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A short review on nitrosamine impurities

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Abstract
Since 2018, N-nitrosamine impurities have become a widespread concern in the global regulatory landscape of pharmaceutical products. This concern arises due to their potential for contamination, toxicity, carcinogenicity, and mutagenicity and their presence in many active pharmaceutical ingredients, drug products, and other matrices. These include carcinogenic effects, metabolic disruptions, reproductive harm, liver diseases, obesity, DNA damage, cell death, chromosomal alterations, birth defects, and pregnancy loss. They are particularly known to cause cancer (tumors). Additionally, N-nitrosamine impurities may contribute to the development of Alzheimer's and Parkinson's diseases and type-2 diabetes. Therefore, it is very important to control or avoid them by enhancing effective analytical methodologies using cutting-edge analytical techniques such as LC-MS, GC-MS, CE-MS, SFC, etc. Moreover, these analytical methods need to be sensitive and selective with suitable precision and accuracy, so that the actual amounts of N-nitrosamine impurities can be detected and quantified appropriately in drugs. Regulatory agencies such as the USFDA, EMA, ICH, WHO, etc. need to focus more on the hazards of N nitrosamine impurities by providing guidance and regular updates to drug manufacturers and applicants. Similarly, drug manufacturers should be more vigilant to avoid Nitrosating agents and secondary amines during the manufacturing processes. Numerous review articles have been published recently by various researchers, focusing on N-nitrosamine impurities found in previously notified products, including Sartans, metformin, and ranitidine. These impurities have also been detected in a wide range of other products. Consequently, this review aims to concentrate on products recently reported to contain N-nitrosamine impurities. These products include rifampicin, Champix, famotidine, nizatidine, atorvastatin, bumetanide, itraconazole, Diovan, enalapril, propranolol, lisinopril, duloxetine, rivaroxaban, pioglitazones, Glifizones, cilostazol, and sunitinib.

Keywords: Nitrosamine impurities, Liquid Chromatography, Mass Spectroscopy, Gas Chromatography

Introduction

Impurities are unwanted substances or contaminants that are present in a product, material, or chemical compound, which may affect its purity, quality, or safety. These substances can be introduced during the manufacturing, storage, or handling processes, and they can come from a variety of sources, including raw materials, production processes, environmental exposure, or degradation over time. Impurities can be chemical, physical, or biological in nature and can impact the performance or safety of the product in which they are found. In many industries, such as pharmaceuticals, food, and chemicals, controlling and minimizing impurities is crucial to meet regulatory standards and ensure the product is safe for use or consumption [1].

Different Approaches for Quantification of Impurities

Quantifying impurities in various products (such as pharmaceuticals, food, chemicals, or cosmetics) is a critical step to ensure quality and compliance with regulatory standards. Different approaches are used depending on the nature of the impurities, the product type, and the required sensitivity. Below are the most common approaches for quantifying impurities:

1. Chromatographic Techniques
   - High-Performance Liquid Chromatography (HPLC):
   - Gas Chromatography (GC)
   - Thin-Layer Chromatography (TLC)
2. Mass Spectrometry (MS)

Types of MS

   - GC-MS (Gas Chromatography-Mass Spectrometry)
   - LC-MS (Liquid Chromatography-Mass Spectrometry)
   - HRMS (High-Resolution Mass Spectrometry)
3. Spectroscopic Techniques
   - UV-Vis Spectroscopy
   - Fourier Transform Infrared (FTIR) Spectroscopy
   - Nuclear Magnetic Resonance (NMR) Spectroscopy
4. Titration Methods
5. Electrochemical Methods
6. Enzyme-Linked Immunosorbent Assay (ELISA)
7. Optical Methods
8. Gravimetric Methods [2].

Nitrosamine Impurities

The term nitrosamine describes a class of compounds having the chemical structure of a nitroso group bonded to an amine (R1N(-R2)-N=O), as shown in Figure 1. The compounds can form by a nitrosating reaction between amines (secondary, tertiary, or quaternary amines) and nitrous acid (nitrite salts under acidic conditions). A different class of precursor is 1,1-disubstituted hydrazine, which can be oxidized to form a nitrosamine. The compounds 1-cyclopentyl-4-nitrosopiperazine and 1-methyl-4-nitrosopiperazine are formed via this hydrazine oxidation process (Horne *et al*. 2023). Nitrosamine compounds are potent genotoxic agents in several animal species and some are classified as probable or possible human carcinogens by the International Agency for Research on Cancer.19 They are referred to as cohort of concern compounds in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidance for industry M7(R2) Assessment and Control of DNA Reactive

(Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk (July 2023). ICH M7(R2) recommends control of any known mutagenic carcinogen, such as nitroso-compounds, at or below a level such that there would be a negligible human cancer risk associated with the exposure to the compound. [3, 4, 5]

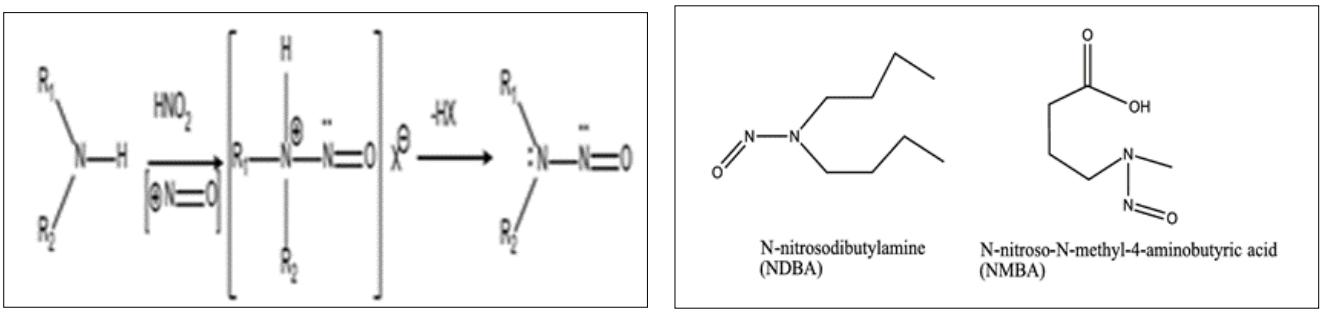

Fig 1: Representive reaction to form Nitrosamines

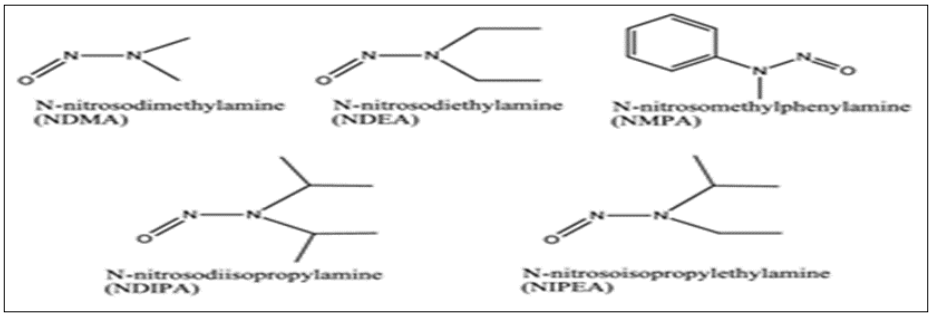

Fig 2: Chemical structures of Potential Small Molecule Nitrosamine Impurities in API's and Drug Products

Applications of Analytical Methods for Quantification of Nitrosamine Impurities Present in Drug Molecules

The quantification of nitrosamine impurities in drug molecules is critical due to the potential carcinogenic risk associated with these compounds. Regulatory bodies, such as the U.S. FDA and the European Medicines Agency (EMA), have set guidelines to limit the levels of nitrosamines in pharmaceutical products, making the detection and quantification of these impurities a key component of quality control in drug manufacturing.

Several analytical methods are applied to identify and quantify nitrosamine impurities in drug molecules. Here are the most commonly used methods:

1. Gas Chromatography-Mass Spectrometry (GC-MS)

Application: GC-MS is one of the most widely used techniques for detecting and quantifying volatile nitrosamine impurities in drug molecules.

- Application Example: Metformin was found to have nitrosamine contamination in some batches, and GC-MS was used to identify and quantify the nitrosamines in the drug product.

2. Liquid Chromatography-Mass Spectrometry (LC-MS)

Application: LC-MS is particularly useful for detecting non-volatile or thermally labile nitrosamines, which may not be suitable for GC-MS analysis.

- Application Example: LC-MS has been used for quantifying nitrosamine impurities such as N-nitrosodimethylamine (NDMA) in products like ranitidine and valsartan.

3. High-Performance Liquid Chromatography (HPLC)

Application: HPLC is widely used in the pharmaceutical industry for detecting and quantifying nitrosamine impurities, especially when coupled with UV detection or mass spectrometry.

- Application Example: HPLC has been used to detect trace levels of NDMA and other nitrosamine impurities in pharmaceutical products.

4. Enzyme-Linked Immunosorbent Assay (ELISA)

Application: ELISA is a more rapid and cost-effective technique used for screening nitrosamines, particularly for larger-scale, routine analysis.

- Application Example: ELISA can be used for rapid screening of NDMA in pharmaceuticals like metformin or losartan during batch production.

5. Fourier Transform Infrared (FTIR) Spectroscopy

Application: FTIR can be used for the identification of nitrosamine impurities, particularly for qualitative analysis in the pharmaceutical industry.

- Application Example: FTIR has been used in research to study the molecular structures of nitrosamines in pharmaceutical formulations.

6. Gas Chromatography (GC)

Application: GC, when used with detectors such as flame ionization detection (FID) or nitrogen-phosphorus detection (NPD), is employed for quantifying volatile nitrosamines in pharmaceutical products.

- Application Example: GC is often employed for the analysis of volatile nitrosamines such as NDMA in drug products.

7. Headspace Gas Chromatography (HS-GC)

Application: Headspace GC is a specific type of GC used to analyze volatile impurities in pharmaceutical products.

- Application Example: NDMA has been quantified in pharmaceutical products like valsartan using headspace GC.

- Metformin (anti hyperglycemic)/Liquid Chromatography- Electrospray Ionization-High Resolution Mass Spectrometry (LC-ESI-HRMS) Method. [5,6,7]

Method and Conditions

HPLC Column	Phenomenex Kinetex® 2.6 µm Biphenyl 100 Å, 150 x 3.0 mm (Part No. 00F-4622-Y0)	Sheath Gas Flow Rate	55 arbitary units
Column Temp.	40 °C	Aux Gas Flow Rate	15 arbitary units
Flow rate	0.4 mL/min	Sweep Gas Flow Rate	0 units
Mobile phase A	0.1% formic acid in water	Spray Voltage	3.5 kV
Mobile phase B	0.1% formic acid in methanol	Capillary Temp.	400 °C
Injection volume	3 µL	S-Lens	55 (applied to Q ExactiveTM)
Autosampler temperature	21 °C (Room Temperature)	Aux Gas Heater Temp.	350 °C
Need wash	80:20, Methanol: Water with 0.1% Formic Acid		

Agilent GC/MS/MS Instrumentation [8,9,10]

MMI injection mode	Pulsed splitless: 12.285 psi until 0.5 min	Mode	Electron ionization, 70 eV
inlet temperature	250 °C	Source temperature	250 °C
Oven temperature progarm	40 °C (1.5 min) 20 °C/min to 200 °C (0 min) 60 °C/min to 250 °C (3 min)	Quadrupole temperature	Q1 and Q2 = 150 °C
Total run time	13.33 minutes		
MS transfer line temperature	250 °C		
Injection volume	2 µL		
Carrier gas	Helium, 1 mL/min		

Transfer line (˚C)	250	Transfer line (˚C)	250
Ion source (˚C)	300	Ion source (˚C)	300
Acquisition method	Timed-SIM	Acquisition method	Timed-SIM
T-SIM parameters (compound, retention time and SIM ions)	NDMA: 6.08 min, 74, 42 m/z DMF: 6.35 min, 73, 44, 42 m/z NDEA: 6.8 min,102, 57, 42 m/z	T-SIM parameters (compound, retention time and SIM ions)	NDMA: 6.08 min, 74, 42 m/z DMF: 6.35 min, 73, 44, 42 m/z NDEA: 6.8 min, 102, 57, 42 m/z

Valsartin/ Headspace GC-MS [11]

Rifampicin(antibiotic)/ Liquid Chromatography-High Resolution Mass Spectrometry (LC-ESI-HRMS) Method [12]

Incubation (˚C)	150	Carrier gas (mL/min)	He, 1.0	Transfer line (˚C)	250
Incubation time (min)	15	Injection mode	Split ratio 5:1, Split flow 5 mL/min	Ion source (˚C)	300
Loop/sample path (˚C)	180	Column	TG-WaxMS 30 m x 0.25 mm x 0.25 µm (P/N: 26088-1420)	Acquisition mode	Timed-SIM
Injection volume (µL)	1000	Oven parameter	45 ˚C (Hold 1 min), 15 ˚C/min to 180 ˚C, 20 ˚C/min to 250 ˚C (hold 1 min)	T-SIM parameters (compound, retention time, SIM ions)	NDMA: 6.08 min, 74, 42 m/z NDEA: 6.8 min, 102, 57, 42 m/z

HPLC Column	HPLC column: Ace Ultracore SuperPhenylHexyl, 2.5 µm 90 Å, 50 x 4.6 mm (Mac-Mod, Part No. CORE25B0546U)	Sheath gas flow rate	55 arbitary units
Column temperature	30 °C	Aux gas flow rate	15 arbitary units
Flow rate	0.5 mL/min	Sweep gas flow rate	0
Mobile phase A	10 mM Ammonium Formate in water, pH = 9.0	Spray voltage	3.5Kv
Mobile phase B	methanol	Capillary temperature	300 °C
Injection volume	3 µL	Aux gas heater temperature	300
Autosampler temperature	4 – 8 °C		
Needle wash	methanol		

Sartans (angiotensin 2 receptor blockers)/ GC-MS Method [13]

Column temperature	38°C (1 min) → 12°C/min to 160°C → 5°C/min to 200°C (1 min)	Ion source temperature	230°C
Injection conditions	Splitless, sampling time 2 min; High pressure injection (250 kPa, 2 min)	Interface temperature	280°C
Injection volume	2 µl	Acquisition mode	MRM (multiple reaction monitoring)
Carrier gas	Helium	Ionisation type	Electron ionisation (EI)
Carrier gas control	42.6 cm/sec (Constant linear velocity)		

Varenicline/ Liquid Chromatography-High Resolution Mass Spectrometry (LC-ESI-HRMS) Method [14]

HPLC column	XSelect CSH Phenyl-Hexyl XP, 2.5 µm 130 Å, 150 x 4.6 mm (Waters Part # 186006735 or equivalent)	Sheath gas flow rate	50 arbitary units
Column rate	30 °C	Aux gas flow rate s	15 arbitary units
Flow rate	0.5 mL/min	Sweep gas flow rate	0 units
Mobile phase A	Water, 0.1% Formic Acid	Spray voltage	3.5 kV
Mobile phase B	Methanol, 0.1% Formic Acid	Capillary temperature	350°C
Injection volume	5 µL	Aux gas heater temperature	350°C
Autosampler temperature	4 – 8 °C		
Needle wash	Methanol		

Ranitidine (H2 blocker)/LC-MS/MS

Column	C18 column (e.g., 100 mm × 2.1 mm, 1.7 µm particle size)
Mobile phase A	Water with 0.1% formic acid
Mobile phase B	Methanol or acetonitrile with 0.1% formic acid
Gradient dilution	Increasing organic phase over time
Flow rate	~0.3 mL/min
Column temperature	~30–40°C
Injection volume	10–20 µL
Run time	~10–20 minutes

GC-MS [15]

Column	DB-624 or DB-5MS (30 m × 0.25 mm, 1.4 µm)
Carrier gas	Helium at ~1 mL/min
Oven program	Ramp from ~40°C to 280°C
Injection mode	Splitless
ionization	EI (Electron Ionization)
Detection mode	Selected Ion Monitoring (SIM)

Quinapril (ACE inhibitor)/ LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry) [16]

Column	C18 (e.g., Waters XBridge C18, 2.1 × 100 mm, 3.5 µm)
Mobile phase A	0.1% Formic acid in water
Mobile phase B	0.1% Formic acid in methanol or acetonitrile
Gradient program	Start with 5% B, increase to 95% B over 10–15 min, hold for 5 min, then return to 5% B.
Flow rate	0.3–0.5 mL/min
Column temperature	30–40°C
Injection volume	5–20 µL

Nizatidine (H2 blocker)/ GC-MS/MS (Gas Chromatography-Tandem Mass Spectrometry) [17]

Ionization Mode	Positive/Negative Electrospray Ionization (ESI)
Analyte-Specific MRM Transitions	NDMA (N-Nitrosodimethylamine): m/z 75 → 43 NDEA (N-Nitrosodiethylamine): m/z 103 → 45 NMBA (N-Nitroso-N-methyl-4-aminobutyric acid): m/z 147 → 117
Capillary Voltage	3.5 kV
Collision Energy:	Optimized for each nitrosamine
Nebulizer Gas	Nitrogen
Source Temperature	350°C

Sitagliptin((DPP-4) inhibitor)/ LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry) [18]

Column	C18 or CSH Fluoro-Phenyl column (e.g., 100 mm × 2.1 mm, 1.7 µm particle size).
Mobile phase A	0.1% formic acid in water
Mobile phase B	0.1% formic acid in acetonitrile
Gradient elution	Increasing organic phase (B) over time.
Flow rate	~0.3 mL/min
Column temperature	30–40°C
Injection volume	5–10 µL

Dronedarone (Antiarrhythmic Drug)/ GC-MS Method (Gas Chromatography-Mass Spectrometry) [19, 20]

Column	DB-624 (30 m × 0.25 mm, 1.4 µm)	Ionization Mode:	Electron Ionization (EI)
Carrier gas	Helium	Detection Mode	Selected Ion Monitoring (SIM)
Oventemperature program	Initial: 40°C, hold for 2 min, Ramp to 250°C at 10°C/min Hold for 5 min	Typical Ions:	NDMA: m/z 75, NDEA: m/z 103
Injection mode	Initial: 40°C, hold for 2 min, Ramp to 250°C at 10°C/min, Hold for 5 min	LOD & LOQ	~0.01–0.1 ppm

Conclusion

Nitrosamine impurities have emerged as a critical quality and safety concern in pharmaceuticals due to their potential carcinogenicity. The FDA guidance for industry recommends that for products with an MDD of less than 880 mg/day, the LOQ should be ≤ 0.03 ppm. For products with an MDD above 880 mg/day, the LOQ should be as low as reasonably practical. Regulatory agencies like the FDA, EMA, and WHO have established strict guidelines for their detection, control, and mitigation to ensure patient safety. To minimize risks, manufacturers must implement robust risk assessments, advanced analytical techniques, and effective control strategies throughout the drug development and production process. Continuous monitoring and regulatory compliance are essential to prevent contamination and maintain drug quality. Addressing nitrosamine impurities requires collaboration among regulatory bodies, pharmaceutical companies, and research institutions to develop innovative solutions for long-term control and mitigation.

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