Challenges and considerations for an LC-MS method transfer: Case Study

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OVERVIEW

1. Current methods
2. Difficult transfer
   - Considerations/Challenges
   - Approaches to dealing with the challenges
3. Considerations in method transfers
4. Conclusions

Method Transfer: The transfer of a method between species and matrix

CURRENT METHODS

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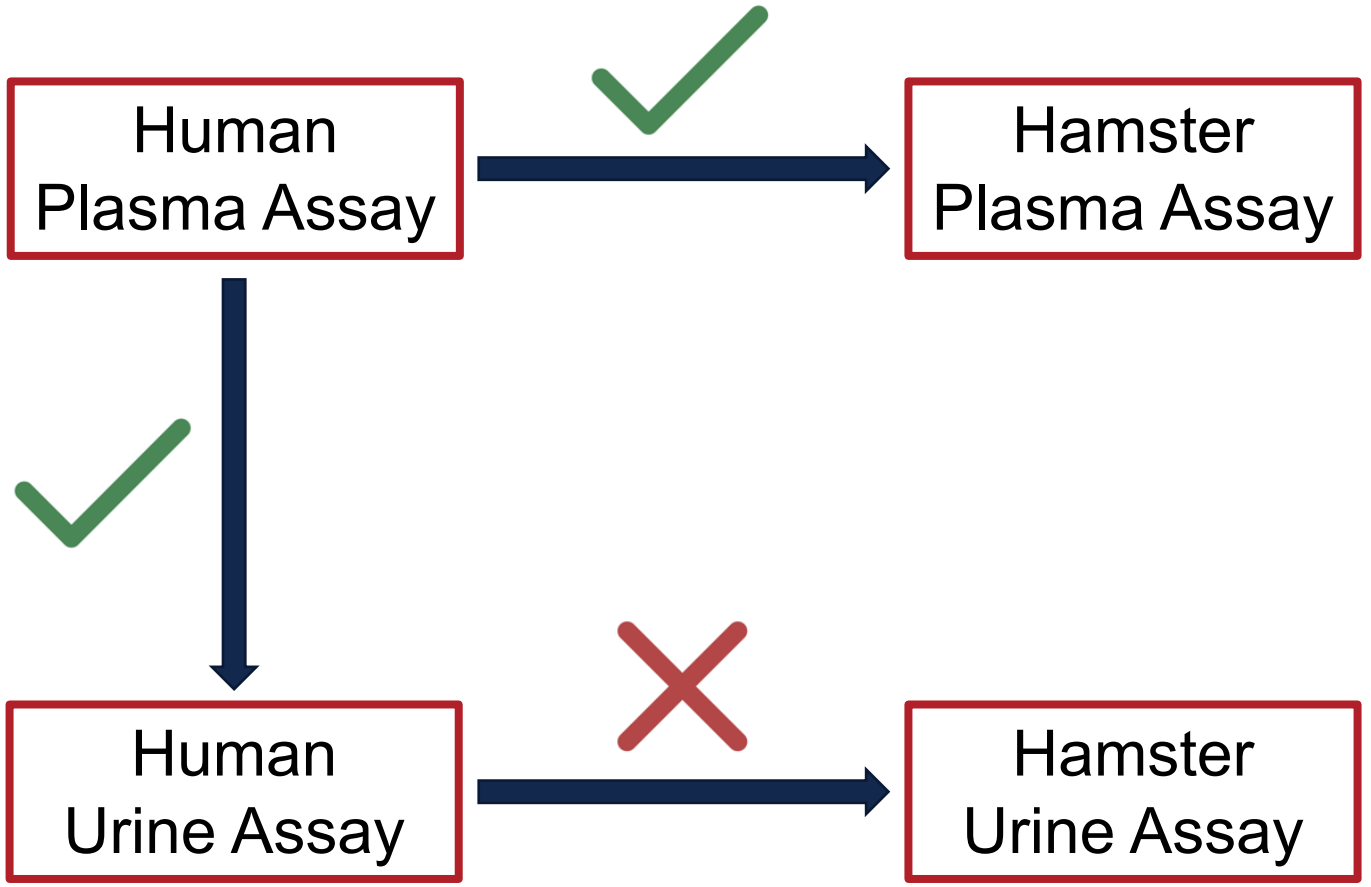

- Both Human assays are protein precipitation/dilution assays, used to support multiple projects
- Sponsor requested a transfer into Hamster plasma & urine

HAMSTER URINE TRANSFER

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- Peak at RT of analyte in all matrix blanks and samples containing matrix
- What is the peak and how do we solve it?

Figure 1: QC LLOQ in Hamster urine

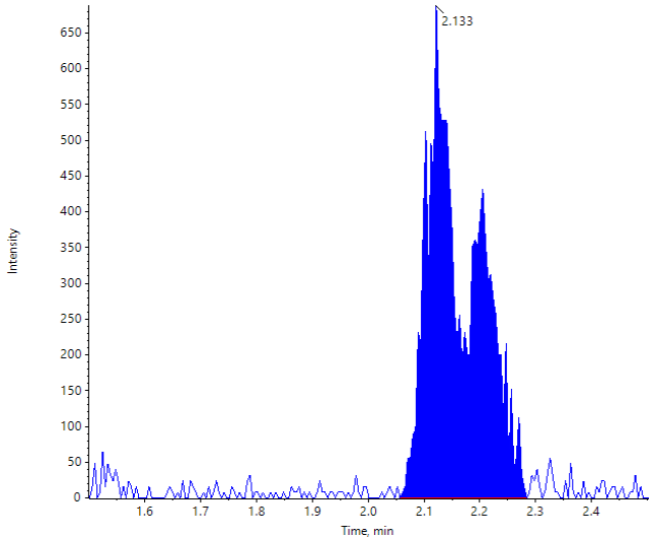
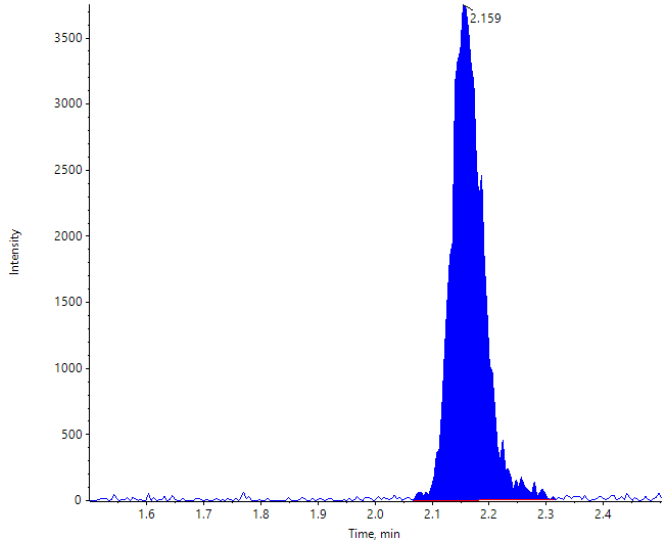


Figure 2: SIL-internal standard in Hamster urine



SYSTEM CONTAMINATION?

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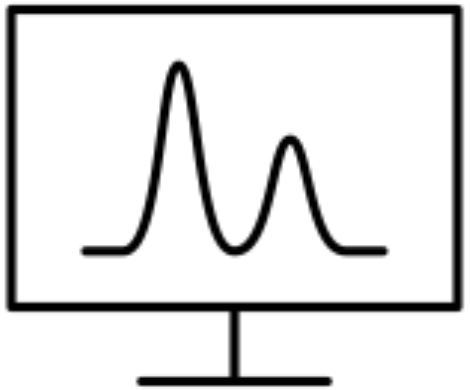
- New Phases
  - Ultra pure organic
  - Fresh pH phase
- New Column
  - Waters Acquity BEH C18, 50 x 2.1 mm, 1.7 µm
- New System
  - LC Acquity
  - Mass spectrometer



Conclusion: The peak at the RT was not due to instrument set up or performance
Next Steps: Investigate potential matrix contamination

CONTAMINATION OF THE MATRIX?

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- 12 Individual lots of hamster urine tested
  - 75% of lots showed a peak at the expected RT
- Multiple pools of hamster urine tested
  - All showed a peak at the expected RT

Conclusion: The peak at the RT was not due to contaminated matrix
Next Steps: Investigate Chromatography & MS

Figure 3: Matrix Blank in Hamster Urine

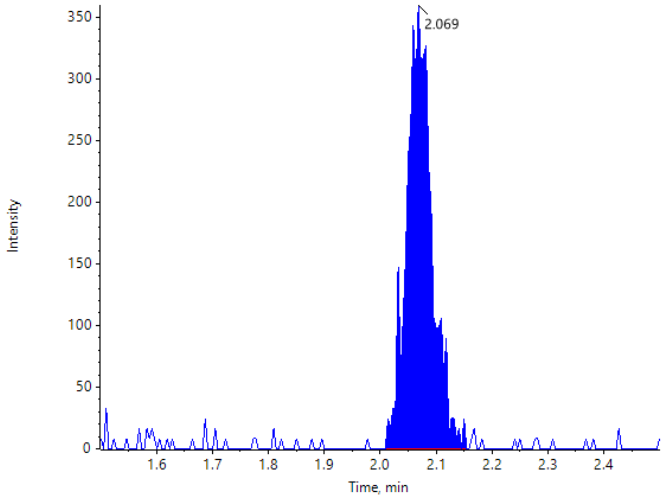


Figure 4: SIL Internal Standard in Hamster Urine

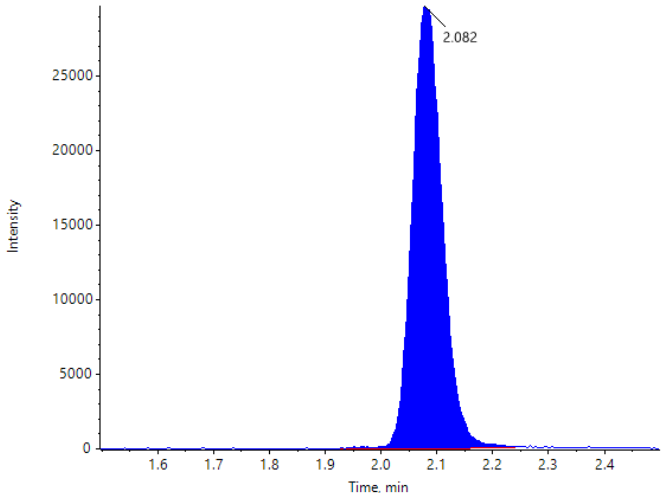
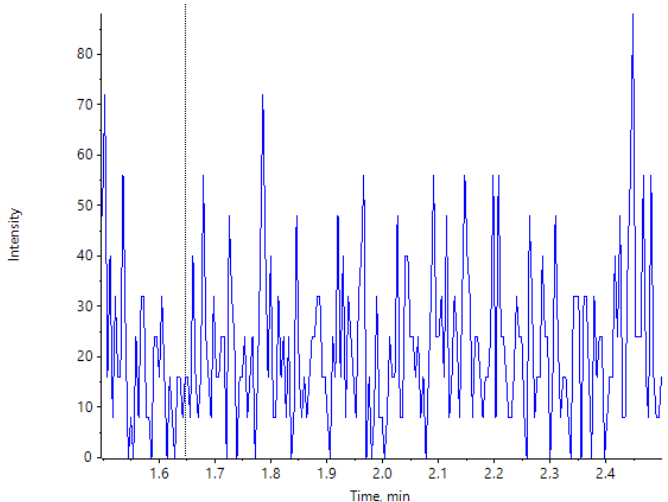


Figure 5: Matrix Blank in Hamster Plasma



RESOLVING INTERFERENCE

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- Chromatographic/MS Approaches
  - Alternative transitions assessed
  - Mobile phase screen
  - Column chemistry

- Extraction
  - Decrease sample volume
  - Increase dilution

ALTERNATIVE TRANSITIONS

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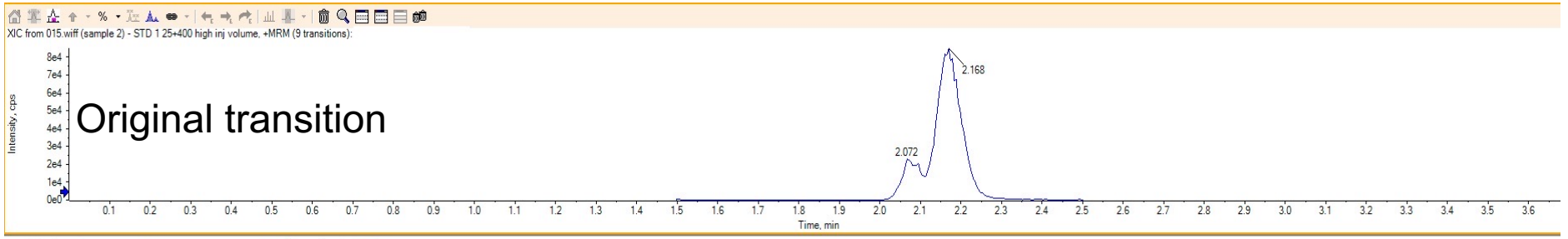

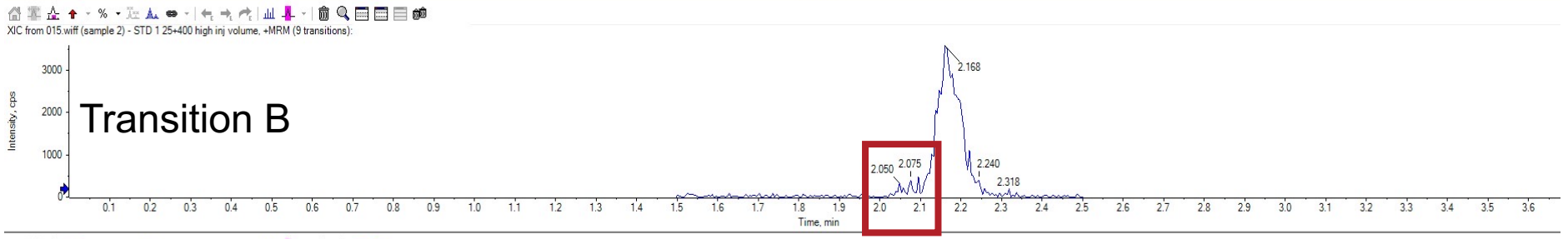

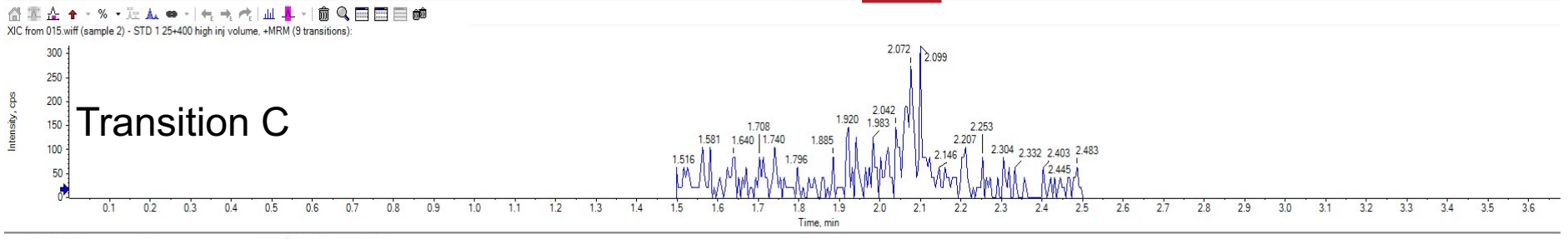

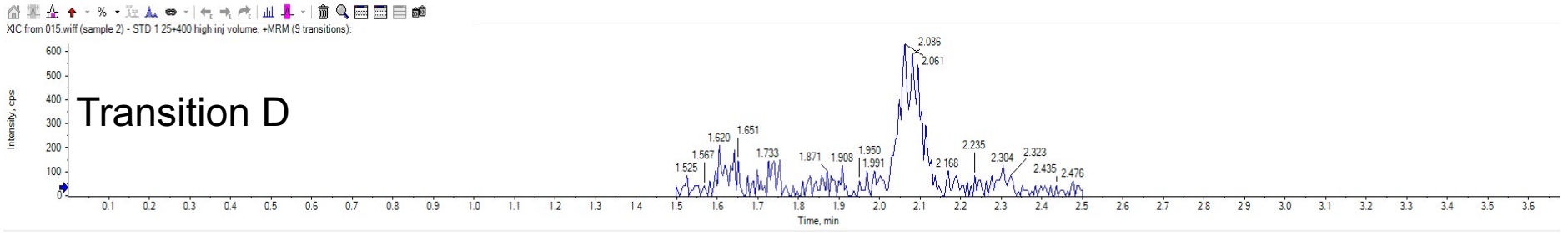

Conclusion:
Transition B had less interference, however still a small hump

Next Steps:
Optimise chromatography to further resolve the hump

MOBILE PHASE SCREEN

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	Original	Updated
Mobile Phase A	Acetonitrile	Methanol
Mobile Phase B	10mM Ammonium Formate (aq) pH3	10mM Ammonium Formate (aq) pH3
Weak Wash	MeOH:H_2O (30:70, v/v)	MeOH:H_2O (30:70, v/v)
Strong Wash	Acetonitrile	Acetonitrile

- ACN and MeOH have different elution power so this could resolve the interference
- ACN to MeOH requires some modifications to LC method to reduce backpressure
  - Decrease flow rate
  - Increase column temperature

MOBILE PHASE SCREEN

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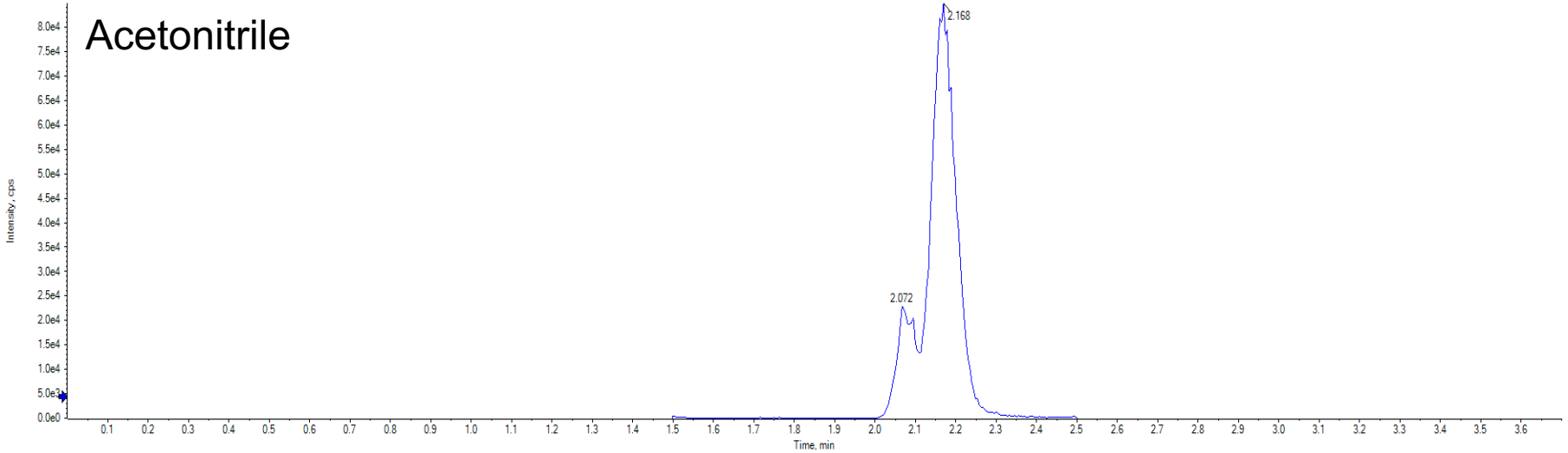

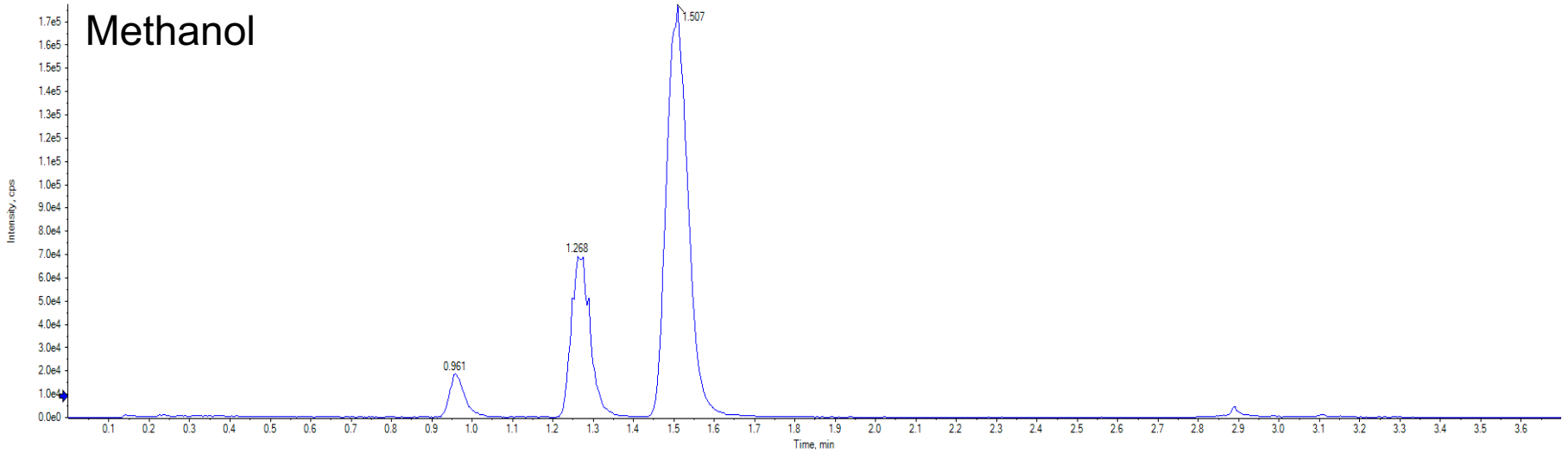

Conclusion: Switching from ACN to MeOH enabled baseline separation

Next Steps: Move on to validation

CONSIDERATIONS WITH METHOD TRANSFERS

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1. Storage temperatures
   - Stability

2. Differences in matrix
   - Composition (proteins, salts etc.)
   - Sample volumes

3. Resources
   - Instrumentation

4. Processes

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CONCLUSIONS

- Try to leverage on information you already have to target specific areas
- Targeted troubleshooting allows for faster establishments in a different matrix/species
- Do your research with different matrices

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Thank you for your attention

Any further question?

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