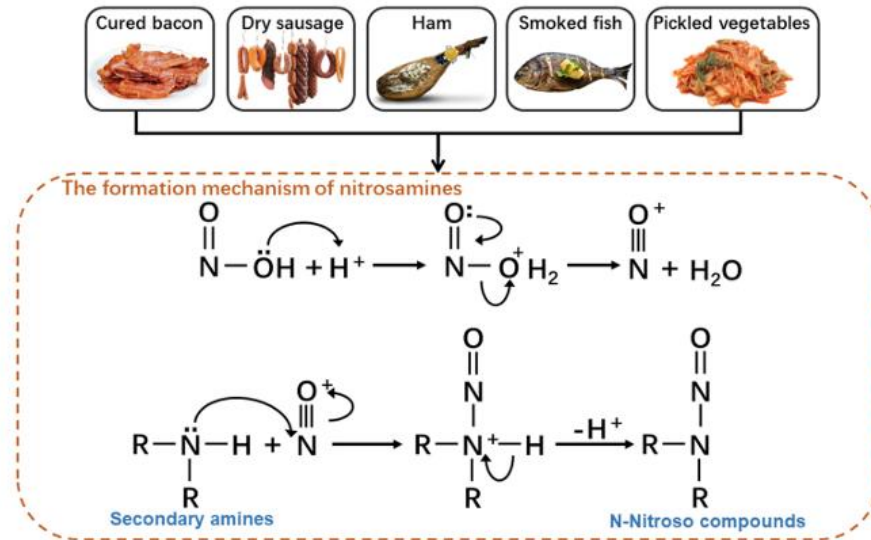


Stanovení nitrosaminů v uzeninách

Simona Wawroszová
Senior Application Chemist

Nitrosaminy: Vznik a výskyt v masných výrobcích

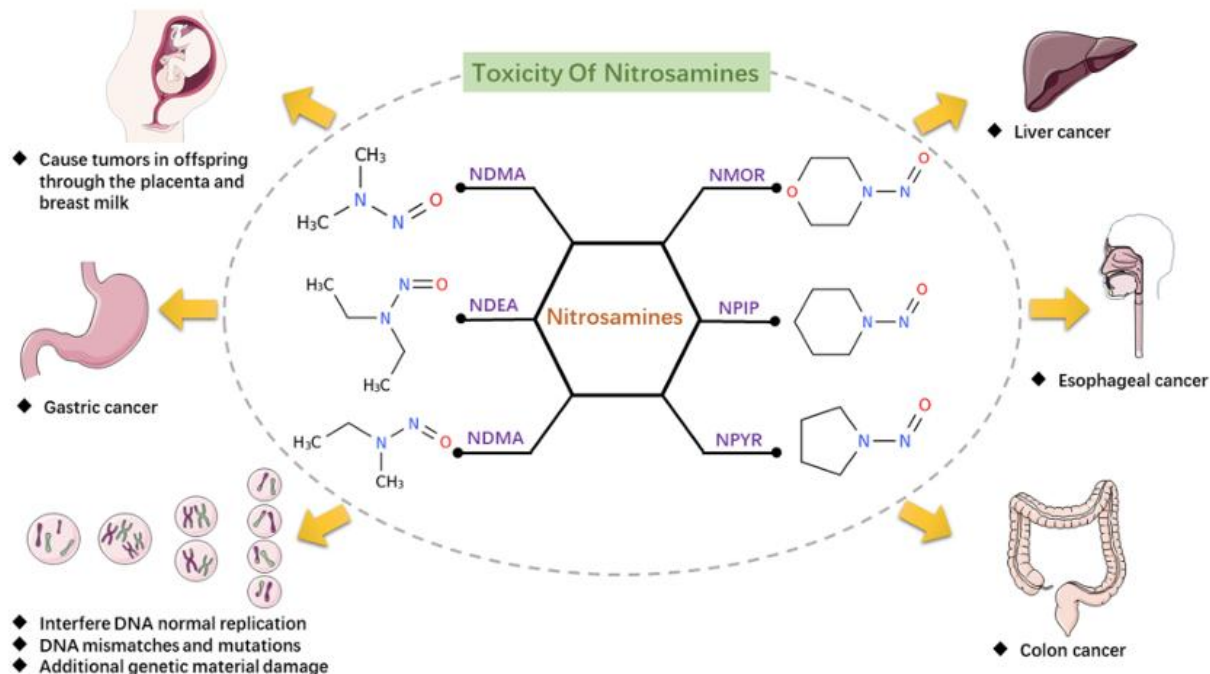
- Nitrosaminy vznikají reakcí dusitanů a dusičnanů (používaných jako konzervanty) s aminy
- K tvorbě dochází za kyselých podmínek, při tepelném zpracování (pečení, smažení) nebo při uzení masa
- Typické zdroje: fermentované a sušené salámy, slanina, klobásy, uzené maso
- Nejčastější nitrosaminy: NDMA, NDEA, NPIP, NPYR, NMOR a NDMA
- Studie z r.2023 udává průměrné koncentrace v uzeninách:
 - NDMA = 0.7 – 2.7 µg/kg; NDEA = 0.04 – 0.9 µg/kg ⁽¹⁾



(1) Xie et al. 2023

Nitrosaminy: Toxicita a zdravotní dopady

- V organismu mohou tvořit reaktivní diazoniové soli, které reagují s DNA → poškození genetického materiálu a mutace⁽¹⁾
- Nejčastěji poškozují játra, ale také jícen, žaludek, tlusté střevo, slinivku⁽²⁾
- V roce 2015 IARC (mezinárodní agentura pro výzkum rakoviny) zařadila zpracované masné výrobky do kategorie 1 (prokazatelně karcinogenní), mimo jiné kvůli riziku vzniku nitrosaminů⁽²⁾



(1) Xie et al. 2023

(2) FoodSafety.Institute. 2024

- EU:
 - Není stanoven přímý limit pro nitrosaminy → regulace přídavku dusitanů a dusičnanů
 - 120 mg/kg NaNO_2 – většina tepelně opracovaných masných výrobků
 - 82 mg/kg NaNO_2 – vybrané neopracované/krátce opracované produkty
 - Stanoven akceptovatelný denní příjem (ADI)
 - Dusitan sodný: 0-0.07 mg/kg tělesné hmotnosti/den (JECFA/EFSA)
- USA:
 - Stanoven limit pro sumu těkavých nitrosaminů → 10 ug/kg pro slaninu (pumped bacon)
- Kanada:
 - 10 ug/kg – NDMA, NDEA, NDPA, NDBA, NPIP
 - 15 ug/kg – NPYR→ uzené masné výrobky
- Čína:
 - NDMA v masných výrobcích: max 3 ug/kg

LC-MS/MS analýza Nitrosaminů v masných výrobcích

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- Nitrosaminy se v masných výrobcích vyskytují ve velmi nízkých koncentracích → nutná vysoká citlivost MS instrumentace
- Xevo TQ Absolute umožňuje dosažení velmi nízkých limitů kvantifikace
- Nutná selektivní analýza → MRM mod pro správnou kvantifikaci a confirmaci
- Masné výrobky = komplexní matrice → Jednoduchá a rychlá QuEChERS extrakce

The 9 nitrosamines analyzed were from the commercially available EPA 8270 Nitrosamine standard mix

NDMA	N-Nitrosodimethylamine
NMEA	N-Nitrosomethylethylamine
NPYR	1-Nitrosopyrrolidine
NDEA	N-Nitrosodiethylamine
NPIP	1-Nitrosopiperidine
NMOR	Nitrosomorpholine
NDPA	N-Nitrosodipropylamine
NDBA	N-Nitrosodibutylamine
NDPhA	N-Nitrosodiphenylamine

Sample Preparation – based on QuEChERS

QuEChERS

5 g of homogenised sample + 10 mL H₂O



Add 10 mL 1% FA in ACN



Add QuEChERS salts (p/n 186006813) and shake 30 min
(1 g Trisodium Citrate Dihydrate, 0.5 g Disodium Hydrogencitrate
Sesquihydrate, 1 g NaCl and 4 g MgSO₄)



Centrifuge for 10 min at 6000 rpm at room temperature



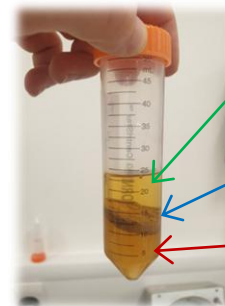
Put in freezer (-20°C) overnight



Centrifuge for 10 min at 6000 rpm at -5°C



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Acetonitrile layer (+ **Analytes**)

Solid sample

Aqueous layer (+ **saturated
buffer salts + ionic/polar
components**)

Sample Preparation – based on QuEChERS

Waters™

QuEChERS

5 g of homogenised sample + 10 mL H₂O

Add 10 mL 1% FA in ACN

Add QuEChERS salts (p/n 186006813) and shake 30 min
(1 g Trisodium Citrate Dihydrate, 0.5 g Disodium Hydrogencitrate Sesquihydrate, 1 g NaCl and 4 g MgSO₄)

Centrifuge for 10 min at 6000 rpm at room temperature

Put in freezer (-20°C) overnight

Centrifuge for 10 min at 6000 rpm at -5°C

Transfer 1 mL of supernatant into dSPE (p/n 186004832)

Vortex for 1 min

Centrifuge for 10 min at > 6000 rpm

Dilute 200 µL supernatant with 800 µL of H₂O

Filter through 13 mm 0.45 µm PTFE syringe filter directly into LC vial



Acetonitrile layer (+ Analytes)



dSPE

150 mg MgSO₄

25 mg PSA

25 mg C18

Conversion factor between ng/mL and µg/kg:

$$\frac{\text{ng}}{\text{mL}} * \frac{10 \text{ mL}}{5 \text{ g}} * \frac{1000 \text{ µL}}{200 \text{ µL}} = 10 \text{ ng/g}$$



Clean-up

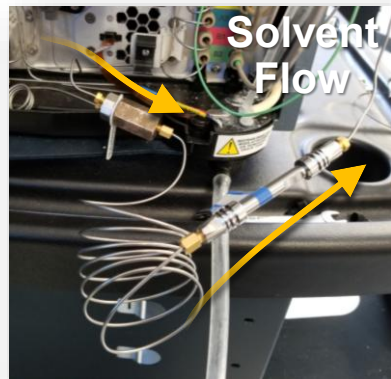
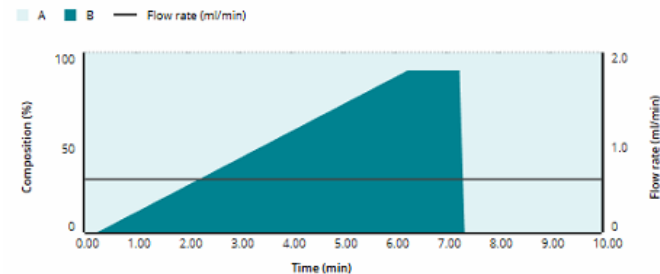
UPLC Conditions

Waters™



UPLC Conditions

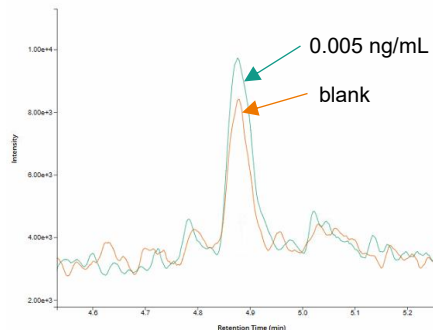
Column	ACQUITY UPLC CSH Phenyl-Hexyl, 1.7 µm, 3.0 x 100 mm
Column Temp	40 °C
Sample Temp	10 °C
Injection volume	4.0 µL sample
Flow Rate	0.6 mL/min
Mobile Phase A	Water + 0.1% formic acid
Mobile Phase B	Methanol + 0.1% formic acid



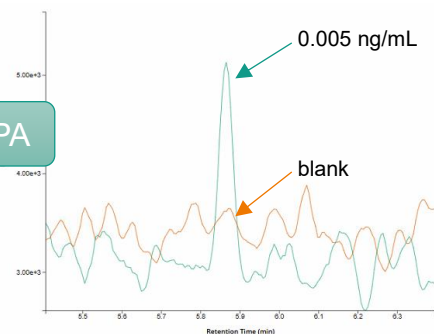
Isolator column:

Atlantis Premier BEH C18 AX,
5 µm, 4.6 x 50 mm

without Isolator column



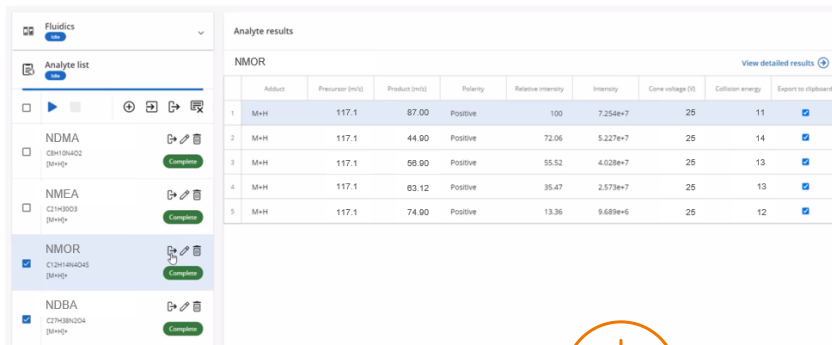
with Isolator column



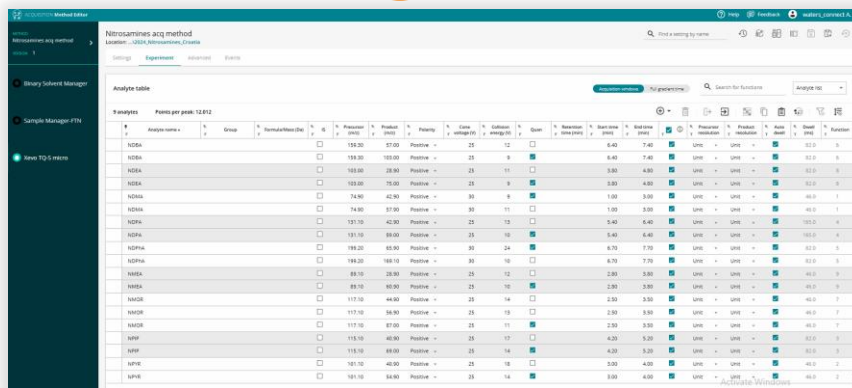
Waters™



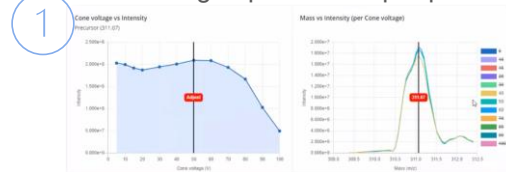
MRM Optimization Tool



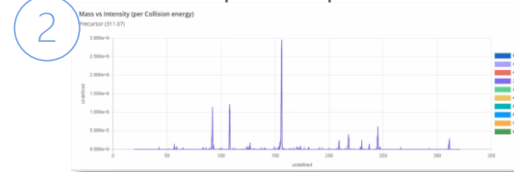
Acquisition Method Editor



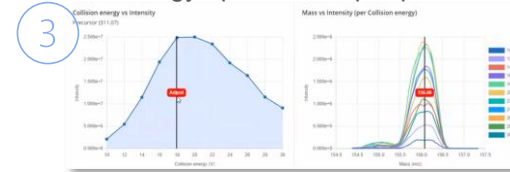
Cone voltage optimisation per precursor ion



Product ion spectrum per collision energy



Collision energy optimisation per product ion



Optimization of MS parameters in waters_connect

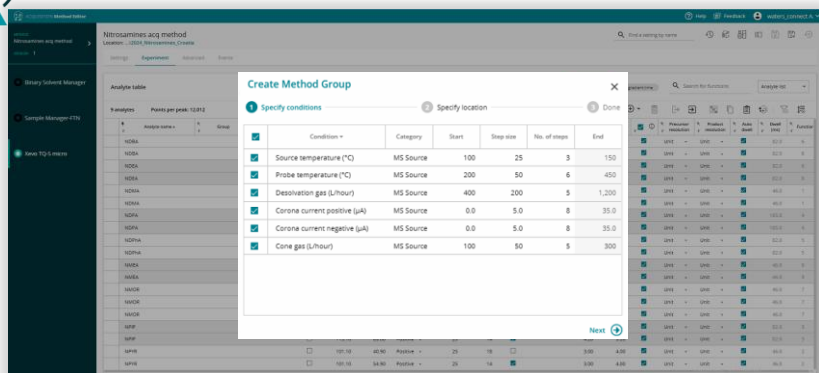
Waters™



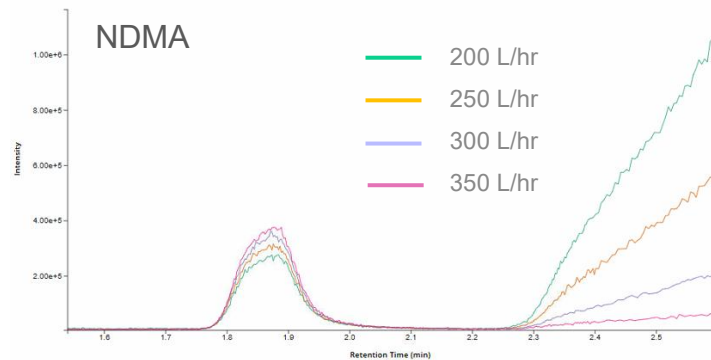
Acquisition Method Editor



LC-MS Toolkit



Cone gas flow



Sample Submission

James Lovely Data from Kate Foster

View full status in System Console

6 mix_Kates Method Method

Sample Trays

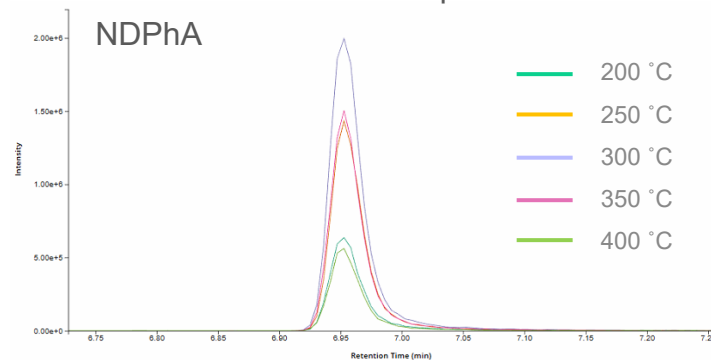
Sample Submission

Sample List Queue Real Time Data

Kates brilliant source optimisation

	Item Name	Item Description	Injection Volume (uL)	Sample Position	Replicates	Acquisition Method	Run Time (min)
1	Capillary Voltage 0.5kV	Capillary Voltage 0.5kV	10.00	1.8,1	1	CapPos-0.50 (6 mix_Kates Method)	2.50
2	Capillary Voltage 1kV	Capillary Voltage 1kV	10.00	1.8,1	1	CapPos-1.00 (6 mix_Kates Method)	2.50
3	Capillary Voltage 1.5kV	Capillary Voltage 1.5kV	10.00	1.8,1	1	CapPos-1.50 (6 mix_Kates Method)	2.50
4	Capillary Voltage 2.0kV	Capillary Voltage 2.0kV	10.00	1.8,1	1	CapPos-2.00 (6 mix_Kates Method)	2.50
5	Capillary Voltage 2.5kV	Capillary Voltage 2.5kV	10.00	1.8,1	1	CapPos-2.50 (6 mix_Kates Method)	2.50
6	Capillary Voltage 3kV	Capillary Voltage 3kV	10.00	1.8,1	1	CapPos-3.00 (6 mix_Kates Method)	2.50
7	Capillary Voltage 3.5kV	Capillary Voltage 3.5kV	10.00	1.8,1	1	CapPos-3.50 (6 mix_Kates Method)	2.50

Probe temperature



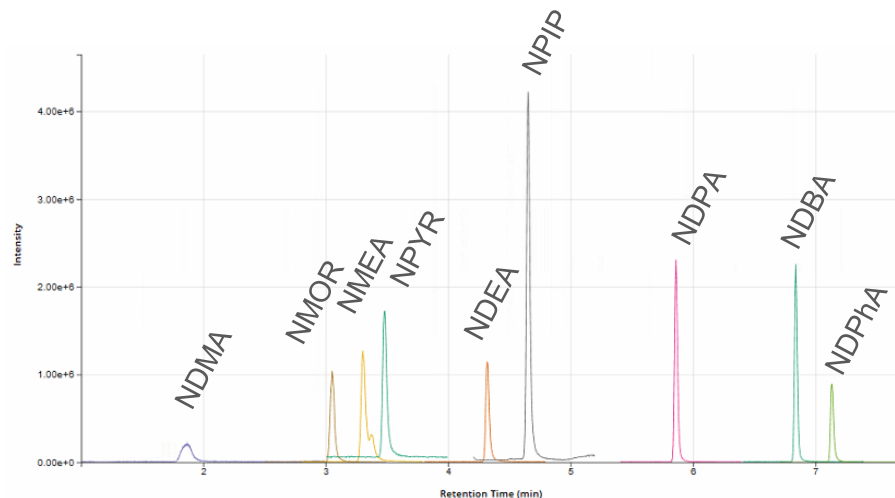
MS Settings – APCI

Xevo™
TQ ABSOLUTE



MS Conditions

APCI - Current Mode (positive)	1.4 μ A
APCI Probe Temp	300°C
Source Temp	130 °C
Desolvation Gas Flow	1000 L/hr
Cone Gas Flow	400 L/hr
Nebuliser Gas Flow	300 L/hr



Waters™

Compound	Parent	Daughter	Cone (V)	Collision (eV)	Soft transmission
NDMA	74.9	42.9	30	9	
NDMA	74.9	57.9	30	9	
NMEA	89.1	28.9	25	12	☒
NMEA	89.1	60.9	25	10	☒
NPYR	101.1	40.9	25	18	
NPYR	101.1	54.9	25	14	
NDEA	103.0	28.9	25	11	☒
NDEA	103.0	75.0	25	9	☒
NPIP	115.1	40.9	25	17	
NPIP	115.1	69.0	25	14	
NMOR	117.1	44.9	25	14	
NMOR	117.1	56.9	25	13	
NMOR	117.1	87.0	25	11	
NDPA	131.1	42.9	25	13	☒
NDPA	131.1	89.0	25	10	☒
NDBA	159.3	57.0	25	12	☒
NDBA	159.3	103.0	25	9	☒
NDPhA	199.2	65.9	30	24	☒
NDPhA	199.2	169.1	30	10	☒

MS Settings – APCI

Xevo™
TQ ABSOLUTE

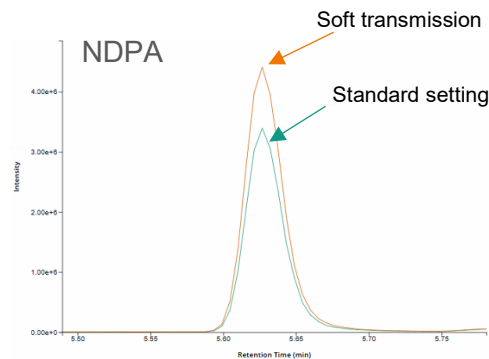
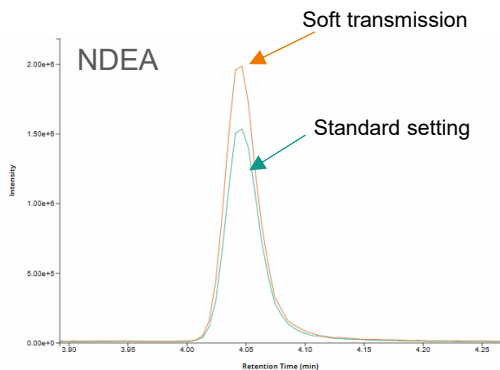


MS Conditions

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APCI Probe Temp	300°C
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Waters™

Compound	Parent	Daughter	Cone (V)	Collision (eV)	Soft transmission
NDMA	74.9	42.9	30	9	
NDMA	74.9	57.9	30	9	
NMEA	89.1	28.9	25	12	☒
NMEA	89.1	60.9	25	10	☒
NPYR	101.1	40.9	25	18	
NPYR	101.1	54.9	25	14	
NDEA	103.0	28.9	25	11	☒
NDEA	103.0	75.0	25	9	☒
NPIP	115.1	40.9	25	17	
NPIP	115.1	69.0	25	14	
NMOR	117.1	44.9	25	14	
NMOR	117.1	56.9	25	13	
NMOR	117.1	87.0	25	11	
NDPA	131.1	42.9	25	13	☒
NDPA	131.1	89.0	25	10	☒
NDBA	159.3	57.0	25	12	☒
NDBA	159.3	103.0	25	9	☒
NDPhA	199.2	65.9	30	24	☒
NDPhA	199.2	169.1	30	10	☒

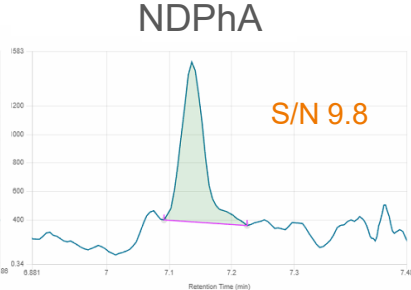
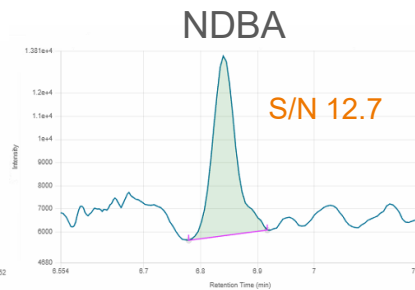
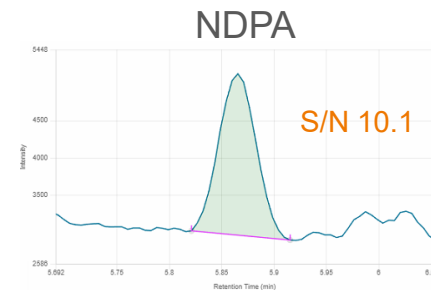
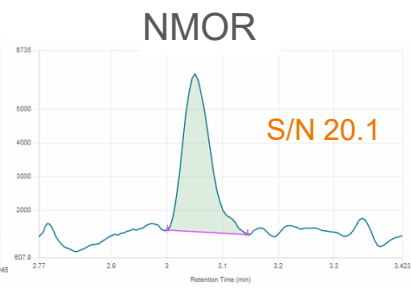
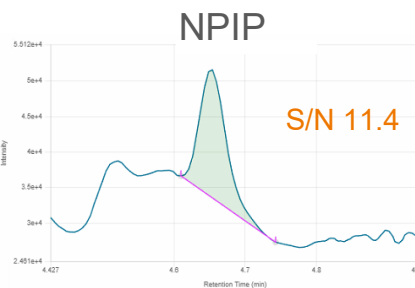
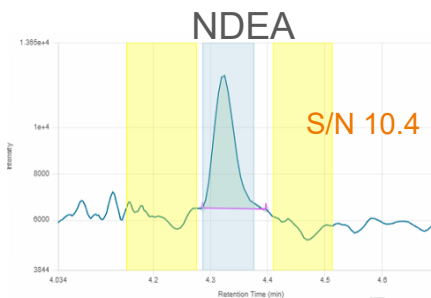
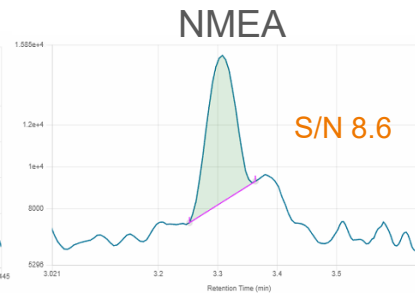
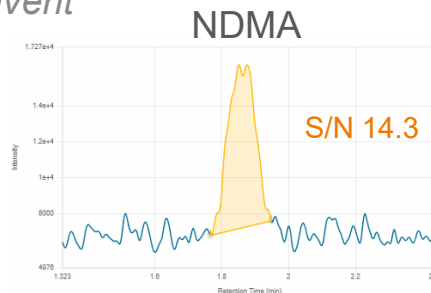


Solvent calibrations

Chromatograms at LOQ levels in solvent

Waters™
NPYR

Compound	LOQ (ng/mL)	Calibration range (ng/mL)	Correlation coefficient
NDMA	0.25	0.25 - 50	0.9998
NMEA	0.05	0.05 - 50	0.9999
NPYR	0.1	0.1 - 50	0.9998
NDEA	0.025	0.025 - 50	0.9998
NPIP	0.025	0.025 - 50	0.9998
NMOR	0.025	0.025 - 50	0.9998
NDPA	0.005	0.005 - 50	0.9998
NDBA	0.01	0.01 - 50	0.9997
NDPhA	0.01	0.01 - 50	0.9993



Solvent calibrations

Linearity up to 50 ng/mL

Waters™

Compound	LOQ (ng/mL)	Calibration range (ng/mL)	Correlation coefficient
NDMA	0.25	0.25 - 50	0.9998
NMEA	0.05	0.05 - 50	0.9999
NPYR	0.1	0.1 - 50	0.9998
NDEA	0.025	0.025 - 50	0.9998
NPPI	0.025	0.025 - 50	0.9998
NMOR	0.025	0.025 - 50	0.9998
NDPA	0.005	0.005 - 50	0.9998
NDBA	0.01	0.01 - 50	0.9997
NDPhA	0.01	0.01 - 50	0.9993

Cals & QCs

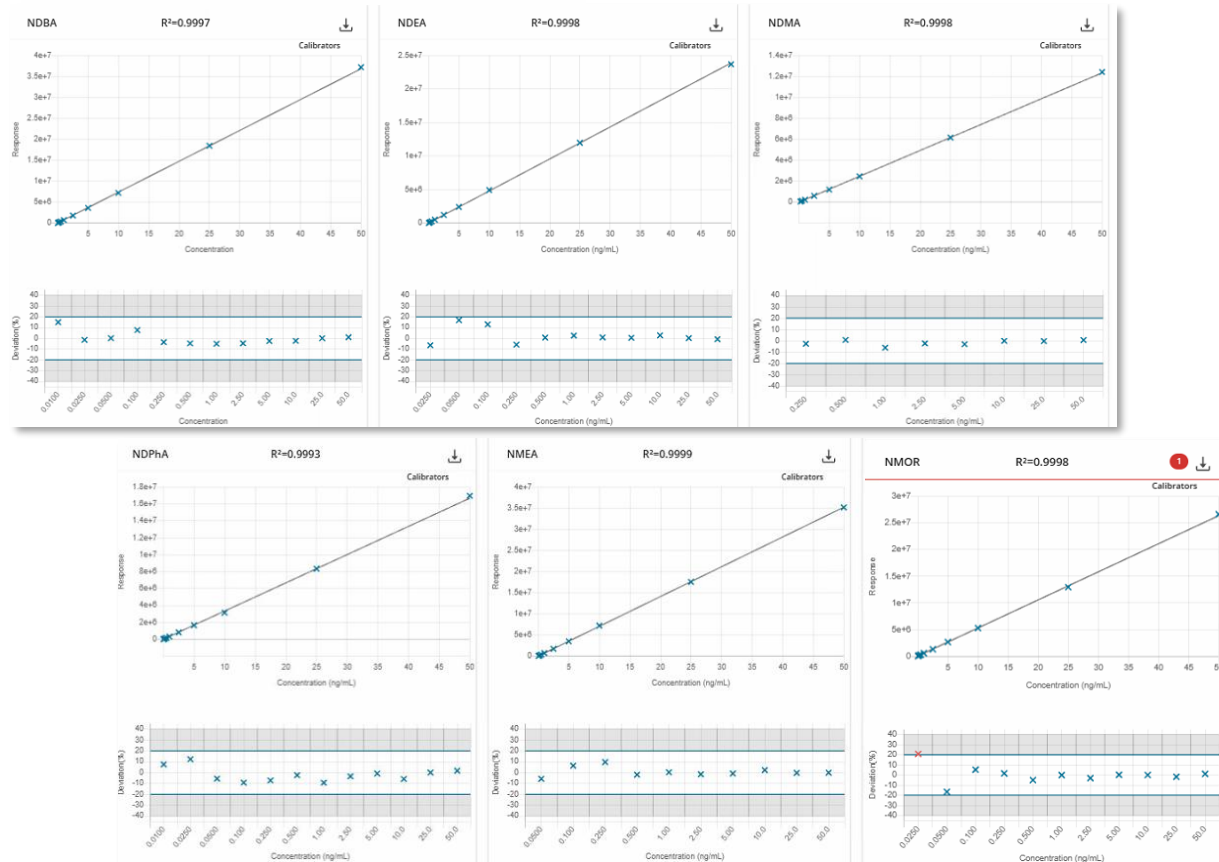
Exception filter

R Squared (0)

Cal Deviation (1)

QC Deviation (0)

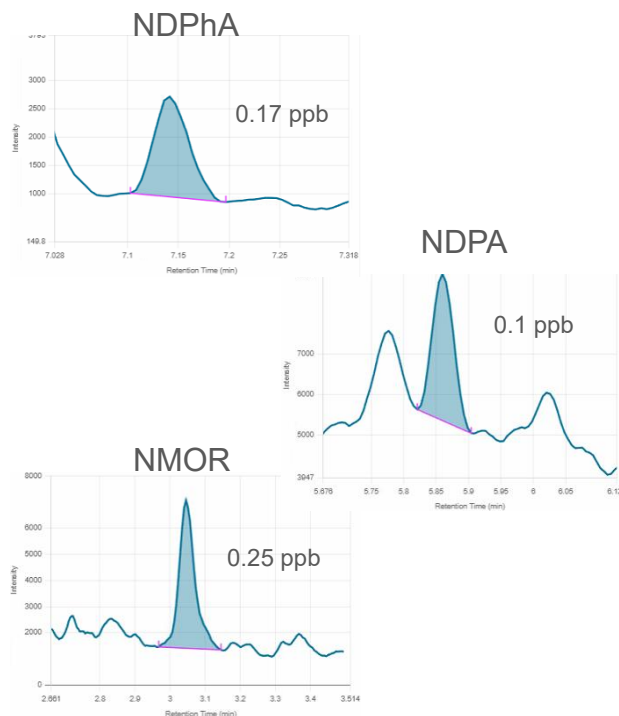
Signal to Noise (0)



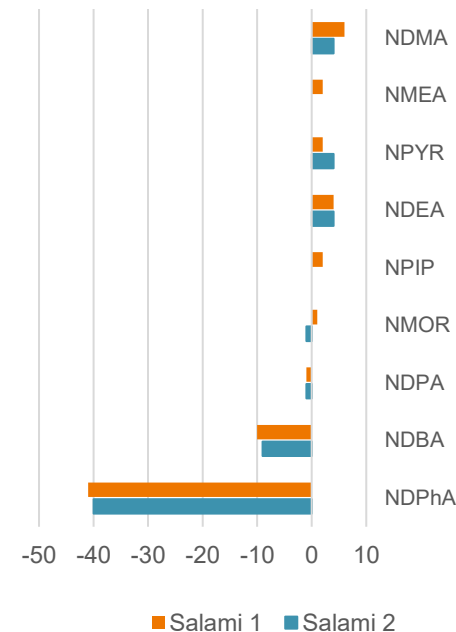
Nitrosamines in salami samples

Compound	Sample	Recovery (%)	Extrapolated LOQ in salami samples µg/kg
NDMA	Salami 1	96	2.5
	Salami 2	89	2.5
NMEA	Salami 1	92	0.5
	Salami 2	90	0.5
NPYR	Salami 1	100	2.5
	Salami 2	94	1
NDEA	Salami 1	100	0.35
	Salami 2	97	0.35
NPIP	Salami 1	100	0.25
	Salami 2	99	0.25
NMOR	Salami 1	96	0.28
	Salami 2	97	0.28
NDPA	Salami 1	99	0.08
	Salami 2	100	0.08
NDBA	Salami 1	101	0.15
	Salami 2	97	0.15
NDPhA	Salami 1	100	0.17
	Salami 2	97	0.17

- Testováni dva druhy fermentovaných salámů
- Dosaženo nízkých LOQ od 0.08 ug/kg
- Výtěžnosti metody v rozmezí 89 – 101%



Matriční efekty



An abstract graphic featuring a complex network of interconnected nodes and lines, resembling a molecular structure or a data network. The nodes are represented by small circles in various shades of blue and grey, connected by thin, light blue lines. The background is a gradient of blue, transitioning from a lighter shade on the left to a darker shade on the right. A solid dark blue horizontal band runs across the middle of the image, serving as a backdrop for the company name.

Waters™