Performance Characteristics of Mass Spectrometry-Based Methods for Quantitation of Nitrosamines: Insight from an Inter-laboratory Study

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Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.
A quality product of any kind consistently meets the expectations of the user.

Drugs are no different.

www.fda.gov
Patients expect safe and effective medicine with every dose they take.
Pharmaceutical quality is assuring every dose is safe and effective, free of contamination and defects.
It is what gives patients confidence in their next dose of medicine.
Participating Laboratories

The National Agency for the Safety of Medicines and Health Products (France)

Therapeutic Goods Administration (Australia)

Bavarian State Office for Health and Food Safety (LGL) (Germany)

Health Canada (Canada)

Swissmedic (Switzerland)

Food and Drug Administration (United States)

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Cynthia Sommers; Jason Rodriguez;
David Keire
Nitrosamines

- Nitrosamines are common contaminants present in low amounts (ppm) in foods, beverages, cosmetics, water waste, tobacco products, and many other consumer goods
- Many nitrosamines are probable or possible carcinogens. “Cohort of concern” in ICH M7 (mutagenic impurities in pharmaceuticals)
Since 2018, nitrosamine contamination has led to the recall and even the market withdrawal of several widely used medicines. It has become one of the main focuses of pharmaceutical manufacturing.
Common Nitrosamines Found or Predicted to be Present in Pharmaceuticals

- **N-nitrosodimethylamine (NDMA)**
  - Chemical Formula: $C_2H_6N_2O$
  - Exact Mass: 74.0480

- **N-nitrosodiethylamine (NDEA)**
  - Chemical Formula: $C_4H_{10}N_2O$
  - Exact Mass: 102.0793

- **N-Nitrosoisopropylethylamine (NIPEA or NEIPA)**
  - Chemical Formula: $C_5H_{10}N_2O_3$
  - Exact Mass: 146.0691

- **N-Nitrosodiisopropylamine (NDIPA)**
  - Chemical Formula: $C_6H_{14}N_2O$
  - Exact Mass: 130.1106

- **N-Nitrosodibutylamine (NDBA)**
  - Chemical Formula: $C_8H_{18}N_2O$
  - Exact Mass: 158.1419

- **N-Nitrosomethylphenylamine (NMPA)**
  - Chemical Formula: $C_7H_8N_2O$
  - Exact Mass: 136.0637

- **N-Nitrosodipropylamine (NDPA)**
  - Chemical Formula: $C_6H_{14}N_2O$
  - Exact Mass: 130.1106

- **N-Nitroso-N-methyl-4-aminobutyric Acid (NMBA)**
  - Chemical Formula: $C_8H_{16}N_2O_3$
  - Exact Mass: 146.0691
FDA Guidance: Acceptable Intake Limits for Nitrosamines

<table>
<thead>
<tr>
<th>Nitrosamine</th>
<th>Acceptable Intake Limit (ng/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMA</td>
<td>96</td>
</tr>
<tr>
<td>NDEA</td>
<td>26.5</td>
</tr>
<tr>
<td>NMBA</td>
<td>96</td>
</tr>
<tr>
<td>NMPA</td>
<td>26.5</td>
</tr>
<tr>
<td>NIPEA</td>
<td>26.5</td>
</tr>
<tr>
<td>NDIPA</td>
<td>26.5</td>
</tr>
</tbody>
</table>

“Manufacturers of APIs and drug products should use methods with LOQs at or below 0.03 ppm. Manufacturers should establish methods for which the LOQ and limit of detection (LOD) are as low as reasonably practical for products for which the maximum daily dose is high (e.g., greater than 1 g)”

## Nitrosamine Determination by Mass Spectrometry

<table>
<thead>
<tr>
<th>Ion source</th>
<th>Scan Mode</th>
<th>APCI</th>
<th>ESI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMA</td>
<td>75.1 → 43.1; 58.1 (+)</td>
<td>MS2 (+) (75.1); EIC 75.0553</td>
<td></td>
</tr>
<tr>
<td>NDEA</td>
<td>103.1 → 75.1; 47.1 (+)</td>
<td>SIM (+) (103.1); EIC 103.0866</td>
<td></td>
</tr>
<tr>
<td>NEIPA</td>
<td>117.1 → 75.1; 47.1 (+)</td>
<td>MS2 (+) (117.1); EIC 75.0553</td>
<td></td>
</tr>
<tr>
<td>NDIPA</td>
<td>131.1 → 89.1; 43.1 (+)</td>
<td>SIM (+) (131.1); EIC 131.1179</td>
<td></td>
</tr>
<tr>
<td>NDPA</td>
<td>131.1 → 89.1; 43.1 (+)</td>
<td>SIM (+) (131.1); EIC 131.1179</td>
<td></td>
</tr>
<tr>
<td>NMPA</td>
<td>137.1 → 107.1; 66.1 (+)</td>
<td>SIM (+) (137.1); EIC 137.0709</td>
<td></td>
</tr>
<tr>
<td>NDBA</td>
<td>159.1 → 103.1; 57.1 (+)</td>
<td>MS2 (+) (159.1); EIC 103.0866</td>
<td></td>
</tr>
<tr>
<td>NMBA</td>
<td>147.1 → 117.1; 44.1 (+)</td>
<td>SIM (-) (145.1); EIC 145.0619</td>
<td></td>
</tr>
</tbody>
</table>
Example Chromatogram
(Losartan Drug Substance Spiked With 0.1 ppm Nitrosamine)
Questions to be Addressed and Study Objectives

• A variety of analytical procedures have been developed to address the needs.
• What performance should be expected when evaluating a nitrosamine analytical procedure?
• Is a 0.03 ppm (or lower) quantitation limit possible to achieve?

Inter-laboratory Study

• To understand the performance characteristics of nitrosamine analytical procedures:
  • Quantitation limit
  • Precision, accuracy
  • Areas for improvement

• To evaluate and recommend appropriate performance criteria (precision and accuracy).
Study Design

- Fully validated;
- ≥ NDMA and NDEA

Valsartan

Losartan

- LC-MS/MS
- LC-HRMS
- GC-MS; LC-MS/MS
- LC-MS/MS
- GC-MS
- LC-HRMS

FDA

ANSM

LGL

Health Canada

Swissmedic
Samples for the Study: Spiked Sample

- 4 sample matrices
  - 2 drug substance
  - 2 drug products
- No detectable nitrosamines
- NDMA, NDEA, NDIPA, NEIPA, NDBA and NMBA were spiked.
- 5 spiking levels
- 3 replicates each level
- Total number of samples: 72
Samples for the Study: Contaminated Samples

- NDMA, NDEA and/or NMBA at a concentration range of 0.003 ppm to 50 ppm.
- 6 lots of drug substance
  - 3 lots of valsartan;
  - 3 lots of losartan
- 6 lots of drug products
  - 3 lots of valsartan;
  - 3 lots of losartan
- 3 replicates each sample
- Total number of samples: 36
Samples for the Study

- Sample preparation
- Instrument analysis
- Data analysis and reporting

Spiked samples

Contaminated samples

✓ Additional sample matrices
## Overview of Analytical Procedures Used in This Study

<table>
<thead>
<tr>
<th>Sample preparation</th>
<th>Instrument analysis</th>
<th>Quantitation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LC-HRMS</strong></td>
<td>Extraction by methanol</td>
<td>ESI</td>
</tr>
<tr>
<td><strong>LC-HRMS</strong></td>
<td>Extraction by methanol</td>
<td>APCI</td>
</tr>
<tr>
<td><strong>LC-MS/MS</strong></td>
<td>Extraction by 25% methanol in water</td>
<td>APCI</td>
</tr>
<tr>
<td><strong>LC-MS/MS</strong></td>
<td>Extraction by 10% methanol in water</td>
<td>APCI</td>
</tr>
<tr>
<td><strong>GC-MS/MS</strong></td>
<td>Extraction by 1 M NaOH (10 mL) → dichloromethane (4 mL) extraction</td>
<td>EI</td>
</tr>
<tr>
<td><strong>GC-MS/MS</strong></td>
<td>Extraction by 1 M NaOH (5 mL) → dichloromethane (1 mL) extraction</td>
<td>EI</td>
</tr>
</tbody>
</table>
Data Analysis

**Precision**

\[ S = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x)^2}{n-1}} \]

**Repeatability**

\[ S_R = \sqrt{S_L^2 + S_r^2} \]

**Reproducibility**

- \( S_L^2 \) between-laboratory
- \( S_r^2 \) within-laboratory

\[ S_r = \sqrt{\frac{\sum_{i=1}^{p} s^2}{p}} \]

\%RSD = Standard deviation / average \times 100

**Accuracy**

\[ \% \text{ Difference} = \frac{x - c}{c} \times 100 \]

- \( x_i \): Single measurement;
- \( x \): Average value;
- \( n \): Number of replicates;
- \( p \): Number of laboratories;
- \( c \): Spiked - spiked concentration;

Reference: ASTM E691-20 Standard Practice for conducting an interlaboratory study to determine the precision of a test method
Spiked Samples: Data Overview

**Repeatability %RSD**
- Total Number of Values: 562
- 98% of the Values: < 20

**Reproducibility %RSD**
- Total Number of Values: 120
- 97% of the Values: < 35

**%Difference**
- Total Number of Values: 562
- 96% of the Values: [-30, 30]
Repeatability %RSD for Spiked Samples

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Sample Matrices</th>
<th>Nitrosamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 ppm</td>
<td>L-DP-0</td>
<td>NDMA</td>
</tr>
<tr>
<td>0.03 ppm</td>
<td>L-DS-0</td>
<td>NMBA</td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>V-DP-0</td>
<td>NDEA</td>
</tr>
<tr>
<td>0.3 ppm</td>
<td>V-DS-0</td>
<td>NEIPA</td>
</tr>
<tr>
<td>1 ppm</td>
<td></td>
<td>NDIPA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NDBA</td>
</tr>
</tbody>
</table>
Repeatability %RSD for Spiked Samples

Laboratory and Nitrosamine

Analytical Technique

- NDMA
- NMBA
- NDEA
- NEIPA
- NDIPA
- NDBA

% RSD

A B C D E F

LC-HRMS (A, D)
LC-MSMS (C, E)
GC-MS (B, F)
Reproducibility %RSD for Spiked Samples

Concentration

Sample Matrices

Nitrosamine

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</tr>
<tr>
<td>1 ppm</td>
<td></td>
<td>NDIPA</td>
</tr>
</tbody>
</table>
Comparison to Predicted Repeatability and Reproducibility

- **Horwitz Equation**

\[
\text{%RSD (reproducibility)} = 2^{(1 - 0.5 \log C)}
\]

%RSD (repeatability) is 2/3 of %RSD (reproducibility)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Reproducibility</th>
<th></th>
<th>Repeatability</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicted</td>
<td>From study</td>
<td>Predicted</td>
<td>From study</td>
</tr>
<tr>
<td>0.01 ppm</td>
<td>32</td>
<td>30</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>0.03 ppm</td>
<td>27</td>
<td>19</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>22</td>
<td>22</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>0.3 ppm</td>
<td>19</td>
<td>19</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>1 ppm</td>
<td>16</td>
<td>21</td>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>

Accuracy Evaluation: % Difference for Spiked Samples

Concentration

Sample Matrices

Nitrosamine

%Difference

(ppm)

0.01 0.03 0.1 0.3 1

L-DP-0 L-DS-0 V-DP-0 V-DS-0

NDMA NMBN NDEA NEIPA NDIPA NDBA
Accuracy Evaluation: % Difference for Spiked Samples

Individual Laboratory & Nitrosamine

![Box plot showing % difference for spiked samples across different laboratories and nitrosamines.](image-url)
Evaluation of Detectability, LOQ and LOD

Quantitation limit (LOQ): defined by repeatability %RSD and % recovery

0.01 ppm
Repeatability %RSD < 20;
Reproducibility %RSD < 30;
% Recovery 100 ± 30%

Detection limit (LOD): between ‘not detectable’ and LOQ

0.0006 – 0.03 ppm (reported by some participating laboratories for their procedures)
Nitrosamines in Contaminated Samples

**NDMA 0.01 – 61 ppm**

**NDEA 0.01 – 0.9 ppm**

**NMBA 3 – 16 ppm**
Contaminated Samples: Repeatability and Reproducibility

- Repeatability is consistent with the spiked samples while the reproducibility shows greater variability.

**Repeatability %RSD**

Total Number of Values: 94
96% of the values: < 20

**Reproducibility %RSD**

Total Number of Values: 20
60% of the values: < 35 (vs 97%)
Potential Causes of the Large Variation for Reproducibility

- Deviation of individual laboratory results
- Sample preparation: extraction efficiency and consistency?
- Sample preparation: artifact?
  - Dichloromethane (DCM) favors NDMA formation from dimethylamine (1)
- Sample matrix: Lack of selectivity and specificity for some sample matrices
  - Some drug products contain additional API
  - \( N, N \)-Dimethylformamide may be present and interfere NDMA identification and quantitation in metformin (2)

Summary

• Trace levels of nitrosamine can be measured with accuracy and precision by a variety of mass spectrometry based analytical techniques.

• LOQ: 0.01 pm and lower
  – Repeatability %RSD < 20;
  – Reproducibility %RSD < 30;
  – % Recovery 100 ± 30%

• Suggested performance criteria
  – Repeatability % RSD: ≤ 18 at 0.03 ppm and lower for higher concentrations
  – Reproducibility % RSD: ≤ 22 - 30;
  – Spiked recovery: 100 ± 30%
  – A procedure needs to be validated for each unique sample matrix

• Areas for improvement
  – Effective and consistent sample preparation
  – Challenging nitrosamine (NMBA)