EUROPEAN BIOANALYSIS FORUM –
YOUNG SCIENTIST SYMPOSIUM

The Unwanted Guest

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Agenda

- Introduction
- Background of what we do
- The issue we encountered
- The step we took to counteract this issue
- Resolution

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LC-MS/MS

- Chromatography is the separation of a mixture passing through a medium in which components move at different rates
- LC-MS/MS: We use mass spectrometry to detect, measure and quantify different components movements
- But how can we determine the difference between what we expect to see, and what we deem as an unwanted guest?

Background of the work

- Single compound assay with an internal standard
- The assay was developed and validated in 2016 to support both preclinical and clinical sample analysis
- For this clinical sample analysis, solid phase extraction was used
  - SPE helps to prepare and purify our desired analyte prior to chromatographic analysis
  - The use of SPE can help to clean up the matrices, concentrate analytes for increased sensitivity and help remove interference that can cause high background or misleading peaks

What was the issue we faced?

- During clinical sample analysis, we started encountering unwanted guests in our analysis
- This made it difficult to integrate our desired peaks at our lower levels of quantification
- Led to difficulties in the acceptance of data
- Which in turn, led to delays in the study while we tried to fix these issues

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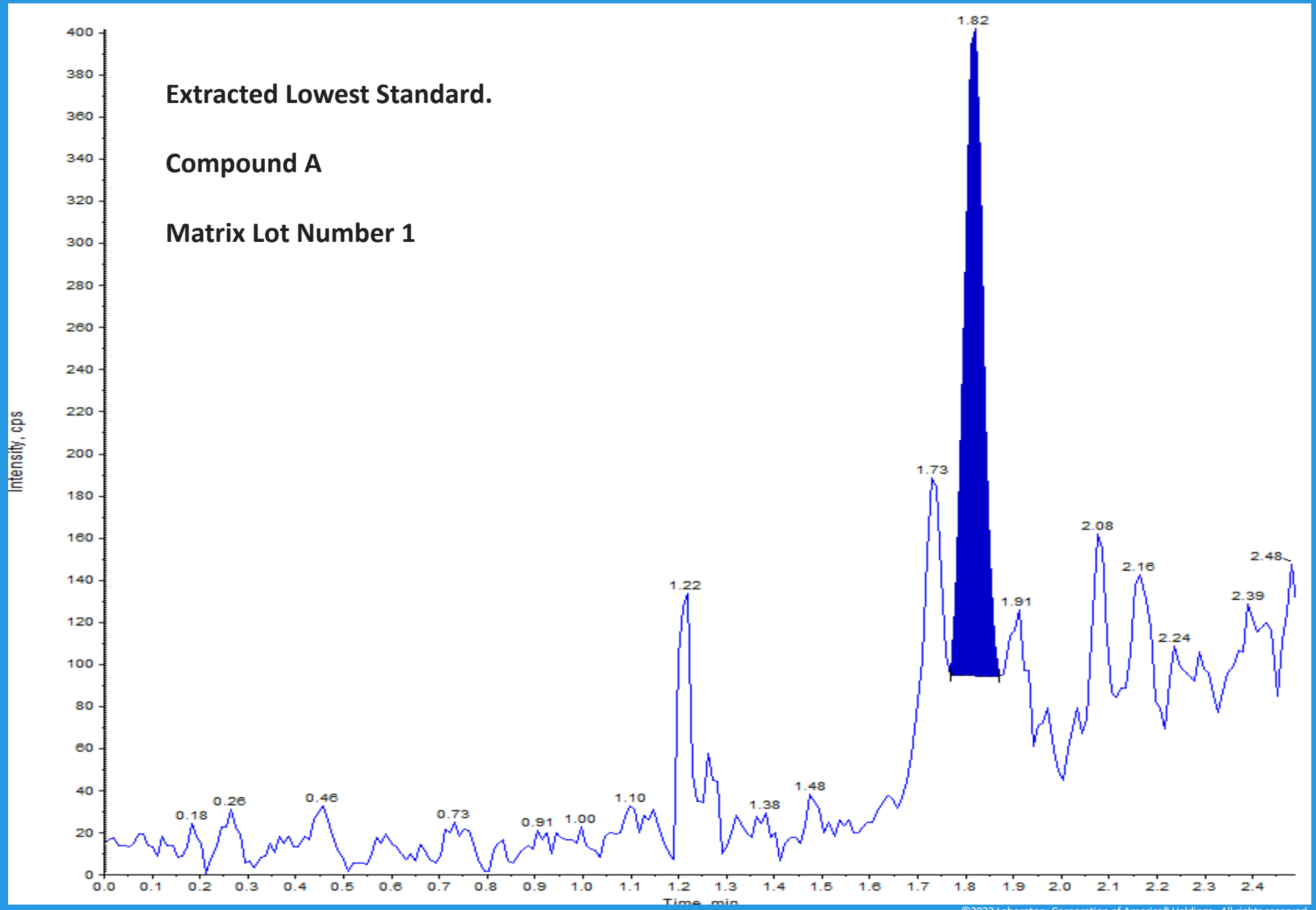
Extracted Lowest Standard.

Compound A

Matrix Lot Number 1



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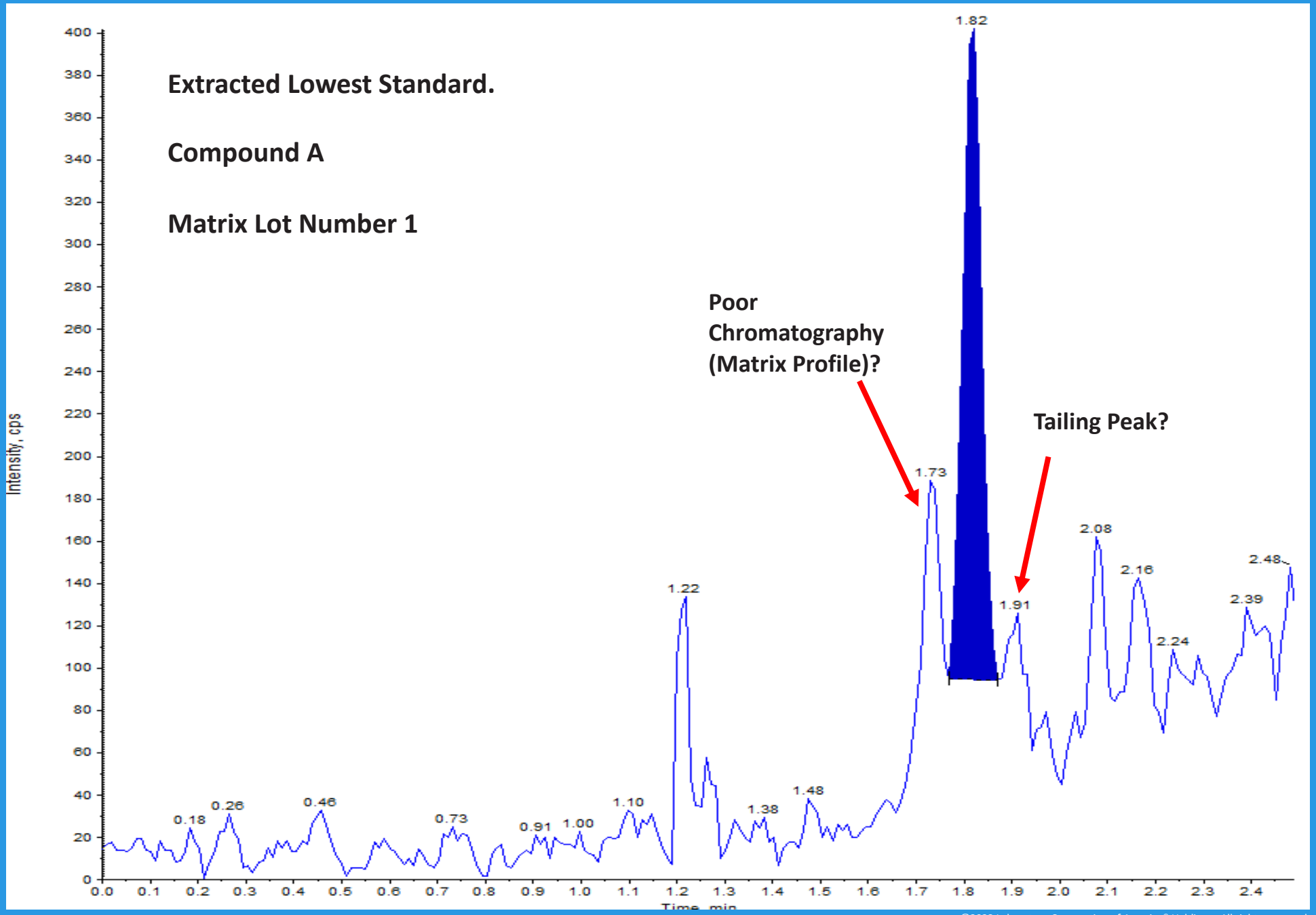

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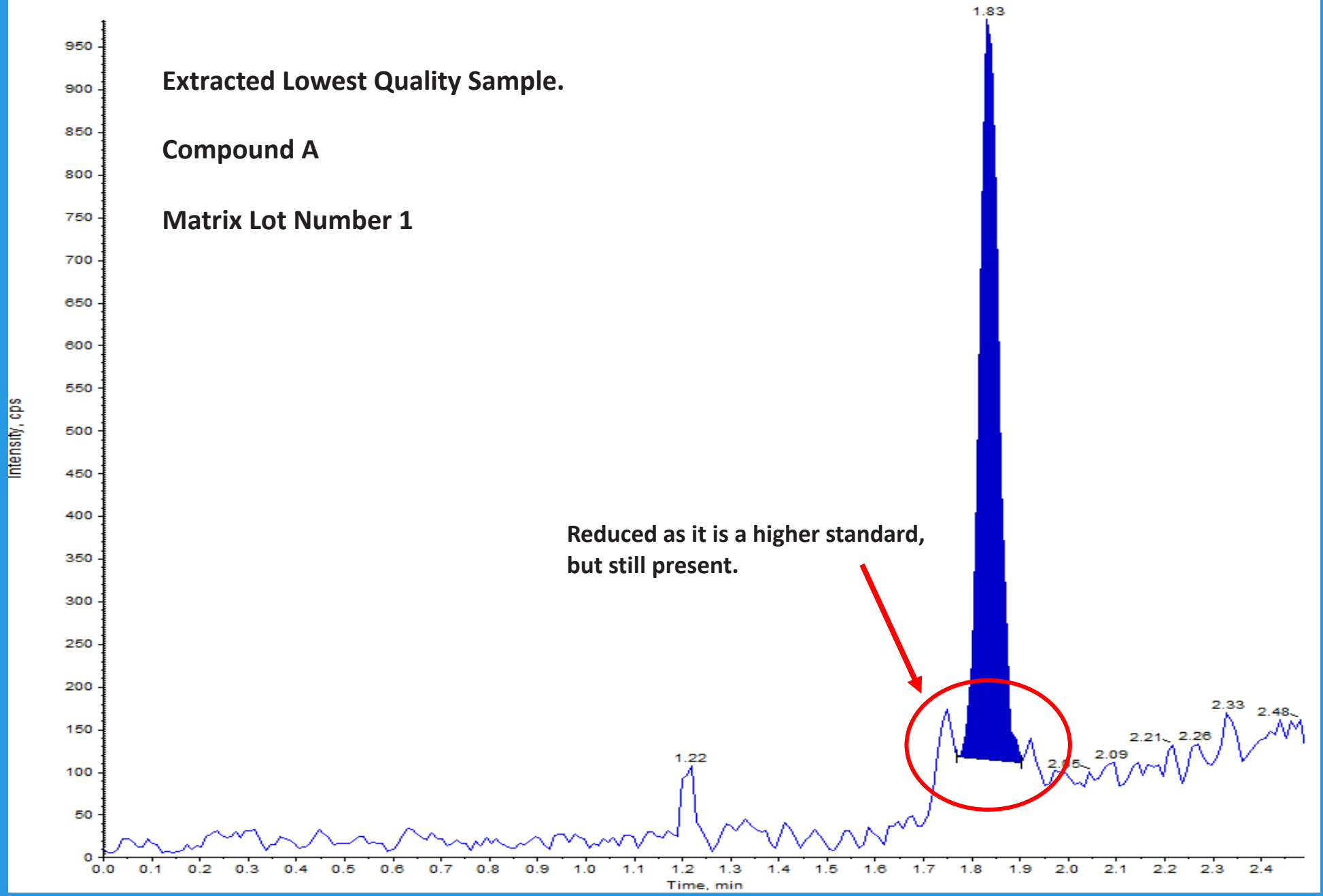
Extracted Lowest Quality Sample.

Compound A

Matrix Lot Number 1



Reduced as it is a higher standard, but still present.

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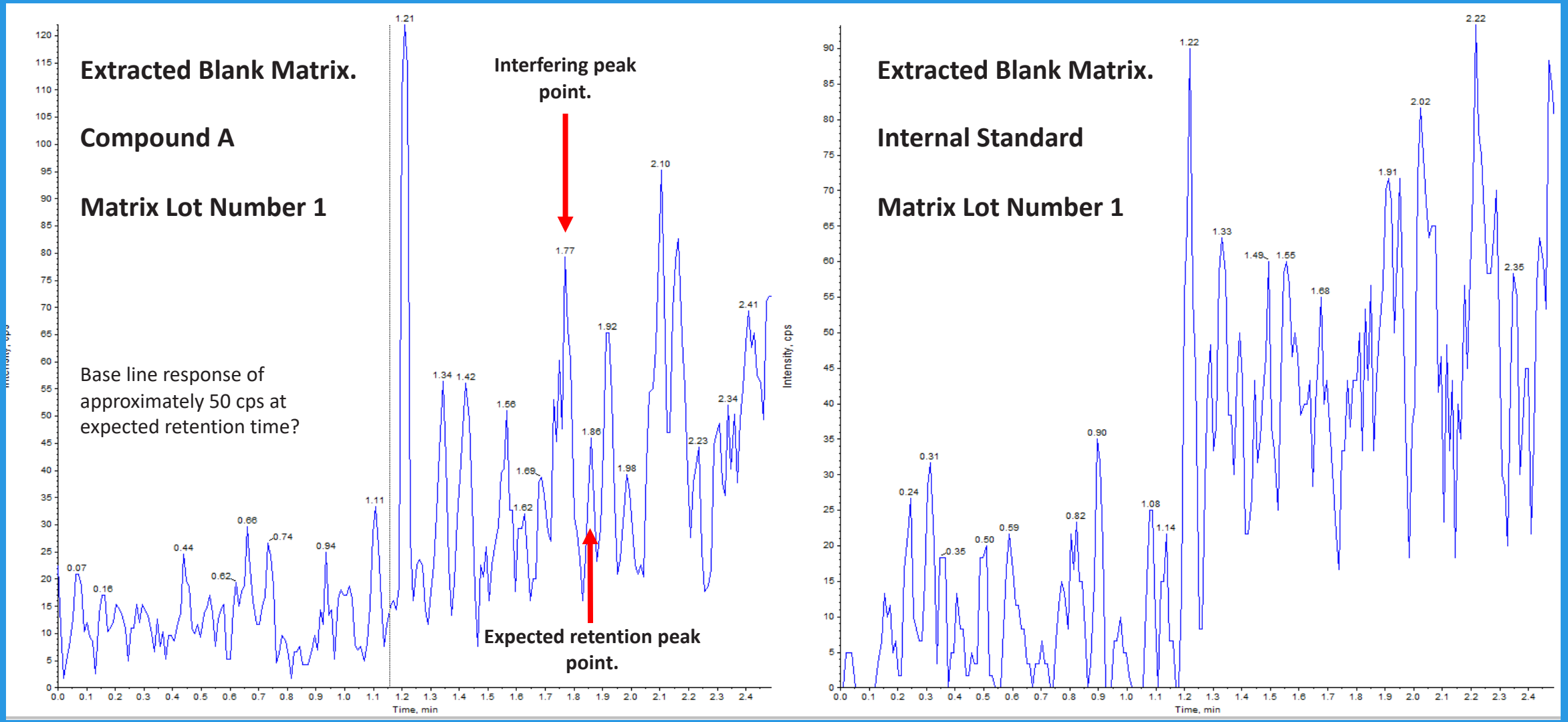

How can we try to solve this?

Determine what the problem is

Work towards addressing the issue

Work effectively and efficiently to meet deadlines

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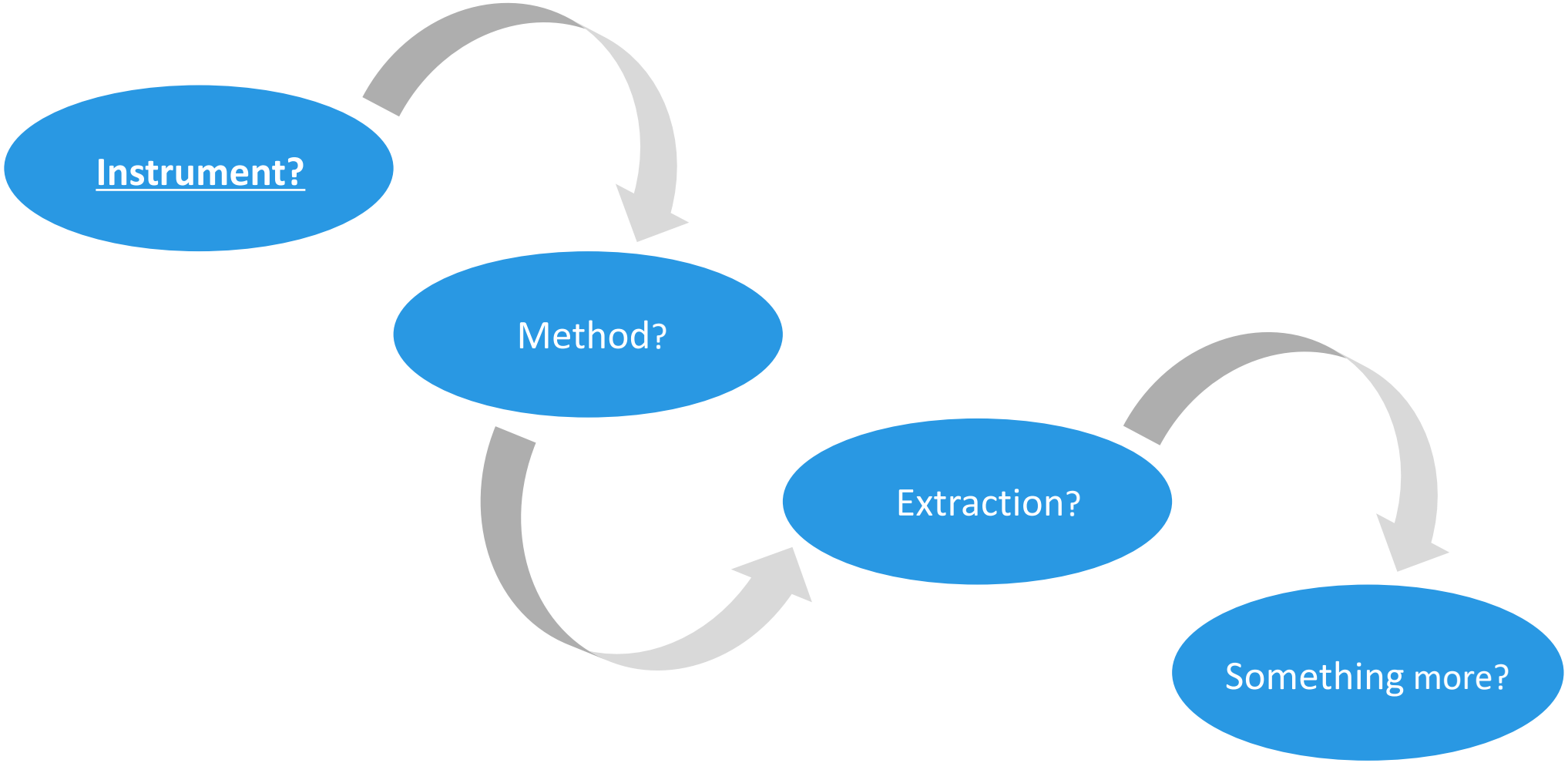

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Instrument issues?

- Are there any mechanical issues with the mass spectrometer?
  - Any leaks in the instrument?
  - Pressure/pumps?
  - Contaminants in the phases?
  - Could there be an issue with the column?
- Use of alternative instrument

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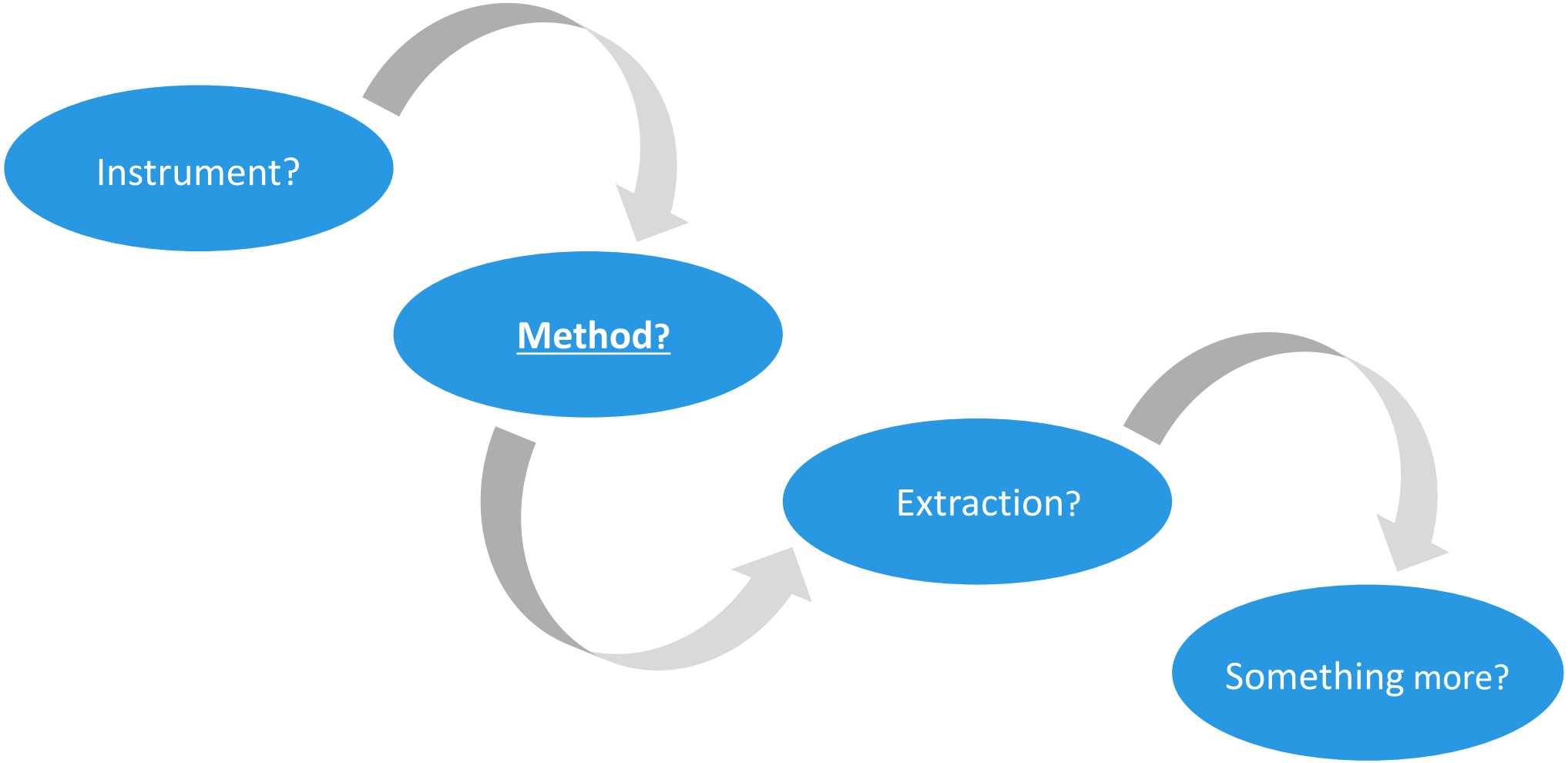

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Method?

- Check whether the method appears to be correct
- Correct reagents during the extraction?
- Correct gradient in the method?
  - Flow rate, flush time, etc.
- Injecting from the correct plate?
  - We are only human

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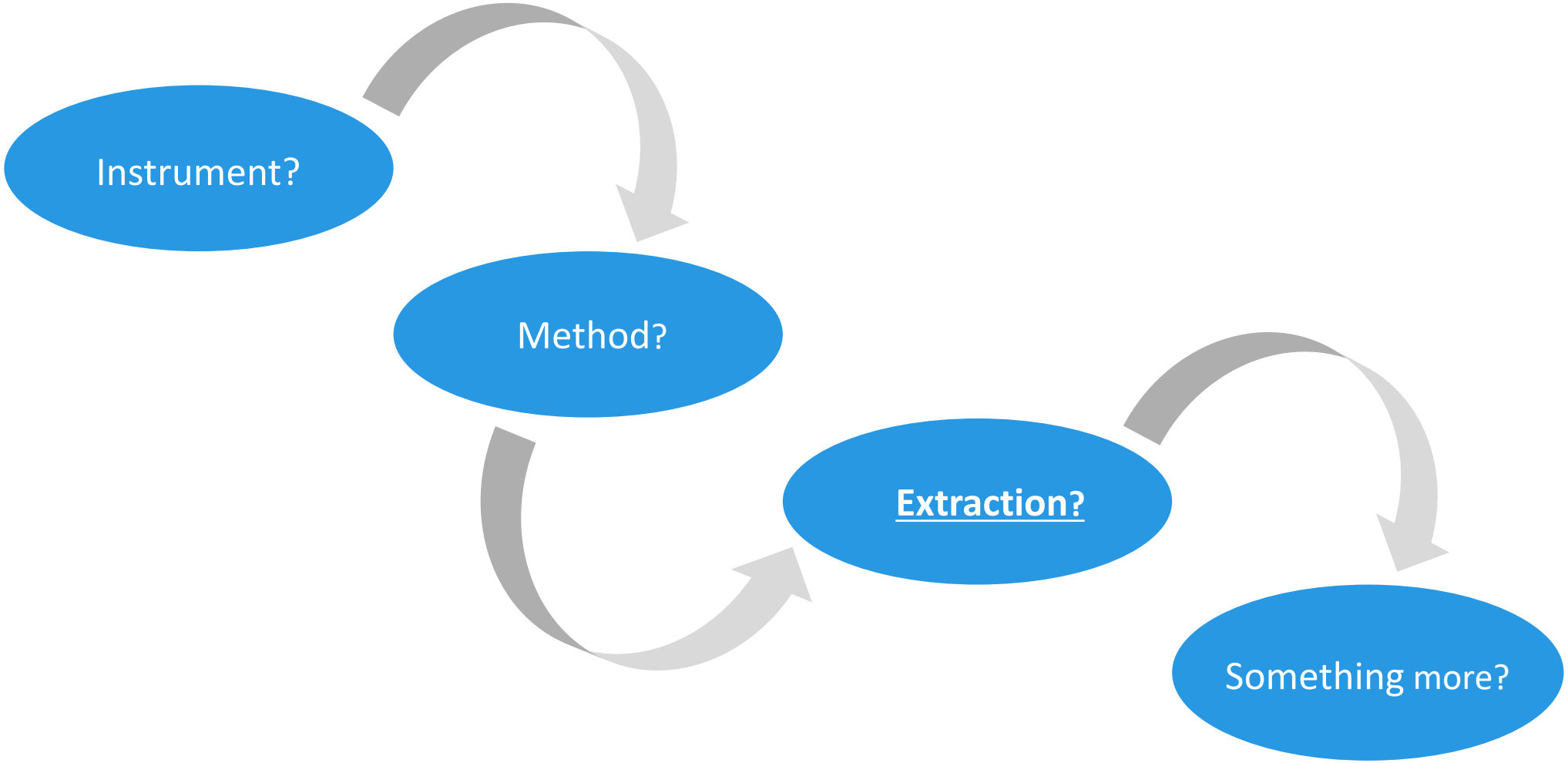

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Extraction?

- What kind of things could cause issues during the extraction?
  - What equipment are we using during the extraction?
  - Is there a build up of contaminants in the glassware?
  - Could there be a plastic leeching effect occurring?
  - Do we need to consider protein binding?

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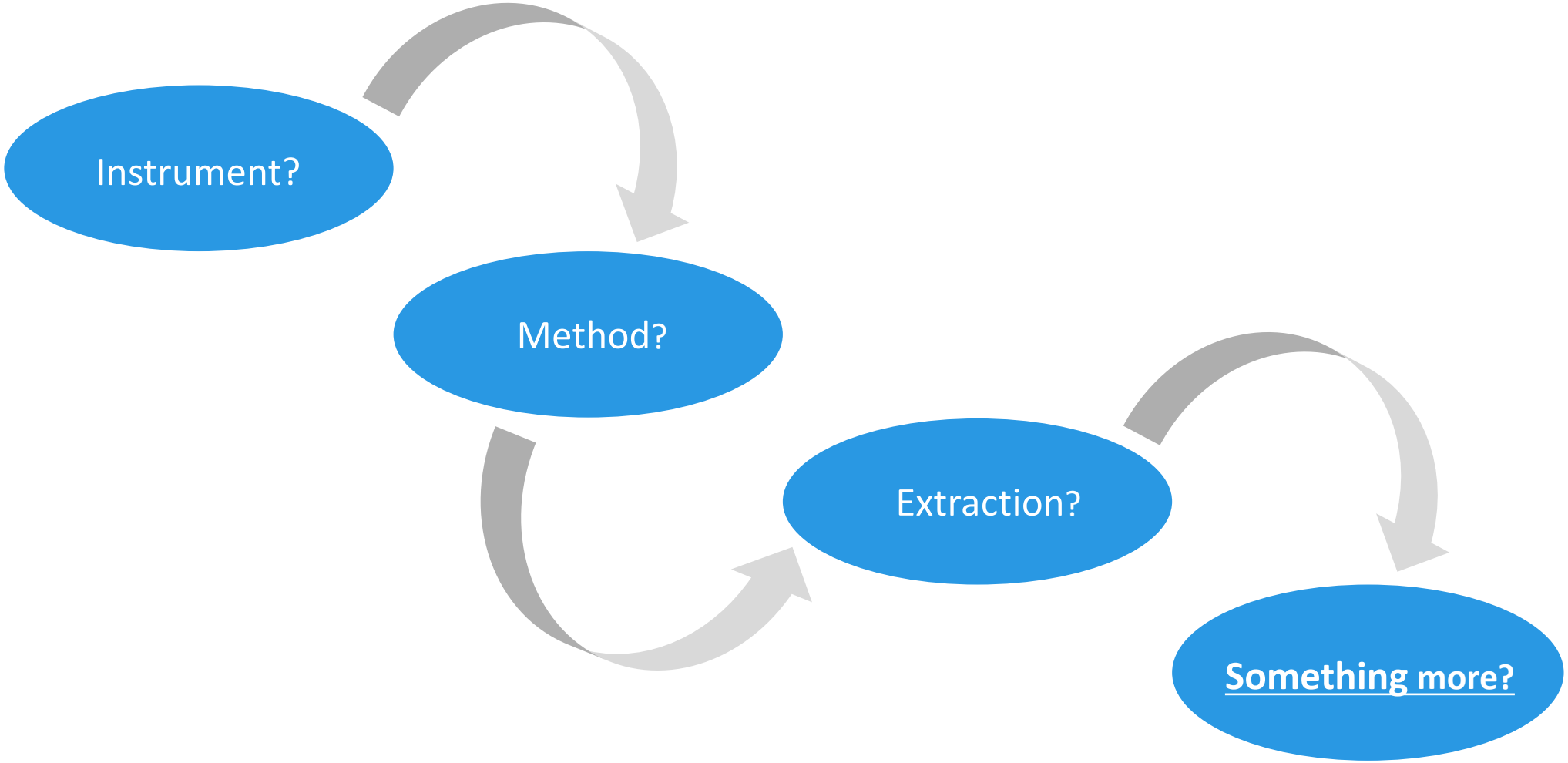

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Something more?

- Could it be something else?
  - Plasma selectivity?
    - Plasma screening
    - Hormonal imbalances
    - Age, race, etc.
  - Time
    - How long it takes to do the extraction?
    - Expiry dates?

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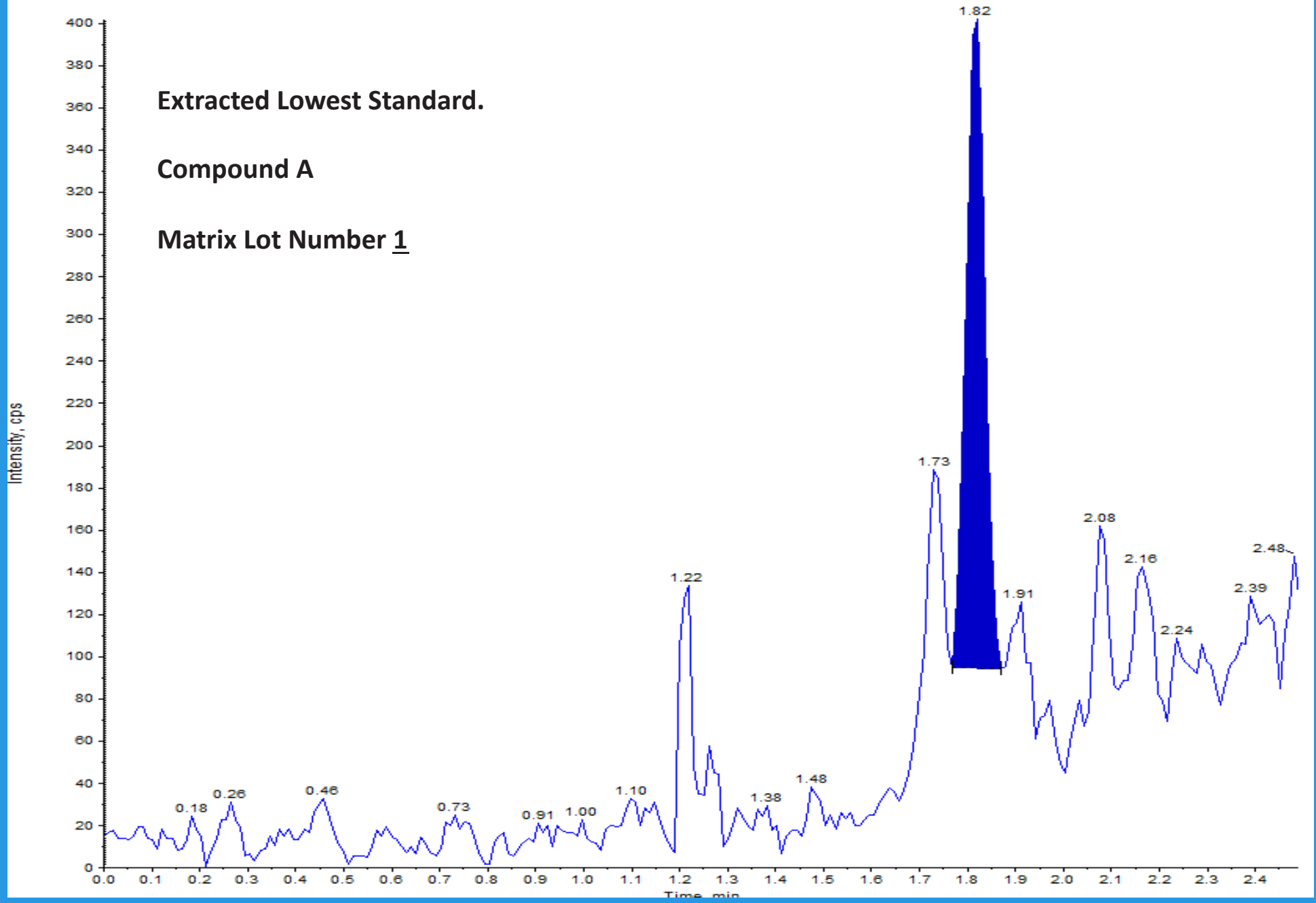
Extracted Lowest Standard.

Compound A

Matrix Lot Number 1



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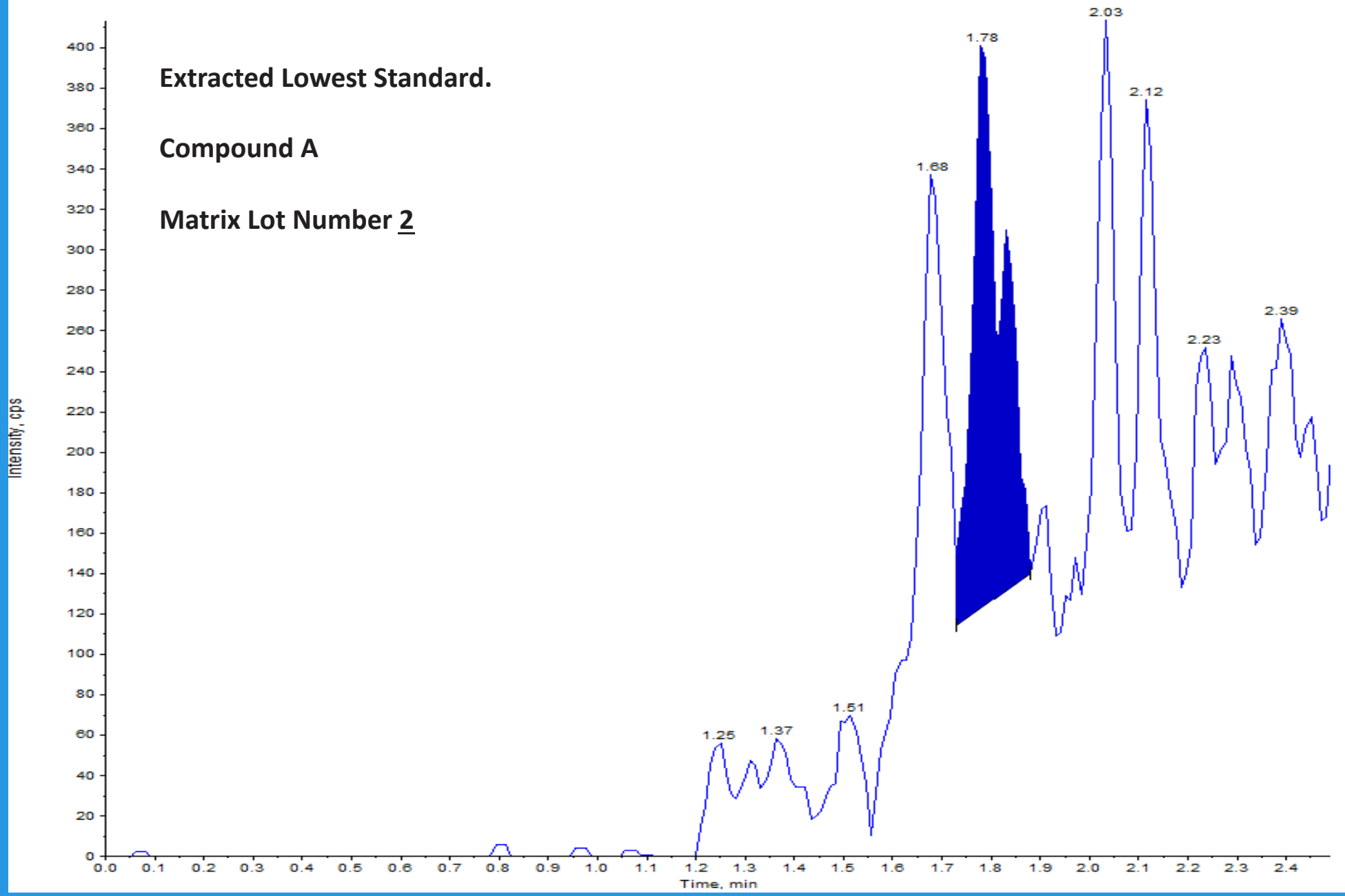
Extracted Lowest Standard.

Compound A

Matrix Lot Number 2



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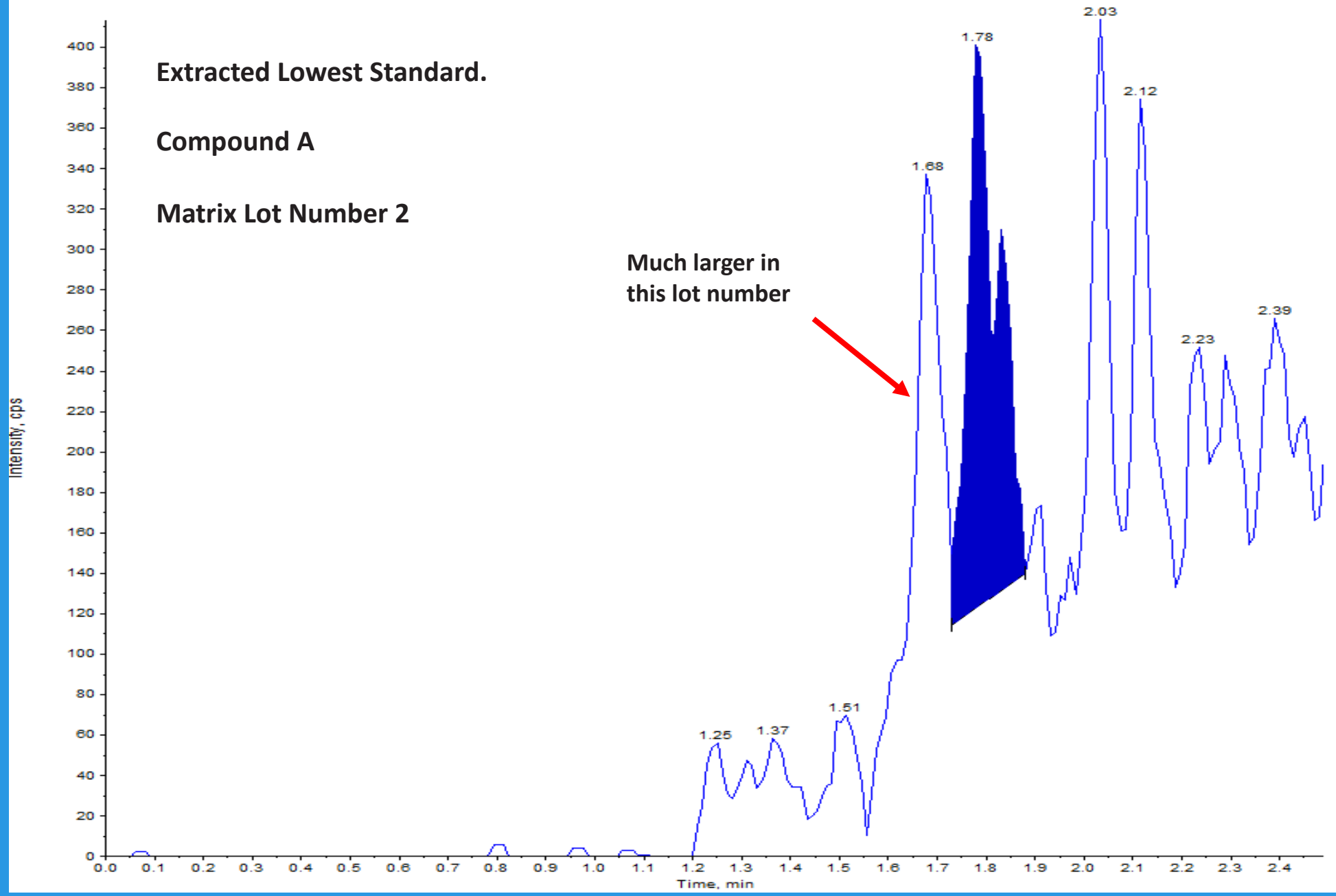
Extracted Lowest Standard.

Compound A

Matrix Lot Number 2

Much larger in this lot number



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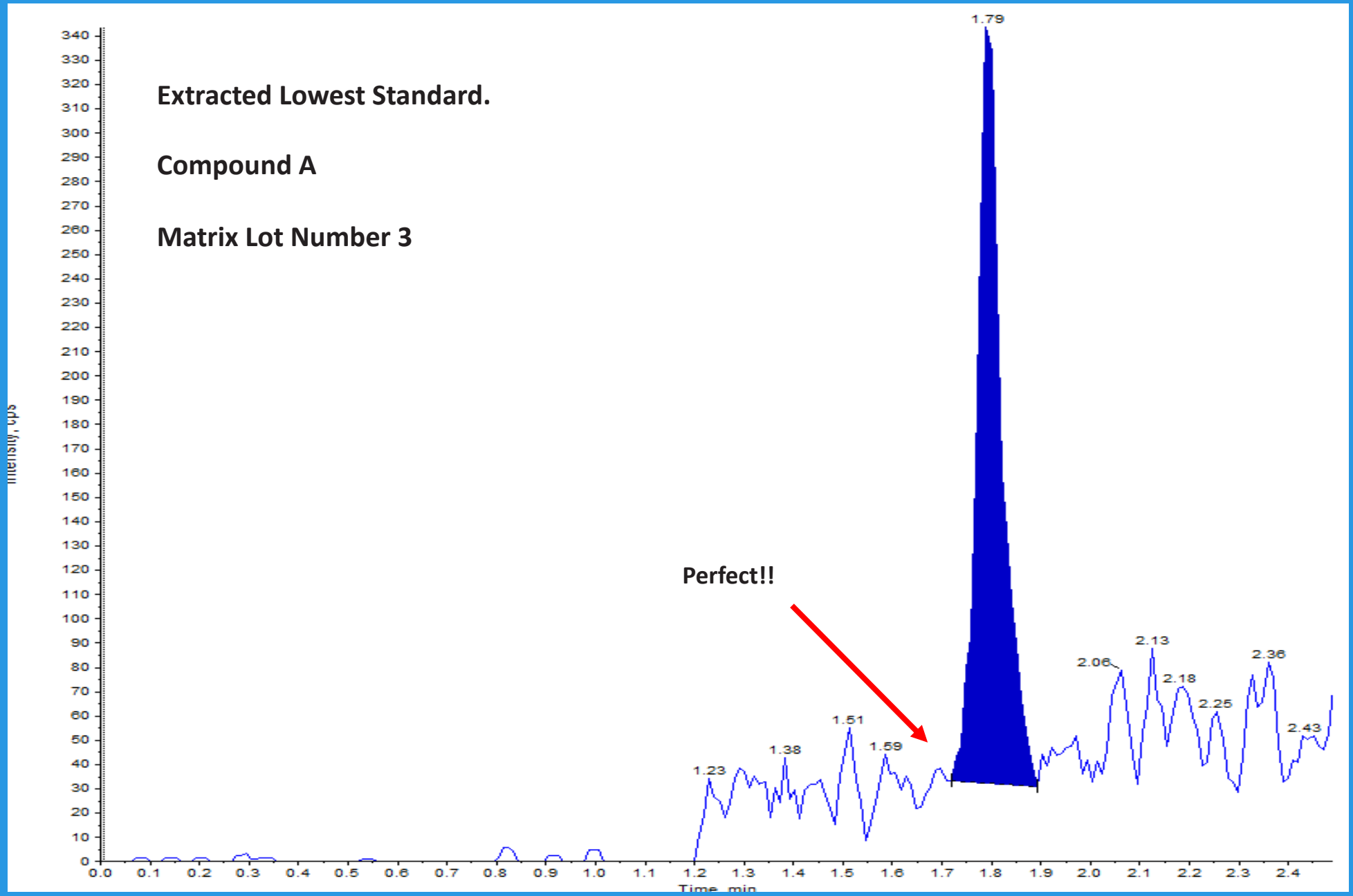
Extracted Lowest Standard.

Compound A

Matrix Lot Number 3

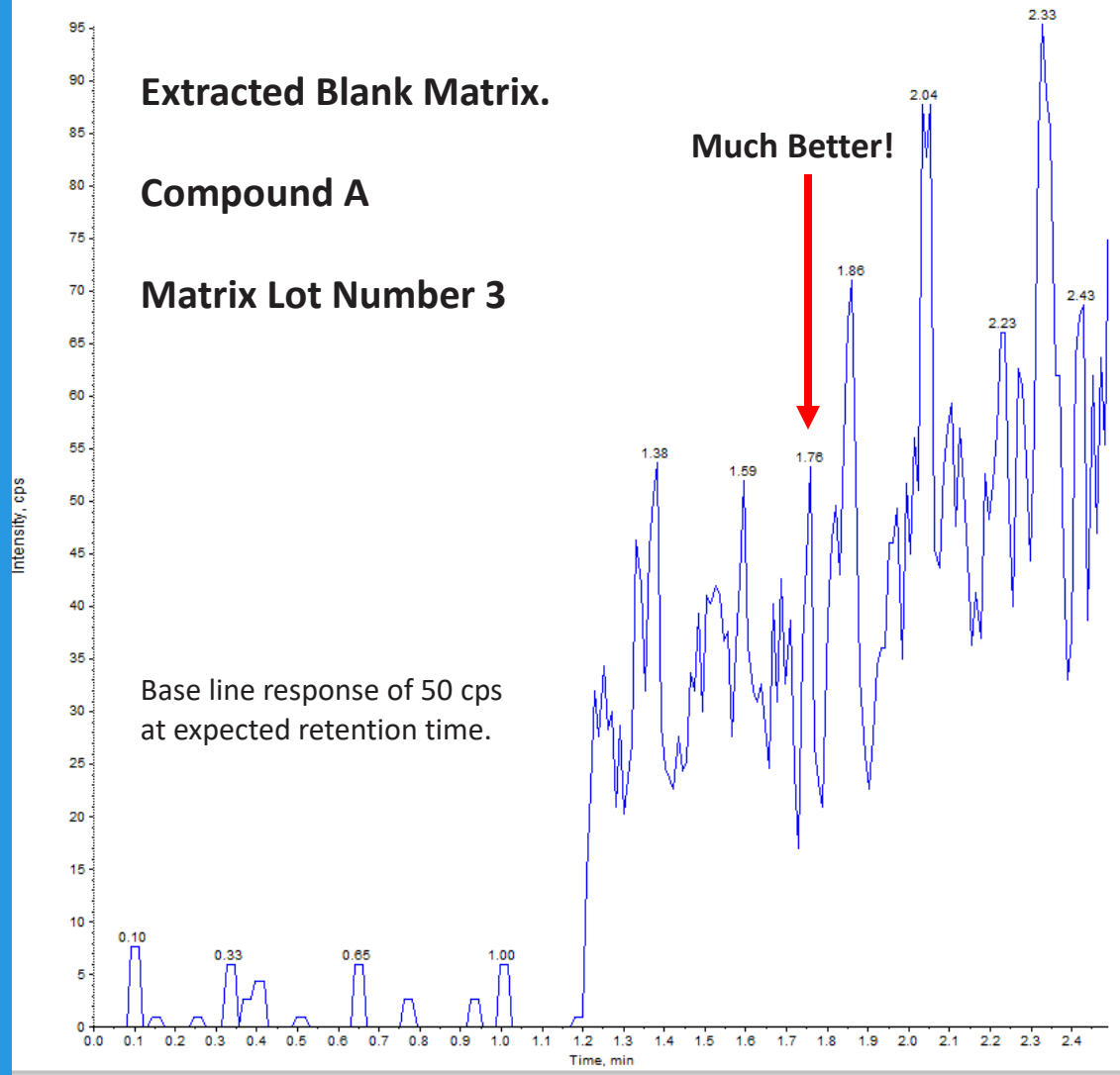


Extracted Blank Matrix.

Compound A

Matrix Lot Number 3

Much Better!

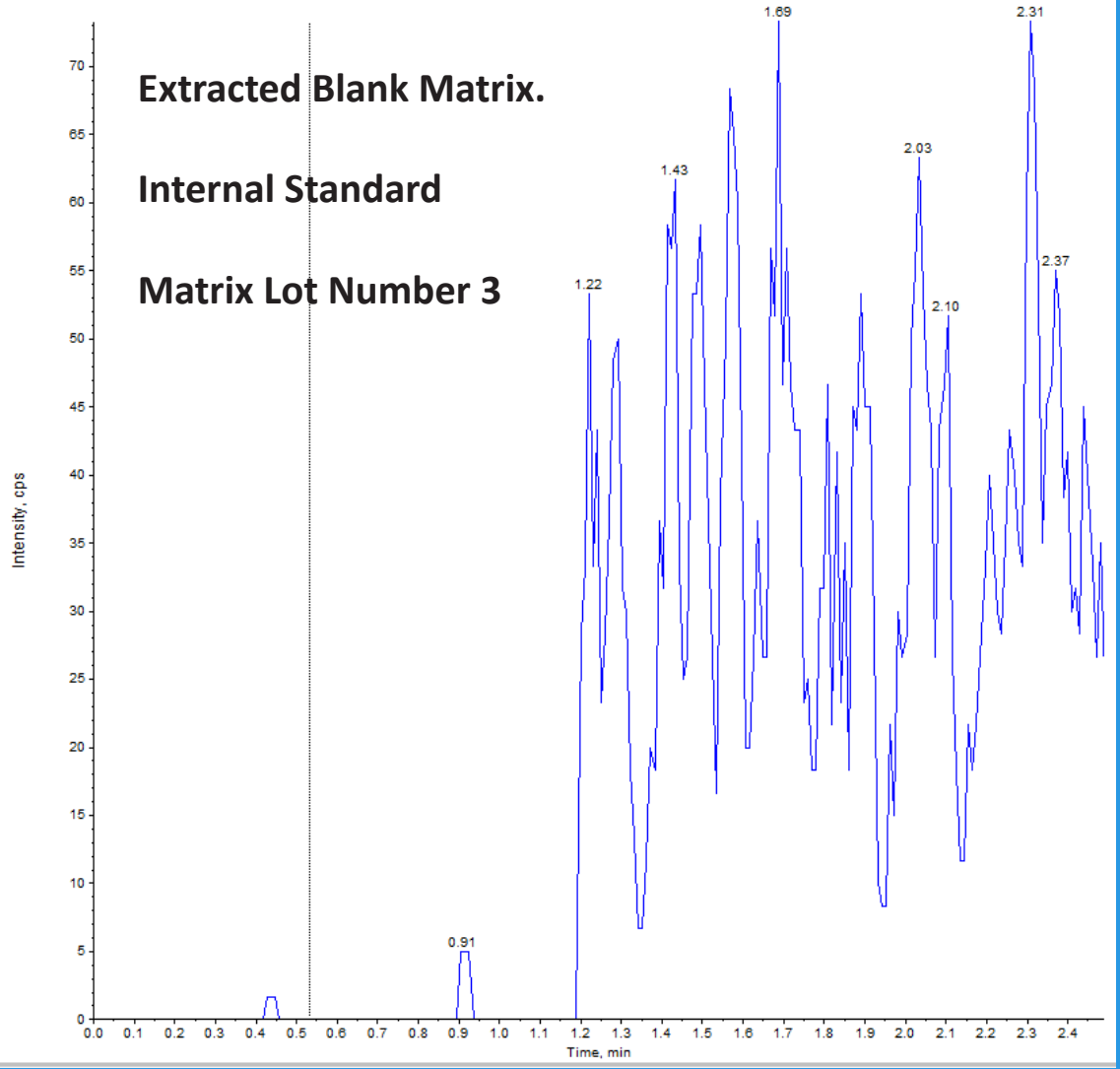


Base line response of 50 cps at expected retention time.

Extracted Blank Matrix.

Internal Standard

Matrix Lot Number 3



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Resolution

- Implementing a combination of changes seemed to give an improved result
- These changes included:
  - Injecting batches on same day of extraction
  - Rinsing glassware with intended solutions and reducing expiry date to 3 days
  - The use of Low binding plates during the elution step
- Include all changes to the method to improve on the repeatability of the assay

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Conclusion

Even though it took a lot of work, we managed to get there in the end

Combination of troubleshooting techniques has lead to improving this assay not only for this study, but other studies using this compound

We ultimately managed to complete all the sample analysis effectively and efficiently to meet deadlines

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Thank you for listening **Any Questions?**

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