Industry Training Workshop

Management of Nitrosamine Impurities in Therapeutic Products

30 Oct 2024 | 2 to 4:30 PM (Singapore Time) | Online via Zoom

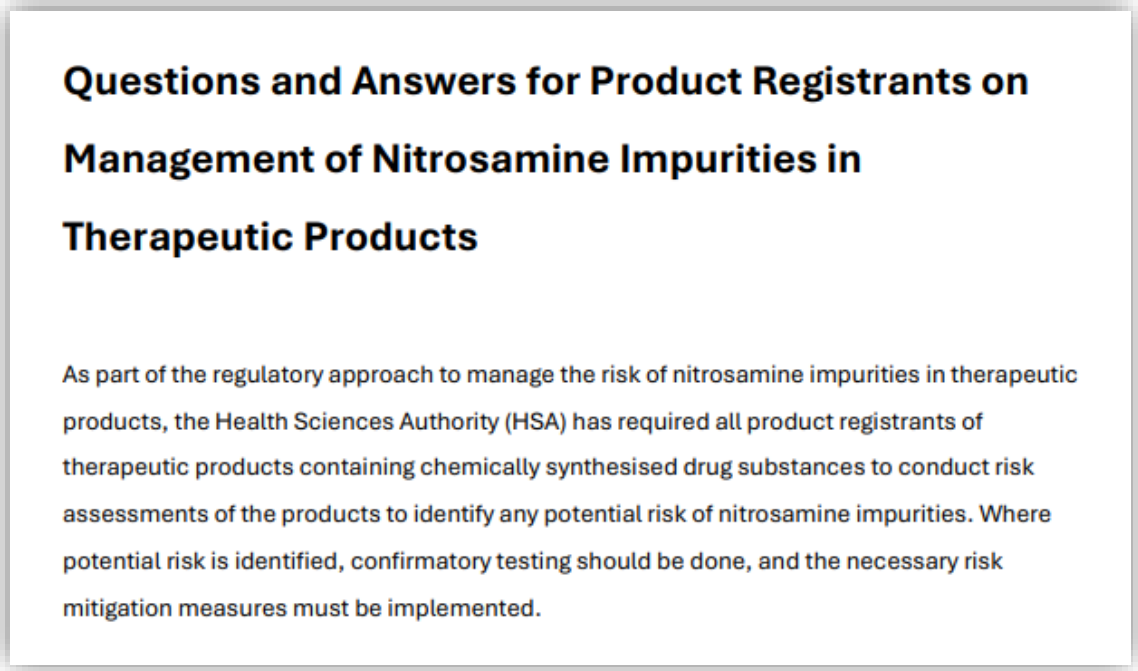
Health Products Regulation Group
Health Sciences Authority, Singapore

Caveat

This presentation is intended to provide general guidance. Every effort has been made to ensure that the information is up to date and factual, we do not, however, warrant its completeness. For specific concerns or questions, we recommend that companies consult with HSA directly.

Aim of the Training Workshop

1 Highlight key aspects of our recently published question-and-answer document that guides product registrants on managing nitrosamine impurities in therapeutic products



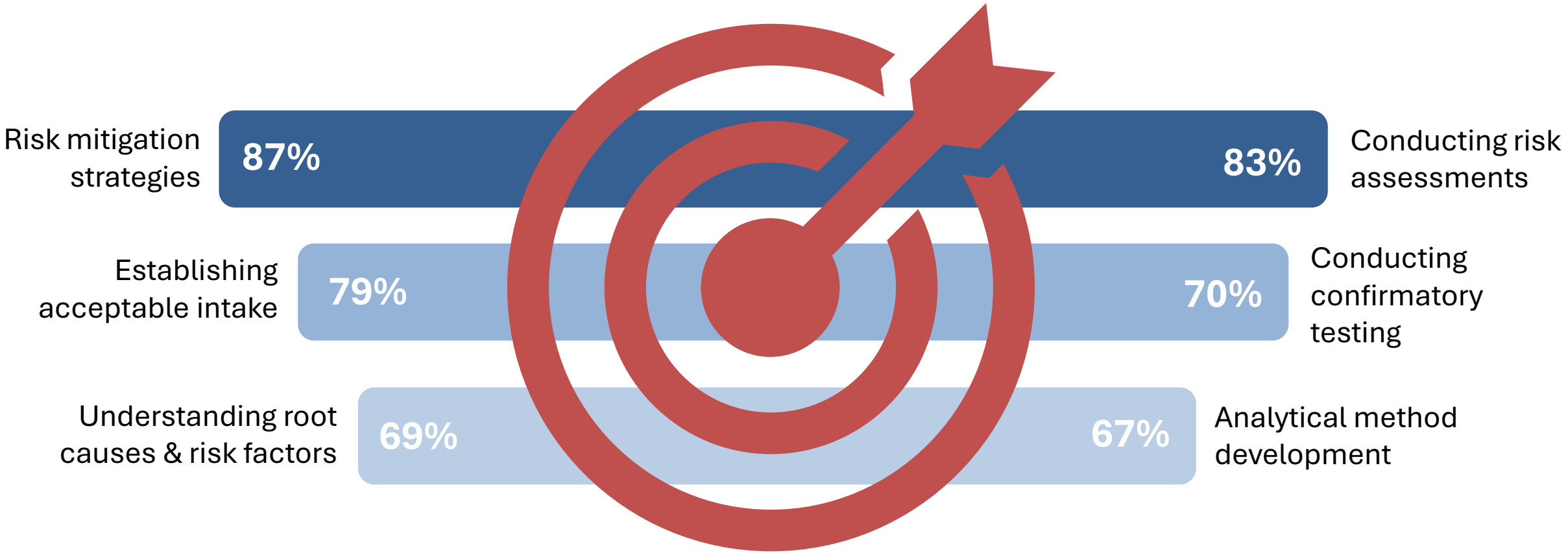
Questions and Answers for Product Registrants on Management of Nitrosamine Impurities in Therapeutic Products

As part of the regulatory approach to manage the risk of nitrosamine impurities in therapeutic products, the Health Sciences Authority (HSA) has required all product registrants of therapeutic products containing chemically synthesised drug substances to conduct risk assessments of the products to identify any potential risk of nitrosamine impurities. Where potential risk is identified, confirmatory testing should be done, and the necessary risk mitigation measures must be implemented.

https://go.gov.sg/qna-management-of-nitrosamine-impurities-in-therapeutic-products

2 Address some common concerns that you may encounter when managing nitrosamine impurities in your products

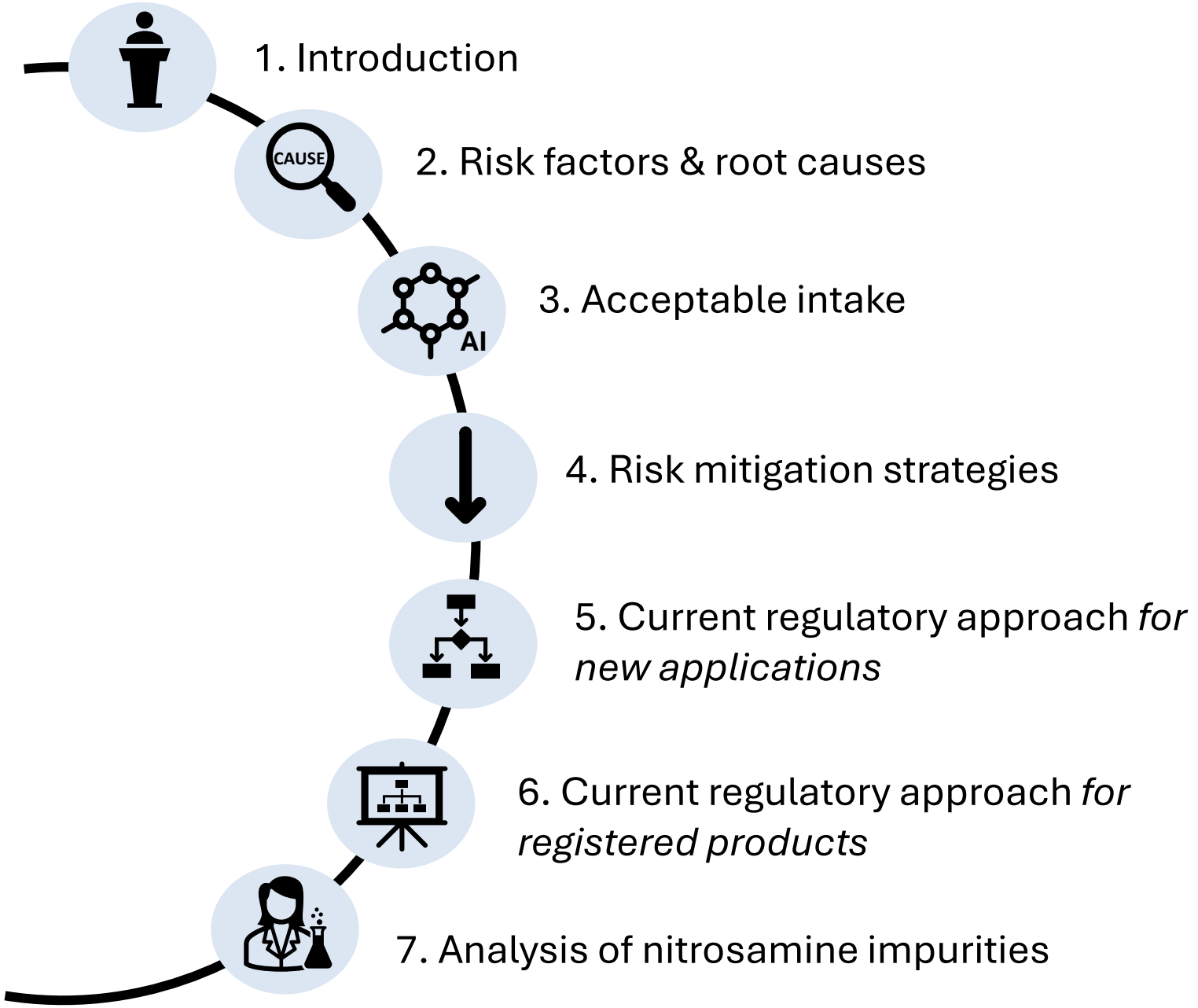
Aim of the Training Workshop



Results based on the 'Industry Survey on Therapeutic Product Defect Reporting and Recall Procedures' conducted from 9 July to 16 August 2024

Agenda

30 October 2024



Introduction to managing nitrosamines in therapeutic products

By: Filina Tan
Vigilance & Compliance Branch

- Main goals
- Timeline of events
- 3-steps approach in reviewing nitrosamine risk
- HSA nitrosamine resources
- International collaboration

Main Goals

of managing nitrosamine impurities in therapeutic products

Safeguard patient's health

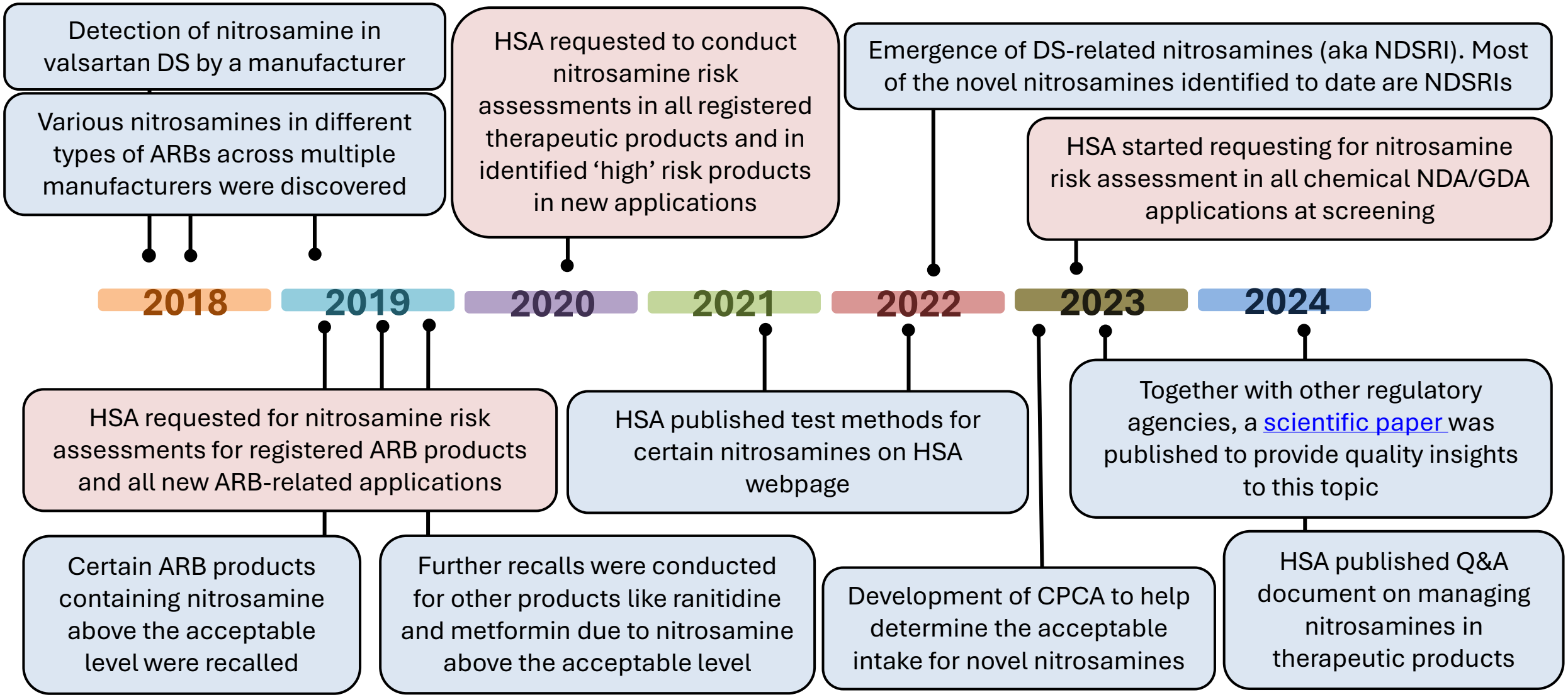
- Animal studies suggest nitrosamines are likely carcinogenic to humans
- To safeguard patients from unnecessary exposure, nitrosamines in medicines should be either eliminated or reduced to the lowest possible levels

Minimise supply disruption

- Complex and dynamic issue affecting numerous products
- Involving more than over 100 nitrosamines (and mostly novel) to date
- Addressing this issue requires substantial time, resources and effort

Timeline of Events

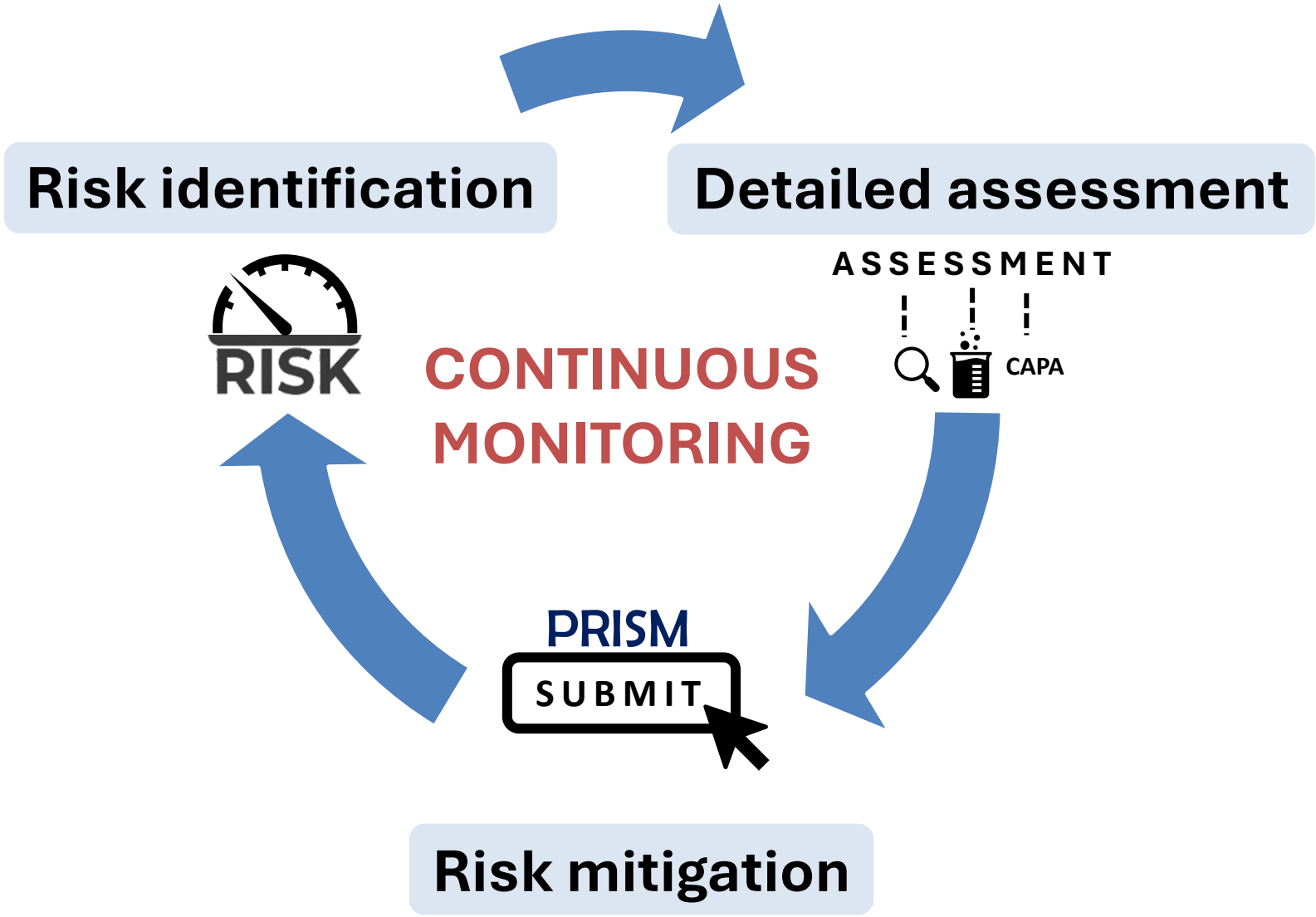
Regulatory measures (in red boxes) implemented to address evolving events (in blue boxes) over time



ARB: Angiotensin II receptor blockers; NDSRI: Nitrosamine drug substance related impurity; DS: Drug substance; CPCA: Carcinogenic Potency Categorisation Approach

3-steps Approach in Reviewing Nitrosamine Risk

An **ongoing process** throughout the lifecycle of the product

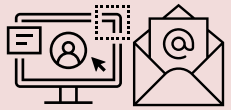


3-steps Approach in Reviewing Nitrosamine Risk

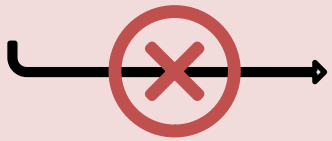
Risk identification

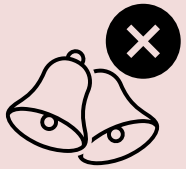
STEP 1

Conduct risk assessment to identify any risk for nitrosamine in the drug substance or drug product manufacturing process



Notify HSA via online Product Defect Reporting Form or via email at HSA_ProductDefect@hsa.gov.sg with the risk assessment documents





HSA does not need to be notified. However, retain the risk assessment documents and submit upon request

Detailed assessment

STEP 2

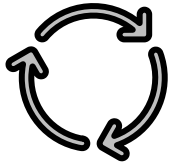
Conduct detailed assessment to identify the root cause, carry out product testing and develop appropriate CAPA (if needed)

Submit the detailed risk assessment report that includes the investigation outcome, test results and CAPA (if needed) to HSA

Risk mitigation

STEP 3

Submit the appropriate variation application for the required changes to mitigate the risk



Continue to monitor the developments and conduct re-assessments with discovery of any new root causes or implementation of any changes that could potentially alter the nitrosamine risk of the product

HSA Nitrosamine Webpage

HSA nitrosamine webpage
Provides general information on the background and the regulatory approach on the nitrosamine impurities issue

List of impacted medicines
Provides information on the list of therapeutic products recalled due to nitrosamine impurities as well as the list of drug substances for which HSA has temporarily allowed the use of a higher interim acceptable intake to avoid supply disruptions to patients

Test methods
Provides the test methods that have been developed by HSA for the identification and determination of nitrosamine impurities in some therapeutic products

Guidance for product registrants
Provides information on the actions required by product registrants to manage the issue

Q&A document
Provides further details and clarifies on HSA's current approach, recommendations, and regulatory requirements for managing nitrosamine impurities in therapeutic products

1 **Appendix 1**
HSA recommended acceptable intake for certain known nitrosamines

2 **Appendix 2**
Assessment aid for assessing nitrosamine risk

3 **Appendix 3**
Guidance on submission of nitrosamine risk assessment where a risk is identified

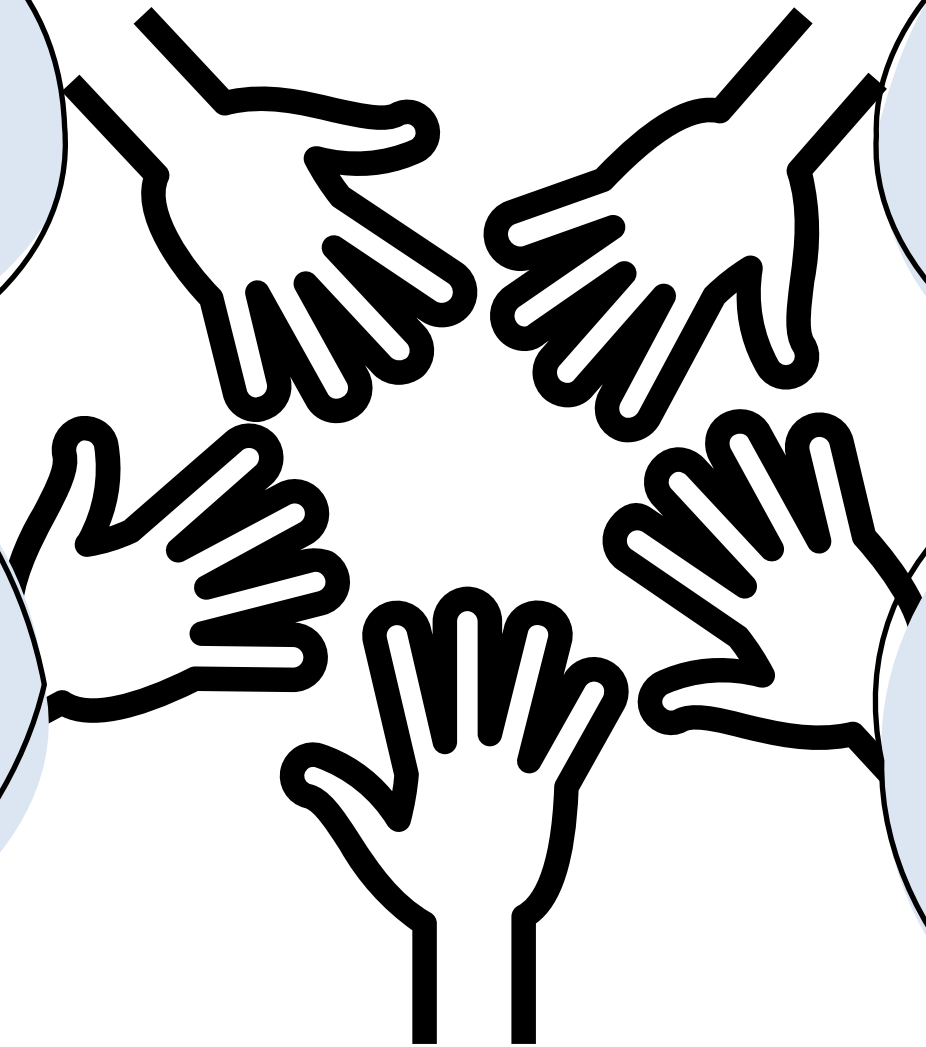
4 **Appendix 4**
Carcinogenic Potency Categorisation Approach (CPCA) for nitrosamines

5 **Appendix 5**
Enhanced Ames test (EAT) conditions for nitrosamines

International Collaboration

HSA collaborates with regulatory bodies from Australia TGA, Brazil ANVISA, Health Canada, EDQM, EMA, Japan MHLW, SwissMedic, the US FDA, and the WHO as part of the NISG/NITWG

This collaboration has led to the development of the scientific paper 'Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals'



This international collaboration strives to harmonise the:

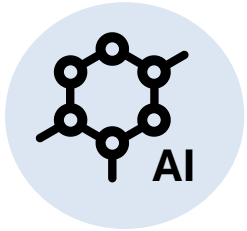
- Regulatory approaches for risk assessment
- Scientific principles for determining acceptable intake, and
- Risk mitigation strategies (wherever possible)

This paper provides an insight into the current scientific understanding of risk factors and potential root causes for nitrosamine impurities from a quality perspective, as well as recommendations for risk mitigation and control strategies

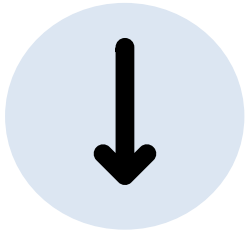
NISG: Nitrosamines International Strategic Group
NITWG: Nitrosamines International Technical Working Group

By: Choong Si Lan
Therapeutics Products Branch

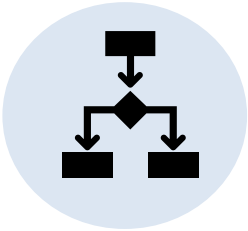
Risk factors & root causes



Acceptable intake



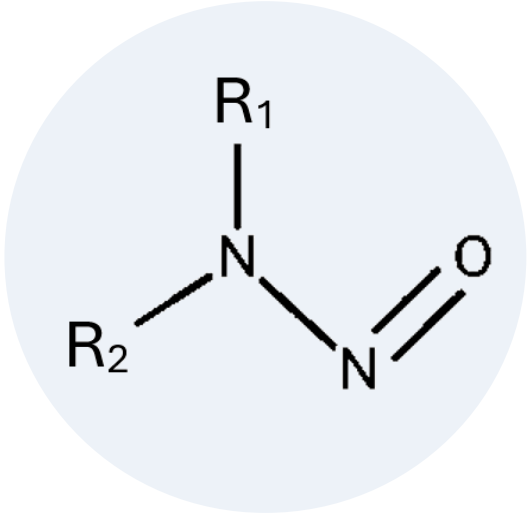
Risk mitigation strategies



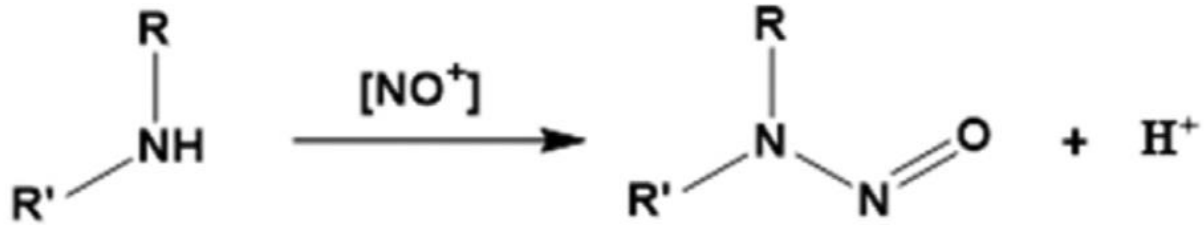
Current regulatory approach for <u>new</u> applications

- Nitrosamine risk assessment requirements

Nitrosamine



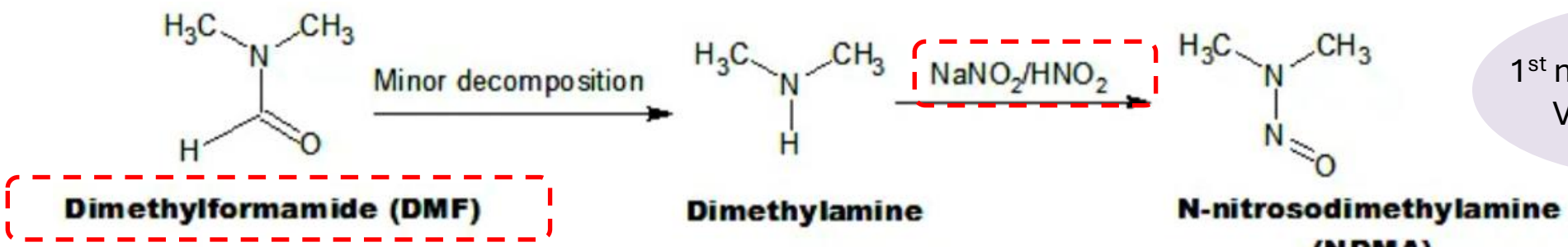
- Highly potent mutagenic carcinogens, ‘ cohort of concern’ (ICH M7 (R2))
- Some are classified as Group1, 2A or 2B by the International Agency for Research on Cancer (IARC)
- Impurities
- Formed by reaction between vulnerable amines and nitrosating agents



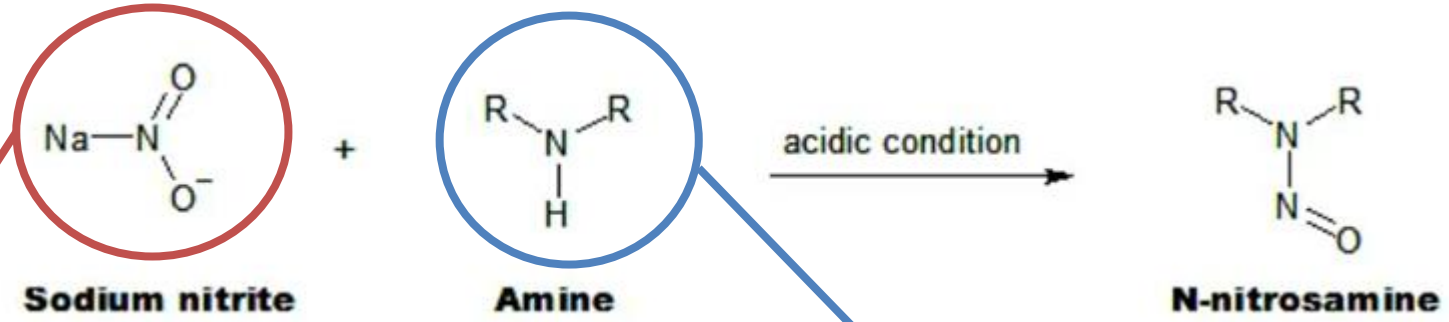
Scheme 1. Generalised nitrosation of secondary amines under acidic conditions.

Horne, S., et.al. (2023). Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals. *Journal of Pharmaceutical Sciences*, *112*(5), 1166–1182. https://doi.org/10.1016/j.xphs.2022.12.022
ICH M7(R2) Guideline on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk
https://monographs.iarc.who.int/list-of-classifications/

Common Risk Factors – In Drug Substance



1st nitrosamine alert: Valsartan 2018



Nitrite sources

- Nitric acid
- Nitrite salts, e.g., $NaNO_2$
- Contaminants in azides

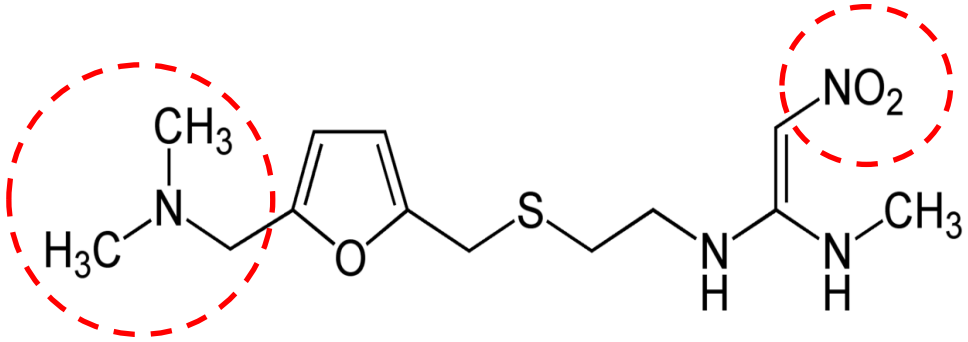
Amine sources

- Reagents, solvents
- Starting materials/Intermediates

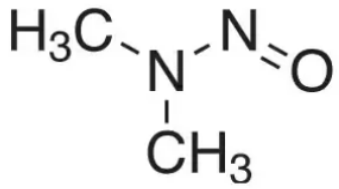
Common Risk Factors - In Drug Substance

- Presence of both an amine and nitro group as structural components of the DS
- Fragment under specific reactive and storage conditions

Example

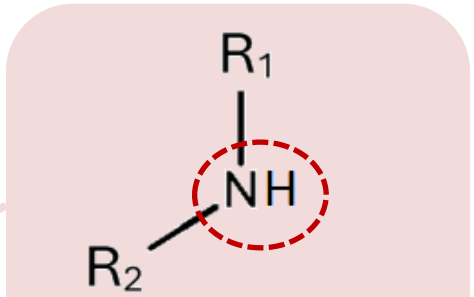


Ranitidine



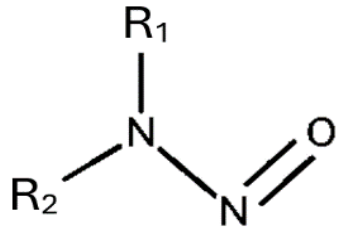
Nitroso-dimethylamine
(NDMA)

Common Risk Factors - In Drug Product



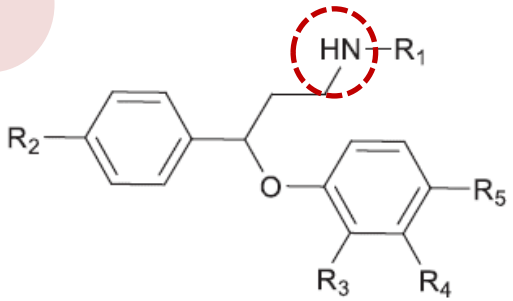
DS or DS fragment

Nitrites in excipients



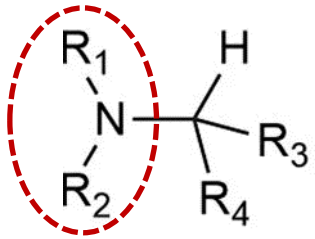
Nitrosamine Drug Substance-Related Impurities (NDSRI)

1



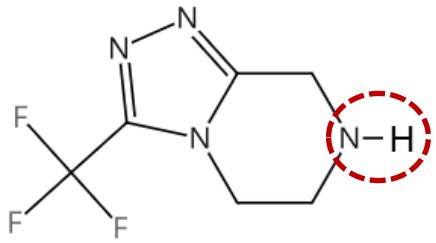
Secondary amine in DS structure

2



Tertiary amines can undergo dealkylative pathway to give secondary amine

3



Degradation impurity of DS containing a secondary amine

Schlingemann, J. et.al. (2022). The landscape of potential small and drug substance related nitrosamines in pharmaceuticals. Journal of Pharmaceutical Sciences, 112(5), 1287–1304. https://doi.org/10.1016/j.xphs.2022.11.013

Common Risk Factors – In Drug Product

Nitrites in excipients

- Nitrite levels present in some commonly used excipients

R. Boetzel et al. / Journal of Pharmaceutical Sciences 00 (2022) 1–10

Table 8
Estimation of Nitrite Contribution from Excipients in a Tablet Formulation.

Component	Function	Tablet Formulation Example		
		Mean nitrite [μg/g] ± SD	*Composition in Tablet [%]*	*Total Nitrite Contribution [μg/g] ± SD*
Active ingredient	Active	NA	15.0	NA
Microcrystalline cellulose	Diluent	0.70 ± 0.55	50.0	0.35 ± 0.28
Mannitol	Diluent	0.42 ± 0.19	22.5	0.09 ± 0.04
Hypromellose	Stabilizer	0.80 ± 0.75	5.0	0.04 ± 0.04
Crospovidone	Disintegrant	6.5 ± 4.7	3.0	0.19 ± 0.14
Colloidal silicon dioxide	Glidant	1.3 ± 0.71	1.0	0.013 ± 0.007
Sodium stearyl fumarate	Lubricant	0.39 ± 0.71	3.0	0.012 ± 0.02
Magnesium stearate	Lubricant	2.6 ± 1.8	0.5	0.013 ± 0.01
			TOTAL	***0.71 ± 0.23***

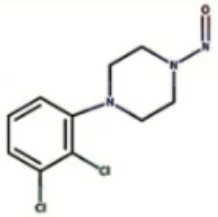
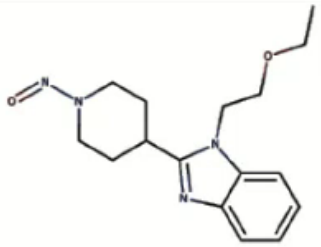
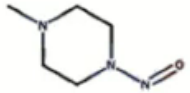
- Can act as nitrosating agents to react with amines in DS or DS fragments even if present in trace amounts
- Same excipient from different suppliers may contain nitrite at different levels depending on the manufacturing process
- There is batch-to-batch variability

Boetzel, R. et.al. (2022b). A Nitrite excipient database: a useful tool to support N-Nitrosamine risk assessments for drug products. Journal of Pharmaceutical Sciences, 112(6), 1615–1624. https://doi.org/10.1016/j.xphs.2022.04.016

Appendix 1: Acceptable Intake

Appendix 1: HSA recommended acceptable intake for certain known nitrosamines

The acceptable intakes (AI) below are recommended by the Health Sciences Authority and are determined using the Carcinogenic Potency Categorisation Approach (CPCA) where applicable. The source refers to drug substances that may potentially contain the nitrosamine impurity. However, this does not mean that the impurity will be found in all products or pharmaceutical forms containing the named drug substance. This list and the source examples provided are not exhaustive and will be updated as more information becomes available.

Structure	Name	Source (non-exhaustive)	CPCA Category (if applicable)	Recommended AI (ng/day)
	1-(2,3-dichlorophenyl)-4-nitrosopiperazine	Aripiprazole	3	400
	1-(2-ethoxyethyl)-2-(1-nitrosopiperidin-4-yl)-1H-benzo[d]imidazole	Bilastine	3	400
	1-methyl-4-nitrosopiperazine	Multiple sources	3	400

Last updated: 01 August 2024

Page **1** of **43**

Source: Appendix 1: HSA recommended acceptable intake for certain known nitrosamines

Acceptable Limit Calculation

- The acceptable limit (in ppm) for a single known nitrosamine can be calculated as follows:

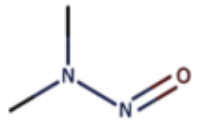
$$\frac{Acceptable\ intake\ of\ the\ nitrosamine\ (in\ ng/day)}{Maximum\ daily\ dose\ of\ the\ product\ (in\ mg)}$$

Example

To calculate the acceptable limit (AL) of the nitrosamine, NDMA, in metformin products:

Recommended AI for NDMA = 96 ng/day (refer to HSA Appendix 1)

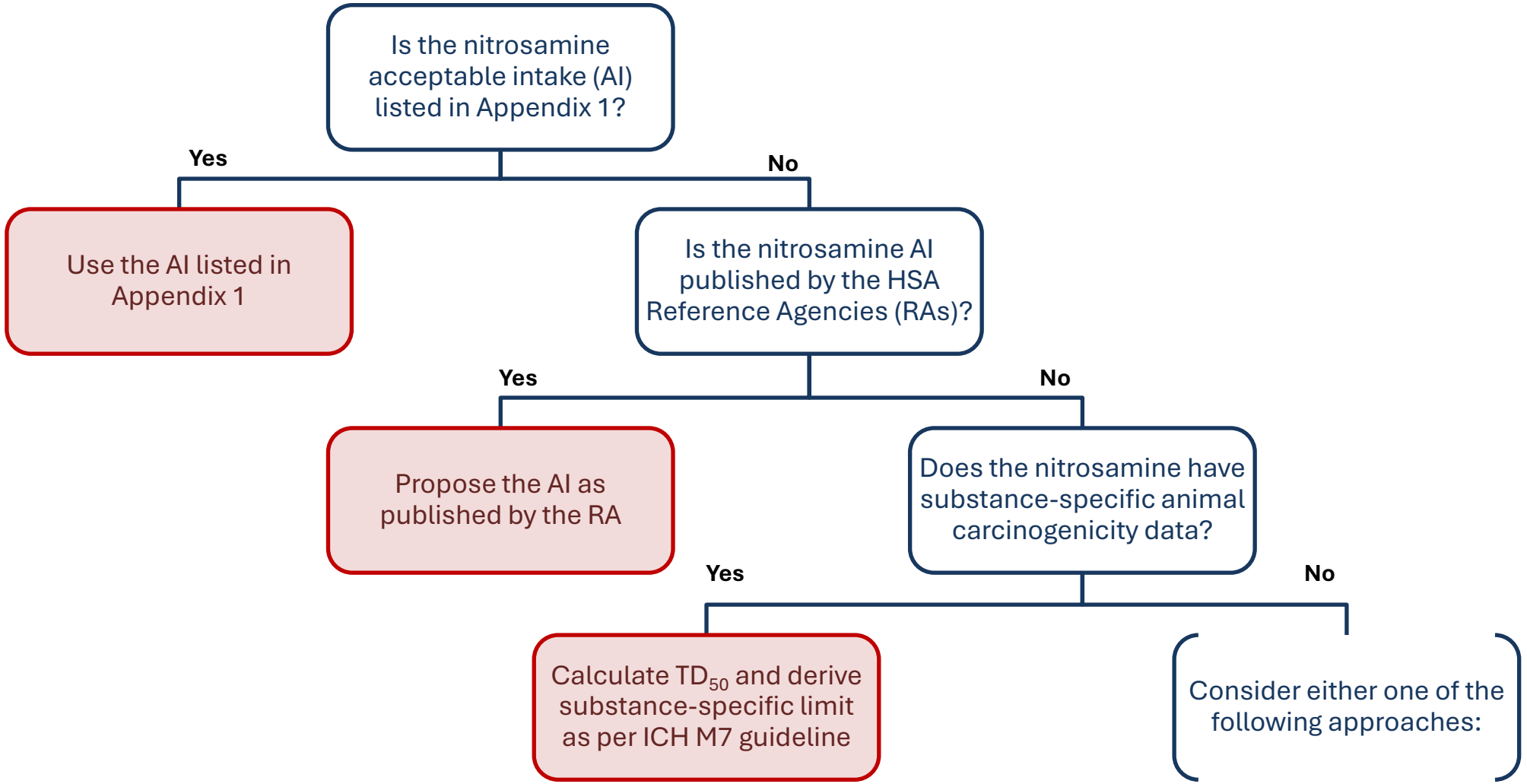
Appendix 1: HSA recommended acceptable intake for certain known nitrosamines

Structure	Name	Source (non-exhaustive)	CPCA Category (if applicable)	Recommended AI (ng/day)
	N-nitroso-dimethylamine	Multiple sources	-	96

- Maximum daily dose for metformin = 3000 mg
- Therefore, AL (in ppm) for NDMA in Metformin = $\frac{96\ ng/day}{3000\ mg/day} = 0.032\ ppm$

** For multiple nitrosamines, please refer to calculation in the Q&A document Question 21*

Establish Acceptable Intake - A Stepwise Approach



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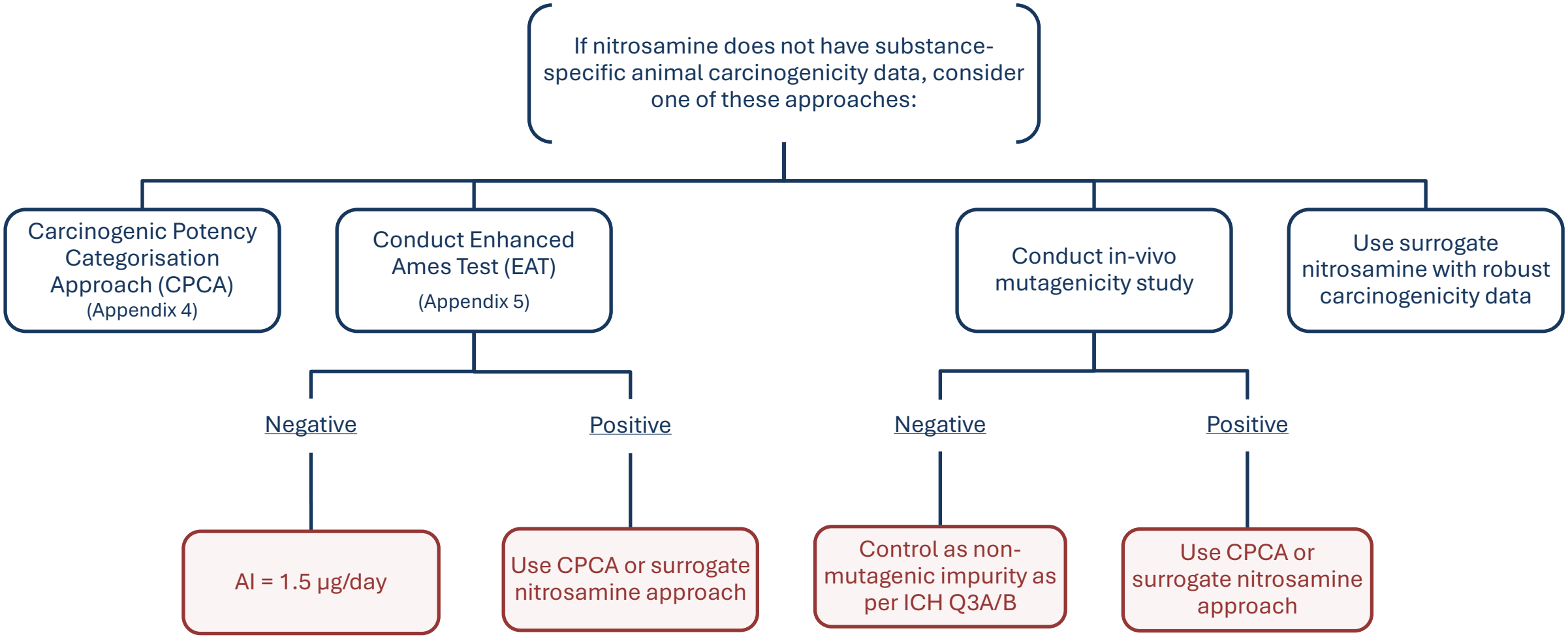
Establish Acceptable Intake - A Stepwise Approach



Appendix 2 – Assessment Aid

Download as a MS Word document

Product name	**ABC Tablet 100mg**
Name of drug substance manufacturer	**Maple-leaf Laboratories Co.**
Drug substance manufactured	**Sitagliptin Phosphate**
Manufacturing site address	**Northern Light Road**
Drug substance section is registered with HSA based on (select as appropriate):	☐ CEP Number: ☒ DMF Number: **015: 001** ☐ CTD
	☐ Drug substance manufacturer information is not captured in PRISM (for existing products only)

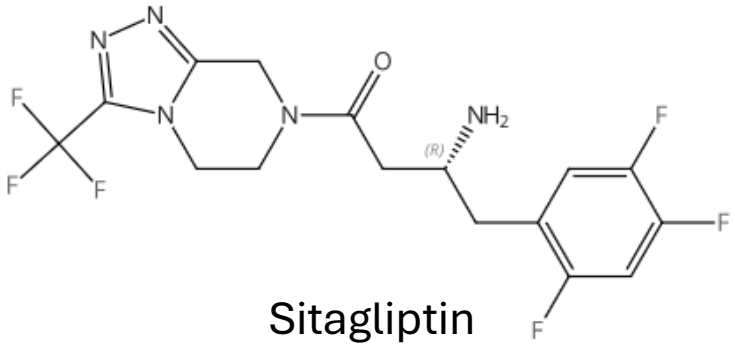
Tip:
Click to repeat for each DS manufacturer

Source: Appendix 2: Assessment aid for assessing nitrosamine risk

Appendix 2 – Assessment Aid

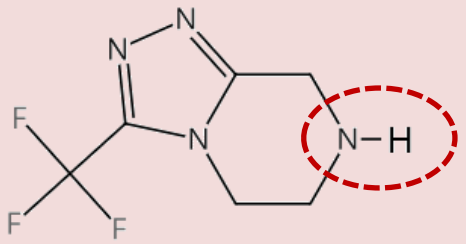
No.	Question	Yes	No
1	Is inorganic or organic nitrite used in the drug substance synthesis (including in the manufacture of starting materials/ intermediates)?	☐	☒
2	Are potential nitrite sources present in the drug substance synthesis (including in the manufacture of starting materials/ intermediates), or could impurities with nitrites or nitrite sources be present in the starting materials, reagents, catalysts/ processing aids or solvents? * * *e.g., nitrates + reducing agents, HNO_3 + reducing metals, urea/ammonium + hypochlorite/chlorine*	☐	☒
3	Are secondary or tertiary amines used in the drug substance synthesis (including the manufacture of starting materials/precursors/intermediates), or could amines or amine sources be present as impurities in starting materials, reagents, catalysts/processing aids or solvents (e.g., dimethylformamide)? *For drug substance manufacturers supported by CEP, does any of the specified impurities or residual solvents contain a secondary or tertiary amine functional group?*	☒	☐
4	Could nitrosamines be present as impurities in the starting materials, reagents, catalysts, processing aids or solvents?	☐	☒
Overall risk assessment for drug substance manufacturer			

If the answer is "Yes" to ANY of the questions above, the manufacturing process of the drug substance is deemed to be at risk for nitrosamine impurity. Please provide a detailed risk assessment.



Sitagliptin

Degrade



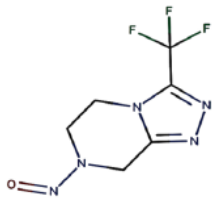
Precursor to NTTP

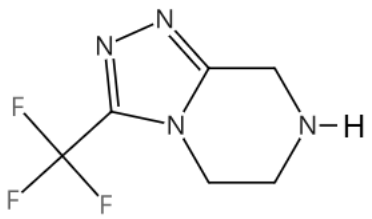
3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4] triazolo[4,3-a]pyrazine

Appendix 2 – Assessment Aid

No.	Question	Yes	No
1	Are there known nitrosamine drug substance related impurities (NDSRI) associated with your drug product? *References:* • *Appendix 1 - HSA Recommended Acceptable Intake for Certain Known Nitrosamines* • *List of acceptable intakes established for nitrosamines that is published by any of HSA's reference agencies (refer to Question 33 of the Question-and-answer document)*	☒	☐
2	Does the drug substance or its related impurities/degradants contain a secondary or tertiary amine?	☒	☐
3	Are residual nitrites known to be present in any of the excipients or solvents? *Reference:* • *Paper 'Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals'.*	☒	☐

Appendix 1: HSA recommended acceptable intake for certain known nitrosamines

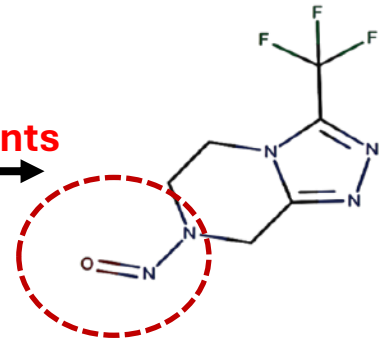
Structure	Name	Source (non-exhaustive)
	7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3-a]pyrazine (NTTP)	Sitagliptin



Precursor to NTTP

3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4] triazolo[4,3-a]pyrazine

Nitrites in excipients

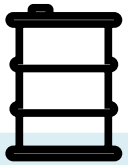


NTTP

- Assess increase during storage

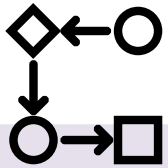
Risk Mitigation Strategies

– In Drug Substance



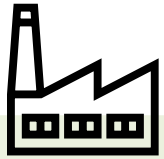
CHOICE OF RAW MATERIALS

- Avoid the use of amines/ amides as a solvent or reagent, where possible
- Residual nitrites in reagents used should be understood and controlled where appropriate (e.g., nitrate salts)



PROCESS DESIGN

- Ensure upstream nitrites are effectively purged before the introduction of downstream amines
- If formation of nitrosamines is unavoidable, ensure effective purging process is in place



CROSS CONTAMINATION CONTROL

- Ensure effective cleaning to remove any residual nitrites from previous process
- Limit the use of recovered materials

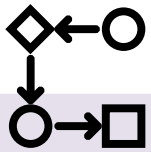
U.S. Food & Drug Administration. Control of Nitrosamine Impurities in Human Drugs Guidance for Industry (SEPTEMBER 2024)
Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities for Pharmaceuticals

Risk Mitigation Strategies

– In Drug Product

NITRITE CONTROLS IN EXCIPIENTS

- Select excipients with lower nitrite content
- Well-designed drug-excipient compatibility studies
- Choose suppliers with better nitrite controls
- Comprehensive supplier qualification program
- Include control of nitrites in excipient specification



PROCESS/ FORMULATION DESIGN

- Optimization of process (e.g., fluid bed drying)
- Addition of nitrite scavenger in the formulation (e.g., ascorbic acid, propyl gallate)

U.S. Food & Drug Administration. Control of Nitrosamine Impurities in Human Drugs Guidance for Industry (SEPTEMBER 2024)
Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities for Pharmaceuticals
Bayne, A.-C. et.al. (2023). N-nitrosamine mitigation with nitrite scavengers in oral pharmaceutical drug products. Journal of Pharmaceutical Sciences, 112(7), 1794–1800. https://doi.org/10.1016/j.xphs.2023.03.022
Nanda, K. K. et.al. (2021). Inhibition of N-Nitrosamine formation in drug products: a model study. Journal of Pharmaceutical Sciences, 110(12), 3773–3775. https://doi.org/10.1016/j.xphs.2021.08.010

Control Strategies

ROUTINE TESTING

- Required when:
  - Risk of nitrosamine formation is high, or
  - Nitrosamine level is found to be at >30% of the AI, or
  - Nitrosamine is detected in the drug product and the root cause is unknown, regardless of the level detected
- LoQ of the test method must minimally correspond to the AI of the nitrosamine

SKIP TESTING

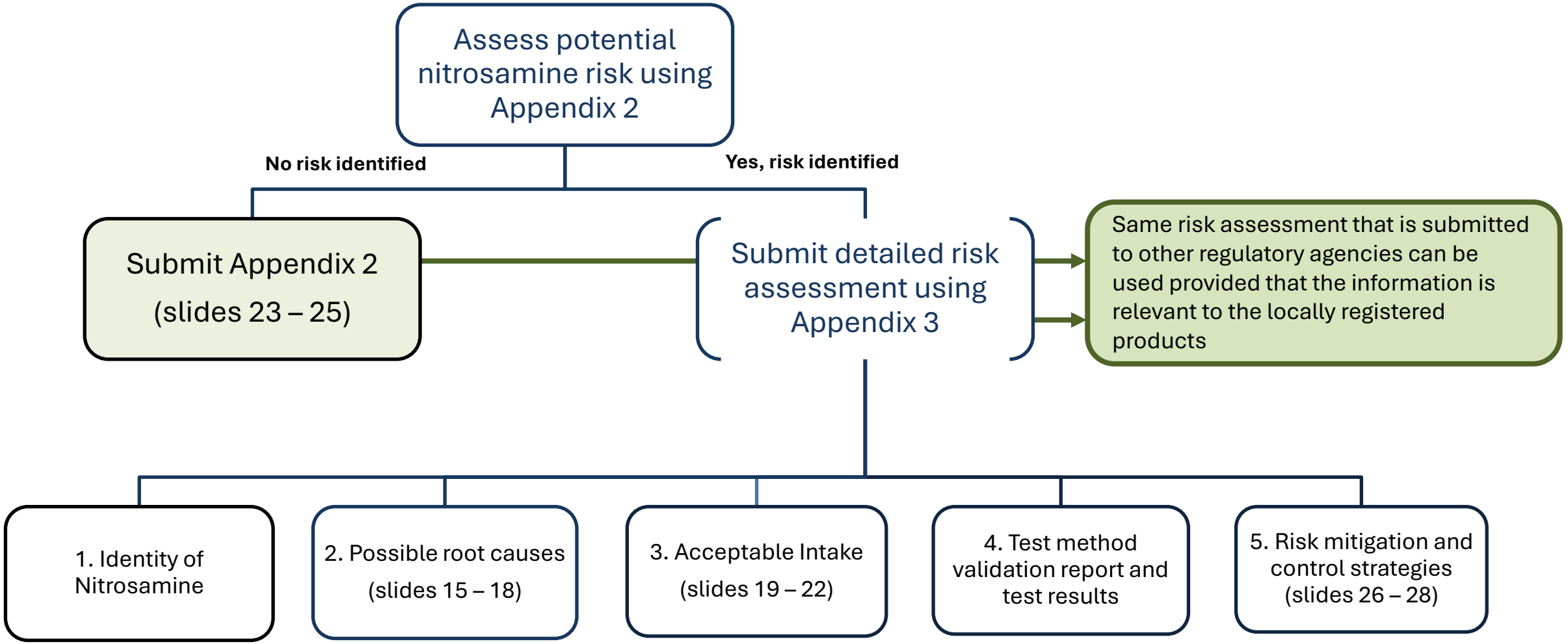
- Required when:
  - Root cause of the nitrosamine formation is known, and
  - Nitrosamine level is consistently ≤30% of the AI
- LoQ of the test method must minimally correspond to 30% of the AI of the nitrosamine

NO TESTING

- Possible when:
  - Nitrosamine level is consistently ≤10% of the AI, and
  - Root cause of the nitrosamine formation is known such that appropriate measures can be established to ensure that the nitrosamine level will consistently be at ≤10% of the AI throughout the product shelf life
- LoQ of the test method must minimally correspond to 10% of the AI of the nitrosamine

Risk Assessment Requirements at Submission

- For new GDA, NDA & MIV applications



Risk Assessment Requirements at Submission

- For new GDA, NDA & MIV applications

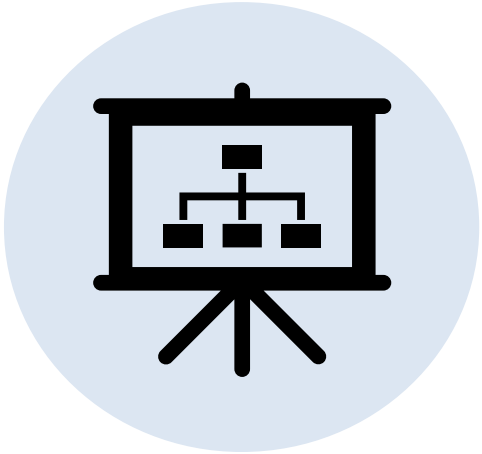
The risk assessment is required for all chemically synthesised drug substance

Must be provided at point of submission for any new GDA and NDA, and any MIV where the change made could alter nitrosamine formation risk
CTD 3.2.S.3.2 (Drug Substance); CTD 3.2.P.5.5 (Drug Product)

Includes the confirmatory test data when nitrosamine risk is identified
For products with high risk for nitrosamine formation: Test results are required before the application can be accepted for evaluation

The level of nitrosamines present should meet the AI specified in Appendix 1, with the necessary controls (e.g., testing) in place where required

Current regulatory approach for registered products



By: Filina Tan
Vigilance and Compliance Branch

- Risk assessment requirements
- Similarities & differences between the requirements for new applications & registered products
- Interim acceptable intake

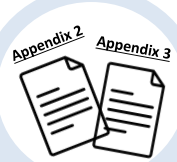
Risk Assessment Requirements

- For registered products

NITROSAMINE RISK ASSESSMENT

Same approaches as new applications

Required for all therapeutic products containing chemically-synthesised drug substance



Use Appendices 2 & 3

to help identify the risk for nitrosamine formation and to guide the risk assessment

Same risk assessment that is submitted to other regulatory agencies can be used

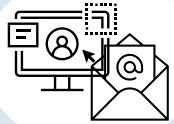
Provided that the information is relevant to the locally registered products (e.g., with the same registered manufacturers, product formula, storage conditions and specifications)

Risk Assessment Requirements

- For registered products

SUBMISSION OF RISK ASSESSMENT

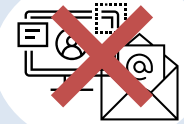
New applications: Risk assessment should be provided at point of application submission regardless of risk presence



Notify HSA if risk is identified during initial and re-assessments

via online Product Defect Reporting Form or via email at HSA_ProductDefect@hsa.gov.sg with the risk assessment documents containing:

- (Preliminary) root cause
- Confirmatory test results
- Interim/Final CAPA plan
- Health hazard assessment
- An assessment on whether the product is medically necessary or important
- Market action proposed (if any)
- An assessment on whether any disruption to product supply is expected



HSA do not need to be notified if risk is not identified during initial and re-assessments

Retain the risk assessment documents and submit upon request

Current Regulatory Approach

- For registered products

NEED FOR MARKET ACTION

If test results exceeds AI

- Consider if market action is required by balancing:
  - The risk to patient safety
  - The medical need of the product
  - Market availability of the product
- As with all other product defects involving impurities:
  - Investigate the root cause, establish and implement CAPA to reduce the nitrosamine levels to ≤AI to prevent unnecessary long-term exposure and safety risk to patients

Current Regulatory Approach

- For registered products

INTERIM ACCEPTABLE INTAKE

Takes substantial time to address

- To prevent disruption of supply of registered products (particularly essential medicines), an interim AI can be proposed for consideration. Please contact us to discuss further

- The interim AI proposed should be:

  - Scientifically sound; and
  - The theoretical excess cancer risk does not exceed 1 in 100,000 during the CAPA implementation period

New applications: An interim AI is generally not allowed for products in new applications. The level of nitrosamines present in products in new applications should meet the AI specified in Appendix 1 before the application can be approved

Current Regulatory Approach
- For registered products

INTERIM ACCEPTABLE INTAKE

- One way to calculate the interim AI:
  - Apply the less-than-lifetime (LTL) approach described in the ICH M7 guideline using the two most conservative adjustment factors:

Treatment duration	Up to 12 months	>12 months
Interim AI	13.3 x AI*	6.7 x AI*

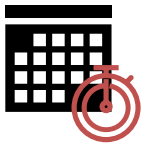
**In any case the AI should not exceed 1.5 µg/day unless the AI (in Appendix 1) is > 1.5 µg/day, or the nitrosamine is placed in Potency Category 5 according to CPCA, or the nitrosamine is shown to be negative in an enhanced Ames test (EAT).*

  - CAPA implementation period:
    - Complete as soon as practically possible
    - Start promptly to buffer against potential unforeseen delays in your CAPA implementation process
    - Provide us with the projected time frame for completing the CAPA and we can discuss on the appropriateness of the proposed timeline

Current Regulatory Approach

- For registered products

INTERIM ACCEPTABLE INTAKE



Time-limited use
It can only be used for a pre-defined period as agreed with HSA because it is only a temporary measure to ensure continuity of medicine supply while the CAPA is being implemented

Additional monitoring
Additional monitoring measures may be required during period of use to ensure nitrosamine levels remain within acceptable levels. For example, mandatory routine testing of every batch of drug product prior to supply in Singapore may be required

Applies to single nitrosamine only
The interim AI cannot be used for products with multiple nitrosamines. Please contact us to discuss on the approach to be taken for such cases

Conclusion

- Nitrosamines are a subset of impurities that are routinely identified in CTD S3.2 and P5.5. Therefore, like other impurities, they need to be:
  - Evaluated during product applications; and
  - Reported if detected in a product post-registration
- In line with our standard product defect reporting procedure, please:
  - Provide as much information as possible
  - Do not postpone your report if certain details are not immediately available. You can always submit additional information later when it becomes available
- For confirmatory testing, please remember to:
  - Test for products that are stored under our locally registered conditions; and
  - Include batches that are near the end of their locally registered shelf life to provide insight into nitrosamine levels in products stored under our climatic conditions
- Information shared is current as of today. Given the dynamic nature of this issue, please keep abreast of the developments and conduct re-assessments if new information arises that could potentially alter the nitrosamine risk of your product

FAQ

<u>Question #1:</u> Does the risk assessment exercise apply to biological therapeutic products?

- A nitrosamine risk assessment is still required to be submitted to HSA if nitrosamine risk factors are present

- For example: It should be conducted for biological therapeutic products containing chemically synthesised fragment such as antibody-drug-conjugates

FAQ

Question #2: Can HSA align the acceptable intake and risk assessment template with other regulatory agencies?

- Continual harmonisation effort through international collaborative activities are on-going

- HSA collaborates closely with regulatory bodies from Australia TGA, Brazil ANVISA, Health Canada, EDQM, EMA, Japan MHLW, SwissMedic, the US FDA, and the WHO as part of the NISG/NITWG to harmonise (as much as possible) the regulatory approaches for risk assessment and the scientific principles for determining acceptable intake

- The regulatory approach in terms of risk assessment requirements as well as establishing of acceptable intakes for nitrosamines (as listed in Appendix 1) are largely internationally aligned. For nitrosamines which are not listed in Appendix 1, product registrants may propose the acceptable intakes published by our reference agencies for discussion

- Risk assessment templates that is submitted to other regulatory agencies can be used, provided that the information is relevant to the locally registered product

FAQ

<u>Question #3:</u> Can HSA provide flexibility and consider acceptance of product registrant's proposed acceptable intakes based on scientific/technical justification and data?

- Product registrants may propose alternative acceptable intakes (AI), provided they are supported by robust scientific and technical justifications with relevant data. The acceptability of alternative proposals will be assessed and determined during evaluation

- For example: The AI of N-nitroso-diclofenac was revised from 1.5 µg/day (based on CPCA) to 78 µg/day via structure-activity relationship using N-nitroso-diphenylamine (NDPh) as reference. The revision was supported by showing NDPh can be considered as a conservative surrogate compound

FAQ

Question #4: Given the challenges of finding a laboratory in Singapore to perform nitrosamine testing and the high costs associated with developing an in-house test method, how can product registrants overcome these challenges?

- HSA has been publishing the test methods that we have developed when available. Product owners/manufacturers may also refer to the available methods published by reference regulatory agencies

- These methods can be used as a starting point for the development and validation of the product owners/manufacturer's own methods. It is important to validate the method before use to ensure its suitability for the intended purpose

- While regulators can help by providing guidance for method development, it is still the responsibility of the product owner/manufacturer to ensure that adequate resources are available so that their products continue to meet safety, quality and efficacy standards

- Product owners/manufacturers are strongly encouraged to utilise these available resources to develop their own testing capabilities

FAQ

<u>Question #5:</u> Given that confirmatory testing is time-consuming, it may impact marketing plans. Can product registrants initiate the submission process first so that HSA can start evaluation while awaiting the results of confirmatory tests?

- HSA requires the complete risk assessment and the test results, where applicable, at the time of application submission for the evaluation to be carried out in a timely and efficient manner

- Where the product registrant is unable to meet the above requirements and the product is critical for meeting Singapore's healthcare needs, please contact HSA to discuss

FAQ

Question #6: Will the registration timeline be affected when a new nitrosamine is identified during evaluation? Can HSA approve the application first with the product registrant committing to provide test results post approval?

- Product registrants are required to demonstrate effective, adequate control of impurities before product applications (NDA, GDA) can be considered for approval. This approach applies to all impurities including newly identified nitrosamines. Hence, if the product has been assessed to have a high risk of the new nitrosamine, the final registration timeline for the application will be dependent on the time taken by the product registrant to provide the test results

- This regulatory approach for new applications is necessary to:

  - Protect public health by limiting the introduction of medicines with nitrosamine levels above acceptable limits into the market
  - Reduce the need for post-market interventions like recall

- Considerations may be given to products that have high medical needs in Singapore, where the need for the product is assessed to outweigh the potential risks of the nitrosamine

FAQ

<u>Question #7:</u> Can product registrants omit data on nitrosamine formation under Zone IVb conditions if the information is unavailable due to the manufacturer primarily serving markets in different climatic zones?

- For registered products
  - HSA would need to understand the risk of nitrosamines in these products as soon as possible as the products are already on the market. Hence, HSA may accept other forms of justification if they are supported by robust scientific data (e.g., extrapolation from stressed/accelerated testing). This will be subject to further assessment
- For new applications
  - Stability data at Zone IVb is required in accordance with current product registration guidelines

FAQ

Question #8: Can HSA provide a longer timeline to respond to post-market queries?

- Extension to timelines can be requested provided that:
  - The reasons for delay are well-justified, and
  - There are no urgent matters requiring immediate action
- HSA encourages product registrants to work together with their manufacturers to conduct the assessments promptly. Should more time be required, please submit a written request for an extension, clearly explaining the reasons for it

FAQ

<u>Question #9</u>: Why is the Less Than Lifetime (LTL) approach for acceptable intake not applicable to new applications but only to registered products?

- As nitrosamines are generally highly potent and classified as a cohort of concern in ICH M7 guideline, it was assessed that the LTL approach is not applicable to establish the acceptable intake

- This approach is only considered as an interim measure to minimise supply disruption during CAPA implementation for certain marketed products

- It is applied on a case-by-case basis to balance risk mitigation while ensuring continuity of medicines to patients

Analysis of nitrosamine impurities in therapeutic products

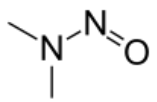
By: Dr Zeng Yun
Pharmaceutical Lab, HSA

Outline

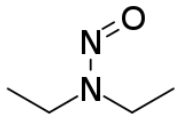
- **Introduction of the analysis of Nitrosamine impurities**
- **Key Consideration of method development and validation**
  - Case study I: Determination of N-Nitroso Fluoxetine in Fluoxetine products by LC-MS/MS
  - Case study II: Determination of NDMA in metformin products by HRAM-GCMS
- **Challenges and the future of Nitrosamine impurities testing**

Nitrosamine Impurities

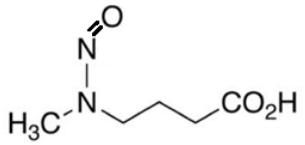
Small-Molecule Nitrosamine Impurities



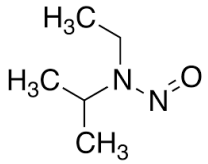
NDMA



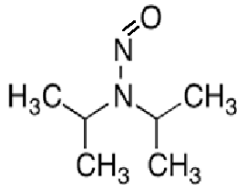
NDEA



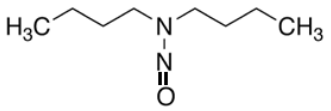
NMBA



NEIPA



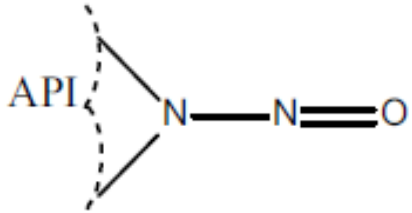
NDIPA



NDBA

Nitrosamine Drug Substance-Related Impurities (NDSRIs)

NDSRIs are a class of nitrosamines sharing structural similarity to the API (having the API or an API fragment in the chemical structure) and are generally unique to each API.



Safeguarding Patients: The Role of Nitrosamine Impurities Analysis

Accurate and reliable analytical methods are essential for detecting Nitrosamine impurities in drug products.

Early detection allows for corrective actions to be taken, such as process adjustments or product recalls.

This helps safeguard patient health by preventing exposure to potentially harmful NDSRIs.

Regulatory Recommendations for Confirmatory Testing

As part of the regulatory approach to manage the risk of nitrosamine impurities in therapeutic products, the Health Sciences Authority (HSA) has required all product registrants of therapeutic products containing chemically synthesized drug substances to conduct risk assessments of the products to identify any potential risk of nitrosamine impurities. Where potential risk is identified, confirmatory testing should be done, and the necessary risk mitigation measures must be implemented.

Control of Nitrosamine Impurities in Human Drugs
Guidance for Industry

U.S. FOOD & DRUG ADMINISTRATION

Guidance for product registrants on management of nitrosamine impurities in therapeutic products

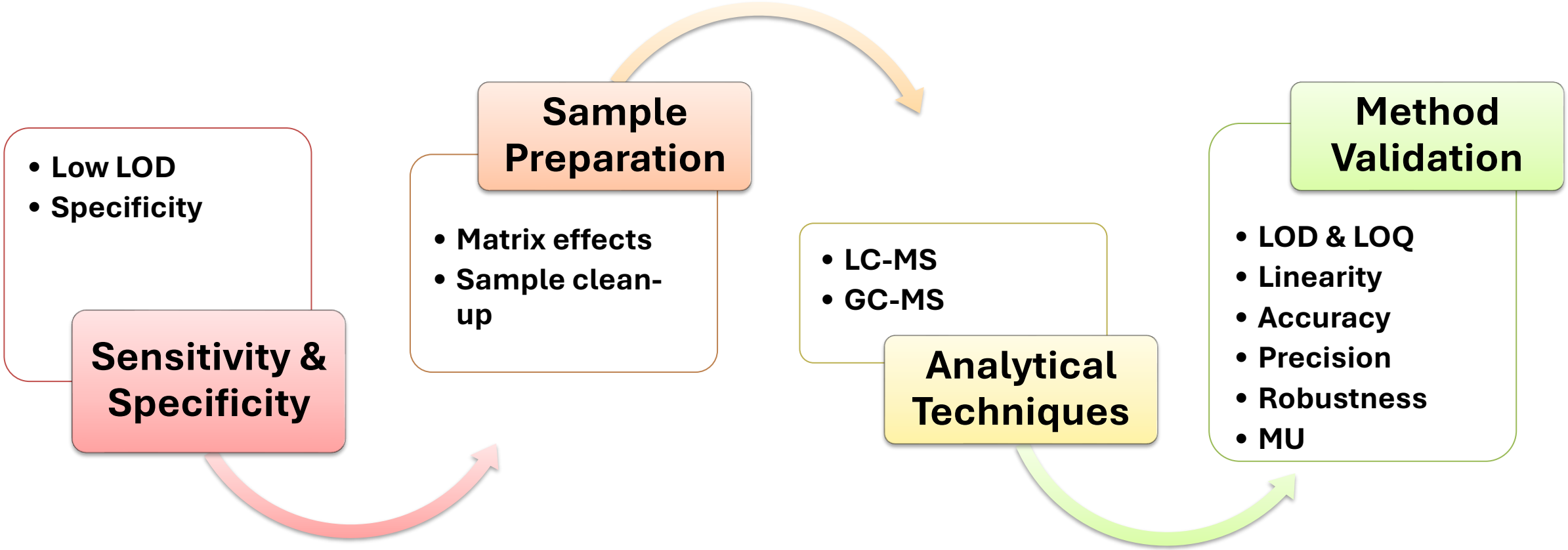
Information on actions required to manage this issue

HSA Health Sciences Authority

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

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Key Consideration for Method Development



Method Development - Sensitivity

Nitrosamines are often present in trace amounts

High sensitivity is essential for accurate detection and quantification.

Low LOD/LOQ

LC-MS and GC-MS offer excellent sensitivity due to their ability to detect and quantify compounds at low concentrations.

Instrument settings

Optimizing parameters such as ionization source, mass spectrometer settings, and chromatographic conditions can improve sensitivity.

Sample preparation

Proper sample preparation, including clean-up and concentration steps, can enhance sensitivity.

Method Development - Sample Preparation

The method should be able to analysis **multiple analyte** simultaneous and eliminate the interference from the sample.

Sample extraction

Choose a suitable extraction method based on the nature of the sample (e.g., liquid, solid, or formulation). Common methods include liquid-liquid extraction (LLE), solid-phase extraction (SPE), or direct dilution.

Clean up

If necessary, implement a cleanup step to remove interfering components that might mask or suppress the signal of the nitrosamine impurities. This can involve techniques like precipitation, filtration, or additional SPE.

Method Development – Analytical Techniques

LC-MS and GC-MS offer excellent sensitivity due to their ability to detect and quantify compounds at low concentrations.

Liquid Chromatography-Mass (LC-MS)

Advantages:
- Suitable for polar compounds.
- Reduced thermal degradation
- Versatility

Disadvantages:
- Lower sensitivity
- Matrix effects

Gas Chromatography-Mass Spectrometry (GC-MS)

Advantages:
- High sensitivity
- High specificity:
- Wide applicability
- Relatively simple sample preparation

Disadvantages:
- Limited applicability for polar compounds.
- Thermal degradation

Chromatographic Conditions

Column Selection

- Choose a chromatographic column with appropriate selectivity and retention for the target nitrosamine impurities. Reversed-phase columns are commonly used.

Mobile Phase

- Select a mobile phase that provides adequate separation between the nitrosamine impurities and other components in the sample. A combination of aqueous buffer and organic solvent is often used.

Gradient Elution

- Consider using gradient elution to improve peak shape and resolution for complex samples.

Mass Spectrometry Conditions

Ionization Mode

- Electrospray ionization (ESI) is generally preferred for nitrosamine impurities using LC-MS/MS due to its sensitivity and compatibility with polar compounds.
- Atmospheric Pressure Chemical Ionization (APCI) is used in LC-MS/MS for small molecular nitrosamines (e.g. NDMA) analysis.
- Electron ionization (EI) mode is wildly used in GC/MS

Source Parameters

- Optimize source parameters (e.g., capillary voltage, cone voltage, temperature) for efficient ionization and fragmentation of the nitrosamine impurities.

MRM Transitions

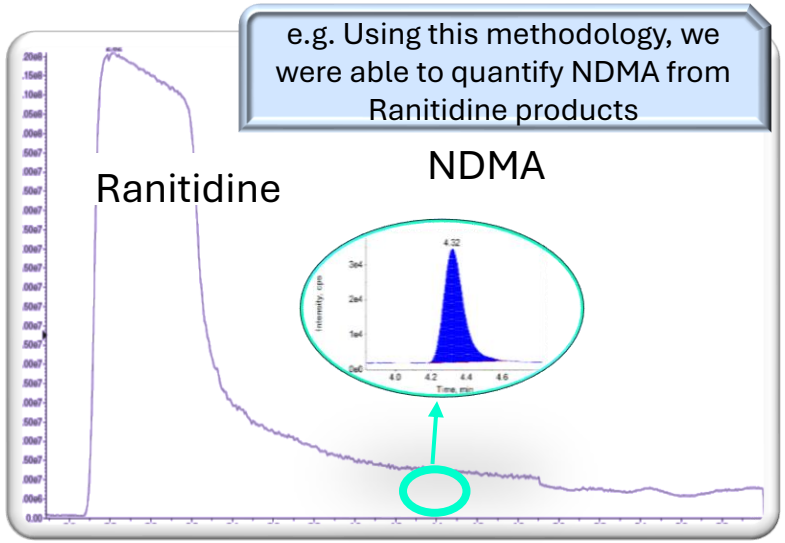
- Select appropriate multiple reaction monitoring (MRM) transitions for each nitrosamine impurity based on their fragmentation patterns. This provides selectivity and sensitivity for quantification.

Internal Standard

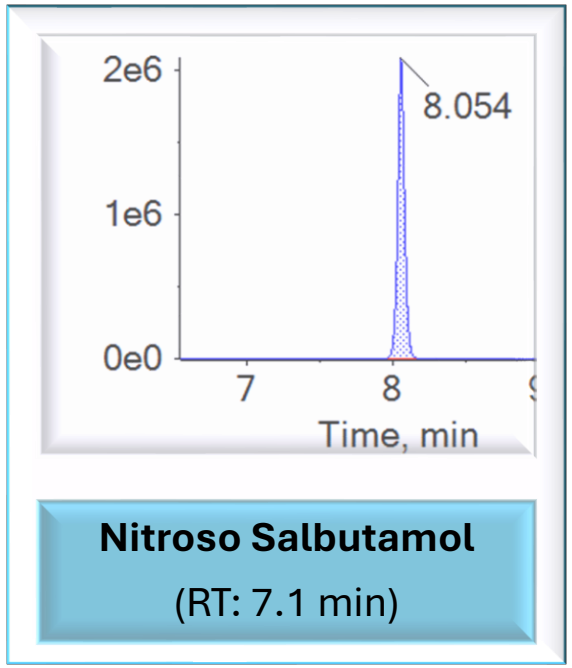
- Internal standards are often preferred to account for matrix effects and variations in recovery.

Matrix Effects and Mitigation Strategies for Nitrosamine Impurities Analysis

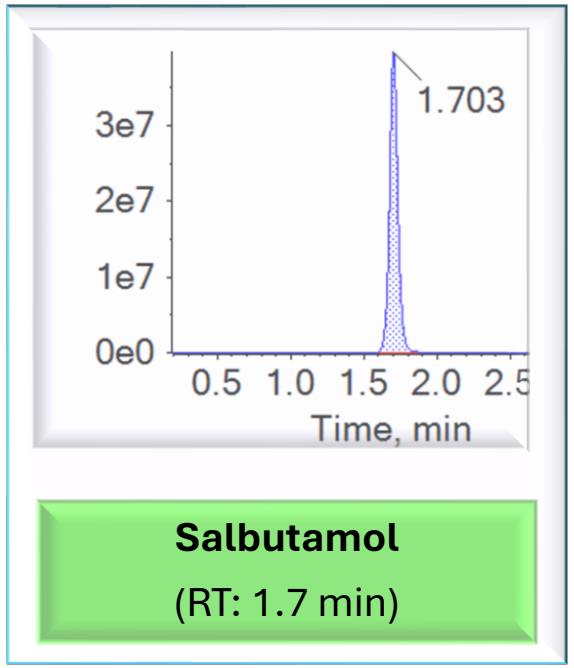
- Matrix effects can arise from co-extracted components in the drug matrix.
  - These can interfere with NDSRI ionization or detection, leading to inaccurate quantification.
- Strategies to mitigate matrix effects include:
  - Sample clean-up techniques (e.g., SPE) to remove interferences.
  - Isotope dilution mass spectrometry for more accurate quantification.
  - Matrix-matched calibration standards to compensate for matrix effects.



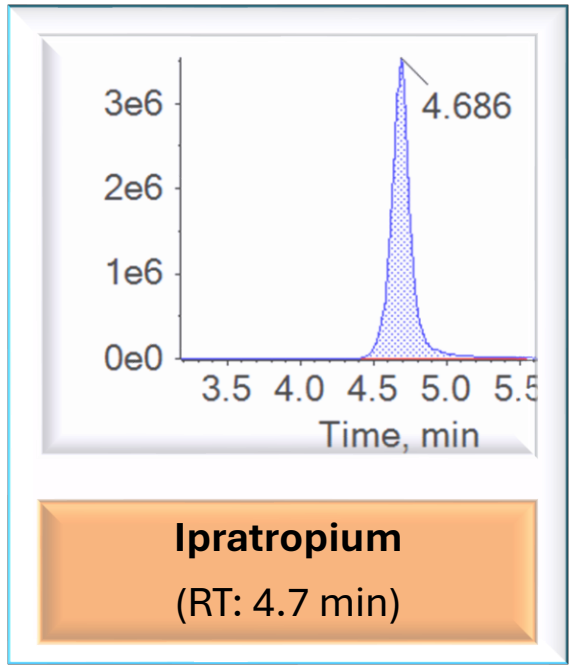
Chromatographic Separation of Nitrosamine Impurities Analysis



Nitroso Salbutamol
(RT: 7.1 min)



Salbutamol
(RT: 1.7 min)

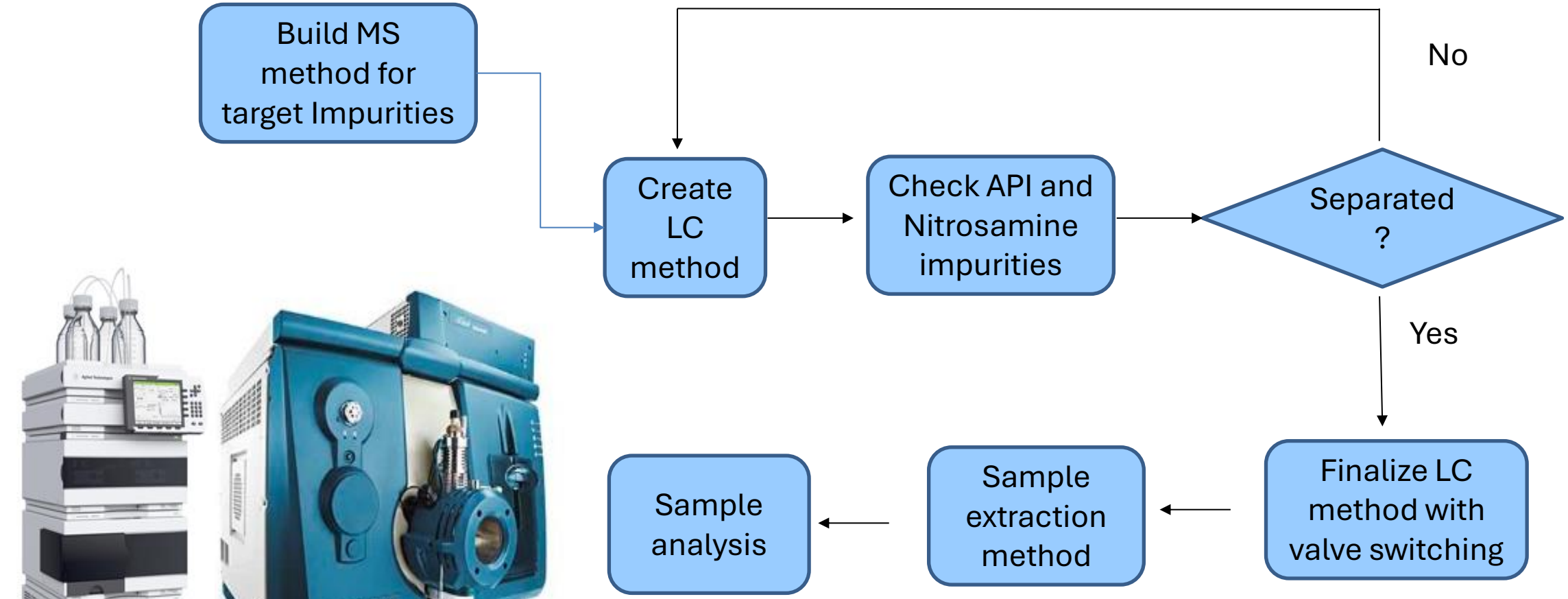


Ipratropium
(RT: 4.7 min)

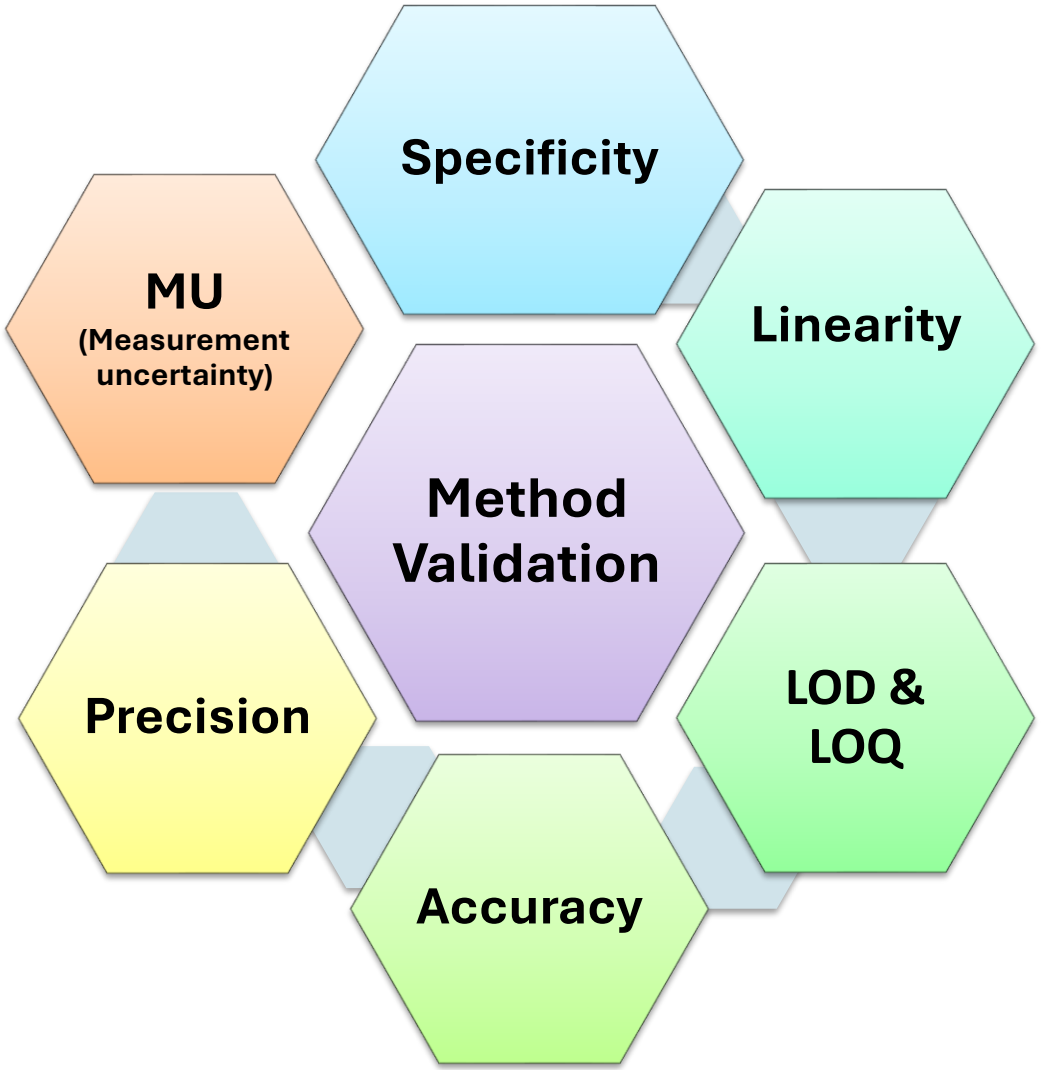
Chromatographic separation is crucial for accurate NDSRI identification and quantification.

Key factors influencing separation include:
- Column selection (stationary phase, particle size, length)
- Mobile phase composition (solvents, gradients)
- Flow rate and temperature.

Workflow of LC-MS/MS Method Development



Method Validation



After the analytical procedure description was finalized, a validation study was planned and completed according to the recommendations in ICH Q2.

ICH harmonisation for better health

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

VALIDATION OF ANALYTICAL PROCEDURES
Q2(R2)

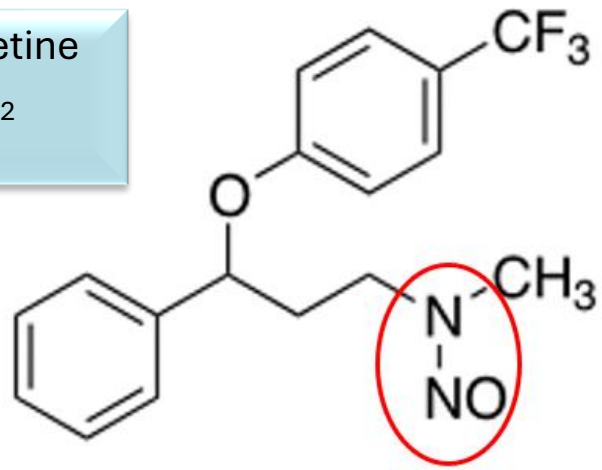
Final Version

Adopted on 1 November 2023

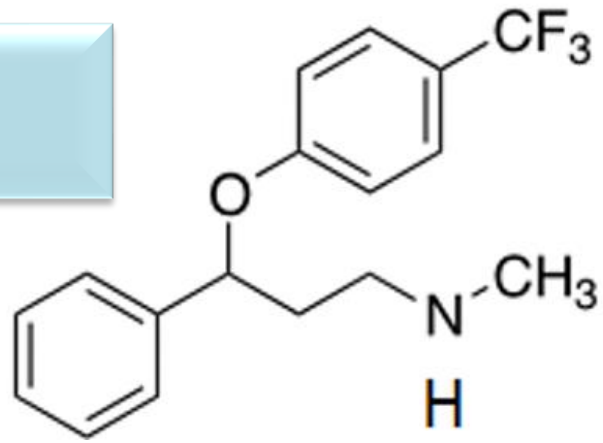
The user should perform proper method validation/verification before adopting an available method to ensure its suitability for the intended use.

Case Study I
Determination of N-Nitroso Fluoxetine in Fluoxetine Products

N-Nitroso Fluoxetine
$C_{17}H_{17}F_3N_2O_2$
MW: 338.32



Fluoxetine
$C_{17}H_{18}F_3NO$
MW: 309.33



- Fluoxetine is an antidepressant medication that belongs to a class of drugs known as selective serotonin reuptake inhibitors (SSRIs). Fluoxetine is commonly prescribed to treat depression, anxiety disorder, obsessive-compulsive disorder, eating disorder, panic disorder and premenstrual dysphoric disorder.
- N-Nitroso fluoxetine is a nitrosamine impurity found in fluoxetine products. It is a potent carcinogen compound.

Determination of N-Nitroso Fluoxetine by LC-MS/MS

QTrap 7500 LC-MS/MS

Q-Exactive HRMS

Sensitivity & Specificity

- Recommended AI limit for N-Nitroso Fluoxetine: 100 ng/day
- Max daily dose of Fluoxetine: 80 mg
- Allowed Conc: 100 ng/80 mg (1.25 µg/g)

➢ LOQ: 0.1 µg/g

Sample Preparation

- A homogenized sample equivalent to 50 mg of fluoxetine was extracted using 5 mL of MeOH : DI water (8:2, v/v).

➢ 10 ng/mL of IS (N-Nitroso fluoxetine-D5) was applied.

Analytical Techniques

- LC-MS/MS

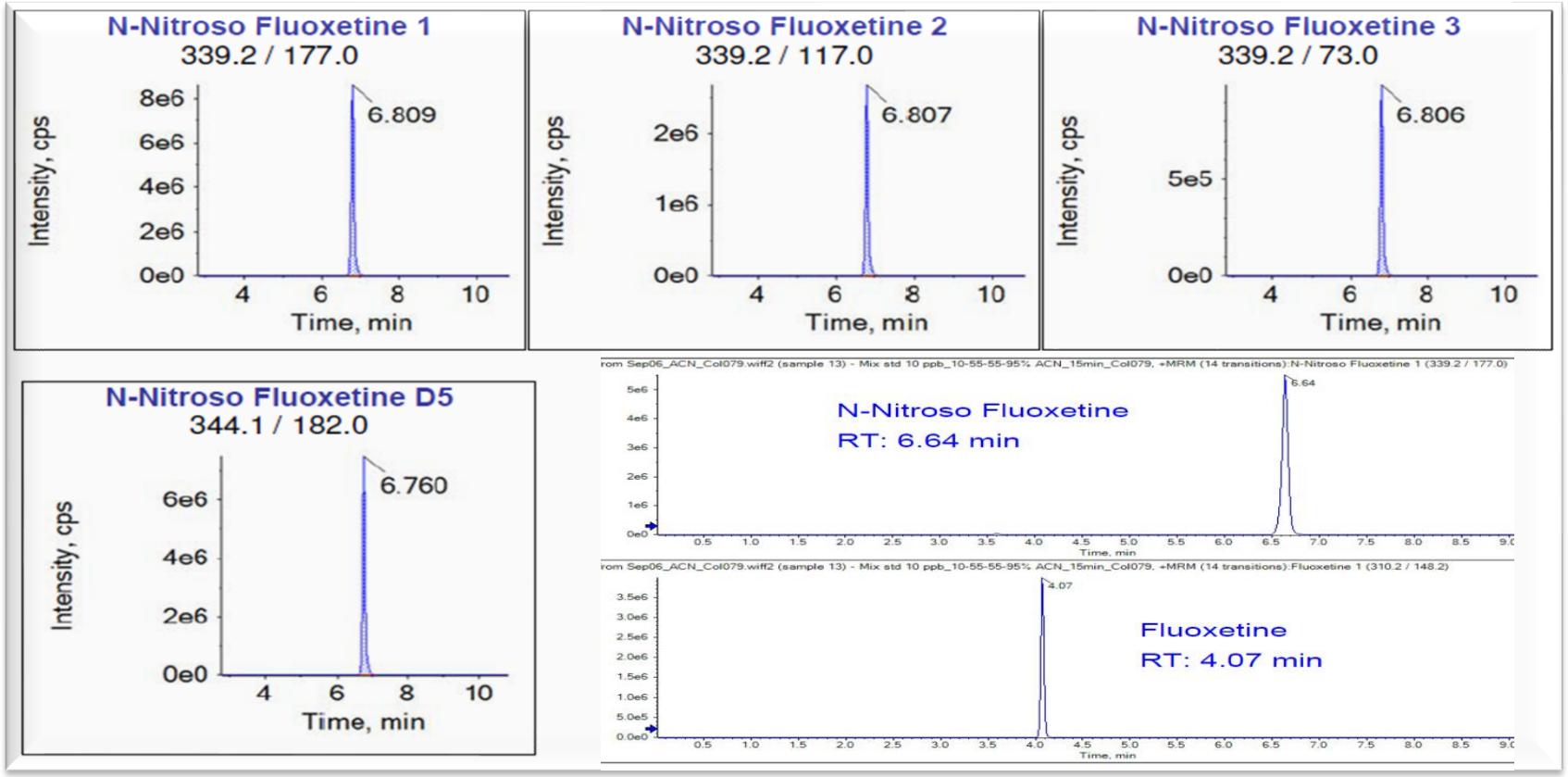
➢ QTrap 7500 LC-MS/MS

➢ Q-Exactive HRMS

Method Validation

➢ LOD & LOQ
➢ Linearity
➢ Accuracy
➢ Precision
➢ Robustness
➢ MU

Method Validation of Determination of N-Nitroso Fluoxetine by LC-MS/MS



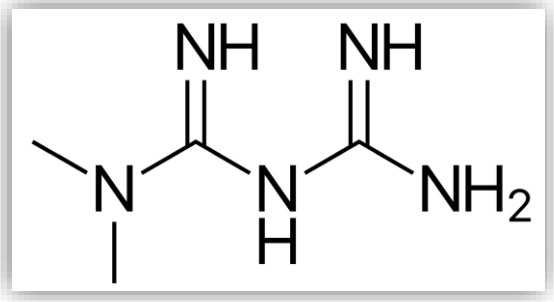
MRM Chromatograms of N-Nitroso Fluoxetine and internal standard (10 ng/mL)

- A 50 mg fluoxetine sample was extracted using 5 mL of MeOH:DI water containing 10 ng/mL of N-Nitroso fluoxetine-D5.
- Vortex-mixed, sonicated, centrifuged, filtered,
- Analyzed by LC-MS/MS

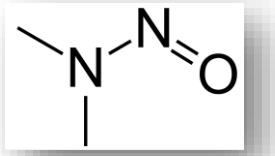
Method Validation of Determination of N-Nitroso Fluoxetine by LC-MS/MS

Linearity range	1-30 ng/mL
R^2	1.0000
Accuracy (20 µg/g) (n=9)	94-104%
Accuracy (50 µg/g) (n=9)	97-106%
Accuracy (100 µg/g) (n=9)	99-108%
Precision (n=7)	RSD 3%
Instrument LOD	0.2 ng/mL
Instrument LOQ	1.0 ng/mL
Method LOD	0.02 µg/g
Method LOQ	0.1 µg/g

Case Study II
Determination of NDMA in Metformin by HRAM-GCMS



Metformin



NDMA

Possible formation of NDMA in Metformin:

- Formed by a reaction of an APIs by-product with nitrite from excipients.
- During packaging process.

Determination of NDMA in Metformin by HRAM-GCMS

High resolution accurate mass- gas chromatography mass spectrometry (HRAM-GCMS)

Sensitivity & Specificity

- Recommended AI limit for NDMA 96 ng/day
- Max daily dose of Metformin: 3 g
- Allowed Conc: 96 ng/3 g (32 ng/g)

➢ LOQ: 20 ng/g

Sample Preparation

- Both NDMA and Metformin has good solubility in general organic solvent and water

➢ Extraction method using CH_2Cl_2 (DCM) and 1N HCl.

Analytical Techniques

- GC-MS provide better sensitivity

➢ HRAM GCMS

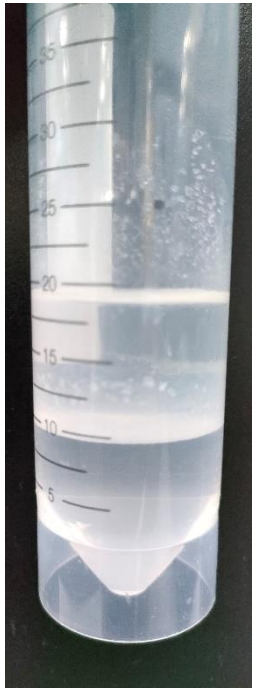
Method Validation

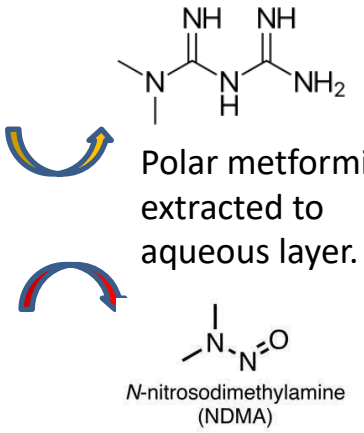
➢ LOD & LOQ
➢ Linearity
➢ Accuracy
➢ Precision
➢ Robustness
➢ MU

Determination of NDMA in Metformin by HRAM-GCMS – Sample Preparation

- Extraction solution: Dichloromethane (DCM) and 1N HCl containing internal standard.

  Results showed both immediate and extended released formulation produced well separated layers after the mixing and centrifuge process, allowing the direct withdrawal of DCM layer for analysis without any other clean-up like SPE.





Determination of NDMA in Metformin by HRAM-GCMS - Overall workflow

Sample
(equivalent to
500 mg API)

Internal
Standard
NDMS-D6

Sample extraction, centrifugation

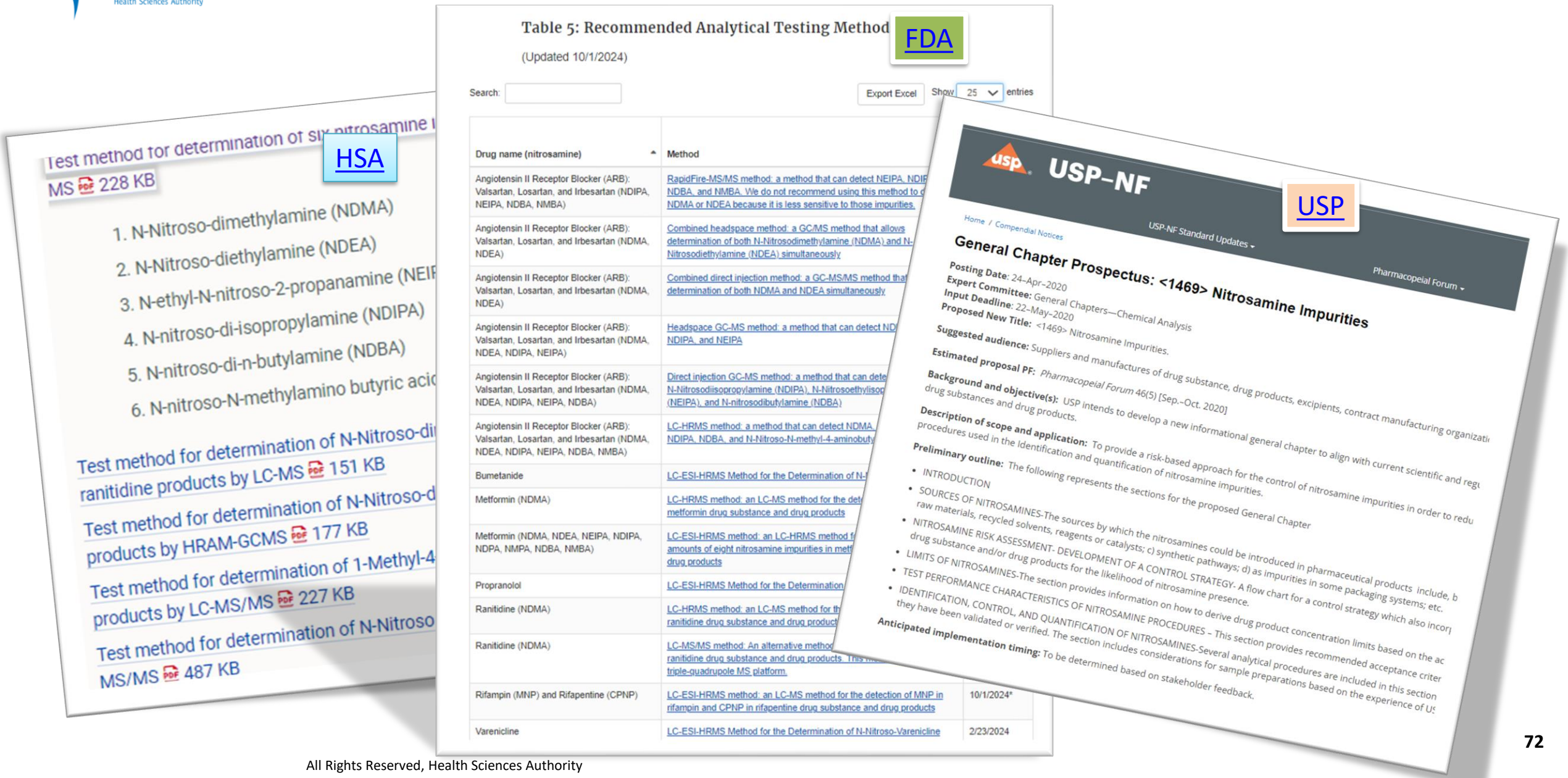
Withdraw and filter the DCM layer for analysis

Data Analysis

Determination of NDMA in Metformin by HRAM-GCMS - Method Validation

Linearity range	1-100 ng/mL
R^2	0.999
Accuracy	88-118 %
Precision (n=7)	RSD < 15%
Instrument LOD	0.5 ng/mL
Instrument LOQ	1.0 ng/mL
Method LOD	10 ng/g
Method LOQ	20 ng/g

Available Test Method



Challenges

Matrix effects

- Complex pharmaceutical matrices can interfere with the ionization and detection of NDSRIs.

Data interpretation

- The analysis generates large datasets that require sophisticated data processing and interpretation tools.

Reference standards

- The availability of reference standards for the potential NDSRIs may be limited.

The Future: Ensuring Patient Safety and Drug Quality

Nitrosamine impurities analysis is a crucial aspect of ensuring drug safety and quality.

Advancements in analytical techniques will continue to enhance sensitivity and accuracy.

Ongoing research is vital for addressing new Nitrosamine impurities analysis and developing robust methods.