Testing Methods for *N*-Nitrosamines Monitoring in Pharmaceuticals

Mrunal Jaywant, Ph.D.
Senior Director – R&D
U.S. Pharmacopeia
mxj@usp.org

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The standard of trust

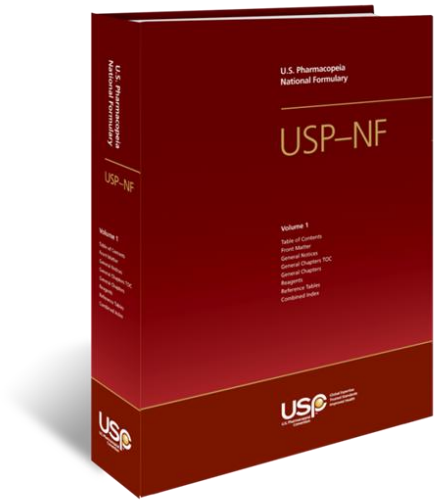
Agenda

- Overview of USP Nitrosamine activities
- Method Performance Characteristics & Factors which impact sensitivity and selectivity
- GC <1469> Nitrosamine Impurities: Testing Methods & Analytical Challenges

Overview of USP Nitrosamine activities

1 Documentary Standard

To address the nitrosamine impurities safety concern from a pharmacopeia perspective, USP Joint Expert Subcommittee (JSC) was convened since February 2020 to develop *General Chapter <1469> Nitrosamine Impurities.*



USP's Nitrosamine Program

2 Reference Standard

Eight USP Reference Standards have been established to support *General Chapter <1469> Nitrosamine impurities*

- *N*-Nitrosodimethylamine (NDMA) (1 mg/mL in MeOH)
- *N*-Nitrosodiethylamine (NDEA) (1 mg/mL in MeOH)
- *N*-Nitrosodiisopropylamine (NDIPA) (1 mg/mL in MeOH)
- *N*-Nitrosodibutylamine (NDBA) (1 mg/mL in MeOH)
- *N*-Nitrosoethylisopropylamine (NEIPA) (1 mg/mL in MeOH)
- *N*-Nitrosomethylaminobutyric Acid (NMBA) (1 mg/mL in ACN)
- *N*-Nitrosomethylphenylamine (NMPA)
- Deutero *N*-Nitrosodimethylamine (NDMA-d_6)

- Additional Impurities to be developed
  - 1-Cyclopentyl-4-NitrosPiperazine (CPNP)
  - 1-Methyl-4-NitrosoPiperazine (MNP)
  - *N*-Nitroso pyrrolidine (NPYR)
  - *N*-Nitrosodiethanolamine (NDELA)
  - *N*-Nitrosodipropylamine (NDPA)
  - *N*-Nitrosomethylethylamine (NMEA)
  - *N*-Nitrosomorphpline (NMOR)
  - *N*-Nitrosopiperidine (NPIP)

Overview of USP Nitrosamine activities

Timeline: GC <1469> Nitrosamines Impurities

1 — 01 Sep 2020

GC <1469> publication in the PF

<1469> published in Pharmacopeial Forum 46 Issue 5, available on-line

2 — 30 Nov 2020

End commentary

Comments period end (ALL stakeholders are encouraged to participate)

3

JSC address comment and review proposal

Sub-committee address public comments and revise chapter

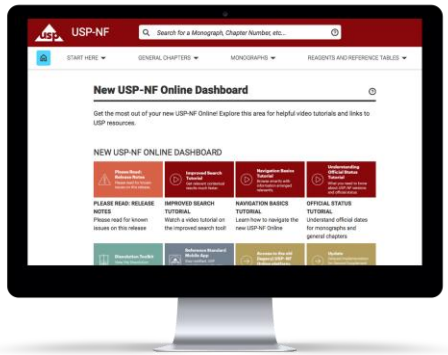
4

Standard is balloted

GC is balloted for approval by Chemical Analysis General Chapter Expert committee

5 — 1st June 2021

Publish to USP-NF 2021 Issue 3



6 — 1st Dec 2021

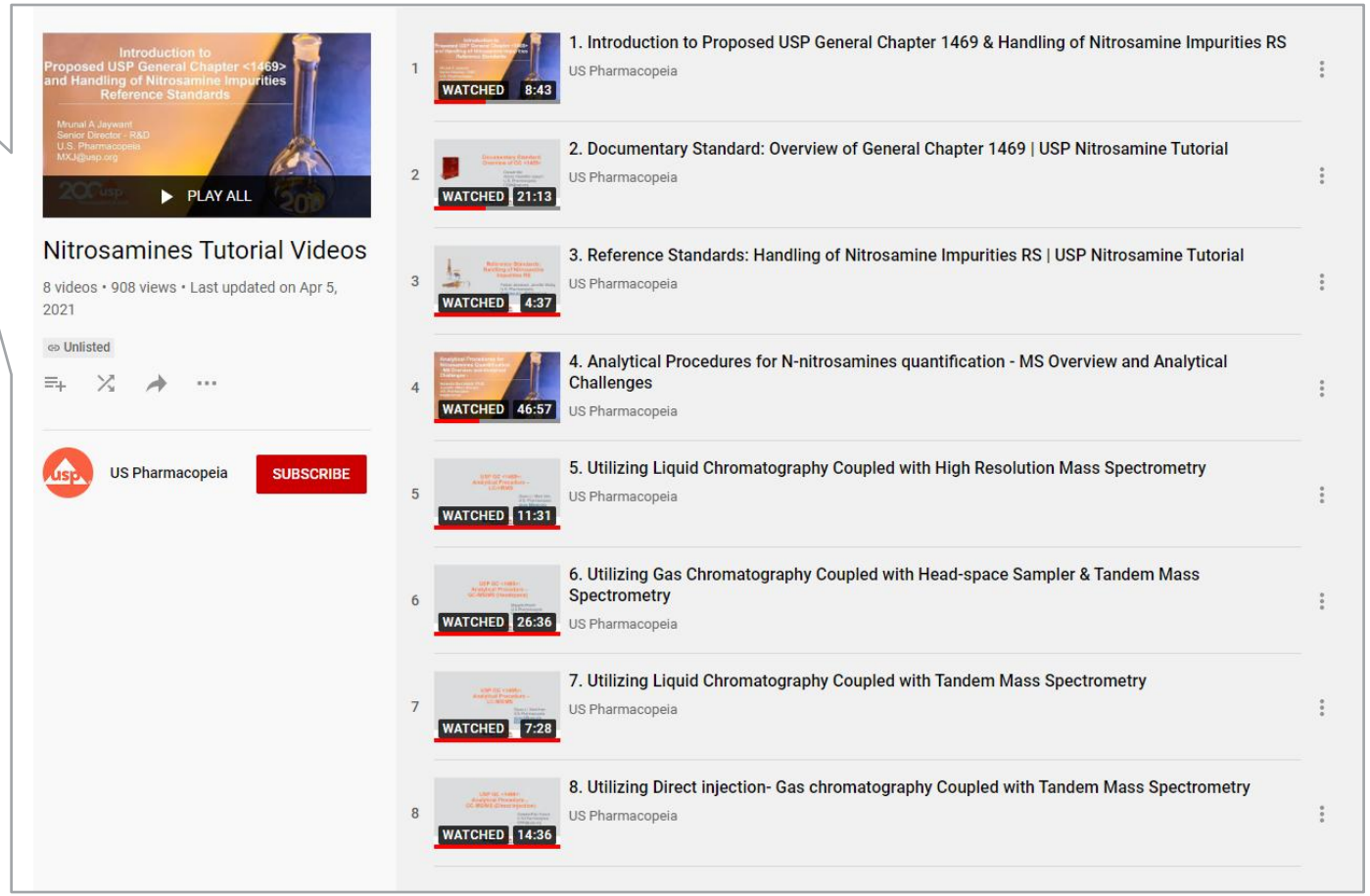
GC <1469> becomes official

On-going development and future considerations

USP is supporting manufacturers and regulators with standards as well as other tools and solutions to test for and assess the risk of nitrosamine impurities

1. SCIENTIFIC DISCUSSIONS & PUBLICATIONS

- Scientific Webinars
- Nitrosamine Tutorial Videos – Youtube: Playlist
- Review article on Testing Methods (2021)



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1. SCIENTIFIC DISCUSSIONS & PUBLICATIONS

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- Nitrosamine Tutorial Videos – Youtube: Playlist
- Review article on Testing Methods (2021)

The Playlist is consisted of 8 modules covering the following topics:

1. Introduction to Proposed USP GC <1469> & Handling of Nitrosamines Impurities
2. Documentary Standard: Overview of General Chapter <1469>
3. Reference Standards: Handling of Nitrosamines Impurities RS
4. Analytical Procedures - MS Overview and Analytical Challenges
5. USP GC <1469> Procedure 1: LC-HRMS
6. USP GC <1469> Procedure 2: Headspace GC-MS/MS
7. USP GC <1469> Procedure 3: LC-MS/MS
8. USP GC <1469> Procedure 4: Direct Injection GC-MS/MS

2. USP EDUCATION

- Addressing Impurities in Drug Products and Drug Substances: ICH Guidelines Q3-A, B, C, D and M7 (Available on demand)
- **USP Course on Nitrosamines GC <1469>** **(15 and 16 June 2021)**

3. TEST PROCEDURES

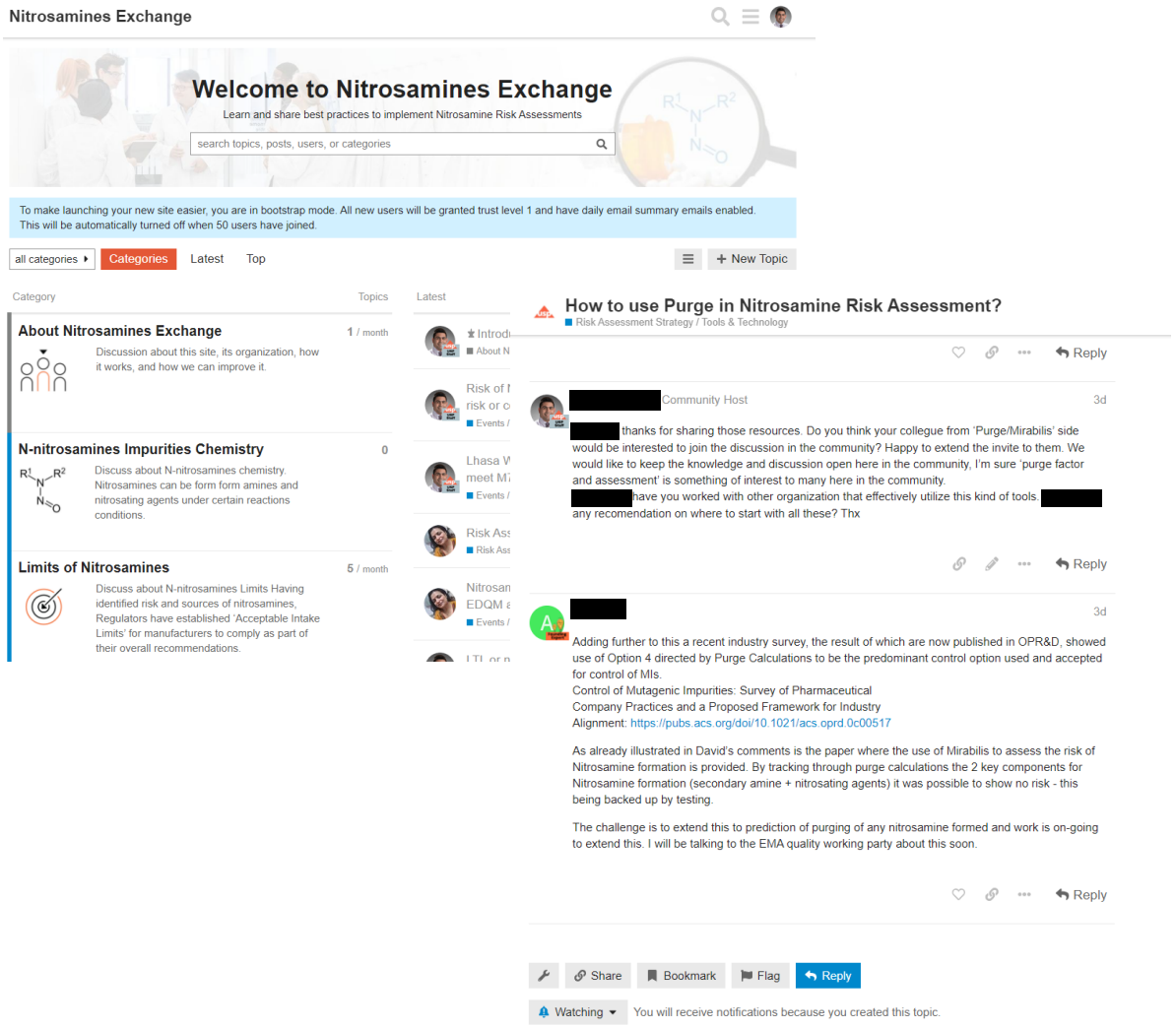
- For Drug Products: Ranitidine, Metformin, Rifapentine
- For detection of nitrosamine impurities in solvents and water used in the manufacturing process
- PF 45 (6) Stimuli Article including methods to determine *N*-nitrosamines and PAHs in elastomeric components for inhalation packaging/delivery systems."

4. OTHER ACTIVITIES

- Nitrosamine Exchange Knowledge Community - Nitrosamines Risk Assessment

Nitrosamine Exchange Community

Nitrosamine Exchange
Risk Assessment Community

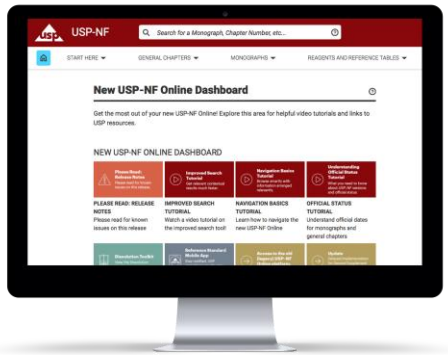


Join us at: http://nitrosamines.usp.org

GC <1469> Nitrosamines Impurities

Content and Rationale

1. INTRODUCTION
2. NITROSAMINE IMPURITIES
3. SOURCES OF NITROSAMINES
4. NITROSAMINE RISK ASSESSMENT - DEVELOPMENT OF A CONTROL STRATEGY
5. LIMITS OF NITROSAMINE
6. TESTING FOR THE PRESENCE OF NITROSAMINES
7. TEST METHOD PERFORMANCE CHARACTERISTICS OF NITROSAMINE METHODS
8. ANALYTICAL PROCEDURES
9. ADDITIONAL SOURCES OF INFORMATION



Analytical Procedure for *N*-Nitrosamines

What should be the required sensitivity for analytical methods?

Nitrosamine Level ≤ **Acceptable Intake (AI)**
toxicologically required limit

e.g.: NDMA in Valsartan (96ng/day / 320mg/day)
AI= 0.3ppm (0.0003mg/mL)

- Limit of Quantification (LOQ) - ICH Q2 (R1): provides the minimum level at which an analyte can be quantified with acceptable accuracy and precision
  - LOQ preferred over LoD for impurity testing and decision-making
  - LOQ should be used to define the required analytical sensitivity for impurity testing.
- Limit of Detection (LOD) - ICH Q2 (R1): LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value
  - Experts recommended not to use LOD for setting limits and not to use technical limits as nitrosamines may not be avoidable completely in many cases

As a minimum requirement the method should have: LOQ ≤ AI and meet regulatory recommendations...

Analytical Procedure for *N*-Nitrosamines

Required sensitivity for analytical methods

- FDA Control of Nitrosamines Impurities in Human Drugs - Guidance for Industry - FDA February 2021
  - Products with MDD < 880 mg/day: LOQ ≤ 0.03ppm
  - Products with MDD > 880 mg/day: LOQ as low as reasonably practical
  - LOQ < Test Result ≤ Acceptable Intake
- EMA Assessment Report - Nitrosamines Impurities in human medicinal products - EMA 25June20
  - LOQ ≤ Acceptable limit for the respective nitrosamine impurities, taking into account the purpose of testing
    - Routine control: LOQ ≤ Acceptable Limit
    - Justify skip testing: LOQ ≤ 30% of AL
    - Justify omission from the specification: LOQ ≤ 10% of AL
  - Exceptions may be needed depending on the maximum daily dose (MDD) or if more than one nitrosamine is expected to be present. Such cases should be discussed with the relevant competent authorities.

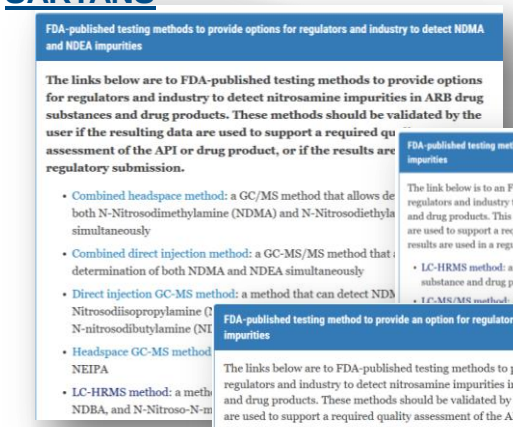
Analytical Procedure for Nitrosamines Quantification

Published Methods for Nitrosamines monitoring in APIs and DP

The low levels at which the nitrosamine impurities occur creates challenges for testing

To assist in the testing of samples the US FDA, Official Medicines Control Laboratories (OMCLs) Network of the Council of Europe, Health Canada and have also published several testing methods for nitrosamines

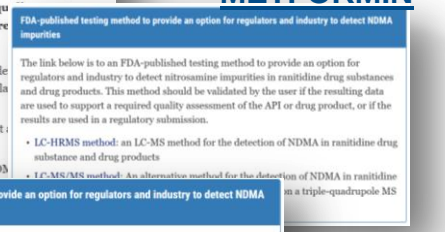
SARTANS


FDA-published testing methods to provide options for regulators and industry to detect NDMA and NDEA impurities

The links below are to FDA-published testing methods to provide options for regulators and industry to detect nitrosamine impurities in ARB drug substances and drug products. These methods should be validated by the user if the resulting data are used to support a required qu... assessment of the API or drug product, or if the results are ... regulatory submission.

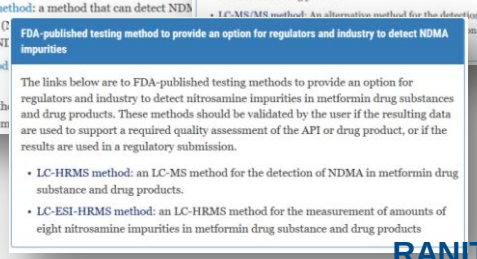
- Combined headspace method: a GC/MS method that allows de... both N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethyla... simultaneously
- Combined direct injection method: a GC-MS/MS method that ... determination of both NDMA and NDEA simultaneously
- Direct injection GC-MS method: a method that can detect NDM... Nitrosodiisopropylamine (... N-nitrosodibutylamine (NI...
- Headspace GC-MS method ... NEIPA
- LC-HRMS method: a meth... NDBA, and N-Nitroso-N-m...

METFORMIN


FDA-published testing method to provide an option for regulators and industry to detect NDMA impurities

The link below is to an FDA-published testing method to provide an option for regulators and industry to detect nitrosamine impurities in ranitidine drug substances and drug products. This method should be validated by the user if the resulting data are used to support a required quality assessment of the API or drug product, or if the results are used in a regulatory submission.

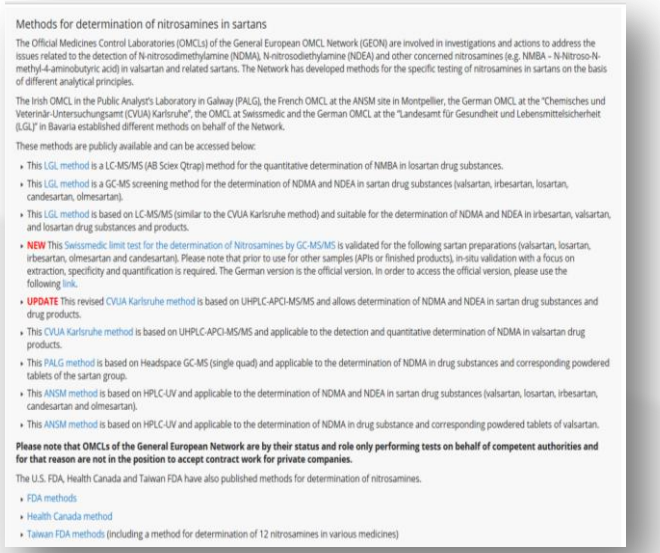
- LC-HRMS method: an LC-MS method for the detection of NDMA in ranitidine drug substance and drug products
- LC-MS/MS method: An alternative method for the detection of NDMA in ranitidine ... a triple-quadrupole MS


FDA-published testing method to provide an option for regulators and industry to detect NDMA impurities

The links below are to FDA-published testing methods to provide an option for regulators and industry to detect nitrosamine impurities in metformin drug substances and drug products. These methods should be validated by the user if the resulting data are used to support a required quality assessment of the API or drug product, or if the results are used in a regulatory submission.

- LC-HRMS method: an LC-MS method for the detection of NDMA in metformin drug substance and drug products.
- LC-ESI-HRMS method: an LC-HRMS method for the measurement of amounts of eight nitrosamine impurities in metformin drug substance and drug products

RANITIDINE


Methods for determination of nitrosamines in sartans

The Official Medicines Control Laboratories (OMCLs) of the General European OMCL Network (GEON) are involved in investigations and actions to address the issues related to the detection of N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA) and other concerned nitrosamines (e.g. NMBA - N-Nitroso-N-methyl-4-aminobutyric acid) in valsartan and related sartans. The Network has developed methods for the specific testing of nitrosamines in sartans on the basis of different analytical principles.

The Irish OMCL in the Public Analyst's Laboratory in Galway (PALG), the French OMCL at the ANSM site in Montpellier, the German OMCL at the "Chemisches und Veterinär-Untersuchungsamt (CVUA) Karlsruhe", the OMCL at Swissmedic and the German OMCL at the "Landesamt für Gesundheit und Lebensmittelsicherheit (LGL)" in Bavaria established different methods on behalf of the Network.

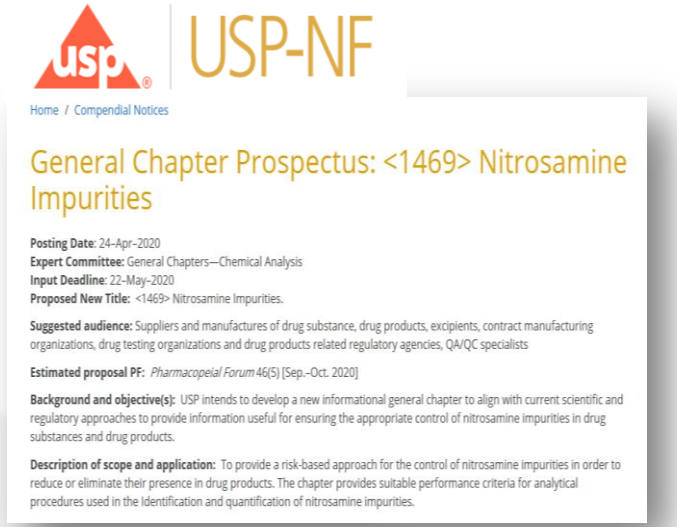
These methods are publicly available and can be accessed below:

- This LGL method is a LC-MS/MS (AB Sciex Qtrap) method for the quantitative determination of NMBA in losartan drug substances.
- This LGL method is a GC-MS screening method for the determination of NDMA and NDEA in sartan drug substances (valsartan, irbesartan, losartan, candesartan, olmesartan).
- This LGL method is based on LC-MS/MS (similar to the CVUA Karlsruhe method) and suitable for the determination of NDMA and NDEA in irbesartan, valsartan, and losartan drug substances and products.
- NEW This Swissmedic limit test for the determination of Nitrosamines by GC-MS/MS is validated for the following sartan preparations (valsartan, losartan, irbesartan, olmesartan and candesartan). Please note that prior to use for other samples (APIs or finished products), in-situ validation with a focus on extraction, specificity and quantification is required. The German version is the official version. In order to access the official version, please use the following link.
- UPDATE This revised CVUA Karlsruhe method is based on UHPLC-APCI-MS/MS and allows determination of NDMA and NDEA in sartan drug substances and drug products.
- This CVUA Karlsruhe method is based on UHPLC-APCI-MS/MS and applicable to the detection and quantitative determination of NDMA in valsartan drug products.
- This PALG method is based on Headspace GC-MS (single quad) and applicable to the determination of NDMA in drug substances and corresponding powdered tablets of the sartan group.
- This ANSM method is based on HPLC-UV and applicable to the determination of NDMA and NDEA in sartan drug substances (valsartan, losartan, irbesartan, candesartan and olmesartan).
- This ANSM method is based on HPLC-UV and applicable to the determination of NDMA in drug substance and corresponding powdered tablets of valsartan.

Please note that OMCLs of the General European Network are by their status and role only performing tests on behalf of competent authorities and for that reason are not in the position to accept contract work for private companies.

The U.S. FDA, Health Canada and Taiwan FDA have also published methods for determination of nitrosamines.

- FDA methods
- Health Canada method
- Taiwan FDA methods (including a method for determination of 12 nitrosamines in various medicines)


USP-NF

Home / Compendial Notices

General Chapter Prospectus: <1469> Nitrosamine Impurities

Posting Date: 24-Apr-2020
Expert Committee: General Chapters—Chemical Analysis
Input Deadline: 22-May-2020
Proposed New Title: <1469> Nitrosamine Impurities.

Suggested audience: Suppliers and manufactures of drug substance, drug products, excipients, contract manufacturing organizations, drug testing organizations and drug products related regulatory agencies, QA/QC specialists

Estimated proposal PF: *Pharmacopeial Forum* 46(5) [Sep.–Oct. 2020]

Background and objective(s): USP intends to develop a new informational general chapter to align with current scientific and regulatory approaches to provide information useful for ensuring the appropriate control of nitrosamine impurities in drug substances and drug products.

Description of scope and application: To provide a risk-based approach for the control of nitrosamine impurities in order to reduce or eliminate their presence in drug products. The chapter provides suitable performance criteria for analytical procedures used in the Identification and quantification of nitrosamine impurities.

US FDA
LC-ESI-HRMS, GC-MS/MS, LC-APCI-QqQ

NDMA, NDEA, NDIPA, NDBA, NEIPA, NMBA, NMPA, NDPA

Health Canada/EMA
GC-MS/MS, LC-APCI-QTrap, LC-APCI-QqQ

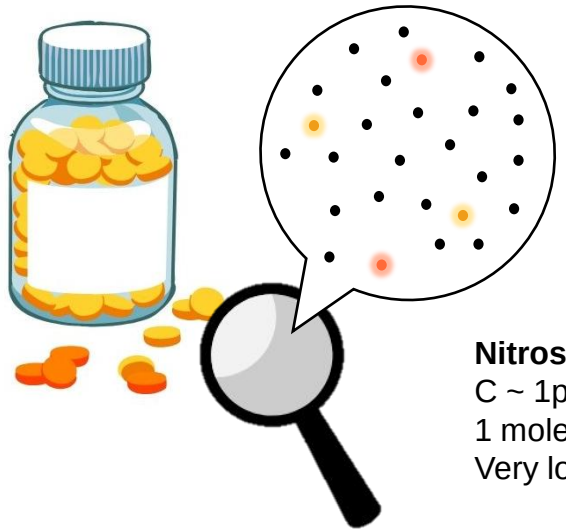
NDMA, NDEA

USP General Chapter <1469> Nitrosamine Impurities – USP/NF- 1st June 2021
(LC-ESI-HRMS, GC-MS/MS, LC-APCI-QqQ)

NDMA, NDEA, NDIPA, NDBA
NEIPA, NMBA, NMPA

Analytical Procedure for *N*-Nitrosamines

Performance Characteristics*



- Active ingredient
- Nitrosamine impurity
- Endogenous Matrix Compound

Nitrosamine Impurity
C ~ 1ppm
1 molecule out of 1,000,000 molecules
Very low concentration!

1. Sensitivity:

- Sensitive to detect and quantify *N*-nitrosamines in DPs and APIs at ppm level
- Sensitive enough to meet the proposed regulatory recommendations
- Neither LOD nor LOQ are constant values and can change over time depending on: equipment, laboratories, personnel, sample preparation and many other factors.

2. Selectivity:

- Selective for target* nitrosamines (Same method applicable to different DP? And APIs?) **those possible to be formed during the API/DP manufacturing process, storage etc – after risk assessment*

3. Accuracy and Precision: Challenges: extraction efficiency suitable recovery of trace impurities, matrix effects

4. Linearity:

- Linear within the proposed concentration range. Ex.: LOQ, 50%, 75%, 100%, 125%, 150% (including the AI)
- Note: LOQ as low as reasonably practical or ≤ 10% of AI if possible)

5. Robustness: ensure consistent/reproducible results (presence of the drug substance and the extraction efficiencies are the key factors affecting robustness)

ICH guideline Q2(R1) - VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY

*"**Specificity** is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.*

** for quantitative analysis*

Analytical Procedure for *N*-Nitrosamines

Analytical Procedure Development

Risk assessent for method development

1. **ANALYTICAL TARGET PROFILE (ATP)**
   Predefined objective that stipulates the performance requirements for the analytical procedure

2. **COMPOUNDS PROPERTIES EVALUATION** (Volatility, Solubility, Polarity, pKa, UV-Vis light absorption, Thermally labile? etc...)

METHOD DESIGN

METHOD UNDERSTANDING/ KNOWLEDGE

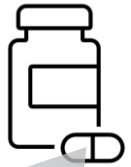
e.g.: ATP - **Selective** and **Robust** method for nitrosamines **quantification** in DS/DP **sensitive enough** to meet the proposed reporting limit threshold (e.g.: LOQ ≤ 0.03ppm, AI=0.3ppm) by LC-MS

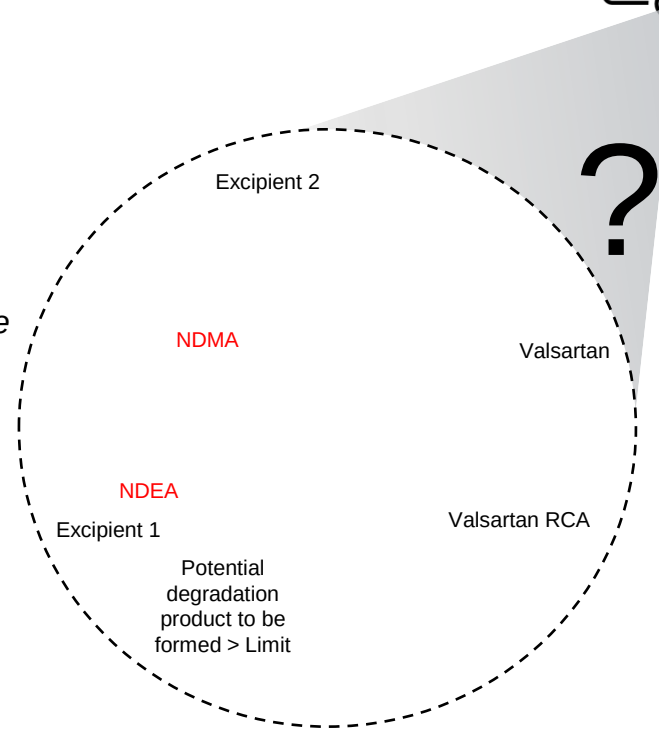
Testing methods main challenges:

- Selectivity and sensitivity
- broad coverage of *N*-Nitrosamines*
- applicability to different APIs/DP

**based on risk assessment of the manufacturing process*
*** Pilot stress testing?*
*** In-silico tools to predict degradation products?*

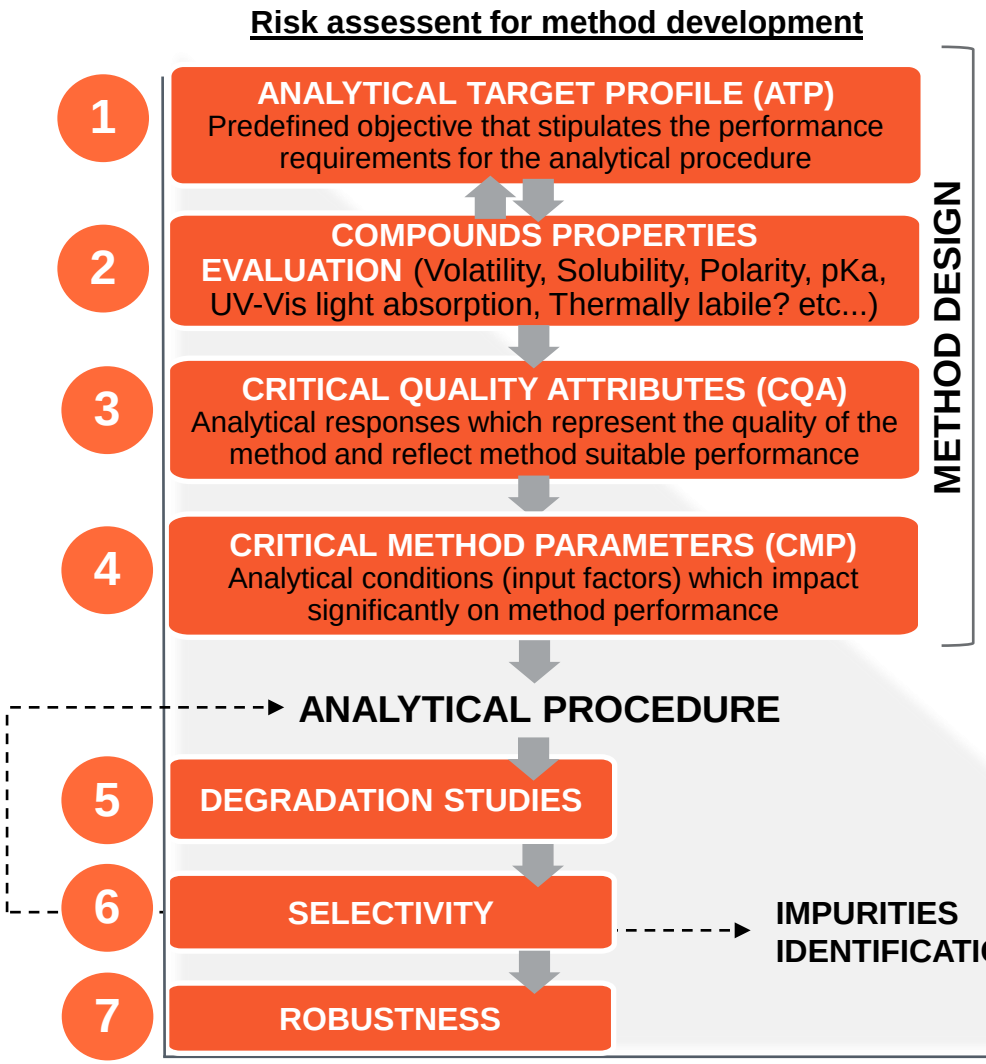
Risk assessment for method development:
Prior knowledge on potential nitrosamines impurities presence* excipients, degradation products** and other impurities





Analytical Procedure for *N*-Nitrosamines

Analytical Procedure Development



CQA

1. Selectivity

- Resolution between target compounds and adjacent peaks (chromatography)
- Resolution between isobaric compounds (MS)

2. Sensitivity

- Sensitive to detect at ppb level

3. Accuracy & Precision

4. Retention Factor (k):

- Ideal: $1 < k < 10$,
- Acceptable: $0.5 < k < 20$

CMP

SAMPLE PREPARATION

- Selective extraction of target compounds *x* Total API/target compounds dissolution?
- Sonication, extraction time, extraction repetition?
- Solid Phase Extraction (SPE) to concentrate trace impurities enhancing sensitivity?
- Reduce/minimize matrix effect

CHROMATOGRAPHY*

- Stationary phase: chromatographic columns
- Organic solvent
- pH of mobile phase
- Use of additives (ion-pair reagents, acids etc)
- Column temperature (especially for ionizable compouds)

** Primary conditions which impact on Selectivity and Retention Factor*

MASS SPECTROMETRY

- Ionization Source/Parameters (sensitivity and selectivity)
- MS resolving power (selectivity)
- Acquisition data strategies (enhance sensitivity and selectivity)
- Reduce/minimize matrix effect

DATA PROCESSING

- Data Processing: mass tolerance settings

Analytical Procedure for *N*-Nitrosamines Quantification

Mass Spectrometry - Overview

SAMPLE INTRODUCTION

- LC
- GC
- ICP
- CE
- Direct Injection
- Flow Injection Analysis (FIA)

IONIZATION SOURCE

- EI *(Electron Ionization)*
- CI *(Chemical ionization)*
- API *(Atmospheric Pressure Ionization)*
  - ESI *(Electrospray Ionization)*
  - APCI *(Atm. Pressure Chemical Ionization)*
  - APPI *(Atm. Pressure Photoionization)*
- MALDI
- *DESI *(Desorption Electrospray Ionization)*
- **DART *(Direct Analysis in Real Time)*
- FAB *(Fast Atom Bombardment)*

ANALYZER

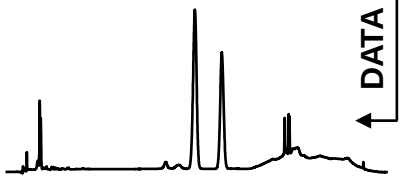
- Quadrupolo
- Ion Trap
- ToF *(Time-of-Flight)*
- *FT-ICR (Fourier Transformed-Ion Cyclotron Ressonance)*
- Orbitrap
- Tandem MS
  - Triple Quadrupole (QqQ)
  - Ion-Trap (IT): 3D or linear
  - QToF/IT-ToF
  - Q-Orbitrap
  - LIT-Orbitrap
  - Qq-Linear Ion Trap
  - Q-Orbitrap-LIT

DETECTOR

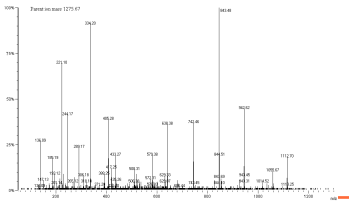
DATA SYSTEM

DATA PROCESSING

CHROMATOGRAMS



MASS SPECTRUM

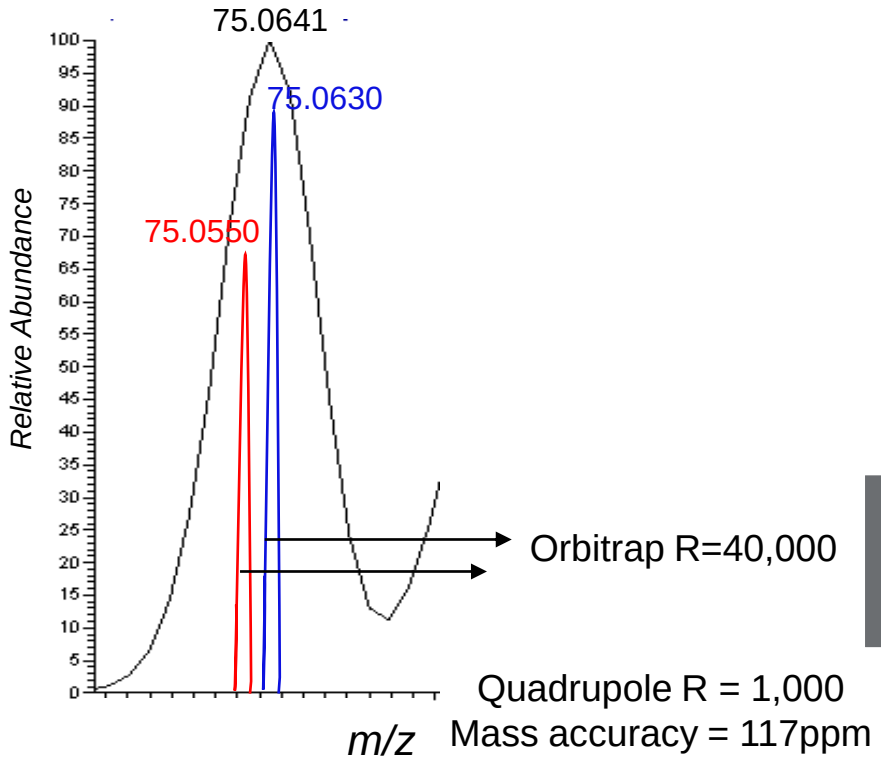


DATA ACQUISITON STRATEGIES

Data-Dependent Acquisition (DDA)
Data-Independent Acquisition (DIA)
Targeted Data Acquisition (TDA**)**
etc...

Resolving Power and Mass Accuracy: Impact on Selectivity

Example: NDMA with DMF



Higher resolving power = greater selectivity

- With low mass, resolving power only peaks differing by 1 mass unit can be separated and the recorded masses are then the nominal masses
- Mass spectrometers with insufficient mass resolving power do not allow distinguishing ions having the same nominal mass but different exact masses (i.e., isobaric ions).
- The increase in the mass resolving power narrows the peak width, allowing:
  - Peaks differing by a small *m/z* increment to be resolved
  - Better accuracy of the mass measurement
- With sufficient high mass resolving power, the contaminant could be determined from the experimentally measured accurate mass

Analytical Procedure for *N*-Nitrosamines Quantification

Mass Analyzer: Lack of selectivity

Yang J. et al. A Cautionary Tale: Quantitative LC-HRMS Analytical Procedures for the Analysis of *N*-Nitrosodimethylamine in Metformin. The AAPS Journal. 2020

Table 2. Comparison of mass spectrometry (MS) conditions used in this study (FDA) and the private laboratory method description

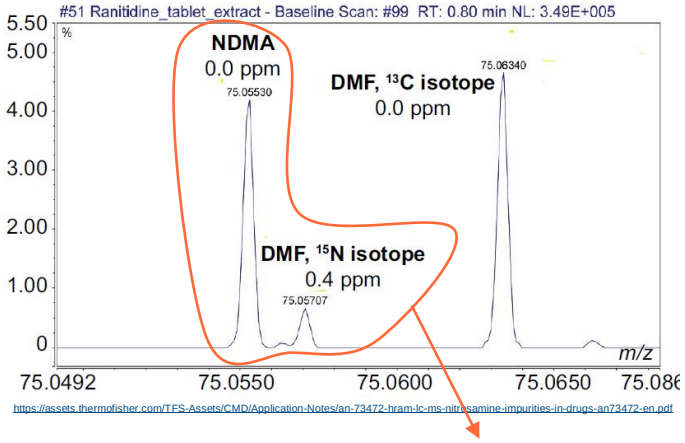
MS Conditions	Private laboratory	FDA
Instrument	QToF	Orbitrap
Ionization mode	APCI, positive	APCI, positive
Data acquisition	MRMHR	Targeted MS2
MS scan	50–450 m/z	40–90 m/z
Mass resolution	>25,000[a]	45,000[b]
Transition(s)	75.0553 → 75.0553	75.0553 → 75.0553
	83.0997 → 83.0997	83.0997 → 83.0997

- High content of NDMA was reported by a private laboratory in metformin drug products
- FDA analyzed the same DPs using MS system with higher resolution
- presence of an **interfering substance (DMF)** which **coeluted** with **NDMA**:
  - insufficient mass resolution or accuracy in data acquisition
  - inappropriate mass tolerance setting in data processing: A mass tolerance window of ±15 ppm or ±30 ppm was applied to obtain the EICs

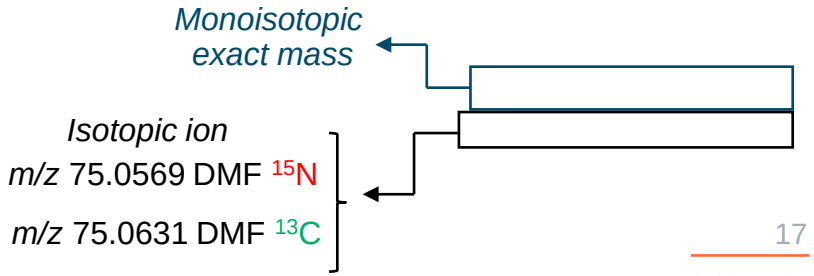
Table 1. NDMA Amounts in metformin samples reported by FDA (using FDA-1 and FDA-2 methods) and the private laboratory

Sample #	Metformin dosage and formulation	Manufacturer name as per private laboratory	Lot #	FDA-1[a,b] (ng/mg)	FDA-2 (ng/mg)	Private lab (ng/mg)
1	500 mg IR	ACI Healthcare USA, Inc.	D105061	ND[c]	ND	0.062
2	500 mg IR	ACI Healthcare USA, Inc.	C105019A	ND	ND	ND
3	500 mg IR	ACI Healthcare USA, Inc.	D105019	ND	ND	ND
4	500 mg ER	Actavis Pharma, Inc.	1376339 M	0.021[d]	0.021	0.364

INSUFICIENT MASS RESOLUTION



A minimum resolution of **45,000** and maximum mass tolerance of **15 ppm** are required to prevent overestimation of NDMA when quantifying NDMA using the monoisotopic ion.



Analytical Procedure for *N*-Nitrosamines Quantification

Mass Analyzer: Lack of selectivity

Yang J. et al. A Cautionary Tale: Quantitative LC-HRMS Analytical Procedures for the Analysis of *N*-Nitrosodimethylamine in Metformin. The AAPS Journal. 2020

Table 2. Comparison of mass spectrometry (MS) conditions used in this study (FDA) and the private laboratory method description

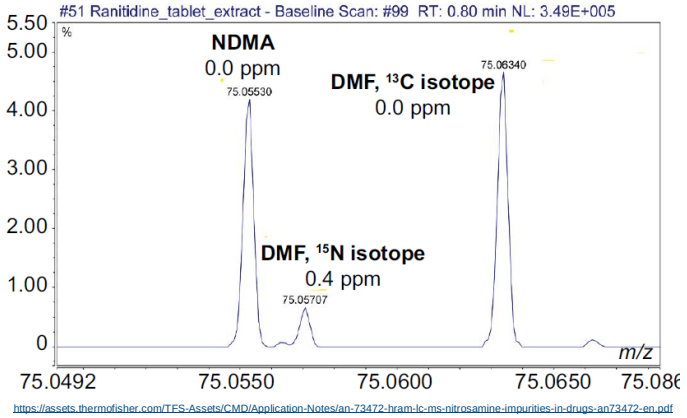
MS Conditions	Private laboratory	FDA
Instrument	QToF	Orbitrap
Ionization mode	APCI, positive	APCI, positive
Data acquisition	MRMHR	Targeted MS2
MS scan	50–450 m/z	40–90 m/z
Mass resolution	>25,000[a]	45,000[b]
Transition(s)	75.0553 → 75.0553	75.0553 → 75.0553
	83.0997 → 83.0997	83.0997 → 83.0997

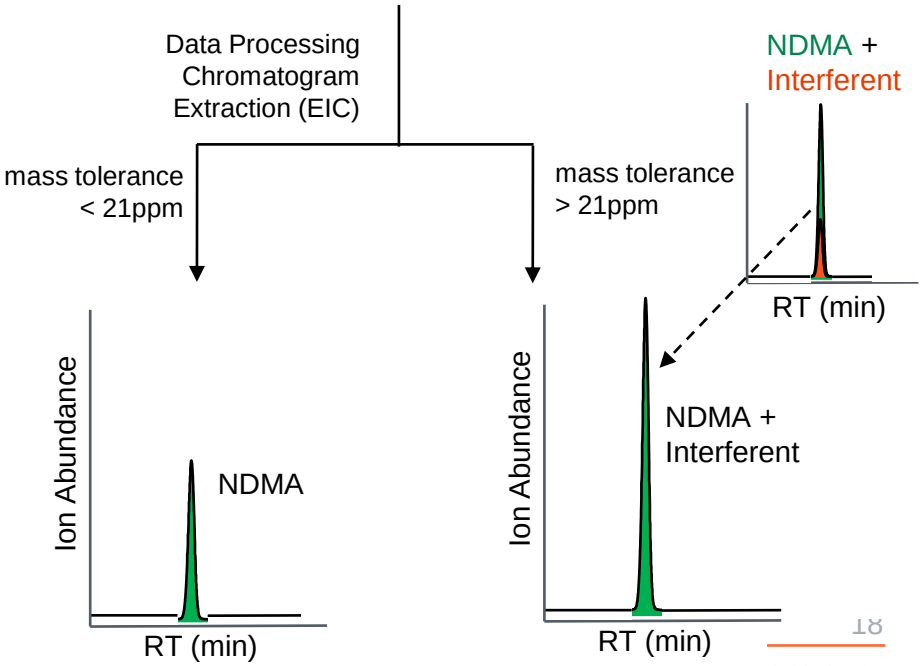
- High content of NDMA was reported by a private laboratory in metformin drug products
- FDA analyzed the same DPs using MS system with higher resolution
- presence of an **interfering substance (DMF)** which **coeluted** with **NDMA**:
  - insufficient mass resolution or accuracy in data acquisition
  - inappropriate mass tolerance setting in data processing: A mass tolerance window of ±15 ppm or ±30 ppm was applied to obtain the EICs

Table 1. NDMA Amounts in metformin samples reported by FDA (using FDA-1 and FDA-2 methods) and the private laboratory

Sample #	Metformin dosage and formulation	Manufacturer name as per private laboratory	Lot #	FDA-1[a,b] (ng/mg)	FDA-2 (ng/mg)	Private lab (ng/mg)
1	500 mg IR	ACI Healthcare USA, Inc.	D105061	ND[c]	ND	0.062
2	500 mg IR	ACI Healthcare USA, Inc.	C105019A	ND	ND	ND
3	500 mg IR	ACI Healthcare USA, Inc.	D105019	ND	ND	ND
4	500 mg ER	Actavis Pharma, Inc.	1376339 M	0.021[d]	0.021	0.364

INNAPROPRIATE MASS TOLERANCE: DATA PROCESSING





GC <1469> Nitrosamines Impurities

Section 7. Test Method performance characteristics of nitrosamines methods

- Section 7: presents the recommended method performance characteristics for quantitative analysis and limit test
- 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.
- For additional guidance on validation of alternative methods for nitrosamines, see Validation of Compendial Procedures ⟨1225⟩.

Require validation:

- existing procedures with modifications changing critical parameters
- existing method is used outside of its original purpose

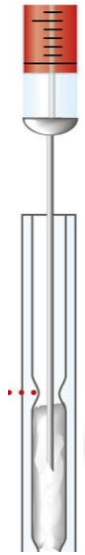
Require verification:

- existing method is used within its original purpose/scope:verify the suitability of the procedures
- For more information on how to conduct verification you can refer to USP chapter 1226 Verification of Compendial Procedures

GC <1469> Nitrosamines Impurities

7.1. CONSIDERATION 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.



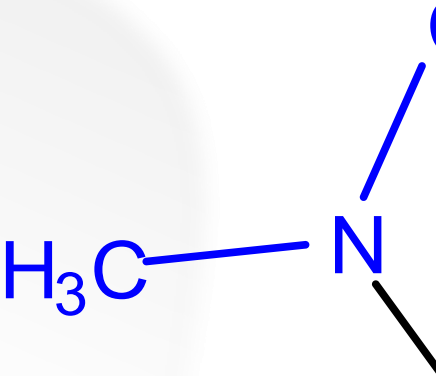
Injection port (GC System): High Temperature

In-situ formation of NDMA in GC-MS analysis

Dialkylamines (dimethylamine):

- degradation product of the API: *total dissolution of the DS containing dimethylamino-group should be avoided when applying GC techniques.*
- *High concentration of the API, when injected in the GC can generate nitrosamines in the injection port if a nitrosating agent is present: sample extractions should be modified to prevent the solubilization of the API while maintaining the extraction efficiency for nitrosamines present in the material.*
- process impurity
- counter ion of the salt form of the API

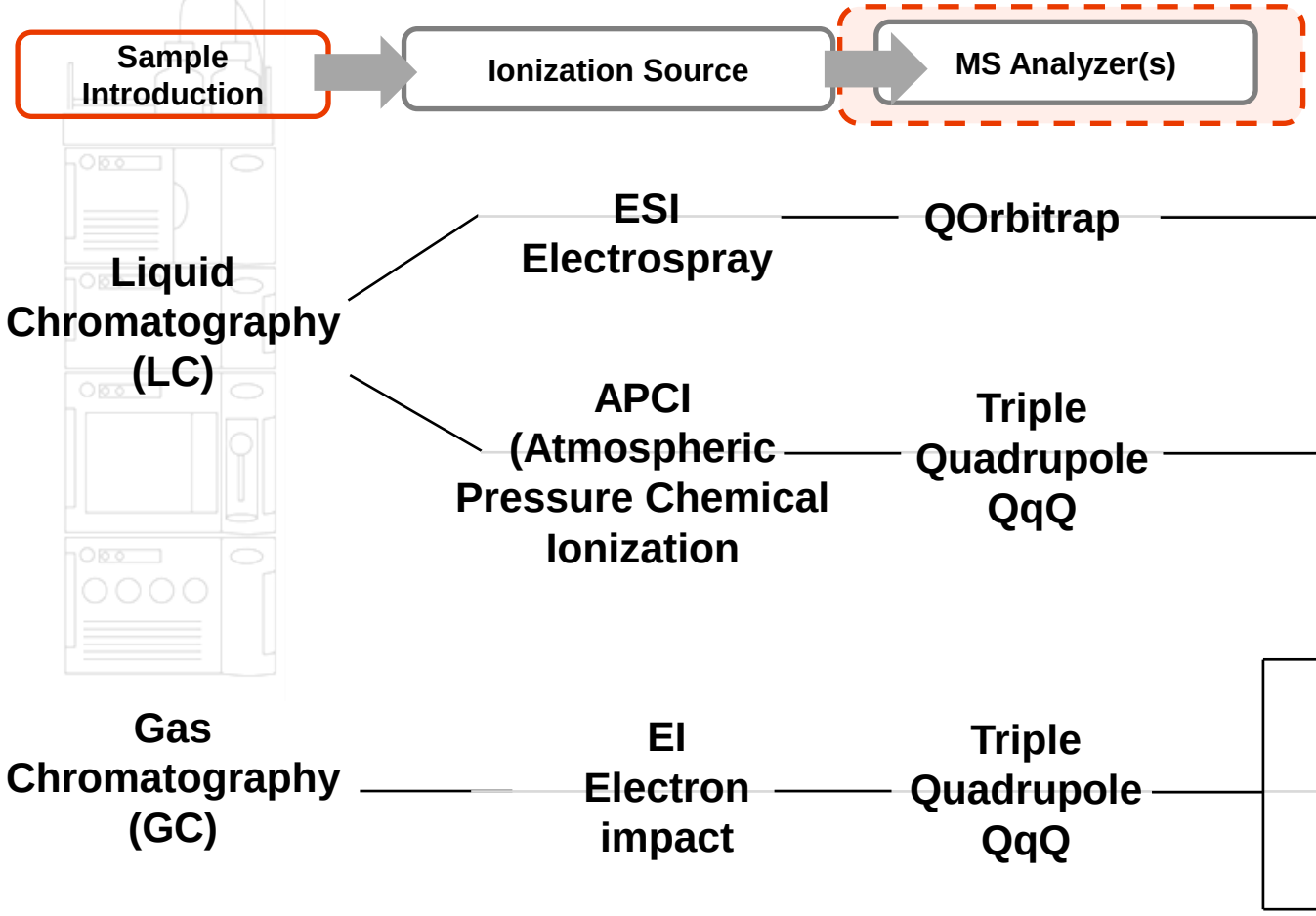
...in the presence of nitrite and acid can lead to *in situ* formation of nitrosamines as an **artifact**, especially in GC analyses.



- It is highly recommended that **LC-MS** be used for determination of NDMA in Ranitidine DS and DP.

GC <1469> Nitrosamines Impurities

8. ANALYTICAL PROCEDURES



Methods

HPLC-HRMS (QOrbitrap)
Procedure 1: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA in selected sartans by HPLC–HRMS
LC Column: L43 - PFP

HPLC-APCI-QqQ
Procedure 3: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA in selected sartans by HPLC–MS/M (Using Internal standards NDMA-d6, NMBA-d3, NDEA-D10 NDBA-D18
LC Column: L1 – C18

GC-MS/MS (Headspace) GC-EI-QqQ
Procedure 2: Quantitation of NDMA, NDEA, NDIPA, and NEIPA in selected sartans by GC–MS (using Internal Standard NDMA-d6)

GC-MS/MS (Direct injection) GC-EI-QqQ
Procedure 4: Quantitation of NDMA, NDEA, NDIPA, NEIPA, and NDBA in selected sartans by GC– MS/MS (triple-quad) – (Using internal standards NDMA-C_{13}d6)

GC <1469> Procedure 1: HPLC-ESI-HRMS

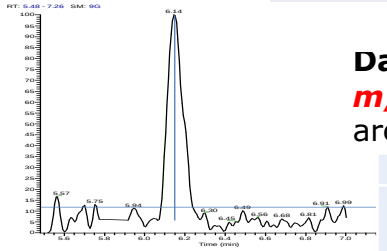
Quantitation of six nitrosamines (NDMA, NDEA, NDIPA, NEIPA, NMBA, NDBA and NMPA) in selected sartans by HPLC-HRMS*

Diluent: Methanol
Standard solution: 6.0 ng/mL(0.3ppm) each in *Diluent* of USP Nitrosamine Reference Standard (NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA)
Sensitivity solution: 1.0 ng/mL (0.05ppm) each in *Diluent* of USP Nitrosamine Reference Standard (NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA) from *Standard stock solution.*
Sample solution: 20 mg/mL of DS in *Diluent.*

Chromatographic system:
Mode: LC
Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in methanol
Column: 4.6 mm x 10-cm, 2.6 µm packing *L43 (PFP)*
Column Temperature: 40º,
Flow Rate: 0.6 mL/min
Injection Volume: 3 µL
Autosampler Temperature: 4º

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

EIC



Detector: High resolution mass spectrometer
MS conditions:
Ionization: Electrospray Ionization (ESI)
Data Acquisition Strategy: Selected Ion Monitoring (SIM)/Parallel Reaction Monitoring (PRM)

Scan settings **[Note -** Divert the API from the MS source during the elution**.]**

Impurity	NDMA	NMBA	NDEA	NEIPA	NDIPA	NDBA
Scan Type	SIM	SIM	PRM	SIM	SIM	PRM
Polarity	POS	NEG	POS	POS	POS	POS
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 Isolated for PRM	N/A	N/A	103.0866	N/A	N/A	159.1492
Resolution	30000	60000	30000	60000	60000	30000
Isolation Window	N/A	N/A	1.5 m/z	N/A	N/A	1.5 m/z
Scan Range	m/z 74.3-75.8	m/z 144.3 - 145.8	m/z 50.0-114.0	m/z 116.4-117.9	m/z 130.4 - 131.9	m/z 50.0-170.0

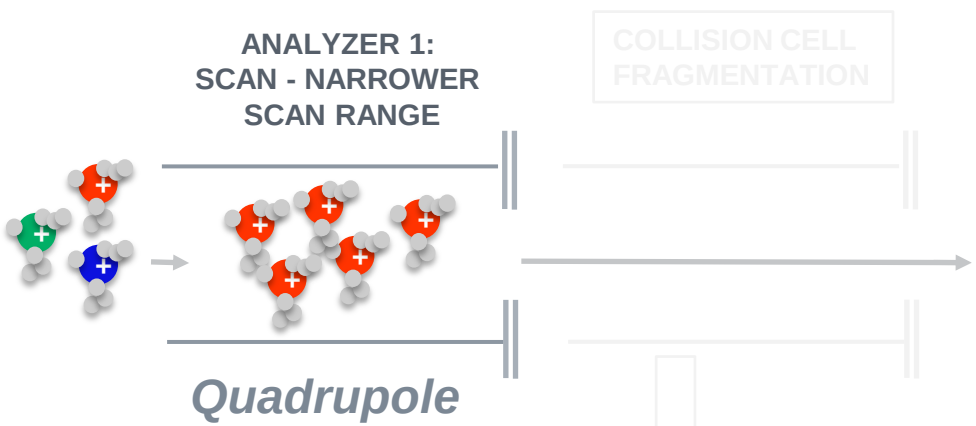
Data Processing: Peak areas in the extracted ion chromatograms (EIC) with a ***m/z* tolerance of 15 ppm** are used for quantitation. The *m/z* values extracted are listed below.

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

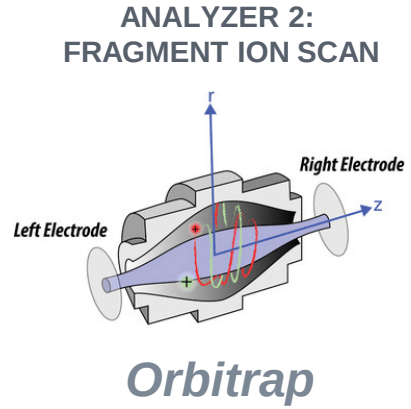
*Adapted from the LC-HRMS method published by the US FDA: https://www.fda.gov/media/125478/download

Mass Analyzer: QOrbitrap (Tandem MS) – High Resolution

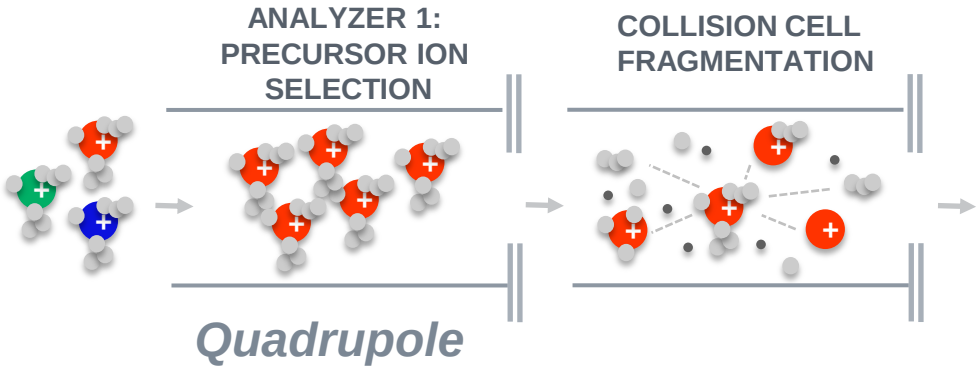
SELECTED ION MONITORING (SIM)



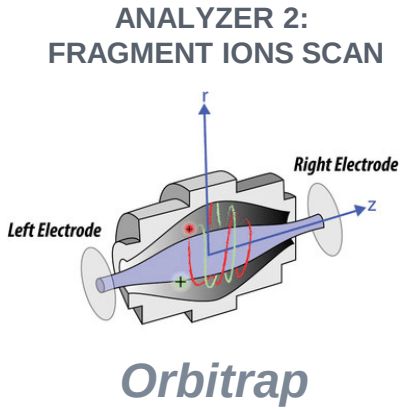
Narrower Scan Range
e.g.: NDMA *m/z* 74.3-75.8 - Peak Elution Window 2.6 -2.8 min



PARALLEL REACTION MONITORING (PRM))



NDEA
***m/z* Isolated for PRM** 103.0866
Scan Range m/z 50.0-114.0
Isolation Window 1.5 *m/z*



QOrbitrap:

- High resolution (Selectivity!)
  - –higher accuracy in mass measurements
- Higher cost: not widely available
- Data aquisition strategies for quantification:

(a) Selected Ion Monitoring (SIM):

- records the abundance of one or more specific *m/z* values that are characteristic of the compound of interest in an expected retention time window.
- In this mode, the MS does not spend time scanning the entire mass range, but rapidly changes between *m/z* values for which characteristic ions are expected: ↑ sensitivity

(b) Parallel Reaction Monitoring (PRM)

- quadrupole selects the precursor ion (selection window is usually *m/z*≤2); the precursor ion is fragmented in the collision cell; Orbitrap scans all product ions with high resolution and high accuracy.
- ↑ sensitivity and selectivity
- Since PRM does not require prior selection of transitions is relatively easier to build a PRM assay

GC <1469> Procedure 3: HPLC-APCI-QqQ

Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA in selected sartans by HPLC-APCI-QqQ

Diluent: 1% formic acid in water

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

Standard stock solution: 10 ng/mL each of USP Nitrosamine RS (NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA) in methanol

Standard solutions: Prepare Standard solutions at the concentration levels (L#) given in Table 13.
0.02 – 1.35ppm NDMA, NDIPA, NEIPA, NMBA, and NDBA
0.01 – 0.89ppm NDEA

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:
Mode: LC
Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in methanol
Column:3.0-mm × 15-cm; 2.7-µm packing L1

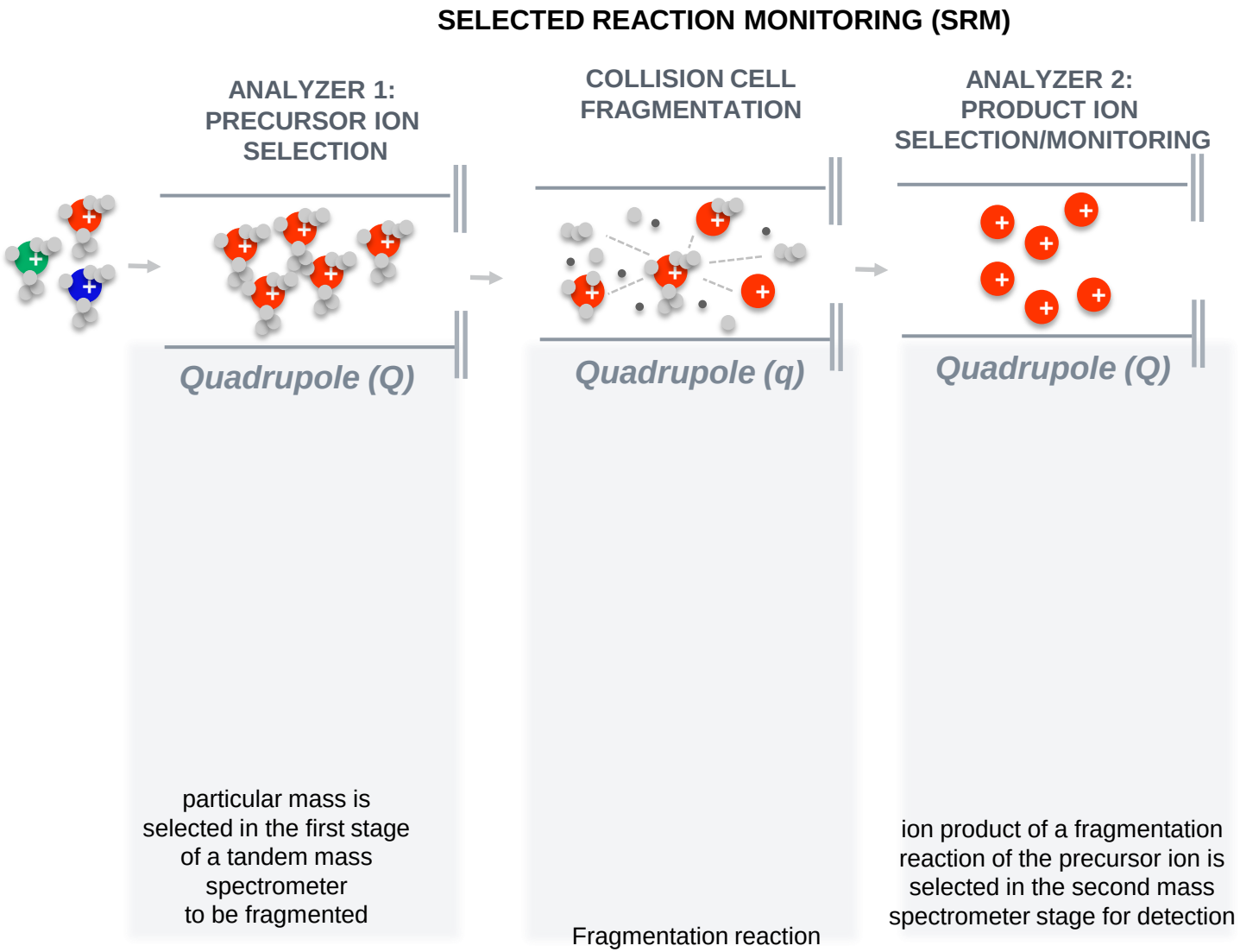
Chromatographic system:
Temperatures
Autosampler:18°
Column:60°
Flow rate: 0.5 mL/min
Flow rate to ion source: 0.5 mL/min
Injection volume: 20 µL

Detector: MS/MS (triple quadrupole mass spectrometer)
MS conditions:
Ionization: Atmospheric pressure chemical ionization (APCI)
Scan Settings:

Impurity	NDMA	NDMA-d6	NDEA	...
Acquisition mode	MRM	MRM	MRM	
Polarity	Positive	Positive	Positive	
MRM-1	*m/z* 75→43	*m/z* 81.2→46	*m/z* 103.1→75.1	
MRM-2	*m/z* 75→44.1	*m/z* 81.2→64.1	*m/z* 103.1→47.1	

System suitability requirements
Generate the response versus concentration standard curve for each nitrosamine impurity under test using the corresponding Standard solutions and perform the linear regression analysis.
Correlation coefficient:NLT 0.99
y-Intercept:NMT 25%, Standard solution L4

MS Analyzer: QqQ (Tandem MS) - Low Resolution



QqQ:

- Low resolving power
  - Lower ability to distinguish between two peaks at *m/z* values differing by a small amount
  - Lower accuracy in mass measurements
- Lower cost: triple quadrupole MS platform is more widely available than the LC-HRMS platform

- Data aquisition modes:

(a) Selected Reaction Monitoring (SRM):

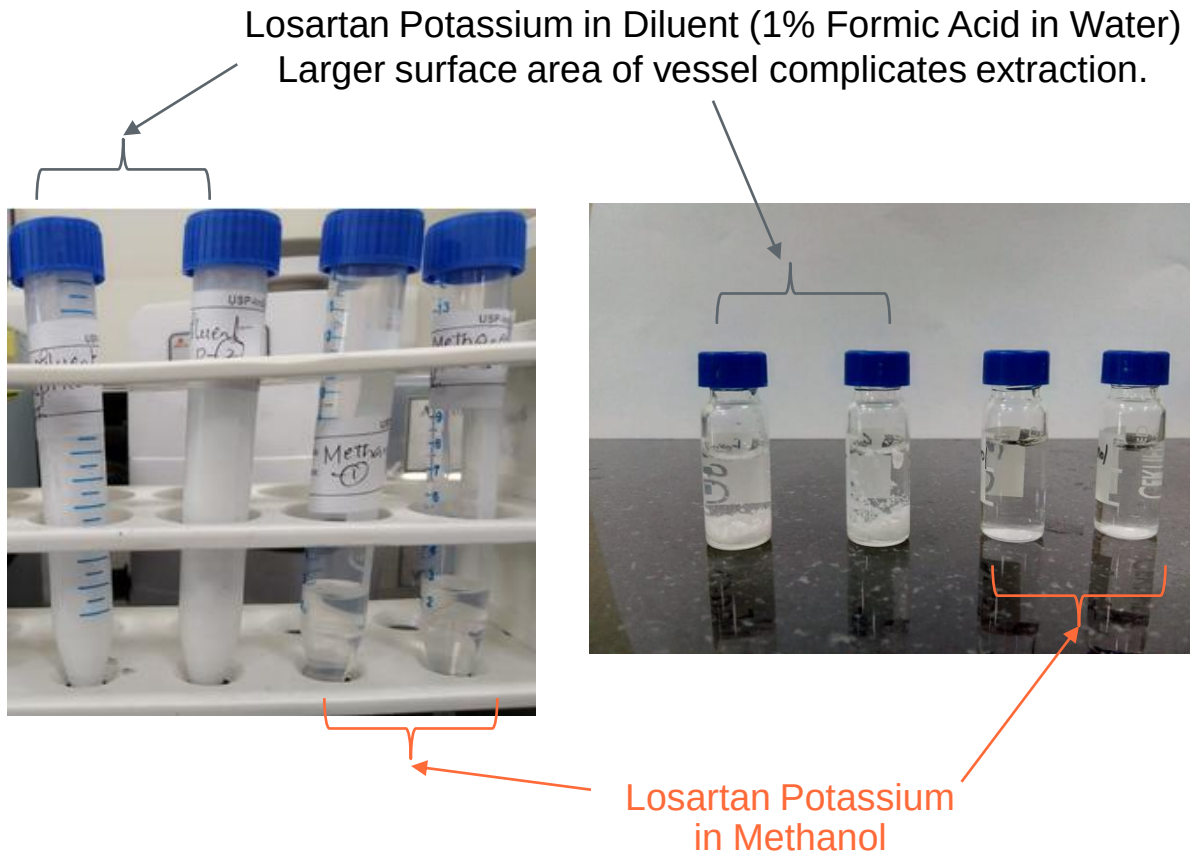
- ↑ selectivity
  - less interference of co-eluting compounds and matrix
  - works like a double mass filter which drastically reduces noise and increases selectivity
- ↑ sensitivity:
  - Better Signal-to-Noise ratio (S/N) allowing quantitation with lower limits of quantitation
  - Wider linear range of quantitation

(b) Multiple Reaction Monitoring (MRM):

- Multiple SRM transitions are measured within the same experiment
- ↑ Selectivity: allows additional selectivity by monitoring the chromatographic coelution of multiple transitions for a given analyte.

Challenges and Recommendations

- **Sample Preparation – Dispersion in Extraction Solvent**
  - The sartans are insoluble in the diluent (1% formic acid in water).
  - The sartans must be fully dispersed for efficient extraction.
  - Clumps or dry spots will cause artificially lower quantification results.
  - Using larger vessels allows for more of the sartans to deposit on the walls, limiting the interaction with solvent.



Challenges and Recommendations

Sample Preparation – Extraction Efficiency Considerations

- Drug substance is **soluble** in extraction solvent.
  - No extraction efficiency issues.
  - Must prevent the API from entering the MS by diverting LC flow to waste during elution.
- Drug substance is **somewhat soluble** in extraction solvent.
  - Extraction time and mixing are critical for proper extraction.
  - Accuracy spike experiments will be lower than target if the nitrosamines are adsorbing onto the drug substance.
- Drug substance is **insoluble** in extraction solvent.
  - Caution must be exercised due to the high potential for nitrosamines to adsorb onto the API yielding lower quantification results. This may be corrected by use of an internal standard and appropriate equilibration. Inefficient extraction will affect LOD/LOQ.
- Extraction efficiency issues? Use of different calibration procedures (Internal standards/ Matrix-matched calibration) can minimize issues?

Challenges and Recommendations

- **Sample Preparation – The Matrix Precipitation Strategy**
  - Dissolve the drug substance in an appropriate solvent to achieve the desired high concentration, then add an “anti-solvent” to precipitate the drug substances.
  - Advantages of matrix precipitation:
    - Full dissolution of the drug substance guarantees nitrosamines are transferred into solution.
    - Precipitation of the drug substance prevents potential contamination of or damage to the mass spectrometer, since it is removed from the sample solutions.
    - Can be more reproducible and less time-consuming than extraction using a solvent in which the drug substance is somewhat soluble or insoluble.
  - Sample preparation in this manner must be validated before use.

https://pubmed.ncbi.nlm.nih.gov/25576043/

Challenges and Recommendations

Use of Internal Standard

- The analyte-to-internal standard response ratio can compensate the matrix effect and ion suppression during analysis providing for a more accurate and precise method.

- ISTD can be used:
  - during sample preparation (account for extraction AND ionization efficiency issues)
  - or prior to sample injection into the LC–MS or GC-MS (account for ionization efficiency issues due to matrix effects)

- ISTD must have ionization properties and retention time similar to the analyte:
  - isotopically labelled compounds
  - structural analogue or
  - another compound that is similar to the analyte under investigation.

USP Deutero *N*-Nitrosodimethylamine RS (NDMA-d_6)

Challenges and Recommendations

Use of Internal Standard

- Isotopically labelled compounds (IL-STD):
  - IL-STD will behave almost identically to the analyte during sample preparation, chromatographic separation and MS ionization
  - Same degree of ion suppression* or enhancement will be observed for the target analyte and its isotopically labeled analogue: the ratio of the two signals should not be affected, and correct quantification can still be achieved

- Challenges for using IL-STD:
  - high cost and difficult to obtain and/or synthesis (often unavailable)
  - lack of confidence in the isotopic purity and integrity

USP GC <1469>: Analytical Procedures

- **Procedure 2:** GC-MS/MS (Headspace) GC-EI-QqQ, ISTD: NDMA-d6
- **Procedure 3:** HPLC-APCI-QqQ
  ISTD: NDMA-d6, NMBA-d3, NDEA-D10
  NDBA-D18
- **Procedure 4**: GC-MS/MS (Direct injection)
  GC-EI-QqQ
  ISTD: NDMA-13C2-d6

Panuwet, P. et al. Critical Reviews in Analytical Chemistry, (2016) 46:2, 93-105
A. Furey et al. Talanta 115 (2013) 104-122

Heavy Isotope Effect on Chromatography*

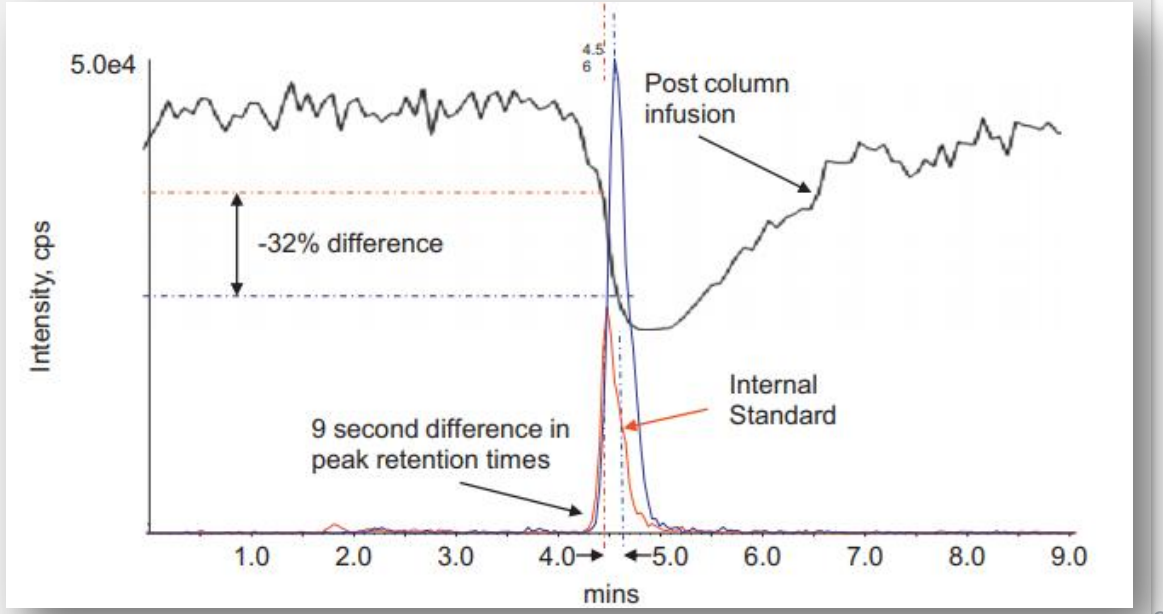


Fig. 5. Illustration of when a standard and internal standard are affected by different levels of ion suppression. This shows a 32% difference in signal response over a 9 second period of analysis.

GC <1469> Procedure 2: (HS) GC-MS/MS (QqQ)

Headspace (HS) GC-MS/MS (QqQ) - Quantitation of NDMA, NDEA, NDIPA, and NEIPA in selected sartans.

Sample solution: 200 ± 10 mg of DS and 100 mg of imidazole in a headspace vial. Add 1.0 mL of Internal standard solution (0.016 µg/mL of NDMA-d6 in methanol) and 1.0 mL of acetonitrile. Apply the stopper, cap and crimp tightly.

Chromatographic conditions:
Mode: GC
Injector: Headspace
Injection type: Split (Split ratio, 1:1 or 1:3)
[Note—Split ratio can be modified to optimize sensitivity.]
Detector: MS/MS (QqQ mass detector)
Column: 0.32-mm × 30-m fused-silica coated with a 1.0-µm layer of phase *G16*
Column temperature:

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

Carrier gas: Helium
Gas Flow: Constant flow at 1.8 mL/min (adjustment and verification are necessary for other carrier gases)
Purge Flow: 3.0 mL/min or default value

MS conditions:
Ionization: Electron Impact
Scan Settings:

Impurity	NMDA	NDMA-d6	NDEA	NEIPA	NDIPA
Acquisition mode	MRM	MRM	MRM	MRM	MRM
MRM 1	*m/z* 74 → 44	*m/z* 80 → 50	*m/z* 102 → 85.1	*m/z* 116 → 99.1	*m/z* 130.0 → 42
MRM 2	*m/z* 74 → 42		*m/z* 102 → 56.1	*m/z* 99.0 → 44.1	*m/z* 130.0→ 43.1

System suitability:
Suitability requirements
Relative standard deviation: NMT 20.0% for the ratios of the impurity standard peak response to the internal standard peak response from six replicate injections, Standard solution
Signal-to-noise ratio: NLT 10 for each nitrosamine,
Sensitivity solution Blank: No interfering peaks from the blank

Analysis
Standard solution and Sample solution Calculate the concentration (ppm) of each specified nitrosamine impurity in the portion of Drug Substance taken:
Result = (1/W) x (RU/ RST) x CST

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

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 (50 ng/mL of NDMA:13C2-d6 in methylene chloride). 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 conditions:
Mode: GC
Injector: Split/splitless
Injection type: Splitless with purge, **Purge time:**0.5 min
Column:0.25-mm × 30-m; fused-silica coated with a 1.0-µm layer of phase G16
Carrier gas: Helium, **Flow rate:** Constant flow at 1.0 mL/min
Injection volume:2 µL
Temp. Injector: 250°
Temp. Transfer line to MS detector:220°
Temp. Ionization source:250°
Column: See Table 17.

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

Detector: MS/MS (triple quadrupole mass spectrometer)
MS conditions
Ionization: Electron impact
Scan Settings:

Impurity	NMDA	NDMA-C13d6	NDEA	NEIPA	NDIPA	NDBA
Acquisition mode	MRM	MRM	MRM	MRM	MRM	
MRM 1	*m/z* 74 → 44	*m/z* 82 → 48	*m/z* 102 → 85.1	*m/z* 116 → 99	*m/z* 130 → 88	*m/z* 158 → 99
MRM 2	*m/z* 74 → 42		*m/z* 102 → 56	*m/z* 99.0 → 44.1	*m/z* 130.0→ 42	*m/z* 84→ 56

System suitability Samples: Generate the response versus concentration standard curve for each nitrosamine impurity under test using the corresponding Standard solutions and perform the linear regression analysis.

Suitability requirements
Correlation coefficient: NLT 0.98
Signal-to-noise: NLT 10 for the impurity peak, Standard solution Cal 2

Summary of Factors Impacting Sensitivity & Selectivity

SELECTIVITY

MASS ANALYZER

Low Resolution MS

- Poor accuracy in mass measurement may lead to false detection of nitrosamines
- Multiple Reaction Monitoring (MRM) will enhance selectivity
- Chromatographic method with good resolution between target compounds and adjacent peaks!

High Resolution MS

- High accuracy in mass measurement
- QOrbitrap hybrid systems: Parallel Reaction Monitoring mode (PRM) QOrbitrap MS systems: provides greatly increased selectivity and confidence compounds identification
- QToF: Multiple Reaction Monitoring

DATA PROCESSING

- Targeted data extraction strategy = Extract the ion Chromatogram for the specific ion (*m/z*)
- LC-HRMS: Use a suitable mass tolerance window to obtain the EICs (not so broad window in ppm/amu)

SENSITIVITY

MASS ANALYZER

QqQ

- Improve analysis sensitivity by acquiring data in Multiple Reaction Monitoring (MRM) mode allowing quantitation with lower limits of quantitation

High Resolution MS

- Improve analysis sensitivity by acquiring data in: PRM/SIM (eg.: QOrbitrap) / MRM (QToF)

IONIZATION SOURCE

*Chromatographic method: may improve ionization efficiency

ESI

- Majority of nitrosamines are not basic - lower ionization efficiency compared to APCI, exception: NMBA (acidic group) great ionization efficiency in ESI(-) mode, MNP/CPNP: ESI+
- Less tolerant to Matrix effects (ME). Is ME impacting on ionization efficiency?

APCI

- Higher ionization efficiency for SOME nitrosamines when compared to ESI (exception: NMBA in ESI- mode and with basic moeity:MNP/CPNP)
- More tolerant to matrix effect

SENSITIVITY and ACCURACY

SAMPLE PREPARATION PROTOCOL

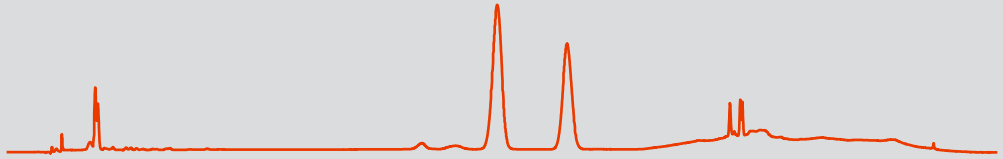
- Matrix effects? Can we improve sample preparation to reduce matrix effects? Are they being totally extracted from matrix?
  - Selective extraction *x* total dissolution extraction
  - Concentration step using SPE after selective extraction?
- Good repeatabilty? Good recovery?

ACCURACY/PRECISION

- GC-MS/MS: Inlet: high temperature - Degradation of API? Formation of nitrosamines in-situ (inlet/headspace)
- LC-MS/MS: broader range of applicability; preferred if the API may degrade due to high temperature (APCI: use ↓ temperature)

Use of internal standard!

- Compensate for extraction issues: Account for possible losses during workup or due to the thermal instability inherent to several N-nitrosamines
- Compensate for ionization efficiency due to matrix effect
- Minimize repeatability issues



Thank You

mxj@usp.org

https://www.usp.org/chemical-medicines/nitrosamine-impurities

200 usp
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