BRIEFING

⟨ **1469**⟩ **Nitrosamine Impurities.** Starting in July 2018 the World Health Organization (WHO), the FDA, the European Directorate for the Quality of Medicines (EDQM), and other regulatory and global health agencies issued guidance documents and public health alerts regarding the presence of nitrosamine impurities in several drug products. To protect patients from the adverse effects of nitrosamines as impurities in drug products, USP's General Chapters–Chemical Analysis Expert Committee, Chemical Medicines Monographs 2 Expert Committee, and Chemical Medicines Monographs 3 Expert Committee are proposing this new general chapter. This chapter is aligned with current scientific and regulatory approaches developed to ensure the appropriate control of nitrosamine impurities in drug substances and drug products. The objective of this standard is to provide a science-based approach for the control of nitrosamine impurities, eliminating or reducing their presence in drug products. The approach described thereby ensures the quality of the product as it relates to safety.

1. The *1. Introduction* presents the concern of nitrosamine presence and summarizes the current industry and regulatory thinking. It is followed by a compilation of the possible sources of nitrosamines.
2. The *2. Nitrosamine Impurities* section provides a list of nitrosamines of concern in the pharmaceutical industry, compiled from the information shared by multiple global health authorities and personal experience of members of the joint subcommittee. It includes additional chemical information for each entry. It also positions nitrosamines from the ICH M7 perspective, which includes the *N*-nitroso compounds in the "Cohort of Concern".
3. The *3. Sources of Nitrosamines* section provides a general overview on how nitrosamines are formed and could end up in pharmaceutical products. It includes a comprehensive table that lists each potential source of nitrosamines and the associated observed or assessed risk for that source.
4. The *4. Nitrosamine Risk Assessments—Development of a Control Strategy* section provides recommendations on how the risk assessment for nitrosamines is performed and the development of a relevant control strategy to ensure that the nitrosamine presence is avoided or limited to levels below acceptable intake.
5. The *5. Limits of Nitrosamines* section details the approach that is used for establishing specific nitrosamines daily acceptable intake (AI) and how the concentration limits are calculated based on it and the maximum daily dose of the drug substance.
6. The *7. Test Method Performance Characteristics of Nitrosamine Methods* section provides guidance on the verification process, the procedures being implemented in the laboratory, and the validation of alternative procedures.
7. The *8. Analytical Procedures* section contains procedures that have been validated or verified in the USP laboratories.
    - *Procedure 1* is based on analyses performed with the Phenomenex Kinetex brand of 2.6-µm F5 100Å, 100 × 4.6-mm column with L43 packing. The Orbitrap Fusion Lumos Tribrid brand of mass spectrometer (ThermoFisher Scientific) was used during validation.
    - *Procedure 2* is based on analyses performed with the following fused-silica columns with phase G16: a) Supelcowax 10 (Supelco #24211) (30 m × 0.32 mm × 1.0 µm); b) SH-Stabilwax (Shimadzu) (30 m × 0.32 mm × 1.0 µm); or c) DB-Wax (Agilent #123-7032) (30 m × 0.32 mm × 0.25 µm). The MS detectors used were TSQ9000 VPI-HS 500 Triple Quadrupole GC–MS (ThermoFisher Scientific), TQ8040 NX mass spectrometer (Shimadzu), or the 7010B Triple Quadrupole GC/MS (Agilent).
    - *Procedure 3* is based on analyses performed with the Restek Raptor brand of 2.7-µm ARC-18, 150-mm × 3.0-mm column with L1 packing. The LCMS-8050 Triple Quadrupole brand of liquid chromatograph mass spectrometer (Shimadzu) was used during method validation. The Xevo TQD Triple Quadrupole brand of mass spectrometer (Waters) was used during method verification.
    - *Procedure 4* is based on analyses performed with the J&W VF-WAXms GC brand of fused-silica column (30 m × 0.25 mm × 1 µm) with phase G16. The TSQ 9000 GC-MS/MS brand of spectrometer (ThermoFisher Scientific) was used during method validation, and the 7000 Series Triple Quad GC/MS brand of spectrometer (Agilent) was used during method verification.
8. The *9. Additional Sources of Information* section presents a compilation of references to analytical procedures that are currently on the websites of regulatory agencies in the United States (FDA) and Europe (EDQM). Users can verify alternative procedures by meeting the requirements recommended in this chapter.

(GCCA: E. Biba)
Correspondence Number—C263095

Add the following:

▲⟨ 1469⟩ NITROSAMINE IMPURITIES

1. INTRODUCTION

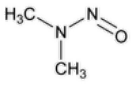
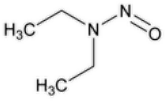
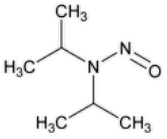
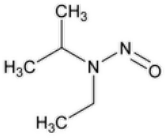
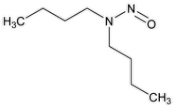
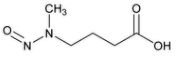
The presence of nitrosamine impurities has been detected recently in several drug substances and drug products. In 2018, *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) were detected in some valsartan drug substances and the drug products manufactured from drug substances using specific synthetic routes. This observation triggered extensive synthetic route assessments and development of analytical procedures to quantify these two nitrosamine impurities. As additional pharmaceuticals were evaluated and in some cases tested, other nitrosamines beyond NDMA and NDEA were added as impurities of concern. Given the potential broad impact of the presence of this class of carcinogenic chemicals, this chapter has been developed to provide guidance in the assessment of materials to ensure that the potential presence of nitrosamines is identified, provide recommendations regarding the establishment of controls, and to provide initial guidance on analytical procedure performance criteria for procedures used to monitor nitrosamine levels.

2. NITROSAMINE IMPURITIES

Nitrosamines addressed in this general chapter are listed in *Table 1* by their common names and chemical names. This list is a compilation of the information shared by multiple global health authorities. The potential presence of any one or more of these impurities is dependent on the reaction chemistries and processes. It is unlikely that all of the listed nitrosamines will be anticipated or observed as impurities in any single material. The list is not intended to be exhaustive but represents the most commonly expected or observed nitrosamines.

N-nitroso compounds are listed as Class 1 mutagens in ICH M7: *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* (*1*). ICH M7 also includes *N*-nitroso compounds in the cohort of concern, a designation that carries with it a recommendation to control the impurities at or below the acceptable cancer risk. In addition, some *N*-nitroso compounds are listed as Class 2 compounds. As a result of the significant potential toxicity associated with these impurities, it is recommended to take steps to control and limit their presence in pharmaceutical materials.

Table 1. Nitrosamines Found as Contaminants in Drug Substances and Drug Products

Common Name and Chemical Name	Acronym	CAS #	Structure	Chemical Formula	Molecular Weight
Nitrosodimethylamine *N*-Methyl-*N*-nitrosomethanamine	NDMA	62-75-9		$C_2H_6N_2O$	74.08
Nitrosodiethylamine *N*-Ethyl-*N*-nitrosoethanamine	NDEA	55-18-5		$C_4H_{10}N_2O$	102.14
Nitrosodiisopropylamine *N*-Isopropyl-*N*-nitrosoisopropylamine	NDIPA	601-77-4		$C_6H_{14}N_2O$	130.19
Nitrosoethylisopropylamine *N*-Ethyl-*N*-nitroso-2-propanamine	NEIPA	16339-04-1		$C_5H_{12}N_2O$	116.16
Nitrosodibutylamine *N*-Butyl-*N*-nitroso-1-butanamine	NDBA	924-16-3		$C_8H_{18}N_2O$	158.25
Nitrosomethylaminobutyric 4-[Methyl(nitroso)amino]butanoic acid	NMBA	61445-55-4		$C_5H_{10}N_2O_3$	146.15

3. SOURCES OF NITROSAMINES

In acidic conditions, secondary or tertiary amines react with nitrites to form nitrosamines. There are a number of pathways by which nitrosamines can be introduced into or generated as impurities in pharmaceutical drug products. Examples of sources/pathways identified empirically or reported in the literature (*2–3*) include (but are not limited to) the following:

- API (active pharmaceutical ingredient) processing under specific conditions and in the presence of certain reagents, solvents, raw materials, and processing aids. There is evidence that despite processing and purification steps, reactive species, whether intentionally added to or formed during the process/reaction sequence (e.g., nitrites and secondary amines in the presence of acidic conditions), can carry over to subsequent steps (see *3.1. Nitrosamine Formation Reaction*). Special attention should be given to the formation of nitrogen-containing heterocycles by employing azide followed by quenching with nitrite to remove excess azide.
- The API itself, which may degrade under some conditions resulting in the formation of nitrosamines (e.g., ranitidine).
- Degradation of solvents (e.g., dimethylformamide [DMF]) leading to the formation of dialkyl amines.
- Impurities in raw materials, solvents (including recycled solvents), reagents, or catalysts.
- Impurities in materials and intermediates, reagents, and solvents used to prepare the starting materials or intermediates.
- Impurities in water, excipients, or processing aids used in the production of the finished drug product.

- During drug product manufacture under certain reaction conditions and in the presence of requisite precursors necessary for the formation of nitrosamines.
- Impurities in the container–closure system for the finished drug product, which may include impurities capable of forming nitrosamines, especially if associated with materials containing amines and potential sources of a nitrosating agent (e.g., nitrite, nitrocellulose).

A risk assessment should be conducted to determine the materials that contribute to the potential for inclusion of nitrosamines in the drug product. All potential sources for the introduction of nitrosamines should be considered in the risk assessment including, for example, the drug substance, excipients, water, solvents, the manufacturing process, packaging components, and formation on stability. See *Figure 1* for a diagram of some potential sources to be considered.

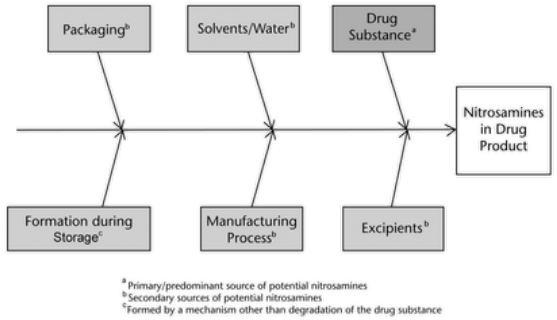


Figure 1. Potential sources of nitrosamine impurities in drug product

Ongoing assessments and evaluations have identified risks associated with several of the potential sources of nitrosamines. These are summarized in *Table 2*.

Table 2

Potential Source of Nitrosamines	Observed Risk[a]
Solvents	• Presence of residual dialkyl amines or tri-substituted amines that can degrade to form dialkyl amines (e.g., triethylamine) • Presence of nitrites or other nitrosating agents • Presence of acid • Limited controls/specification limits for recycled solvents • Poor quality water or solvents
Water	• Presence of residual dialkyl amines or impurities that can degrade to form dialkyl amines • Presence of nitrites or other nitrosating agents • Presence of acid
Excipients	• Presence of nitrites or other nitrosating agents
Drug substance	• Use of sodium azide and nitrite for azide quenching in the synthesis in acid media • Use of di- or tri-alkylamines and amides (e.g., dimethylformamide [DMF], dimethylamine [DMA], triethylamine [TEA], *N*-methylpyrrolidone [NMP]) in the presence of nitrites and acid media • Use of recycled solvents that may contain nitrosamines or their precursors • Use of sanitized water (e.g., chloramines) • Need of additional purification steps (e.g., crystallization)
Manufacturing process	• Contamination • Use of poor quality or recycled solvents that may contain nitrosamines or their precursors • Poor quality solvents • Presence of nitrous oxides in air used to dry the API or drug product
Drug product (including stability)	• Secondary or tertiary amine group in molecule • Presence of nitrate counter ions (potentially as an impurity) • Potential reactions within the formulation matrix during stability/shelf life (e.g., presence or generation of acidic conditions, moisture, and heat)
Container–Closures	• Thermal decomposition of nitrocellulose to produce nitrites followed by migration to the drug product • Biodegradation of nitrocellulose to produce nitrites followed by migration to the drug product

[a] General chemical reactions leading to formation of nitrosamines can be found in *3.1 Nitrosamine Formation Reaction*.

3.1 Nitrosamine Formation Reaction

The general reaction responsible for the formation of nitrosamines is described in *Figure 2*. Examples of representative reactions are described in the scientific literature (2–3).

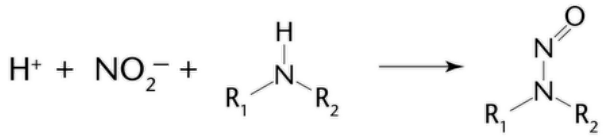


Figure 2. General example of formation of nitrosamines.

If the potential for the presence of nitrosamines is identified, an appropriate control strategy should be developed. If nitrosamines are identified as impurities in ingredients, they may be controlled as appropriate in the ingredients (e.g., manufacture of the drug substance or controls placed on the drug substance). If nitrosamines are identified as degradation products (i.e., being formed during manufacturing of the drug product or formed during product storage), they should be controlled as appropriate in the drug product. In some cases, changes to the manufacturing process(es) or ingredients may be required to achieve acceptable levels or the elimination of nitrosamine impurities in the drug product.

4. NITROSAMINE RISK ASSESSMENTS—DEVELOPMENT OF A CONTROL STRATEGY

In order to determine the level of control, if any, which requires ensuring that levels of nitrosamines are at or below the provisional acceptable intake (AI) if their presence could not be avoided, the components of drug products should be assessed for the potential to form nitrosamines or to be contaminated with nitrosamines. Although one of the sources with the highest potential for nitrosamines is the API synthetic route, the API manufacturing process, drug product manufacturing process, and excipients and raw materials should also be subjected to a risk assessment to establish the level of control needed. The high level process flow for evaluating materials is shown in *Figure 3*.

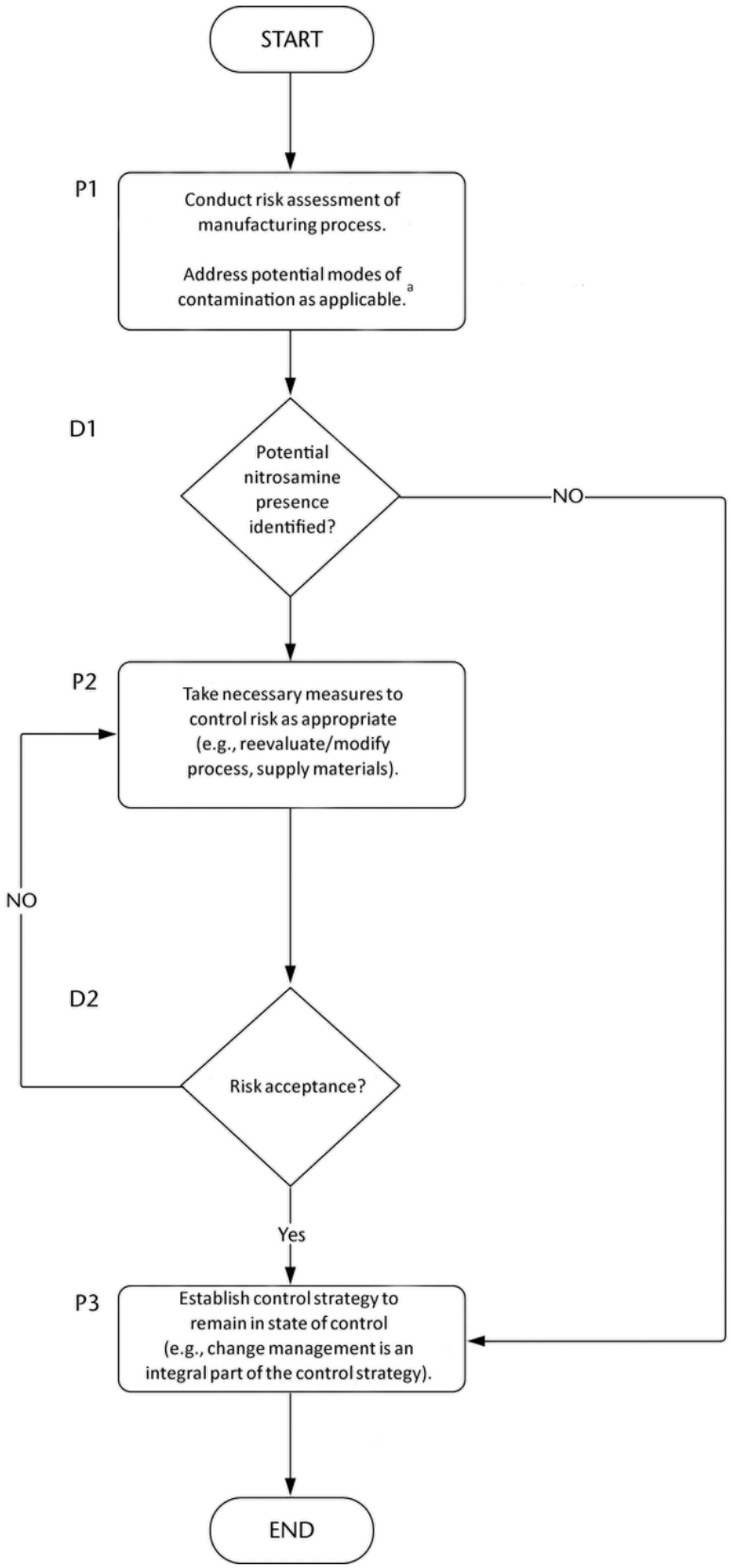


Figure 3. High level process for development of a nitrosamine impurity control strategy. ([a]Refer to *Table 2*; P1, P2, P3 = Process 1, 2, 3; D1, D2 = Decision 1, 2)

In all cases, if nitrosamines are predicted in the risk assessment or confirmed to be present through testing in the drug product, the control strategy should define the approach to ensure that the nitrosamine levels comply with the established interim AIs.

5. LIMITS OF NITROSAMINES

Nitrosamine impurities identified in this chapter have significant toxicity with no therapeutic value. Because nitrosamines are classified as Class 1 mutagenic impurities, rather than applying a Threshold of Toxicological Concern (TTC), the available safety data should be used to establish a material-specific AI. The AI is defined as an intake level that poses a negligible health risk.

5.1 Derivation of the Interim Limits for AI

There are a number of methodologies that toxicologists have applied in establishing AIs. Considering the toxicity of NDMA and NDEA as representative nitrosamines and the available non-clinical (animal model) data that are available, the interim limits published by FDA (at the time this document was submitted for publication) were based on the dose giving a 50% tumor incidence (TD50) with a 1:100,000 safety factor applied (decreasing the potential cancer risk to 1 in 100,000).

For the official AI values, refer to FDA Updates and Press Announcements on Angiotensin II Receptor Blocker (ARB) Recalls (Valsartan, Losartan, and Irbesartan).

The AIs in nanograms per day and the maximum daily dose (MDD) of the drug substance (DS) from the drug label in milligrams per day can then be used to calculate the maximum nitrosamine concentration limits, in ppm, for individual drug products using the equation below.

$$\text{Concentration} = \text{AI}/\text{DS}_{\text{dose}}$$

Since the exposure to nitrosamines is related to the MDD of the drug, different concentrations of nitrosamines (ng/g) may be acceptable for each material evaluated. The acceptable concentration in the material can be calculated using the equation below.

$$\text{Acceptable nitrosamine content} = \text{AI}/\text{MDD}$$

AI = acceptable intake of the nitrosamine (ng/day)

MDD = maximum daily dose of the API (g/day)

Table 3. Example Using an AI of 96 ng/day for the Target Nitrosamine

Name	Acceptable Concentration (ng/g)			
Nitrosamine 1	**0.050 (50 mg dose)**	**0.100 (100 mg dose)**	**0.250 (250 mg dose)**	**1.00 (1000 mg dose)**
	1920	960	384	96

6. TESTING FOR THE PRESENCE OF NITROSAMINES

Upon completion of the risk assessment, exploratory testing may need to be performed to confirm the conclusions of the risk assessment and proposed control strategy. On the basis of the controls identified (e.g., incoming material testing or specification limit; API or drug product specification limit), it may be necessary to implement routine testing for nitrosamines. If testing is applied to ensure that the nitrosamine(s) concentration(s) do not exceed the AI, methods should be established following the recommendations detailed in this section. Example analytical procedures can be found in *7. Test Method Performance Characteristics of Nitrosamine Methods*.

6.1 Presence of Two or More Nitrosamines

The current published recommended AIs reflect limits for the presence of a single nitrosamine. If multiple nitrosamines are possible and are determined analytically to be present at levels above the limit of quantitation (LOQ) and below the AI, the relevant health authority should be consulted to determine a specific path forward.

7. TEST METHOD PERFORMANCE CHARACTERISTICS OF NITROSAMINE METHODS

The AIs associated with nitrosamines require the application of sensitive analytical procedures. In many cases, the most reliable procedures take advantage of the sensitivity and selectivity of chromatographic separation techniques coupled with quantitation by mass spectrometry (e.g., HPLC–MS/MS, GC–MS/MS). For additional guidance on validation of alternative methods for nitrosamines, see *Validation of Compendial Procedures* ⟨1225⟩.

7.1 Considerations for Sample Preparation

Appropriate sample preparation is a critical step in trace impurity analyses such as those required to evaluate the levels of nitrosamines in drug substances and drug products. This is particularly critical to prevent the loss or generation of nitrosamines as artifacts of the analytical procedure itself, as in the following circumstances.

- The presence of dialkyl amine (dimethylamine) as a process impurity or counter ion of the salt form of the active ingredient in the presence of nitrite and acid can lead to in situ formation of nitrosamines as an artifact, especially in GC analyses.
- Total solubilization versus selective extraction: If the active ingredient contains a dimethylamino group, total dissolution of the drug substance should be avoided when applying GC techniques. High concentration of the active ingredient, when injected in the GC instrument can generate nitrosamines in the injection port if a nitrosating agent is present. In these situations, sample extractions should be modified to prevent the solubilization of the active ingredient while maintaining the extraction efficiency for nitrosamines present in the material.

In selecting the appropriate analytical procedure, the performance characteristics for a quantitative analytical procedure shown in *Table 4* are recommended to ensure that the method is suitable for its intended purpose. Examples of quantitative analytical procedures are included in *8. Analytical Procedures*.

Table 4. Recommended Quantitative Analytical Procedure Performance Criteria

Parameter	Recommended Range
Range	50%–150% of the limit corresponding to AI
Accuracy	Recovery 70%–130%
Repeatability	Relative standard deviation (%RSD) $\leq$ 25% (n = 6)
Intermediate precision	%RSD $\leq$ 30% (n = 12)
Limit of quantitation[a] (See ⟨1225⟩.)	Dependent on material MDD and AI

[a] If LOQ is the concentration limit corresponding to AI, then a system suitability test should be added to the procedure for sensitivity verification.

If a limit test is selected for the analytical procedure, the performance criteria shown in *Table 5* are recommended.

Table 5. Recommended Limit Test Analytical Procedures Performance Acceptance

Parameter	Acceptance Criteria
Results	$R_{U(i)}/R_{ST(i)}$ = NMT 0.5[a]

Parameter	Acceptance Criteria
Specificity	The procedure must be able to unequivocally assess (see ⟨1225⟩ each target compound in the presence of components that may be expected to be present, including other target compounds and matrix components.
Recovery	70%–130%
Detectability	The minimum concentration at which the analyte can reliably be detected is established (signal-to-noise ratio 10:1).
Solution stability	The detectability should meet the requirements throughout the testing period.

[a] $R_{U(i)}$ = Peak response ratio of the respective target *N*-nitrosamine to the internal standard from the *Sample solution*; $R_{ST(i)}$ = Peak response ratio of the respective target *N*-nitrosamine to the internal standard from the *Spiked sample solution*

8. ANALYTICAL PROCEDURES

The following procedures have been established as suitable for their intended (specified) purpose. Use of these methods should be verified by the user for the specific materials for which they are intended to be applied. The objective of verification is to demonstrate the suitability of a test procedure under actual conditions of use (see *Verification of Compendial Procedures* ⟨1226⟩).

8.1 Quantitative Procedures

Procedure 1: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA in selected sartans by HPLC–HRMS

Diluent: Methanol

Solution A: 0.1% formic acid in water

Solution B: 0.1% formic acid in methanol

Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	90	10
1.5	90	10
7.0	45	55
17.0	45	55
17.1	10	90
21.0	10	90
21.1	90	10
25.0	90	10

Sensitivity solution: 1.0 ng/mL each of USP *N*-Nitrosodimethylamine RS, USP *N*-Nitrosodiethylamine RS, USP *N*-Nitrosoethylisopropylamine RS, USP *N*-Nitrosodiisopropylamine RS, USP *N*-Nitrosodibutylamine RS, and USP *N*-Nitrosomethylaminobutyric Acid RS in *Diluent*

Standard solution: 6.0 ng/mL each of USP *N*-Nitrosodimethylamine RS, USP *N*-Nitrosodiethylamine RS, USP *N*-Nitrosoethylisopropylamine RS, USP *N*-Nitrosodiisopropylamine RS, USP *N*-Nitrosodibutylamine RS, and USP *N*-Nitrosomethylaminobutyric Acid RS in *Diluent*

Sample solution: 20 mg/mL of drug substance prepared as follows. Transfer 100 mg of drug substance into a suitable container. Add 5.0 mL of *Diluent* and vortex until fully dispersed or dissolved. Pass the solution through a suitable filter of 0.2-µm pore size. Use the filtrate for analysis.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: High resolution mass spectrometer

MS conditions

Ionization: Electrospray ionization (ESI)

Scan settings: See *Table 7*.

Table 7

Nitrosamine Impurity	NDMA	NMBA	NDEA	NEIPA	NDIPA	NDBA
Scan type	SIM[a]	SIM	PRM[b]	SIM	SIM	PRM
Polarity	positive	negative	positive	positive	positive	positive

Scan start–end (min)	1.0–3.5	3.5–5.5	5.5–7.0	7.0–8.5	8.5–10.0	13.0–15.5
m/z[c] isolated for PRM	N/A	N/A	103.0866	N/A	N/A	159.1492
NCE[d]	N/A	N/A	25	N/A	N/A	20
Scan range (m/z)	74.3–75.8	144.3–145.8	50.0–114.0	116.4–117.9	130.4–131.9	50.0–170.0
Microscans	3	3	3	3	3	3
Resolution	30,000	60,000	30,000	60,000	60,000	30,000
AGC target value (%)[e]	250	250	250	250	250	250

[a] SIM = selected ion monitoring.
[b] PRM = parallel reaction monitoring.
[c] m/z = mass to charge ratio.
[d] NCE = normalized collision energy.
[e] AGC = automatic gain control.

[NOTE—Divert the API from the MS source during the elution.]

Data processing: Peak areas in the extracted ion chromatograms (EIC) with an m/z tolerance of 15 ppm are used for quantitation. The m/z values extracted are listed in *Table 8*.

Table 8

Nitrosamine Impurity	NDMA	NMBA	NDEA	NEIPA	NDIPA	NDBA
m/z to be extracted	75.0553	145.0619	75.0553, 103.0866	117.1022	131.1179	57.0704, 103.0872, 159.1492

Column: 4.6-mm × 10-cm; 2.6-µm packing L43
Temperatures
Autosampler: 4°
Column: 40°
Flow rate: 0.6 mL/min
Flow rate to ion source: 0.6 mL/min
Injection volume: 3 µL

System suitability
Samples: *Sensitivity solution* and *Standard solution*
[NOTE—The relative retention times for NDMA, NMBA, NDEA, NEIPA, NDIPA, and NDBA are 0.20, 0.31, 0.46, 0.57, 0.66, and 1.00, respectively.]

[NOTE—NMBA and NEIPA exist as syn- and anti-conformers due to the restricted rotation of the N–N bond. These conformers are partially separated by the method's chromatographic conditions. The NMBA peak is observed as a doublet. Integrate both of the NMBA peaks and use the combined peak areas for calculation of the NMBA concentration. The NEIPA peak may appear as a doublet or a single peak with a tailing shoulder. For NEIPA, if the conformers are resolved, integrate both peaks and combine the peak areas for the calculation of the NEIPA concentration. If the NEIPA conformers are not fully resolved (e.g., evidence of a shoulder is present), integrate the main peak and shoulder as a single peak and use the combined peak area to calculate the NEIPA concentration.]

Suitability requirements
Relative standard deviation: NMT 20.0% from 6 replicate injections, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, in ppm, of each specified nitrosamine impurity in the portion of drug substance taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 10^6$$

r_U = peak response of the individual specified nitrosamine impurity from the *Sample solution*

r_S = peak response of the corresponding nitrosamine impurity from the *Standard solution*

C_S = concentration of USP *N*-Nitrosodimethylamine RS, USP *N*-Nitrosodiethylamine RS, USP *N*-Nitrosoethylisopropylamine RS, USP *N*-Nitrosodiisopropylamine RS, USP *N*-Nitrosodibutylamine RS, or USP *N*-Nitrosomethylaminobutyric Acid RS in the *Standard solution* (µg/mL)

C_U = concentration of the drug substance in the *Sample solution* (µg/mL)

Report the nitrosamine impurity concentration in the drug substance in ppm (µg/g).

Procedure 2: Quantitation of NDMA, NDEA, NDIPA, and NEIPA in selected sartans by GC–MS
Diluent: Methanol

Internal standard stock solution: 0.4 µg/mL of NDMA-d6 in *Diluent*

Internal standard solution: 0.016 µg/mL of NDMA-d6 prepared as follows. Transfer 2.0 mL of *Internal standard stock solution* into a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Nitrosamine RS stock solution: 0.4 µg/mL each of USP *N*-Nitrosodimethylamine RS, USP *N*-Nitrosodiethylamine RS, USP *N*-Nitrosoethylisopropylamine RS, and USP *N*-Nitrosodiisopropylamine RS prepared as follows. Transfer an appropriate amount of USP *N*-Nitrosodimethylamine RS, USP *N*-Nitrosodiethylamine RS, USP *N*-Nitrosoethylisopropylamine RS, and USP *N*-Nitrosodiisopropylamine RS into a suitable volumetric flask, and dilute with *Diluent* to the volume.

Standard stock solution: 0.016 µg/mL each of USP *N*-Nitrosodimethylamine RS, USP *N*-Nitrosodiethylamine RS, USP *N*-Nitrosoethylisopropylamine RS, and USP *N*-Nitrosodiisopropylamine RS prepared as follows. Transfer 2.0 mL of *Nitrosamine RS stock solution* and 2.0 mL of *Internal standard stock solution* into a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Standard solution: Transfer 1 mL of *Standard stock solution* to an appropriate headspace vial containing about 100 mg of imidazole and 1.0 mL of acetonitrile. Apply the stopper, cap, and crimp tightly.

Sensitivity stock solution: 0.004 µg/mL each of USP *N*-Nitrosodimethylamine RS, USP *N*-Nitrosodiethylamine RS, USP *N*-Nitrosoethylisopropylamine RS, and USP *N*-Nitrosodiisopropylamine RS in *Diluent* prepared as follows. Transfer 0.5 mL of the *Nitrosamine RS stock solution* and 2.0 mL of *Internal standard stock solution* into a 50-mL volumetric flask and dilute with *Diluent* to volume. Transfer 1 mL of this solution to an appropriate headspace vial containing about 100 mg of imidazole. Apply the stopper, cap, and crimp tightly.

Sensitivity solution: Transfer 1 mL of *Sensitivity stock solution* to an appropriate headspace vial containing about 100 mg of imidazole and 1.0 mL of acetonitrile. Apply the stopper, cap, and crimp tightly.

Sample solution: Transfer 200 ± 10 mg of drug substance and about 100 mg of imidazole into a headspace vial, and then add 1.0 mL of *Internal standard solution* and 1.0 mL of acetonitrile. Apply the stopper, cap, and crimp tightly.

Blank: Transfer about 100 mg of imidazole into a headspace vial, and then add 1.0 mL of *Internal standard solution* and 1.0 mL of acetonitrile. Apply the stopper, cap, and crimp tightly.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Injector: Headspace (see *Table 9* for parameters)

Table 9

Equilibration temperature	95°–110°
Loop temperature	150°
Rate 1	10°/min
Transfer line temperature	160°
Pressurizing gas pressure	20.00 psi
Equilibration time	10.00 min
Pressurizing time	2.00 min
Load time	2.00 min
Injection time	1.00 min
Vial size	20 mL

Injection type: (Split, Split ratio 1:1 or 1:3)

[Note—Split ratio can be modified to optimize sensitivity.]

Detector: Mass spectrometer

Column: 0.32-mm × 30-m fused-silica, coated with a 1.0-µm layer of phase G16

Column temperature: See *Table 10*.

Table 10

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
45	0	45	3
45	10	130	3
130	130	15	—
190	40	240	10

Carrier gas: Helium

Flow rates

Gas: Constant flow at 1.8 mL/min (adjustment and verification are necessary for other carrier gases)

Purge: 3.0 mL/min or default value

MS conditions: See *Table 11*.

Table 11

Ionization mode	Electron impact (EI)
Polarity	positive
Event 1	
Name	NDMA
Start time	10.0 min
End time	12.0 min
Acquisition mode	multiple reaction mode (MRM)
Ch 1 m/z	74.00 > 44.00
CH 1 collision energy	4.00 V
Ch 2 m/z	74.00 > 42.00
CH 2 collision energy	15.00 V
Event 2	
Name	NDMA-d6
Start time	10.0 min
End time	12.0 min
Acquisition mode	MRM
Ch 1 m/z	80.00 > 50.00
CH 1 collision energy	5.00 V
Event 3	
Name	NDEA
Start time	12.00 min
End time	12.75 min
Acquisition mode	MRM
Ch 1 m/z	102.00 > 85.1
CH 1 collision energy	6.00 V
Ch 2 m/z	102.00 > 56.1
CH 2 collision energy	15.00 V
Event 4	
Name	NEIPA
Start time	12.75 min
End time	13.35 min
Acquisition mode	MRM
Ch 1 m/z	116.00 > 99.10
CH 1 collision energy	6.00 V
Ch 2 m/z	99.00 > 44.10
CH 2 collision energy	9.00 V

Event 5	
Name	NDIPA
Start time	13.35 min
End time	14.00 min
Acquisition mode	MRM
Ch 1 m/z	130.00 > 42.00
CH 1 collision energy	10.00 V
Ch 1 m/z	130.00 > 43.10
CH 2 collision energy	18.00 V

System suitability

Samples: *Standard solution*, *Sensitivity solution*, and *Blank*

[NOTE—The relative retention times for NDMA, NDMA-d6, NDEA, NEIPA, and NDIPA are 0.80, 0.80, 0.90, 0.96, and 1.00, respectively.]

Suitability requirements

Relative standard deviation: NMT 20.0% for the ratio of the impurity standard peak response to the internal standard peak response from 6 replicate injections, *Standard solution*

Signal-to-noise ratio: NLT 10 for the impurity peak, *Sensitivity solution*

Interference: There should be no interfering peak in the *Blank*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, in ppm, of each specified nitrosamine impurity in the portion of drug substance taken:

$$\text{Result} = 1/W \times (R_U/R_S) \times C_S$$

W = weight of the drug substance in the *Sample solution* (g)

R_U = peak response ratio of the specified nitrosamine impurity to that of the internal standard from the *Sample solution*

R_S = peak response ratio of the specified nitrosamine impurity to that of the internal standard from the *Standard solution*

C_S = concentration of USP *N*-Nitrosodimethylamine RS, USP *N*-Nitrosodiethylamine RS, USP *N*-Nitrosoethylisopropylamine RS, and USP *N*-Nitrosodiisopropylamine RS in the *Standard solution* (µg/mL)

Report the nitrosamine impurity concentration in the drug substance in ppm (µg/g).

Procedure 3: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA in selected sartans by HPLC–MS/MS

Diluent: 1% formic acid in water

Solution A: 0.1% formic acid in water

Solution B: 0.1% formic acid in methanol

Mobile phase: See *Table 12*.

Table 12

Time (min)	Solution A (%)	Solution B (%)
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

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, *N*-nitrosoethylisopropylamine, *N*-nitrosodiisopropylamine, *N*-nitrosodibutylamine, and *N*-nitrosomethylaminobutyric acid by mixing appropriate volumes of the respective USP Reference Standards and dilute with water.

[CAUTION—Prepare *Nitrosamine standard stock solution* in amber vials and store at −18° to −20°).]

NDEA standard stock solution:

Prepare a solution of 132 ng/mL of *N*-nitrosodiethylamine 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 from *Table 13* from the *Nitrosamine standards stock solution mixture* and *NDEA standard stock solution* by mixing specified volumes of each solution as indicated. [NOTE—*Table 13* represents an example for preparing solutions for constructing the calibration curve. Other dilution schemes may be used for preparing the set of 5 linearity solutions covering the range of interest. L1 is used only for NDEA when applicable. For others, linearity starts with L2.]

Table 13

Linearity Solution #	Concentration Level	Concentration of NDMA, NMBA, NDBA, NEIPA, NDIPA (ng/mL)/NDEA (ng/mL)	Content of NDMA, NMBA, NDBA, NEIPA, NDIPA (ppb)/NDEA (ppb)	Volume of Nitrosamine Standard Stock Solution Mixture (µL)	Volume of NDEA Standard Stock Solution (µL)	Volume of Water (µL)	Volume of 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

Sample solution: Transfer about 80 mg of the drug substance into a 2-mL lidded centrifuge tube. Add 1188 µL of *Diluent* and 12 µl of the *Internal standard solution*. Vortex at 2500 rpm for 20 min, except for losartan (vortex losartan NMT 5 min). Centrifuge at about 10,000 rpm for 10 min, and filter into a vial using a hydrophilic polytetrafluoroethylene (PTFE) filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: MS/MS (triple quadrupole mass spectrometer)

MS conditions

Ionization: Atmospheric pressure chemical ionization (APCI)

Scan settings: See *Table 14*.

Table 14

Nitrosamine Impurity	Acquisition Mode	Polarity	Transitions	
			MRM-1	MRM-2
NDMA	MRM	Positive	+75.0 amu[a] → +43.0 amu	+75.0 amu → +44.1 amu
NDMA-d6	MRM	Positive	+81.2 amu → +46.0 amu	+81.2 amu → +64.1 amu
NDEA	MRM	Positive	+103.1 amu → +75.1 amu	+103.1 amu → +47.1 amu
NDEA-d10	MRM	Positive	+113.2 amu → +34.2 amu	+113.2 amu → +49.1 amu
NMBA	MRM	Positive	+147.1 amu → +44.1 amu	+147.1 amu → +117.1 amu
NMBA-d3	MRM	Positive	+150.1 amu → +47.1 amu	+150.1 amu → +120.2 amu
NDBA	MRM	Positive	+159.2 amu → +41.1 amu	+159.2 amu → +29.1 amu

Nitrosamine Impurity	Acquisition Mode	Polarity	Transitions	
			MRM-1	MRM-2
NDBA-d18	MRM	Positive	+177.3 amu → +66.2 amu	+177.3 amu → +46.2 amu
NEIPA[b]	MRM	Positive	+117.1 amu → +75.1 amu	+117.1 amu → +47.2 amu
NDIPA[b]	MRM	Positive	+131.2 amu → +89.1 amu	+131.1 amu → +47.1 amu

[a] The abbreviation amu is atomic mass unit.
[b] NDEA-d10 is used as internal standard for NDIPA and NEIPA.

Column: 3.0-mm × 15-cm; 2.7-µm packing L1
Temperatures
Autosampler: 18°
Column: 60°
Flow rate: 0.5 mL/min
Flow rate to ion source: 0.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solutions*
Generate the peak response ratio of the specified impurity to that of the internal standard versus concentration standard curve for each nitrosamine impurity under test using the corresponding selected *Standard solutions* and perform the linear regression analysis.
[NOTE—The relative retention times for NDMA, NMBA, NDEA, NEIPA, NDIPA, and NDBA are 0.20, 0.31, 0.46, 0.57, 0.66, and 1.00, respectively.]
Suitability requirements
Correlation coefficient: NLT 0.99
***y*-Intercept:** NMT 25% of the response of the medium concentration solution used in standard curve generation
Analysis
Samples: *Standard solutions* and *Sample solution*
Calculate the concentration, in ppm, of each specified nitrosamine impurity in the *Sample solution* using the corresponding calibration curve:

$$\text{Result} = [(R_U - y_{int})/a] \times (1/C_U) \times 10^3$$

R_U = peak response ratio of the specified nitrosamine impurity to that of the internal standard from the *Sample solution*

y_{int} = *y*-intercept of the calibration curve for the specified nitrosamine impurity from the *Standard solutions*

a = slope of the calibration curve for the specified nitrosamine impurity from the *Standard solutions*

C_U = concentration of the drug substance in the *Sample solution* (mg/mL)

Report the nitrosamine impurity concentration in the drug substance in ppm (µg/g).

Procedure 4: Quantitation of NDMA, NDEA, NDIPA, NEIPA, and NDBA in selected sartans by GC–MS/MS (triple-quad)

Internal standard solution: 50 ng/mL of NDMA:C13-d6 in methylene chloride

Standard solution: Prepare a mixture of 0.1 µg/mL each of *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosoethylisopropylamine, *N*-nitrosodiisopropylamine, and *N*-nitrosodibutylamine by mixing appropriate volumes of respective USP Reference Standards and diluting with *Internal standard solution*.

Calibration solutions: Depending on the targeted nitrosamine concentration in the sample, prepare a set of five consecutive solutions from *Table 15* that are used for generating the calibration curve by following the preparation scheme shown in *Table 15*. Volumes may be adjusted to prepare larger quantities of the calibration solutions as needed, maintaining final concentrations of the nitrosamines. For each calibration solution, transfer the designated aliquot of *Standard solution* to the designated volumetric flask, and adjust the volume with the *Internal standard solution*.

Table 15

Calibration Solution ID	Standard Solution Aliquot (µL)	Final Volume (mL)	Final Nitrosamine Concentration (µg/mL)	Equivalent Nitrosamine Concentration (µg/g)
Cal 1	50	10	0.0005	0.005
Cal 2	100	10	0.001	0.010
Cal 3	200	10	0.002	0.020
Cal 4	300	10	0.003	0.030
Cal 5	400	10	0.004	0.040
Cal 6	500	10	0.005	0.050
Cal 7	1000	10	0.010	0.100
Cal 8	1500	10	0.015	0.150

Sample solution: Transfer 500 mg of the drug substance into a disposable 10- to 15-mL glass centrifuge tube. Add 5.0 mL of the *Internal standard solution*. Cap the tube. Vortex the sample for 1 min, and then place in the centrifuge. Centrifuge the sample at 4000 rpm for 2.5 min. Transfer 2 mL of the bottom methylene chloride layer to a 5-mL syringe fitted with a 0.45-µm nylon filter. Filter 1 mL of sample extract into a 2-mL GC autosampler vial and cap.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Injector: Split/splitless

Injection type: Splitless with purge

Purge time: 0.5 min

Detector: MS/MS (triple quadrupole mass spectrometer)

MS conditions

Ionization: Electron impact

Scan settings: See *Table 16*.

Table 16

Nitrosamine Impurity	Acquisition Mode	Polarity	Transitions	
			MRM-1[a]	MRM-2
NDMA	MRM	Positive	74 amu → 44 amu	74 amu → 42 amu
NDMA:c13-d6	MRM	Positive	82 amu → 48 amu	–
NDEA	MRM	Positive	102 amu → 85 amu	102 amu → 56 amu
NEIPA	MRM	Positive	116 amu → 99 amu	71 amu → 56 amu
NDIPA	MRM	Positive	130 amu → 88 amu	130 amu → 42 amu
NDBA	MRM	Positive	158 amu → 99 amu	84 amu → 56 amu

[a] MRM-1 is used for quantitation.

MS1 and MS2 resolution: Q1: normal; Q3: wide (1.5)

Minimum window: 1 min

Emission current: 50 µA

Column: 0.25-mm × 30-m; fused-silica coated with a 1.0-µm layer of phase G16

Temperatures

Injector: 250°

Transfer line to MS detector: 250°

Ionization source: 250°

Column: See *Table 17*.

Table 17

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	0.5
40	20	200	0
200	60	250	3

Carrier gas: Helium

Flow rate: Constant flow at 1.0 mL/min (adjustment and verification are necessary for other carrier gases)

Injection volume: 2 µL

System suitability

Samples: *Calibration solutions*

Generate the peak response ratio of the specified impurity to that of the internal standard versus concentration standard curve for each nitrosamine impurity under test using the corresponding *Calibration solutions* and perform the linear regression analysis.

[Note—The relative retention times for NDMA, NDEA, NEIPA, NDIPA, and NDBA are 0.74, 0.80, 0.83, 0.85, and 1.00, respectively.]

Suitability requirements

Correlation coefficient: NLT 0.98

Signal-to-noise: NLT 10 for the impurity peak of the lowest concentration *Calibration solutions* used in the calibration curve

Analysis

Samples: *Calibration solutions* and *Sample solution*

Calculate the concentration, in ppm, of each specified nitrosamine impurity in the *Sample solution* using the corresponding calibration curve:

$$\text{Result} = 5 \times (1/W) \times [(R_U - y_{int})/a]$$

W = weight of the drug substance in the *Sample solution* (g)

R_U = peak response ratio of the specified nitrosamine impurity to that of the internal standard from the *Sample solution*

y_{int} = *y*-intercept of the calibration curve for the specified nitrosamine impurity from the corresponding *Calibration solutions*

a = slope from the calibration curve for the specified nitrosamine impurity from the corresponding *Calibration solutions*

Report the nitrosamine impurity concentration in the drug substance in ppm (µg/g).

8.2. Limit Test Procedures

While a limit test analytical procedure for nitrosamines content is not currently available, recommended sample preparation procedures are shown in *Table 18*.

Table 18

Solutions	Solution Preparation
Internal standard solution	Prepare a suitable *Internal standard solution* that, when added to the *Sample solution*, will have the resultant peak response at the highest appropriate target limit of the nitrosamines of interest in the sample.
Sample solution	Prepare a solution of the article to be examined, spiked with the internal standard, and prepared as described in the sample preparation. The amount of substance to be examined is chosen in such a way that the amount, in ppm, of a target *N*-nitrosamine, if present at its limit concentration for that substance, would be equal to the contribution of the respective spiking solution.
Spiking solution	A solution of target *N*-nitrosamine(s) of a concentration that, if added to the amount of article used for the preparation of the *Sample solution*, would result in the acceptance limit.
Spiked sample solution	Prepare a solution of the article to be examined spiked with a) appropriate *Spiking solution*(s) and b) *Internal standard solution* prepared as described in the *Sample solution*.

9. ADDITIONAL SOURCES OF INFORMATION

Several test procedures have been developed for the specific testing of nitrosamines in sartans and/or other official articles based on different scientific principles and are publicly available from many regulatory agencies. The hyperlinks in this section direct the user to the respective regulatory agencies' procedures. These can be used as alternative procedures and must be validated under actual use to meet the respective performance characteristics acceptance criteria set forth in *7. Test Method Performance Characteristics of Nitrosamine Methods*.

1. FDA-published testing methods to provide options for regulators and industry to detect NDMA and NDEA impurities: https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan/?utm_campaign=UPDATE%20on%20angiotensin%20II%20receptor%20blocker%20%28ARB%29%20recalls%20-%20FDA%20publishes%20LC-HRMS%20and%20RapidFire-MS%2FMS&utm_medium=email&utm_source=Eloqua#testingmethods.
2. *Ph. Eur.* 2.4.36 *N*-Nitrosamine impurities in active substances: https://pharmeuropa.edqm.eu/app/phpa/content/issue32-2/20436E.htm?highlight=on&terms=2.4.36.
3. EDQM projects on sampling strategies and testing methods with the Official Medicines Control Laboratory (OMCL) Network: https://www.edqm.eu/en/ad-hoc-projects-omcl-network.

10. *USP REFERENCE STANDARDS* ⟨11⟩

USP *N*-Nitrosodibutylamine RS
USP *N*-Nitrosodiethylamine RS
USP *N*-Nitrosodiisopropylamine RS
USP *N*-Nitrosodimethylamine RS
USP *N*-Nitrosoethylisopropylamine RS
USP *N*-Nitrosomethylaminobutyric Acid RS

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2. Williams DLH, Chapter 1: Reagents effecting nitrosation. In: *Nitrosation Reactions and the Chemistry of Nitric Oxide*. Amsterdam, Netherlands: ElsevierScience; 2004:1–34.
3. Ogata Y, Sawaki Y, Kuriyama Y. The reaction of trialkylamine with nitric acid in a mixture of acetic acid and acetic anhydride. *Tetrahedron*. 1968;24(8):3425–3435.

▲ (USP 1-May-2022)

Auxiliary Information- Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee

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<1469> NITROSAMINE IMPURITIES	Edmond Biba Senior Scientific Liaison	GCCA2020 General Chapters - Chemical Analysis 2020

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