



# Implementation of SFC as an Analytical Tool in a GMP Regulated Environment: A Three Vendor Evaluation

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# Method Validation: Criteria

- Regulatory Bodies – FDA and ICH
  - *Validation of Analytical Procedures: Text and Methodology Q2(R1)*
  - *Q2B Validation of Analytical Procedures: Methodology*
    - Specificity
    - Linearity
    - Range
    - Accuracy
    - Precision
      - Repeatability
      - Intermediate Precision
    - Limit of Detection
    - Limit of Quantitation



# The Experiment

- Flurbiprofen – Nominal Conc. 1 mg/mL
  - Column: AD-H, 250x4.6mm, 5 $\mu$ m
  - Flow Rate: 4 mL/min
  - Isocratic @ 20% MeOH
  - Pressure: 100 bar
  - Temperature: 40°C
  - Injection Volume: 10  $\mu$ L (full loop)
  - Detection @ 254 nm
- % Nominal Conc.
    - 0.0002 mg/mL = 0.02%
    - 0.0005 mg/mL = 0.05%
    - 0.001 mg/mL = 0.1%
    - 0.005 mg/mL = 0.5%
    - 0.01 mg/mL = 1%
    - 0.05 mg/mL = 5%
    - 0.5 mg/mL = 50%
    - 0.75 mg/mL = 75%
    - 1.0 mg/mL = 100%
    - 1.25 mg/mL = 125%
    - 1.5 mg/mL = 150%



# Acceptance Criteria

- High Level Linearity
  - 5 standard preparations ranging from 50 – 150% of nominal concentration (1 mg/mL)
  - $R^2 = 0.999$
  - Y-intercept  $< 5\%$  of the nominal concentration
- Low Level Linearity
  - 5 standard preparations ranging from 0.05 – 5.0% of nominal concentration (1 mg/mL)
  - $R^2 = 0.995$
  - Y-intercept  $< 10\%$  of the 0.1% concentration
  - The difference between the slopes of the high level and low level linearity plots  $< 10\%$



# Acceptance Criteria Continued

- LOQ

- Determined using low level linearity values and should be at least 0.05% of nominal concentration
- $RSD \leq 10\%$  for 6 injections at 0.05%
- $S/N \geq 10$

- LOD

- Determined using low level linearity values and should be at least 0.02% of nominal concentration
- $RSD \leq 30\%$  for 6 injections at 0.02%
- $S/N \geq 3$



# Acceptance Criteria Continued

- Precision
  - Nominal concentration: Area & RT %RSD  $\leq 2.0\%$  for 6 injections
- Resolution
  - $R_s \geq 2.0$
- Asymmetry
  - $0.7 \leq \text{Asym} \leq 2.0$



# The Instruments

- **Thar**
  - Method Station II
  - Detection: Waters PDA 2998
    - Sampling Rate: 5 pts/sec; Reference  $\lambda$ : 300-340nm
- **Jasco**
  - Analytical System
  - Detection: UV/VIS, Model UV-2075
- **Aurora**
  - Fusion A5
  - Agilent 1200 HPLC
  - Detection: Agilent DAD SL
    - 0.05 peak width; 16nm slit width; Reference  $\lambda$ : 360nm



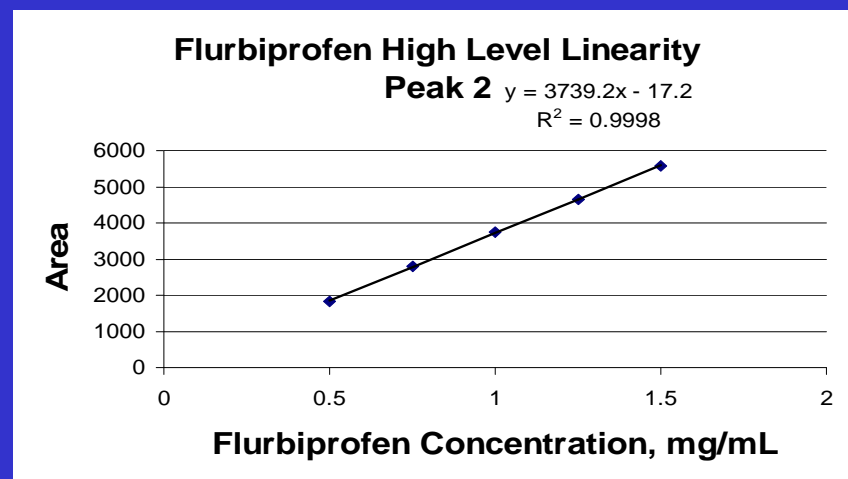
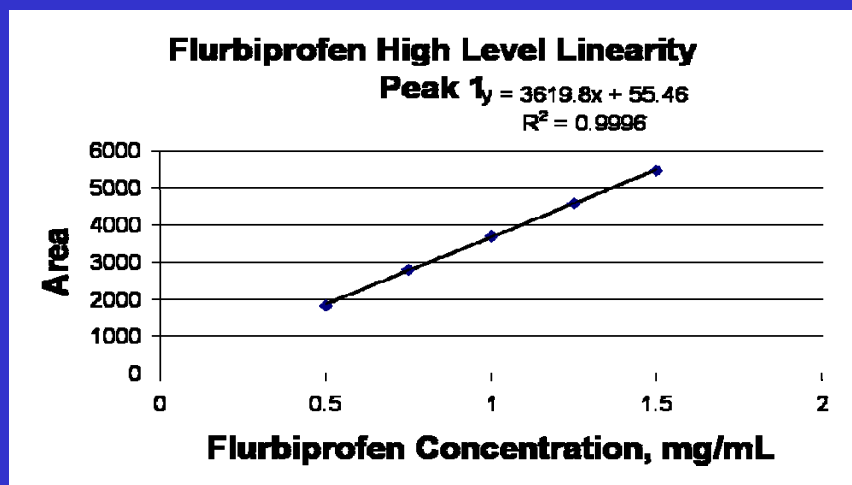
# Linearity – High Level

<b>Regulatory Criteria</b>	<b>Internal Criteria</b>
<p>Report correlation coefficient, y-intercept, slope of the regression line and residual sum of squares. A plot of the data should be included. Five concentration levels are recommended for the establishment of linearity.</p>	<p>70 – 130% of the nominal sample concentration (minimum of 5 pts) <math>R \geq 0.9990</math> Y-intercept <math>\leq 2.0\%</math> of response of nominal sample concentration Each response factor is 98 to 102% relative to the nominal sample concentration response factor</p>



# High Level Linearity

	Aurora		Jasco		Thar	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
R <sup>2</sup>	0.9996	0.9998	0.9993	0.9994	0.9999	0.9999
y=mx+b	y=3619.8x + 55.46	y=3739.2x - 17.2	y=24267x + 44089	y=24786x + 16038	y=81848x	y=81511x



Data complements of Aurora SFC



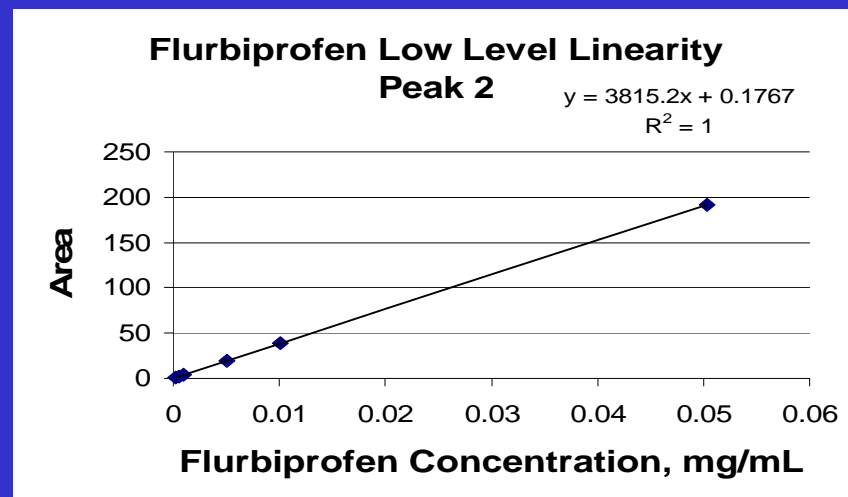
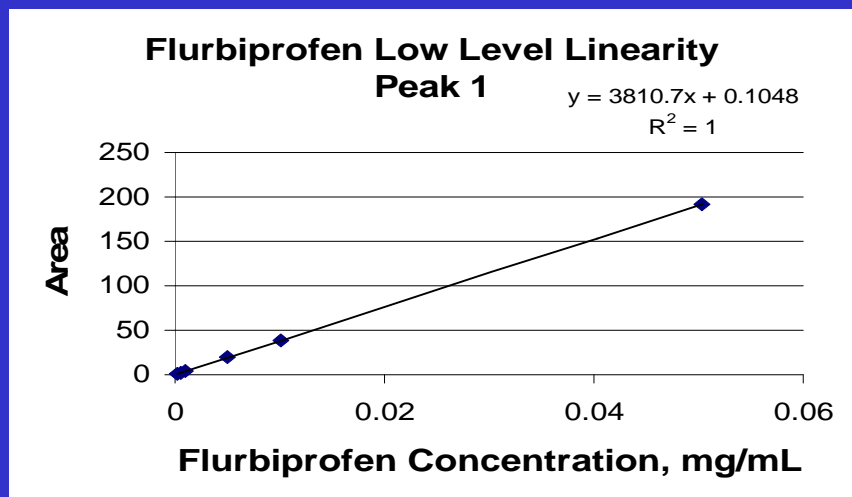
# Linearity – Low Level

<b>Regulatory Criteria</b>	<b>Internal Criteria</b>
<p>Report correlation coefficient, y-intercept, slope of the regression line and residual sum of squares. A plot of the data should be included. Five concentration levels are recommended for the establishment of linearity.</p>	<p>Reporting level to 2X specification level (minimum of 5 pts)</p> <p><math>R \geq 0.9950</math></p> <p>Y-intercept <math>\leq 10.0\%</math> of response of maximum concentration</p> <p>Response factor of impurity standards across the range must be <math>\pm 10\%</math> relative to the expected specification level response factor</p>



# Low Level Linearity

	Aurora		Jasco		Thar	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
R <sup>2</sup>	1.000	1.000	0.9986	0.9989	1.000	0.9999
y=mx+b	y=3810.7x + 0.1048	y=3815.2x - 0.1767	y=25434x - 6.2952	y=25001x + 310.43	y=82353x	y=81053x



Data complements of Aurora SFC



# Precision

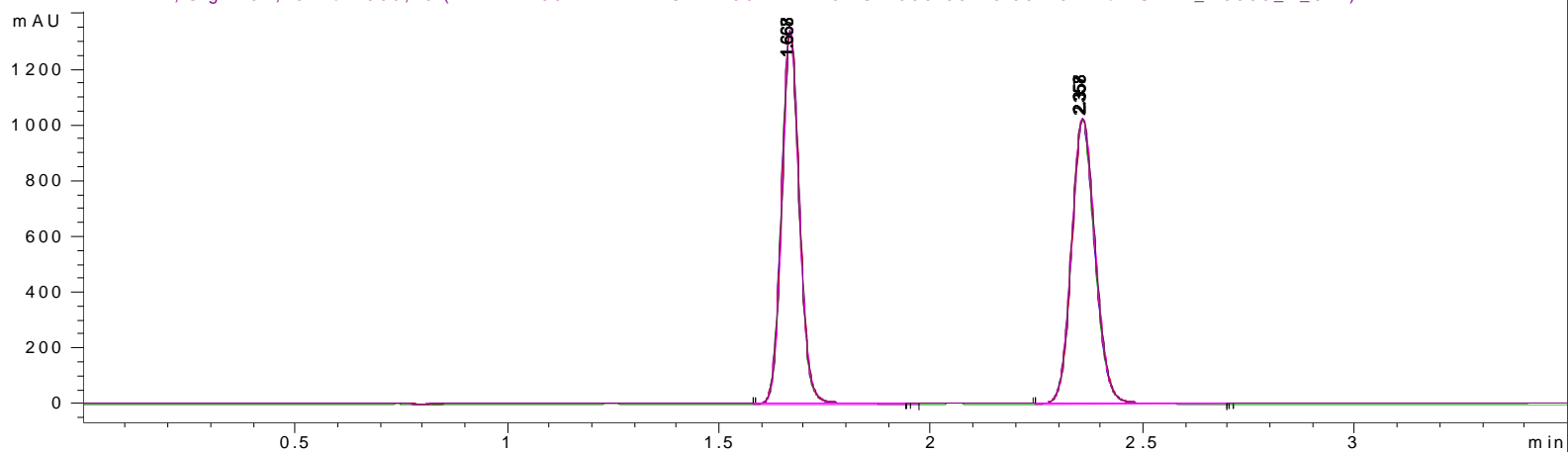
<b>Parameter</b>	<b>Regulatory Criteria</b>	<b>Internal Criteria</b>
Precision – High Level Repeatability	A minimum of 6 determinations at 100% test concentration.  The standard deviation, relative standard deviation and confidence interval should be reported.	6 injections of a standard at the nominal sample concentration  $RSD \leq 1.0\%$ for peak response of main band
Precision – Low Level Repeatability		6 injections of one sample at the RL  $RSD \leq 10\%$ for peak response



# Precision – 1mg/mL

	Aurora		Jasco		Thar	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
% RSD Area	0.214	0.221	0.376	0.379	0.477	0.297
% RSD RT	0.024	0.022	0.109	0.112	0.088	0.090

DAD1 A, Sig=254,16 Ref=360,40 (PFIFER 05-... AND LOW 100 BAR 20PC 2009-05-20 09-25-21\FLURBL\_10000\_2\_1.D)  
DAD1 A, Sig=254,16 Ref=360,40 (PFIFER 05-... AND LOW 100 BAR 20PC 2009-05-20 09-25-21\FLURBL\_10000\_2\_2.D)  
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DAD1 A, Sig=254,16 Ref=360,40 (PFIFER 05-... AND LOW 100 BAR 20PC 2009-05-20 09-25-21\FLURBL\_10000\_2\_4.D)  
DAD1 A, Sig=254,16 Ref=360,40 (PFIFER 05-... AND LOW 100 BAR 20PC 2009-05-20 09-25-21\FLURBL\_10000\_2\_5.D)  
DAD1 A, Sig=254,16 Ref=360,40 (PFIFER 05-... AND LOW 100 BAR 20PC 2009-05-20 09-25-21\FLURBL\_10000\_2\_6.D)



Data complements of Aurora SFC



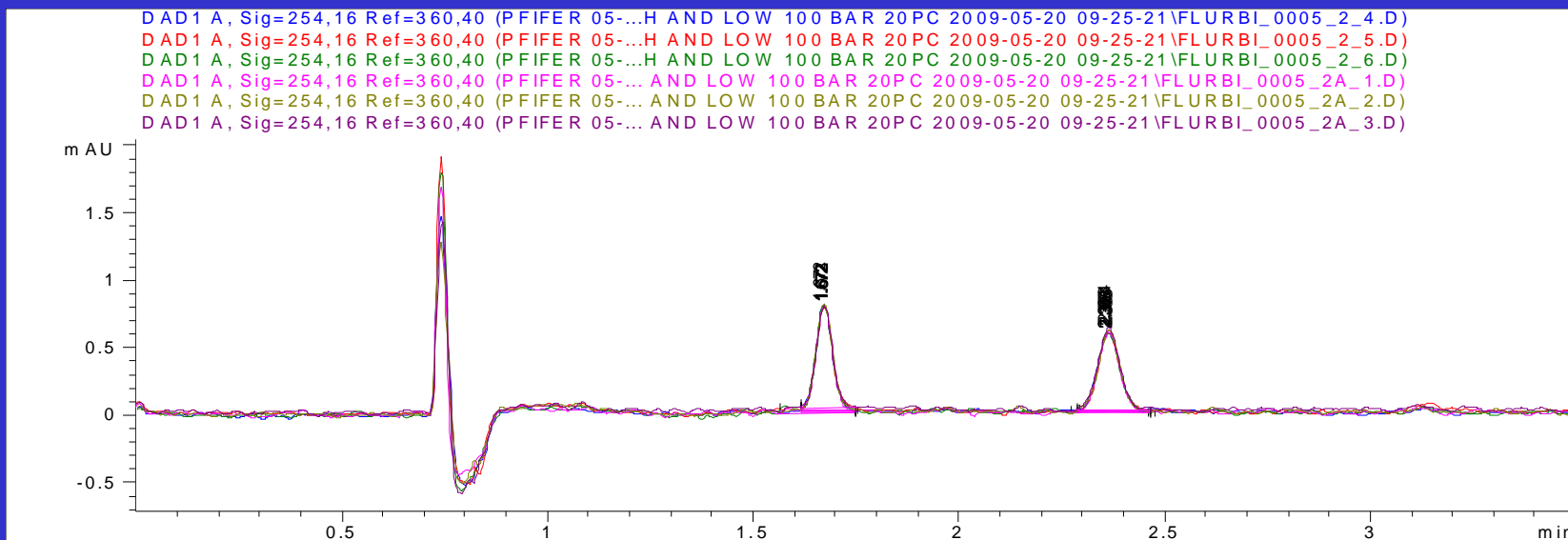
# Limit of Quantitation

<b>Regulatory Criteria</b>	<b>Internal Criteria</b>
<p>The quantitation limit and the method used for determining the quantitation limit should be presented.</p> <p>Signal-to-Noise</p> <p>The minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.</p>	<p>QL: 10 X S/N</p> <p>The QL should be approximately 50% lower than the RL</p> <p>LOQ should be validated by the analysis of samples known to be near or prepared at the limit</p>



# LOQ Repeatability

	Aurora		Jasco		Thar	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
% RSD Area	2.65	1.72	5.44	4.10	6.64	6.87
% RSD RT	0.02	0.04	0.34	0.49	0.22	0.23



Data complements of Aurora SFC

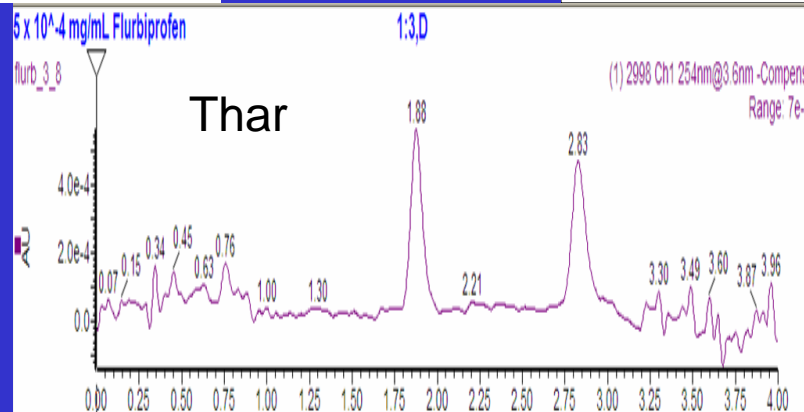
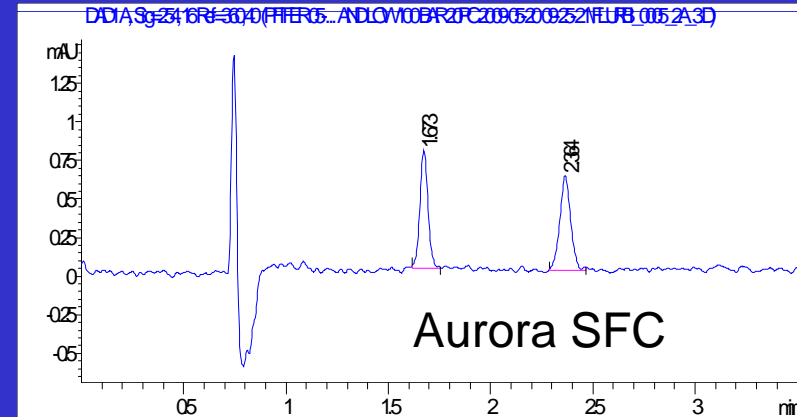
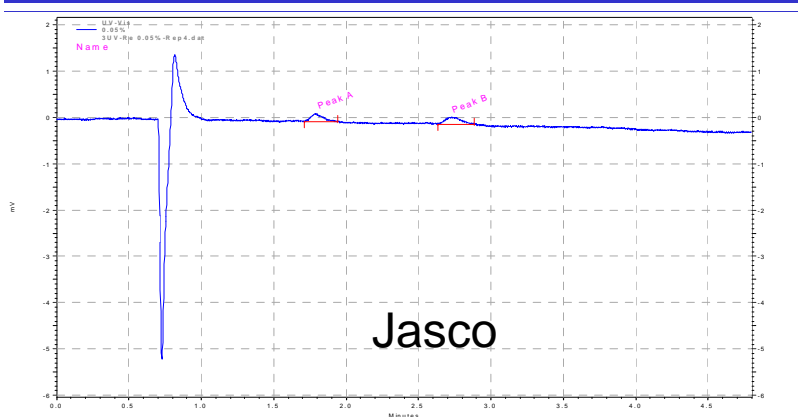


# Limit of Quantitation

0.05% of Nominal

	Aurora		Jasco		Thar	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
S/N	19.6	14.9	8.5	7.8	44.5	36.8

Signal-to-Noise assessed as peak to peak noise





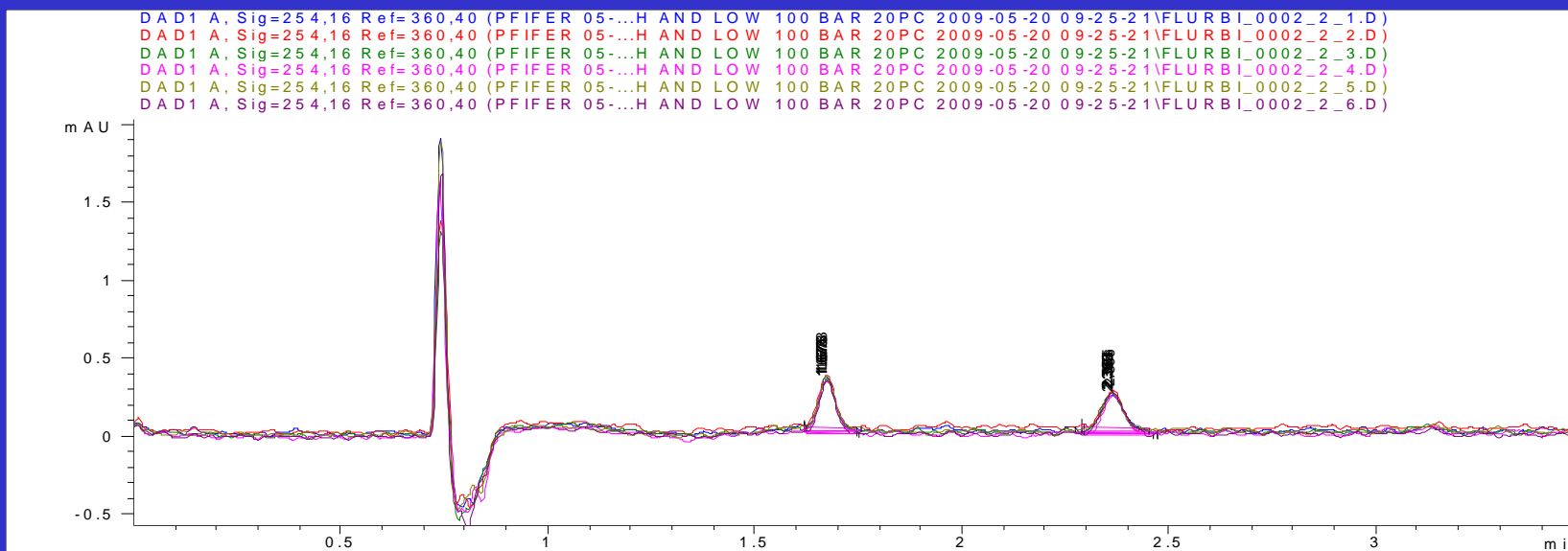
# Limit of Detection

<b>Regulatory Criteria</b>	<b>Internal Criteria</b>
<p>The detection limit and the method used for determining the detection limit should be presented.</p> <p>Signal-to-Noise</p> <p>The minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable.</p>	<p>DL: Report the DL calculated as 2-3 X S/N or by using linearity data</p> <p>LOD should be validated by the analysis of samples known to be near or prepared at the limit</p>



# LOD Repeatability

	Aurora		Jasco		Thar	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
% RSD Area	2.85	9.92	7.05	9.33	6.76	4.34
% RSD RT	0.07	0.05	0.61	0.43	0.13	0.26



Data complements of Aurora SFC

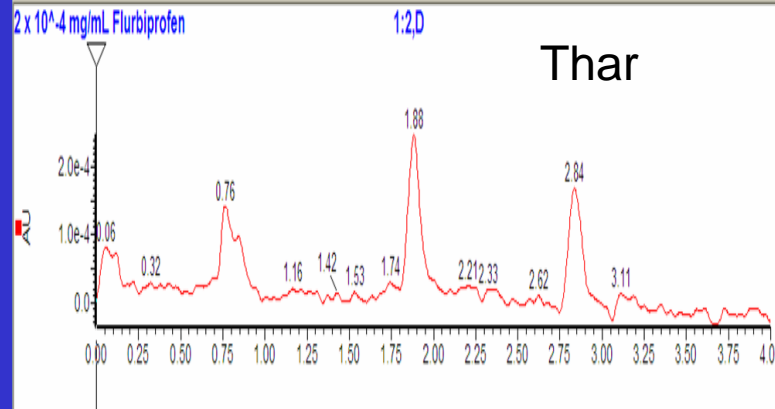
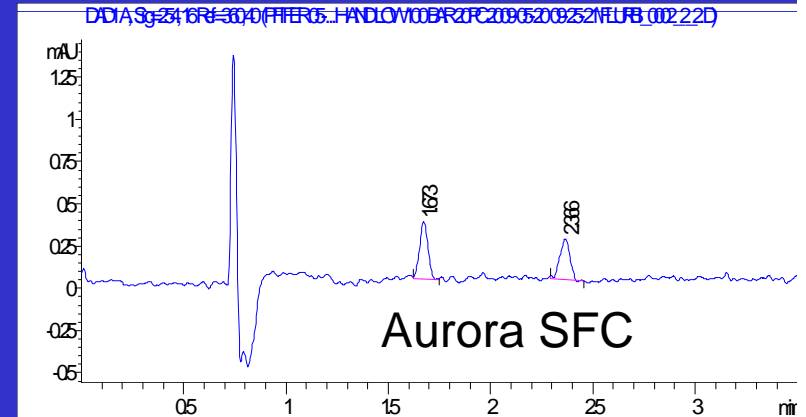
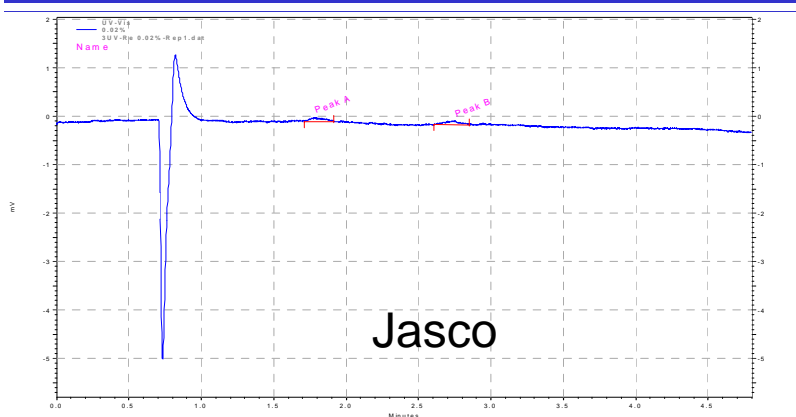


# Limit of Detection

0.02% of Nominal

	Aurora		Jasco		Thar	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
S/N	8.7	6.5	4.1	3.9	17.6	12.8

Signal-to-Noise assessed as peak to peak noise





# Specificity and Range

<b>Parameter</b>	<b>Regulatory Criteria</b>	<b>Internal Criteria</b>
Specificity	For critical separations, specificity can be demonstrated by the resolution of the two components which elute closest to each other	$R_s \geq 1.5$ from main band $R_s \geq 1.0$ for separation of specified impurities from each other
Range	Range is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision	This parameter is set by the linearity, accuracy & precision data



# Accuracy

## Regulatory Criteria

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range.

Accuracy should be reported as a percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence interval.

## Internal Criteria

3 replicates at 3 levels  
70%, 100%, & 130% of the nominal sample concentrations  
Individual Recoveries: 97.0 – 103.0%

3 replicates at 3 levels (Reporting level, specification, & 2X specification)

Level (%)	Mean recovery at each level
$\leq 0.15$	100 ± 15%
$> 0.15$	100 ± 10%



# Additional Tests

- Intermediate Precision
  - Not a requirement for US filing
- Other tests we consider during validation
  - Filter Suitability
    - Drug Product Only
  - Repeatability
    - Taken from accuracy data
  - Solution Stability



# Conclusions

- Aurora SFC and Thar exceed the criteria for LOD and can reliably detect lower limits. Jasco met the LOD criteria, but fall slightly below the accepted LOQ S/N
  - Jasco is introducing a new PDA this fall – MD2018
- Detection limits and system reproducibility that once impeded SFC from entering the mainstream are achievable
- Detection has increased dramatically in the last year and it continues to be the point of emphasis in product development



# Where Do We Go From Here?

- We have the capability to validate analytical methods on a SFC
- Instrument Qualification and Operational Verification protocols are required to move SFC into manufacturing environments (GMP)
- The regulatory requirements for an instrument qualification are vague – no specific criteria
  - “The calibration of instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event accuracy and/or precision limits are not met. Instruments, apparatus, gauges, and recording devices not meeting established specifications shall not be used.”  
-21CFR211.160(b)(4)

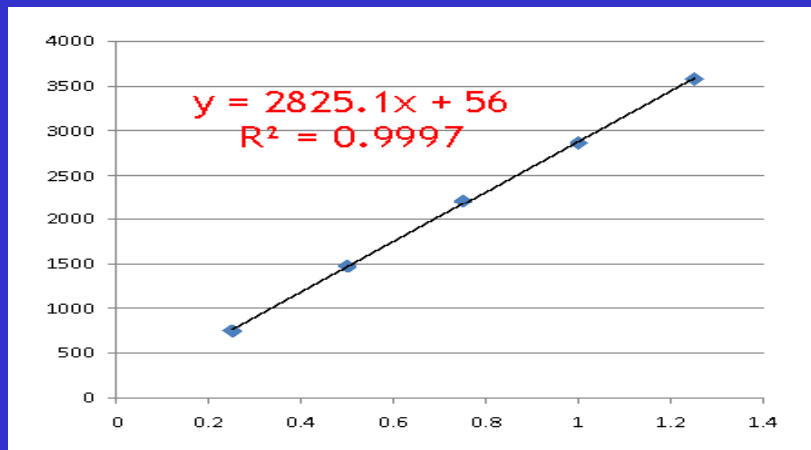
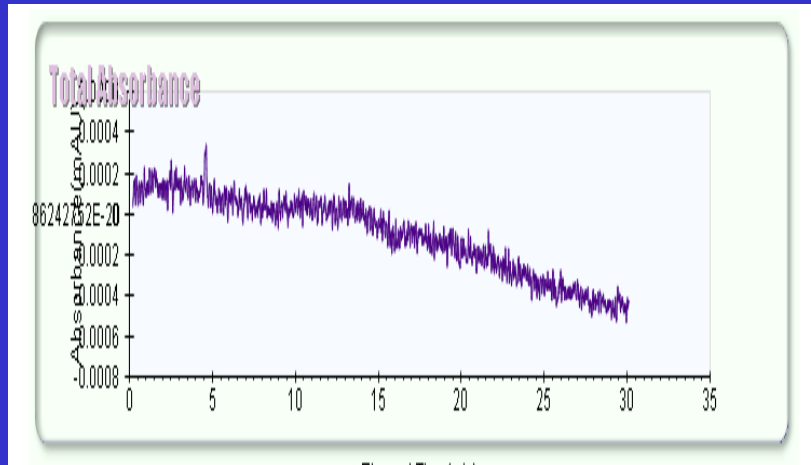


# Instrument Qualification

- Similar verification parameters to HPLC
  - Detector
    - Noise and Drift, Linearity, Wavelength Accuracy
  - Modifier Pump
    - Linear Gradient, Step Gradient, Accuracy and Precision
  - Injector
    - Precision, Linearity, Carryover
  - Column Oven
    - Accuracy



# Detector Verification

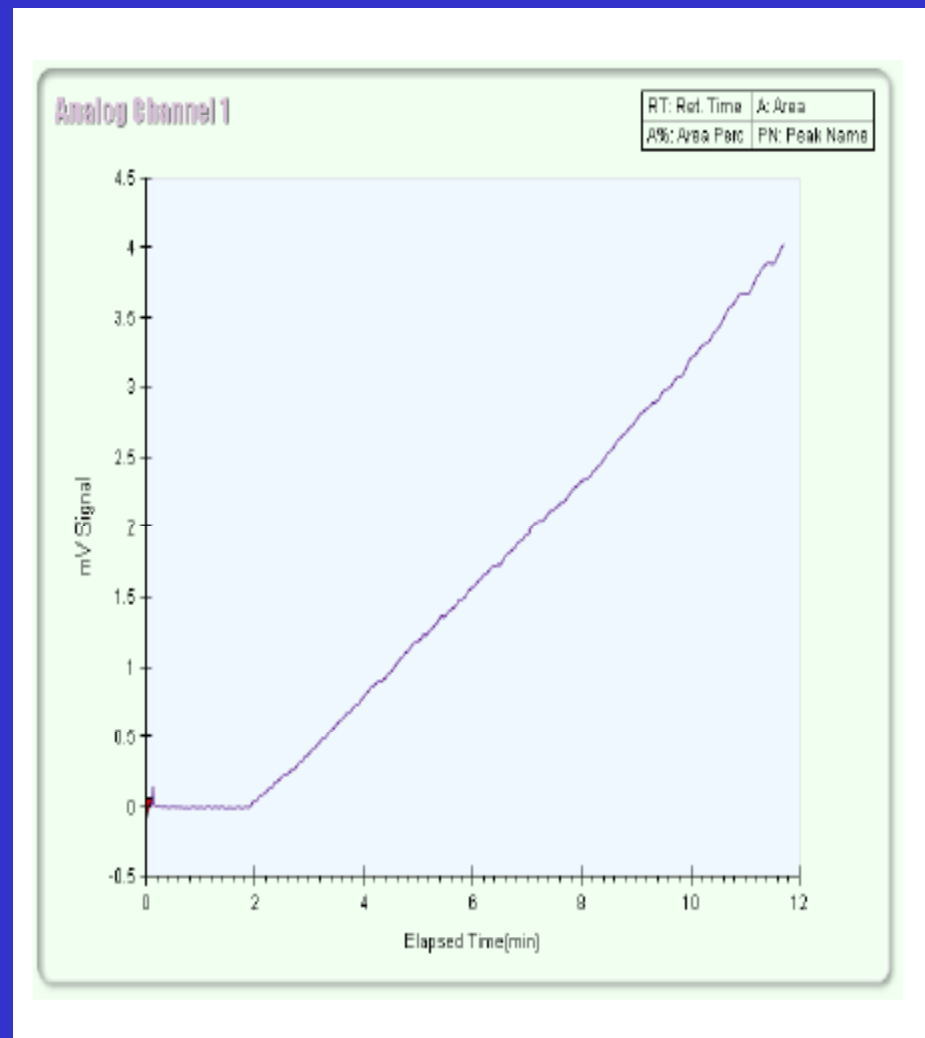


Data complements of Thar

- Determine noise and drift – Two different wavelengths
- Determine linearity using dilutions of a standard
- Check wavelength accuracy against a standard i.e. Holmium oxide
- Follow similar protocol for PDA or VWD

# ○ Modifier Pump – Linear Gradient

- Set up a linear gradient to pump 0.1% acetone in methanol solution
- Evaluate at two flow rates i.e. 2 and 5 mL/min

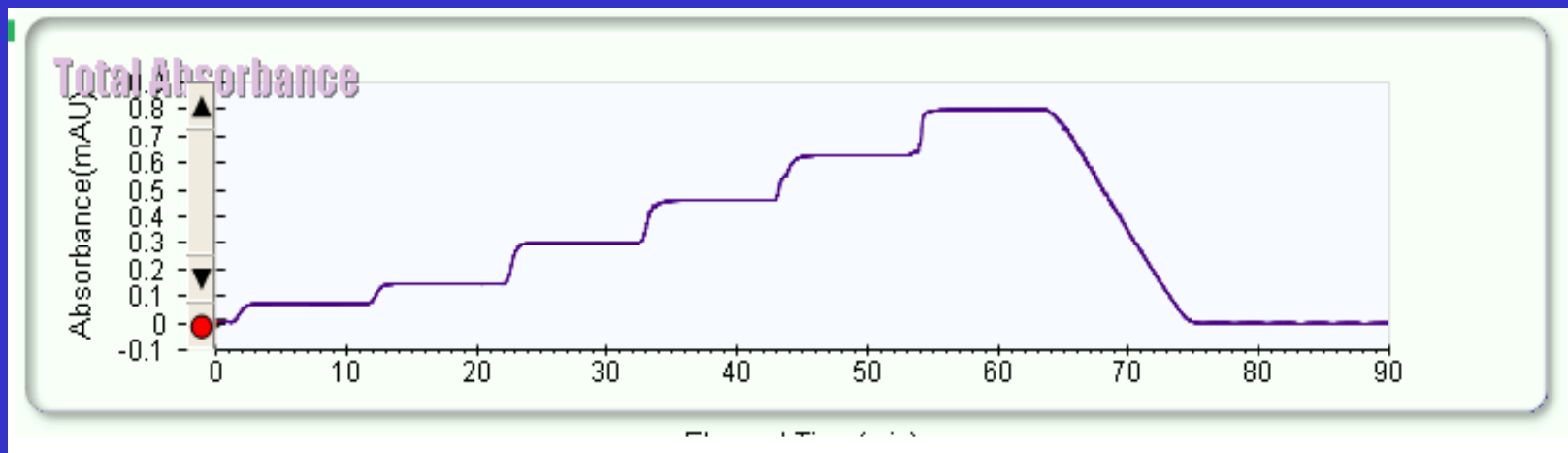


Data complements of Thar



# Modifier Pump – Step Gradient

- Set up a step gradient to pump 0.1% acetone in methanol solution
- Evaluate at two flow rates i.e. 2 and 5 mL/min



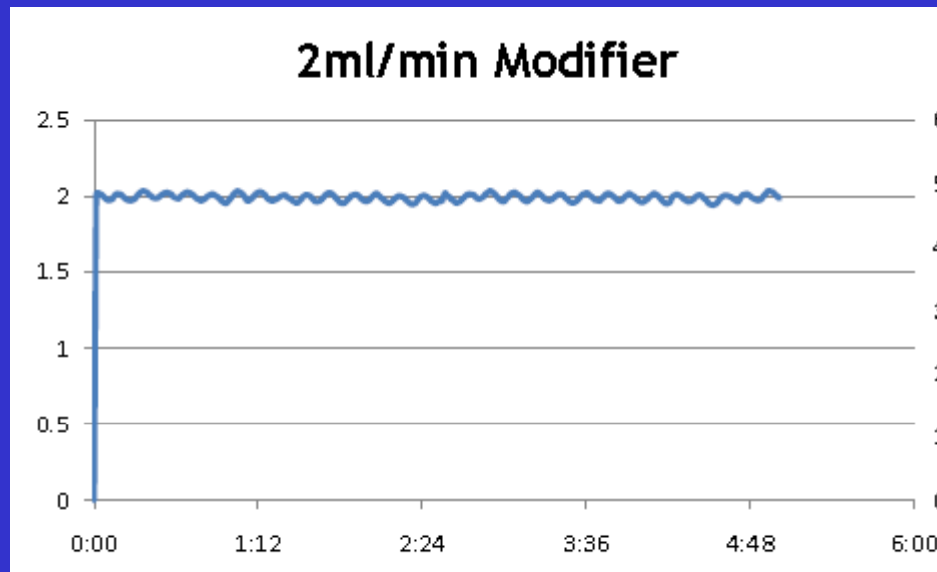
Data complements of Thar



# Modifier Pump

## Accuracy and Precision

- Plumb a calibrated flow meter inline with the modifier flow
- Measure the flow rate of the modifier flow at two different flow rates. Take five separate readings at each flow



**2 ml/min measurement**

Measurement	Mass (g)	Volume (ml)	Flow rate (ml/min)
1	7941.8	10.03006	2.006012
2	7894.3	9.970068	1.994014
3	7893.1	9.968553	1.993711
4	7872.74	9.942839	1.988568
5	7876.2	9.947209	1.989442
<b>Average</b>			<b>1.994349</b>
<b>RSD%</b>			<b>0.3492</b>



# Injector Precision

- Precision of injector for a full and partial loop
  - Using a 1mg/mL caffeine standard

Calculated RSD% for partial loop 5 $\mu$ L injection	
Log #	Peak Area
1	0.4155
2	0.4149
3	0.4152
4	0.4172
5	0.4191
6	0.4146
Standard Deviation	0.0017
Average	0.4161
RSD%	0.4

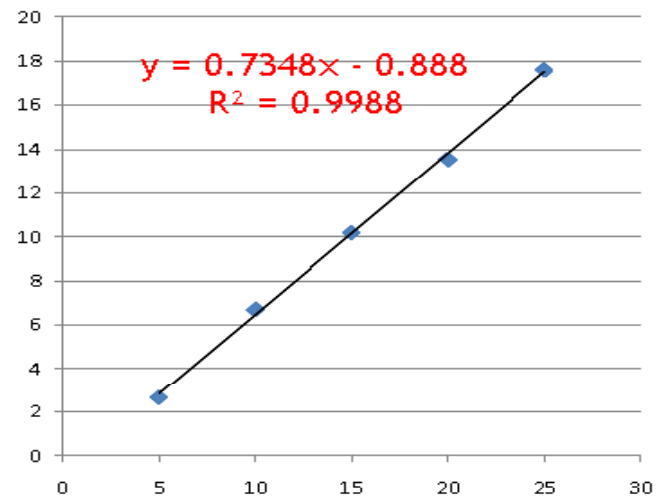
Calculated RSD% for full loop 10 $\mu$ L injection	
Log #	Peak Area
1	0.7084
2	0.7115
3	0.7050
4	0.7060
5	0.7078
6	0.7065
Standard Deviation	0.0023
Average	0.7075
RSD%	0.3

Data complements of Thar



# Injector Linearity

- Inject solution using varying injection volumes
- Caffeine, 1mg/mL



Injection Linearity	
Injection Volume (uL)	Peak Area
5	2.64
10	6.7
15	10.2
20	13.54
25	17.59

Data complements of Thar



# Injector Carryover

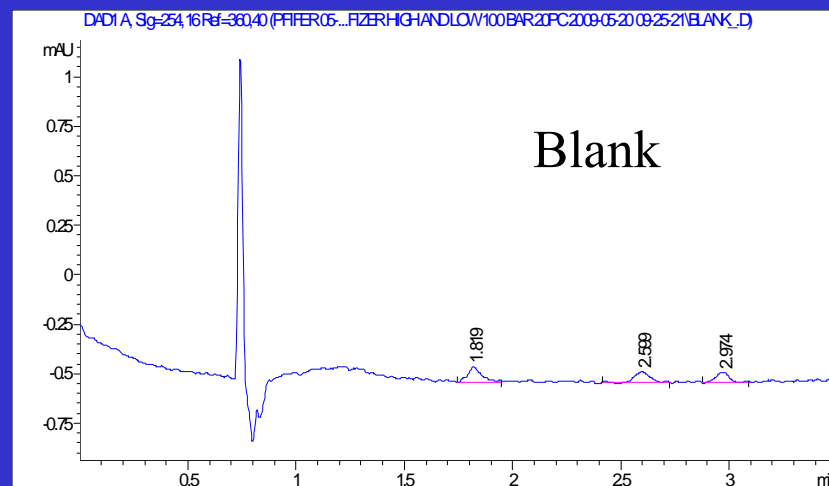
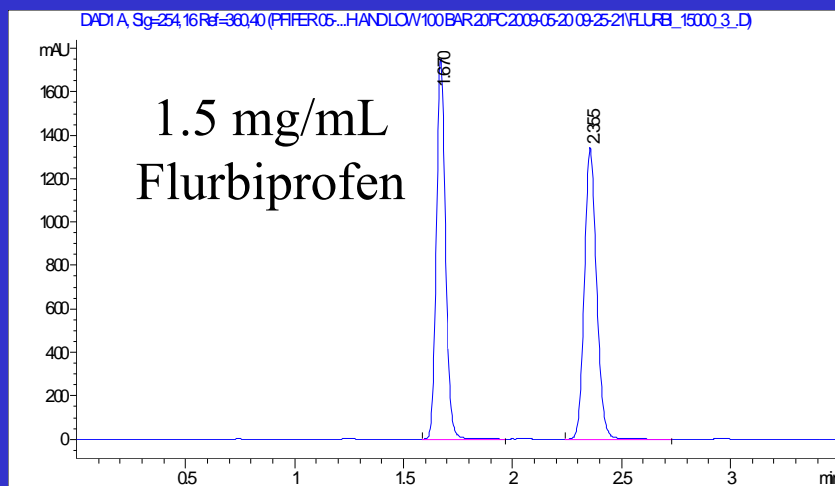
- Carryover determined from blank after sample injection
  - Peak A: 0.007% Carryover
  - Peak B: 0.005% Carryover

Peak 1 Area: 5194

Peak 2 Area: 5283

Peak 1 Area: 0.35

Peak 2 Area: 0.25



Data complements of Aurora SFC



# Column Oven

- Evaluate the temperature in the column compartment at two positions. Conduct the experiment at two different temperatures

Column Temperature 30°C

Reading	Position 1	Position 2
1	30.9	30.6
2	30.9	30.6
3	30.9	30.6
4	30.9	30.6
5	30.9	30.6
6	30.9	30.6
Average	30.9	30.6
Std Dev	0	0
% RSD	0	0

Column Temperature 50°C

Reading	Position 1	Position 2
1	50.6	49.7
2	50.6	49.7
3	50.6	49.7
4	50.6	49.7
5	50.6	49.7
6	50.6	49.7
Average	50.6	49.7
Std Dev	0	0
% RSD	0	0



# Special Considerations in SFC

- Parameters that require special consideration with SFC
  - CO<sub>2</sub> Delivery Pump
    - How do you measure CO<sub>2</sub> flow to ensure accurate delivery? Mass flow meter?
  - Back Pressure Regulator
    - Ensure that an accurate pressure is being maintained by the BPR
- All 3 instruments under consideration deliver CO<sub>2</sub> differently and use different BPR devices...



# Conclusion

- Instrument verification is the last obstacle for SFC to hurdle to be accepted in a GMP regulated environment
- SFC is the next building block in our “method development toolbox” at Pfizer
- SFC is perfect for chiral and preparative applications. We are currently pushing the technology for achiral and polytide applications.



# Acknowledgements

- Pfizer
  - Frank Riley
- Thar
  - Harbaksh Sidhu
  - Rui Chen
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  - Rick Wikfors
  - Dave Boulton
- Jasco
  - John Pollard
  - Jeff D'Agostino



Thank you!

And

Questions?