Technische Universität Braunschweig

Wätzig et al.
Institute of Medicinal and Pharmaceutical Chemistry



Peak Integration in Separation Sciences

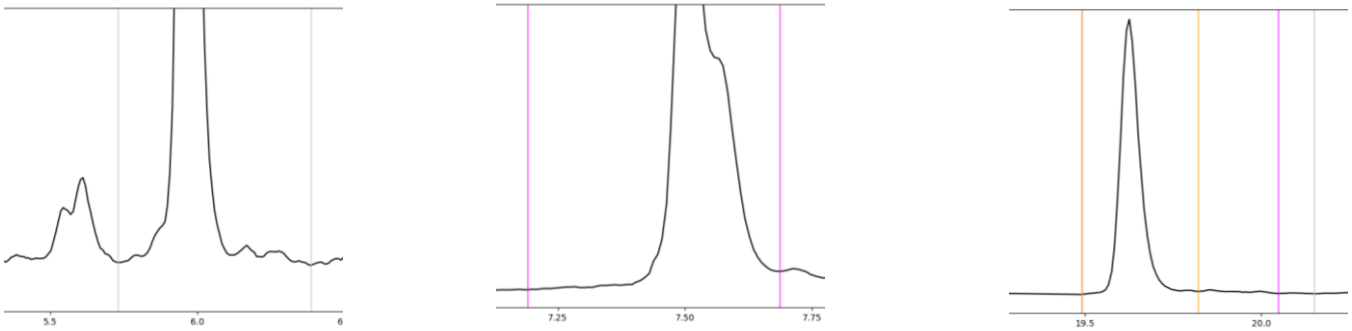
Reference Data and its Power for Accurate Quantitative Analysis

Marlon Krompholz, Institute for Medicinal and Pharmaceutical Chemistry, 30 September 2026

Difficulties in Peak Integration

Would you manually correct these software integration limits?

Each color represents different software



➢ Necessity for improved software solutions

Intercompany Collaboration in CE and LC

Many efforts to improve automatic integration

None can show that they are real improvements

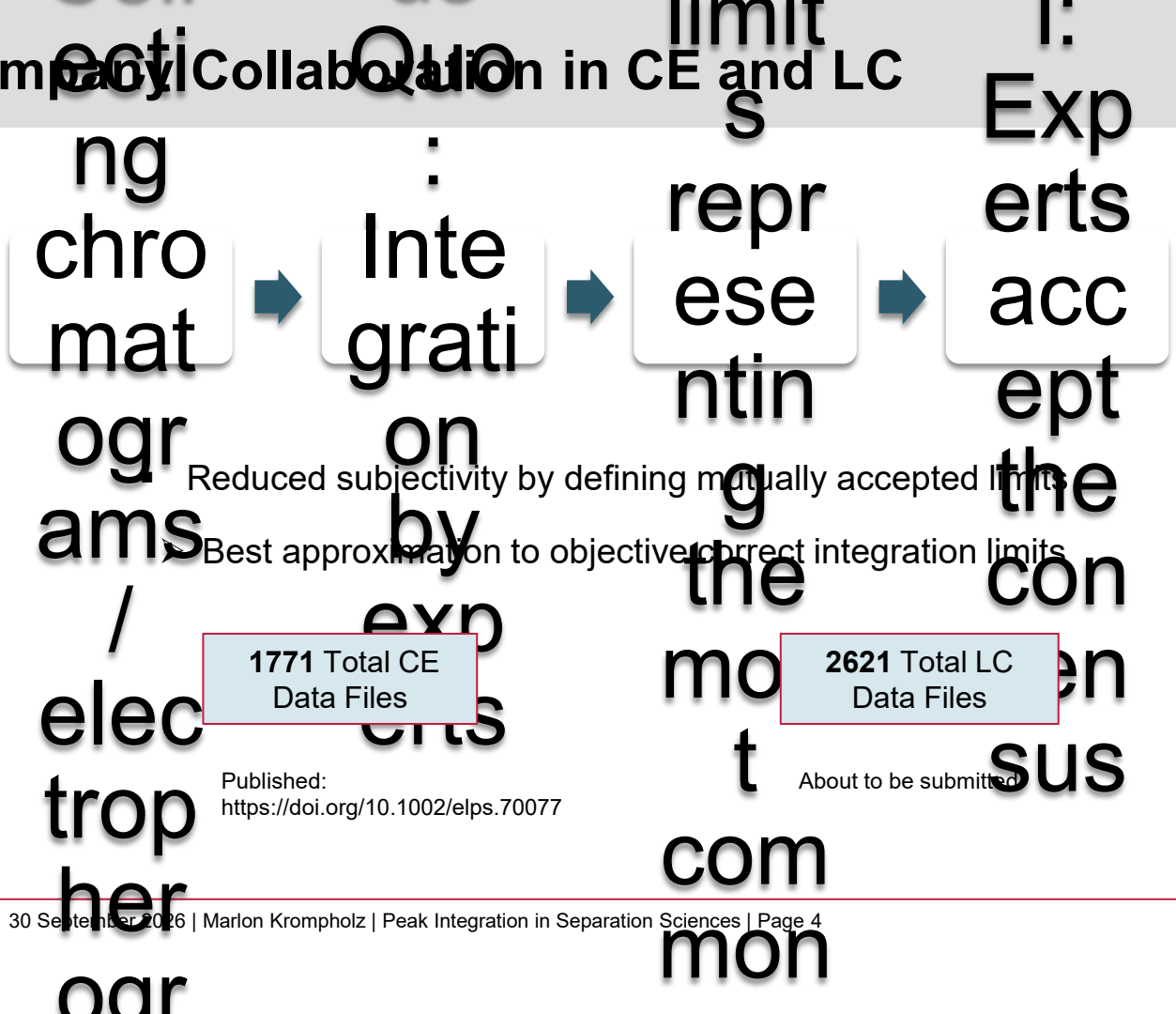


Reference data needed

(Ideal:
100 % accurate peak areas)

Most feasible:
Consensus among experienced experts in the field

Intercompany Collaboration in CE and LC



- Reduced subjectivity by defining mutually accepted limits
- Best approximation to objective, correct integration limits

1771 Total CE Data Files

Published: https://doi.org/10.1002/elps.70077

2621 Total LC Data Files

About to be submitted

Intercompany Collaboration in CE and LC

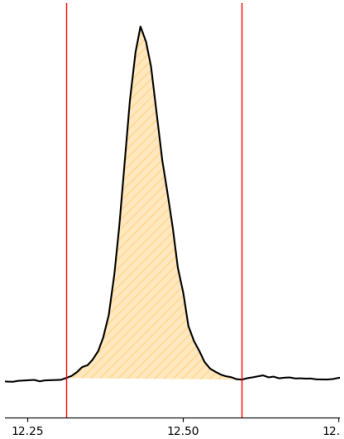
CE Data Set

Mostafa A. Atia, Knut Baumann, Tim Blanc, Tao Bo, Patricia Christensen, Miyuru de Silva, Tara Enda, Gábor Járvás, Frédéric Ginot, Philip Hoang, Andrei Hutanu, Ryan Hylands, Steffen Kiessig, Marlon Krompholz, Rick Linkous, Huixin Lu, Jane Luo, Will McElroy, Nathalie Montel, Matthew Myers, Trang D. Nguyen, Jeremy D. Osko, Ashley Prout, Timothy Riehlman, David Ripley, Cari Sänger-van de Griend, Carlie M Schaeffer, Adam Sutton, Amanda Torelli, Ewoud van Tricht, Carolina G. Vega, Yun Wang, Hermann Wätzig, Brian Wei, Yuling Zhang

HPLC Data Set

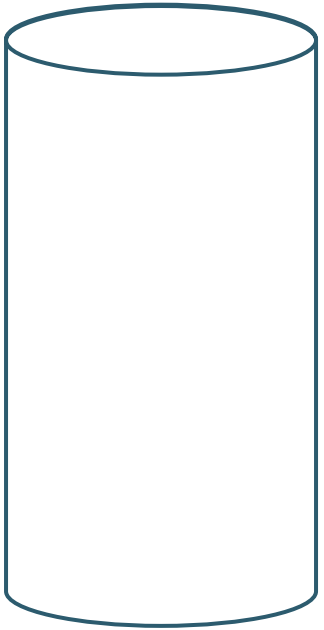
Tim Blanc, Patricia Christensen, Marianne Fillet, Paul Gottschalk, Davy Guillarme, Jana Haegner, Christina Heinz, Takayuki Kawai, Silvia Kindler, Juliane Kramer, Marlon Krompholz, Michael Lämmerhofer, Anna Laurent, Huixin Lu, C.V.L. Nelly Luong, Jonathan Maurer, Trang D. Nguyen, Lucie Nováková, Matthew Osborn, Vít Pavelka, Kateřina Pražáková, Ashley Prout, Mareike Pruefer, Pau Rubirola Vila, Serge Rudaz, Cari Sänger-van de Griend, Athanasios Tsalmpouris, Hermann Wätzig, Jufang Wu Ludvigsson, Christian Zeigler

Peak Area Accuracy: Difficulty

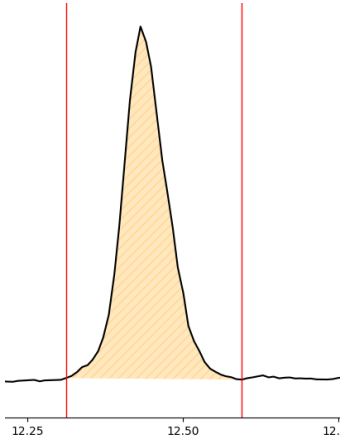


Measured peak area: 98% of the expected area

Bias: 2%

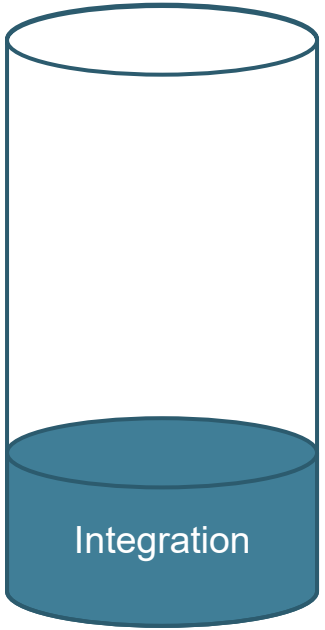


Peak Area Accuracy: Difficulty

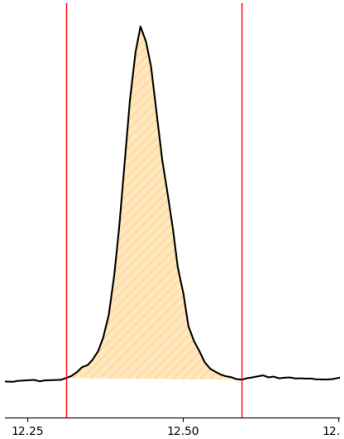


Measured peak area: 98% of the expected area

Bias: 2%

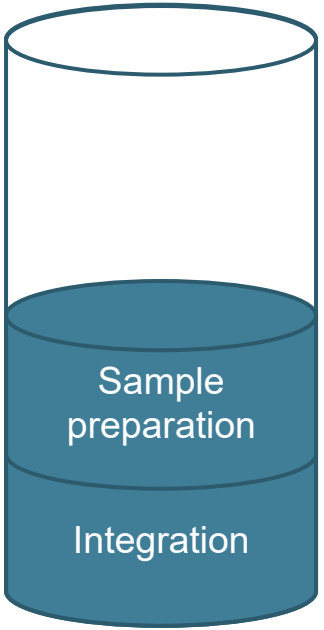


Peak Area Accuracy: Difficulty

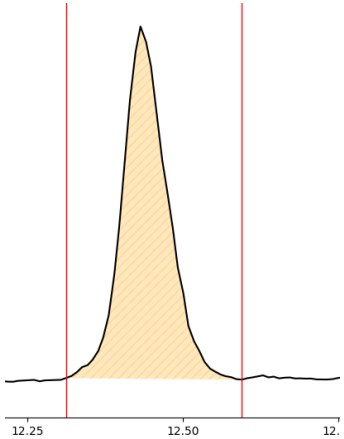


Measured peak area: 98% of the expected area

Bias: 2%

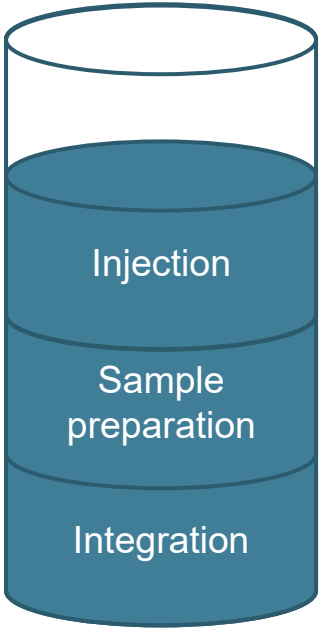


Peak Area Accuracy: Difficulty

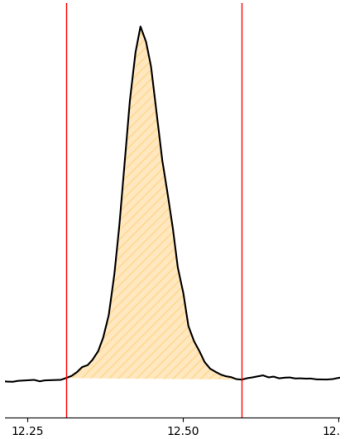


Measured peak area: 98% of the expected area

Bias: 2%



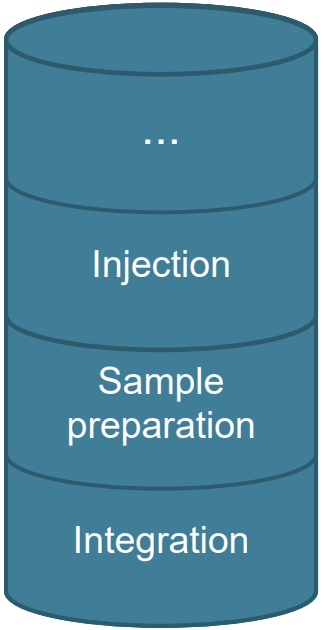
Peak Area Accuracy: Difficulty



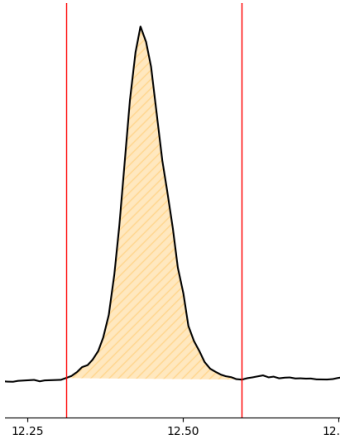
Measured peak area: 98% of the expected area

Integration being 98% accurate? ☹

Bias: 2%

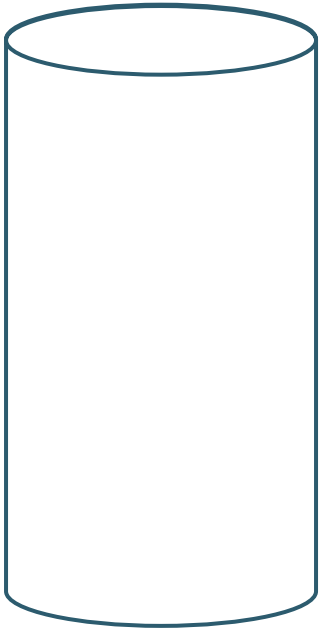


Peak Area Accuracy: Difficulty

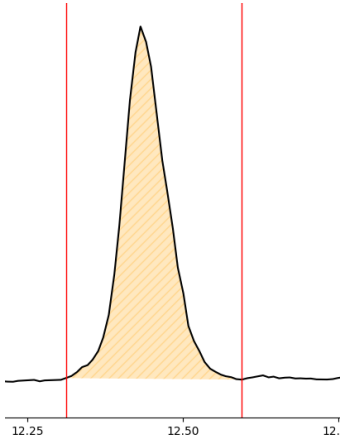


Measured peak area: 98% of the expected area

Bias: 2%



Peak Area Accuracy: Difficulty



Measured peak area: 98% of the expected area

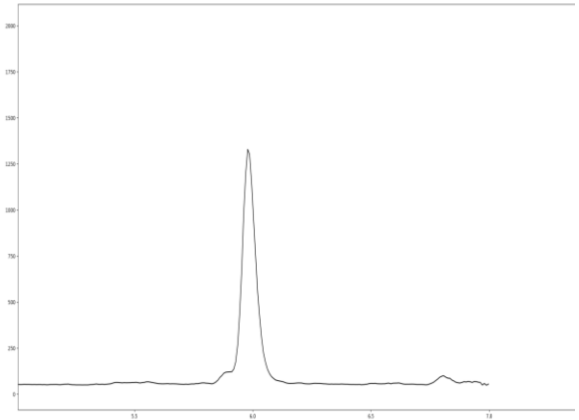
Integration being 98% accurate? ✓ ☺

Bias: 2%

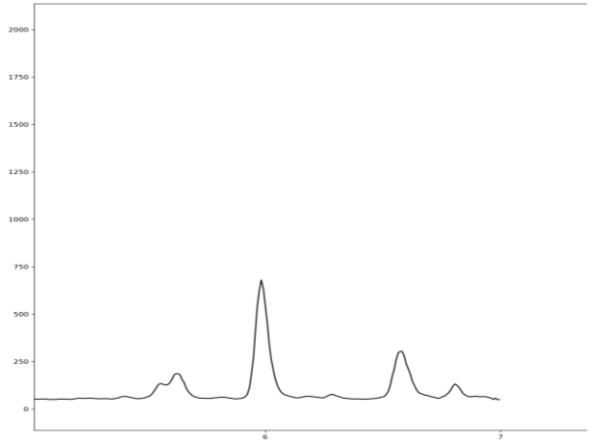


Peak Area Accuracy: MS-Data-Based Approach

Extracted Ion Chromatograms (EICs)



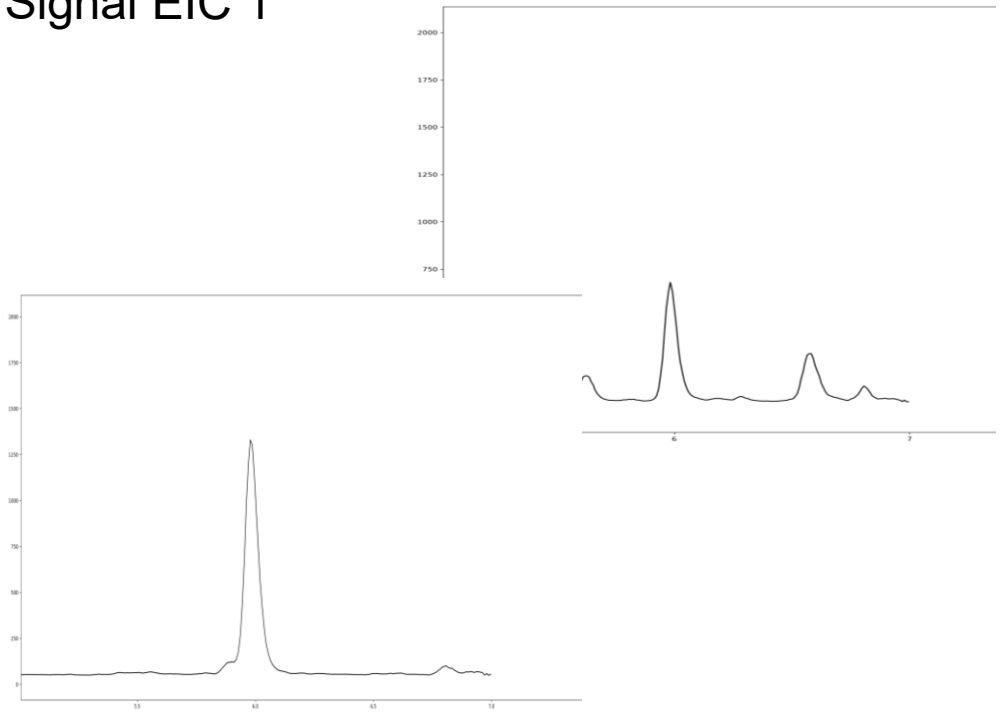
<u>EIC 1.1:</u>
Substance 1
Mass transition 1



<u>EIC 1.2:</u>
Substance 1
Mass transition 2

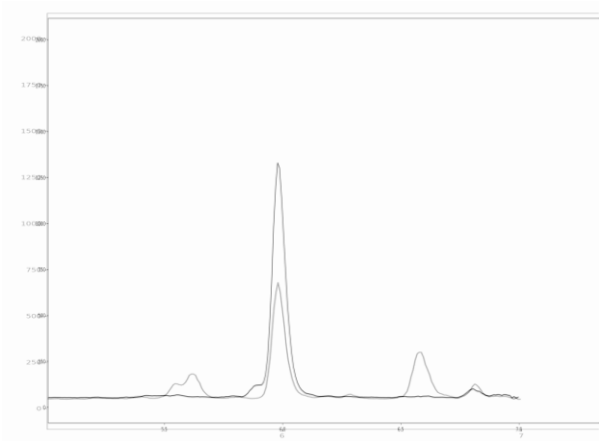
Peak Area Accuracy: MS-Data-Based Approach

Signal EIC 1



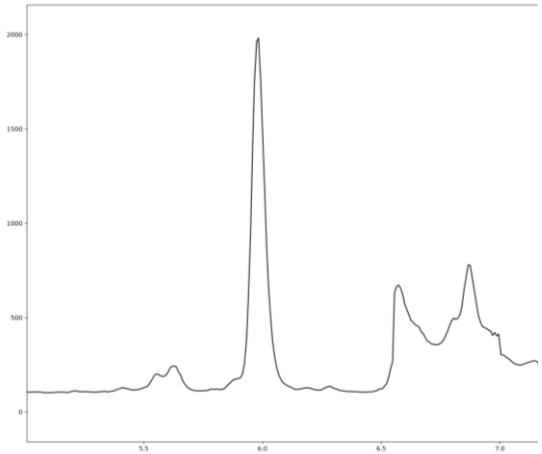
Peak Area Accuracy: MS-Data-Based Approach

Signal EIC 1 + Signal EIC 2



Peak Area Accuracy: MS-Data-Based Approach

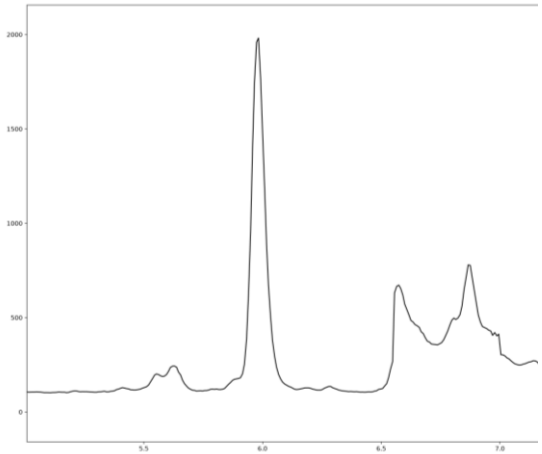
Signal EIC 1 + Signal EIC 2



Total Ion Chromatogram

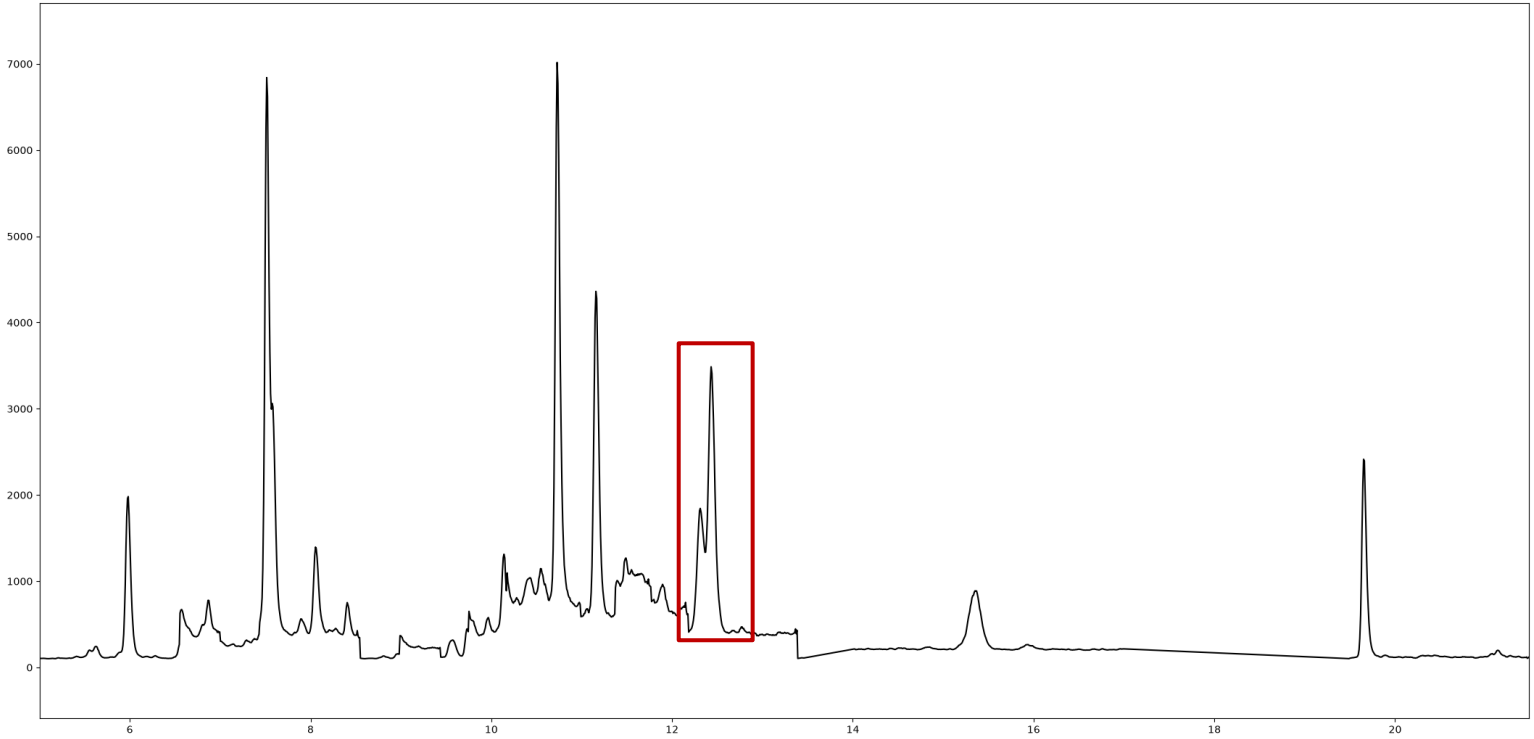
Peak Area Accuracy: MS-Data-Based Approach

Total Ion Chromatogram (TIC) = Sum of all EIC signals



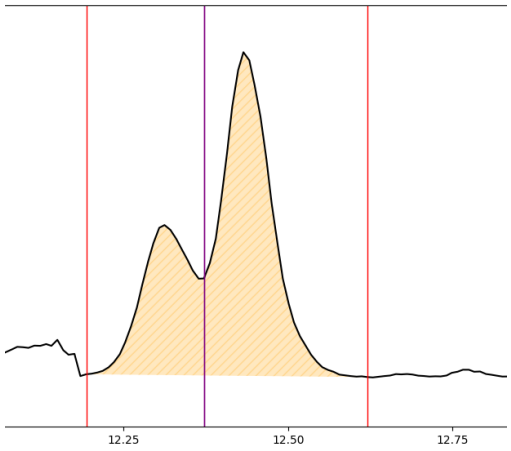
Total Ion Chromatogram

Peak Area Accuracy: MS-Data-Based Approach



Peak Area Accuracy: MS-Data-Based Approach

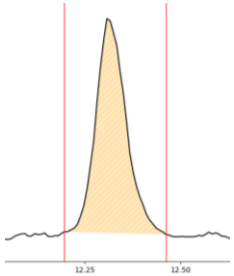
Area Comparison: TIC peaks and sum of the underlying EIC peaks



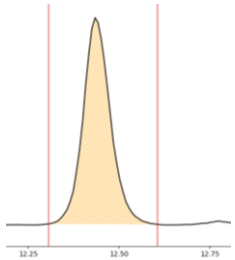
TIC

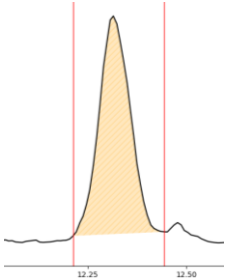
⇄

EIC 1.1

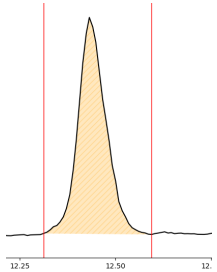


EIC 2.1





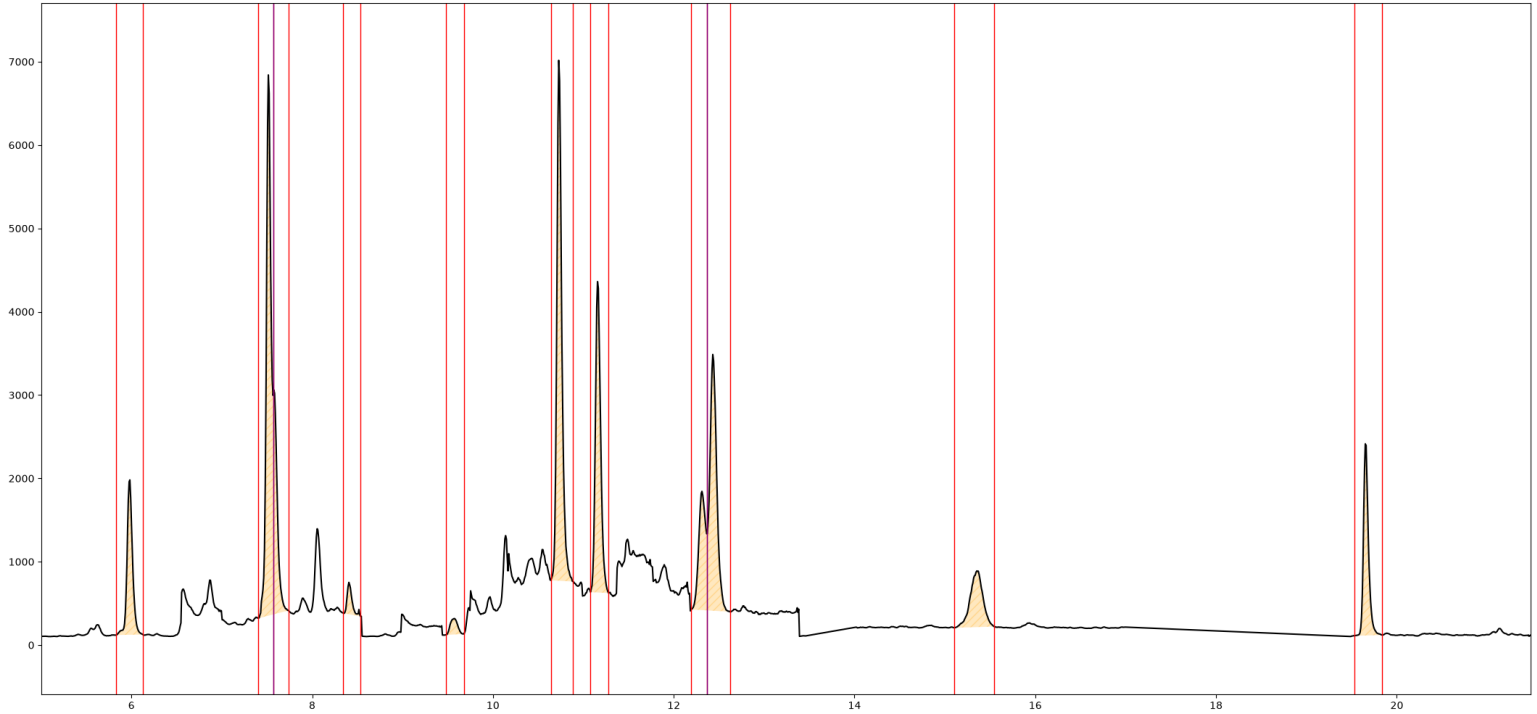
EIC 1.2



EIC 2.2

Correct integration limits ⇒ Minimal difference in area

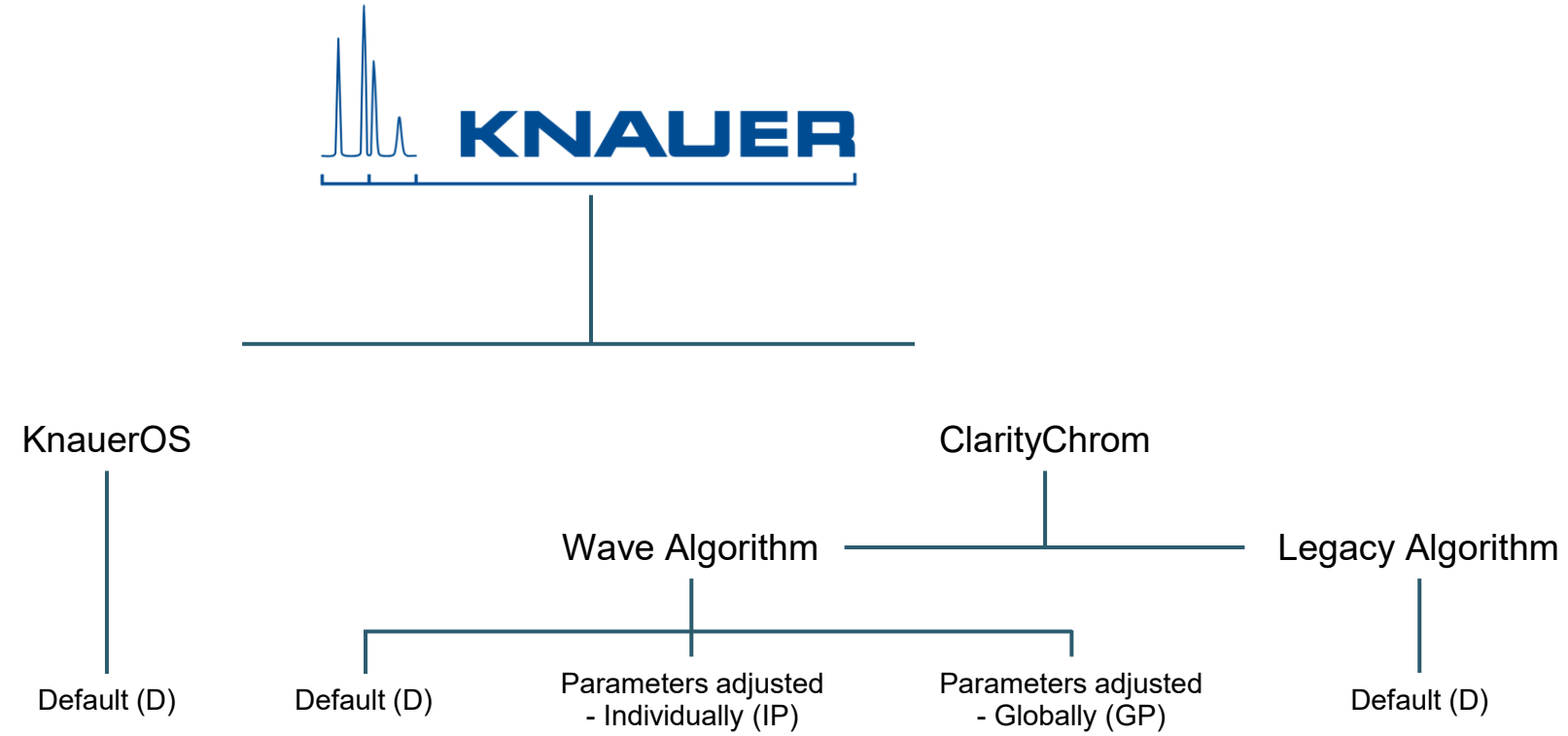
Investigation: Integration Accuracy of Established Software



Series: n = 10 Chromatograms

11 Peaks representing the main components

Investigation: Integration Accuracy of Established Software



Investigation: Integration Accuracy of Established Software

	Peak	KnauerOS (D)	ClarityChrom - Wave (D)	ClarityChrom - Wave (IP)	ClarityChrom - Wave (GP)	ClarityChrom - Legacy (D)
		Mean	Mean	Mean	Mean	Mean
Accuracy	1	94.9%	98.8%	98.5%	98.1%	96.1%
	2	97.9%*	98.5%*	98.3%*	98.3%*	96.7%*
	3					
	4	72.7%	31.4%	31.4%	31.4%	24.9%
	5	99.0%	99.3%	99.0%	99.3%	99.0%
	6	98.8%	98.5%	97.8%	97.8%	94.6%
	7	98.6%	98.3%	98.3%	98.3%	97.8%
	8	99.0%	99.0%	99.0%	99.0%	99.2%*
	9	99.3%	99.4%	99.4%	99.4%	
	10	93.0%	98.2%	96.1%	95.0%	93.5%
	11	95.7%	99.7%	98.8%	99.6%	99.2%

Series: n = 10 Chromatograms

* The peaks were not recognized as two separate peaks in at least one of the chromatograms.

Investigation: Integration Accuracy of Established Software

	Peak	KnauerOS (D)	ClarityChrom - Wave (D)	ClarityChrom - Wave (IP)	ClarityChrom - Wave (GP)	ClarityChrom - Legacy (D)
		Mean	Mean	Mean	Mean	Mean
Accuracy	1	94.9%	98.8%	98.5%	98.1%	96.1%
	2	97.9%*	98.5%*	98.3%*	98.3%*	96.7%*
	3					
	4	72.7%	31.4%	31.4%	31.4%	24.9%
	5	99.0%	99.3%	99.0%	99.3%	99.0%
	6	98.8%	98.5%	97.8%	97.8%	94.6%
	7	98.6%	98.3%	98.3%	98.3%	97.8%
	8	99.0%	99.0%	99.0%	99.0%	99.2%*
	9	99.3%	99.4%	99.4%	99.4%	
	10	93.0%	98.2%	96.1%	95.0%	93.5%
	11	95.7%	99.7%	98.8%	99.6%	99.2%

Series: n = 10 Chromatograms

* The peaks were not recognized as two separate peaks in at least one of the chromatograms.

Investigation: Integration Accuracy of Established Software

	Peak	KnauerOS (D)	ClarityChrom - Wave (D)	ClarityChrom - Wave (IP)	ClarityChrom - Wave (GP)	ClarityChrom - Legacy (D)
		Mean	Mean	Mean	Mean	Mean
Accuracy	1	94.9%	98.8%	98.5%	98.1%	96.1%
	2	97.9%*	98.5%*	98.3%*	98.3%*	96.7%*
	3					
	4	72.7%	31.4%	31.4%	31.4%	24.9%
	5	99.0%	99.3%	99.0%	99.3%	99.0%
	6	98.8%	98.5%	97.8%	97.8%	94.6%
	7	98.6%	98.3%	98.3%	98.3%	97.8%
	8	99.0%	99.0%	99.0%	99.0%	99.2%*
	9	99.3%	99.4%	99.4%	99.4%	
	10	93.0%	98.2%	96.1%	95.0%	93.5%
	11	95.7%	99.7%	98.8%	99.6%	99.2%

Series: n = 10 Chromatograms

* The peaks were not recognized as two separate peaks in at least one of the chromatograms.

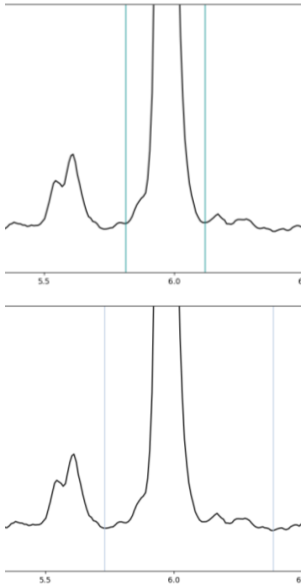
Investigation: Integration Accuracy of Established Software

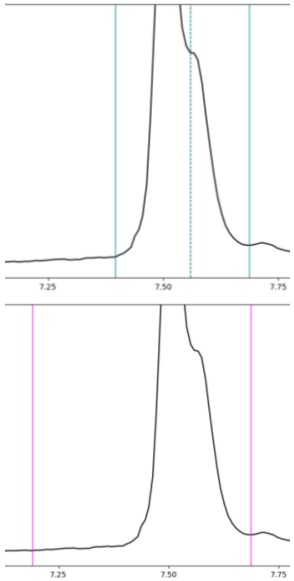
	Peak	KnauerOS (D)	ClarityChrom - Wave (D)	ClarityChrom - Wave (IP)	ClarityChrom - Wave (GP)	ClarityChrom - Legacy (D)
		Mean	Mean	Mean	Mean	Mean
Accuracy	1	94.9%	98.8%	98.5% ↘	98.1% ↘	96.1%
	2	97.9%*	98.5%*	98.3%* ↘	98.3%* ↘	96.7%*
	3					
	4	72.7%	31.4%	31.4%	31.4%	24.9%
	5	99.0%	99.3%	99.0% ↘	99.3%	99.0%
	6	98.8%	98.5%	97.8% ↘	97.8% ↘	94.6%
	7	98.6%	98.3%	98.3%	98.3%	97.8%
	8	99.0%	99.0%	99.0%	99.0%	99.2%*
	9	99.3%	99.4%	99.4%	99.4%	
	10	93.0%	98.2%	96.1% ↘	95.0% ↘	93.5%
	11	95.7%	99.7%	98.8% ↘	99.6% ↘	99.2%

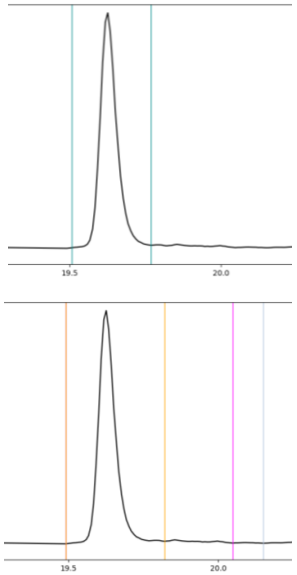
Series: n = 10 Chromatograms

* The peaks were not recognized as two separate peaks in at least one of the chromatograms.

Investigation: Integration Accuracy of Established Software







Accuracy:

94.1%

KnauerOS (D)

99.8%*

Wave (IP)

98.4%, Wave (GP)

93.3%, Wave (IP)

92.6% KnauerOS (D)

* The shoulder has not been recognised.

Thank you very much!

Many thanks to all collaborators and supporters of the CE and LC projects!

CASSS
SHARING SCIENCE SOLUTIONS







Tim Blanc, Cari Sänger-van de Griend and Huixin Lu



Jana Haegner, Nelly Luong and Hermann Wätzig