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# **Evaluation and Qualification of Agilent HPLC with Aurora Fusion A5 Module as a GMP SFC System**

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# Overview

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- Analytical SFC in pharmaceutical industry
- Qualification strategy and process overview
  - USP general chapter <1058>
  - Amgen approaches
- Instrument readiness and quality assessments for GMP purposes
  - Hardware and software considerations
  - Evaluation methodology and pre-qualification testing
  - Maintaining GMP status
- Setting test parameters and acceptance criteria in hardware qualification protocol
  - Modular tests and holistic tests
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- Overcoming technical difficulties in qualification tests
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  - Gradient accuracy and precision
  - Noise and signal-to-noise ratio
  - Wavelength accuracy of UV detector
- Hardware OQ results summary
- Conclusions

# Analytical SFC in pharmaceutical industry

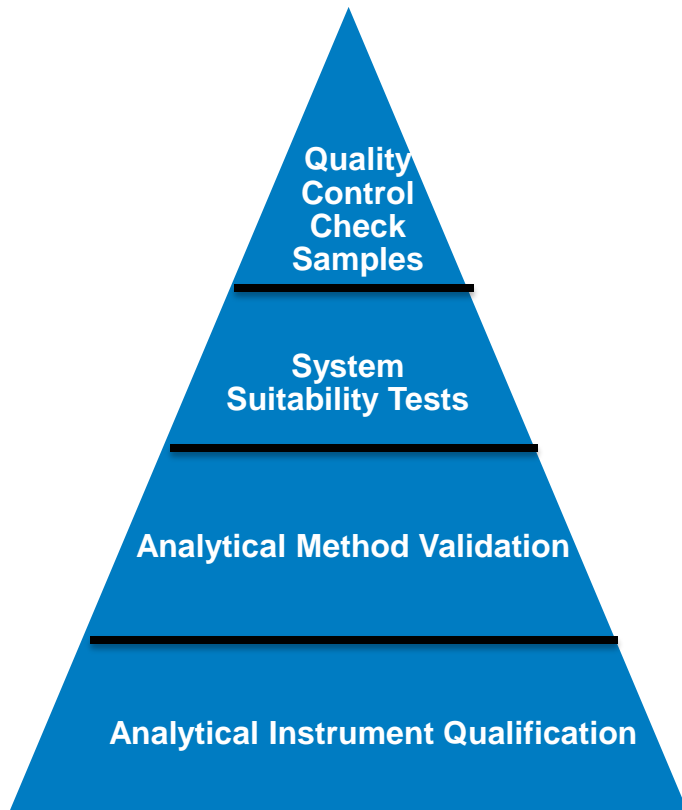
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- Fast chromatography and green chemistry have been the most compelling trends in today's pharmaceutical industry
- SFC can meet both the 'green' and 'fast' expectations
- SFC has superior performances in chiral separation and more chiral compounds are added to pharmaceutical product pipelines
- Analytical SFC has limited applications in the highly regulated pharmaceutical industry before it could be qualified and meet GMP requirements
  - Used primarily in the high throughput screenings of drug candidates at discovery stage
  - Cannot be used in regulated environments for product release or stability testing
  - Lack of qualified instrument discourages SFC method development and method transfer
    - Reversed phase, normal phase, or polar organic phase chromatography methods have to be developed from sketch
    - SFC method could not be transferred even though the contract manufacturer or the test laboratory has the SFC instrument

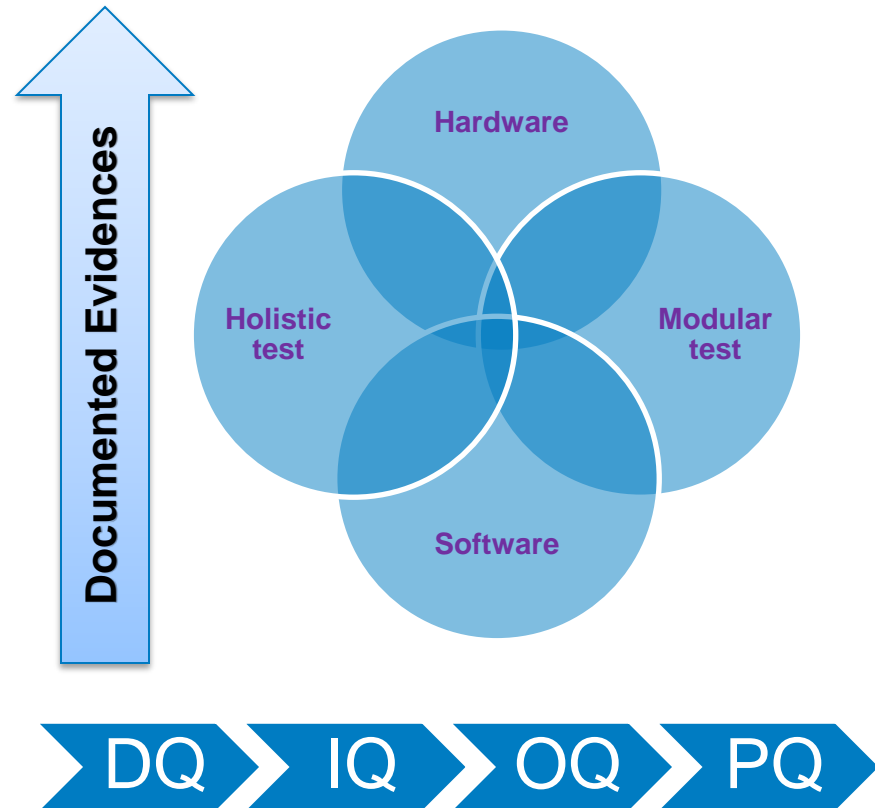
# Qualification strategy and process overview

- USP general chapter <1058> analytical instrument qualification

## COMPONENTS OF DATA QUALITY

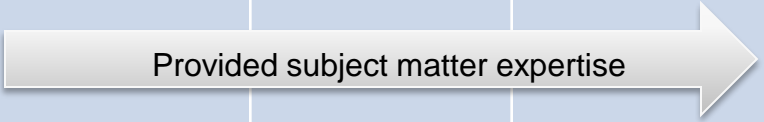


## QUALIFICATION PHASES



# Qualification strategy and process overview

- Amgen approaches
  - Form a SFC qualification team consists of subject matter experts, technical operations, and quality assurance
  - Collaborate with Agilent in preparing documents and executing qualification

	Pre-purchase	DQ	IQ	OQ	PQ
Vendor (Aurora)	Demo	<ul style="list-style-type: none"> <li>•DQ of Fusion A5</li> <li>•Provide doc</li> </ul>			
User (Amgen)	Evaluation	Doc review	<ul style="list-style-type: none"> <li>•Site prep</li> <li>•Review and approve doc</li> </ul>	<ul style="list-style-type: none"> <li>•Review and approve doc</li> </ul>	<ul style="list-style-type: none"> <li>•Schedule</li> <li>•Performance check</li> </ul>
Executor (Agilent)	Prepare HPLC	Provide HPLC DQ doc	<ul style="list-style-type: none"> <li>•Doc prep</li> <li>•Installation</li> <li>•PM and IQ</li> </ul>	<ul style="list-style-type: none"> <li>•Doc prep</li> <li>•OQ</li> </ul>	<ul style="list-style-type: none"> <li>•Doc prep</li> <li>•PM and PQ</li> </ul>
Documents	Evaluation reports	<ul style="list-style-type: none"> <li>•Warranty</li> <li>•Certificate</li> <li>•User manual</li> </ul>	<ul style="list-style-type: none"> <li>•Site prep guidelines</li> <li>•PM and IQ checklists</li> <li>•IQ reports</li> </ul>	<ul style="list-style-type: none"> <li>•OQ protocols: One HW Separate SW</li> <li>•OQ reports</li> </ul>	<ul style="list-style-type: none"> <li>•PM/PQ checklist</li> <li>•PQ internal guidelines</li> <li>•PQ reports</li> </ul>

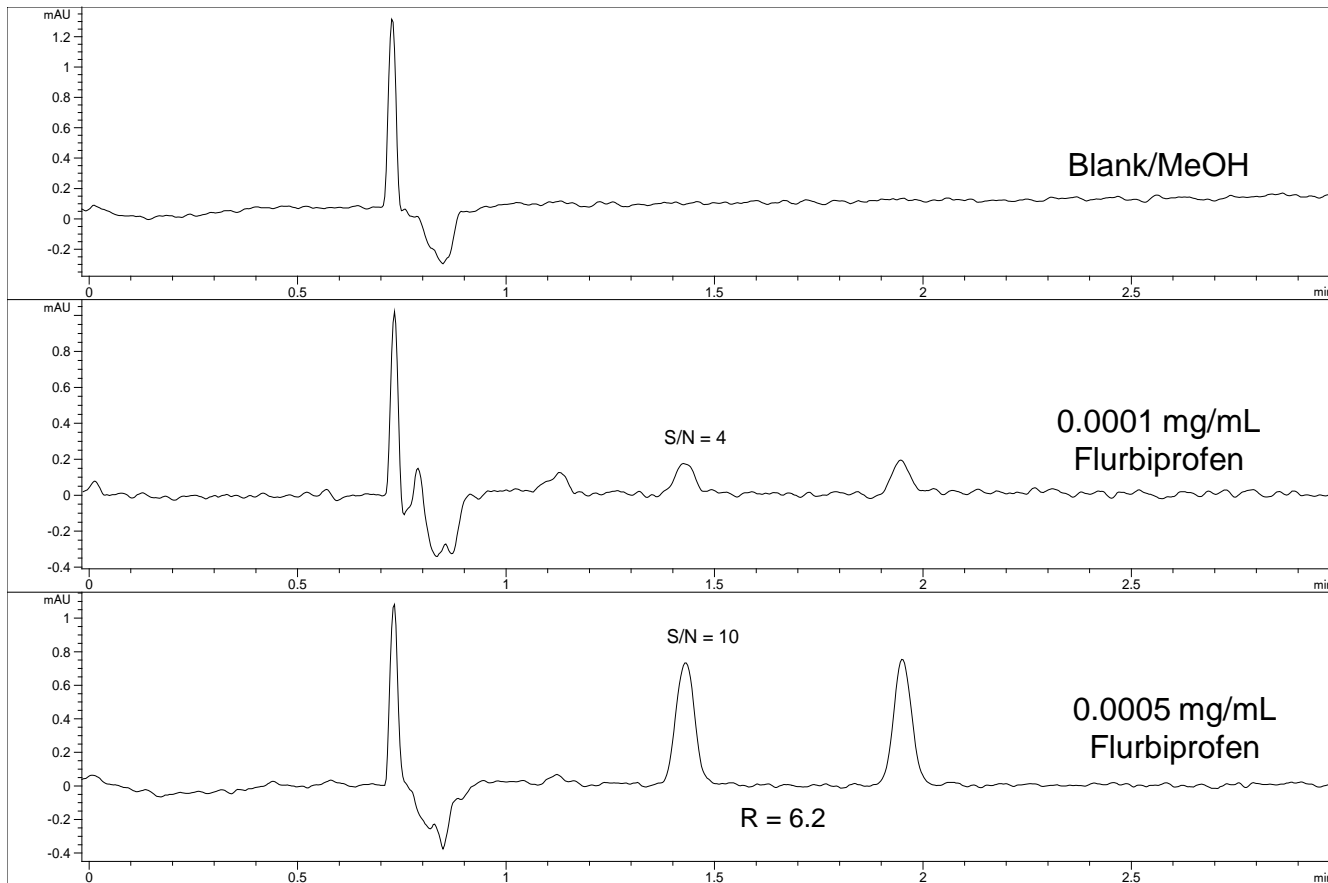
# Instrument readiness and quality assessments for GMP purposes

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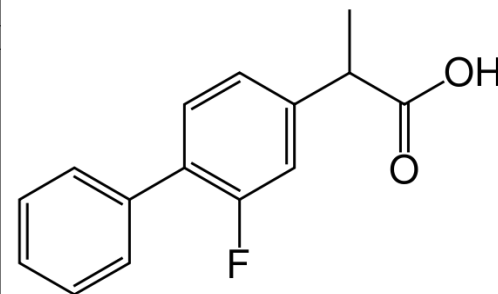
- Evaluate SFC for GMP purposes
  - Identify the 'qualifiable' SFC instruments
    - Hardware – components integration and control
    - Software – data processing and reporting
  - Assess the 'soft elements'
    - Vendor services/supports – consulting, repair and maintenance
    - Instrument lifecycle – durability and obsolescence
    - Operation costs
    - Easy of use – learning curve
    - Easy of end user maintenance
    - Training
    - Change control – maintaining GMP status
  - Demo evaluation
    - Evaluation plan/protocol
      - Linearity: high levels (50% -150%) and low levels (0.05% - 5%)
      - Injection precision: 6 injections of high level (100%) and of low level (0.05%)
      - Retention time precision by gradient elution: 10 injections
      - LOQ: S/N  $\geq 10$
      - LOD: S/N  $\geq 3$
      - System suitability: resolution, peak asymmetry, and carryover
      - Orthogonal screening by different modes of chromatography
  - Recommend for purchase
    - Evaluation report
    - Vendor quotes
    - Potential applications and drivers – purchase justification

# Instrument readiness and quality assessments for GMP purposes

- Assess detection limits



By excising instrument control, data acquisition, processing and reporting, and system suitability verification, this analysis provided valuable data regarding the readiness of a specific SFC system for GMP qualification.

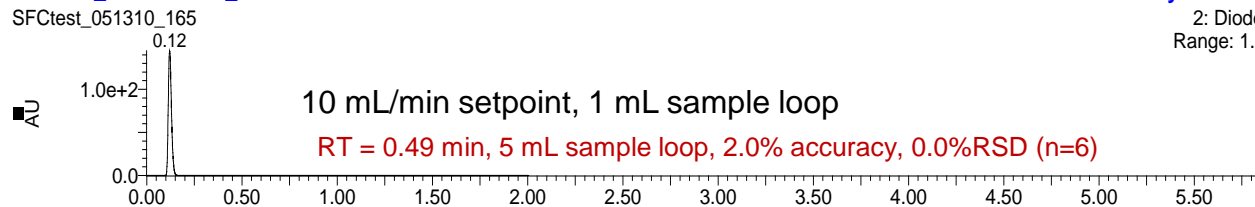


AD-H column, 250 x 4.6 mm, 4 mL/min, 200 bar, CO<sub>2</sub>:MeOH 80:20, 40 °C, 254 nm, 10 µL

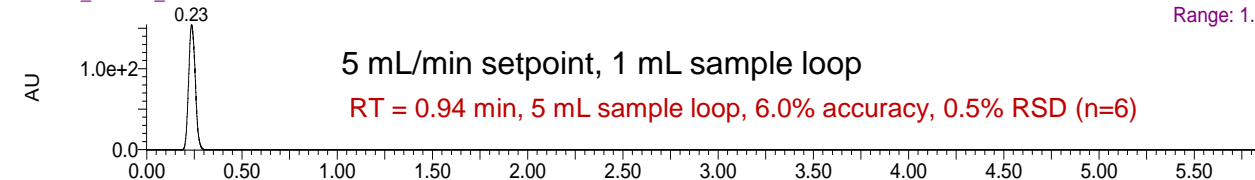
# Instrument readiness and quality assessments for GMP purposes

- Verify CO<sub>2</sub> flow rate (= sample loop volume / acetone RT)

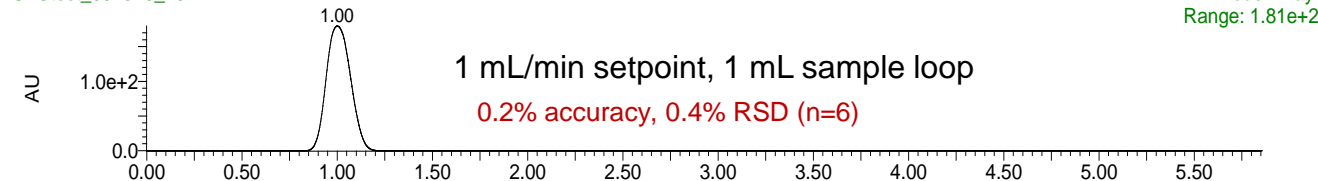
Acetone\_10.0mL/min\_200bar



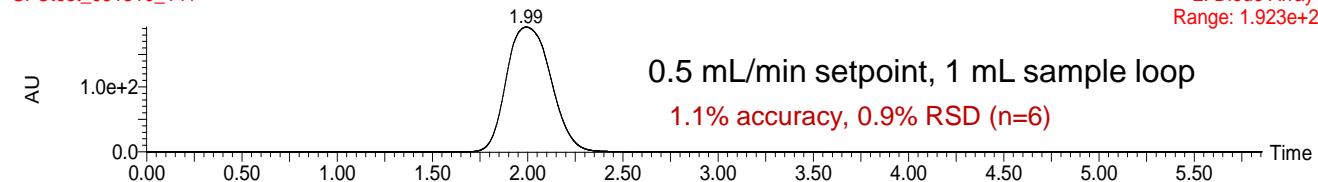
SFCtest\_051310\_158



SFCtest\_051310\_151



SFCtest\_051310\_141



Injection: 1 µL of acetone; Detection: 215 nm – 350 nm; Backpressure: 200 bar

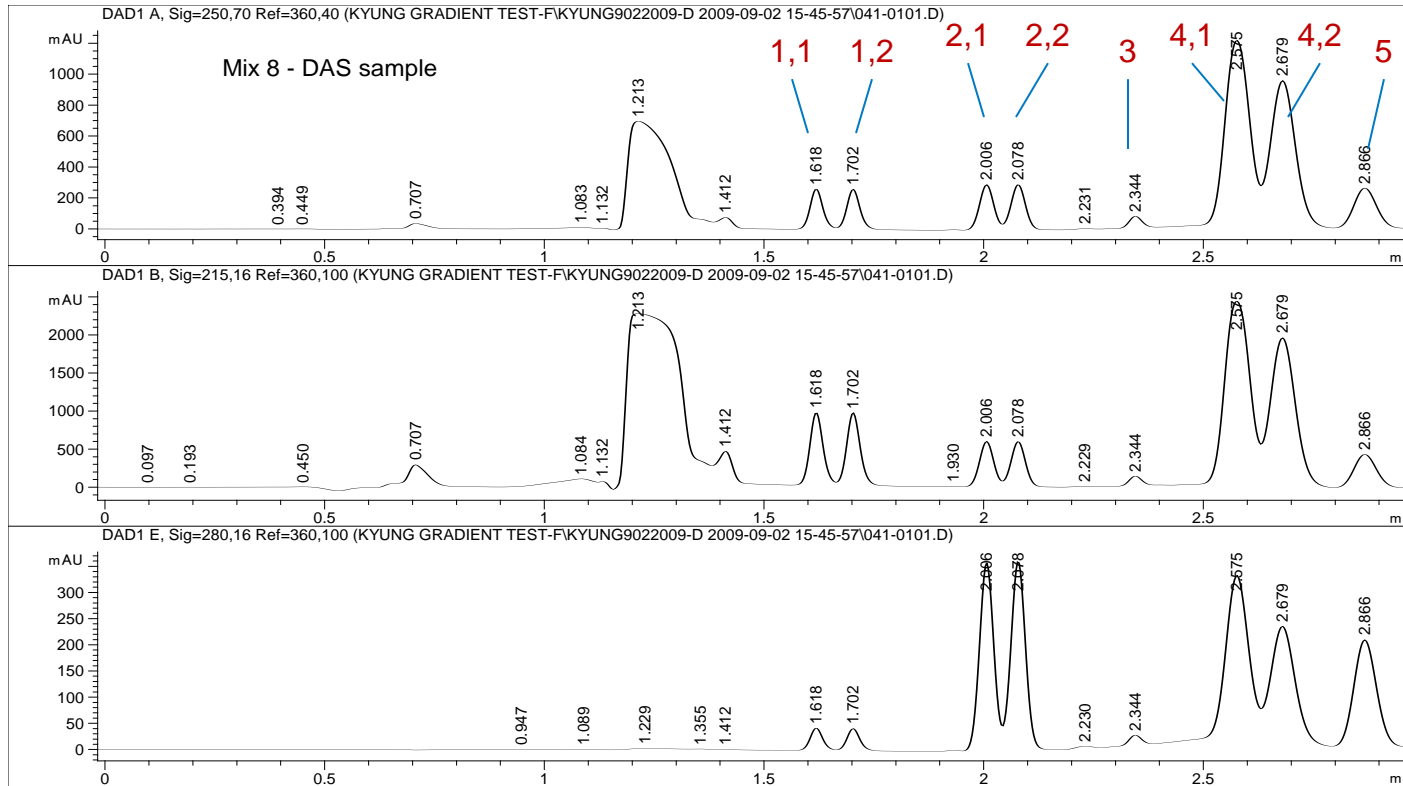
- Cost of certified high pressure flow meter: > \$10K

- Using appropriate sample loop – 1 mL for 0.5 and 1 mL/min; 5 mL for 5 and 10 mL/min

- Depending on autosampler configuration and flow path, the flow rate determined by this procedure can deviate from the low flow rate setpoints

# Instrument readiness and quality assessments for GMP purposes

- Retention time reproducibility (courtesy of Kyung Gahm)



Peak Name	RT (min)	%RSD (n=10)
1,1	1.63	0.5
1,2	1.72	0.5
2,1	2.01	0.3
2,2	2.08	0.3
3	2.35	0.4
4,1	2.58	0.3
4,2	2.69	0.2
5	2.86	0.2

AD-H 150 x 4.6, 4 mL/min, 0.2% DEA in MeOH

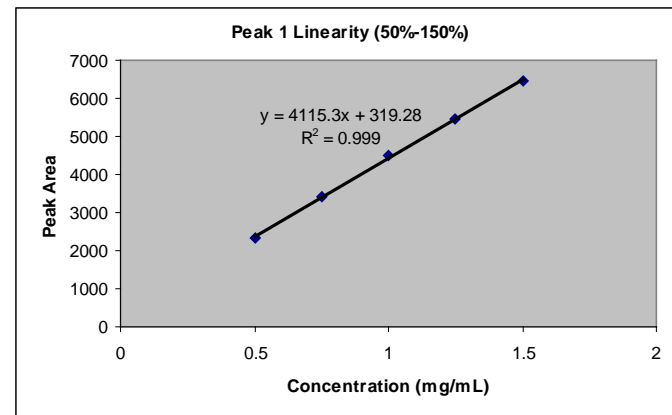
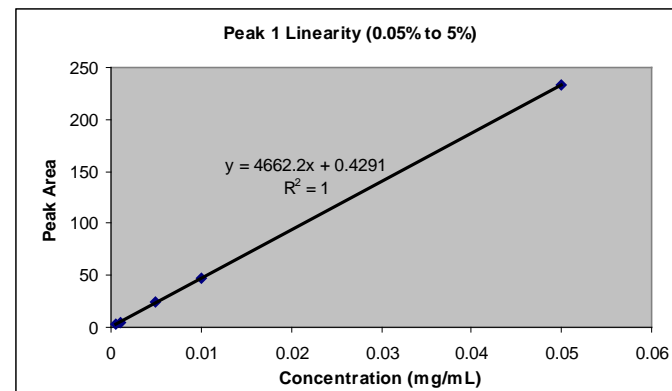
Gradient: hold at 5%B for 0.1 min, increase to 60%B in 1.9 min and hold at 60%B for 1 min

# Instrument readiness and quality assessments for GMP purposes

- Summary of evaluation results

Test Parameters	Acceptance Criteria	Results
Linearity	High levels: 50%, 75%, 100% (1mg/mL), 125%, 150% - $R^2 \geq 0.999$ Low levels: 0.05%, 0.1%, 0.5%, 1%, 5% - $R^2 \geq 0.99$	$R^2 = 0.9990$ (peak 1) $R^2 = 0.9991$ (peak 2) $R^2 = 1.0000$ (peak 1) $R^2 = 1.0000$ (peak 2)
Precision	100% level: %RSD $\leq 2.0\%$ - peak areas of 6 injections 0.05% level: %RSD $\leq 10.0\%$ - peak areas of 6 injections All levels: %RSD $\leq 2.0\%$ - retention times of 6 injections	%RSD = 0.9 (peak 1) %RSD = 0.8 (peak 2) %RSD = 2.2 (peak 1) %RSD = 4.0 (peak 2) 100% level: %RSD = 0.1 (peak 1) %RSD = 0.1 (peak 2) 0.05% level: %RSD = 0.2 (peak 1) %RSD = 0.1 (peak 2)
LOQ	0.05% level, S/N $\geq 10$	S/N = 10 (peak 1) S/N = 10 (peak 2)
LOD	$\leq 0.02\%$ level, S/N $\geq 3$	0.01% level: S/N = 4 (peak 1) S/N = 4 (peak 2)
System suitability	Resolution: $\geq 2.0$ Asymmetry: $0.7 \leq \text{Asym} \leq 2.0$ Carryover: $\leq 0.01\%$ after the 100% level injection	100% level: Resolution = 6.2 USP tailing = 1.0 (peak 1) 1.1 (peak 2) Carryover = 0.04% (peak 1)* 0.04% (peak 2)*

Injection volume = 10  $\mu\text{L}$



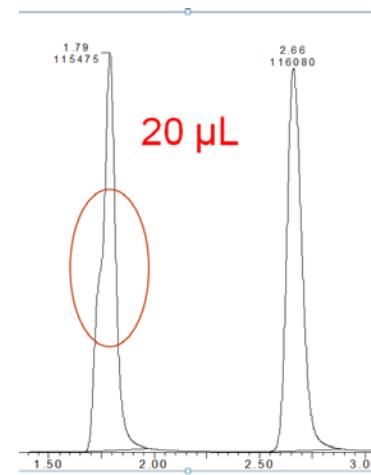
Note: \*In first blank after the 100% flurbiprofen injection; 0.007% (peak 1) and 0.004% (peak 2) of flurbiprofen were detected in the second blank injection; no flurbiprofen was detected in the third blank injection.

# Setting test parameters and acceptance criteria in hardware qualification protocol

- Critical parameters and limits

Test Name	Setpoints and Limits in Initial Protocol	Setpoints and Limits in Final Protocol
CO <sub>2</sub> Pump Flow Accuracy and Precision	Flow rate 1 = 0.5 mL/min @ 200 bar Flow rate 2 = 5.0 mL/min @ 200 bar Flow rate 3 = 10.0 mL/min (if applicable) Accuracy = +/- 5.0% from setpoint Precision <= 0.5 % RSD	Flow rate 1 = Pump upper limit @ 100 bar Flow rate 2 = 0.5 mL/min @ 100 bar Accuracy = +/- 10% from setpoint 1 Accuracy = +/- 20% from setpoint 2 Precision <= 5.0 % RSD
Modifier Pump Flow Accuracy and Precision	Flow rate 1 = 5.0 mL/min Flow rate 2 = 0.1 mL/min Accuracy = +/- 5 % from setpoint Precision <= 0.5 % RSD from setpoint	Flow rate 1 = 5.0 mL/min Flow rate 2 = 0.2 mL/min Accuracy = +/- 5% from setpoint Precision <= 0.5% RSD @ > 1 mL/min Precision <= 1.0% RSD @ <= 1 mL/min
CO <sub>2</sub> Pump Pressure Regulation	Pressure 1 = 90 bar @ 2 mL/min Pressure 2 = 300 bar @ 2 mL/min Accuracy = +/- 5.0% from setpoint	Pressure 1 = 90 bar @ 2 mL/min Pressure 2 = 300 bar @ 2 mL/min Accuracy = +/- 5.0% from setpoint
Response Linearity (UV/Vis)	10 µL injection of flurbiprofen at levels of 0.001, 0.05, 0.5, 1.0, 1.5 mg/mL R <sup>2</sup> >=0.999 R/F precision <=5.0% RSD	5 µL injection of flurbiprofen at levels of 0.005, 0.05, 0.5, 1.0, 1.5 mg/mL R <sup>2</sup> >=0.99 R/F precision <=20.0% RSD
UV/Vis Detector Signal to Noise (S/N)	S/N = Signal height/ASTM Noise of 10 µL injection of 0.001 mg/mL flurbiprofen S/N >= 10	S/N = Signal height/ASTM Noise of 5 µL injection of 0.005 mg/mL flurbiprofen S/N >= 10
Auto sampler Injection Precision (UV-Vis)	1 µL and 20 µL injections of 0.5 mg/mL flurbiprofen Area and Height RSD @ 1 µL <= 5.0% Area and Height RSD @ 20 µL <= 2.0%	1 µL and 10 µL injections of 0.5 mg/mL flurbiprofen Area and Height RSD @ 1 µL <= 5.0% Area and Height RSD @ 10 µL <= 2.0%
Auto sampler Injection Carryover (UV-Vis)	20 µL injection of 1.0 mg/mL flurbiprofen Area carryover <=0.1% Height carryover <=0.4%	10 µL injection of 1.0 mg/mL flurbiprofen Area carryover <=0.1% Height carryover <=0.1%

- Modular tests: based on specs
- Holistic tests: driven by practical needs
- Two mockup qualifications prior to the major revisions
- Separate limit for each setpoint
- One detection limit is set for all SFC instruments
- 5µL/10µL is used in final protocol



Note: N = 6 in all precision tests.

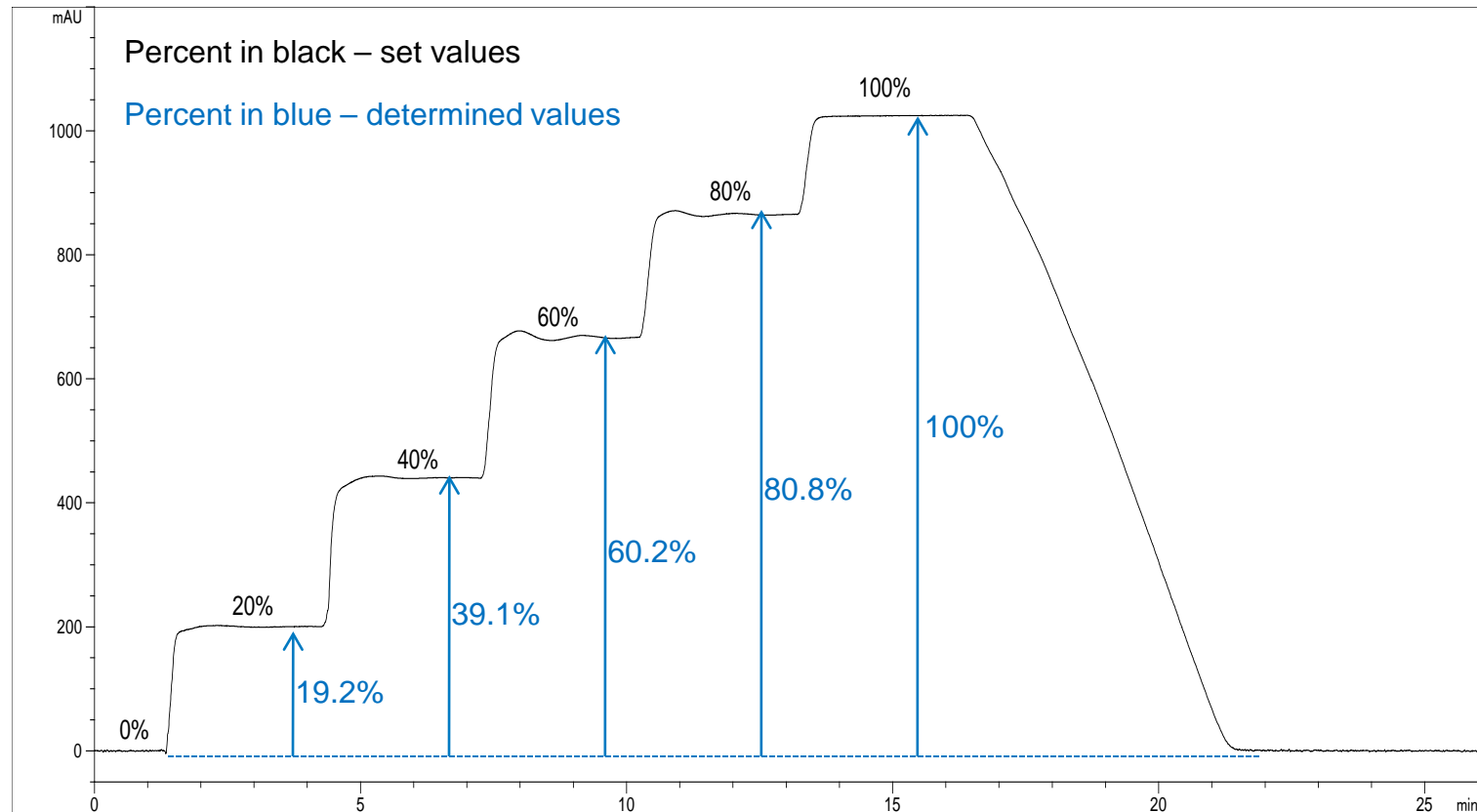
# Overcoming technical difficulties in qualification tests

- Flow rate accuracy and precision of supercritical CO<sub>2</sub> fluid by custom-made Coriolis flow meter (up to 300 bar)

	Flow rate 1: 5mL/min	Flow rate 2: 0.5 mL/min
Reading 1	5.18	0.51
Reading 2	5.22	0.49
Reading 3	5.19	0.49
Reading 4	5.16	0.50
Reading 5	5.20	0.51
Reading 6	5.19	0.51
Average	5.19	0.50
Accuracy (as %error)	3.8% (<=10.0%)	0.3% (<=20.0%)
Precision (%RSD)	0.4% (<=5.0%)	2.0% (<=5.0%)

# Overcoming technical difficulties in qualification tests

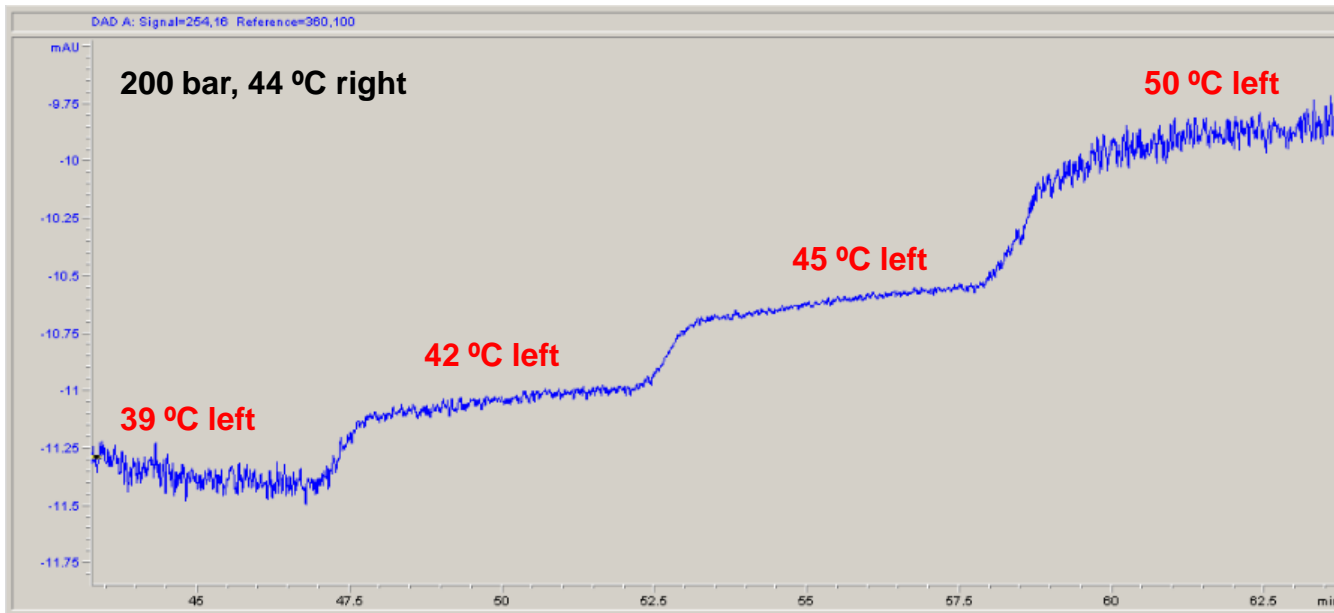
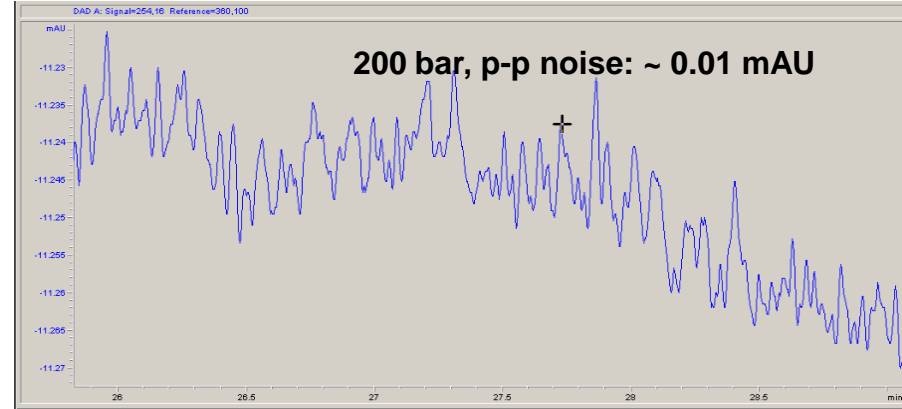
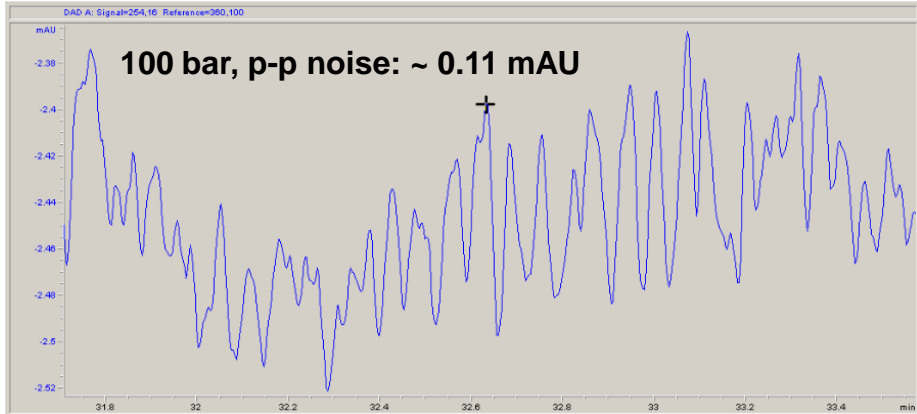
- Gradient accuracy and precision



Zero-volume union, 0.5% acetone in methanol, 4 mL/min, 200 bar, 265 nm, 0  $\mu$ L

# Overcoming technical difficulties in qualification tests

- Noise and S/N ratio



AD-H column, 150 x 4.6 mm

CO<sub>2</sub>:MeOH 80:20

254 nm

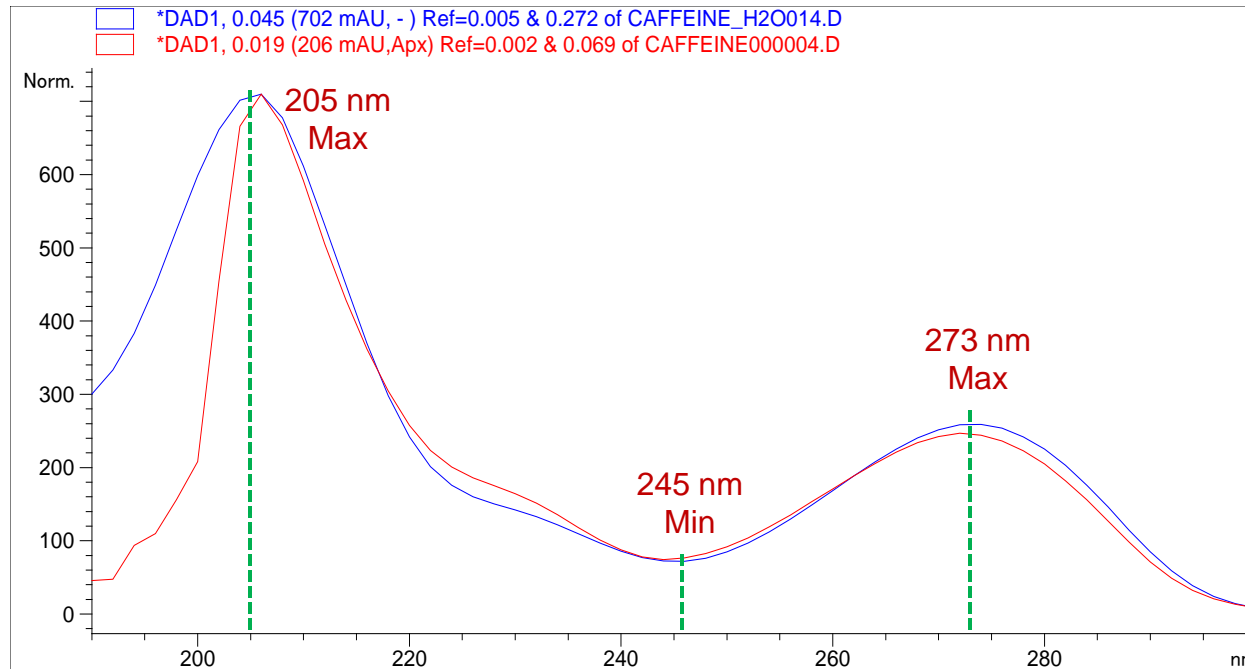
In the thermostatted Agilent column compartment:

- Right (6  $\mu$ L) to column inlet

- Left (3  $\mu$ L) to detector inlet

# Overcoming technical difficulties in qualification tests

- Wavelength accuracy



Wavelength accuracy can be affected by:

- Sample diluent
- Mobile phase compositions

Options:

- 1) Revise protocol – redefine test procedures and acceptance criteria
- 1) Force caffeine aqueous solution into the off-line flow cell (Amgen/Agilent approach)

Blue – UV spectrum of caffeine in water by HPLC, mobile phase: water

Red – UV spectrum of caffeine in methanol by SFC, mobile phase: CO<sub>2</sub>:MeOH 80:20

Caffeine concentration = 27 µg/mL in both experiments

# OQ results summary

Test parameters	Test results (limits)
<b>CO<sub>2</sub> pump flow rate accuracy and precision</b>	
Flow rate 1: 5 mL/min @ 100 bar Flow rate 2: 0.5 mL/min @ 100 bar	Accuracy (%error): 3.8 (<=10.0); Precision (%RSD): 0.4 (<=5.0) Accuracy (%error): 0.3 (<=20.0); Precision (%RSD): 2.0 (<=5.0)
<b>Modifier pump flow rate accuracy and precision</b>	
Flow rate 1: 5 mL/min Flow rate 2: 0.2 mL/min	Accuracy (%error): 0.5 (<=5); Precision (%RSD): 0.01 (<=0.5) Accuracy (%error): 0.7 (<=5); Precision (%RSD): 0.8 (<=1)
<b>CO<sub>2</sub> pump pressure regulation</b>	
Pressure 1: 90 bar @ 2 mL/min Pressure 2: 300 bar @ 2 mL/min	93.71 bar (90 bar +/- 5.0%) 309.86 bar (300 bar +/- 5.0%)
<b>Wavelength accuracy</b>	
Wavelength 1: 205 nm (max) Wavelength 2: 245 nm (min) Wavelength 3: 273 nm (max)	Accuracy (error in nm): 1 (<=2) Accuracy (error in nm): 1 (<=2) Accuracy (error in nm): 0 (<=2)
<b>Column temperature accuracy and stability</b>	
Temperature 1: 60°C Temperature 2: 30°C	Diff. from setpoint (°C) : 0.2 (<=2.0) Diff. from setpoint (°C): 0.1 (<=1.0); Stability (in °C): 0 (<=1.0)
<b>Signal noise and drift</b>	
ASTM baseline noise Slope of regression fit for drift	Noise (ASTM, in mAU): 0.1 (<=0.2) Drift (mAU/Hr): 0.08 (<=5)

# OQ results summary (cont'd)

Test parameters	Test results (limits)
<b>Signal to noise ratio</b>	
Signal height is divided by ASTM baseline noise for 5 µL injection of 0.005 mg/mL flurbiprofen standard	Signal to noise: 17 ( $\geq 10$ )
<b>Autosampler injection precision</b>	
Injection volume 1: 10 µL Injection volume 2: 1 µL Injections made with 0.5 mg/mL flurbiprofen standard	Area %RSD: 0.5 ( $\leq 2.0$ ); Height %RSD: 0.8 ( $\leq 2.0$ ) Area %RSD: 1.2 ( $\leq 5.0$ ); Height %RSD: 1.3 ( $\leq 5.0$ )
<b>Autosampler injection carryover</b>	
Injection volume of 10 µL of the 1.0 mg/mL flurbiprofen standard	Area carryover (%): 0.02 ( $\leq 0.1$ ) Height carryover (%): 0.01 ( $\leq 0.1$ )
<b>System suitability – retention time precision</b>	
Injection volume 1: 10 µL Injection volume 2: 1 µL Injections made with 0.5 mg/mL flurbiprofen standard	Retention time %RSD: 0.02 ( $\leq 2.0$ ) Retention time %RSD: 0.05 ( $\leq 2.0$ )
<b>System suitability – enantiomer resolution</b>	
Injection volume 1: 10 µL Injection volume 2: 1 µL Injections made with 0.5 mg/mL flurbiprofen standard	Resolution: 5.5 ( $\geq 2$ ) Resolution: 6.8 ( $\geq 2$ )

# OQ results summary (cont'd)

Test parameters	Test results (limits)
<b>System suitability – tailing factor</b>	
Injection volume 1: 10 µL Injection volume 2: 1 µL Injections made with 0.5 mg/mL flurbiprofen standard	Tailing: 0.6 (<=2.0) Tailing: 1.4 (<=2.0)
<b>Response linearity</b>	
5 µL injection of flurbiprofen @ 5 concentrations of certified reference standard (0.005, 0.05, 0.5, 1.0, 1.5 mg/mL)	R <sup>2</sup> : 0.99973 (>=0.99) R/F precision (%RSD): 2.4 (<= 20.0)
<b>Gradient composition accuracy, noise and drift</b>	
20% step 40% step 60% step 80% step	0.8% error; 1.9% noise; 1.1% drift (<=2.0% for all) 0.9% error, 1.0% noise and 0.5% drift (<=2.0% for all) 0.2% error, 0.3% noise and 0.05% drift (<=2.0% for all) 0.8% error, 0.5% noise and 0.1% drift (<=2.0% for all)
<b>Gradient linearity</b>	
Linear gradient from 100% to 0%	Range 95 to 75%: R <sup>2</sup> = 0.99978 (>=0.99) Range 75 to 25%: R <sup>2</sup> = 0.99998 (>=0.99) Range 25 to 5%: R <sup>2</sup> = 0.99976 (>=0.99)
<b>Sample temperature accuracy</b>	
Temperature: 4 °C (four vials of water in different tray positions)	Positions 1, 2, 3: 7.6°C; Diff from setpoint: 3.6°C (-1.0 to 5.0°C) Position 4: 7.7°C; Diff from setpoint: 3.7°C (-1.0 to 5.0°C)

# Conclusions

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- The IQ and OQ of both hardware and software were successfully completed by March 2011
- Through the qualification process, evidence were collected demonstrating that Aurora SFC system performs suitably for its intended purpose and meet the GMP requirements
- The critical quality attributes of a SFC system must be closely examined and assessed prior to qualification
- The test devices including high-pressure mass flow meter and pressure meter need to be refined and standardized
- As the SFC instrumentation especially software continues to improve, the test procedures and qualification protocols should be retuned accordingly to meet new expectations
- The success of SFC qualification will further extend its applications across many fronts and establish SFC as one of the essential separation technologies in pharmaceutical industry

# Acknowledgements

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- Amgen:

David Semin, Donna Norton, Joey Ling, Hue Lu, Janet Cheetham, Kyung Gahm, Larry Miller

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