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Canadian-Based
Global Health Company

ANALYTICAL METHODS FOR NDSRIS: CHALLENGES & TROUBLESHOOTING

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AGENDA

1. Framework & Regulatory Foundation
2. Analytical Target Profile -Challenges
3. Method Validation
4. Key Challenges & Mitigations

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FRAMEWORK AND REGULATORY FOUNDATION

Specification

Recommended Acceptable Intake Limits for NDSRIs Guidance:

- Structural methodology for predicting carcinogenic potency (using the **CPCA**) to calculate allowable daily intake
- Establishing specifications in drug products: **if a nitrosamine impurity is detected above the LOQ**, the manufacturer or applicant should develop a strategy to ensure that the nitrosamine level remains within the recommended AI limit
- The control strategy should include specification limits for the identified nitrosamine impurities in the drug product if the confirmatory testing **finds levels of the nitrosamine impurity above 10 percent of the recommended AI limit,** including in stability samples at expiry.

Analytical Procedure

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TECHNOLOGY / PLATFORM SELECTION

	NITROSAMINE TYPE	PREFERRED PLATFORM	RATIONALE	Methods
	Small-Molecule Volatile e.g., NDMA, NDEA, NMPA, NMBA	**GC-MS/MS** Headspace or direct injection	Proven high sensitivity for volatile, low-MW compounds with well-established regulatory methods	Published platform methods available for small-molecule nitrosamines
	Small-Molecule Non-Volatile / Polar	**LC-MS/MS (triple quad)**	Versatile platform offering high sensitivity with broad availability across laboratories	Published platform methods available for small-molecule nitrosamines
	NDSRIs	**LC-MS/MS or LC-HRMS** Q-TOF, Orbitrap	Non-volatile profile favours LC-based detection; HRMS enables structural confirmation with enhanced specificity Full-scan accurate mass supports identification of unanticipated nitrosamine species	Each NDSRI requires a compound-specific method; no universal approach available

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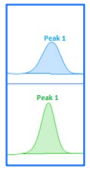
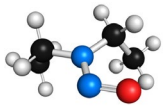
THE ANALYTICAL TARGET PROFILE

Analytical Target	Nitrosamine-Specific Requirement
Purpose	Quantitation of specific nitrosamine impurities (NDSRIs) in DS and/or DP
Target Analytes	Identified nitrosamines from risk assessment (NDSRIs per API structure)
Reportable Range	From LOQ (≤10% of AI limit) to 120% or 150% of specification limit
Accuracy	Mean recovery at LOQ; and at higher levels
Precision	Repeatability with RSD criteria at LOQ; at specification level (Repeatability and Intermediate Precision)
Specificity	Resolution from API, degradants, excipient peaks, and isobaric compounds
Sensitivity (LOQ)	Sufficient to quantify at ≤10% of AI (often sub-ppb for low-AI nitrosamines)
Robustness	Method must perform within defined MODR under routine lab variability

THE ANALYTICAL TARGET PROFILE

Nitrosamine-Specific Risk Factors/Challenge

Risk Category	Critical Parameters	Impact
 Artifact Formation	pH, nitrite, acidic conditions, contact time, temperature	False positives → regulatory risk
 Matrix Effects / Ion Suppression	Sample concentration, excipient profile, mobile phase composition	Sensitivity loss, quantitation bias
 Analyte Instability	Storage conditions, solvent selection, light exposure	Recovery loss

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THE ANALYTICAL TARGET PROFILE

Nitrosamine-Specific Risk Factors/Challenge

Risk Category	Critical Parameters	Impact
Reference Std Availability	NDSRI synthesis complexity, purity characterization	Quantitation uncertainty
Chromatographic Selectivity	Column chemistry, gradient profile, mobile phase pH	Co-elution with isobaric species
Solvent/Reagent Purity	Nitrite in water, NOx in lab air, reagent grade	Elevated blanks

Critical: Artifact formation is a challenge for nitrosamine methods.

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SAMPLE PREPARATION OPTIMIZATION

Technique	When to Use	Key Considerations
Direct Dilution	Drug Substance testing, simple matrices	Simplest approach; minimizes artifact risk; maximize API loading for sensitivity
Liquid-Liquid Extraction	Drug Products with complex excipients	Selective cleanup; risk of analyte loss for polar nitrosamines
Solid-Phase Extraction	Matrix suppression	Reduce matrix interference; sorbent chemistry must match analyte properties

Artifact Mitigation & Trouble shooting:

- ✅ Use ultra-pure water/solvents
- ✅ Minimize acidic sample solvents during sample preparation
- ✅ Keep preparation time short; prepare samples fresh
- ✅ Control sample preparation temperature (ambient or below)
- ✅ Evaluate stability storage of sample solutions
- ✅ Evaluate reference standard stability

CHROMATOGRAPHIC & MS/MS OPTIMIZATION

Category	Specific Parameters
Sample Preparation ✓	*Solvent, extraction time, sample concentration*
Chromatography Conditions	Column temp, gradient slope, mobile phase pH, flow rate
MS Source	temperature, cone voltage, capillary voltage

Some examples:

Chromatographic Parameters:

Column: e.g. UHPLC (1.7 µm, 2.1 × 100 mm) — screen C18/phenyl/amide

Mobile Phase: Organic/formic acid

Gradient: optimize slope

Flow Rate: 0.3–0.5 mL/min

Column Temperature: 30–40°C range

Injection Volume: 1–20 µL

Critical: Ensure resolution from C-nitro/O-nitroso isobaric interferences

MS/MS Parameters:

Ionization Mode: ESI+ (most common);

MRM Transitions: Quantifier + qualifier ion; confirm NO loss specificity

Source Parameters: capillary voltage, temp/gas, cone voltage

Dwell Time: Balance sensitivity

Divert Valve: Divert high-concentration API from MS source to protect sensitivity

LOQ: achieve ≤10% AI

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ANALYTICAL METHOD VALIDATION…

Method Validation	Testing	Changes in AI

LOQ/LOD	• LOQ ≤ 10% of AI limit; LOD ≤ 3% of AI limit
Specificity	• No interference from matrix • MRM selectivity confirmed
Accuracy	• Recovery at LOQ, mid, high levels
Precision	• Repeatability • Intermediate Precision
Linearity	• Established from LOQ to above specification • Range
Robustness	• MODR

- ≤10% AI (through shelf life) ✅
- ≤ Acceptable Intake ✅

- Change in AI: potential method to be redeveloped
- Differences in AI in different regions:
  Example:
  FDA: 1500ng/day
  EMA: NMI follow
  ICH Q3B

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IN CONCLUSION: CHALLENGES & MITIGATION

Challenge	Mitigation Strategy
Ultra-trace sensitivity (ppb or lower detection limits)	Optimized MS source parameters via DoE; maximize API loading
Artifact formation (in-situ nitrosamine generation)	Control sample preparation; ultra-pure reagents; minimal acidic conditions
Matrix complexity (suppression, co-elution)	Matrix-specific methods; orthogonal sample preparation (LLE/SPE)
Structural diversity (NDSRIs, varying physiochemistry)	Platforms (LC-MSMS; LC-HRMS); product-specific method development
Lack of reference standards/Unstable reference standards	NDSRIs not commercially available Reference standard storage and handling
Instrument contamination	Solvent/reagent controls; environmental controls; rigorous blank monitoring
Different AI limits – different regions	Difference in AI limits results in additional method development/validation; changing from HPLC (UV method) to LC-MSMS or vice versa

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THANK YOU

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