Technische Universität Braunschweig

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Institut für Medizinische und Pharmazeutische Chemie

Reference data for peak integration in capillary electrophoresis: another successful CASSS project

Marlon Krompholz[1], Huixin Lu[2], Cari Sänger-van de Griend[1,3,4] , Hermann Wätzig[1]

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[2]*Health Canada*
[3]*Kantisto BV, The Netherlands*
[4]*Department of Medicinal Chemistry, Faculty of Pharmacy, Uppsala Universitet, Sweden*

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Integration of Electropherograms in GMP Labs Under Increasing Scrutiny Due to Data Integrity Intensive Inspections

Tim Blanc[1], Hermann Wätzig[2], Cari Sänger-van de Griend[2, 3, 4]

[1]*Eli Lilly and Company*
[2]*Institute of Medicinal and Pharmaceutical Chemistry, University of Braunschweig, Germany*
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[4]*Department of Medicinal Chemistry, Faculty of Pharmacy, Uppsala Universitet, Sweden*

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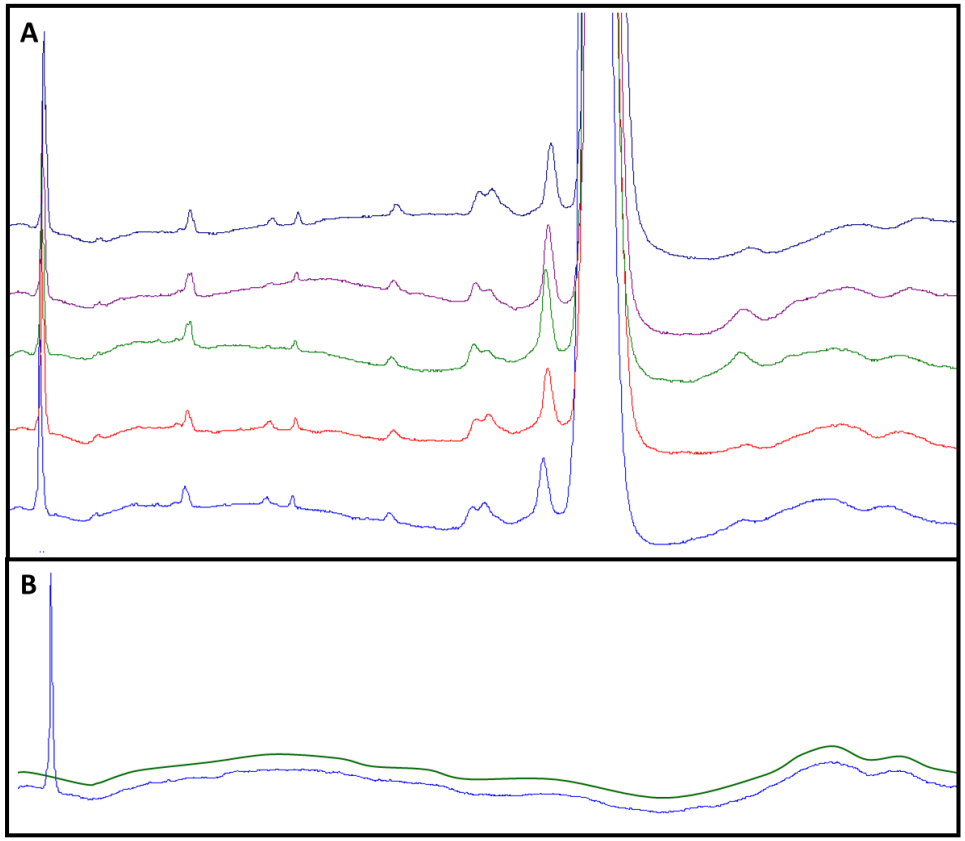
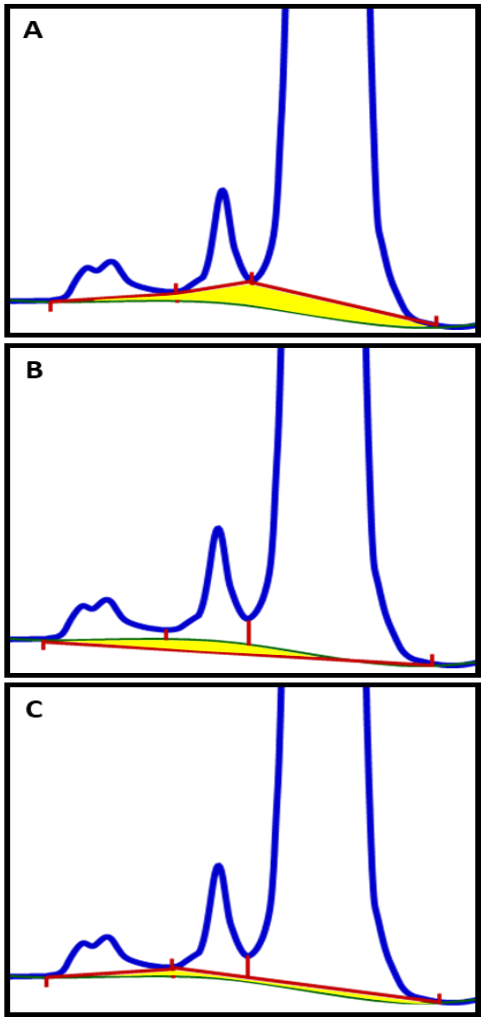
Peak integration: critical examples

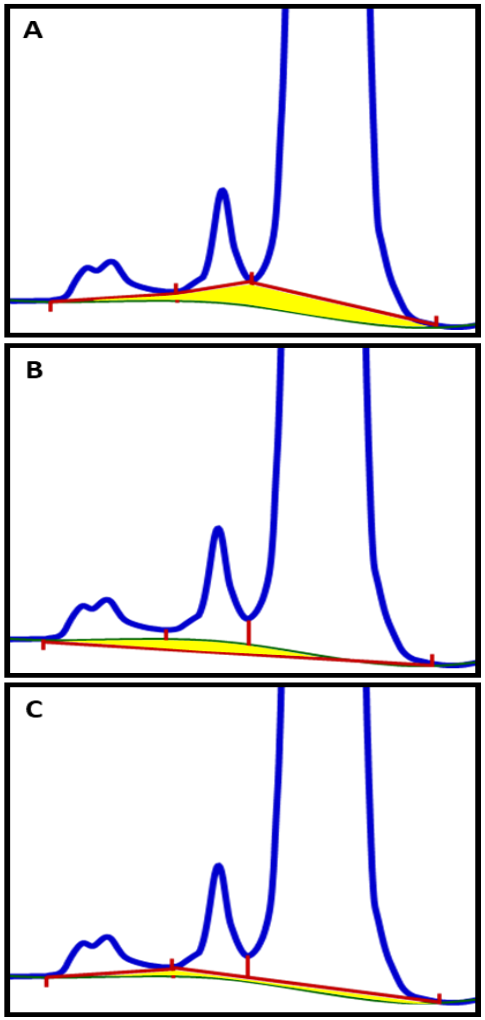




Peak integration: critical examples

Tim Blanc

Cari Sänger



Peak integration

- Integration in GMP laboratories is critically important
  - has become focus of data integrity-centric regulatory inspections

- Data systems developed for chromatograms rather than electropherograms, and the increased regulatory scrutiny call for a resolution.

- This extends to R&D, clinical, and academic labs

Tim Blanc, Hermann Wätzig and Cari Sänger – van de Griend,
Peak Integration of Electropherograms in GMP and Research Labs: Navigating Increased Scrutiny Amid Data Integrity Audits and Inspections
Electrophoresis, 2025; 46:653–668
http:// dx.doi.org/10.1002/elps.70002

Peak integration: Conclusions from this review

- We need APs and SOPs
- We need detailed method descriptions, defaults, parameter lists, illustrations, training
- We need to keep manual integration at the present time
- Collaboration within the industry for generally accepted standards
- Benchmark data sets could be an important step
- Based on this, considerable improvements in integration algorithms are possible
- So probably less manual integration in the future, but at present it is still a must

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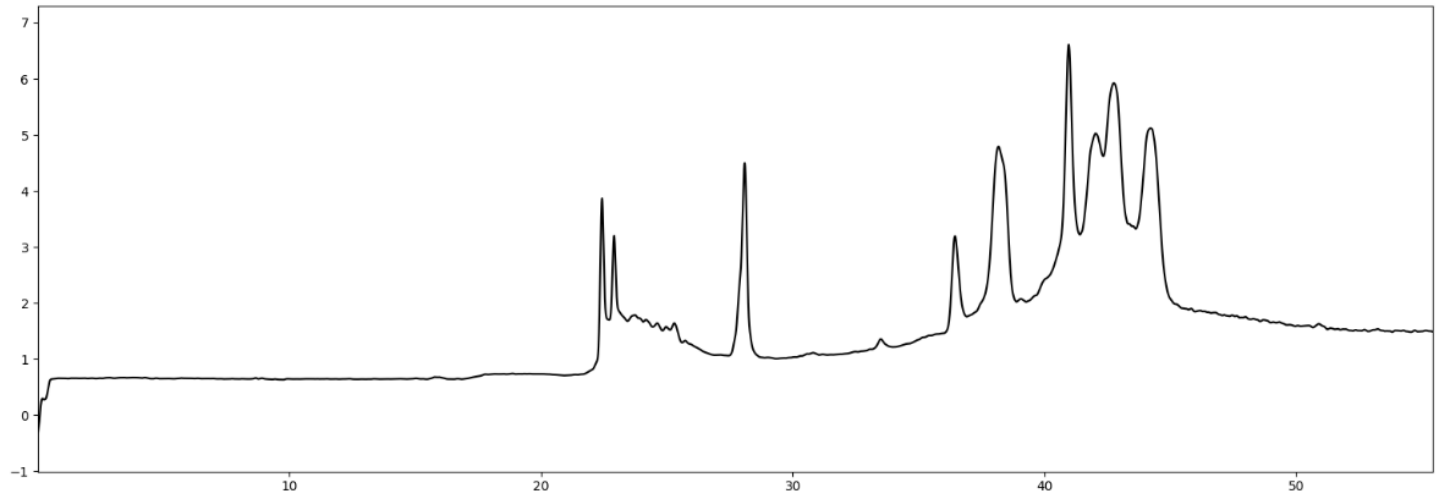
Interesting Reference Data for Peak Integration



- Broad, asymmetrical peaks
- Fronting/Tailing
- Baseline irregularities

[3]

→ Existing softwares can not properly face these challenges

Intercompany Collaboration on Reference Data

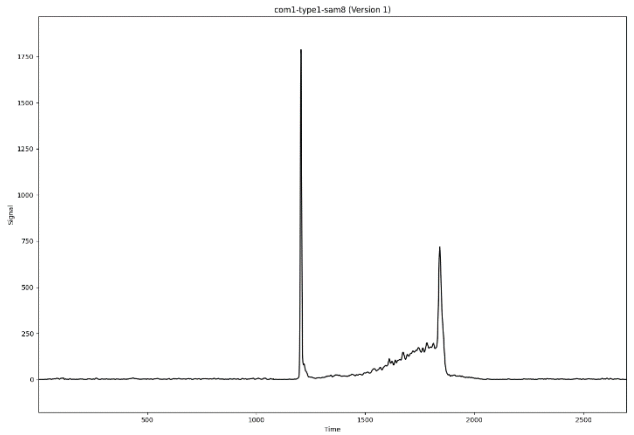
Marlon Krompholz

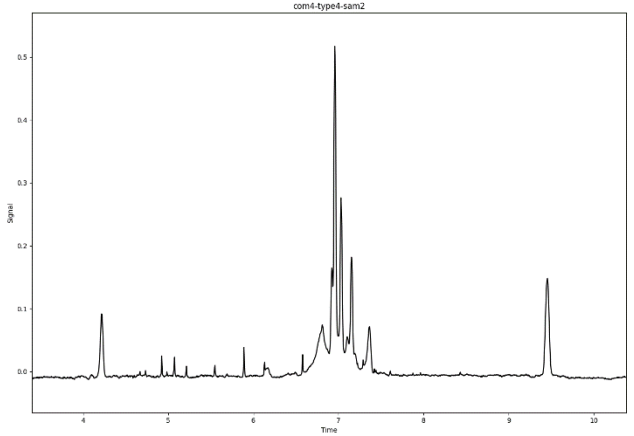
- Multinational collaboration of CE experts
- Data collection
- Experts set peak limits
- Comparison and Discussion
- Refined integration rules
- Pre-integration by Marlon
- Approval/Confirmation by experts
- Approval by the community
- Reference Data Set CE Integration #1

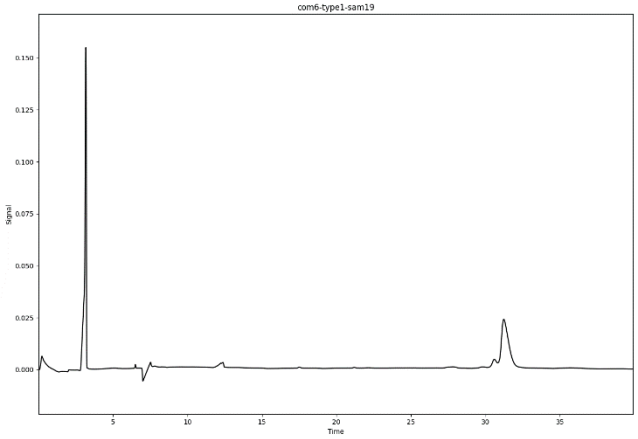
Intercompany Collaboration: a CASSS initiative

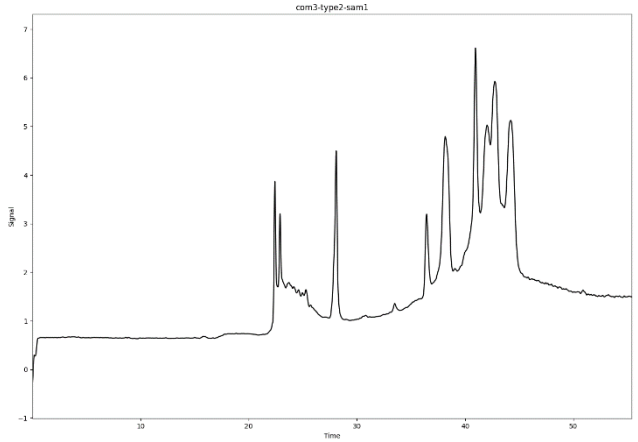
Mostafa Abuzeid, **Timothy Blanc, Tao Bo**, M Chan, **Patricia Christensen**, Adriana Coll de Peña, **Miyuru de Silva**, Hans Dewald, Gangadhar Dhulipala, Tara Enda, Joachim Ermer, **Jarvas Gabor**, James Geiger, **Frederic Ginot**, Mervin Gutierrez, Jakob Haglöf, Mark Haverick, Philip Hoang, Robert Hong, Christopher Hood, Philipp Huber, **Andrei Hutanu**, Ryan Hylands, Yun Hwang, **Pilsoo Kang, Steffen Kiessig, Marlon Krompholz**, Andreas Krupke, David Lasar, **Rick Linkous**, **Huixin Lu**, Jane Luo, **Will McElroy**, **Chris Heger, Paul S Michael-Butler**, Jana Molitor, Cristina Montealegre Dondarza, **Bernd Moritz**, Matthew Myers, Ryan Nai, **Trang D Nguyen,** John Orlet, **Rebecca Osborne**, Jeremy Osko, David O'Sullivan, **Ashley Prout**, **Timothy Riehlman**, David Ripley, Jacob Rostkowski, Shauna Salem, **Cari Sänger-van de Griend**, Emineguel Sakka, Carlie M Schaeffer, Johannes Schlecht, Kristin Schultz-Kuszak, **Jana Steflova**, **Christian Streng,** Adam Sutton, Lalin Theverapperuma, Amanda Torelli, Ewoud van Tricht, **Carolina G. Vega**, **Yun Wang**, **Denise Warzak**, Brian Wei, Katharina Yandrofski, Tao Zao, Yuling Zhang

Intercompany Collaboration: Data Collection

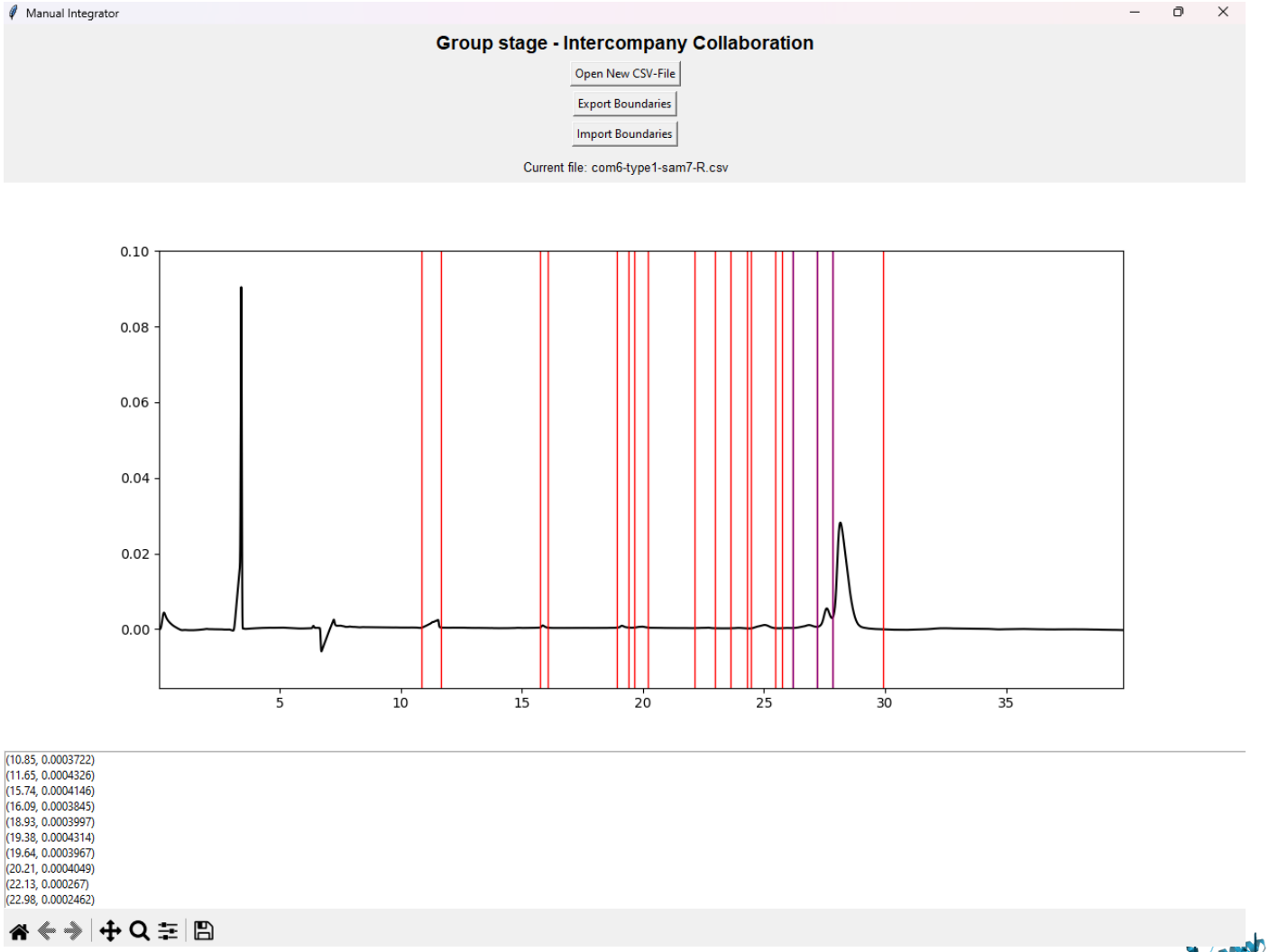








Intercompany Collaboration: Experts set peak limits



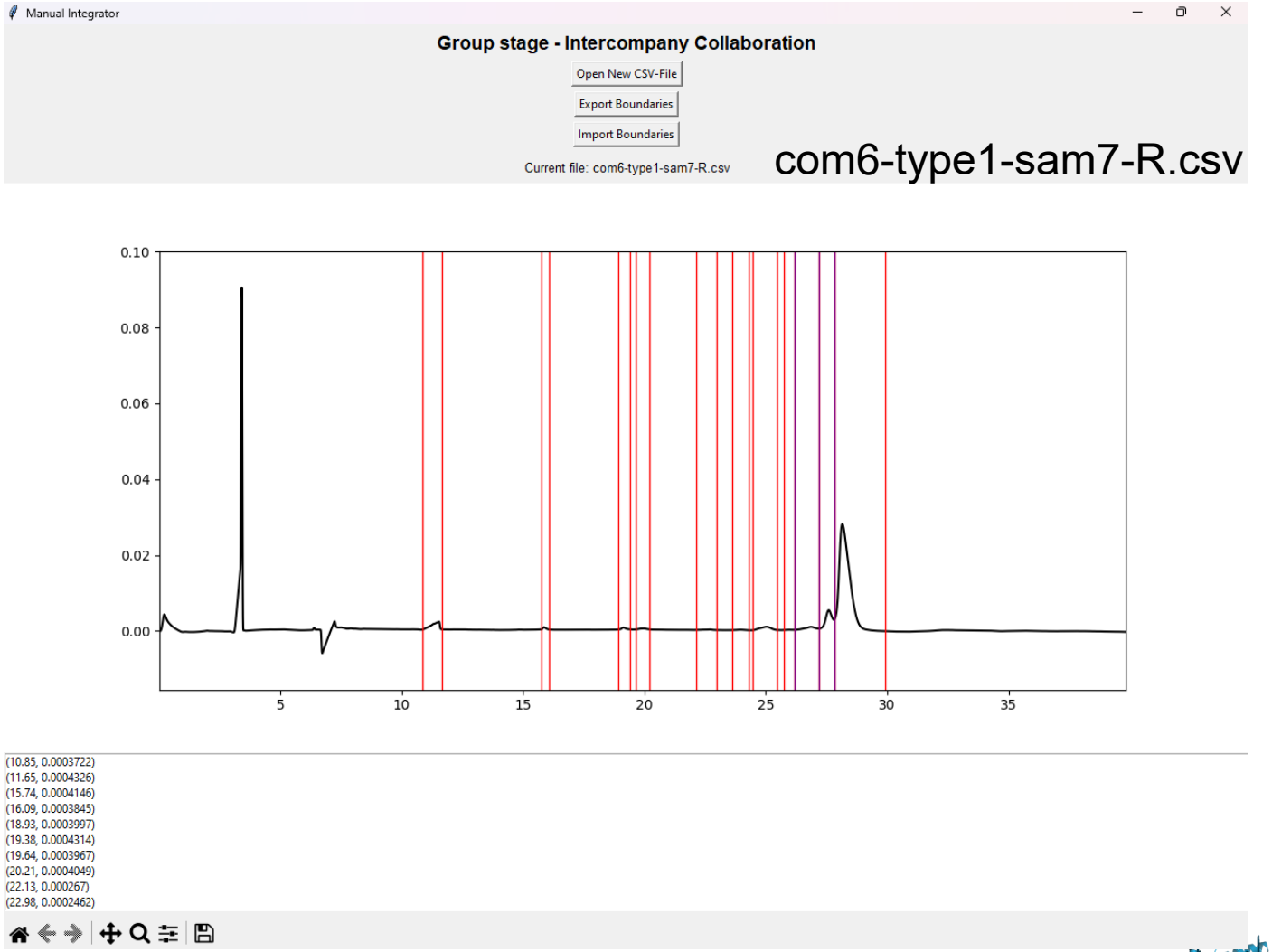
Software tool:

Developed with

Python using the

OpenAI o3 model

Intercompany Collaboration: Experts set peak limits



Software tool:

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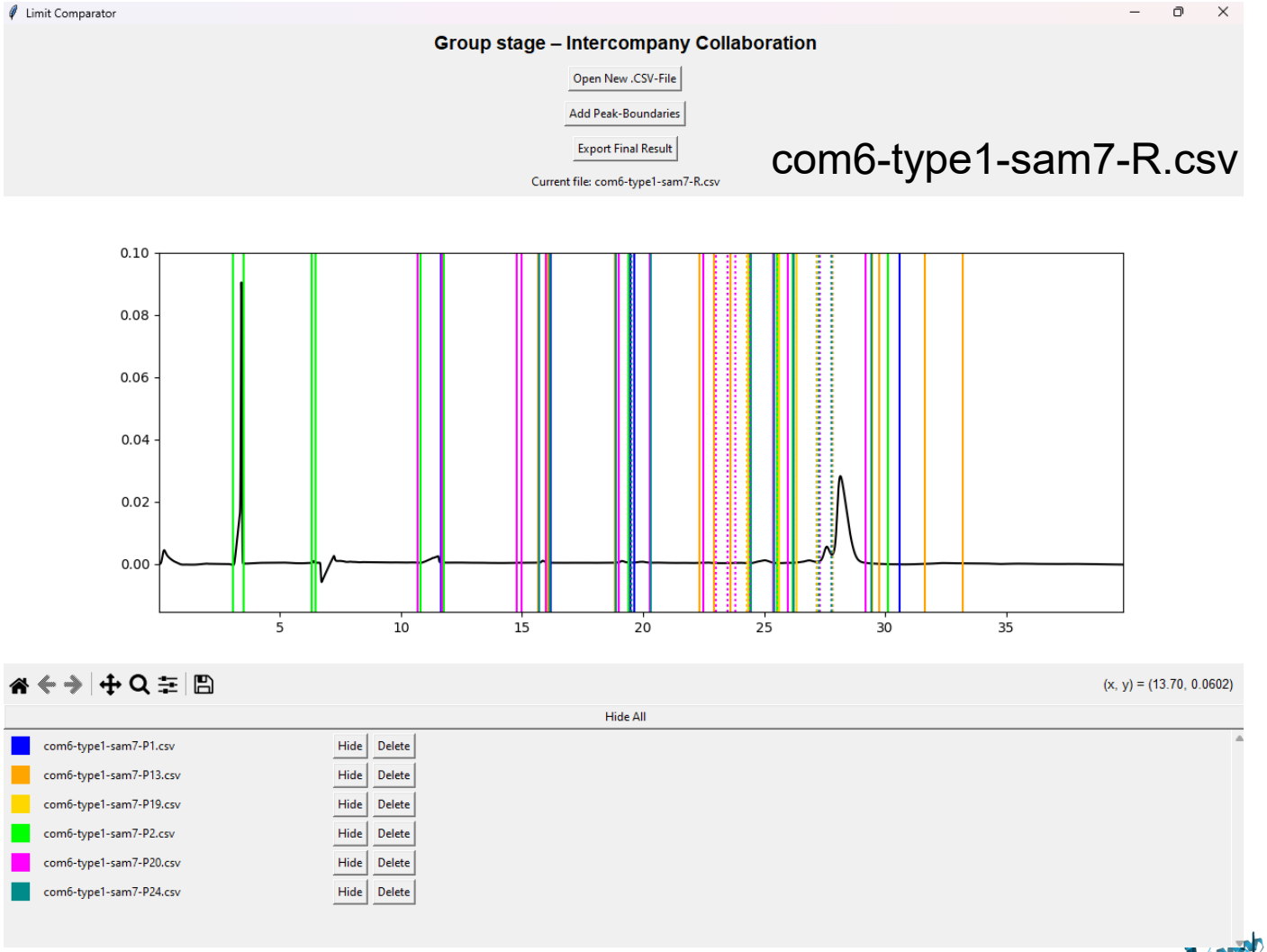
Intercompany Collaboration: Comparison and Discussion

<u>Software tool:</u>

Developed with

Python using the

OpenAI o3 model



Intercompany Collaboration: Comparison and Discussion

<u>Software tool:</u>

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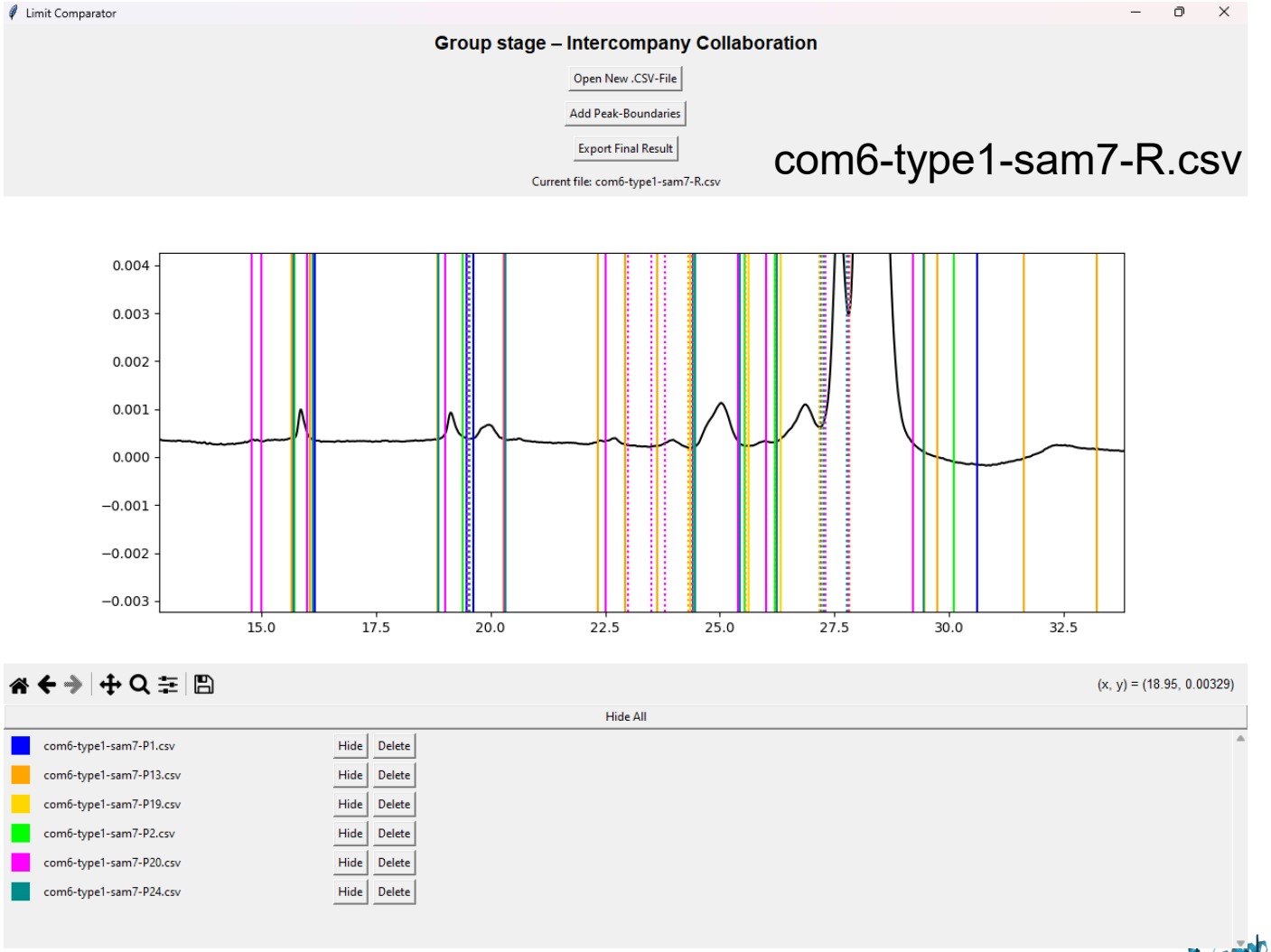
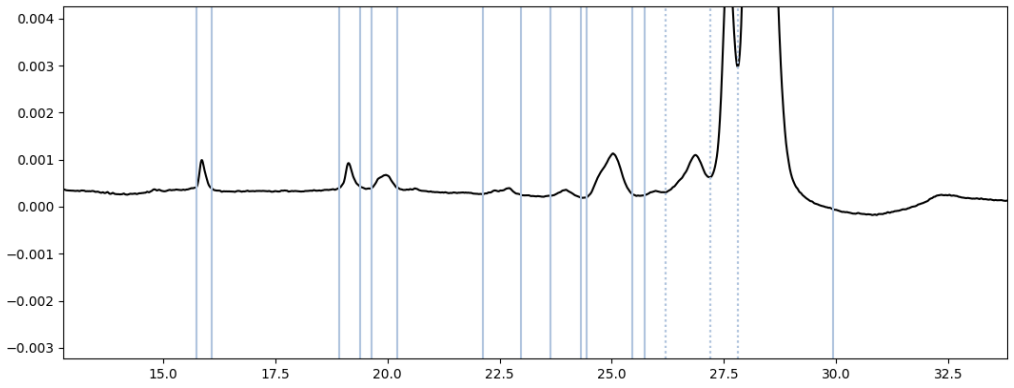
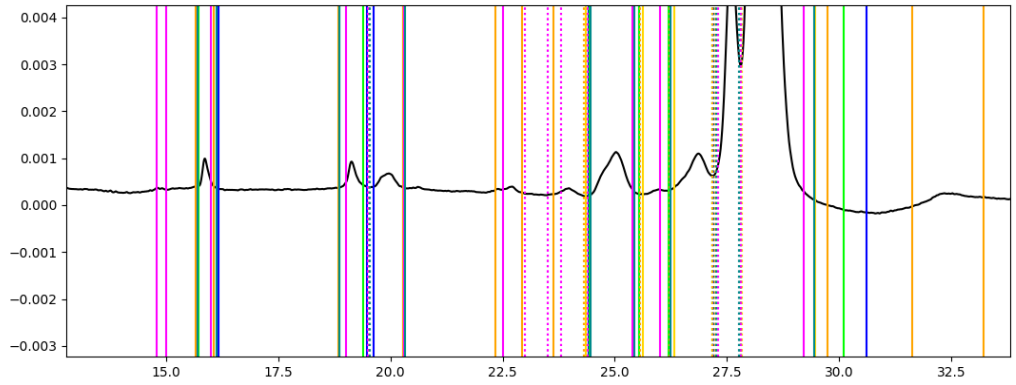


Intercompany Collaboration: Comparison and Discussion

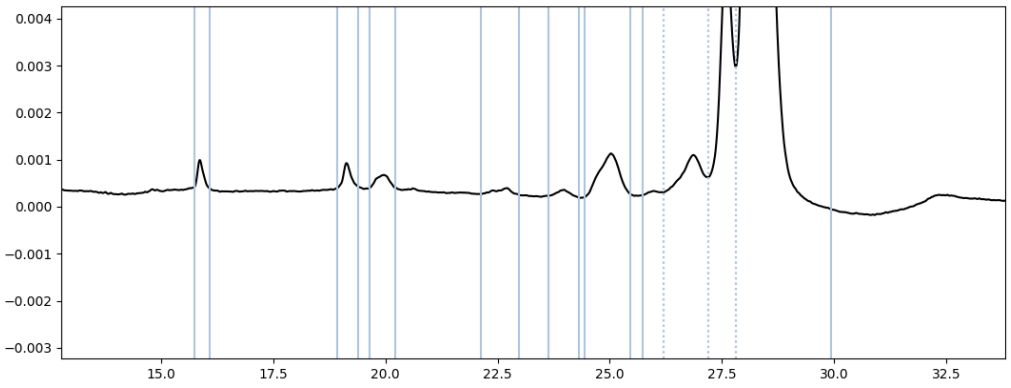


Re-set boundaries

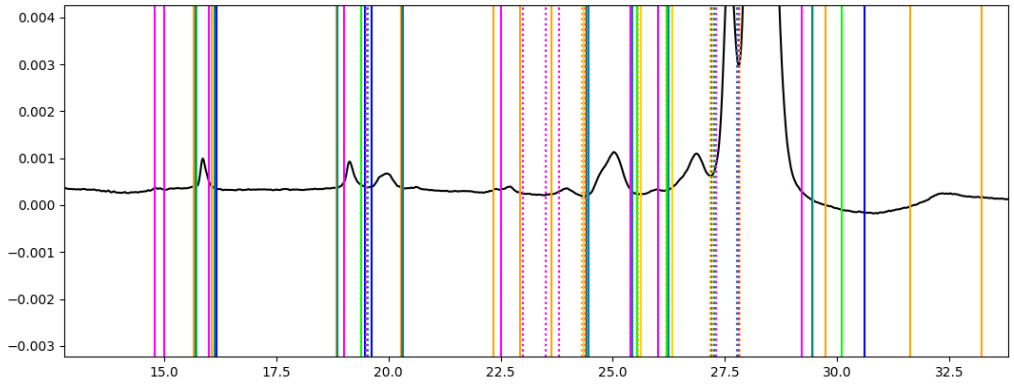


Experts‘ boundaries

Refining Integration Rules



Re-set boundaries



Experts‘ boundaries

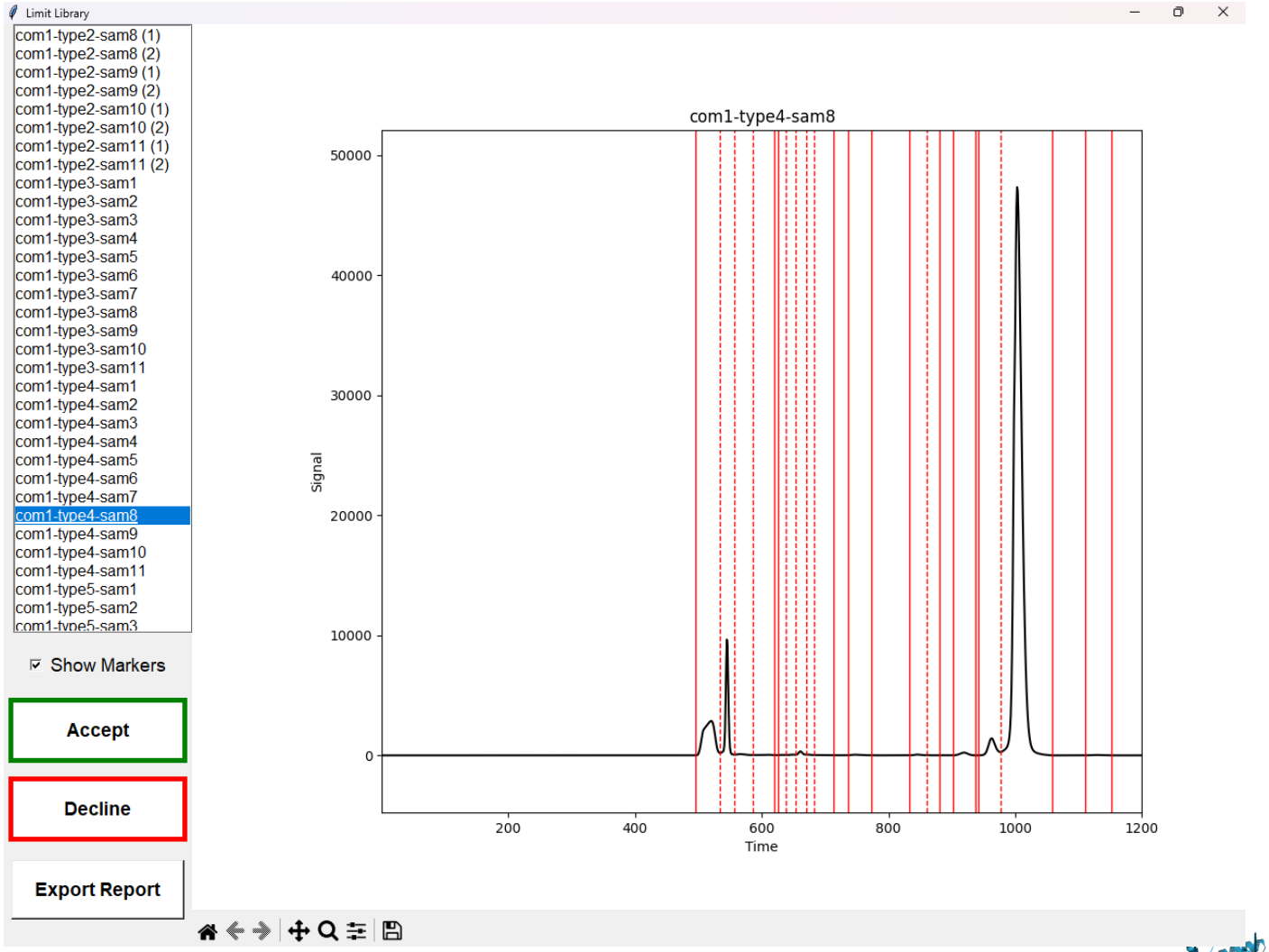
Pre-Integration by Marlon

Software tool:

Developed with

Python using the

OpenAI o3 model



Intercompany Collaboration: Confirmation by the Experts

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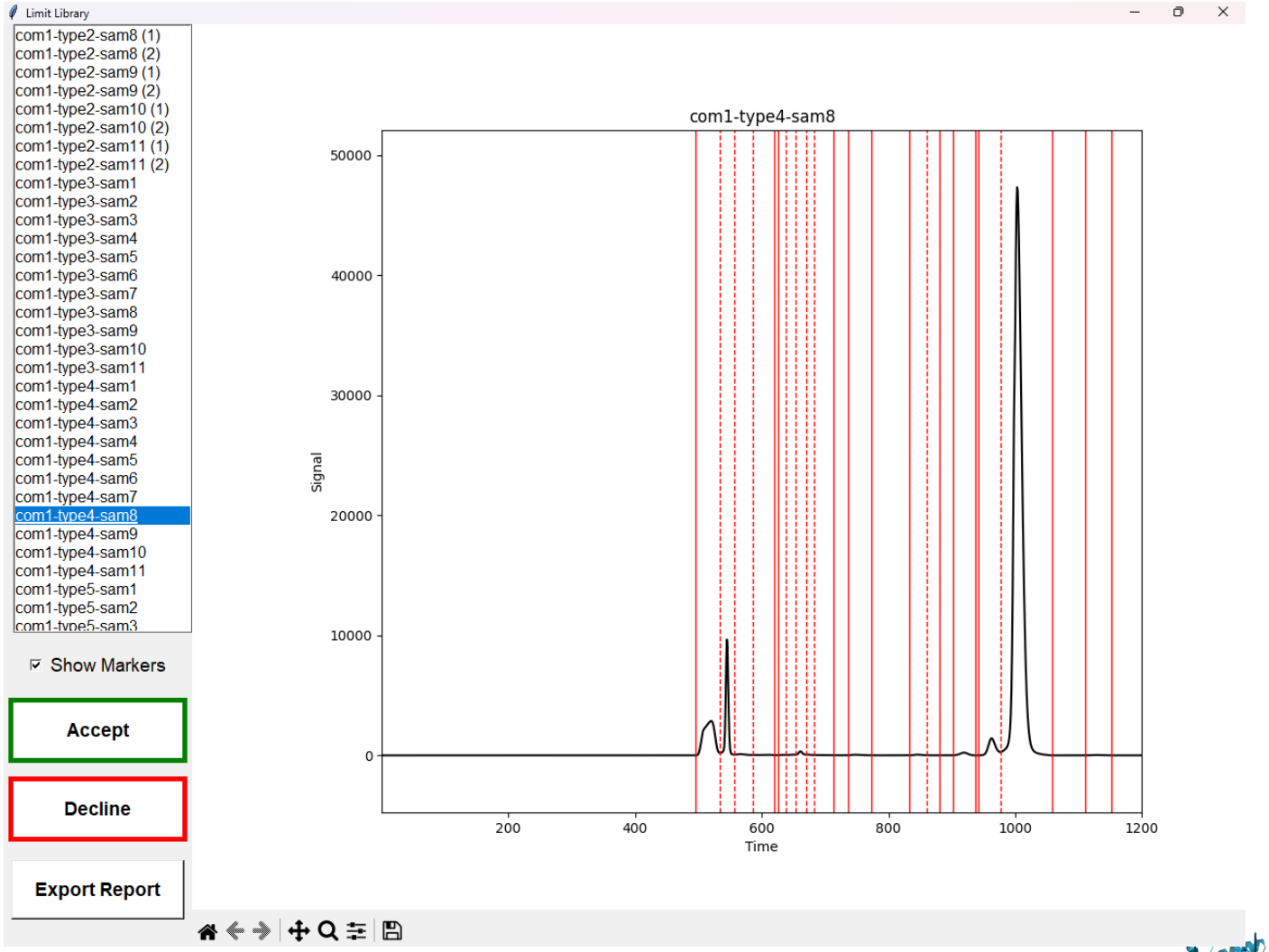
Developed with

Python using the

OpenAI o3 model



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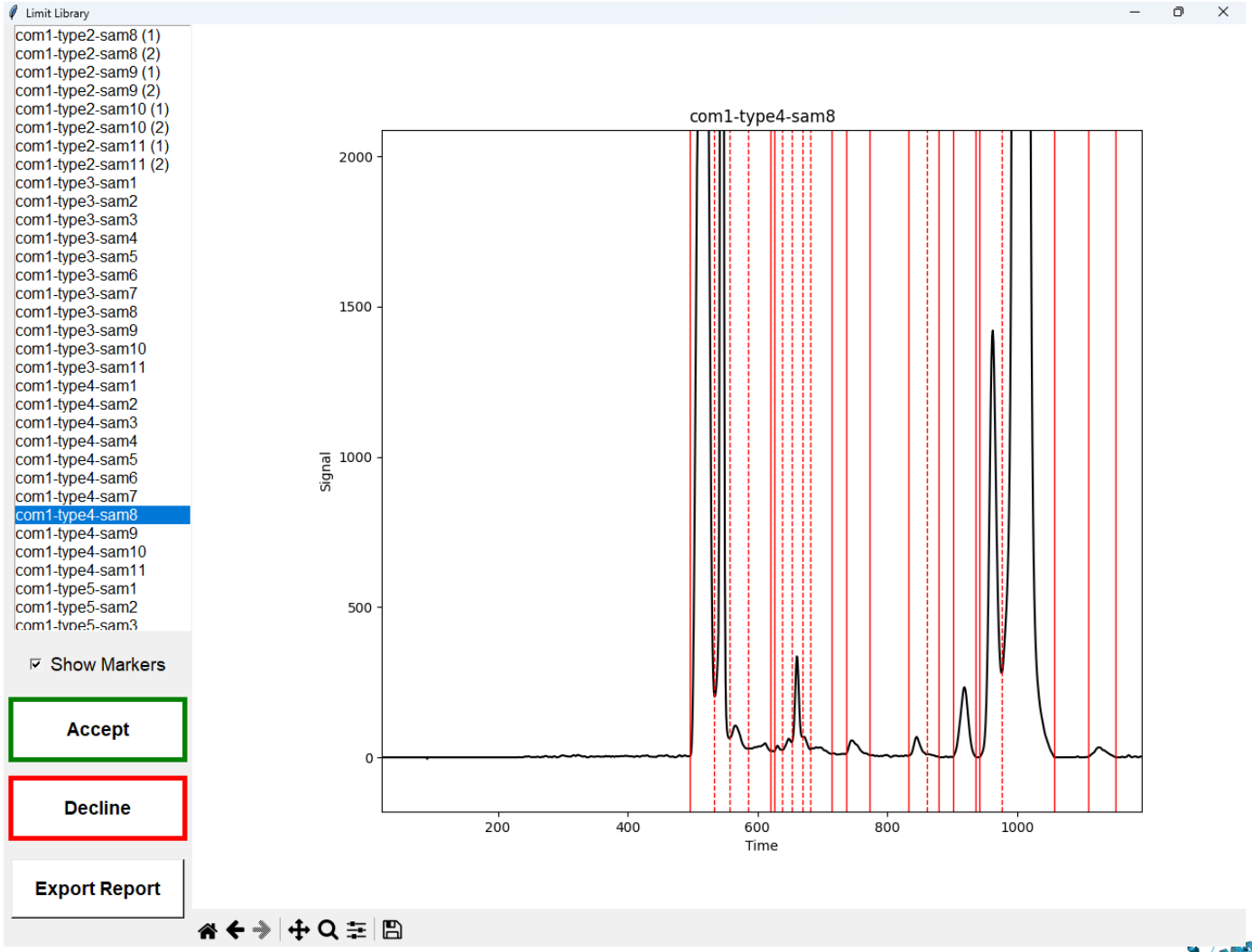
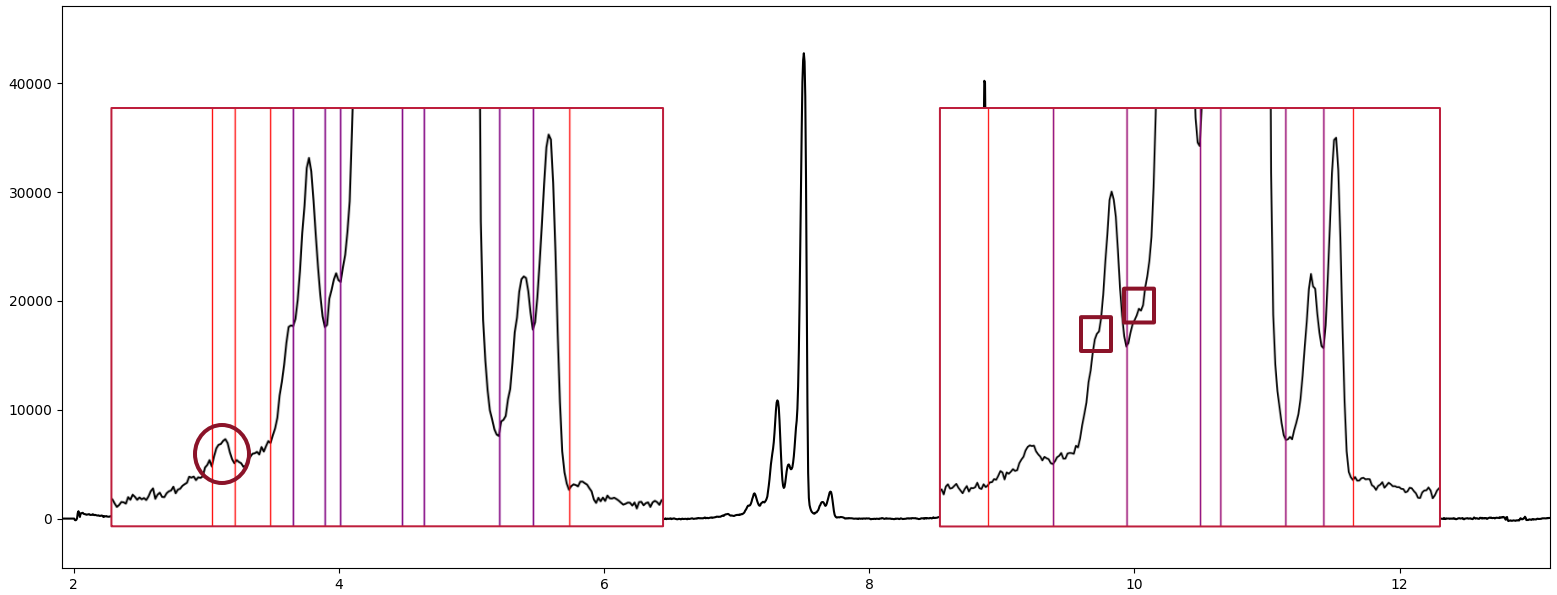
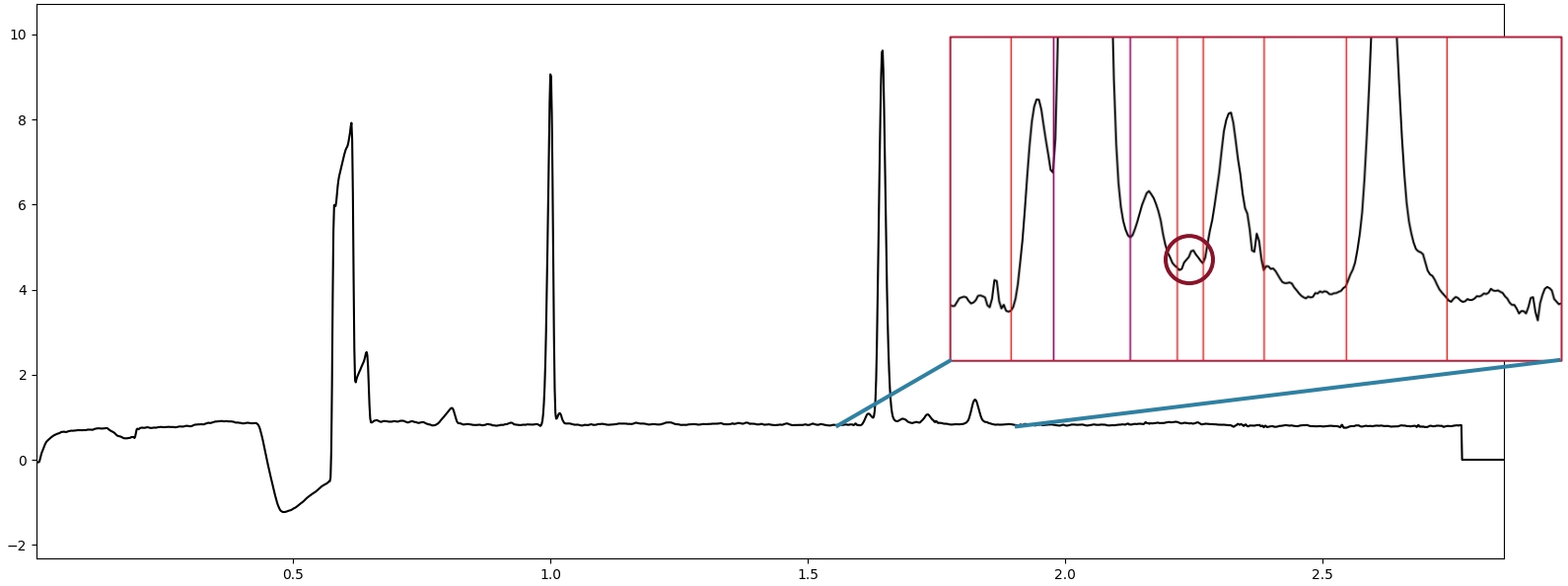


Debatable and context-dependant cases



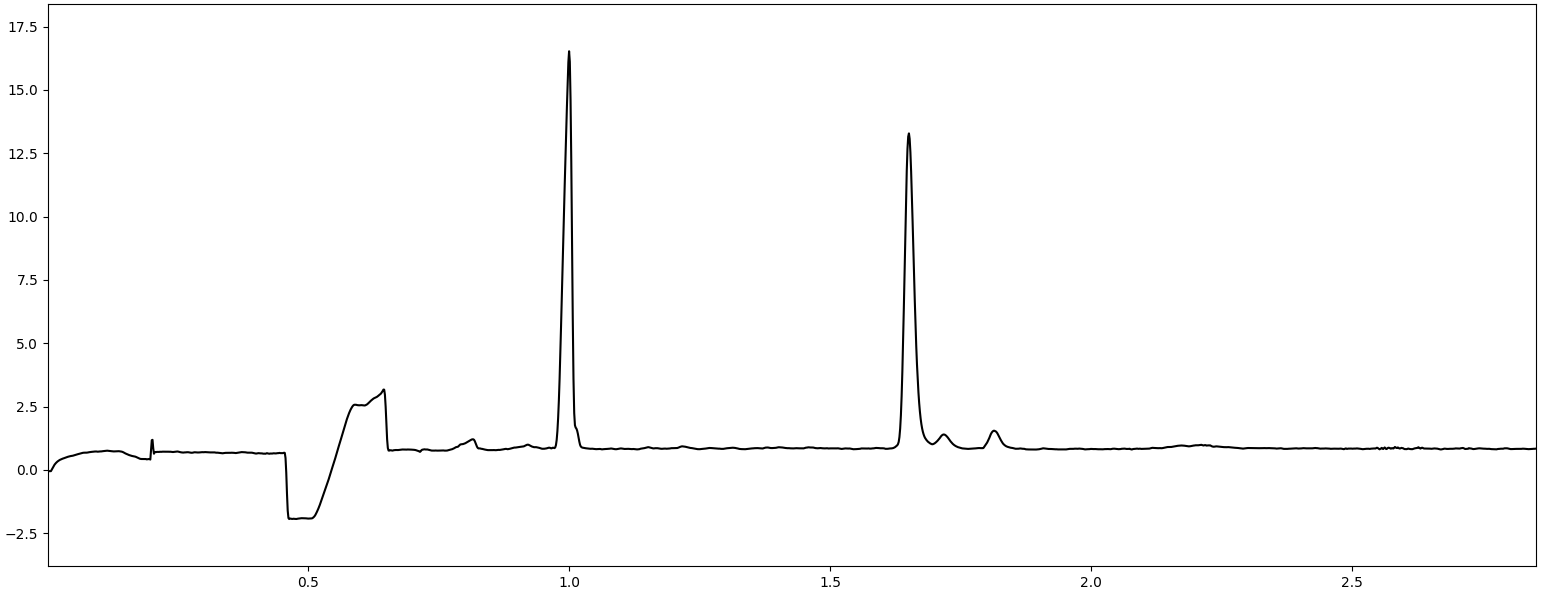
com2-type1-sam15 vs. com2-type1-sam2

- Is circle marked area a peak or noise?
- If peak: Which approach is better?
- Should square marked points be integration limits due to the comparison to the left image section?



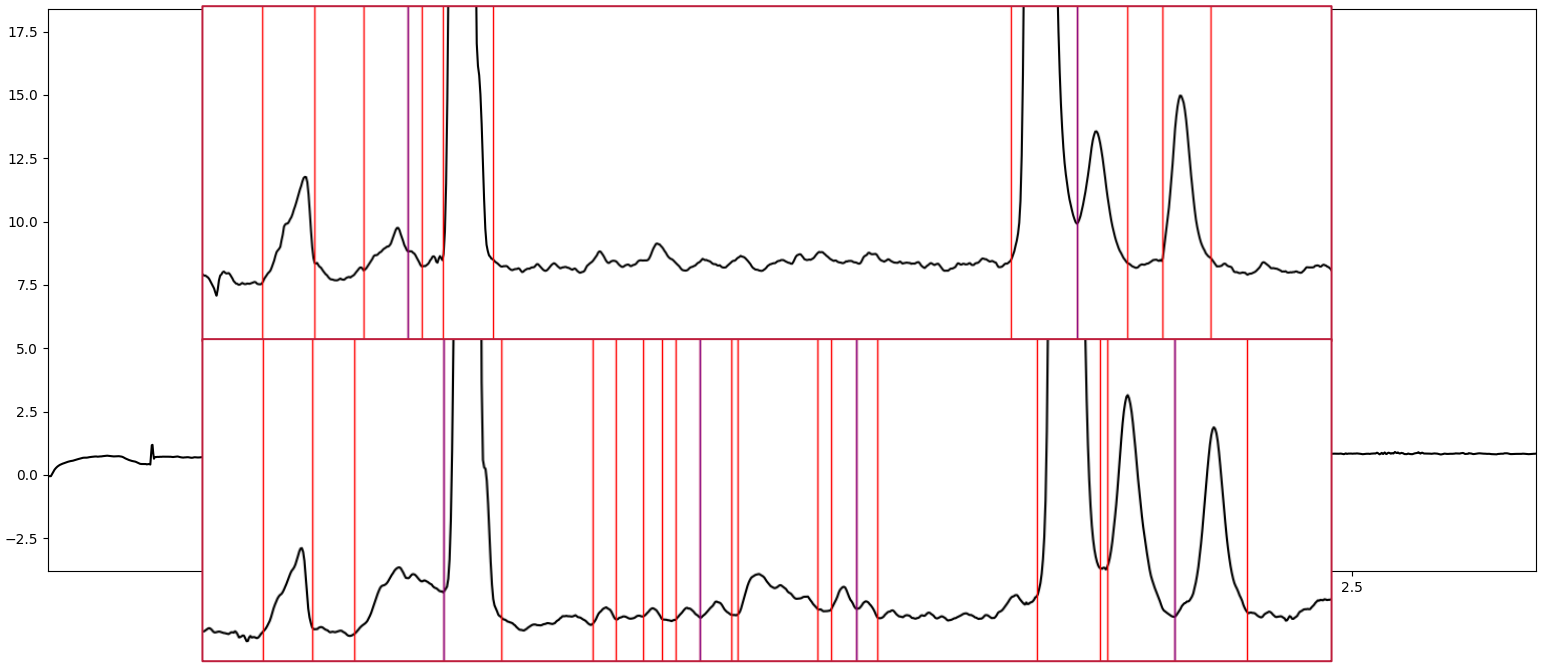
com2-type2-sam2

- Does the marked area belong to the baseline or does it lay above and therefore form a peak?



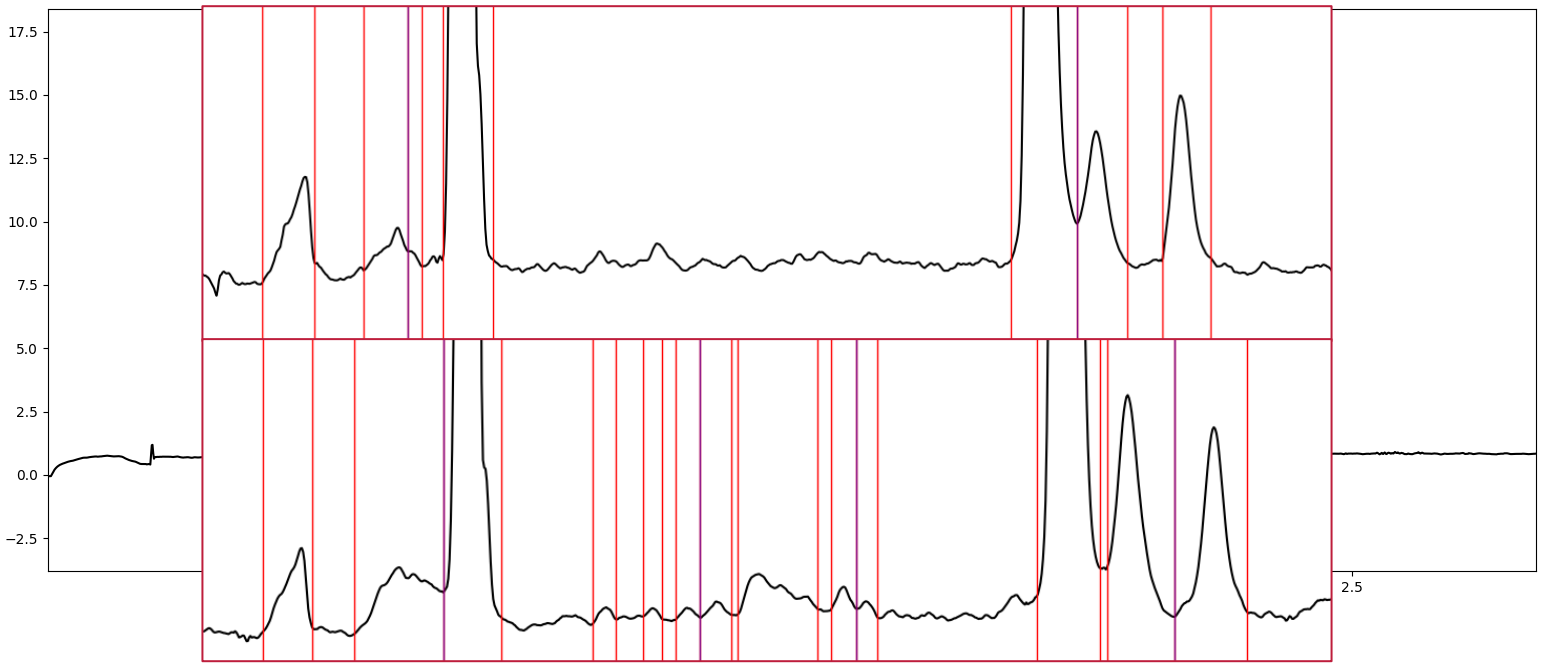
com2-type2-sam25 and com2-type2-sam5

- Are there any peaks to be considered between the main peaks or is it just noise?



com2-type2-sam25 (top) vs. com2-type2-sam5 (bottom)

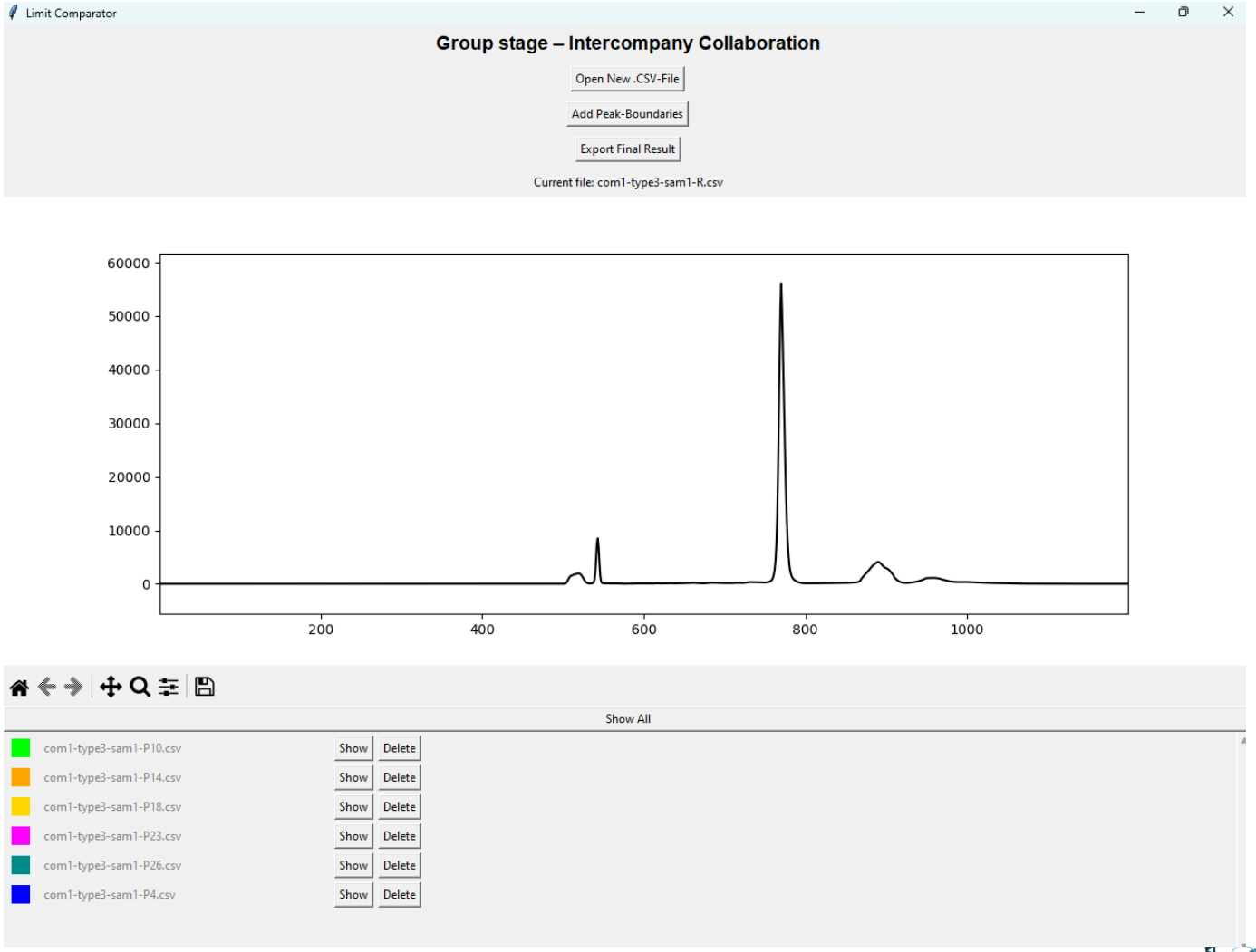
- Are there any peaks to be considered between the main peaks or is it just noise?



Suggestion, related to the European Pharmacopoeia 2.2.47, Capillary Electrophoresis:

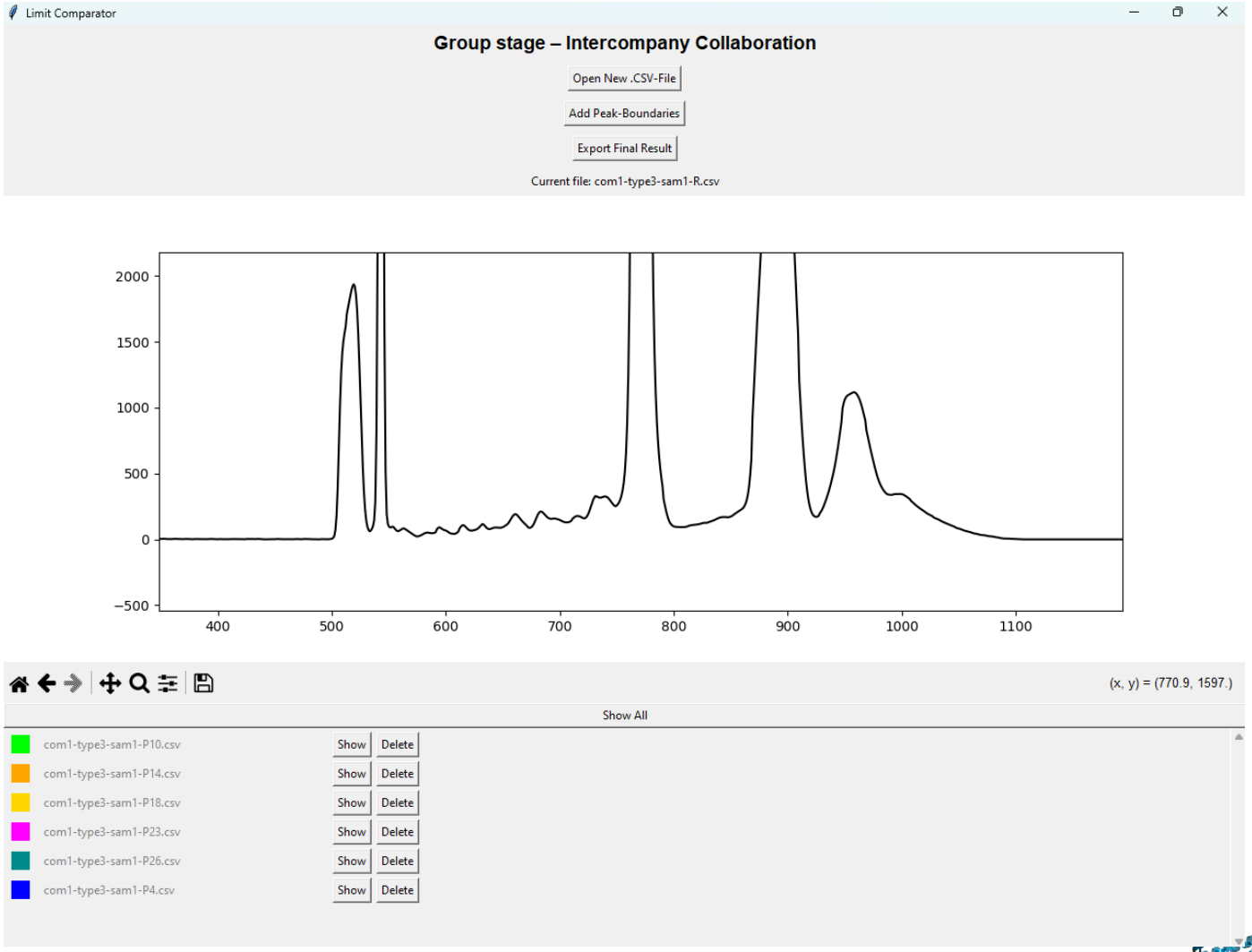
Find baseline as close as possible to the possible peaks of interest, 20 times as long as a major peak´s $h_{0.5}$, estimate peak-to-peak noise. All signal S/N > 3 considered as peaks

Interesting Findings

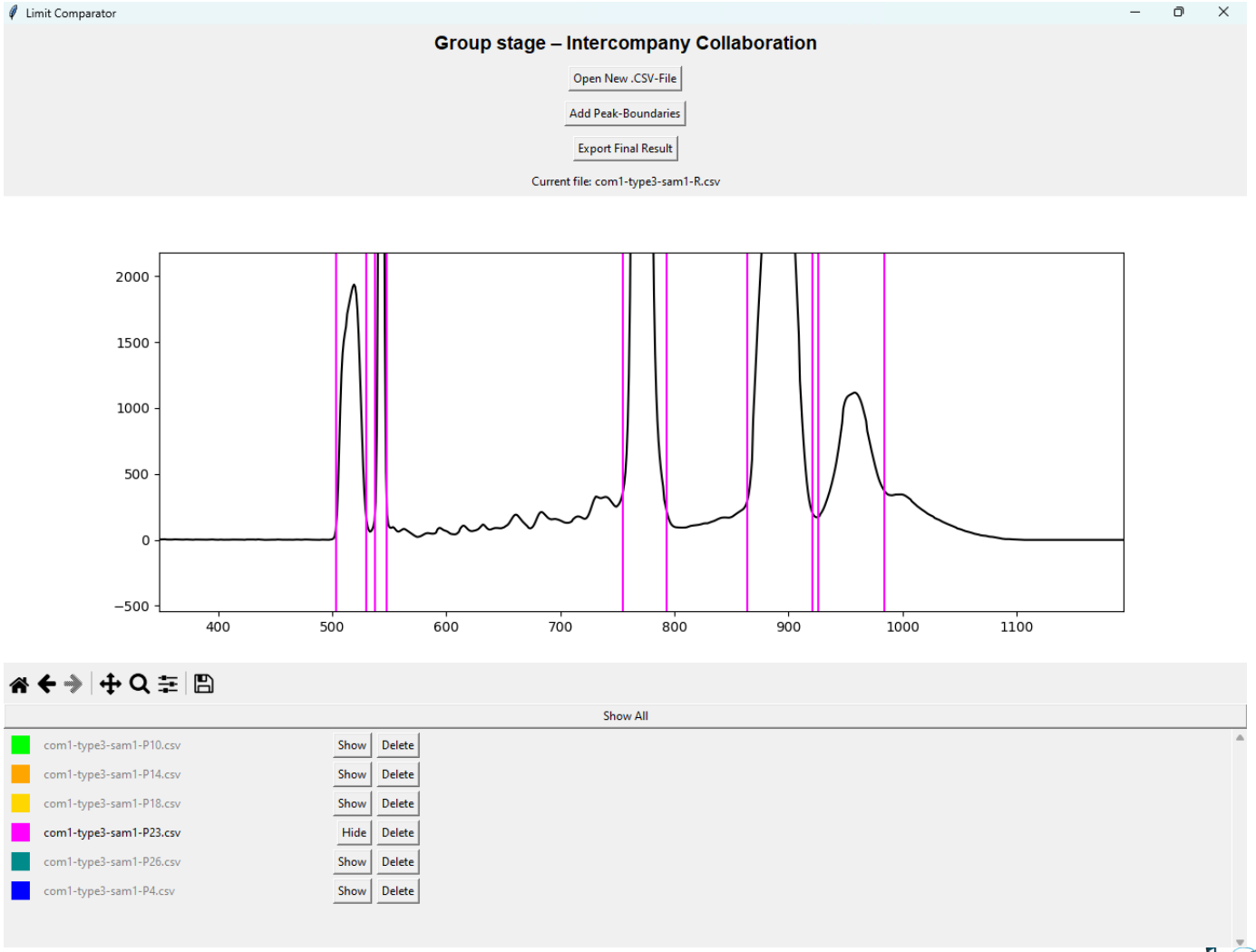


Interesting Findings

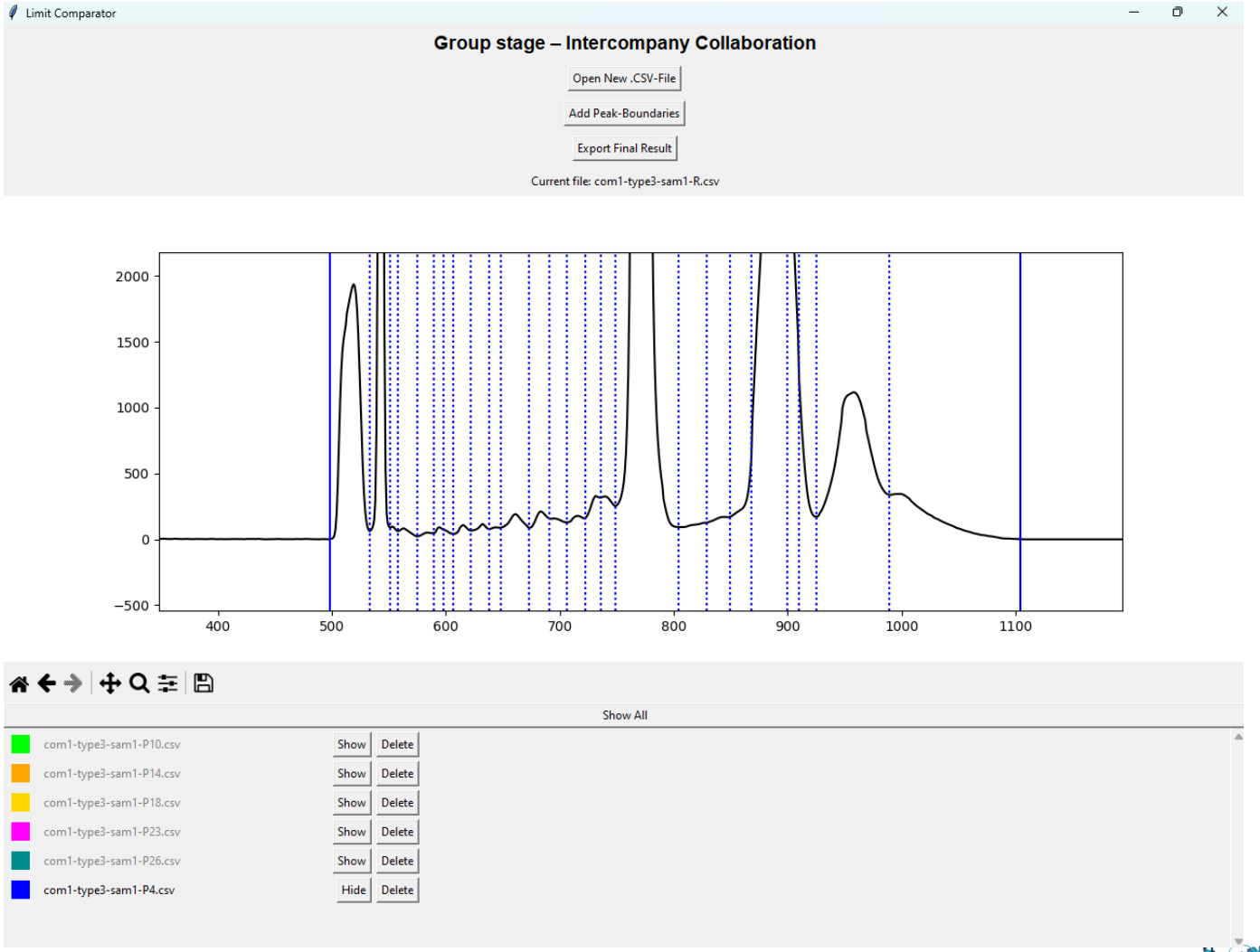
Zoom level
is crucial



Interesting Findings



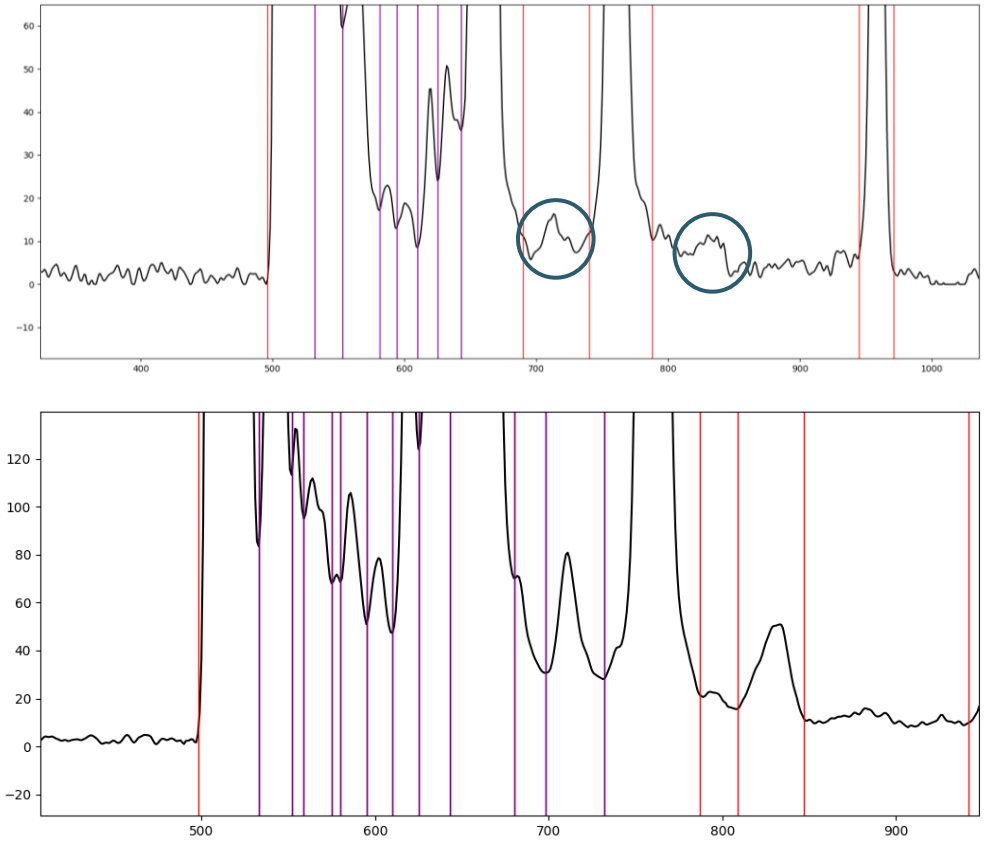
Interesting Findings



Interesting Findings

Integration depends on the context:

Peak or baseline?

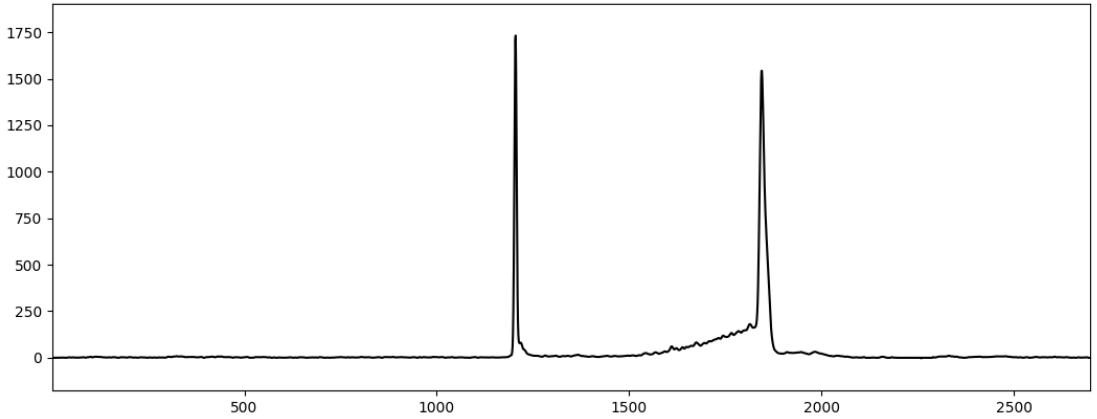


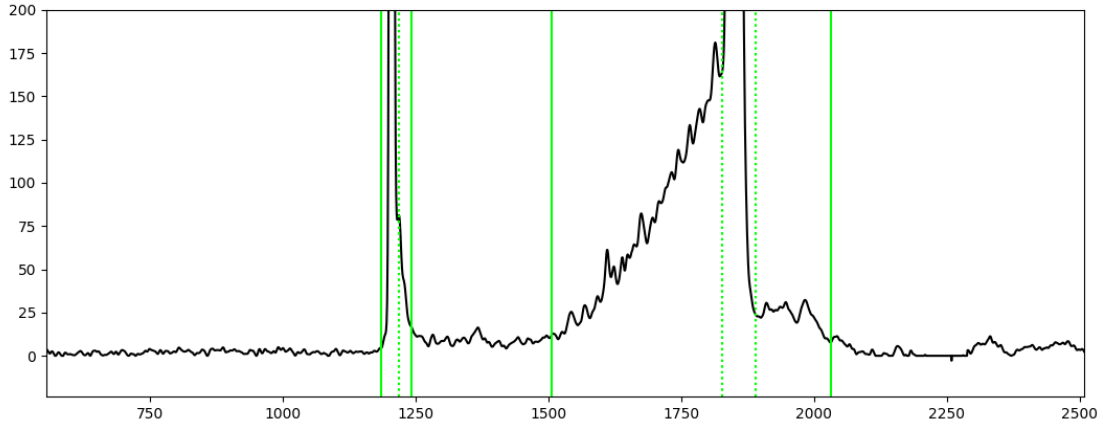
→ Clear peaks after comparing to other injection

Interesting Findings

Integration depends on the context:

RNA degradation measurement





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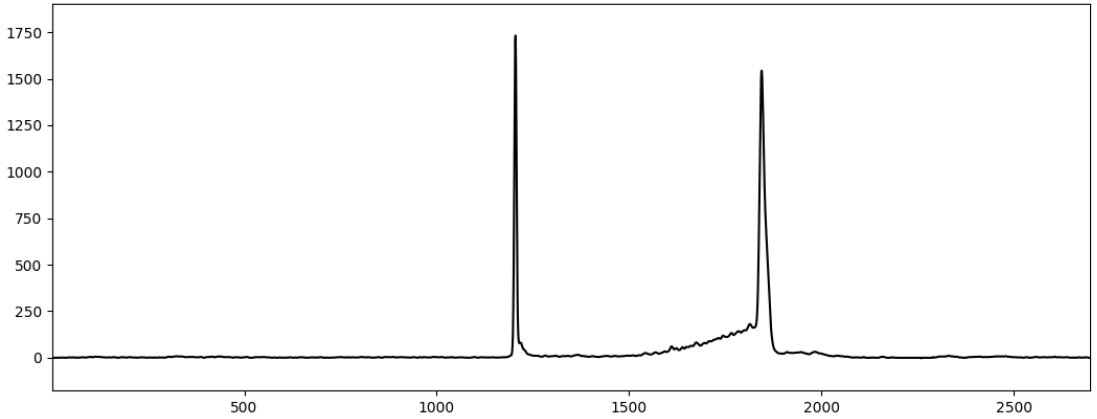

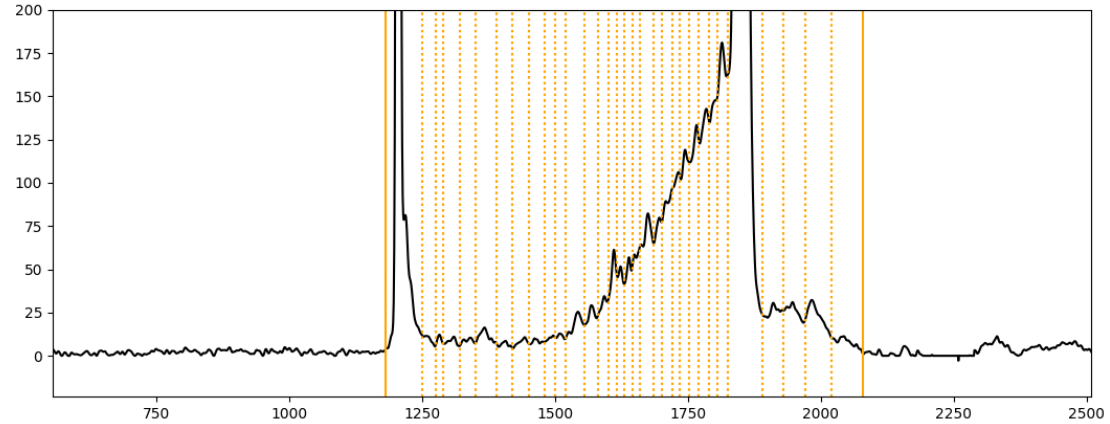

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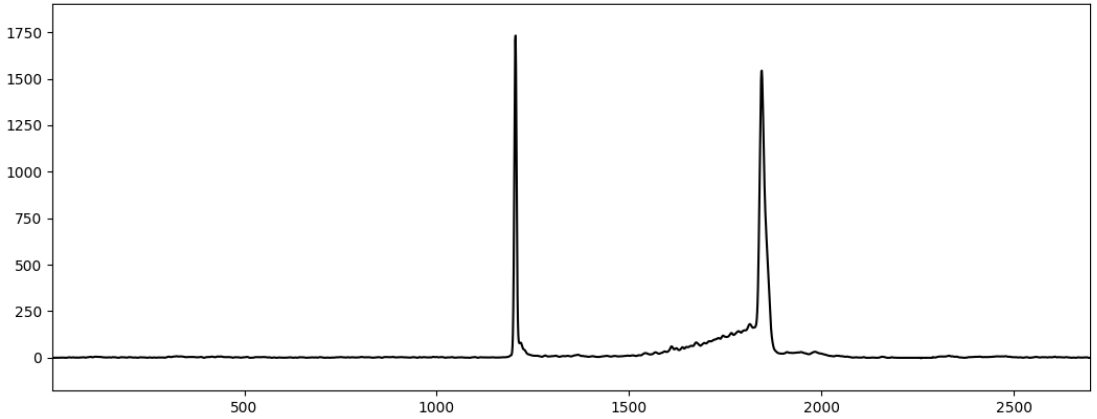
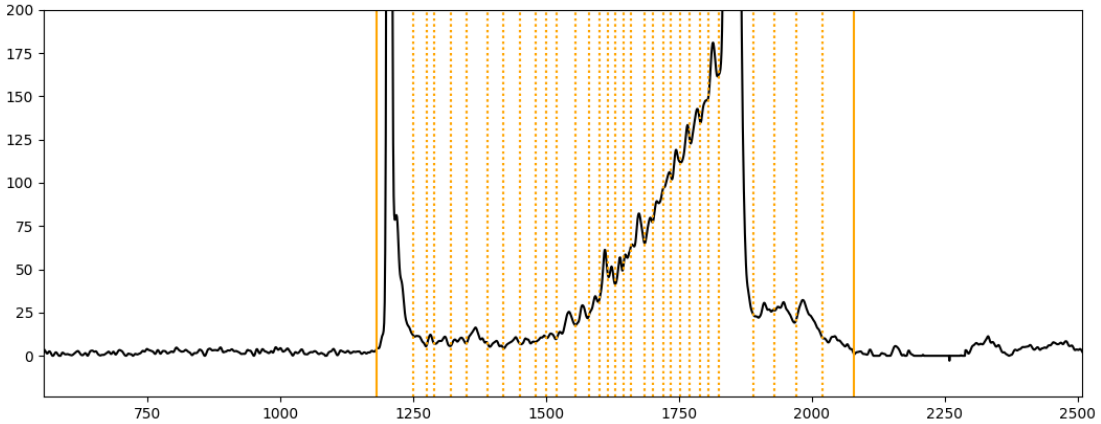


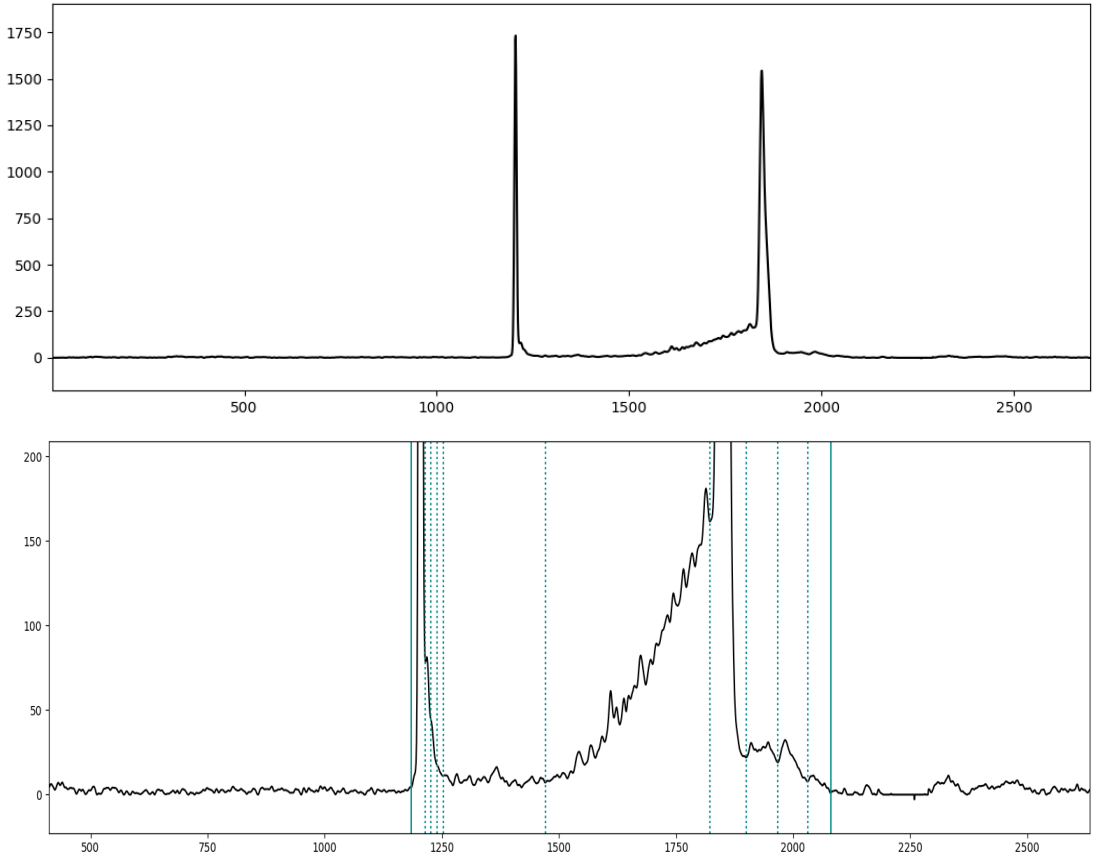


→ Cutting the front might not be necessary for the analysis

Interesting Findings

Integration depends on the context:

RNA degradation measurement



More Examples => Marlon´s Poster

Marlon Krompholz

Technische Universität Braunschweig

CASSS

Wätzig et al.
Institute of Medicinal and Pharmaceutical Chemistry

Peak Integration of Electropherograms: Reference data sets as a landmark for real improvement

Marlon Krompholz[1], Dr. Huixin Lu[2], Dr. Gábor Járvás[3], Prof. Dr. Cari Sänger – van de Griend[4,5], Prof. Dr. Hermann Wätzig[1]
[1]University of Technology Brunswick | Institute of Medicinal and Pharmaceutical Chemistry | Brunswick, Germany
[2]Health Canada | Ottawa, Canada
[3]University of Pannonia | Institute of Biomedical and Chemical Engineering | Veszprém, Hungary
[4]Kantisto BV | Haaksbergen, The Netherlands
[5]Uppsala Universitet | Department of Medicinal Chemistry | Uppsala, Sweden
marlon.krompholz@tu-braunschweig.de

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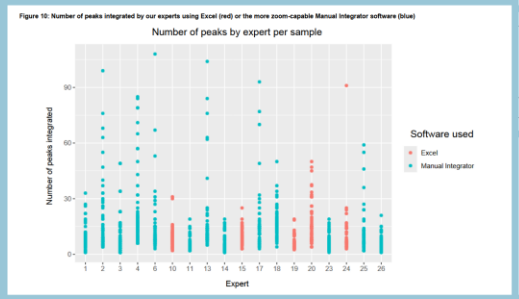
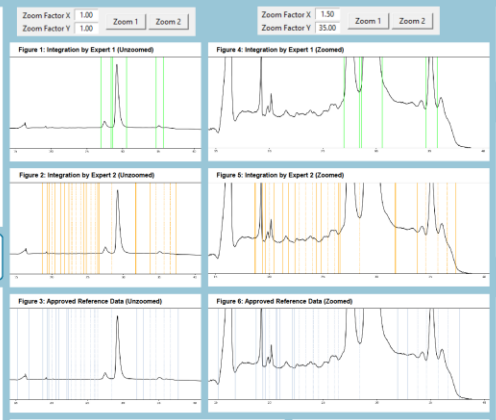
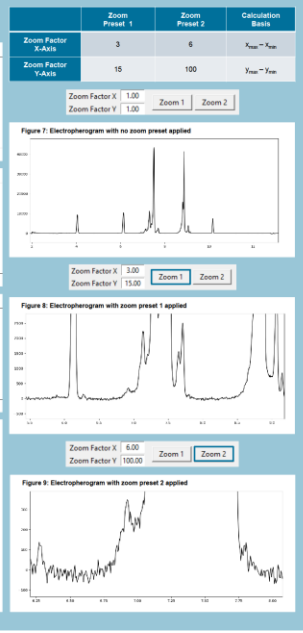
Abstract

Capillary electrophoresis (CE) often offers superior separation relative to chromatography for macromolecules like monoclonal antibodies (mAbs), a major pharmaceutical class. However, electropherogram baselines pose challenges that traditional chromatography integration algorithms cannot address, necessitating complex integration processes [1]. There are numerous ideas for improving peak integration, ranging from simple algorithms to improve the S/N ratio to more sophisticated AI solutions. However, none of these can demonstrate that they are genuine improvements, as they cannot be compared with each other or with previous algorithms using the same reference datasets. This is why a multinational collaboration between industry and universities was initiated. Data sets were collected, brought into a standardized format and then integrated by teams of experts. Once all the experts have reached a consensus on reasonable peak boundaries, this consolidated data is now available to the scientific public.
Discussions among the experts have revealed, that the used zoom factor appears to have a notable influence on the way people integrate, emphasising the necessity of its documentation and standardisation.

Collecting electrophero-grams → Integration by experts → Discussing the integrations → Re-integration → Approval as reference data







Conclusion

- By the end of the collaboration, we will publish a total of over 229 electropherograms with approved reference peak boundaries.
- To ensure comprehensibility, we recommend the documentation of the zoom level currently applied whenever manual integration is carried out, as well as the utilisation of pre-defined zoom factors.

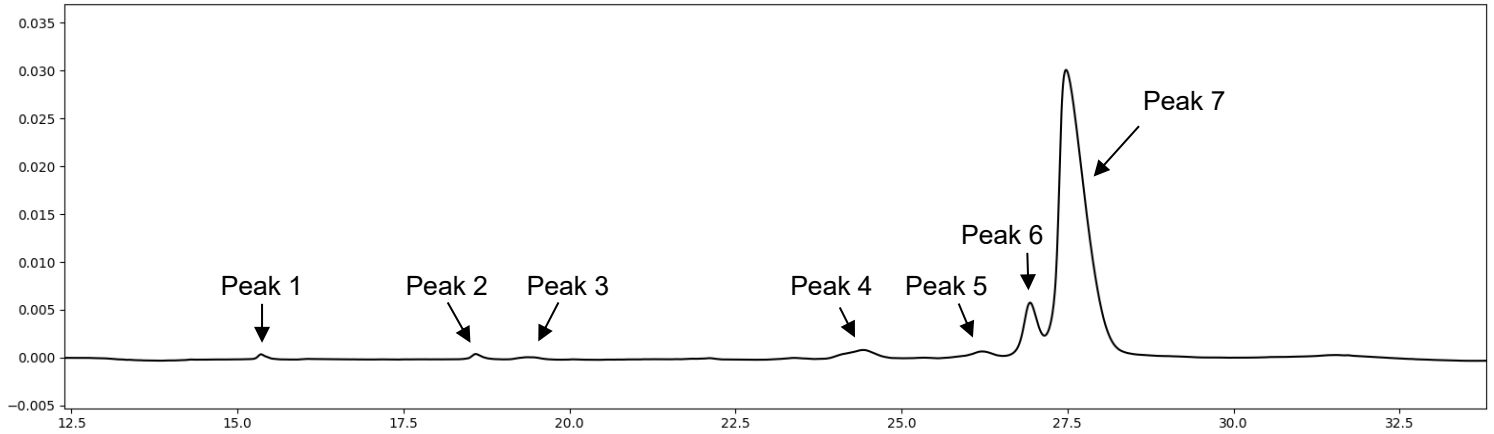
Acknowledgements

M Abuzeid, T Blanc, T Bo, M Chan, P Christensen, A Coll de Peña, M de Silva, H Dewald, C Montealegre Dondarza, G Dhulipala, T Enda, J Ermer, J Geiger, P Ginot, M Gutierrez, J Haglöf, M Haverick, P Hoang, R Hong, C Hood, P Huber, A Hutanu, R Hylands, Y Hwang, P Kang, S Kiessig, A Krupka, D Lasar, R Linkous, J Luo, W McElroy, C Heger, P S Michael-Butler, J Molitor, B Moritz, M Myers, R Nai, T D Nguyen, J Oriet, R Osborne, J Osko, D O'Sullivan, A Prout, T Riehlman, D Ripley, J Rostkowski, S Salem, E Sakka, C M Schaefer, J Schlecht, K Schultz-Kuszak, J Steflova, C Streng, A Sutton, L Thevarapperuma, A Torelli, E van Tricht, C G. Vega, Y Wang, D Warzak, B Wei, K Yandrofski, T Zao, Y Zhang

[1] Blanc, T. Wätzig, H. Sänger – van de Griend, C. *Peak Integration of Electropherograms in GMP and Research Labs: Navigating Increased Scrutiny Amid Data Integrity Audits and Inspections.* Electrophoresis. 2025; 10.1002/elps.70002

Key Facts Data Aquisition	Contribution of 229 Electropherograms	Contributed by 7 Companies	Integrated by 19 Experts	Approved by 8 Experts

Potential



Series of n = 21 injections

	Peak Area RSD%						
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
Empower	15.43	8.13	25.56	11.96	23.04	3.20	1.36
Manual Integration*	12.40	5.75	10.08	9.94	19.16	3.19	1.01

Intercompany Collaboration: a CASSS initiative

Mostafa Abuzeid, **Timothy Blanc, Tao Bo**, M Chan, **Patricia Christensen**, Adriana Coll de Peña, **Miyuru de Silva**, Hans Dewald, Gangadhar Dhulipala, Tara Enda, Joachim Ermer, **Jarvas Gabor**, James Geiger, **Frederic Ginot**, Mervin Gutierrez, Jakob Haglöf, Mark Haverick, Philip Hoang, Robert Hong, Christopher Hood, Philipp Huber, **Andrei Hutanu**, Ryan Hylands, Yun Hwang, **Pilsoo Kang, Steffen Kiessig, Marlon Krompholz**, Andreas Krupke, David Lasar, **Rick Linkous**, **Huixin Lu**, Jane Luo, **Will McElroy**, **Chris Heger, Paul S Michael-Butler**, Jana Molitor, Cristina Montealegre Dondarza, **Bernd Moritz**, Matthew Myers, Ryan Nai, **Trang D Nguyen,** John Orlet, **Rebecca Osborne**, Jeremy Osko, David O'Sullivan, **Ashley Prout**, **Timothy Riehlman**, David Ripley, Jacob Rostkowski, Shauna Salem, **Cari Sänger-van de Griend**, Emineguel Sakka, Carlie M Schaeffer, Johannes Schlecht, Kristin Schultz-Kuszak, **Jana Steflova**, **Christian Streng,** Adam Sutton, Lalin Theverapperuma, Amanda Torelli, Ewoud van Tricht, **Carolina G. Vega**, **Yun Wang**, **Denise Warzak**, Brian Wei, Katharina Yandrofski, Tao Zao, Yuling Zhang

The next steps

- **Approval by the community**
- **Reference Data Set CE Integration #1**

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- **Approval by the community**
- **Reference Data Set CE Integration #1**

Software development and qualification

- Performance tests of existing softwares: precision & accuracy
- Development of algorithmic and AI strategies
- Investigations on Human errors in integration

Extending the concept to LC (HPLC 2025)

Thank you very much!

Jana Haegner, Nelly Luong, Marlon Krompholz, Hermann Wätzig