

Detection Method Considerations

Sampling Rates and Filter Constants



Importance of Sampling Rate

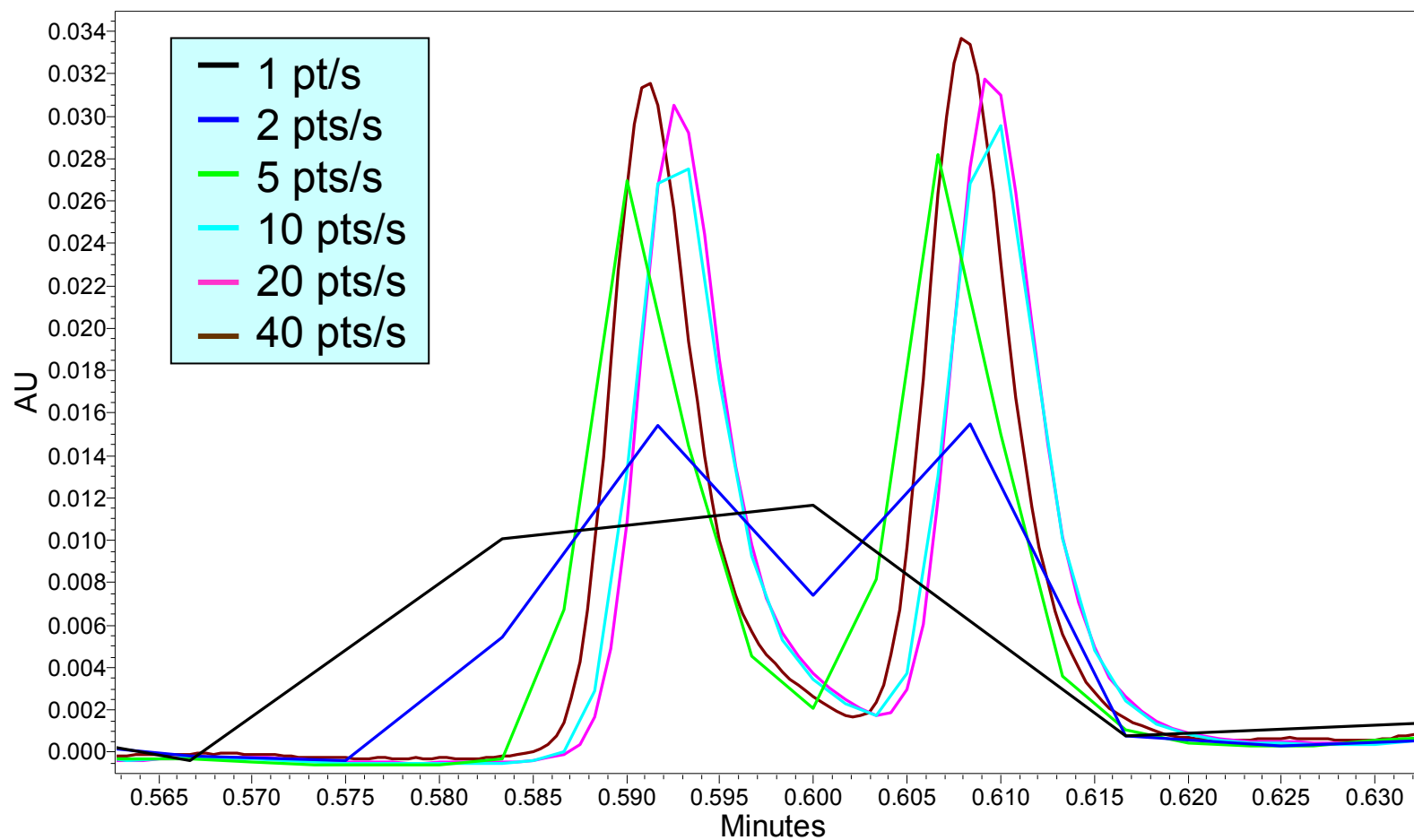
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Must ensure enough points are collected across a peak to adequately define the peak shape.
- Peak detection algorithms require a minimum number of points across a peak to distinguish it from baseline noise and correctly determine peak lift off and touch down.
- A peak which does not have enough data points will be difficult to integrate and therefore have irreproducible peak areas and heights.

Effect of Sampling Rate on Peak Shape

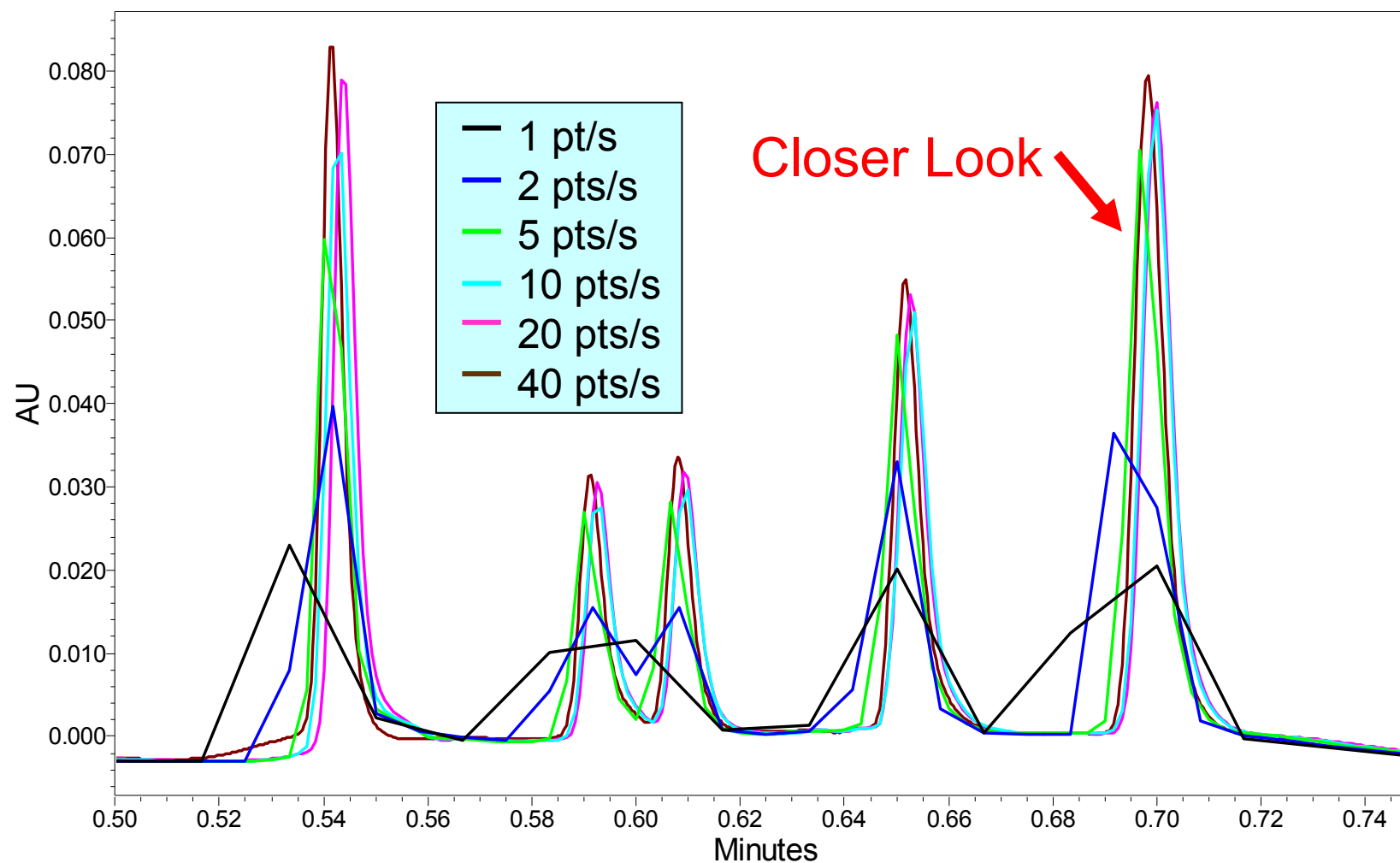
Waters

THE SCIENCE OF WHAT'S POSSIBLE.™



Effect of Sampling Rate on Chromatography

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



5 component Analgesic Mix on 2.1x50mm ACQUITY UPLC™ Column, 30s gradient

Effect of Sampling Rate on Reproducibility

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Sampling Rate	Points Across Peak	Peak Area %RSD	Peak Height %RSD
1 pt/s	2	2.436	15.515
2 pts/s	4	1.790	13.455
5 pts/s	7	0.971	3.962
10 pts/s	13	1.129	1.015
20 pts/s	25	0.603	1.156
40 pts/s	49	0.284	1.127

- Reproducible integration requires 15 points across a peak.
- Reproducible quantitation requires 25 – 50 points across a peak.
- Why not collect as many data points as possible to always ensure good quantitation? Noise.

Data Rate Considerations

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

- Detectors have a base sampling rate.
- To get lower data rates, points are averaged resulting in some filtering.
- The noise at 10Hz and 2Hz will be different, even with the same filtering constant.

Example

Data Rate	1pts/s	2pts/s	5pts/s	10pts/s	20pts/s	40pts/s
Noise Level	1x	1.3x	1.5x	1.6x	2.0x	2.0x

What is Digital Filtering and Why is it Important?

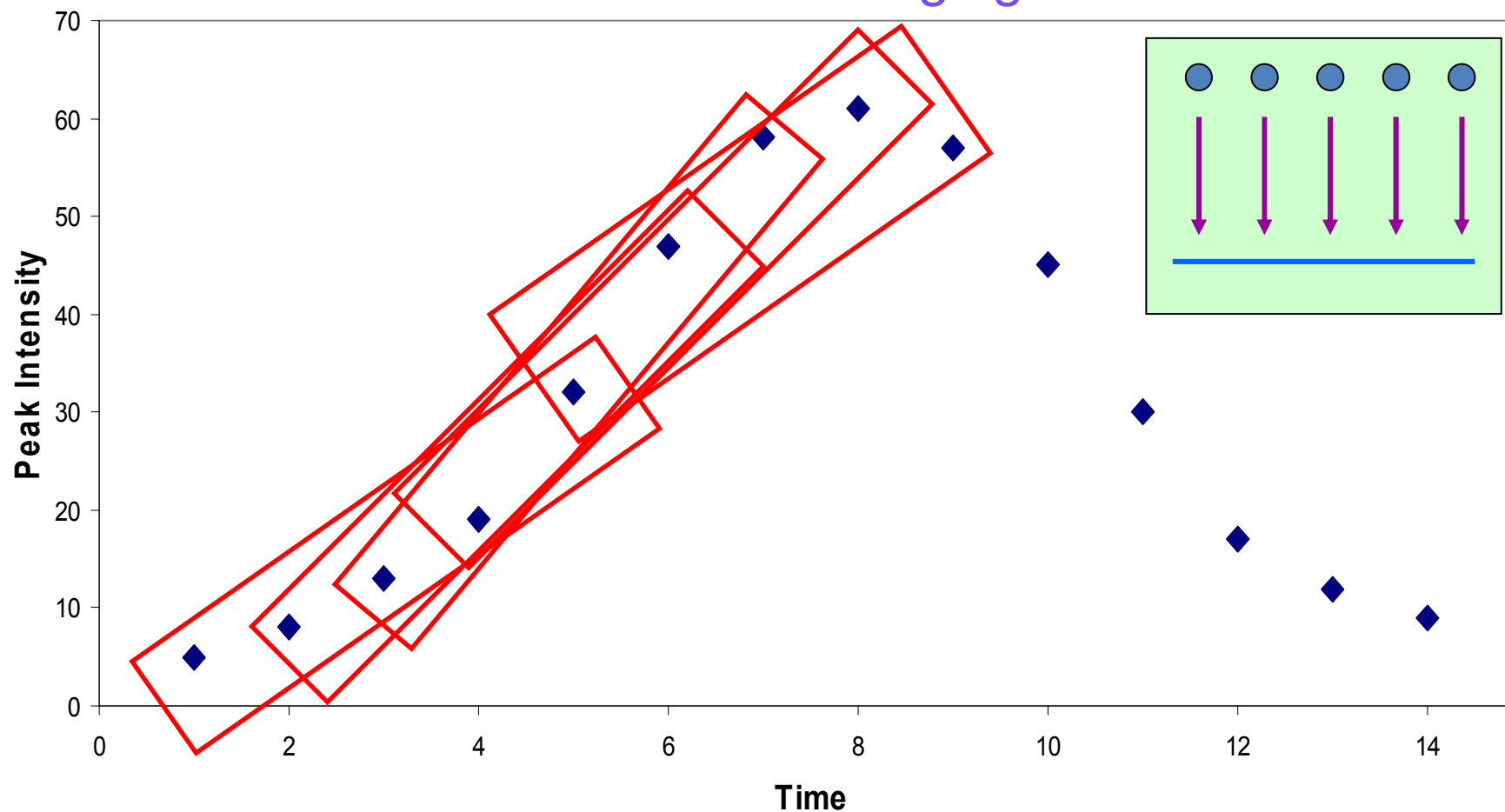
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Digital Filtering is a mathematical algorithm applied to a data set that smoothes out higher frequency noise.
- The desired result is reduced baseline noise with minimal impact on peak intensities so as to increase signal-to-noise.
- The degree of filtering (time constant) is crucial, too much filtering can dramatically impact peak shapes and resolution.

Visual Representation of How Digital Filtering Works

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

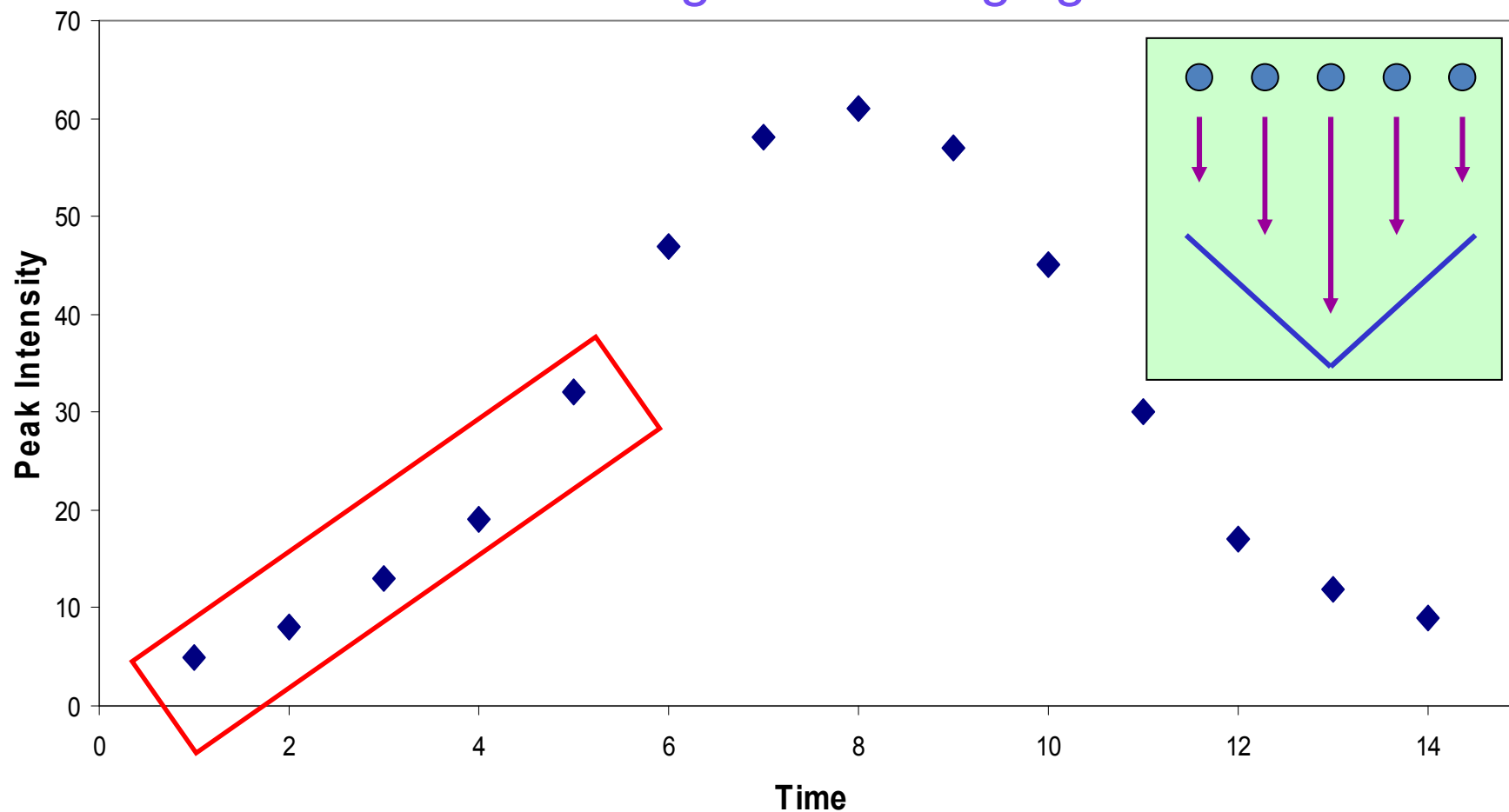
Boxcar Averaging



Visual Representation of How Digital Filtering Works

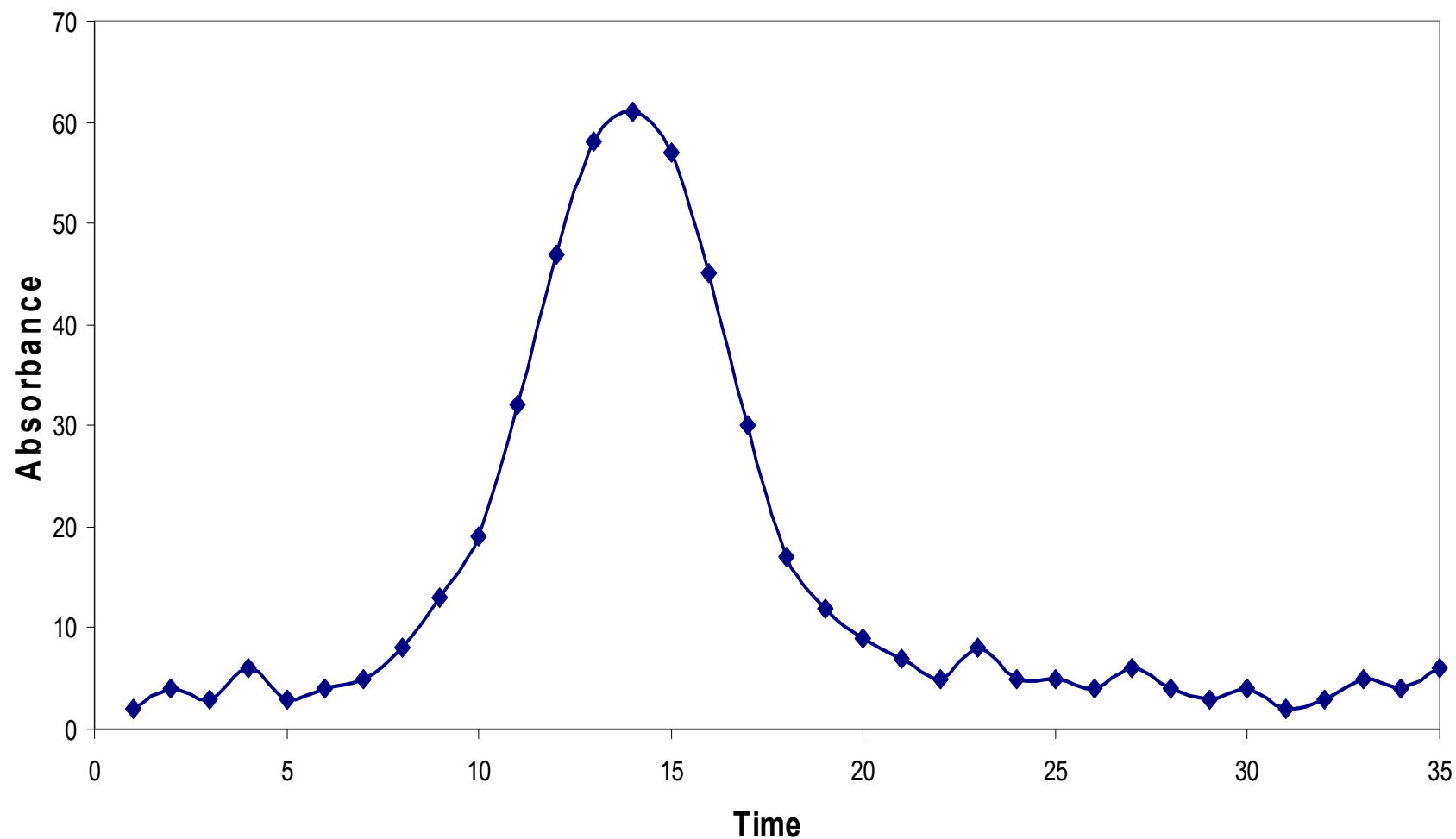
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Weighted Averaging



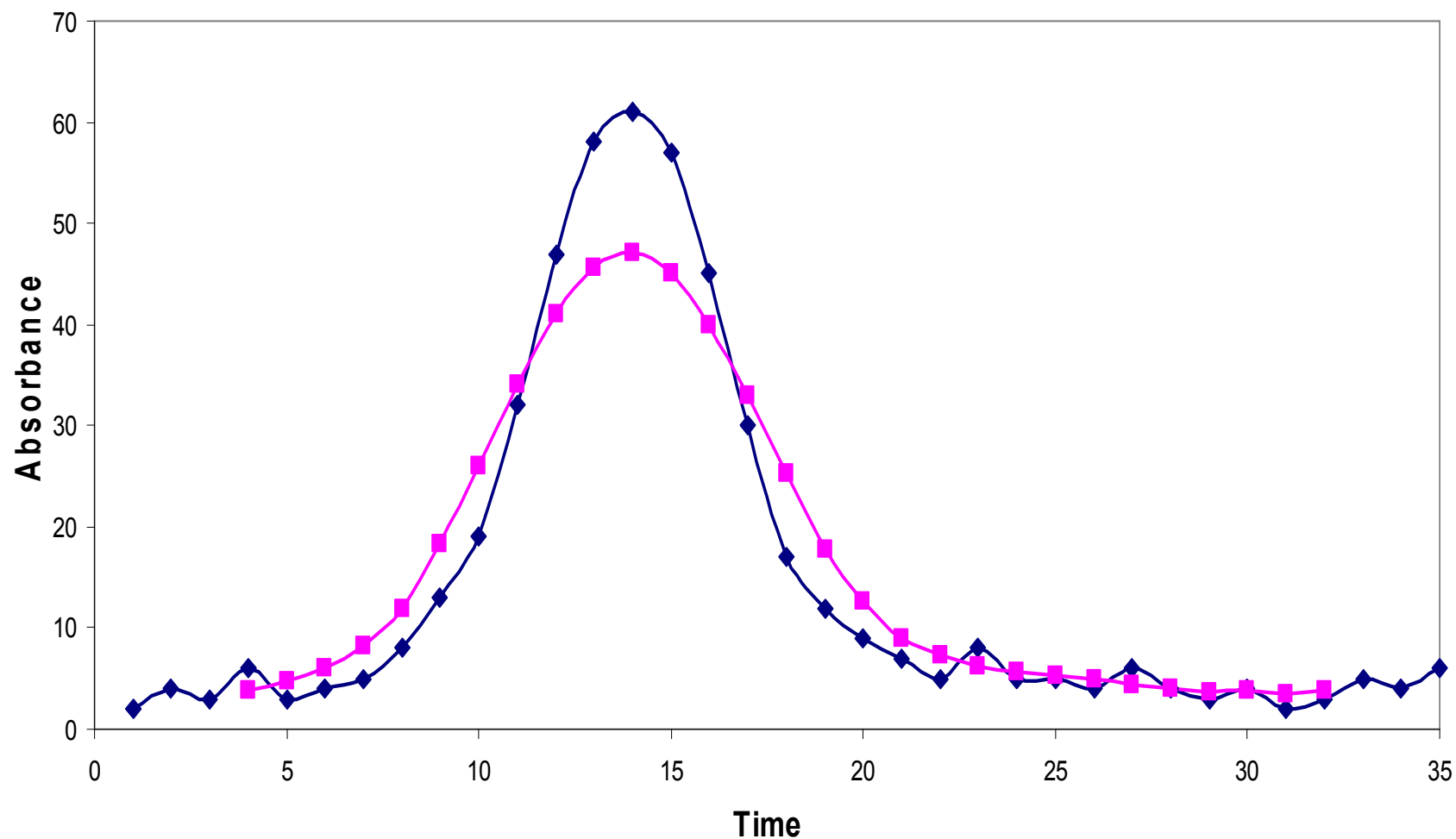
Application of Boxcar and Weighted Averaging to Example Data

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



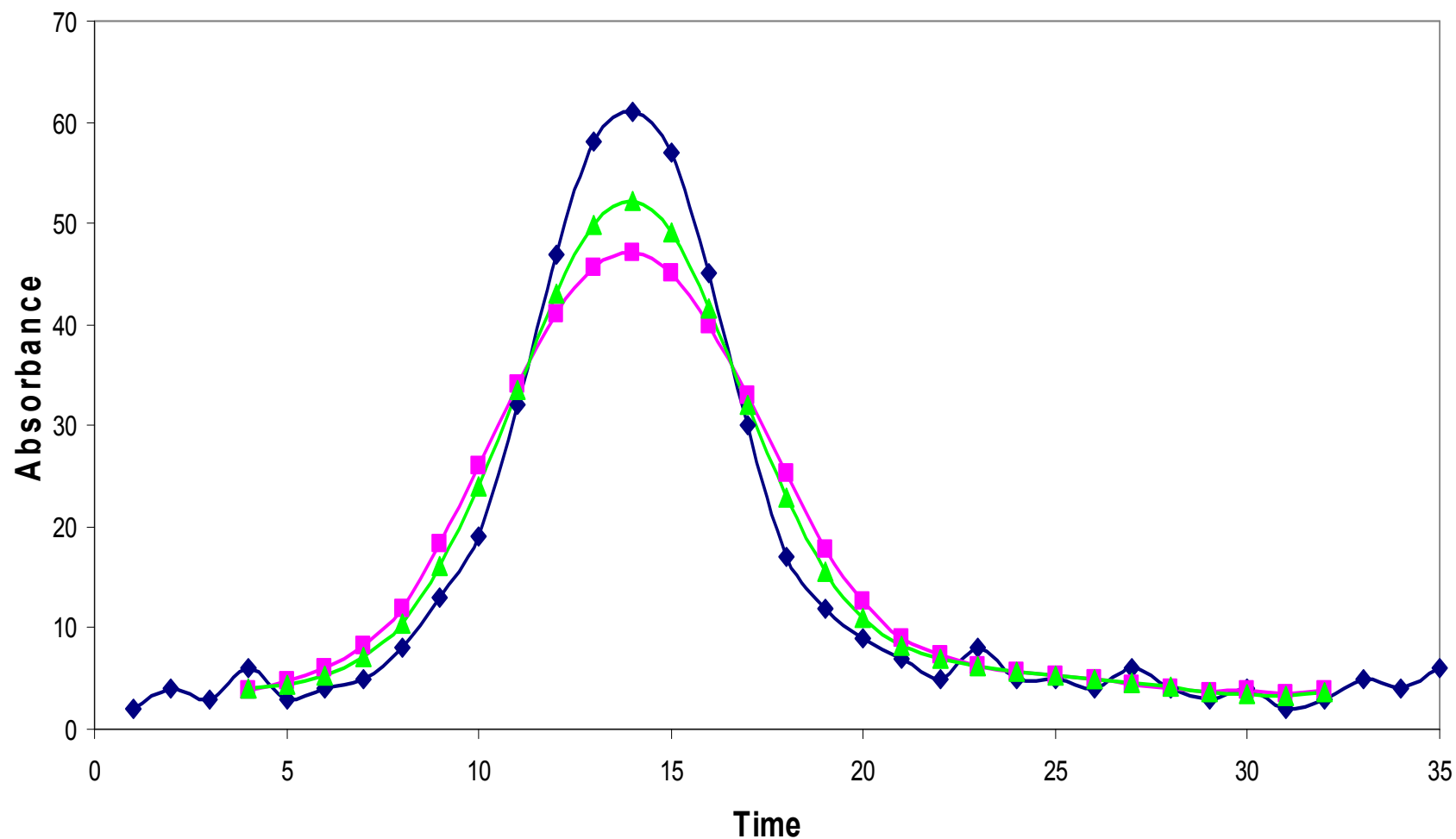
Application of Boxcar and Weighted Averaging to Example Data

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



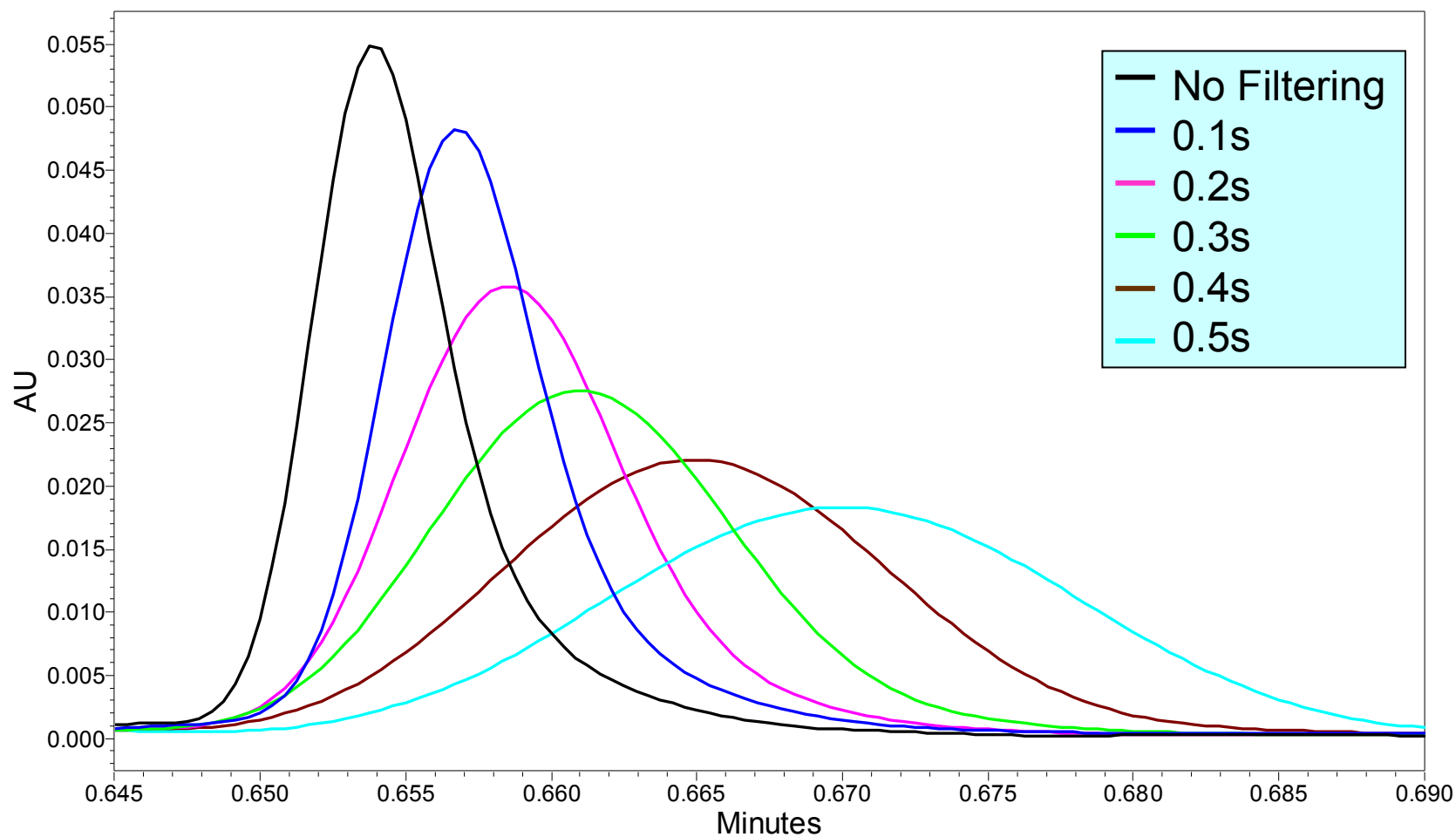
Application of Boxcar and Weighted Averaging to Example Data

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



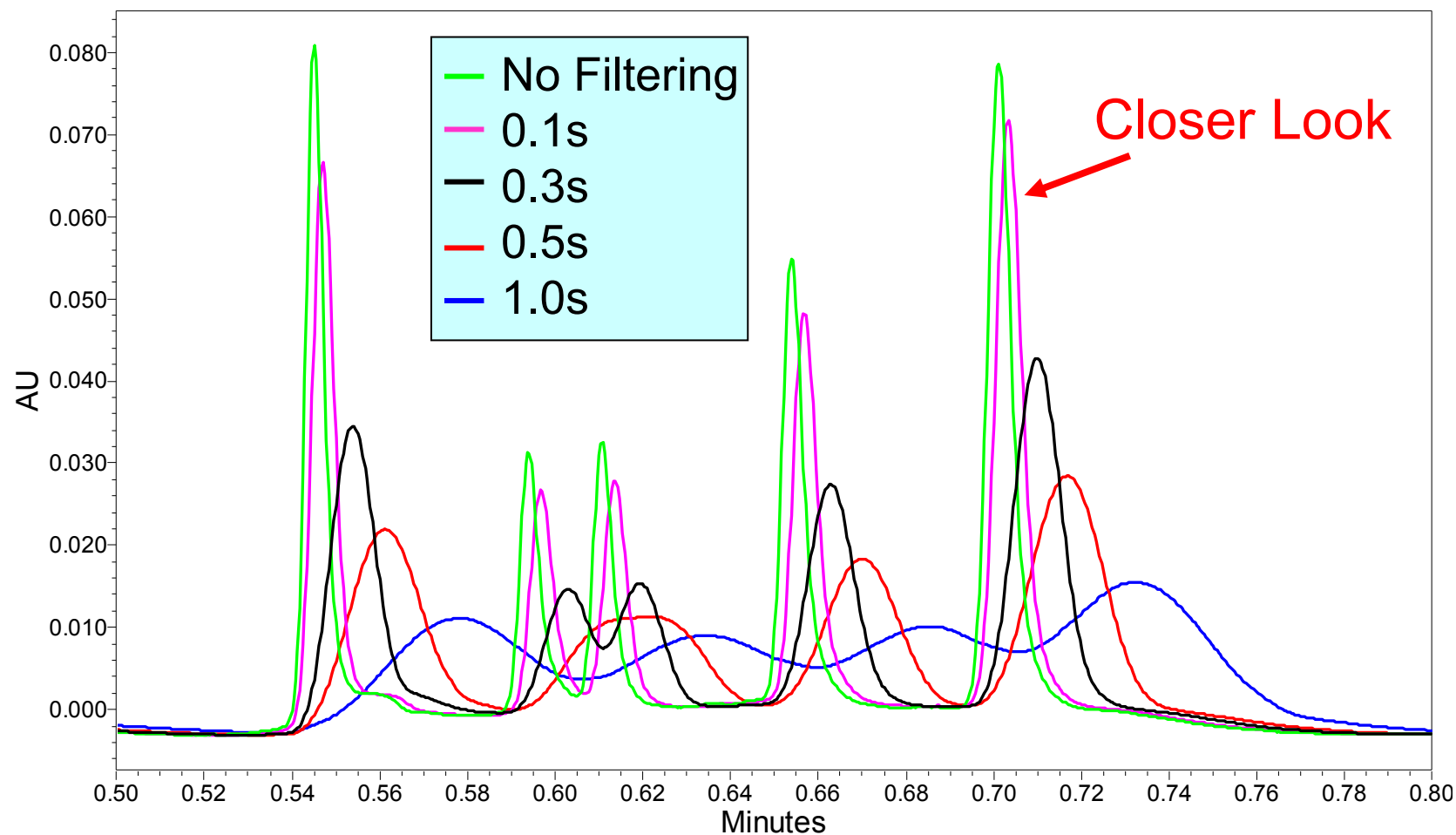
Effect of Filtering Constant on Peak Shape

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Effect of Filtering Constant on Chromatography

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



5 component Analgesic Mix on 2.1x50mm ACQUITY UPLC™ Column, 30s gradient

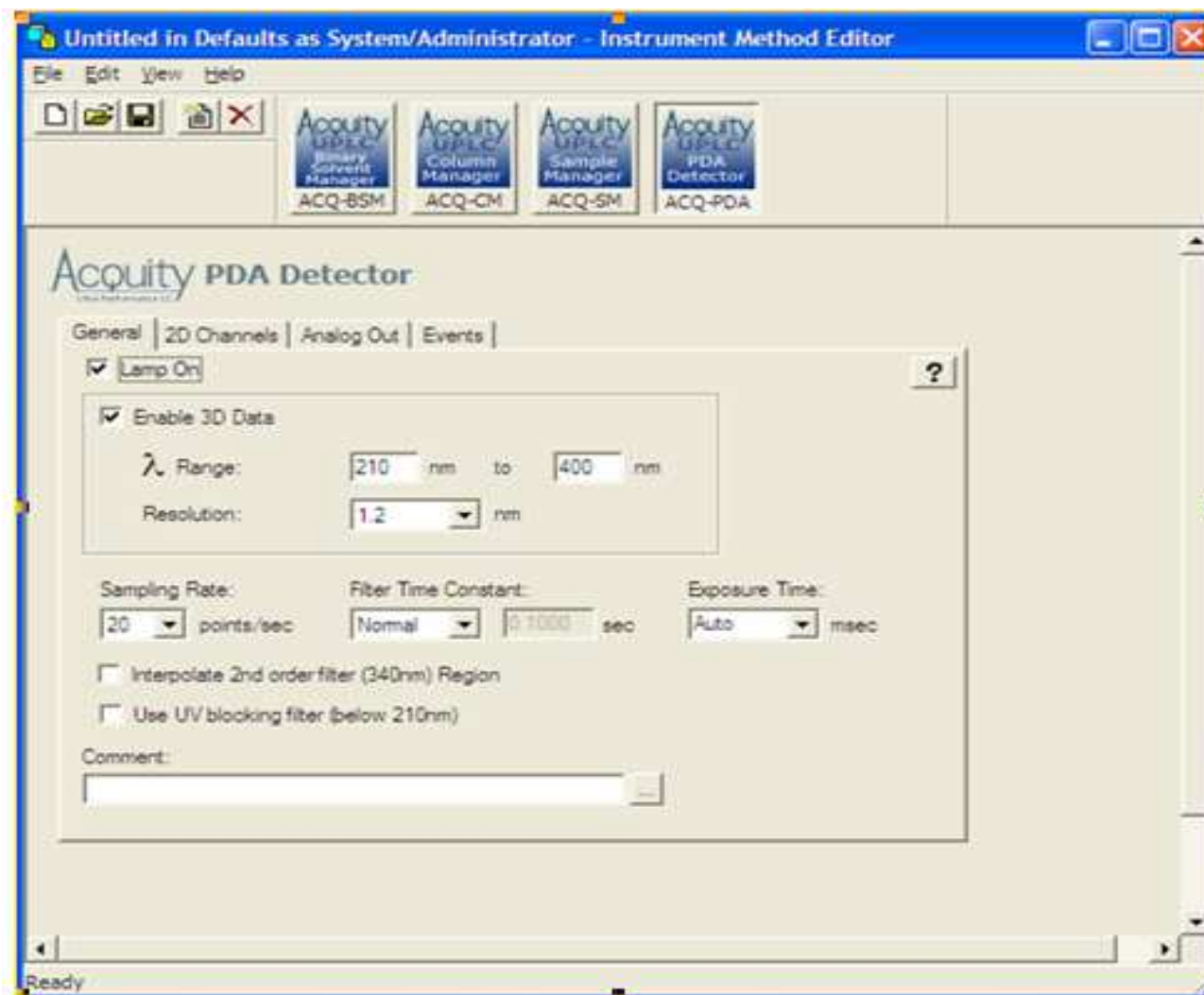
Effect of Filtering Constant on Resolution and Signal-to-Noise

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Filter Constant	Peak Width (min)	Resolution	Signal-to-Noise
No Filtering	0.0147	4.934	431
0.1	0.0162	4.232	524
0.2	0.0202	3.079	543
0.3	0.0253	2.302	512
0.4	0.0411	1.795	458
0.5	0.0569	1.453	400

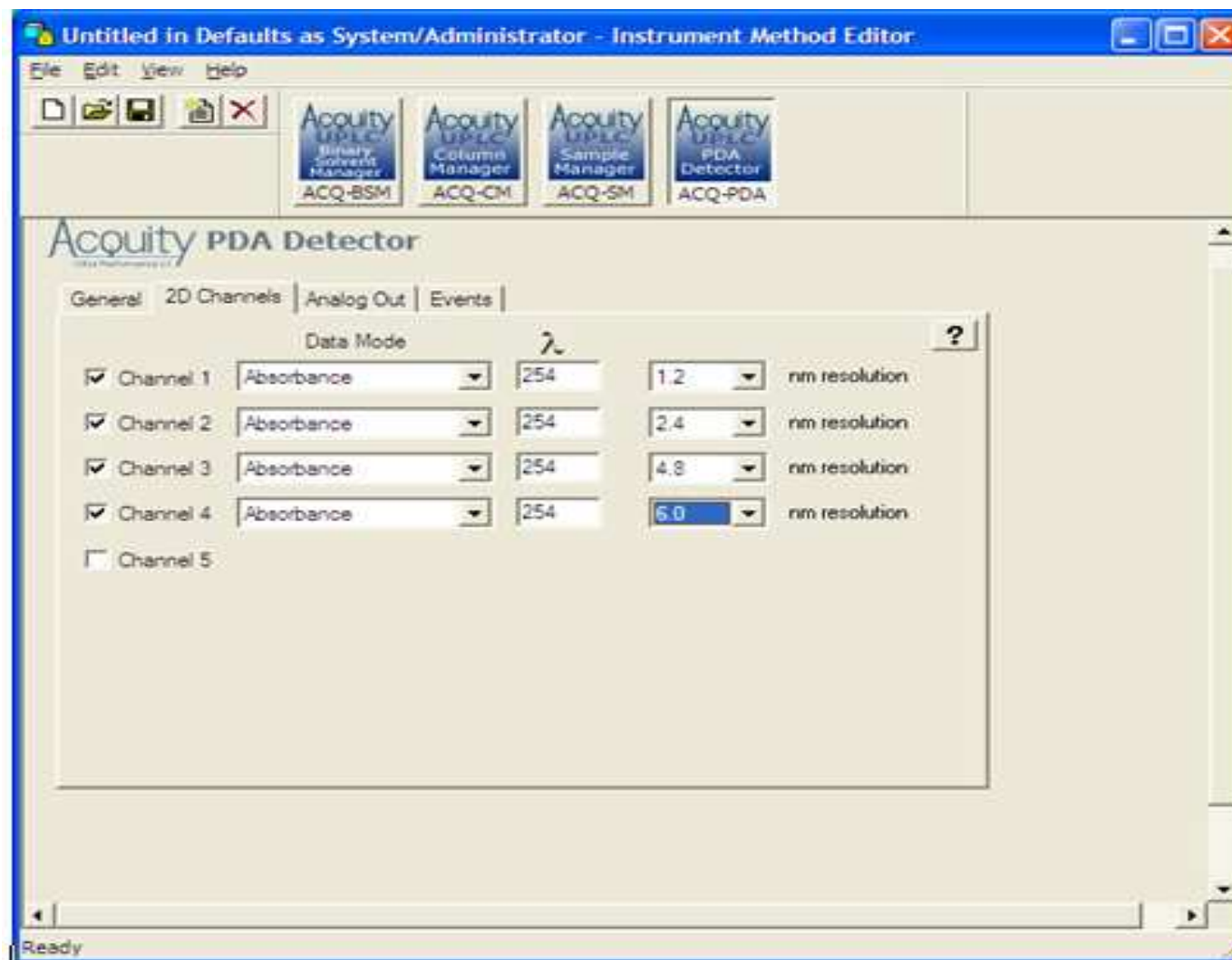
How to Select the Best Filtering Constant

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



How to Select the Best Filtering Constant

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



How to Select the Best Filtering Constant

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Ensure that the data rate is set to collect 25 – 50 points.
- Start with the inverse of the sampling rate

$$\text{Filtering Time Constant} = 1/\text{Sampling Rate}$$

- Is increased sensitivity needed? Is baseline noise interfering with integration?

↑ *Filtering Time Constant*

- Is increased resolution needed?

↓ *Filtering Time Constant*

Fast Data Rates for UPLC™ with Independent Data Filter Optimization

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Fast Filter Constant

Filter Constant = $1/\text{Data Rate}$

Normal Filter Constant

Filter Constant = $4 * (1/\text{Data Rate})$

Data Rate	Fast Time Constant	Normal Time Constant
10	0.1	0.4
20	0.05	0.2
40	0.025	0.1
80	0.0125	0.05

Noise Comparison at Different Data Rates

Data Rate	1.2 nm	2.4 nm	4.8 nm	8.4 nm	12 nm
1	4.8	4.4	3.8	3.7	3.6
2	9.5	8.1	6.4	6.3	6.3
5	17.6	15.7	11.9	10	9.2
10	28.4	24.9	16	13.4	12
20	49.9	43.2	26.6	19.6	17
40	88.5	75.9	44.8	30.1	25.6
80	136	118.6	72.5	43.3	39.8

Normal Time Constant