CIRM

LIÈGE université
Laboratory of Pharmaceutical Analytical Chemistry

# Simultaneous detection of unspecific trace N-nitrosamine impurities by LC-MS/MS in a pharmaceutical formulation


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# N-nitrosamines? What are they?

- Possibly carcinogenic
- Various matrices:



- N-nitrosamines
  - Unspecific impurities: NDMA, NDEA, NDPA, NDIPA, NEIPA, etc
  - N-nitrosamine drug substance-related impurities (NDSRIs): N-nitroso-varenicline, N-nitroso-enalapril, etc

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# EMA's investigation procedure

- Step 1 - Documentary risk assessment
- Step 2 - Confirmatory testing
- Step 3 - Implementation of risk mitigation measures

*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's investigation procedure

- Step 1 - Documentary risk assessment
- **Step 2 - Confirmatory testing**
  - Validated methods
  - Requirements for method sensitivity
- Step 3 - Implementation of risk mitigation measures

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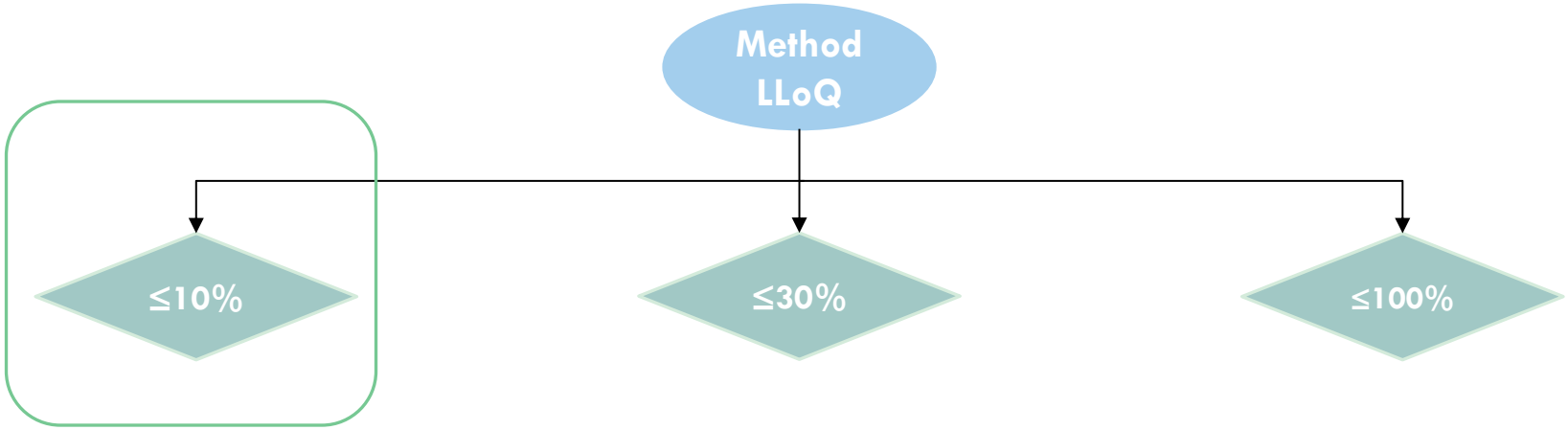
# Step 2 - Confirmatory testing: scenarios

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***AI: Acceptable intake, defined by the EMA***
***MDD: Maximum daily dose***

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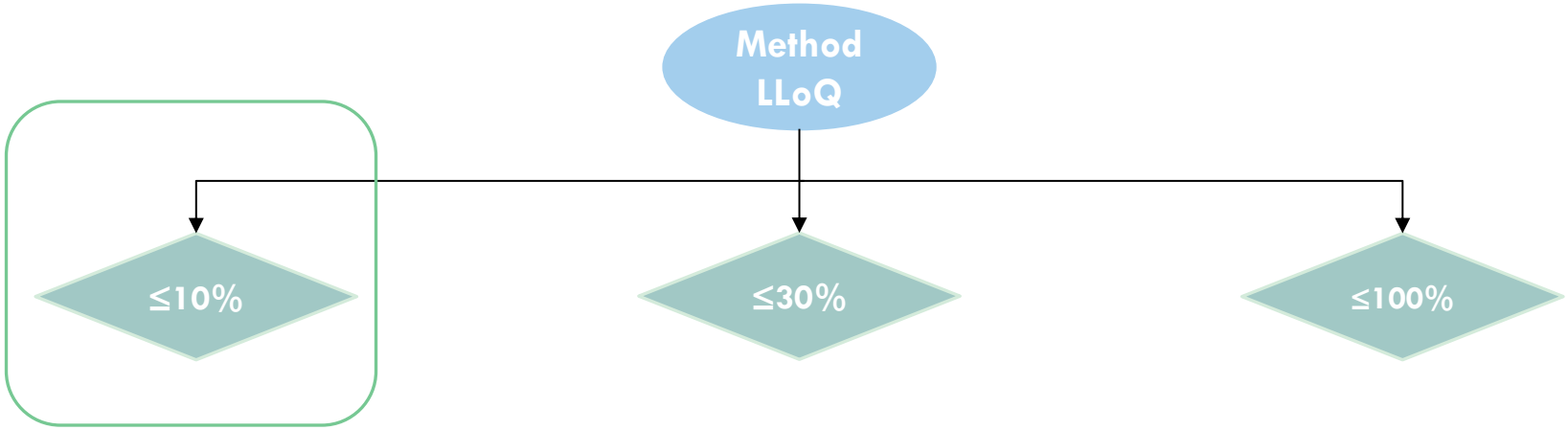


## Goal 1:

- Development and optimization of generic methods for simultaneous determination of unspecific N-nitrosamine impurities in a commercialized pharmaceutical formulation for Quality Control

# LC-MS/MS instrumentation and methods

- RP-LC/APCI (+)-MS/MS

Acquity® Premier UPLC® (Waters)

Mobile phase 1: pH 2.7 (FA 0.1%)

Mobile phase 2: pH 5.0 (AmAc 10 mM)

Xevo® TQ-Absolute (Waters)

APCI probe T°1: 150°C

APCI probe T°2: 250°C

- ✓ Method 1: pH 2.7 / 150°C
- ✓ Method 2: pH 2.7 / 250°C
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**Single-run analysis to detect up to 18 N-nitrosamines**

# Specification limit – N-nitrosamines

| N-nitrosamine | AI (ng/day) | MDD (mg/day) | 100% specification limit (ppm) | 30% specification limit (ppm) | 10% specification limit (ppm) |
|---|---|---|---|---|---|
| NMBA | 96.0 | 16 | | | |
| NDPhA | 78000.0 | 16 | | | |
| NMOR | 127.0 | 16 | | | |
| NNK | 100.0 | 16 | | | |
| NMPA | 34.3 | 16 | | | |
| NDPA | 26.5 | 16 | | | |
| NDIPA | 26.5 | 16 | | | |
| NEIPA | 26.5 | 16 | | | |
| NDBA | 26.5 | 16 | | | |

AI: Acceptable intake, defined by the EMA
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$$Specification\ limit\ (ppm) = \frac{AI\ (ng/day)}{MDD\ (mg/day)} = \frac{96\ (ng/day)}{16\ (mg/day)} = 6.00\ ppm$$

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|---|---|---|---|---|---|
| NMBA | 96.0 | 16 | 6.00 | 1.80 | |
| NDPhA | 78000.0 | 16 | | | |
| NMOR | 127.0 | 16 | | | |
| NNK | 100.0 | 16 | | | |
| NMPA | 34.3 | 16 | | | |
| NDPA | 26.5 | 16 | | | |
| NDIPA | 26.5 | 16 | | | |
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|---|---|---|---|---|---|
| NMBA | 96.0 | 16 | 6.00 | 1.80 | 0.60 |
| NDPhA | 78000.0 | 16 | | | |
| NMOR | 127.0 | 16 | | | |
| NNK | 100.0 | 16 | | | |
| NMPA | 34.3 | 16 | | | |
| NDPA | 26.5 | 16 | | | |
| NDIPA | 26.5 | 16 | | | |
| NEIPA | 26.5 | 16 | | | |
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| N-nitrosamine | AI (ng/day) | MDD (mg/day) | 100% specification limit (ppm) | 30% specification limit (ppm) | 10% specification limit (ppm) |
|---|---|---|---|---|---|
| NMBA | 96.0 | 16 | 6.00 | 1.80 | 0.60 |
| NDPhA | 78000.0 | 16 | 4875.00 | 1462.50 | 487.50 |
| NMOR | 127.0 | 16 | 7.94 | 2.38 | 0.79 |
| NNK | 100.0 | 16 | 6.25 | 1.88 | 0.63 |
| NMPA | 34.3 | 16 | 2.14 | 0.64 | 0.21 |
| NDPA | 26.5 | 16 | 1.66 | 0.50 | 0.17 |
| NDIPA | 26.5 | 16 | 1.66 | 0.50 | 0.17 |
| NEIPA | 26.5 | 16 | 1.66 | 0.50 | 0.17 |
| NDBA | 26.5 | 16 | 1.66 | 0.50 | 0.17 |

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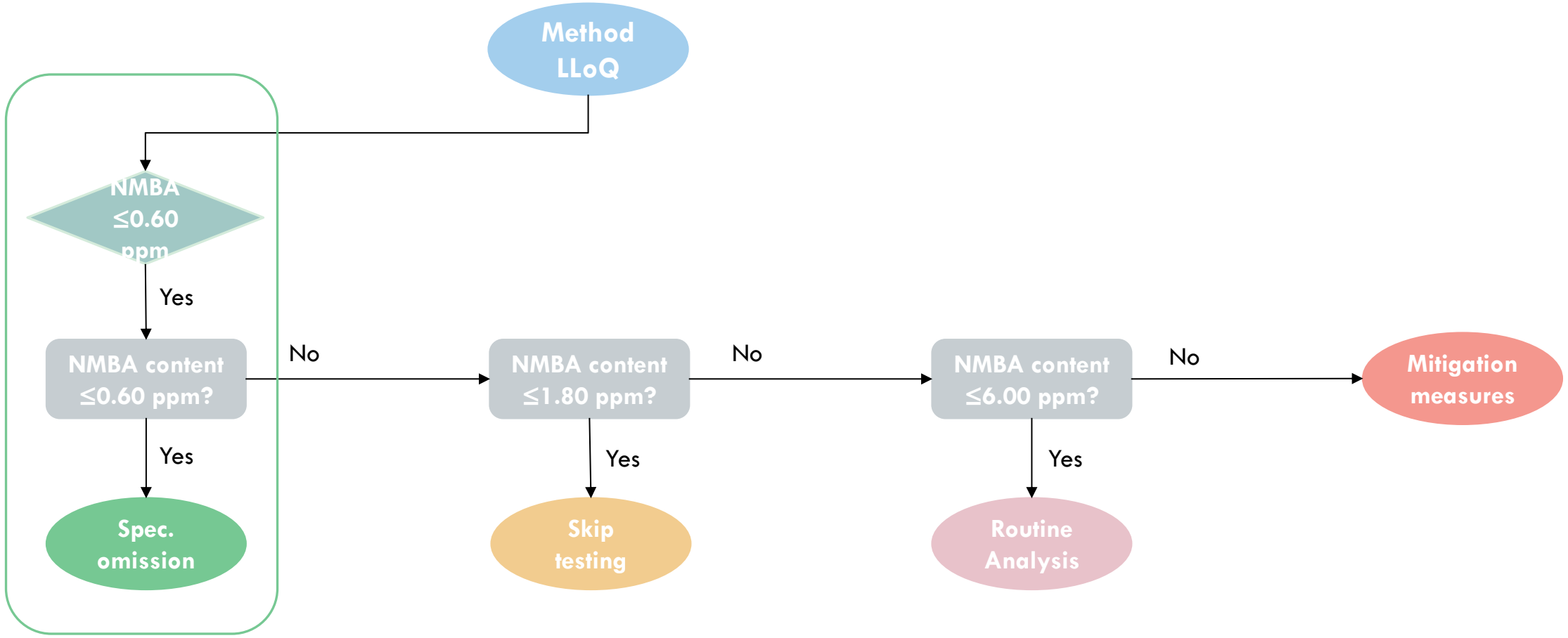
# Scenarios


Method LLoQ
NMBA ≤0.60 ppm
Yes
NMBA content ≤0.60 ppm?
No
NMBA content ≤1.80 ppm?
No
NMBA content ≤6.00 ppm?
No
Mitigation measures
Yes
Spec. omission
Yes
Skip testing
Yes
Routine Analysis


Omission
Skip testing
Routine analysis
0%
10%
30%
100%

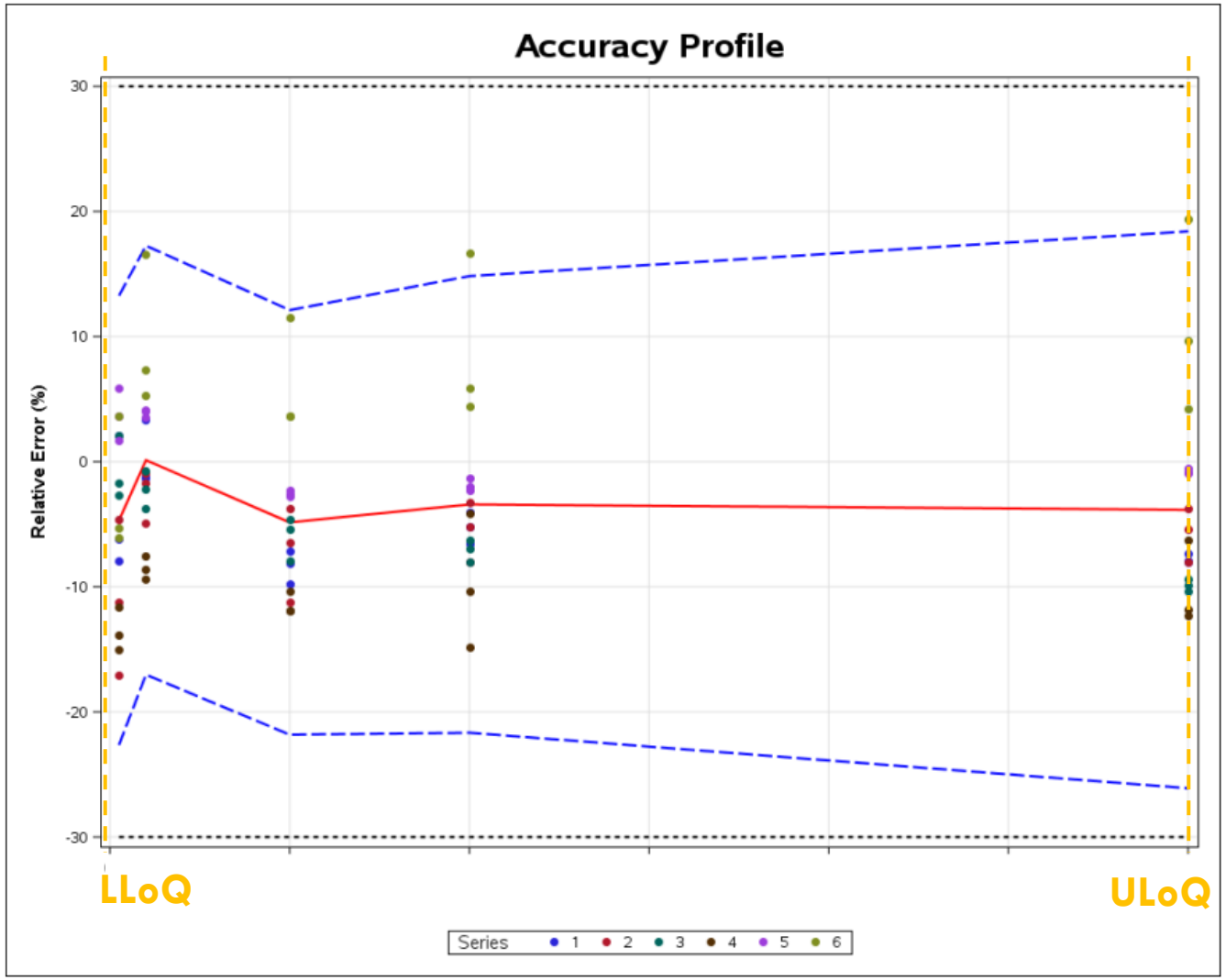
# Goal 2: Validation – ICH Q2(R2)

- Limit test for impurity
  - Selectivity / specificity
  - LoD
- Quantative analysis of trace impurities
  - Range
    - ✓ Response function
    - ✓ Linearity
    - ✓ Lower range limits: LoD/LLoQ
  - Combined approach
    - ✓ Total error – accuracy profile

# 3-level limit test

- Specification limit
  - 10%
  - 30%
  - 100%
- Support in decision-making during confirmatory testing
  - If content consistently ≤ 10% : Specification omission
  - If content consistently > 10% and ≤ 100% : Quantitative testing
    - ✓ **Skip testing (≤ 30%):** 1 batch out of every *x* batches must be tested
    - ✓ **Routine analysis (≤ 100%):** every batch must be tested

# NMBA (linear regression through 0, fitted level 3)




LoD: 8.62 ppb
**LLoQ: 0.10 ppm**
ULoQ: 11.72 ppm

..... Acceptance limit
- - - β-expectation tolerance interval
——— Bias

# System suitability tests (SSTs)

- Equipment readiness (AHP 50 ng/mL ; n=3)
  - Specifications: mean intensities ≥ 8,000,000 counts and RSD (%) ≤ 5%

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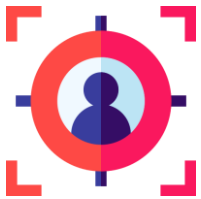
- Equipment readiness (AHP 50 ng/mL ; n=3)
  - Specifications: mean intensities ≥ 8,000,000 counts and RSD (%) ≤ 5%

- $Ion\ ratio = \frac{less\ intense\ AUC}{most\ intense\ AUC}$
  - LC-MS/MS specificity
  - Maximum permitted tolerance for LC-MS/MS technique



| Ion ratio | Maximum permitted tolerance |
|---|---|
| > 0.50 | ± 20% |
| > 0.20 to 0.50 | ± 25% |
| > 0.10 to 0.20 | ± 30% |
| ≤ 0.10 | ± 50% |

*Journal of the European Communities, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002*

# Goal 3: Confirmatory testing (step 2)

- *"Testing should be conducted on **10% of annual batches**, or **3 per year, whichever is highest**. This includes testing not only of **newly produced batches** but also **retained samples of batches still within expiry date**. If fewer than 3 batches are manufactured annually, then all batches should be tested."*
- 12 batches of drug product, including release and ongoing stability samples over T36M
  - 2024: 3 batches → Release
  - 2023: 3 batches → T0M – T12M
  - 2022: 3 batches → T12M – T24M
  - 2021: 3 batches → T24M – T36M
  - 2020: …
- 3-level limit test approach

# Goal 3: Confirmatory testing (step 2)

- 2024: Release
  - Only one nitrosamine detected, with content **< 10%** of the specification limit
- 2023: T0M – T12M
  - Only one nitrosamine detected, with content **> 10% but < 30%** of the specification limit
- 2022: T12M – T24M
  - Only one nitrosamine detected, with content **> 10% but < 30%** of the specification limit
- 2021: T24M – T36M
  - Only one nitrosamine detected, with content **> 30% but < 100%** of the specification limit

→ This N-nitrosamine shows an evolving profile in the finished product during storage.

# Key messages

- Generic methods for simultaneous detection of N-nitrosamine impurities
- Methods validated with a LLoQ ≤ 10% specification limit
- Confirmatory testing
  - Routine analysis required for only one N-nitrosamine

# Acknowledgement


**Supervisors:**

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