Using novel stationary phase selectivity to address potential NDMA over-quantification due to isobaric interference in the LC-MS/MS analysis of nitrosamines

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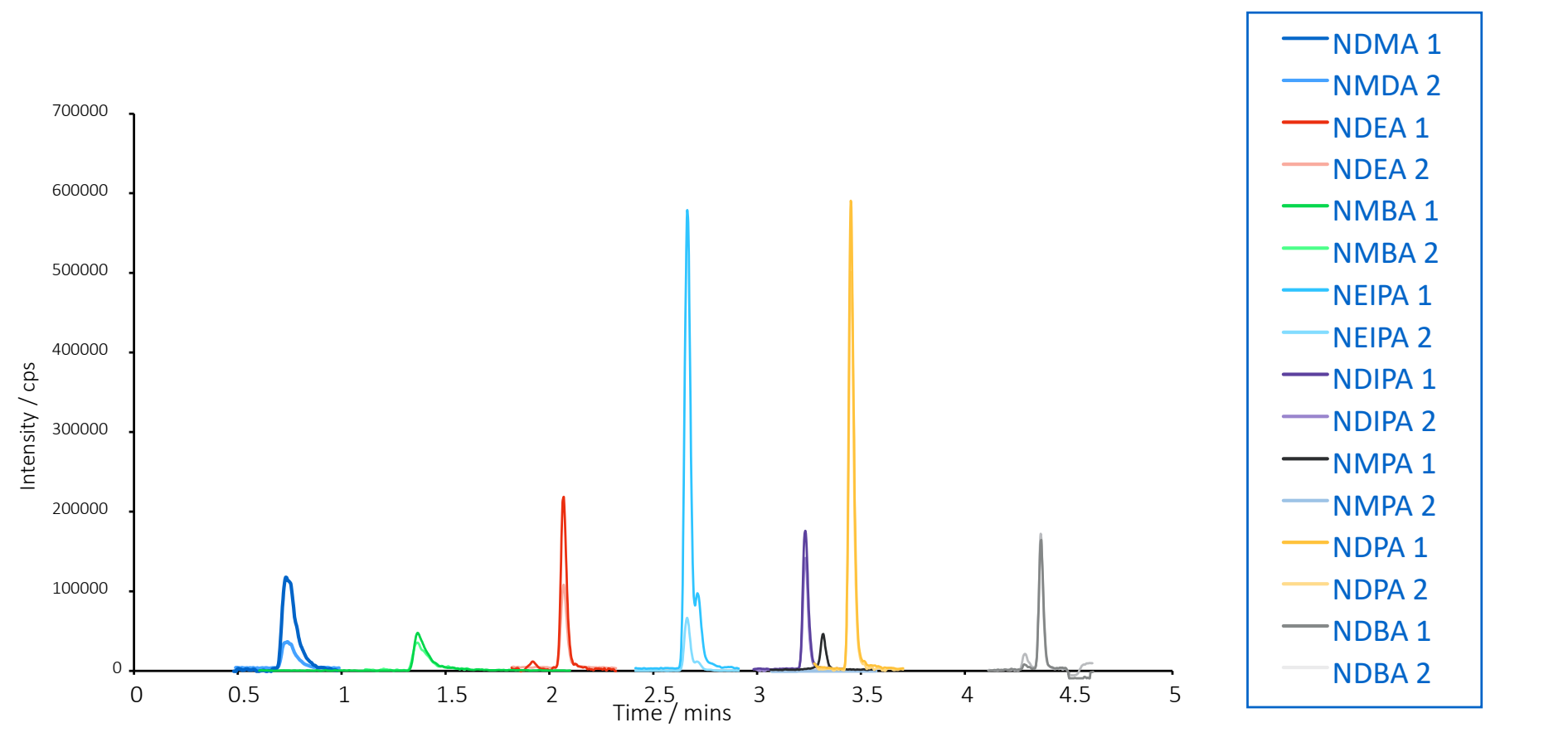
1. Background

- Nitrosamines are potent mutagens and require LC-MS quantification in potentially contaminated drug substances and products.
- LC-MS/MS method developed on an Avantor® ACE® UltraCore C18 column, providing rapid separation and low-level (ppb) quantification of nitrosamines highlighted by FDA and EMA.
- Interference from N,N-dimethylformamide (DMF) has been reported as a potential issue in the quantification of NDMA by LC-HRMS.
- In this work, isobaric interference from DMF was observed by LC-MS/MS.
- The following solutions have been identified:
  - Use of alternative NDMA quantifier transition
  - Use of alternative LC stationary phases

2. Initial Method

- Proposed USP method used as starting point for method development.[1]
- 8 nitrosamines and 4 deuterated internal standards.
- Calibration performed according to USP method
  - 1.33–30 ng/mL (20-450 ppb), 0.66-19.8 ng/mL (10-297 ppb) for NDEA
  - Excellent linearity ($r^2 > 0.997$), % accuracy (94.1 – 105.9 % and % CV (<7.6% RSD)


A. Avantor® ACE® UltraCore C18

Column: **Avantor® ACE® UltraCore C18**
Dimensions: 100 x 2.1 mm
Particle size: 3.5 µm

Mobile Phases: A: 0.1% formic acid in H_2O
B: 0.1% formic acid in MeOH
Flow Rate: 0.5 mL/min
Temperature: 40 °C
Injection vol: 40 µL
LC system: ExionLC AD UHPLC
Detector: Sciex QTRAP® 6500+.
Ionisation mode: APCI, positive mode
Source temp: 300 °C
Curtain gas: 33 psig
Ionspray™ source: 5500 V
Ion source gas: 30 psig
Needle current: 2 µA
Sample: Valsartan (66.67 mg/mL) spiked at 1 ng/mL

Gradient:

Time (mins)	% B
0	2.5
0.2	2.5
4.2	80
4.5	80
4.6	2.5
7	2.5

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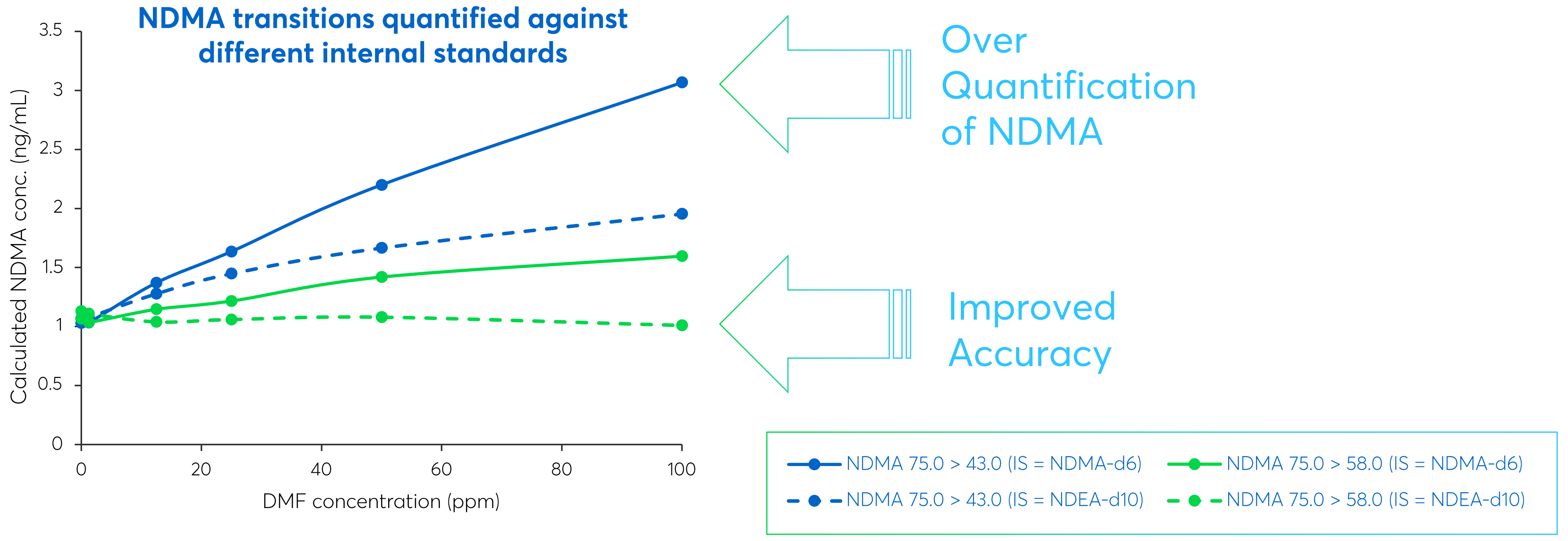
[1] Procedure 3, USP General Chapter <1469> "Nitrosamine Impurities" (Accessed February 2021)

Acknowledgement: This method was first published in *LCGC Europe* (2021), 34 (7) pp267-276

3. Investigating Isobaric Interference

- DMF was found to co-elute with NDMA.
- DMF Spiking experiments (0-100 ppm) to assess impact on NDMA quantification (1 ng/mL).
- NDMA quantifier (75.0 → 43.0) overquantified NDMA in presence of DMF.
- NDMA qualifier transition (75.0 → 58.0) was less affected.

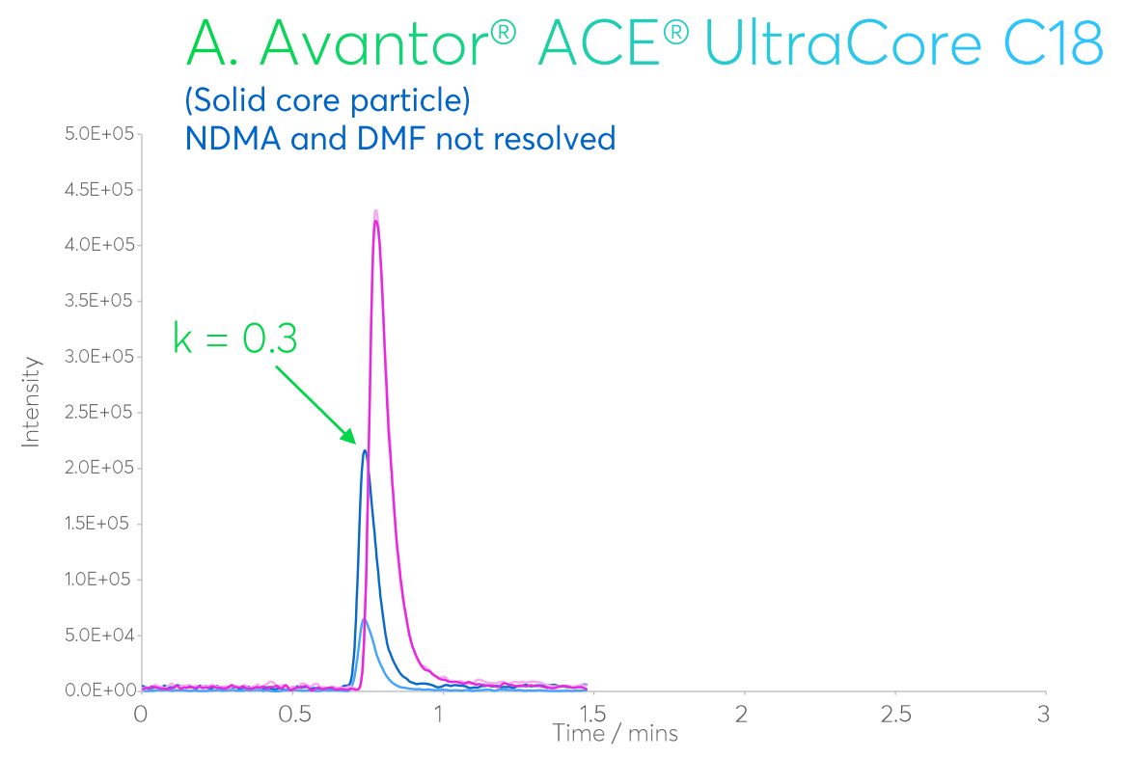

NDMA transitions quantified against different internal standards

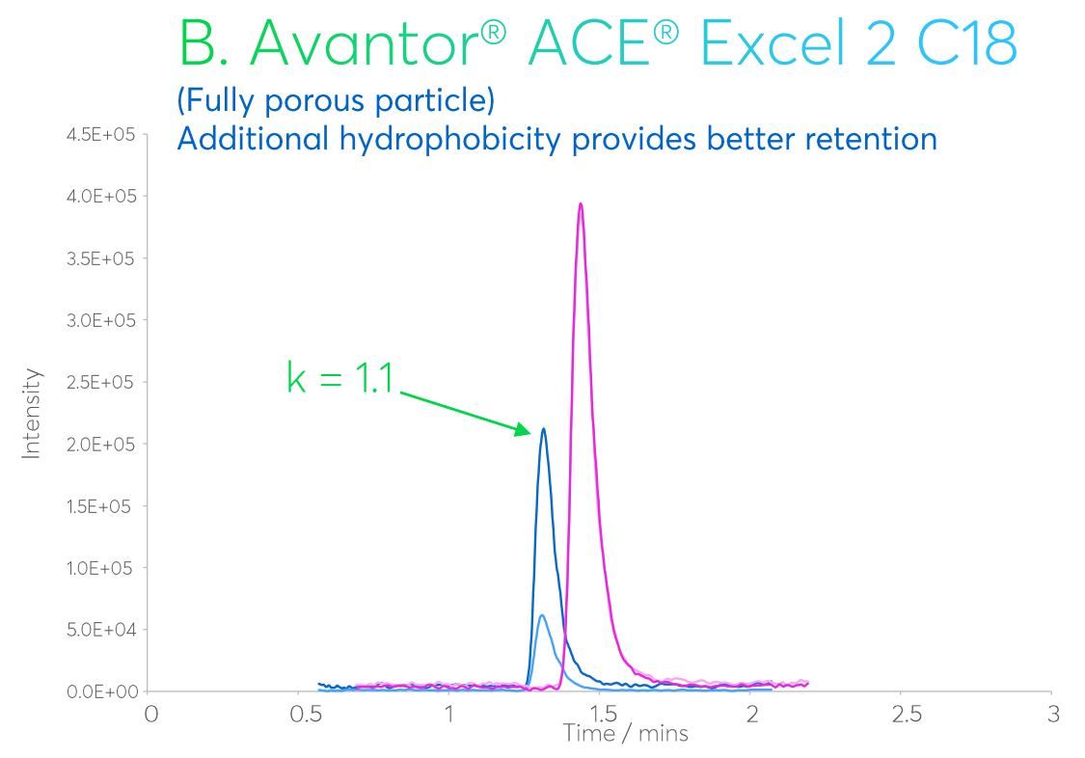
Over Quantification of NDMA

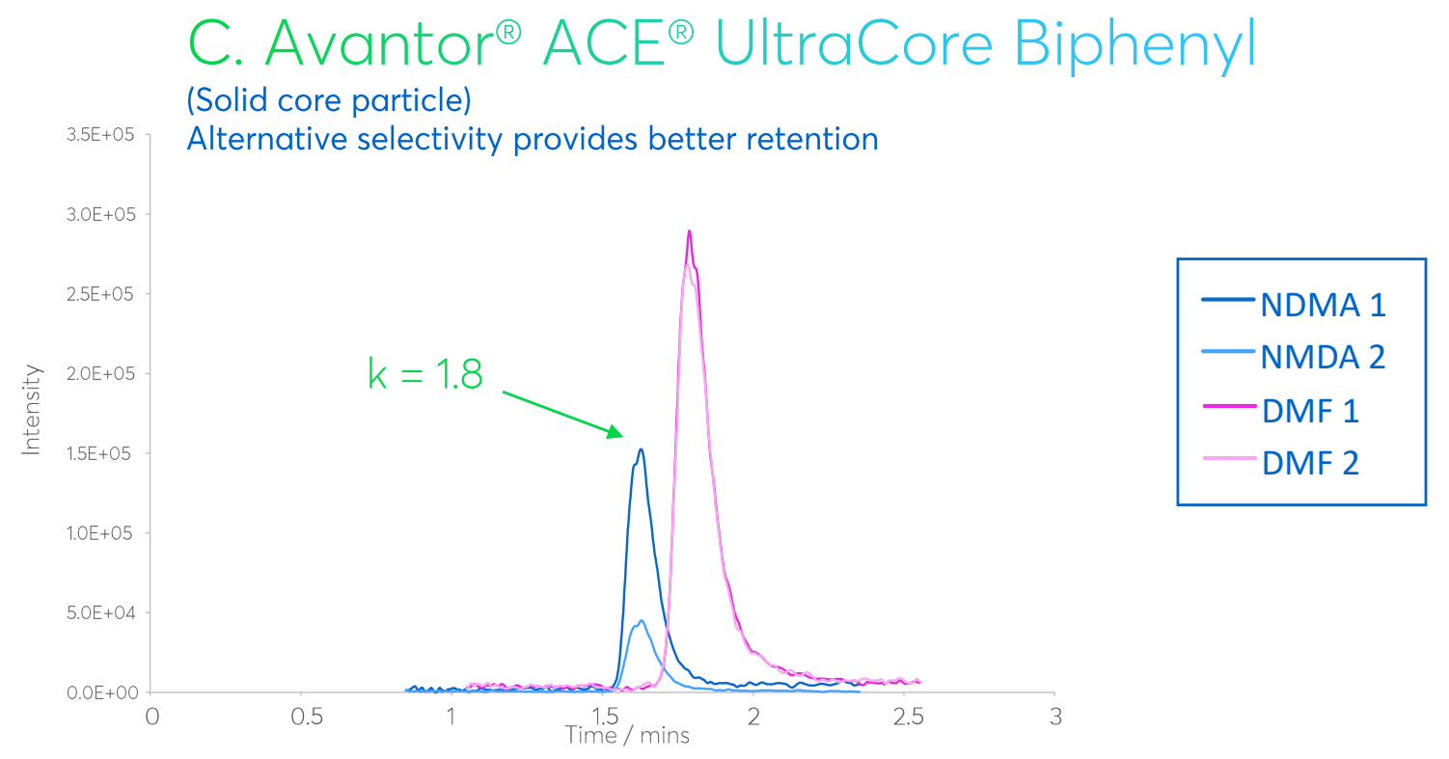
Improved Accuracy

4. Solution: Chromatographic Separation

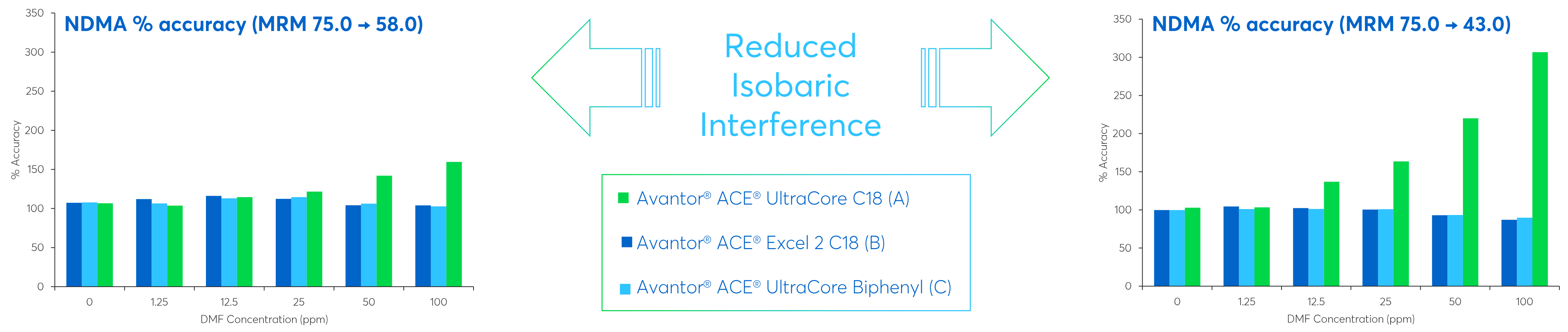
- Chromatographic separation of NDMA and DMF is clearly desirable.
- Low retention obtained for NDMA precluded separation from DMF.
- Alternative column selectivities and particle morphologies were screened to separate DMF and NDMA.


A. Avantor® ACE® UltraCore C18
(Solid core particle)
NDMA and DMF not resolved


B. Avantor® ACE® Excel 2 C18
(Fully porous particle)
Additional hydrophobicity provides better retention


C. Avantor® ACE® UltraCore Biphenyl
(Solid core particle)
Alternative selectivity provides better retention

- Both columns showed improved quantitative performance, with no evidence of interference.

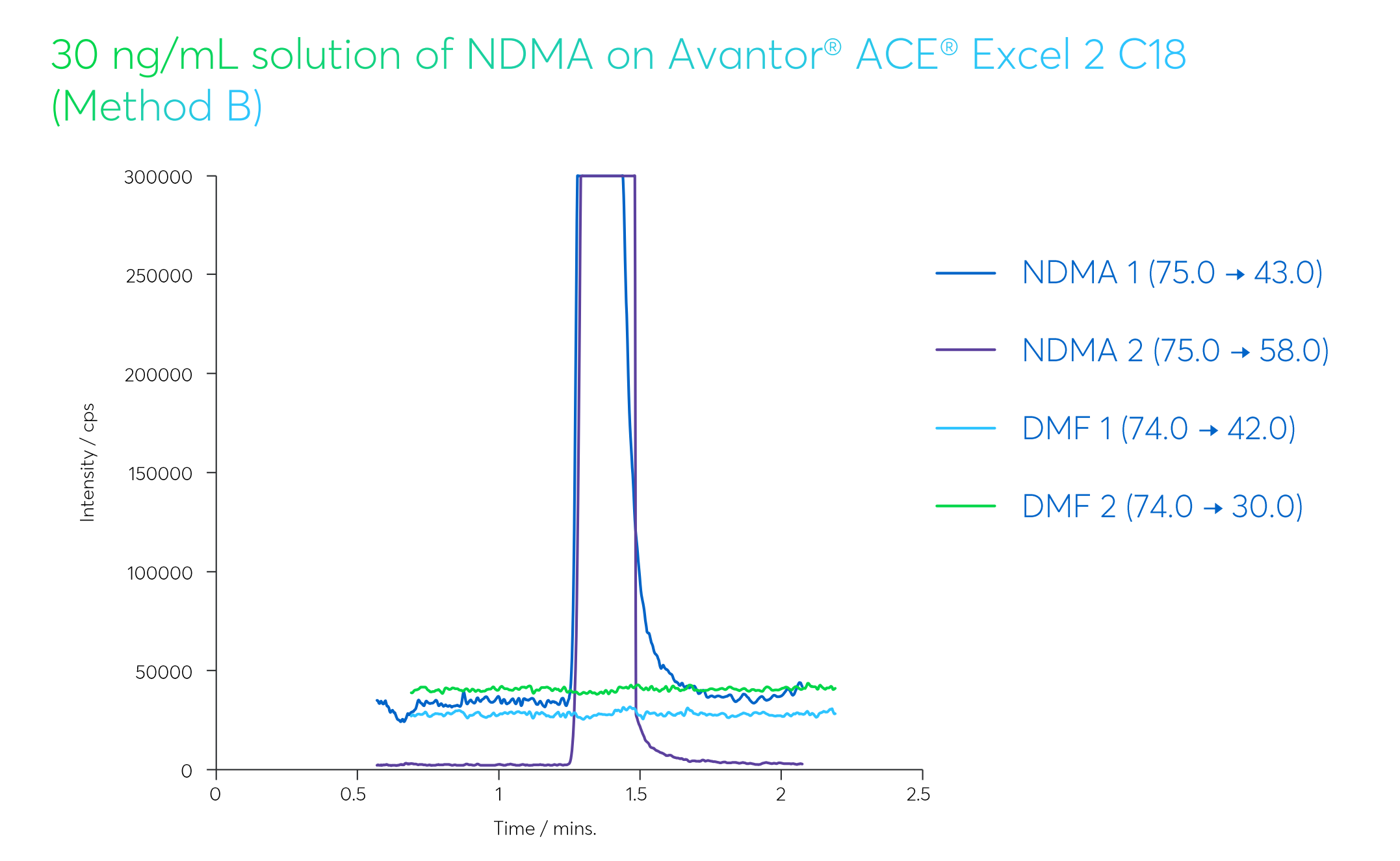


Reduced Isobaric Interference

- 🟩 Avantor® ACE® UltraCore C18 (A)
- ■ Avantor® ACE® Excel 2 C18 (B)
- ■ Avantor® ACE® UltraCore Biphenyl (C)

5. DMF Screening

- DMF MRM transitions (74.0 → 42.0 and 74.0 → 30.0) were also established.
- For a sample containing a high concentration of NDMA (30 ng/mL), no response was observed in either transition.
- Highly selective, so can be used to monitor for presence of DMF in real-world samples and safeguard against risk of NDMA over-quantification.


30 ng/mL solution of NDMA on Avantor® ACE® Excel 2 C18 (Method B)

6. Summary and Conclusions

- Rapid LC-MS/MS separation developed using an Avantor® ACE® UltraCore C18 column.
- Excellent linearity, accuracy and precision demonstrated.
- LOD/LOQ values below those sought by regulatory authorities.
- Co-elution of NDMA with DMF resulted in over-quantification of NDMA.
- Quantification using the NDMA qualifier transition and the NDEA-d10 internal standard was found to reduce this problem.
- Alternative methods using Avantor® ACE® UltraCore Biphenyl and Avantor® ACE® Excel C18 stationary phases provided enhanced retention and separation of DMF and NDMA and avoid interference.