CIRM

LIÈGE université
Center for Interdisciplinary Research on Medicines

In-silico-assisted development of LC-MS/MS methods for the determination of 17 N-nitrosamines in a drug matrix

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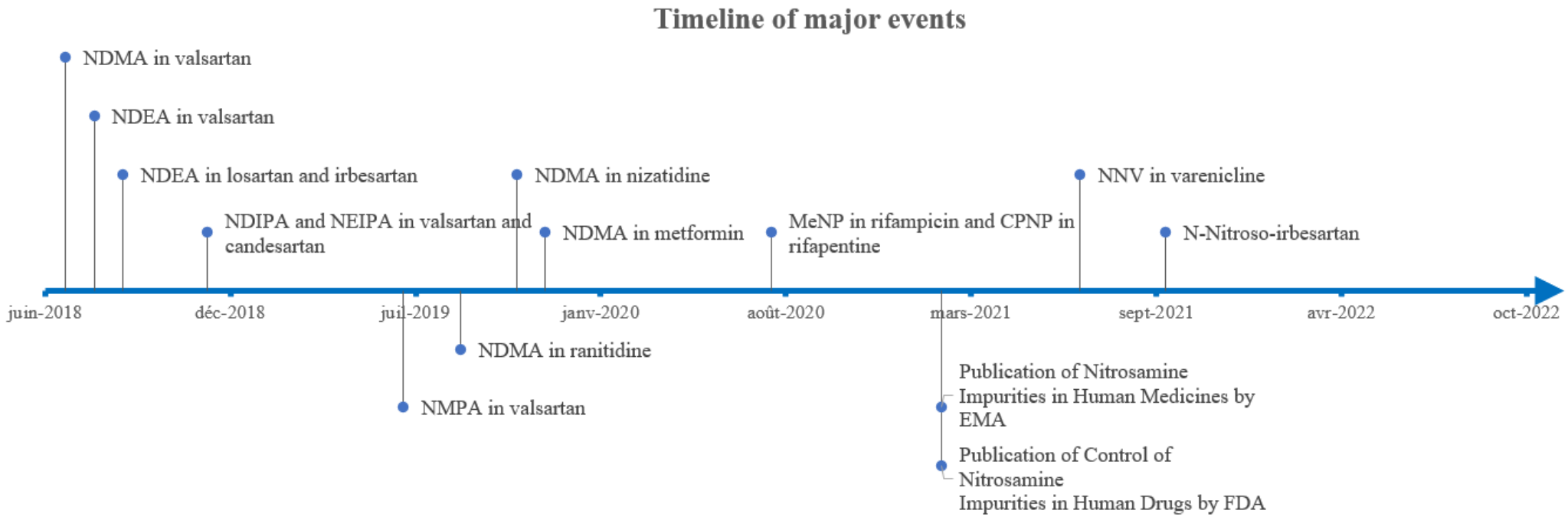
Agenda

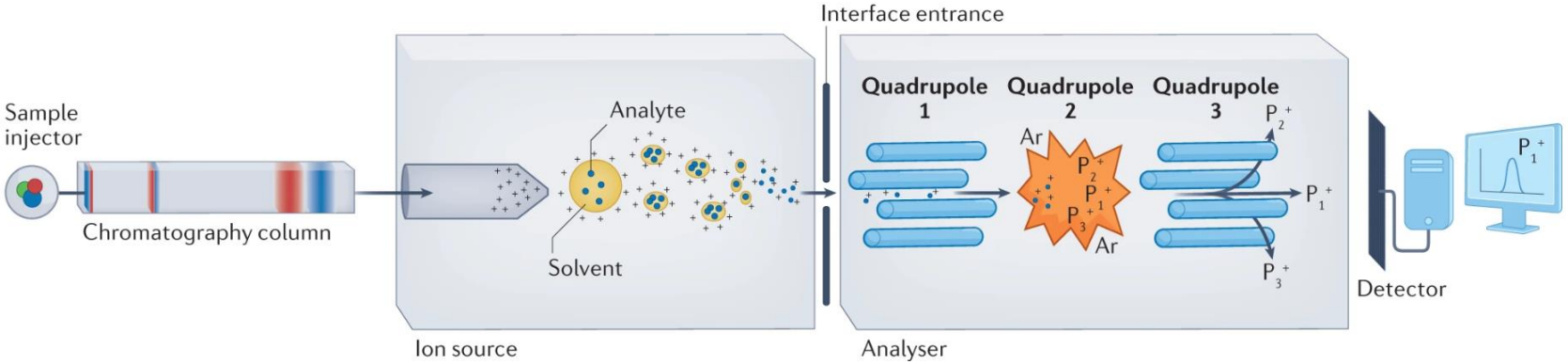
- Introduction
- Materials and methods
- Results
- Conclusions



- Some N-nitrosamines (NAs) classified in 2A or 2B groups by IARC
- EMA investigation procedure:
  - Risk assessment of presence of NAs
  - Confirmatory testing
  - Minimizing and monitoring the risk of contamination

Choice of analytical technique

- Need of the development and implementation of highly sensitive and specific methods as analytical tools
  - NAs detection and quantitation
  - Decision-making support
  - Ensuring public health
- Hyphenated technique – LC-MS/MS
  - LC: separation of volatile and non-volatile molecules
  - MS/MS detector: high sensitivity and fragmentation-based specificity



LC-MS/MS system

In-silico-assisted development of LC conditions

- EOS project

QSRR model

↓

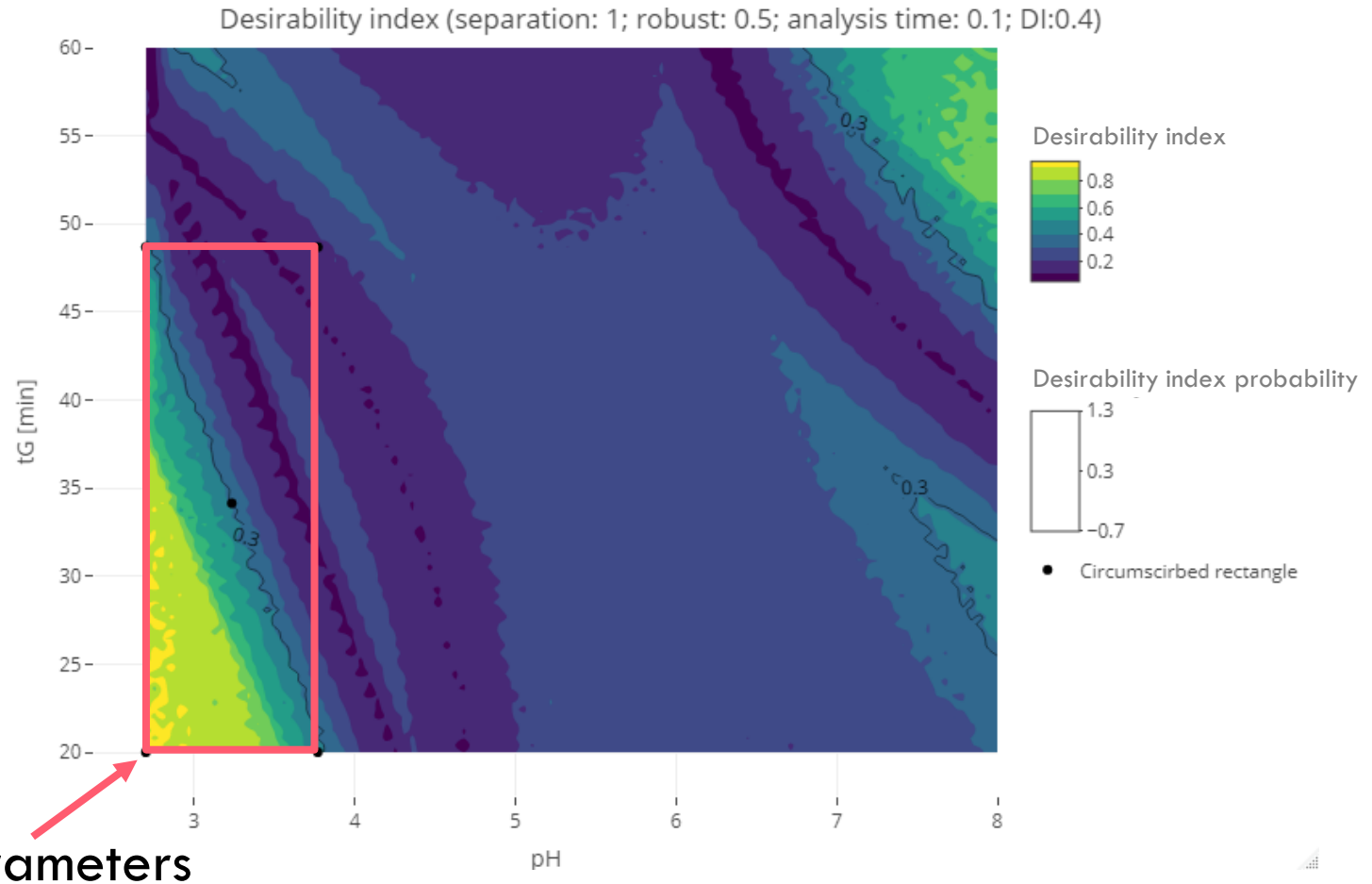
Response surface model

↓

DI graph

↓

Circumscribed rectangle



- Predicted optimal LC parameters
  - pH 2.7
  - tG 20.0 min

LC method

- Stationary phase: Acquity HSS T3 Premier, 100 X 2.1 mm (1.8 µm)
- Mobile phase
  - 0.1% FA in water, **pH 2.7**
  - 0.1% FA in MeOH
- **Gradient**

Time (min)	Flow rate (mL/min)	%A	%B
Initial	0.400	100.0	0.0
1.00	0.400	100.0	0.0
10.90	0.400	5.0	95.0
13.50	0.400	5.0	95.0
13.60	0.400	100.0	0.0
17.00	0.400	100.0	0.0

- Injection volume: 5 µL
- Column temperature: 45°C
- Autosampler temperature: 10°C

tG (time of gradient)

Geometric transfer

HPLC column 20.0 min → UPLC column 9.90 min

Acquity® Premier

Optimized MS/MS method

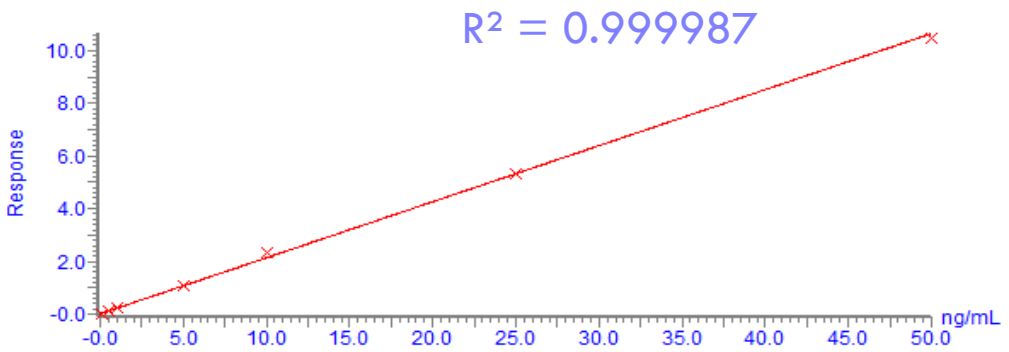
- Ionization mode: APCI +
- Corona current: 1.5 µA
- Gas flow
  - Desolvatation: 950 L/h
  - Nebulizer: 250 L/h
  - Cone: 250 L/h
  - Collision: 0.15 mL/min
- Source temperature: 120°C
- APCI probe temperature: 150°C or 250°C
- Collision energy: compound- and fragment-specific
- Cone voltage: compound-specific

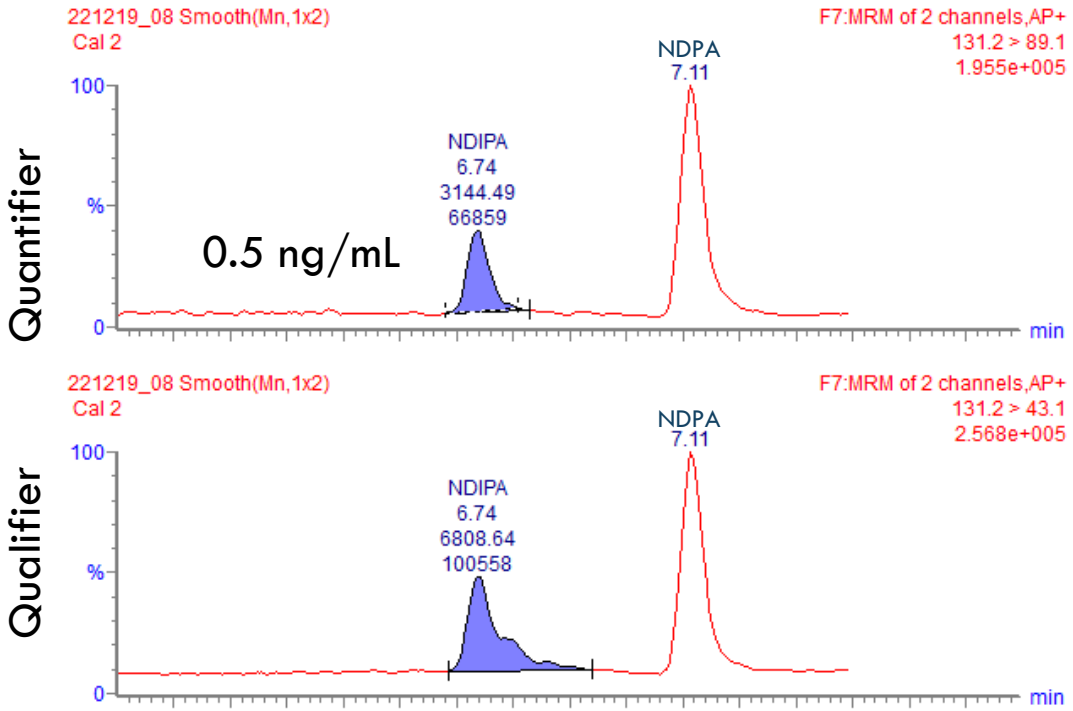
Xevo® TQ-Absolute

Chemicals

- 17 N-nitrosamine impurities
  - Two mass transitions monitored for each compound: a quantifier and a qualifier
- 8 deuterated internal standards
  - To cover the entire retention time range
  - To compensate for variability in the ionization process
  - To compensate for sample preparation variations

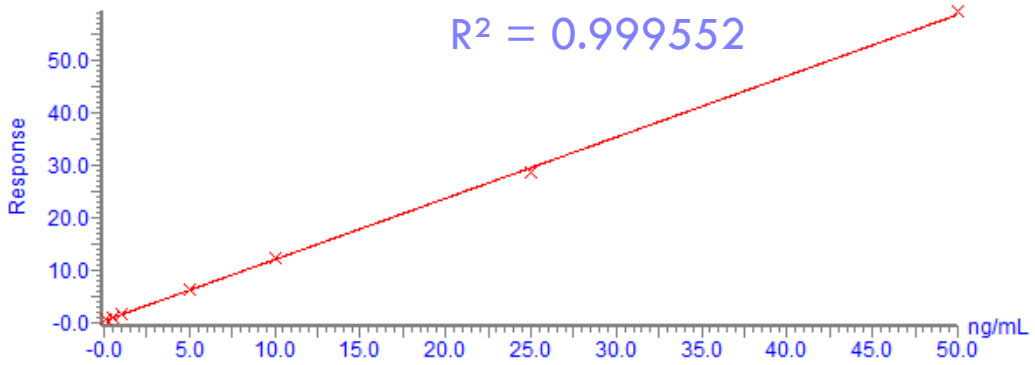
NDIPA – N-nitrosodiisopropylamine

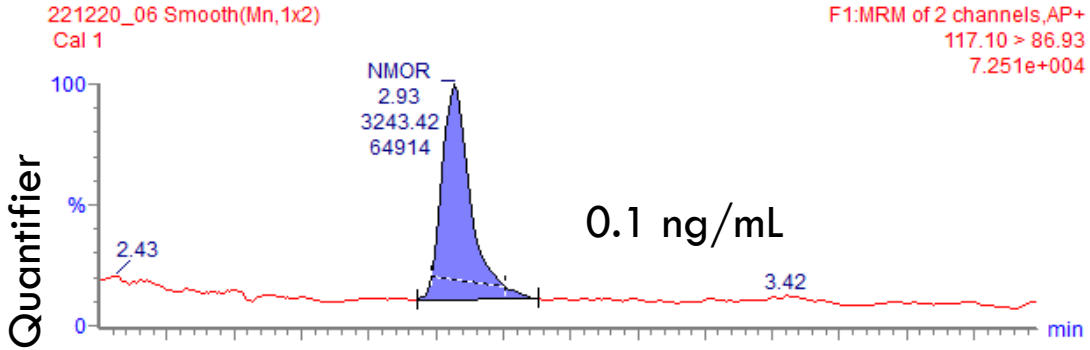


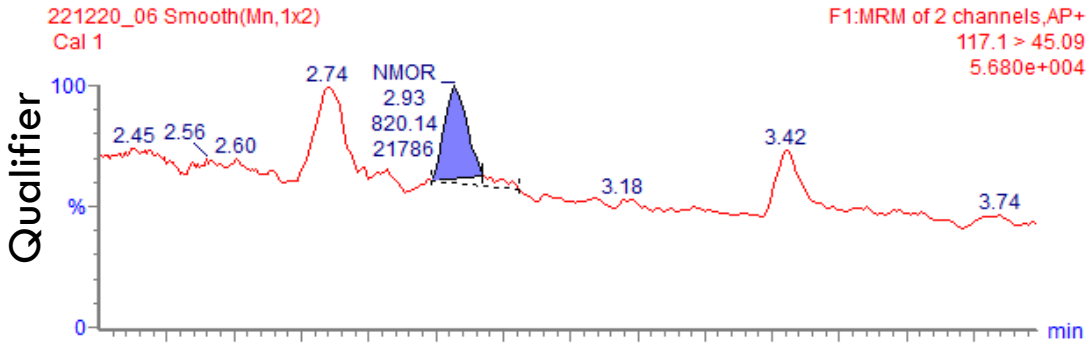


- Impossible to distinguish between NDIPA and NDPA despite MS/MS specificity
- Isobaric pair of NDIPA and NDPA chromatographically separated

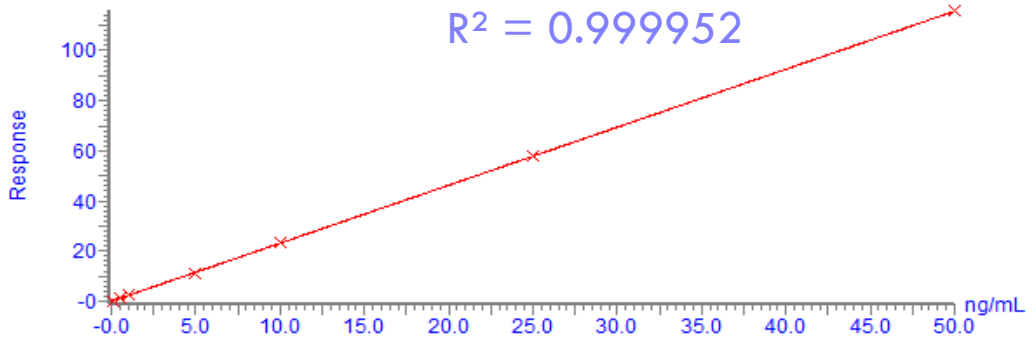
NMOR – N-nitrosomorpholine

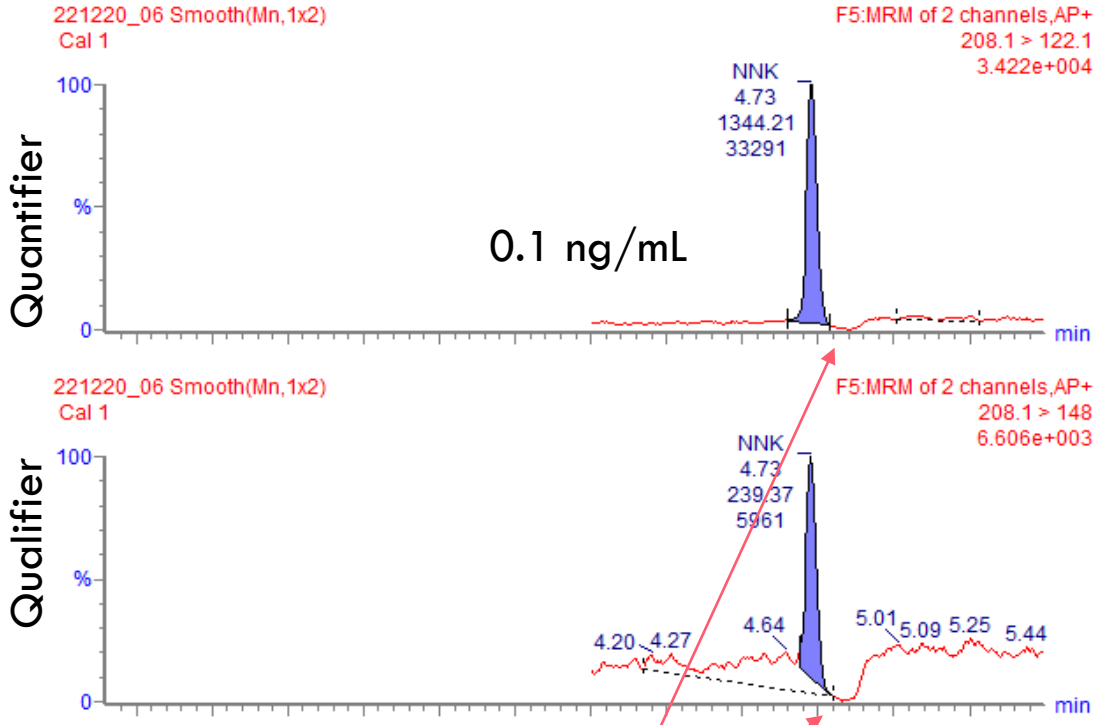






NNK – 4-(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone

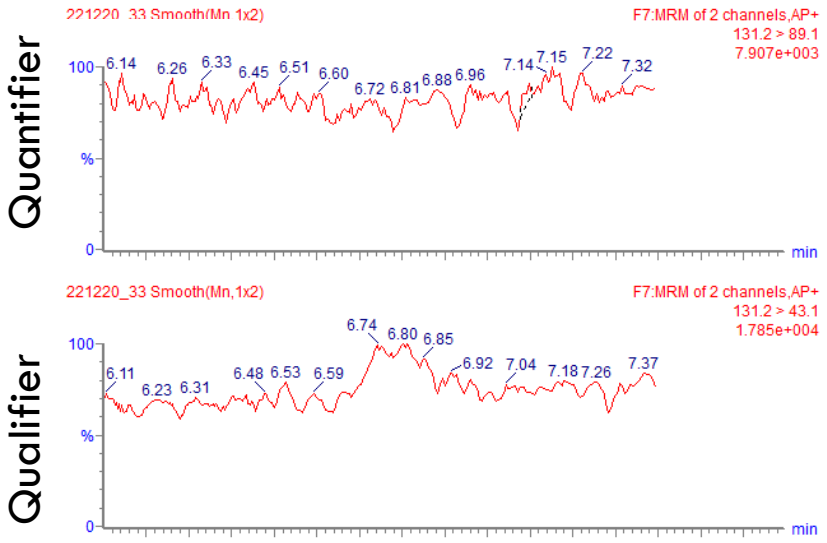




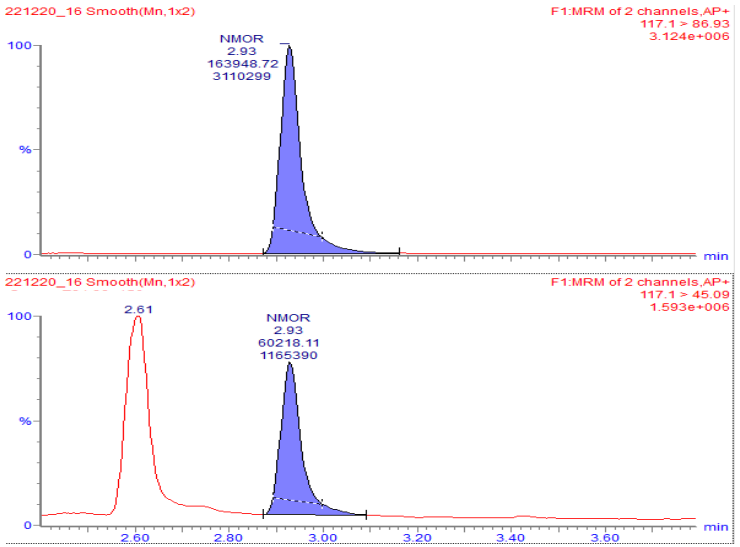
- Close to ion suppression zone

Analysis of real tablets

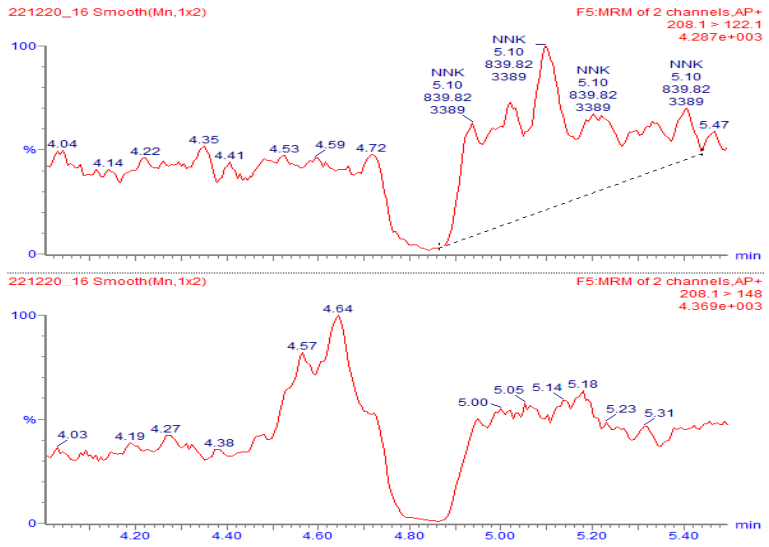
NDIPA & NDPA



NMOR



NNK



Ion suppression zone

Estimated NMOR content:

- 20.7 ng/mL or 8.09 ppm
- 8.09 ppm > 7.94 ppm (specification limit)

Specification limit, LLoQ to reach and estimated for each NA

N-nitrosamine (EMA watchlist)	Specification limit (ppm)	LLoQ to reach (ppm)	LLoQ to reach (ng/mL)		LLoQ estimated on Xevo TQ-Abs (ng/mL)	S/N ratio (Eur. Pharm.)
NDMA	6.00	0.600	1.536		0.500	24
NDEA	1.66	0.166	0.424	<	0.500	29
NEIPA	1.66	0.166	0.424		0.100	48
NDIPA	1.66	0.166	0.424	<	0.500	70
NDPA	1.66	0.166	0.424		0.100	24
NMBA acid	6.00	0.600	1.536		0.100	45
NDBA	1.66	0.166	0.424		0.100	123
NMOR	7.94	0.794	2.032		0.100	138
NMPA	2.14	0.214	0.549		0.100	197
NPIP	81.25	8.125	20.800		0.500	43
NTHP	2.31	0.231	0.592		0.100	11
NNK	6.25	0.625	1.600		0.100	184
NPYR	106.25	10.625	27.200		0.500	32
NDPhA	4875.00	487.500	1248.000		0.100	139

Specification limit = AI (Acceptable intake) / MDD (maximal daily dose)
LLoQ to reach (ppm) in order to omit the specification limit: LLoQ ≤ 10% specification limit
LLoQ to reach (ng/mL) calculated from the current sample preparation procedure

Conclusions

- Good linearity from 0.1 ng/mL to 50 ng/mL in spiked drug matrix
- Some NAs close to the ion suppression zone

  → But still show good linearity in the working concentration zone

  → Possibility to use a mobile phase at pH 5.0 to delay the retention time of the API
- LLoQ estimated for NDEA and NDIPA superior to required LLoQ
  - NDEA: 0.500 ng/mL > 0.424 ng/mL
  - NDIPA: 0.500 ng/mL > 0.424 ng/mL

  → Increase the injection volume up to 10 µL or set the lowest calibration point at 0.25 ng/mL
- Method validation

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Thank you

For Your Attention

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