Stanovení nitrosaminů v uzeninách

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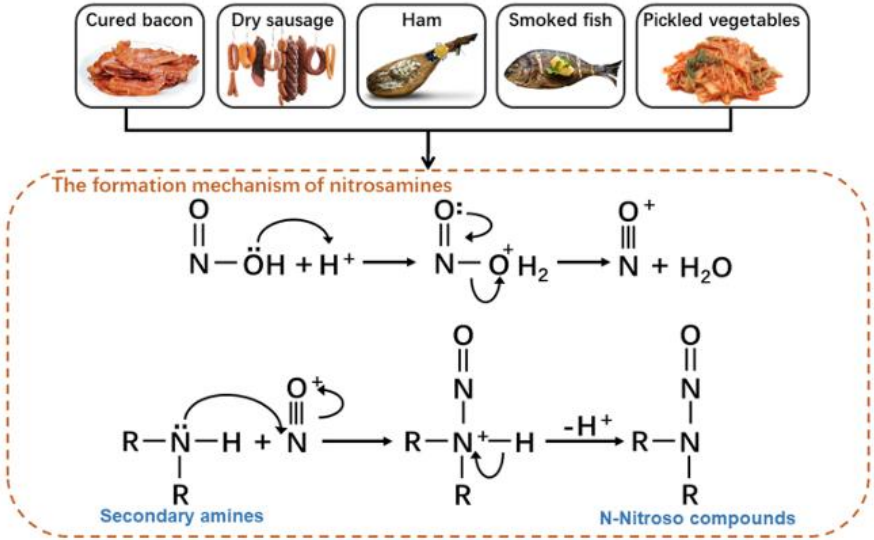
Nitrosaminy: Vznik a výskyt v masných výrobcích

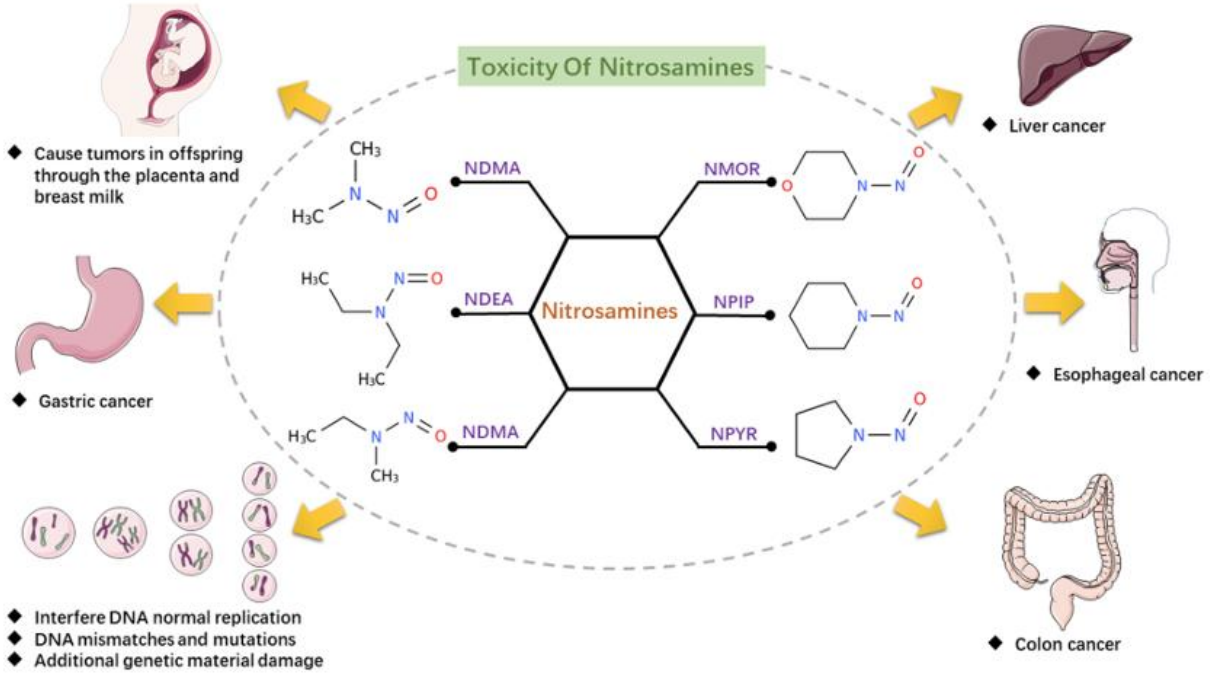
- Nitrosaminy vznikají reakcí dusitanů a dusičnanů (používaných jako konzervanty) s aminy
- K tvorbě dochází za kyselých podmínek, při tepelném zpracování (pečení, smažení) nebo při uzení masa
- Typické zdroje: fermentované a sušené salámy, slanina, klobásy, uzené maso
- Nejčastější nitrosaminy: NDMA, NDEA, NPIP, NPYR, NMOR a NDBA
- 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

Nitrosaminy: Legislativa

- 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 konfirmaci
- 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 H_2O

↓

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 Sesquilhydrate, 1 g NaCl and 4 g MgSO4)

↓

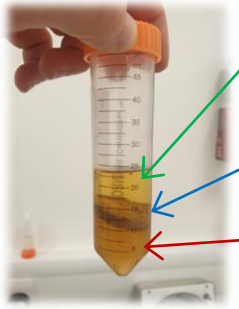
Centrifuge for 10 min at 6000 rpm at room temperature

↓

Put in freezer (-20°C) overnight

↓

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



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 H_2O

↓

Add 10 mL 1% FA in ACN

↓

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↓

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

Acetonitrile layer (+ **Analytes**)

Clean-up

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 H_2O

↓

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

dSPE

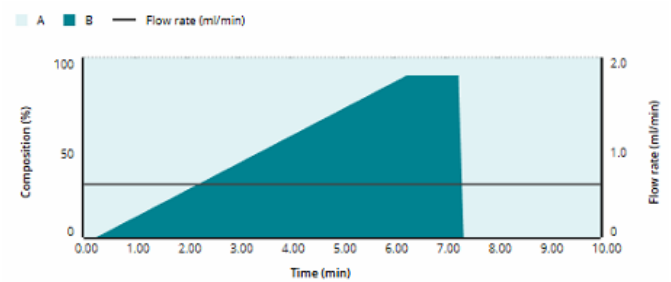
- 150 mg MgSO4
- 25 mg PSA
- 25 mg C18

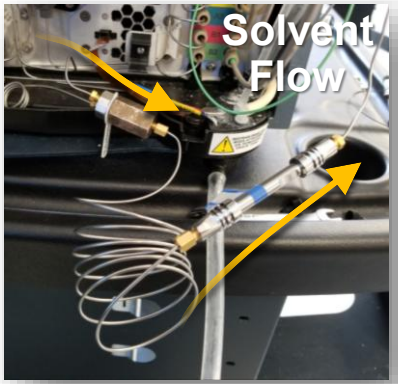
Conversion factor between ng/mL and µg/kg:

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

UPLC Conditions

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

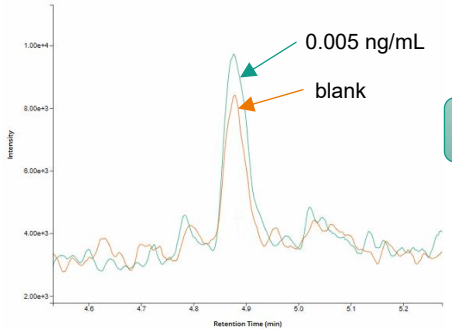




Isolator column:

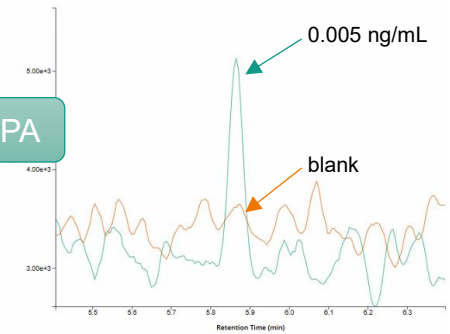
Atlantis Premier BEH C18 AX, 5 µm, 4.6 x 50 mm

without Isolator column



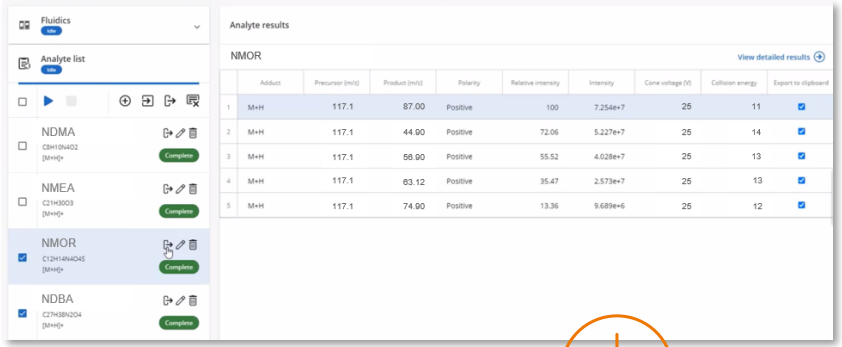
with Isolator column

NDPA



Optimization of MS parameters in waters_connect

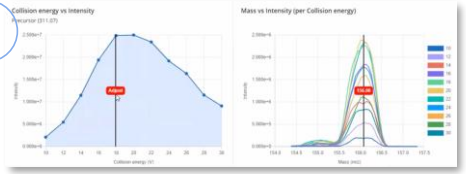
MRM Optimization Tool



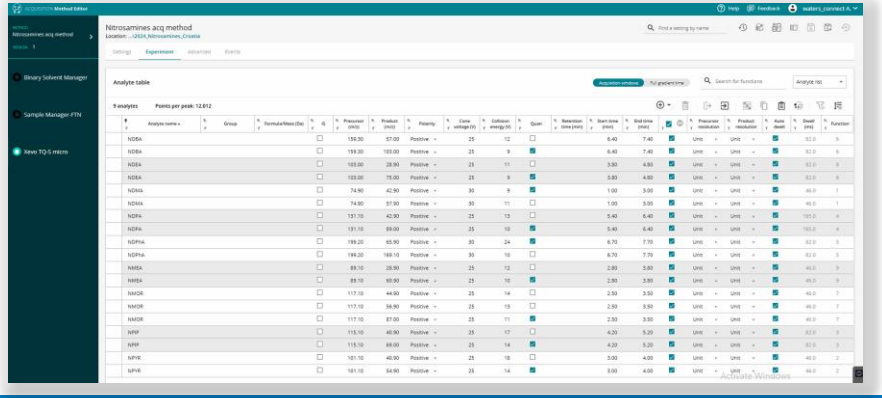
1 Cone voltage optimisation per precursor ion

2 Product ion spectrum per collision energy

3 Collision energy optimisation per product ion



Acquisition Method Editor



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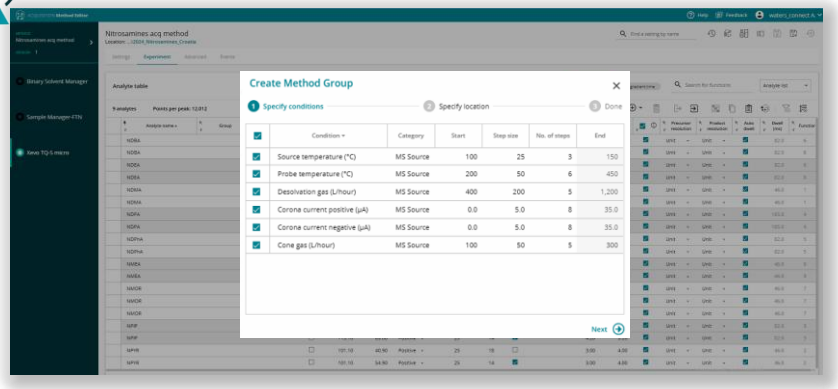
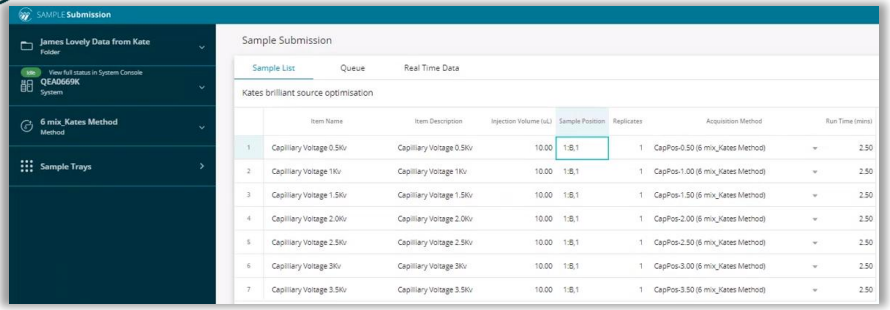
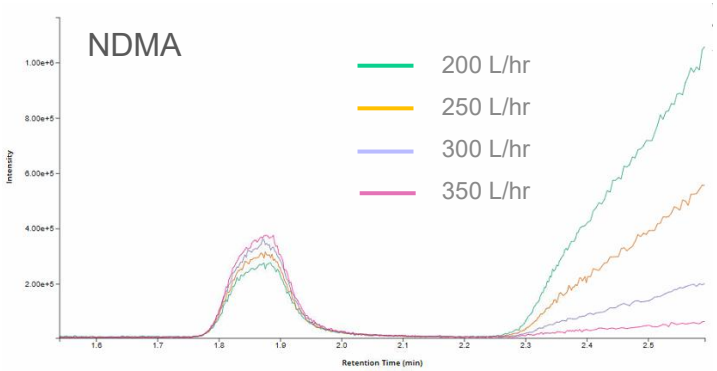


Sample Submission

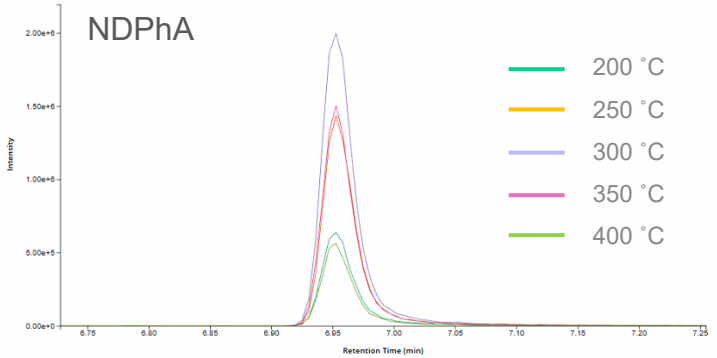


LC-MS Toolkit

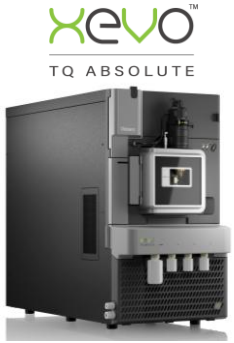
Cone gas flow



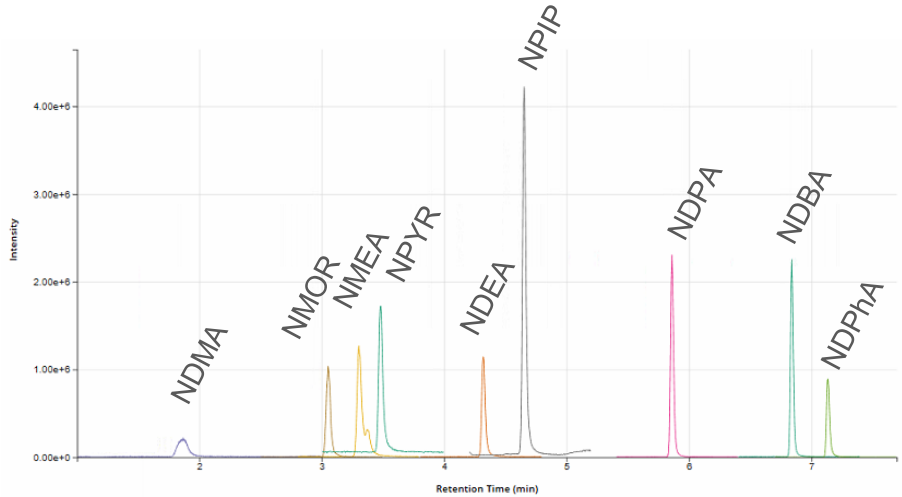
Probe temperature



MS Settings – APCI

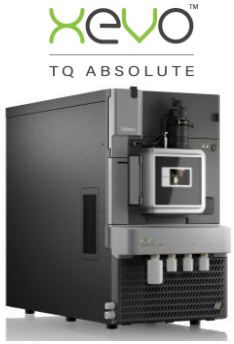


MS Conditions	
APCI - Current Mode (positive)	1.4 µA
APCI Probe Temp	300°C
Source Temp	130 °C
Desolvation Gas Flow	1000 L/hr
Cone Gas Flow	400 L/hr
Nebuliser Gas Flow	300 L/hr



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



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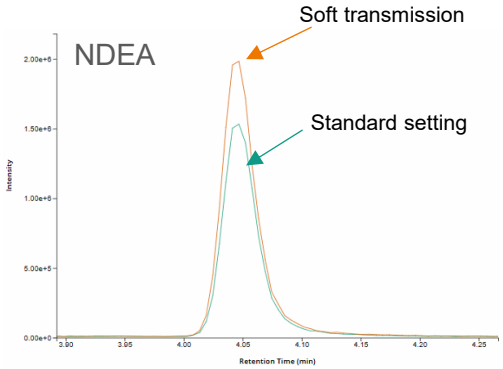

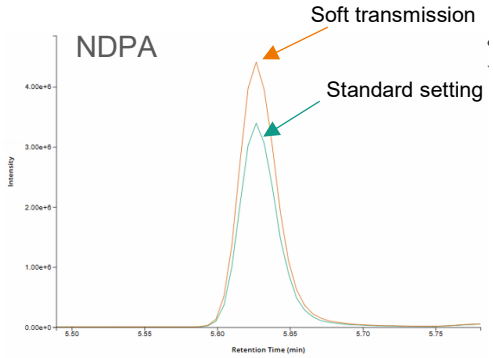

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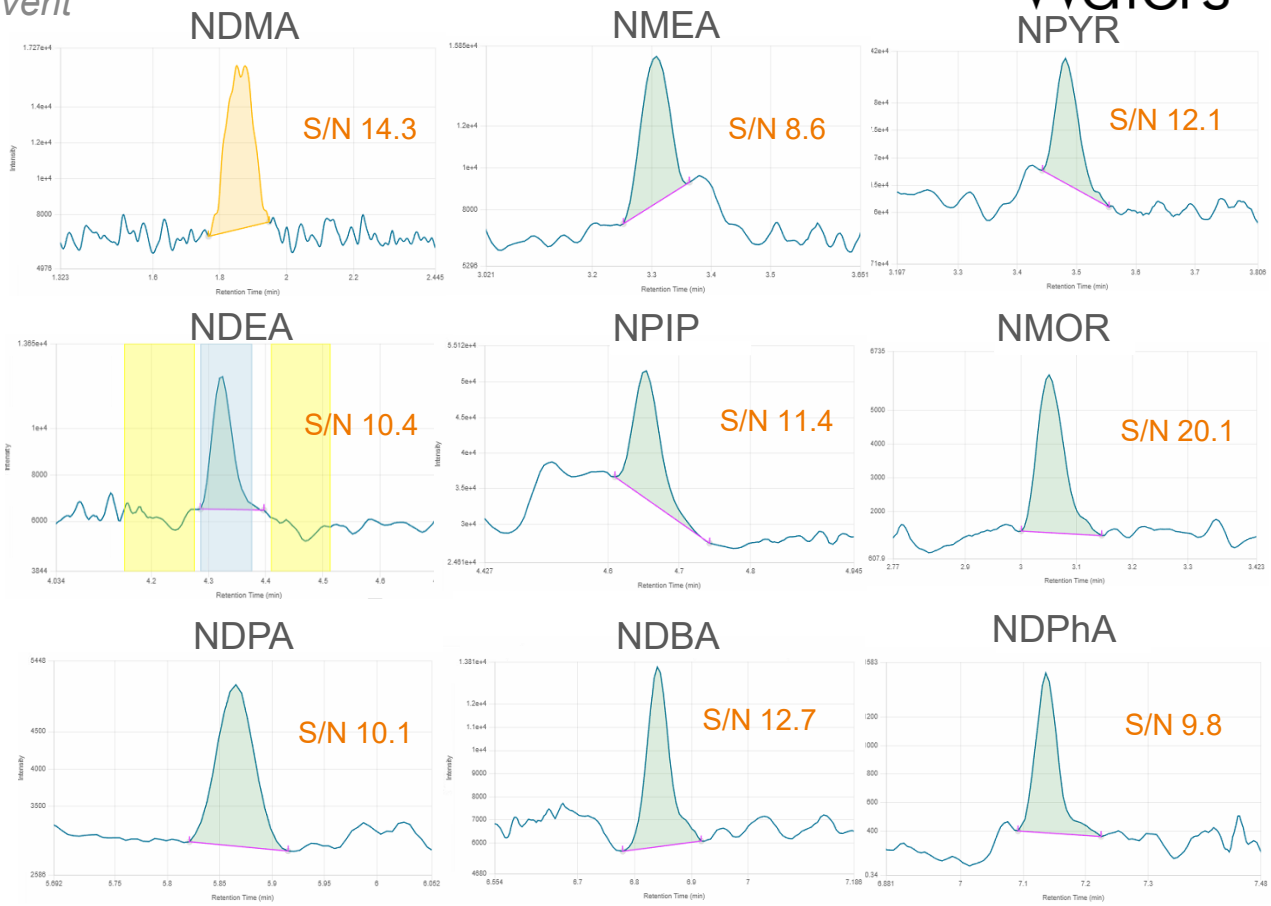
NDPhA	199.2	65.9	30	24	☑
NDPhA	199.2	169.1	30	10	☑

Solvent calibrations

Chromatograms at LOQ levels in solvent

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

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

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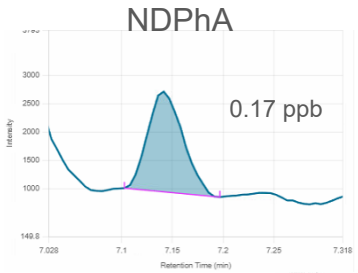
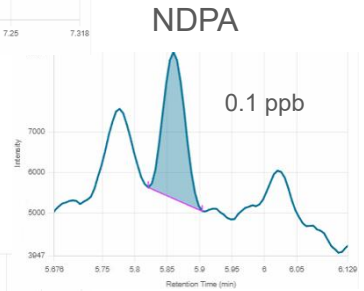
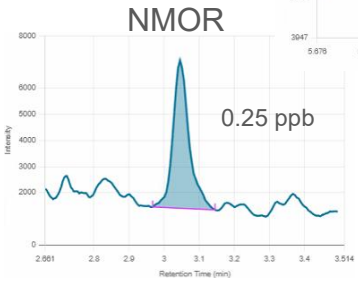
Nitrosamines in salami samples

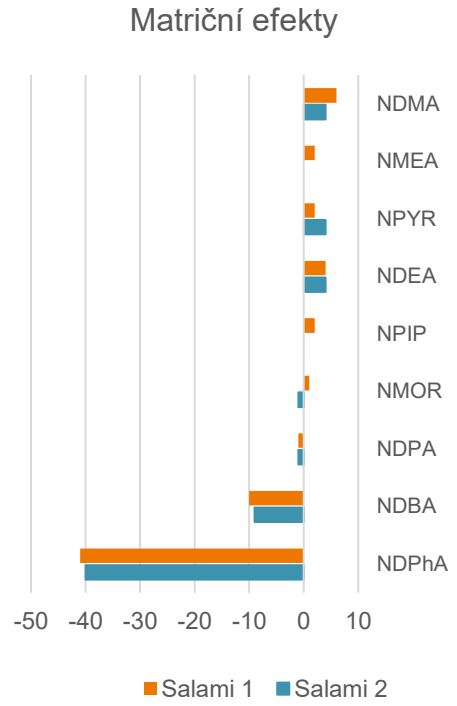
Compound	Sample	Recovery (%)	Extrapolated LOQ in salami samples µg/kg
NDMA	Salami 1	96	2.5
	Salami 2	89	2.5
NMEA	Salami 1	92	0.5
	Salami 2	90	0.5
NPYR	Salami 1	100	2.5
	Salami 2	94	1
NDEA	Salami 1	100	0.35
	Salami 2	97	0.35
NPIP	Salami 1	100	0.25
	Salami 2	99	0.25
NMOR	Salami 1	96	0.28
	Salami 2	97	0.28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

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









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