Nitrosamine impurities: Update on USP Tools and Solutions

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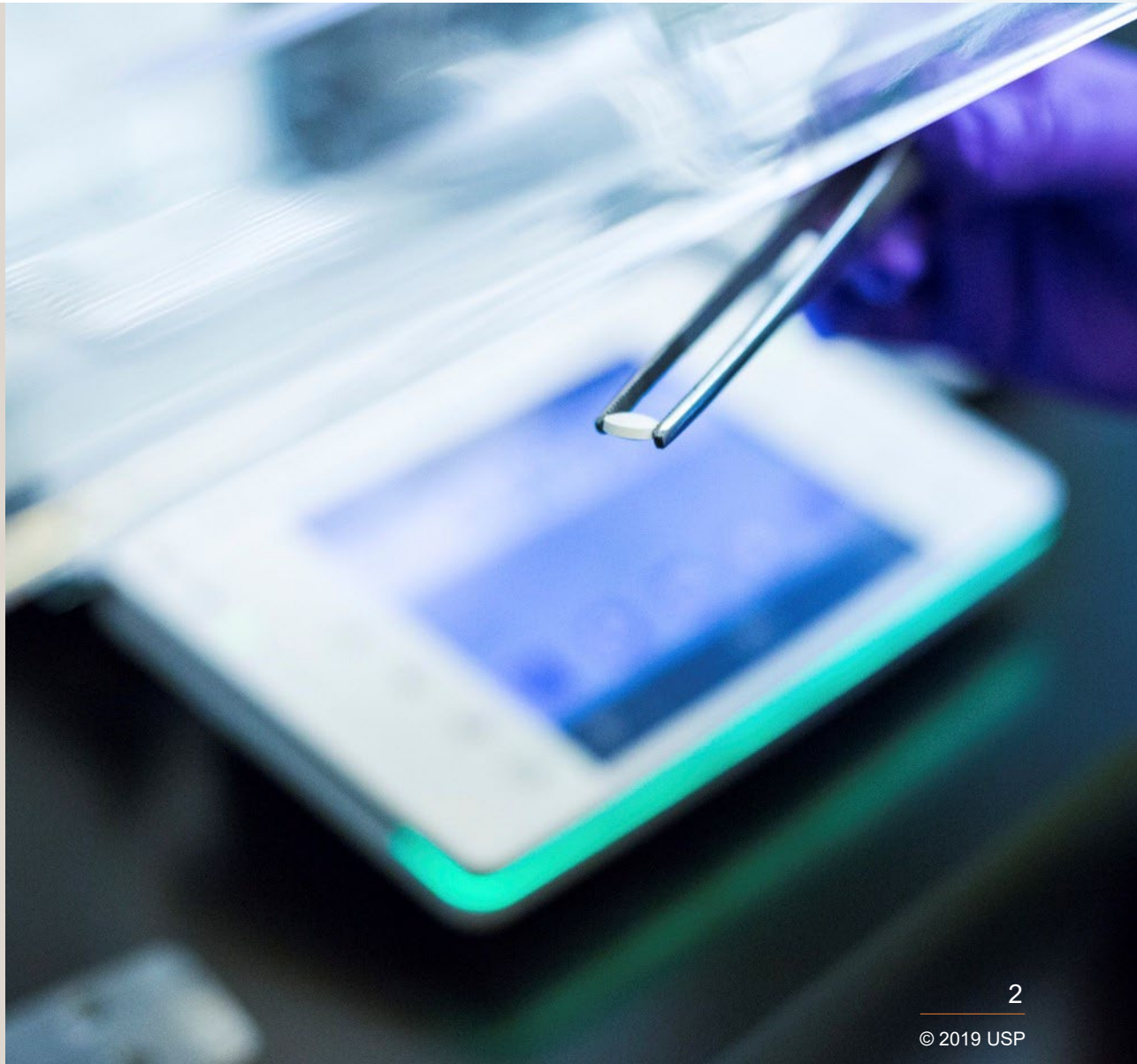
Outline

- Complex Nitrosamines
  - Introduction of NDSRIs and Product Recalls
- Regulatory Landscape
  - FDA, EMA and Others
- USP's Current Strategy
  - USP's Nitrosamine Program
  - USP's Tools and Solutions
  - Non-compendial solutions
  - Pharmaceutical Analytical Impurities
  - Strategy for Excipients
- Future Roadmap



Introduction of Complex Nitrosamine Drug-Substance Related Impurities

INNOVATION. QUALITY. GLOBAL REACH.

June 2021

Varenicline

Health Canada requested recall of the 5 impacted lots of CHAMPIX (varenicline) with levels of a nitrosamine impurity, N-nitrosovarenicline, above the acceptable intake limit established by Health Canada.

EMA: Recalled several batches of Champix from Europe region.

July 2021

Varenicline

FDA announced voluntary recall of varenicline (Chantix) drug products. FDA did not object to certain manufacturers temporarily distributing varenicline tablets containing N-nitroso-varenicline above the FDA's acceptable intake limit of 37 ng per day but below the interim acceptable intake limit of 185 ng per day. In May 2022, FDA announced the new acceptable intake limit is 37 ng per day.

March 2022

Propranolol hydrochloride

Voluntary recall of Inderal-LA (propranolol hydrochloride) capsules in Canada due to the presence of a nitrosamine impurity (N-nitroso-propranolol) above the acceptable level.

March 2022

Orphenadrine Citrate

Voluntary recall of Orphenadrine Citrate 100 mg Extended Release (ER) Tablets due to potentially unacceptable levels of NMOA or Nitroso-orphenadrine impurity

March 2022

Quinapril and Hydrochlorothiazide Tablets

Voluntary recall of Quinapril and Hydrochlorothiazide Tablets due to the presence of a nitrosamine, N-nitroso-quinapril, above the Acceptable Daily Intake (ADI) level.

Aug 2022

Sitagliptin

FDA recently became aware of a nitrosamine impurity, Nitroso-STG-19 (known as NTTP), in certain samples of sitagliptin. To avoid a shortage, FDA allowing temporary distribution of Sitagliptin products with NTTP impurity above the acceptable intake limit of 37 ng per day, and up to 246.7 ng per day.

Reference:

https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamine-varenicline-chantix#60f8a171a4486

https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts

https://www.fda.gov/drugs/drug-safety-and-availability/

https://recalls-rappels.canada.ca/en/alert-recall/pfizer-recalls-accupril-blood-pressure-tablets-due-nitrosamine-impurity

Recent Recalls Due to NDSRIs

- In December 2022 the FDA announced a voluntary recall of four lots of Quinapril Tablets due to the presence of a nitrosamine impurity observed in testing above FDA's proposed interim limit, adding to the growing number of recalls due to nitrosamine impurities in recent years.

- The March 2023 voluntary recall of **Dabigatran Etexilate Capsules** due to presence of **NDAB or N-nitroso-dabigatran impurity** from the U.S. market is putting nitrosamine drug substance related impurities (NDSRI) in the spotlight once again.

- Amidst the latest recall related to NDSRIs, USP continues to lead the charge by providing quality standards-based solutions, organizing workshops and training courses and hosting a forum for the exchange of crucial knowledge to help keep our medicine supply chain strong and protect patient health.

Reference:
https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/aurobindo-pharma-usa-inc-initiates-voluntary-nationwide-recall-two-2-lots-quinapril-and
https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/lupin-pharmaceuticals-inc-issues-voluntary-nationwide-recall-four-lots-quinapril-tablets-due

FDA Guidance: Control of Nitrosamine Impurities in Human Drugs - Updated guidance - Feb. 2021

Acceptable Intake Limits by FDA

- FDA recommends the following acceptable intake (AI) limits for the nitrosamine impurities for NDMA, NDEA, NMBA, NMPA, NIPEA, and NDIPA in Drug Products

Table 1. AI Limits

Nitrosamine (Small Nitrosamines)	AI Limit (ng/day)
NDMA	96
NDEA	26.5
NMBA	96
NMPA	26.5
NIPEA	26.5
NDIPA	26.5

No limits for NDSRIs

(Ref: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/control-nitrosamine-impurities-human-drugs)

FDA's Post on 'Possible Mitigation Strategies'

Updates on possible mitigation strategies to reduce the risk of nitrosamine drug substance related impurities in drug products

FDA issued a guidance for industry, *Control of Nitrosamine Impurities in Human Drugs*, in September 2020 to help ensure the safety of the U.S. drug supply by recommending steps manufacturers of active pharmaceutical ingredients (API) and drug products should take to detect and prevent objectionable levels of nitrosamine impurities in pharmaceutical products. The guidance also described conditions that may introduce nitrosamine impurities and described a three-step mitigation strategy.

Recently, FDA has received additional reports of certain types of nitrosamine impurities that formed in several drug products. These nitrosamine drug substance-related impurities (NDSRIs) are a class of nitrosamines sharing structural similarity to the API. NDSRIs can be generated during manufacturing or during the shelf-life storage period of the drug product. In some cases, the root cause of NDSRI formation has been attributed to nitrite impurities present in excipients at parts-per-million amounts.[1] Nitrite impurities have been observed in a range of commonly used excipients (as well as water) and may lead to the formation of NDSRIs in certain drug products.

Posted on 11/18/2021

Introduced NDSRIs first time

https://www.fda.gov/drugs/drug-safety-and-availability/updates-possible-mitigation-strategies-reduce-risk-nitrosamine-drug-substance-related-impurities

FDA's Post on 'Possible Mitigation Strategies'

Nitrite impurities present in excipients at parts-per-million amounts:

A supplier qualification program that takes into account potential nitrite impurities across excipient suppliers and excipient lots to reduce the risk of nitrosamine formation in the drug product.

Mitigation strategies related to formulation design:

The formation of nitrosamines typically occurs under acidic conditions, whereas, in a neutral or basic environment, the kinetics of these reactions are significantly reduced. Thus, formulation designs that incorporate excipients such as sodium carbonate that modify the micro-environment to neutral or basic pH, should in principle inhibit the formation of NDSRIs.

The addition of antioxidants to formulations may significantly inhibit the formation of NDSRIs in drug products.

FDA encourages manufacturers to consider these as well as other innovative strategies to reduce the formation of NDSRIs to acceptable levels in drug products.

FDA's Post on 'Possible Mitigation Strategies'

- FDA will consider meeting requests, as appropriate, to discuss innovative mitigation strategies with prospective applicants or manufacturers.
- The data in the NDA/BLA and ANDA meeting package should include, at a minimum, the following:
  - Description of the formulation design strategy employed to reduce the formation of NDSRIs in the drug product.
  - Supporting manufacturing information and, at a minimum, three months of accelerated stability data demonstrating control of NDSRI.
  - For approved NDAs and ANDAs that require reformulation as part of a mitigation strategy, in vitro or in vivo bioequivalence bridging studies.

https://www.fda.gov/drugs/drug-safety-and-availability/updates-possible-mitigation-strategies-reduce-risk-nitrosamine-drug-substance-related-impurities

Recent FDA Notice on NDSRI

NATIONAL ARCHIVES

FEDERAL REGISTER

The Daily Journal of the United States Government

Notice

Identification, Assessment, and Control of Nitrosamine Drug Substance-Related Impurities in Human Drug Products; Establishment of a Public Docket; Request for Comments

A Notice by the Food and Drug Administration on 05/04/2023

This document has a comment period that ends in 16 days. (07/03/2023)

SUBMIT A FORMAL COMMENT

2 comments received. View posted comments

https://www.federalregister.gov/documents/2023/05/04/2023-09526/identification-assessment-and-control-of-nitrosamine-drug-substance-related-impurities-in-human-drug

Recent FDA Notice on NDSRI (contd..)

AGENCY:

Food and Drug Administration, HHS.

ACTION:

Notice; establishment of a public docket; request for comments.

SUMMARY:

The Food and Drug Administration (FDA, Agency, or we) is announcing the establishment of a docket to solicit public comments on the identification, assessment, and control of N-nitrosamine (nitrosamine) drug substance-related impurities (NDSRIs) that may be considered by the Agency in its regulation of these types of impurities in drug products. This notice identifies scientific and regulatory considerations regarding the identification, assessment, and control of NDSRIs, including areas that may benefit from collaborative efforts, and requests comments on these topics. This notice is not intended to communicate FDA's regulatory expectations on these issues but is instead intended to seek input from the public to inform scientific and/or regulatory approaches as appropriate.

DATES:

Either electronic or written comments must be submitted by July 3, 2023.

DOCUMENT DETAILS

Printed version:
PDF

Publication Date:
05/04/2023

Agencies:
Food and Drug Administration

Dates:
Either electronic or written comments must be submitted by July 3, 2023.

Comments Close:
07/03/2023

Document Type:
Notice

Document Citation:
88 FR 28557

Page:
28557-28562 (6 pages)

Agency/Docket Number:
Docket No. FDA-2023-N-1585

Document Number:
2023-09526

DOCUMENT DETAIL

https://www.federalregister.gov/documents/2023/05/04/2023-09526/identification-assessment-and-control-of-nitrosamine-drug-substance-related-impurities-in-human-drug

Recent FDA Notice on NDSRI (contd..)

Important Dates:

- Publication Date: 05/04/2023
- Comments Close: 07/03/2023

Safety Assessments of the Potential for regarding nitrosamines, and NDSRIs are a subcategory of these impurities that share structural similarity with the active pharmaceutical ingredient in drug products.

Background:

- Nitrosamines, Including NDSRIs, in Human Drug Products
- Safety Assessments of the Potential for Mutagenic and Carcinogenic Risk
  - Assessment of Potential Mutagenicity and Carcinogenicity
  - Computational Toxicology
  - Determining AI Limits for NDSRIs
- FDA's Ongoing Work on Nitrosamine Risk Assessment and Mitigation
- Regulatory Challenges
- Collaborative Efforts To Develop NDSRI Data

https://www.federalregister.gov/documents/2023/05/04/2023-09526/identification-assessment-and-control-of-nitrosamine-drug-substance-related-impurities-in-human-drug

Recent updates to EMA Q&A Document

- Introduction of Q&A 22 on approach to control presence of N-nitrosamine exceeding the AI while CAPAs are being implemented.
  - This approach is considered in accordance with the regulatory steps taken by authorities following the identification of an N-nitrosamine exceeding the AI.
  - Less-than lifetime (LTL) concept or the use of interim limits may be considered by the lead authority and NCAs on a temporary basis.
  - Applicability:
    - A duration of treatment not exceeding 10 years.
    - CAPA implementation timeline of up to 3 years from the establishment and publication of the AI (nevertheless MAHs are expected to expedite CAPAs implementation).
  - Approach/Limits:
    - The interim limits are based on the LTL approach outlined in the ICH M7 guideline, using the two most conservative adjustment factors (6.7 and 13.3 x AI).

Treatment duration	Up to 12 months	>12 months up to 10years
Interim limit	13.3 x AI*	6.7xAI*

*In any case the limit should not exceed 1.5 µg/day unless the established AI (Table 1, Q10) is > 1.5 µg/day

  - Non-Applicability:
    - A duration of treatment exceeding 10 years
    - CAPA implementation exceeding 3 years from the establishment and publication of the AI
    - New/ongoing regulatory applications

Conclusion:

- Amendment of Q&A 22 to indicate that no variation should be submitted to implement temporary above AI limits in specifications

(**Ref** Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products EMA/409815/2020 Rev.15, 30 March 2023)

Regulatory Landscape: Recommended AIs

N-Nitrosamine (CAS Number)	FDA AI Limit (ng/day)	EMA AI Limit (ng/day)/ Source
N-Nitrosodimethylamine, NDMA[3,4] (62-75-9)	96	96
N-Nitrosodiethylamine, NDEA[3,4] (55-18-5)	26.5	26.5
N-Nitrosoethylisopropylamine, EIPNA[3,5] (16339-04-1)	26.5	26.5
N-Nitrosodiisopropylamine, DIPNA[3,5] (601-77-4)	26.5	26.5
N-Nitroso-N-methyl-4-aminobutyric acid, NMBA[3,6] (61445-55-4)	96	96
1-Methyl-4-nitrosopiperazine, MeNP[5] (16339-07-4)	0.16 ppm	26.5 (Rifampicin)
N-Nitroso-di-n-butylamine, NDBA[3,5] (924-16-3)		26.5
N-Nitroso-N-methylaniline, NMPA[3,4] (614-00-6)	26.5	34.3
N-Nitrosomorpholine, NMOR[3,7] (59-89-2)		127
N-Nitrosovarenicline, NNV[8]	37	37 (Varenicline)
N-Nitrosodipropylamine, NDPA (621-64-7)[3,5]		26.5

(Ref Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal EMA/409815/2020 Rev.15, 30 March 2023 **and** https://www.fda.gov/regulatory-information/search-fda-guidance-documents/control-nitrosamine-impurities-human-drugs **Rev, 1, Feb. 2021)**

Regulatory Landscape: Recommended AIs

N-Nitrosamine (CAS Number)	FDA AI Limit (ng/day)	EMA AI Limit (ng/day)/ Source
N-Nitrosomethylphenidate[9], NMPH, (55557-03-4)		1300 (Methylphenidate)
N-Nitrosopiperidine[3] (100-75-4)		1300
N-Nitrosorasagiline[10]		18 (Rasagiline)
7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3- a]pyrazine[11]	37	37 (Sitagliptin)
N-Nitroso-1,2,3,6-tetrahydropyridine, NTHP[3] (55556-92-8)		37
N-Nitrosonortriptyline[12]		8 (Amitryptylin, Nortryptylin)
N-Methyl-N-nitrosophenethylamine, NMPEA[3] (13256-11-6)		8
N-Nitrosodabigatran[10]		18 (Dabigatran)
4-(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK)[7]		100

(**Ref** Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products EMA/409815/2020 Rev.15, 30 March 2023)

Regulatory Landscape: Recommended AIs

N-Nitrosamine (CAS Number)	FDA AI Limit (ng/day)	EMA AI Limit (ng/day)/ Source
N-nitrosoduloxetine[13]		100 Duloxetine
N-nitroso-fluoxetine[13]		100 Fluoxetine
N-nitrosoparoxetine[9]		1300 Paroxetine
N-nitroso-diphenylamine NDPh[14] (86-30-6)		78000
N-nitroso-mefenamic acid[15]		78000 Mefenamic acid
N-nitroso-pyrrolidine NPYR[3,7] (930-55-2)		1700
N-nitroso-diethanolamine NDELA[3,7](1116-54-7)		1900
Nitroso-Orphenadrine (NMOA)	26.5	
1-cyclopentyl-4-nitrosopiperazine (CPNP)	0.1 ppm	

(**Ref** Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products EMA/409815/2020 Rev.15, 30 March 2023)

Regulatory Resources on Nitrosamines

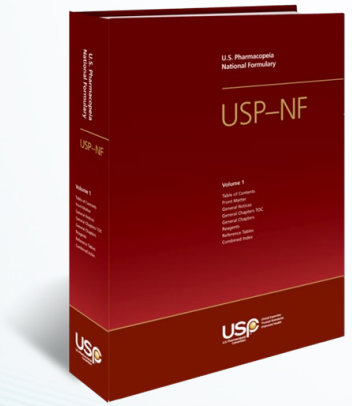
- **United States Pharmacopeia (USP) : https://nitrosamines.usp.org/**
- **World Health Organization (WHO) :** https://www.who.int/news/item/20-11-2019-information-note-nitrosamine-impurities
- **U.S. Food and Drug Administration (FDA) :** https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities-medications
- **European Medicines Agency (EMA):**https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities
- **European Directorate for the Quality of Medicines and HealthCare (EDQM) :**https://www.edqm.eu/en/n-nitrosamine-contamination-in-brief
- **Health Canada (HC) :** https://www.canada.ca/en/health-canada/services/drugs-health-products/compliance-enforcement/information-health-product/drugs/nitrosamine-impurities.html
- **Pharmaceuticals and Medical Devices Agency (PMDA-Japan) :** https://www.pmda.go.jp/english/safety/info-services/drugs/safety-measures/0001.html
- **Therapeutic Goods Administration (TGA –Australia)** : https://www.tga.gov.au/news/safety-alerts/nitrosamine-impurities
- **Medicines & Healthcare products Regulatory Agency (MHRA - UK) :** https://www.gov.uk/guidance/medicines-marketing-authorisation-holders-submission-of-nitrosamine-risk-evaluation#full-publication-update-history

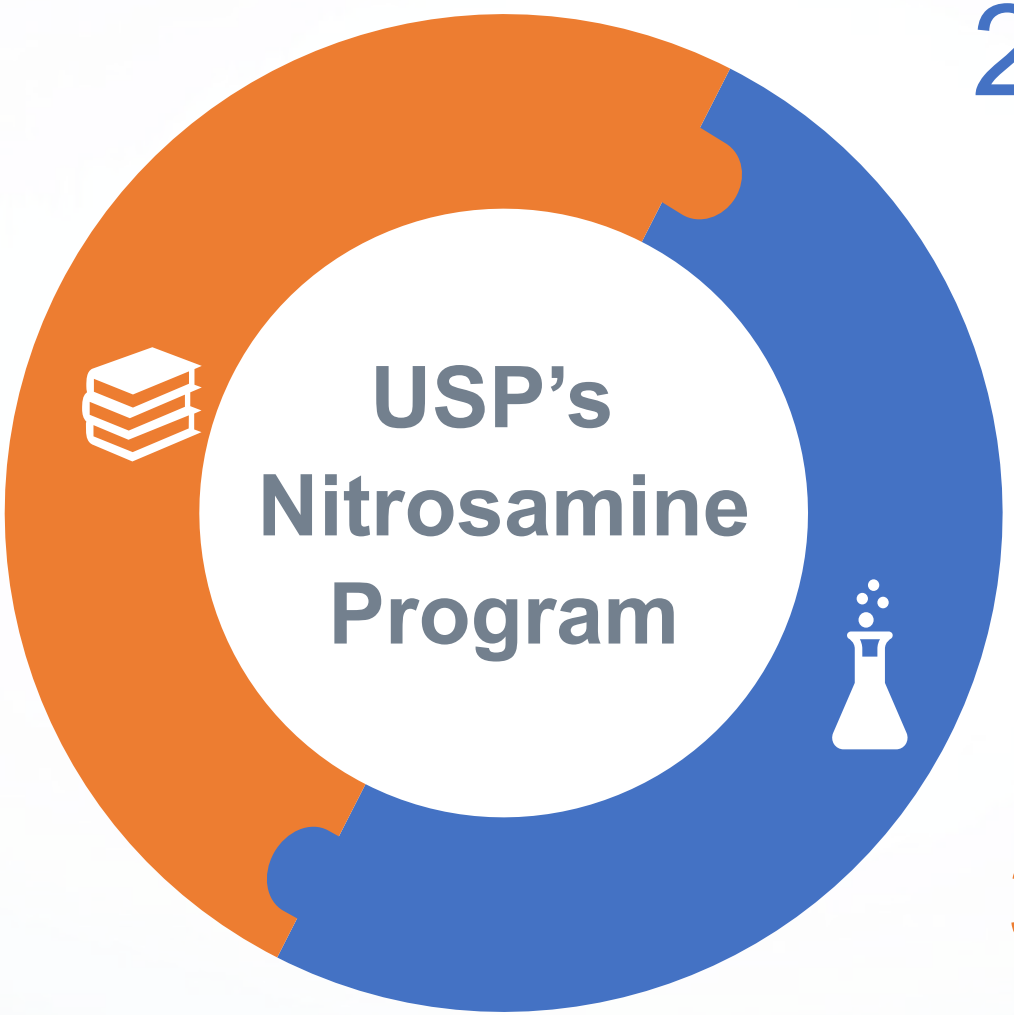
USP's Nitrosamine Program: Accomplishments so far…

INNOVATION. QUALITY. GLOBAL REACH.

1 Documentary Standard

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





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)

3 Advocacy and capability building

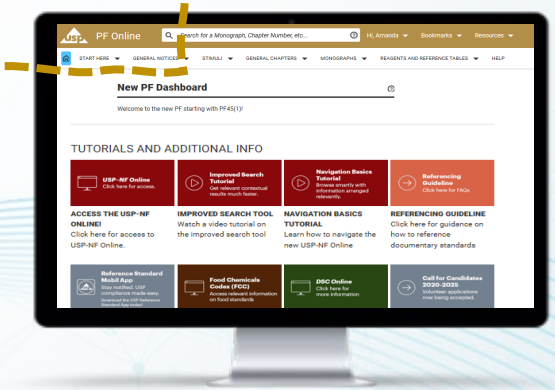
USP Education course

Webinar, Round Table Discussion, Workshop, User Forums

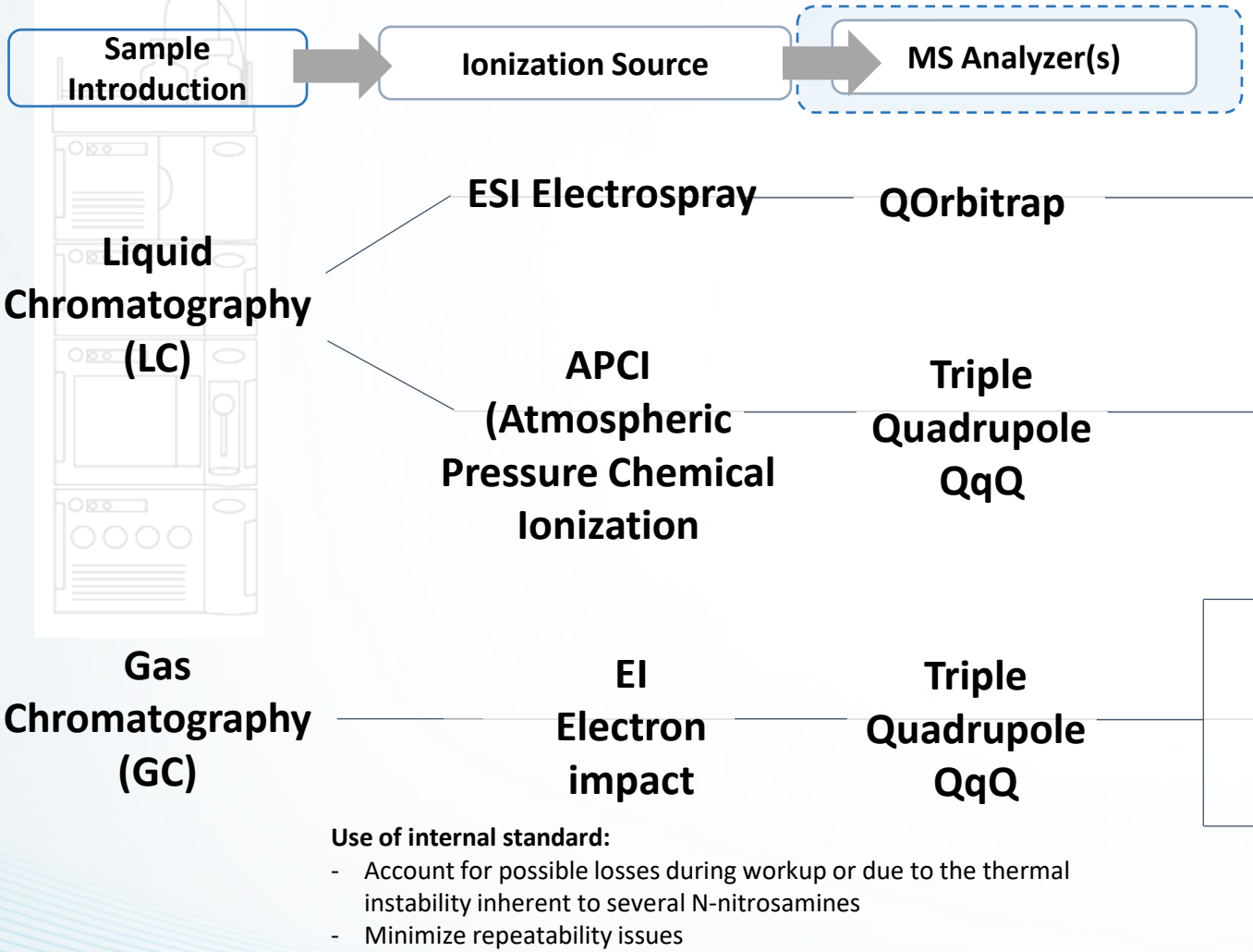
Trainings to Regulators

GC <1469> Nitrosamines Impurities

CONTENT



GC <1469> Test Procedures



Use of internal standard:

- Account for possible losses during workup or due to the thermal instability inherent to several N-nitrosamines
- Minimize repeatability issues

Methods

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

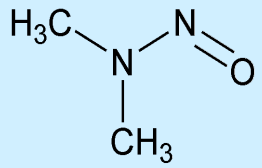
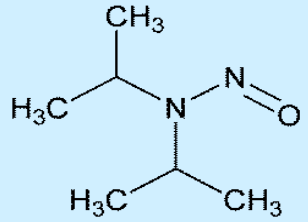
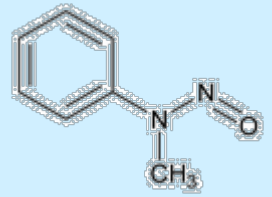
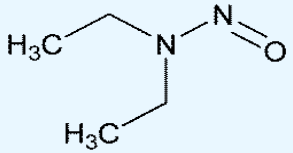
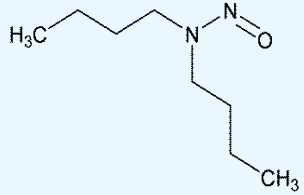
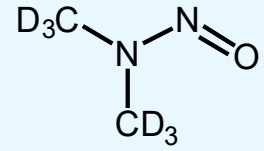
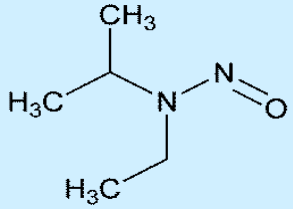
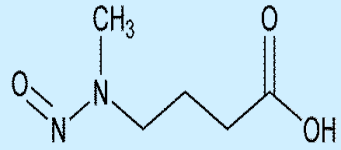
HPLC-APCI-QqQ
Procedure 3: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA in selected sartans by HPLC–MS/MS (Using Internal standards NDMA-d6, NMBA-d3, NDEA-D10, NDBA-D18
LC Column: L1 – C18

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, NMPA, and NDBA in selected sartans by GC– MS/MS (triple-quad) – (Using internal standards NDMA-13C-d6)

USP Nitrosamine Reference Standards

- USP developed eight USP Nitrosamine Reference Standards for use with General Chapter <1469> Nitrosamine Impurities

Catalog # Name Label value	**Structure**	**Catalog # Name Label value**	**Structure**	Catalog # Name Label value	**Structure**
1466674 N-Nitroso dimethylamine (NMDA) 1.00 mg/mL in Methanol		1466663 N-Nitroso diisopropylamine (NDIPA) 1.00 mg/mL in Methanol		1466607 N-Nitrosomethyl phenylamine (NMPA) 1.00 mg/mL in Methanol	
1466652 N-Nitroso diethylamine (NDEA) 1.00 mg/mL in Methanol		1466641 N-Nitroso dibutylamine (NDBA) 1.00 mg/mL in Methanol		1175800 Deutero N-Nitrosodimethylamine (NDMA-d6) 1.00 mg/mL in Methanol	
1466685 N-Nitroso ethylisopropylamine (NEIPA) 1.00 mg/mL in Methanol		1466696 N-Nitroso methtylamino butyric acid (NMBA) 1.00 mg/mL in Acetonitrile			

Nitrosamine Training Materials

Introduction to Proposed USP General Chapter <1469> and Handling of Nitrosamine Impurities Reference Standards: Posted on YouTube in Nov. 2020

Modules

USP <1469> Nitrosamines Impurities: Launched in June 2021

Module 1—Nitrosamines Overview
Module 2—Risk Assessment
Module 3— Testing Methods - Mass Spectrometry Fundamentals, Analytical Challenges and Validation of Procedures
Module 4 — Analytical Procedures

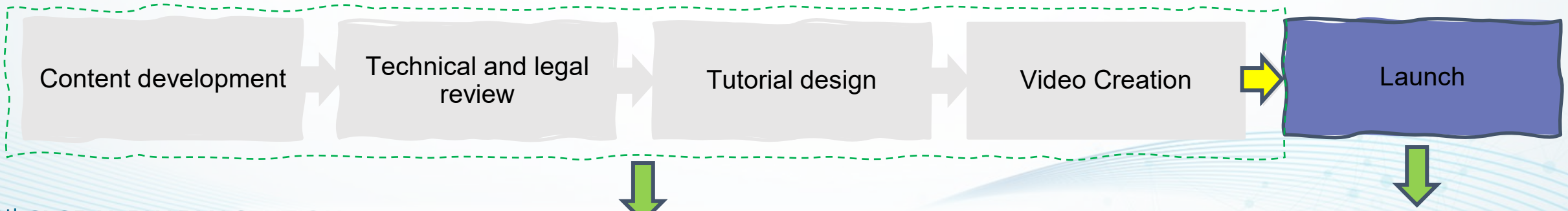
Nitrosamine Lab Demonstration Videos

Scope:

To design and create lab demonstration videos on USP <1469> procedures highlighting critical troubleshooting points in nitrosamine methods (LC-MS/MS & GC-MS/MS).

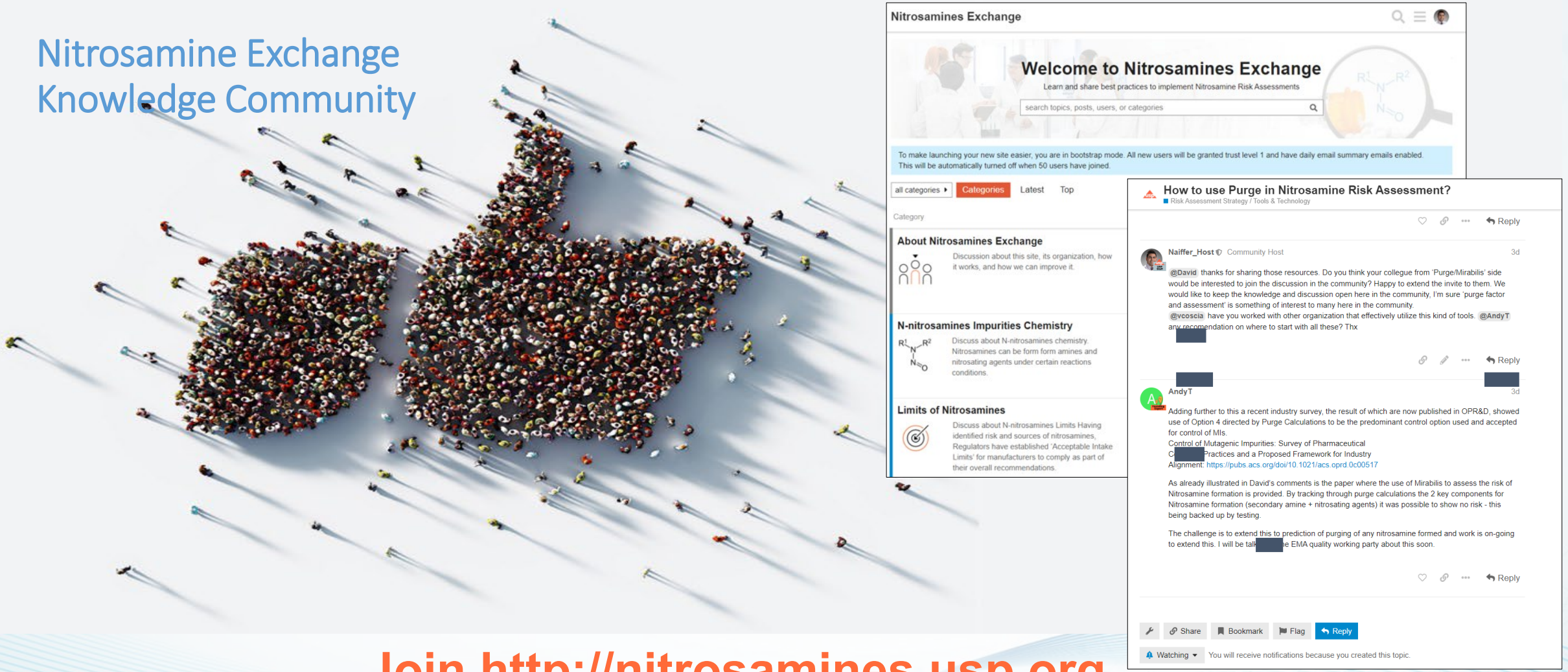
Work Plan:

Status:



Nitrosamine Exchange – Online Community

Nitrosamine Exchange Knowledge Community



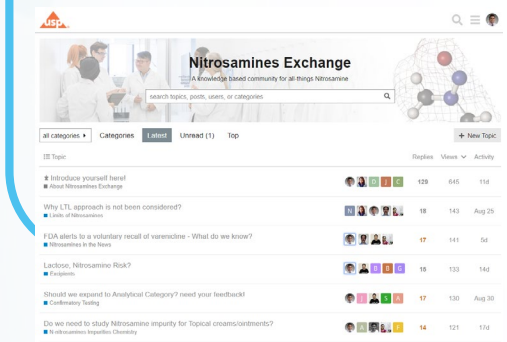
Join http://nitrosamines.usp.org

8th GLOBAL PHARMACEUTICAL QUALITY SUMMIT 2023

Learnings Nitrosamines Exchange – Can we do it?

Apr'21

Launch

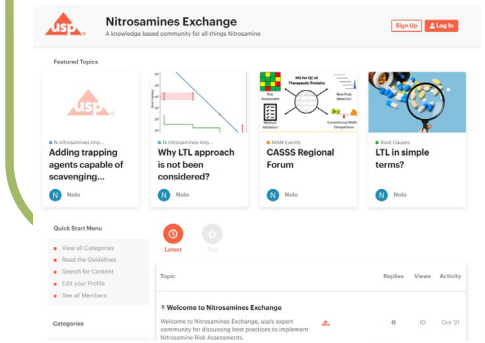


Oct'21

Analytical expansion

Feb'22

Redesign & Multi-language



Today

Collaboration Hub

- 2800+ members, 90 countries
- 70% new to USP; 86% outside U.S.
  - *new in 2022: ability to translate text between 22 languages*
- ~500K page views
- 60% give 4 or 5 on usefulness (scale 1-5)

Nitrosamine Impurities Survey 2.0

Research Goal

Understanding current challenges and practices for controlling & testing Nitrosamines' impurities. Identifying priorities to support work in this space.

What? Online survey distributed through Qualtrics via email and online intercepts.

When?

Fieldwork date range: Mar– April 2023

Who?

Targets included USP stakeholders involved with Nitrosamines such as USP-NF users and reference standard purchasers, Nitrosamine Exchange Knowledge Hub Community

Total sample for analysis and reporting ~223

Key findings:

- ***There are clear challenges to addressing nitrosamines, both operationally and in a regulatory setting***
- ***To address these challenges, industry is interested in seeking support from USP and others, as well as the potentially open to forming a consortium***
- ***It's common for industry working with Nitrosamines to be testing for NDSRIs, but not universal***
- ***It's nearly unanimous that access to RS/Materials for NDSRIs would be beneficial***
- ***Knowing limits for new and emerging Nitrosamines as an unaddressed challenge.***

USP's In- Progress Activities

- Non-compendial solutions:
  - Publications
  - Analytical Hub
  - Pharmaceutical Analytical Impurities (PAI)
- Strategy for Excipients:
  - Nitrite and Nitrate in Excipients
- Development of Risk Assessment Tool

Publication: Method for Nitrosamines in commercially used solvents

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

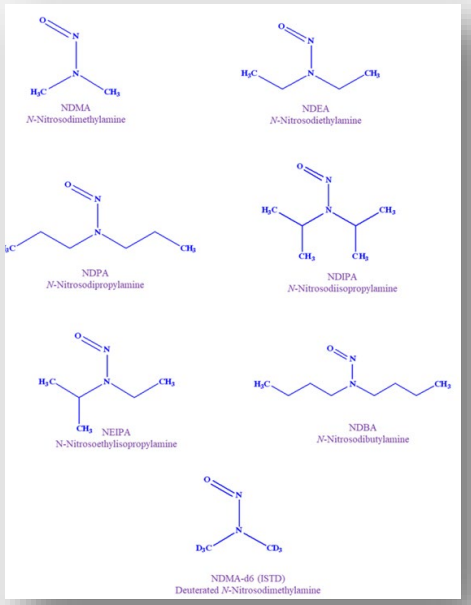
Pharmaceutical Biotechnology

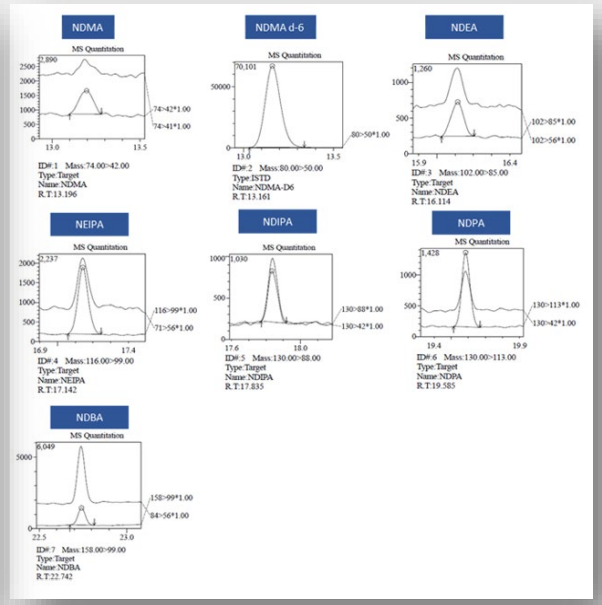
A GC-MS/MS method for trace level quantification of six nitrosamine impurities (NDMA, NDEA, NEIPA, NDIPA, NDPA, and NDBA) in commercially used organic solvents: Dichloromethane, ethyl acetate, toluene, and o-xylene

Eswara Raju Kosuri[a,*], Mayank Bhanti[a], Mrunal A Jaywant[a], Mark Han[b], Xiaochun Wang[b], Marcus Obeng[b]

[a] United States Pharmacopeia India Pvt Ltd, Hyderabad, Telangana, India
[b] United States Pharmacopeial Convention, Rockville, MD, USA

DOI:https://doi.org/10.1016/j.xphs.2022.11.024





- **Solvents** are widely used as a **reaction media** and other steps in the **production** of drug substances and products in pharmaceutical **industries**.
- Nitrosamine **contamination** can occur when fresh solvents (ortho-xylene, toluene, and methylene chloride) get contaminated during **shipment** from vendors (e.g., during **transfer between storage vessels**).
- The determination of **nitrosamines** in **solvents** plays an important control in the **manufacturing** of quality drug substances and drug products.
- The current study provides a **highly sensitive** procedure with a **LOQ of 5 ppb** (for NDMA, NDEA, NEIPA, NDIPA, NDPA) and **13 ppb** (for NDBA) for the determination of **six nitrosamines** in widely used solvents: **dichloromethane**, **ethyl acetate**, **toluene**, and **o-xylene**.

https://www.fda.gov/media/141720/download

Publication: Analysis of USP GSRS Database

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

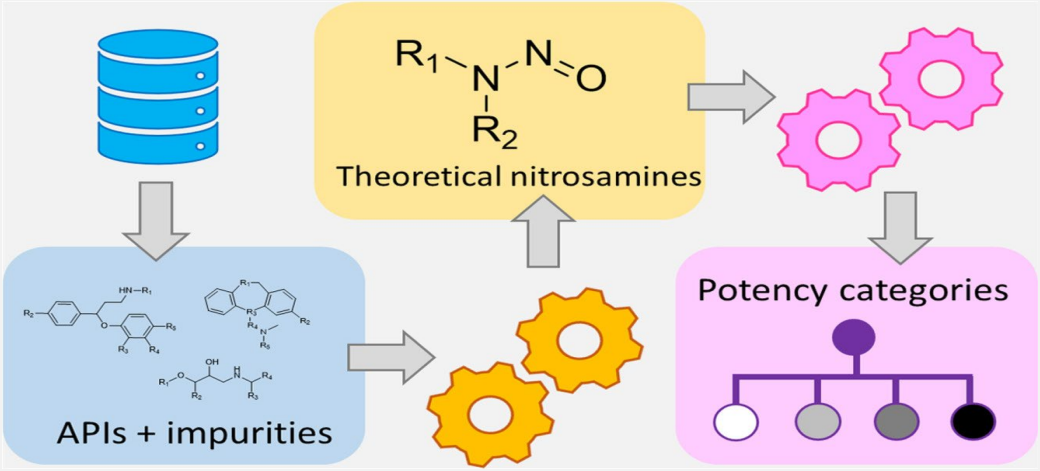
journal homepage: www.jpharmsci.org

Global Health

The Landscape of Potential Small and Drug Substance Related Nitrosamines in Pharmaceuticals

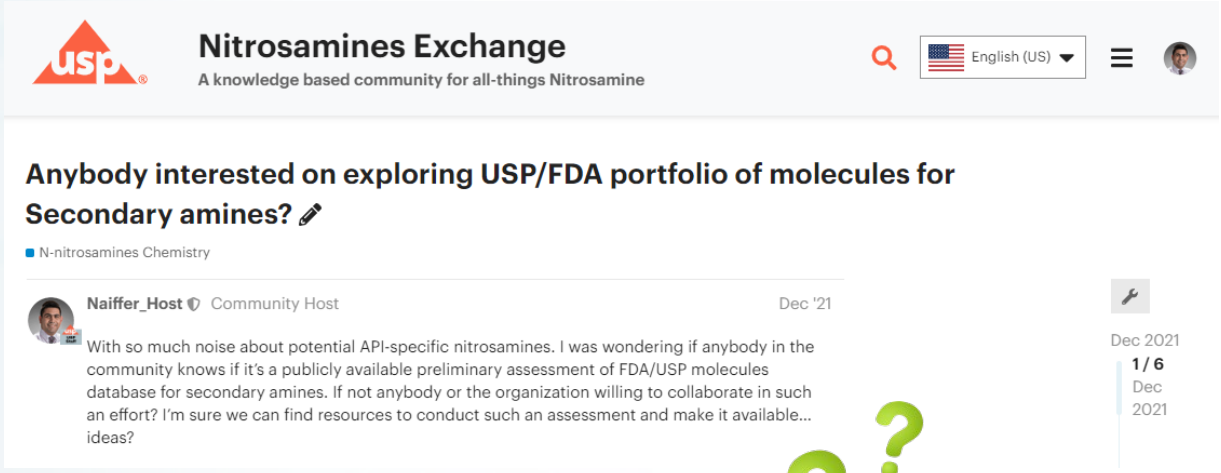
Joerg Schlingemann[a,1,*], Michael J. Burns[b,1,*], David J. Ponting[b], Carolina Martins Avila[b,f], Naiffer E. Romero[c], Mrunal A. Jaywant[c], Graham F. Smith[d], Ian W. Ashworth[e], Stephanie Simon[a], Christoph Saal[a], Andrzej Wilk[c]

[a] Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany
[b] Lhasa Limited, Granary Wharf House, 2 Canal Wharf, Leeds, United Kingdom
[c] U.S. Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD, USA
[d] AstraZeneca, Data Science and AI, Clinical Pharmacology and Safety Sciences, R&D, Cambridge, United Kingdom
[e] Chemical Development, Pharmaceutical Technology & Development, Operations, AstraZeneca, Macclesfield, United Kingdom
[f] Current affiliation: Sai Life Sciences Limited, Basement A, Block 33, Alderley Park, Macclesfield, United Kingdom



- An **in-silico analysis** of more than **12,000** small molecule drugs and drug impurities.
- In total, **41.4 %** of the **APIs** and **30.2 %** of the **API impurities** listed in the GSRS database are **potential nitrosamine precursors.**
- Most structures identified through this workflow could form **complex API-related nitrosamines (NDSRIs)**.
- **Analytical standards** that would allow for quantification in the pharmaceuticals concerned are currently only available for **less than 5 %** of all potential NDSRIs.

Scale of the problem?



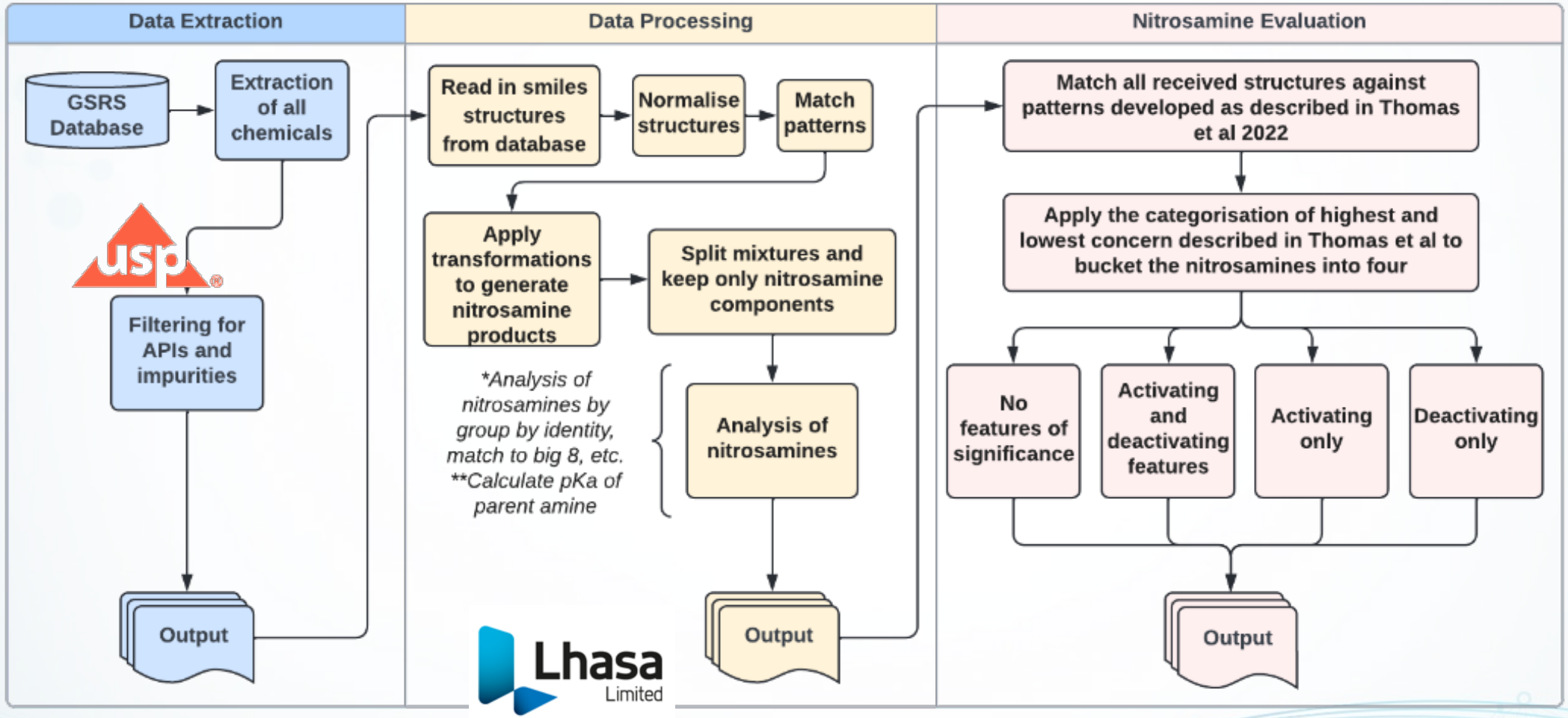
Data Set available:

- United States Pharmacopeia – Global Substance Registration System APIs and registered impurities
- FDA Orange book
- Top200 small molecule drugs by sales
- WHO Essential Medicines list

Plan:

- Analyze the databases and identify amines that could present a nitrosamine risk
- Identify trends in structural properties of the nitrosamines
- Assess likely toxicology trends based on current understanding

How to approach the data?



Theoretically generate Nitrosamines

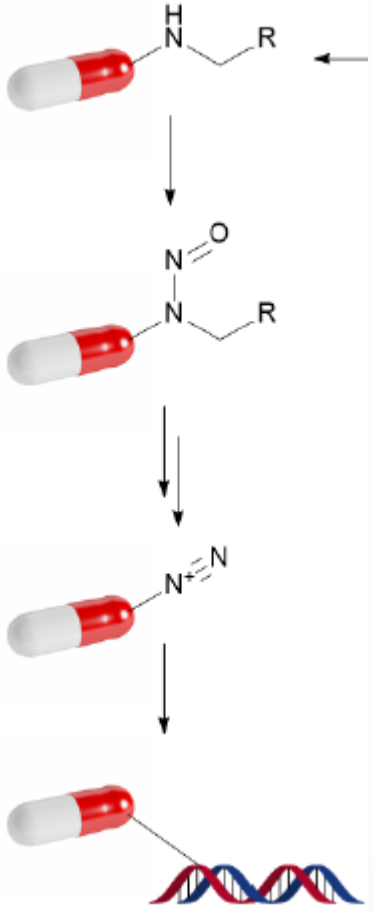
- Identify all amines at risk of forming nitrosamines
  The focus is on chemical potential, not the toxicity

- Generate all the unique nitrosamines which may theoretically form
  Identify all at-risk amines
  Transform to the corresponding nitrosamines

Secondary Amine **Tertiary Amine**

= *aromatic or single bonds only*
C_1/C_2 = *single bonds only, hydrogen count > 0*
C_3
N = single bonds only (excludes aromatic)

Outcome…



12,000 USP DB

4,848 APIs (40.4%)

3,552 Impurities (29.6%)

Journal of Pharmaceutical Sciences 000 (2022) 1–18

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The Landscape of Potential Small and Drug Substance Related Nitrosamines in Pharmaceuticals

Joerg Schlingemann[a,1,*], Michael J. Burns[b,1,*], David J. Ponting[b], Carolina Martins Avila[b,f], Naiffer E. Romero[c], Mrunal A. Jaywant[c], Graham F. Smith[d], Ian W. Ashworth[e], Stephanie Simon[a], Christoph Saal[a], Andrzej Wilk[c]

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NDMA
In-silico
GSRS

ABSTRACT

This article reports the outcome of an *in silico* analysis of more than 12,000 small molecule drugs and drug impurities, identifying the nitrosatable structures, assessing their potential to form nitrosamines under relevant conditions and the challenges to determine compound-specific AIs based on data available or read-across approaches for these nitrosamines and their acceptance by health authorities. Our data indicate that the presence of nitrosamines in pharmaceuticals is likely more prevalent than originally expected. In total, 40.4 % of the analyzed APIs and 29.6 % of the API impurities are potential nitrosamine precursors. Most structures identified through our workflow could form complex API-related nitrosamines, so-called nitrosamine drug substance related impurities (NDSRIs), although we also found structures that could release the well-known small and potent nitrosamines NDMA, NDEA, and others. Due to common structural motifs including secondary or tertiary amine moieties, whole essential drug classes such as beta blockers and ACE inhibitors are at risk. To avoid the risk of drug shortages or even the complete loss of therapeutic options, it will be essential that the well-established ICH M7 principles remain applicable for nitrosamines and that that the industry and regulatory authorities keep an open communication not only about the science but also to make sure there is a good balance between risk and benefit to patients.

Introduction

The recent discovery of small-molecule nitrosamine impurities in marketed drugs, starting with *N*-nitrosodimethylamine (NDMA) in batches of Valsartan in 2018, has led to significant regulatory response, including drug recalls and regulatory guidance that requires the re-evaluation of all synthetic and formulation routes for the potential presence of nitrosamine impurities.[1]

Due to the wide range of potential routes of formation for nitrosamines, many active pharmaceutical ingredients (APIs) and impurities are themselves liable to be nitrosated, either during the later stages of the synthetic process of the API, during drug product manufacturing, or in the finished and packaged drug product. Several recent drug recalls were conducted due to contamination with such API-derived complex nitrosamines, also called Nitrosamine Drug Substance Related Impurities (NDSRIs) (Fig. 1), e.g., Nitroso-Varenicline,[2,3] Nitroso-Propranolol,[4] Nitroso-Orphenadrine,[5] and Nitroso-Quinapril.[6] Nitrosamines are of concern as some of them have been reported to be potent rodent mutagens and carcinogens and have been categorized as probable or possible carcinogens by the WHO IARC. Because of this higher potency, nitrosamine impurities are considered to be members of the "cohort-of-concern" according to the ICH M7 guideline,[7] and need to be controlled at or below compound-specific limits. These might be much lower as compared to the limit of 1.5 μg/day acceptable intake (AI) for other potentially

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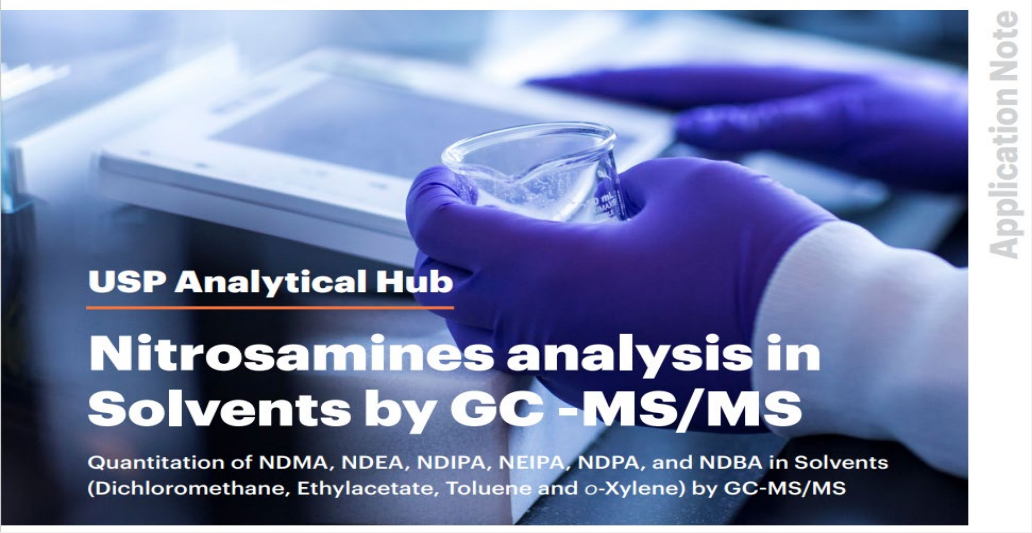
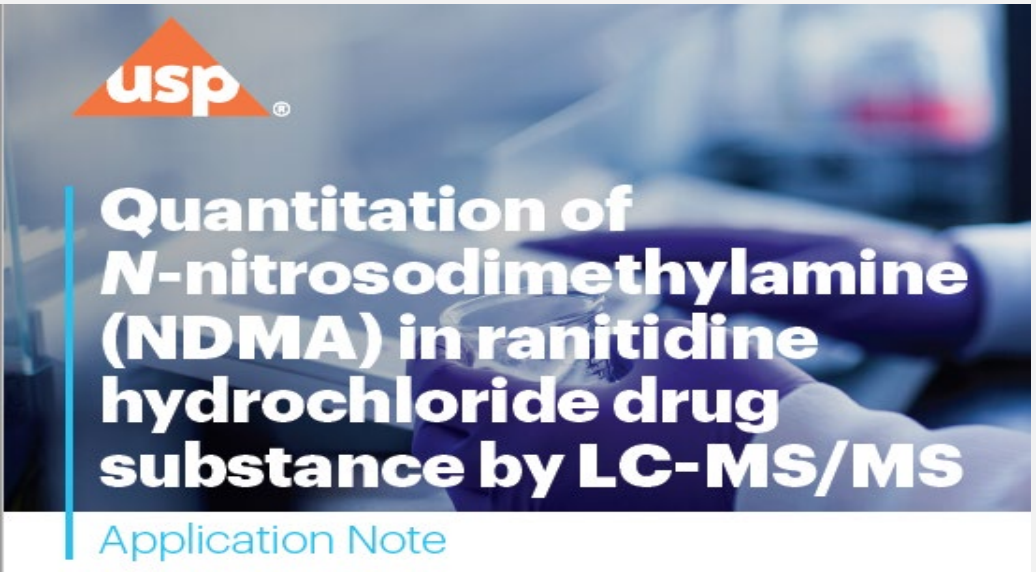
The Landscape of Potential Small and Drug Substance Related Nitrosamines in Pharmaceutical. Journal of Pharmaceutical Science Nov'23 - https://doi.org/10.1016/j.xphs.2022.11.013

USP Analytical Hub: Additional Methods

- Launched in December 2022



- Published in June 2023

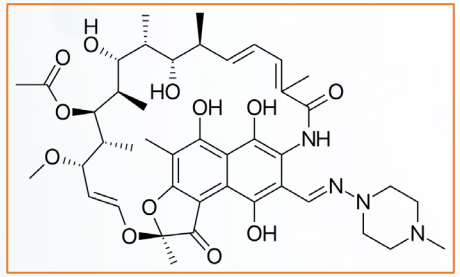


- Public **online repository** containing **non-compendial** analytical procedures (analytical notes) for the testing of nitrosamine impurities and related substances.
- USP's scientists curate these analytical procedures through **internal development/validation** or through scientific review of non-compendial donations. They are **NOT** compendial standards.
- The **procedures** contained in the analytical notes should be **validated** by the user. USP is **not** and will not be responsible for the use or implementation of the procedures.
- Hosted in **The Nitrosamine Exchange**. The Analytical Hub allows keyword searches and the view of key analytical procedure parameters and chromatograms.

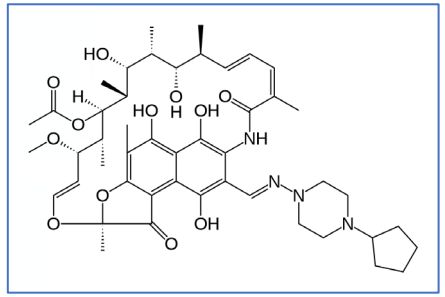
1. https://nitrosamines.usp.org/t/nitrosamines-analysis-in-solvents-by-gc-ms-ms/4556
2. https://nitrosamines.usp.org/t/quantitation-of-ndma-in-ranitidine-ds-by-lc-ms-ms/6352

Nitrosamines in Rifapentine and Rifampin

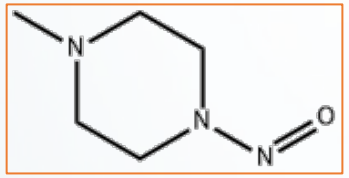
- Method development and validation for determination of MNP and CPNP in API and drug products to support the GPH program. Potential opportunity for Collaboration with WHO.



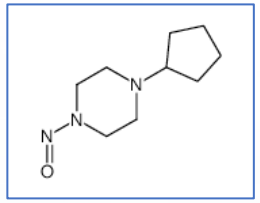
Rifampin



Rifapentine



1-methyl-4-nitrosopiperazine (MNP)



1-Cyclopentyl-4-nitrosopiperazine (CPNP)

- Rifampin and Rifapentine are antibacterial drugs used to treat **tuberculosis**; rifampin is also used to treat or prevent other serious infections.
- The **acceptable intake limits (in terms of concentration in ppm) are 0.16 ppm for MNP** in rifampin and **0.1 ppm for CPNP in rifapentine**
- The two **LC-MS/MS** methods were validated in the range of **0.05 – 10 ppm** for **CPNP** in rifapentine drug substance and products and **0.05 – 5 ppm** for **MNP** in rifampin drug substance and products.
- Accuracy was within **70% – 130%** and reproducibility was below **20%** RSD.
- **Matrix effects** were shown to be **critical** considerations and should be effectively mitigated in trace-level quantification of nitrosamine impurities in pharmaceuticals by LC-MS/MS

Reference: FDA Updates and Press Announcements on Nitrosamines in Rifampin and Rifapentine https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamines-rifampin-and-rifapentine#:~:text=8%2F26%2F2020%3A%20FDA,or%20prevent%20other%20serious%20infections.

Common Method for Small Nitrosamines

Approach

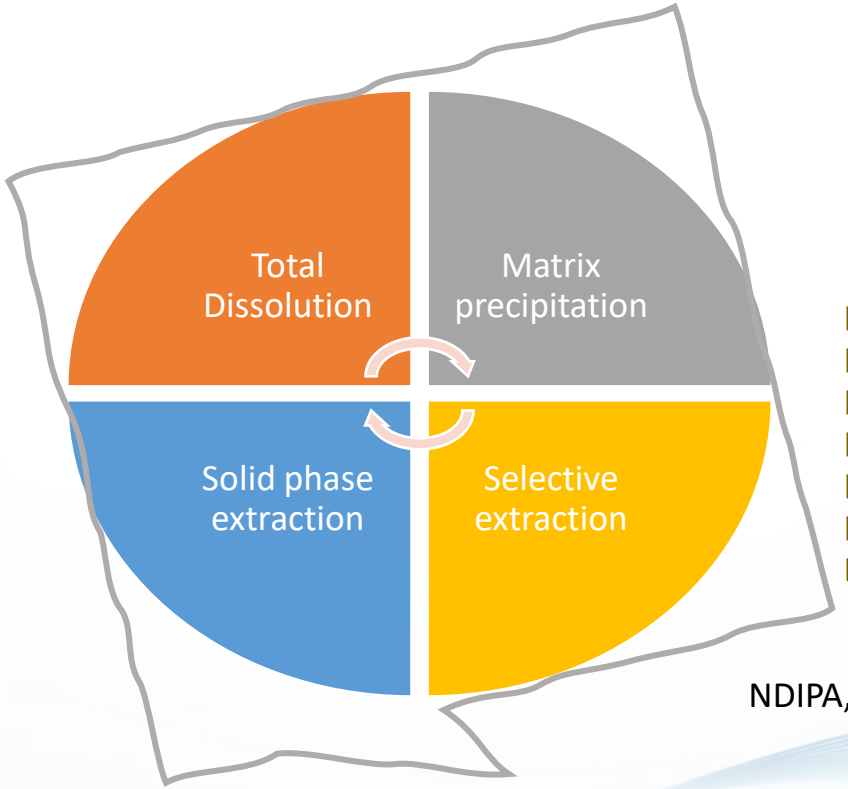
Different class of drugs → Sample preparation optimization → Mass spectrometric detection

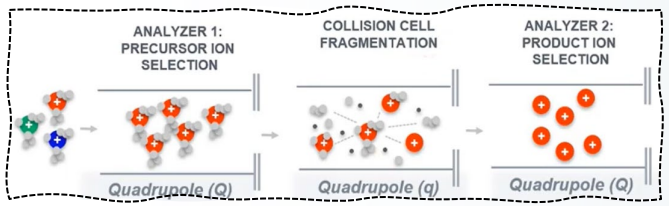
Angiotensin II Receptor Blockers
(Sartans)

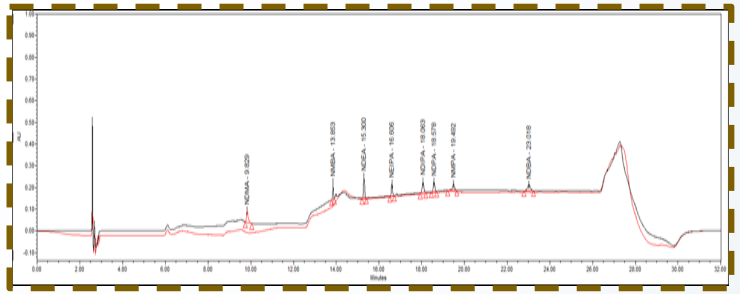
Histamine-2 Receptor Antagonists
(Ranitidine and Nizatidine)

Antidiabetic Agents
(Metformin Hydrochloride)

Antimicrobial Agents
(Rifampin and Rifapentine)







NDMA, NMBA, NDEA, NEIPA,
NDIPA, NDPA, NMPA, NDBA, and additional nitrosamines

Optimization in-process

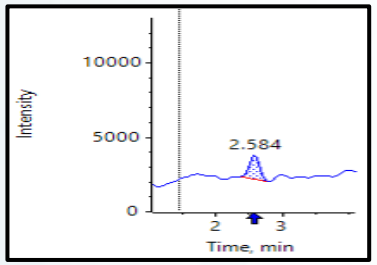
Common Method for Small Nirosamines

INNOVATION. QUALITY. GLOBAL REACH.

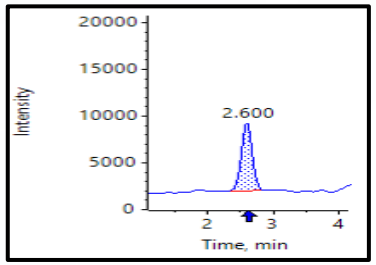
Nitrosamines	APIs	Different Diluents
N-Nitrosodimethylamine (NDMA)	Losartan Potassium	Methanol,
N-Nitrosomethylaminobutyric (NMBA)	Valsartan	Methanol: Water (50:50 % v/v),
N-Nitrosodiethylaminae (NDEA)	Irbesartan	0.1% Formic acid in Methanol: Acetonitrile (50:50 % v/v),
N-Nitrosoethylisopropylamine (NEIPA)	Olmesartan Medoxomil	Methanol: Water (80:20% v/v)
N-Nitrosodiisopropylamine (NDIPA)	Candesartan Cilexetil	
N-Nitrosodipropylamine (NDPA)	Telmisartan	
N-Nitrosomethylphenylamine (NMPA)		
N-Nitrosodibutylamine (NDBA)		
N-Nitrosopiperidine (NPIP)		
N-Nitrosopyrrolidine (NPYR)		
1-Methyl-4-nitrosopiperazine (MeNP)		
2-(4-Nitrosopiperazin-1-yl) pyrimidine		
4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone		
2-(4-Nitrosopiperazin-1-yl) ethanol		
N-Nitroso morpholine (NMOR)		

Chromatogram of Sensitivity solution

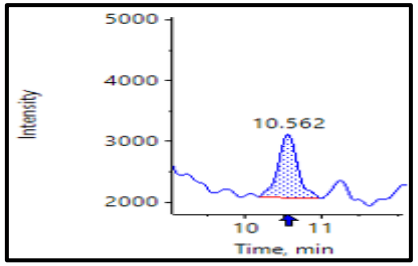
(10 ppb or 0.01 ppm w.r.t sample concentration: 20 mg/mL)



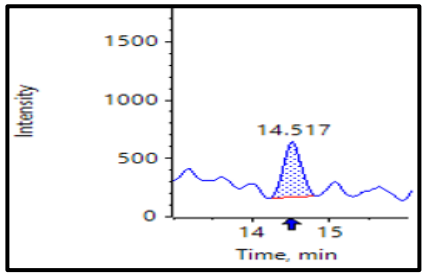
1-Methyl-4-nitrosopiperazine
MRM: 129.9→100



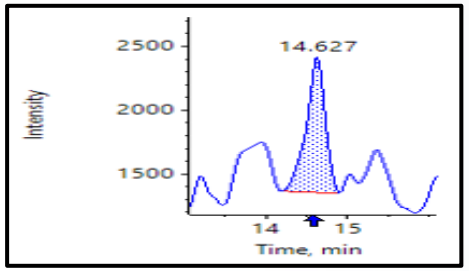
2-(4-Nitrosopiperazin-1-yl) ethanol
MRM: 160 → 99.0



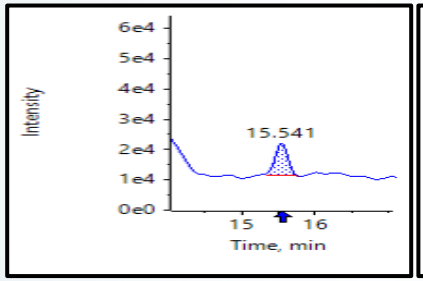
N-Nitrosodimethylamine (NDMA)
MRM: 75 → 58



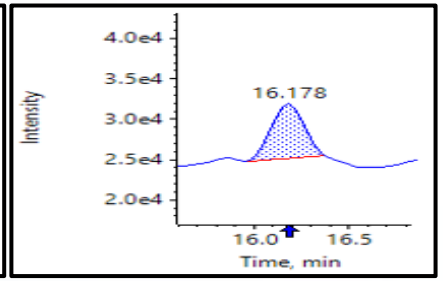
N-Nitroso morpholine
MRM:116.9 → 85.9



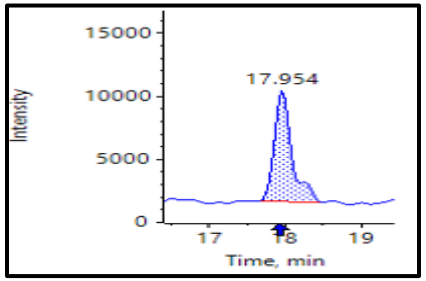
N-Nitrosomethylaminobutyric (NMBA)
MRM:146.9 → 117



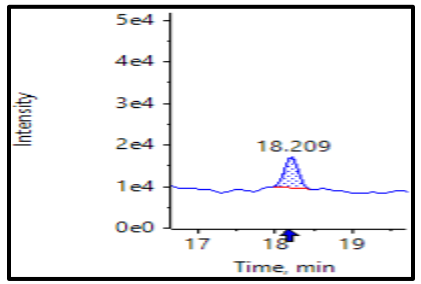
N-Nitrosopyrrolidine
MRM: 100.9→54.9



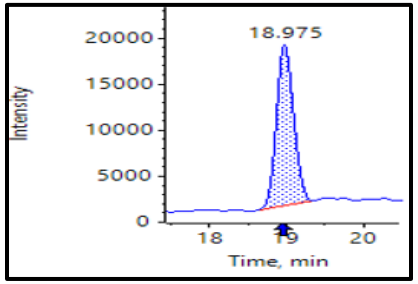
N-Nitrosodiethylamine (NDEA)
MRM: 103 → 75



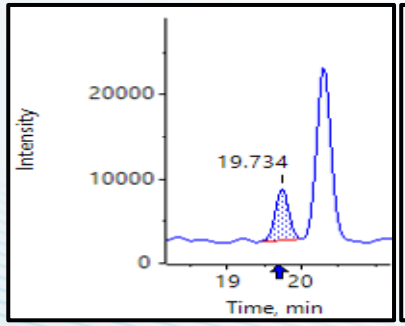
N-Nitrosoethylisopropylamine (NEIPA)
MRM: 117.1 → 75.0



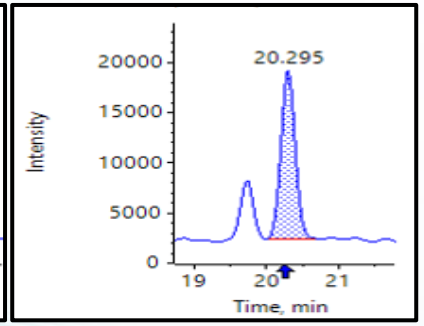
N-Nitrosopiperidine
MRM: 115 → 41



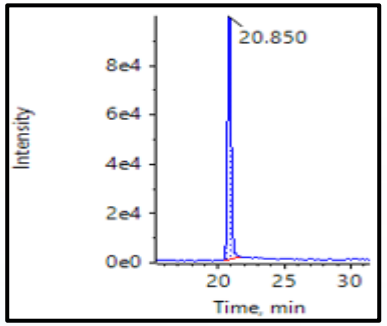
4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone
MRM: 208 → 121.8



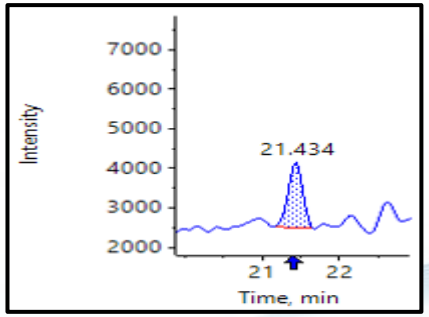
N-Nitrosodiisopropylamine (NDIPA)
MRM: 131.1 → 88.9



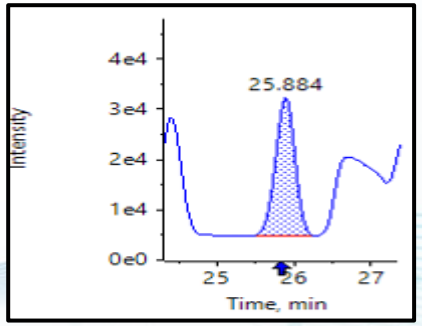
N-Nitrosodipropylamine (NDPA)
MRM: 131.1 → 88.9



2-(4-Nitrosopiperazin-1-yl) pyrimidine
MRM: 194→120.9



N-Nitrosomethylphenylamine (NMPA)
MRM: 137→107



N-Nitrosodibutylamine (NDBA)
MRM: 159→103

Method For NDSRIs: Work In-progress

Scope:

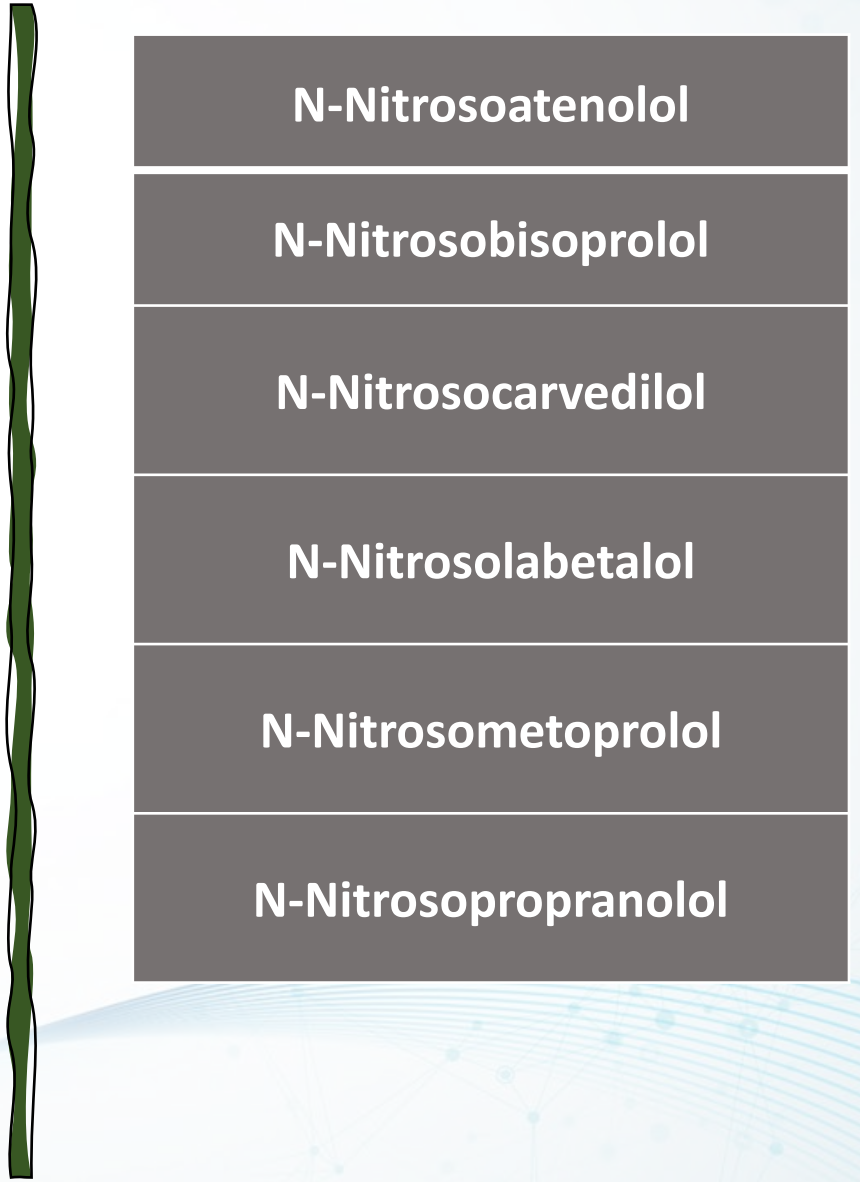
To develop analytical procedure for determination of NDSRIs.

Work Plan:

Identify a specific class of drug products.
Synthesis and characterization of reference materials.
Develop sensitive and robust analytical procedures (LC-MS/MS).

Status:

- Synthesis and characterization completed
- Method development is in progress



Pharmaceutical Analytical Impurities (PAI)

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Available in June and later

RFI CAS	Impurity name or Chemical formula	API	Molecular Formula
621-64-7	N-Nitrosodipropylamine Solution (NDPA)	–	C6H14N2O
61379-66-6	1-Cyclopentyl-4-nitrosopiperazine Solution	Rifapentine	C9H17N3O
16339-07-4	1-Methyl-4-nitrosopiperazine Solution	Rifampin	C5H11N3O
930-55-2	N-Nitrosopyrrolidine Solution	--	C4H8N2O
25081-31-6	N-Nitrosoiminodiacetic acid	--	C4H6N2O5
2219339-64-5	1-(2-Methoxyphenyl)-4-nitrosopiperazine Solution	Piperazine	C11H15N3O2
138768-62-4	N-Nitroso Metoprolol Solution	Metoprolol	C15H24N2O4
100-75-4	N-Nitrosopiperidine Solution	--	C5H10N2O
84418-35-9	N-Nitroso Propranolol Solution	Propranolol	C16H20N2O3
2820170-74-7	N-Nitroso Labetalol Solution	Labetalol	C19H23N3O4
134720-04-0	N-Nitroso Atenlol Solution	Atenolol	C14H21N3O4
2820170-76-9	N-Nitroso Bisoprolol Solution	Bisoprolol	C18H30N2O5

Pharmaceutical Analytical Impurities (PAI)

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Available in June and later

RFI CAS	Impurity name or Chemical formula	API	Molecular Formula
48121-20-6	4-Nitrosopiperazine-1-ethanol Solution	--	C6H13N3O2
1698-25-5	1-Benzhydryl-4-nitrosopiperazine Solution	Piperazine	C17H19N3O
1A04140*	4-Nitroso-1-(4-fluorobenzoyl)piperazine Solution	Piperazine	C11H12FN3O2
2470278-90-9	N-Nitroso Rasagiline Solution	Rasagline	C12H12N2O

Additional PAIs being prepared and coming soon!

- Includes a mix of both simple nitrosamine impurities and **N**itrosamine **D**rug **S**ubstance **R**elated **I**mpurities (NDSRI)
- Therapeutic categories of medicines with the potential to be affected by these impurities include:
  - Antidotes, Deterrents, and Toxicologic Agents
  - Central Nervous System Agents
  - Cardiovascular Agents
  - Genitourinary Agents
  - Blood Products/Modifiers/Volume Expanders
  - Antidepressants
  - Antiparkinson Agents

Strategy for Nitrosamines in Excipients

Scope:

To develop a strategy for the control of Nitrosamines in Excipients in collaboration with the Excipients Test Method Expert Committee

Work plan:

Determination of Nitrates and Nitrites in at risk excipients

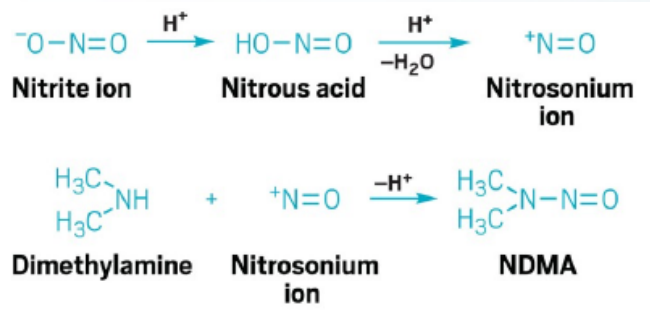
Preliminary Findings:

- Challenging sample preparation
- Interferences from other ions
- Inconsistent recoveries

Status:

- Work in-progress to establish a sensitive and robust method

Formation of NDMA from Nitrite



Nitrite in Excipients

- As part of risk assessment, the level of Nitrites and Nitrates in excipients needs to be evaluated and a control strategy needs to be established by the drug product manufacturers.

Nitrite and Nitrate in Excipients

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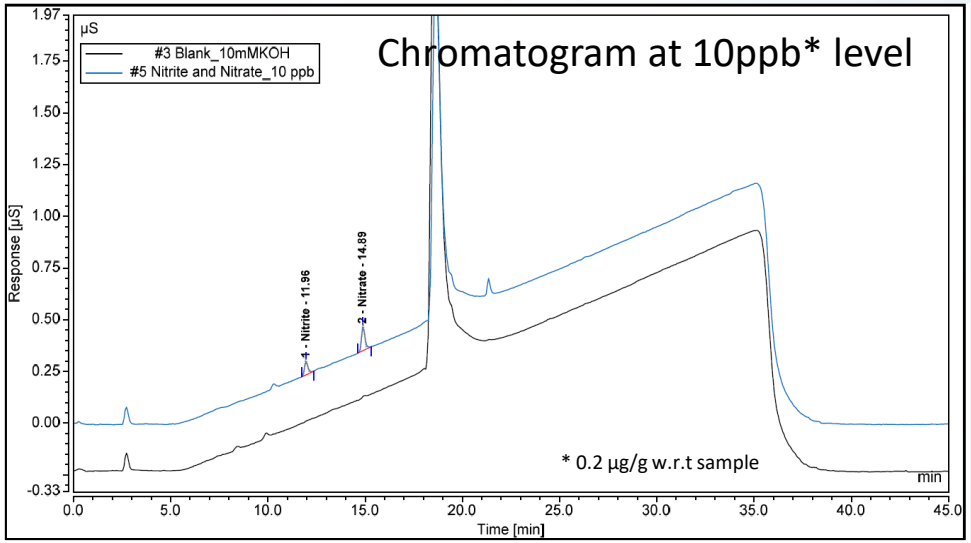
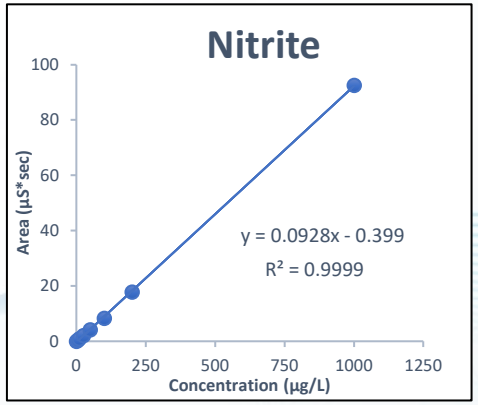
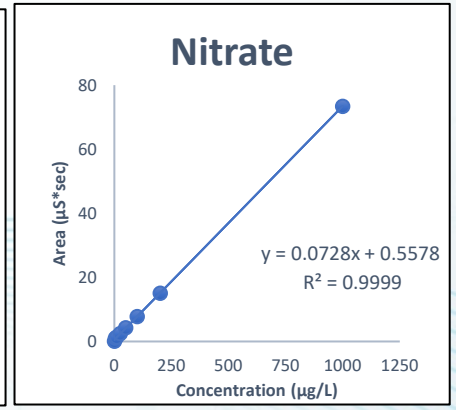
Excipients → **Sample preparation** → **Ion chromatography Conductivity detection**

- Lactose Monohydrate
- Crospovidone
- Povidone
- Colloidal Silicon Dioxide
- Hydroxypropyl cellulose
- Croscarmellose Sodium
- Pregelatinized Starch
- Stearic Acid
- Magnesium Stearate
- Microcrystalline Cellulose
- Sodium Starch Glycolate

- Total dissolution with Water ; Water : ACN/ MeOH
- Vortex/ Shaking
- Matrix Precipitation
- Filtration / Centrifugation
- Warming up in Water bath
- Extraction with Water & Organic solvent







Nitrite and Nitrate in Excipients

Excipients	Orthogonal approach	Challenges

Excipients
Lactose Monohydrate
Crospovidone
Povidone
Colloidal Silicon dioxide
Hydroxypropyl cellulose
Croscarmellose Sodium
Pregelatinized Starch
Stearic Acid
Magnesium Stearate
Microcrystalline Cellulose
Sodium Starch Glycolate

01 IC-Conductivity detection

Separation by Anionic exchange and followed by Conductivity detection

- Suppressor (sample load)
- Contamination-Vial, Water, Flask
- Analyte recovery
- Baseline drift due to Carbonate
- Sample swelling in diluent

02 HPLC-Fluorescence detection

Derivatization with 2,3-diaminonaphthalene (DAN)

- Reagent concentration
- Analyte recovery
- Sample pH
- Sample swelling in diluent

03 HPLC-UV Vis detection

Derivatization with Griess reagent

- Reagent concentration
- Analyte recovery
- Sample pH
- Sample swelling in diluent

Risk Assessment Tool

Scope:

To develop a ‘practical’ guidance document for conducting Risk Assessment [What ← → How]

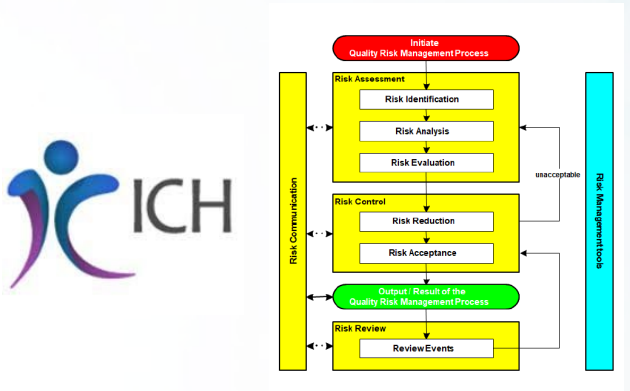
Work Plan:

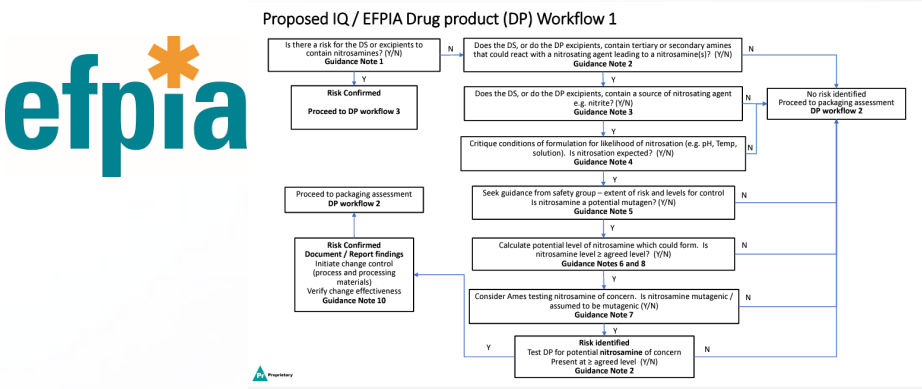
Development through a collaborative process with Nitrosamine Exchange community members

Inputs from Expert Committee and FDA liaisons

Publication of final guidance document (White Paper, Stimuli Article, Peer-review article)

Status: Draft proposal submitted to Expert Committee.





Overview of USP Nitrosamine activities

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Documentary Standards

<1469>- Nitrosamine Impurities

USP Reference Standards

- NDIPA
- NDMA
- NDBA
- NDEA
- NMBA
- NEIPA
- NMPA
- NDMA –d6

Training material/ Education course

Launched a tutorial, education course, lab demonstration videos on Nitrosamine impurities

USP Workshops / Webinars / Conferences

Scientific Webinars/ Workshops

Round table discussions/ stakeholder forums

Industry connect forums

Consortium

Non-Compendial Solutions

Nitrosamine Exchange

Analytical Hub

Pharmaceutical Analytical impurities

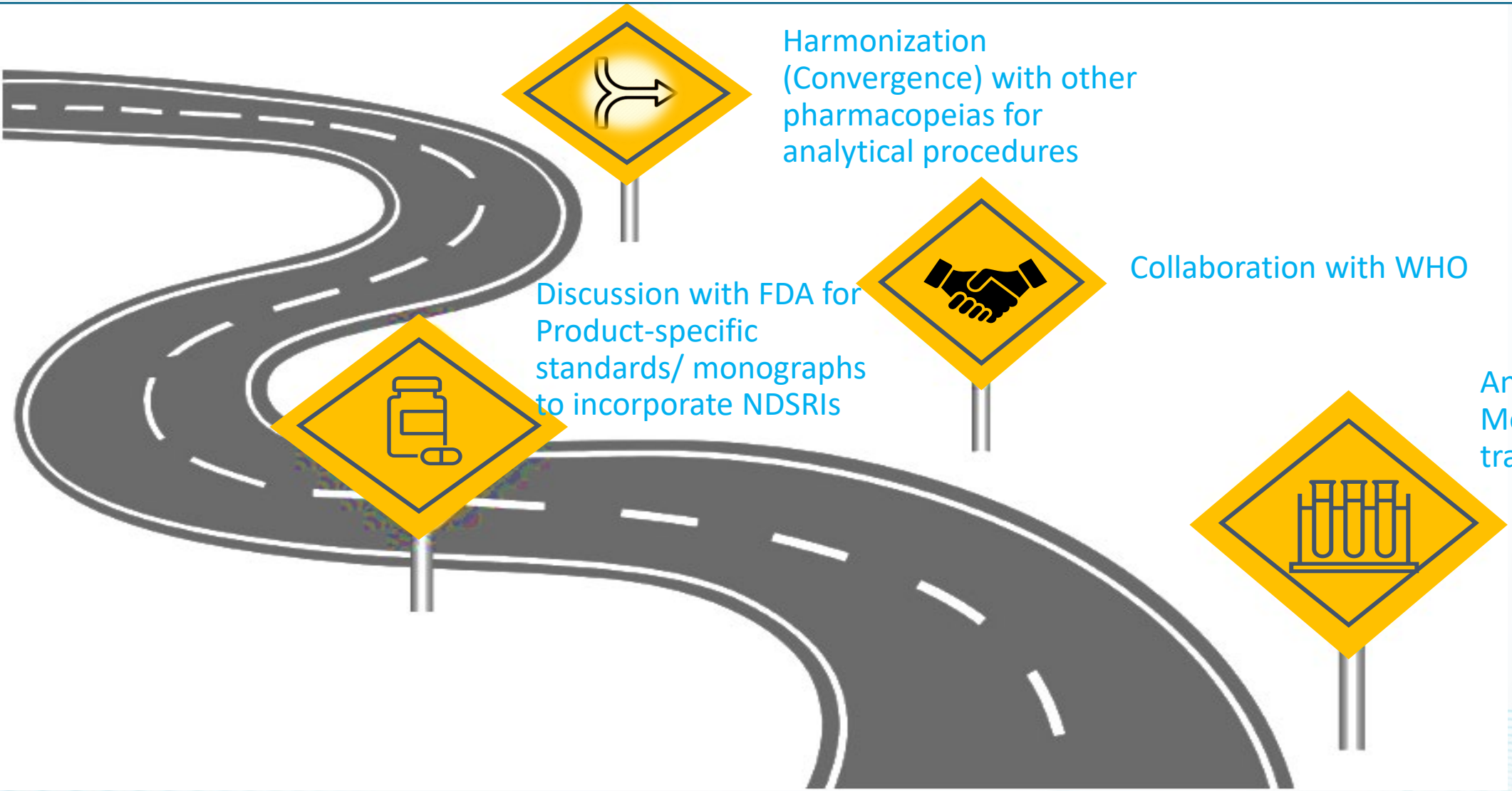
Publications

Survey

FDA Efforts

- **AAM/ CHPA/ PhRMA Questions** for May 4th FDA-Industry Meeting to Discuss Nitrosamine Impurities in Pharmaceuticals https://www.fda.gov/media/150864/download
- **FDA/HESI Workshop**: *Building A Research Roadmap for Hazard and Risk Assessment of Nitrosamine Impurities in Drugs*
- **Published a paper** with tittle *"Revisiting the mutagenicity and genotoxicity of N-nitroso propranolol in bacterial and human in vitro assays*".
- **FDA Notification for Public Comments:** Identification, Assessment, and Control of Nitrosamine Drug Substance-Related Impurities in Human Drug Products;
    - Federal Register :: Identification, Assessment, and Control of Nitrosamine Drug Substance-Related Impurities in Human Drug Products; Establishment of a Public Docket; Request for Comments

Future projects under consideration…



Harmonization (Convergence) with other pharmacopeias for analytical procedures

Collaboration with WHO

Discussion with FDA for Product-specific standards/ monographs to incorporate NDSRIs

Analytical services ??? Method development, training, etc.

Questions?

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Thank You

Stay Connected

mxj@usp.org