Interlaboratory Validation Study for the Analysis of Nitrosamines in Drinking Water using GC-MS/MS

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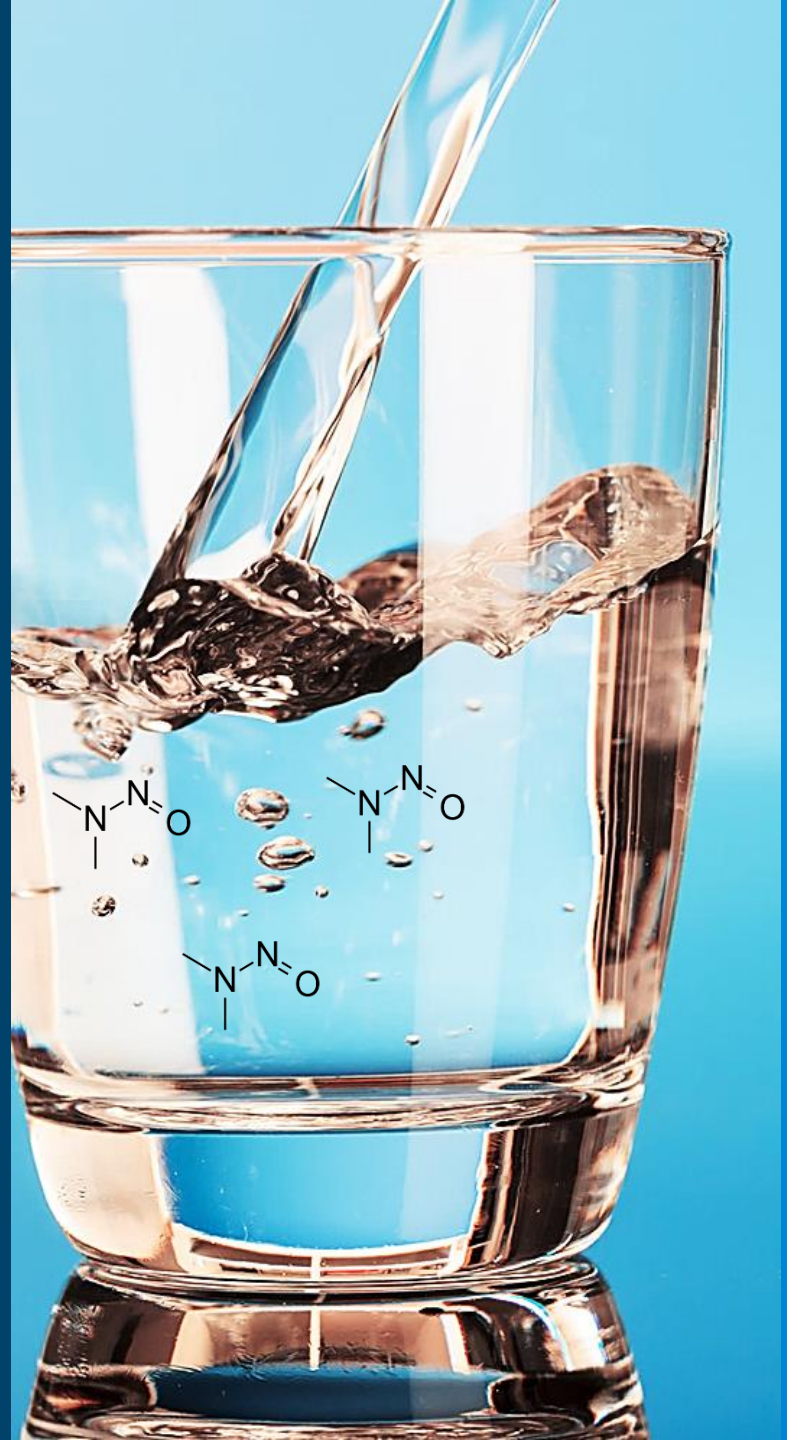
National Environmental Monitoring Conference
Washington, D.C.
August 7, 2017



Agilent
Trusted Answers

Outline

- Background
- Purpose of Project
- Phase I
  - GC/MS Ion Trap versus GC/MS Triple Quad (GC-IT vs GC-MS/MS)
- Phase II
  - Results of Interlaboratory validation study (ILS) with GC-MS/MS
- Conclusion

Background and Purpose of Project

- EPA Method 521 (2004): “Determination of nitrosamines in drinking water by **solid phase extraction** and capillary column **gas chromatography** with **large volume injection** and **chemical ionization tandem mass spectrometry**”
- Ion Trap GC/MS is the approved instrumentation for Method 521 but it is being obsoleted
- EPA might regulate nitrosamines due to the occurrence in drinking water and wastewater (particular NDMA)
- EPA Office of Ground Water/Drinking water (OGWDW) considers alternate detection techniques without changing the guidelines for sample preparation
- Purpose of the project is to directly compare Triple Quadrupole GC/MS (GC-MS/MS) and the currently used Ion Trap GC/MS (GC-IT) method using split samples set
- Phase I: Varian 4000 GC-IT vs Agilent 7010 GC-MS/MS
- Phase II: Three Lab Validation Studies of GC-MS/MS Method

Phase I

LAB A evaluates nitrosamines in drinking water using GC-IT and GC-MS/MS

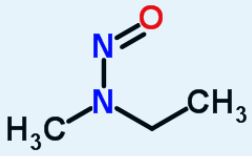
- Which nitrosamines are investigated
- Sample Preparation
- Optimized parameters for GC-MS/MS
- Compare GC-IT vs GC-MS/MS Results

Nitrosamines Investigated

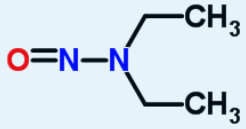
NMOR and NDPhA were evaluated in addition to all nitrosamines in Method 521

Analytes in EPA Method 521

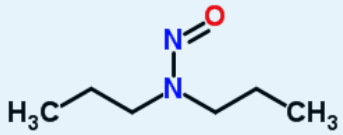
NDMA
N-Nitrosodimethylamine



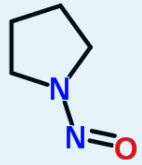
NMEA
N-Nitrosomethylethylamine



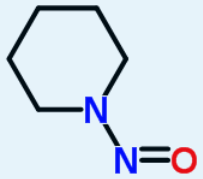
NDEA
N-Nitrosodiethylamine



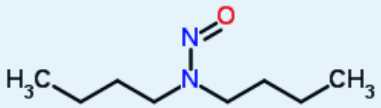
NDPA
N-Nitrosodi-n-propylamine



NPYR
N-Nitrosopyrrolidine

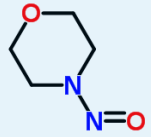


NPIP
N-Nitrosopiperidine

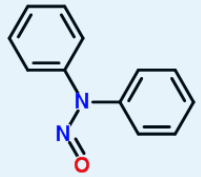


NDBA
N-Nitrosodi-n-butylamine

Addition

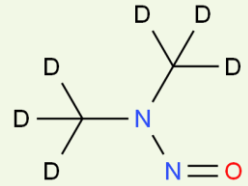


NMOR
N-Nitrosomorpholine



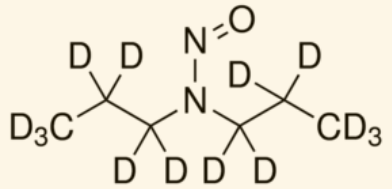
NDPhA
N-nitrosodiphenylamine

Surrogate



NDMA-d6
N-Nitrosodimethylamine-d6

Internal Standard



NDPA-d14
N-Nitrosodipropylamine-d14

Note: Method 521 (2004) evaluated NMOR but was not included in the method due to contamination problems

Drinking Water Extraction

All water samples were extracted manually. No changes made to Method 521 sample preparation

SPE Procedure

Condition Cartridge

Methylene Chloride
Methanol
Reagent water

Extract Sample

500-mL water sample

Elute Cartridge

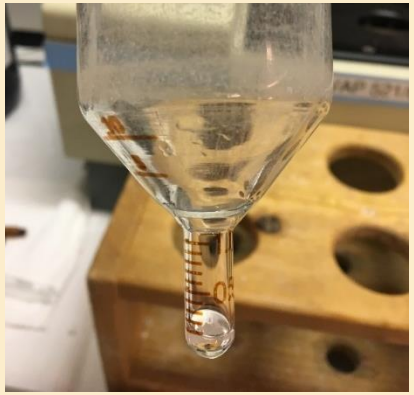
Methylene chloride
Soak
Collect

Concentration

Remove residual water

Sodium Sulfate
(anhydrous)

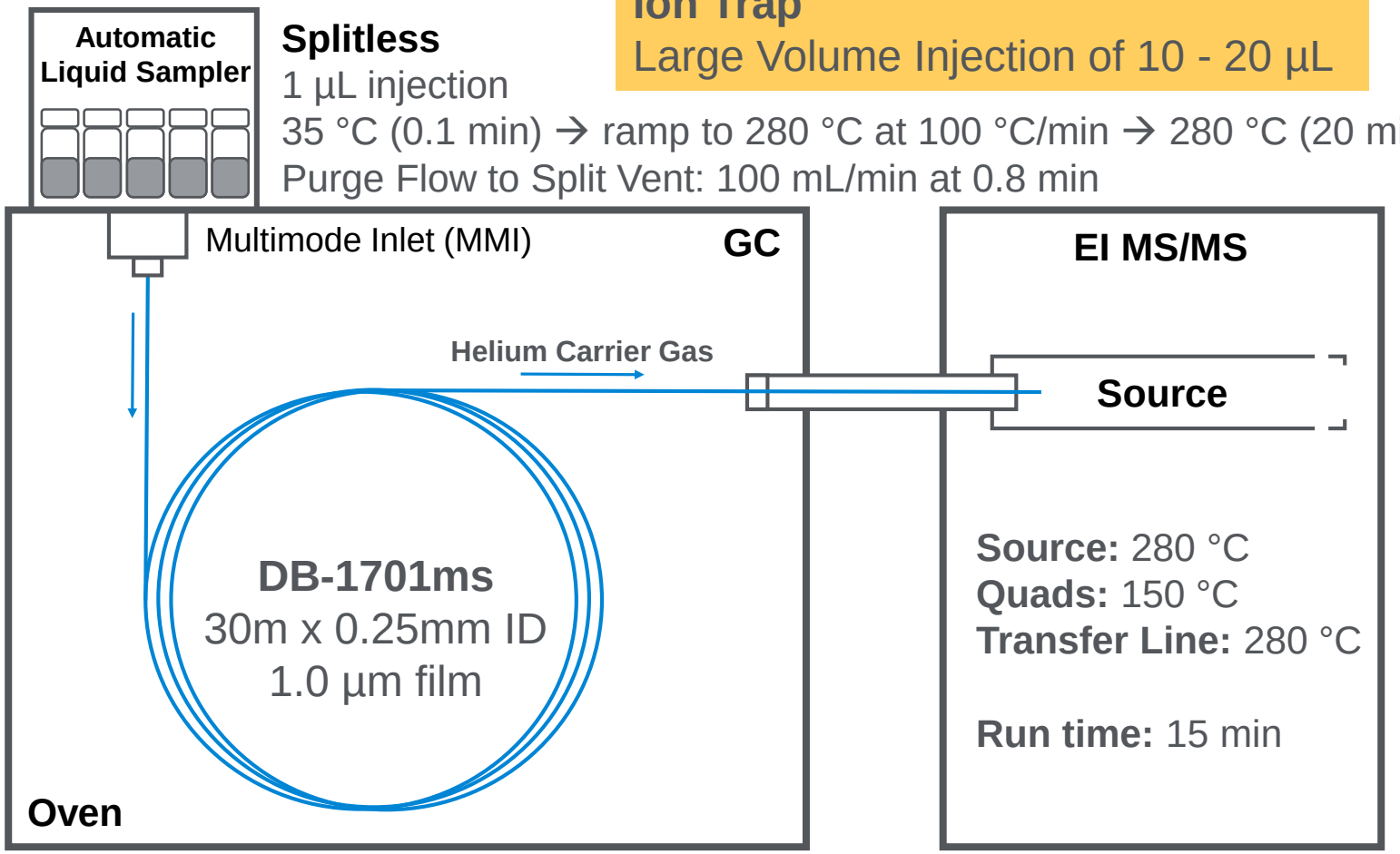
Concentration



Water Bath
1mL sample

GC-MS/MS System Parameters

Ion Trap
Large Volume Injection of 10 - 20 µL



Ion Trap
Similar Oven Parameters

Ion Trap
Chemical Ionization
Run time = 40 min

Inlet liner
4mm double-tapered, UI

GC Parameters
MMI Inlet → MSD
Constant Flow
Flow 1.2 mL/min

Column
DB-1701ms UI
14% cyanopropylphenyl
86% dimethylpolysiloxane

Oven program:
33 °C (1min)
35 °C/min to 80 °C (2 min)
10 °C/min to 140 °C (0 min)
50 °C/min to 280 °C (2 min)
50 °C/min to 300 °C (3min)

MRM Transitions using GC-MS/MS

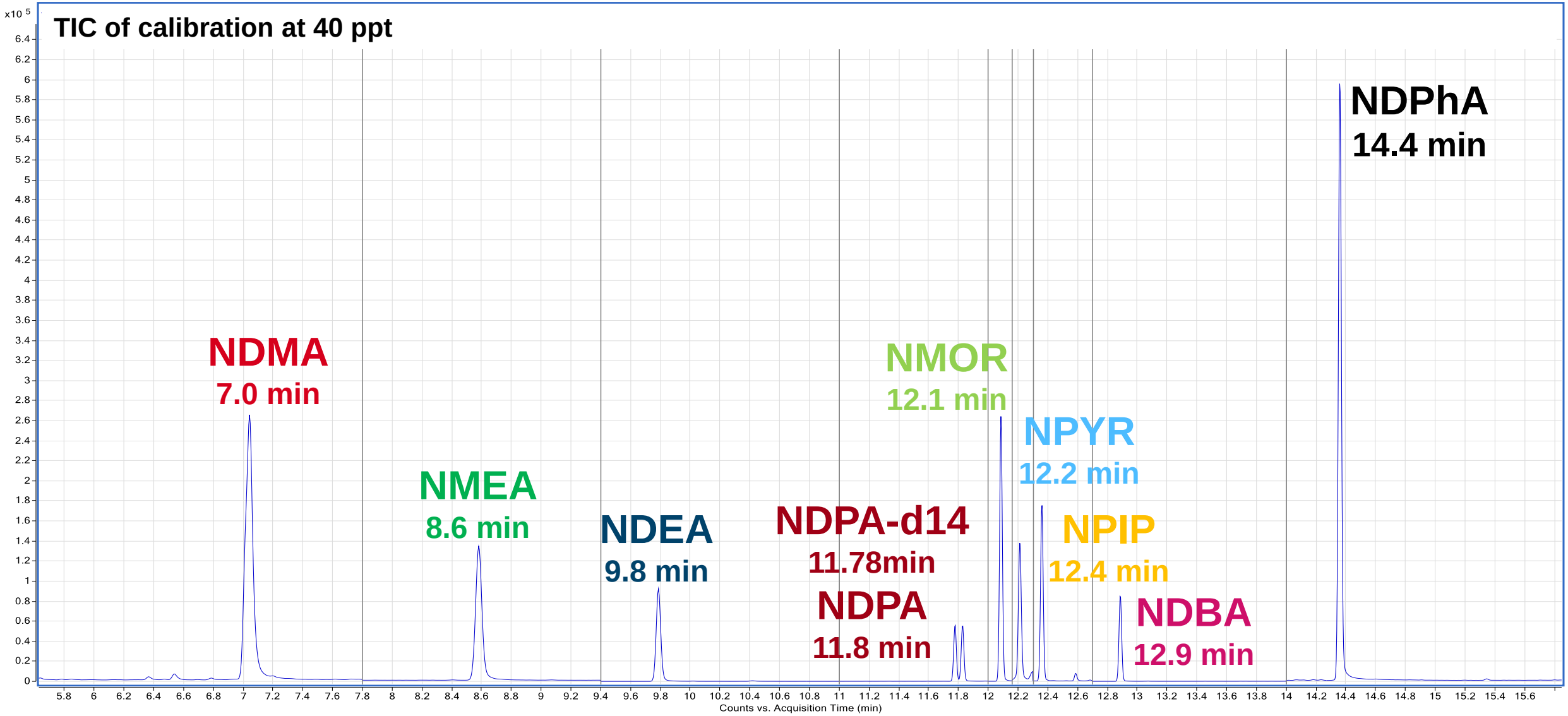
Optimized using MS1 Scan, Product Ion Scans, and Multiple Reaction Monitoring (MRM)

Sample Information		MRM Transitions					
Type	Analyte	Quantifier	CE	Qualifier	CE	Qualifier	CE
Surrogate	NDMA-d6	80→50	8	80→46	25		
Target	NDMA	74→44	6	74→42	22		
Target	NMEA	88→71	4	88→42	23	88→42	23
Target	NDEA	102→85	4	102→44	12		
ISTD	NDPA-d14	144→126	3	144→50	13		
Target	NDPA	130→43	12	101→70	5		
Target	NMOR	116→86	2	116→56	15		
Target	NPYR	100→55	7	100→70	7	100→43	10
Target	NPIP	114→84	7	114→55	25		
Target	NDBA	158→141	3	158→99	8	116→99	2
Target	NDPhA	169→167	35	169→66	30	169→77	40

ISTD = Internal Standard

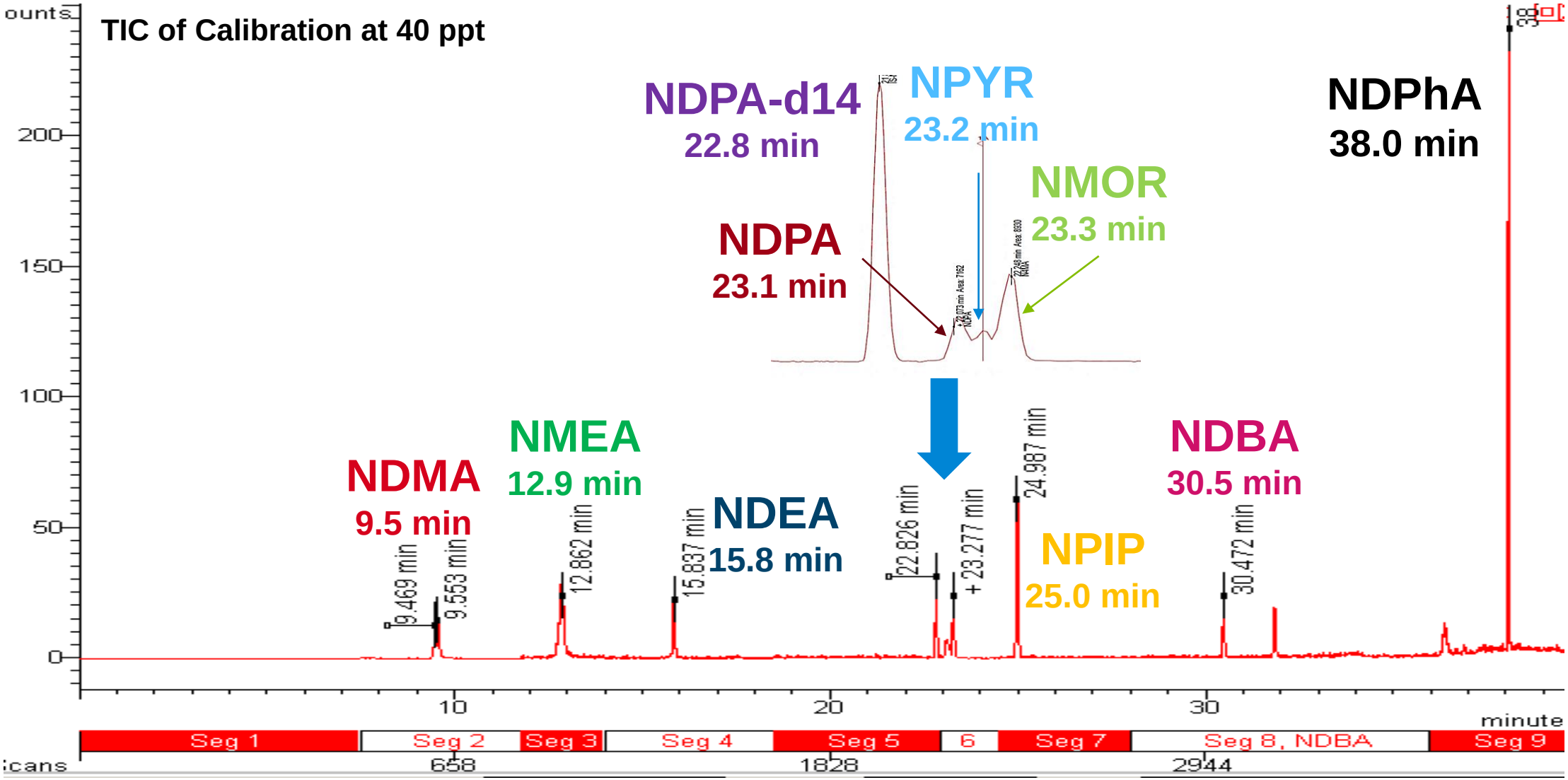
Nitrosamines analysis using GC-MS/MS

Triple Quad Run Time is 15 min, Baseline separation observed for NDPA, NPYR, and NMOR



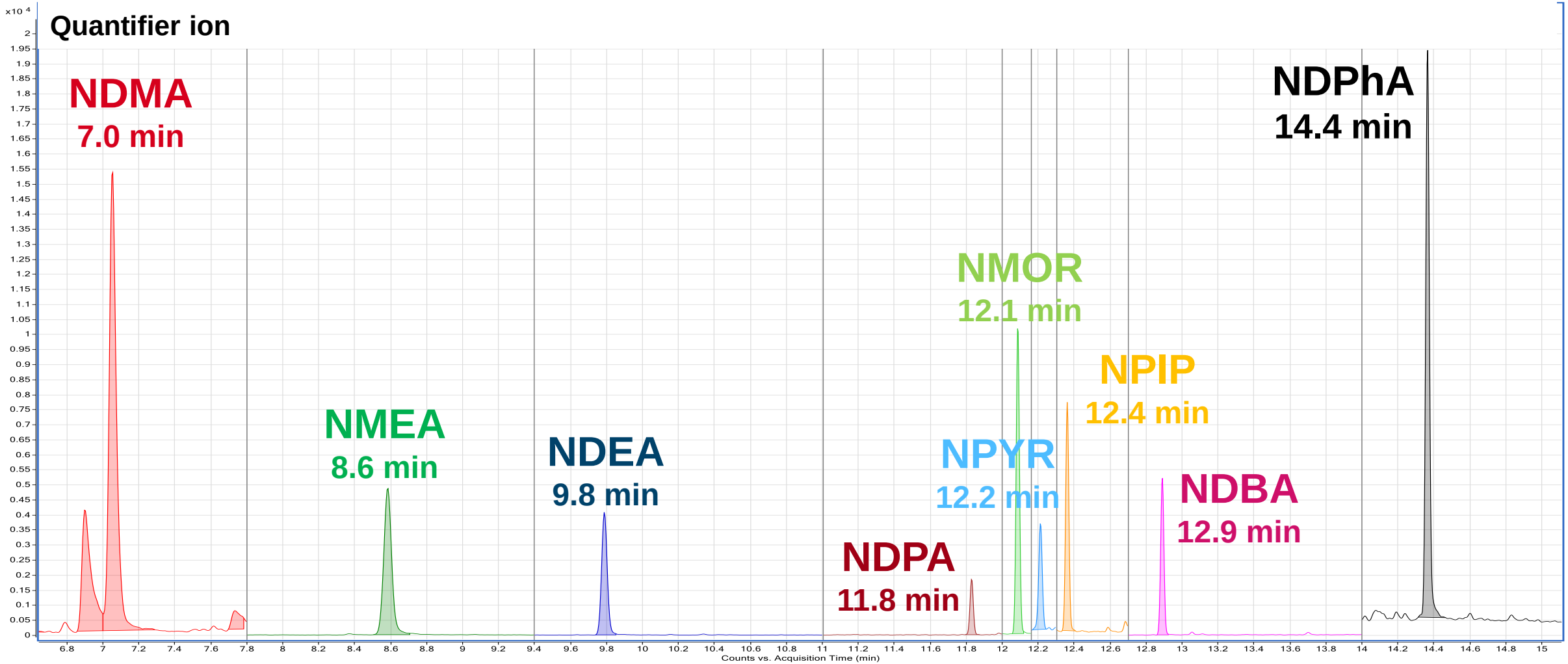
Nitrosamine analysis using GC-IT

40 min run-time, Poor baseline separation for NDPA, NPYR, and NMOR



Nitrosamine Analysis in Sample Water Extracts using GC-MS/MS

0.5 ppt nitrosamines in Sample Water Extract

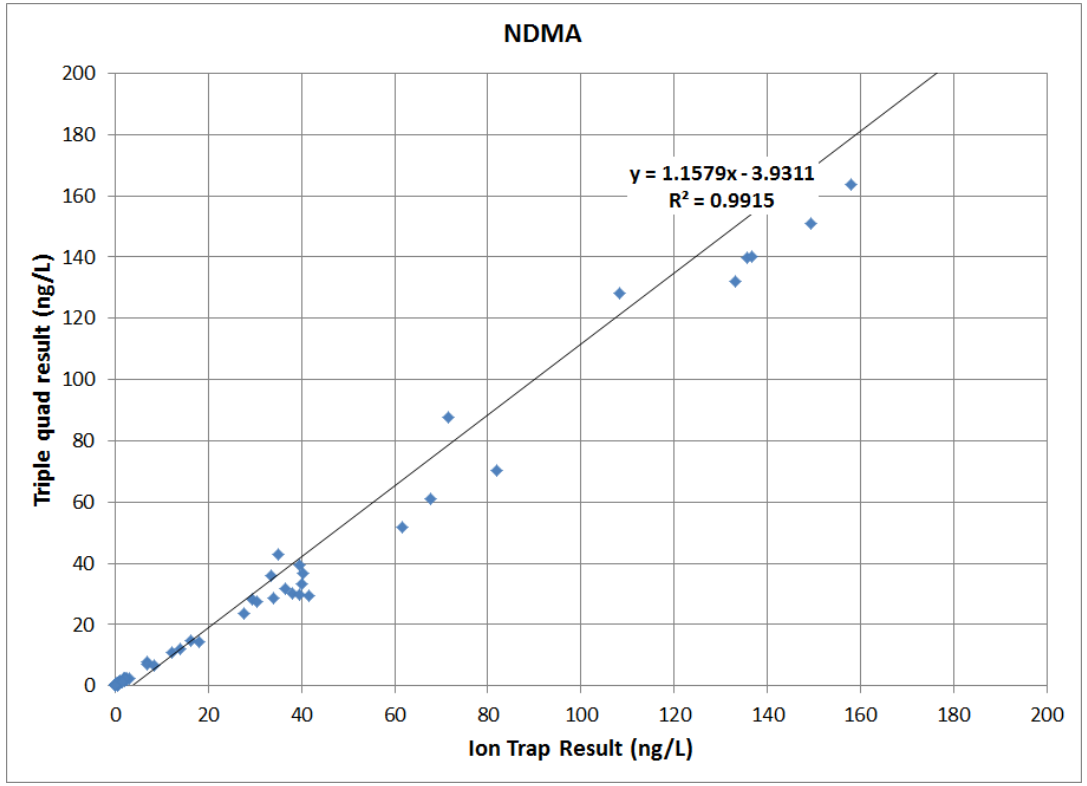


Internal standard is not plotted as 20 ppt overwhelms TIC when plotted with 0.5ppt analytes in extract.

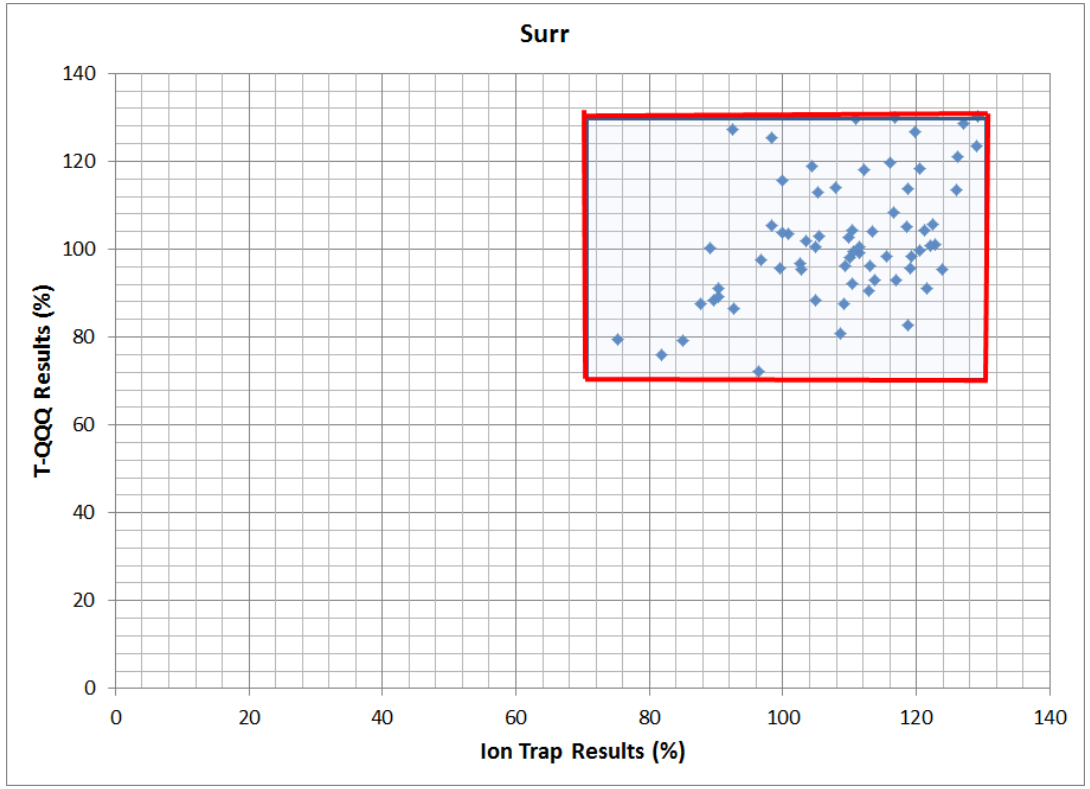
Field Sample Comparison (GC-IT vs GC-MS/MS)

Correlation observed in samples and surrogates

Real Extracted WaterSamples



Surrogate recoveries are within limits



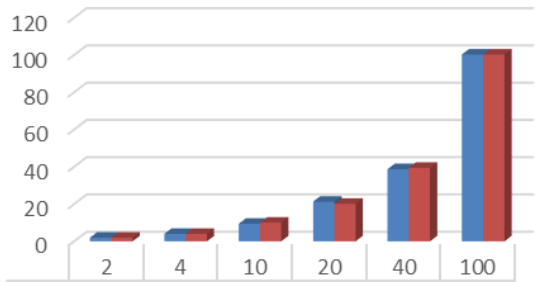
Note:

- Real Extracted Samples were analyzed using GC-IT and GC-QQQ
- Same holding time, standards, extraction process, mixes

Calibration Comparison (GC-IT vs GC-MS/MS)

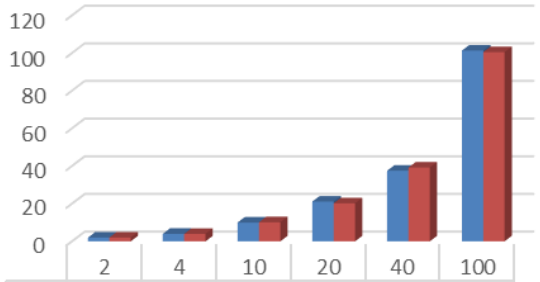
Correlation observed at 2.0 to 100 ppt for Extracted Water Sample Set

NDMA comparison TQ to IT



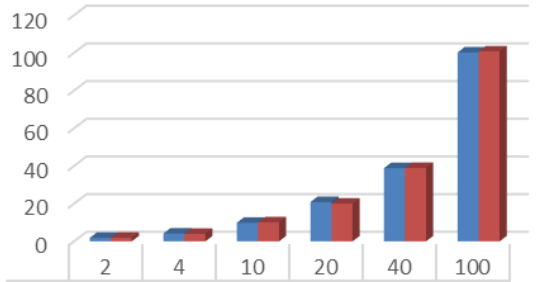
	2	4	10	20	40	100
IT	1.993	4.062	9.484	21.324	38.793	100.31
TQ	1.999	3.995	10.043	20.150	39.459	100.34

NMEA comparison TQ to IT



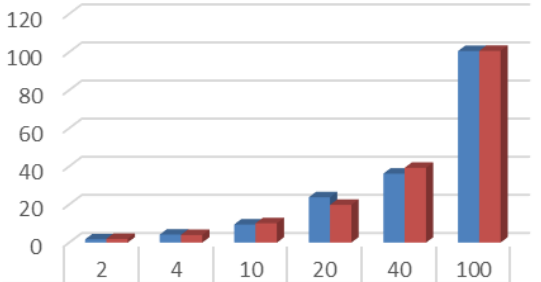
	2	4	10	20	40	100
IT	1.992	3.997	9.977	21.123	37.495	101.32
TQ	2.008	3.942	10.139	20.230	39.207	100.46

NDEA comparison of TQ to IT



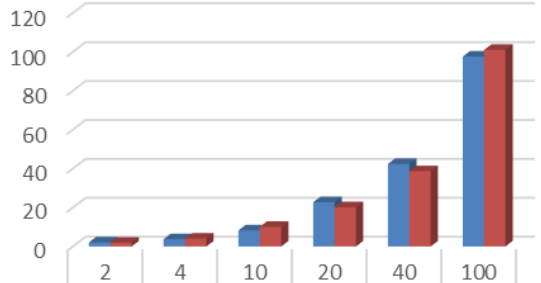
	2	4	10	20	40	100
IT	1.898	4.153	9.897	20.926	38.873	100.25
TQ	1.990	4.025	10.134	20.055	38.979	100.81

NPYR comparison TQ to IT



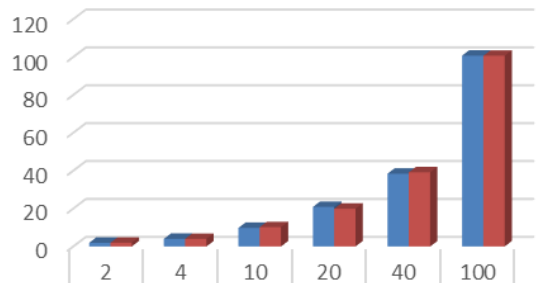
	2	4	10	20	40	100
IT	1.743	4.234	9.546	23.818	36.215	100.56
TQ	1.987	4.040	10.162	19.838	39.277	100.67

NDPA comparison TQ to IT



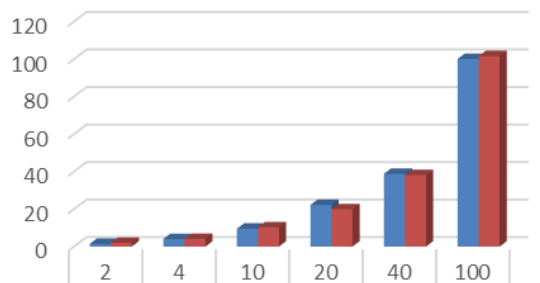
	2	4	10	20	40	100
IT	2.09	3.762	8.294	22.76	42.398	97.558
TQ	1.983	4.063	10.066	20.183	38.692	100.98

NPIP comparison TQ to IT



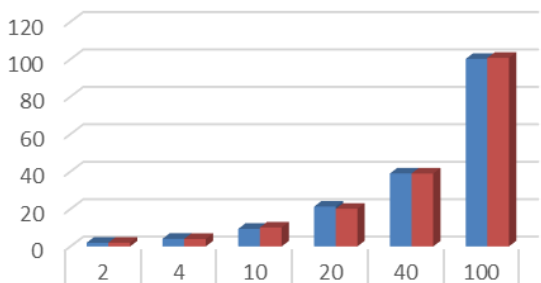
	2	4	10	20	40	100
IT	1.986	4.05	9.823	20.895	38.41	100.72
TQ	1.994	4.003	10.191	19.951	39.158	100.67

NDBA comparison TQ to IT



	2	4	10	20	40	100
IT	1.441	4.034	9.547	22.227	38.798	100.09
TQ	1.971	4.089	10.288	19.876	38.154	101.55

NMOR comparison TQ to IT

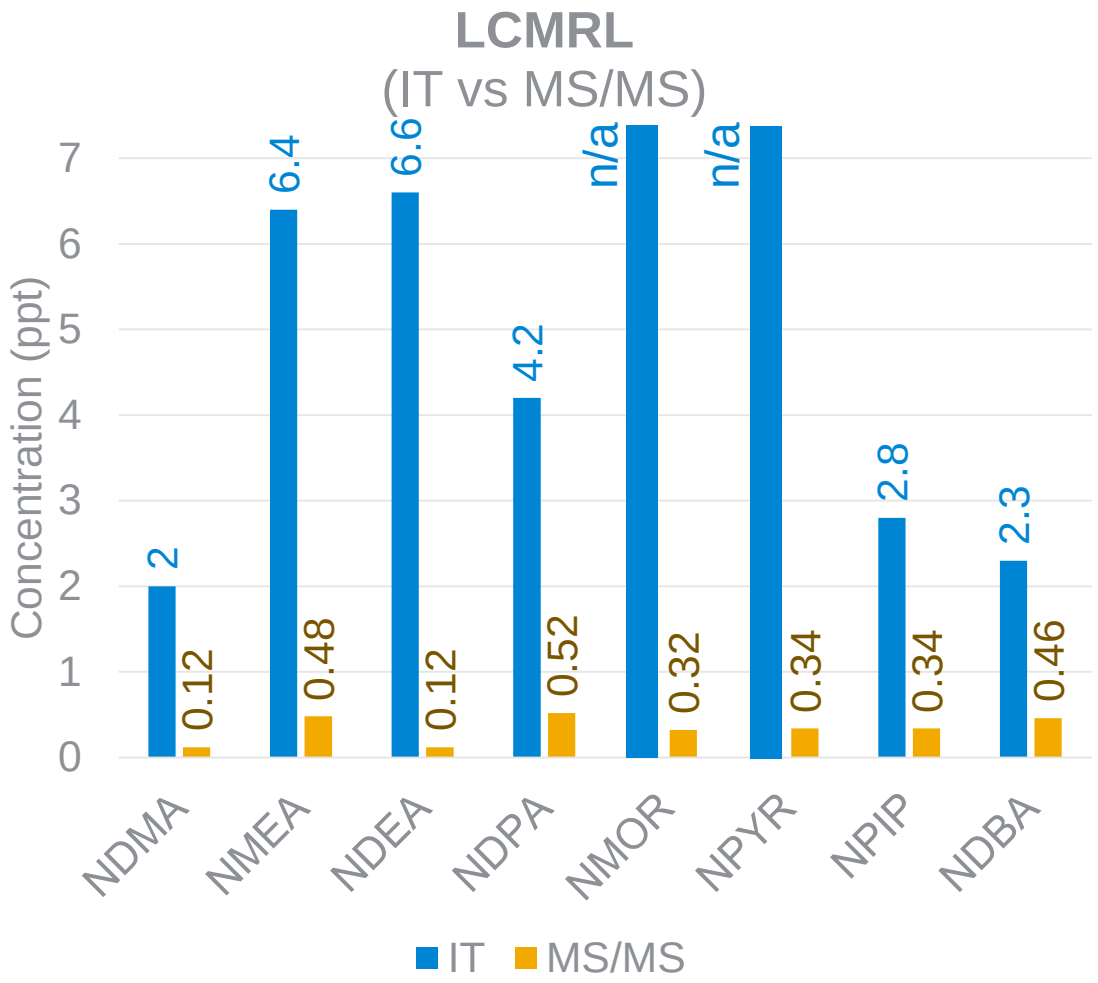


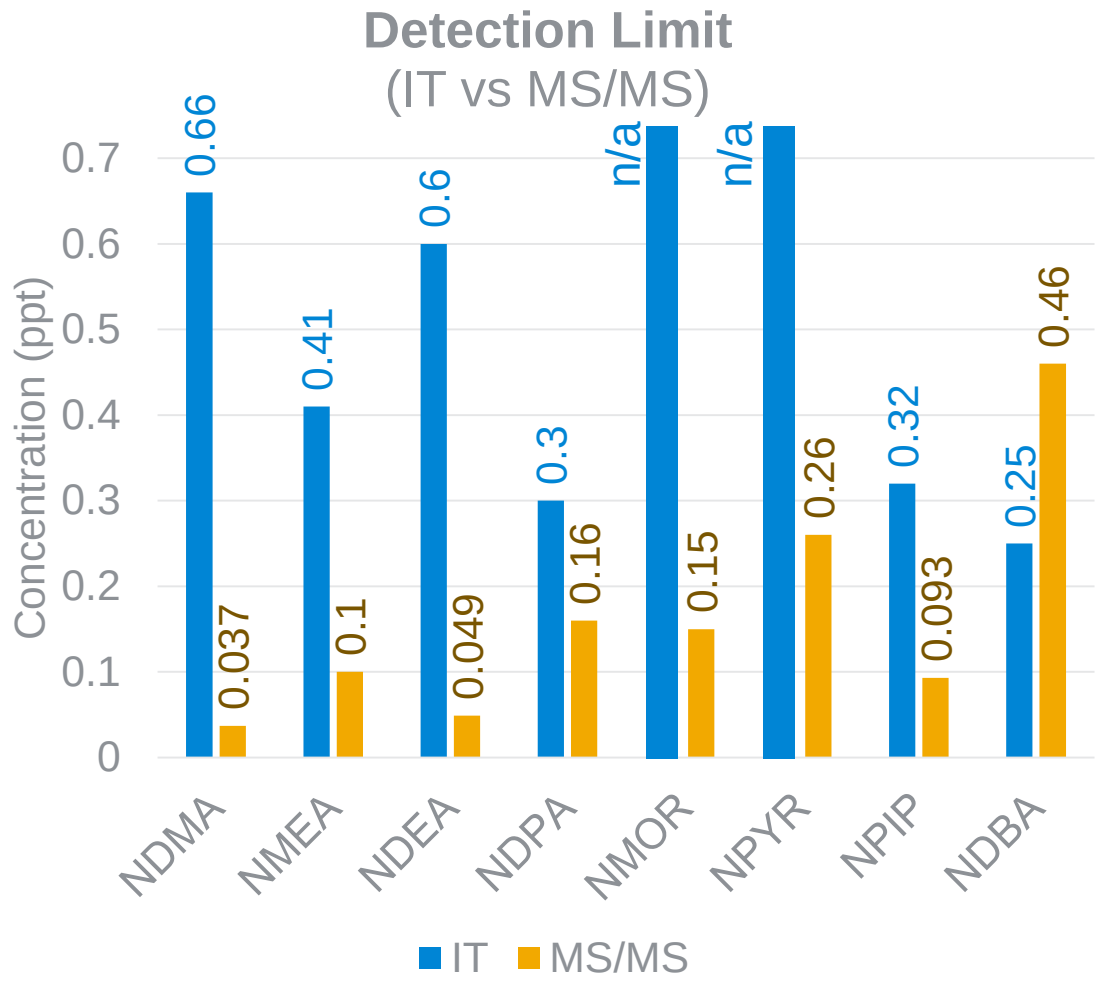
	2	4	10	20	40	100
IT	1.988	4.088	9.416	21.308	38.998	100.19
TQ	1.997	3.992	10.158	20.124	38.971	100.74

Initial calibrations points were plotted

LCMRL and DL of water extract (GC-MS/MS vs GC-IT)

GC-MS/MS achieves lower DL and LCMRL





Note: n/a LCMRL and DL on GC-IT is above the highest spiking level or spiking level exceeds working range for NMOR and NPYR. Spiking levels Range 0.1 to 10 ppt

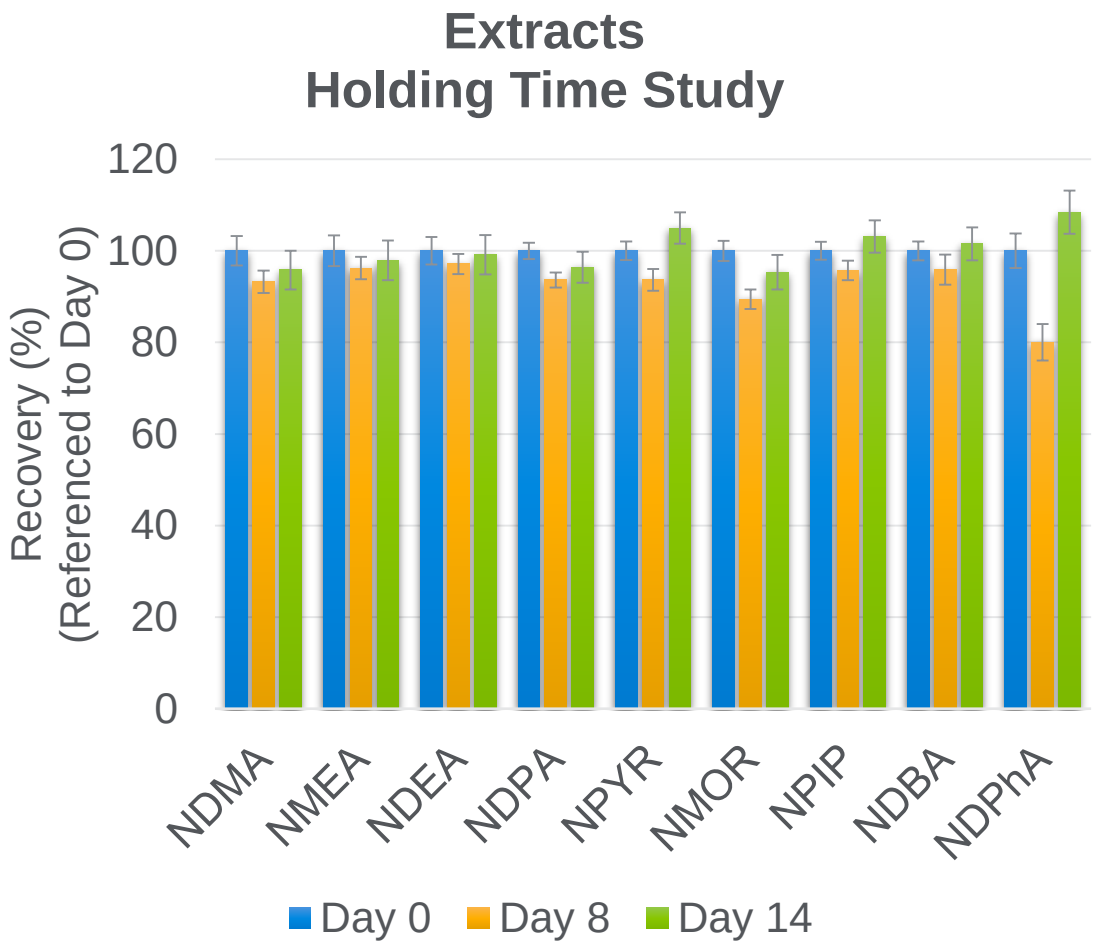
Phase II

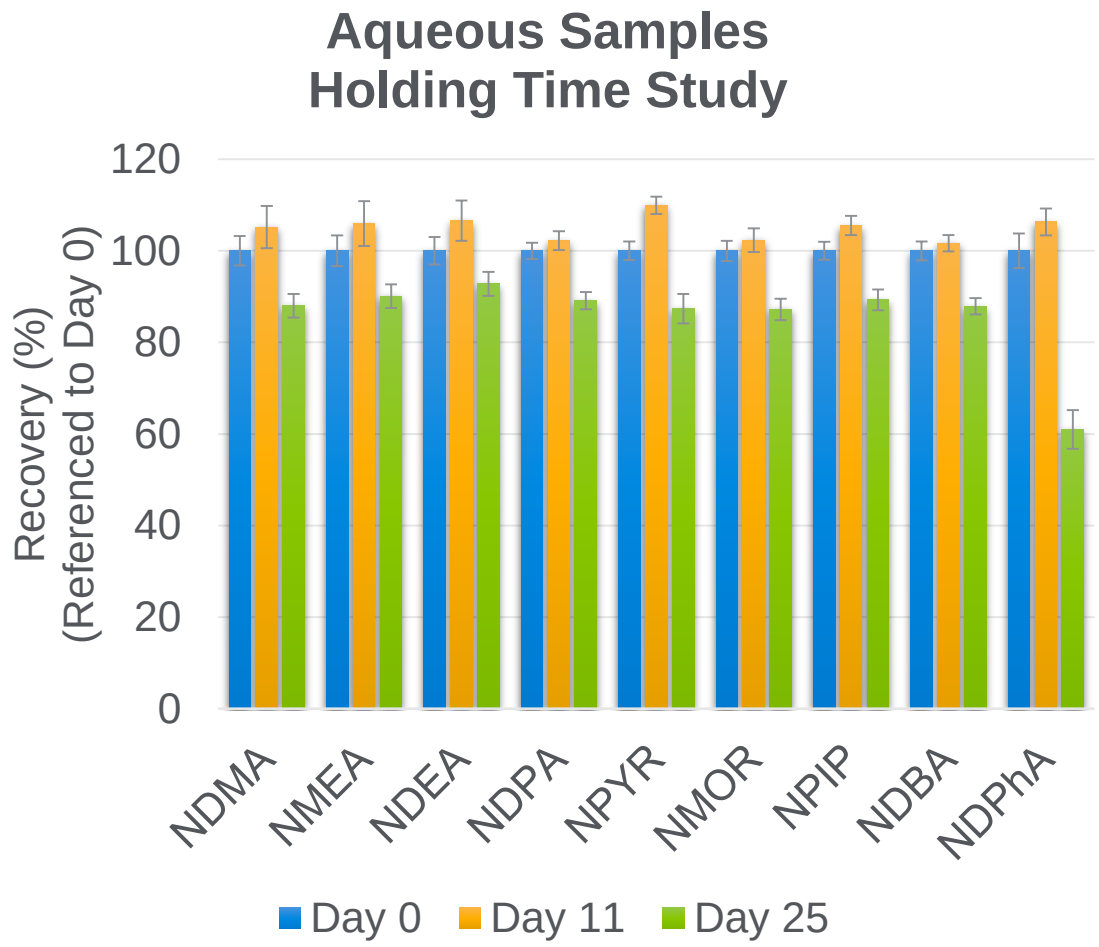
Interlaboratory Validation Study (ILS)

- LAB A
  - performs analyte stability study
  - performs precision and accuracy analysis in Fortified Water Sample Extracts
  - extracts drinking water
  - splits extract for analysis by LAB B and LAB C
- Results from interlaboratory study

Stability of Nitrosamines in Drinking Water Extracts (LAB A)

Most nitrosamines are stable. NDPhA should be analyzed within 11-14 days

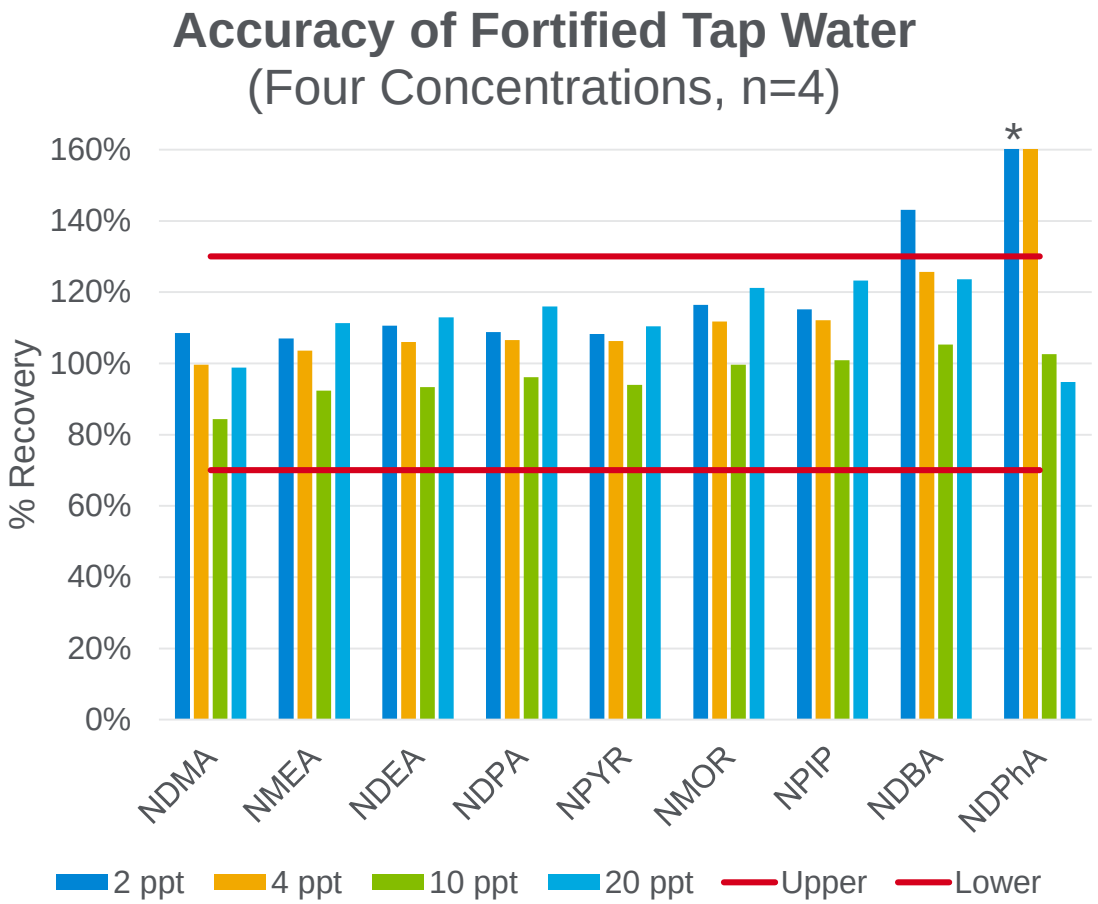


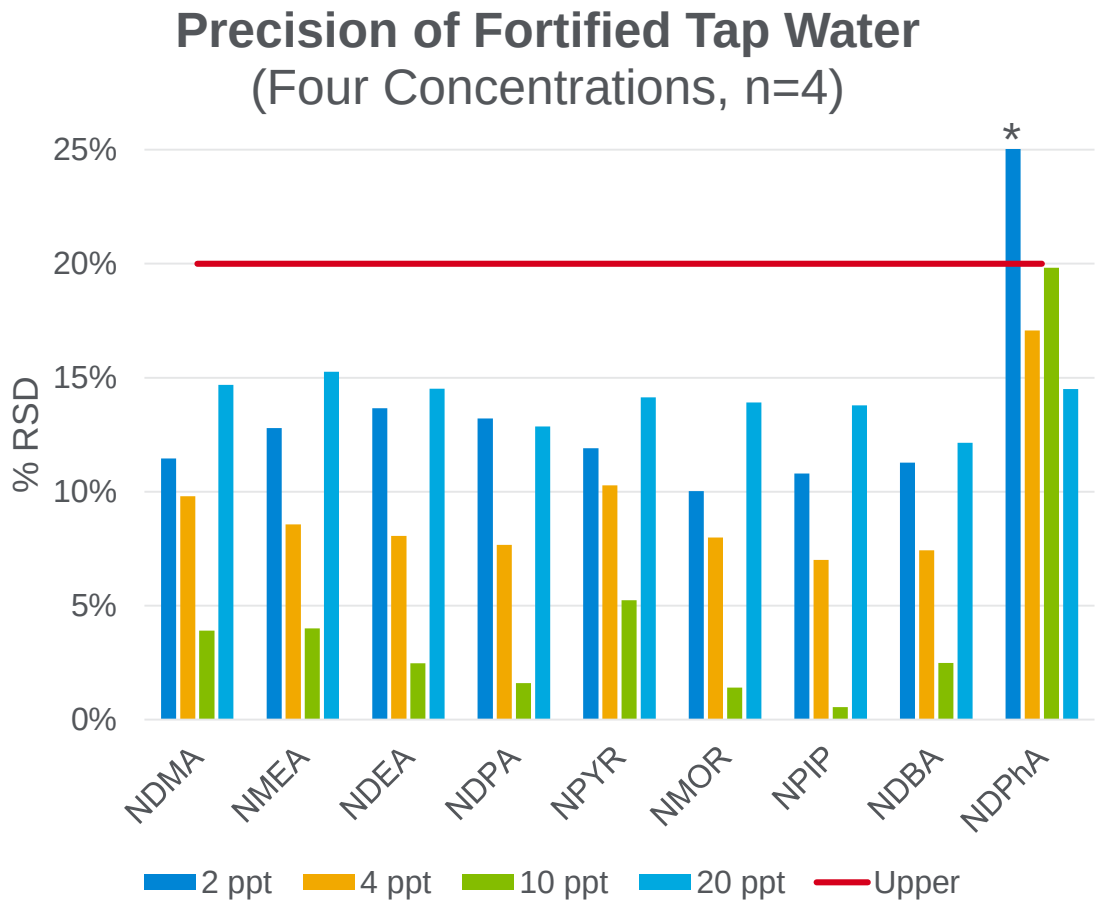


Error bars represent standard deviation in 7 replicates. Aqueous sample extracts are tap water fortified with 40 ng/L of nitrosamines.

Precision and Accuracy of Fortified Tap Water (LAB A)

Most nitrosamines are within limits for precision and accuracy.



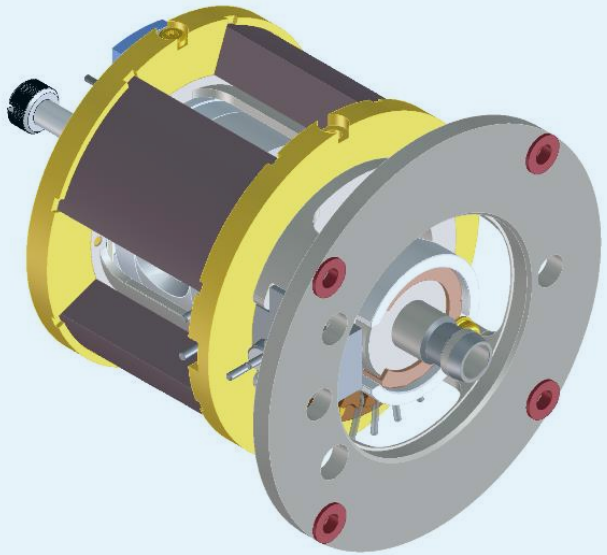


Sect. 9.2.2 Initial Demonstration of Precision (IDP) Analyze 4-7 replicate LFBs fortified at 10-20 ng/L, or other mid-range concentration. RSD must be ≤20% for all analytes.
Sect. 9.2.3 Initial Demonstration of Accuracy (IDA) Calculate average recovery for replicates used in IDP Mean recovery 70-130% of true value
*NDPhA contamination

GC-MS/MS used in Interlaboratory Validation Study

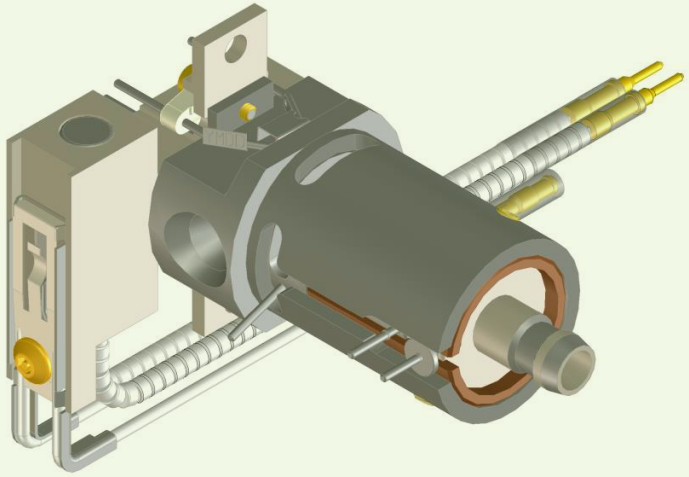
LAB A and LAB B

7010 GC-MS/MS
High Efficiency Source



LAB C

7000 GC-MS/MS
Extractor Source



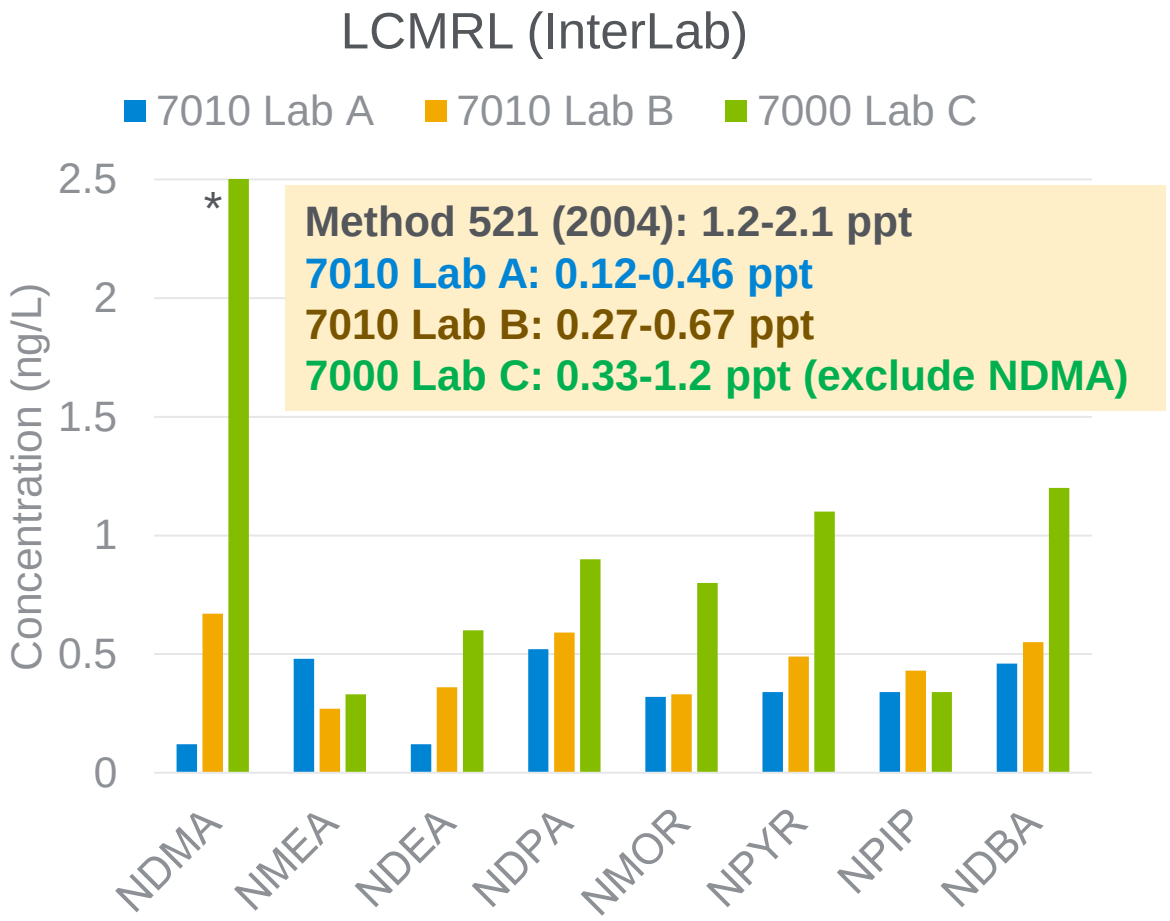
Complete Source Redesign
on the 7010 GC-MS/MS

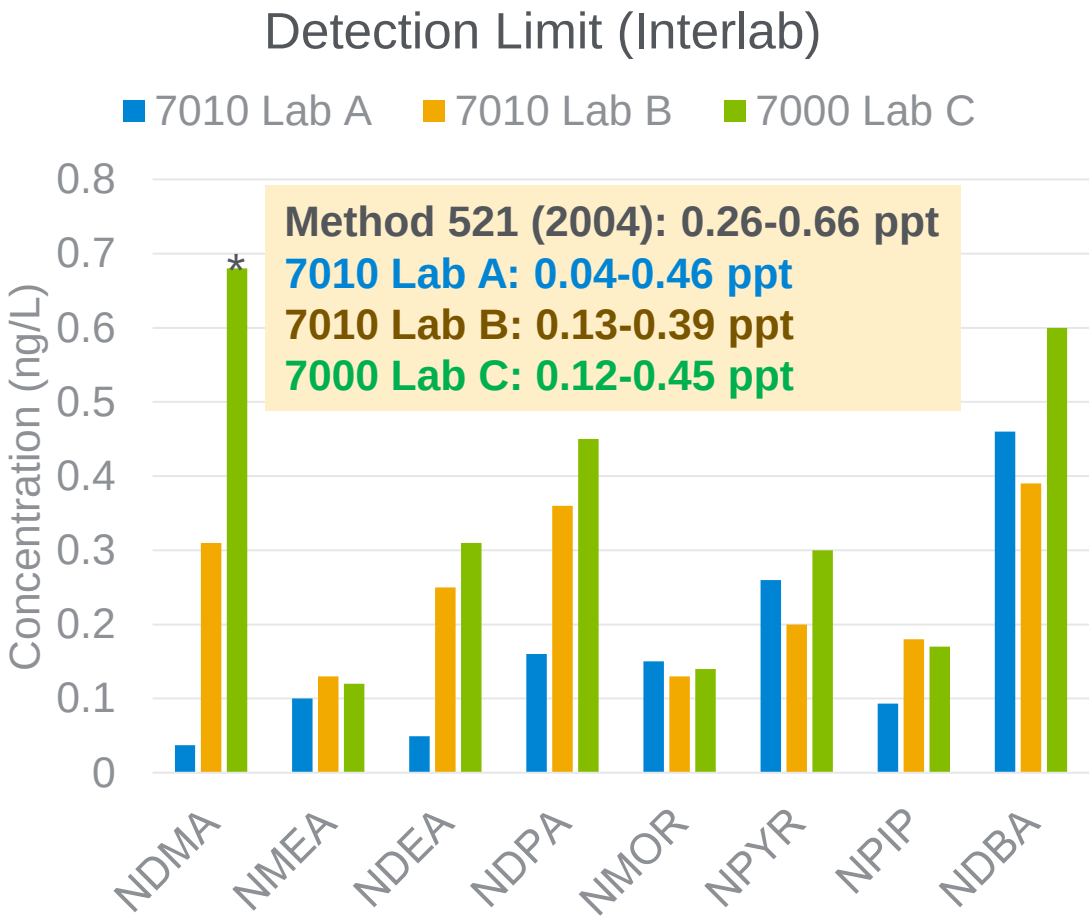
20x more ions

Is the
High Efficiency Source
required to meet the
LCMRL?

LCMRL Results from Interlaboratory Validation Study

Both GC-MS/MS systems achieved lower LCMRL and DL than Method 521 (2004)





Four replicates at 0.1, 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 5.0, 8.0, and 10.0 ppt

*Lab C has on outlier in at 3 and 4 ppt

Calibration Curve of ILS

$R^2 \geq 0.99$ for both 7000 and 7010 GC-MS/MS

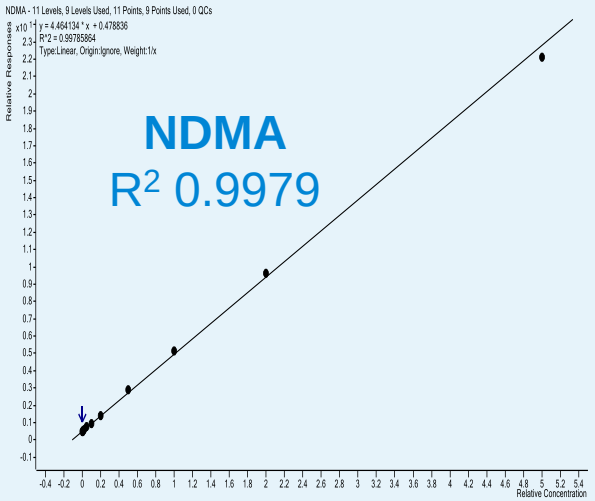
Analyte	7010 Lab A	7010 Lab B	7000 Lab C
NDMA	0.9999	0.9979	0.9935
NMEA	0.9999	0.9983	0.9988
NDEA	0.9999	0.9993	0.9986
NDPA	0.9998	0.9987	0.9965
NMOR	1.0000	0.9993	0.9992
NPYR	0.9981	0.9994	0.9976
NPIP	0.9999	0.9993	0.9979
NDBA	0.9996	0.9990	0.9985
NDPhA	0.9992	0.9985	0.9979

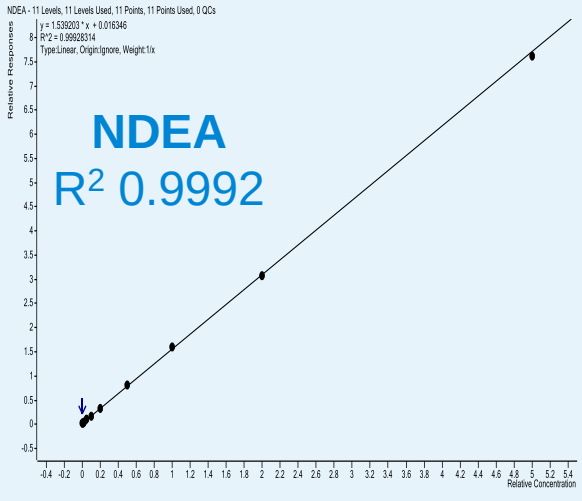
Linear, 1/x weight, 11 calibration points (0.0625,0.125,0.25,0.5,1.0,2.0,4.0,10,20,40,100 ppt)

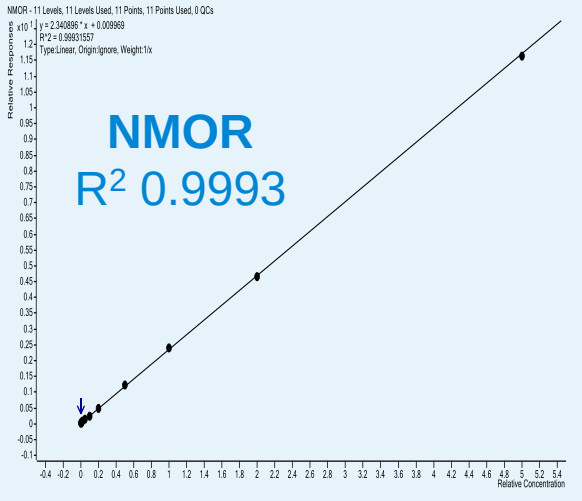
Calibration Curves using GC-QQQ

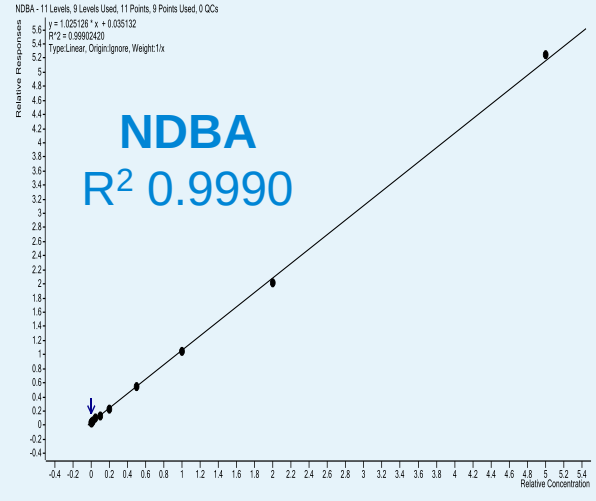
$R^2 \geq 0.999$ for both 7010 and 7000 Triple Quad GC/MS System

7010 GC-MS/MS

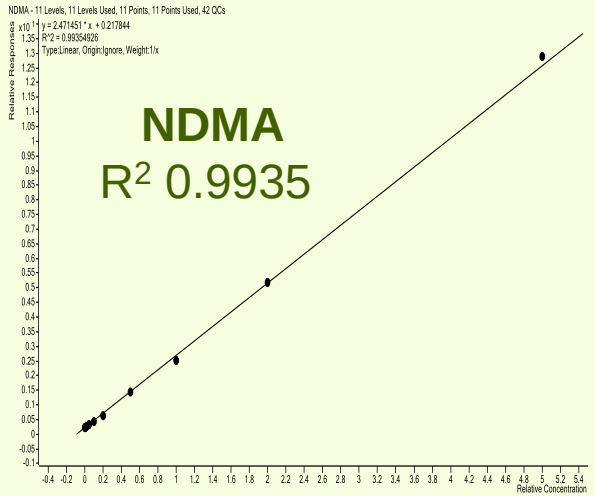


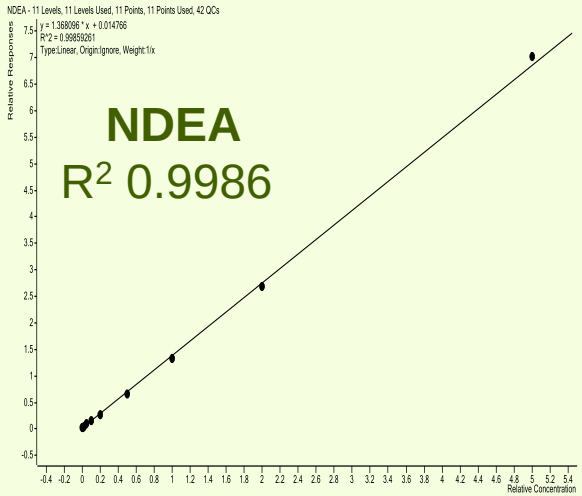


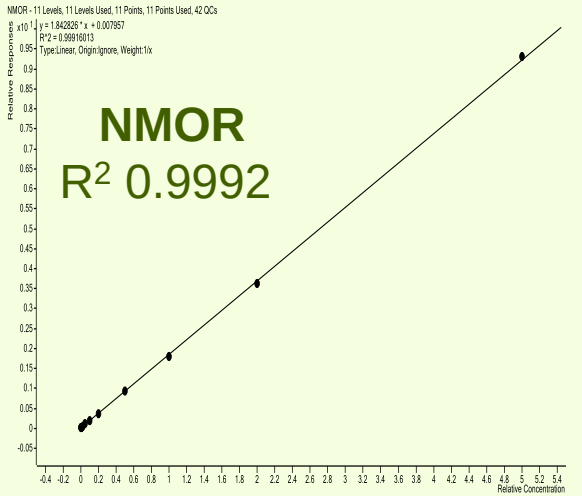


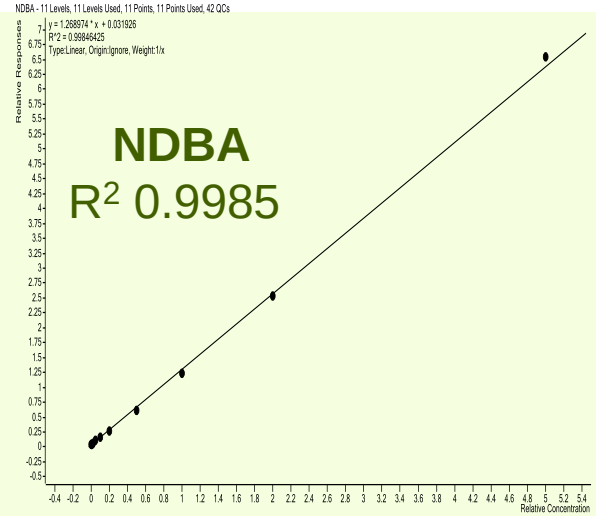


7000 GC-MS/MS









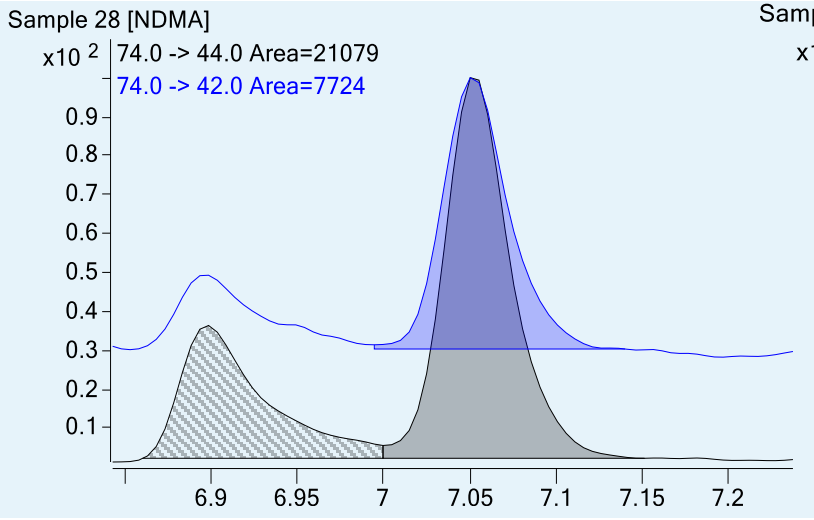
Linear, 1/x weight, 11 calibration points (0.0625,0.125,0.25,0.5,1.0,2.0,4.0,10,20,40,100 ppt)

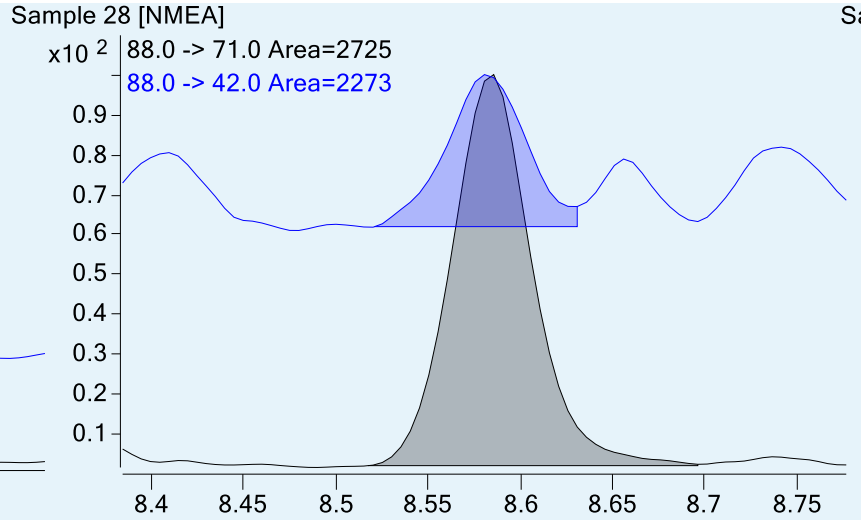
Peak Shape at 0.5 ppt Nitrosamines in Water Sample Extracts

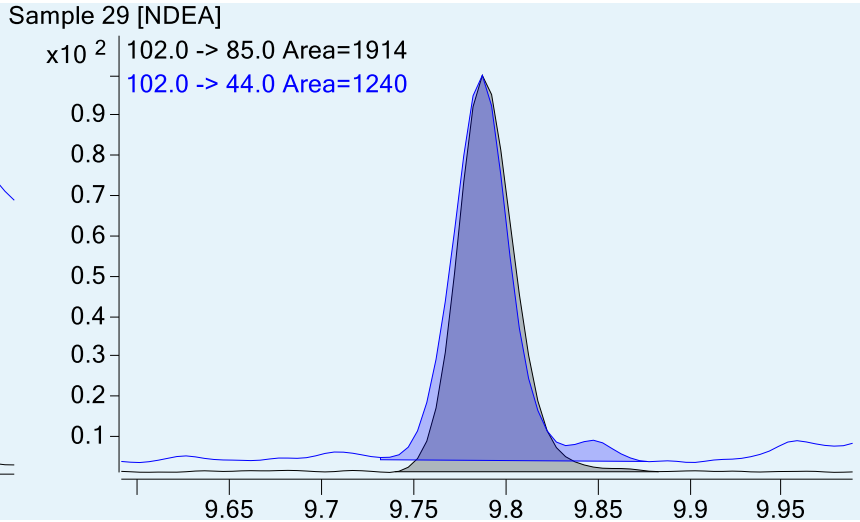
Both systems can detect low levels of nitrosamines. Peak areas are greater on the 7010 GC-MS/MS.

NDMA | **NMEA** | **NDEA**

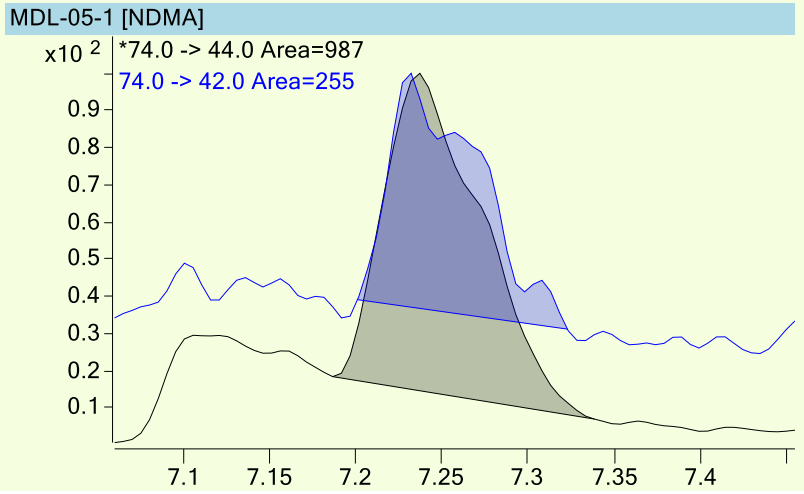
7010 GC-MS/MS

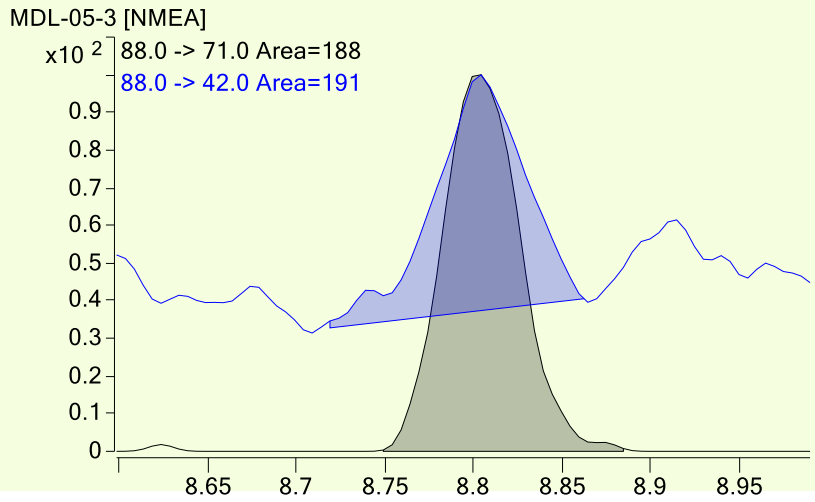


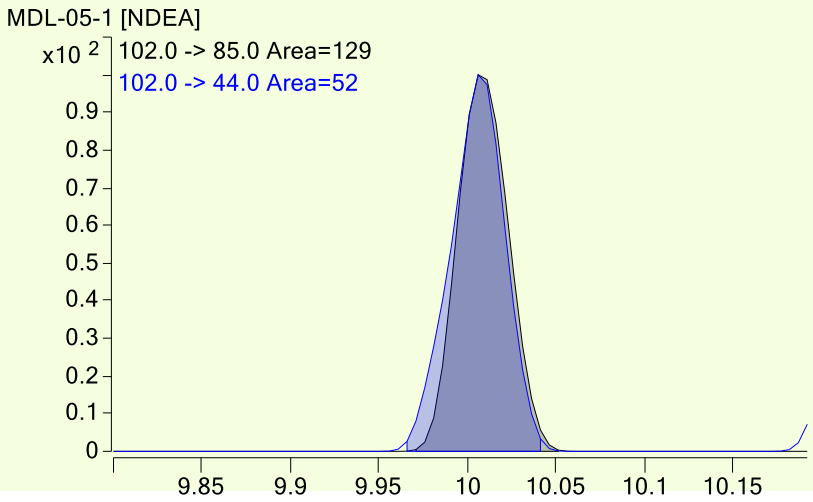




7000 GC-MS/MS





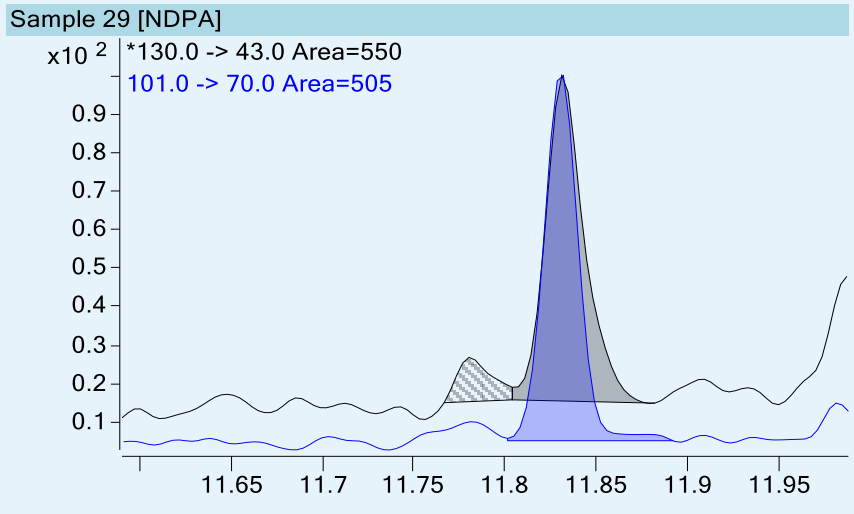


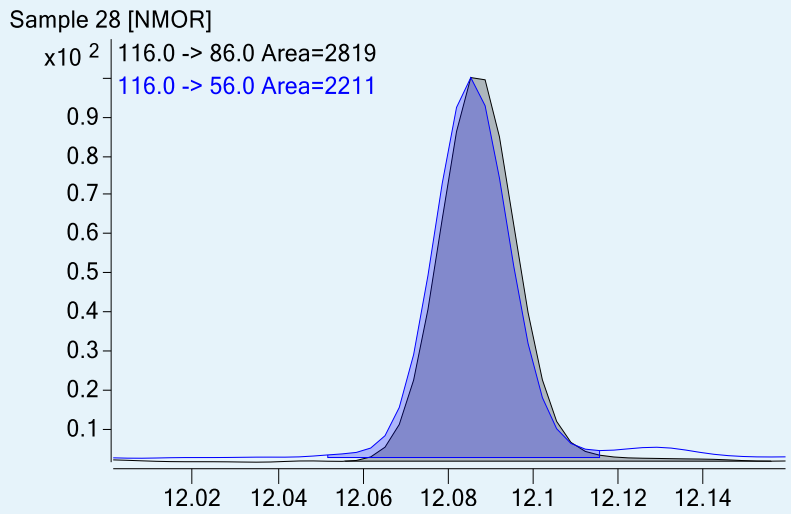
Peak Shape of 0.5 ppt Nitrosamine in Extract

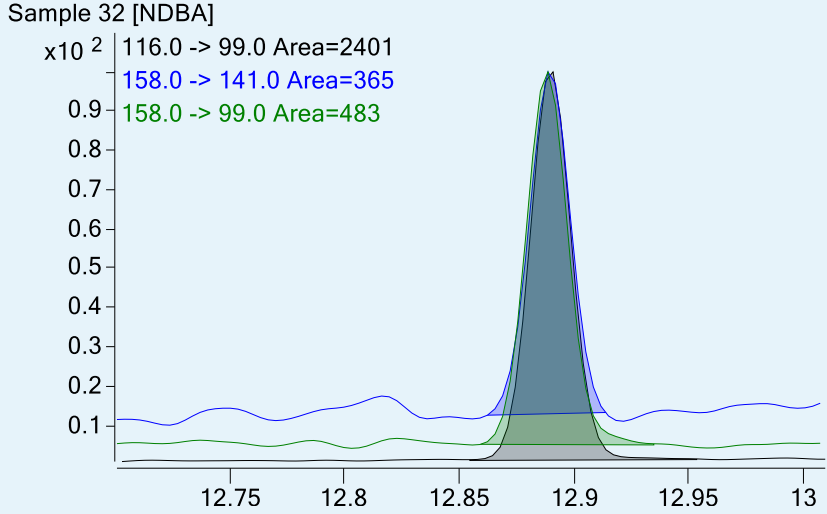
Both systems can detect low levels of nitrosamines. Peak areas are greater on the 7010 GC-MS/MS.

NDPA | **NMOR** | **NDBA**

7010 GC-MS/MS







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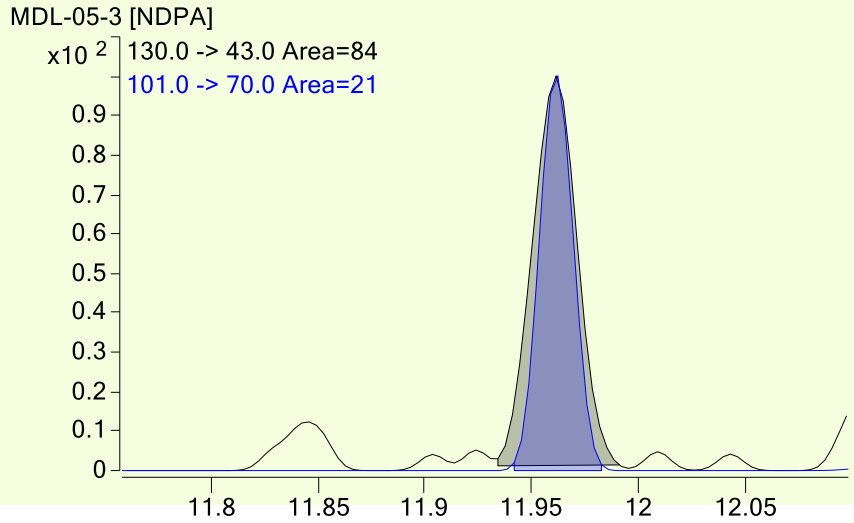
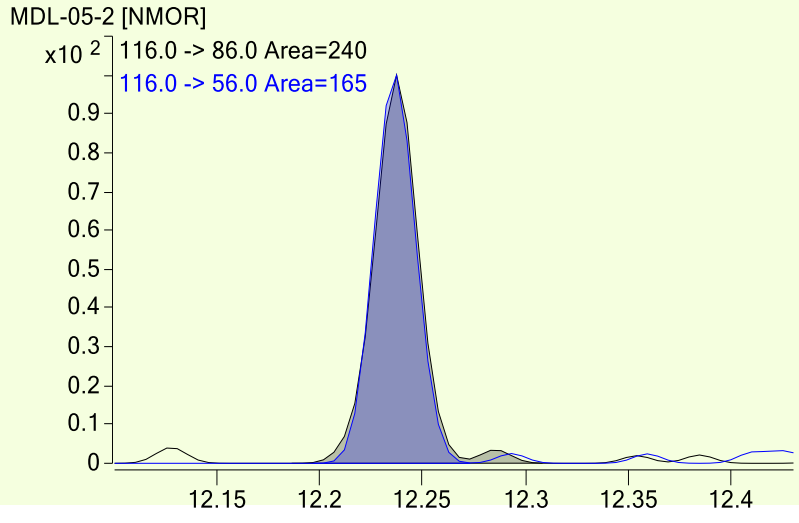
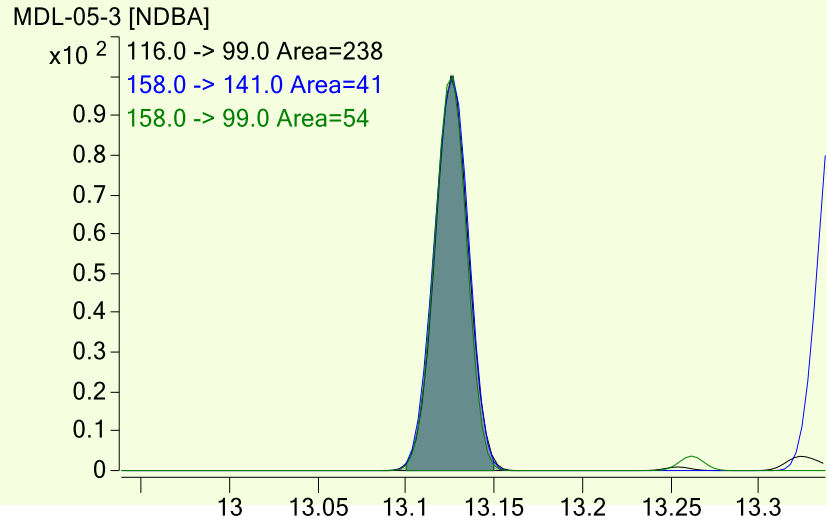
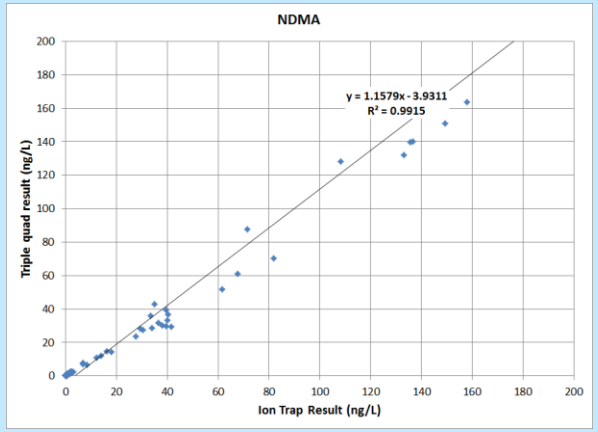


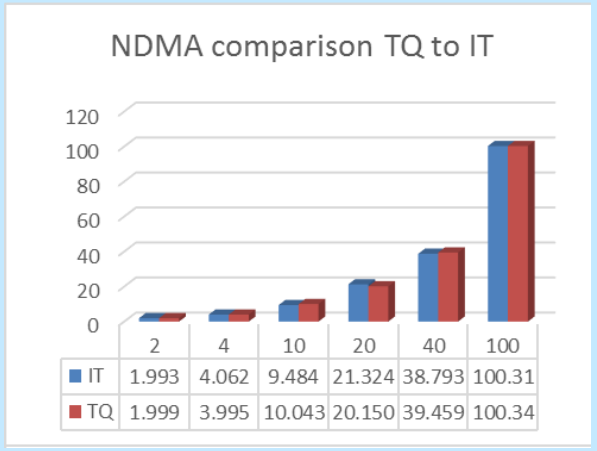




Phase I Summary – GC-MS/MS Advantages

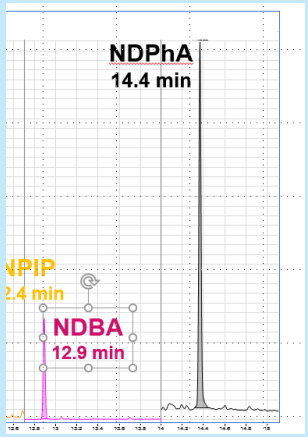
Good Correlation



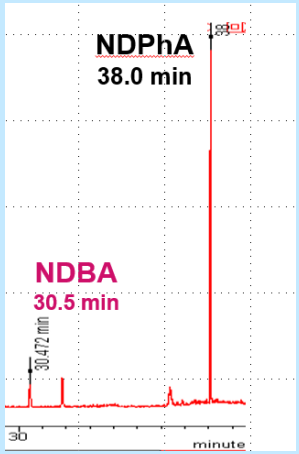


Shorter Run Time

GC-MS/MS

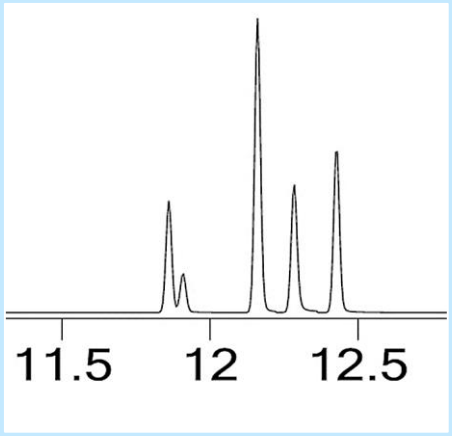


GC-IT

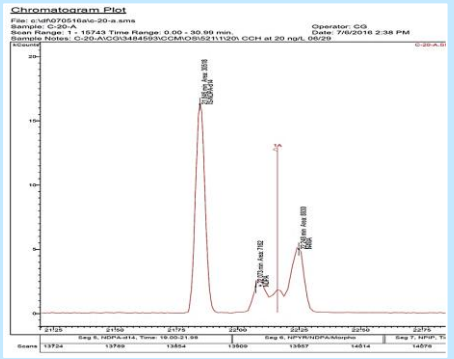


Better Separation (NDPA, NPYR, NMOR)

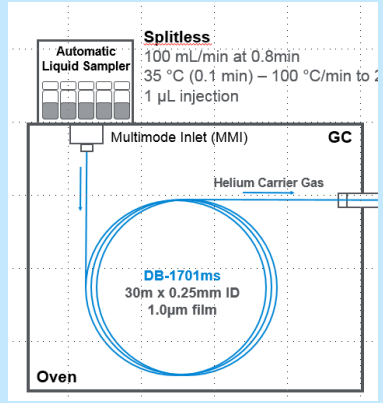
GC-MS/MS



GC-IT



Lower injection volume



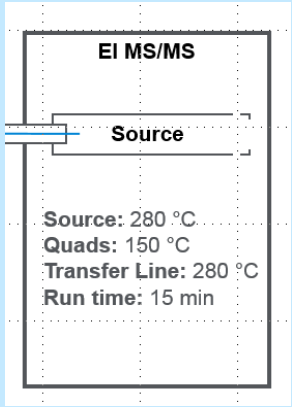
1 µL (GC-MS/MS)
vs
10-20 µL (GC-IT)

10-20X
Lower Volume

EI vs CI mode

Easier Operation

Increase Reliability

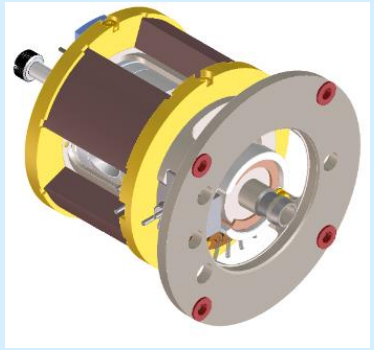


Phase II Summary – Interlaboratory Validation

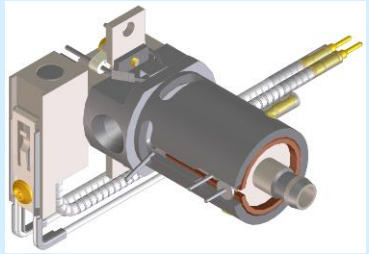
Method Compliance

Both Systems Work!

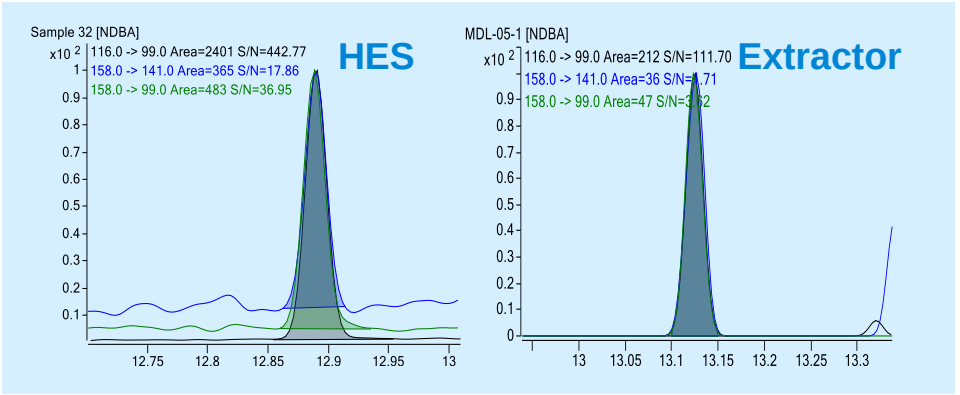
7010 GC-MS/MS
High Efficiency Source



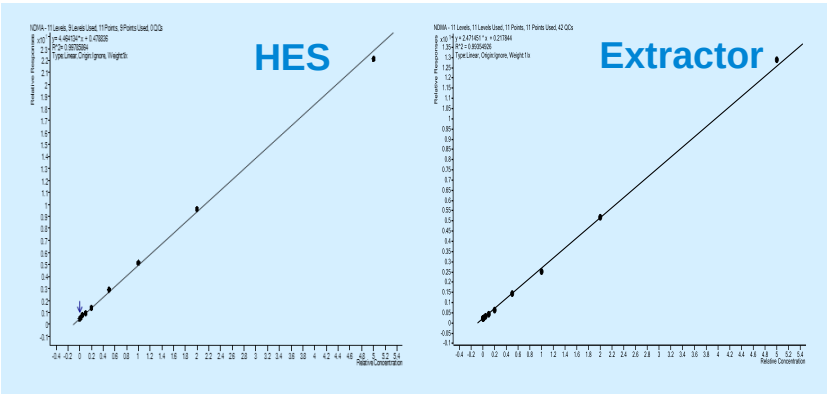
7000 GC-MS/MS
Extractor Source



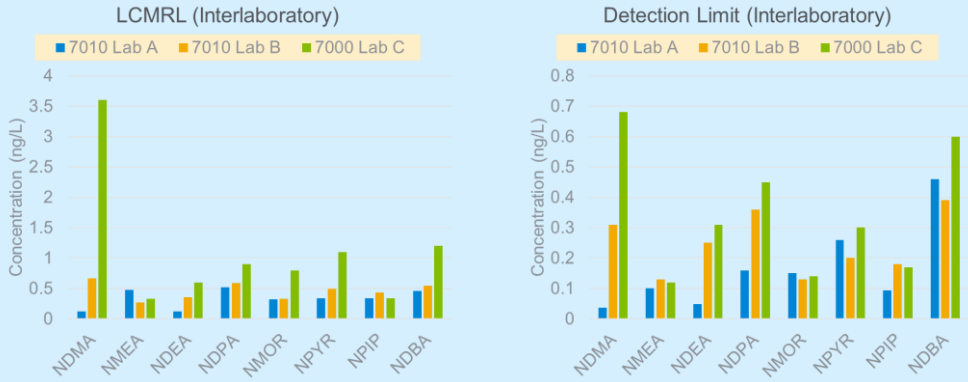
Baseline Separation and Sensitivity



$R^2 > 0.99$



LCMRL and Detection Levels



LCMRL
Method 521 (2004): 1.2-2.1ppt
Lab A: 0.12-0.46 ppt
Lab B: 0.27-0.67 ppt
Lab C: 0.33-1.2 ppt (exclude NDMA)

Detection Level
Method 521 (2004): 0.26-0.66 ppt
Lab A: 0.04-0.46 ppt
Lab B: 0.13-0.39 ppt
Lab C: 0.12-0.45 ppt

Current Status

- Phase I is Reviewed and Accepted by the EPA Office of Drinking Water
- Preparation of Alternate Test Procedure (ATP) update for Method 521
- Recommending new version of EPA Method 521.1 based on GC-MS/MS
- EPA deployment upon approval

Acknowledgements – Interlaboratory Validation Study

Eurofins Eaton Analytical
Monrovia, California (USA)

Andy Eaton, PhD
Chuck Grady, PhD
Konjit Tadigo

Eurofins Eaton Analytical
South Bend, Indiana (USA)

Bruce Li, PhD
Bill Davis

Vogon Labs
Cochrane, Alberta (Canada)

Ralph Hindle, PhD
Kathy Hunt

Acknowledgement

Agilent Technologies

Craig Marvin

Ron Honnold, PhD

Bruce Quimby, PhD

Tom Doherty, PhD

Paul Salverada

Jessica Lehman

Dave Speltz

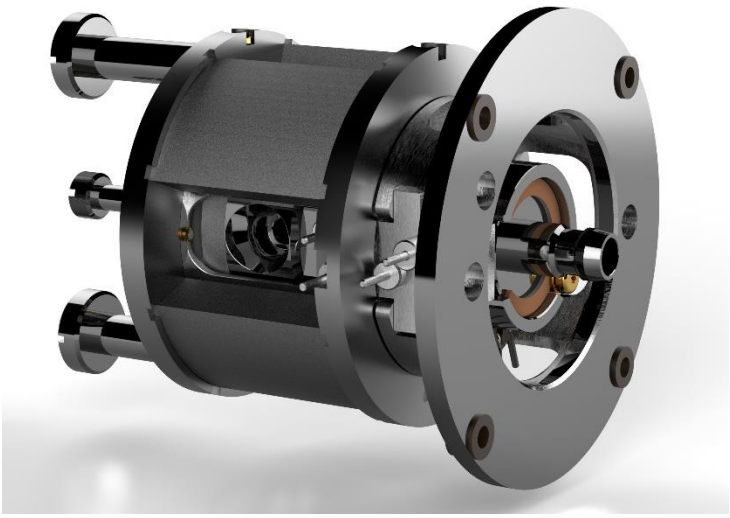
Fred Pisarski

Dave Warren

Tarun Anumol, PhD

7890 Gas Chromatograph
7010 Triple Quadrupole
Mass Spectrometer

High Efficiency Source (HES)



Thank you NEMC!

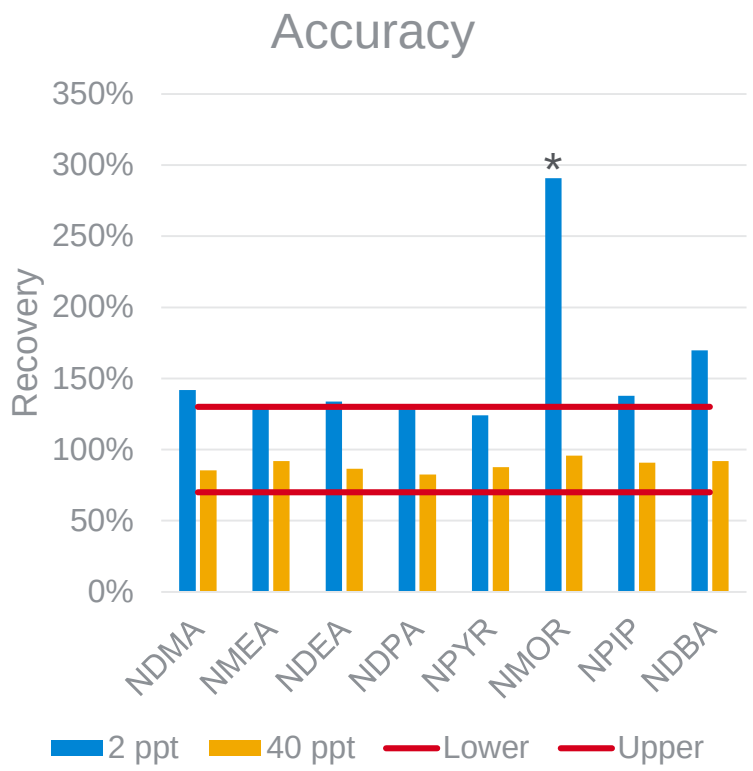
Questions

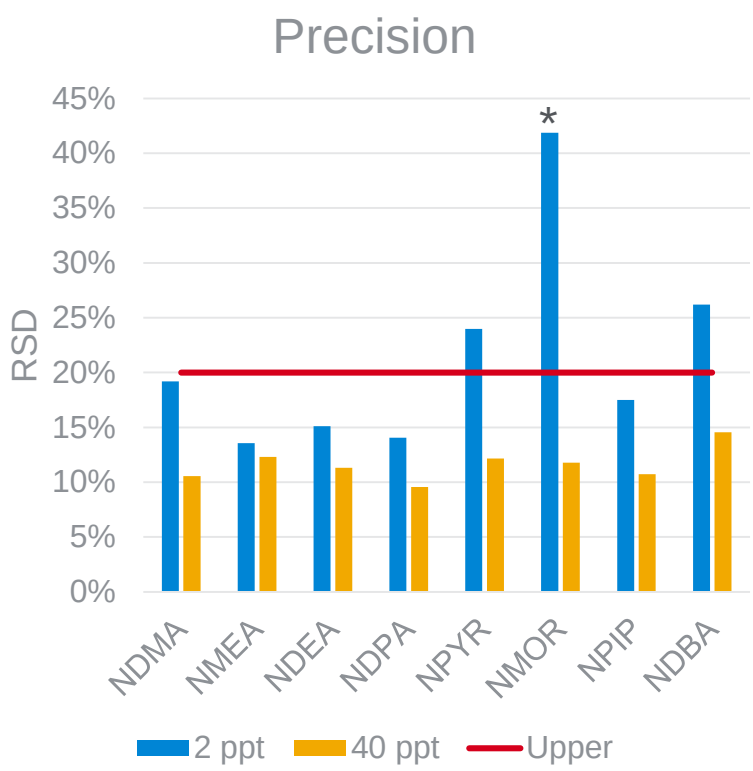
Diana Wong, PhD
GC/MS Market Development Scientist
diana.wong@agilent.com

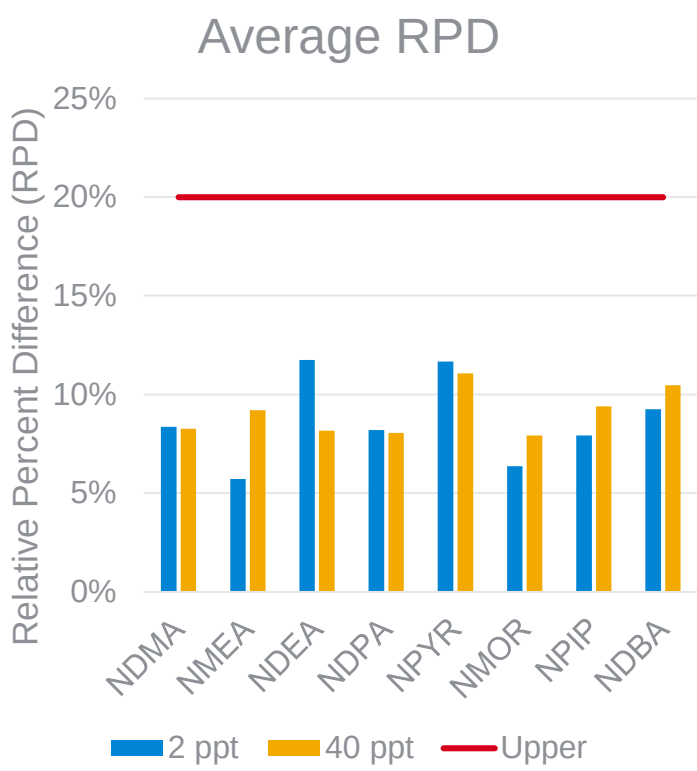
APPENDIX

Precision and Accuracy of Various Drinking Water and Recycled Water Samples (LAB A)

Most nitrosamines are within limits. NMOR is occasionally found in trace amounts.







Various recycled water and drinking water were fortified at 2 and 40ppt (24-26 samples per concentration total).
*NMOR is occasionally found in trace amount used in matrix spike and matrix spike duplicate

Varian 4000 GC/MS Ion Trap System Parameters

EPA Method 521

Inlet Parameters
Large Volume Injection
20 µL injection

Temperature Program
37 °C (0.72 min)
100 °C/min to 250 °C (2.13 min)
250 °C (40 min)

Oven program:
35 °C (4 min)
4 °C/min to 130 °C (2 min)
40 °C/min to 280 °C (0.5 min)