



A Feasibility Study for Implementation of Chiral SFC as the Primary Chiral Purity Tool for API Release and Stability Testing Through All Clinical Phases

*Yun Huang[‡], Fengzhen Wang[#], Duc Vicki. Vuong[‡],
Mark Hardink[#], Karen Alsante[‡],
[‡]Research Analytical, [#]Analytical Development
Pfizer Global Research and Development,
Groton Laboratories*

- SFC overview
 - Advantages over HPLC
 - Pharmaceutical application
 - Feasibility on applying chiral SFC for clinical release and stability testing
 - HPLC and SFC methods comparison
 - Different SFC instruments comparison
 - Berger and Aurora
 - Method transferability in different departments and different SFC systems
-



Why SFC: Advantages of SFC over HPLC

- Faster – 3 to 10 times faster than HPLC
 - Supercritical CO₂ as the mobile phase –
 - Faster diffusion; Lower viscosity
 - higher flow rates, resulting in faster analysis

- Superior for chiral separation
 - Similar to normal phase LC.
 - Complementary to reversed phase HPLC for its orthogonality.
 - Chosen as a primary choice for chiral separation.

- Cost effective and green
 - CO₂ is recycled from air and cheap
 - Less organic mobile phase waste and disposal,
 - Extremely suitable for large scale separation and isolation



SFC in Pharmaceutical Industry

- Historically, SFC most used in drug discovery and early development
 - As the primary preparative separation/purification tool
 - Used in > 95% chiral separations, purification and impurity isolation
 - Not commonly used in API clinical release testing
 - Due to less sensitivity compared to HPLC

- ▣ Currently, Chiral SFC is rapidly replacing HPLC
 - Sensitivity enhanced dramatically during recent years
 - Instrument improvement
 - Noise reduction
 - Gaining more popularity to serve as the fast automated screening and method development tool for chiral compounds.
 - Not only chiral but also achiral are used in analytical support for process development and in-process control of GMP API manufacture.
 - **Potential to be used in clinical release testing.**



Feasibility Study on Chiral SFC for Clinical Release and Stability Testing

Objectives:

- Demonstrates SFC can be the primary method to replace conventional HPLC method for chiral purity analysis in clinical release and stability testing
- Demonstrates chiral SFC can meet the same requirements for HPLC method and thus can be used in API release and stability testing.
- Improves business efficiency by shortening method development time without compromising on method performance.

Approaches:

- Selected two chiral APIs (under development).
- Developed chiral HPLC method (if not available) and alternative SFC methods.
- Compared both methods (HPLC and SFC) performance through validating per current ICH guidance for registration.
- Compared the method comparability among different SFC instruments (Berger and Aurora 1100/1200).



Experiment - Instrumentations

Analytical HPLC Systems:

Agilent 1100 equipped with DAD

Analytical SFC Systems:

1: Berger SFC equipped with Agilent DAD /MSD SL

2: Berger SFC equipped with Agilent DAD

3: Aurora SFC Fusion A5 - Agilent 1100 DAD

4: Aurora SFC Fusion A5 - Agilent 1200 DAD



Compounds information

	PF-00981823	PD-0348292
Current chiral spec	0.5%	0.1%
Target LOQ	0.1%	0.05%
Complexity	Low , only API with its enantiomer PF 03507819	High , enantiomer, diastereoisomers, and other process impurities
HPLC availability	No	Yes



Validation Protocols

Parameter	Acceptance Criteria
Specificity	$R_s \geq 1.5$ from main band; $R_s \geq 1.0$ for separation of specified impurity from each other
Linearity /Range	80%-120% of nominal (5 points): $r \geq 0.9990$, y-intercept $\leq 2.0\%$, RL (reporting level) to 2X specification (5 points), $r \geq 0.9950$, y-intercept $\leq 10.0\%$ of response of maximum (2X specification); Each RRF: 98-102% to nominal
Accuracy	3 replicates at 3 levels (LOQ, Spec and 2X of Spec) -Level($\%$) $\leq 0.15\%$, mean recovery 100 \pm 15%; -Level($\%$) $> 0.15\%$, mean recovery 100 \pm 10%
System precision	6 injections of Nominal: $RSD \leq 1.0\%$ for peak response 6 injections of LOQ, $RSD \leq 10\%$ for peak response
Repeatability	$RSD \leq 10\%$ (use accuracy data)
LOQ	S/N at least 10 (0.05% LOQ), at least 20 (0.1% LOQ)



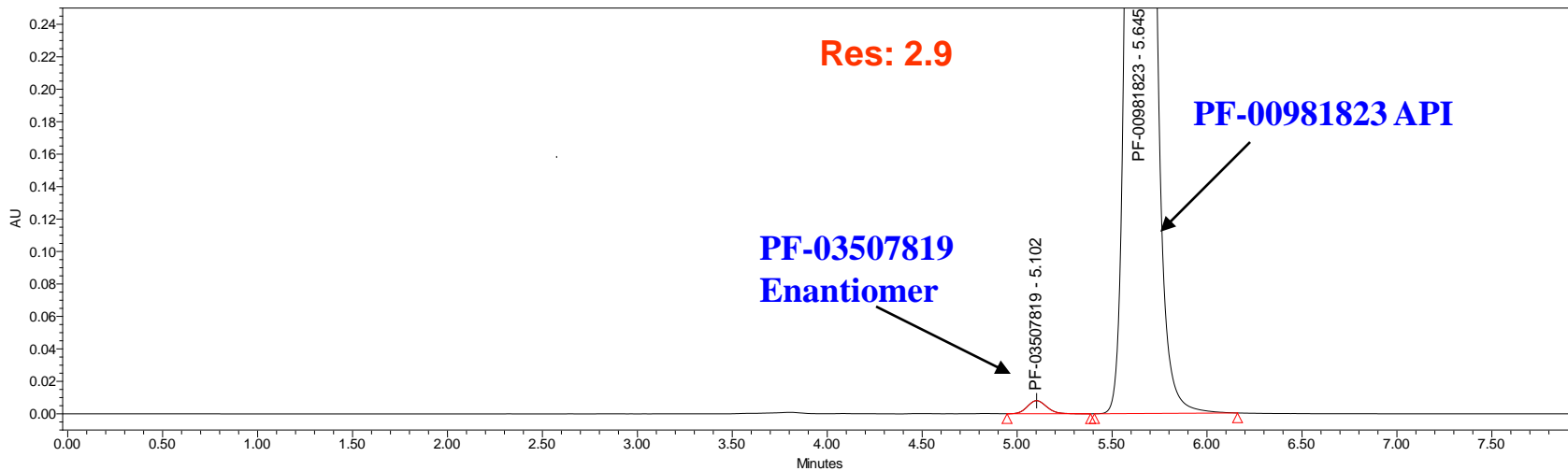
Case 1 Results: PF-00981823

	NP-HPLC (Agilent 1100)	SFC (Berger)
Column	Chiralpak AD-H, 5 μ m, 4.6x250 mm	
Detection	240 nm	
Sample Conc.	0.7 mg/mL	
Column Temp	25 °C	40 °C
Mobile phase	Heptane:IPrOH (60:40)	CO ₂ with 9% IPrOH modifier
Flow rate	0.8 mL/min	4.0 mL/min; (Pressure: 120 bar)
Injection volume	5 μ L	10 μ L
Isocratic Run time	8 min	3 min

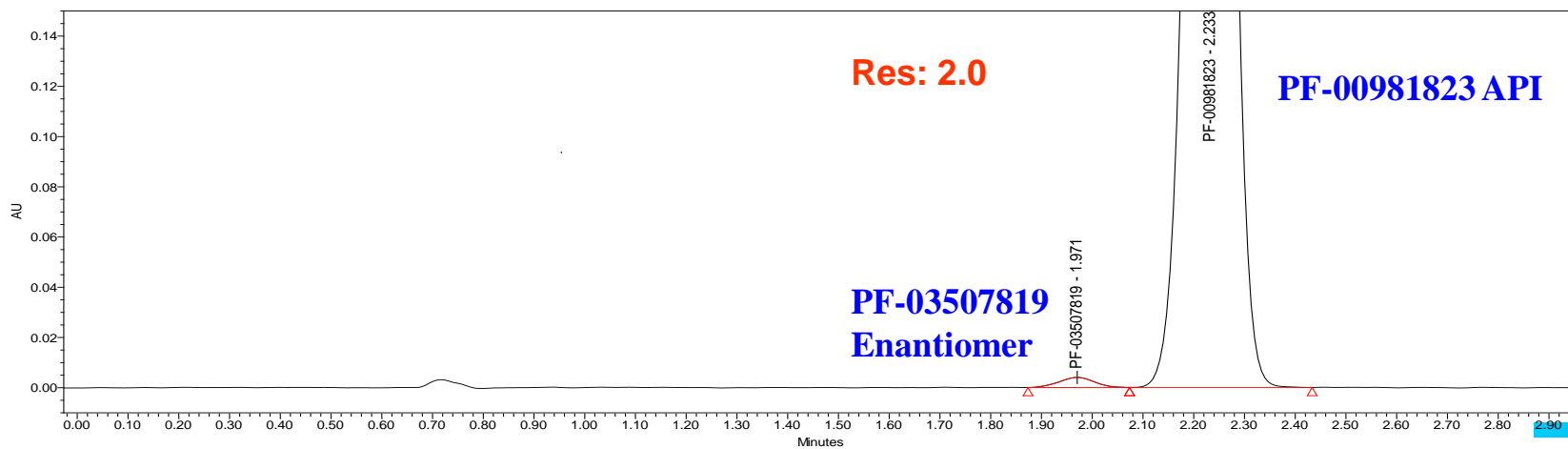


Case 1: PF-00981823 (Chromatograms)

HPLC method



SFC method





Case 1: Comparison for NP-HPLC and SFC

<u>Parameter</u>	<u>Acceptance Criteria</u>	<u>NP-HPLC</u>	<u>SFC (Berger)</u>
Specificity	Res \geq 1.5 from API	Res: 2.9	Res: 2.0
Linearity	r=0.9950 Y-int%: < 10% of max conc.	r=0.9999, Y-int%: 0.79	r=0.9985, Y-int%: 0.97
Accuracy	Mean recovery 100 15% (\leq 0.15%) 100 10% ($>$ 0.15%)	105% at 0.1% level 106% at 0.5% level 106% at 1.0% level	114% at 0.1% level 97% at 0.5% level 99% at 1.0% level
System precision	RSD \leq 10 % (LOQ)	2.4	6.6
Repeatability	RSD \leq 10%	2.4	8.6
LOQ at 0.1%	S/N \geq 20	S/N = 180	S/N = 20

Conclusion:

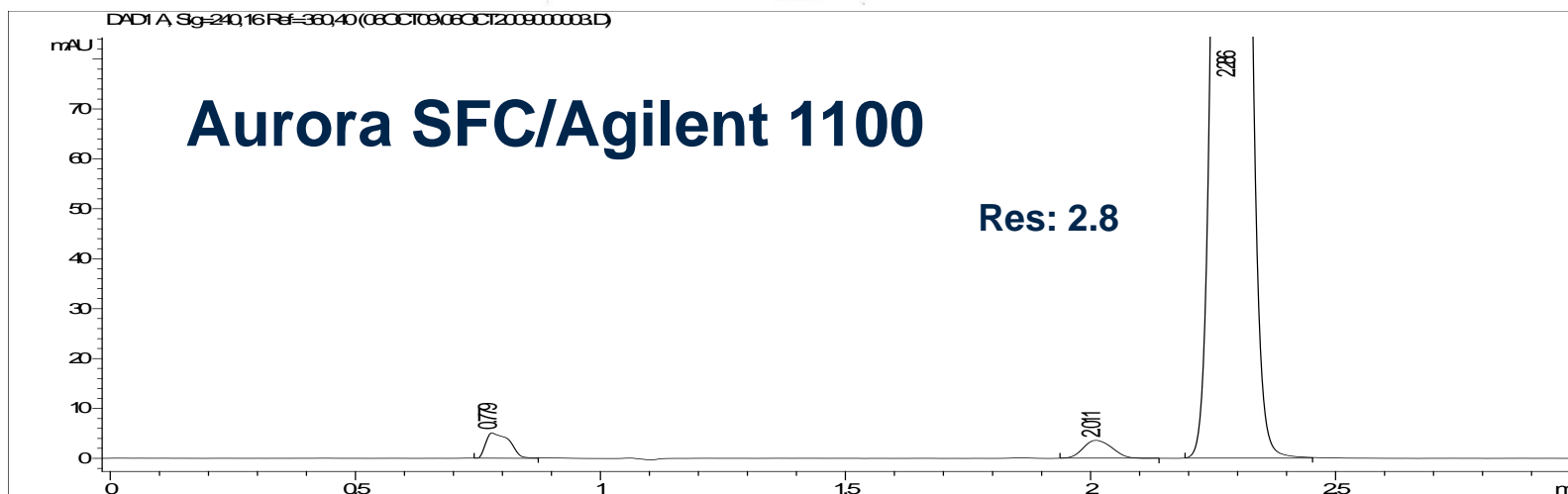
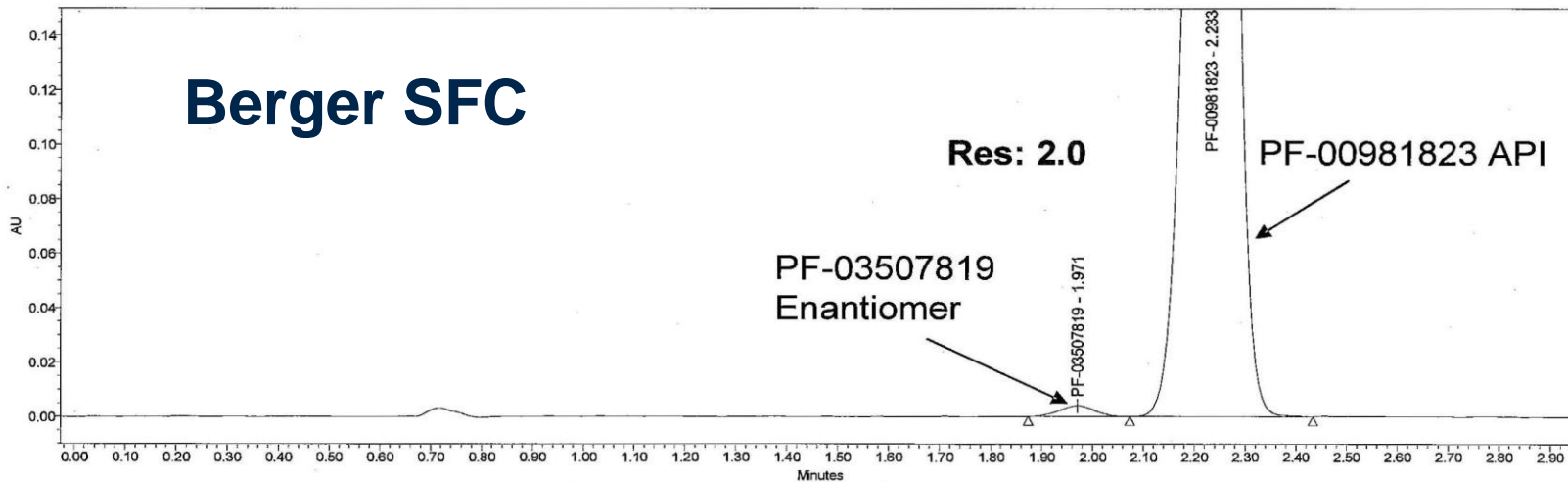
1: NP-HPLC showed better sensitivity

2: However, both methods all passed acceptance criteria and suitable for intended use



Case 1: Comparison Between Berger and Aurora SFC

PF-00981823 SFC Chromatograms at nominal concentration (0.7mg/mL)





Case 1: Comparison Between HPLC, Berger and Aurora SFC

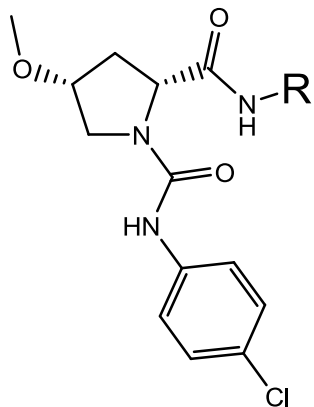
Parameter	NP-HPLC	SFC – Aurora/1100	SFC – Berger
Specificity	Res = 2.9	Res = 2.8	Res = 2.0
Linearity (0.1 -1.0%)	r = 0.9999 Y-int% = 0.79	r = 0.9999 Y-int% = 1.0	r = 0.9985 Y-int% = 0.97
Accuracy	0.1% = 105%	0.1% = 111%	0.1% = 114%
	0.5% = 106%	0.5% = 109%	0.5% = 97%
	1.0% = 106%	1.0% = 107%	1.0% = 99%
System Precision	2%	4%	7%
Repeatability	2%	2%	9%
LOQ @ 0.1%	S/N = 180	S/N = 20	S/N = 20

Conclusion:

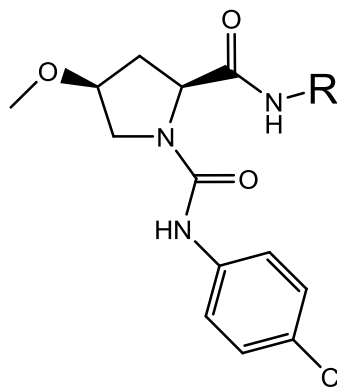
- 1: NP-HPLC still showed better sensitivity than SFCs, however, SFC sensitivities are sufficient for intended use
- 2: Aurora system demonstrated advanced SFC performance which is close to NP-HPLC.

Case 2: PD-0348292

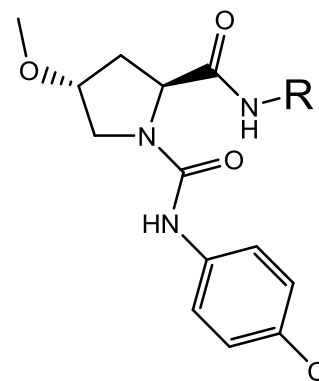
API and Impurities (chiral, non-chiral)



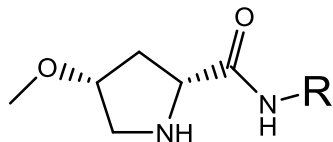
PD-0348292 API Mass:484.1



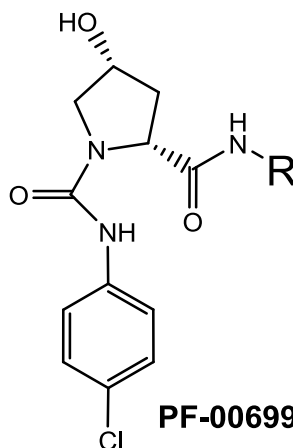
PF-00520809 Enantiomer
Mass:484.1



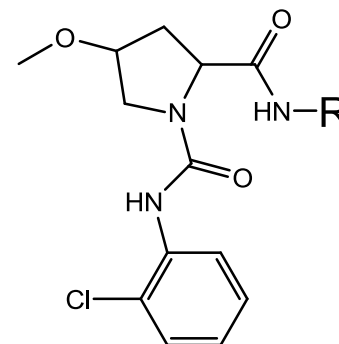
PF-0074434, Diastereomer
Mass:484.1



PF-00483932, Chiral precursor
Mass 331.1



PF-00699235, Mass 470.1

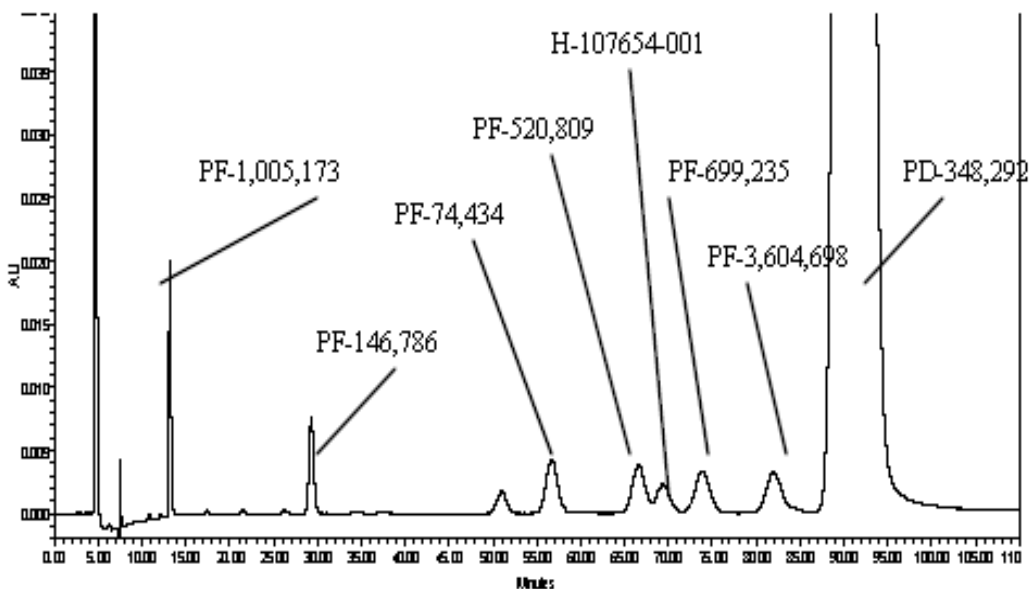


PF-3604698, impurity, Mass 484.1
Same mass as API



Case 2: In-use NP-HPLC method (validated)

- Column: **2 coupled** Chiralcel OD columns, 10 μ m, 4.6x250 mm
 - Temperature: ambient
 - Mobile Phase: Hexanes/MeOH/EtOH (880:90:30, v/v/v)
 - Flow rate: 1.2 mL/min; UV at 245 nm
 - Sample concentration: 3.0mg/mL Injection volume: 20 μ L
 - Run time: **Isocratic, 110** min



	Peak	Source
1	PF-1005173	Starting Material
2	PF-146786	Starting Material
3	not assigned	Diastereomer
4	PF-00074434	Diastereomer
5	PF-00520809	Enantiomer
6	H-107654-001	Process impurity
7	PF-00699235	Process impurity
8	PF-03604698	Process impurity
9	PD-0348292	API coeluted with precursor
10	PF-0483932*	



Case 2: NP-HPLC method complexity

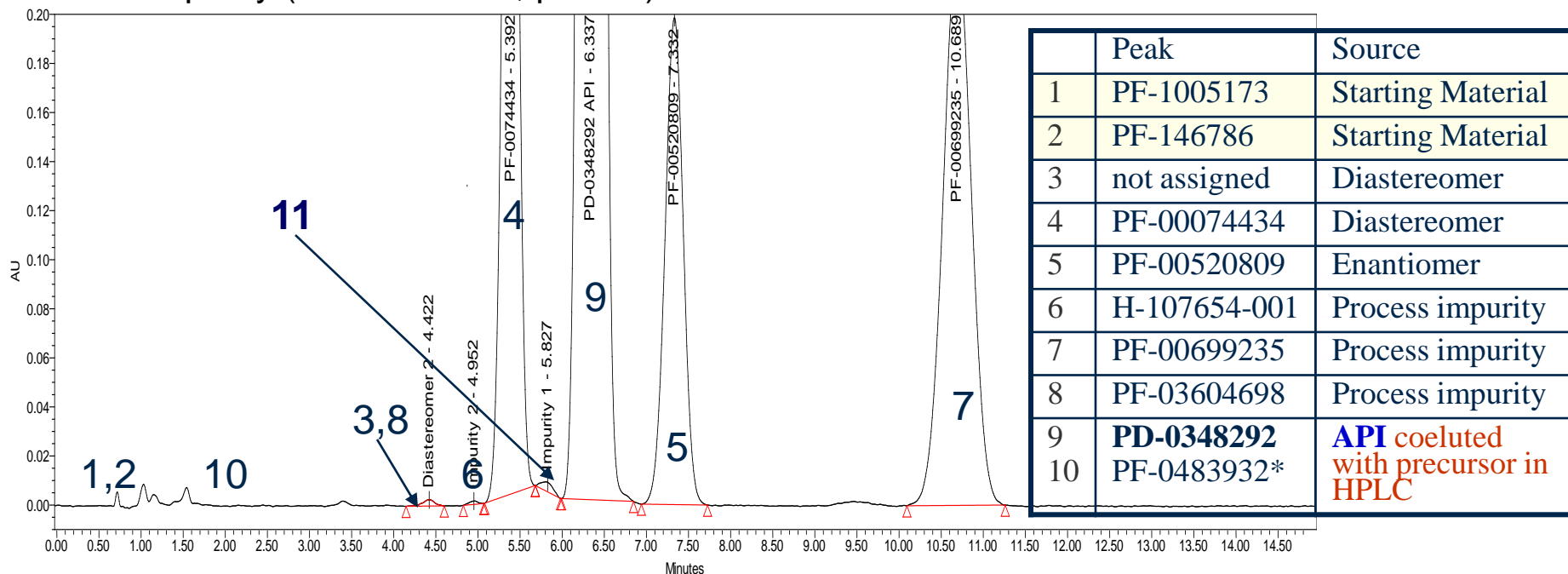
- 2 chiral centers, resulting in 3 chiral impurities (enantiomer and two diastereomers).
- Difficulty in separating enantiomer from the process impurity (H-107654-001) - critical pair
 - Even used **coupled columns**, resolution only 1.0
 - Not easy to integrate and quantitate the enantiomer.
- Precursor PF-00483932 co-elutes with API, thus enantiomer quantitation has to be performed by using external enantiomer standard instead of area% of the API
- 110 minutes run time



Case 2: SFC (Berger) Method

- **Column:** Chiralpak OJ-H, 5 μ m, 4.6x250 mm **Temperature:** 40 °C
- **Mobile Phase:** CO₂ + modifier (16% of 0.1% IPAm in MeOH)
- **Flow rate:** 4.0 mL/min; **Pressure:** 150 bar; **UV** at 245 nm **Injection volume:** 10 μ L
- **Run time:** Isocratic, 15 min

PD-0348292 spiked with **enantiomer** (peak 5), one available diastereomer (peak 4), and an impurity (PF-00699235, peak 7)

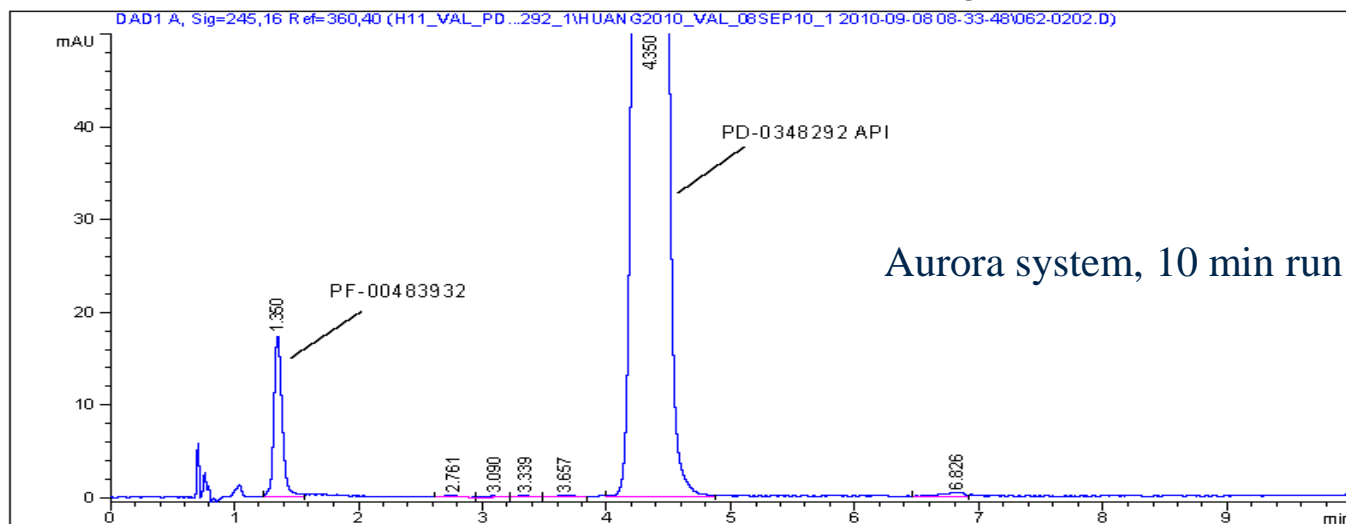


Peak 1-10 found in HPLC method are all well separated in SFC, and labeled with the same peak #. An additional peak 11 was also separated and identified.



Case 2: SFC Method Highlights

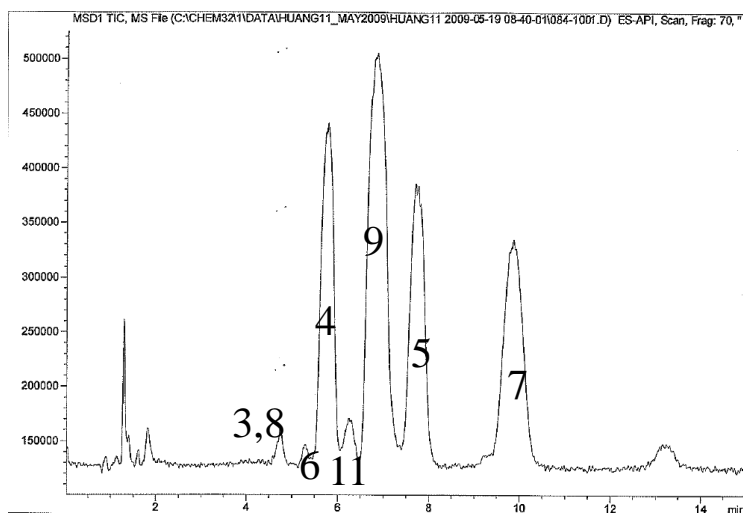
- Single column is used. API and 3 chiral impurities (isomers) are all well resolved.
- Enantiomer is well separated from API and other impurities (Res = 2.6), making the quantitation easier and more accurate.
- No peak co-eluted with API. The co-eluted impurity (PF-00483932) in HPLC is well separated from API in SFC (as Chromatogram below). Enantiomer can be quantitated by area% API, no need to prepare enantiomer external standard.
- Shorten the run time from 110 min to 15 min on Berger SFC and 10 min on Aurora SFC



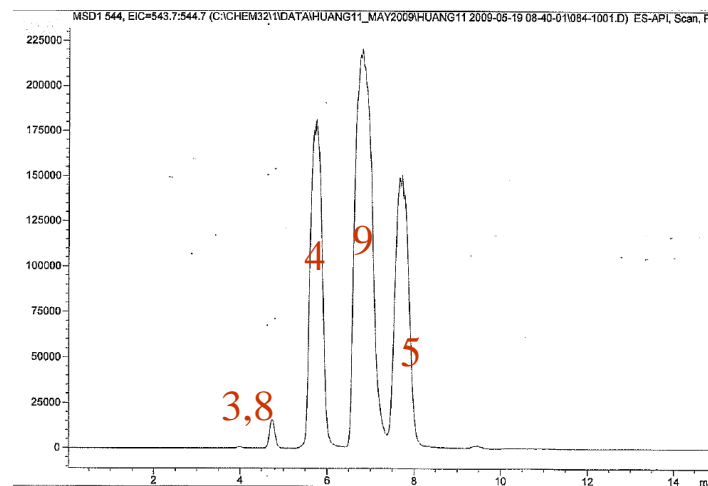


Case 2: SFC/MS Identification of Chiral Isomers and Impurities

TIC mass spec for sample – All peaks



SIM at 544 [M+60 (isopropylamine)] – single mass to show API and its isomers



- SFC is compatible with mass spec to become a convenient and rapid identification tool
- Mass spec analysis confirmed that API and enantiomer peaks are pure without the existence of precursor PF-00483932, which was confirmed by spiked PF-00483932 in API chromatogram
- SFC/MS also identified peak 11, which was not observed in HPLC method. It has the same mass as PF-00699235, thus suspected to be its chiral isomer
- With all other peaks identified, peak 6 is most likely H-107654-001 (the critical pair with enantiomer) in HPLC method



Case 2: SFC method, Peak assignment

Peak #	RRT	Compound	Description	ID method	Resolution ^b
3 or 8	0.70	Diastereomer or PF-03604698	Diastereomer of API or process related impurity	SFC-MS	NA
6	0.78	H-107654-001	Process Impurity	Note 1	2.2
4	0.85	PF-00074434	Diastereomer	AM ^a / SFC-MS	1.7
11	0.92	Chiral isomer of PF-00699235	Process Impurity	SFC-MS	1.4
9	1.0	PD-0348292	API	-	1.5
5	1.16	PF-00520809	Enantiomer	AM / SFC-MS	2.6
7	1.69	PF-00699235	Process impurity	AM / SFC-MS	6.5

^aAM: authentic material

^bResolution between the closest peak

Note 1: As no authentic material and mass data available, this peak is assigned as the last peak after all other peaks have been positively identified.



Case 2: Method Validation Results for NP-HPLC and SFC Methods

3.0 mg/mL as nominal conc.

<u>Parameter</u>	<u>*NP-HPLC</u>	<u>SFC (Berger)</u>
Specificity	¹ Res = 1.0	¹ Res = 2.6
Linearity/ Range	r=0.9995 Y-int%:0.90 (0.05 – 0.4%)	r=0.9999 Y-int%:0.8 (0.05 – 0.5%)
Accuracy	93% to 107% at 0.05% LOQ 93% to 107% at 0.1% (spec) 93% to 102% at 0.2%	99% at 0.05% LOQ level; 107% at 0.1% spec level; 96% at 0.2% level
System precision (LOQ)	2.2	6.1
Repeatability	4.8	6.7
LOQ (0.05%)	S/N = 100 (20µL injection)	S/N = 12 (10µL injection)

*: **NP-HPLC**: Validation data was cited from the validation summary report

¹: **Resolution**: Between PF-00520809 (enantiomer) and its closest peak;



Method Transferability

Objectives:

- Use case 2 compound (PD-0348292) to study the method transferability between partner lines (Research Analytical – up to phase IIa; Analytical Development – after phase IIa, GMP lab – all phases release and stability testing) .
- Integrate SFC platforms within all analytical communities to establish an efficient and effective work stream.

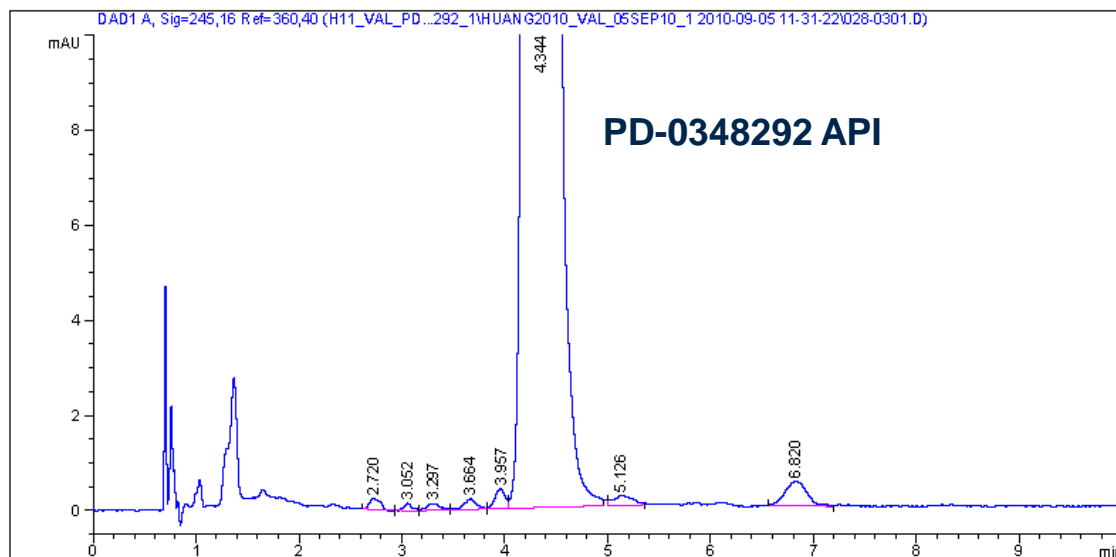
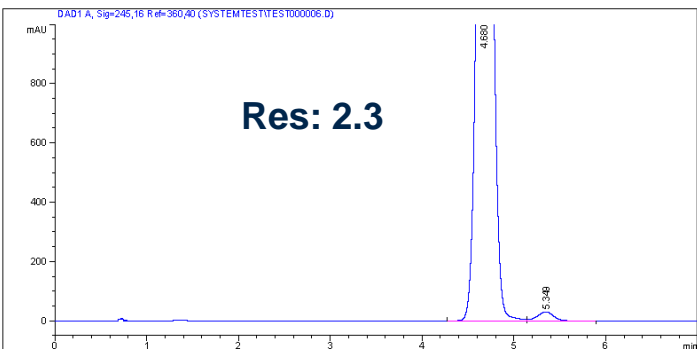
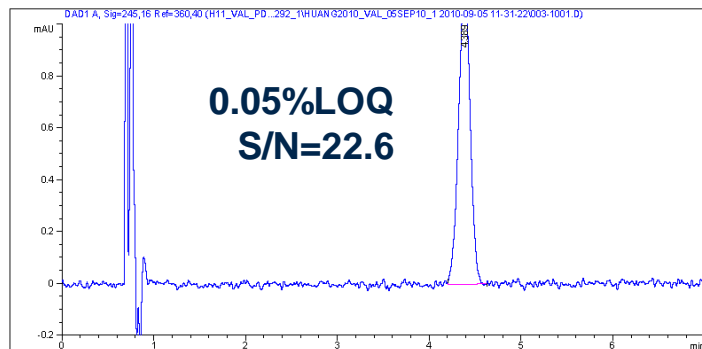
Approaches:

- Modified the chiral SFC method to simplify the analytical procedure for enantiomeric purity determination.
 - Conduct a co-validation with 3 function lines' colleagues on 2 Aurora SFC systems.
-



Transfer: Chromatograms on Aurora Fusion A5 - Agilent 1100/1200

Nominal concentration, 2.0 mg/mL





Results: Specificity Comparison Between Berger SFC and Aurora SFC

<u>Compound</u>	<u>Description</u>	<u>Resolution to the closest peak on Berger</u>	<u>Resolution to the closest peak on Aurora 1100</u>
PD-0348292	API	1.5	1.9
PF-00520809	Enantiomer	2.6	2.3

All peaks have been proven to be pure by SFC/MS

The resolution for peaks of interest (API and enantiomer) and their closest peak are not less than 1.5.



Results: Linearity of PD-0348292 on Aurora SFC/Agilent 1200 System

Low Level Linearity (0.05% - 1.0% of nominal concentration (2.0 mg/mL))

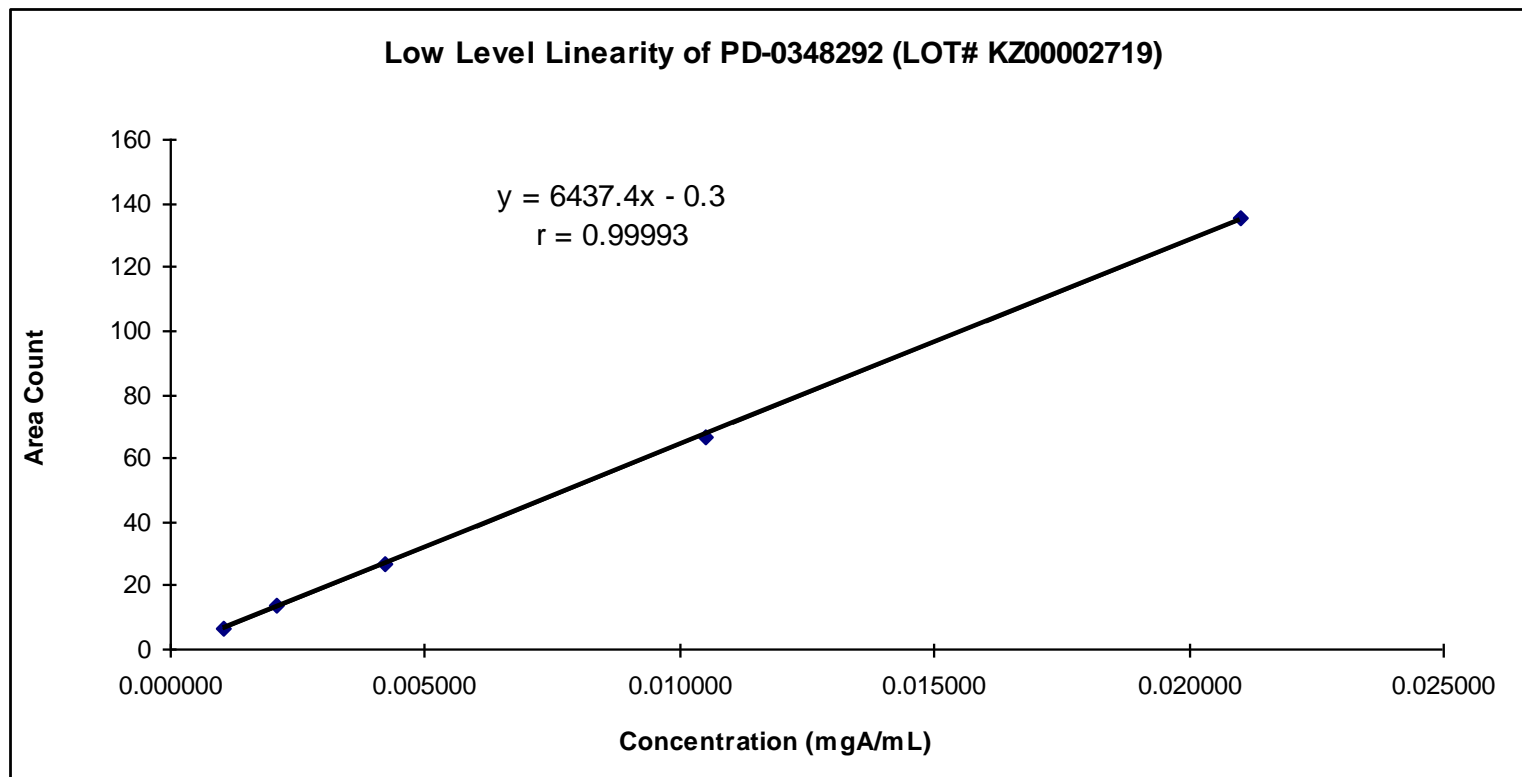
Percent of Nominal	Concentration (mgA/mL)	Area Count	RF	%RF*
0.05%	0.001051	6.755230	6427.43	101.43
0.1%	0.002102	13.51712	6430.60	101.46
0.2%	0.004204	26.74640	6362.05	100.38
0.5%	0.010510	66.31318	6309.53	99.55
1.0%	0.010510	135.49426	6445.97	101.60

* RF: relative to 100% response



Results: Validation of PD-0348292 on Aurora SFC/Agilent 1200 System

Low Level Linearity (0.05% - 1% of nominal concentration, 2.0 mg/mL)



y-intercept of the 0.5% nominal assay response: 0.5%



Results: Linearity of PD-0348292 on Aurora SFC/Agilent 1200 System

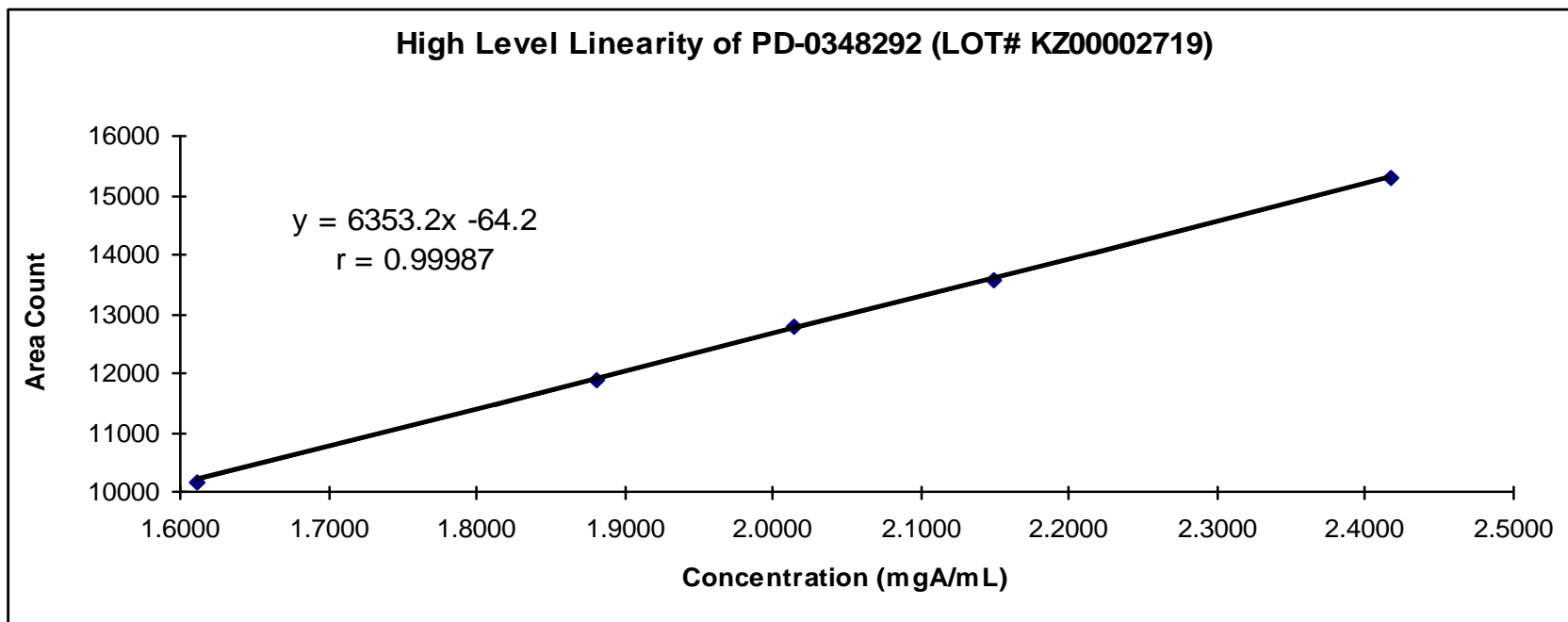
High Level Linearity (80% - 120% of nominal concentration (2.0 mg/mL))

Percent of Nominal	Concentration (mgA/mL)	Area Count	RF	%RF
80.0%	1.6120	10149	6296.06	99.24
90.0%	1.8806	11895	6325.11	99.70
100.0%	2.015	12784	6344.42	100.00
107.0%	2.1493	13582	6319.27	99.60
120.0%	2.4179	15276	6317.88	99.58



Results: Linearity of PD-0348292 on Aurora SFC/Agilent 1200 System

High Level Linearity (80% - 120% of nominal concentration, 2.0 mg/mL)



y-intercept of the nominal assay response: 0.5%

Slope (6353) agrees with low level slope (6437) within 1.3%, confirming the good linearity across low to high level.



System Precision

System Precision Solution used	Aurora/1100	Aurora/1200
Nominal (2.0 mg/mL)	0.29	0.76
0.5% of Nominal (0.01 mg/mL)	0.27	0.22
0.1% of Nominal (0.002 mg/mL)	0.74	0.64
0.05% of nominal (LOQ,0.001 mg/mL)	0.87	0.95



% Recovery/Accuracy

Std. to be used		Aurora 1100			Aurora 1200		
		LOQ 0.05%	Spec 0.1 %	2xSpec 0.2%	LOQ 0.05%	Spec 0.1 %	2xSpec 0.2%
Nominal (2 mg/mL)	Rep 1	102.8%	103.2%	101.1%	104.2%	101.3%	99.9%
	Rep 2	102.9%	102.3%	102.1%	101.1%	101.7%	100.0%
	Rep 3	102.2%	101.8%	101.3%	101.9%	102.0%	100.9%
0.5% (0.01 mg/mL)	Rep 1	100.3%	100.7%	98.6%	103.0%	100.1%	98.7%
	Rep 2	99.7%	99.8%	99.6%	100.0%	100.5%	98.8%
	Rep 3	100.3%	99.3%	98.8%	100.7%	100.8%	99.7%
0.1% (0.002 mg/mL)	Rep 1	100.3%	100.7%	98.6%	101.2%	98.3%	97.0%
	Rep 2	99.7%	99.8%	99.6%	98.2%	98.7%	97.1%
	Rep 3	100.3%	99.3%	98.8%	99.0%	99.0%	97.9%



System comparability among SFC Systems vs. NP-HPLC

Parameter	NP-HPLC	Berger	Aurora/1100	Aurora/1200
Nominal conc.	3.0 mg/mL	3.0 mg/mL	2.0 mg/mL	2.0 mg/mL
Specificity ^a	Res 1.0	Res = 2.6	Res = 2.3	Res = 2.5
Linearity (low)	r = 0.9995 Y-int% = 0.90	r = 0.9999 Y-int% = 0.8	r = 99995 Y-int% = 0.7	r = 0.99999 Y-int% = 0.4
Accuracy	0.05%: 93-107%	99% at 0.05%	103% at 0.05%	102% at 0.05%
	0.1%: 93-107%	107% at 0.1%	102% at 0.1%	102% at 0.1%
	0.2%: 93-102%	96% at 0.2%	102% at 0.2%	100% at 0.2%
Sys. Precision ^b	2.2%	6.1%	0.87%	0.95%
Repeatability	4.8%	6.7%	0.7%	1.3%
S/N@ LOQ	100 (20 µL inj.)	12 (10 µL inj.)	23 (10 µL inj.)	14 (10 µL inj.)
^a resolution: peak of interest (API and the enantiomer) with their closest peaks				
^b 6 injections of LOQ @ 0.05%				

Conclusion: SFC method is transferable between different systems and vendors.



Conclusions

- # Chiral SFC demonstrated to be a practical technique to replace conventional HPLC as the primary chiral analysis tool throughout all phases of development
 - Recent SFC instrumentation improvement has enhanced its sensitivity and system robustness.
 - From the two case studies, data demonstrated that chiral SFC can achieve 0.1% or 0.05% LOQ and also passed all validation requirements, thus comparable to their counterpart NP-HPLC method.
 - In case 2 high complexity sample separation, SFC showed much better separation, shorter analysis, and better validation results (when using Aurora system) compared to NP-HPLC method.

- # SFC is a more efficient and faster method than HPLC, significantly shortening the method development, validation and release testing analysis time.
 - Case 1: 8 min for HPLC, 3 min for SFC (60% reduction in time)
 - Case 2: 110 min for HPLC, 15 or 10 min for SFC (85-90% reduction in time)



Conclusions

- Chiral SFC methods can be transferred from one system to the other as well as different vendor's.
 - Aurora SFC Fusion A5 with either Agilent 1100 and Agilent 1200 have demonstrated a sufficient sensitivity (signal to noise), and shown greater system precision, accuracy and repeatability.
 - SFC/MS is a helpful analytical tool to identify chiral isomers and impurities during method development.
-



ACKNOWLEDGMENTS

- # Frank Riley for his support towards this study and helpful discussion.
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 - # Lynne Kalmbach: PF-00981823 (Case 1) SFC validation on Aurora Fusion/Agilent 1100 system.
 - # Yanqiao Xiang: PD-0348292 (Case 2) materials, HPLC validation data.
-