


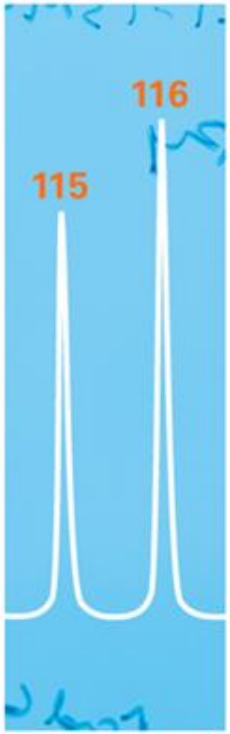

High Sensitivity LC/MS/MS Quantification of Corticosteroids in Dried Blood Spots and Evaluation of Software Saturation Correction for Extending Dynamic Range

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AB SCIEX

Co-Authors

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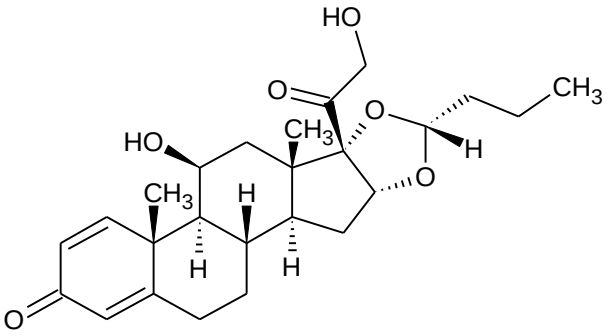
- Neil Spooner, Ph.D.

Outline

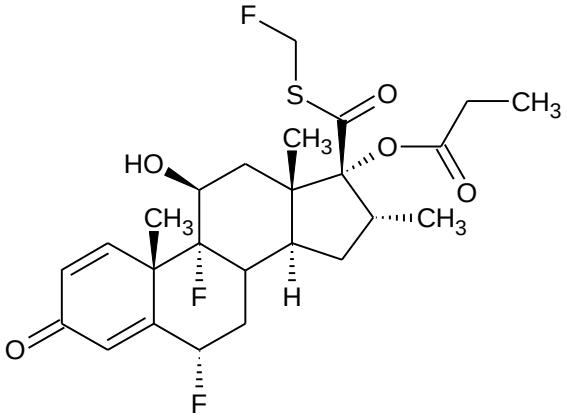
- Challenge of performing DBS bioanalysis on inhalation drugs with low systemic circulation
- Case study: Budesonide and Fluticasone Propionate in asthma
- Evaluation of a high sensitivity hybrid triple quadrupole for achieving required LOQ's in DBS
- Evaluation of a new integration algorithm with software saturation correction for extending dynamic range and overcoming dilution integrity issues

Corticosteroids for Asthma – Powder Inhalation



Budesonide

MW 430.2



Fluticasone Propionate

MW 430.2

Compound	Mean C_{max} (Plasma) (pg/mL)	Dose (Inhaled Dry Powder) (mg)
Fluticasone Propionate	130	1.0
Budesonide	2,432	1.2

T. W. Harrison and A. E. Tattersfield; *Thorax* 2003 58: 258-260

Bioanalytical Challenges of Using DBS for Inhaled Corticosteroids

- High sensitivity required (at least 10 – 50 pg/mL LOQ's)
- Small sample volumes
- In case of Budesonide, wider dynamic range is needed due to higher C_{max} and shorter half-life
  - Unlike plasma assays, DBS poses challenges in validation of dilutions and verification of dilution integrity

➢ Assay challenges at both ends of the calibration curve

Evaluation of High Sensitivity and Software Saturation Correction

- Can sufficient sensitivity be achieved with QTRAP® 5500 system and fast LC ?
- Can linear dynamic range (LDR) be extended using software saturation correction (SignalFinder™ integration algorithm) ?

Experimental - Sample Preparation

- Fluticasone propionate and Budesonide
- Sprague-Dawley Rat whole blood
- Curve range: 5 – 250,000 pg/mL
- ID Biologics 226 card
- 50 µL spot volume
- Overnight drying at room temperature
- 2 x 8 mm punch size
- Sonicate for 60 minutes in 250 µL methanol
- Centrifuge, decant 200 µL, and dry down
- Reconstitute in 100 µL 50/50 MeOH / Water

Experimental - Chromatography

- Waters® ACQUITY UPLC® system
- BEH C-18 column 1.7 µm, 2.1 x 50 mm
- Mobile Phase
  - A: Water w/ 0.1% NH4OH
  - B: Methanol
- Flow Rate: 0.6 mL/min
- Column Temp: 60˚ C
- Run time: 2.5 minutes
- Injection volume: 20 µL

Time (min)	Flow Rate (ml)	%A	%B
0	0.6	95	5
0.5	0.6	95	5
1	0.6	0	100
1.5	0.6	0	100
2.0	0.6	95	5
2.5	0.6	95	5

J. Mather *et al,* Application Note, 2009.

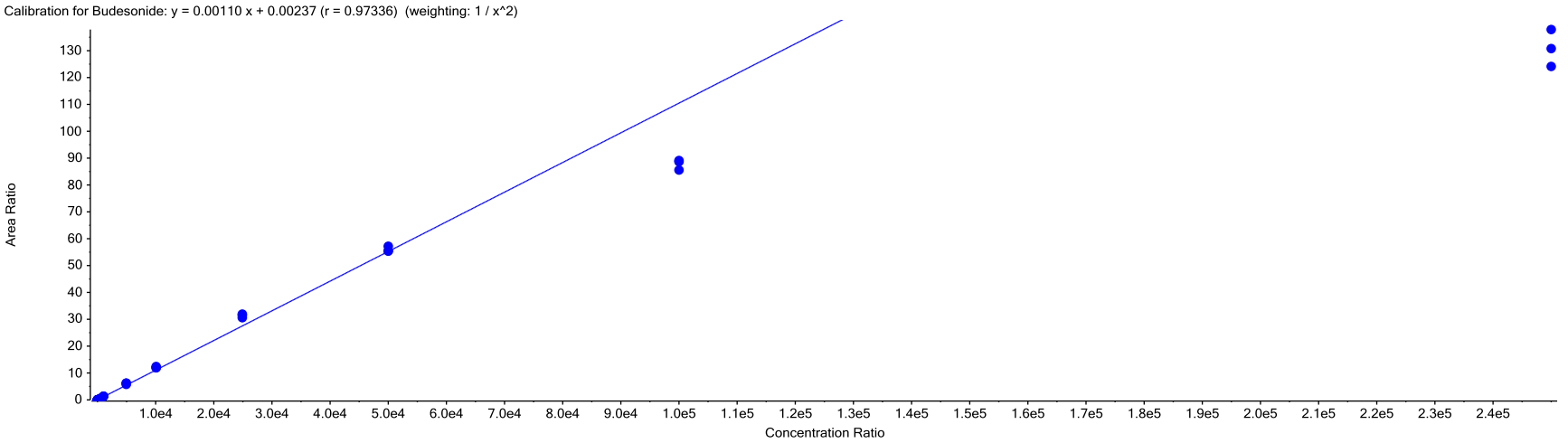
Experimental - LC/MS/MS

- AB SCIEX QTRAP® 5500 System
- Turbo V™ source in ESI Mode
- Budesonide: 431.3 → 323.2
- Fluticasone Propionate: 501.2 → 313.1
- Q1 = Unit, Q3 = Low
- Dwell time: 100 ms per transition
- Data processing: MultiQuant™ software version 2.0
  - SignalFinder™ Algorithm with saturation correction
  - MQ4 Algorithm for comparison

Results

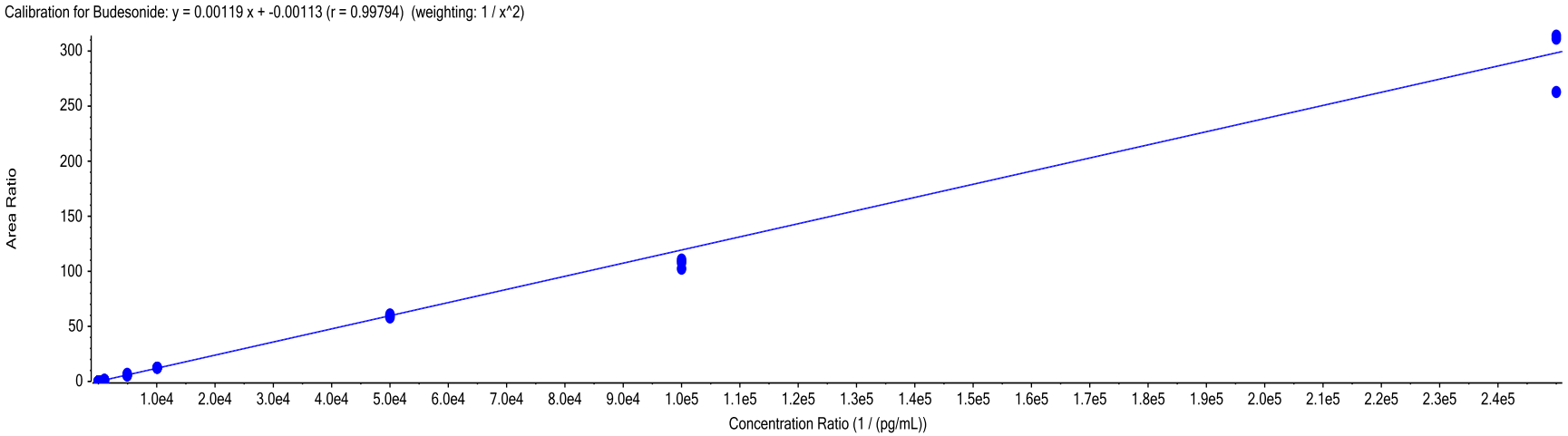
- Sensitivity evaluation
- Saturation Correction evaluation
- Peak deconvolution and Integration consistency in the SignalFinder™ algorithm

Budesonide Standard Curve – MQ4 Algorithm



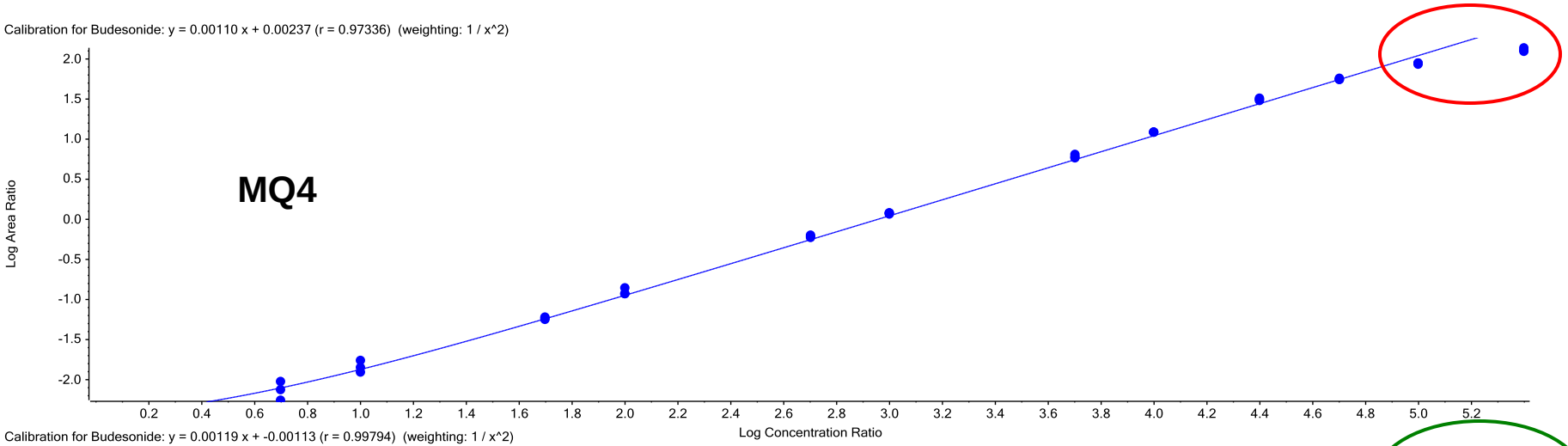
Component Name	Actual Concentration (pg/mL)	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
Budesonide	5	3 of 3	4.67E+00	1.83E+00	39.2	93.4	2.88E+00	4.60E+00	6.53E+00
Budesonide	10	3 of 3	1.11E+01	2.29E+00	20.6	111.4	9.19E+00	1.37E+01	1.06E+01
Budesonide	50	3 of 3	5.09E+01	1.59E+00	3.1	101.9	5.26E+01	5.08E+01	4.94E+01
Budesonide	100	3 of 3	1.11E+02	9.82E+00	8.8	111.2	1.23E+02	1.06E+02	1.05E+02
Budesonide	500	3 of 3	5.58E+02	1.73E+01	3.1	111.6	5.77E+02	5.44E+02	5.53E+02
Budesonide	1000	3 of 3	1.08E+03	1.46E+01	1.4	107.7	1.09E+03	1.06E+03	1.08E+03
Budesonide	5000	3 of 3	5.55E+03	2.26E+02	4.1	110.9	5.79E+03	5.34E+03	5.51E+03
Budesonide	10000	3 of 3	1.10E+04	2.02E+02	1.8	110.3	1.09E+04	1.13E+04	1.09E+04
Budesonide	25000	3 of 3	2.83E+04	6.80E+02	2.4	113.3	2.90E+04	2.77E+04	2.83E+04
Budesonide	50000	3 of 3	5.07E+04	1.07E+03	2.1	101.4	5.00E+04	5.01E+04	5.19E+04
Budesonide	100000	3 of 3	7.95E+04	1.65E+03	2.1	79.5	8.04E+04	7.76E+04	8.06E+04
Budesonide	250000	3 of 3	1.19E+05	6.22E+03	5.2	47.4	1.12E+05	1.19E+05	1.25E+05

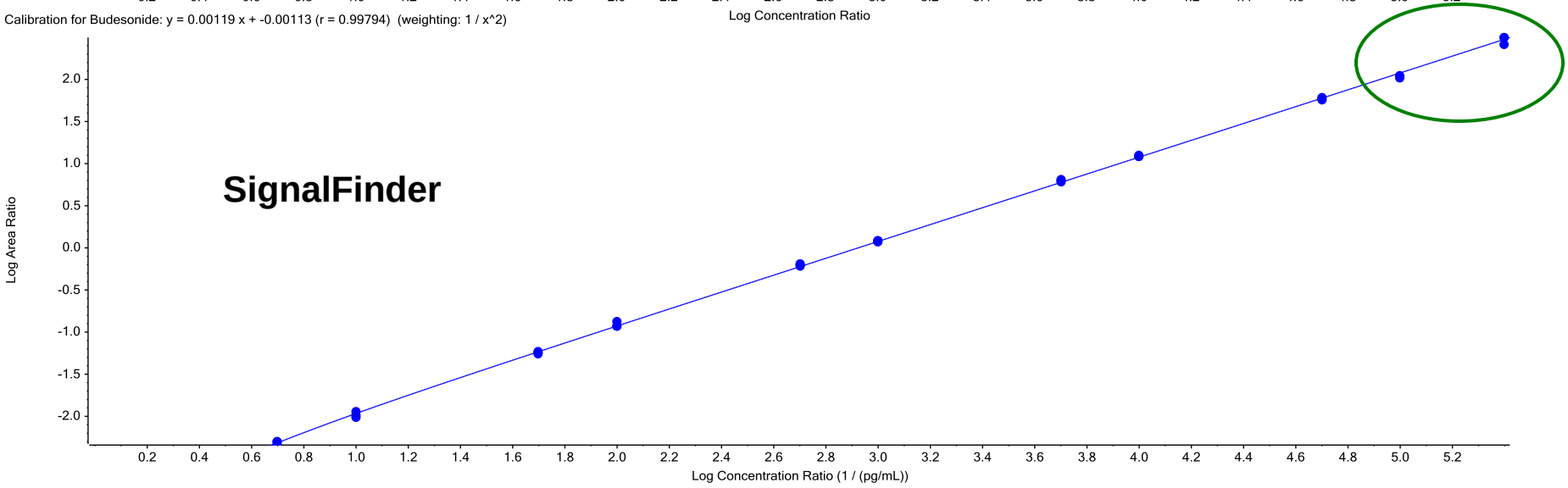
Budesonide Standard Curve – SignalFinder™ Algorithm



Component Name	Actual Concentration (pg/mL)	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
Budesonide	5	3 of 3	5.08E+00	1.64E-01	3.23	101.55	4.89E+00	5.16E+00	5.18E+00
Budesonide	10	3 of 3	9.71E+00	6.50E-01	6.7	97.08	9.14E+00	9.56E+00	1.04E+01
Budesonide	50	3 of 3	4.84E+01	1.38E+00	2.85	96.87	4.85E+01	4.98E+01	4.70E+01
Budesonide	100	3 of 3	1.03E+02	7.61E+00	7.37	103.36	1.12E+02	9.87E+01	9.93E+01
Budesonide	500	3 of 3	5.24E+02	1.64E+01	3.13	104.85	5.11E+02	5.19E+02	5.43E+02
Budesonide	1000	3 of 3	1.01E+03	2.09E+01	2.08	100.47	1.02E+03	9.81E+02	1.01E+03
Budesonide	5000	3 of 3	5.20E+03	1.74E+02	3.34	103.98	5.38E+03	5.03E+03	5.19E+03
Budesonide	10000	3 of 3	1.04E+04	1.79E+02	1.72	103.71	1.03E+04	1.06E+04	1.03E+04
Budesonide	50000	3 of 3	4.95E+04	1.14E+03	2.31	99.04	5.07E+04	4.94E+04	4.84E+04
Budesonide	100000	3 of 3	8.98E+04	3.67E+03	4.08	89.83	9.32E+04	8.59E+04	9.03E+04
Budesonide	250000	3 of 3	2.48E+05	2.42E+04	9.74	99.26	2.20E+05	2.63E+05	2.61E+05

The Effect of Saturation Correction

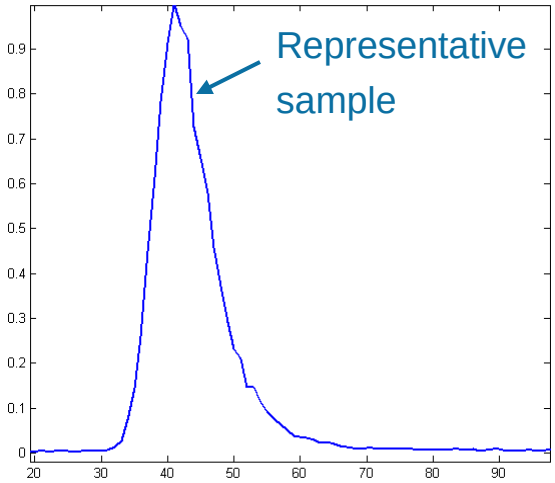




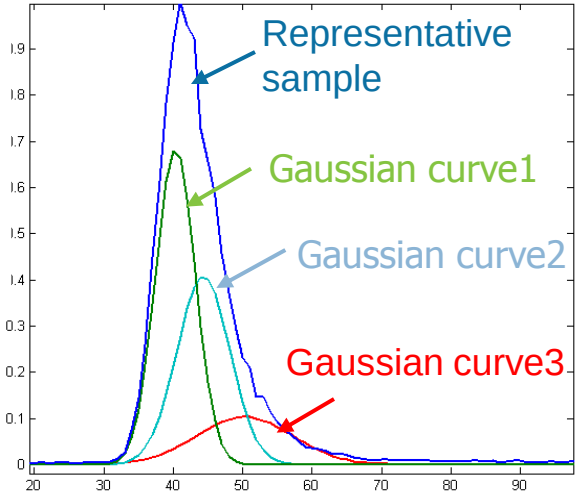
How Does Saturation Correction Work ?

- Peak Modeling
- Saturation Correction

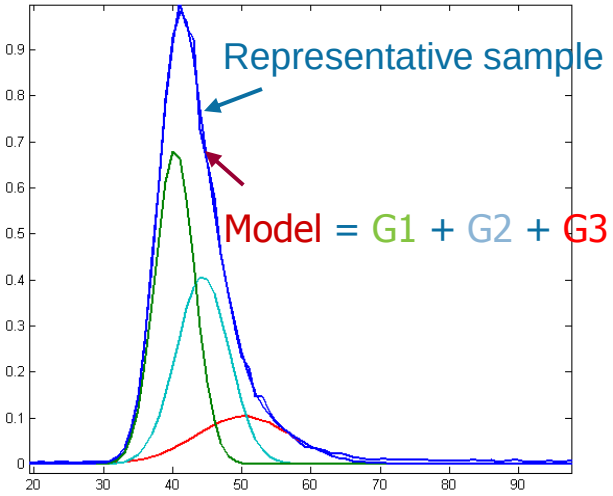
How Does Peak Modeling Work ?



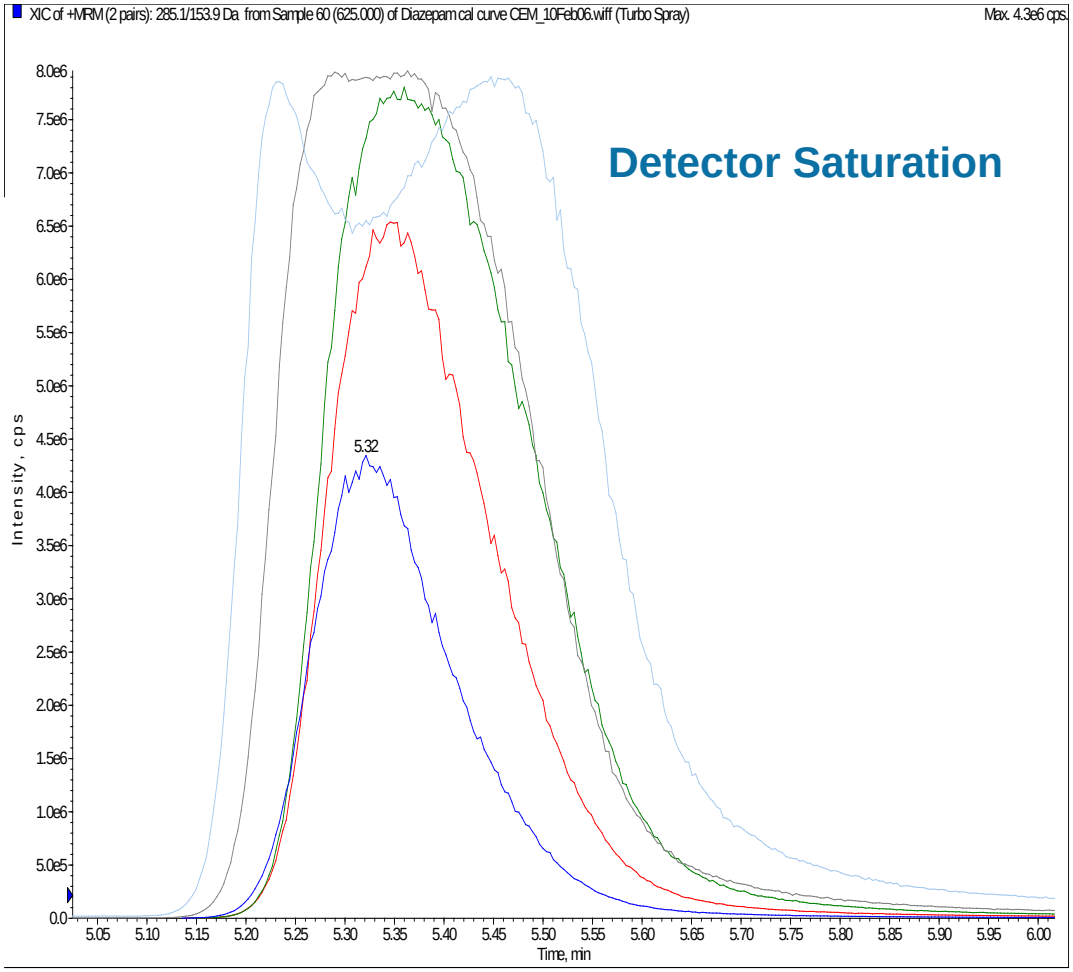
Model is constructed based on a representative peak from the actual raw data and takes into account the entire peak shape.



Representative peak is simulated by a sum of 3 Gaussian curves. The sum of those curves becomes the peak model.



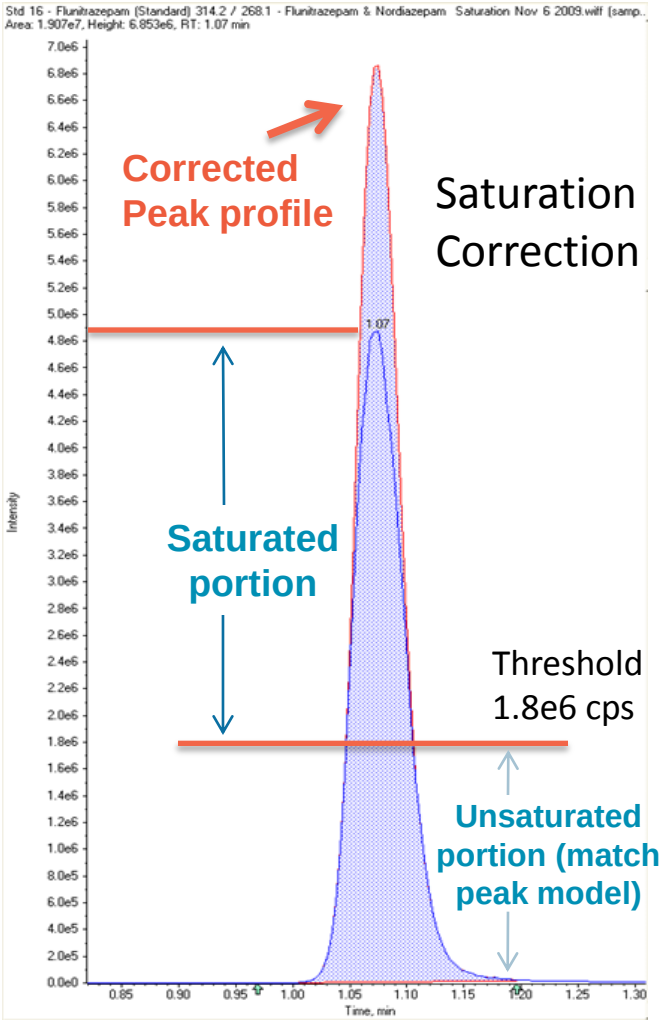
Detector Saturation

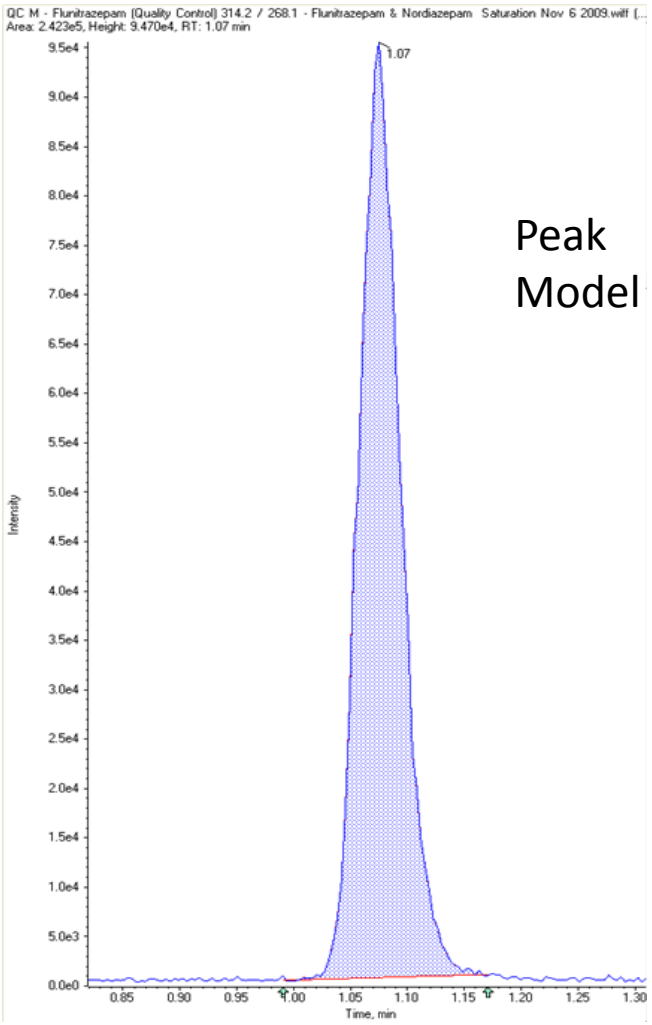


- Response flattens as concentration increases
- Peak width also increases
- Peak modeling can account for both phenomena

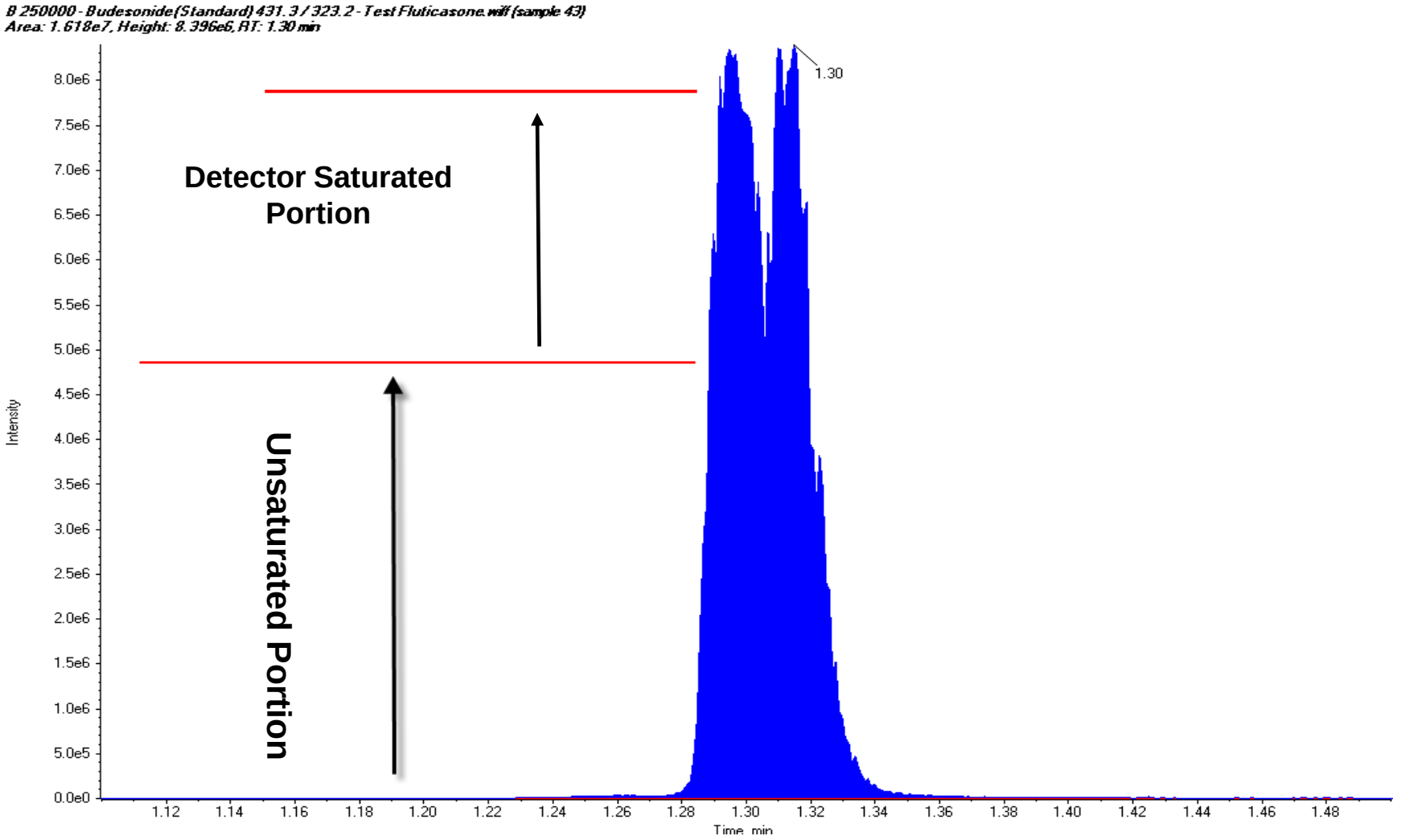
Detector Saturation Correction

Using SignalFinder™ Integration Algorithm

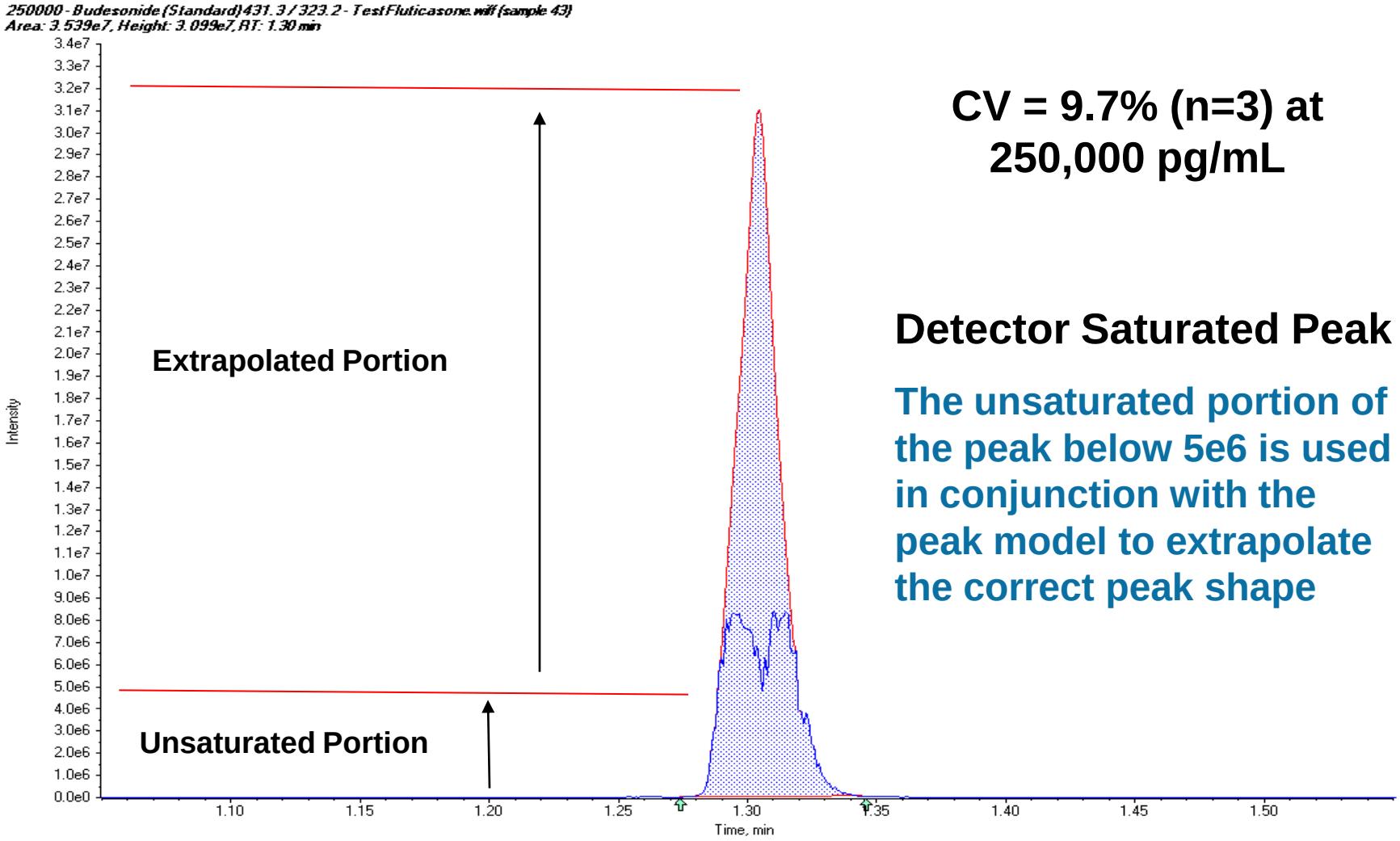




Saturated Peak – Budesonide 250,000 pg/mL



Saturation Correction with SignalFinder

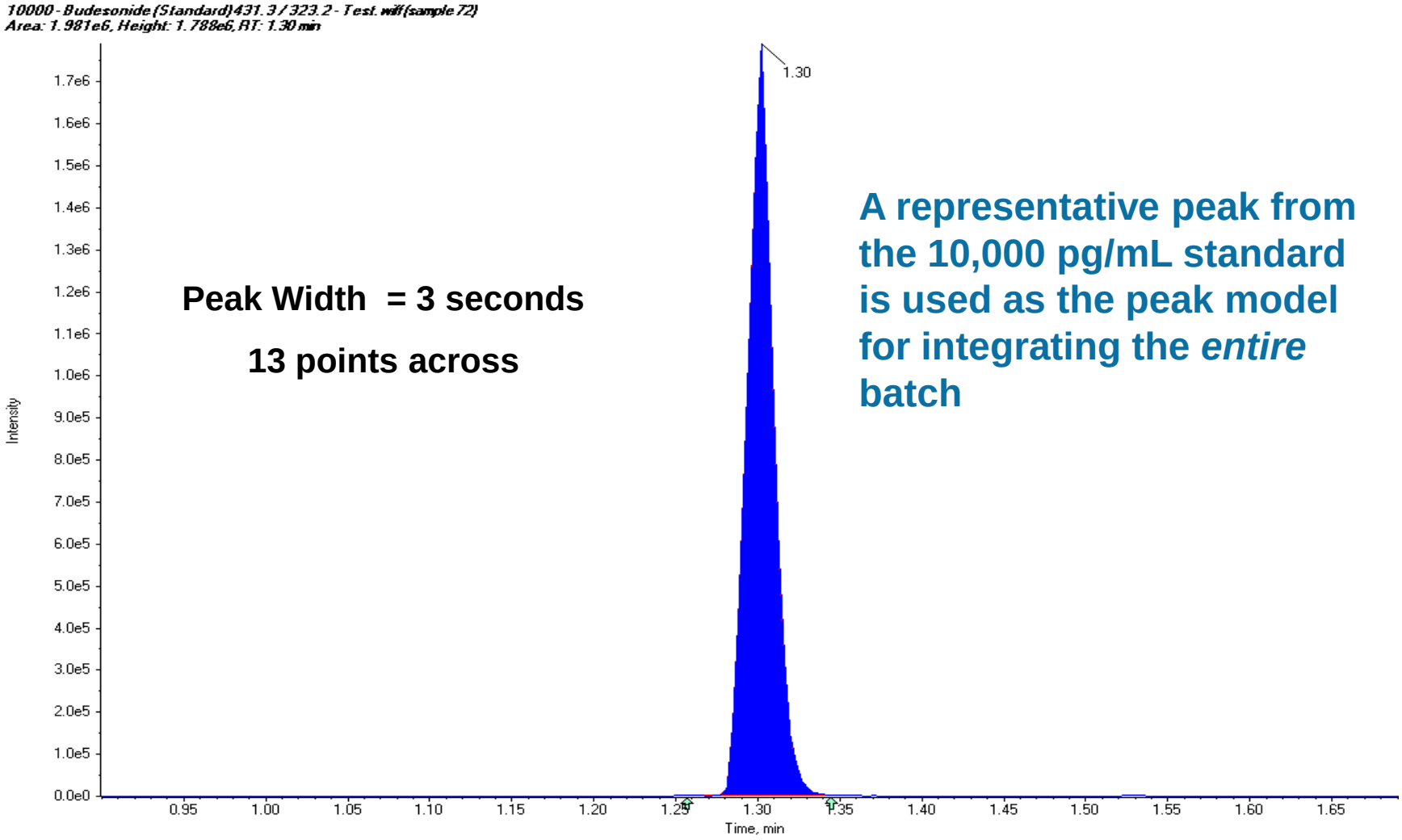


CV = 9.7% (n=3) at 250,000 pg/mL

Detector Saturated Peak

The unsaturated portion of the peak below 5e6 is used in conjunction with the peak model to extrapolate the correct peak shape

Peak Used for Model – 10,000 pg/mL

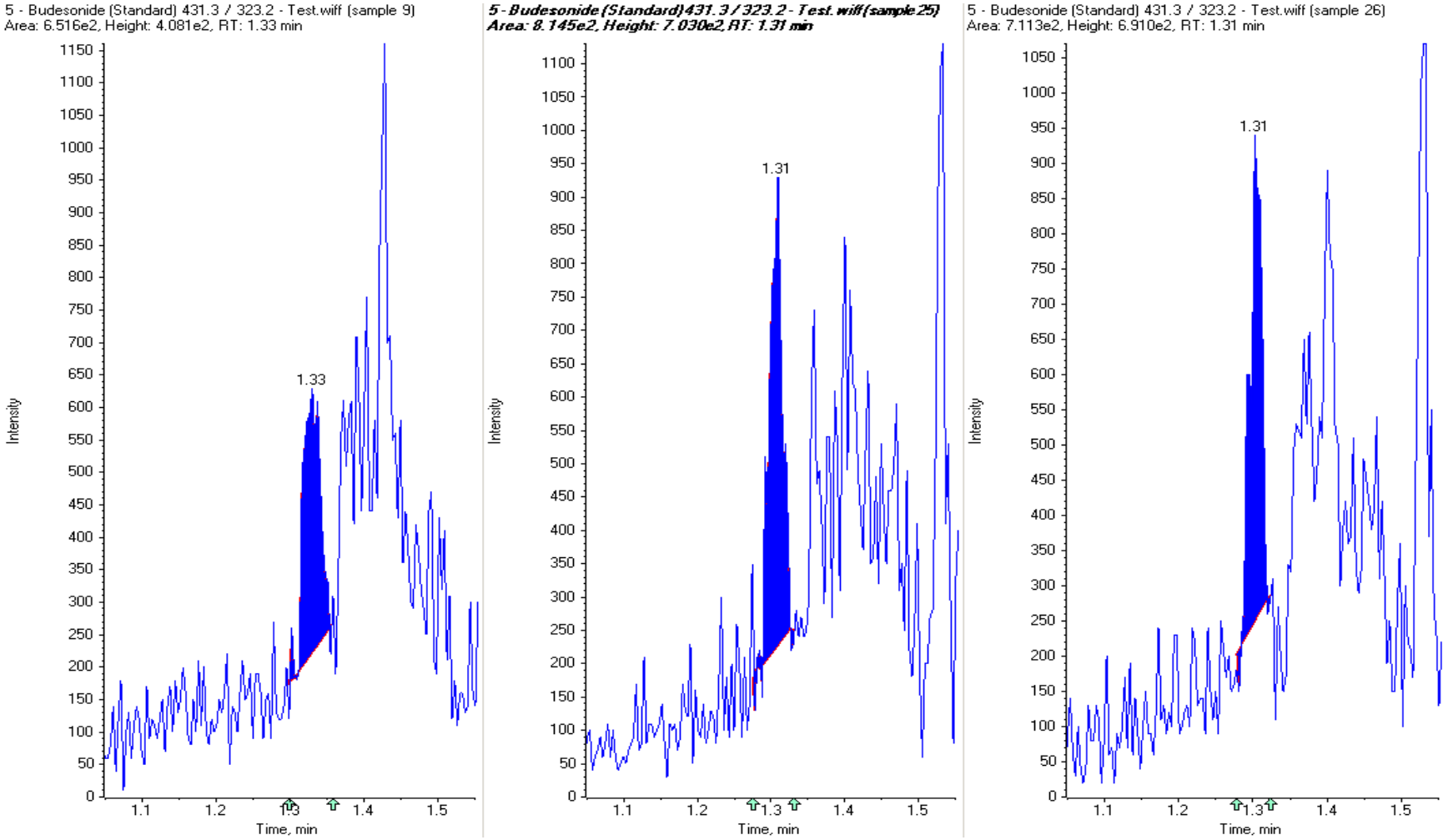


Peak Width = 3 seconds

13 points across

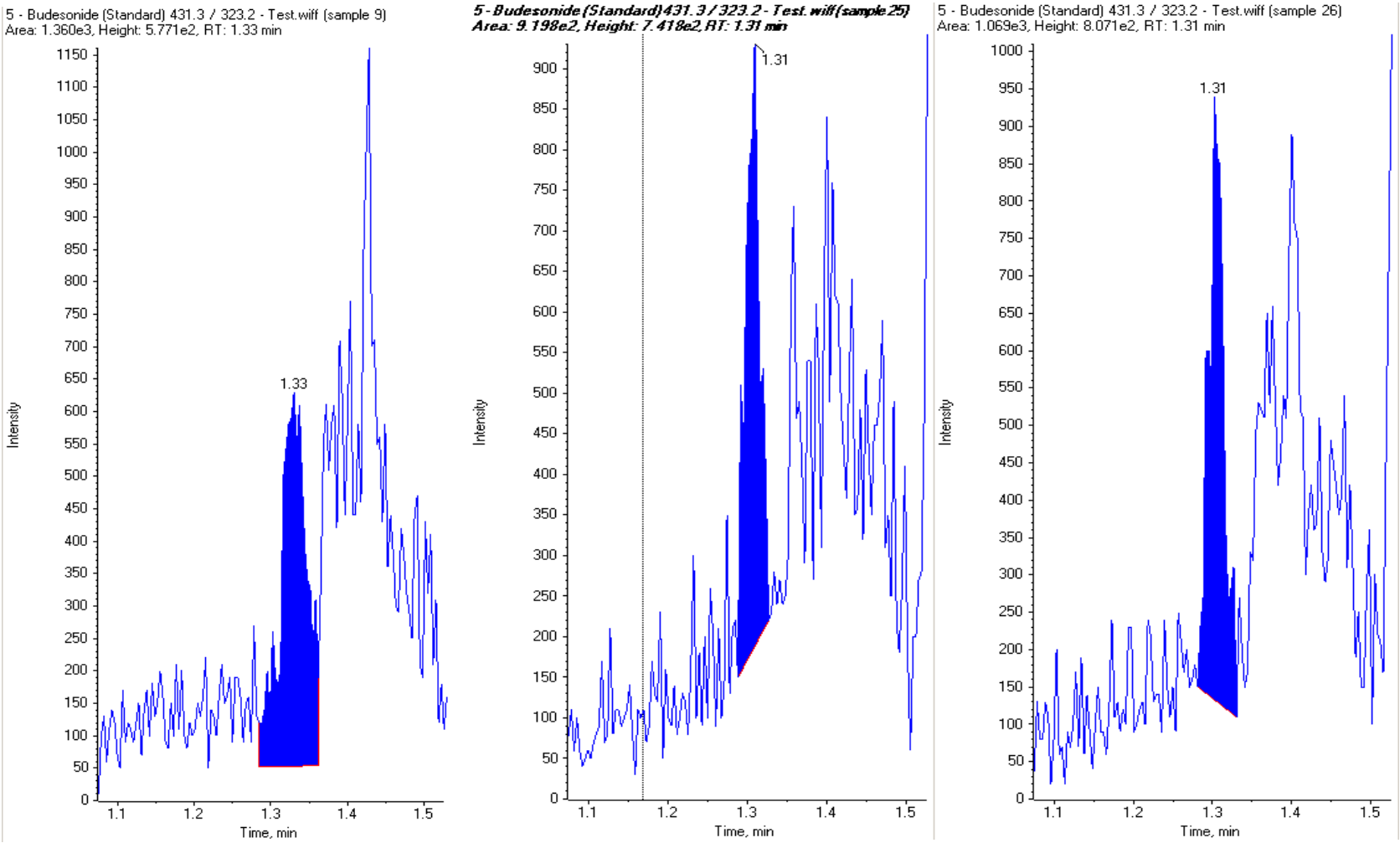
A representative peak from the 10,000 pg/mL standard is used as the peak model for integrating the *entire* batch

Peak Deconvolution at LOQ – More Consistent Integration with Peak Modeling Approach



CV = 3% (n=3) at 5 pg/mL

Integration Consistency at LOQ Using Conventional Integration



CV = 39% (n=3) at 5 pg/mL

Peak Modeling and Saturation Correction
Advantages and Potential Issues

- Advantages
  - Linear range extension has the potential to reduce the need for dilutions and overcome DBS dilution integrity issues. This takes on additional importance with online direct analysis approaches.
  - Peak modeling provides for more consistent integration of weak or poorly resolved peaks.
  - Better precision and accuracy at the LOQ.
  - Reduces the need for manual adjustment of integration parameters.

- Potential Issues
  - No precedent for using software saturation correction in regulated bioanalysis (to the best of the authors' knowledge).
  - SignalFinder™ algorithm can only correct for detector saturation at the present time. Source and column saturation are not corrected for.
  - The amount of linear range extension is analyte and assay dependent.
  - Obtaining a sufficient number of points *across the unsaturated portion* of the peak is critical for accurate extrapolation.

Conclusions

- Quantification of inhalation drugs with low systemic circulation is feasible with DBS
- High sensitivity triple quadrupole instruments with UPLC can achieve sufficient sensitivity for 5-10 pg/mL LOQ's
- Two large punches (8 mm) had to be used to achieve sufficient sensitivity. Additional sensitivity would be highly beneficial to reduce punch size.
- Software saturation correction can achieve significant linear range extension in DBS bioanalysis
- By increasing linear range, the need to perform dilutions is reduced and dilution integrity issues can be overcome
- Peak modeling based integration algorithm also demonstrated more consistent integration of weak or poorly resolved peaks

Acknowledgements

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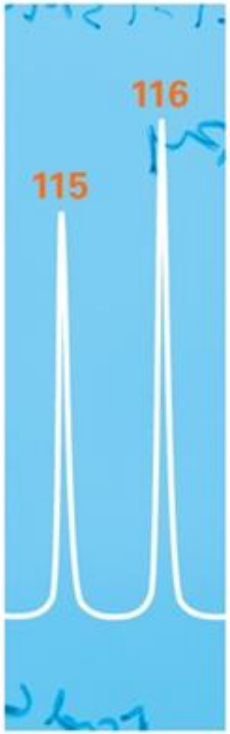

Thank You

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