Analysis of nitrosamines in APIs

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Analysis of Nitrosamines in APIs

01
- Introduction to nitrosamines

02
- Recent timeline

03
- Development of assay

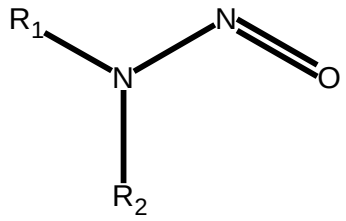
04
- Accuracy, precision and linearity

05
- Ion suppression

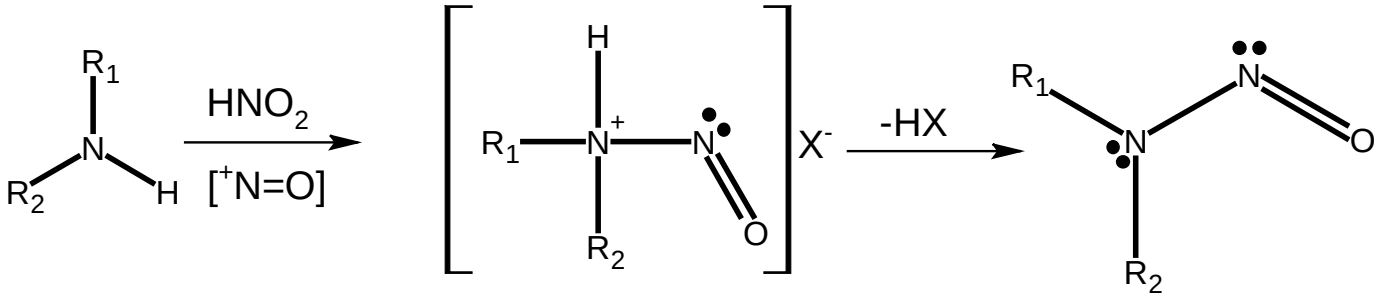
06
- Conclusions

Nitrosamines

- Compounds having the chemical structure of a nitroso group bonded to an amine.

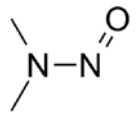


- Formed by a nitrosating reaction between amines (secondary, tertiary, or quaternary amines) and nitrous acid (nitrite salts under acidic conditions).

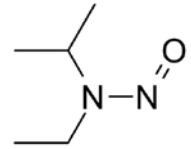


Why are we interested?

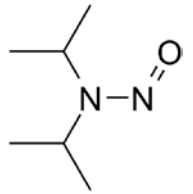
- Nitrosamines are known mutagenic agents
- Classified as probable human mutagens/carcinogens
- May be formed in manufacture/processing of:
  - Cosmetics
  - Rubber
  - Meat
  - Beer
  - Consumer goods
  - Rivers
  - Sewerage plants
  - Pharmaceuticals
- Regulatory authorities (ICH, FDA, EMA) have identified several nitrosamine impurities that theoretically could be present in drug products



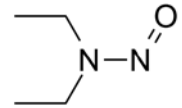
NDMA
N-nitrosodimethylamine



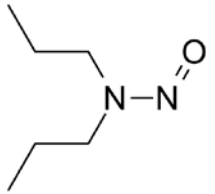
NEIPA
N-nitrosoethylisopropylamine



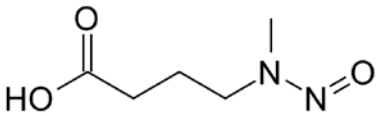
NDIPA
N-nitrosodiisopropylamine



NDEA
N-nitrosodiethylamine

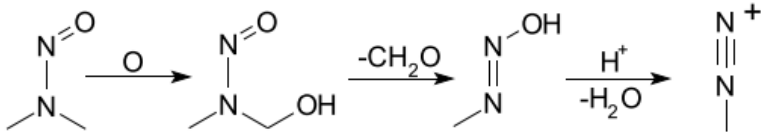


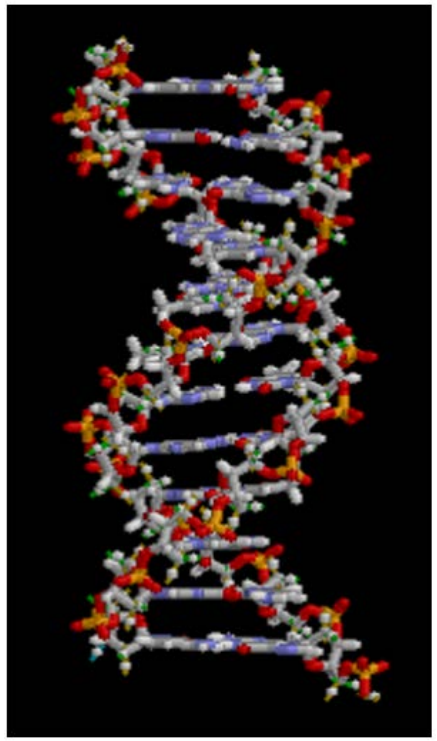
NDPA
N-nitroso-di-n-propylamine



NMBA
N -nitroso-N-methyl-4-aminobutyric acid

Why are we interested?





with kind permission from brian0918&#153

- Nitrosamines are mutagenic
  - Can potentially cause cancer in lung, brain, liver, kidney, bladder, stomach, oesophagus, and nasal sinus.
- N-Nitrosamines are activated metabolically to form diazonium ions
  - These are precursors of reactive carbenium ions that form stable adducts with DNA
  - Specific alkylating agents vary with the nitrosamine

History

- 1950s — John Barnes and Peter Magee, reported that dimethylnitrosamine produced liver tumours in rats.

  Subsequent studies showed that approximately 90% of the 300 nitrosamines tested were carcinogenic in a wide variety of animals[1].

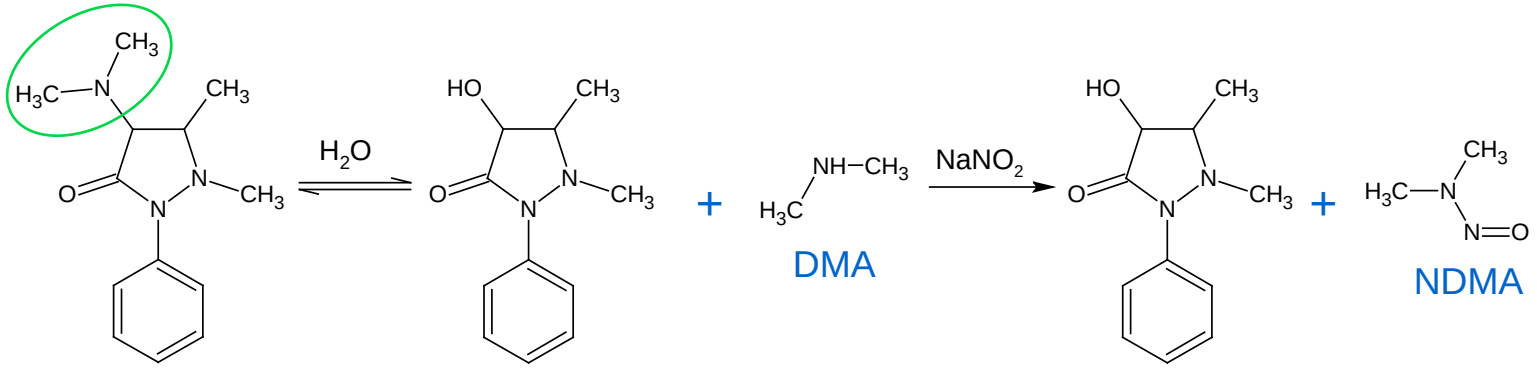
- 1960s — Concerns raised over the excessive use of nitrite in food preservation resulting in NDMA formation in bacon and other meat products

- 1970s — Researchers discovered a link between animal diet of fishmeal and formation of cancer in farm animals.

  Fishmeal had been preserved with sodium nitrite, which led to the formation of NDMA.

- 1977 — First recording of nitrosamines in a drug product

  Aminophenazone preparations recommended to be withdrawn by German Health Authority



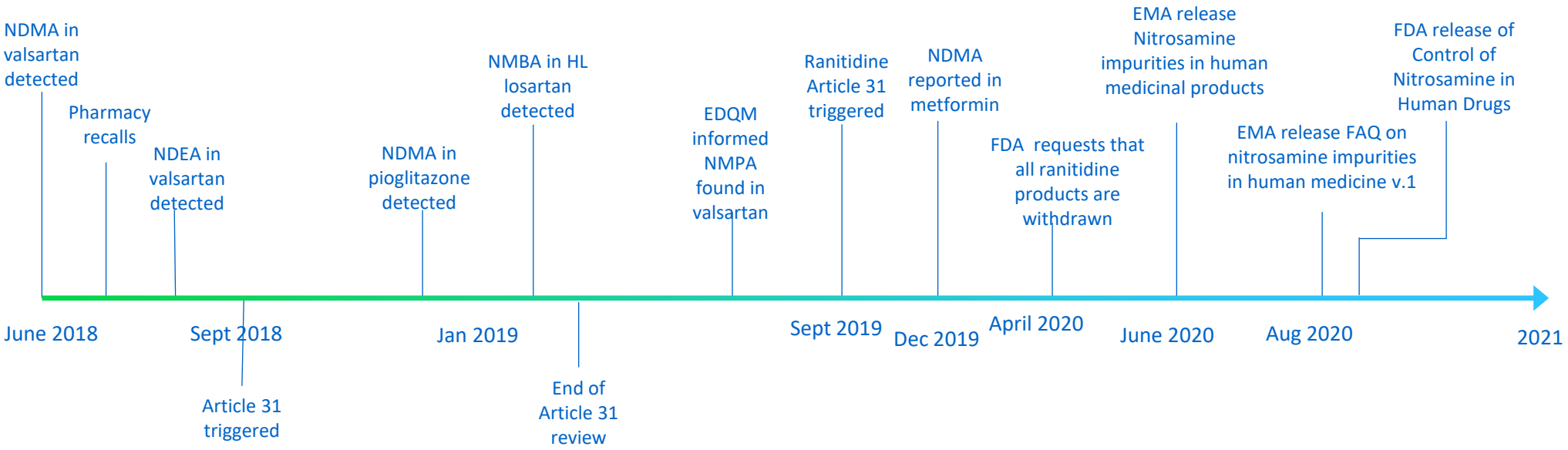
[1]J. Barnes and P. Magee, *Brit. J. Ind. Med.* **11**(3), 167-174 (1954)

History

- 1980's — It was noted that nitrosamines can be formed from disinfectants, which results in environmental pollution
  - Chlorination of nitrite is suspected to be cause
  - Later studies suggested that UV disinfection does remove nitrosamines
- 2007 — Up to 173 APIs noted as forming nitrosamines upon reaction with nitrite *in vivo*[1]
- 2018 — Detection of NDMA in valsartan

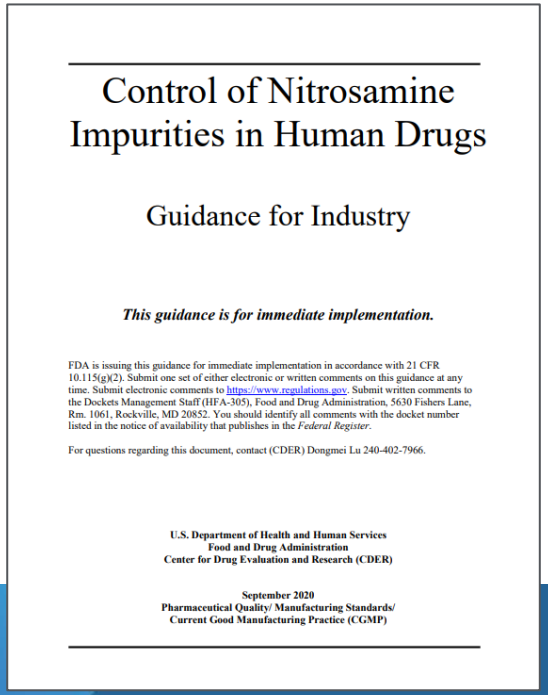
[1] G. Brambilla, A. Martelli, *Mutation Res.* **635** (2007) 17-52

Timeline of recent activity



Regulatory aspects

- FDA guidance – risk assessment strategy for detection and prevention of nitrosamine formation by API/drug product manufacturers
  - Assess processes for various sources –
    - Amine reagents/intermediates + nitrite salts in acidic conditions
    - Use of nitrous acid to quench residual azide in tetrazole ring/azide functional group formation
    - Degradation of amide solvents (e.g. DMF etc.) to form amines
    - Vendor sourced (nitrite impurities, solvent impurities, raw materials, excipients)
    - Recovered materials (e.g. catalysts, solvents)
    - Potable water – nitrite/nitrosamine contamination possible
  - Conduct confirmatory testing when risk identified
  - Consider refining/changing processes to mitigate risk
  - Control and monitor to ensure nitrosamines remain below acceptable limits



Control of Nitrosamine Impurities in Human Drugs

Guidance for Industry

This guidance is for immediate implementation.

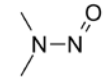
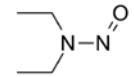
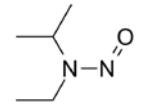
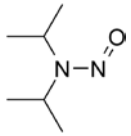
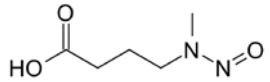
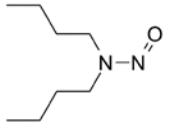
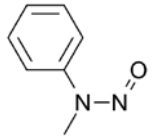
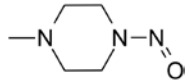
FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(2). Submit one set of either electronic or written comments on this guidance at any time. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this document, contact (CDER) Dongmei Lu 240-402-7966.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

September 2020
Pharmaceutical Quality/ Manufacturing Standards/
Current Good Manufacturing Practice (CGMP)

Potential nitrosamines in drug product/API

	FDA	EMA
NDMA 	✓	✓
NDEA 	✓	✓
NEIPA 	✓	✓
NDIPA 	✓	✓
NMBA 	✓	✓
NDBA 	✓	✓
NMPA 	✓	✓
MeNP 	-	✓

Acceptable intake limits

- FDA recommends the following acceptable intake (AI) 31 limits for the nitrosamine impurities NDMA, NDEA, NMBA, NMPA, NIPEA, and NDIPA
- Daily AI limit approximates 1:100,000 cancer risk after 70 yrs exposure (ICH M7(R1)

N-Nitrosamine	FDA limit	EMA limit
	ng/day	ng/day
NDMA	96.0	96.0
NDEA	26.5	26.5
NEIPA	26.5	26.5
NDIPA	26.5	26.5
NMBA	96.0	96.0
MeNP	N/A	26.5
NDBA	26.5	26.5
NMPA	26.5	34.3

- Acceptable nitrosamine content = AI/MDD

For an AI of 96 ng/day:

50 mg MDD → 1920 ng/g

1000 mg MDD → 96 ng/g

Need for high sensitivity driven by MMD/AI approach

- High MDD APIs require lower analytical detection limits

Drug	Maximum daily dose (mg/day)	Acceptable intake NDMA (ng/day)	Acceptable intake NDMA (ppm)	Acceptable intake NDEA (ng/day)	Acceptable intake NDEA (ppm)	Acceptable intake NMBA (ng/day)	Acceptable intake NMBA (ppm)
Candesartan	32	96	3.0	26.5	0.83	96	3.0
Olmesartan	40	96	2.4	26.5	0.66	96	2.4
Azilsartan	80	96	1.2	26.5	0.33	96	1.2
Telmisartan	80	96	1.2	26.5	0.33	96	1.2
Losartan	100	96	0.96	26.5	0.27	96	0.96
Irbesartan	300	96	0.32	26.5	0.088	96	0.32
Valsartan	320	96	0.3	26.5	0.083	96	0.3
Eprosartan	800	96	0.12	26.5	0.033	96	0.12

Increased analytical sensitivity required

- Note this is for presence of single nitrosamines
- Potential for multiple nirosamines requires low ppb sensitivity

Analytical approaches

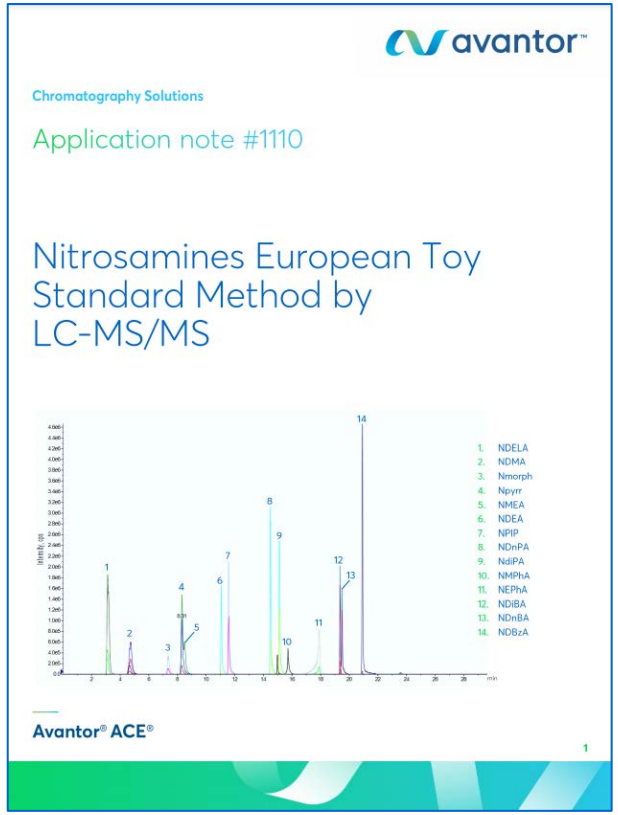
- Low AI limits dictate that methods require low LOD
- Typically use selectivity/sensitivity of mass spectrometry
- New USP general chapter under review (26/11/2020) includes 4 options for sartan drugs:

Method	Technique	Column	Analytes
1	LC-HRMS	L43 (propyl PFP)	NDMA, NMBA, NEIPA, NDIPA, NDBA
2	GC-MS	G16	NDMA, NDEA, NDIPA, NEIPA
3	LC-MS/MS	L1 (2.7 µm)	NDMA, NDEA, NDIPA, NEIPA, NMBA, NDBA
4	GC-MS/MS	G16	NDMA, NDEA, NDIPA, NEIPA, NDBA

Why Mass Spectrometry?

- Mass Spectrometers are expensive
  - Initial purchase, higher than a UV detector
  - Operational costs higher
  - Time to develop detector conditions

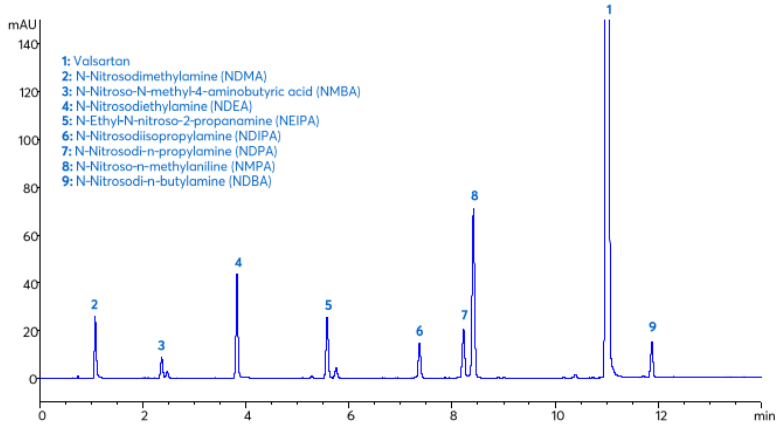
- Mass Spectrometers are
  - Very sensitive, detection limits typically 10 – 100 than UV detector
  - Very specific, the ability to tune into a unique mass / compound
  - Very fast analysis, potentially co-elution is not a problem due to specificity
  - Data collection rates can be limiting



Approach to method development

- Review physiochemical properties of nitrosamines
  - Log D, pK_a
- Develop chromatography based on a previous application note using LC-UV
- Tune MS for nitrosamines: 8 total (NDPA and NMPA also included) + 4 deuterated internal standards
  - Use Infusion & FIA
- Perform calibration according to USP method, determine LOD/LOQ, linearity
- Samples – valsartan drug substance (IS only, spiked at LOQ, and 0.3 ppm).

Nitrosamine Contaminants in Valsartan API



CONDITIONS

Column:	Avantor® ACE® UltraCore C18
Dimensions:	100 x 3.0 mm
Mobile Phases:	A: 20 mM KH_2PO_4 pH 2.7 in H_2O B: 20 mM KH_2PO_4 pH 2.7 in MeCN/H_2O 70:30 v/v

Gradient:

Time (mins)	%B
0	4
1	4
15	95
17	95
17.5	4
25	4

Flow Rate:	0.6 mL/min
Injection:	1 µL
Temperature:	20 °C
Detection:	UV, 254 nm
System Dwell Volume:	204 µL

Physicochemical Properties

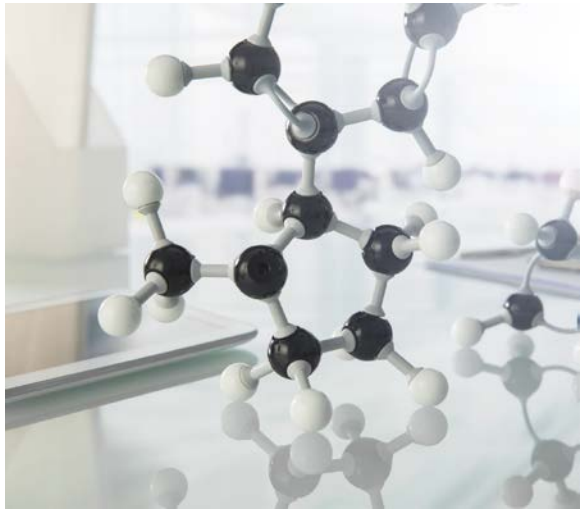
- Uses databases to try and predict physical and chemical properties
  - accuracy dependant on databases
  - databases can be trained by using real experimental data from similar compounds to give more accurate data
- Allows for the predication of log P, log D and pK_a
  - Log D & log D identifies hydrophobicity, allowing extraction and chromatographic conditions to be predicted
  - pK_a allows selection of correct pH for chromatography, extraction and MS ionisation

However will only be an estimate of real properties

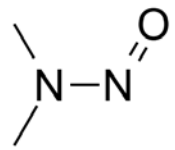
$$logP = \frac{[C_o]}{[C_w]}$$

$$logD = \frac{[C_o]}{[C_w].[C_w^+]}$$

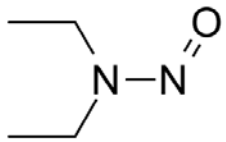
$$logD = \frac{[C_o]}{[C_w].[C_w^-]}$$



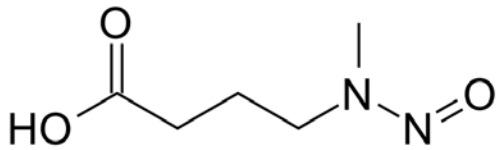
Nitrosamines investigated



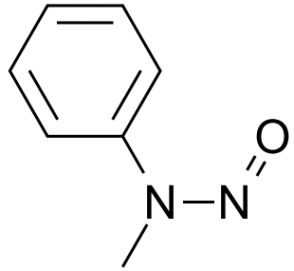
NDMA
N-nitroso-N-dimethylamine



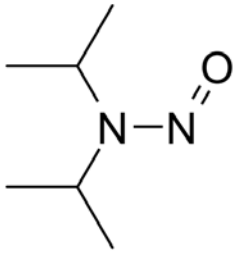
NDEA
N-nitroso-N-diethylamine



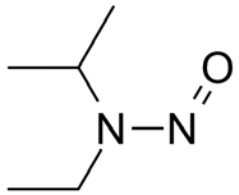
NMBA
N -nitroso-N-methyl-4-aminobutyric acid



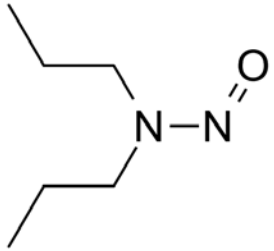
NMPA
N-nitrosomethylphenylamine



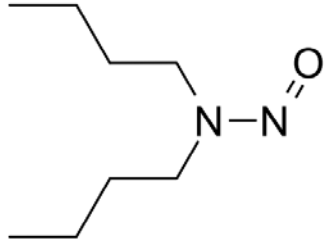
NDIPA
N-nitroso-N-diisopropylamine



NEIPA
N-nitroso-N-ethylisopropylamine

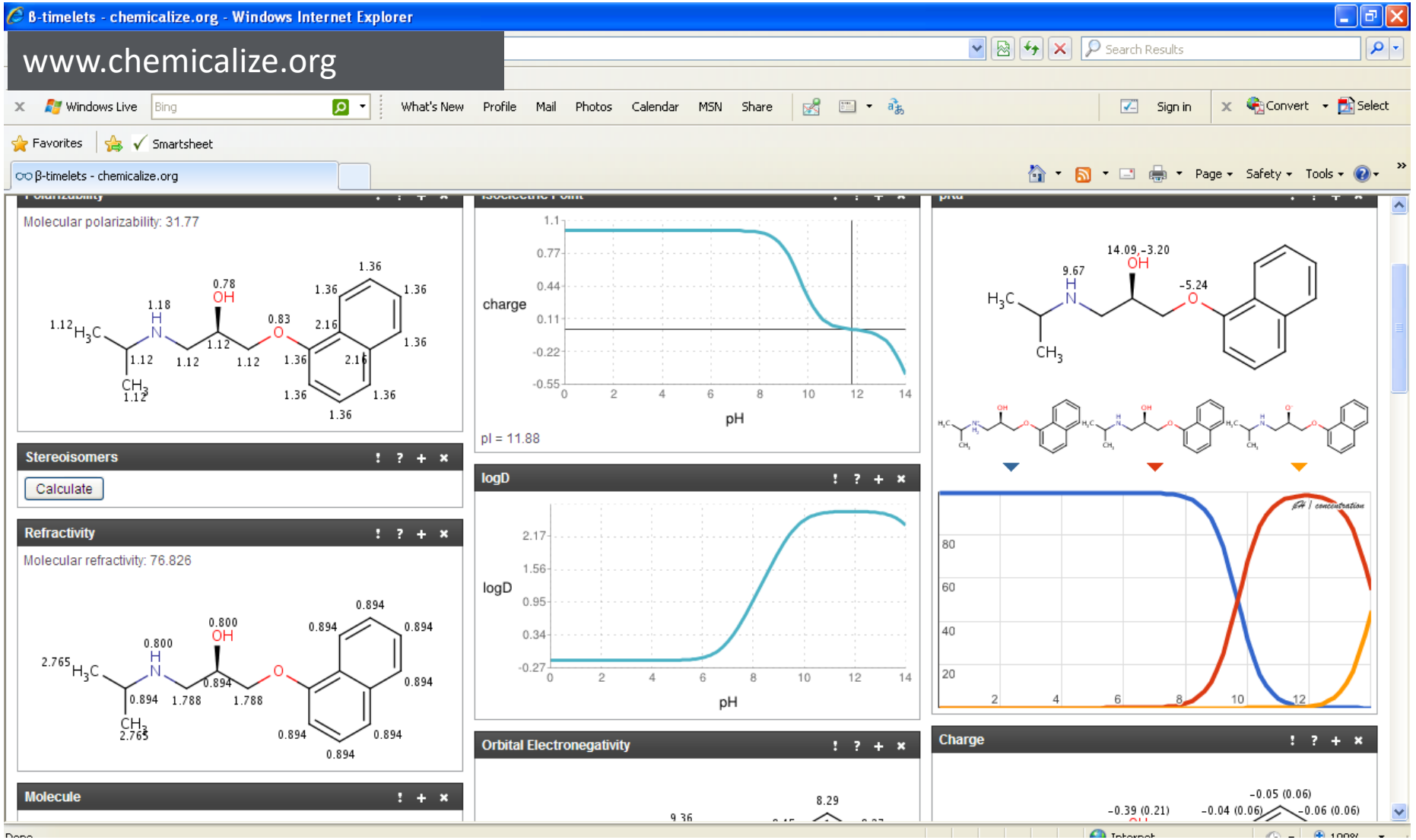


NDPA
N-nitroso-di-n-propylamine



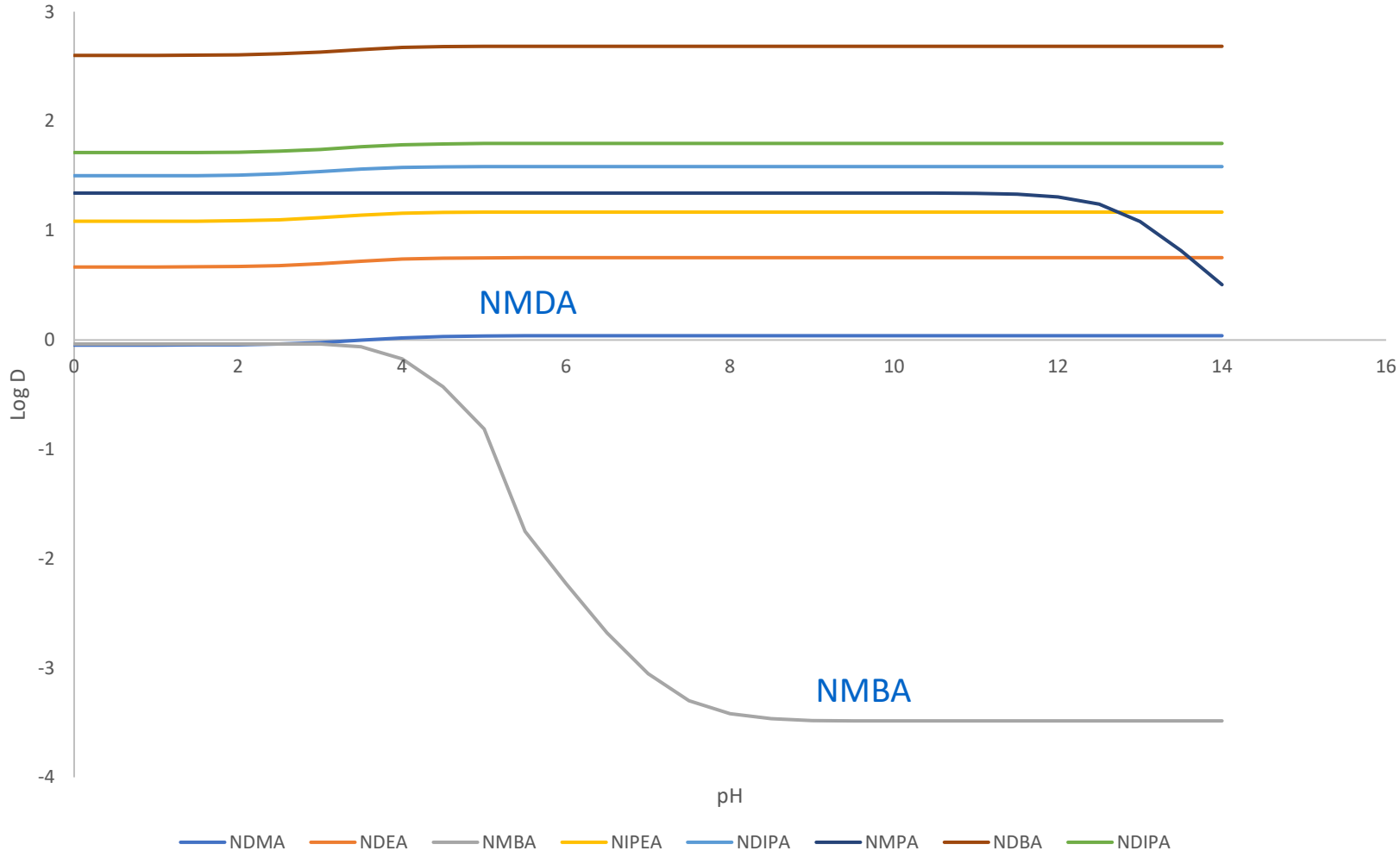
NDBA
N-Nitroso-di-n-butylamine

Calculating chemical properties



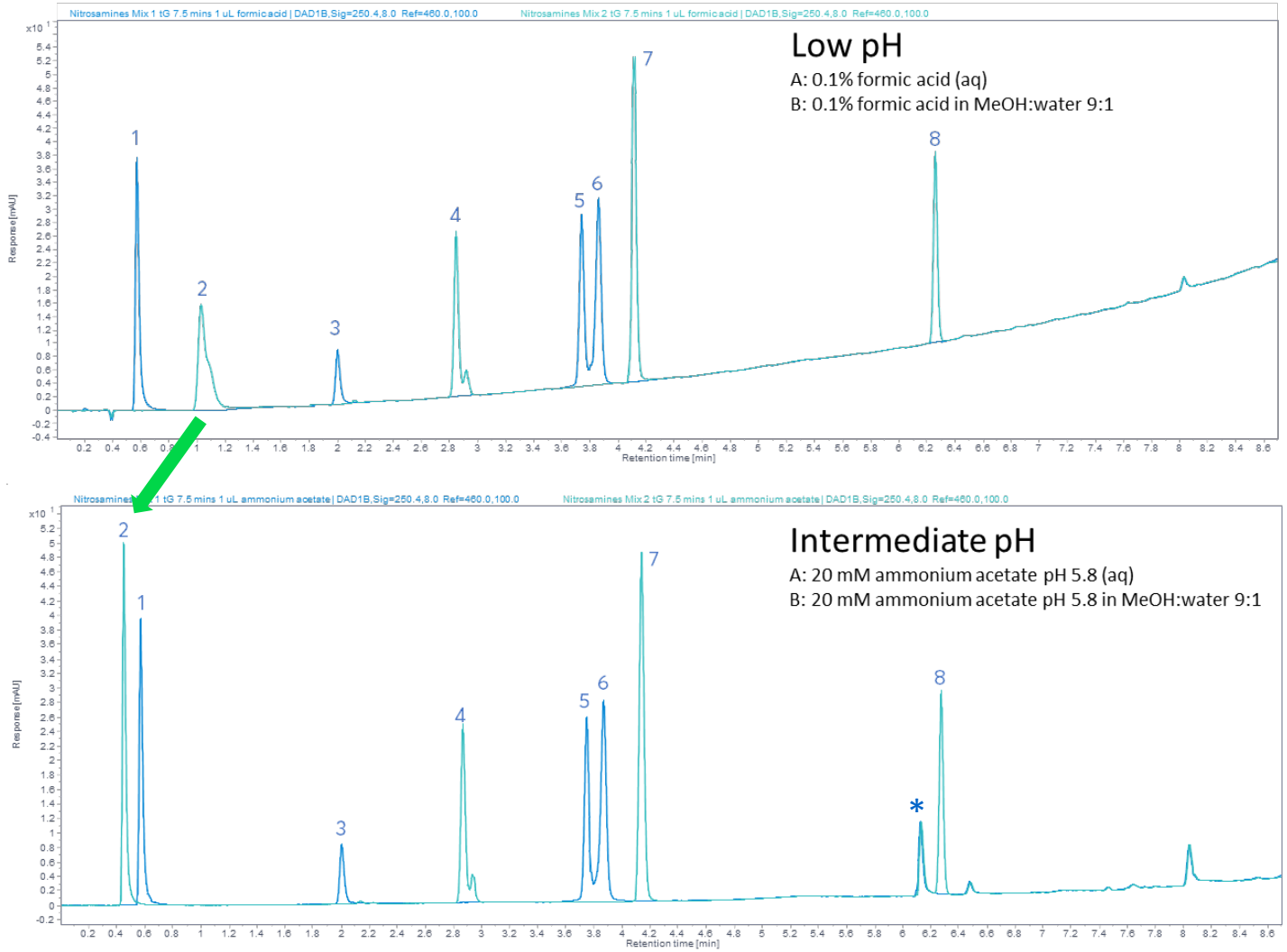
- Many different packages aroun including;
  - ACD, www.chemicalize.org, ChemAxon, SimulationPlus…

Chromatography development



- Broad range of log Ds
  - Broad range of retention times, which should mean getting a separation easier
- Mixture of acidic and basic moieties
  - pH will can a significant effect on retention times
- Some nitrosamines have negative log D's
- NMBA and NMDA most polar
- NMBA most likely to be affected by pH

Nitrosamine separation – Effect of pH



Column:	Avantor® ACE® UltraCore C18, 50 x 3.0 mm, 3.5 µm
Gradient:	3%B for 0.6 mins, then 3 to 100 %B in 7.5 minutes
Flow:	0.5 mL/min
Temp:	40 °C
Inj. vol:	1 µL
Detection:	UV, 250 nm

1. NDMA
2. NMBA
3. NDEA
4. NEIPA
5. NDIPA
6. NMPA
7. NDPA
8. NDBA

* Mobile phase impurity

- Movement of NMDA and NMBA could be predicted from log D
- Other compounds do not move due to focusing effect of gradient
- syn- and anti-conformers not resolved for NEIPA, NMBA

Considerations for LC-MS/MS method

- Increase in MS signal intensity for five out of nine nitrosamines tested over non-buffered mobile phase, when 0.05 or 0.1% (v/v) formic acid was added to the mobile phase,
  - 0.1% proved optimal for lower response nitrosamines
  - Higher concentrations of formic acid were found to compromise signal intensity.

- Electrospray ionisation (ESI) has been used for LC-MS analysis of nitrosamines
  - ESI may be impacted by ion suppression due to matrix effects.

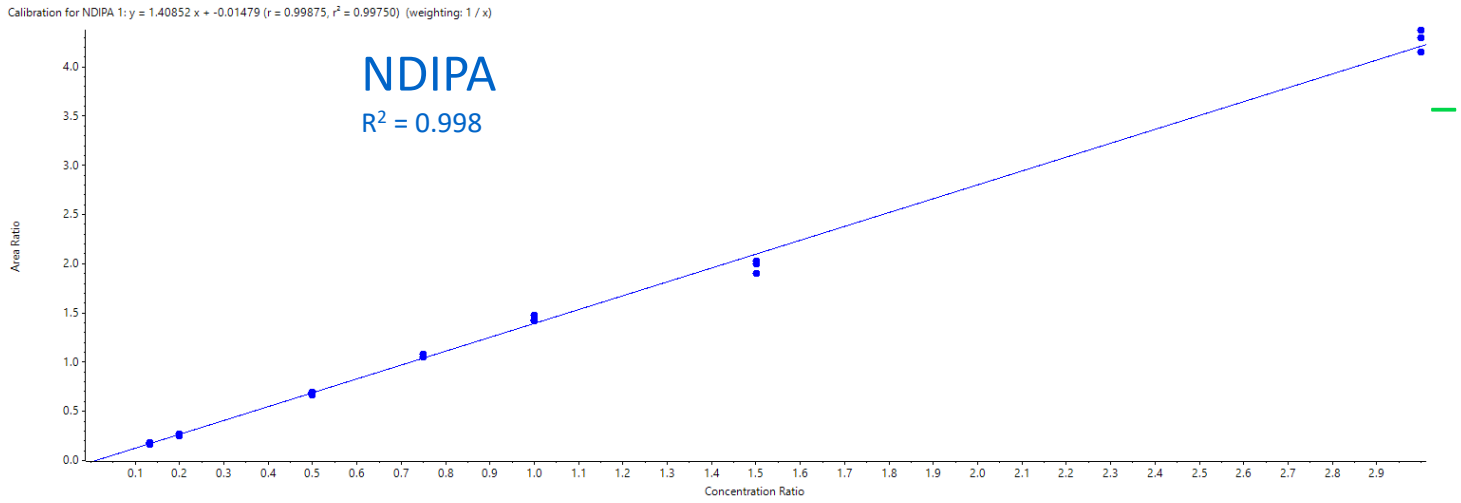
- Positive mode atmospheric pressure chemical ionisation (APCI) is preferential
  - Provides much improved sensitivity

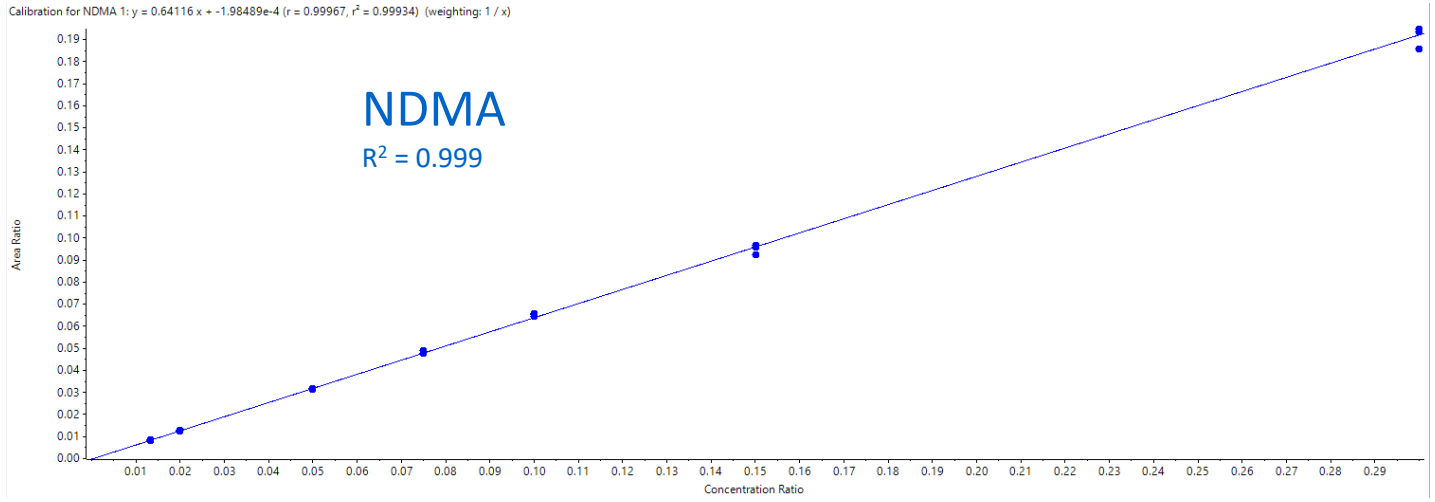
Mass spectrometer conditions

Nitrosamine Impurity	MRM	Optimised MS Parameters		
		Declustering potential (V)	Collision energy (V)	Cell exit potential (V)
NDMA	+75.0 amu → +43.0 amu	11	19	10
	+75.0 amu → +58.0 amu	11	17	28
NDMA-d6	+81.2 amu → +46.0 amu	40	22	11
	+81.2 amu → +64.1 amu	40	17	12
NMBA	+147.1 amu → +117.1 amu	11	11	12
	+147.1 amu → +87.1 amu	11	17	10
NMBA-d3	+150.1 amu → +120.2 amu	16	11	8
	+150.1 amu → +47.1 amu	21	17	8
NDEA	+103.1 amu → +75.1 amu	16	21	10
	+103.1 amu → +47.1 amu	16	23	22
NDEA-d10	+113.2 amu → +34.2 amu	21	33	6
	+113.2 amu → +49.1 amu	6	23	6
NEIPA	+117.1 amu → +75.1 amu	26	17	10
	+117.1 amu → +47.1 amu	21	23	10
NDIPA	+131.1 amu → +89.1 amu	76	15	10
	+131.1 amu → +47.1 amu	71	23	10
NMPA	+137.1 amu →+66.0 amu	21	23	8
	+137.1 amu →+107.1 amu	16	21	12
NDPA	+131.1 amu → +89.1 amu	16	17	10
	+131.1 amu → +43.1 amu	16	21	10
NDBA	+159.2 amu → +57.1 amu	46	17	10
	+159.2 amu → +103.2 amu	51	15	10
NDBA-d18	+177.3 amu → +66.2 amu	46	23	8
	+177.3 amu → +46.2 amu	41	37	22

- MS set up to do
  - Quantitative ion MRM
  - Qualifier ions MRM
- APCI, needle current 2 µA, Source Temp 300 °C
- Mass transitions are for very low molecular masses
  - Results in high levels of noise
  - Difficult to find low noise transition
- SIL IS used throughout
  - Some SILs are used for multiple compounds
- Qualifier ion can be used to ensure better specifi
  - Compare ion ratio

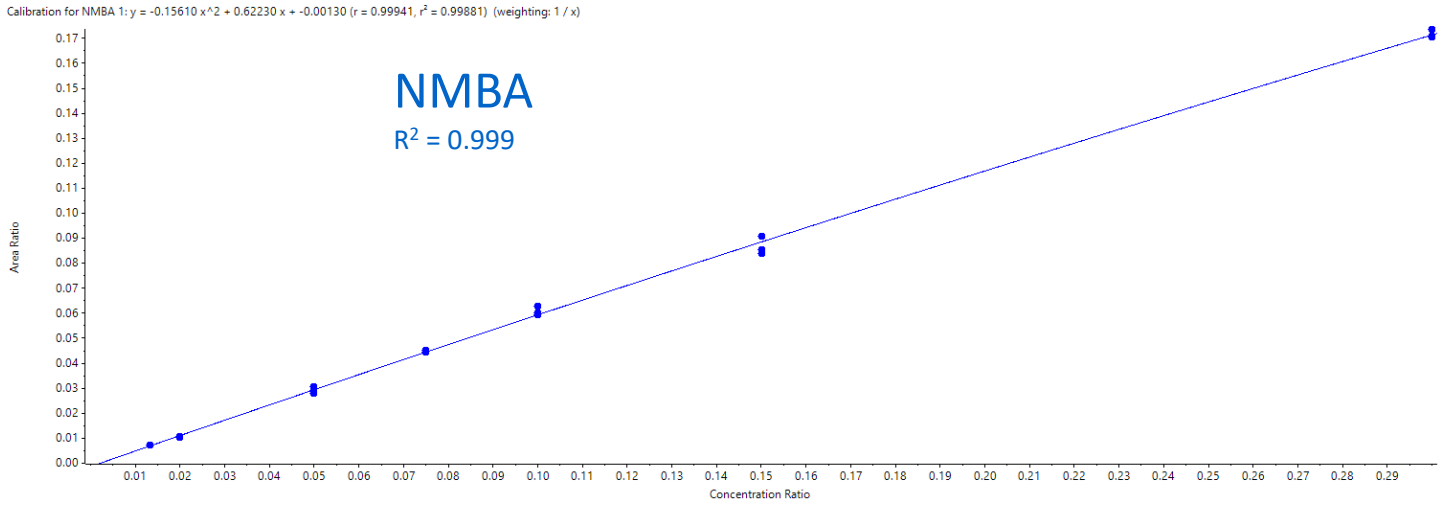
Linearity

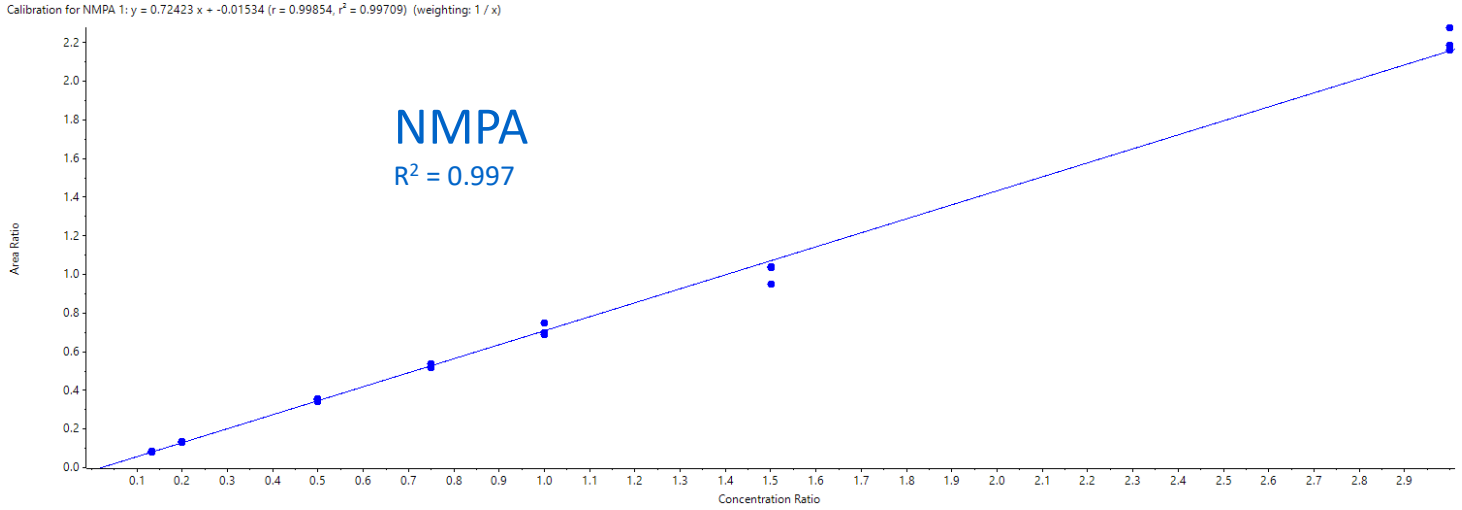




- 3 replicate injections
  - Accuracy and precision all look good
  - R^2 also very good

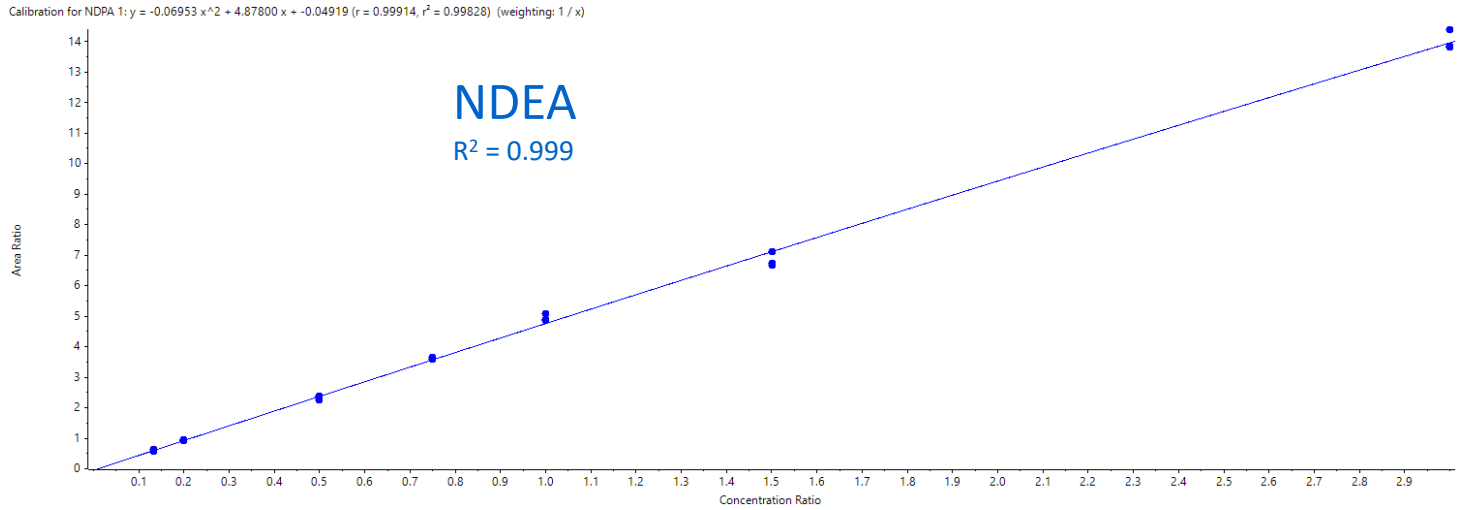
Linearity

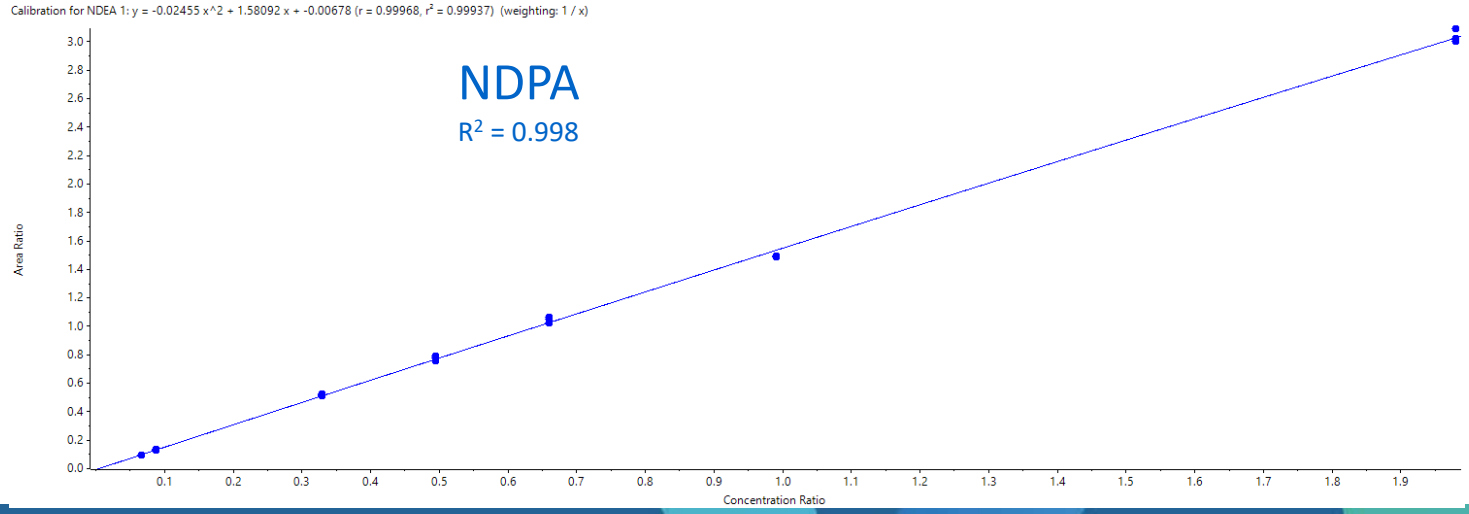




- 3 replicate injections
  - Accuracy and precision all look good
  - R^2 also very good

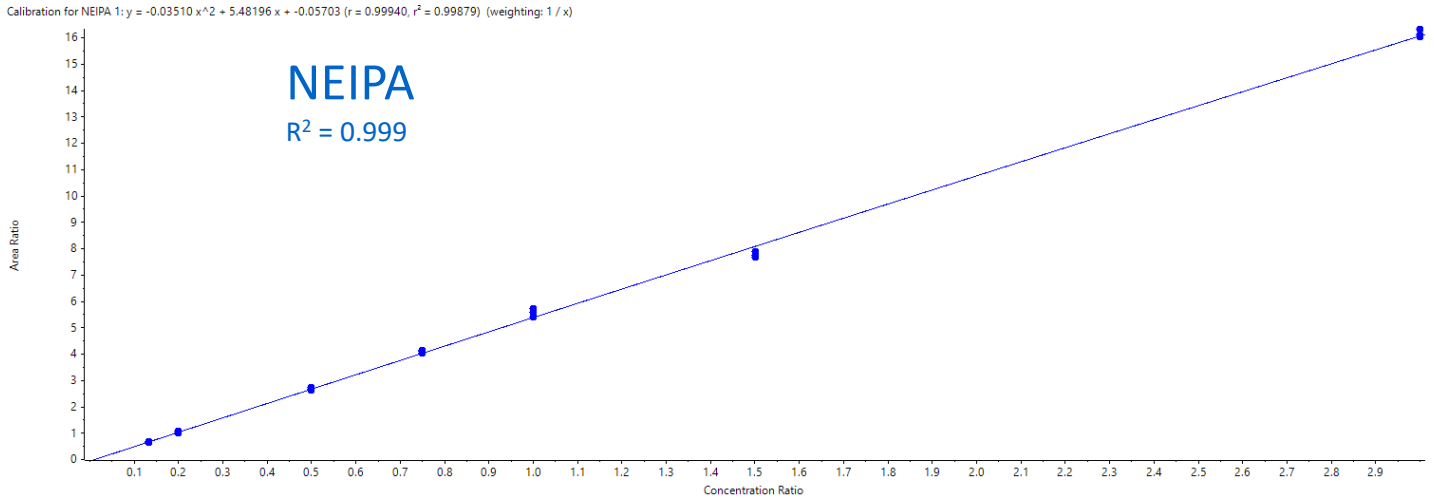
Linearity

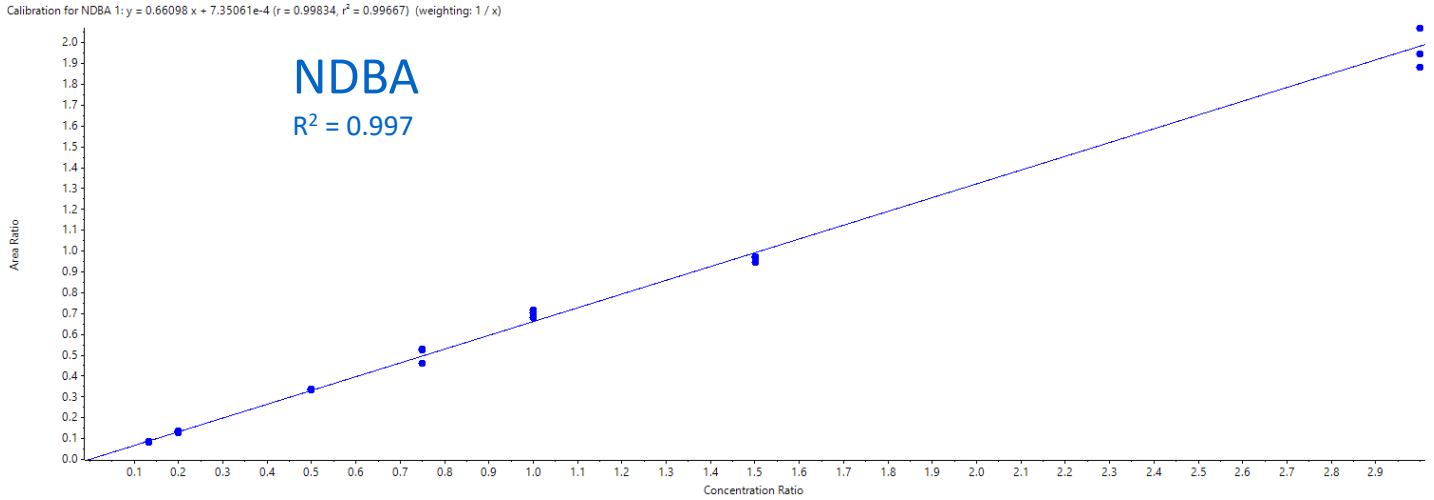




- 3 replicate injections
  - Accuracy and precision all look good
  - R^2 also very good

Linearity





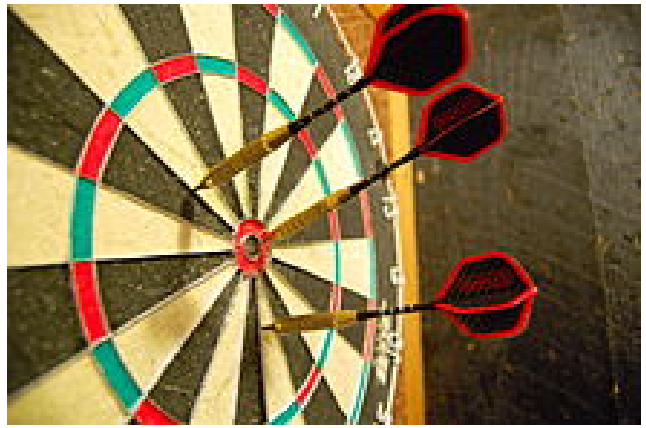
- 3 replicate injections
  - Accuracy and precision all look good
  - R^2 also very good

Accuracy / Precision data

Concentration level	NDMA Accuracy %	NMBA Accuracy %	NDEA Accuracy %	NEIPA Accuracy %	NDIPA Accuracy %	NMPA Accuracy %	NDPA Accuracy %	NDBA Accuracy %
L1	99	103	100	99	101	100	99	95
L2	100	96	99	99	99	102	100	100
L3	99	99	101	100	99	101	98	101
L4	101	101	101	102	102	101	102	102
L5	102	103	102	103	103	101	104	106
L6	99	98	97	96	94	94	96	97
L7	100	100	100	101	102	102	100	99

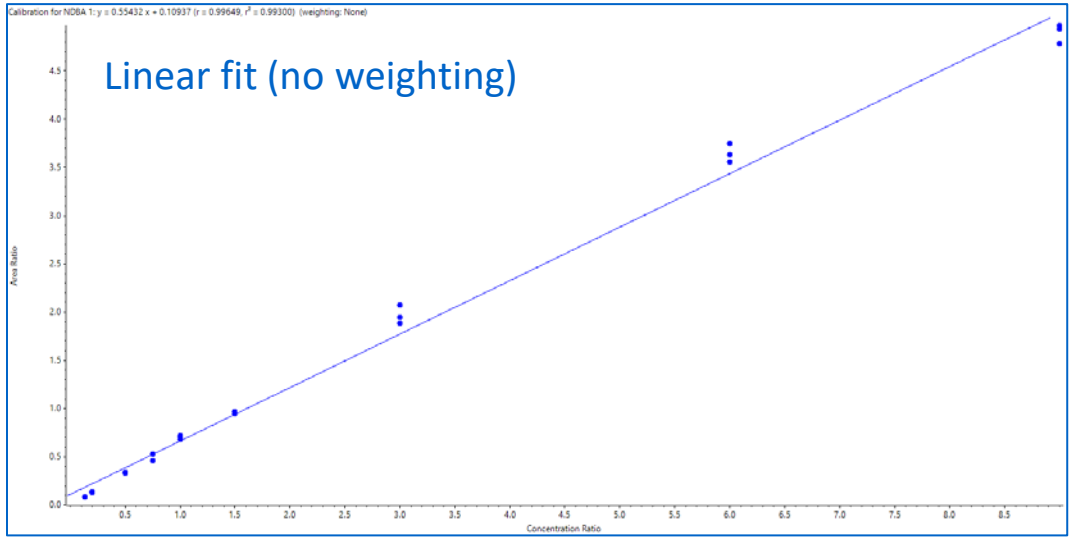
Concentration level	NDMA % CV	NMBA % CV	NDEA % CV	NEIPA % CV	NDIPA % CV	NMPA % CV	NDPA % CV	NDBA % CV
L1	1.9	0.6	1.6	2.9	4.8	2.7	4.9	2.4
L2	1.8	1.7	2.4	4.4	4.3	1.7	1.8	2.9
L3	1.0	4.5	0.8	2.5	1.9	2.2	3.0	1.1
L4	1.7	1.1	2.4	1.4	1.7	2.1	1.1	7.6
L5	1.0	2.9	2.2	2.7	2.0	4.7	2.6	2.8
L6	2.4	4.3	0.4	1.3	3.5	5.1	3.6	1.6
L7	2.5	1.0	1.6	1.0	2.7	2.8	2.4	4.8

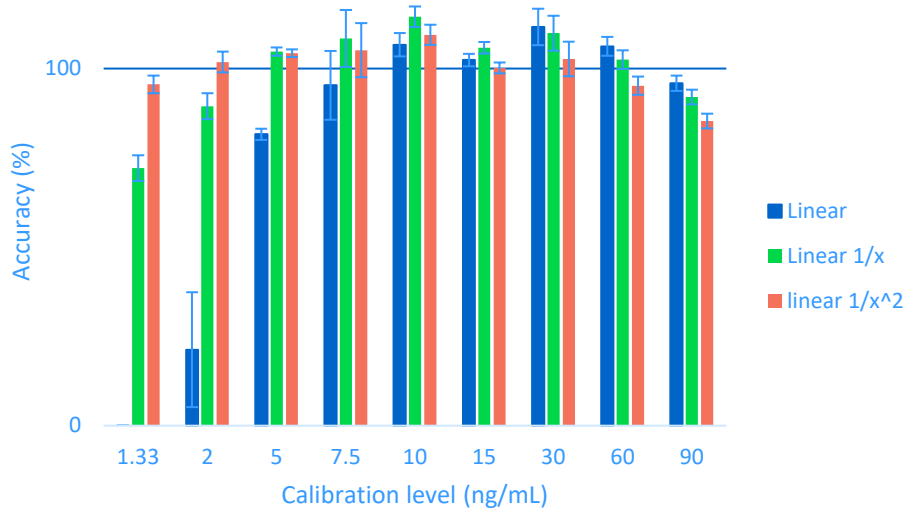
- Accuracy and Precision data very good
- Accuracy ranges from 94% - 106
  - Majority of data between 98 – 102%
- Precision data < 7.6%
  - Majority of data <3%

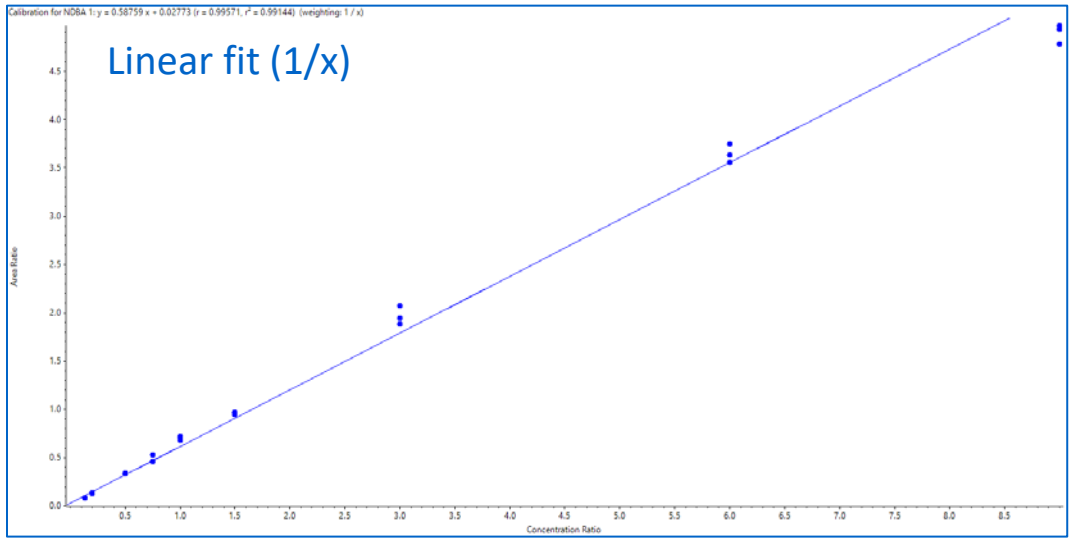


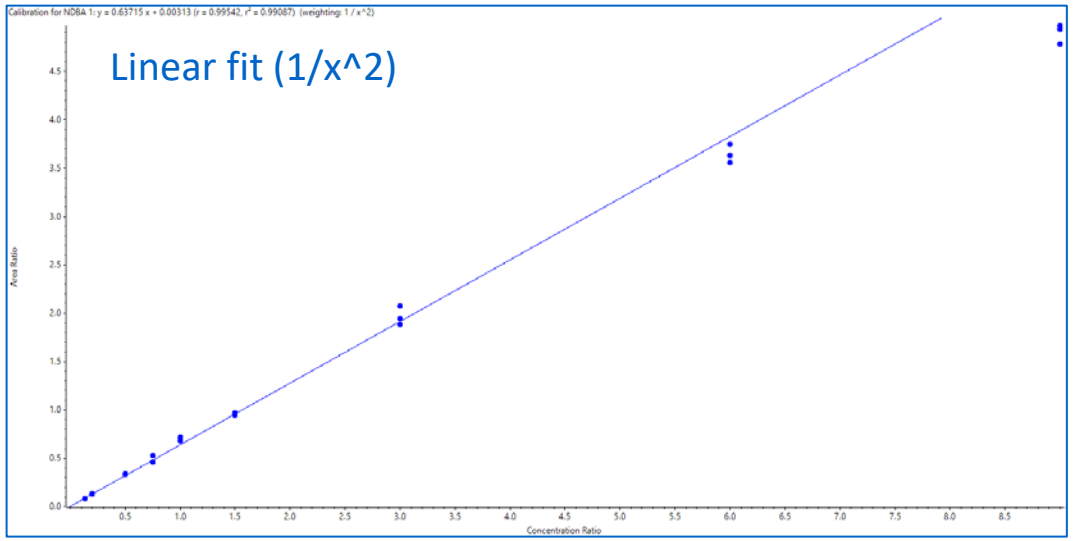
* Reproduced with kind permission from PeterPan23

Impact of changing weight (NDBA - Linear fit)

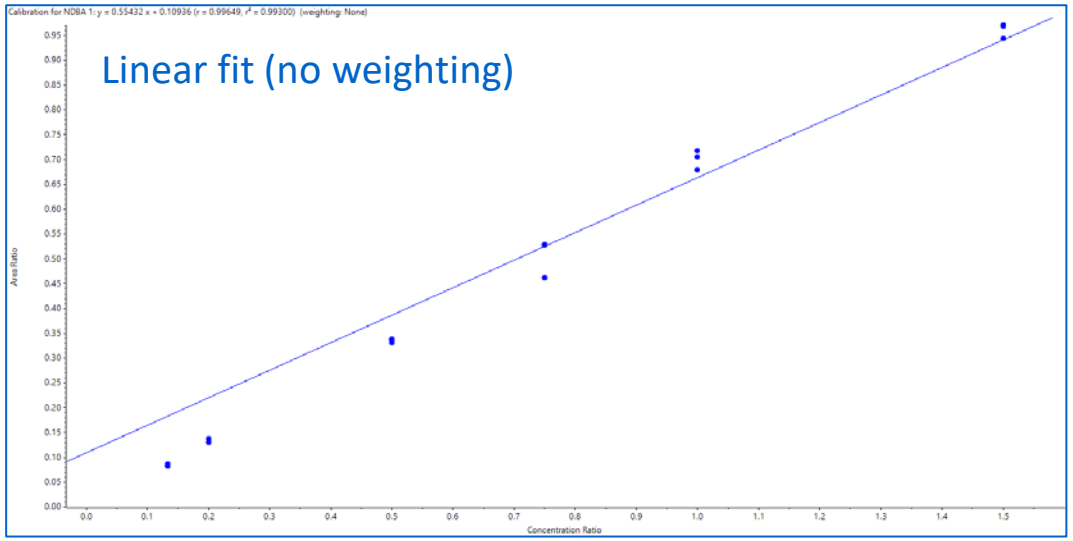




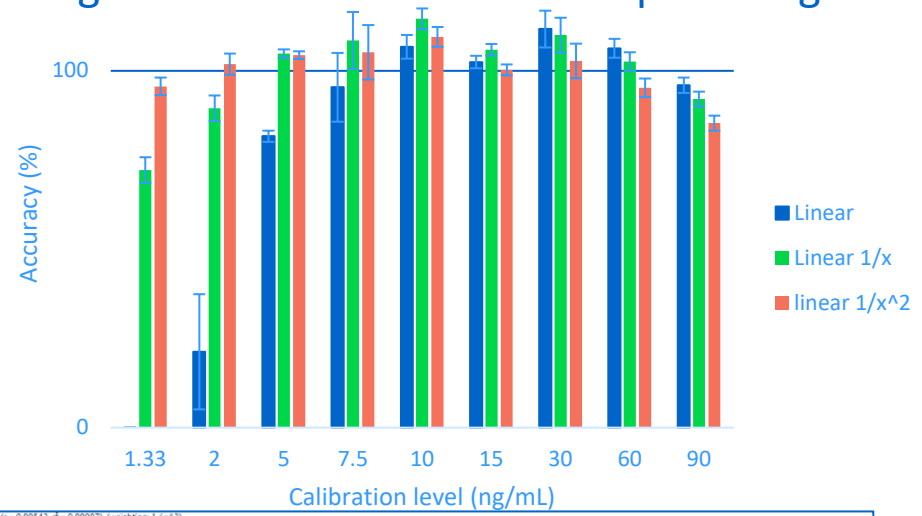


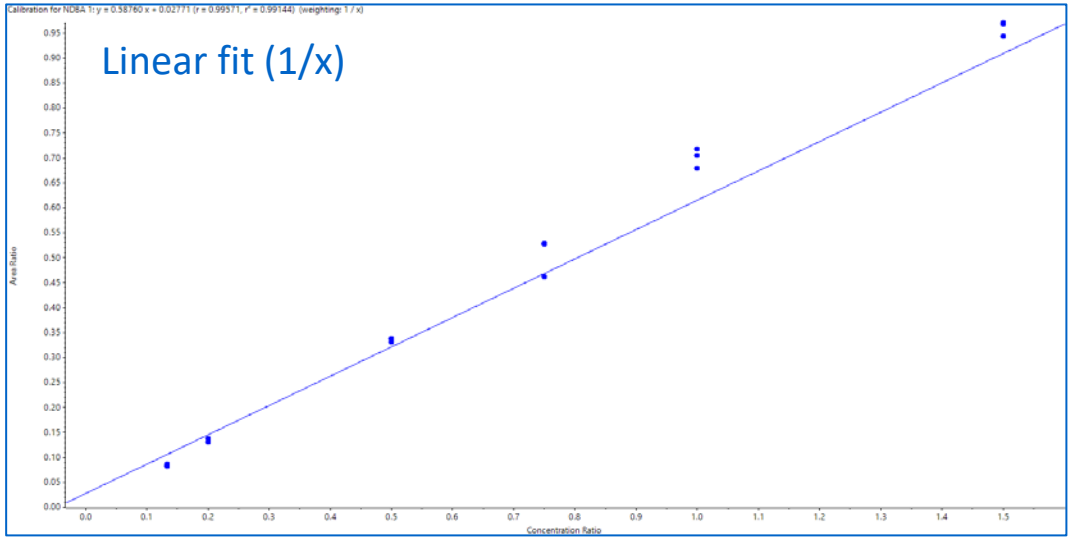


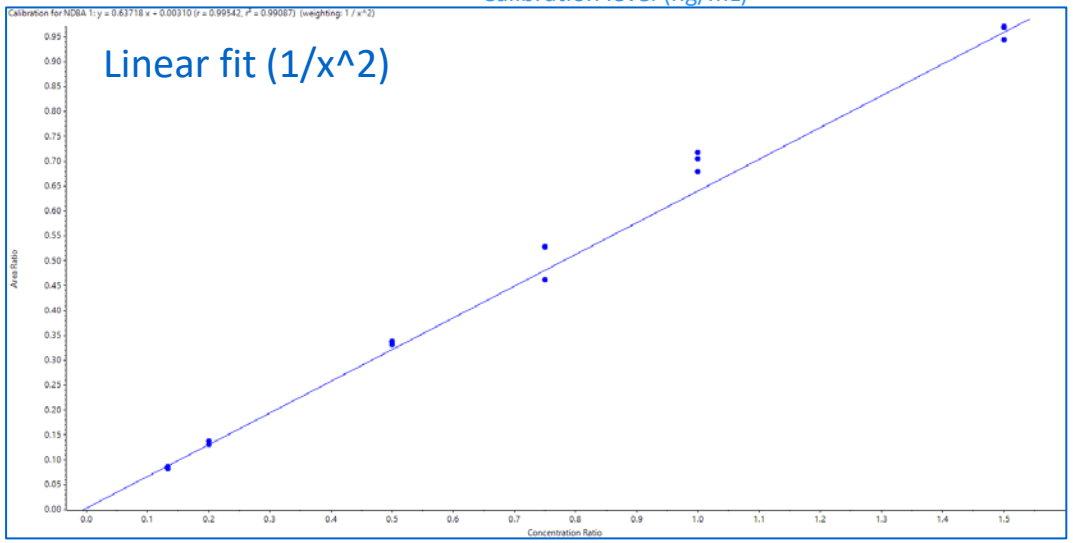
Effect of weighting (NDBA - Linear fit)



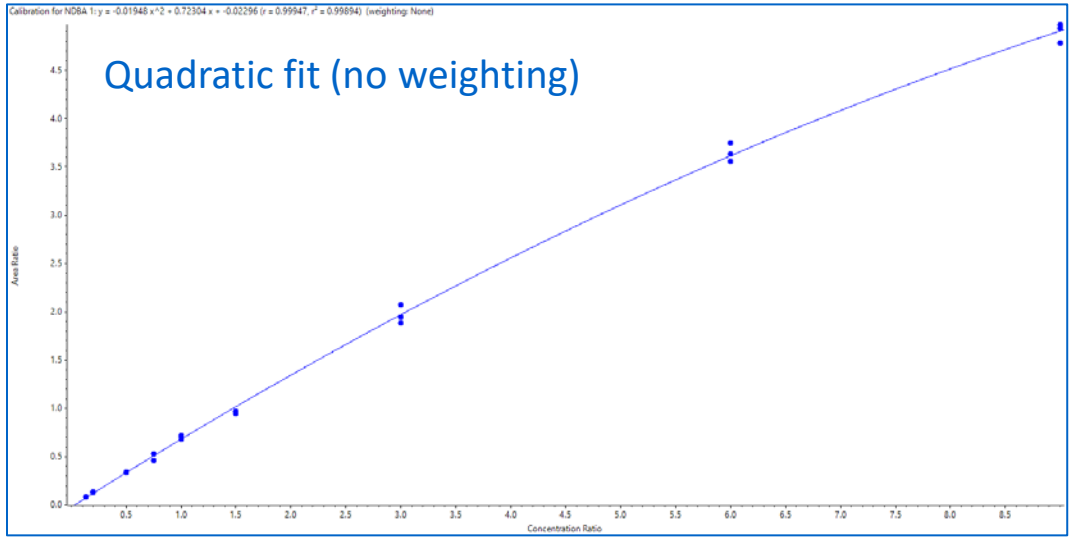
- Weighting affects low end calibration points significantly



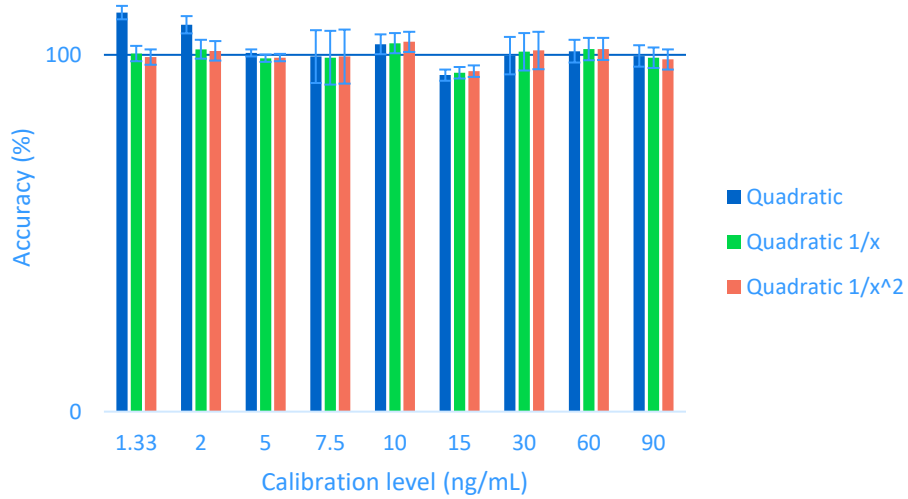


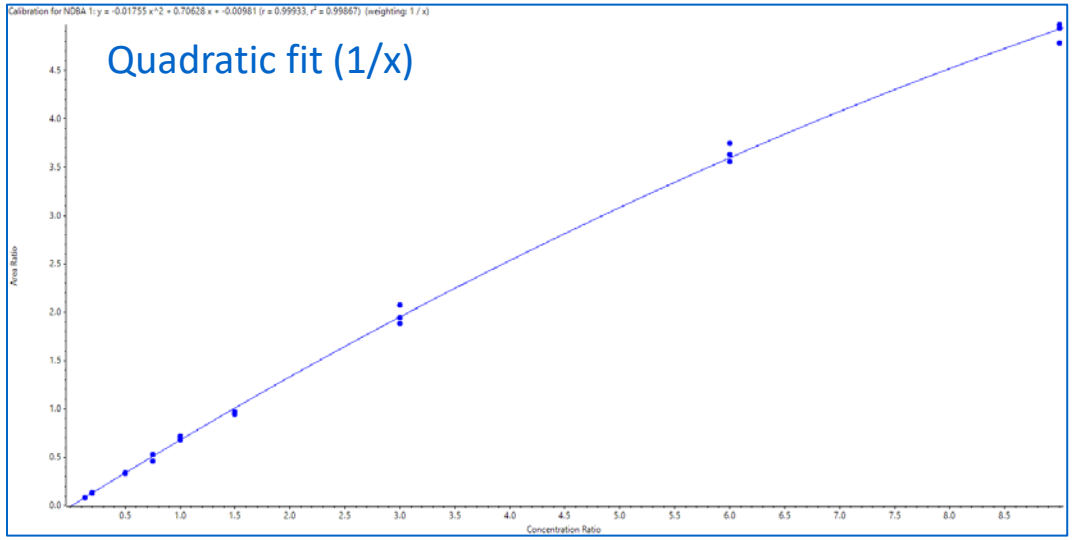


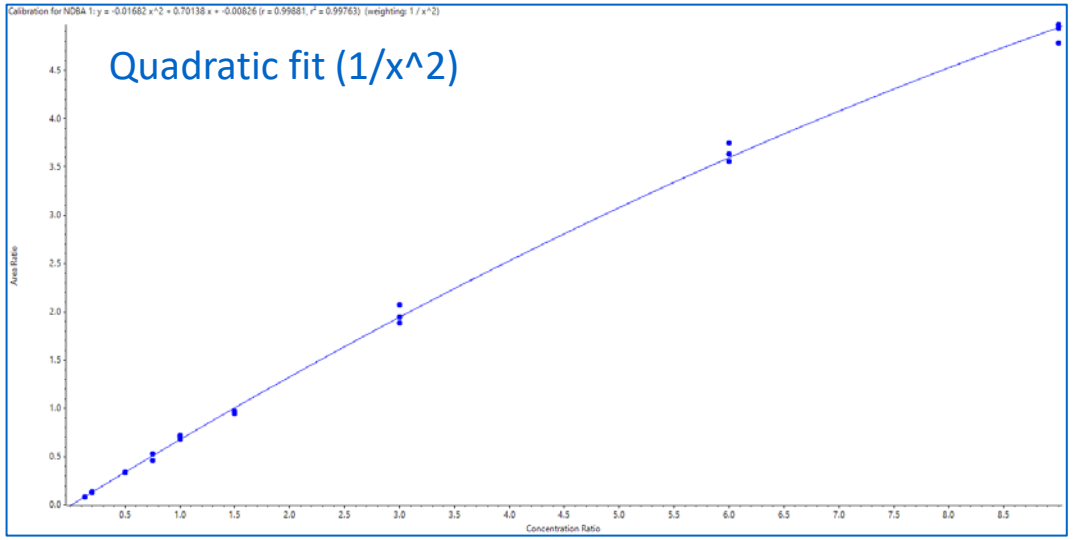
Impact of changing weight (NDBA - Quadratic fit)



- Shape of calibration line







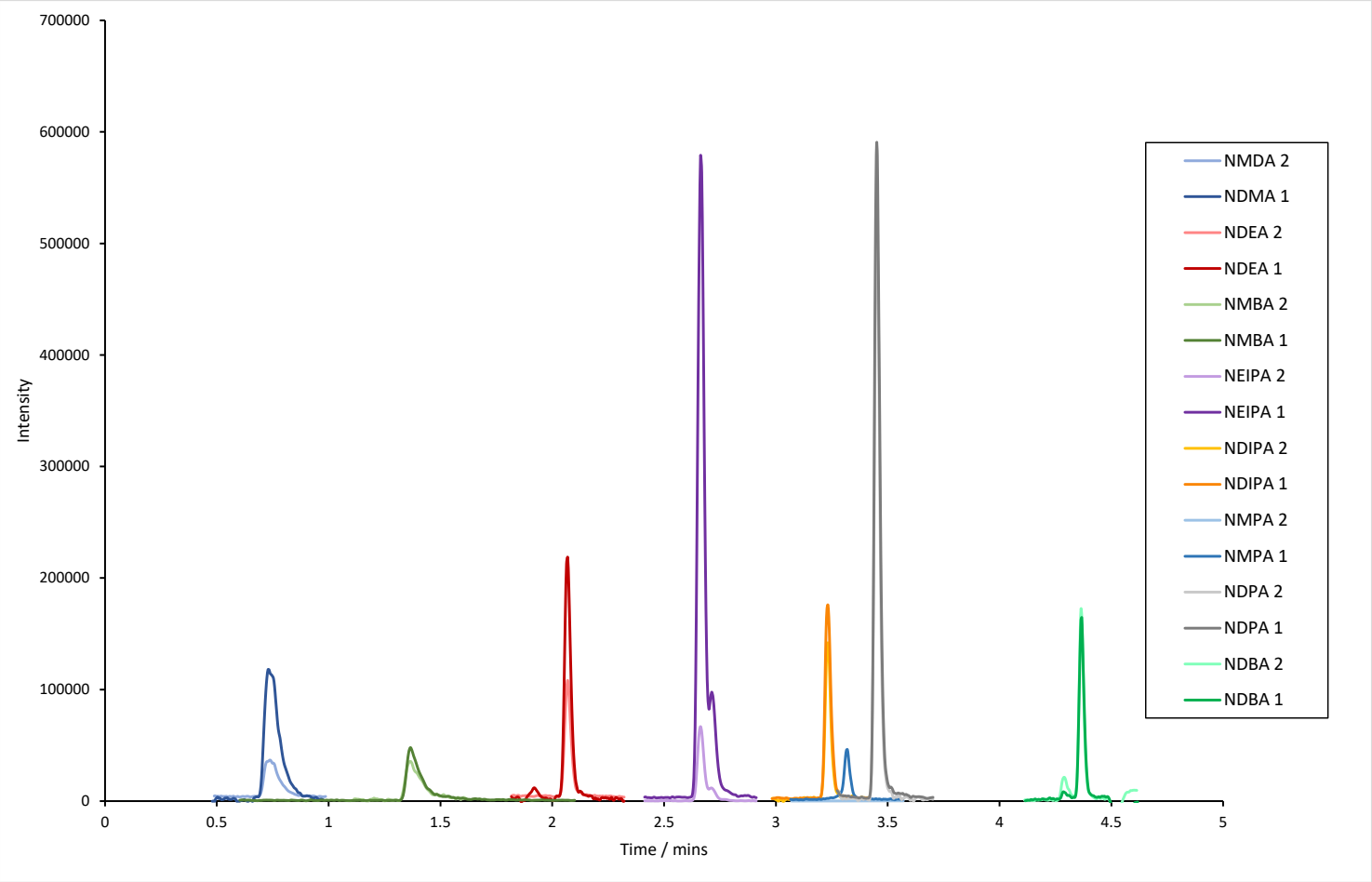
LOQ/LOQ

- low ppb sensitivity that can be achieved using the tested methodology

		ng/mL		ppb*	
	S/N (3σ) at L1 level	LOD	LOQ	LOD	LOQ
NDMA	99.1	0.040	0.134	0.60	2.01
NMBA	433.1	0.009	0.031	0.14	0.46
NDEA	197.7	0.010	0.033	0.15	0.50
NEIPA	3199	0.001	0.004	0.02	0.06
NDIPA	1204	0.003	0.011	0.05	0.17
NMPA	401	0.010	0.033	0.15	0.50
NDPA	3428	0.001	0.004	0.02	0.06
NDBA	629.9	0.006	0.021	0.10	0.32

* with respect to 66.67 mg/mL drug substance

Nitrosamines spiked in valsartan



– 1 ng/mL (15 ppb)*

Column: Avantor® ACE® UltraCore C18
Dimensions: 100 x 2.1 mm, 3.5 µm,
Mobile phase: A: 0.1% formic acid in water,
B: 0.1% formic acid in methanol

Gradient:

Time (min)	% Mobile phase A	% Mobile phase B
0	97.5	2.5
0.2	97.5	2.5
4.2	20	80
4.5	20	80
4.6	97.5	2.5
7	97.5	2.5

Flow rate: 0.5 mL/min
Temperature: 40 °C
Injection volume: 40 µL

* wrt 66.7 mg/mL API, as per USP nitrosamines sample preparation

– NMBA and NEIPA are observed as doublet peaks due to syn- and anti-conformers

Impact of IS

- Overall, good data is obtained for the calibration standards, as expected.
  - But what about in sample matrix?

- No IS

Concentration level	NDMA		NMBA 1		NDEA 1		NEIPA 1		NDIPA 1		NMPA 1		NDPA 1		NDBA 1	
	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV
L1	101.91	2.10	105.29	1.45	101.21	2.91	102.53	0.51	102.45	4.12	97.50	7.26	103.13	2.21	102.32	1.77
L2	100.07	1.24	95.38	3.18	99.61	2.33	98.46	1.19	97.31	5.13	99.77	1.88	98.81	2.39	98.87	1.21
L3	98.08	1.42	99.30	7.10	100.34	1.49	99.32	0.93	99.43	2.20	104.02	1.84	97.46	1.19	98.92	1.13
L4	97.54	0.16	98.87	3.46	97.25	3.49	98.11	1.42	99.94	3.90	99.92	4.56	98.16	0.93	98.04	3.31
L5	101.11	1.16	99.61	2.62	100.25	2.29	100.45	1.46	101.72	3.81	100.67	2.69	101.29	0.29	100.98	3.43
L6	101.65	1.19	101.91	2.08	101.64	2.72	101.42	1.37	99.09	2.03	97.74	1.65	101.52	2.60	101.13	0.73
L7	99.65	0.98	99.65	2.12	99.71	2.26	99.72	1.28	100.06	3.31	100.38	3.54	99.63	1.59	99.75	3.04

- With IS

Concentration level	NDMA		NMBA		NDEA		NEIPA		NDIPA		NMPA		NDPA		NDBA	
	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV	Accuracy %	% CV
L1	99.35	1.91	103.25	0.55	99.53	1.55	99.09	2.92	101.41	4.83	99.74	2.68	99.35	4.93	94.88	2.42
L2	99.56	1.79	95.97	1.71	98.95	2.38	99.24	4.44	98.74	4.30	101.93	1.66	100.00	1.75	99.99	2.87
L3	98.92	1.00	99.18	4.46	101.00	0.84	100.18	2.54	98.88	1.91	100.59	2.23	98.44	3.00	101.33	1.05
L4	101.47	1.72	100.92	1.07	100.87	2.37	101.54	1.36	101.83	1.68	100.65	2.14	101.50	1.10	101.88	7.57
L5	101.84	1.02	102.50	2.92	102.14	2.16	103.37	2.74	103.45	2.01	100.53	4.66	104.05	2.55	105.92	2.78
L6	99.21	2.39	97.98	4.29	97.10	0.38	96.07	1.28	94.18	3.47	94.22	5.11	96.22	3.64	96.86	1.56
L7	99.65	2.52	100.22	1.03	100.42	1.59	100.52	0.97	101.51	2.65	102.33	2.75	100.46	2.38	99.13	4.83

Impact of IS – spiked sample quantification

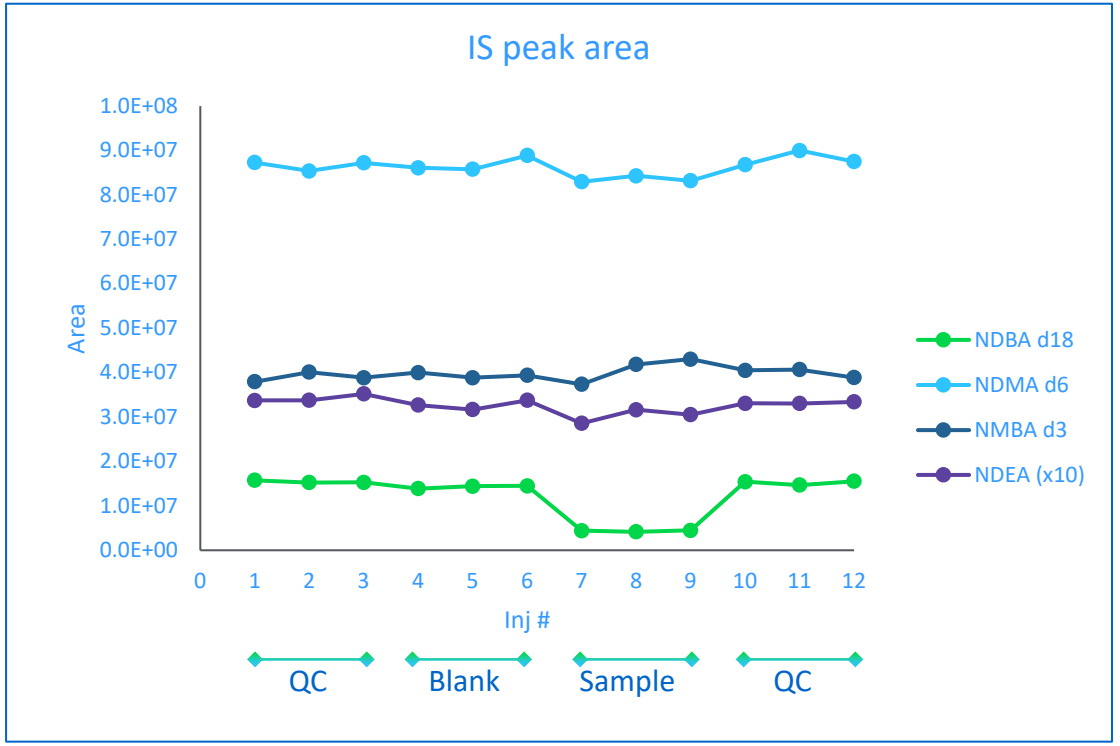
- Data for valsartan spiked sample with and without quantitation against internal standards
- Examination of IS peak areas shows suppression relative to blanks and QC samples for NDBA d18
- Quantifying against IS accounts for suppression effects.

No IS

	Determined concentration (ng/mL)			Accuracy	STDEV	%CV
	Value #1	Value #2	Value #3			
NDMA	1.04	1.09	1.05	105.8	0.029	2.70
NMBA	1.15	1.16	1.17	116.1	0.013	1.08
NDEA	0.87	0.92	0.92	90.2	0.025	2.75
NEIPA	0.83	0.88	0.85	85.6	0.022	2.55
NDIPA	0.81	0.81	0.79	80.4	0.008	1.05
NDPA	0.79	0.78	0.77	77.9	0.011	1.44
NDBA	0.31	0.30	0.33	31.2	0.013	4.06

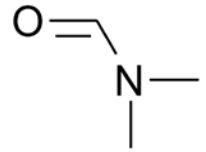
With IS

	Determined concentration (ng/mL)			Accuracy	STDEV	%CV
	Value #1	Value #2	Value #3			
NDMA	1.05	1.08	1.06	106.1	0.013	1.25
NMBA	1.14	1.12	1.06	110.7	0.040	3.65
NDEA	1.04	1.00	1.01	101.5	0.021	2.08
NEIPA	1.00	0.95	0.95	96.6	0.026	2.68
NDIPA	0.98	0.88	0.89	91.7	0.055	6.01
NDPA	0.90	0.80	0.82	84.3	0.053	6.30
NDBA	0.85	0.90	0.88	87.5	0.024	2.78

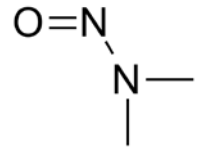


Interference from DMF

- DMF was determined by LC-UV to partially co-elute with NDMA
- NDMA at 1.0 ng/mL was spiked with DMF to assess potential interference



DMF
73.053 Da



NDMA
74.048 Da

Spike level	NDMA (ng/mL)	NDMA (ppm*)	DMF (ng/mL)	DMF (ppm*)
0	1.0	0.015	0	0
1	1.0	0.015	83.3	1.25
2	1.0	0.015	833.3	12.5
3	1.0	0.015	1666.7	25.0
4	1.0	0.015	3333.3	50.0
5	1.0	0.015	6666.7	100.0

- * wrt 66.7 mg/mL API, as per USP nitrosamines sample preparation

- USP 35 residual solvent limits

Table 2. Class 2 Residual Solvents

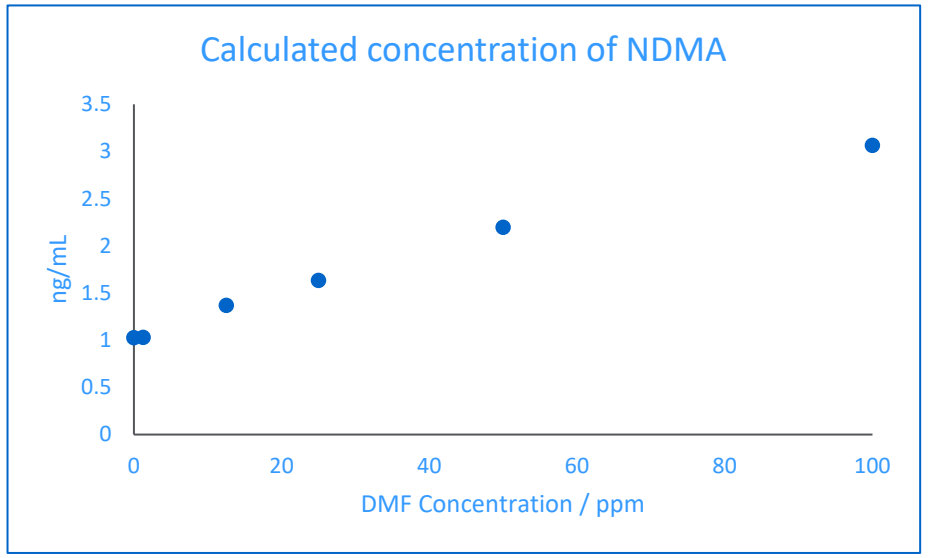
Solvent	PDE (mg/day)	Concentration Limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
1,2-Dimethoxyethane	1.0	100
N,*N*-Dimethylacetamide	10.9	1090
N,*N*-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
Methylene chloride	6.0	600
N-Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200

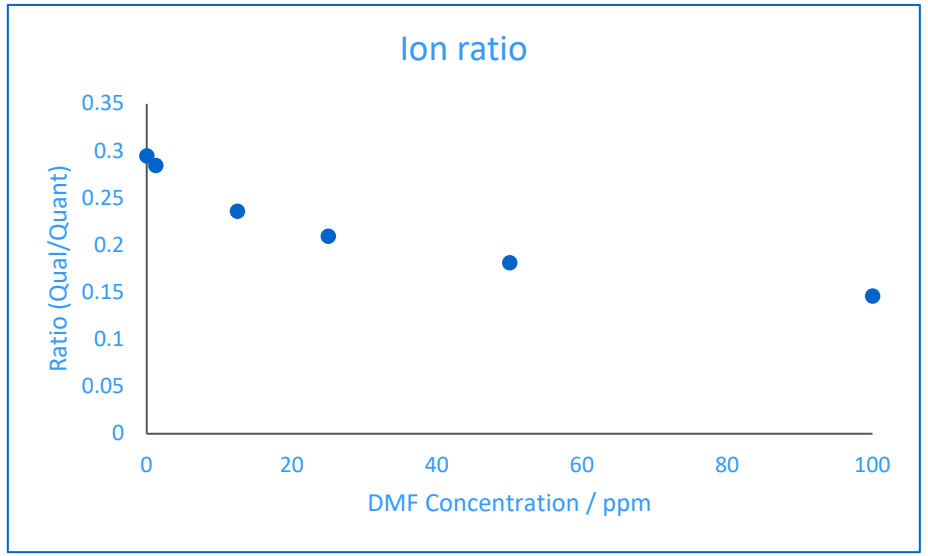
*Usually 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene with 17% ethyl benzene.

Interference from DMF

Spike level	NDMA (ng/mL)	NDMA (ppm*)	DMF (ng/mL)	DMF (ppm*)	Calculated NDMA Conentration (ng/mL)
0	1.0	0.015	0	0	1.03
1	1.0	0.015	83.3	1.25	1.03
2	1.0	0.015	833.3	12.5	1.37
3	1.0	0.015	1666.7	25.0	1.64
4	1.0	0.015	3333.3	50.0	2.20
5	1.0	0.015	6666.7	100.0	3.07

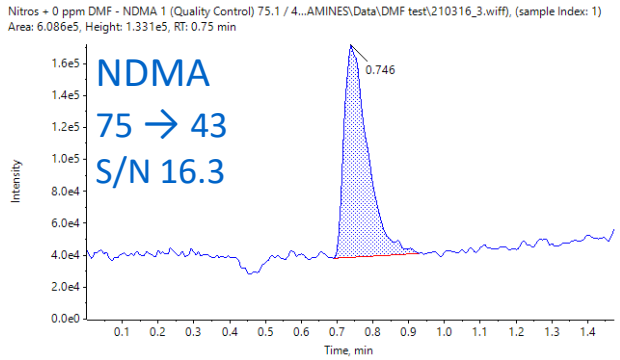
- Presence of DMF may lead to false positives/over quantification of NDMA.
- Monitoring ion ratios is important.

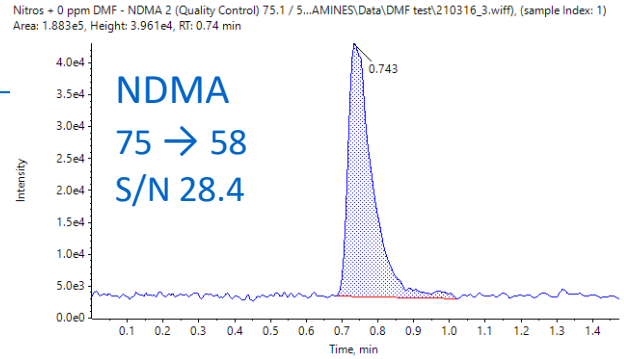


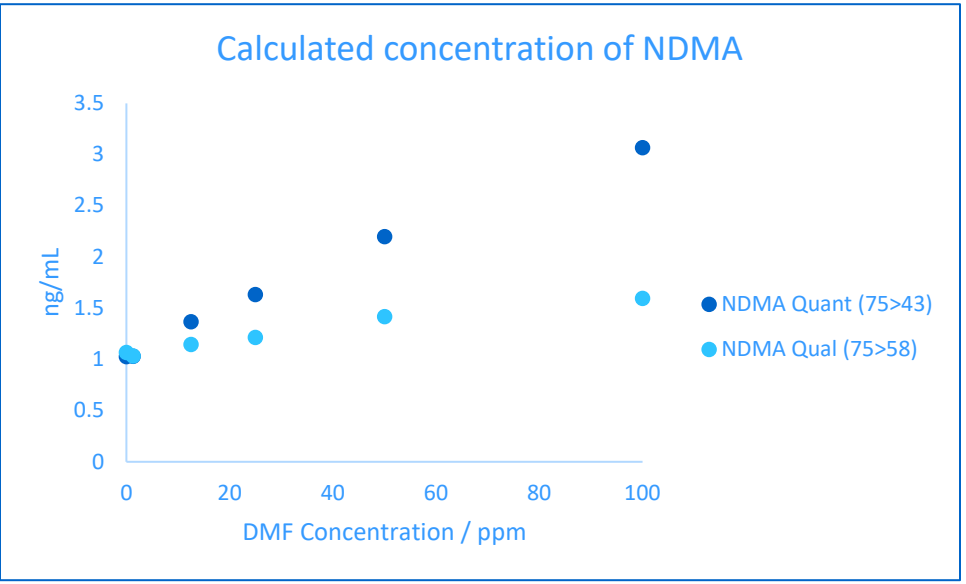


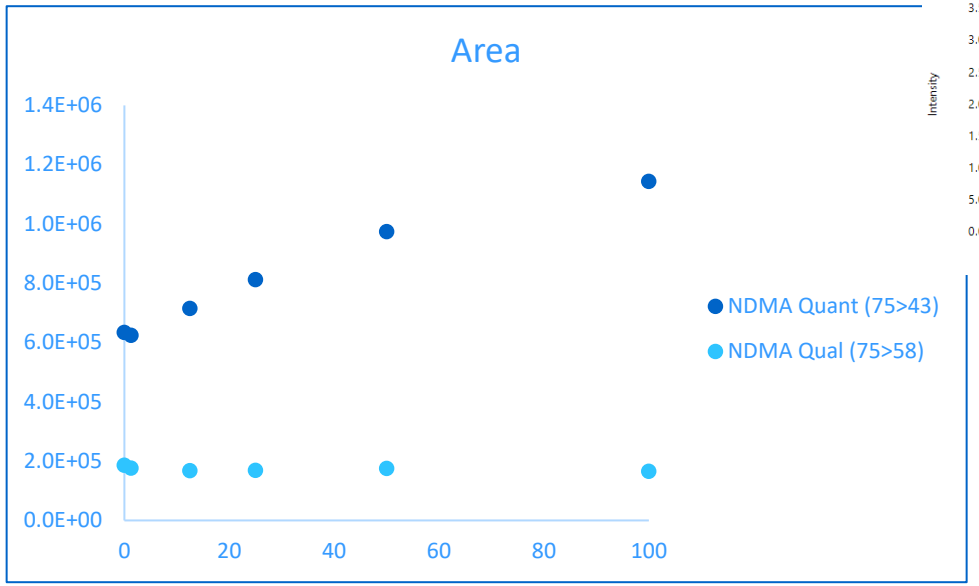
Interference from DMF– Possible solutions

- Qualifier not as affected
- Peak area for qualifier steady
- Suppression of internal standard plus interference with 75 → 43 quantifier transition?
- Can the NDMA qualifier transition be used to quantify in presence of DMF?



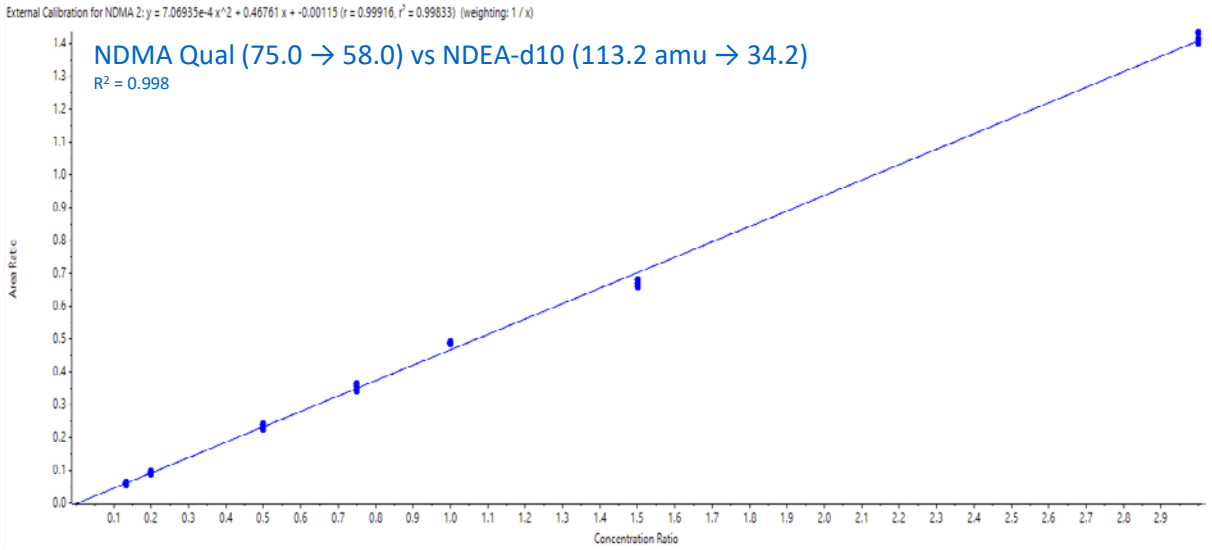


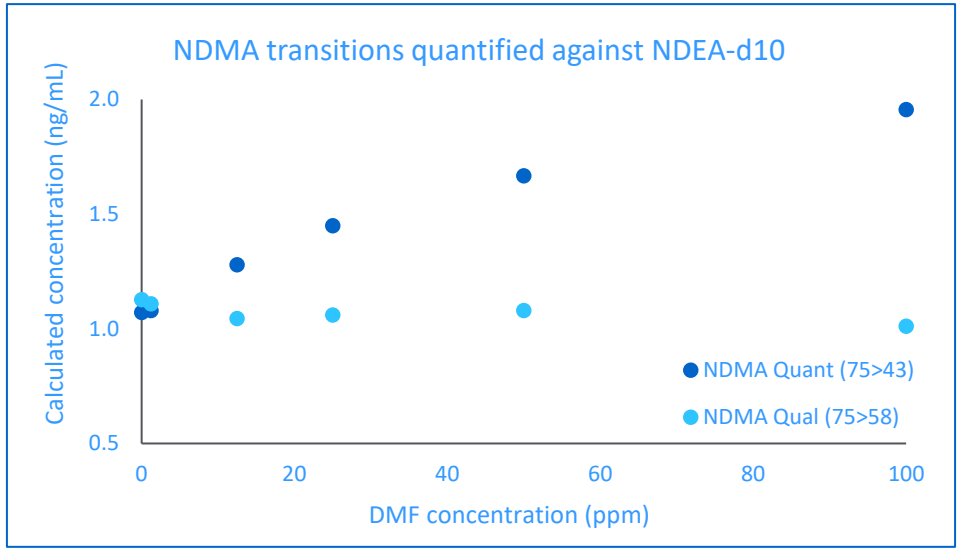




Interference from DMF Calibration vs NDEA d10

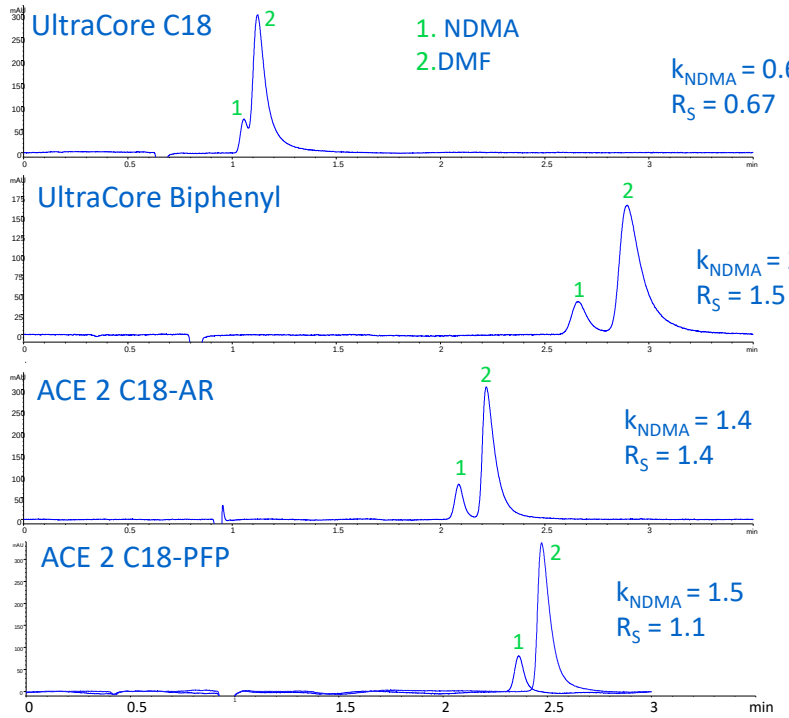
- The NDMA Qualifier ion was calibrated against NDEA-d10





DMF concentration (ppm)	NDMA concentration (ng/mL)	Calculated concentration (ng/mL)	%CV	Accuracy
0	1.00	1.13	4.56	112.7
1.25	1.00	1.11	5.65	110.8
12.5	1.00	1.04	2.59	104.4
25	1.00	1.06	1.59	105.9
50	1.00	1.08	5.45	107.9
100	1.00	1.01	3.54	101

Separation of DMF and NDMA



Column:	Avantor® ACE® UltraCore 3.5 µm, 100 x 2.1 mm Avantor® ACE® ACE Excel 2 µm, 100 x 3.0 mm
Mobile phase	A: 0.1% formic acid (aq) B: 0.1% formic acid in MeOH Isocratic, A:B 98:2
Flow rate:	0.3 mL/min (2.1 mm ID), 0.61 mL/min (3.0 mm ID)
Injection vol:	2.5 µL (2.1 mm ID), 5 µL/min (3.0 mm ID)

- Can we separate NDMA and DMF chromatographically?
- Selectivity screen for NDMA and DMF
  - Assess different stationary phase chemistries
- Potential options identified
  - Future work to apply to LC-MS/MS method

Conclusion

- Review of nitrosamines
  - Initial discovery of genotoxicity
  - Discovery within pharmaceutical industry
- Regulatory overview
  - Understanding landscape
  - Understanding acceptable intake limits
- Development of analytical assay
  - Use of mass spectrometry
  - Development of chromatography
  - Detector considerations (ion suppression & interference from DMF)

Thank you

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