The incentive of dual injection capabilities for method development and quantitation in heart-cut 2D-LC
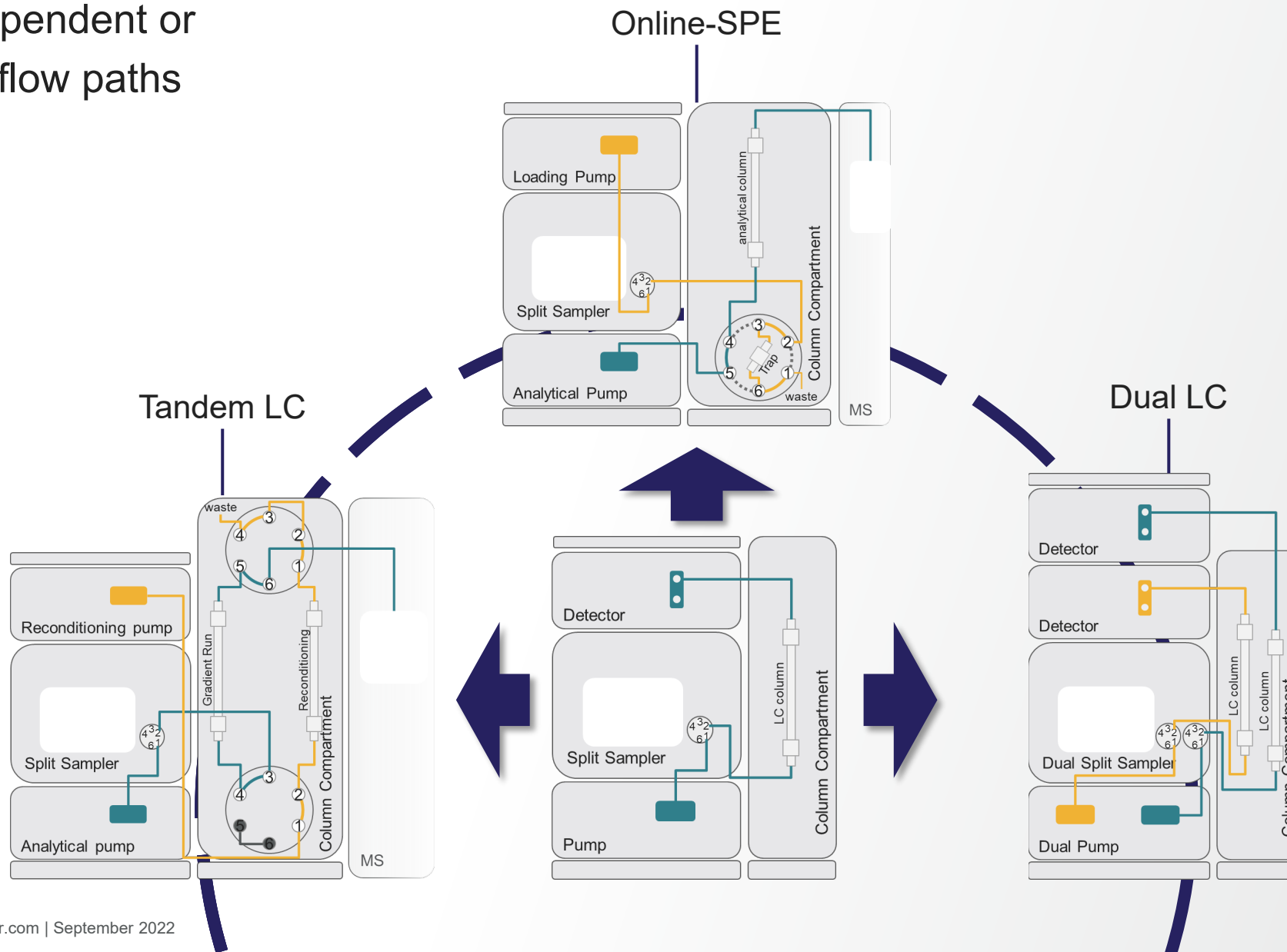
Chris Tuczemskyi, Matthias Schiell, Maria Grübner, Frank Steiner
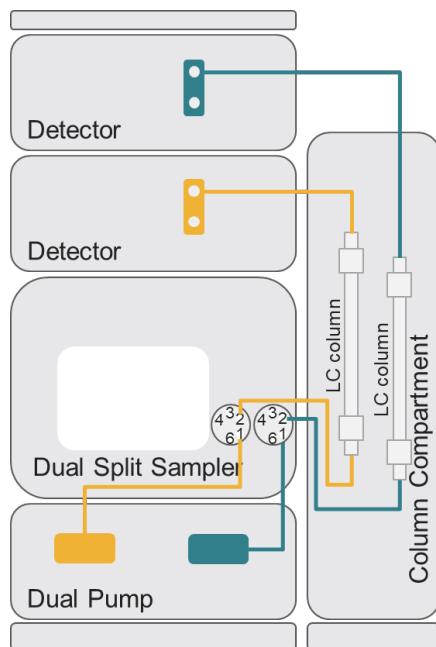
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Application-specific LC systems

…multiple dependent or independent flow paths

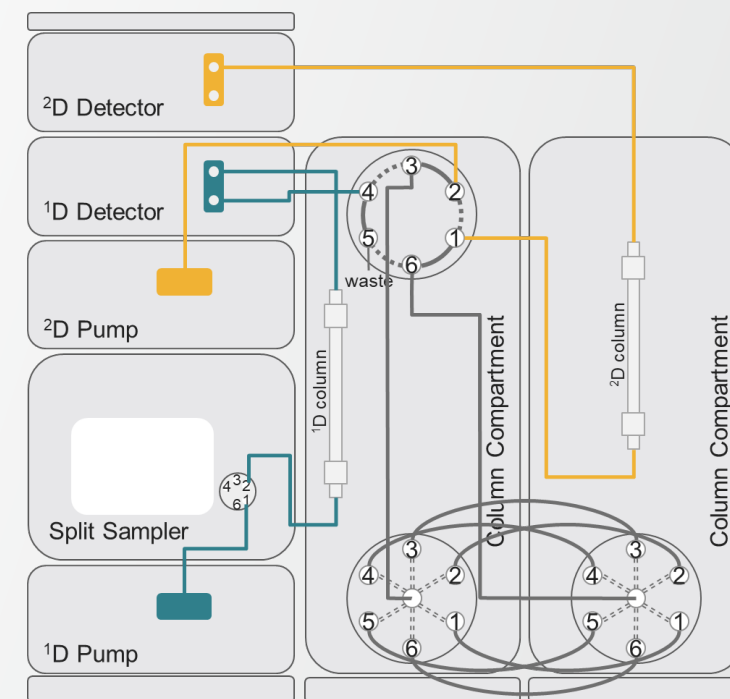# Dependency of LC flow paths

Independent flow paths

Key differentiators:

- Flow path connecting interfaces
- Injection capabilities

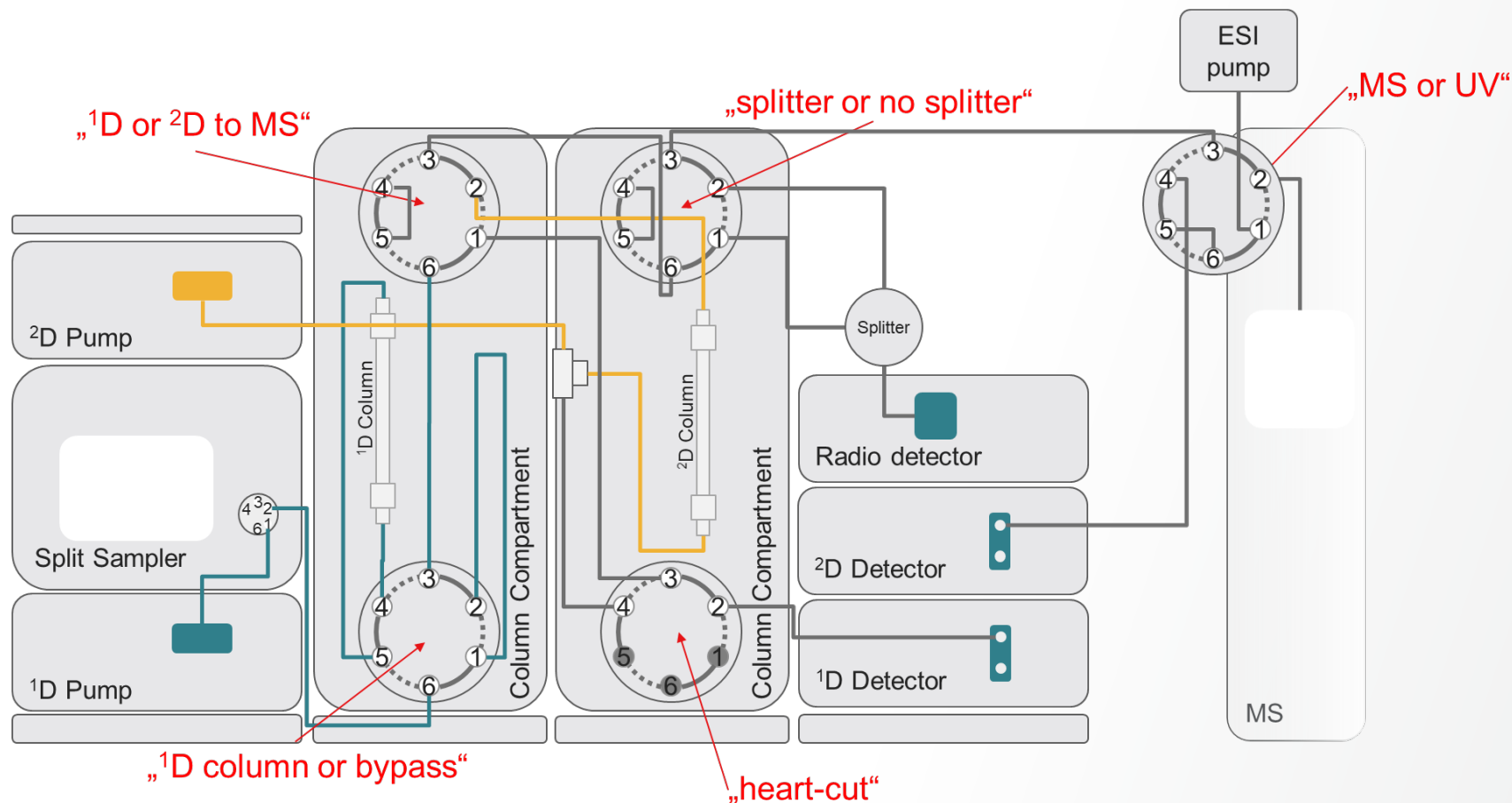Are there LC systems that can be used either in dependent or independent mode?

Dependent flow paths

Dual LC

Heart-cut 2D-LC

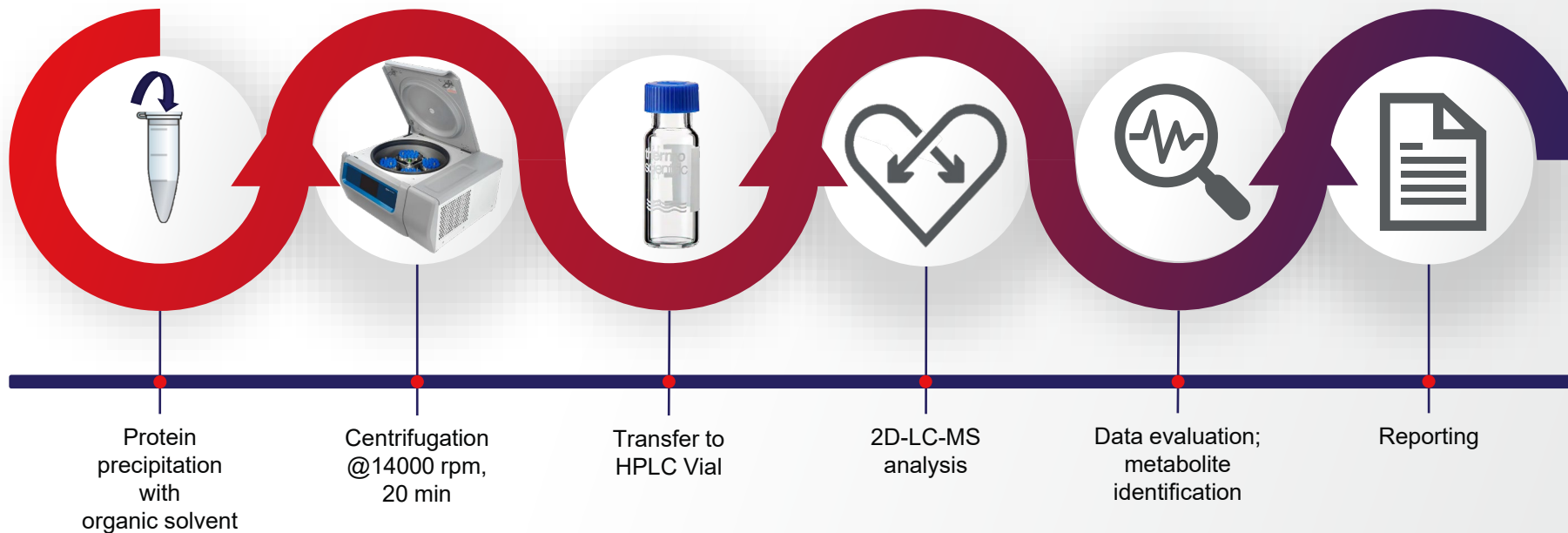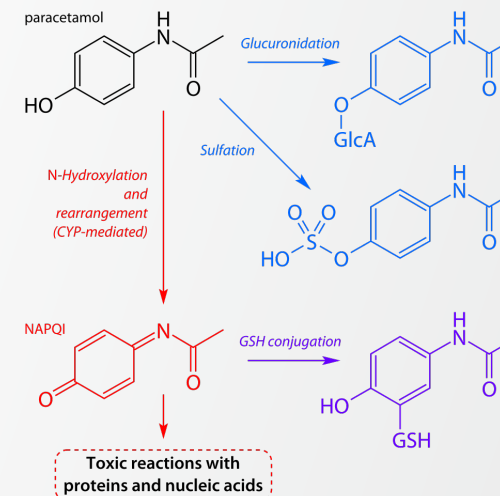# 2D-LC system for drug metabolism and pharmacokinetics

by Matthias Schiell (Sanofi, Frankfurt, Germany)

- Highly complex valve setup
- Shared sampler
- Multiple detection options
- Dilution of ^{1}D flow by ^{2}D flow via T-piece
- Various operation modes

Poster - 1D/2D-UHPLC-MS System and its use in the Field of Drug Metabolism and Pharmacokinetics

Drug metabolism and pharmacokinetics

- Biological matrices from in *in-vitro* studies and in *in-vivo* studies
- Challenging conditions:
  - Low analyte concentrations
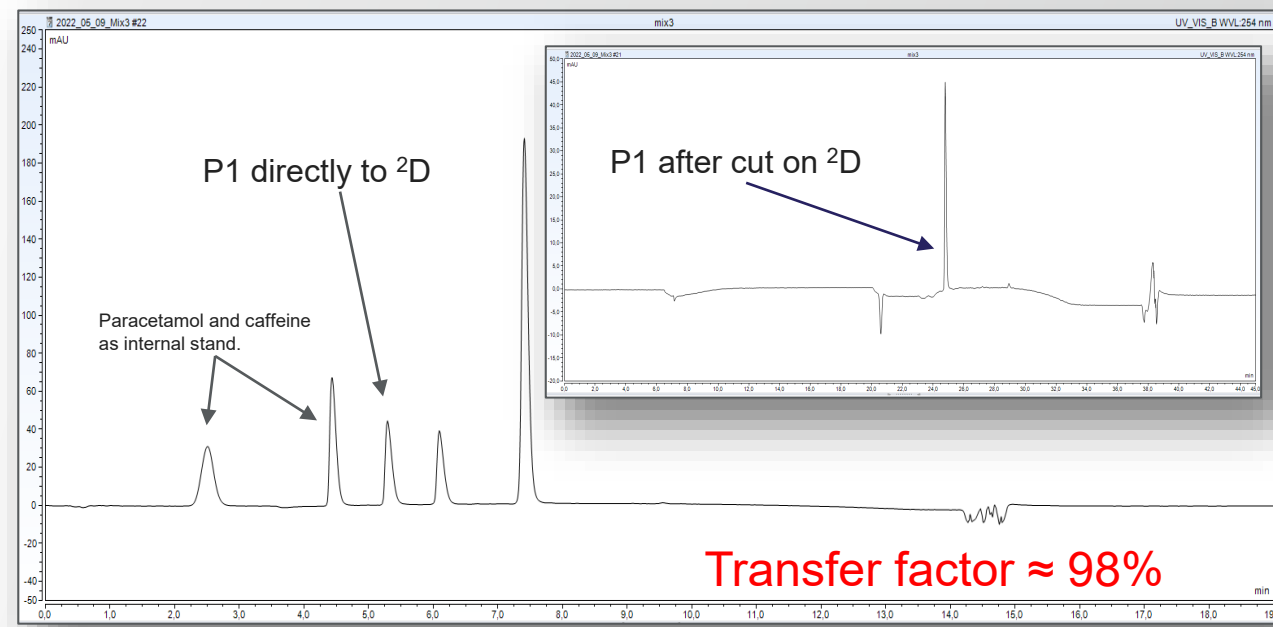 - High injection volumes
  - High organic solvent content

Protein precipitation with organic solvent → Centrifugation @14000 rpm, 20 min → Transfer to HPLC Vial → 2D-LC-MS analysis → Data evaluation; metabolite identification → Reporting

Paracetamol image from: https://en.wikipedia.org/wiki/Paracetamol_poisoning

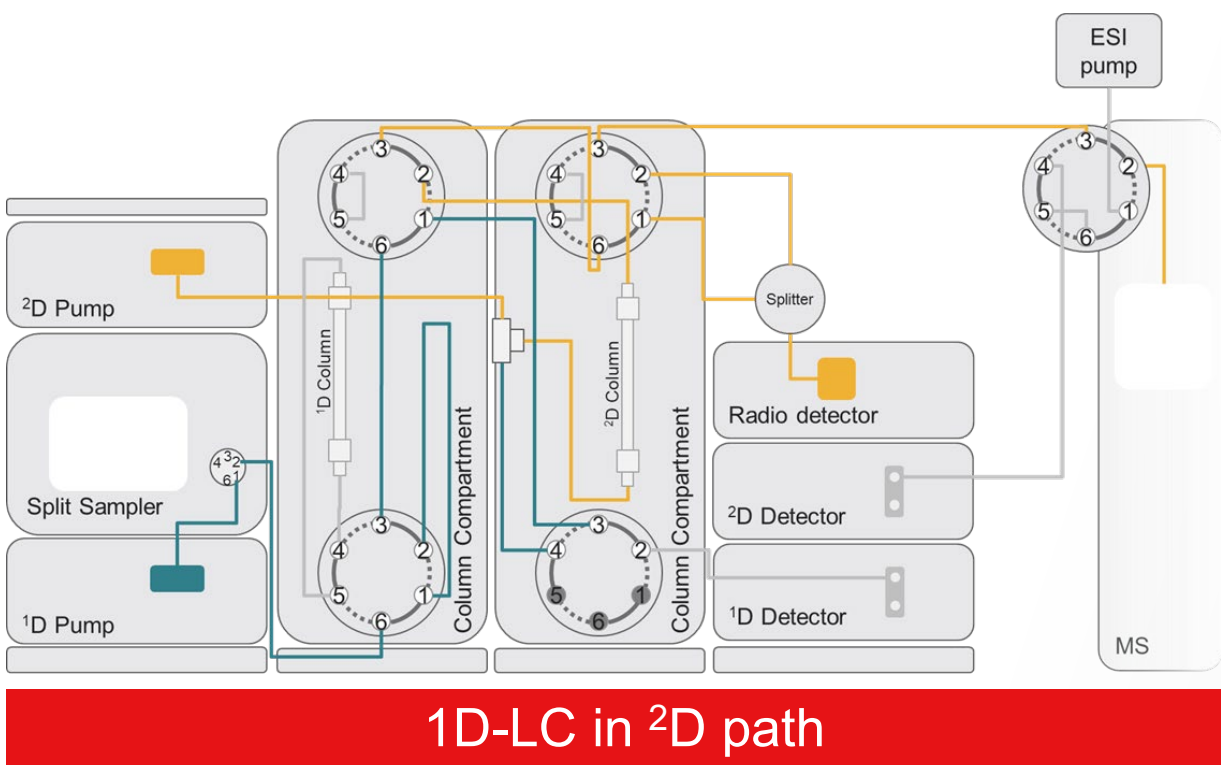chris.tuczemskyi@thermofisher.com | September 2022

A system with various operation modes

- 1D-LC-UV, 1D-LC-MS, 1D-LC-RD/MS (^{1}D path)

- 1D-LC-UV, 1D-LC-MS, 1D-LC-RD/MS method development (^{2}D path)

- Heart-cut 2D-LC with solvent modulation for peak band focusing

- 1D-LC with dilution of large volume/high organic sample plug in ^{2}D path

- 1D-LC in ^{1}D path with MS unfriendly mobile phase and dilution by ^{2}D flow for MS friendliness

- Ternary/quaternary 1D-LC gradients in ^{2}D path by combining two pump flows for alternative elution or ionization
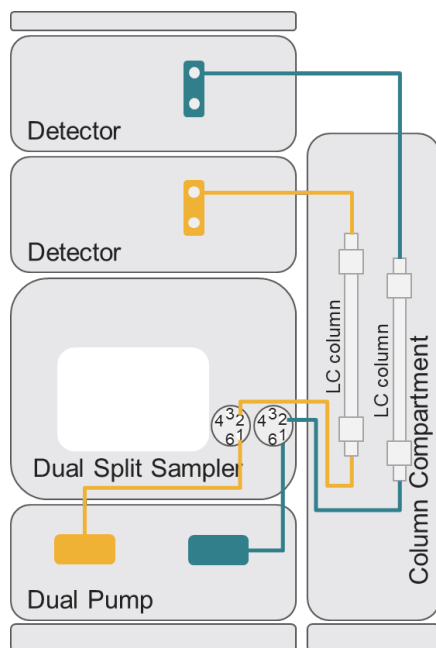

2D-LC during heart-cut

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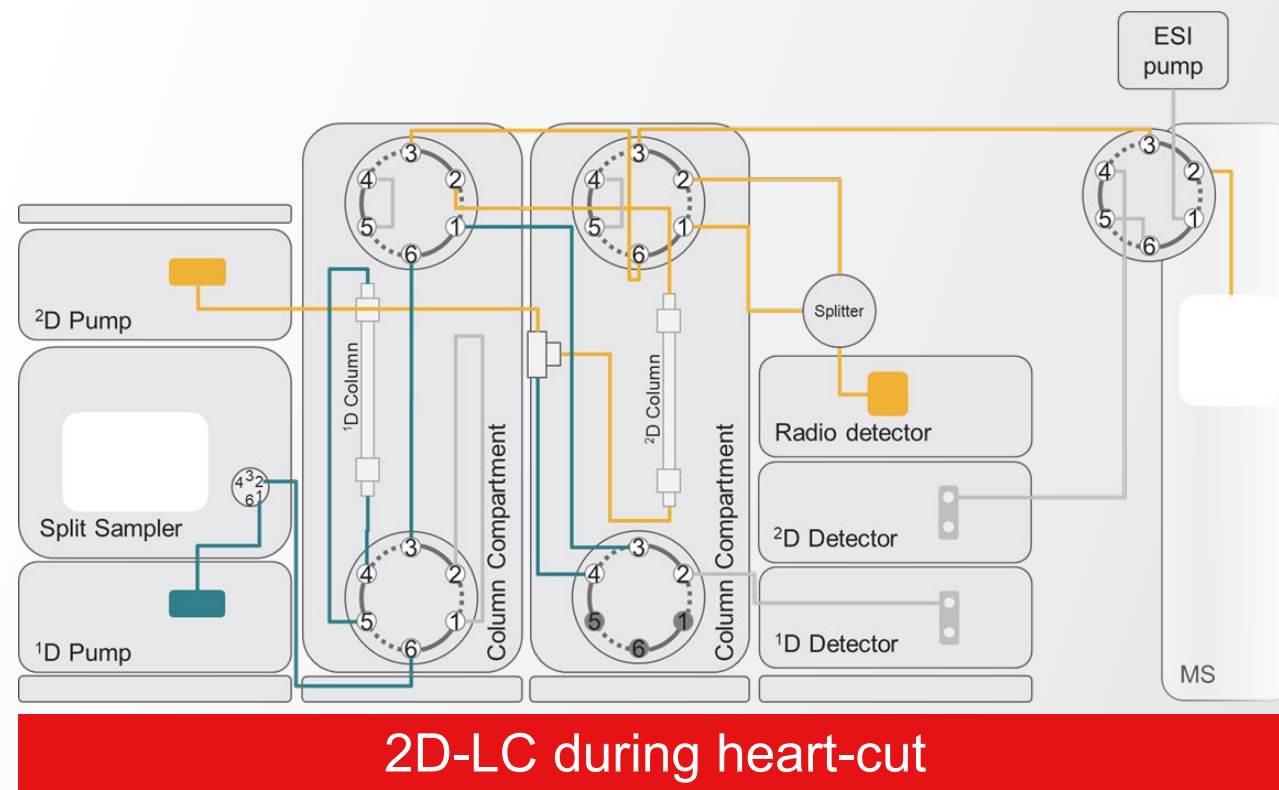
Example: Transfer factor determination

- Transfer factor = recovery of ^1D fraction in ^2D

(Quantity ^2D/ Quantity ^1D) x 100 = % transfer

1D-LC in ^2D path

P1 directly to ^2D

P1 after cut on ^2D

Paracetamol and caffeine as internal stand.

Transfer factor ≈ 98%

Dependency of LC flow paths
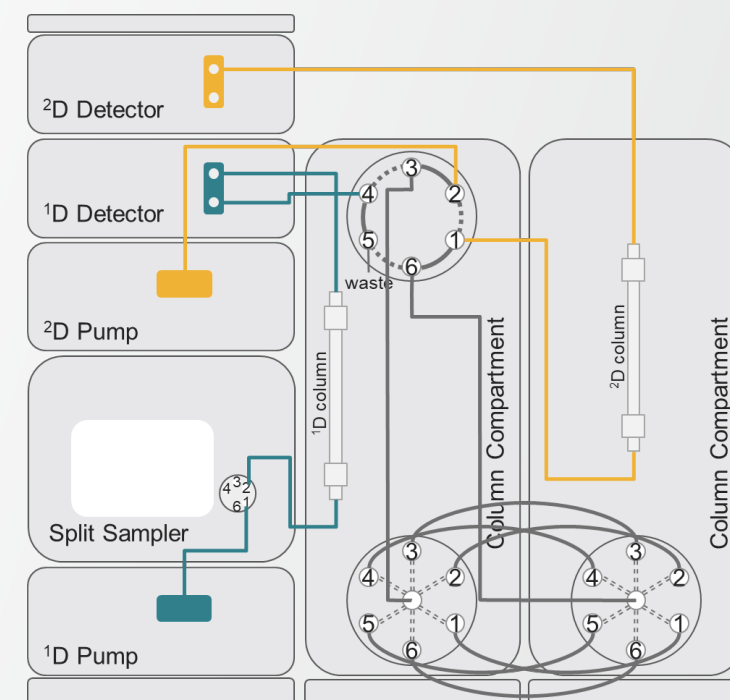

Independent flow paths

Dual LC

Key features:

- Flow path connecting interfaces
- Injection capabilities

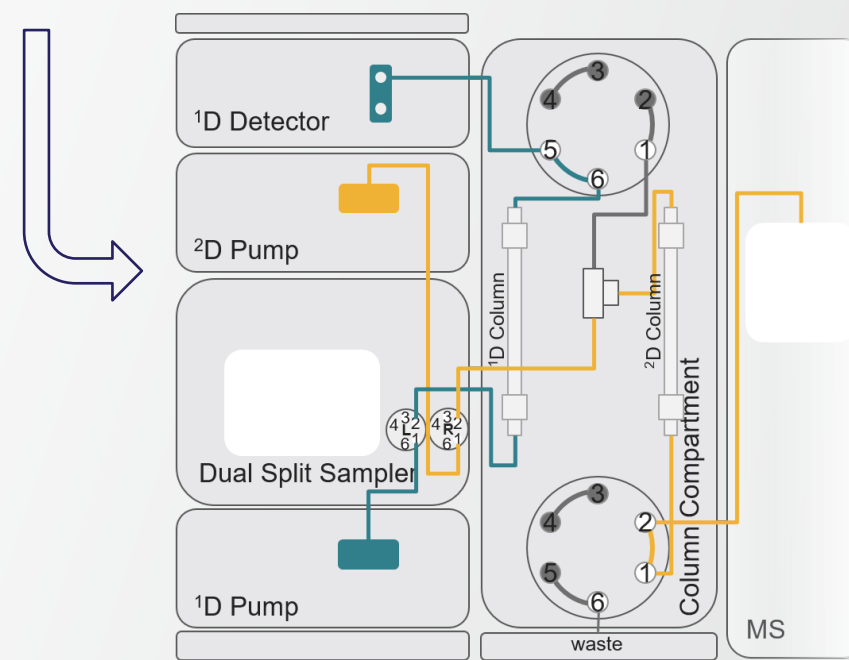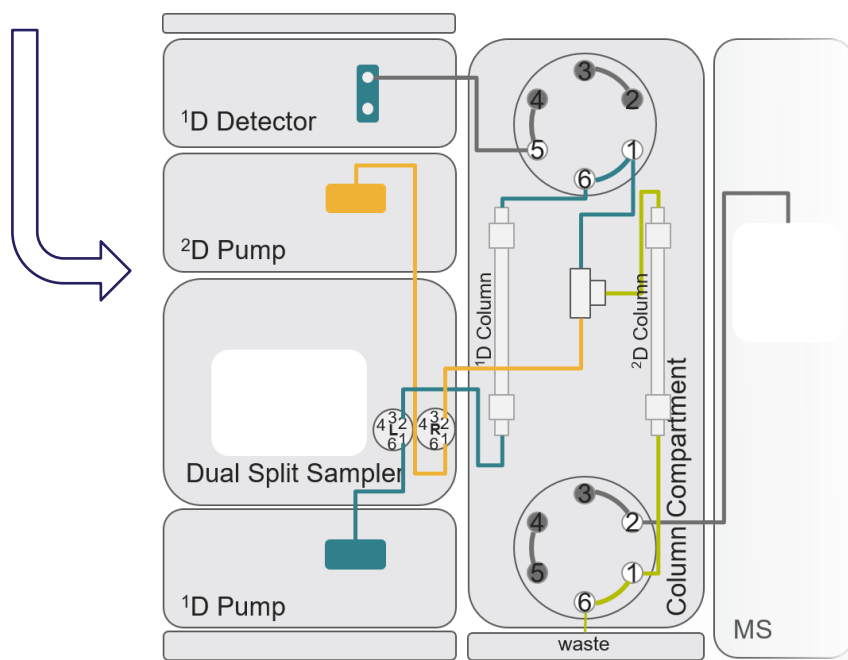**Are there LC systems that can be used either in dependent or independent mode?**
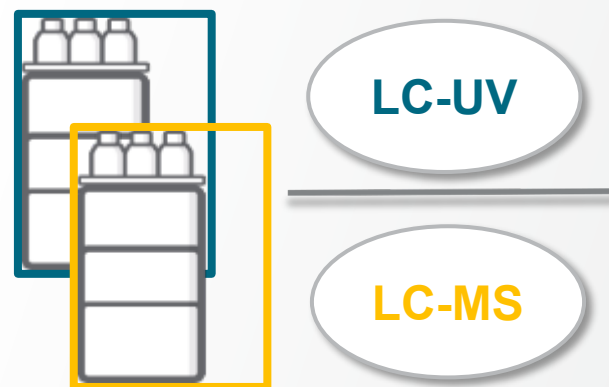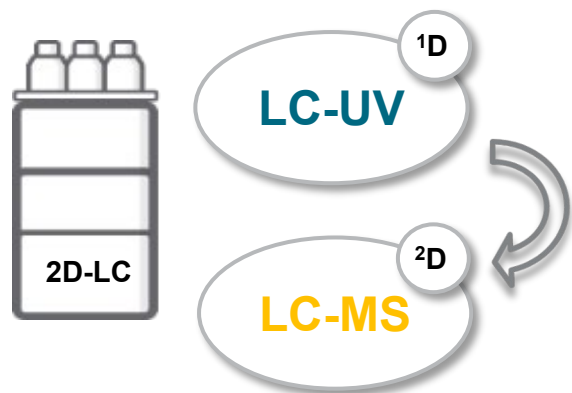
Dependent flow paths

Heart-cut 2D-LC

Thermo Scientific™ Vanquish™ Simple Switch 2D-LC Systems
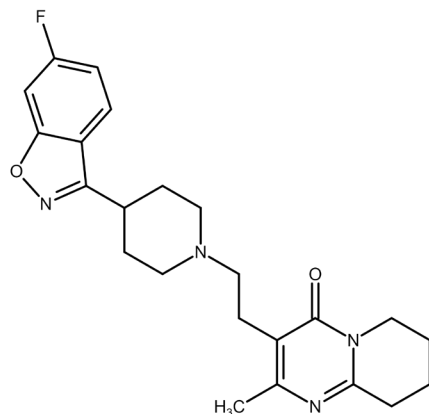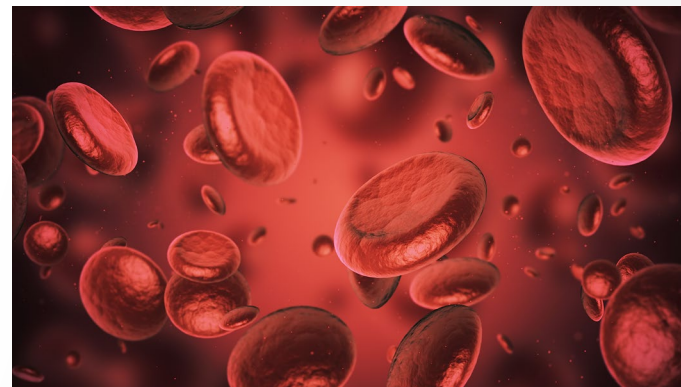
2D-LC systems equipped with dual sampler

Application: Therapeutic drug monitoring of risperidone

TDM
Determination of medication level in blood to optimize and personalize drug therapies

Risperidone
- Antipsychotic drug
- Treatment of schizophrenia, bipolar disorder, autism
- Active metabolite 9-hydroxyrisperidone (paliperidone)
- Target range:
  - Risperidone 2-10 µg/L
  - Hydroxy-risperidone 20-55 µg/L
  - sum 20-60 µg/L
  - toxic >120 µg/L

https://www.labor-lademannbogen.de
Wei Liu, et al., 2020, *Analytical Letters*, 53:12, 2002-2019

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Quantification strategies in LC-MS and TDM

external standard

Calibration

internal standard

isotope labeled standard

- Gold standard in LC-MS
- Isotope dilution assay (IDA)

ECHO standard

- Unlabeled standard of target compound is injected into the analytical sample run (pseudointernal standard)
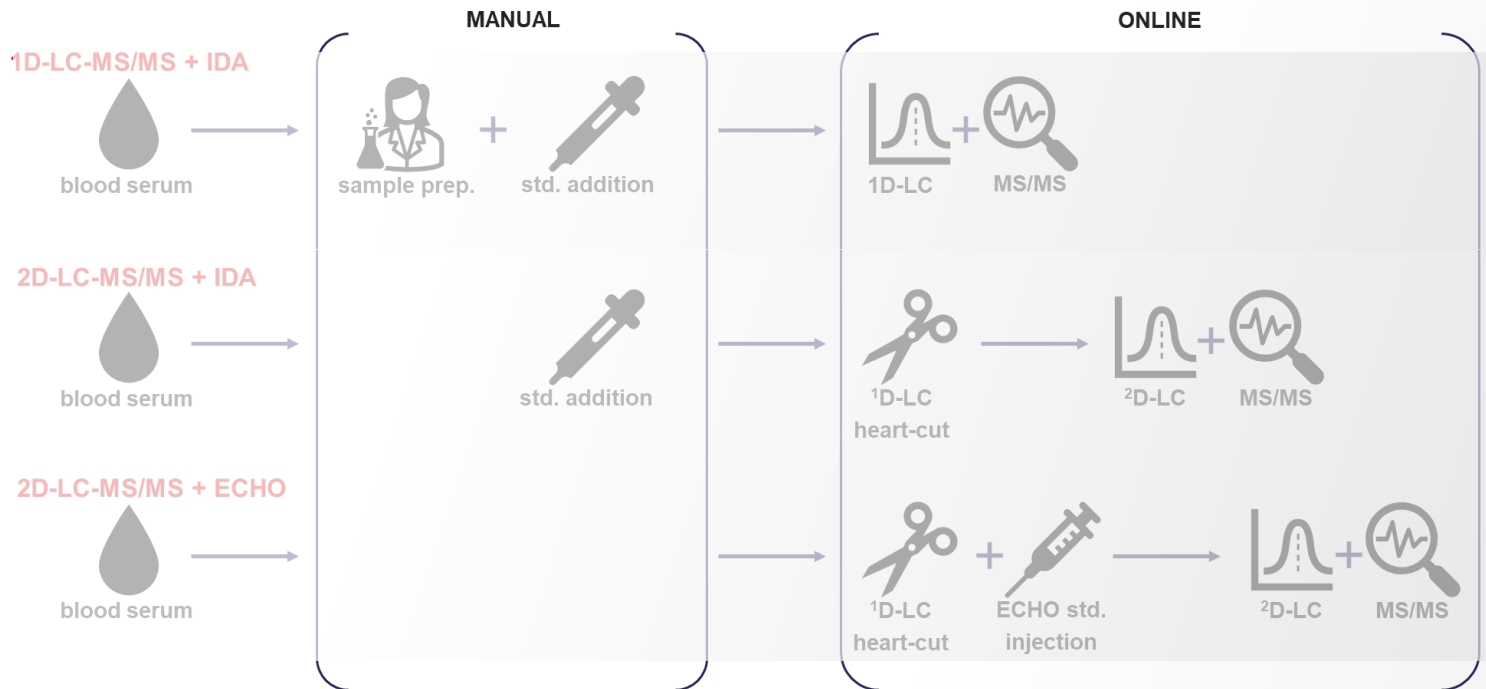- two staggered injections of sample and standard

standard addition
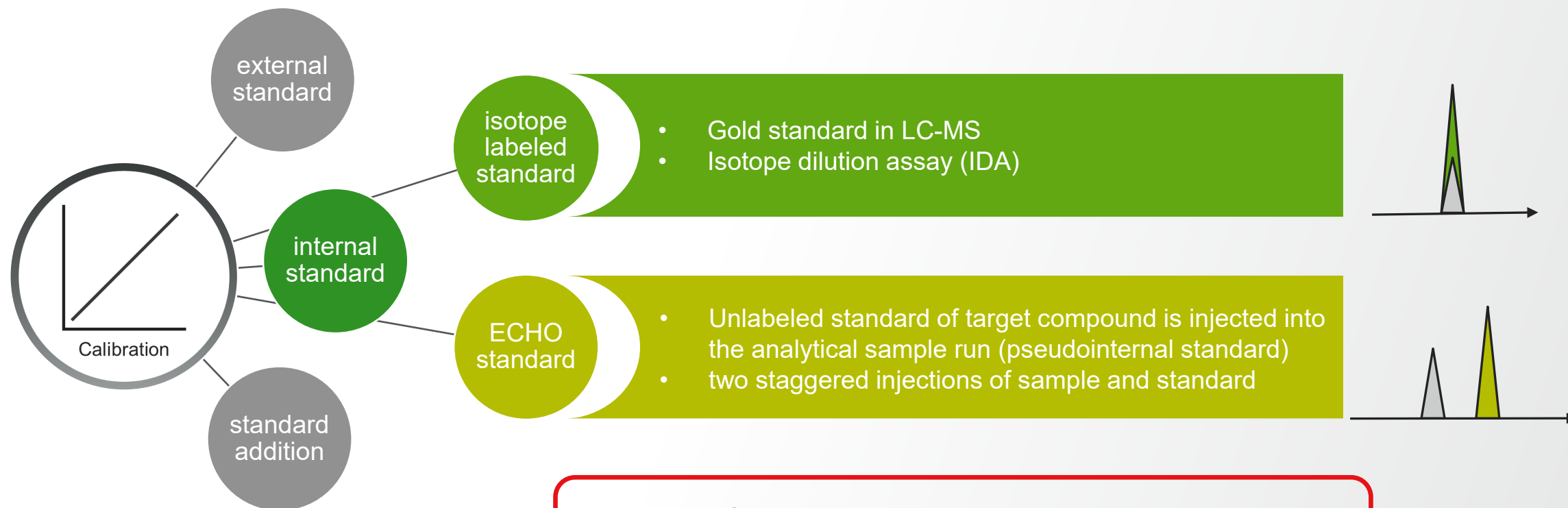
Chromatographic methods in TDM

- Most often LC-MS/MS with IDA or internal std.

- Heart-cut 2D-LC-UV with external standard

- LC-UV requires extensive sample preparation

- Protein precipitation, LLE, SPE

- Usually, no automated sample preparation

Intelmann, D., et al., 2009, *Journal of agricultural and food chemistry*, *57*(4), 1172–1182
Bader, M., et al., 2017, *Journal of agricultural and food chemistry*, *65*(23), 4853–4858
Tuzimski T, Petruczynik A., *Molecules*. 2020; 25(17):4026
Cao, Y., et al., 2020, *Journal of chromatography. B, 1147*, 122129
Ezzeldin, E., et al., 2017, *International journal of analytical chemistry*, *2017*, 1271383
Wei Liu, et al., 2020, *Analytical Letters*, 53:12, 2002-2019

Workflow

Goal:

➤ Automate TDM sample preparation by heart-cut 2D-LC

➤ Evaluate IDA vs. ECHO

| Parameter	¹D	²D
Column	Thermo Scientific™ Hypersil Gold™ C8 Column, 2.1x100mm 1.9µm incl. guard column	Thermo Scientific™ Accucore™ PFP Column, 3 x 50mm, 2.6µm
Mobile phase	A: 0.1% formic acid in water B: acetonitrile	
Flow Rate	0.5 mL/min	0.80 mL/min
Injection Volume	1µl	
Gradient	Linear 10-95% B, 10 min total	Linear 5-95% B
Hear-cut	3.0-3.4min	
ECHO Inject	5.0 min	
Column temp.	40°C (still air)	
Sampler temp.	10°C	
Detection	254 nm, 210nm; 10 Hz	SRM, positive ESI, 3500 V
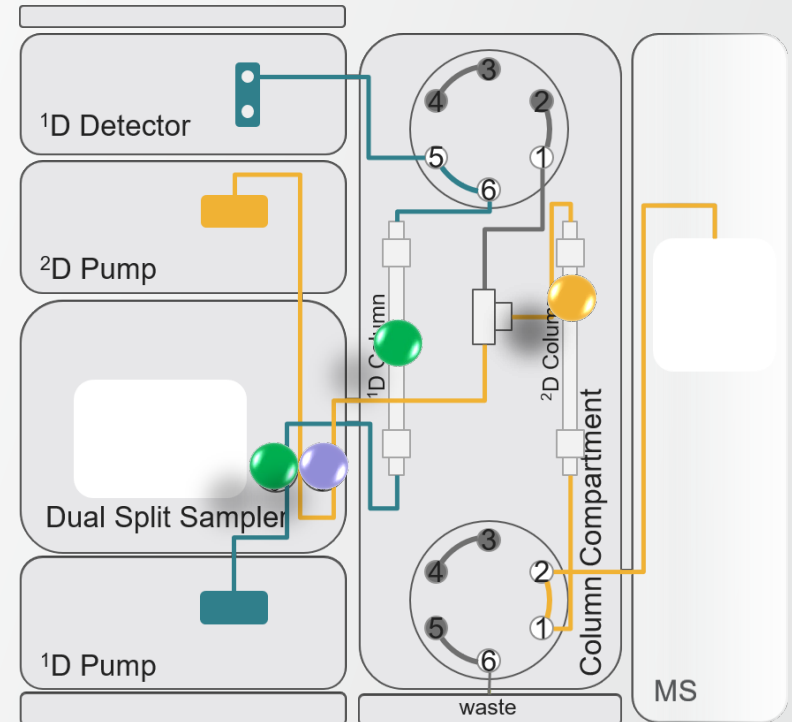
Method development in 1D-LC and 2D-LC

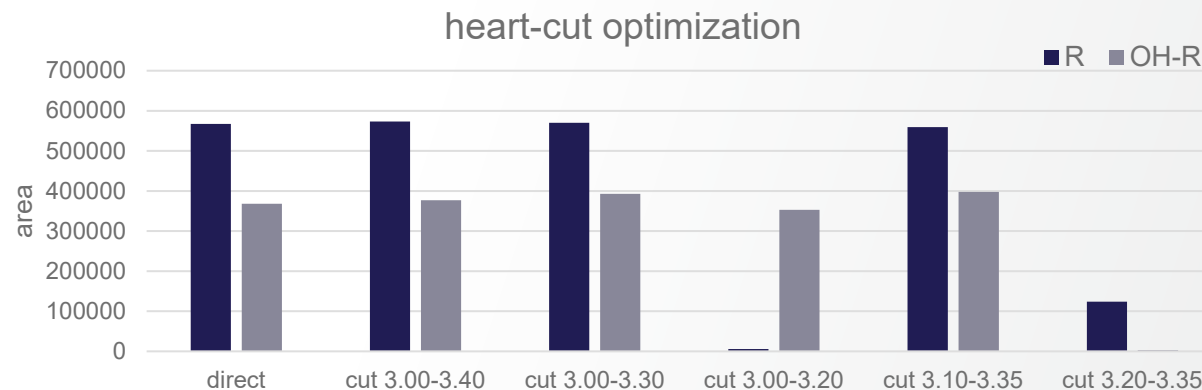## Matrix effect evaluation: 50ppb prepared in solvent vs. in serum matrix
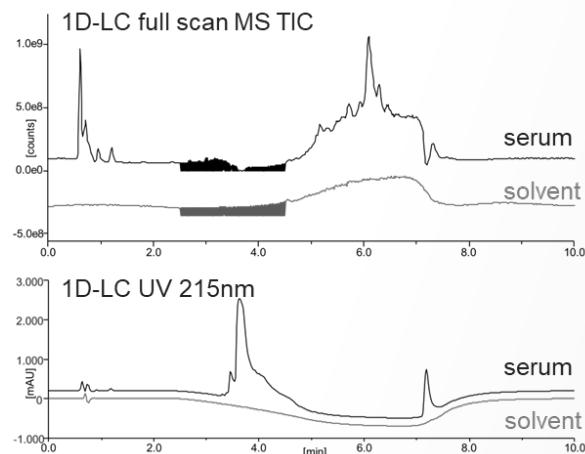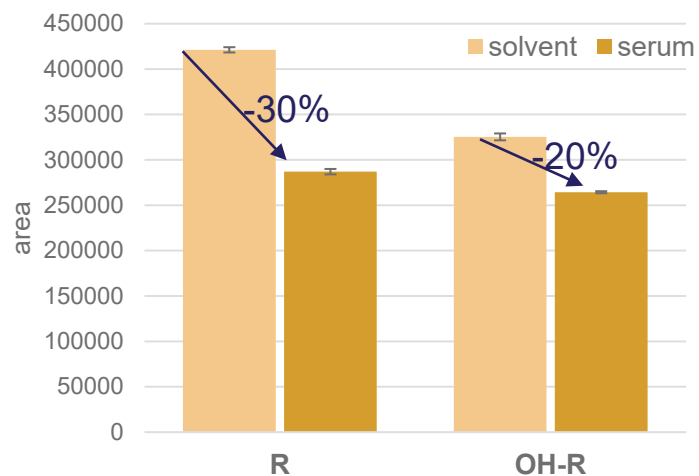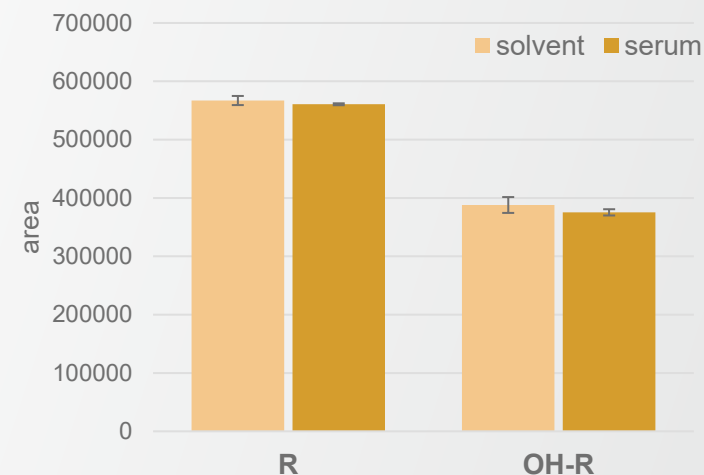
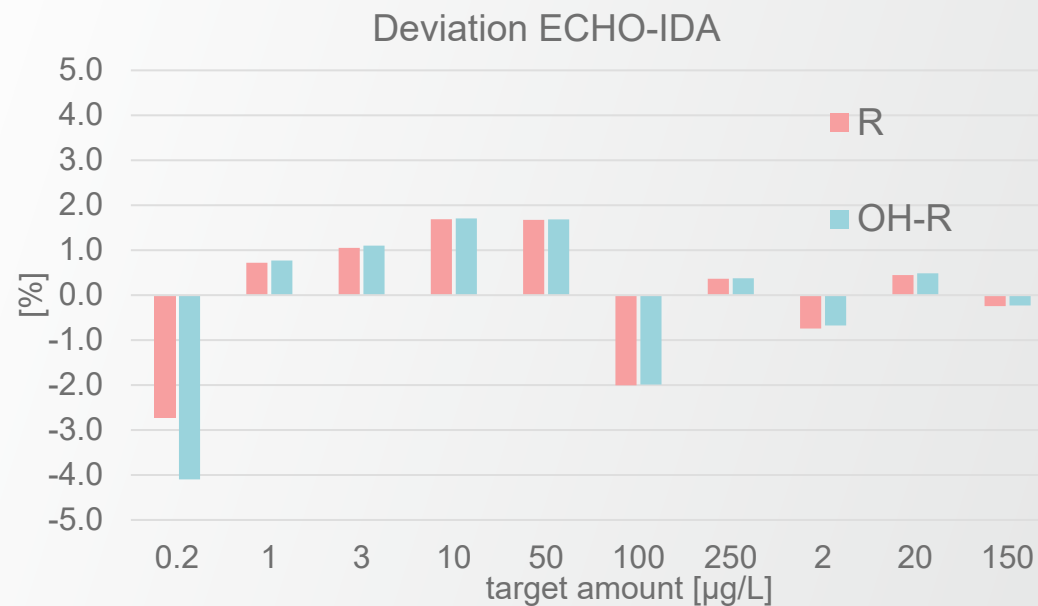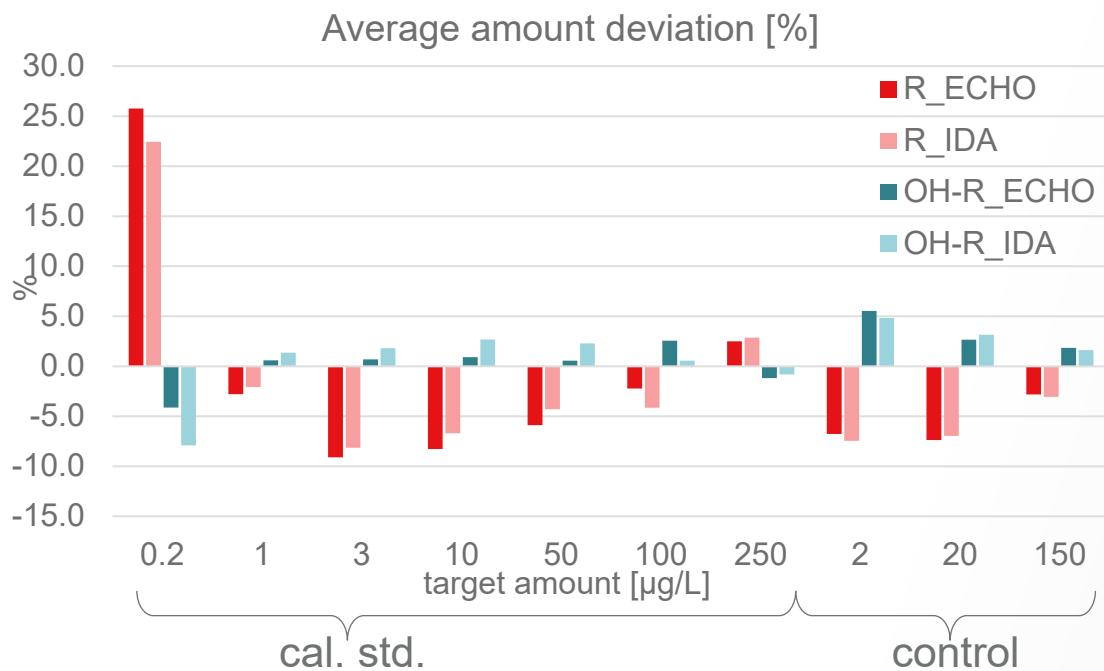## Heart-cut optimization: std. injection in ^{2}D compared to 2D-LC

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IDA and ECHO calibration performance and accuracy

Linear calibration, weighting 1/amount:

R^2	ECHO	IDA
OH-R	0.99955	0.99986
R	0.99862	0.99866

Average amount deviation [%]

Deviation ECHO-IDA
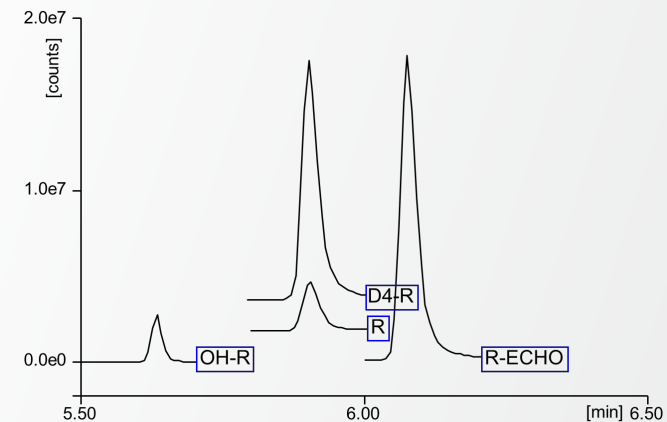
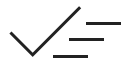# Limitations and benefits of 2D-LC-MS/MS with ECHO

Limitations

- Contamination of ^{1}D column

- Resolution of target and ECHO peak is critical
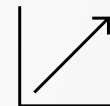
Benefits

- Automated matrix removal

- Standard injected by second sampling unit

- Standards: less expensive, commercially available or from prep. purification

- System available for 1D-LC-MS/MS

- Equivalent quantification performance of ECHO and IDA

Summary

- Application-specific LC setups usually utilize >1 flow path

- Dependency of flow paths is related to the interface between flow paths and injection capabilities

- Flexible operation in dependent or independent mode can be obtained by shared injectors or dual autosamplers

- Dual samplers allow more straightforward setups than shared injectors

Thank you

chris.tuczemskyi@thermofisher.com | September 2022