15TH Multidimensional Chromatography Workshop

# Two-Dimensional Liquid Chromatography for Small Molecule Pharmaceutical Analysis – More Knowledge in Less Time

Qinggang Wang, Yehia Baghdady, Ziqing Lin, Hui Lin, Johnathan Shackman, Brian He, Yiyang Zhou, Catherine Miles and Lianjia Ma

Chemical Process Development
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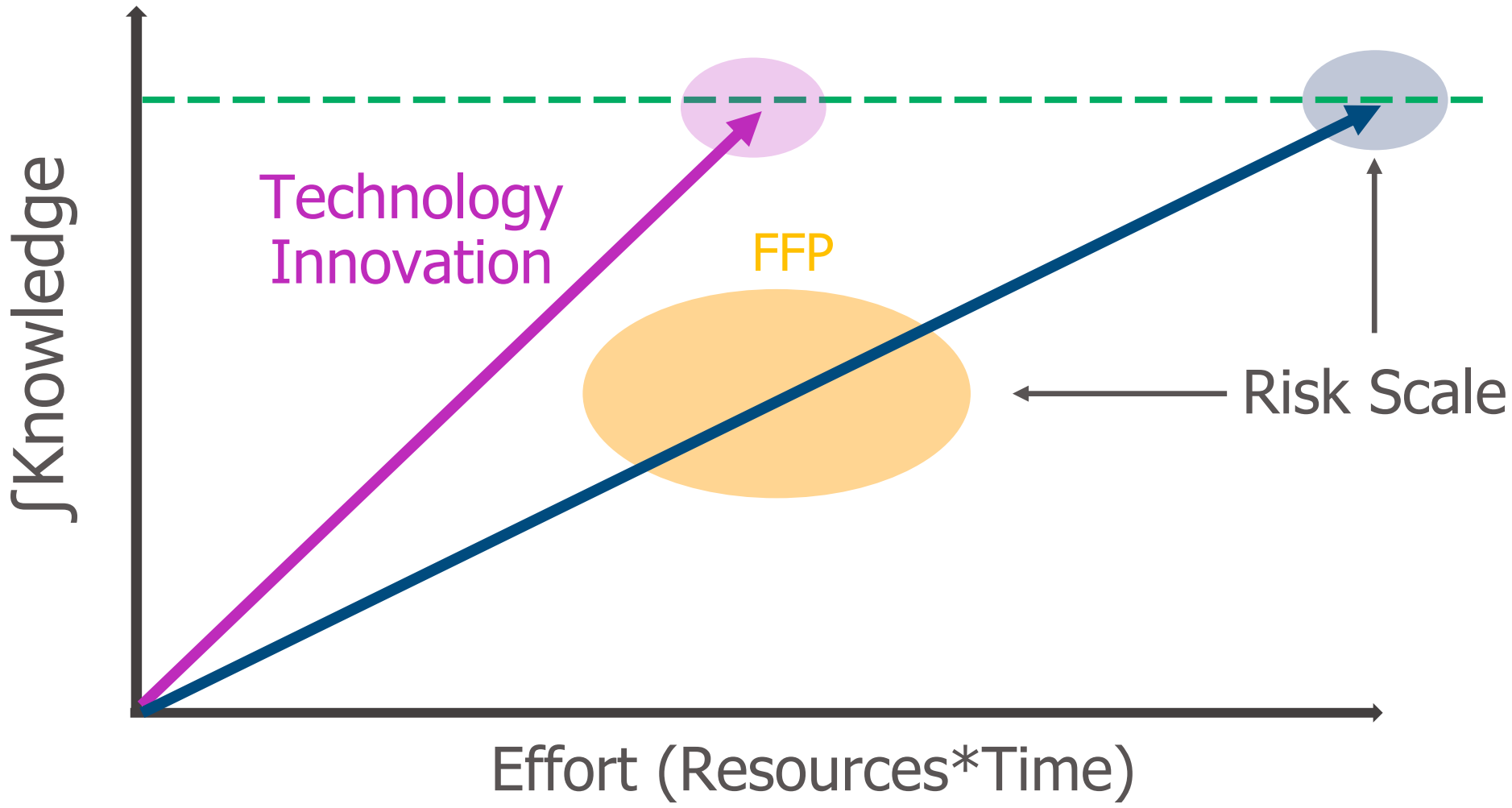
# Outline

- Introduction
- 2D-LC applications in BMS for small molecule pharmaceutical analysis
  - 2D-LC peak purity check
  - Quantitative 2D-LC Impurity analysis
  - Trapping mode 2D-LC
- Summary

# Introduction

- Pharmaceutical companies have been under consistent pressure to bring safe therapeutics to patients faster.
- The structural complexity of synthetic drug candidates has been increasing in the pharmaceutical development pipeline, and complete separation of all relevant peaks can be challenging in LC method development. This is especially true during early-stage process development, when the knowledge of process impurities is still limited.
- These challenges have prompted the evolution of innovative analytical techniques to facilitate knowledge generation in chemical process development.

# Effect of Technology Innovation



# 2D-LC Evolution

- Compared with conventional 1D-LC, 2D-LC can significantly improve separation power through unique selectivity pairings.
- 2D-LC has grown rapidly in both practical and theoretical aspects in the last few decades.  There are ~ 800 publications on 2D-LC since 2000, of which over 200 are published in the last four years.
- Significant progress has been made to address technical challenges with 2D-LC such as solvent mis-match, limitation on $^{2}$D cycle time, $^{2}$D sensitivity, complexity in both hardware and software.
- Turnkey commercial instruments are now available from multiple vendors.

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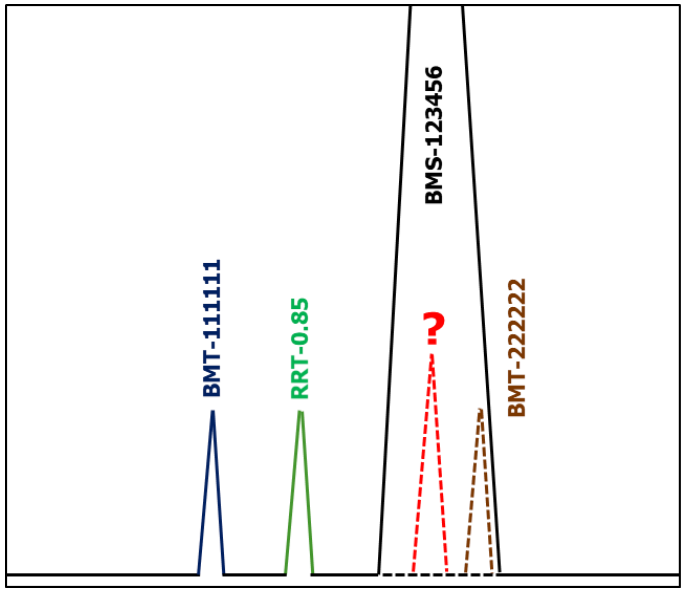
# Method Specificity/Selectivity

- **Impurity control for pharmaceutical compounds is critical for patient safety**
  - Known knowns: Identified impurities separated from the main
  - Unknown knowns: Un-identified impurities separated from the main
  - Known unknowns: Identified impurities not separated from the main
  - Unknown unknowns: Un-identified impurities not separated from the main
- Existing peak purity check tools, such as MS and PDA , have limitations, and are generally not very sensitive to low level impurities.
- Comprehensive column/mobile phase screening is a very powerful tool, but usually very time consuming (long run time and complicated process for impurity tracking).
- 2D-LC, combining with PDA and MS, can greatly improve confidence on detection of “unknown unknowns”.



# 2D-LC Peak Purity Check Workflow (Version 1)

- Use the existing HPLC method as the first dimension ($^{1}$D LC).
- For the second dimension ($^{2}$D LC), select orthogonal columns using **HSM** with on-line column database (http://www.hplccolumns.org).
- Change organic solvents between acetonitrile or acetonitrile with 20% methanol.
- Change mobile phase (MP) pH between acidic (0.05% TFA) and neutral (10 mM NH4OAc).
- Keep the other method conditions the same as 1D-LC.

# Identify Orthogonal LC Conditions

## $^1$D LC

Column: Ascentis Express C18 (2.1 x 150 mm, 2.7 µm)

MPA: 10 mM $NH_4OAc$ in Water: MeCN (90:10)

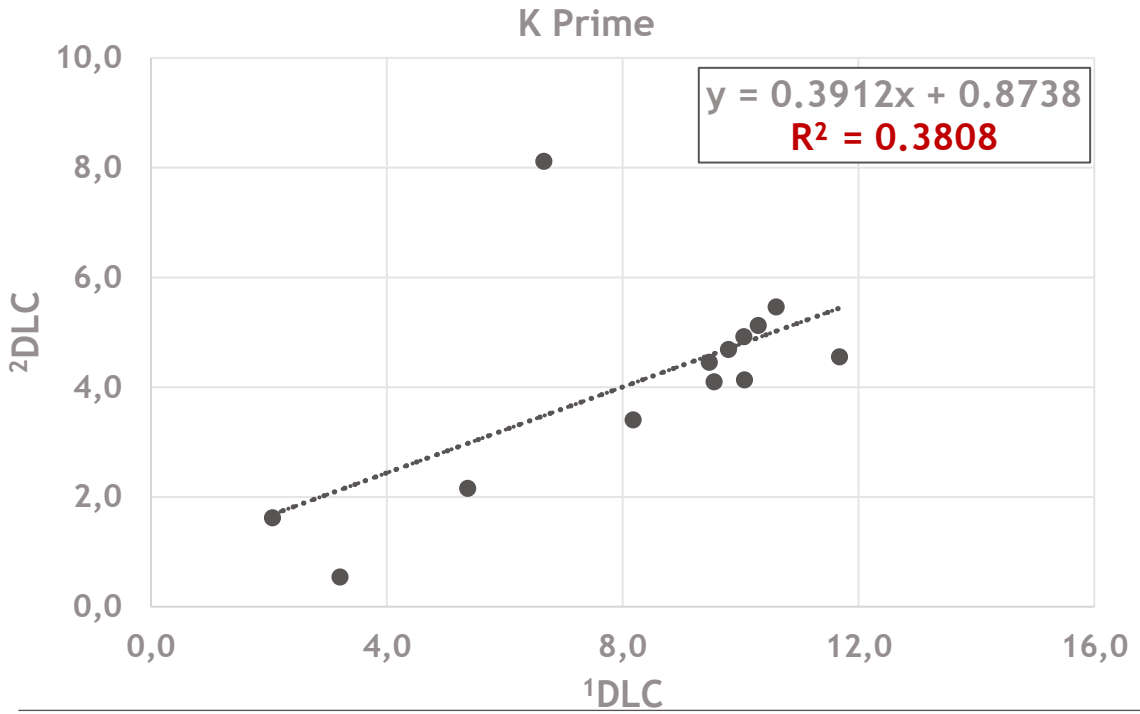
MPB: 10 mM $NH_4OAc$ in Water: MeCN (10:90)

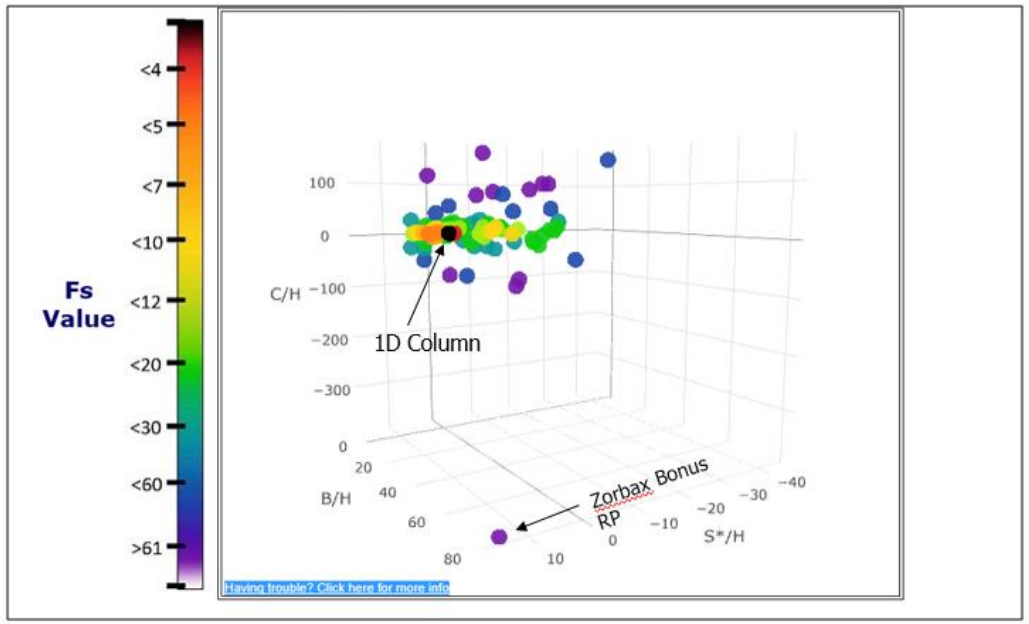
## $^2$D LC

Column: Zorbax Bonus-RP (2.1 x 100 mm, 1.8 µm)

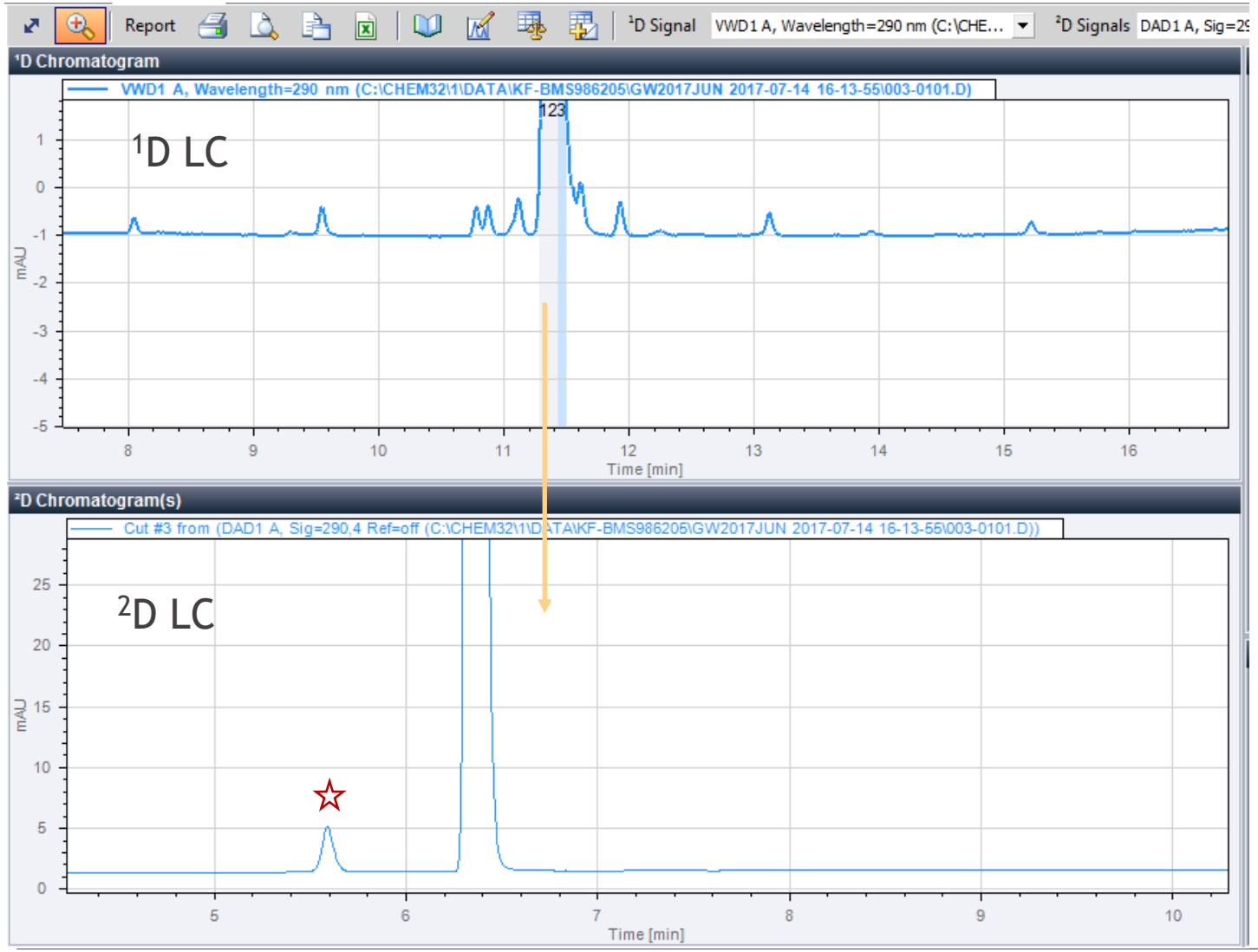
MPA: Water: MeOH (80:20) with 0.1% TFA

MPB: Water: MeOH: MeCN (10:20:70) with 0.1% TFA



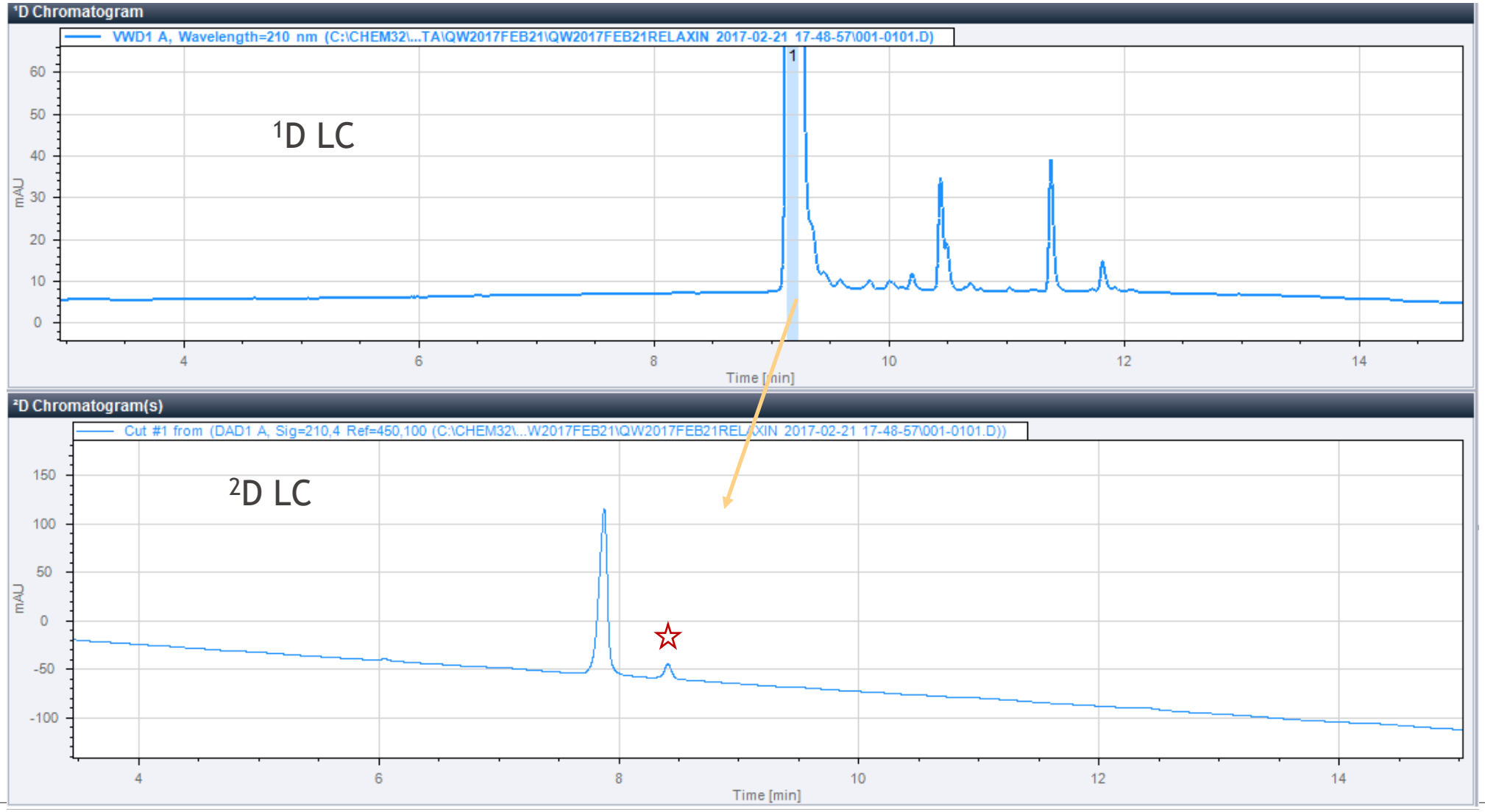



# 2D-LC Peak Purity Check Performance Evaluation



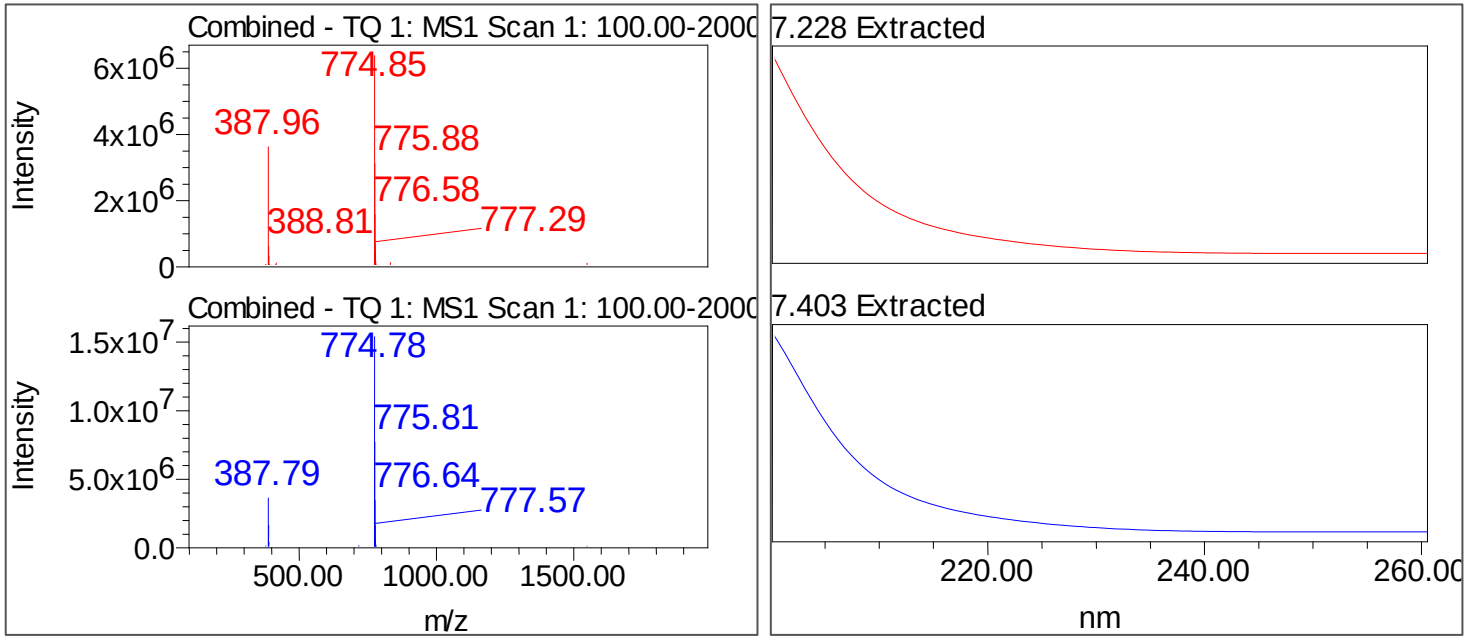

- Co-eluting impurity in ¹D LC was separated in ²D LC.
- No efficiency loss was observed.
- Sensitivity of ²D LC at **0.1AP** could be achieved.

# 2D-LC-MS Peak Purity Check - an Example



# 2D-LC-MS Peak Purity Check – an Example

## Mass Spectra and UV Spectra of Main Peak and Impurity




- Co-eluting isomeric impurity cannot be detected by PDA or MS

Top: Co-eluting isomeric impurity;  Bottom: Main peak

# 2D-LC-MS Peak Purity Check Workflow (Version 4)

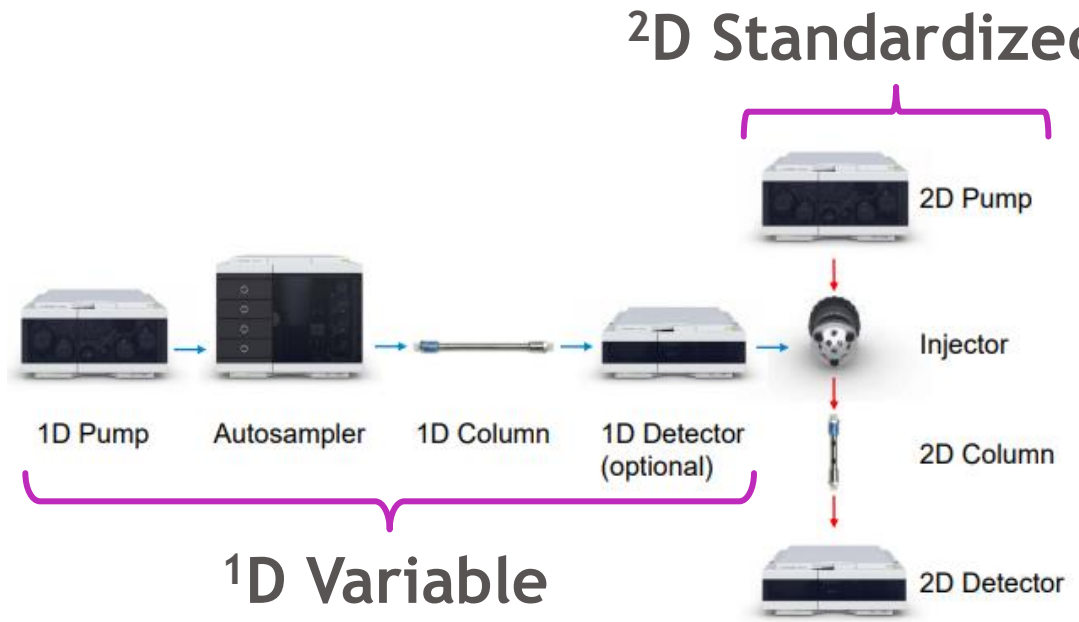
Gather $^{1}$D conditions ← SharePoint Online Submission Link

Collect samples, column and MPs

Select $^{2}$D MPs

Create $^{2}$D methods based on time-based triggering

Run $^{2}$D purity check and generate report



# Standardized $^{2}$D LC Conditions

## $^{2}$D MP selection based on $^{1}$D MPs:

| $^{1}$D MPs | $^{2}$D MP Selection |
|---|---|
| MeCN only, pH ≤ 4 | MeCN w/ MeOH, neutral |
| MeCN only, pH > 4 | MeCN w/ MeOH, acidic |
| MeCN w/ MeOH or MeOH only, pH ≤ 4 | MeCN only, neutral |
| MeCN w/ MeOH or MeOH only, pH > 4 | MeCN only, acidic |

## $^{2}$D Columns:

| Valve Position | Column Description |
|---|---|
| 1 | Zorbax Bonus RP, 4.6×100 mm, 1.8 um |
| 2 | Ascentis Express F5, 4.6×100 mm, 2.7 um |
| 3 | Halo Biphenyl, 4.6×100 mm, 2.7 um |
| 4 | Chiralpak IG-3, 4.6×100 mm, 3 um |
| 5 | Waters HSS C18 SB, 4.6x100 mm, 1.8 um |
| 6 | By-pass |

## Gradient:

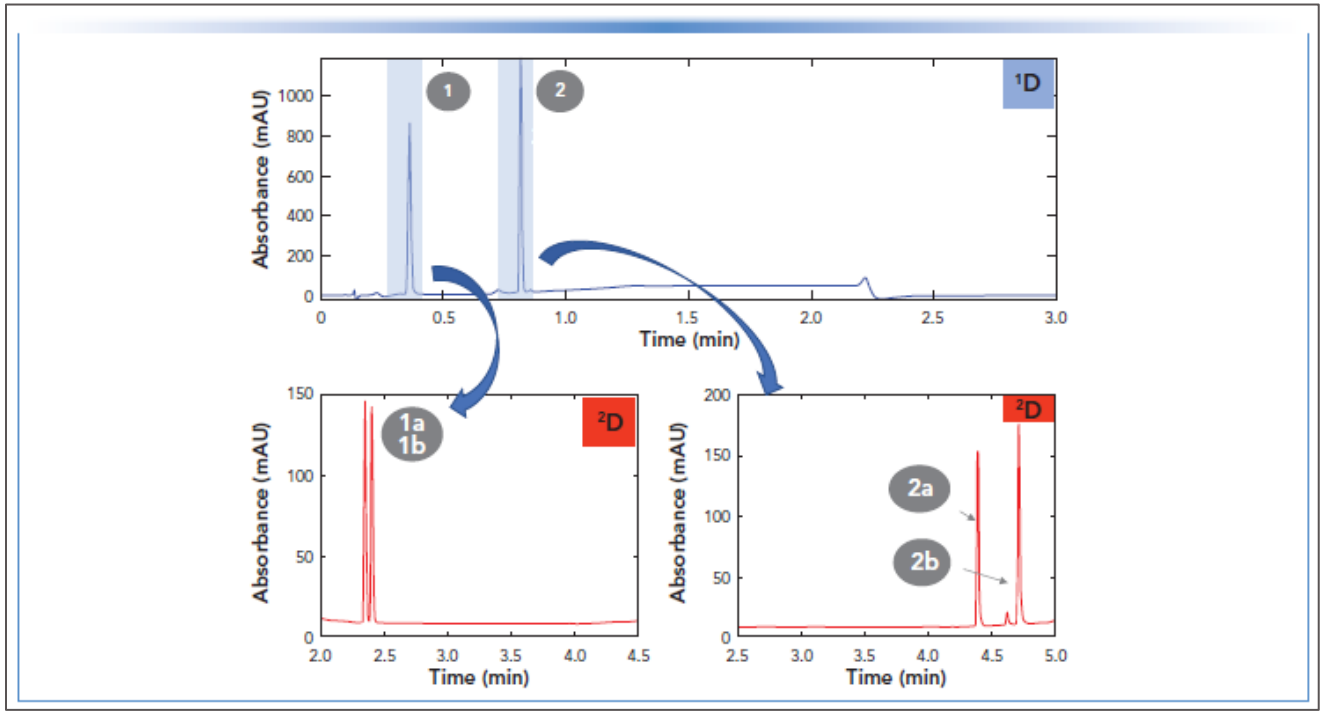
| Time | %A | %B |
|---|---|---|
| 0.00 | 100.0 | 0.0 |
| 1.00 | 100.0 | 0.0 |
| 16.00 | 0.0 | 100.0 |
| 17.00 | 0.0 | 100.0 |

## Other LC Conditions:

| | |
|---|---|
| Flow Rate: | 1.0 mL/min |
| Column Temperature: | 40 °C |
| UV Wavelength: | Same as $^{1}$D |
| MS: | Full Scan |

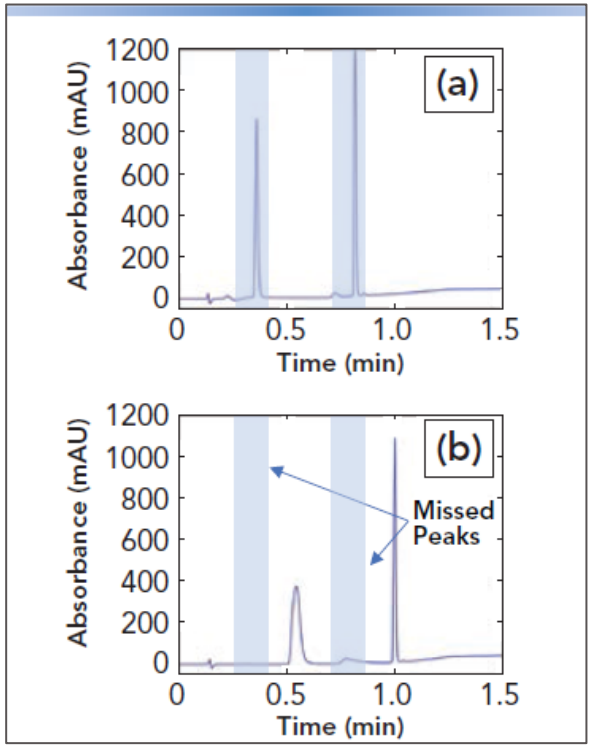
# Overcoming Technical Challenges for Routine Use

## Generic 2DLC system suitability test (SST) to verify if the 2DLC instrument functions properly




Good

Bad




*Baghdady YZ, Stoll DR, LCGC, 2022, 40:292*

# Overcoming Technical Challenges for Routine Use

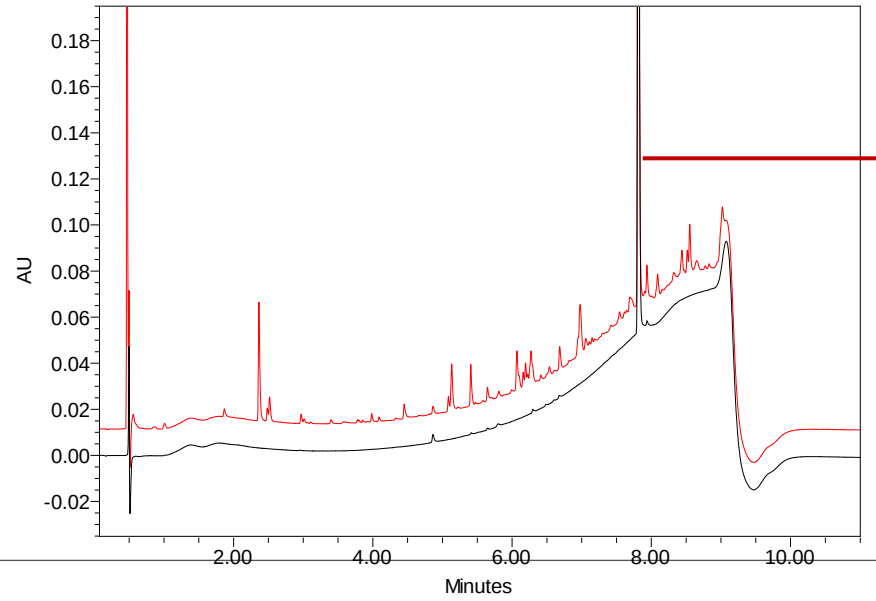
## Complete $^{1}$D peak coverage

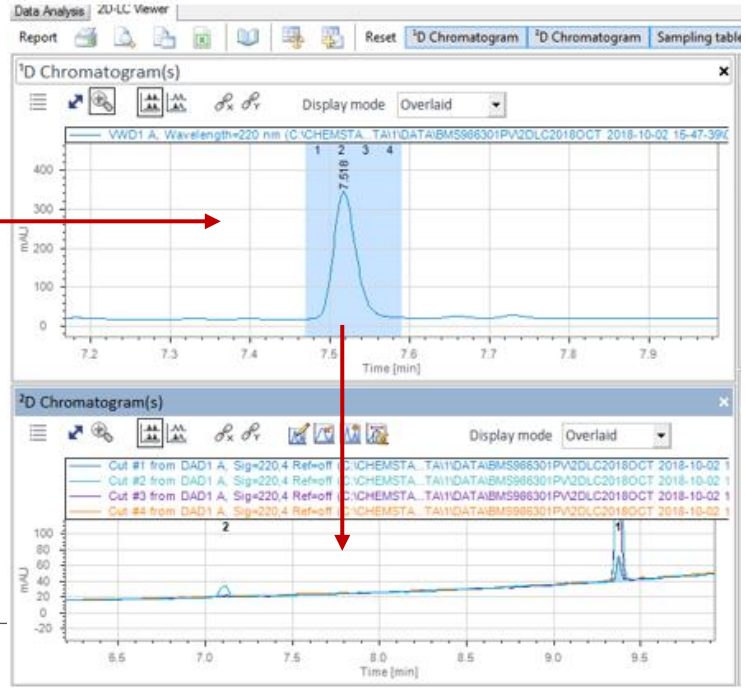
- Large loop size (120-180 µL to replace 40 µL default)
- Multi-Injection feature with new OpenLab CDS software
- Maximize ASM factor (0.12x85 mm ASM capillary, ASM ratio: 5)
- Start gradient with low solvent strength (0-5%)

# Overcoming Technical Challenges for Routine Use

## Detect false positive (In-loop degradation)

- Repeat $^{2}$D LC with 1D-LC-MS to confirm co-elution
- Understand sample stability
  - Repeat 1D-LC with longer initial gradient holding time
  - Multi-injection with OpenLab CDS



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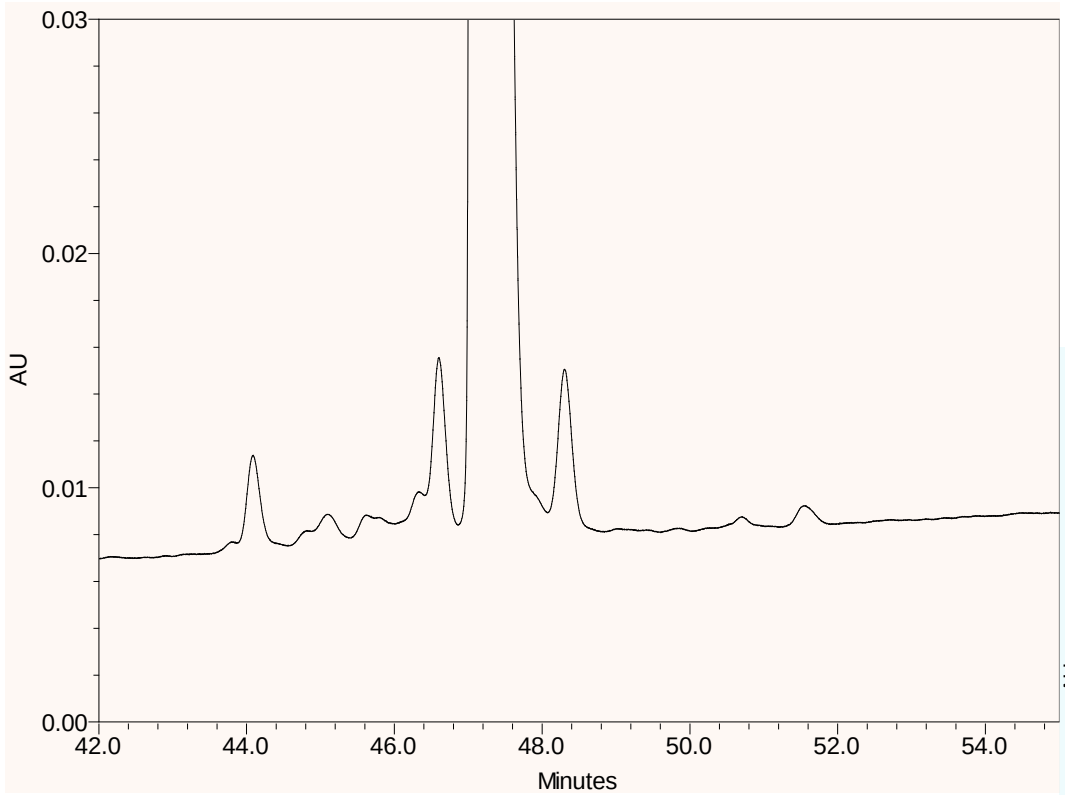
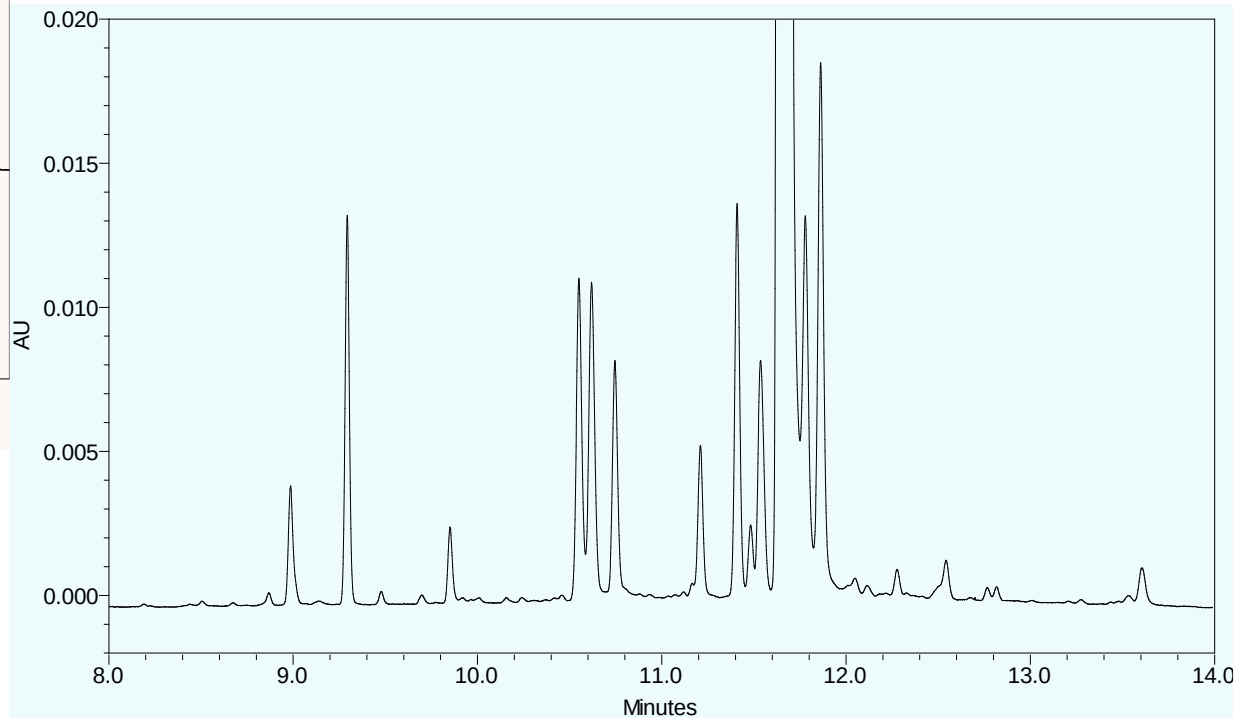
# 2D-LC Quantitative Impurity Analysis

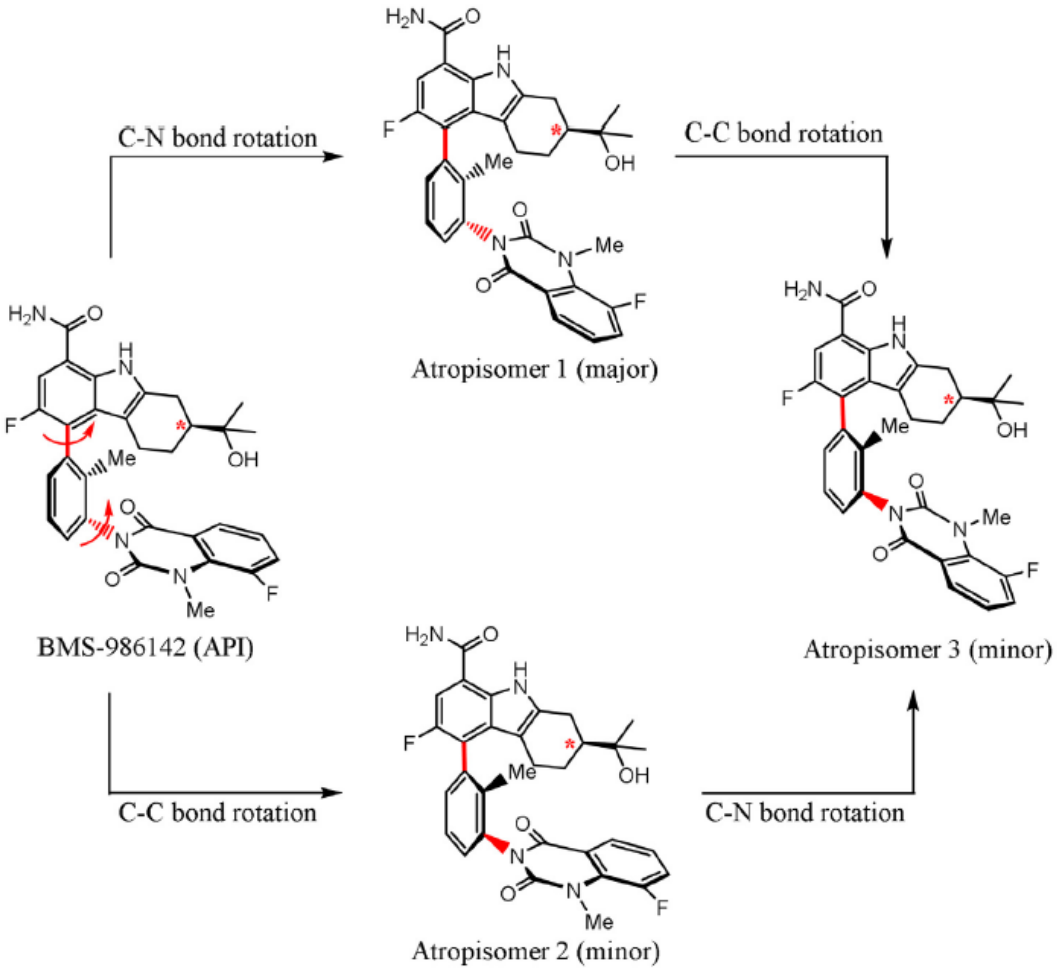






# 2D-LC Quantitative Impurity Analysis – an Example

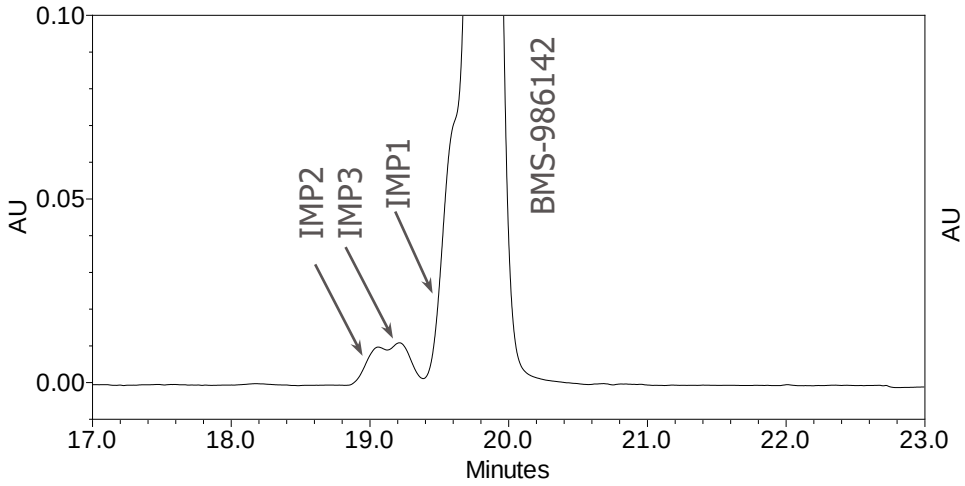
- The test molecule, BMS-986142, contains two rotational axes which results in three potential atropisomeric impurities (IMP1, IMP2 and IMP3).
- Separation of BMS-986142 from these impurities by achiral LC was very challenging. Three atropoisomeric impurities can only be partially resolved from each other and BMS-986142.

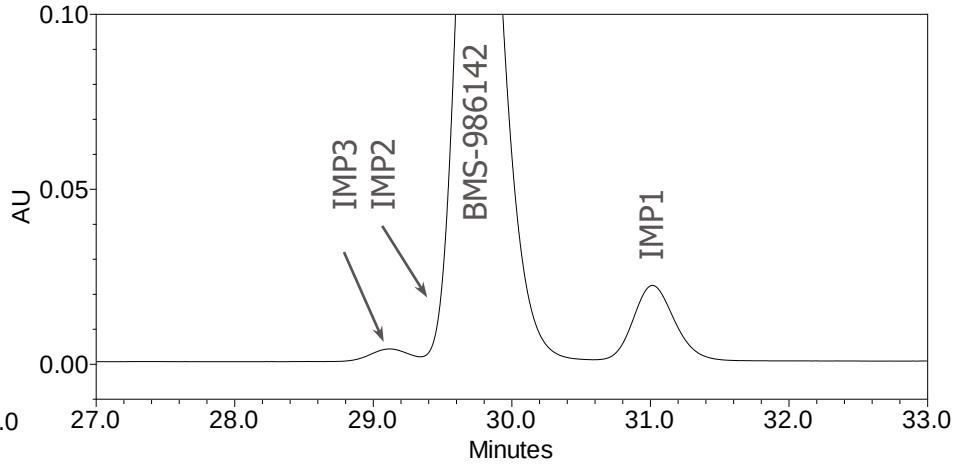



*Wang Q, He B, Shackman J, JPBA, 2021, 193:113730*

# Chromatographic Conditions

| $^{1}$D LC | $^{2}$D LC |
|---|---|
| **Column:** BEH Shield RP18 (100x2.1 mm, 1.7 µm) | Column: Pinnacle DB BiPh (150x4.6 mm, 3 µm) |
| **MPA:** 10 mM NH4HCOO, pH 3 | MPA: 10 mM NH4HCOO, pH 3 |
| **MPB:** MeOH:MeCN 30:70 | MPB: MeOH |
| **Gradient:** 0/30/35 min, 40/65/80 %B | Gradient: 0/40 min, 60/90 %B |
| **Flow rate:** 0.2 mL/min | Flow rate: 0.6 mL/min |
| **Detection:** 320 nm | Detection: 320 nm |



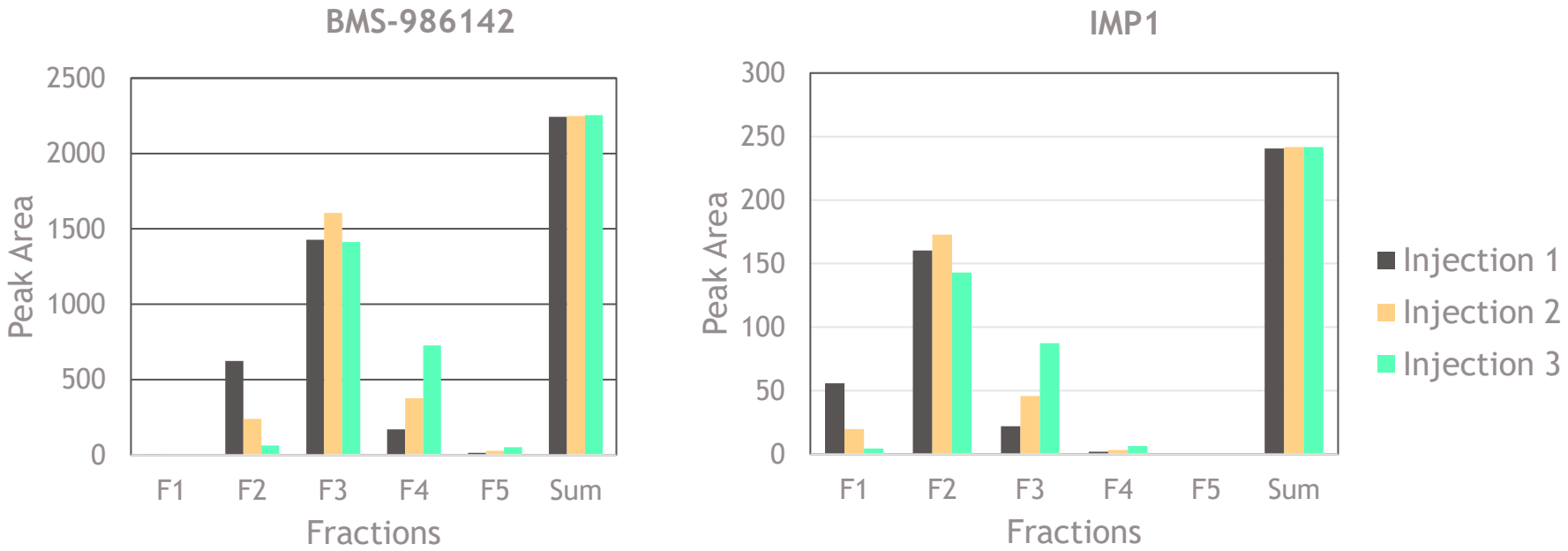

*Wang Q, He B, Shackman J, JPBA, 2021, 193:113730*

# 2D-LC with High Resolution Sampling


*Wang Q, He B, Shackman J, JPBA, 2021, 193:113730*

# 2D-LC with High Resolution Sampling

- **Quantitative Results**

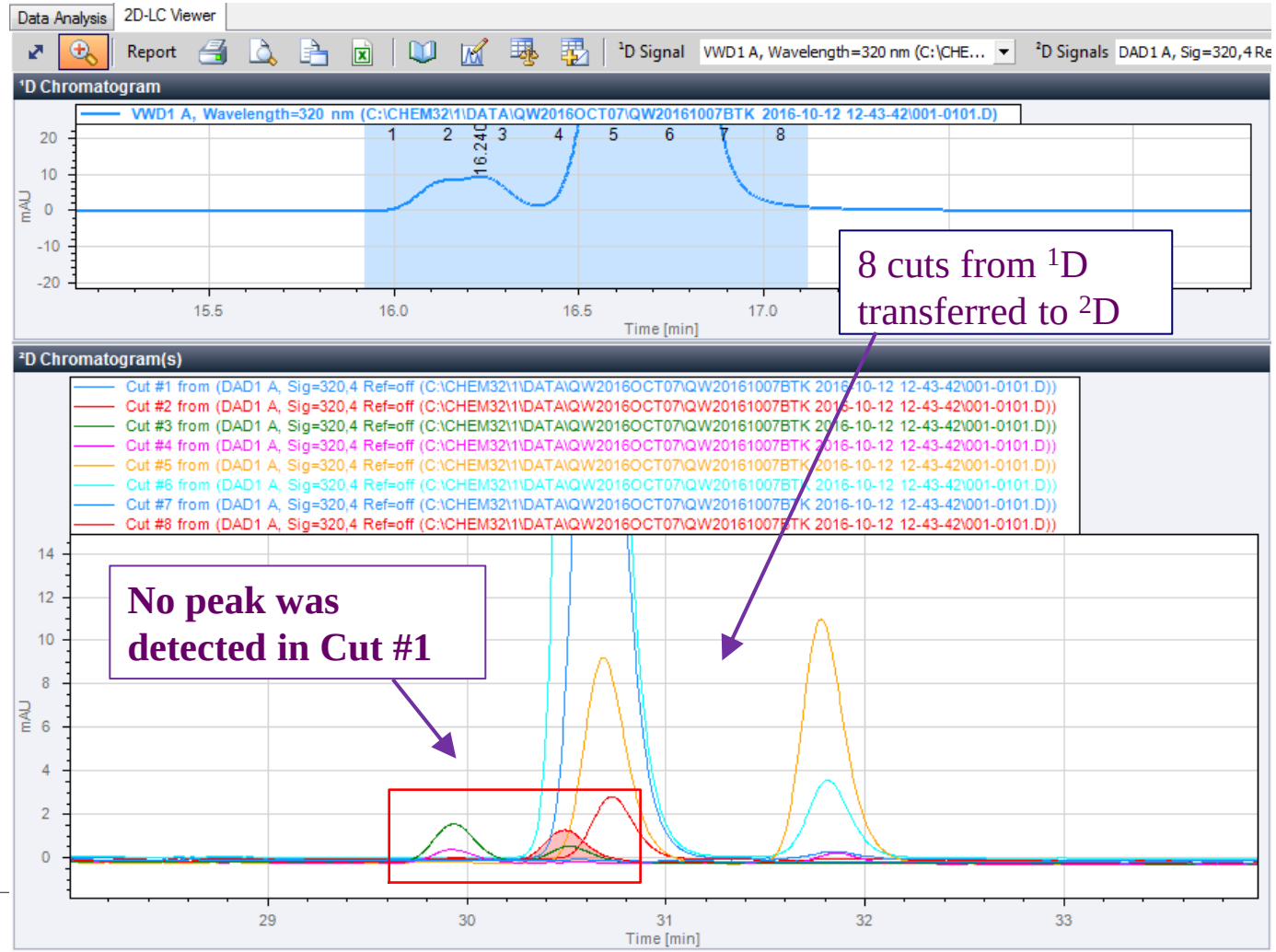



- Large variabilities were observed for individual fractions, however, sum of peak areas were consistent among injections (%RSD=0.3 for both peaks, n=3).
- Average recovery of IMP1 is 101.2% (%RSD=0.2, n=3)

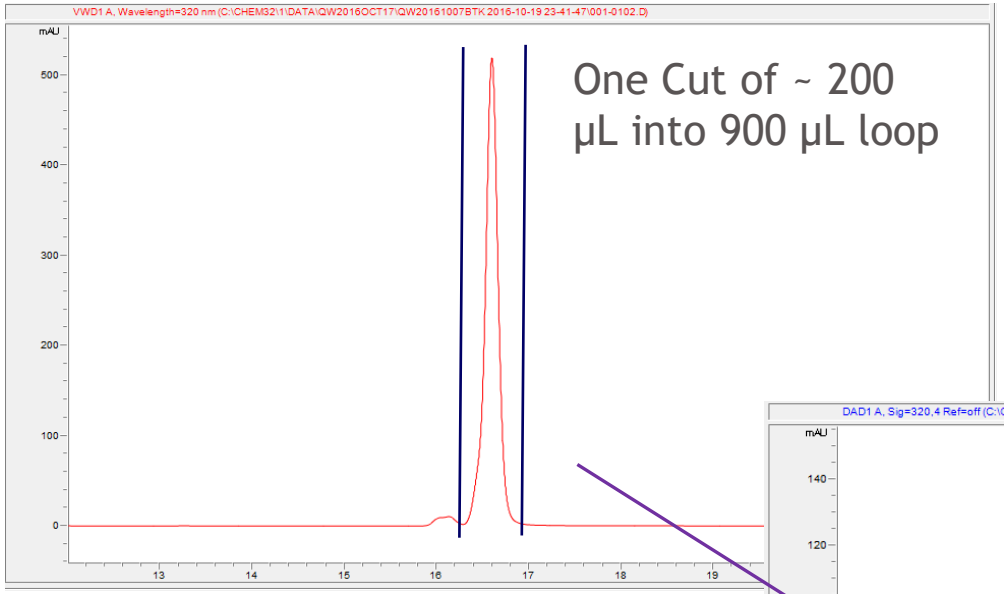
*Wang Q, He B, Shackman J, JPBA, 2021, 193:113730*

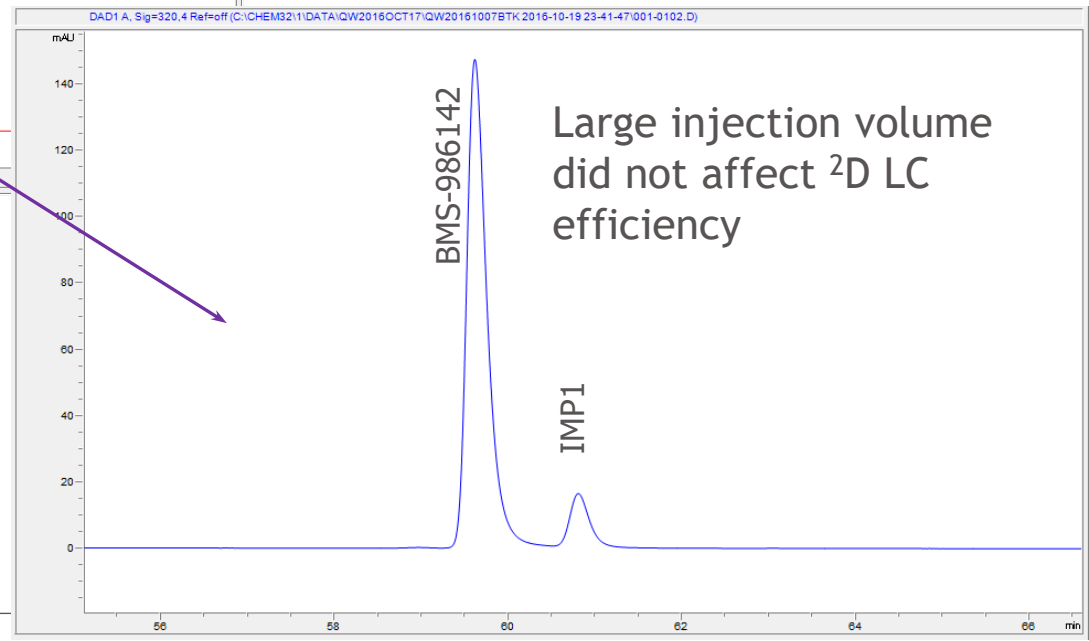
# 2D-LC with High Resolution Sampling - Sensitivity Evaluation

- Multiple consecutive cuts are required to cover the whole $^{1}D$ peak, some levels of sensitivity loss will happen for $^{2}D$ peaks.



# Heart-Cutting with On-line Dilution



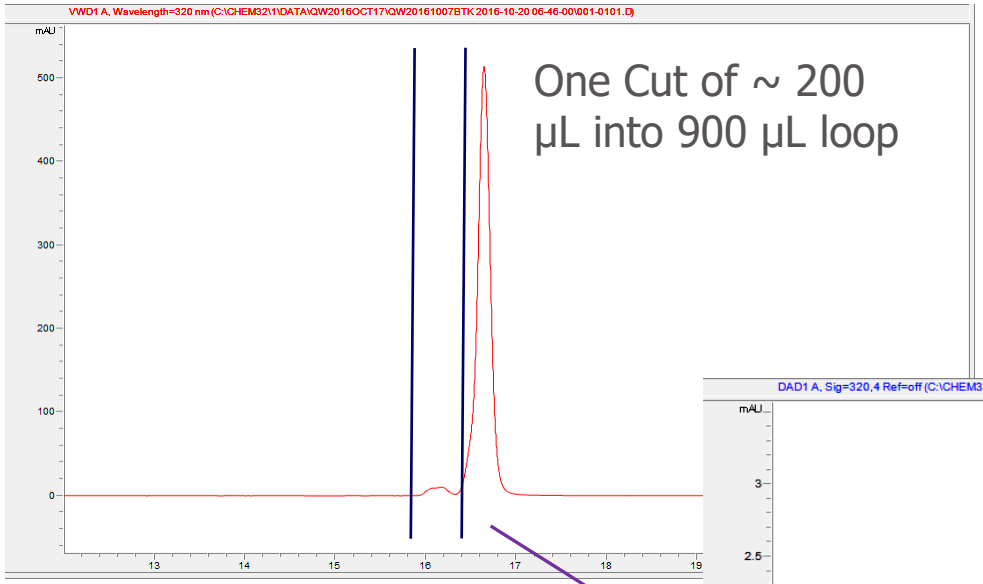



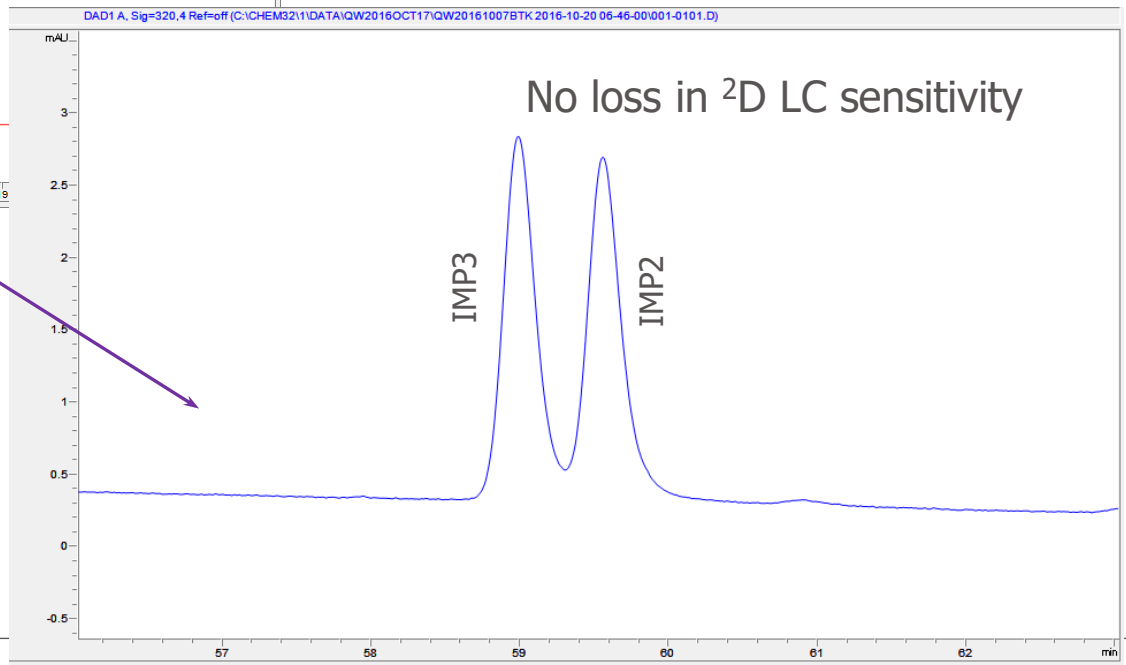

- Peak area %RSD= 0.08% for BMS-986142; and 2.1% for IMP1 (n=4);
- Average recovery of IMP1 is 96.8%.



*Wang Q, He B, Shackman J, JPBA, 2021, 193:113730*

# Heart-Cutting with On-line Dilution




- Peak area %RSD= 0.3% for IMP2; and 3.1% for IMP3 (n=3);
- Average recovery of IMP2 is 98.8%.



*Wang Q, He B, Shackman J, JPBA, 2021, 193:113730*

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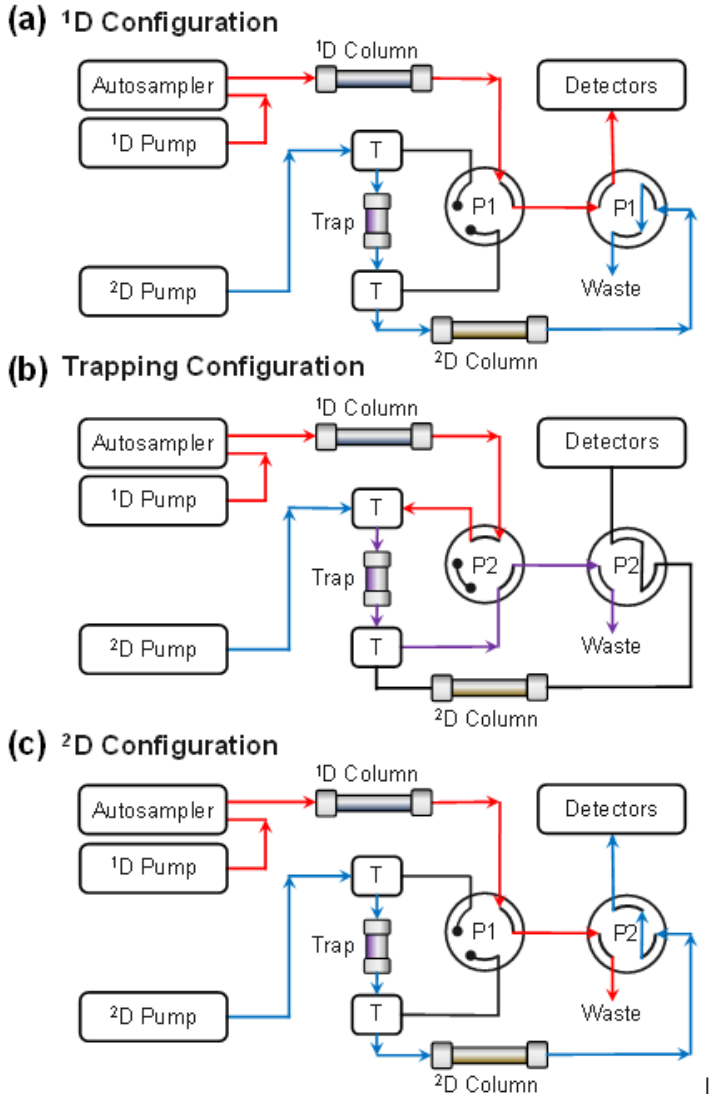
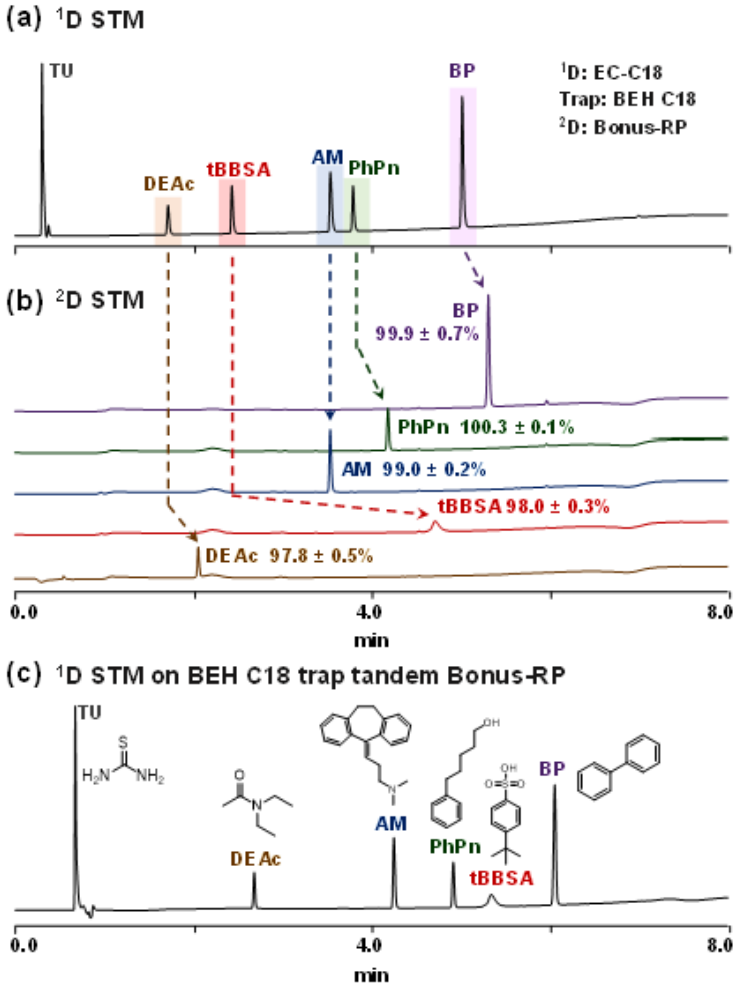
# Schematic Diagram of Trapping Mode 2D-LC

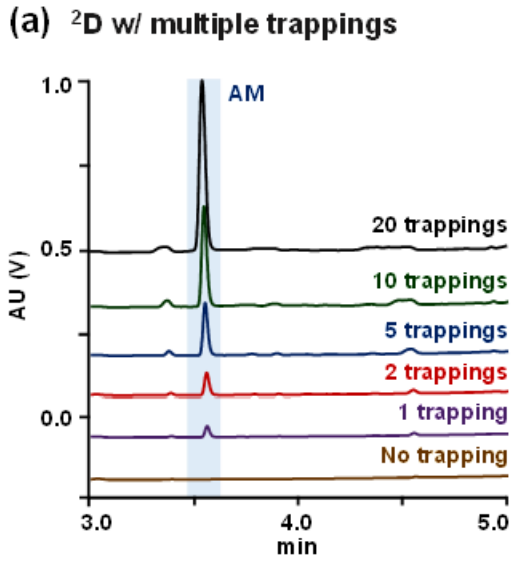
- H-Class UPLC components controlled by Empower
- Quantitative low-level impurity enrichment

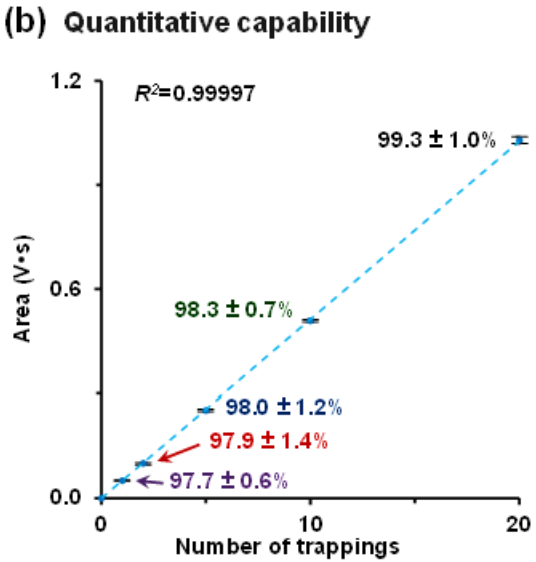



*Lin Z, Wang Q, Zhou Y, Shackman J, JCA, 2023, 1700:464043*

# Performance Verification




- Trapping can be done for analytes with wide range of hydrophobicity
- Quantitative enrichment over 20 trappings

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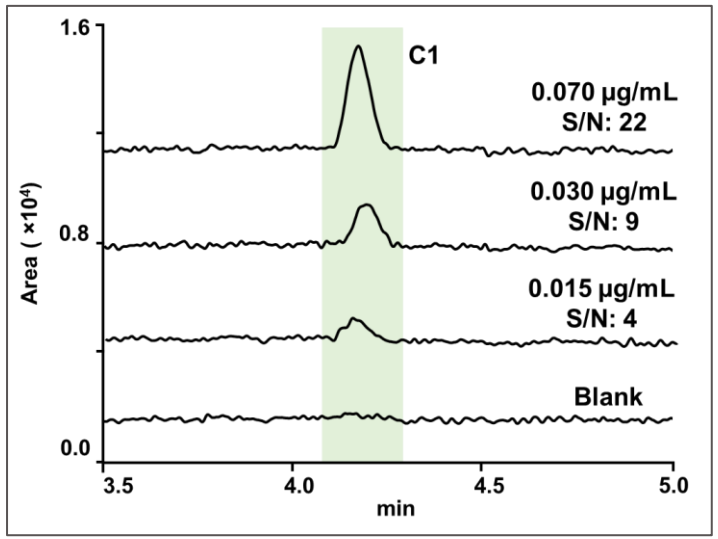
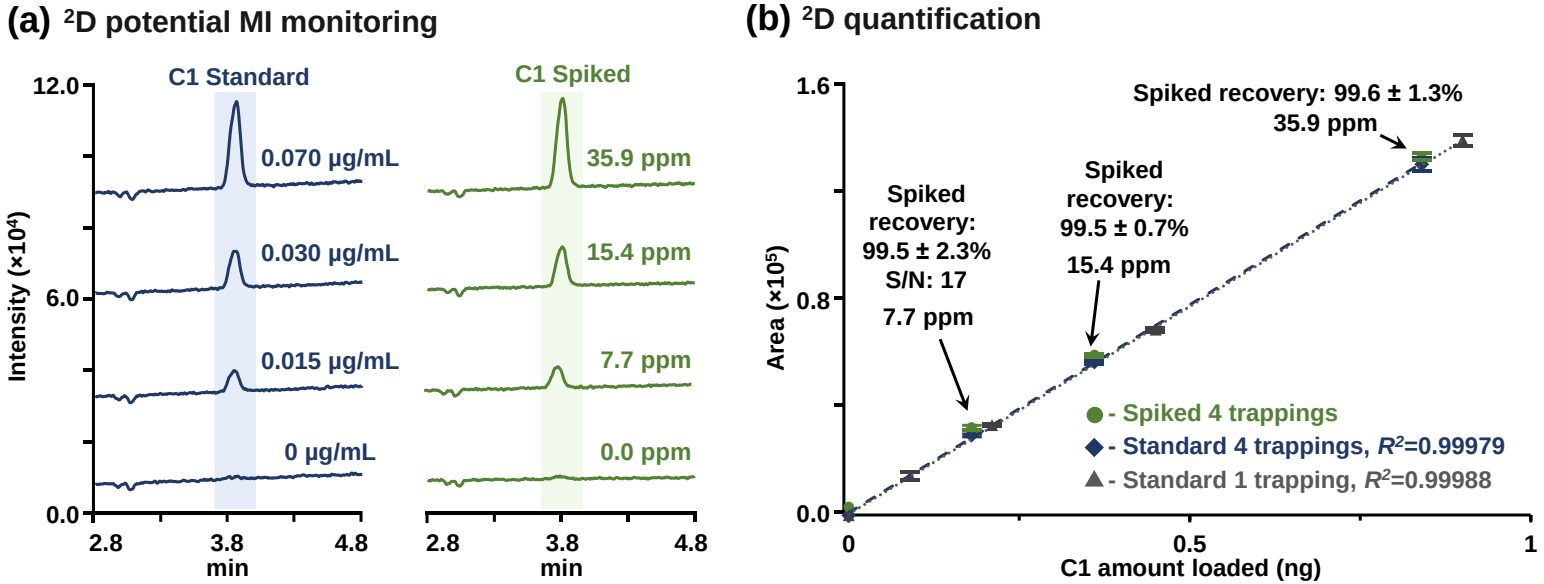
# Quantitative Trapping of a Mutagenic Impurity




Without trapping




With 4 trappings

*Lin Z, Wang Q, Zhou Y, Shackman J, JCA, 2023, 1700:464043*

# Summary

- An overview of BMS efforts in exploring 2D-LC for fast knowledge generation in chemical process development was presented. The examples include peak purity check by orthogonal RPLC-RPLC-MS, 2D-LC quantitative impurity analysis, and quantitative enrichment of trace level impurities by trapping mode 2D-LC.
- The results demonstrate that 2DLC is a powerful tool in understanding chemical processes, as well as ensuring analytical method quality for pharmaceutical development.
- For certain types of synthetic drug candidates with complex impurity profiles, 2D-LC has the potential to become the default technique for impurity analysis.

# Acknowledgement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