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

Jingyue (Jan) Yang

Senior Research Scientist

CDER/OPQ/Office of Testing and Research
US Food and Drug Administration

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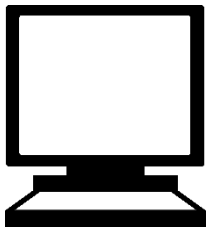
Pharmaceutical Quality

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



Pharmaceutical Quality

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

Drugs are no different.


FDA
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)

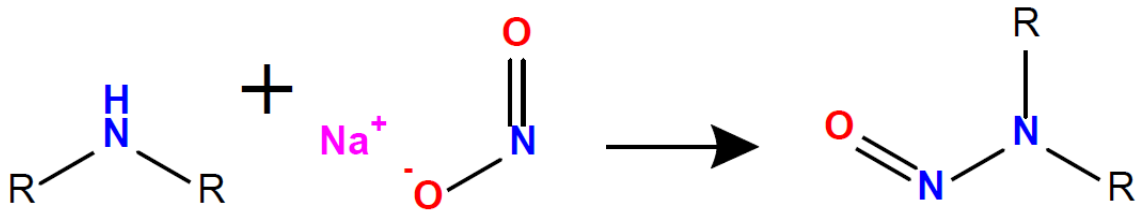
Food and Drug Administration (United States)

Raghavi Kakarla; Tim Marzan;

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)

Nitrosamine Contamination in Pharmaceutical Products

- Since 2018, nitrosamine contamination has led to the recall and even the market withdrawal of several widely used medicines.
- It has become one of main focuses of pharmaceutical manufacturing

Ranitidine (Zantac)

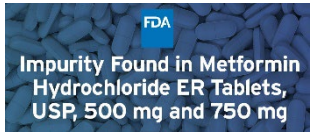
September 2019

Market removal
April 2020

July 2018

High blood pressure medication
(Angiotensin II receptor blocker medicine)
(valsartan, losartan, Irbesartan)

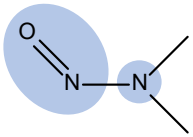
December 2019



Diabetes medication
(Metformin)

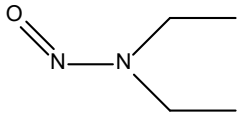
https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities-medications

Common Nitrosamines Found or Predicted to be Present in Pharmaceuticals



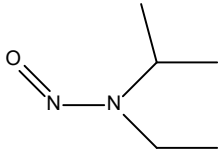
Chemical Formula: $C_2H_6N_2O$
Exact Mass: 74.0480

N-nitrosodimethylamine
(NDMA)



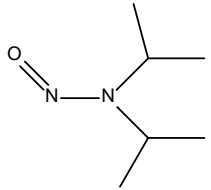
Chemical Formula: $C_4H_{10}N_2O$
Exact Mass: 102.0793

N-nitrosodiethylamine
(NDEA)



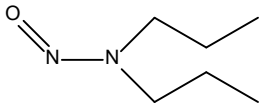
Chemical Formula: $C_5H_{12}N_2O$
Exact Mass: 116.0950

N-Nitrosoisopropylethylamine
(NIPEA or **NEIPA)**



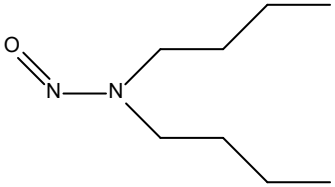
Chemical Formula: $C_6H_{14}N_2O$
Exact Mass: 130.1106

N-Nitrosodiisopropylamine
(NDIPA)



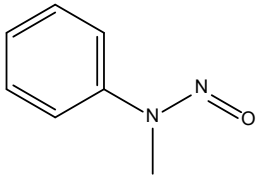
Chemical Formula: $C_6H_{14}N_2O$
Exact Mass: 130.1106

N-Nitrosodipropylamine
(NDPA)



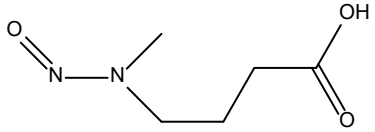
Chemical Formula: $C_8H_{18}N_2O$
Exact Mass: 158.1419

N-Nitrosodibutylamine
(NDBA)



Chemical Formula: $C_7H_8N_2O$
Exact Mass: 136.0637

N-Nitrosomethylphenylamine
(NMPA)



Chemical Formula: $C_5H_{10}N_2O_3$
Exact Mass: 146.0691

N-Nitroso-N-methyl-4
-aminobutyric
Acid **(NMBA)**

Response and Engagement of Regulatory Authorities

GUIDANCE DOCUMENT

Control of Nitrosamine Impurities in Human Drugs

Guidance for Industry

SEPTEMBER 2020

Nitrosamine impurities

Share

Table of contents

- Scientific review on the risk of nitrosamine impurities in human medicines
- Nitrosamine Implementation Oversight Group
- Guidance for marketing authorisation holders

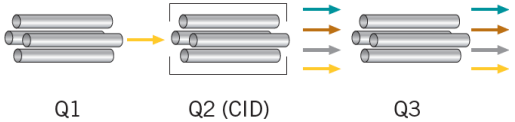
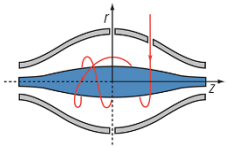
FDA Guidance: Acceptable Intake Limits for Nitrosamines

Nitrosamine	Acceptable Intake Limit (ng/day)
NDMA	96
NDEA	26.5
NMBA	96
NMPA	26.5
NIPEA	26.5
NDIPA	26.5

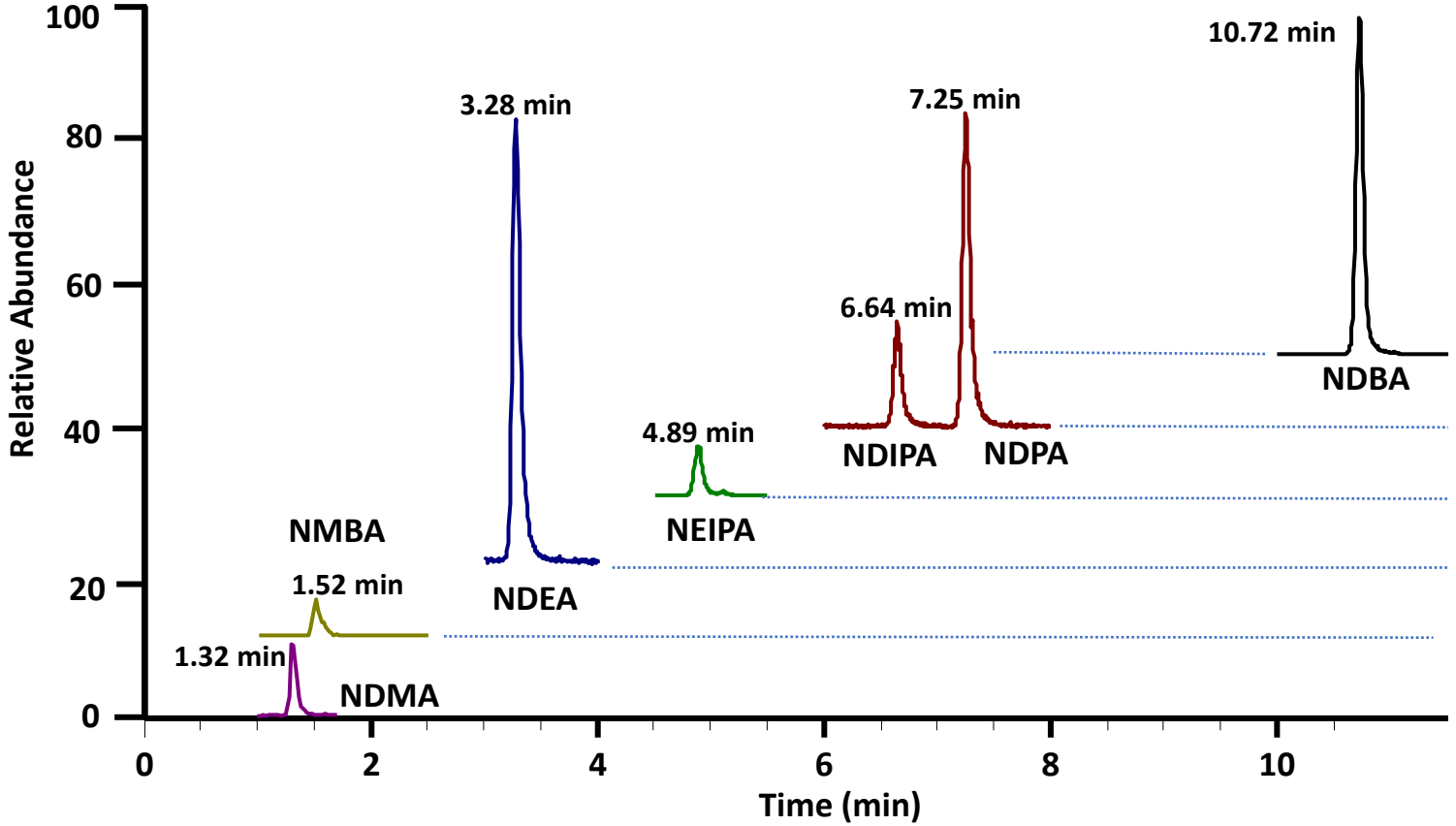
"Manufacturers of APIs and drug products should use methods with **LOQ**s at or below **0.03 ppm** Manufacturers should establish methods for which the LOQ and limit of detection (LOD) are **as low as reasonably practical** for products for which the maximum daily dose is high (e.g., greater than 1 g)"

FDA Guidance for Industry: Control of Nitrosamine Impurities in Human Drugs (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/control-nitrosamine-impurities-human-drugs)

Nitrosamine Determination by Mass Spectrometry

		MS/MS (MRM)	High Resolution MS
Mass Analyzer			 Orbitrap
Ion source		APCI	ESI
Scan Mode	**NDMA**	75.1 → 43.1; 58.1 (+)	MS2 (+) (75.1); EIC 75.0553
	NDEA	103.1 → 75.1; 47.1 (+)	SIM (+) (103.1); EIC 103.0866
	NEIPA	117.1 → 75.1; 47.1 (+)	MS2 (+) (117.1); EIC 75.0553
	NDIPA	131.1 → 89.1; 43.1 (+)	SIM (+) (131.1); EIC 131.1179
	NDPA	131.1 → 89.1; 43.1 (+)	SIM (+) (131.1); EIC 131.1179
	NMPA	137.1 → 107.1; 66.1 (+)	SIM (+) (137.1); EIC 137.0709
	NDBA	159.1 → 103.1; 57.1 (+)	MS2 (+) (159.1); EIC 103.0866
	NMBA	147.1 → 117.1; 44.1 (+)	SIM (-) (145.1); EIC 145.0619

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

ansm .... LC-MS/MS

Australian Government Department of Health Therapeutic Goods Administration .... LC-HRMS

LGL .... GC-MS; LC-MS/MS

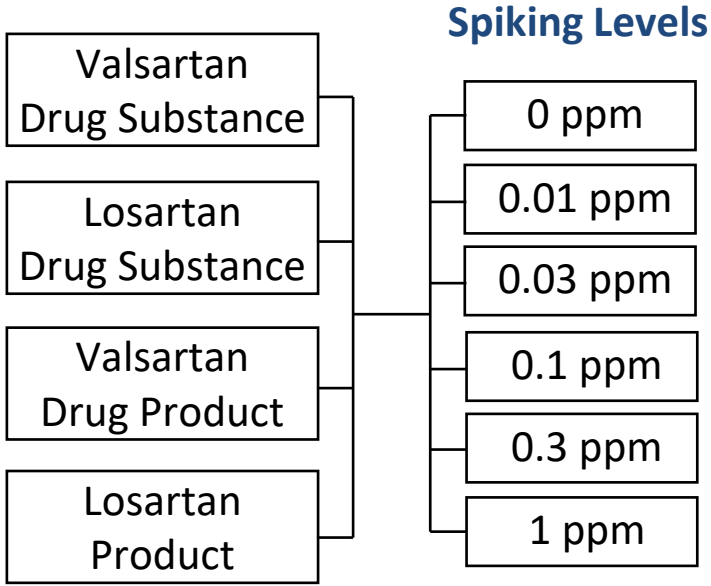
Health Canada .... LC-MS/MS

swissmedic .... GC-MS

FDA .... LC-HRMS

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



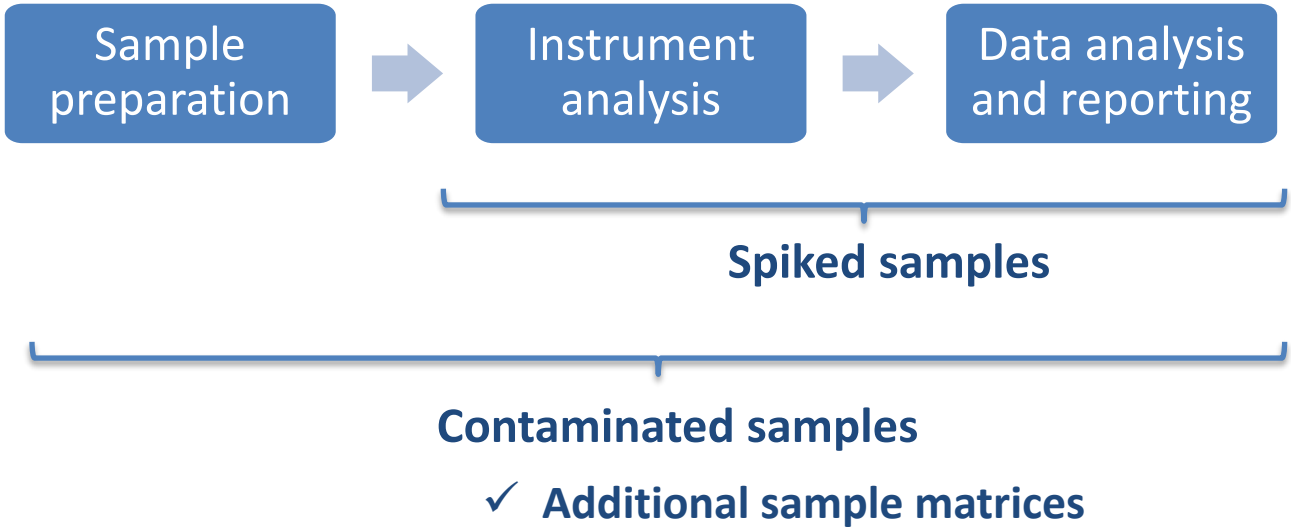
Samples for the Study: Contaminated Samples

- 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

Valsartan Drug Substance	V-DS-1	V-DS-2	V-DS-3
Losartan Drug Substance	L-DS-1	L-DS-2	L-DS-3

Valsartan Drug Product	V-DP-1	V-DP-2	V-DP-3
Losartan Drug Product	L-DP-1	L-DP-2	L-DP-3

Samples for the Study



- ✓ **Additional sample matrices**

Overview of Analytical Procedures Used in This Study

	Sample preparation	Instrument analysis			Quantitation
LC-HRMS	Extraction by methanol	ESI	Orbitrap	SIM/MS^2	External standard
LC-HRMS	Extraction by methanol	APCI	Orbitrap	Full MS	ILIS (isotope labeled internal standard)
LC-MS/MS	Extraction by 25% methanol in water	APCI	Triple quad	MRM	ILIS
LC-MS/MS	Extraction by 10% methanol in water	APCI	Triple quad	MRM	ILIS
GC-MS/MS	Extraction by 1 M NaOH (10 mL) → dichloromethane (4 mL) extraction	EI	Triple quad	MRM	ILIS
GC-MS/MS	Extraction by 1 M NaOH (5 mL) → dichloromethane (1 mL) extraction	EI	Triple quad	MRM	ILIS

Data Analysis

Precision

Repeatability

$$S = \sqrt{\frac{\sum_{i=1}^{n}(xi - x)^2}{n-1}}$$

Reproducibility

$$S_R = \sqrt{S_L^2 + S_r^2}$$

S_L^2 between-laboratory

S_r^2 within-laboratory

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

$$\%\text{RSD} = \text{Standare deviation} / \text{average x } 100$$

Accuracy

$$\%\ Difference = \frac{x - c}{c} \times 100$$

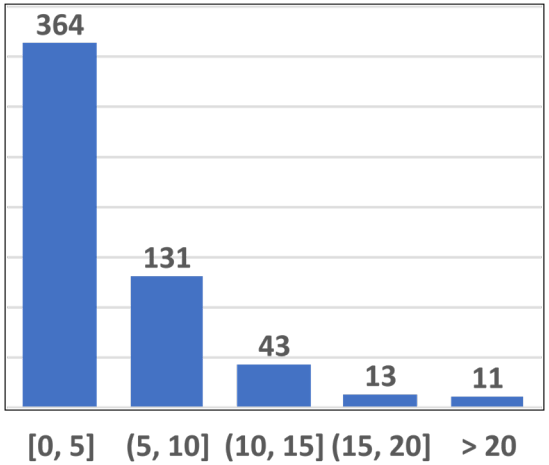
xi: Single measurement; **x**: Average value; **n**: Number of replicates; **p**: Number of laboratories; **c**: Spiked - spiked concentration;

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

Spiked Samples: Data Overview

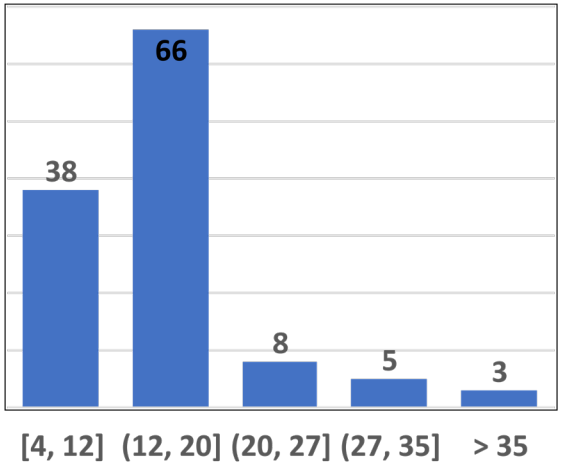
Repeatability %RSD

Total Number of Values: 562
98% of the Values: < 20



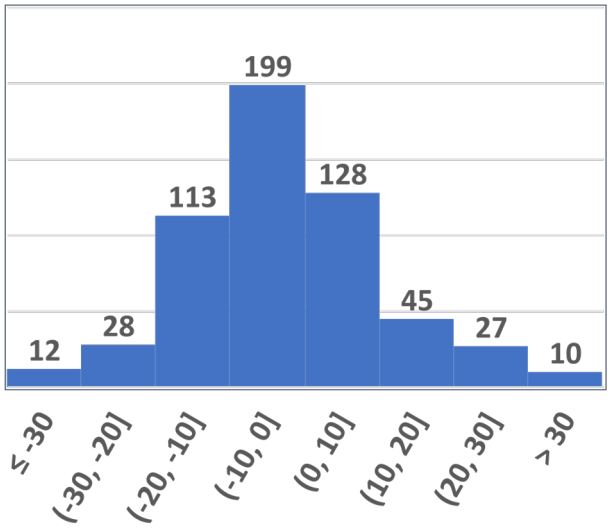
Reproducibility %RSD

Total Number of Values: 120
97% of the Values: < 35

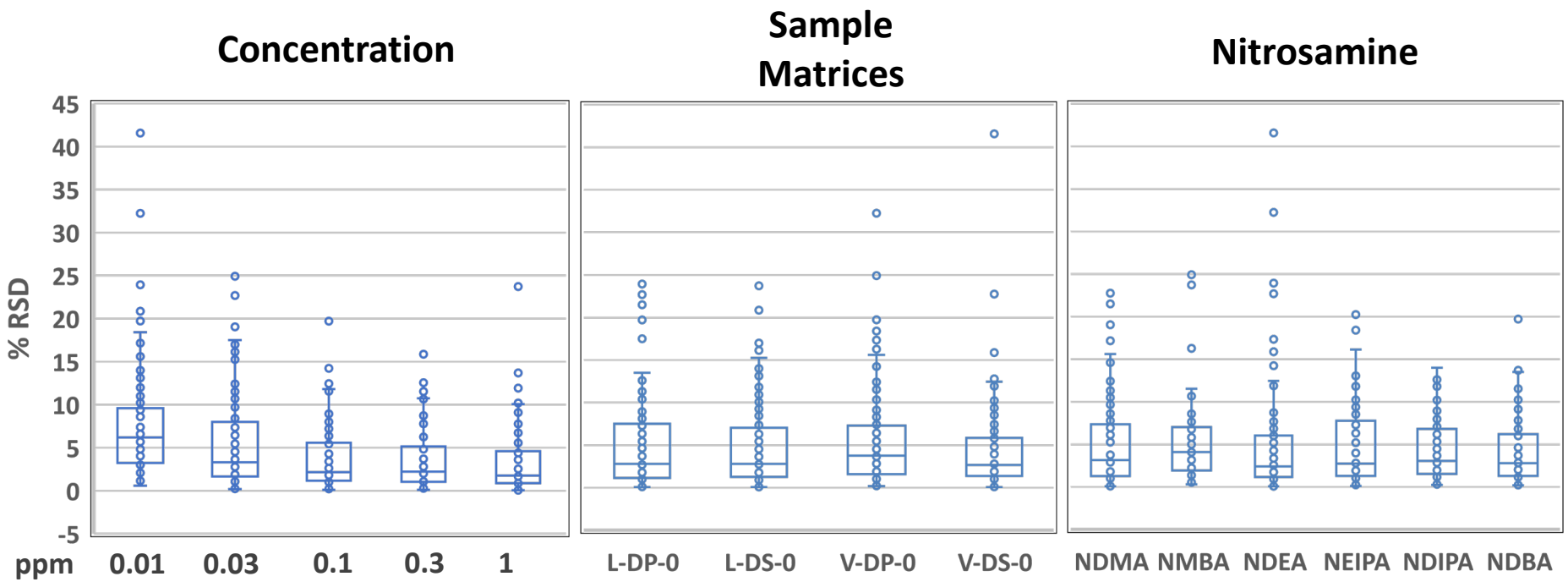


%Difference

Total Number of Values: 562
96% of the Values: [-30, 30]

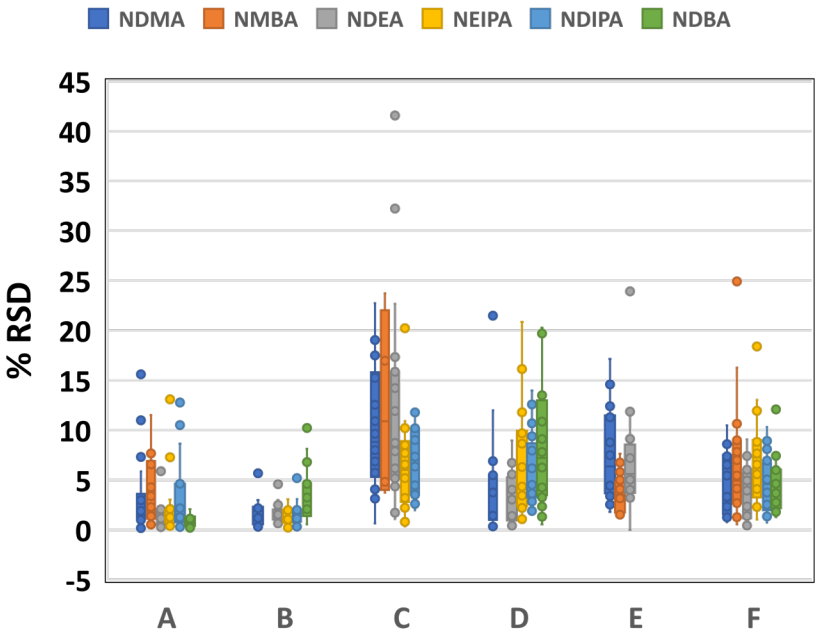


Repeatability %RSD for Spiked Samples

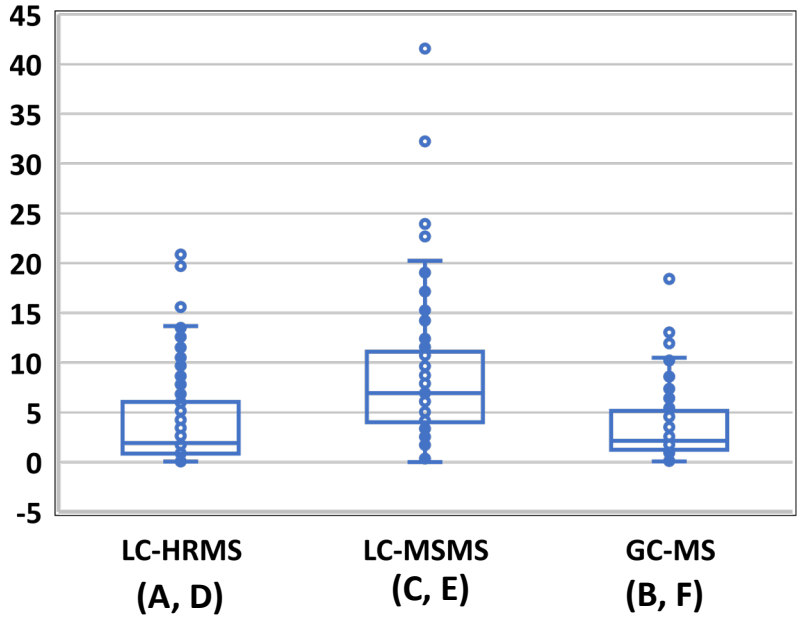


Repeatability %RSD for Spiked Samples

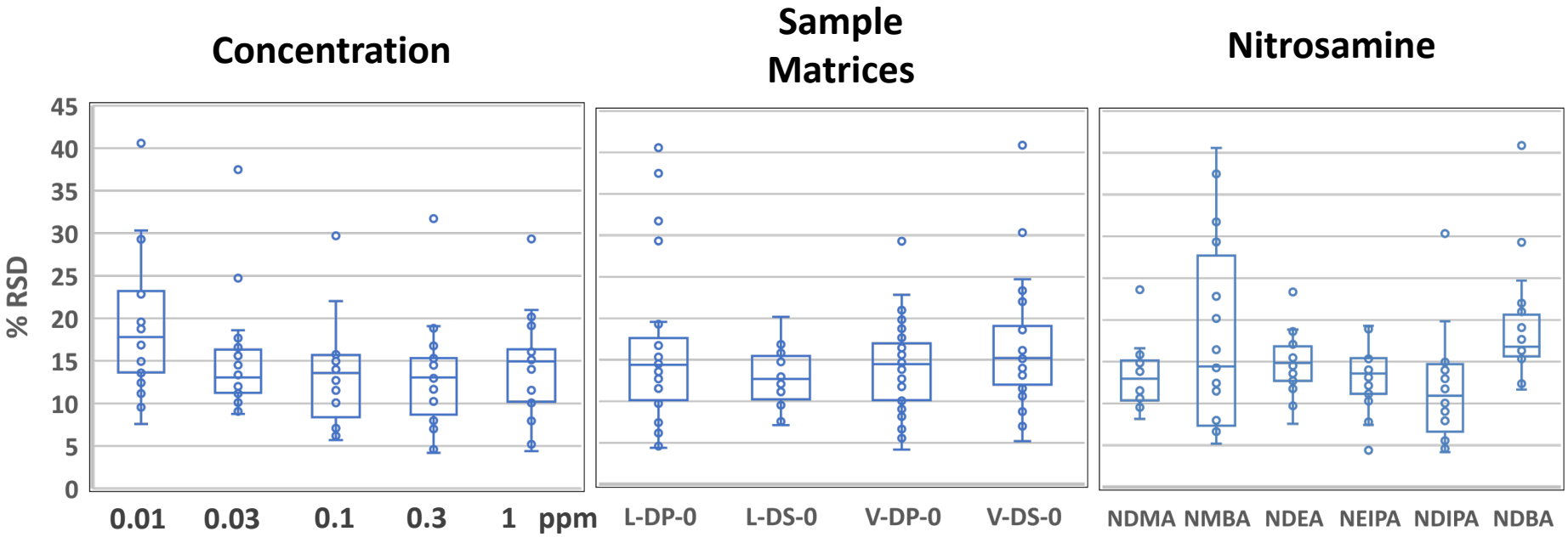
Laboratory and Nitrosamine



Analytical Technique



Reproducibility %RSD for Spiked Samples



Comparison to Predicted Repeatability and Reproducibility

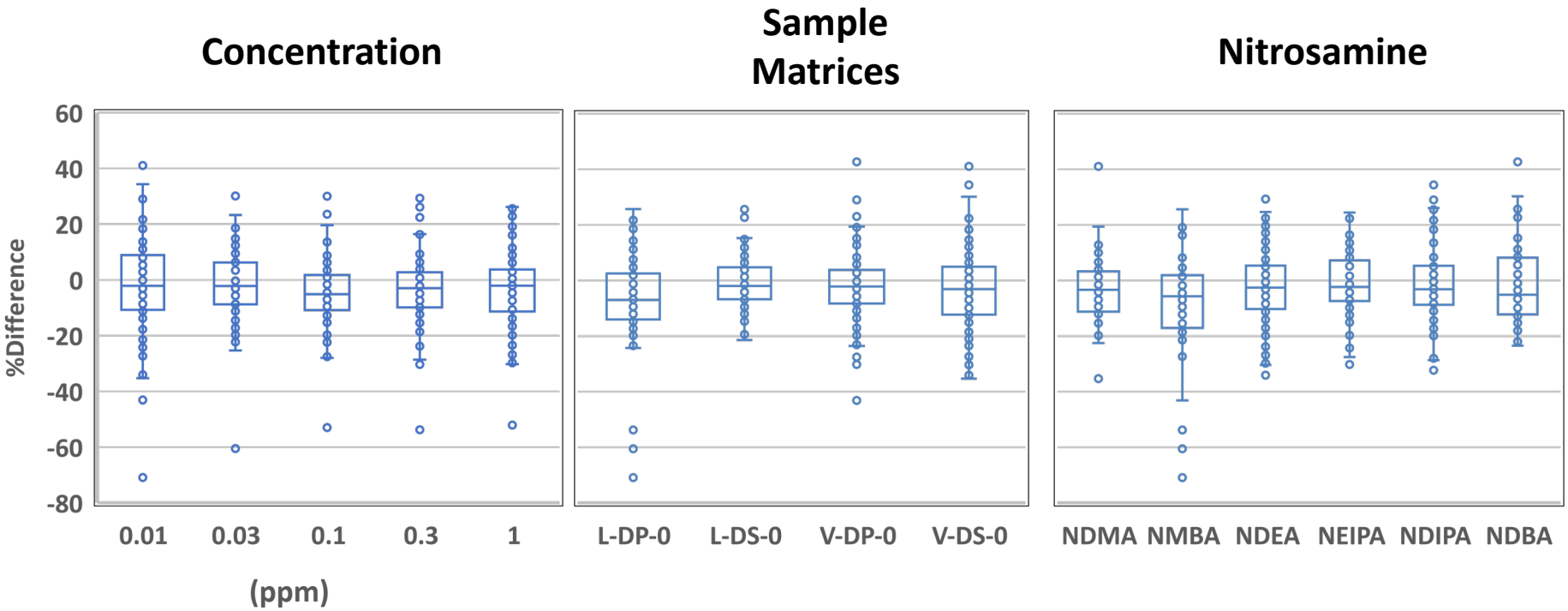
- **Horwitz Equation** %RSD (reproducibility) = $2^{(1-0.5logC)}$

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

Concentration	Reproducibility		Repeatability	
	Predicted	From study	Predicted	From study
0.01 ppm	32	30	21	18
0.03 ppm	27	19	18	18
0.1 ppm	22	22	15	12
0.3 ppm	19	19	13	11
1 ppm	16	21	11	10

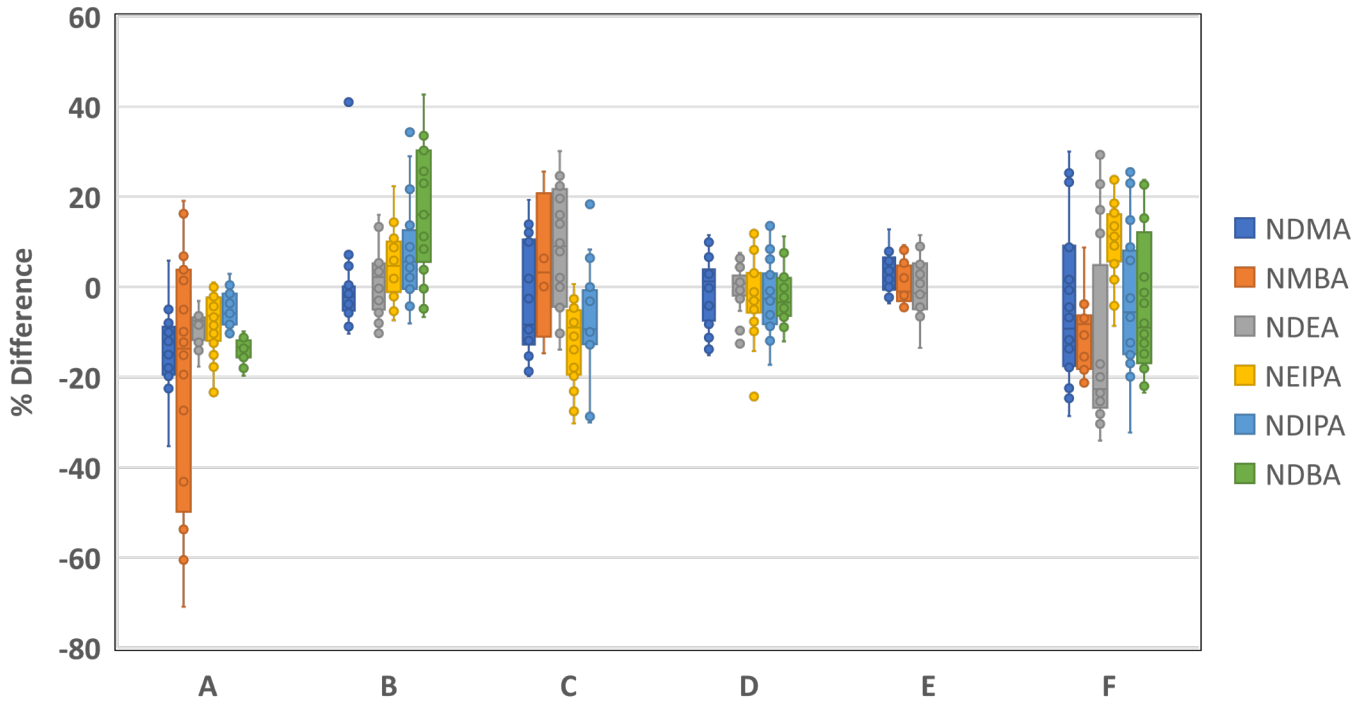
Ref. Horwitz, W., *Evaluation of analytical methods used for regulation of foods and drugs.* Analytical Chemistry, 1982. 54(1): p. 67-76

Accuracy Evaluation: % Difference for Spiked Samples

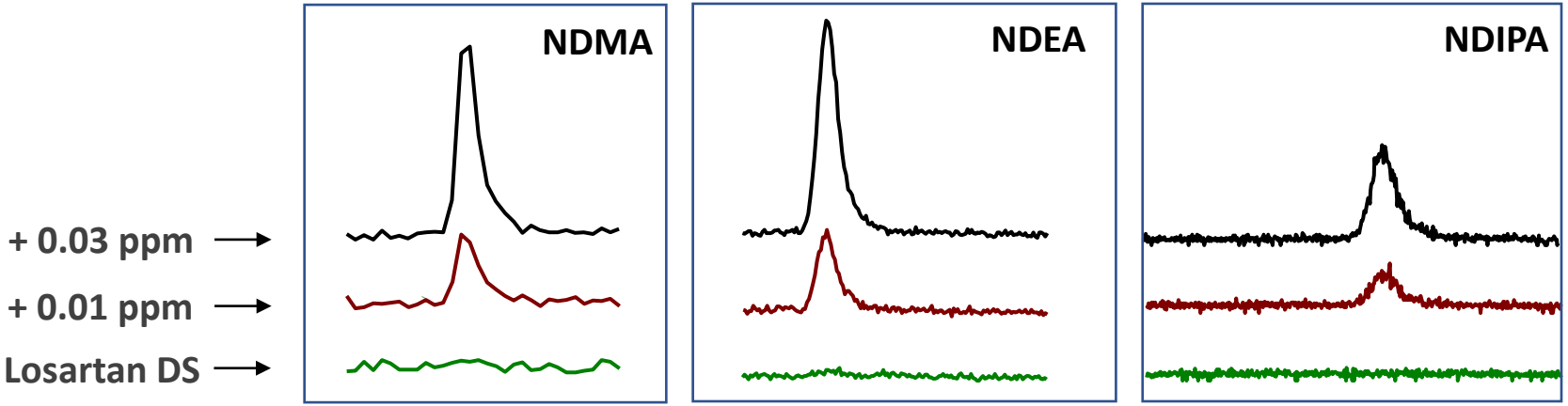


Accuracy Evaluation: % Difference for Spiked Samples

Individual Laboratory & Nitrosamine



Evaluation of Detectability, LOQ and LOD



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

0.01 pm

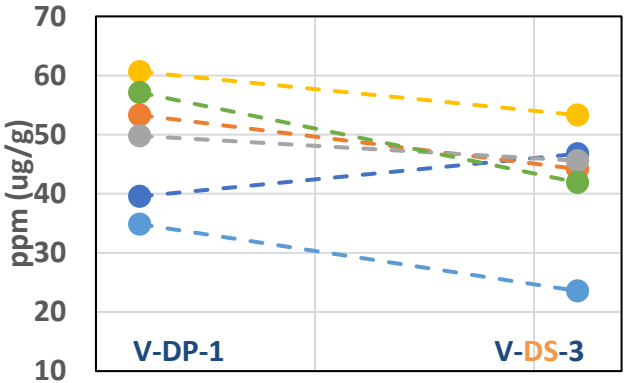
Repeatability %RSD < 20;
Reproducibility %RSD < 30;
% Recovery 100 ± 30%

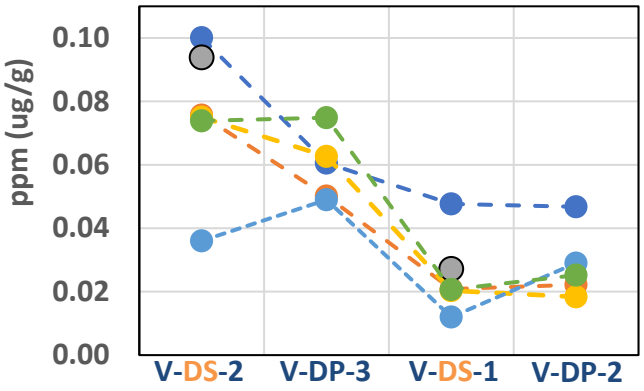
Detection limit (LOD): between 'not detectable' and LOQ

0.0006 – 0.03 ppm (reported by some participating laboratories for their procedures)

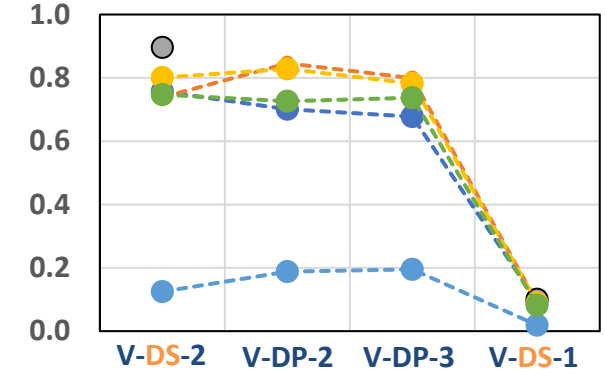
Nitrosamines in Contaminated Samples

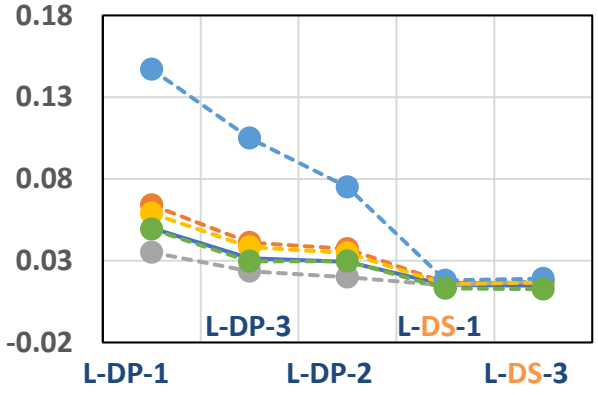
NDMA 0.01 – 61 ppm



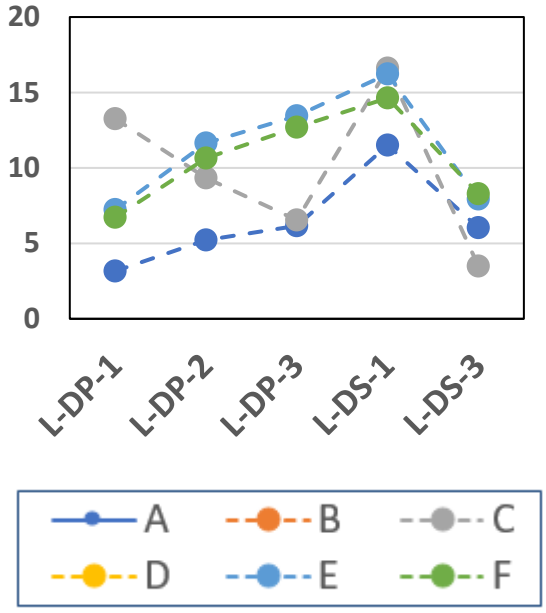


NDEA 0.01 – 0.9 ppm





NMBA 3 – 16 ppm

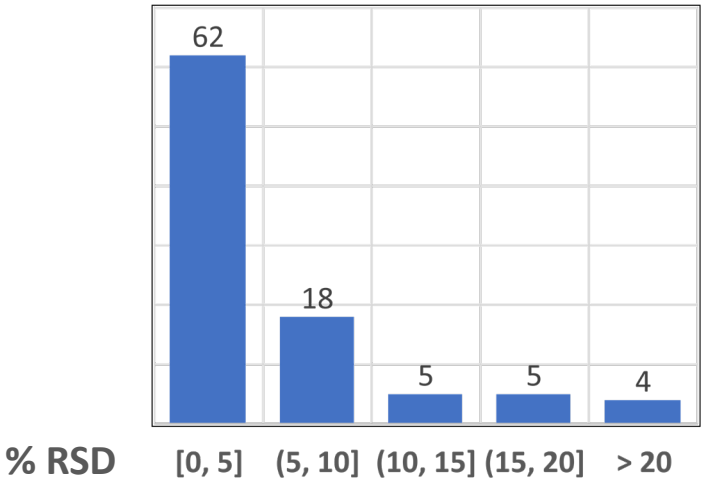


Contaminated Samples: Repeatability and Reproducibility

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

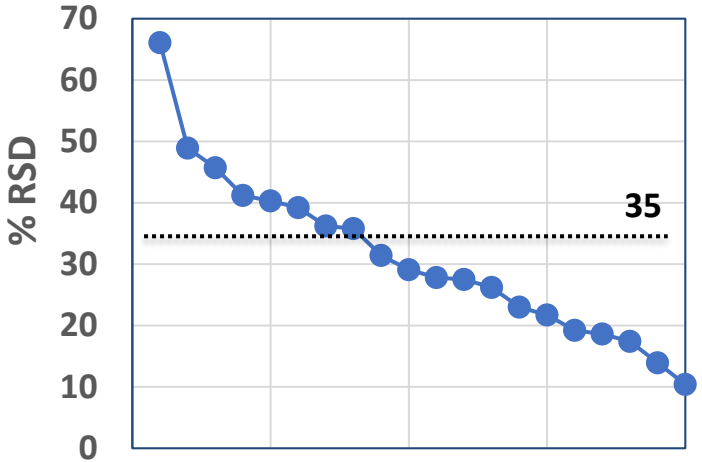
Repeatability %RSD

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



Reproducibility %RSD

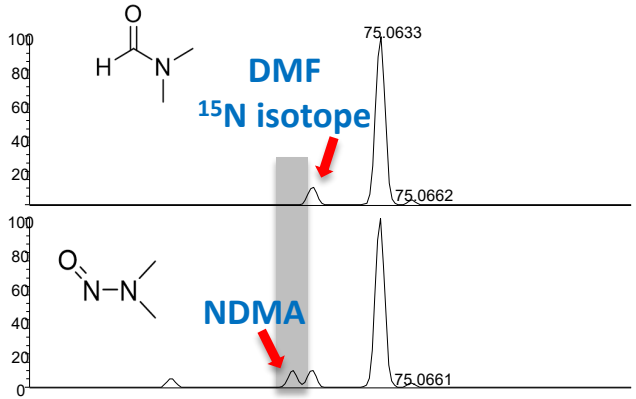
Total Number of Values: 20
60% of the values: < 35 (vs 97%)



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)



1. NDMA analytics in metformin products: comparison of methods and pitfalls. Eur J Pharm Sci. 2021;168:106026

2. A Cautionary Tale: Quantitative LC-HRMS Analytical Procedures for the Analysis of *N*-Nitrosodimethylamine in Metformin; The AAPS Journal volume 22, Article number: 89 (2020)

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)