Introducing automated online multicolumn two-dimensional liquid chromatography screening as a rapid and efficient tool for method development of multiple pipeline modalities

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Outline

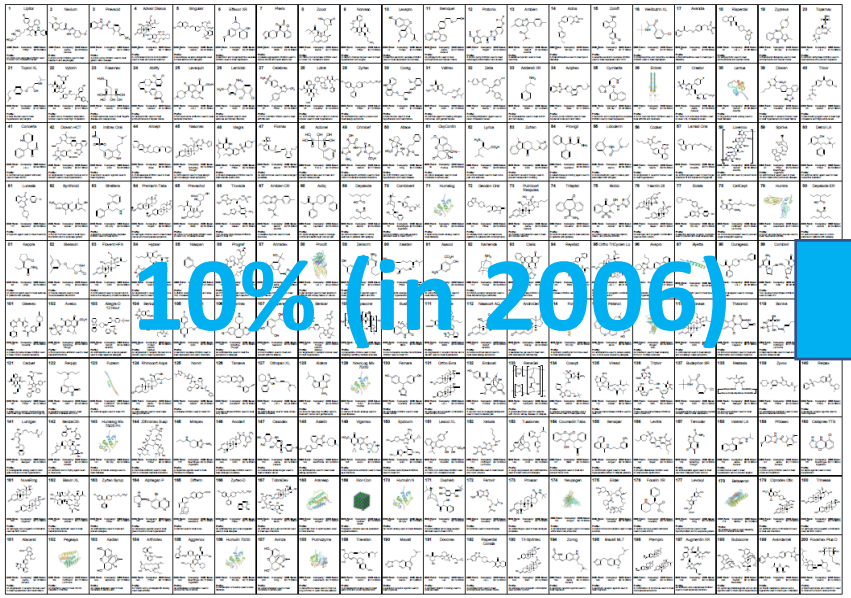
- Therapeutic modalities become more complex
- Challenges of the traditional analytical workflows
- Online multicolumn 2D-LC-DAD-MS screening system
- Applications

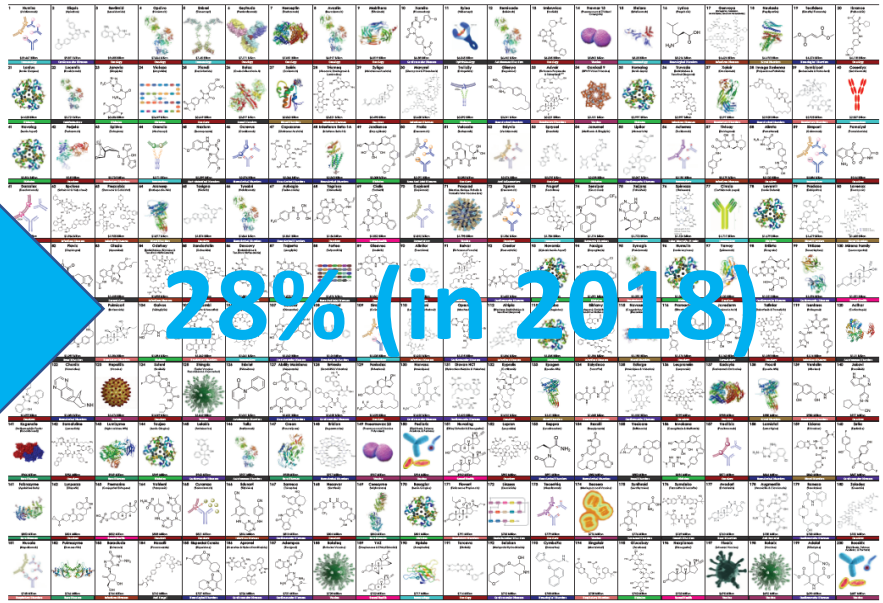
Transitioning from a small molecule–dominated focus to a more balanced portfolio

- Non-SM increased from 10% in 2006 to 28% in 2018
- "non–small molecule" drugs include monoclonal antibodies, engineered proteins, oligonucleotides, and vaccines

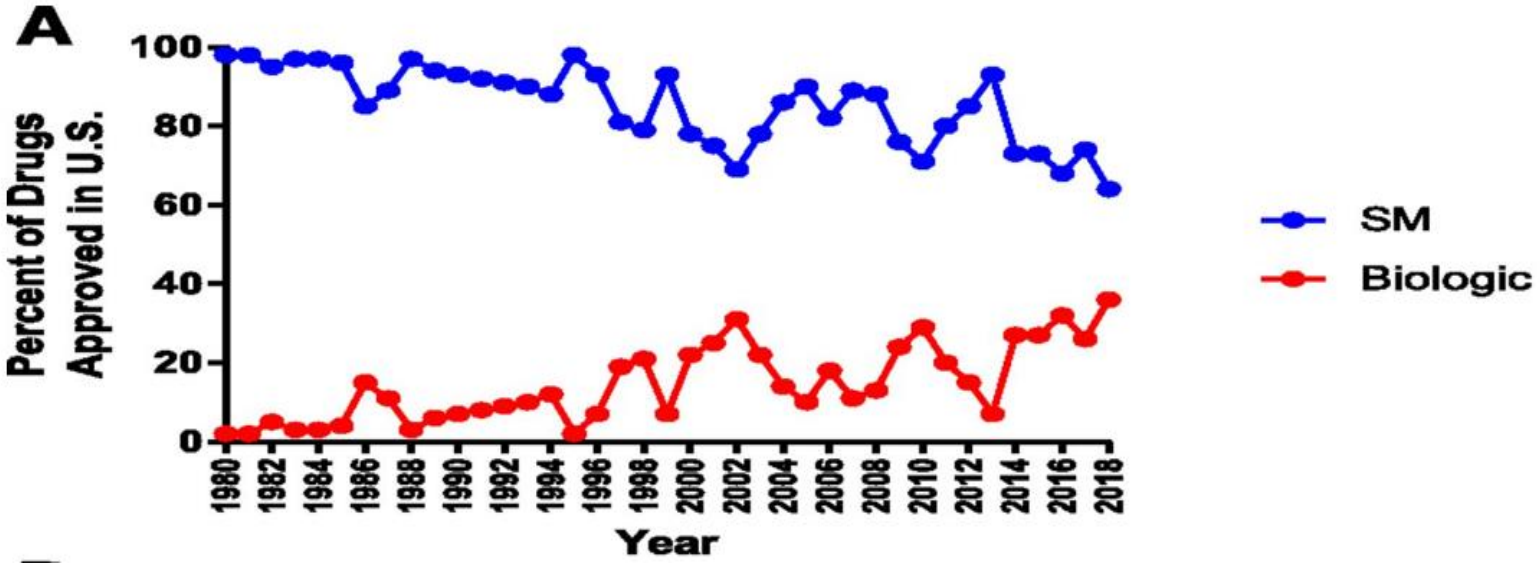
Percentage of Non-SM Drugs in Top 200 Drugs by sales

by Njarðarson Group at University of Arizona

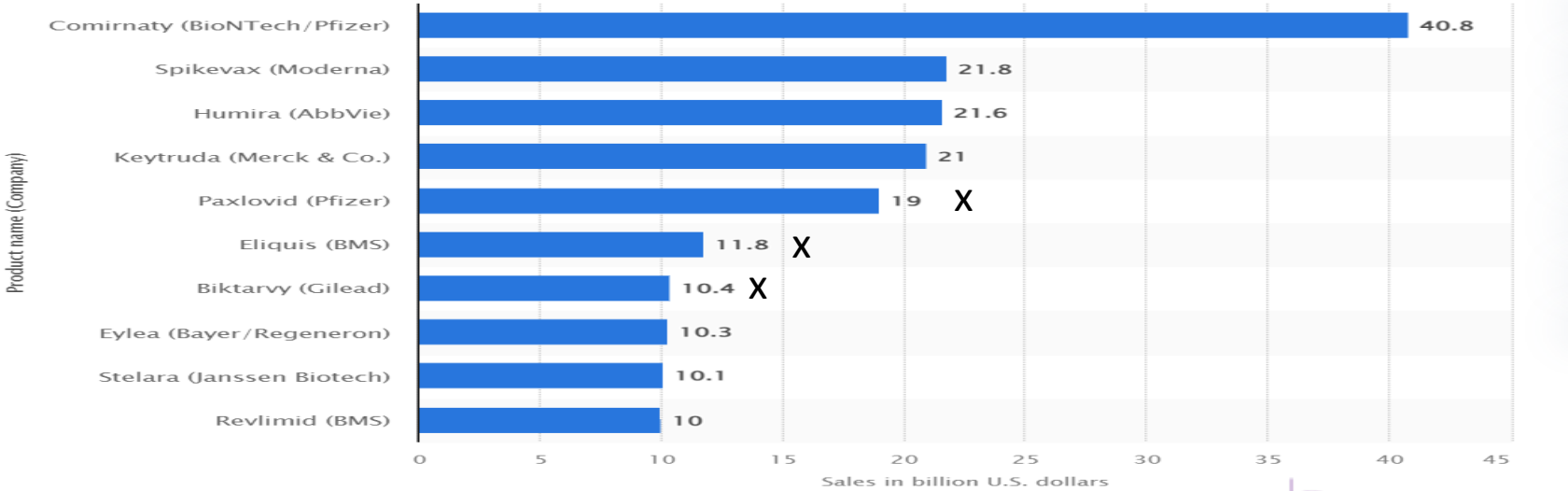




Drug approvals by FDA



Sales of top 10 best-selling drugs in 2022



Brooke M. Rock, and Robert S. Foti Drug Metab Dispos 2019;47:1097-1099

ASPET DRUG METABOLISM AND DISPOSITION

Increasing drug complexity

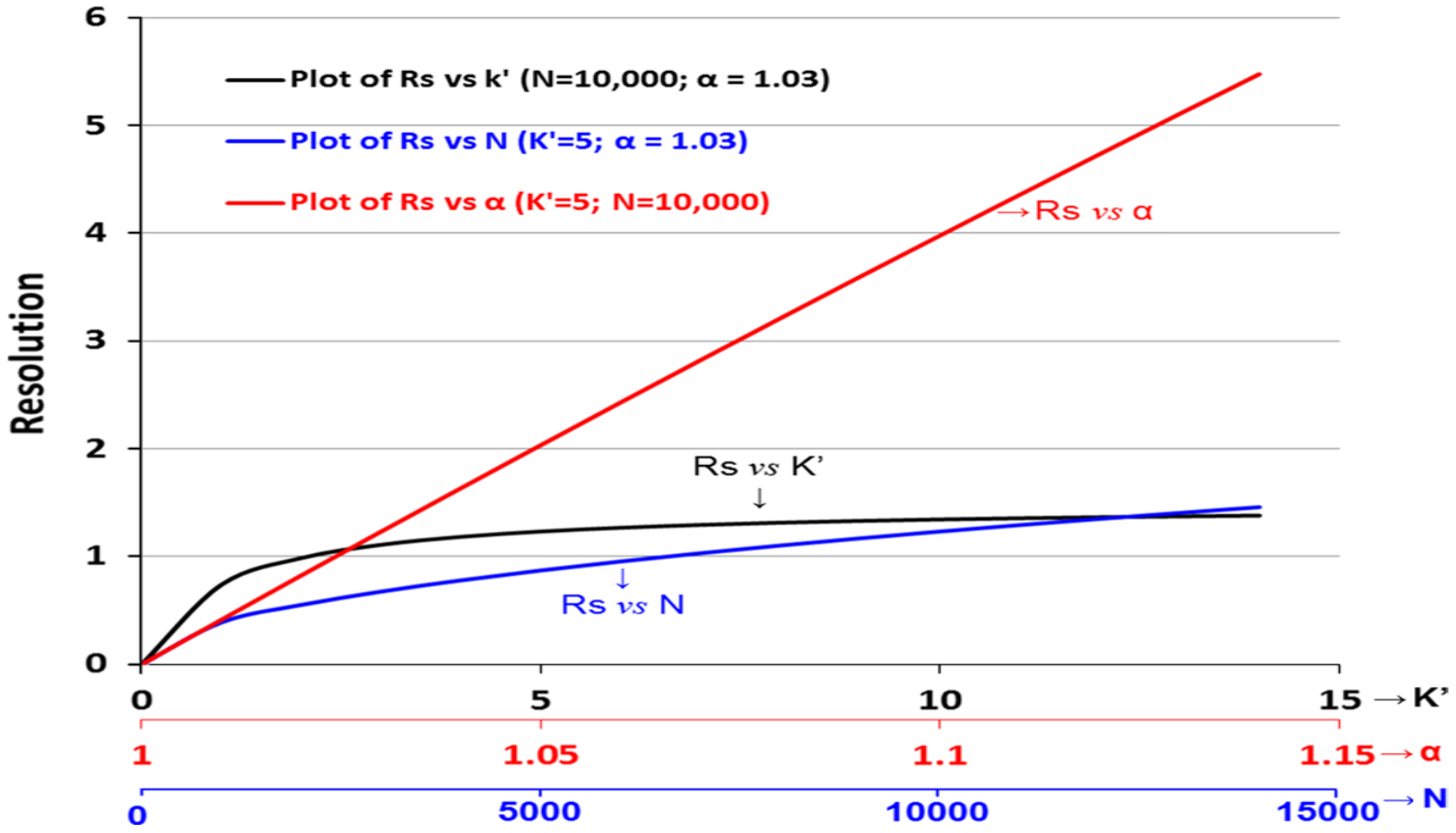
Complex Drugs

Challenge to method development

As more and more large molecule products are being developed, analytical characterization of biologics becomes incredibly challenging.

Goal: Pioneer analytical method development to handle increasingly complex targets

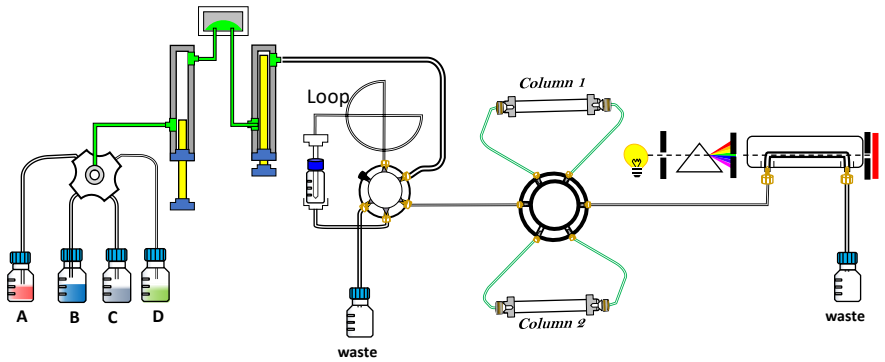
$$R_s = \frac{\sqrt{N_2}}{4} \cdot \left[\frac{\alpha - 1}{\alpha}\right] \cdot \left[\frac{k_2}{k_2 + 1}\right]$$



Standard Method Development Practices

- Following preferred method development scheme based on experiences
- Using method development software (ACD , LC-simulator, etc.)
- Evaluating and screening multiple columns/multiple mobile phases in a manual or automated fashion

Power of screening in method development



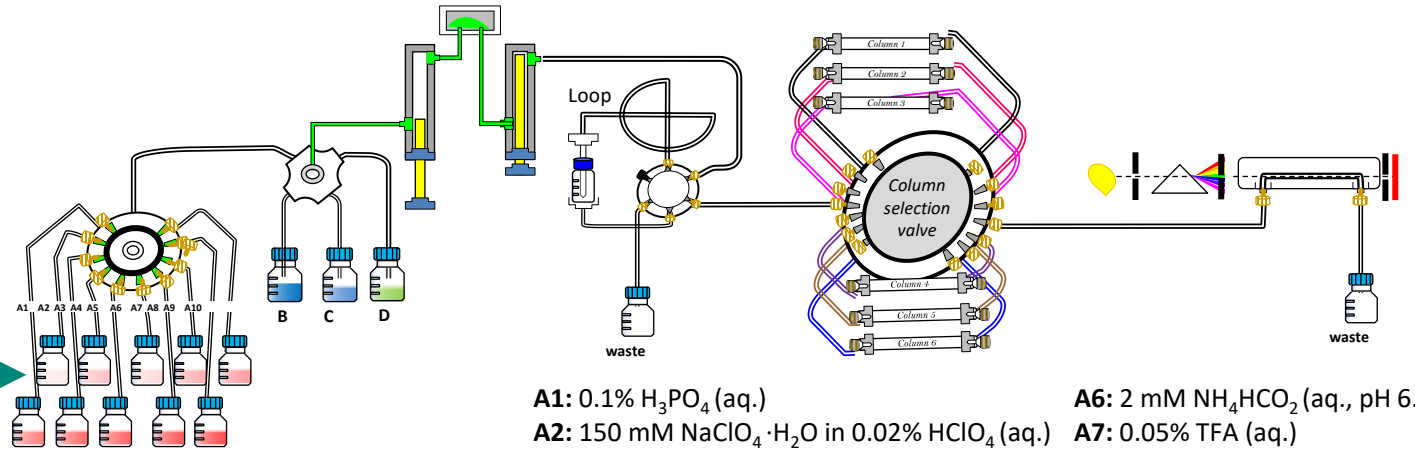
2 x 2 x 2 Permutations

Traditional HPLC system

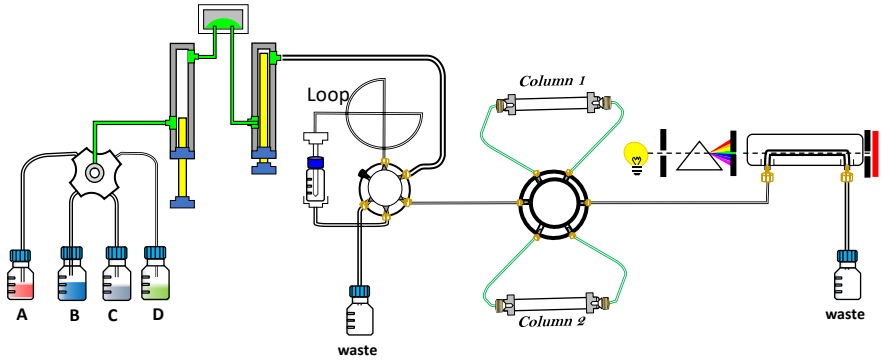
Power of screening in method development

7 x 2 x 6 Permutations

UHPLC screening system



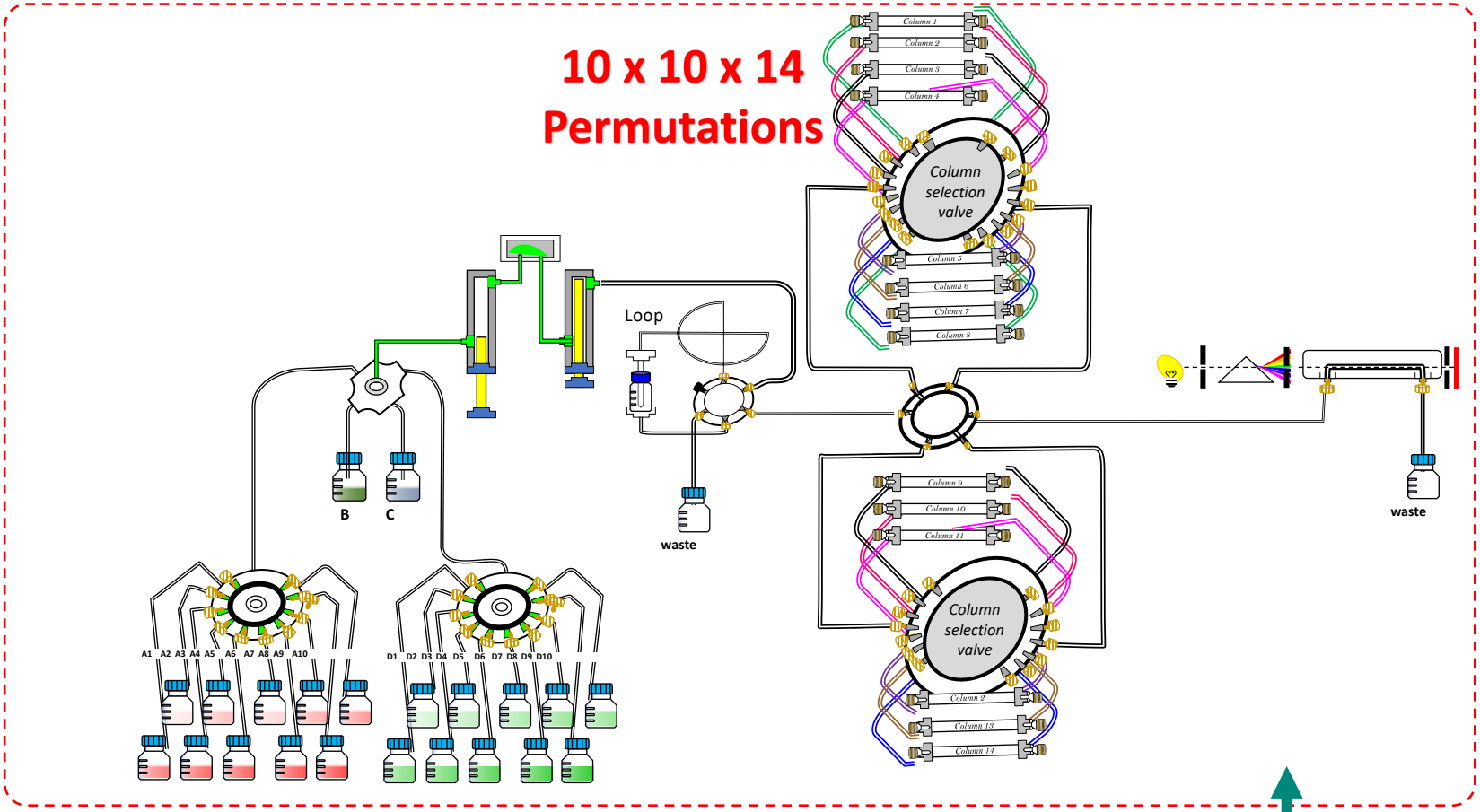
A1: 0.1% H_3PO_4 (aq.)
A2: 150 mM $NaClO_4 \cdot H_2O$ in 0.02% $HClO_4$ (aq.)
A3: 5 mM $(NH_4)_2HPO_4$ (aq.)
A4: 35 mM KPF_6 in 0.1% H_3PO_4 (aq.)
A5: 10 mM CH_3COONH_4 (aq., pH 6.5)
A6: 2 mM NH_4HCO_2 (aq., pH 6.5)
A7: 0.05% TFA (aq.)
B: CH_3CN
C: CH_3OH
D: H_2O



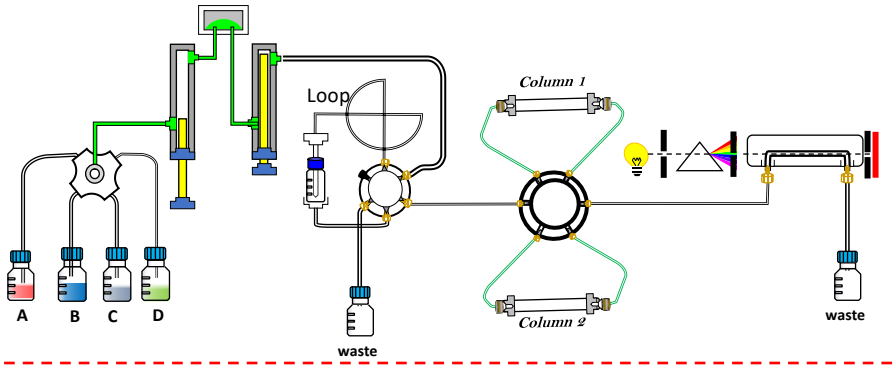
2 x 2 x 2 Permutations

Traditional HPLC system

Power of screening in method development



Screening capability added

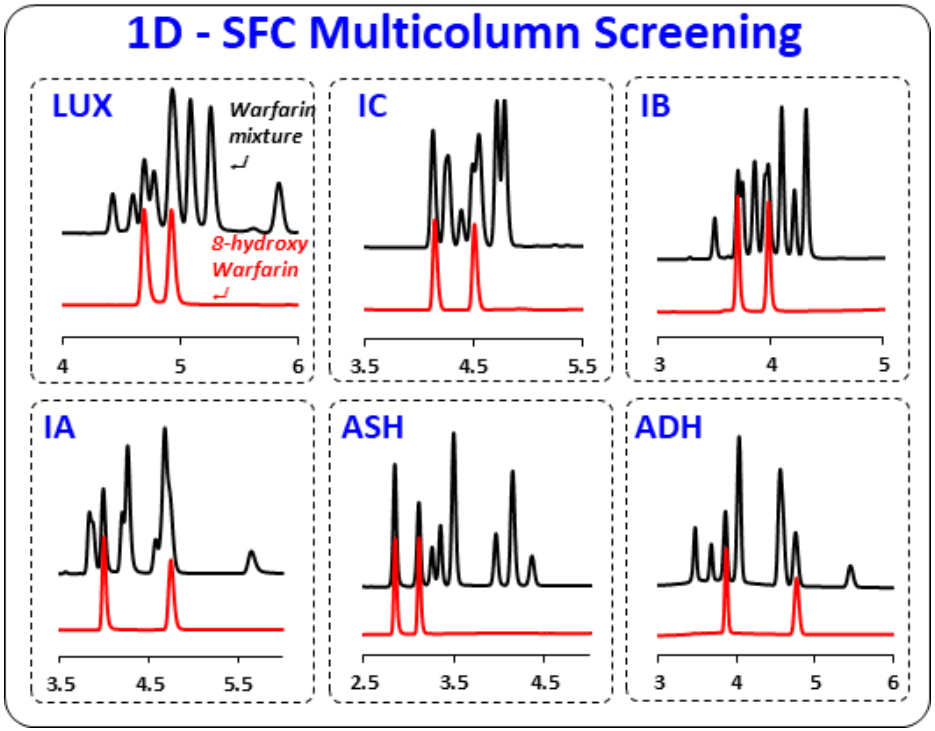


2 x 2 x 2
Permutations

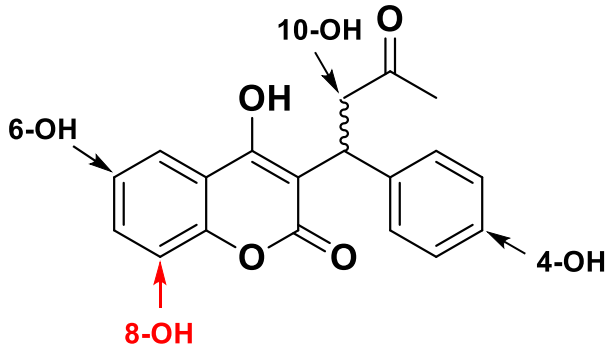
Typical HPLC system

Challenges in One-Dimensional Liquid Chromatography

Challenges exist even with most powerful screening techniques. . .



Warfarin and hydroxylation positions



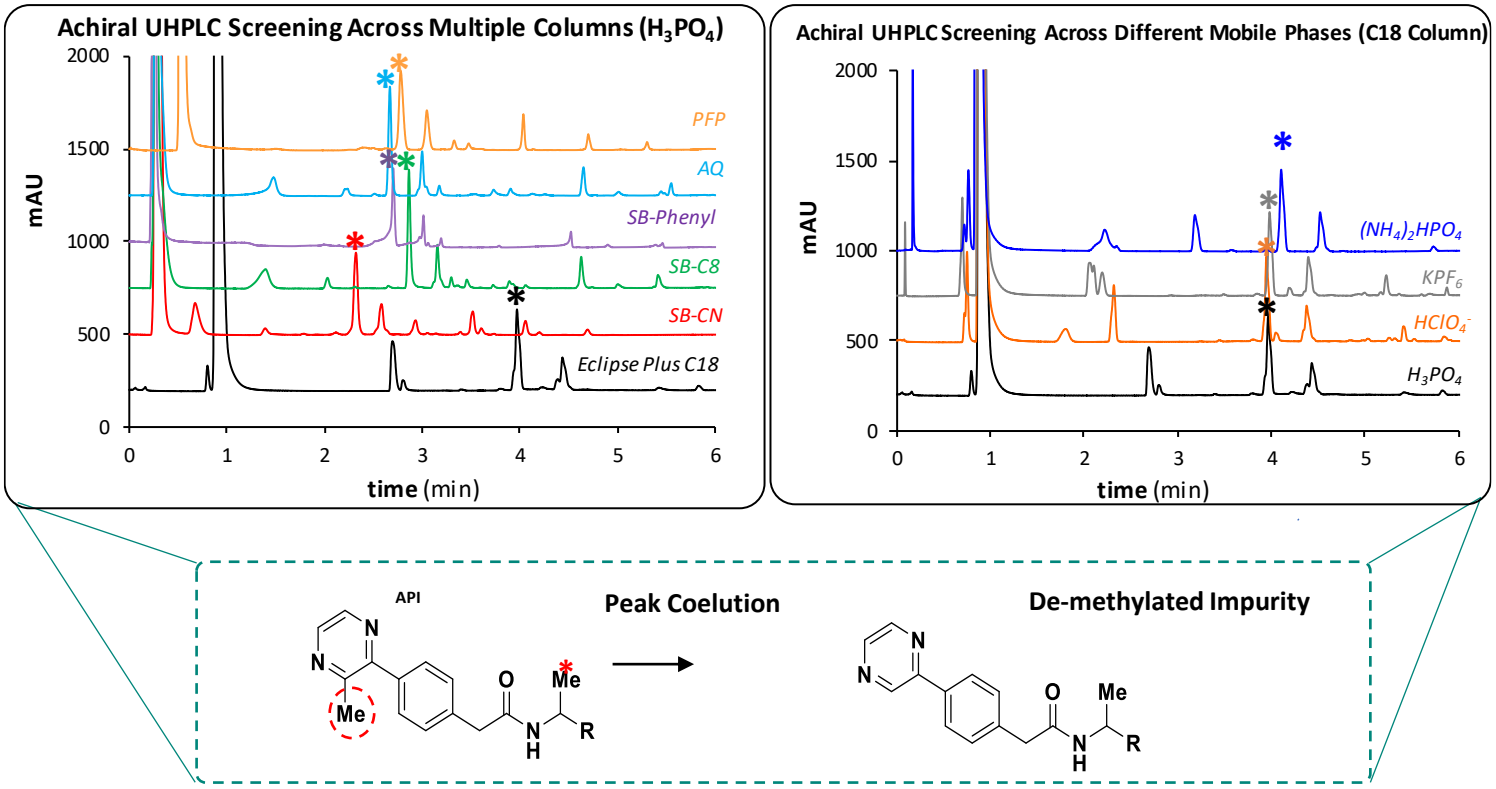
- Mixture contains species of multiple stereoisomers
- With SFC each screening, retention times of the enantiomer pair shifts
- Difficult to identify by peak areas
- MS does not help

Current analytical methods lack selectivity to handle the increasing complexity of pharmaceutical development

Multidimensional separation is a must-have tool

Challenges in One-Dimensional Liquid Chromatography

Challenges exist even with most powerful analytical techniques. . .



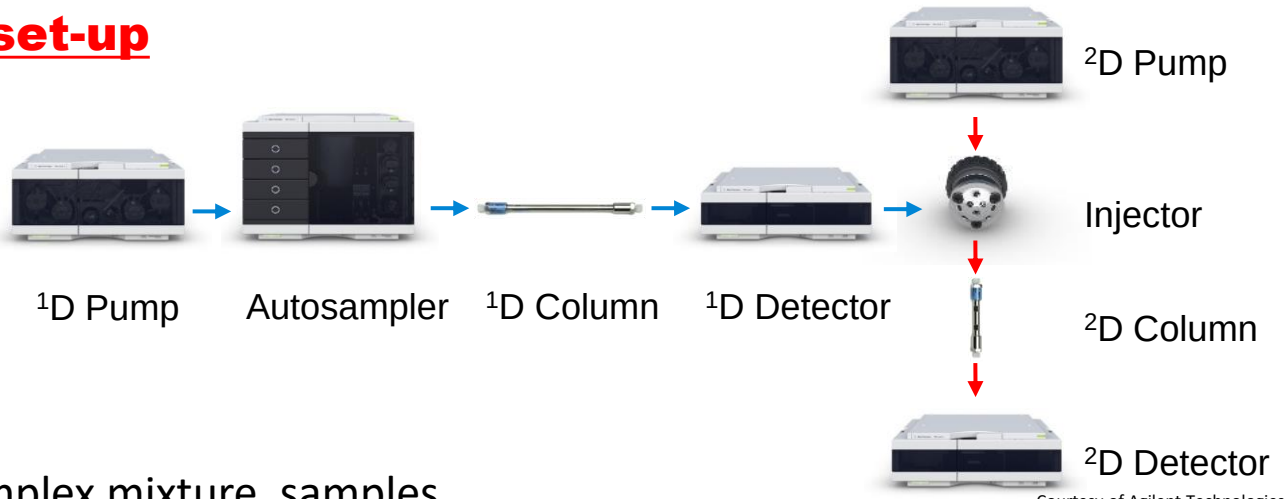
Current analytical methods lack selectivity to handle the increasing complexity of pharmaceutical development

Multidimensional separation is a must-have tool

Two-dimensional Liquid Chromatography

2-D liquid chromatography has become a valuable tool for improving peak capacity and selectivity.

Typical 2D-LC set-up

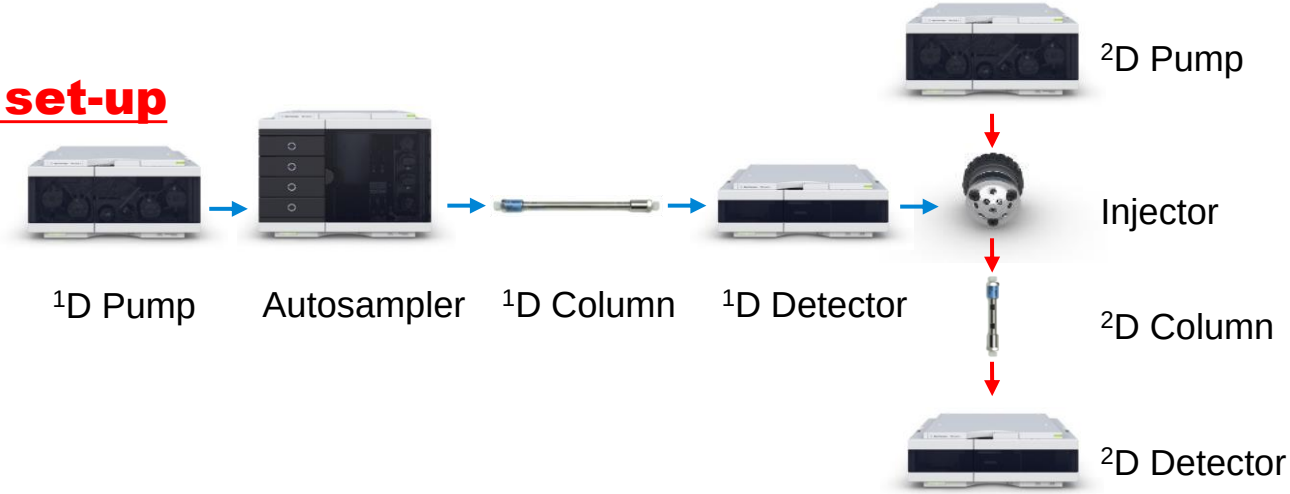


2D LC is for complex mixture samples

Courtesy of Agilent Technologies

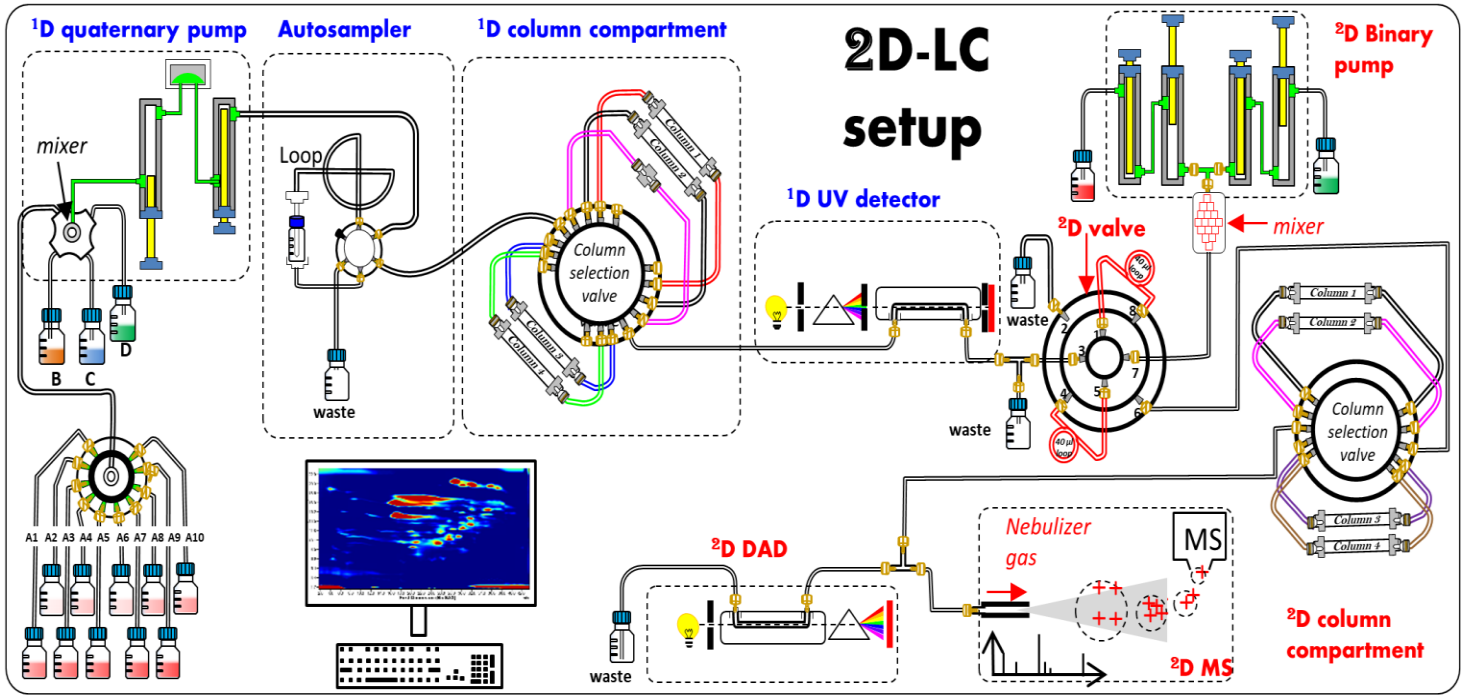
Online Multicolumn Two-dimensional Liquid Chromatography Screening

Typical 2D-LC set-up

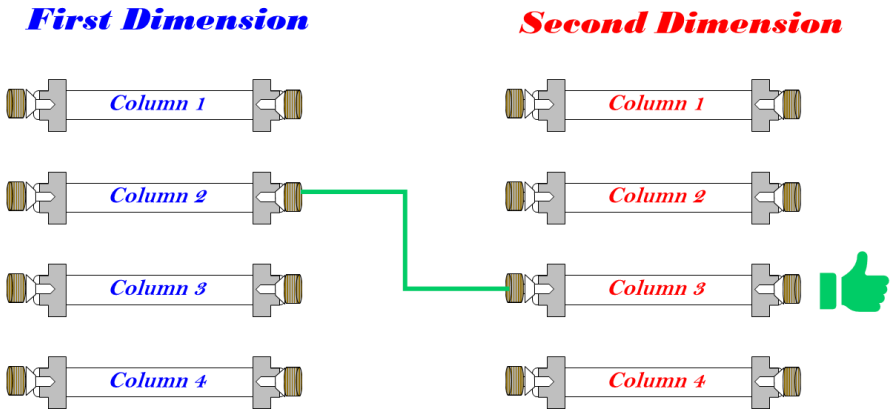


Online Multi-column screening 2D-LC set-up

Courtesy of Agilent Technologies



Online Multicolumn Two-dimensional Liquid Chromatography Screening



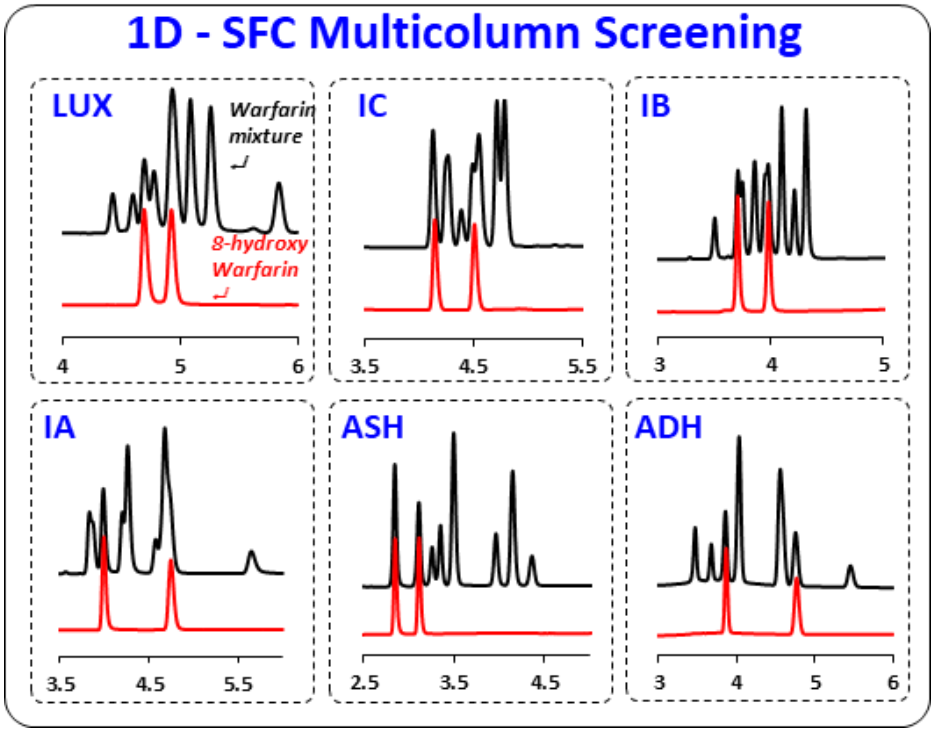
Online Multi-column screening 2D-LC set-up

- Automatic on-line heart cutting or high-resolution sampling
- DAD and MS as detectors
- Multiple column selection on both dimensions
- Multiple mobile phases selections
- In an automated fashion

Solved the challenges we faced with one dimensional LC

Challenges in One-Dimensional Liquid Chromatography

Challenges exist even with most powerful screening techniques. . .



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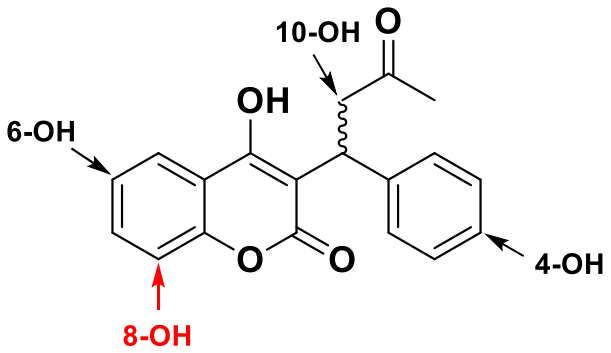


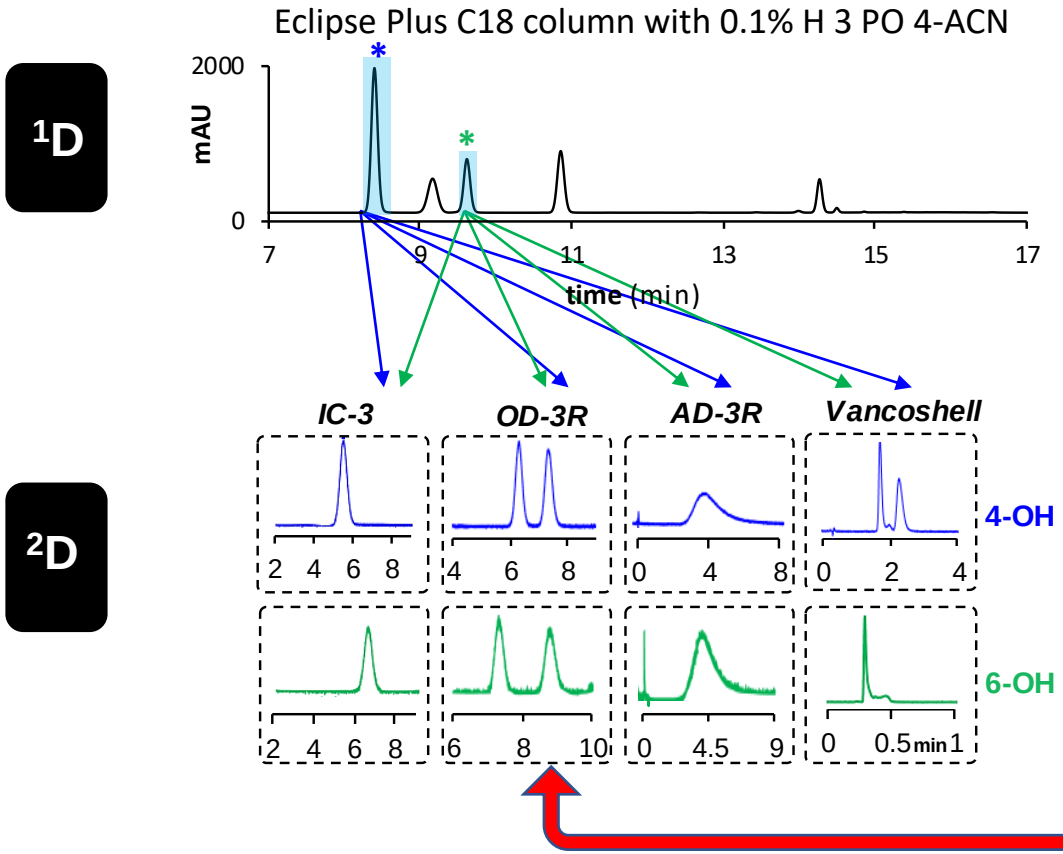
- Mixture contains species of multiple stereoisomer
- With each screening, retention times of the isomers shifts

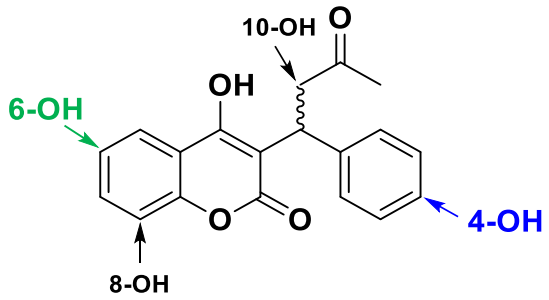
Current analytical methods lack selectivity to handle the increasing complexity of pharmaceutical development

2D-LC set up approach makes this analyses easier

Online Multicolumn 2D-LC-DAD-MS

Analysis of Warfarin and Hydroxylated Isomers

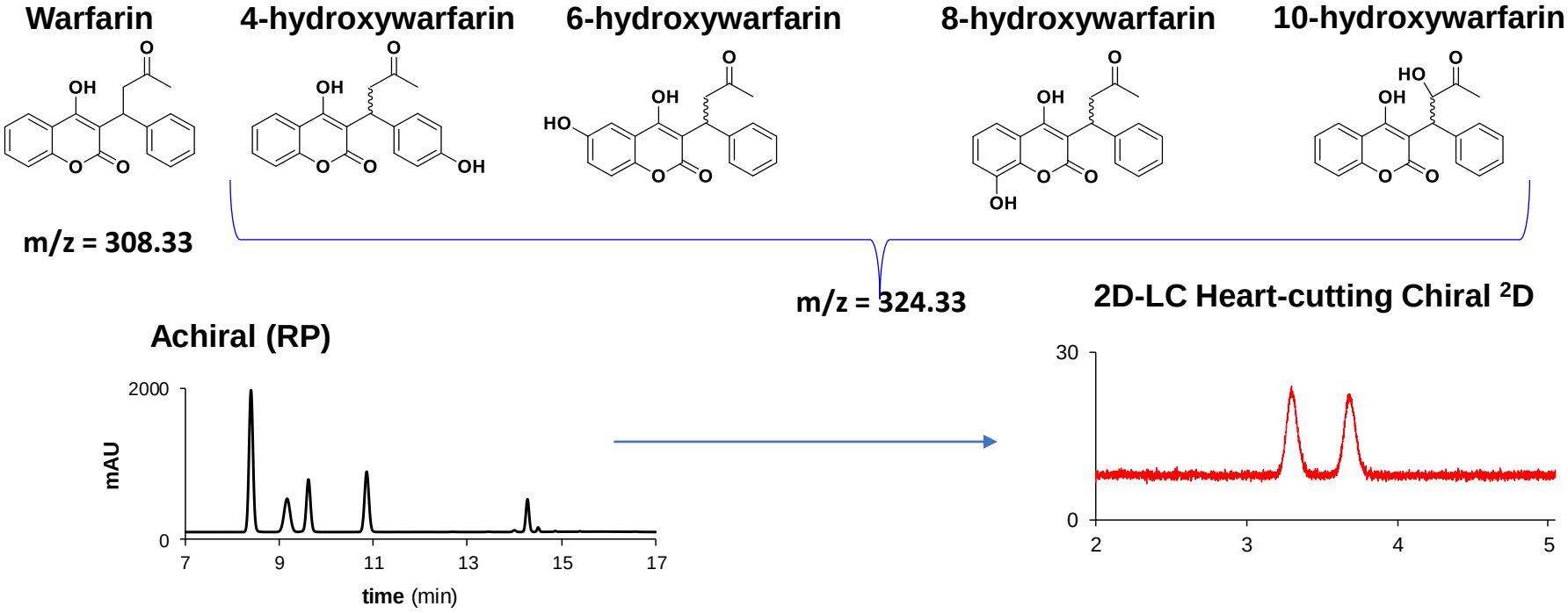




For separation of the enantiomer pairs of both 4-OH and 6-OH, OD-3R outperforms all other chiral columns assessed.

Streamlined method development is only possible with *online multicolumn* functionality!

Heart-cutting Online Multicolumn 2D-LC Analysis of Closely Related Species



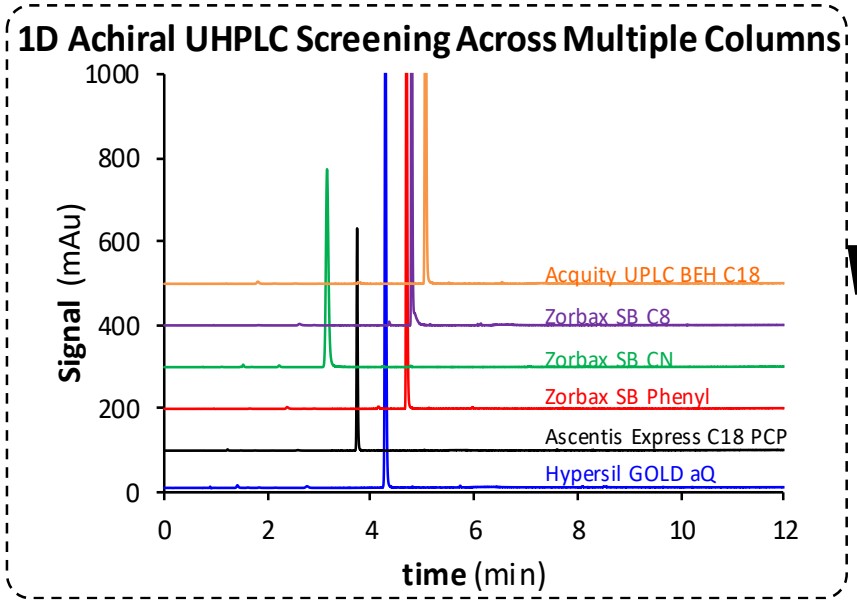
A: MS-incompatible buffers can be used in 1D to improve separation, without affecting MS analysis in the 2D

B: Heart-cuts can now be injected into the 2D using different columns.

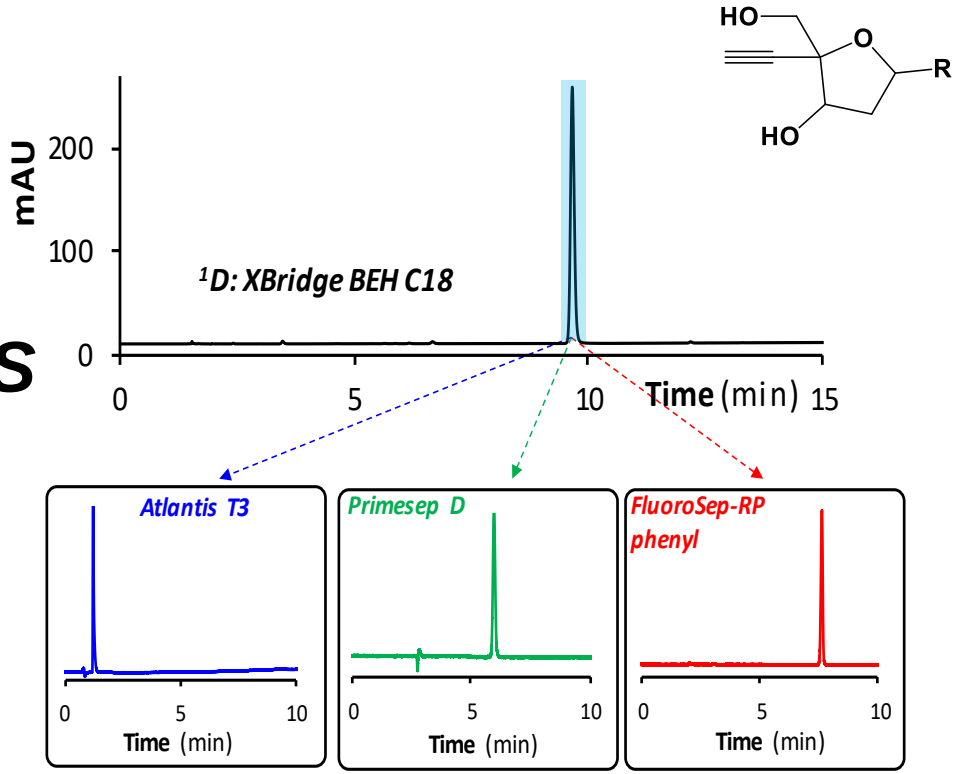
C: Achiral-chiral set up, stereoisomers of interested are selectively isolated from rest of chemical species.

Online Multicolumn 2D-LC-DAD-MS

Confirmation of purity for peak of interest in pipeline drug substance



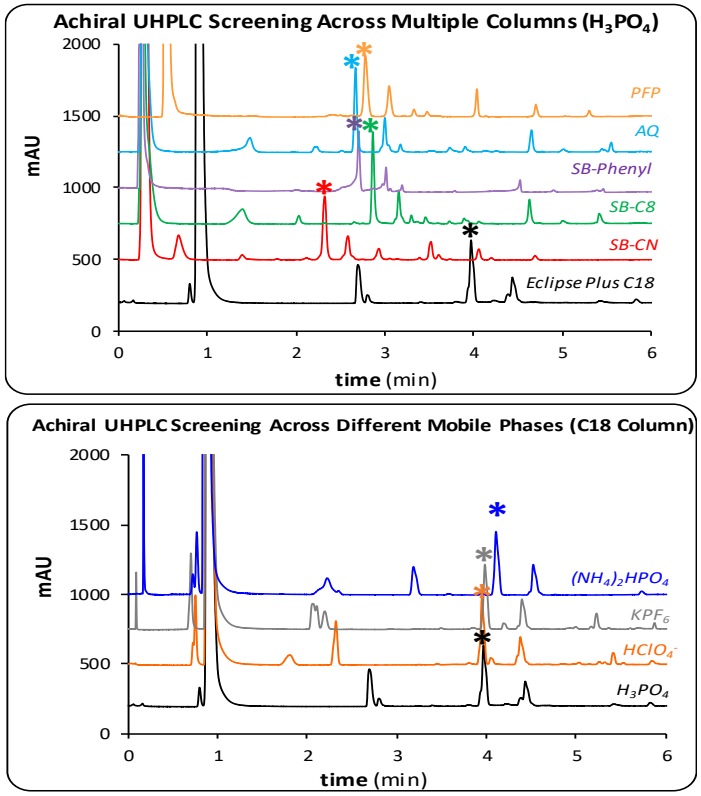
VS



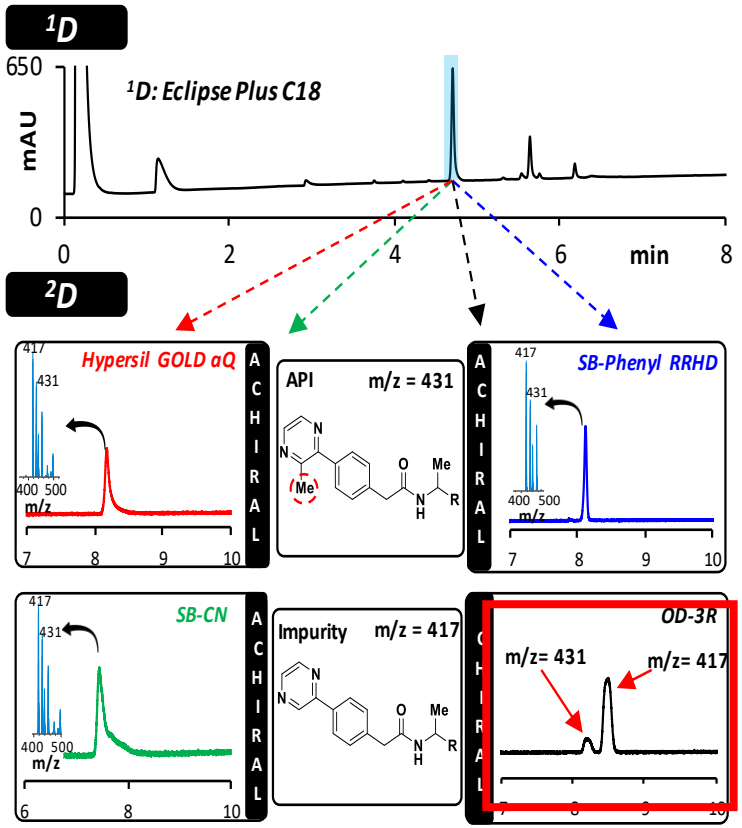
Peak purity confirmed by automated online multicolumn heart-cutting 2D-LC

Online Multicolumn 2D-LC-DAD-MS

Discovery of a de-methylated major impurity

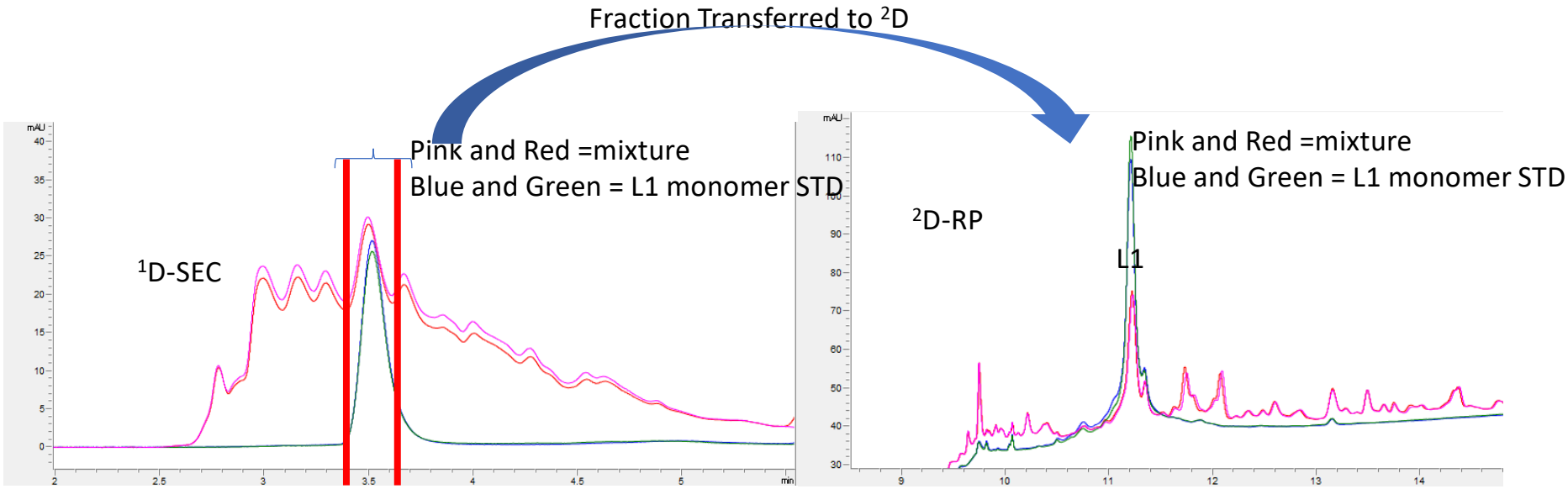


VS.



Heart-cutting Achiral – Chiral is the only method that reveals the major impurity and enables reaction monitoring

Example SEC-RPLC separation for L1 protein



1D condition:
Acquity protein BEH SEC-200A at 25C
MPA: 0.1% SDS 15mM Na phosphate and 150mM NaCl pH3
F=0.3ml/min isocratic for 15minues

Waters Protein BEH C4 300 Å, 1.7um 2.1x100mm

Bioshell lgG 1000A C4 2.7um 2.1x100mm

HALO C4 1000A 2.7um 2.1x75mm at 70C

BEH peptide C18 300A 1.7um 2.1x100mm at 75C

YMC Triart BIO C4 300A 1.9um 150x2.1mm at 70C

Conclusions

- Automated multicolumn online 2D-LC-DAD-ESI-MS approach are developed for the separation and analysis of complex pharmaceutical mixtures.
- Multi-column selection valve technology in both dimensions showed excellent chromatographic performance and repeatability.
- This technique enables rapid and efficient identification of column/eluent combinations, as well as sample analysis across multiple columns in both dimensions overnight with a single click.

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