Is 2D HPLC the Answer for You

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2D-LC: What Is It and Why Use It

What is 2D-LC?

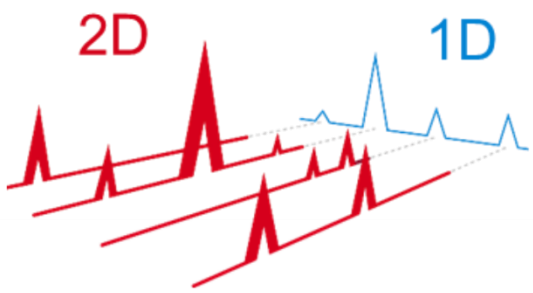
- The selective transfer of a fraction (or fractions) from one chromatographic column to a secondary chromatographic column for further separation

Why might you use it?

- Increase peak capacity
- To further resolve a complex mixture that's not well separated on a single column
- To identify all the impurities in your sample using an orthogonal technique
- To confirm that your main peak is pure
- For sample cleanup; to remove matrix or interfering compounds

Chromatographic Challenges

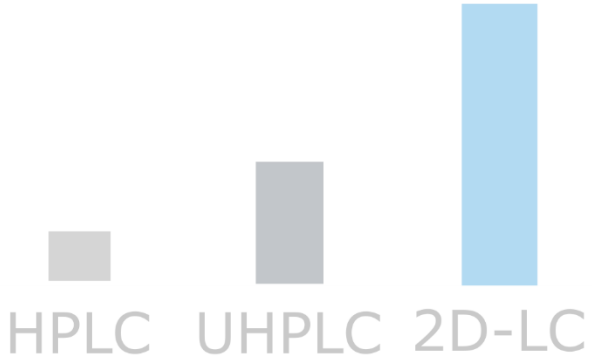
Are you sure?



Avoid suprises and use full orthogonality to prove purity of compounds.

Lack of the full picture?

Peak capacity per 30 min



Resolve more compounds in highly complex samples.

In need for speed?

Substitute manual prefraction or sample desalting with online methods.

Chromatographic Challenges

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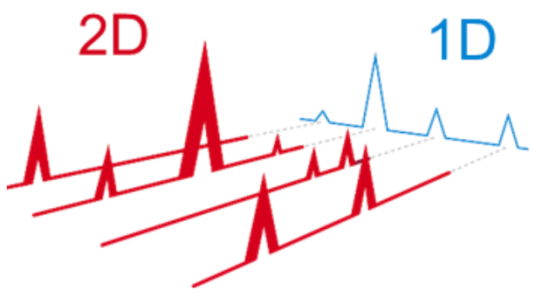

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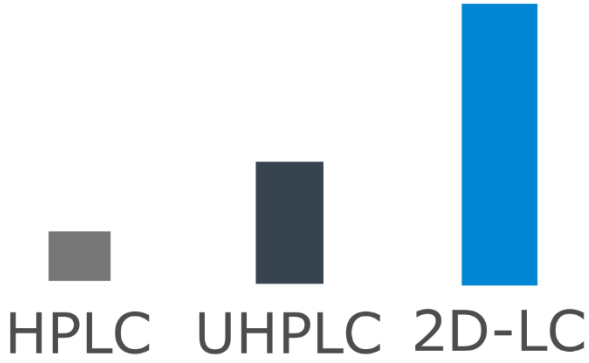

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Chromatographic Challenges

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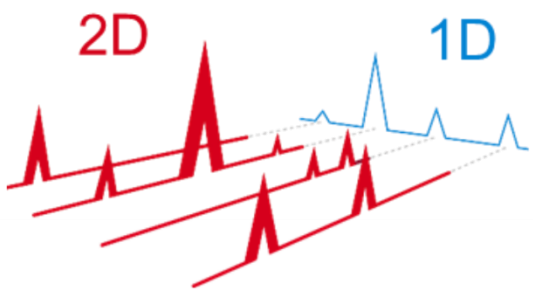

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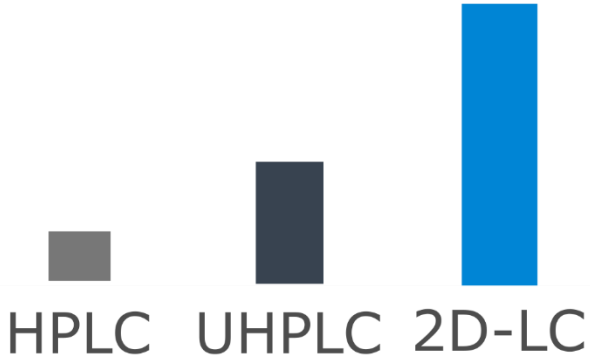

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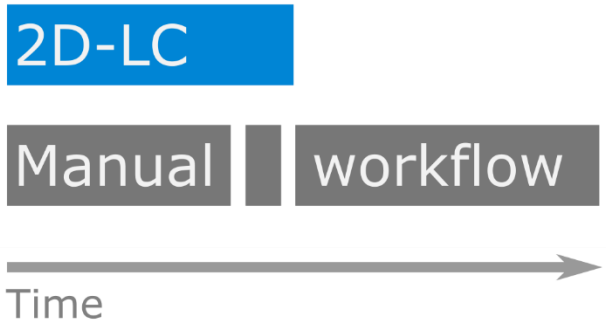
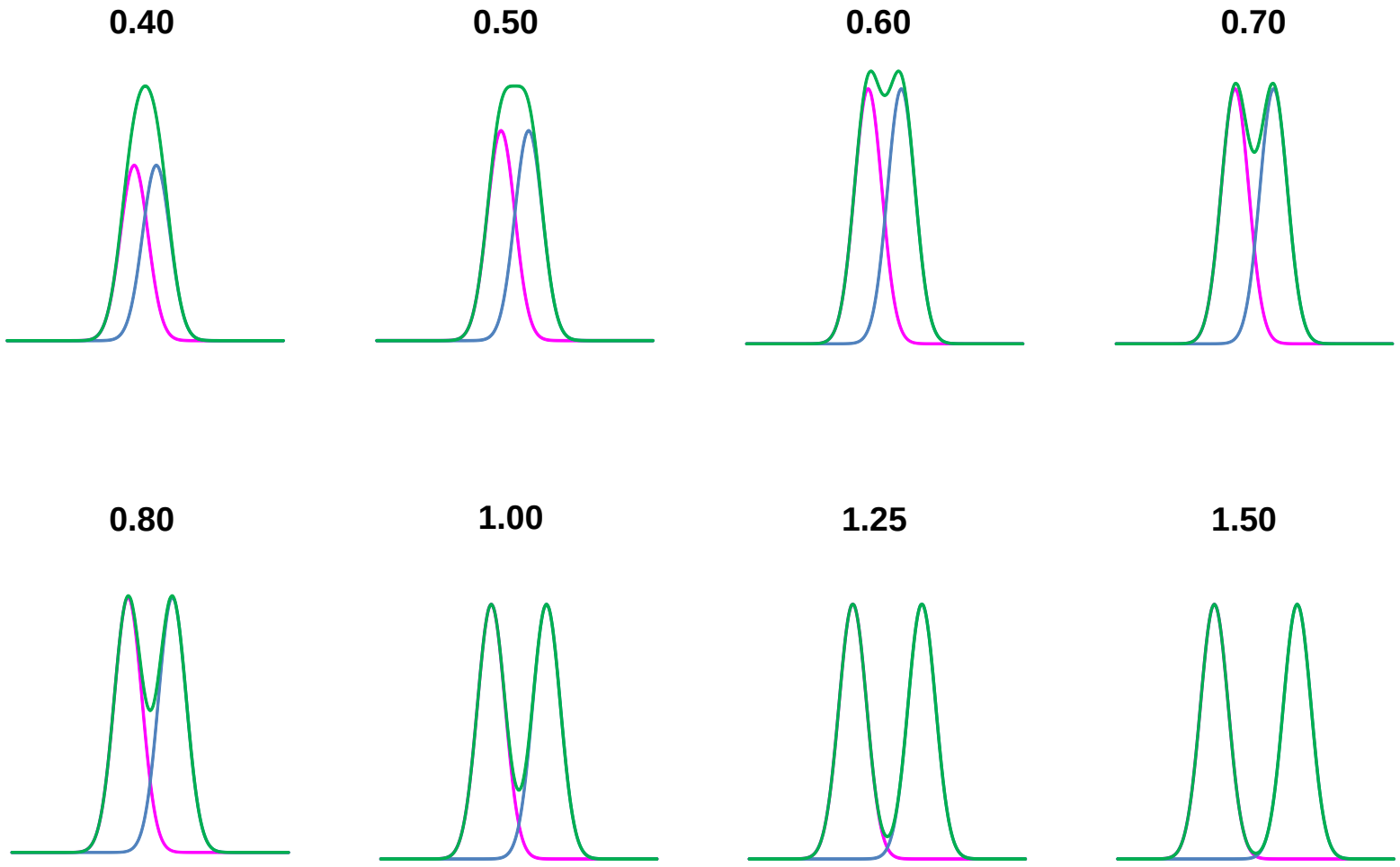
In need for speed?



Substitute manual prefraction or sample desalting with online methods.

Chromatographic Challenges

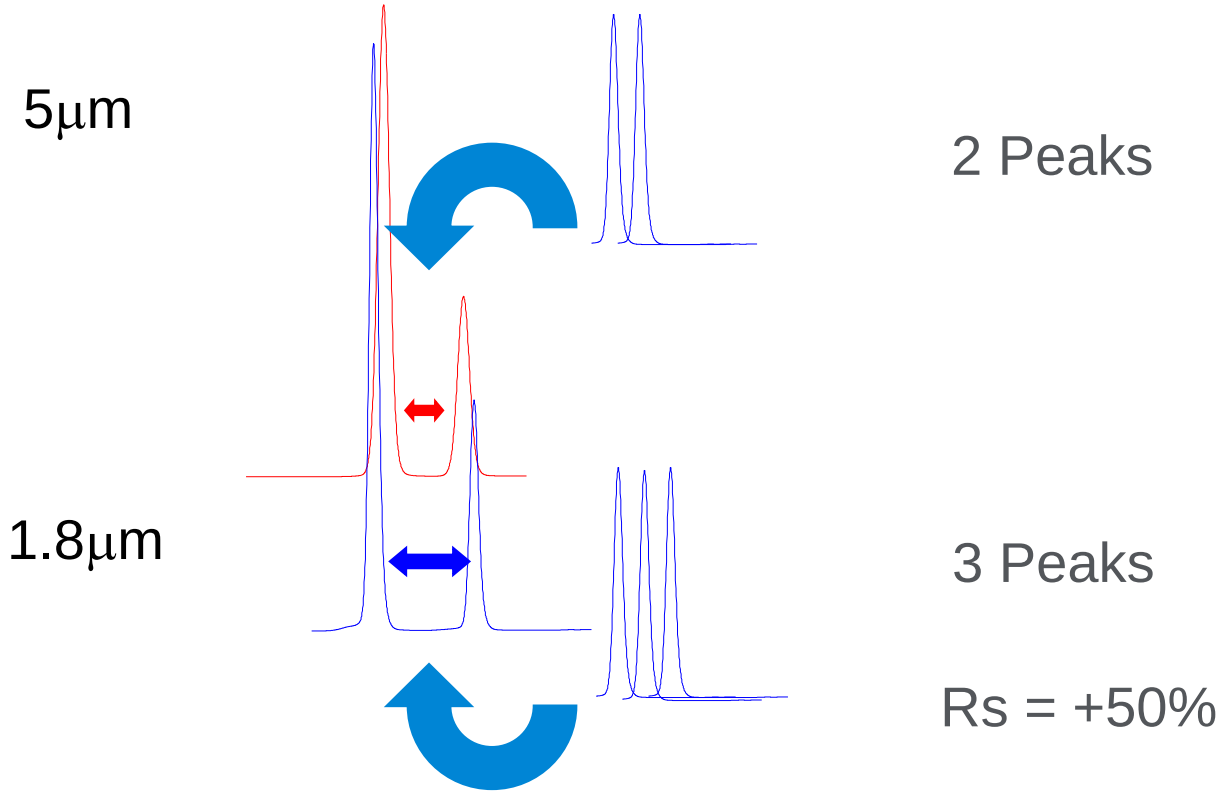
Resolution



For equal peak areas, R = 1.5 gives “baseline” separation.

What is Peak Capacity?

- The number of peaks that can be separated with defined resolution (e.g., Rs=1) in a certain time period for a given system (column length and particle size).
- Another measure of separation efficiency
  - Especially useful for complex chromatograms



Peak Capacity

Calculation of Peak Capacity

Equation for isocratic elution

$$P_c = 1 + \frac{\sqrt{N}}{4R_S} \ln[1+k_{last}]$$

k_{last}: Retention factor for last peak

N: Efficiency

R_s: Minimum required resolution

Equation for gradient elution

$$P_c = 1 + \frac{t_G}{\frac{1}{n}\sum_1^n w}$$

$$= 1 + \frac{t_G}{w_{av}}$$

P_c: Peak Capacity

t_G: Gradient time

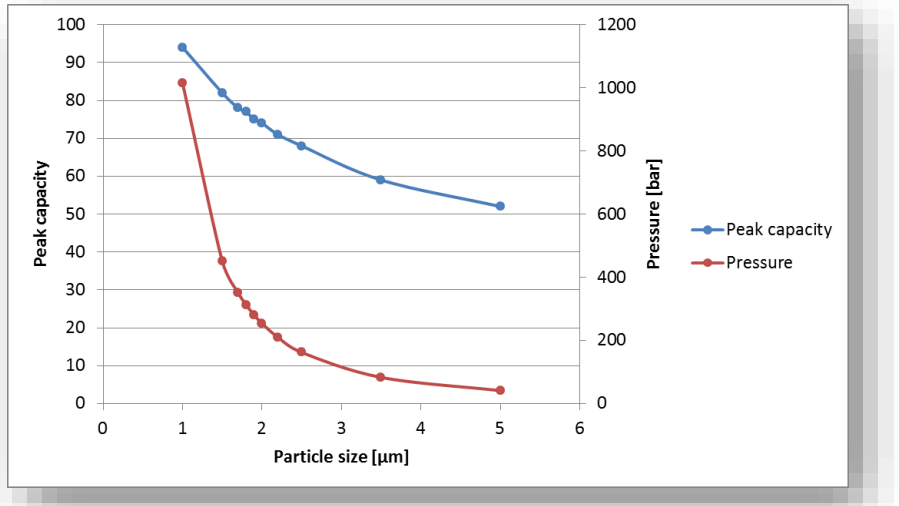
W: Peak width

w is influenced by N
N depends on column length and particle size

Peak Capacity can be Increased by Using…

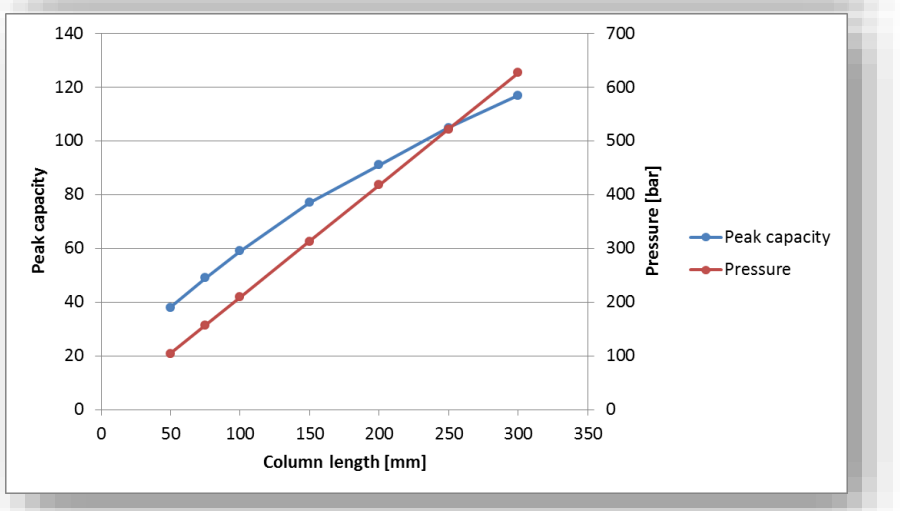
…smaller particles.

Disadvantage: Heavily increased back pressure



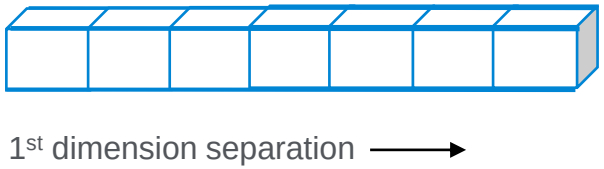
…longer columns.

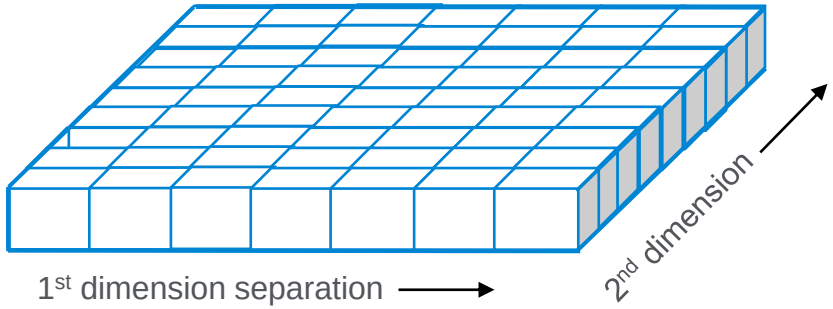
Disadvantage: Increased back pressure and run time



Or We Can Go to a Second Dimension

Schematic Representation of Peak Capacity





1st dimension separation

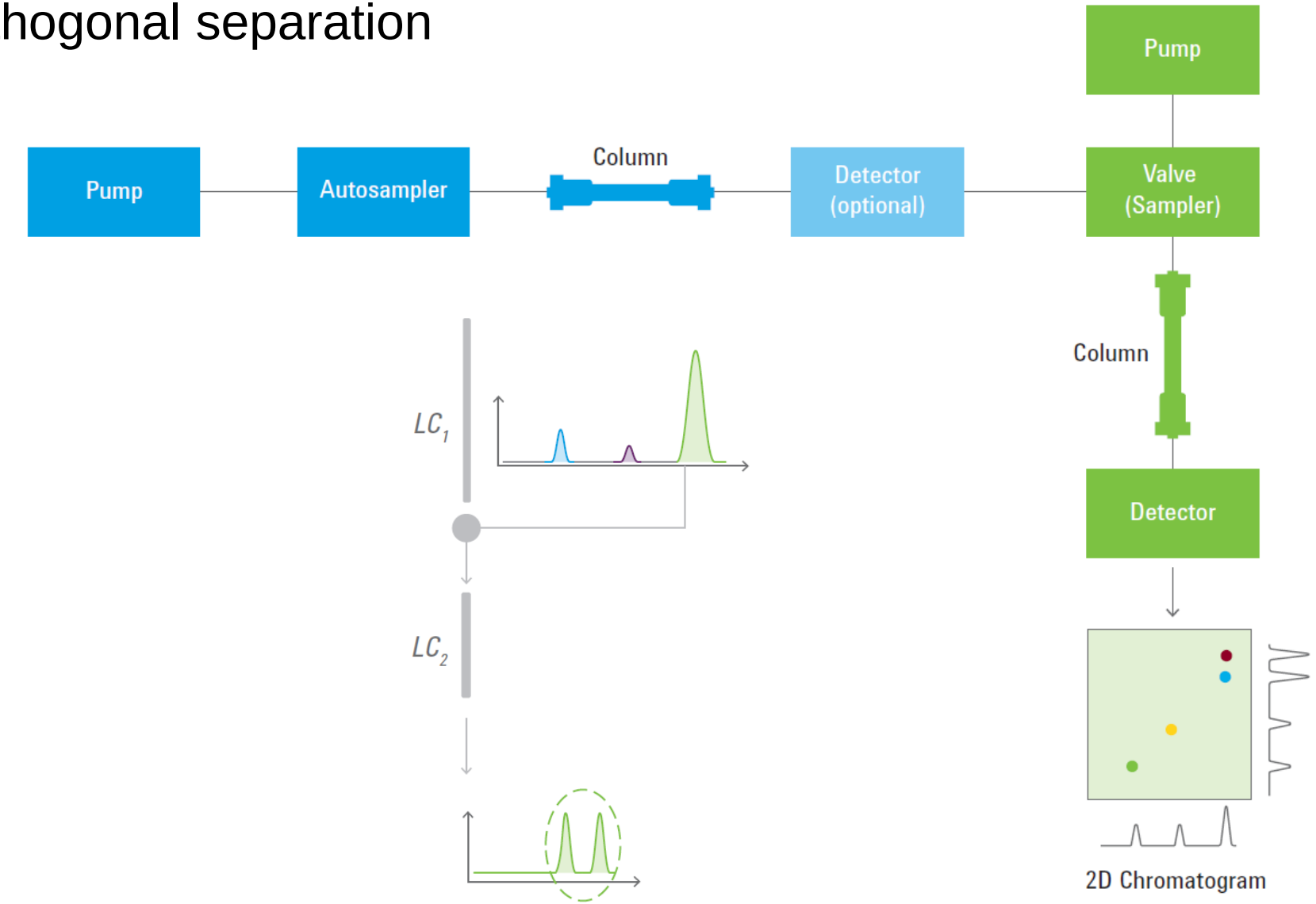
- Each block represents a unit of peak capacity
- Lined up in the same dimension

2nd dimension separation

- Increases the number of blocks/units of capacity

What is 2D Chromatography

Adding orthogonal separation



2D-LC Separation Modes

Hardware

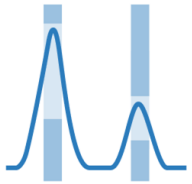
Agilent InfinityLab

1290 Infinity II 2D-LC System

Software

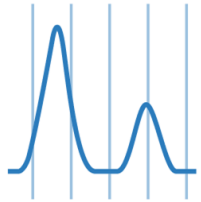
2D-LC software for OpenLab ChemStation

(Multiple) Heart-Cutting 2D-LC (MHC 2D-LC)



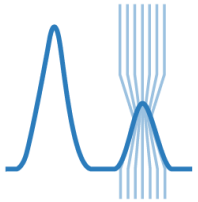
Store one or multiple peaks of interest and further analyze in the second dimension.

Comprehensive 2D-LC (LCxLC)



Send the complete 1D efluent in equal portions to the second dimension and get the full picture.

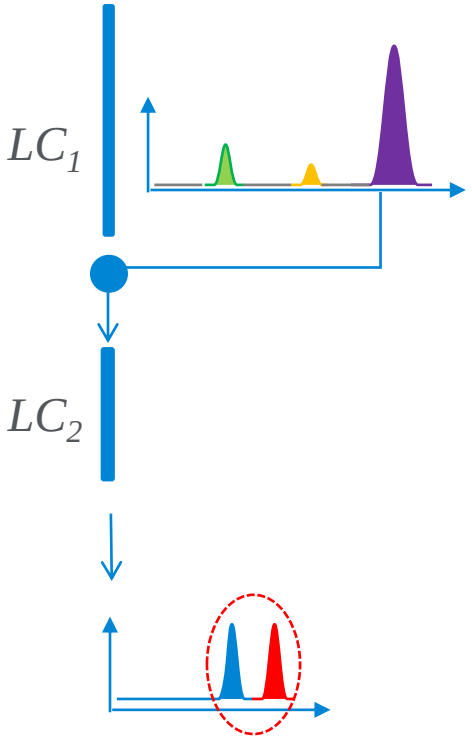
High Resolution Sampling 2D-LC (HiRes 2D-LC)



Make multiple cuts across one peak of interest and compare results at different positions, for example, for reliable quantification.

2D-LC – Heart-cutting vs Comprehensive 2D-LC

Heart-cutting 2D-LC (LCxLC)



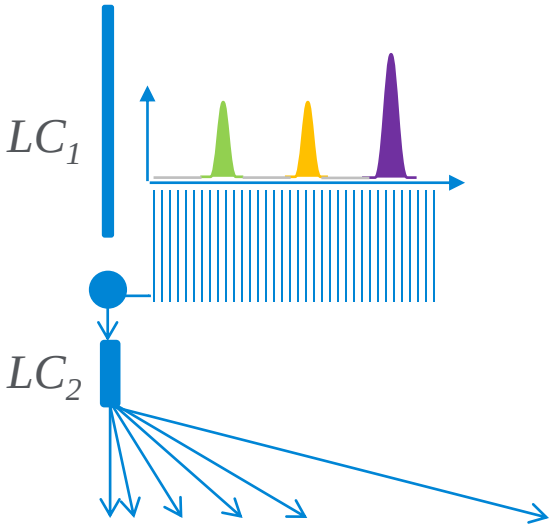
Parts of the 1D effluent are injected onto the 2D system

Long 2D gradients possible → good data quality

Limited 2D information

2D-LC –Comprehensive 2D-LC

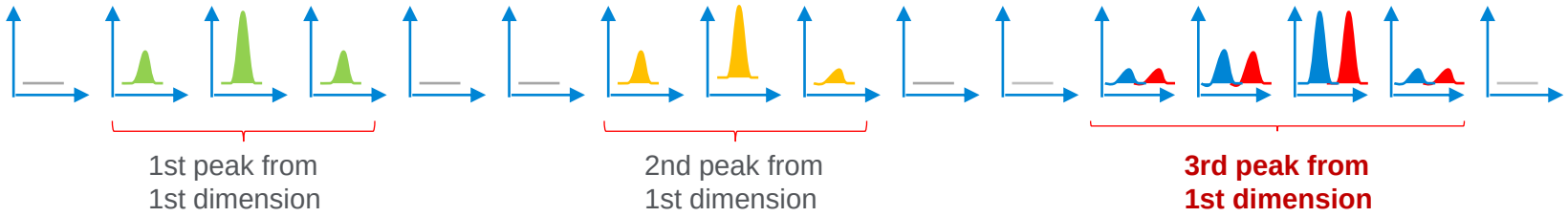
Comprehensive 2D-LC (LCxLC)



The whole 1D effluent is injected onto 2D system

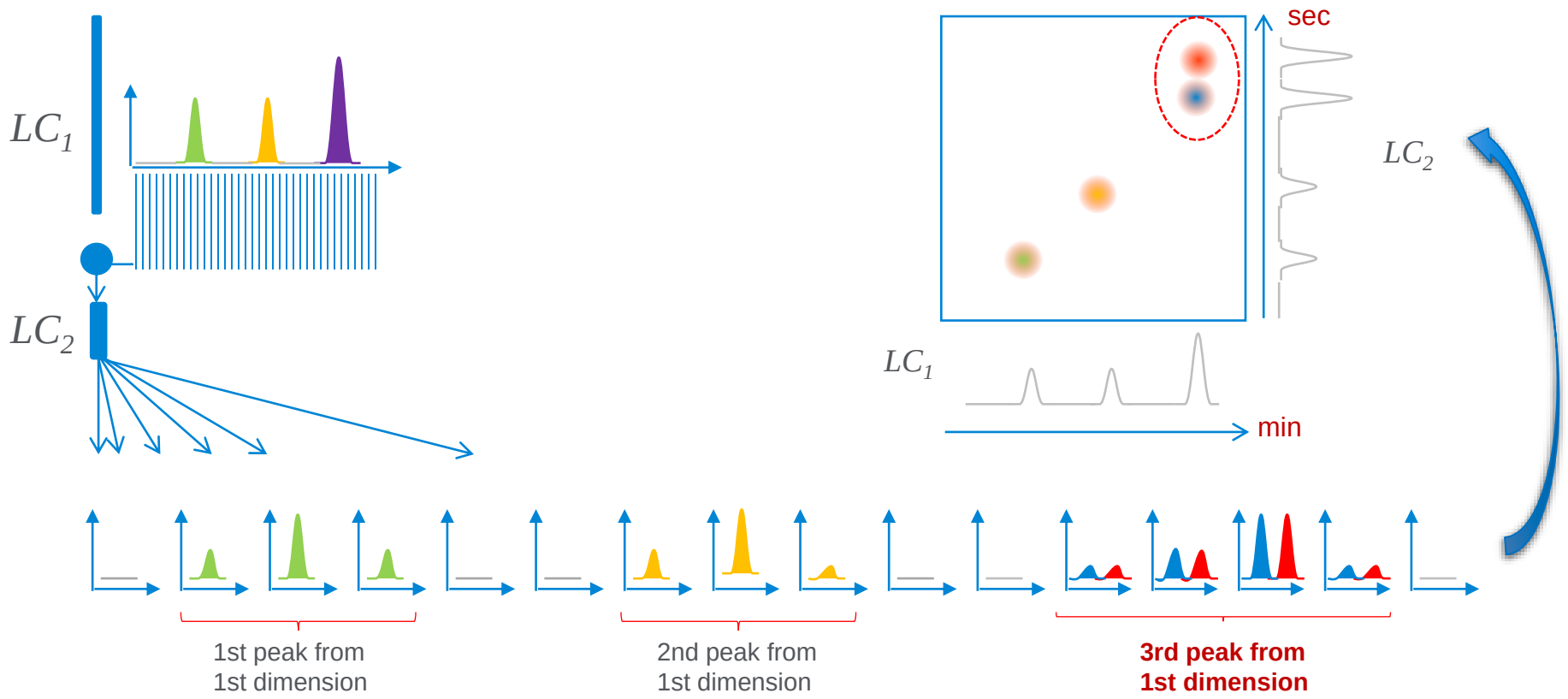
(Ultra)Short 2D gradients necessary → Good data quality with fast pumps & detector

Full (“comprehensive“) 2D information!

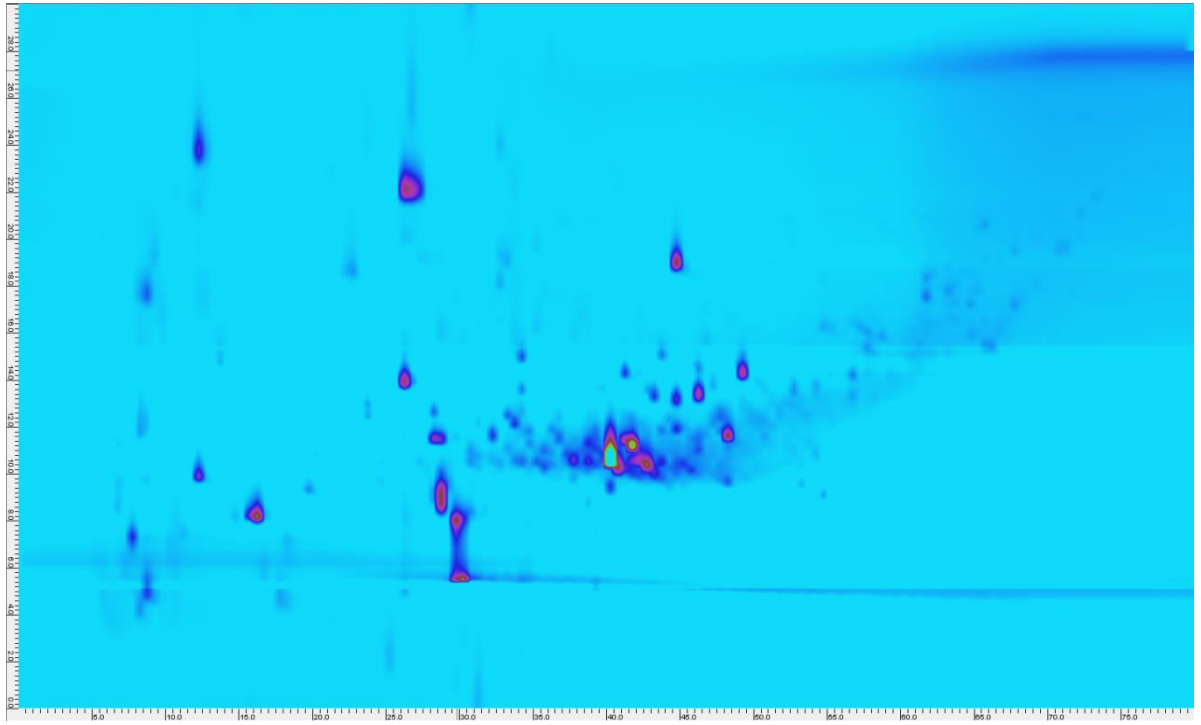


2D-LC –Comprehensive 2D-LC

Comprehensive 2D-LC (LCxLC)



2D-LC – Comprehensive Data View



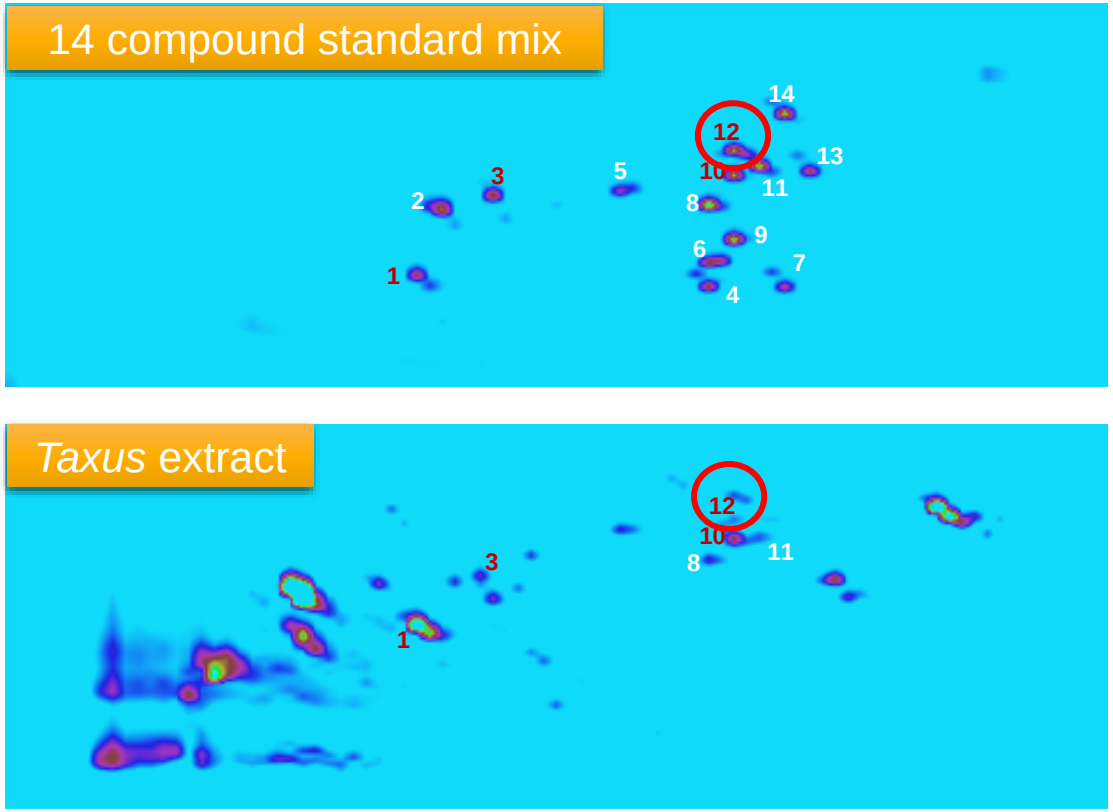
Taxanes from Taxus Extract

Analysis of extract vs. standard sample

Full separation of all taxanes from interfering peaks

- Especially main peak of interest (Paclitaxel, #12); separation fully achieved

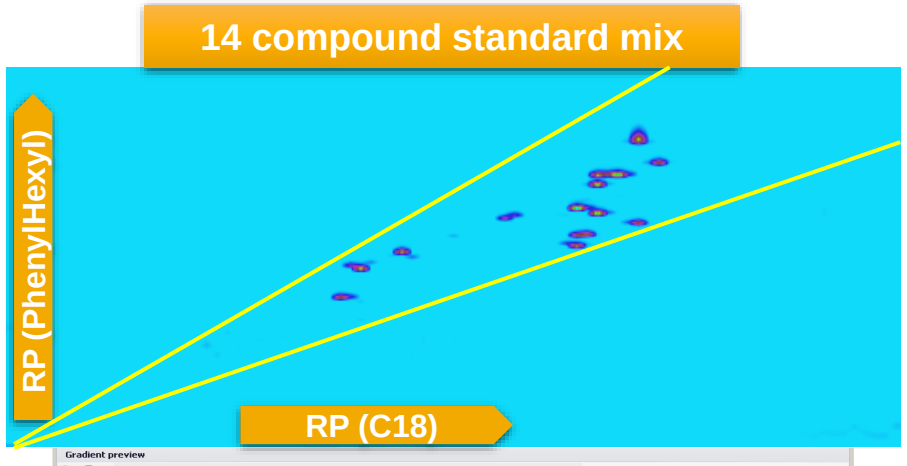
More robust separation of peaks compared to 1D separation

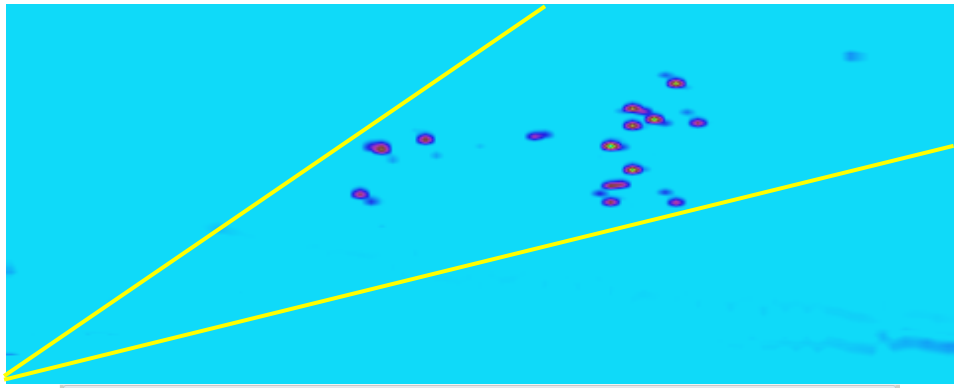


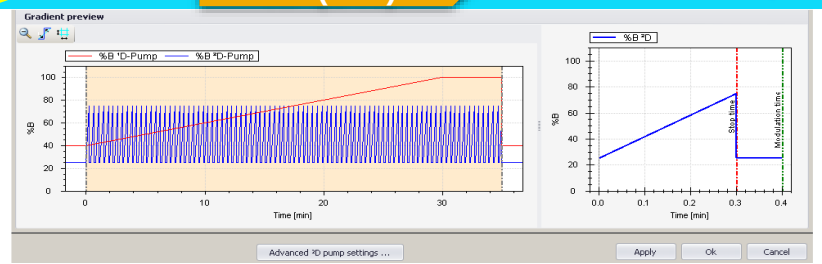
[Application Note 5991-3576EN.pdf]

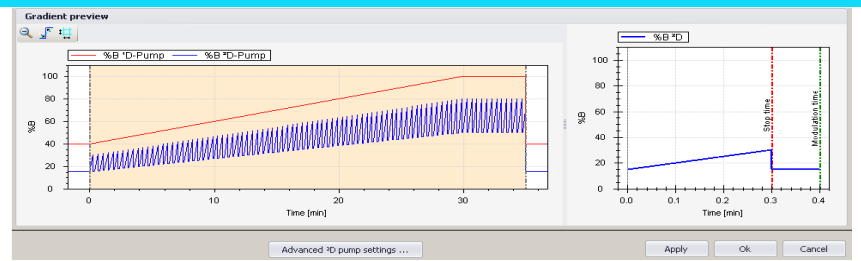
Taxanes from Taxus Extract

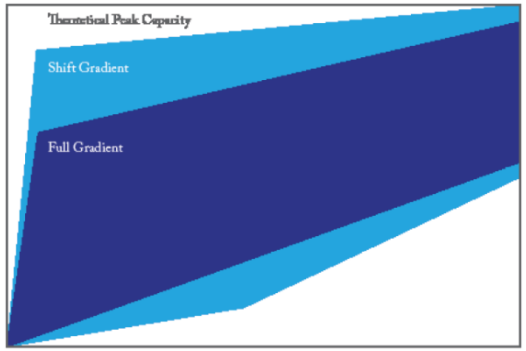
Method optimization using shifted gradient











D. Li and O. J. Schmitz

"Use of Shift Gradient in the Second Dimension to Improve the Separation Space in Comprehensive Twodimensional Liquid Chromatography"

Anal. Bioanal. Chem. 405, 6511-6517 (2013)

Application: Components of Beer Samples

Analysis of two different commercially available Japanese beer samples with different separation modes

System:	
1st dim pump:	1260 Infinity Binary Pump
AutoSampler:	1290 Ininfity Autosampler
2nd dim. pump:	1290 Infinity Binary Pump
TCC:	1290 Infinity Therm. Column Comp. w. 2D-LC valve
Detector:	1290 Infinity Diode-Array Detector G4212A,
Data acquisition:	OpenLAB ChemStation Edition w. 2D-LC Add-on
Data analysis:	LC image software from GC image LLC

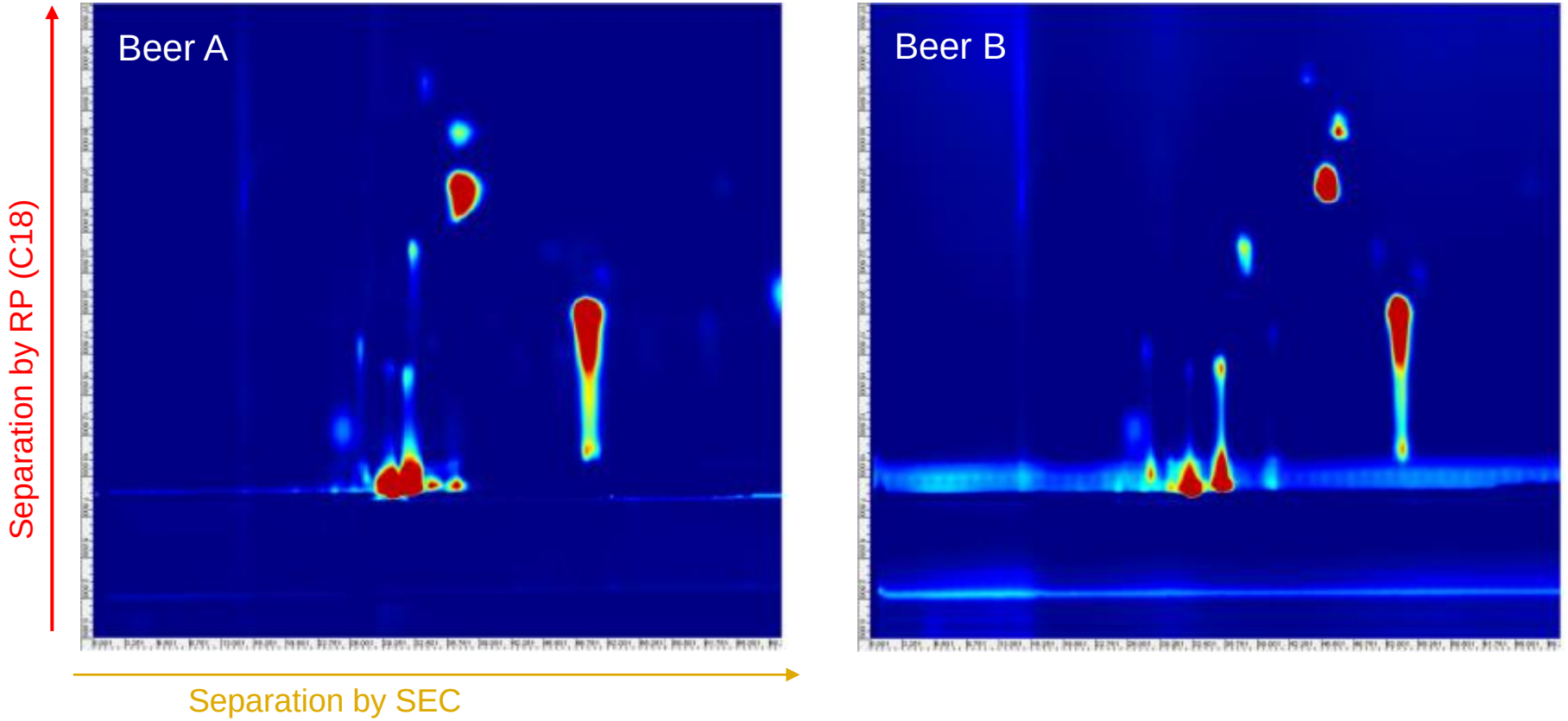
Experiments	1st dimension	2nd dimension
1.	SEC	RP (C18)
2.	IEX	RP (C18)
3.	RP (C18)	RP (Phenyl)

From WikiCommons, File:Selection of Japanese beer.jpg

Components of Beer Samples

SEC - RP

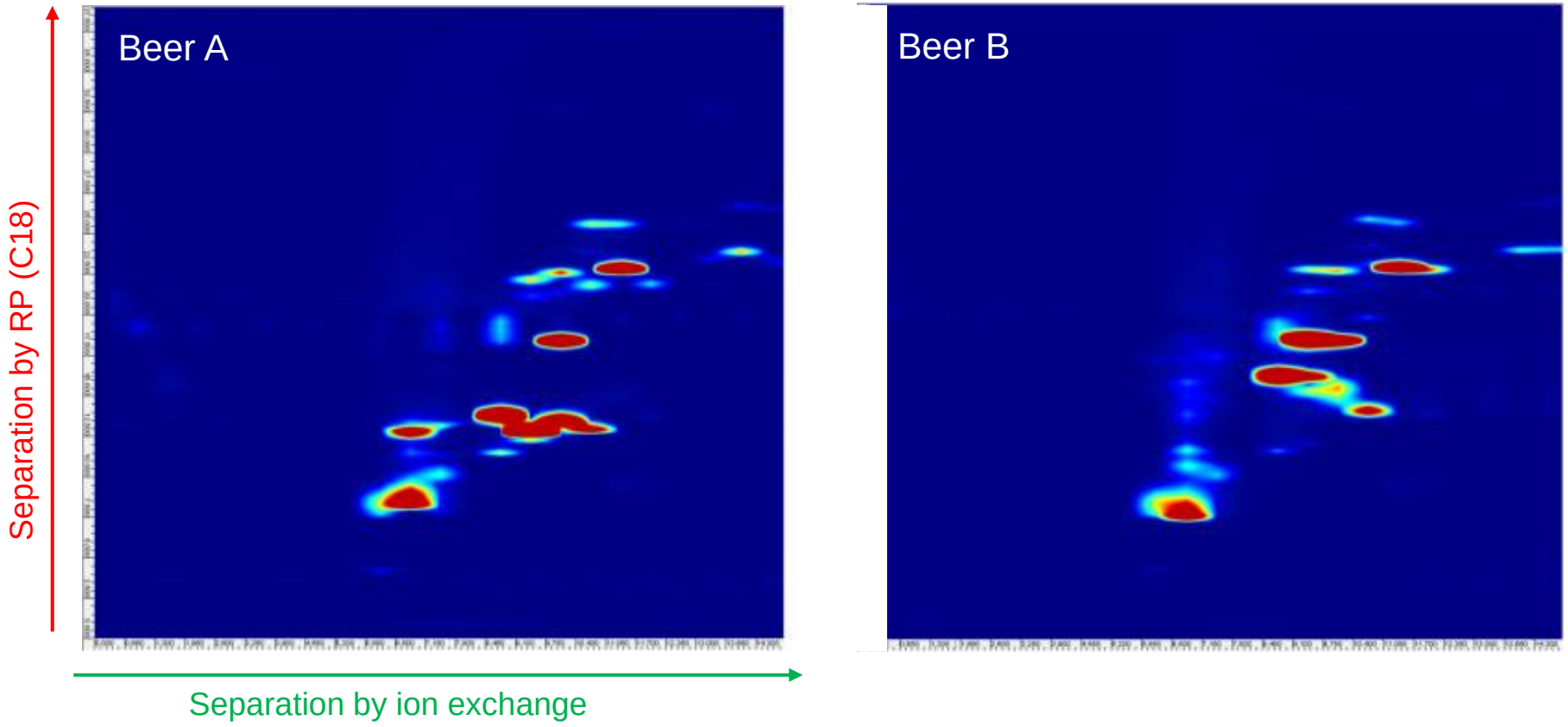
1st dimension - SEC **2nd dimension - Reverse phase (C18)**



Components of Beer Samples

IEX - RP

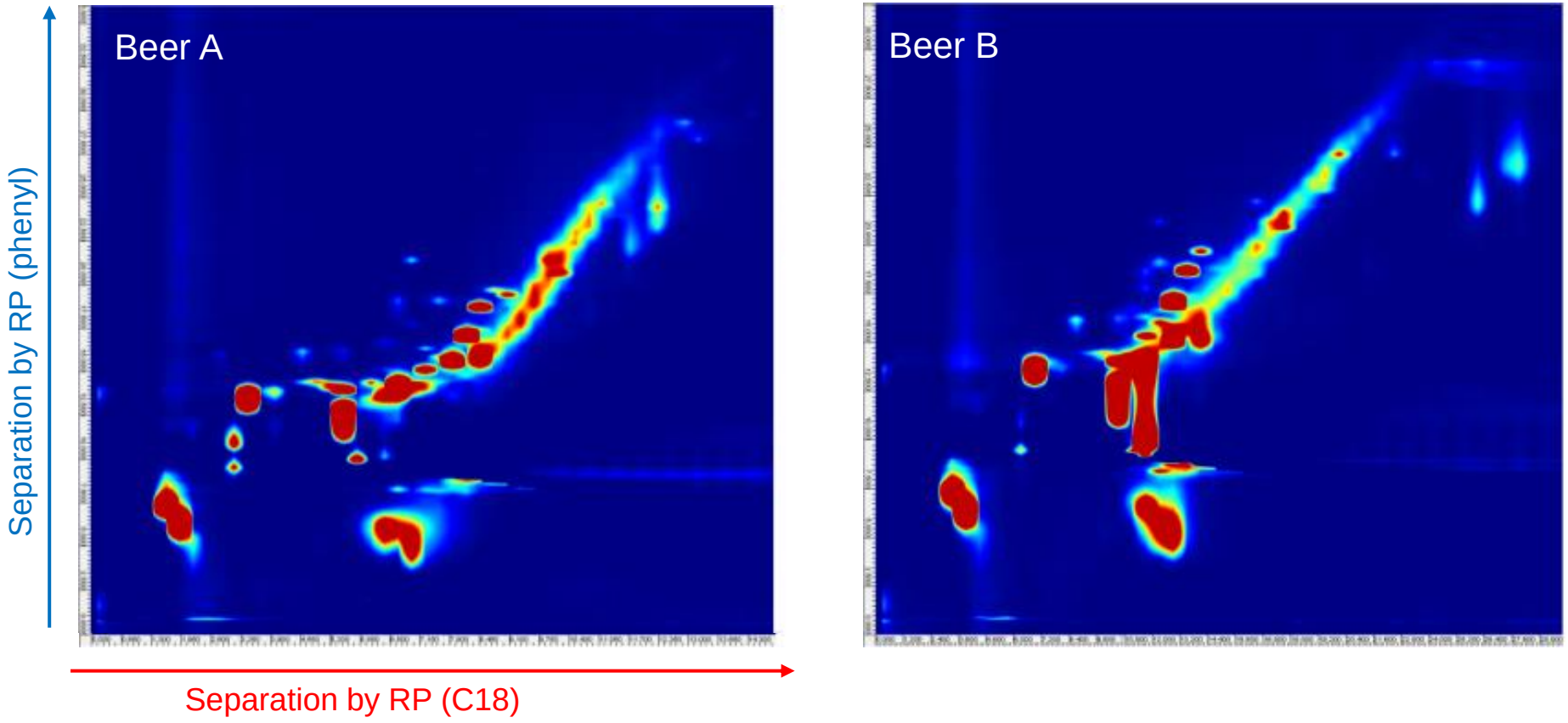
1st dimension - Ion exchange 2nd dimension – Reverse phase (C18)



Components of Beer Samples

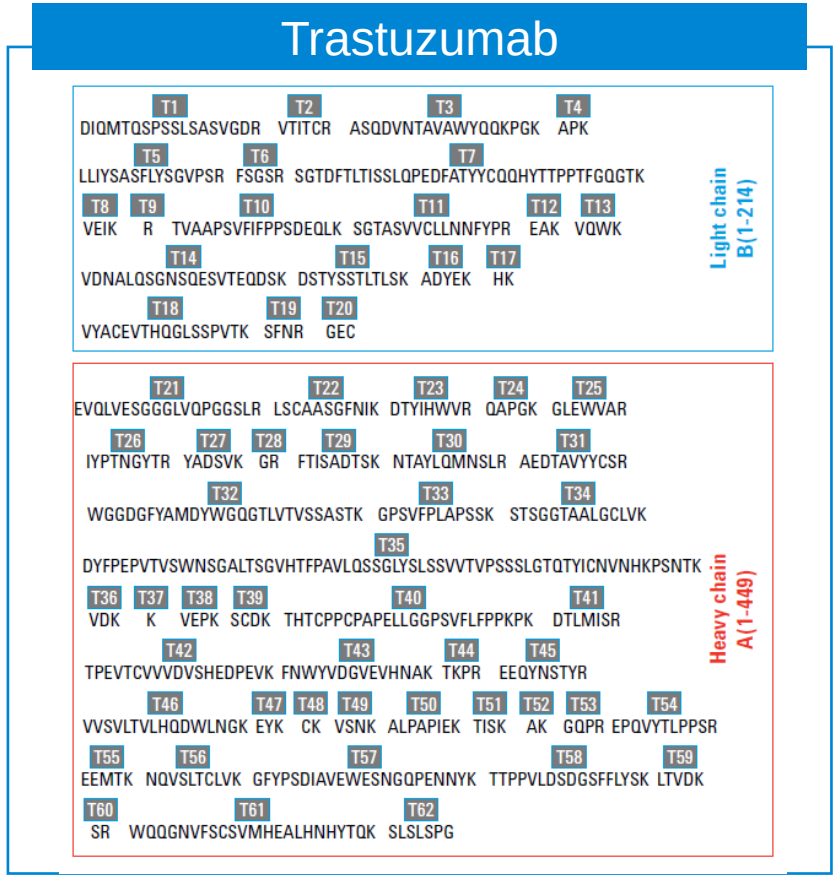
RP - RP

1st dimension - Reverse phase (C18) **2nd dimension - Reverse phase (phenyl)**



Comprehensive 2D-LC

Peptide mapping of nonstressed and stressed mAbs



Objectives

- Analysis of a tryptic digest of trastuzumab (herceptin)
- Use of HILICxRP for increased orthogonality

1D mode: HILIC

- Agilent ZORBAX RRHD 300-HILIC, 2.1 × 100 mm, 1.8 µm
- Flow: 50 µL/min
- Solvents: A:15 mM NH_4Formiate pH 4.5, B: 15 mM NH_4Formiate in 90% ACN, pH 4.5

2D mode: RP

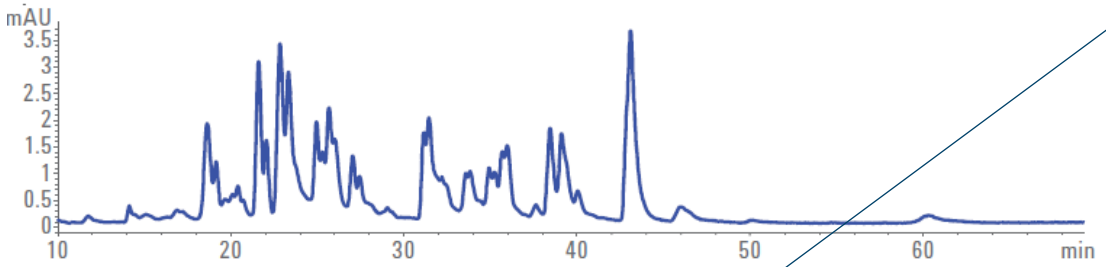
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 3.5 µm
- Solvents: A: H_2O+0.1% FA, B: ACN
- Flow: 4 mL/min

Read more here: Application note 5991-4530EN

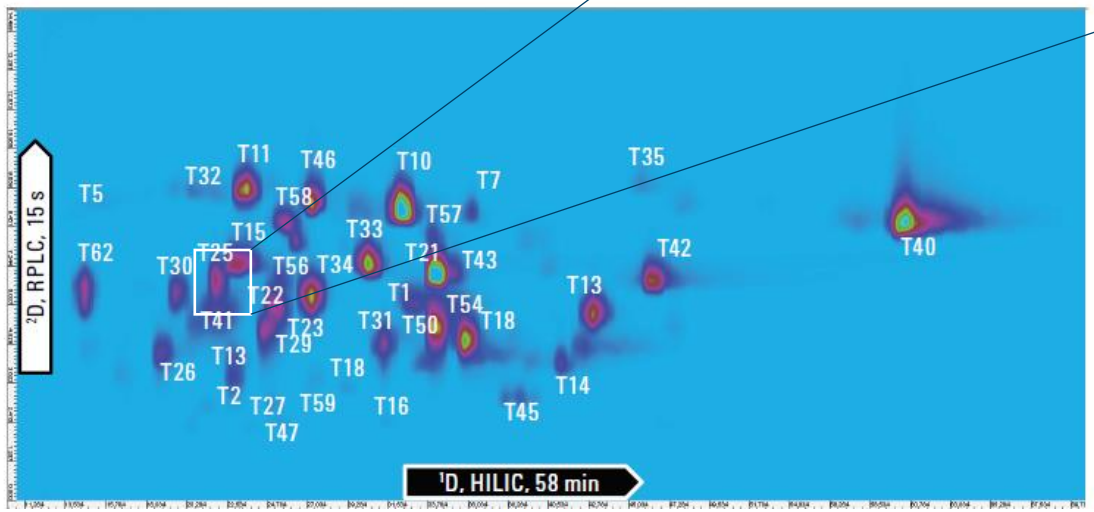
Comprehensive 2D-LC

Peptide mapping of nonstressed and stressed mAbs, application note 5991-4530EN

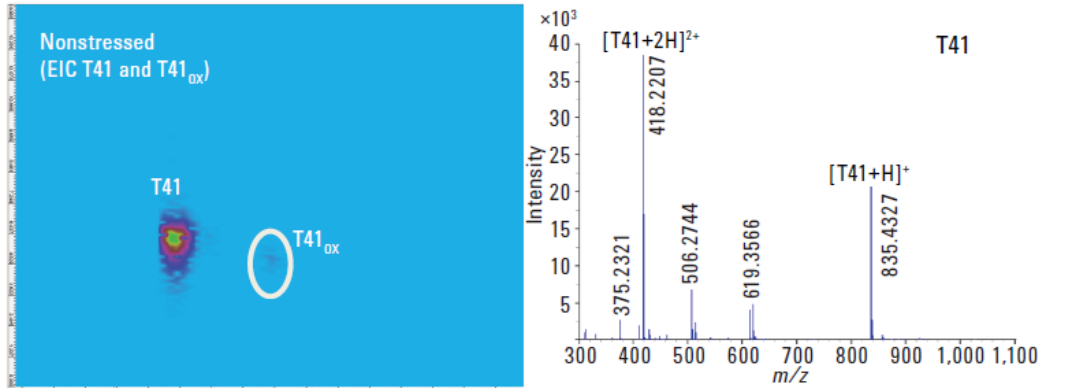
HILIC (^{1}D)



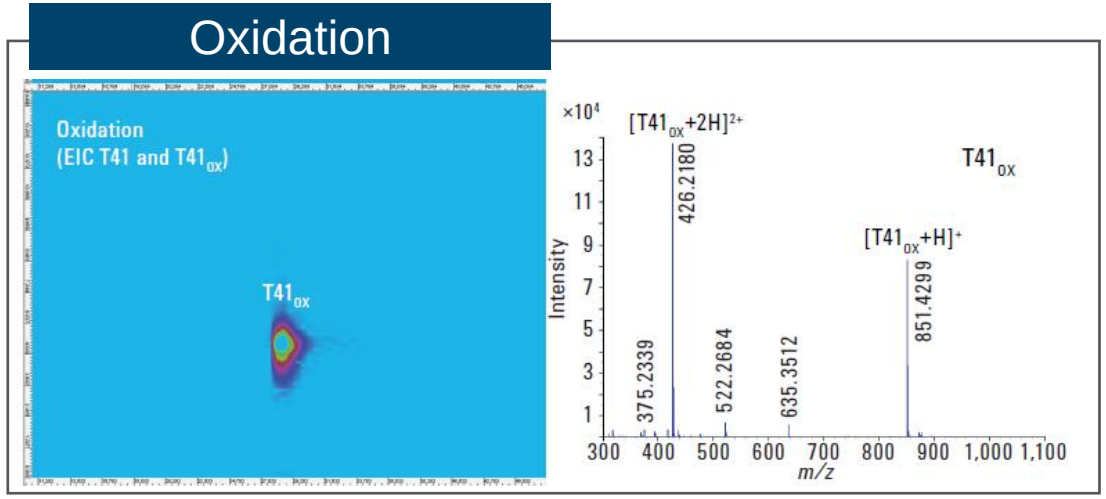
HILICxRP



Nonstressed

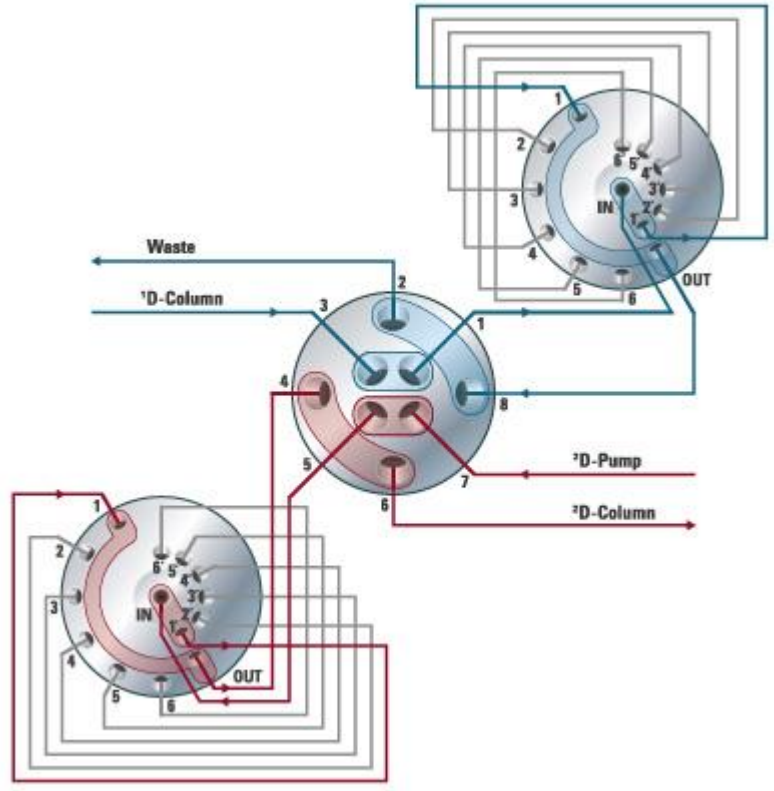


Oxidation

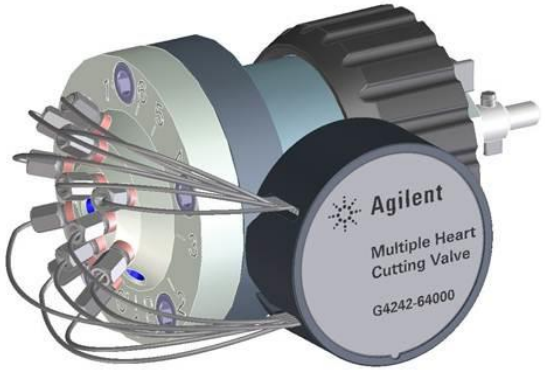


Unmatched Multiple Heart-cutting 2D-LC Usability

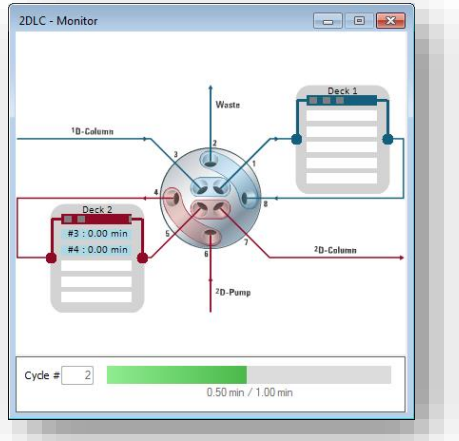
Smart Valve-Loop Setup with 12 loops
→ **2D-LC valve + two 6/14 valves**



Pre-aligned loop-valve kits, just add to the existing 2D-LC system

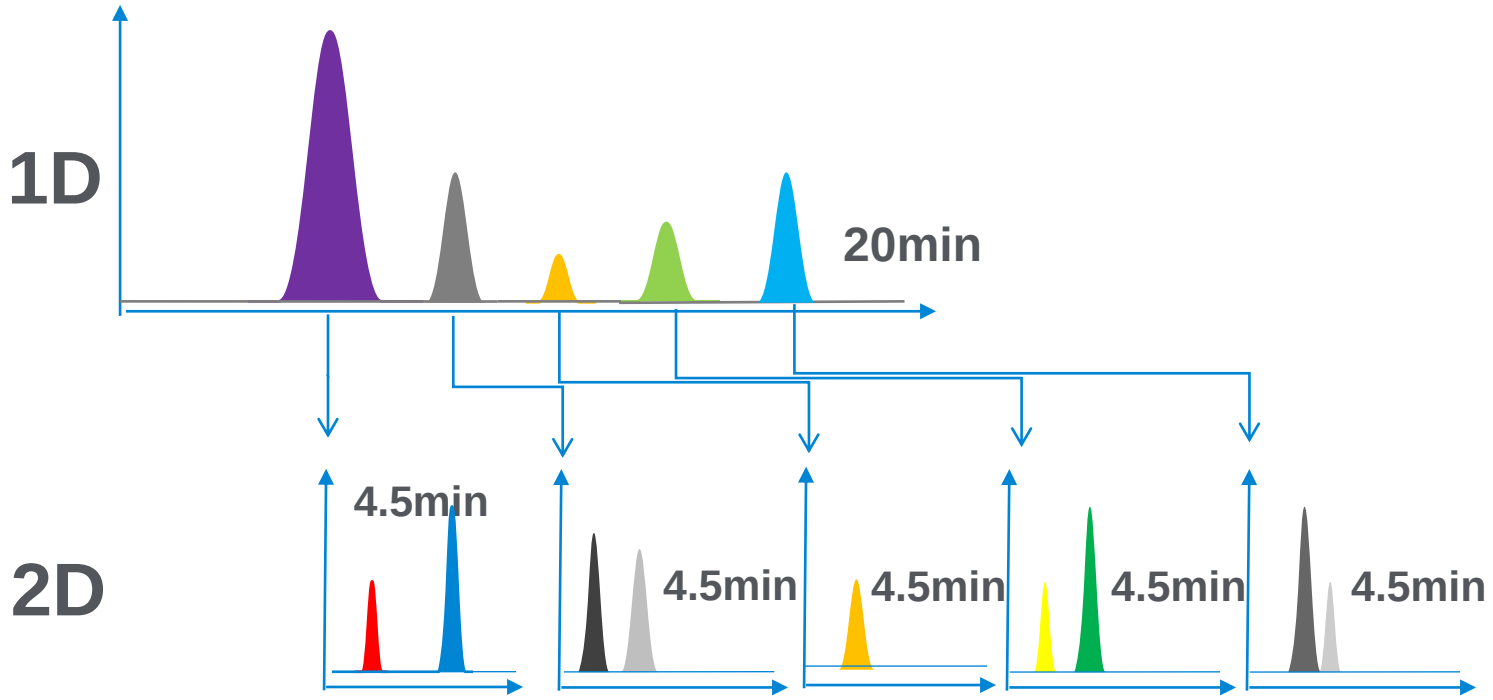


Online status monitoring



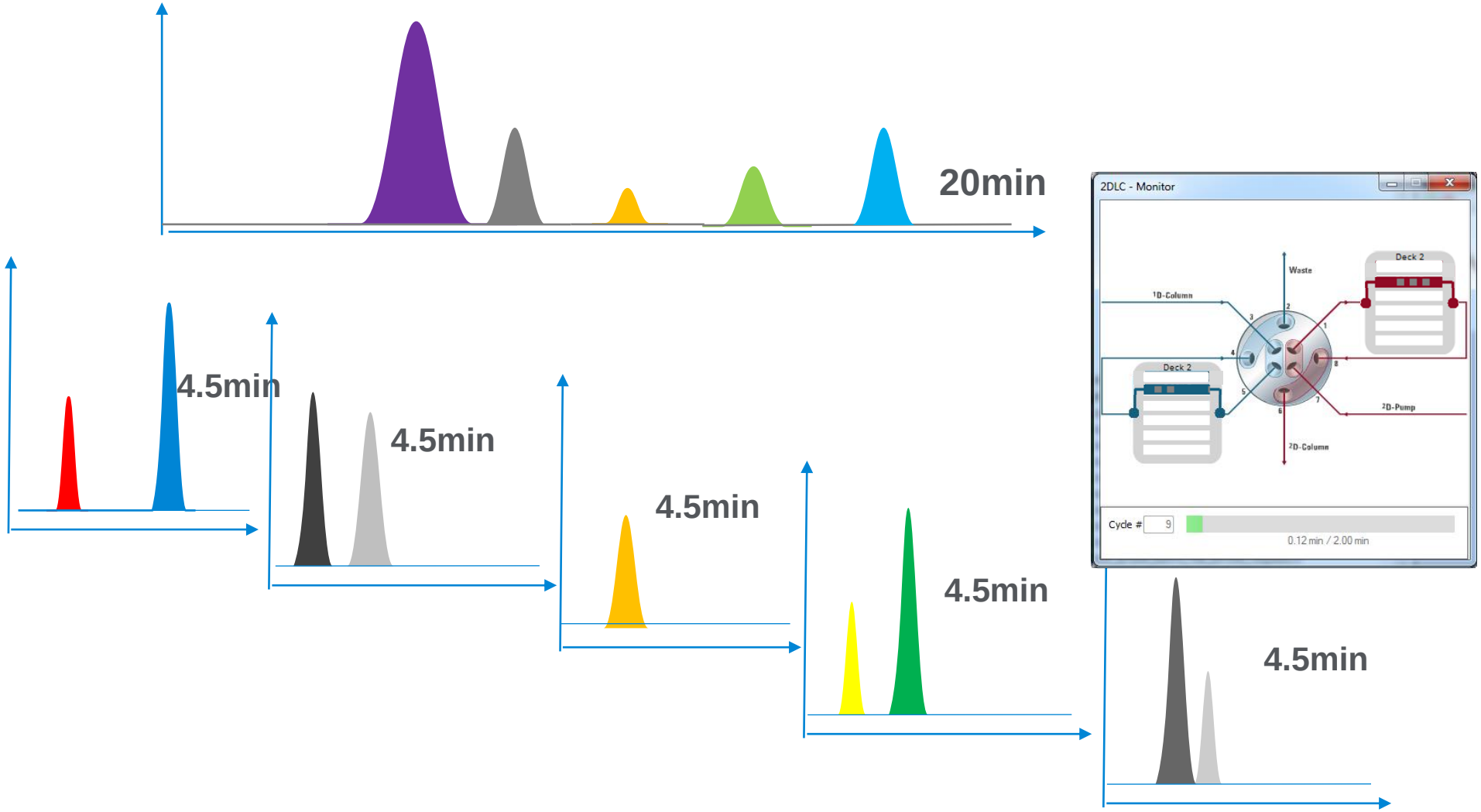
1290 Infinity 2D-LC Solution

Multiple Heart-cutting



1290 Infinity 2D-LC Solution – Scalability

Multiple Heart-cutting



Single Heart-Cutting 2D-LC

Direct Analysis of In-process Oligonucleotides, application note 5991-9490EN

Nucleotide samples

Oligonucleotide resolution standard (p/n 5190-9028):
14 mer:
rCrArCrUrGrArArUrArCrCrArArU
17 mer:
rUrCrArCrArCrUrGrArArUrArCrCrArArU
20 mer:
rUrCrArUrCrArCrArCrUrGrArArUrArCrCrArArU
21 mer:
rGrUrCrArUrCrArCrArCrUrGrArArUrArCrCrArArU

RNA sample (RNA/2'-OMethyl mix; synthesized by Agilent NSAD):
5'-GuGcCaAcCuGaUgCaGcU-3',
upper case: RNA, lower case: OMethyl

Objectives

- 1st dimension: Desalting of oligonucleotides samples
- 2nd dimension: Separation of oligonucleotides from impurities

1D method parameters

- Column: PLRP-S, 2.1 × 50 mm, 3 µm
- Gradient: Depending on sample
- Solvents: A: 50 mM NH4Ac, pH 7
- Flow: 0.4 mL/min

2D method parameters

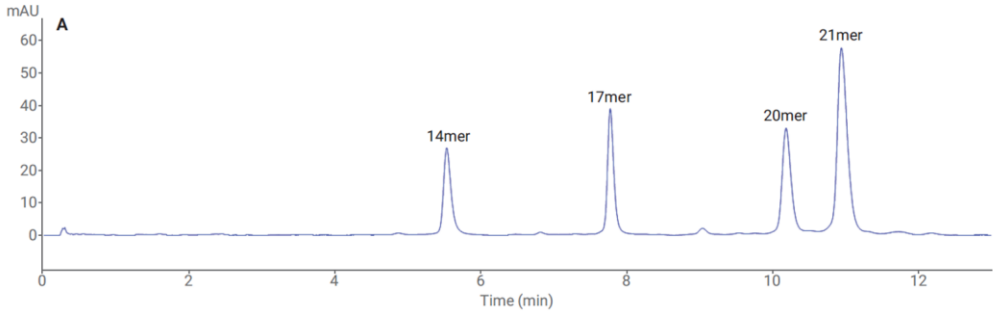
- Column: AdvanceBio Oligonucleotide, 2.1 × 50 mm, 2.7 µm
- Solvent: 400 mM HFIP + 15 mM TEA in water; B: Solvent A/methanol (50:50 v:v)
- Flow: 0.4 mL/min, ASM Factor 5

Single Heart-Cutting 2D-LC

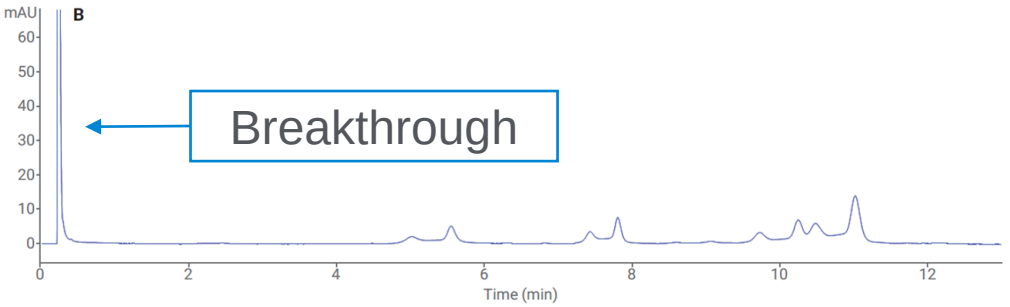
Oligonucleotides in high salt conditions show lack of RP retention, application note 5991-9490EN

1D IP-RP separation

H2O



1M NaCl



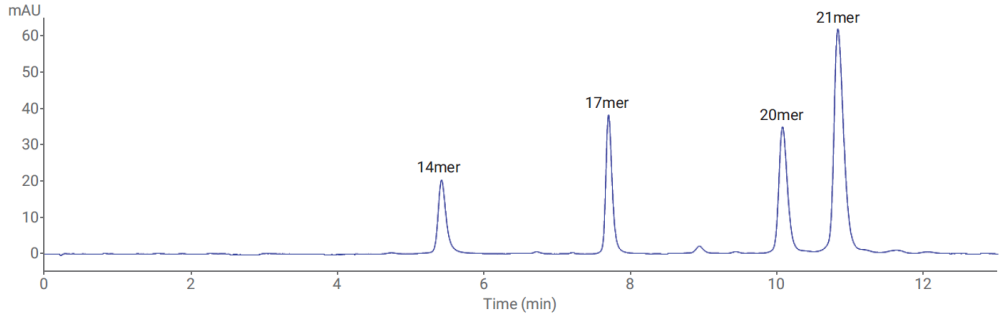
Offline sample preparation workflow

High-salt sample

Manual sample preparation using spin filters

Desalted sample

IP-RP

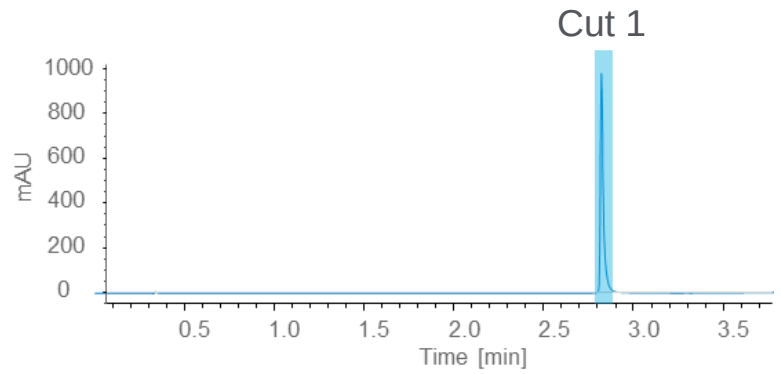


Single Heart-Cutting 2D-LC

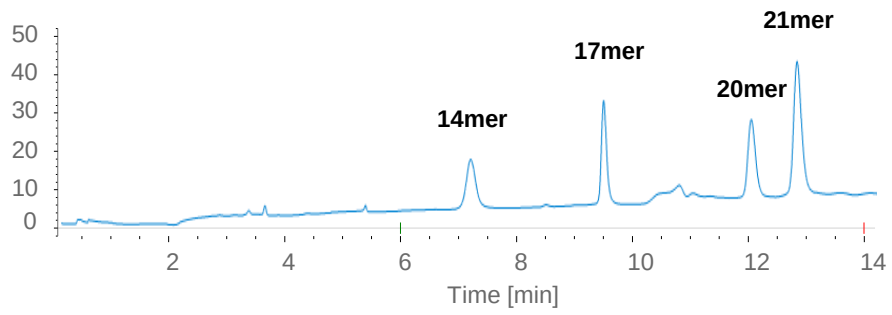
2D-LC Online desalting enables IP-RP separation of oligos

Oligo standard

^{1}D: Online desalting

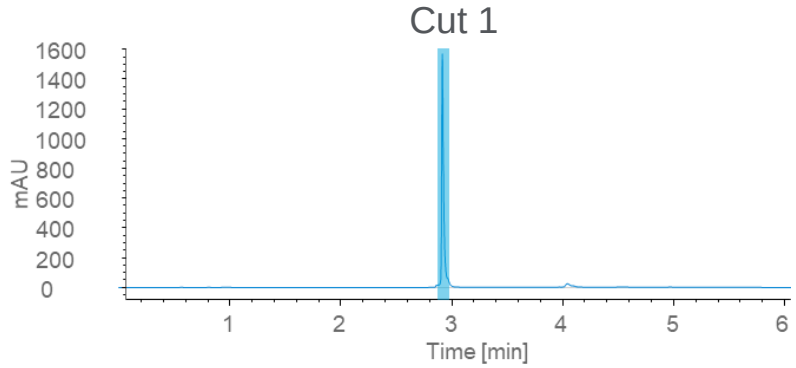


^{2}D: IP-RP

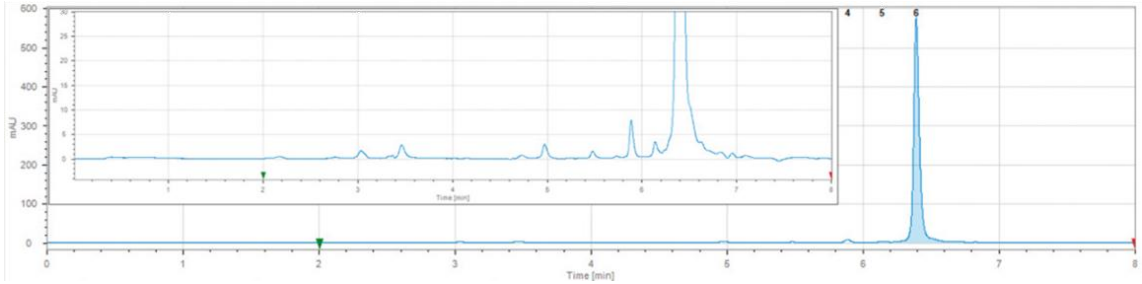


Agilent NSAD RNA sample

^{1}D: Online desalting



^{2}D: IP-RP



Multiple Heart Cutting

Analysis of mAb glycoforms using MHC 2D-LC, application note 5991-6673EN

Rituximab

Rituximab heavy chain

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWV
KQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTADKS
SSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWG
AGTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Rituximab light chain

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKP
GSSPKPWIYATSNLASGVPVRFSGSGSGTSYSLTISRVE
AEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

Objectives

- Compare Rituximab with biosimilars for possible variants with intact proteins
- Use 2D-LC technology to desalt after WCX separation to automate this workflow

1D mode: WCX

- Agilent Bio MAb, nonporous, 2.1 × 250 mm, 5 µm, PEEK
- 1260 Infinity II bio-inert pump (due to high salt concentrations)
- Salt gradient 0 to 200 mM NaCl in phosphate buffer pH 6.2

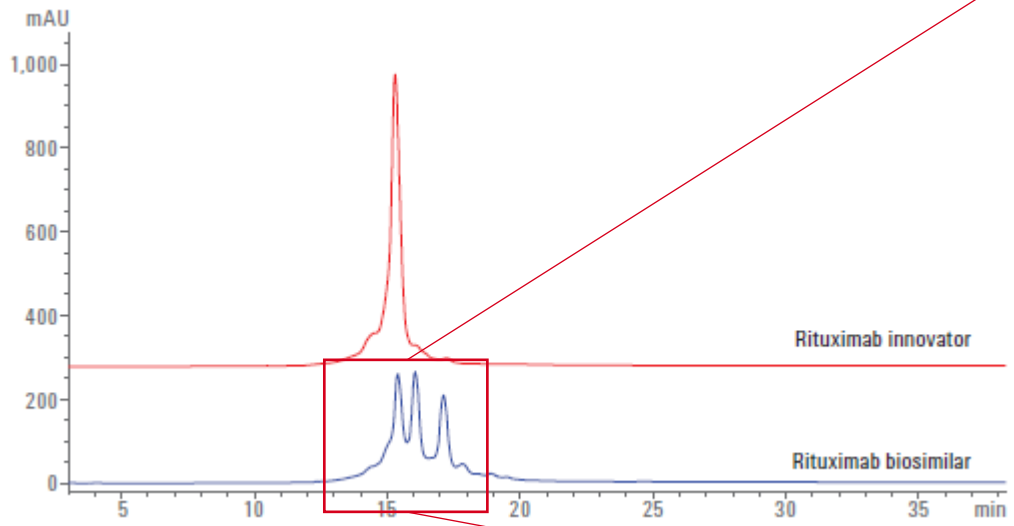
2D mode: RP

- AdvanceBio RP-mAb C4, 2.1 × 75 mm, 3.5 µm
- Solvent: A: H_2O+ 5%FA, B: ACN+5%
- Flow: 1 mL/min

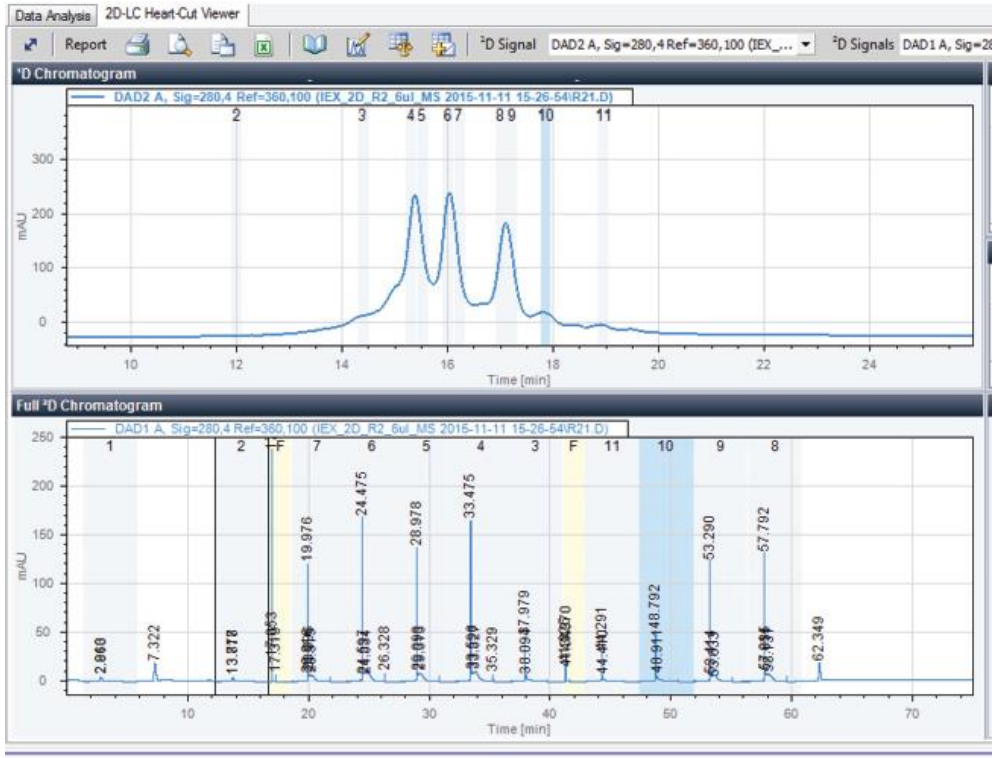
Multiple Heart Cutting

Analysis of mAb glycoforms using MHC

WCX (^{1}D)



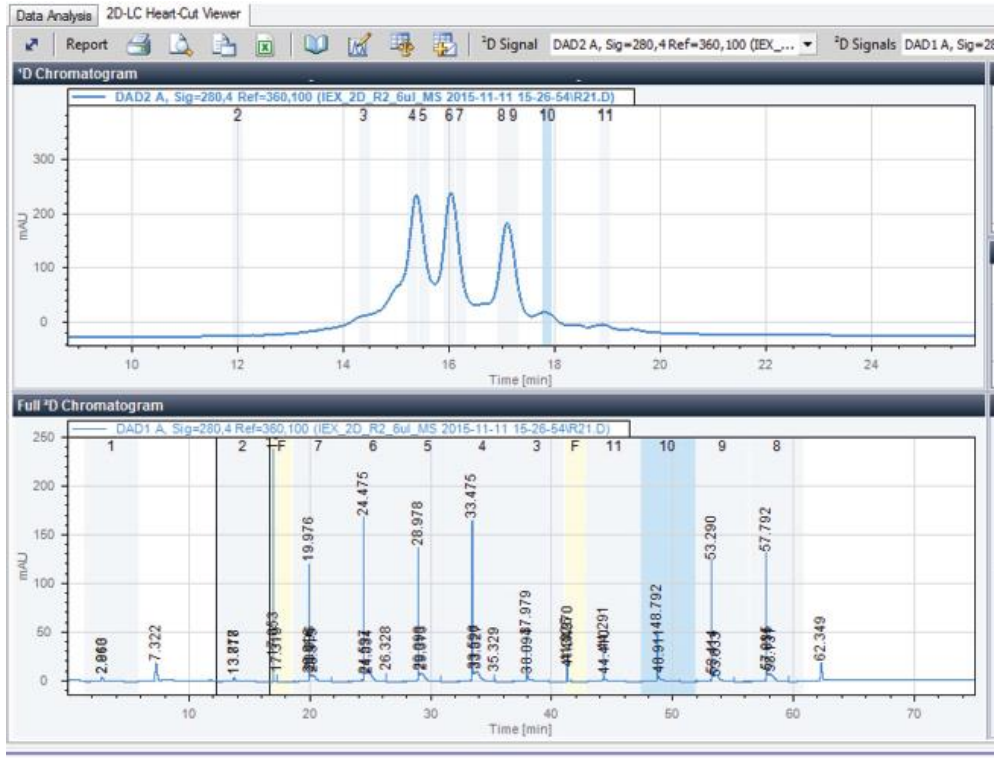
Sample desalting (^{2}D)



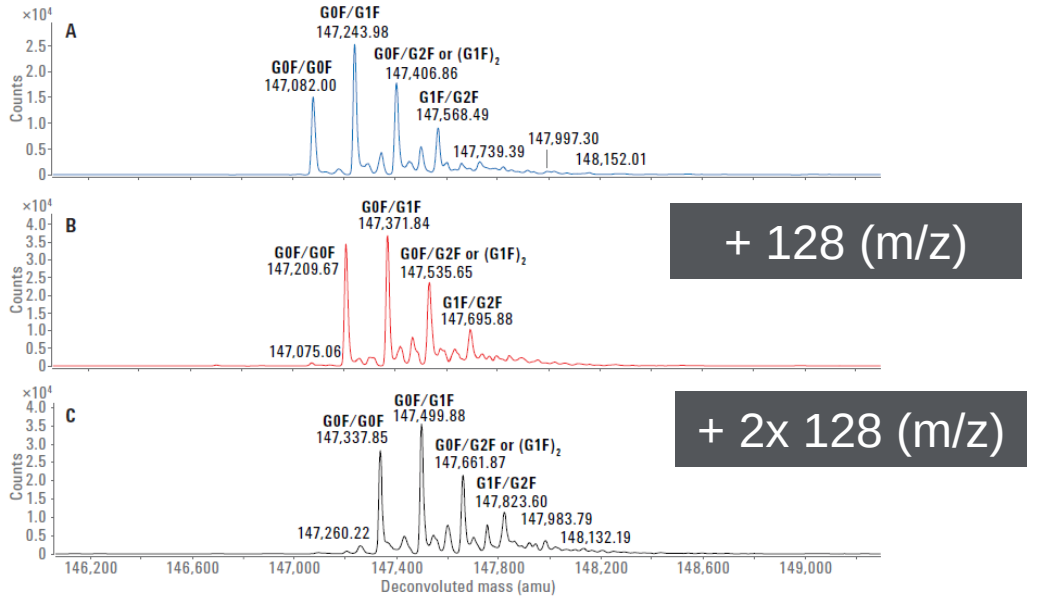
Multiple Heart Cutting

Analysis of mAb charge variants using MHC

Sample desalting (^{2}D)



QTOF analysis



+ 128 (m/z)

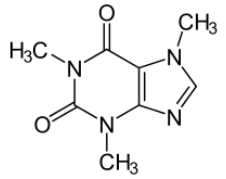
+ 2x 128 (m/z)

2D-LC-QTOF reveals two additional variants (Δ(m/z)=128) with c-terminal lysins in the Rituximap biosimilar

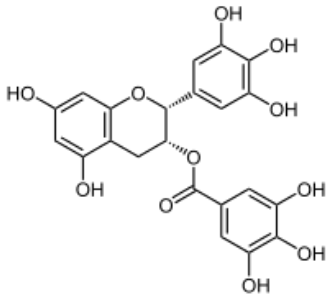
High-Resolution Sampling

Quantification of co-eluting compounds in green tea, application note 5991-7637EN

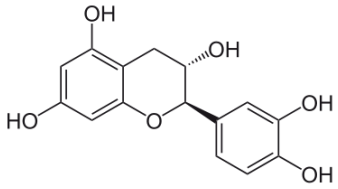
Green Tea Extract



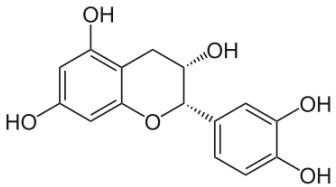
Caffeine



(–)-Epigallocatechin gallate



(+)Catechine



(-)Epi-Catechine

Objectives

- 1st dimension: Separation of compounds in green tea
- 2nd dimension: Quantification of co-eluting compounds caffeine and (–)-epigallocatechin gallate

1D mode: RP

- Column: ZORBAX Eclipse Plus C18 RRHD, 2.1 x 100 mm, 1.8 µm
- Flow: 0.2 mL/min

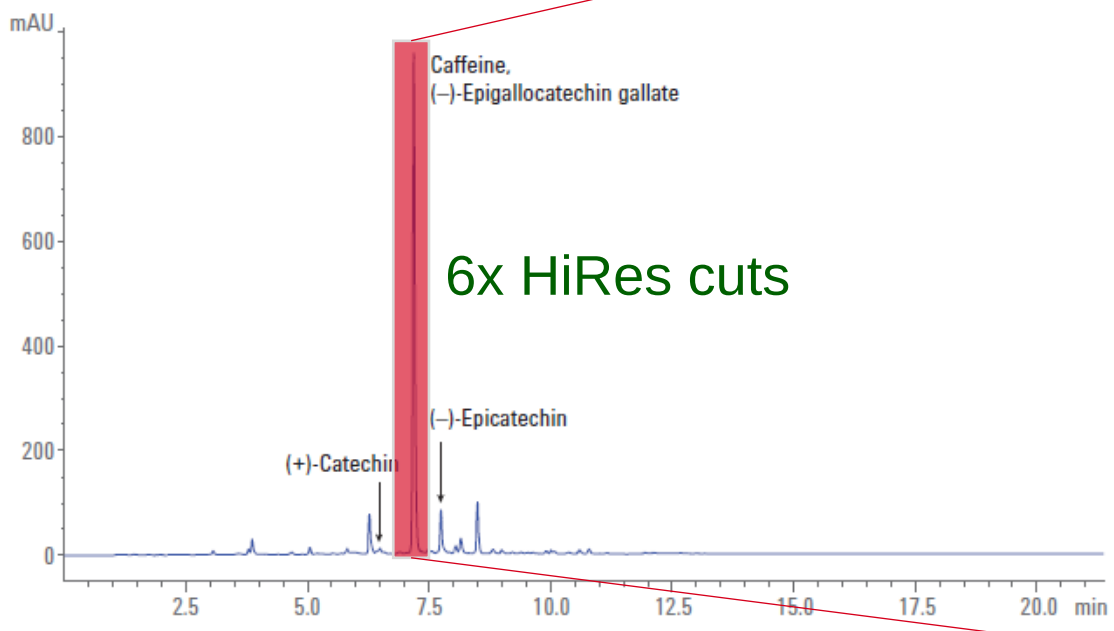
2D mode: RP

- Column: ZORBAX Bonus-RP RRHD, 2.1 × 50 mm, 1.8 µm
- Solvent: Water/methanol (35/65) + 0.1 % FA (isocratic)
- Flow: 1 mL/min

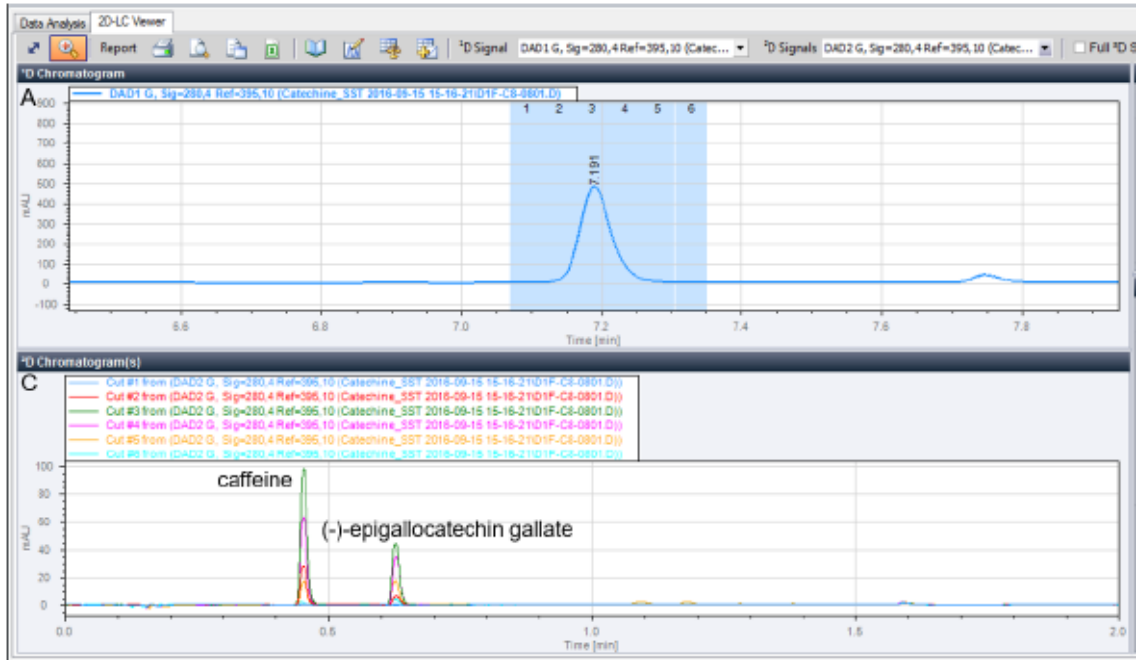
High-Resolution Sampling

High-resolution sampling on Agilent 1290 Infinity II 2D-LC solution

Preseparation (^{1}D)



Complete separation (^{2}D)

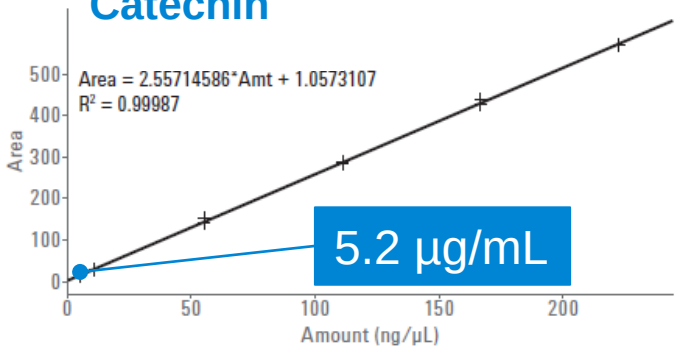


High-Resolution Sampling

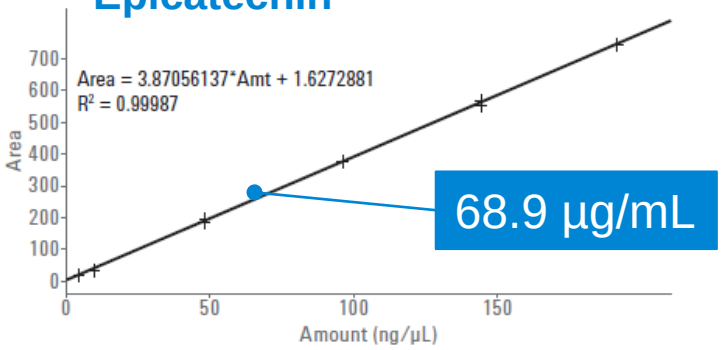
Quantification of four compounds of interest

Quantified in ^{1}D

Catechin

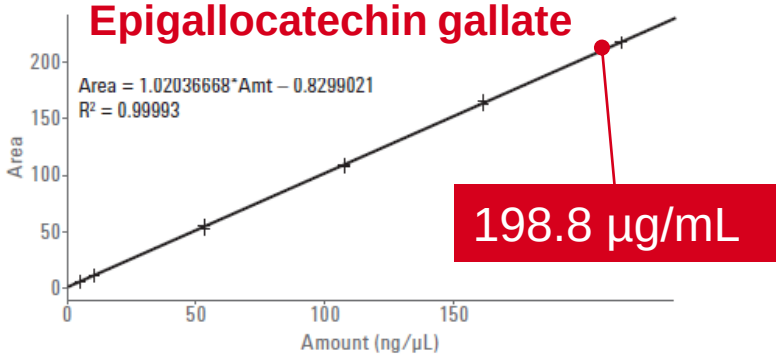


Epicatechin

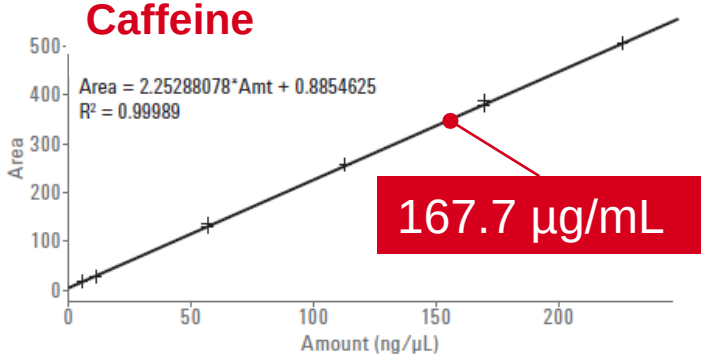


Quantified by HiRes ^{2}D

Epigallocatechin gallate



Caffeine



Hardware
Module flexibility

2. Dimension

1290 Infinity binary pump

1. Dimension

1290 Infinity binary pump

1290 Infinity autosampler or 1260 HiP autosampler

Optional 1260/1290 Infinity Detector

For 1st dimension chromatogram and peak-triggering

1260/1290 Infinity detector

Optional 1260/1290 Infinity Detector

To monitor waste-line

1260 Infinity Capillary Pump

1260 Infinity Autosampler

1260 Infinity Binary or Quaternary Pump

Almost any Agilent pump or autosampler can be used in the 1st dimension
Almost any detectors is supported
A 1290 Infinity binary pump for the 2nd dimension is best.

Agilent Column Portfolio

Part of the InfinityLab family

Column strategies for orthogonal 2D-LC separations

RP-RP (low pH - high pH)

		Orthogonality	Peak capacity
1D	Poroshell SB-(C8/C18/Aq), PFP, Phenyl-Hexyl, CN 1.9 µm, 2.7 µm, 4 µm	+	++
2D	Poroshell HPH C8, C18 1.9 µm (C18) , 2.7 µm, 4 µm		

SEC-RP

		Orthogonality	Peak capacity
1D	Bio SEC 3 100,150, 300 Å 3 µm Bio SEC 5 100, 150, 300, 500, 1000, 2000 Å 5µm	++	+
2D	Poroshell SB-(C8/C18/Aq), PFP, Phenyl-Hexyl, CN 1.9 µm, 2.7 µm, 4 µm		

IEX-RP

		Orthogonality	Peak capacity
1D	Bio MAb, Bio SCX, Bio WCX, Bio SAX, Bio WAX 1.7 µm, 3 µm, 5 µm, 10 µm	++	+
2D	Poroshell SB-(C8/C18/Aq), PFP, Phenyl-Hexyl, CN 1.9 µm, 2.7 µm, 4 µm		

HILIC-RP

		Orthogonality	Peak capacity
1D	Poroshell HILIC, HILIC Z, HILIC OH5 1.9 µm (HILIC), 2.7 µm, 4 µm (HILIC)	+	+
2D	Poroshell SB-(C8/C18/Aq), PFP, Phenyl-Hexyl, CN 1.9 µm, 2.7 µm, 4 µm		

Agilent Column Portfolio

Part of the InfinityLab family

Column strategies for orthogonal 2D-LC separations

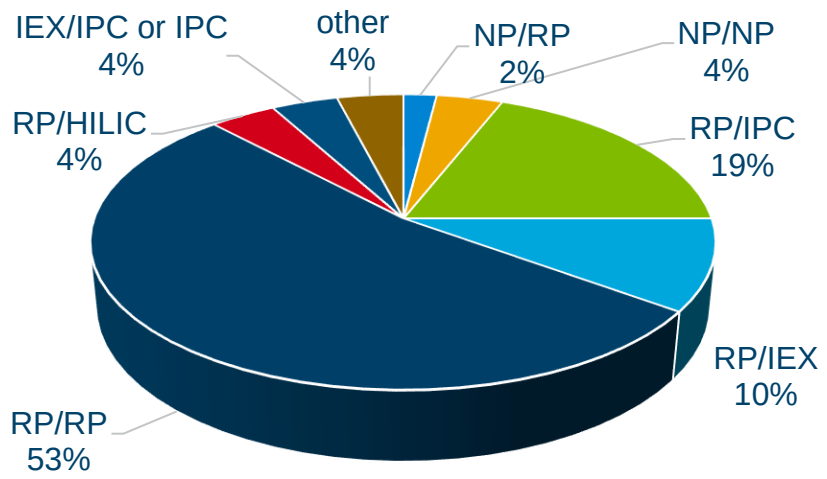
RP-Chiral

		Orthogonality	Peak capicity
^{1}D	Poroshell SB-(C8/C18/Aq), PFP, Phenyl-Hexyl, CN 1.9 µm, 2.7 µm, 4 µm	++	-
^{2}D	Poroshell Chiral-V, Chiral-T, Chiral-CD, Chiral-CF 2.7 µm		

SEC/IEC/AC-desalting

		Solvent compatibility	Peak capicity
^{1}D	Dependent on ^{1}D separation mode	++	-
^{2}D	AdvanceBio Desalting-RP cadridge, 1000 Å		

Publications on 2D-LC in pharmaceutical analysis



Iquiniz et al., J Pharm Biomed Anal. 2017 Oct 25;145:482-503

Agilent

When One Dimension is not Enough…

2D-LC may be the answer

Improve peak capacity, additional selectivity, and resolution

Increase confidence in the data

- Uncover hidden peaks

Higher throughput

- Shorten long 1D gradient, resolve in short 2D gradient

Trace enrichment of compounds of interest

Software compatible Chemstation, Mass Hunter 11 and OL CDS (Nov. release)

Resources

Zorbax Column Portfolio

A proven and reliable portfolio of totally porous HPLC columns

The Agilent ZORBAX family offers all advantages of totally porous particle columns such as increased retention, loadability and resistance to sample solvents. Easily scale your methods all the way from UHPLC to preparative LC.

Agilent InfinityLab

Agilent ZORBAX	Chemistry	Particle Sizes	Pore Size (Å)	Temperature Limit	pH Range	Endcapped	Carbon Load (%)	Surface Area	USP Designation	Benefits and Applications
Eclipse Plus C18		1.8, 3.5, 5	95	60 °C	2–9	Double	9	160 m²/g	L1	General purpose Starting Point for LC method development
Eclipse Plus C8		1.8, 3.5, 5	95	60 °C	2–9	Double	7	160 m²/g	L7	General purpose Lower retention of hydrophobic analytes vs. C18
Eclipse Plus Phenyl-Hexyl		1.8, 3.5, 5	95	60 °C	2–8	Double	9	160 m²/g	L11	Alternative selectivity for aromatic compounds Enhanced pi-pi interactions when using methanol
Eclipse Plus PAH	Polymeric C18	1.8, 3.5, 5	95	60 °C	2–9	Double	14	160 m²/g	L1	Application-specific Designed for the separation of PAHs in LC
Eclipse XDB C18		1.8, 3.5, 5	80	60 °C	2–9	Double	10	180 m²/g	L1	General purpose, higher carbon load Higher hydrophobicity with alternative selectivity for lipophilic analytes
Eclipse XDB C8		1.8 (RRHT) 3.5, 5, 7	80	60 °C	2–9	Double	7.6	180 m²/g	L7	General purpose, higher carbon load Higher hydrophobicity with alternative selectivity for lipophilic analytes but reduced retention vs. XDB-C18
Eclipse XDB Phenyl		3.5, 5	80	60 °C	2–9	Double	7.2	180 m²/g	L11	Alternative selectivity for aromatic compounds Enhanced pi-pi interactions when using methanol
Eclipse XDB CN		3.5, 5	80	60 °C	2–9	Double	4.2	180 m²/g	L10	Polar analytes in RP, low bleed Excellent peak shape of polar and mid-polar compounds
StableBond C18		1.8, 3.5, 5, 7	80	90 °C	0.8–8	No	10	180 m²/g	L1	Low pH and high temperature Excellent stability and peak shape at highly acidic conditions
StableBond C8		1.8, 3.5, 5, 7	80	80 °C	1–8	No	5.5	180 m²/g	L7	Low pH and high temperature Lower retention of hydrophobic analytes vs. C18
StableBond C3		1.8, 3.5, 5	80	80 °C	1–8	No	4	180 m²/g	L56	Low pH and high temperature Reduced retention of hydrophobic analytes
StableBond Aq		1.8, 3.5, 5, 7	80	80 °C	1–8	No	Proprietary	180 m²/g	L96	Polar analytes in RP Excellent peak shape and retention of polar compounds using reversed-phase LC,

Which particle is best for my method?

1.8 µm ZORBAX RRHD: highest UHPLC performance
Maximum pressure: 1200 bar
Ideal for: 1290 Infinity II LC or 1260 Infinity II Prime LC

1.8 ZORBAX RRHT: ultra-fast chromatography at up to 600 bar
Maximum pressure: 600 bar
Ideal for: 1260 Infinity II LC

3.5 µm ZORBAX RR: Higher resolution of HPLC methods
Maximum Pressure: 400 bar
Update of traditional methods on general HPLC instruments

5 µm ZORBAX: Proven and reliable for HPLC methods
Maximum Pressure: 400 bar
Used for traditional methods on general HPLC instruments and in preparative LC

1 bar = 14.5 PSI

psi	1450	2900	4350	5800	7250	8700	10,150	11,600	13,050	14,500	15,950	17,400	18,850	20,300
bar	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300	1400

What column ID and length should I choose?

Format	Comment
Column ID	4.6 mm for legacy methods 3.0 mm for lower solvent use than 4.6 mm 2.1 mm for lowest solvent use and MS applications
Column length	Shorter 30 to 100 mm for fastest separations Longer 150 to 250 mm for increased resolution

The InfinityLab Poroshell 120 Portfolio

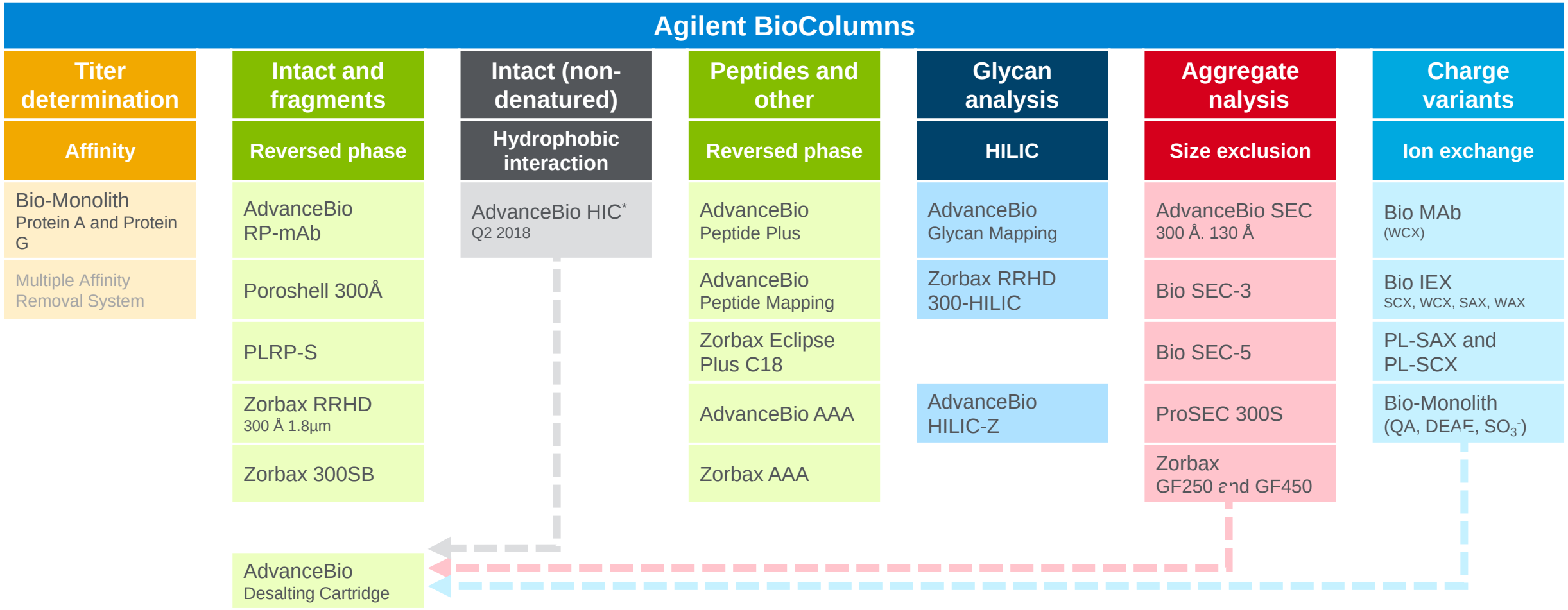
Agilent Poroshell columns are designed for multiple separation modes

Best all around	Best for low pH mobile phases	Best for high pH mobile phases	Best for alternative selectivity	Best for more polar analytes	Chiral
EC-C18 [A] 1.9 µm, 2.7 µm, 4 µm	**SB-C18** [A] 1.9 µm, 2.7 µm, 4 µm	**HPH-C18** [A] 1.9 µm, 2.7 µm, 4 µm	**Bonus-RP** [A,B] 2.7 µm	**SB-Aq** [A,B] 1.9 µm, 2.7 µm, 4 µm	**Chiral-V** [A,C,D] 2.7 µm
EC-C8 [A] 1.9 µm, 2.7 µm, 4 µm	**SB-C8** [A] 2.7 µm	**HPH-C8** [A] 2.7 µm, 4 µm	**PFP** [A,B,D] 1.9 µm, 2.7 µm, 4 µm	**EC-CN** [A,B,C,D] 2.7 µm	**Chiral-T** [A,C,D] 2.7 µm
Phenyl-Hexyl [A] 1.9 µm, 2.7 µm, 4 µm		← **CS-C18** [A] 2.7 µm →		**HILIC** [D,E] 1,9 µm, 2.7 µm, 4 µm	**Chiral- CD** [A,C,D] 2.7 µm
				HILIC-Z [D,E] 1.9 µm, 2.7 µm, 4 µm	**Chiral-CF** [A,C,D] 2.7 µm
				HILIC- OH5 [D,E] 2.7 µm	

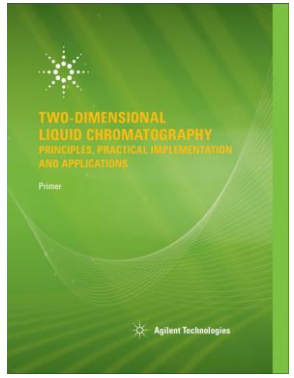
Legend
- [A] Reversed phase
- [B] Can be operated at 100% aqueous
- [C] Normal phase
- [D] SFC
- [E] HILIC

Agilent BioColumns Portfolio

Portfolio overview



2D-LC Primer



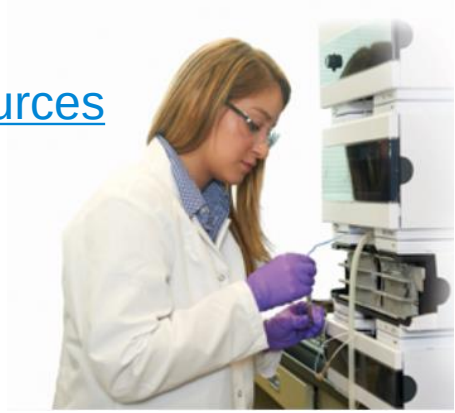
Application	Mode/Stationary phase		
	First	Second	Reference
Small molecule pharmaceuticals	RP/C18 (low pH)	RP/C18 (pH 8.6)	59
Surfactants	HILIC/Zic-HILIC	RP/C8-Aqua	48
Traditional Chinese medicine	RP/CN	RP/C18 (low pH)	60
Lipids	Argentation (Silver ion)	RP/C18	49
Carotenoids	NP/Bare silica	RP/C18	61
Peptides	RP/C18 (pH 1.8)	RP/C18 (pH 10)	62
Peptides	IEX/Phosphate modified zirconia	RP/C18 (low pH)	45
Polymethacrylates	RP/C18	SEC/C18 (critical conditions)	63

Table 4.3 Representative recent applications of LCxLC and the separation modes used.

https://www.agilent.com/cs/library/primers/public/5991-2359EN.pdf

Resources for Support

- LC Troubleshooting poster (5994-0709EN)
- Resource page http://www.agilent.com/chem/agilentresources
  - Quick reference guides
  - Catalogs, column user guides
  - Online selection tools, how-to videos
- Agilent 2D-LC, Heart-Cutting 2D-LC, 1290 Infinity II 2D-LC System | Agilent
- InfinityLab Supplies catalog (5991-8031EN)
- LC handbook (5990-7595EN)
- Your local FSE and specialists
- Agilent community, https://community.agilent.com/community/resources
- Agilent University, http://www.agilent.com/crosslab/university
- Youtube – Agilent channel (maintenance videos)
- Agilent service contracts



Contact Agilent Chemistries and Supplies Technical Support

1-800-227-9770 Option 3, Option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Option 6 for former Prozyme products

Available in the U.S. and Canada 8–5 all time zones

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

advancebio.glycan@agilent.com

Web chat: Product pages of agilent.com

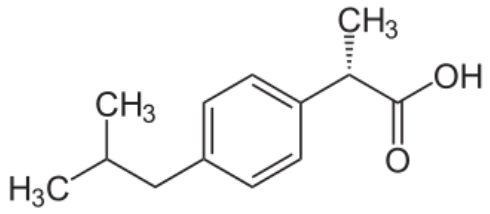
Appendix

Single Heart-Cutting 2D-LC

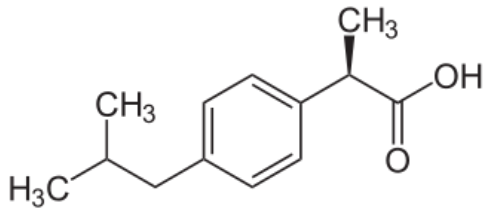
Online *ee* determination of chiral compounds in complex samples

Pharma

Ibuprofen



(S)-Ibuprofen



(R)-Ibuprofen

+ Impurities

Objectives

- 1st dimension: Separation of impurities from racemic ibuprofen
- 2nd dimension: Chiral separation of ibuprofen

1D method parameters

- Column: ZORBAX Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm
- Gradient: 0 min 5%, 20 min 95%
- Solvents: A: Water + 0.1% FA; B: ACN + 0.1% FA
- Flow: 0.25 mL/min

2D method parameters

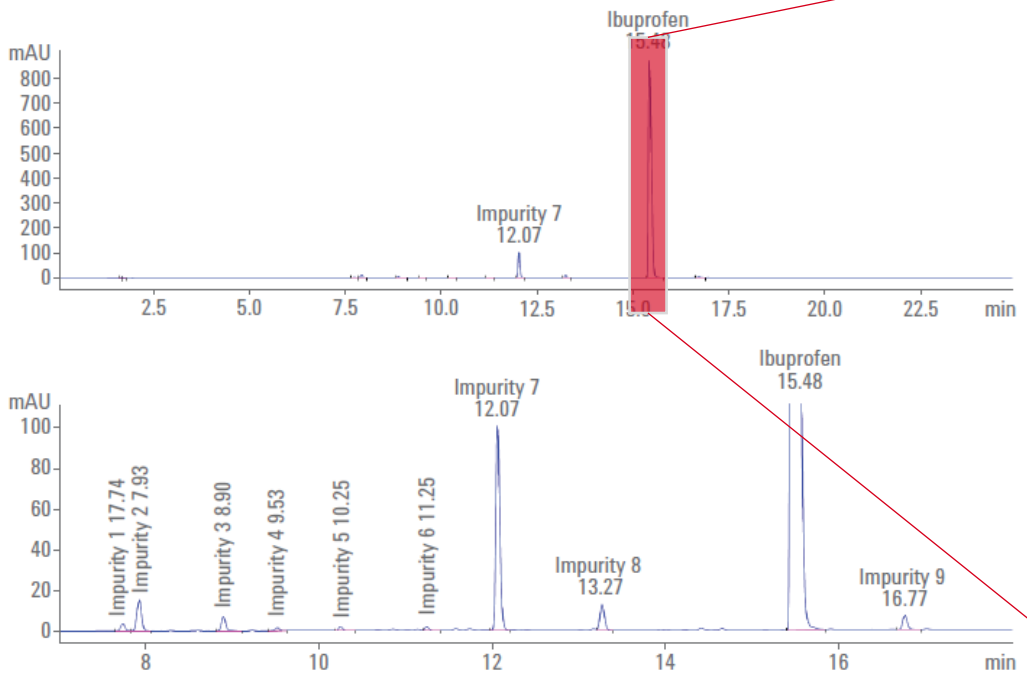
- Column: Chiral column 4.6 × 250 mm, 5 µm
- Solvent: Water/Methanol (35/65) + 0.1 % FA (isocratic)
- Flow: 1 mL/min

Single Heart-Cutting 2D-LC

Online *ee* determination of chiral compounds in complex samples

Pharma

Impurity profiling (^{1}D)



Chiral analysis (^{2}D)

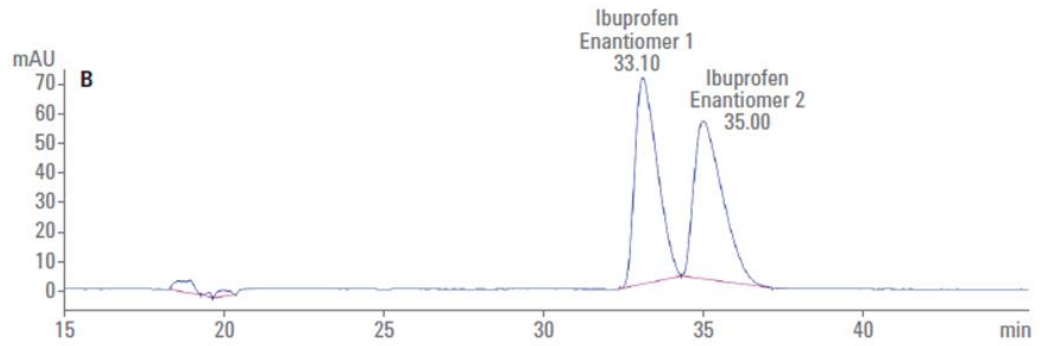
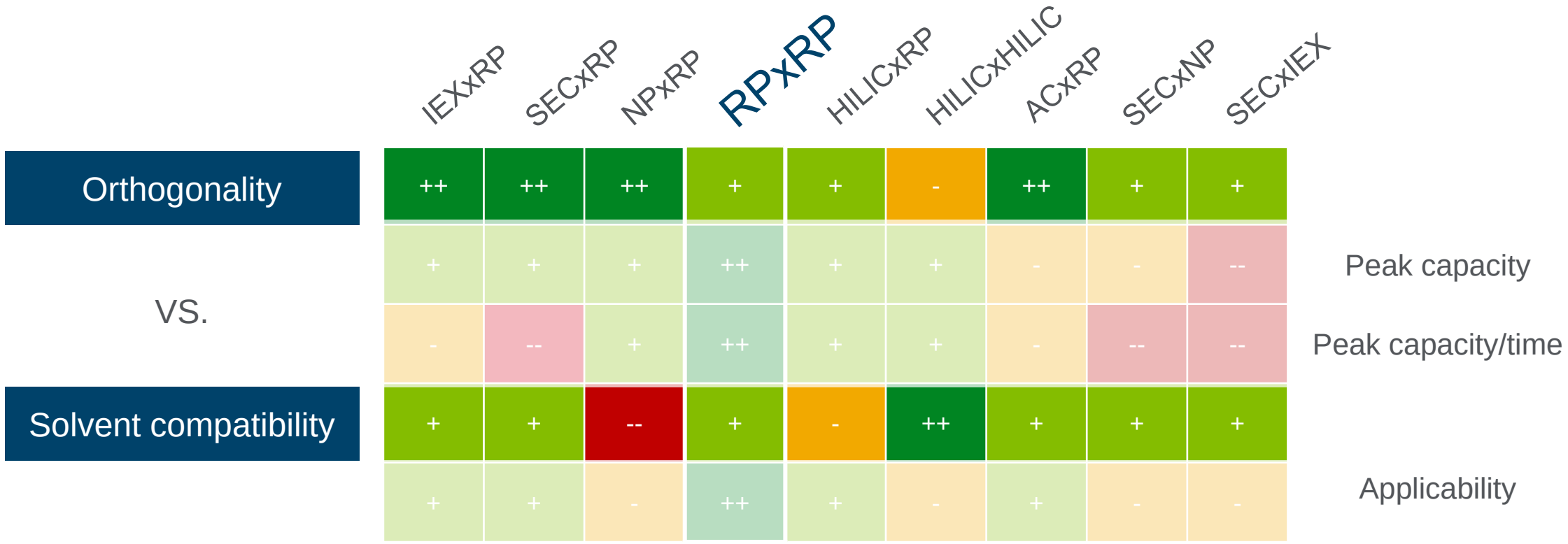


Table 1. Statistical evaluation of the analysis of 1 mg/ mL ibuprofen sodium salt (n = 10).

		Second dimension	
	First dimension	Enantiomer 1	Enantiomer 2
Retention time ± S.D.	15.48 ± 0.01 min	33.10 ± 0.07 min	35.00 ± 0.07 min
RSD	0.07 %	0.2 %	0.2 %
Area ± S.D.	4,697 ± 29	3,360 ± 17	3,290 ± 17
RSD	0.6 %	0.5 %	0.5 %

Fundamentals of 2D-LC

Coupling of orthogonal separation modes can be challenging



Fundamentals of 2D-LC

The 2D-LC valve is the heart of every 2D-LC instrument

Full symmetry of both flow paths

The 2D-LC valve is a 2nd dimension fixed loop injector