
N-Nitrosodimethylamine (NDMA) USP Reference Standard	**1466674**
N-Nitrosodiisopropylamine (NDIPA) USP Reference Standard	**1466663**
N-Nitrosoethylisopropylamine (NEIPA) USP Reference Standard	**1466685**
N-Nitrosomethylaminobutyric Acid (NMBA) USP Reference Standard	**1466696**
Nitrosodibutylamine (NDBA) USP Reference Standard	**1466641**
Nitrosodiethylamine (NDEA) USP Reference Standard	**1466652**
N-Nitrosomethylphenylamine (NMPA)	**PHR3611**
Millex® Syringe Filter LG 0.20 µm hydrophilic PTFE, 13 mm	**SLLGX13**
HPLC	
Ascentis® Express C18, 2.7 µm HPLC column 150 mm x 3.0 mm I.D.	**53816-U**
Methanol hypergrade for LC-MS LiChrosolv®	**1.06035**
Water for chromatography (LC-MS Grade) LiChrosolv®	**1.15333**
Formic acid for LC-MS LiChropur™	**00940**
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Nitrosodibutylamine (NDBA) USP Reference Standard	**1466641**
Nitrosodiethylamine (NDEA) USP Reference Standard	**1466652**
Nitrosodimethylamine-d6 (NDMA-d6)	**591068**
Millex® Syringe Filter LCR 0.45µm hydrophilic PTFE, 13 mm	**SLCRX13**

For a determination of N-nitrosamines in valsartan by GC-MS/MS see the article in Analytix Reporter issue 11 at **SigmaAldrich.com/analytix**

More information on Pharma QC topics can be found at: **SigmaAldrich.com/PharmaQC**

Investigation of Nitrosamine Extractables and Recovery on Different Syringe Filter Devices Using USP <1469>

Introduction

Due to findings in valsartan products, nitrosamines gained attention as possible impurities in pharmaceuticals.[1] There have been multi-national efforts to develop analytical methods for testing nitrosamines in both drug substances and products, including methods from ASTM, EMA, EP[2] and USP[3]. Many GC-MS and LC-MS methods involve a filtration step using syringe filters. To maintain data accuracy, it is important that any consumable used for sample preparation, including syringe filters, does not introduce contaminants or impede analyte recovery. Thus, this study aimed to evaluate nitrosamine extractables in syringe filters from various manufacturers and recovery by spiking valsartan, establishing device suitability for use in nitrosamine testing.

Experimental Conditions

A previously validated method[4] based on USP <1469> Procedure 3 was used and is summarized in **Figure 1**.

Part 1. Extractables in Syringe Filters 0.1% formic acid in water was spiked with 4 isotopically labeled internal standards (IS) according to USP <1469>, vortexed, centrifuged, and approximately 1 mL supernatant filtered using 13 mm diameter syringe filters. The filtrate was analyzed for 6 nitrosamine compounds using LC-MS/MS.

Part 2. Recovery of Nitrosamines 40 mg of valsartan dura® (80 mg dose) was pulverized, diluted with 1% formic acid in water, spiked at low- (L2) concentration according to USP <1469> (**Table 2**) with reference standards, centrifuged, filtered, and analyzed as described in Part 1.

Syringe filters Tested (all 13mm) Two lots, 3 devices per lot for (1) Millex® PVDF 0.22 µm and (2) Millex® PTFE 0.2 µm. One lot, 3 devices per lot for (3) Supplier P PVDF 0.2µm (4) Supplier C H-PTFE 0.2µm (5) Supplier M PVDF 0.2µm and (6) Supplier M PTFE 0.2 µm.

Results and Discussion

Part 1. Extractables The filtrate from the syringe filtration step was analyzed for nitrosamine extractables using an external calibration curve from 1.33-90 ng/mL (NDMA, NMBA, NEIPA, NDIPA and NDBA) and 0.66-69.4 ng/mL (NDEA). The lowest concentration of the calibration curves for each analyte had S/N >10, and no nitrosamine was detected at or above this value in any of the syringe filters tested. (**Table 1**).

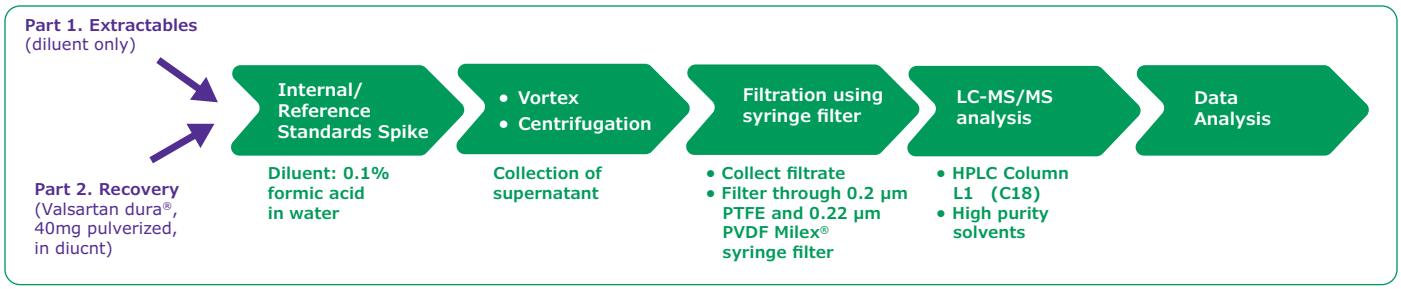

Figure 1. Schematic of the experimental procedure based on USP <1469> Procedure 3.

Table 1. Results of nitrosamine extractables analysis in various syringe filter devices using USP <1469> Procedure 3.

Compound	Lowest Concentration on Calibration Curve (ng/ml)	S/N	Syringe Filters with PVDE Membrane			Syringe Filters with PTFE Membrane		
			Millex®	Vendor P	Vendor M	Millex®	Vendor C	Vendor M
NDMA	1.33	>10	<1.33	<1.33	<1.33	<1.33	<1.33	<1.33
NMBA	1.33	>10	<1.33	<1.33	<1.33	<1.33	<1.33	<1.33
NDEA	0.66	>10	<0.66	<0.66	<0.66	<0.66	<0.66	<0.66
NEIPA	1.33	>10	<1.33	<1.33	<1.33	<1.33	<1.33	<1.33
NDIPA	1.33	>10	<1.33	<1.33	<1.33	<1.33	<1.33	<1.33
NDBA	1.33	>10	<1.33	<1.33	<1.33	<1.33	<1.33	<1.33

ABBREVIATIONS: PVDF = hydrophilic poly(vinylidene) fluoride; PTFE = hydrophilic polytetrafluorethylene

Table 2: Average percent recovery of six nitrosamine compounds. Values represent the mean ± standard deviation of n=3 devices tested per lot.

Compound	Spike Concentration (ng/mL)	Syringe Filters with PVDF Membrane			Syringe Filters with PTFE Membrane		
		Millex®	Vendor P	Vendor M	Millex®	Vendor C	Vendor M
NDMA	2.0 (low)	95.6±2.5	95.6±4.0	91.2±1.2	92.7±0.3	94.6±1.0	94.2±1.2
NMBA	2.0 (low)	101.1±1.5	99.9±1.6	97.8±0.2	98.6±2.0	97.5±2.3	101.1±0.2
NDEA	0.88 (low)	101.4+2.6	101.9±1.2	99.0±3.4	102.4±1.9	100.2+1.9	98.6+1.6
NEIPA	2.0 (low)	95.6±1.1	94.6±3.7	92.1±1.1	99.5±3.1	94.6±2.7	93.1±1.3
NDIPA	2.0 (low)	92.4±3.8	94.8±3.7	91.6±2.5	99.5±0.6	98.1±0.2	99.2±1.5
NDBA	2.0 (low)	83.6±1.8	85.4 5.9	83.6±3.9	82.1±0.9	85.5±1.5	84.213.2

Part 2. Recovery Study The average recoveries of nitrosamine compounds were within the acceptable range[4] of 70-130% as defined by USP <1469> for all syringe filter devices tested, with some minor differences evident across compounds and filter material (**Table 2**). For example, NDBA demonstrated comparatively low recoveries (still within acceptable QC range), potentially due to its hydrophobic interactions with the filter media or ingredients in the drug product. Also, both Millex® hydrophilic PVDF and PTFE showed consistent lot-to-lot recoveries.

Overall, slightly different recoveries were observed for hydrophilic PTFE and hydrophilic PVDF, all within acceptable QC range, indicating that nitrosamine compounds may interact differently with PTFE versus PVDF. Other polymers with polar functional groups and higher non-specific binding tendencies, such as nylon, could demonstrate higher losses of analytes.

Conclusion

All syringe filters tested demonstrated levels of nitrosamine extractables below the limit of quantitation according to USP <1469> Procedure 3. Further, both hydrophilic PVDF and PTFE syringe filters gave acceptable recoveries of spiked nitrosamine analytes, showing only minor differences for different compounds. This demonstrates the suitability of these filter media for use in sample preparation for nitrosamine analytical methods.

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Featured Products

Description	Cat. No.
Millex® Syringe Filter, PVDF 0.22 µm, 13 mm	**SLGVX13**
Millex® Syringe Filter, PTFE 0.2 µm, 13 mm	**SLLGX13**

Related Products

Description	Cat. No.
Ascentis® Express C18, 2.7 µm HPLC column 150 mm x 3.0 mm I.D.	**53816-U**
Methanol hypergrade for LC-MS LiChrosolv®	**1.06035**
Water for chromatography (LC-MS Grade) LiChrosolv®	**1.15333**
Formic acid for LC-MS LiChropur™, 97.5-98.5% (T)	**5.33002**
N-Nitrosodibutylamine (NDBA) United States Pharmacopeia (USP) Reference Standard	**1466641**
N-Nitrosodiethylamine (NDEA) United States Pharmacopeia (USP) Reference Standard	**1466652**
N-Nitrosodiisopropylamine (NDIPA) United States Pharmacopeia (USP) Reference Standard	**1466663**
N-Nitrosodimethylamine (NDMA) United States Pharmacopeia (USP) Reference Standard	**1466674**
N-Nitrosodimethylamine-d6 98 atom % D	**591068**
N-Nitrosoethylisopropylamine (NEIPA) United States Pharmacopeia (USP) Reference Standard	**1466685**
N-Nitrosomethylaminobutyric Acid (NMBA) United States Pharmacopeia (USP) Reference Standard	**1466696**

HPLC Separation of Nitrosamines with Supel™ Carbon LC

HPLC separation of six nitrosamines with porous graphitic carbon particulate column

Eddy Tan, Associate Senior Scientist

Abstract

The Supel™ Carbon LC column is used for the separation of six nitrosamine compounds. This method is a reversed phase gradient separation of the nitrosamines for identification and quantification.

Introduction

Nitrosamines have been known to exist in the environment and within food sources[1,2] with awareness further raised in 2018 when many regulatory authorities for medicines became aware of the dangers of nitrosamines as drug contaminants.[3] This class of compounds is known to be potent carcinogens[4] and can be introduced through multiple introduction pathways.[5] In this application, the 2.7 µm porous graphitic carbon Supel™ Carbon LC column is used to separate six different nitrosamines, followed by UV detection.

Table 1. HPLC Conditions used for nitrosamine determination HPLC Parameters

Experimental	
Column:	Supel™ Carbon, 2.7 µm HPLC column 100 mm x 3.0 mm I.D. (**59993-U**)
Mobile phase:	[A] Water + 0.1% trifluoroacetic acid (TFA): Add 100 µL of TFA to 100 mL of water, [B] Acetonitrile + 0.1% TFA: Add 100 µL of TFA to 100 mL of acetonitrile, add trifluoroacetic acid
Gradient:	**Time (min) / % B** 0.00 / 2.5 0.50 / 2.5 5.00 / 95.0 7.00 / 95.0 7.01 / 2.5 10.00 / 2.5
Flow Rate:	0.5 mL/min
Pressure:	80 – 130 bar
Column Temp:	90 ºC
Detector:	UV, 230 nm
Injection:	5 µL
Samples:	Standard solution (see below)

Nitrosamine Standard Solutions for Linearity, LOD and LOQ Determination

NMBA, NEIPA, NDIPA, NDBA and NMPA

- For NMBA, NEIPA, NDIPA, NDBA and NMPA (standard concentration ~1000 mg/L), transfer 400 µL into a 5 mL volumetric flask.
- Top-up to mark with mobile phase A (water + 0.1% TFA) and mix well. Sonicate for 10 min. This solution is the main working standard (~80 mg/L).
- Dilute to approximately 5, 10, 20, 40, 60 mg/L with mobile phase A in 5 mL volumetric flasks based on serial dilution. (True concentrations for linearity are based on batch CoA of reference materials used.)

NDEA

- For NDEA (Neat liquid ~99.8 % purity), weigh ~50 mg of NDEA CRM into a 5 mL volumetric flask.
- Top-up to mark with acetonitrile and mix well. Sonicate for 10 min. This solution is the stock solution (~10,000 mg/L).
- Dilute to approximately 5, 10, 20, 40, 60, 80 mg/L with mobile phase A in 5 mL volumetric flasks based on serial dilution. (True concentrations for linearity is based on batch CoA of reference material used.)

Linearity data is obtained from linear regression. LOD and LOQ values are calculated based on USP.

Results & Discussion

The analysis of the standard mix containing six nitrosamines (NDEA, NMBA, NEIPA, NDIPA, NDBA, NMPA) at 20 mg/mL each is shown in **Figure 1**. The chromatographic data for the analysis of the standard mix is listed in **Table 2**. All peaks were base line separated.

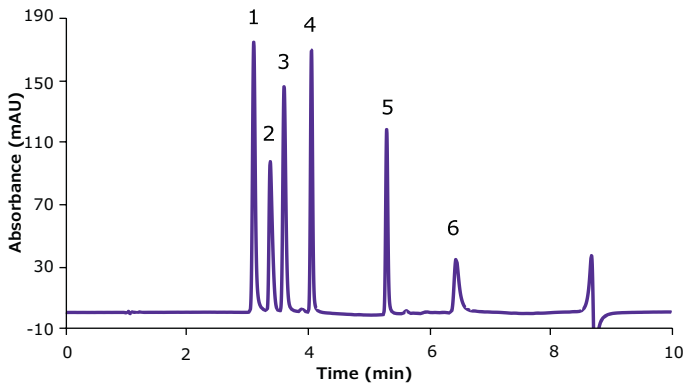

Figure 1. Separation of six nitrosamines standard mix (~20 mg/L each); blank subtracted chromatogram. 1: NDEA, 2: NMBA, 3: NEIPA, 4: NDIPA, 5: NDBA, 6: NMPA.

Table 2. Nitrosamines retention time, resolution and asymmetry for a standard with ~20 mg/L each compound

Peak no.	Compound	Retention Time (min)	*Asymmetry (USP)	*Resolution (USP)
1	N-Nitrosodiethylamine (NDEA)	3.1	1.34	2.92
2	N-Nitroso-N-methyl-4-aminobutyric acid (NMBA)	3.4	1.59	2.36
3	N-Nitrosoethylisopropylamine (NEIPA)	3.6	1.26	5.8
4	N-Nitrosodiisopropylamine (NDIPA)	4.1	1.16	18.95
5	N-Nitrosodibutylamine (NDBA)	5.3	1.19	10.69
6	N-Nitrosomethylphenylamine (NMPA)	6.5	1.54	-

*Asymmetry and resolution values were generated from Chromeleon® 7 CDS software

Table 3. Calibration data summary, limits of detection (LOD), and limits of quanitification (LOQ) for six nitrosamine compounds.

Peak	Compound	Calibration Range (mg/L)	# of Calibrators	R2	LOD (mg/L)	LOQ (mg/L)
1	N-Nitrosodiethylamine (NDEA)	11.03 - 88.24	5	0.9998	1.58	4.79
2	N-Nitroso-N-methyl-4-aminobutyric acid (NMBA)	4.76 - 57.16	5	0.9999	0.73	2.21
3	N-Nitrosoethylisopropylamine (NEIPA)	10.51 - 84.02	5	0.9961	7.18	21.75
4	N-Nitrosodiisopropylamine (NDIPA)	5.00 - 60.00	5	0.9998	1.18	3.56
5	N-Nitrosodibutylamine (NDBA)	4.96 – 59.46	5	1	0.41	1.25
6	N-Nitrosomethylphenylamine (NMPA)	9.48 - 75.79	5	0.9996	2.13	6.46

The calibration curves for all six nitrosamines exhibited excellent linearity. In **Table 3**, the calibration data summary and combined limits of detection (LOD) and limits of quantification (LOQ) of the applied method for the six nitrosamines are listed. LOD and LOQ of these nitrosamines were found to be in the low mg/L range when using UV detection.

Conclusions

The Supel™ Carbon LC column was able to separate six nitrosamines using reversed phase conditions with good reproducibility for repeated analyses (data not shown here). Resolution and peak asymmetry for the six different nitrosamines was greater than 1.5 and less than 2.0 respectively. LODs and LOQs were in the low mg/L range, and it is expected that this could be further improved with the use of mass spectrometry instrumentation.

References

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Description	Cat. No.
Supel™ Carbon, 2.7 µm HPLC column 100 mm x 3.0 mm I.D.	**59993-U**
Trifluoroacetic acid eluent additive for LC-MS, LiChropur™	**80457**
Acetonitrile gradient grade for liquid chromatography LiChrosolv®	**1.00030**
Vials, crimp top, convenience pack, 12 x 32 mm, large opening, pkg 100 (Amber)	**29127-U**
Certified glass inserts for 12 x 32 mm, large opening vials	**29441-U**
Ultrapure water from Milli-Q® IQ 7 series water purification system	**ZIQ7005T0C**
N-Nitrosodiethylamine (NDEA) Pharmaceutical Secondary Standard, neat, 1 g	**PHR2408**
N-Nitrosomethylaminobutyric Acid (NMBA) Pharmaceutical Secondary Standard, 1000 µg/mL in acetonitrile, 1 mL	**PHR3610**
N-Nitrosoethylisopropylamine (NEIPA) Pharmaceutical Secondary Standard, 1000 µg/mL in methanol, 1 mL	**PHR3609**
N-Nitrosodiisopropylamine (NDIPA) Pharmaceutical Secondary Standard, 1000 µg/mL solution in methanol, 1 mL	**PHR3607**
N-Nitrosodibutylamine (NDBA) Pharmaceutical Secondary Standard, 1000 µg/mL solution in methanol, 1 mL	**PHR3608**
N-Nitrosomethylphenylamine (NMPA) Pharmaceutical Secondary Standard, 1000 µg/mL solution in methanol, 1 mL	**PHR3611**

supporting products

for nitrosamine analysis

Description	Cat. No.
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N-Nitrosodimethylamine (NDMA), CRM	**PHR2407**
N-Nitrosodiethylamine (NDEA), CRM	**PHR2408**
N-Nitrosomethylphenylamine (NMPA), CRM	**PHR3611**
N-Nitrosomethylaminobutyric Acid (NMBA), CRM	**PHR3610**
N-Nitrosoethylisopropylamine (NEIPA / NIPEA), CRM	**PHR3609**
N-Nitrosodibutylamine (NDBA), CRM	**PHR3608**
N-Nitrosodiisopropylamine (NDIPA), CRM	**PHR3607**
4-Nitroso Hydrochlorothiazide, CRM	**PHR9223**
United States Pharmacopeia (USP) Reference Standards	
N-Nitrosodimethylamine, USP Reference Standard	**1466674**
N-Nitrosodiethylamine, USP Reference Standard	**1466652**
N-Nitroso-ethyl-isopropylamine, USP Reference Standard	**1466685**
N-Nitroso-diisopropylamine, USP Reference Standard	**1466663**
N-Nitrosodi-n-butylamine, USP Reference Standard	**1466641**
N-nitroso-N-methyl-4-aminobutyric acid, USP Reference Standard	**1466696**
Deutero N-Nitrosodimethylamine (NDMA-d6), USP Reference Standard	**1175800**
N-Nitrosomethylphenylamine (NMPA), USP Reference Standard	**1466607**
European Pharmacopeia (EP) Reference Standards	
N-Nitroso-dibutylamine, EP Reference Standard	**Y0002261**
N-Nitroso-dipropylamine, EP Reference Standard	**Y0002264**
N-Nitroso-diethylamine, EP Reference Standard	**Y0002258**
N-Nitroso-dimethylamine, EP Reference Standard	**Y0002259**
N-Nitrosodiisopropylamine, EP Reference Standard	**Y0002263**
N-Nitroso-N-methyl-4-aminobutyric acid, EP Reference Standard	**Y0002260**
N-Nitroso-ethyl-isopropylamine, EP Reference Standard	**Y0002262**
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SUPELCOWAX 10 Capillary GC Column, 30 m x 0.32 mm I.D., 1.0 µm df	**24211**
SPB® 624 Capillary GC Column, 30 m x 0.25 mm I.D., 1.4 µm df	**24255**
Ascentis® Express F5, 2.7 µm HPLC column 100 mm x 4.6 mm I.D.	**53590-U**
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Chromatographic Columns	
Ascentis® Express C18, 2.7 µm HPLC column 150 mm x 4.6 mm I.D.	**53829-U**
Ascentis® Express C18, 2.7 µm HPLC column 50 mm x 4.6 mm I.D.	**53826-U**
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Dimethylsulfoxide for headspace Gas Chromatography, Suprasolv®	**1.01900**
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Millex® PVDF syringe filter, 33 mm, 0.45 µm	**SLHV033NS**
Millex® hydrophilic PTFE syringe filter, 13 mm, 0.45 µm	**SLCRX13**
Millex® hydrophilic PTFE syringe filter, 13 mm, 0.20 µm	**SLLGX13**
Millex® Nylon syringe filter, 13 mm, 0.45 µm	**slhnx13**
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