Advantageous Application of Preparative SFC Conditions to Meet Purity Specifications for Pharmaceutical Analysis

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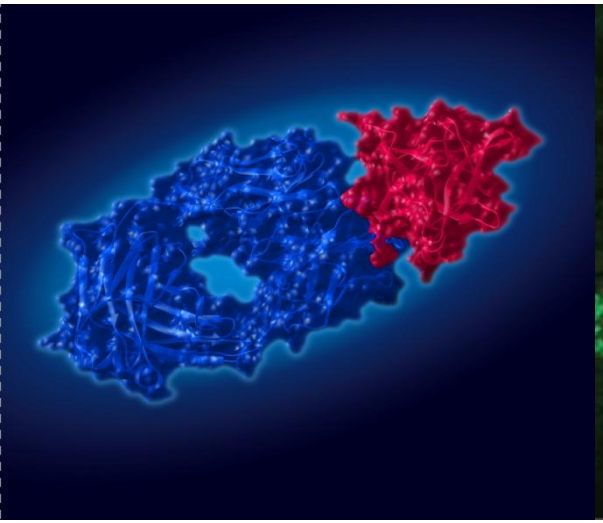

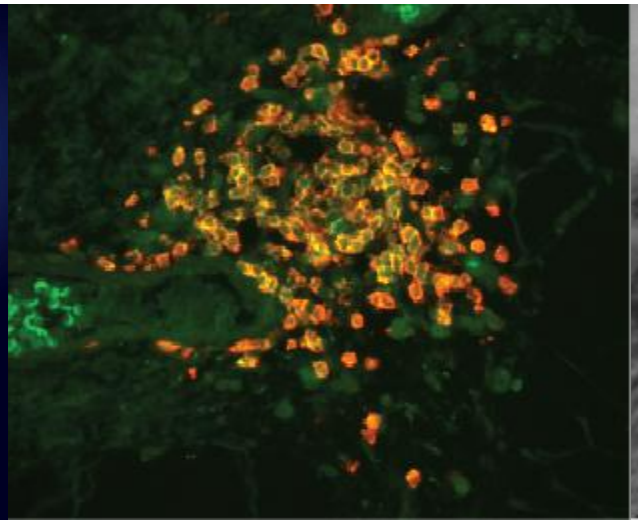

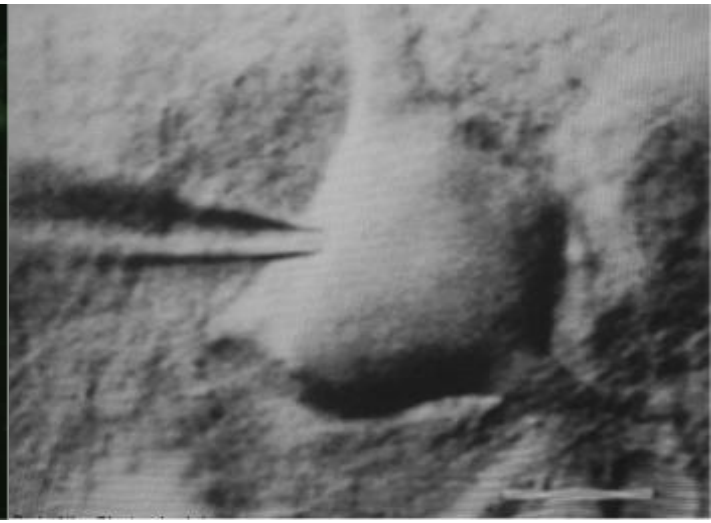

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Introduction

For compounds either to be dosed in humans or for animal in vivo studies, it is critical to isolate the pharmacological effect to the API. The International Conference on Harmonization (ICH), among others, publishes criteria for batch product purity and impurity levels, including levels for specific residual solvents.

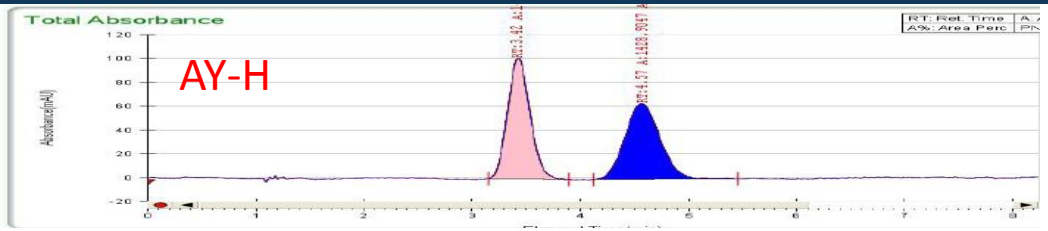
Solvents employed in the synthesis and purification process should be easily removed and ideally non-toxic at low levels, i.e. Class 3. SFC is the chiral separation method of choice in most chromatographic purifications. The final preparative method utilized must possess sufficient chiral resolution in addition to resolution from all other impurities. Only the API and liquid mobile phase component should remain in the final fraction prior to evaporation with only residual mobile phase left after evaporation.

Characterization of the low level impurities introduced from synthesis or formulation in an API batch is also often required. At levels ≤ 0.1% a careful and often challenging preparative purification is required for impurity isolation. SFC purification can be used advantageously for this purpose. Often insufficient resolution of the impurity is achievable with reversed phase methods due to poor selectivity. In these cases SFC can be used as an alternative or complement to such methods to enable successful impurity isolation.

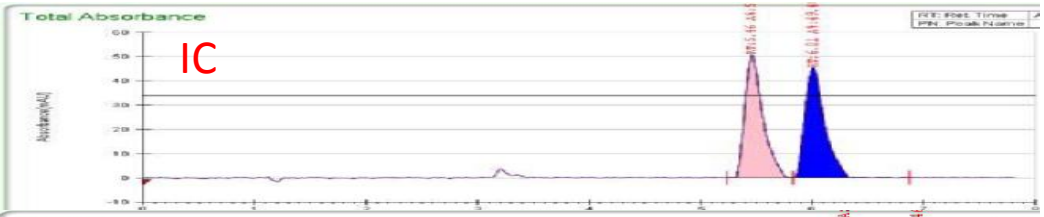
Objective 1

- Client requests 3.5 kg pure enantiomer from 8 kg racemic API batch
- Purity required is 99% EE
- Separation requires neutral conditions
- Sample solubility may be challenging
- No detectable toxic solvent should remain in final batch

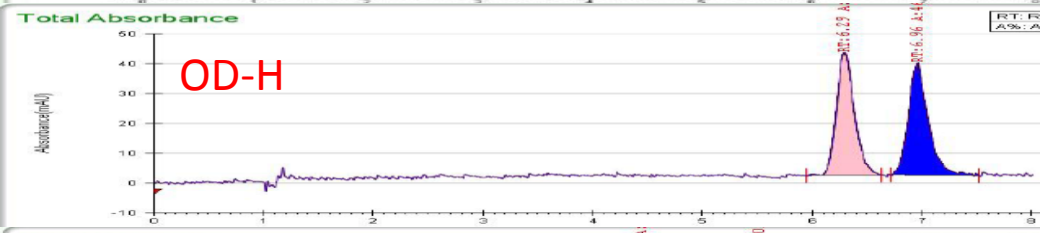
Analytical Method Selection



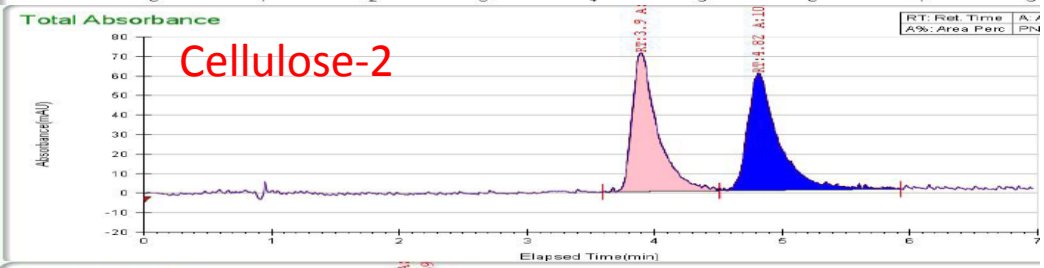
CO_2/MeOH(0.1%DEA)= 80/20



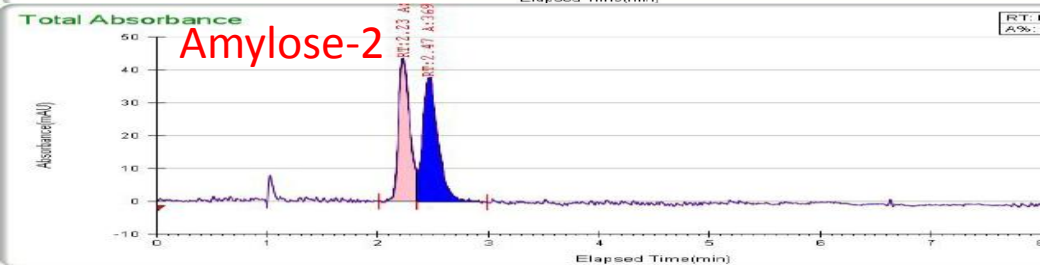
CO_2/MeOH(0.1%DEA)= 75/25



CO_2/MeOH(0.1%DEA)= 85/15



CO_2/MeOH(0.1%DEA)= 70/30



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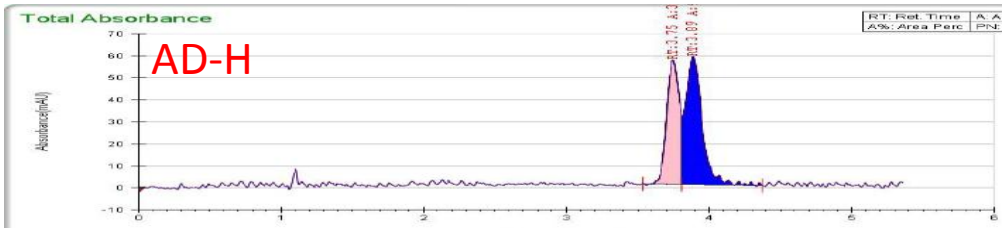

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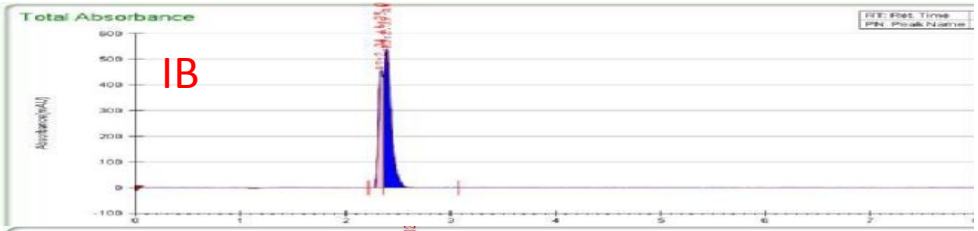

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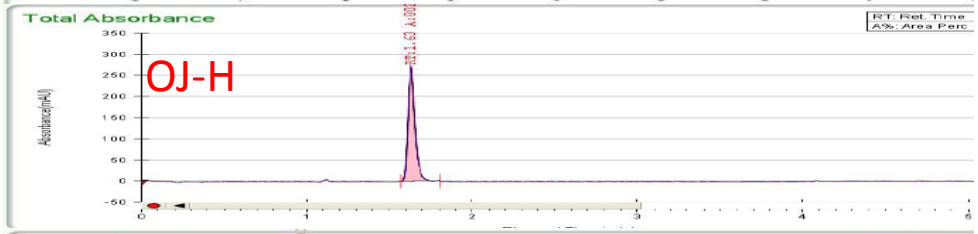

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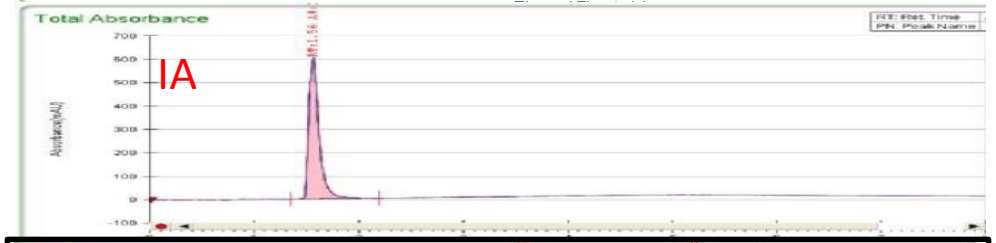

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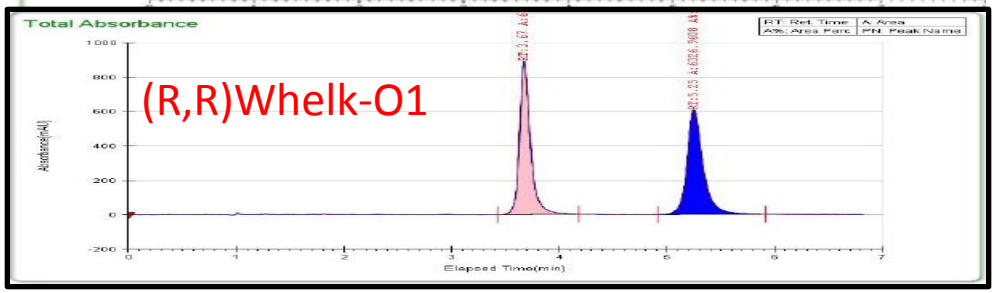
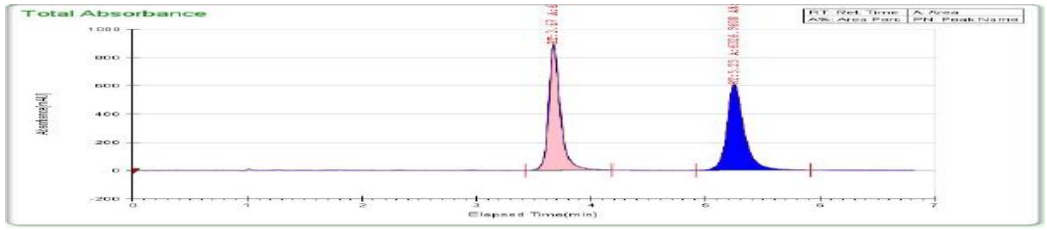


CO_2/MeOH(0.1%DEA)= 60/40

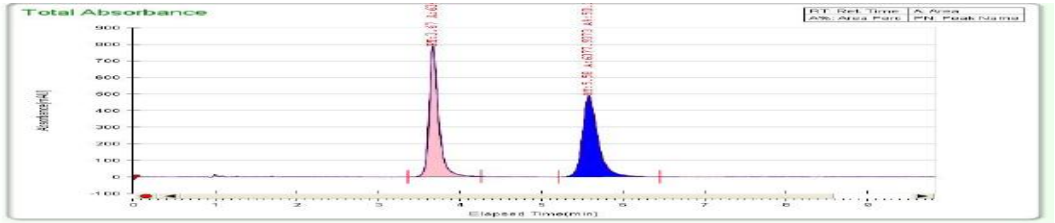
- From the above method/stationary phase evaluation the most optimal for scale up is that which utilizes the Whelk-O1 column.

Mobile Phase Choice

Mobile phase evaluation with (R,R)Whelk-O1



CO_2/MeOH= 60/40



CO_2/EtOH= 55/45

Solvent	Boiling Point	Cost	Concerns
Methanol	64.7^{0}C	￥8.0/L	Toxicity higher than other solvents
Ethanol	78.4^{0}C	￥15.3/L	Moderate cost, boiling point
IPA	82.5^{0}C	￥25.3/L	Highest cost; Highest boiling point

- Analysis shows ethanol also yields an effective separation with similar conditions to methanol (no DEA used.) The choice of ethanol is also helpful as it is a less toxic residual solvent with a lower boiling point, lower cost and lower viscosity than IPA.

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Sample Solubility Comparison

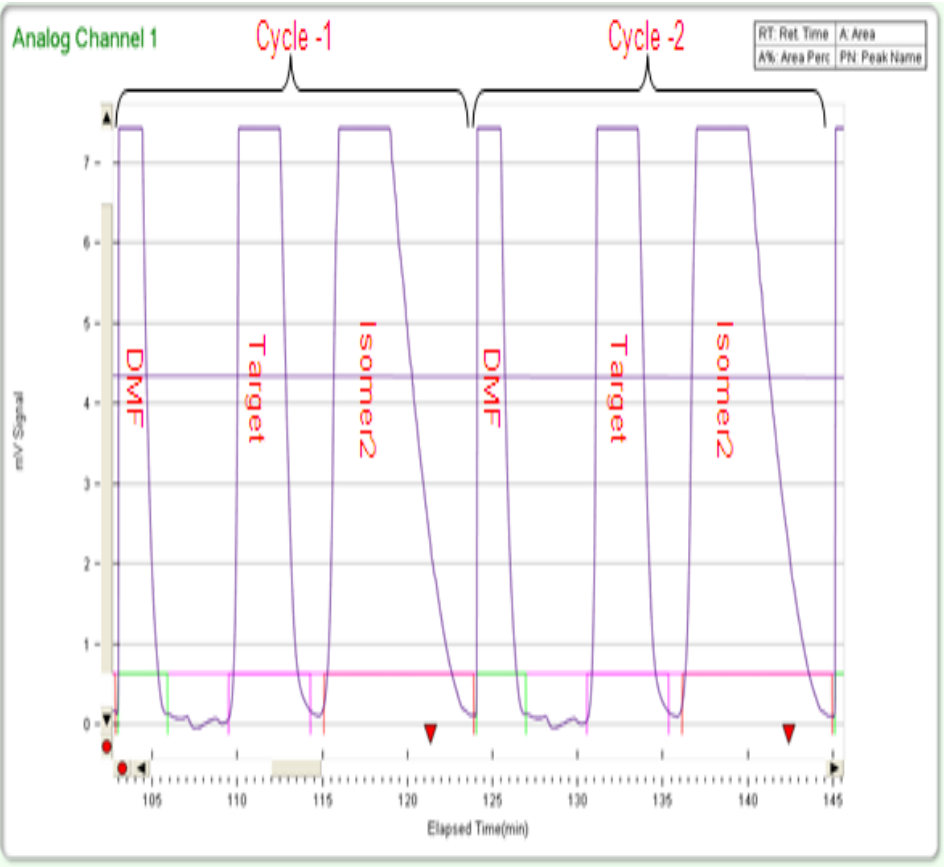
Solvent	Appx. Solubility	Properties
DMF	100mg/ml	Boiling point: 153^{0}C Freezing point:-61^{0}C Viscosity: 0.802
DMSO	>100mg/ml	Boiling point: 189^{0}C Freezing point: 19^{0}C Viscosity: 1.99
CH_2Cl_2/Methanol=2/1(v/v)	33mg/ml*	
THF	50mg/ml*	

*Heating to 40^{0}C

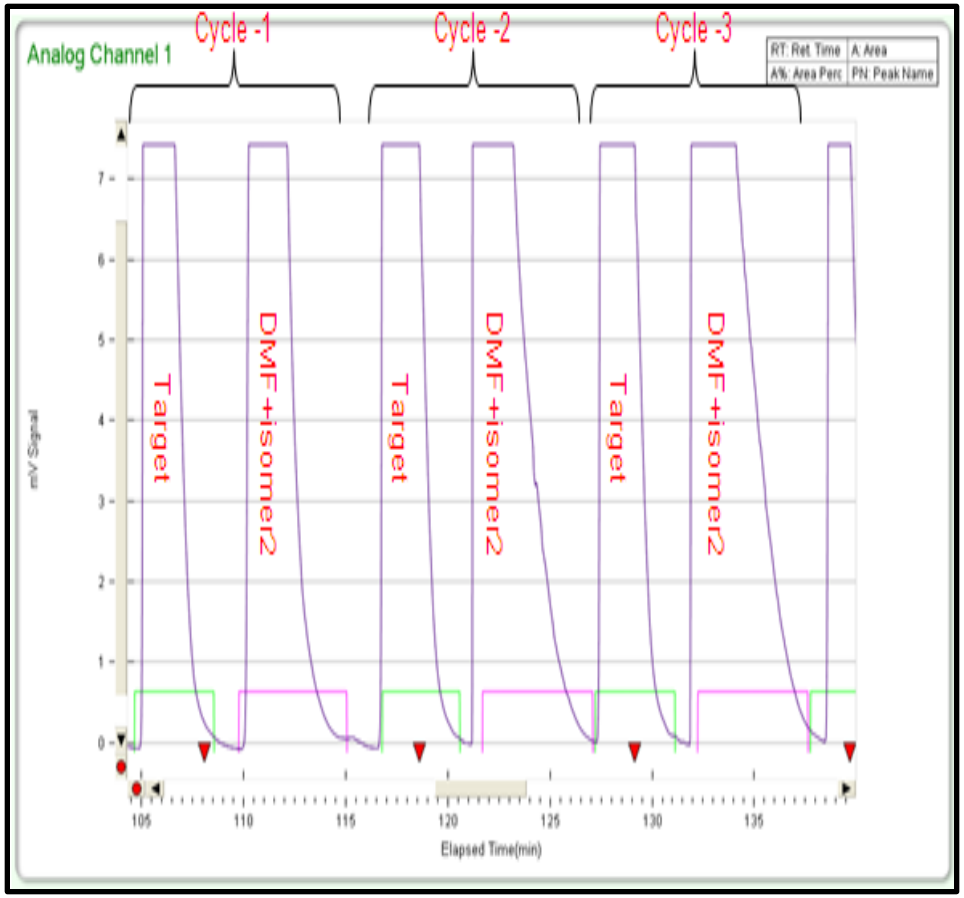
- DMSO is less volatile than DMF and does not freeze upon mobile phase depressurization. DMF is also less viscous that DMSO and therefore preferable for introduction into the SFC mobile phase.
- The preparative sequence below utilized an SFC200 with a 50x250mm (R,R)Whelk-O1 column at 35°C. The mobile phase was 60:40 CO_2:EtOH at 170mL/min at a set backpressure of 100 bar. The sample was prepared to 160mg/mL in DMF; stacked injections of 9mL (1.4g) were made.

Preparative Method Optimization

Method 1



Method 2



- In Method 1, peaks detected are labeled “DMF”, “target” (isomer 1), and “isomer 2”. All three can be separated in a cycle time of 21 min. Since it was not necessary to separate DMF from isomer 2 peaks were set to overlap in Method 2 thus reducing cycle time to12.3 minutes and saving 41% of run time. The 8 kg batch was separated in 2 months.

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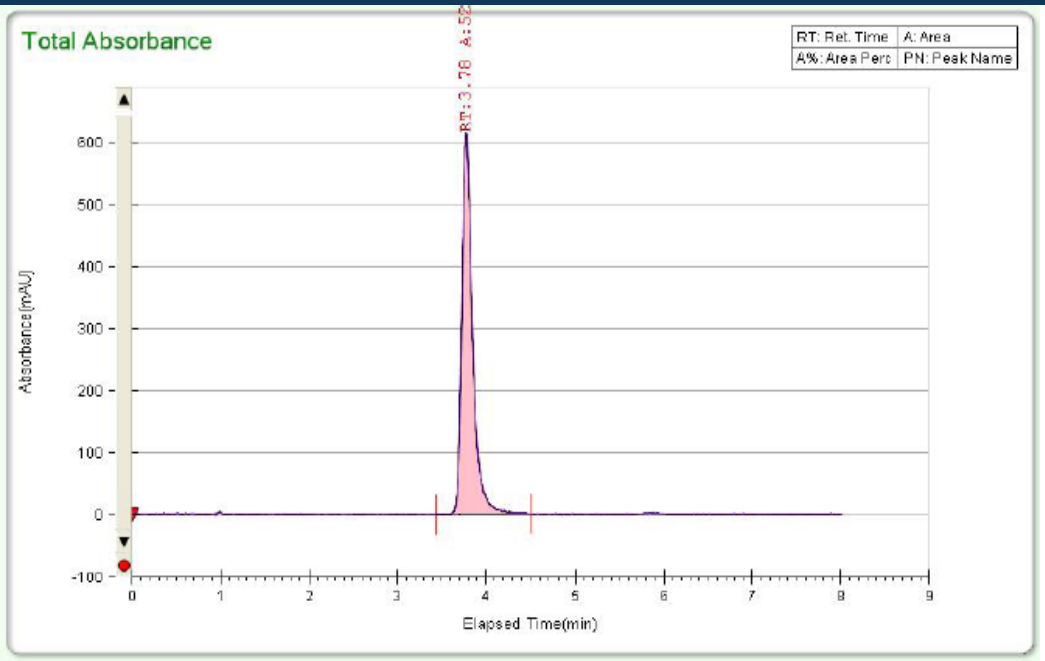
Chiral Purity/Residual Solvent Check after Separation



SFC method

- Column: (R,R)Whelk-O1, 4.6x250mm, 5µm
- Temperature: 35 ºC
- Mobile phase: Ethanol / CO_2= 45/55
- Flow: 3.0mL/min
- Back Pressure: 150bar
- Injection volume: 5µL

Peak Info

Number	RT (min)	Area %	Area
1	3.78	100	5205.4871

Residual solvent check by NMR

Sample	*DMF*	*EtOH (w/w%)*
Material	N/D	N/D
Target isomer	N/D	N/D
Isomer2	N/D	0.39

- A representative final purity check is shown indicating EE > 99%. Some ethanol used in the separation remained with the final product batch, but only at 0.39% (w/w%). This was a sufficiently low level of relatively non-toxic solvent for submission of the batch for the dosing necessary in the customer's next step.

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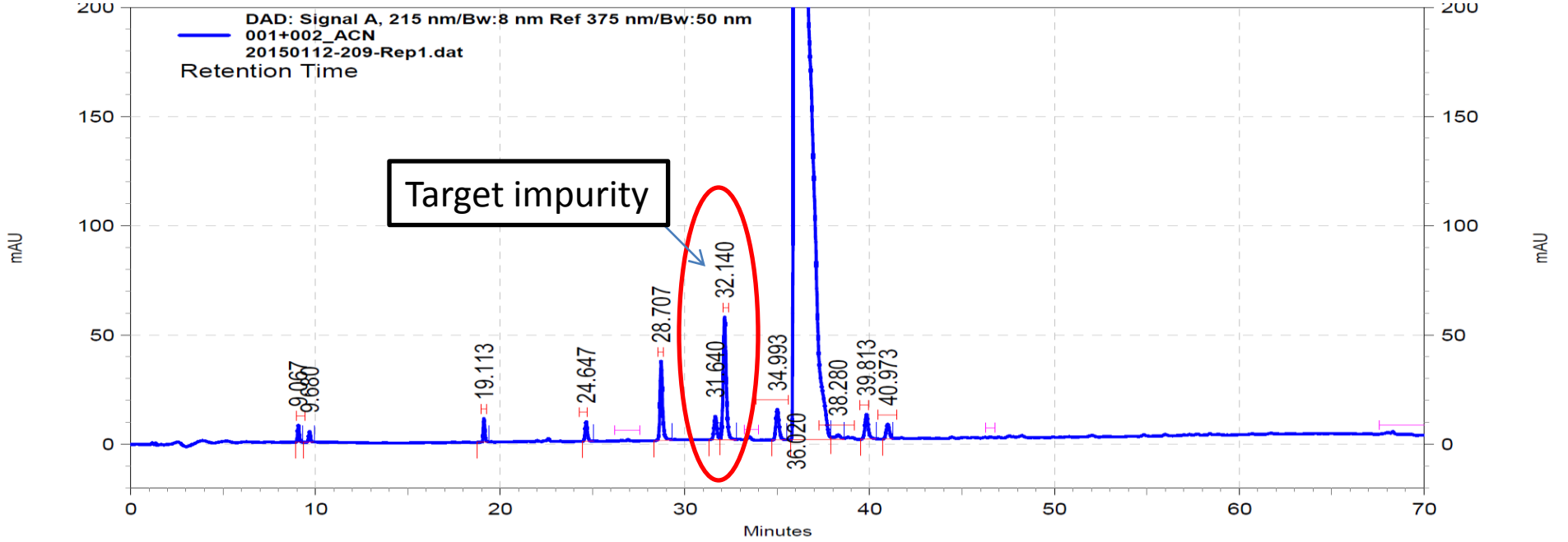
Objective 2

- Client requires isolation and structure elucidation of low level impurity in 100 g batch to be dosed
- At least 10 mg of most abundant impurity necessary for structure elucidation
- Target impurity should be at least 95% chemically pure
- Completion of project within two weeks is requested

Data from Client



Analytical HPLC method

- Instrument: Agilent 1200
- Column: Zorbax Eclipse Plus C18, 4.6x150mm, 3.5µm
- Flow rate: 1.0 mL/min
- Gradient:
  - 5 – 40% ACN in Water(0.1% TFA) : 0 – 20 min.
  - 40–65% ACN in Water(0.1% TFA) : 20 – 50 min.
  - 65–95% ACN in Water(0.1% TFA) : 50 – 70 min.

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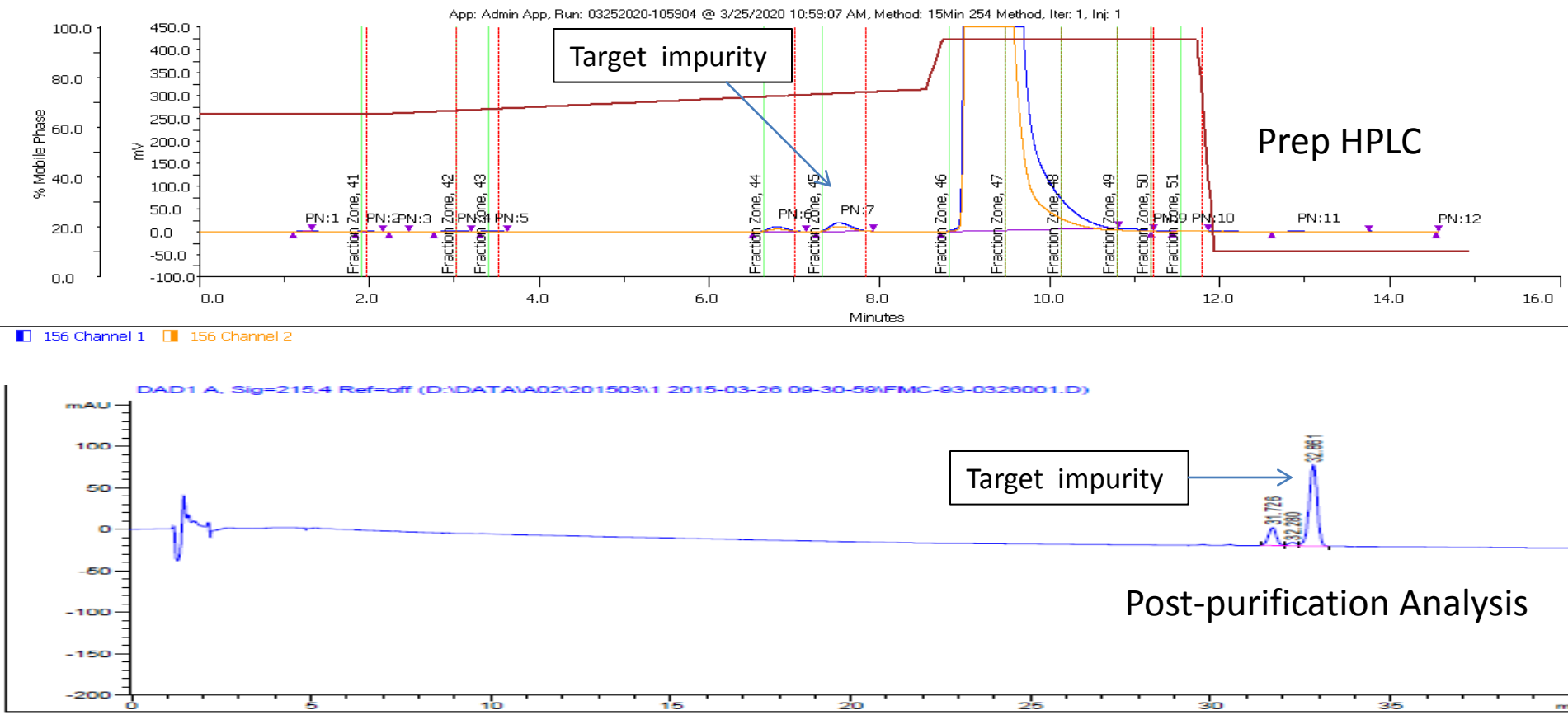
First Stage Separation Step



- Repetitions of the above preparative HPLC method based on the client's data utilizing a C18 column were performed on the batch to isolate the target impurity. Post-purification fraction analysis shows the desired impurity was isolated to ~ 80% purity in the associated fraction. Closely eluting impurities would be time-consuming to remove via further HPLC purification.

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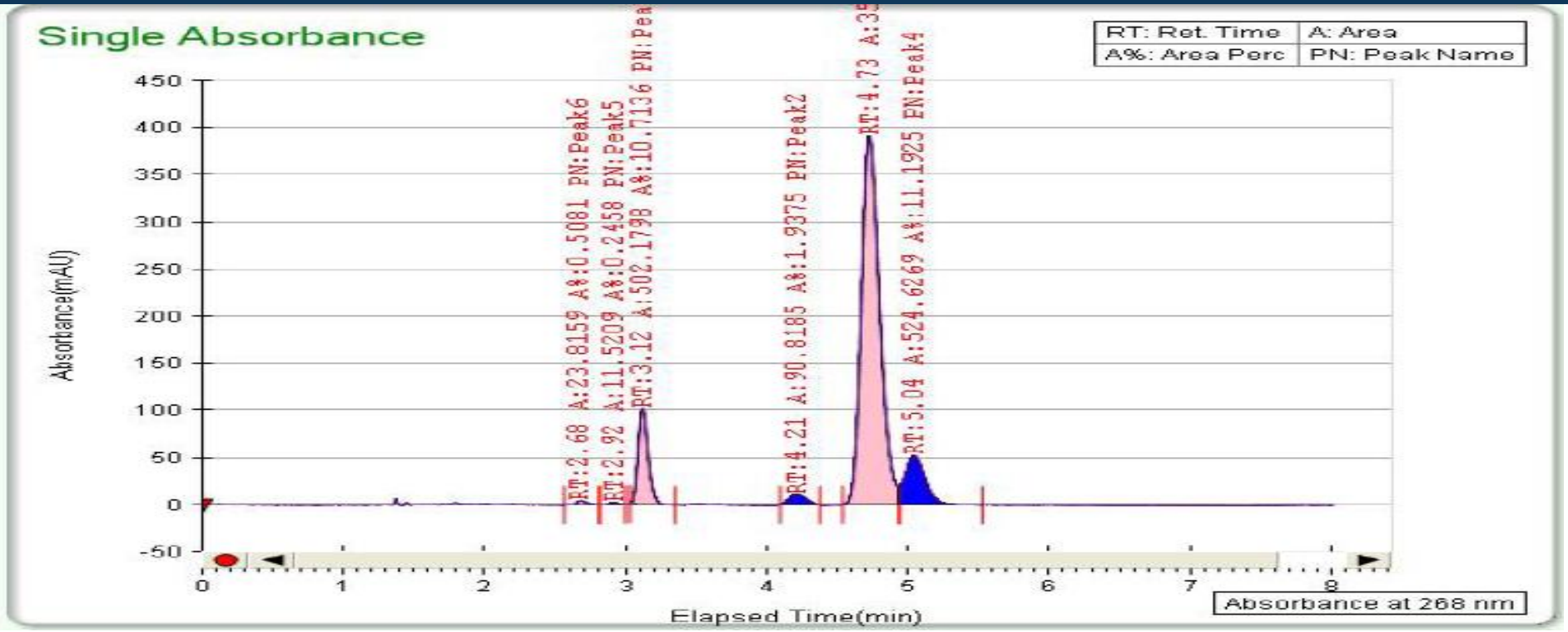
Next Steps toward Isolation of Impurity

- Target impurity was collected by HPLC purification
- Chemical purity in the final mixture was < 80%
- SFC anticipated to yield selectivity from interfering impurities to reach desired purity of target
- Since achiral selectivity is often achieved via chiral stationary phases, chiral SFC screening performed
- Similar screen to that described previously resulted in selection of the Cellulose-4 stationary phase for the purification method

Analytical SFC Method



Analytical SFC method:

- Instrument: SFC Methods Station
- Column: Cellulose-4, 250x4.6 mm, 5μm
- Column Temperature: 35°C
- Mobile phase: CO_2/Methanol = 60/40
- Flow Rate: 3.0mL/min
- Back pressure: 120bar

Peak Info

Number	RT (min)	Area %	Area	Height
1	2.68	0.5081	23.8159	4.5758
2	2.92	0.2458	11.5209	2.5765
3	3.12	10.7136	502.1798	101.8173
4	4.21	1.9375	90.8185	10.6134
5	4.73	75.4025	3534.3604	391.5595
6	5.04	11.1925	524.6269	52.699

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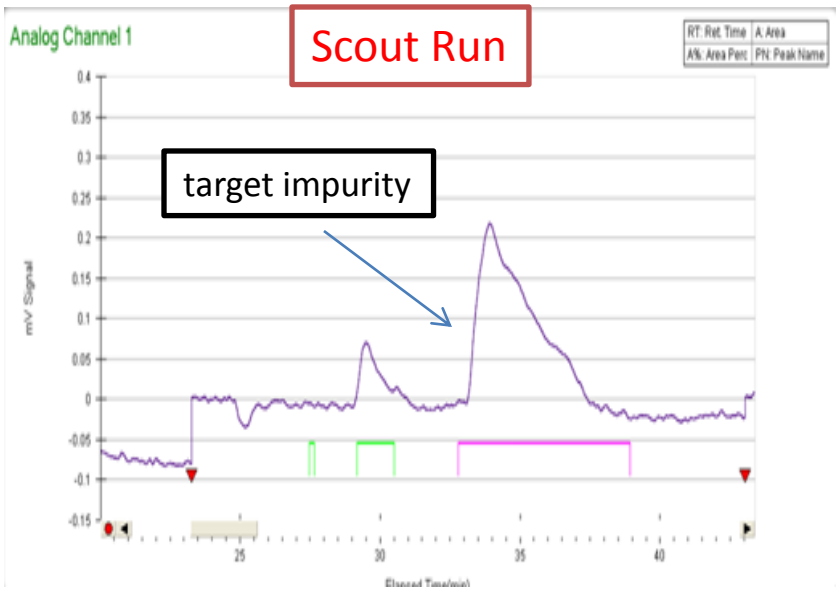
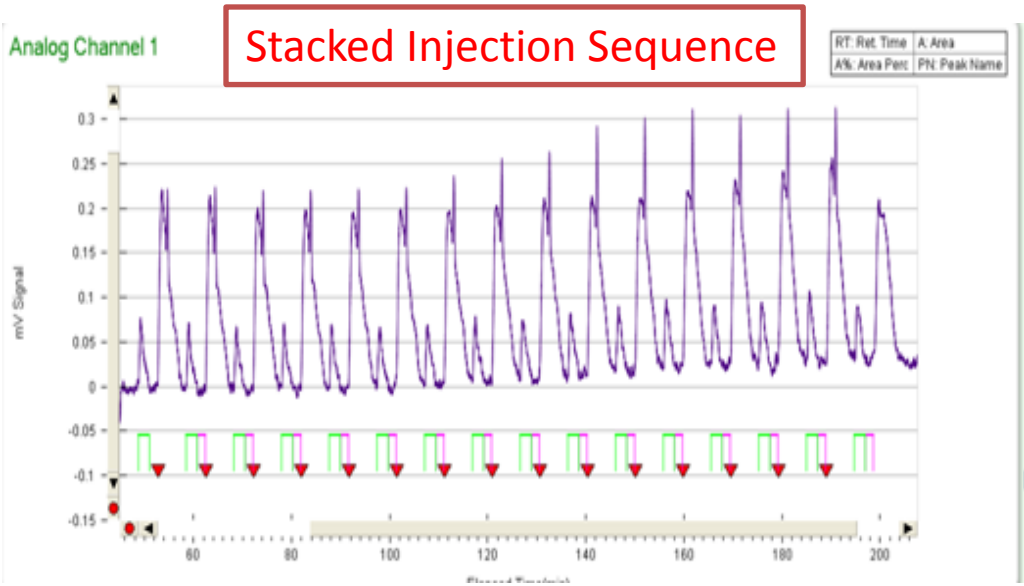
SFC Purification Step





Preparative SFC method:

- Instrument: Prep SFC80
- Column: Cellulose-4, 250x30 mm, 5µm
- Column Temperature: 35°C
- Mobile phase: CO_2/Methanol = 60/40
- Flow Rate: 80mL/min
- Back pressure: 100bar
- Cycle time: 9.7min

The cycle completed with 18 injections < 3 hours. Target fraction evaporated.

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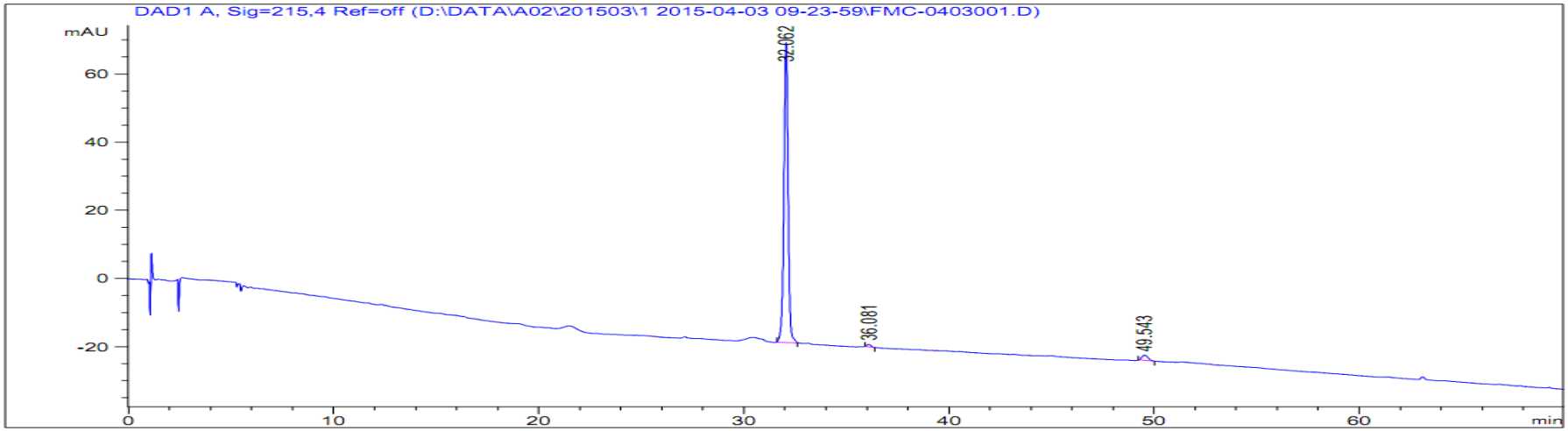
Outcome following SFC Separation



- The HPLC method utilized for analysis of the fraction from reversed phase purification before the SFC stage was used to analyze the final purified fraction after prep SFC. The resulting purity of this fraction was 96.8%. After evaporation the quantity recovered was 20 mg, sufficient for 2D-NMR experiments. The results of HSQC, COSY, HMBC and NOESY were used to positively elucidate the structure of this small molecule impurity. The full purification and structure elucidation was able to be completed in under two weeks.

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Conclusion

Experiments presented demonstrate how preparative SFC is advantageously used at ChemPartner to yield products of sufficient purity and quality required by clients for the next step in their drug development efforts.

Example I showed how chiral SFC purification conditions for a poorly-soluble compound were advantageously chosen so that 1) sufficient purity was obtained in minimal time, 2) undesired solvents were fundamentally excluded from the final batch, and 3) the remaining residual solvent was non-toxic.

Example II showed how preparative SFC was successfully applied to an achiral separation for the purpose of a challenging impurity isolation request. In this case preparative SFC was effectively utilized as the latter stage of a two-step procedure, following HPLC purification. The resulting product isolated was of sufficient quantity and purity to enable a full NMR analysis to elucidate the structure of the previously unknown impurity.