Status: Currently Official on 12-Feb-2025
Official Date: Official as of 01-Dec-2021
Document Type: General Chapter
DocId: GUID-C97F817C-A383-4693-8E0C-2F0A0A371977_2_en-US
DOI: https://doi.org/10.31003/USPNF_M15715_02_01
DOI Ref: peb3u

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 potentially broad implications of the presence of carcinogenic members of this class of chemicals, this chapter has been developed to provide a science- and risk-based approach for the control of nitrosamine impurities to ensure that the potential presence of nitrosamines in drug substances and drug products is identified, assessed, and controlled.

Recommendations are provided regarding: a) the establishment of controls of nitrosamine levels in order to ensure their elimination or reduction; and b) analytical procedure performance characteristics for procedures used to monitor nitrosamine levels.

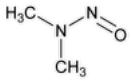
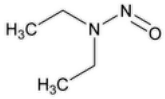
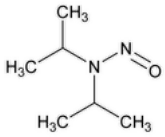
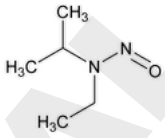
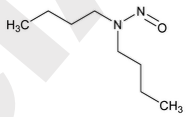
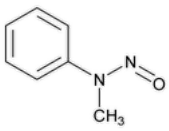
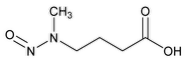
2. NITROSAMINE IMPURITIES

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. As additional nitrosamines are identified as potential concerns, the principles described herein should be applied for the assessment of these nitrosamines. If a manufacturer finds a nitrosamine not listed in *Table 1*, the appropriate regulatory authority should be contacted for determining appropriate AI limits. The potential presence of any one or more of these impurities is dependent on the reaction chemistries and processes. The list of nitrosamines is not intended to be exhaustive but represents those that have been observed and communicated by regulators and manufacturers as being potentially present or observed.

N-nitroso compounds are among the structural groups of high potency mutagenic carcinogens in several animal species, and some are classified as probable or possible human carcinogens referred to as the “cohort of concern" in ICH M7: *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* (*1*), a designation that carries with it a

recommendation to control the impurities at or below the acceptable cancer risk. As a result of the 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
Nitrosomethylphenylamine *N*-Methyl-*N*-nitrosophenylamine	NMPA	614-00-6		$C_7H_8N_2O$	136.15
Nitrosomethylaminobutyric acid 4-[Methyl(nitroso)amino] butanoic acid	NMBA	61445-55-4		$C_5H_{10}N_2O_3$	146.15

3. SOURCES OF NITROSAMINES

There are a number of pathways by which nitrosamines can be introduced into or generated as impurities in pharmaceutical drug products. Specifically, nitrosamines are formed by chemical reaction of secondary or tertiary amines with nitrites (the latter via intermediate degradation) under acidic conditions (see *3.1. Nitrosamine Formation Reaction*). Some examples of the reported sources or pathways leading to the generation of nitrosamines identified empirically or reported in the literature (2–3) include (but are not limited to) the following:

- Drug substance 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 nitrous acid to remove excess azide.
- The drug substance 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.

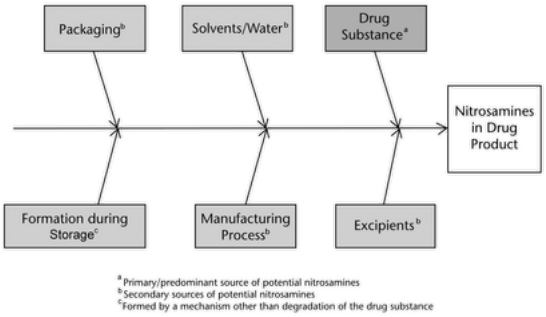


Figure 1. Potential sources of nitrosamine impurities in drug product.

Ongoing assessments and evaluations have identified risks associated with several of the potential sources of nitrosamines. Some of the examples identified 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 intermediates that can further react with nitrosating agents • Presence of nitrites or other nitrosating agents • Presence of acid • Limited controls/specification limits for recycled solvents • Poor quality solvents
Water	• Presence of residual dialkyl amines or impurities that can degrade to form dialkyl amines • Presence of acid and nitrosating agents
Excipients	• Presence of nitrites or other nitrosating agents and/or nitrosamine impurities (if applicable)
Drug substance	• Use of sodium azide in the synthesis followed by use of nitrites in acidic medium (nitrous acid) for quenching excess azides • 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) • Insufficient purification

Potential Source of Nitrosamines	Observed Risk[a]
	• Degradation of drug substances containing functional groups that can then participate in nitrosation reactions
Manufacturing process	• Contamination • Use of poor quality or recycled solvents that may contain nitrosamines or their precursors • Presence of nitrous oxides in air used to dry the drug substance or drug product • Carryover of relevant reactive species into subsequent steps
Drug product (including stability)	• Secondary, tertiary, or quaternary amine group in molecule of drug substance • Presence of nitrate counter ions (potentially containing nitrite 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	• Packaging materials containing vulnerable amines that might react with nitrosating agents present in the packaging material itself (e.g., amines in inks reacting with nitrocellulose print base)

[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 schematic representation of the chemical reaction responsible for the formation of nitrosamines from secondary amines is described in *Figure 2*. Examples of representative reactions are described in the scientific literature (*2–3*).

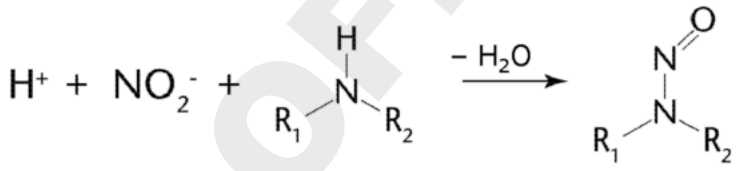


Figure 2. General example of formation of nitrosamines, where R_1 and R_2 are alkyl or functionalized alkyl groups (and only one of them could be an aryl or functionalized aryl group).

If the potential for the presence of nitrosamines is identified, where appropriate, a 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 may be required for ensuring that levels of nitrosamines are at or below the acceptable intake (AI) if their presence cannot be avoided, the components of drug products should be assessed by the drug product manufacturer 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 drug substance synthetic route, the drug substance manufacturing process, drug product manufacturing process, and excipients and raw materials should also be included in a risk assessment to establish if controls or additional controls are needed. An example of high-level process flow for evaluating materials is shown in *Figure 3*.

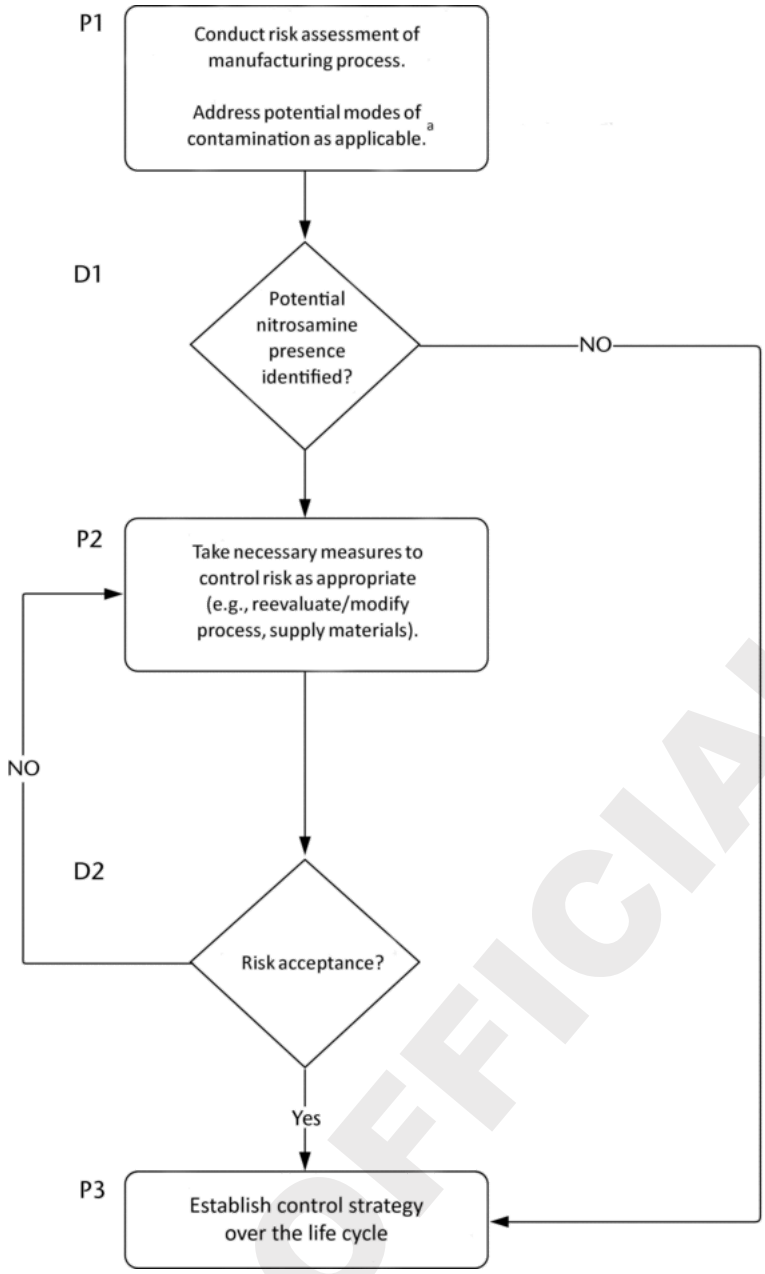


Figure 3. High level process for development of a nitrosamine impurity control strategy. ([a] Refer to *Table 2*; P1, P2, P3 = Process 1, 2, 3; D1, D2 = Decision 1, 2)

In all cases, if nitrosamines are predicted by the risk assessment or confirmed to be present through testing of the drug substance, drug product, or other materials, a control strategy should define an approach to ensure that the nitrosamine levels comply with the established AIs. The control strategy should be aligned with the current regulatory requirements in place.

5. LIMITS OF NITROSAMINES

Nitrosamine impurities identified in this chapter have potential and established toxicity with no therapeutic value. Because nitrosamines are among the structural groups of high potency mutagenic carcinogens of the “cohort of concern” in ICH M7 (*1*), the threshold of toxicological concern (TTC) does not apply. Instead, the available safety data should be used to establish a material-specific AI on a case-by-case basis. The AI is defined as an intake level that poses a negligible health risk.

5.1 Derivation of AI Limits

There are a number of methodologies that toxicologists have applied in establishing AIs. In this case, the median tumorigenic dose (TD_{50}) of NDMA, NDEA, and other nitrosamines was used as representative data in a linear extrapolation to establish an acceptable risk level. The limits have been published in the FDA Guidance for Industry: *Control of Nitrosamine Impurities in Human Drugs* (*4*).

5.2 Example Calculations of Nitrosamine Limits

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 be used to calculate the maximum nitrosamine concentration limits, in ppm, for individual drug products using the following equation:

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

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

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

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

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

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

Name	Acceptable Concentration (μg/g)			
	0.050 (50-mg dose)	**0.100 (100-mg dose)**	**0.250 (250-mg dose)**	**1.00 (1000-mg dose)**
Nitrosamine 1	1.920	0.960	0.384	0.096

[Note—If multiple nitrosamines are identified in the material and the total exceeds 26.5 ng/day, the appropriate regulatory authority should be consulted to determine an acceptable approach.]

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; drug substance 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 chapter. Example analytical procedures can be found in *8. Analytical Procedures*.

6.1 Presence of Two or More Nitrosamines

The current published AIs reflect limits for the presence of a single nitrosamine. If multiple nitrosamines are possible and are determined analytically to be present at levels exceeding the maximum amount permitted by the regulatory authority, the relevant health authority should be consulted to determine a specific path forward. Manufacturers should contact FDA for determining the AI limits if multiple nitrosamine impurities are detected in a drug substance or drug product in which the total nitrosamine level exceeds 26.5 ng/day based on MDD.

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.

The recommended method performance characteristics that need to be evaluated for quantitative analysis of nitrosamines include range, accuracy, repeatability, intermediate precision, and limit of quantitation. If a limit test is intended for use, the recommended method performance characteristics to be assessed include specificity, recovery, detectability, and solution stability. The performance criteria for these parameters should be properly set and confirmed through validation to ensure that the method is suitable for its intended use based on the specific analytes, matrices, and required precision and accuracy of the analytical procedures. Precision and recovery depend highly on concentration and matrix complexity, and the final proposed acceptance criteria need to be justified in the

procedure validation documentation. Higher variability may be tolerated or acceptable at concentrations approaching the limit of quantitation of the procedure while lower variability (higher precision) would be expected at higher concentrations. Examples of quantitative analytical procedures are included in *8. Analytical Procedures*. Additional example methods may be provided as they become available for these additional nitrosamines.

8. ANALYTICAL PROCEDURES

The following procedures have been established as suitable for their intended (specified) purpose. Users should validate these methods while considering the effect of sample solubility and extraction efficiency on the test results for other materials for which they are intended to be applied (see ⟨1225⟩ and *Verification of Compendial Procedures* ⟨1226⟩).

8.1 Quantitative Procedures

Procedure 1: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, NMPA, and NDBA in selected sartans (valsartan, irbesartan, and losartan potassium) 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 4*.

Table 4

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, USP *N*-Nitrosomethylphenylamine 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, USP *N*-Nitrosomethylphenylamine 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 5*.

Table 5

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

Polarity	positive	negative	positive	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	8.5–10.0	13.0–15.5
m/z[c] isolated for PRM	N/A	N/A	103.0866	N/A	N/A	N/A	159.1492
NCE[d]	N/A	N/A	25	N/A	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	136.3-137.8	50.0–170.0
Microscans	3	3	3	3	3 (1)[e]	3 (1)[e]	3
Resolution	30,000	60,000	30,000	60,000	60,000	60,000	30,000
AGC target value (%)[f]	250	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] 1 microscan must be used only if both NDIPA and NMPA are present.

[f] AGC = automatic gain control.

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

Data processing: Peak areas in the extracted ion chromatograms (EIC) with an m/z extraction window of ±15 ppm are used for quantitation. The m/z values extracted are listed in *Table 6*.

Table 6

Nitrosamine Impurity	NDMA	NMBA	NDEA	NEIPA	NDIPA	NMPA	NDBA
m/z to be extracted	75.0553	145.0619	75.0553, 103.0866	117.1022	131.1179	137.0709	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, NMPA, and NDBA are 0.20, 0.31, 0.46, 0.57, 0.66, 0.67, 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, USP *N*-Nitrosomethylphenylamine 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 (valsartan, irbesartan, losartan potassium, olmesartan medoxomil, candesartan cilexetil, and telmisartan) by headspace 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.

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 7* for parameters)

Table 7

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 8*.

Table 8

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
45	0	45	3
45	10	130	3
130	15	190	—
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 9*.

Table 9

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 2 m/z	130.00 > 43.10
CH 2 collision energy	18.00 V

[NOTE—Ch 1 m/z in multiple reaction mode (MRM) is used for quantitation.]

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 stock 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 (valsartan, losartan potassium, olmesartan medoxomil, candesartan cilexetil, and telmisartan) 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 10*.

Table 10

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 standards stock solution mixture* 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 11* from the *Nitrosamine standards stock solution mixture* and *NDEA standard stock solution* by mixing specified volumes of each solution as indicated. [Note—*Table 11* 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 11

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 potassium, which should be vortexed 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 12*.

Table 12

Nitrosamine Impurity	Acquisition Mode	Polarity	MRM Transitions (m/z)	
			MRM-1[a]	MRM-2
NDMA	MRM	Positive	+75.0 → +43.0	+75.0 → +44.1
NDMA-d6	MRM	Positive	+81.2 → +46.0	+81.2 → +64.1
NDEA	MRM	Positive	+103.1 → +75.1	+103.1 → +47.1
NDEA-d10	MRM	Positive	+113.2 → +34.2	+113.2 → +49.1
NMBA	MRM	Positive	+147.1 → +44.1	+147.1 → +117.1
NMBA-d3	MRM	Positive	+150.1 → +47.1	+150.1 → +120.2

Nitrosamine Impurity	Acquisition Mode	Polarity	MRM Transitions (m/z)	
			MRM-1[a]	MRM-2
NDBA	MRM	Positive	+159.2 → +41.1	+159.2 → +29.1
NDBA-d18	MRM	Positive	+177.3 → +66.2	+177.3 → +46.2
NEIPA[b]	MRM	Positive	+117.1 → +75.1	+117.1 → +47.2
NDIPA[b]	MRM	Positive	+131.2 → +89.1	+131.1 → +47.1

[a] MRM-1 is used for quantitation.

[b] NDEA-d10 is used as internal standard for NEIPA and NDIPA.

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 the 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* $[(\mu g/mL)^{-1}]$

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, NMPA, and NDBA in selected sartans (valsartan, losartan potassium, and candesartan cilexetil) 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, *N*-nitrosomethylphenylamine 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 5 consecutive solutions from *Table 13* that are used for generating the calibration curve by following the preparation scheme shown in the table. 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 13

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 14*.

Table 14

Nitrosamine Impurity	Acquisition Mode	Polarity	MRM Transitions (m/z)	
			MRM-1[a]	MRM-2
NDMA	MRM	Positive	74 → 44	74 → 42
NDMA:c13-d6	MRM	Positive	82 → 48	–
NDEA	MRM	Positive	102 → 85	102 → 56
NEIPA	MRM	Positive	116 → 99	71 → 56
NDIPA	MRM	Positive	130 → 88	130 → 42
NMPA	MRM	Positive	106 → 77	77 → 51
NDBA	MRM	Positive	158 → 99	84 → 56

[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 15*.

Table 15

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*

[NOTE—The relative retention times for NDMA, NDEA, NEIPA, NDIPA, NDBA, and NMPA are 0.73, 0.78, 0.81, 0.82, 0.99, and 1.00, respectively.]

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.

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* $[(\mu g/mL)^{-1}]$

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 16*.

Table 16

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.
	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

Analysis

Samples: *Spiked sample solutions* and *Sample solution*

Determine the peak response ratio of the respective target *N*-nitrosamine to the internal standard from the *Sample solution*: $R_{U(i)}$

Determine the peak response ratio of the respective target *N*-nitrosamine to the internal standard from the *Spiked sample solution*: $R_{ST(i)}$

Acceptance criteria: $R_{U(i)}/R_{ST(i)}$ is NMT 0.5

Spiking solution	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
	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 for the respective performance characteristics recommended 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 on angiotensin II receptor blocker (ARB) recalls - FDA publishes LC-HRMS and RapidFire-MS/MS&utm_medium=email&utm_source=Eloqua#testingmethods*.
2. *Ph. Eur.* 2.5.42 *N*-Nitrosamines in active substances: *https://www.edqm.eu/sites/default/files/medias/fichiers/European_Pharmacopoeia/News/european_pharmacopoeia_n-nitrosamines_in_active_substances.pdf*.
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
USP *N*-Nitrosomethylphenylamine RS

REFERENCES

1. International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use. ICH M7: *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*, 2017. *https://www.ich.org/page/multidisciplinary-guidelines*.
2. Williams DLH, Chapter 1: Reagents effecting nitrosation. In: *Nitrosation Reactions and the Chemistry of Nitric Oxide*. Amsterdam, Netherlands: Elsevier Science; 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.
4. Food and Drug Administration. *Control of Nitrosamine Impurities in Human Drugs-Guidance for Industry*, 2021. *https://www.fda.gov/media/141720/download*.

▲ (USP 1-Dec-2021)

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

Topic/Question	Contact	Expert Committee
<1469> NITROSAMINE IMPURITIES	Edmond Biba Senior Scientific Liaison	GCCA2020 General Chapters - Chemical Analysis 2020

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 46(5)

Current DocID: GUID-C97F817C-A383-4693-8E0C-2F0A0A371977_2_en-US
DOI: https://doi.org/10.31003/USPNF_M15715_02_01
DOI ref: peb3u