Control Strategies and Method Development for Nitrosamines in APIs

Shankar Sankaran, Director Analytical
SK pharmteco Small Molecules US

Overview

For late-phase drugs, risk assessments for nitrosamines have become an important supplement for filings with both the FDA and EMA, as well as other regulatory agencies. When these assessments propose a potential risk, even if low risk, a robust testing strategy is required to demonstrate compliance with the issued guidance.

- What are the technologies used for nitrosamine testing?
- How do we establish/validate methods for screening and for routine analysis of nitrosamines?
- What are the challenges associated with nitrosamines method development?
- Strategies for establishing test methods for drug-derived nitrosamines
- Case studies for drug-derived nitrosamines

Nitrosamine Control

Nitrosamine impurities Guidance (FDA and EMA) specifies

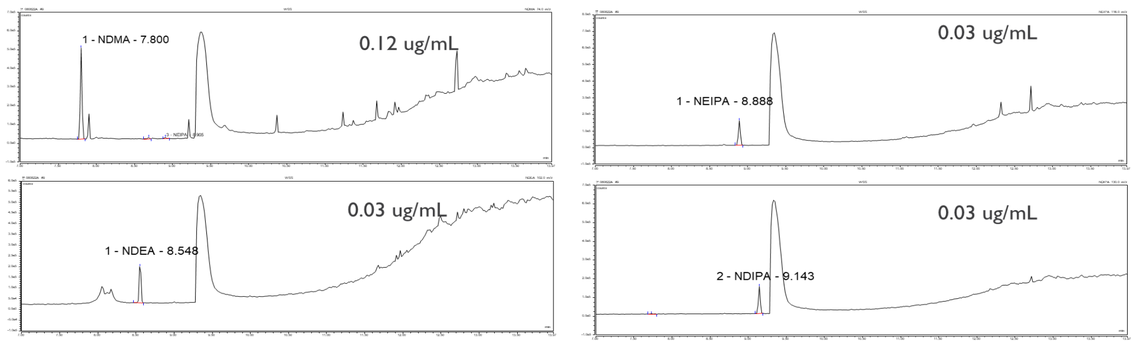
- Very low limits for the typical nitroso impurities individually.
- Total nitrosamines must be no more than the limit for the lowest acceptable intake.
- Product-specific nitroso impurity limits (NDSRIs) can vary, especially if structure-activity-relationship data is available.
- The recommended limit is calculated based on Carcinogenic Potency Categorization Approach (CPCA) risk scoring.

Nitrosamine Impurity	Acceptable Intake Limit	Example Limit for a drug with Max Daily dose (in ppm)	
	(ng/day)	200 mg/day	1,500 mg/day
NDMA (Nitrosodimethylamine)	96	0.48	0.06
NDEA (Nitrosodiethylamine)	26.5	0.13	0.02
NEIPA (Nitrosoethylisopropylamine)	26.5	0.13	0.02
NMBA (Nitrosomethylaminobuytric acid)	96	0.48	0.06
NDIPA (Nitrosodiisopropylamine)	26.5	0.13	0.02
NDBA (Nitrosodibutylamine)	26.5	0.13	0.02
NMPA (Nitrosomethylphenylamine)	26.5	0.13	0.02
Product Specific Nitrosamine	18 – 1500	0.09	0.01

Method Considerations

- Generally, sensitive methods with **limits of quantitation (LOQ) in the parts-per-billion (ppb) range** are needed to meet the low AIs recommended for nitrosamines.
- **Target LOQ** should be 10% of the limit (to justify no routine testing) or 30% of the limit (to justify skip lot testing).
- Testing is typically achieved through **GC-MS or LC-MS/MS**.
- **High-Res Mass-Spec applications** are useful for drug-derived nitroso impurities.
- A robust screening process is created by setting up a set of platform methods by validating orthogonal methods across these technologies.

A platform **GC-MS method** can be a fast and robust option for **smaller nitrosamines** (NDMA, NDEA, NDIPA, NEIPA)



Method Development typically takes ~2 weeks to optimize for accuracy and precision in the specified API matrix and 1-1.5 weeks to validate.

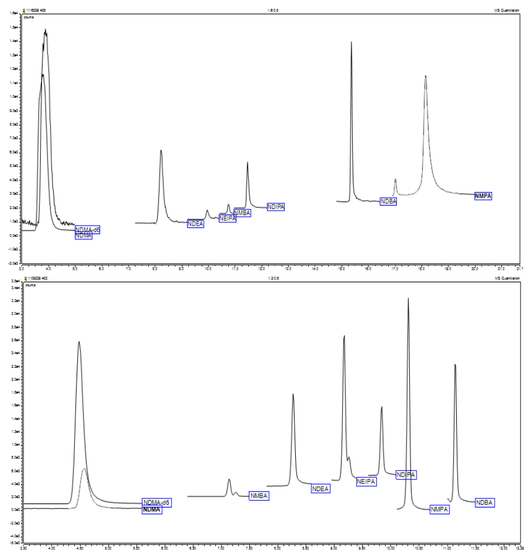
- **Pros**: Minimize sample matrix interference, Flexibility in sample preparation
- **Cons**: Sample concentrations are generally higher as sensitivity is not as low as LC-MS. Potential for *in situ* nitrosamines depending on the sample matrix

A Platform **LC-MS/MS methods** can be used for the wider complement of nitrosamines

This method is established across two orthogonal columns (Thermo Hybercarb and Hypersil GOLD Phenyl)

Method Development typically takes ~2-4 weeks, as matrix-related effects are challenging to control, and 2-3 weeks to validate.

- **Pros**: Easier to optimize sensitivity and specificity. Lower Sample concentrations
- **Cons**: Sample matrix-related issues can be challenging, (solubility and interference).



LC-MS/MS Sensitivity			
Nitrosamine	Chemical Name	Detection Limit (ng/mL)	Quantitation Range (ng/mL)
NDMA	N-Methyl-N-nitrosomethanamine	0.04	0.08 - 2.0
NDEA	N-Ethyl-N-nitrosoethanamine	0.02	0.04 - 1.0
NMBA	4-[Methyl(nitroso)amino] butanoic acid	0.02	0.04 - 1.0
NDIPA	N-Isopropyl-N-nitrosoisopropylamine	0.02	0.04 - 1.0
NEIPA	N-Ethyl-N-nitroso-2-propanamine	0.02	0.04 - 1.0
NDBA	N-Butyl-N-nitroso-1-butanamine	0.02	0.04 - 1.0
NMPA	N-Methyl-N-nitrosophenylamine	0.02	0.04 - 1.0

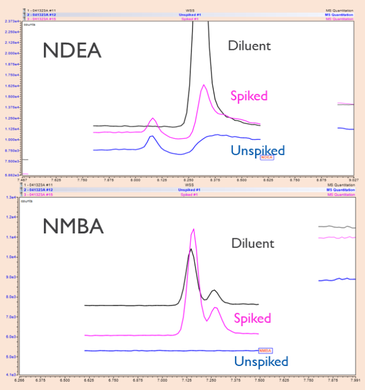
Sensitivity (LOD and LOQ)

Analyte	LOQ	LOQ S/N	LOD	LOD S/N
NDMA	1.5 ng/mL	100	0.75 ng/mL	93
NDEA		42		24
NEIPA		25		8
NDIPA		30		10

Accuracy and Precision

Analysis	Test	Level NDMA / NDEA, NEIPA, NDIPA	API Result	Drug Product Result
Accuracy	%Recovery 50-150%	0.24 / 0.06 ppm	89-139%	79-148%
		2.4 / 0.6 ppm	96-109%	85-89%
		3.6 / 0.9 ppm	100-113%	89-97%
Precision	NMT 25% RSD	0.24 / 0.06 ppm	14-25%	8-10%
		2.4 / 0.6 ppm	1-3%	2-7%
		3.6 / 0.9 ppm	1-3%	3-6%

Case Study 1: Bradykinin, a 9 AA peptide



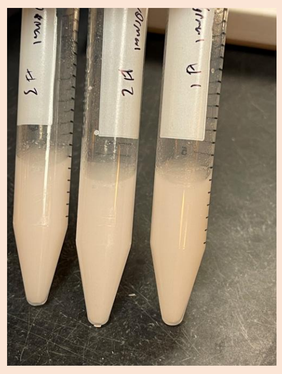
Orthogonal methods on LC-QQQ

Spiked recoveries from sample matrix as expected for all screened nitrosamines except two, NDEA, and NMBA (using Hypersil method)

- NDEA recovery issues due to coelution with API, resolved using an orthogonal method (Hypercarb)
- NMBA issues are matrix/diluent-related effects. All injections after samples, including bracketing standards, show a ~2x response. Further development for NMBA is needed, specifically evaluating sample prep and diluent additives

Case 1: Bradykinin Screening		
Nitrosamine	Recovery (%) Hypersil	Recovery (%) Hypercarb
NDMA	92	93
NDEA	30	93
NDBA	90	71
NDIPA	94	91
NEIPA	92	92
NMBA	200	170
NMPA	105	83

Case Study 2: Method for API and Drug Product



LCMS

- **Poor DP solubility** due to excipients,
  - Slurry extraction from the excipients matrix is efficient, with good recovery for nitrosamines (in the absence of API).
- API no solubility issues with aqueous/formic acid yet, API coeluted with multiple nitrosamines, impacting recovery
- Various orthogonal LCMS methods were evaluated; coelution continued to be an issue
- Switched to GCMS, dissolved in aqueous, and extracted to DCM;

Matrix-related interference issues were resolved.

Case 2: Screening				
Nitrosamine	Recovery (%) LCMS		Recovery (%) GCMS	
	API	DP	API	DP
NDMA	<50%	<50%	96	89
NDEA	<50%	<50%	96	87
NDIPA	<50%	<50%	109	88
NEIPA	<50%	<50%	109	85

Case Study 3: NDSRI Method for API and Drug Product

- **Tablets don't dissolve** in common diluents, extraction in Methanol/Formate Buffer mixture
- NDSRI coelutes with API, baseline resolution of API from the nitroso impurity failed.
- With **QTOF**, leveraged mass resolution to quantify nitroso API, with relatively narrow mass filter (± 0.0025 m/z). Clean instrument and good tune required for accurate analysis.
- Extraction efficiency was demonstrated through increasing volume studies (1x vs 2x) demonstrating adequate recovery of NDSRI

NDSRI could be quantified

Summary

Nitrosamines present an analytical challenge requiring highly sensitive and robust methods. This can only be achieved via MS techniques, such as GC-MS, High-Resolution LCMS and LC-MS/MS.

Platform Methods for typical nitrosamines can be established based on current FDA/USP methods

- Once an accurate and sensitive method is established, chromatography development can be minimal.
- Challenges remain for individual sample matrices as composition of API and Drug Product, solubility, structural similarities to impurities will all play a factor.
- Having orthogonal separations pre-developed can assist in rapid screening for impurities and accelerate the development and validation process.
- Nitrosamine Drug Substance Related Impurities (NDSRI) need high-res MS and MS/MS as a primary testing strategy
- Single Reaction Monitoring and Fragmentation can provide clarity on the identity of impurity peaks observed with masses consistent with NDSRIs.

Want to know more? Contact us at www.skpharmteco.com