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FACULTEIT WETENSCHAPPEN

Enhanced chiral screening of complex samples via aqueous achiral × chiral comprehensive liquid chromatography

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Outline

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1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

2. Achiral TRLC x chiral comprehensive 2D-LC

3. Achiral PALC x chiral comprehensive 2D-LC

4. conclusions

Separation Science Group

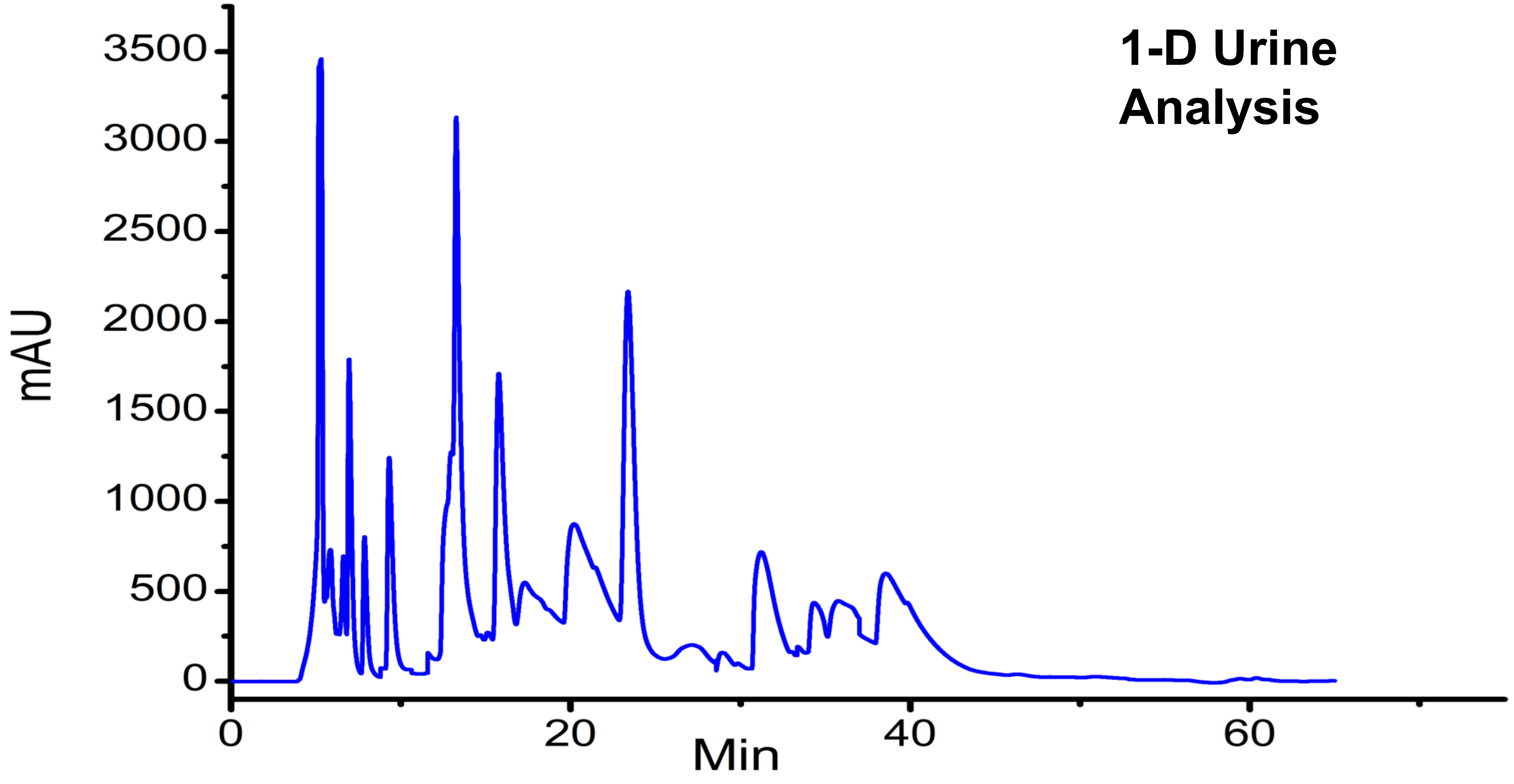
1. Aqueous 1D-based comprehensive 2D-LC: rationale

1. Aqueous ¹D-based comprehensive 2D-LC: rationale

Chiral 1D-LC unsuitable for complex samples

- The complexity of natural mixtures (usually) prohibits efficient collection of the chiral information due to (major) peak overlap

- Chiral 1-D LC-MS less reliable due to “matrix” effects
  - Lower peak capacity of chiral LC compared to RPLC
  - Confusing isomeric information



1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

Solution: 2D-LC for the analysis of complex samples

2D-LC

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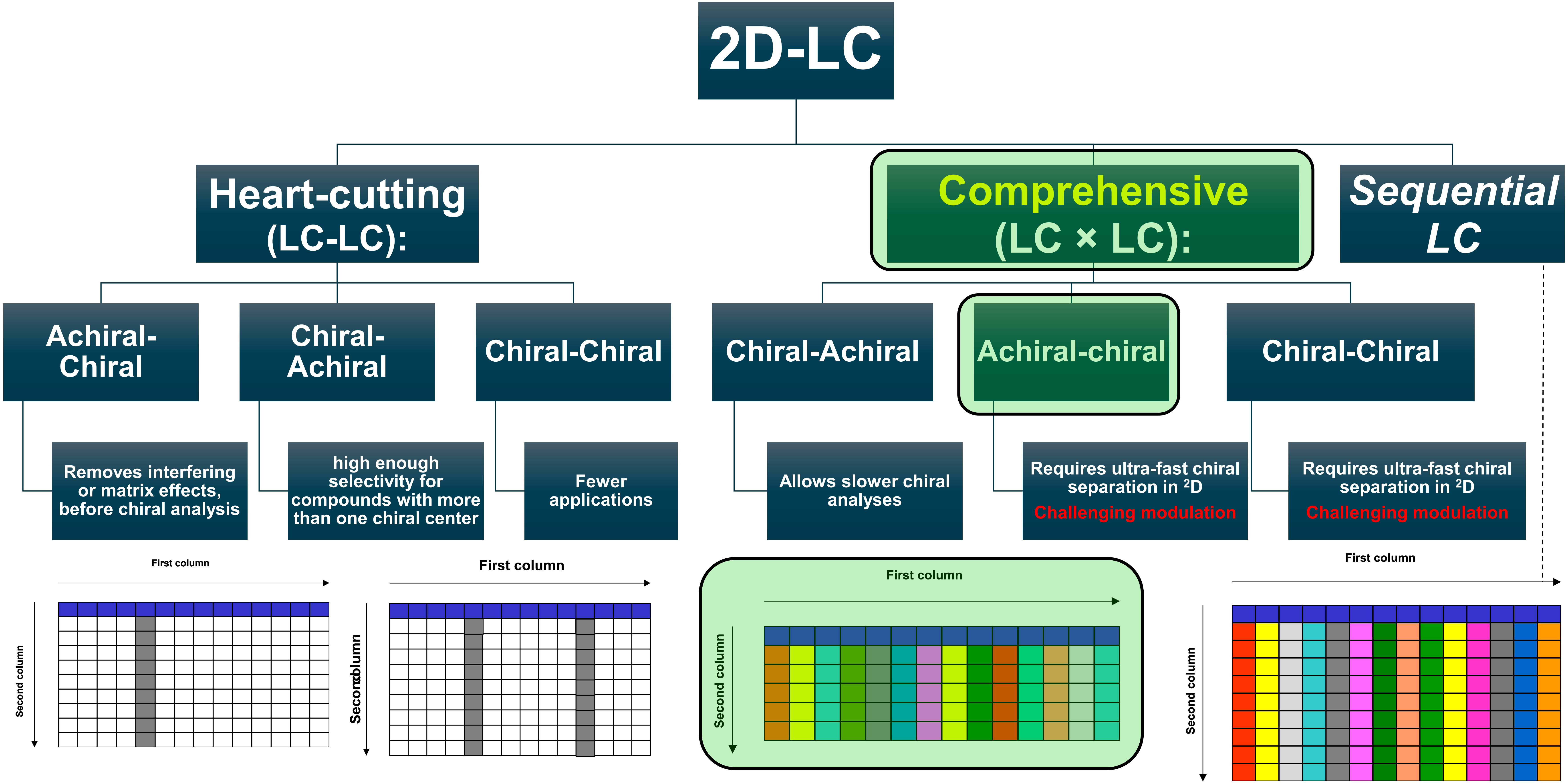
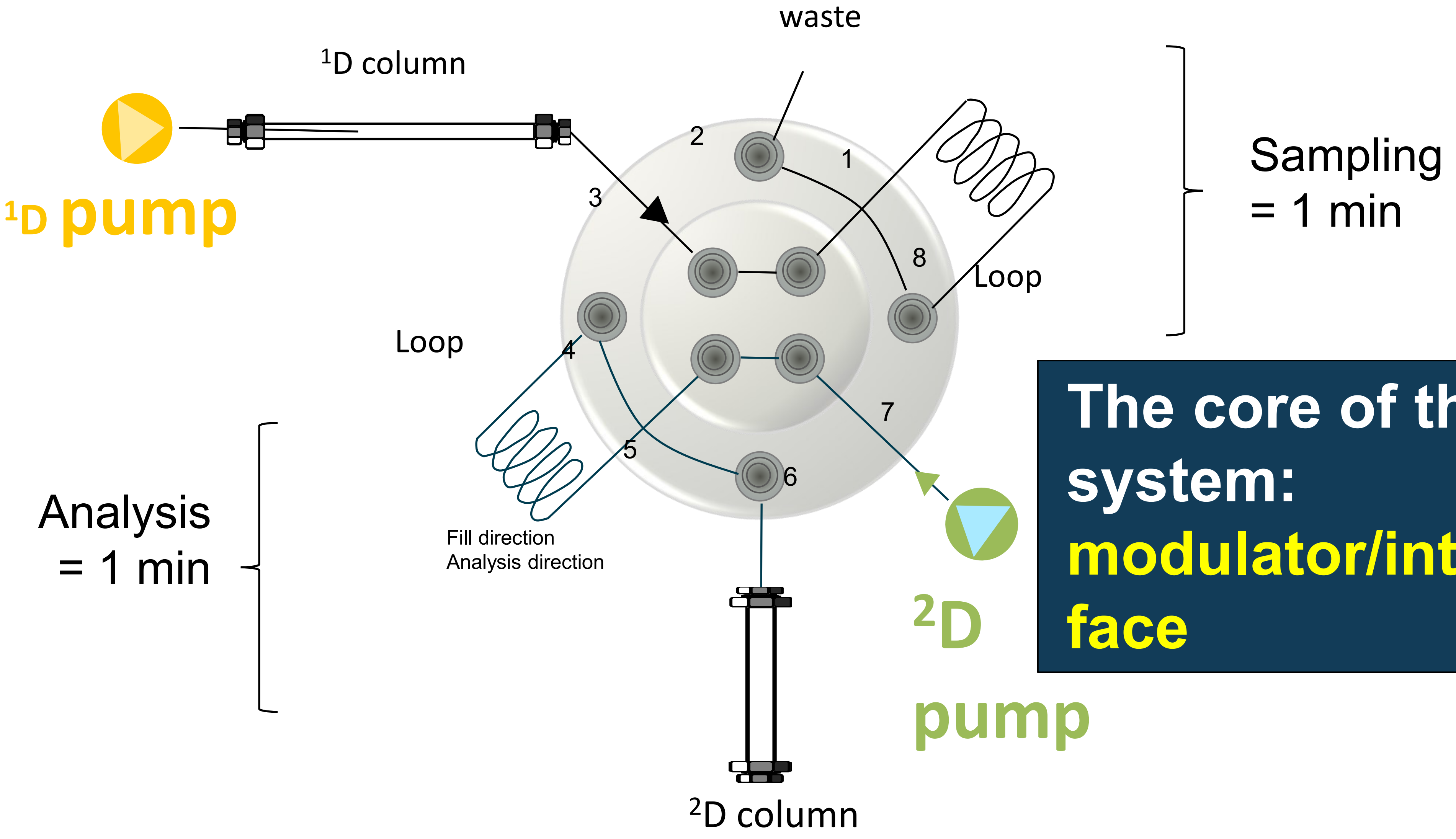


1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

Comprehensive 2D-LC (LCxLC): principles

Automated real-time transfer of fractions from a 1st to a 2nd separation dimension

Analysis of the full effluent content of ^{1}D in real-time



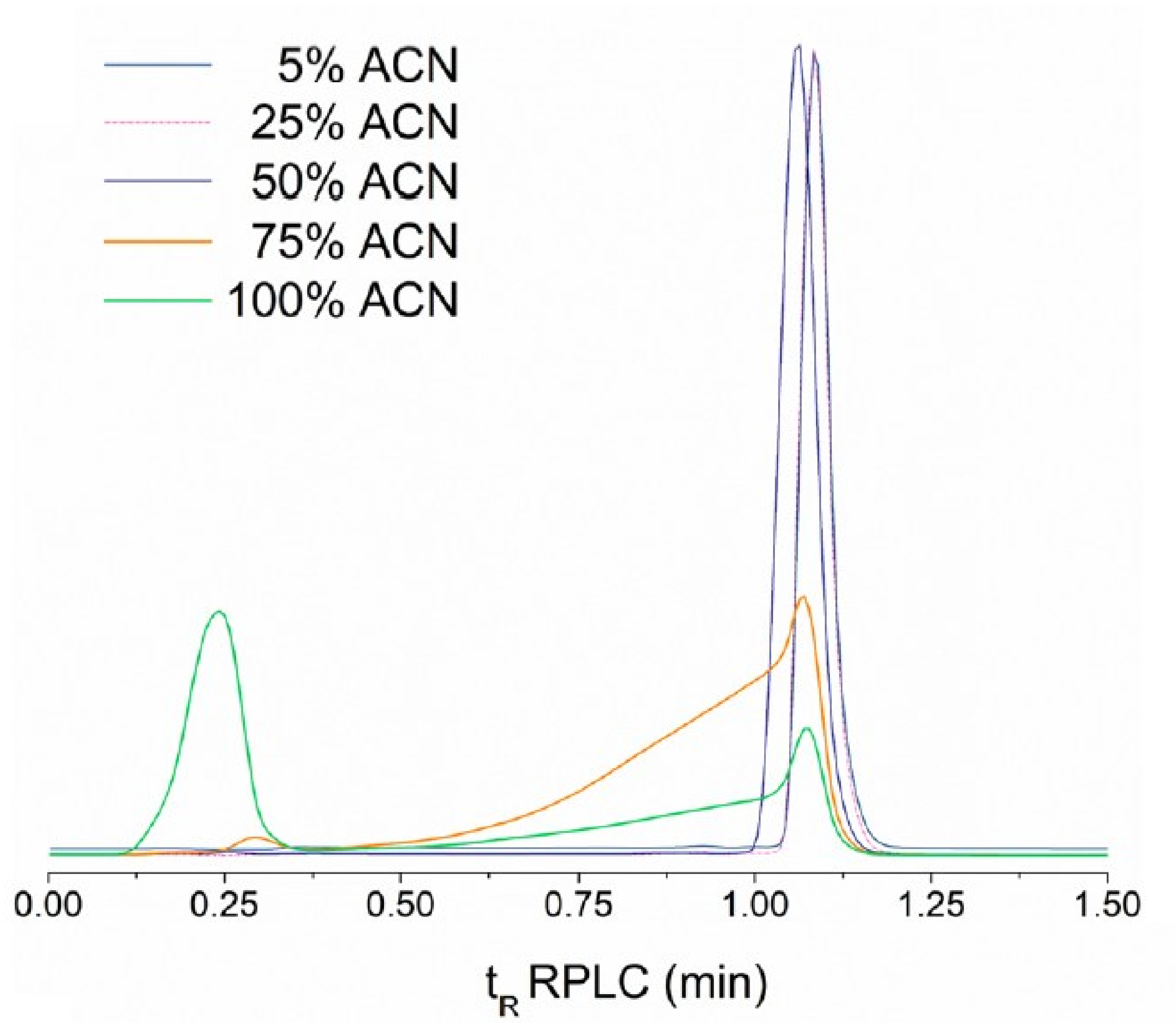
The core of the system: modulator/inter-face

1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

Challenges in LCxLC

Challenges of LCxLC

- Transfer of large loop volumes on ^{2}D
- Too high eluotropic strength of ^{1}D
  - → Peak breakthrough in ^{2}D
  - → Loss of resolution in ^{2}D
  - → Need for dilution in ^{2}D
- Short columns in ^{2}D
- Short (< 1 min) ^{2}D analysis time
- Rapid gradient and equilibration in ^{2}D
- Loss of sensitivity compared to ^{1}D-HPLC
- Overly complex solutions
- Flow splitting required prior to ESI-MS (^{2}D)
- Requires expert users
- Complex data
- ^{2}D column life-time
- Data treatment
- Data collection rate (HRMS)
- …

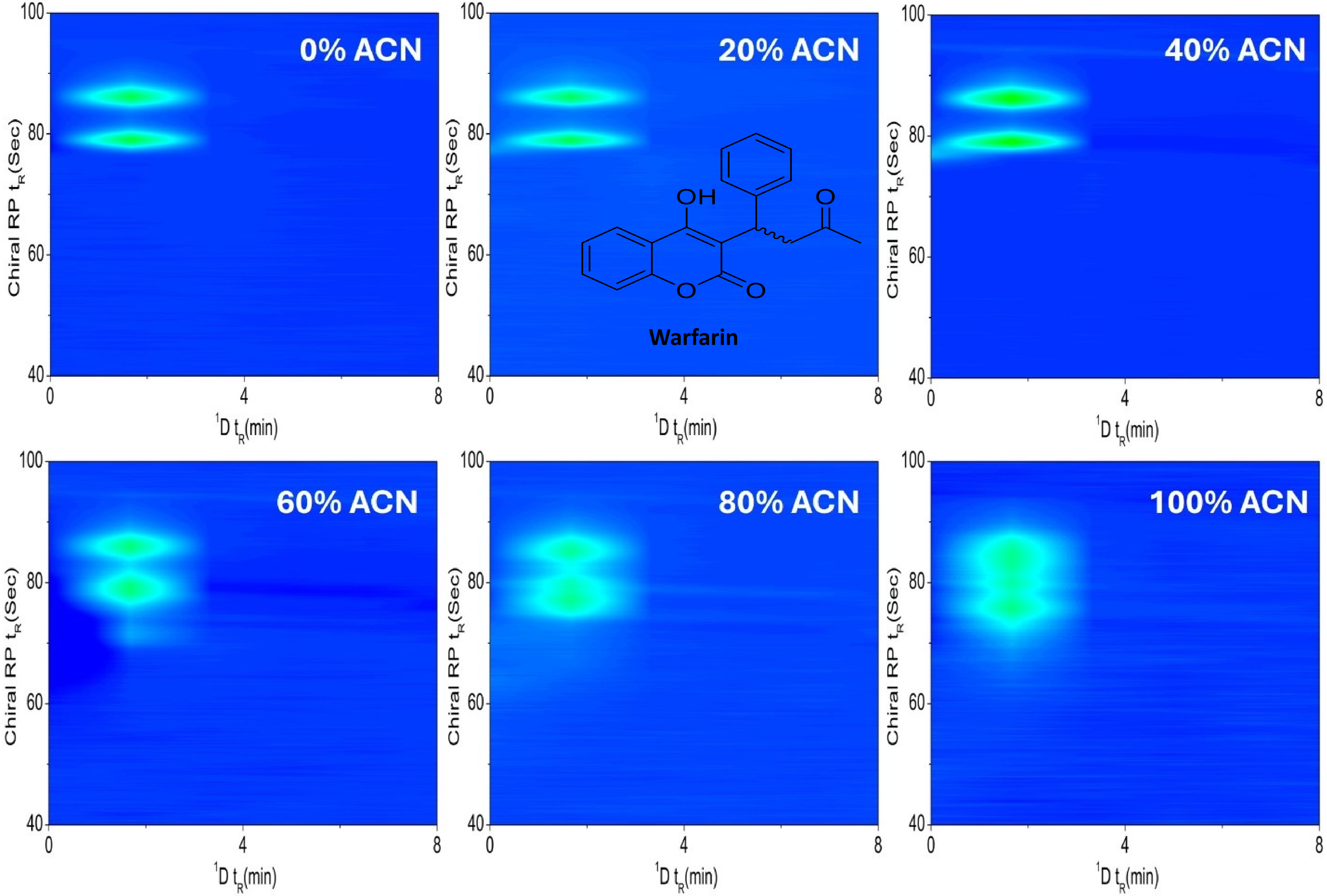


- Injection of 120 µL of a progestogen in acetonitrile rich mobile phase
- column: PFP (50 mmx 3 mm ID)
- gradient 30-70% ACN,
- Flow rate: 1.25 mL/min

Problem exacerbates for the more polar and earlier eluting solutes

1. Aqueous ¹D-based comprehensive 2D-LC: rationale

Challenges in LCxLC: influence of the ¹D solvent on fast chiral separations



¹D method

- No column
- 2 µL injection
- ¹D mobile phase
  A: H_2O (pH 3.2), B: ACN
- Flow rate: 0.1 mL /min

²D method

- Loop volume: 200 µL
- Cellulose 1 column
- ²D mobile phase
  A: H_2O (NH_4TFA, pH 2.9), B: ACN
- Flow rate: 3ml/min
- modulation time:100s
- T=20°C
- Gradient profile

Time (s)	%ACN
0	15
72	70
73	15

LogP warfarin : 2.9

Problem further exacerbates for the more polar (earlier eluting) solutes

1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

The modulation problem (solvent issues)

- **Solvent incompatibility issues undermine reaping the full performance of LCxLC**

- **Often absence of natural re-focusing**

Overview of the possible online LC × LC combination using the most-common forms of LC separations

	^{2}RP F^{+}H^{+}Q^{+}M^{+}	^{2}NP F^{-} Q^{-}	^{2}HILIC M^{+}Q^{-}	^{2}HIC F^{-}H^{-}M^{-}Q^{-}	^{2}IEX M^{-}Q^{-}S^{+}	^{2}SEC-Aq F^{+} H^{-} I	^{2}SEC-Or F^{+}H^{-} I	^{2}Ag F^{-} Q^{-} S^{+}	^{2}Chiral F^{+} I S^{+}	^{2}Affinity H^{-} Q^{-} S^{+}	^{2}SFC F^{+} H^{+} M^{+}
^{1}RP H^{2+}	E O^{+} P^{+} X^{+}	B O^{2+} X^{2-}	B O^{2+} X^{+}	B E O^{-} P^{-} X^{+}	O^{+}	A E O^{+} P^{+} X^{+}	A E O^{+}	B O^{2+} X^{-}	O^{2+}	O^{2+} X^{+}	B O^{2+} X^{-}
^{1}NP H^{+}	B O^{2+} X^{2-}	O^{-} P^{-} X^{+}	O^{-} P^{-} X^{-}	B O^{2+} P^{-} X^{2-}	O^{2+}	O^{2+} X^{2-}	O^{2+} P^{+} X^{+}	O^{+} X^{+}	O^{2+}	O^{+} X^{2-}	O^{-} X^{2+}
^{1}HILIC H^{+}	B O^{2+} P^{+} X^{+}	B O^{-} X^{-}	O^{-} X^{+}	B O^{2+} P^{-} X^{-}	O^{+} X^{+}	O^{2+} P^{+}	A O^{+} X^{+}	B O^{+} X^{-}	O^{2+}	X^{-}	X^{+}
^{1}HIC H^{-}	E O^{-} X^{2+}	B O^{2+} P^{-} X^{2-}	B O^{2+} X^{-}	O^{2-} P^{2-}	B O^{+} P^{-} X^{2+}	O^{2+} P^{-} X^{2+}	A O^{+} P^{-} X^{-}	B O^{2+} P^{-} X^{2-}	O^{2+} P^{2-}	O^{+} X^{+}	O^{+} P^{2-} X^{2-}
^{1}IEX H^{-} S^{+}	E O^{+} P^{+} X^{2+}	B O^{2+} X^{2-}	B O^{+} X^{-}	B O^{+} P^{-} X^{2+}	B X^{-}	O^{+} X^{2+}	A O^{+} P^{-} X^{-}	B O^{+} X^{-}	O^{2+}	O^{+} X^{+}	O^{+} X^{2-}
^{1}SEC-Aq H^{2-}	E O^{+} P^{+} X^{2+}	B O^{2+} X^{2-}	B O^{2+} X^{-}	B O^{+} P^{-}	O^{+} X^{2+}	O^{2-} P^{2-}	A O^{2-} P^{2-} X^{2-}	O^{2-} X^{2-}	O^{2+} P^{-}	O^{2+} X^{+}	E O^{2+} P^{-} X^{-}
^{1}SEC-Or H^{2-}	B^{2-} O^{+} X^{-}	B O^{2+} X^{+}	O^{+} X^{+}	B O^{+} P^{-} X^{2-}	B O^{+} P^{-} X^{-}	O^{2-} P^{2-} X^{-}	O^{2-} P^{2-}	O^{2+} X^{+}	O^{2-} P^{-}	O^{2+} P^{2-} X^{-}	O^{+} P^{-} X^{+}
^{1}Ag H^{+} S^{+}	B O^{2+}	O^{+} X^{+}	O^{+} X^{+}	B O^{2+} P^{-} X^{-}	O^{2+} X^{-}	O^{2+} X^{-}	O^{2+} X^{-}	O^{2-} P^{2-}	O^{2+}	O^{2+} X^{2-}	O^{+} X^{+}
^{1}Chiral I S^{+}	O^{2+}	O^{2+}	O^{2+}	O^{2+} P^{2-}	O^{2+}	O^{2+} P^{-}	O^{2+} P^{-}	O^{2+}	O^{2-} P^{2-}	O^{2+}	O^{2+}
^{1}Affinity H^{-} S^{++}	O^{2+} P^{-} X^{+}	B O^{2+} P^{-} X^{-}	B O^{2+} P^{-}	O^{2+} P^{-}	O^{+} P^{-} X^{+}	O^{2+} P^{-} X^{+}	A O^{2+} P^{2-} X^{2-}	B O^{2+} P^{-} X^{-}	O^{2+} P^{-}	O^{-} P^{2-}	O^{+} P^{-} X^{2-}
^{1}SFC H^{+}	E O^{2+} X^{+}	O^{-} X^{+}	E O^{-}	O^{2+} P^{3-}	O^{2+} X^{+}	O^{2+} P^{2-} X^{2+}	O^{2+} X^{2+}	O^{+} X^{+}	O^{2+}	O^{2+} X^{-}	E O^{-} X^{2+}

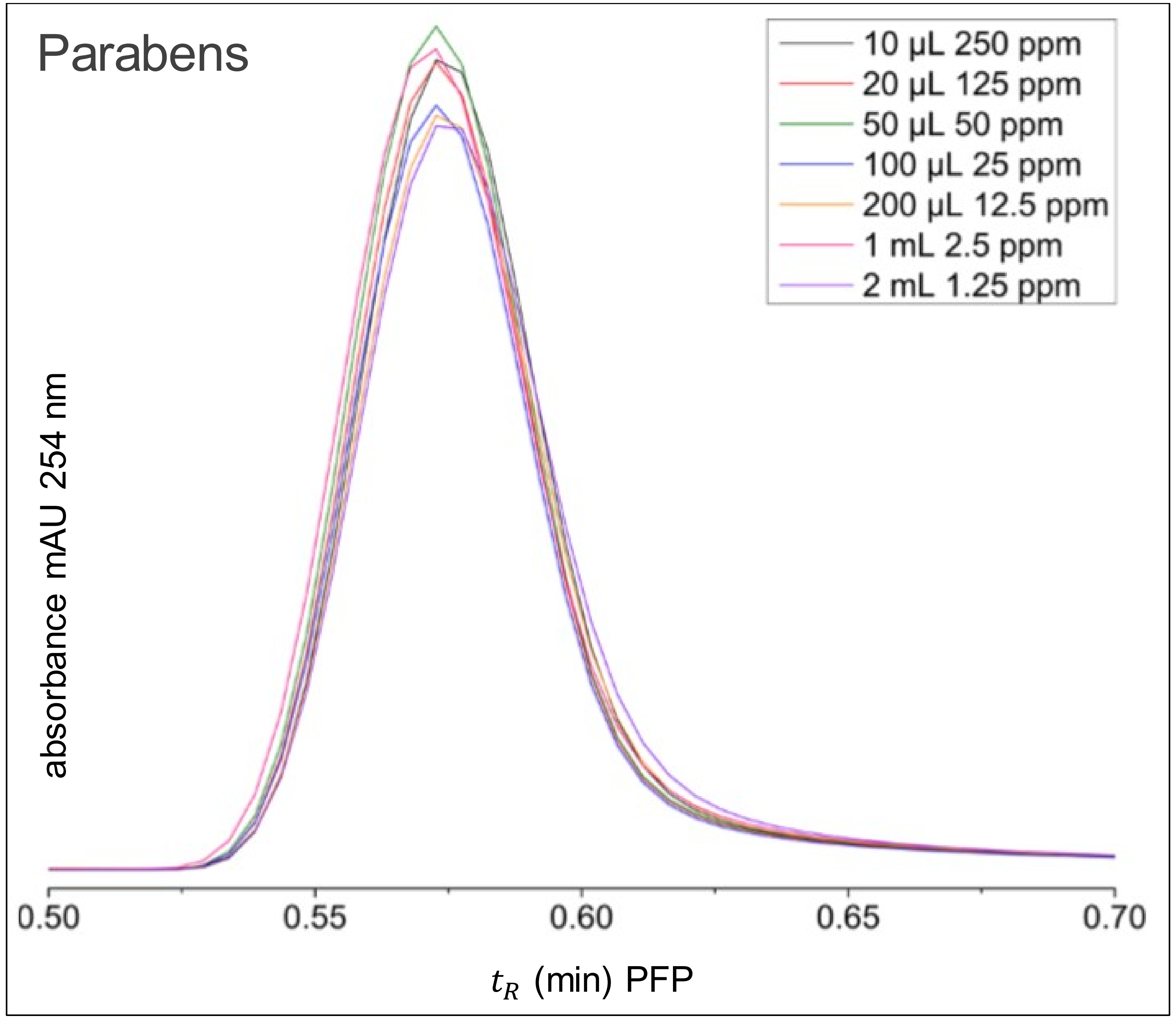
BWJ Pirok; AFG Gargano; PJ Schoenmakers, J. Sep. Sci. 2018,41, 1, 68-98

1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

The modulation problem: the purely aqueous solution

- Complete refocusing on a reversed phase column when injection analyte in water

- Volume-independent, optimal analyte focusing by injection of methylparaben in pure water plugs onto a RP column (10 µL – 2 mL).



1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

The modulation problem: the purely aqueous solution

- **Even RPLCxRPLC requires some dilution**

- **Most compatible combinations**
- **Transfer <u>only water</u> to ^{2}D in the RP mode**

- **Highly compatible combinations**

Overview of the possible online LC × LC combination using the most-common forms of LC separations

	^{2}RP	^{2}NP	^{2}HILIC	^{2}HIC	^{2}IEX	^{2}SEC-Aq	^{2}SEC-Or	^{2}Ag	^{2}Chiral (RP mode)	^{2}Affini-ty	^{2}SFC
^{1}RP											
^{1}NP											
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1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

The modulation problem: the purely aqueous solution

- Even RPLCxRPLC requires some dilution

- Most compatible combinations
- Transfer only water to ^{2}D in the RP mode

- Highly compatible combinations

- Need for new (~) 100% aqueous modes

- Same concepts apply when chiral RP is used in ^{2}D

Overview of the possible online LC × LC combination using the most-common forms of LC separations

	^{2}RP	^{2}NP	^{2}HILIC	^{2}HIC	^{2}IEX	^{2}SEC-Aq	^{2}SEC-Or	^{2}Ag	^{2}Chiral (RP mode)	^{2}Affini-ty	^{2}SFC
^{1}RP											
^{1}NP											
^{1}HILIC											
^{1}HIC											
^{1}IEX											
^{1}SEC-Aq											
^{1}SEC-Or											
^{1}Ag											
^{1}Chiral											
^{1}Affinity											
^{1}SFC											
^{1}TRLC											
^{1}PALC											

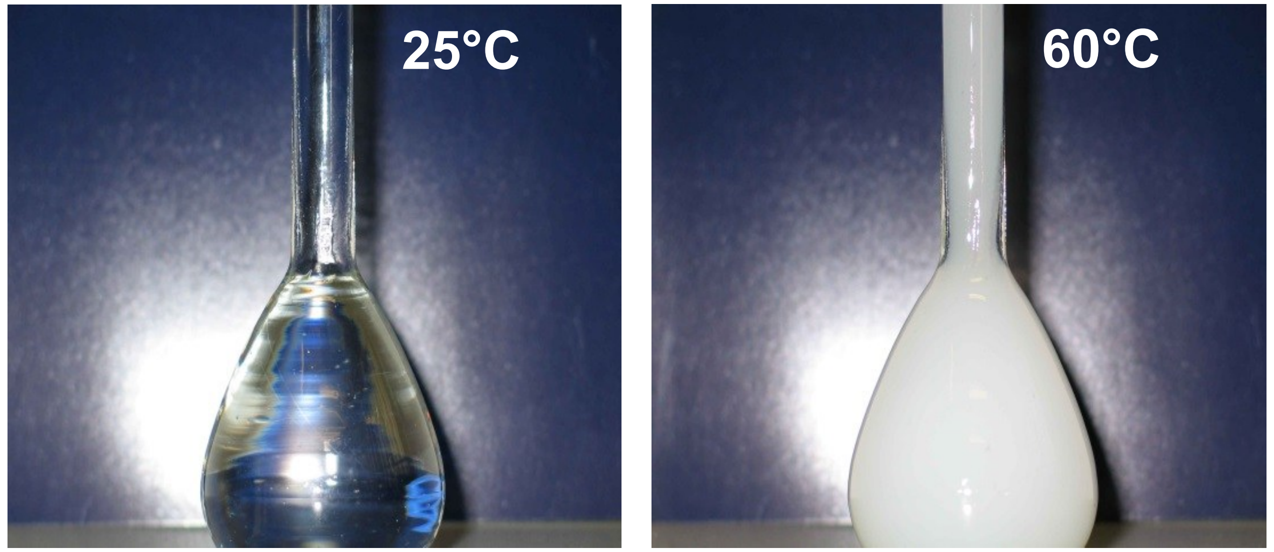
1. Aqueous ¹D-based comprehensive 2D-LC: rationale

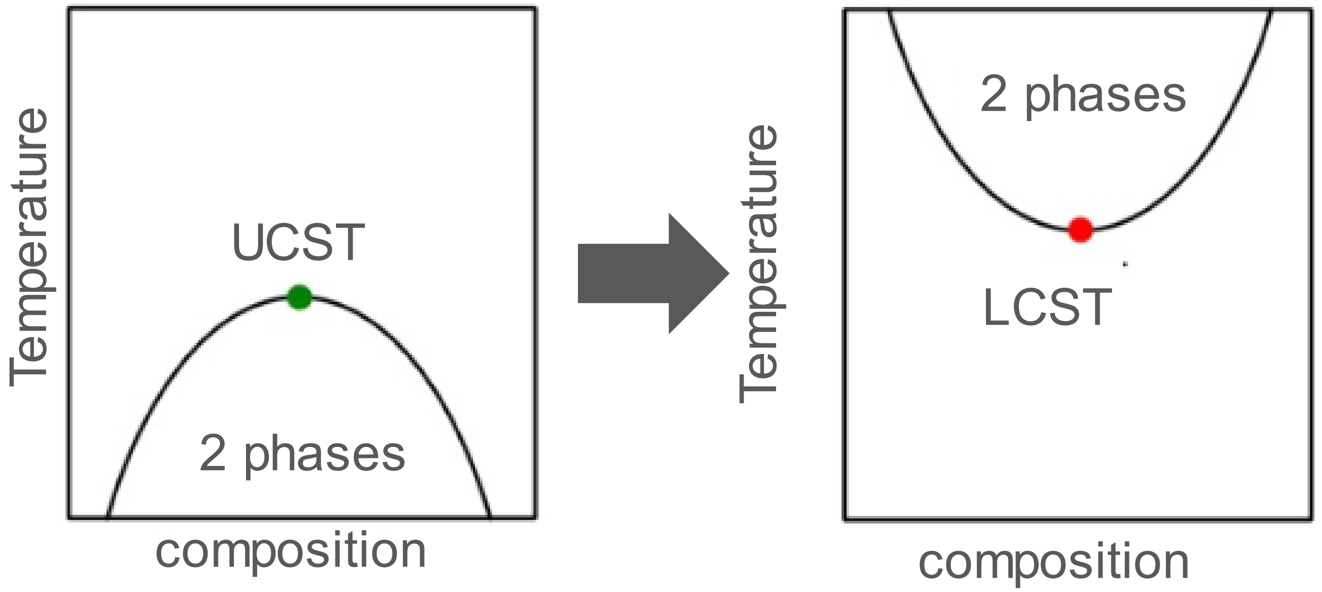
temperature responsive liquid chromatography: principles

Polymers that exhibit a drastic and discontinuous change of their physical properties with temperature

Class of stimuli-responsive materials

polymers display a miscibility gap in their temperature composition diagram





1. Aqueous 1D-based comprehensive 2D-LC: rationale

temperature responsive liquid chromatography: principles

LCST is an entropically driven proces (ΔS)

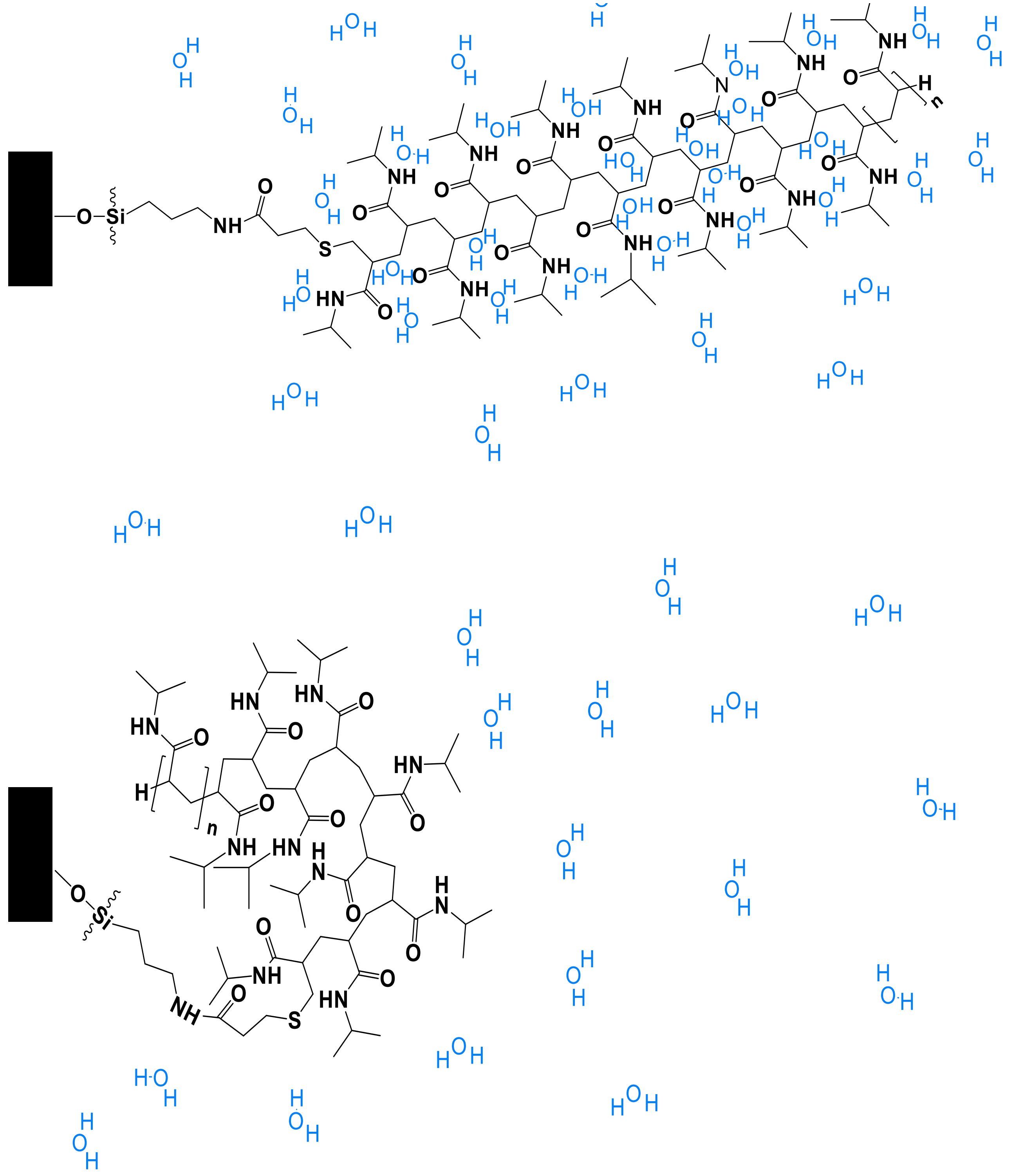
Exothermic enthalpic contribution (ΔH<0) due to the hydrogen-bond formation between water and the polar groups on the polymer

The hydrophobic groups on the polymer lead to loss of entropy as water molecules are forced in a cage like orientation around these moities (ΔS<0)

As temperature increases the Gibbs free energy becomes positve leading to demixing

$$(\Delta G = \Delta H - T\Delta S)$$

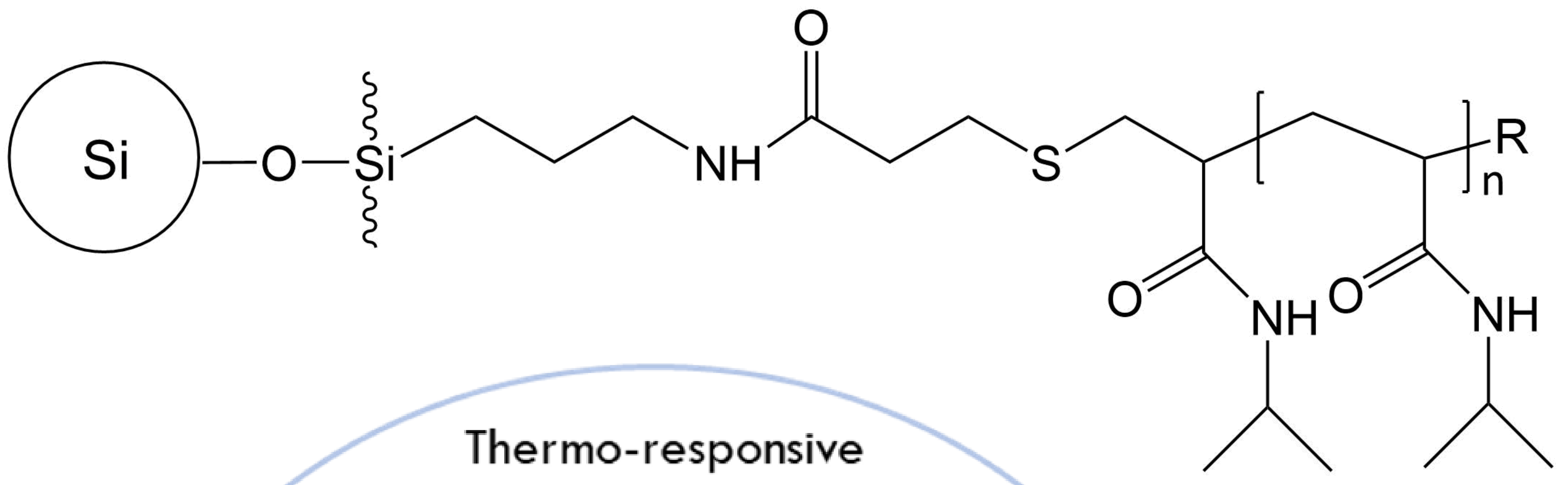
$$\Delta G < 0 \rightarrow \Delta G > 0$$

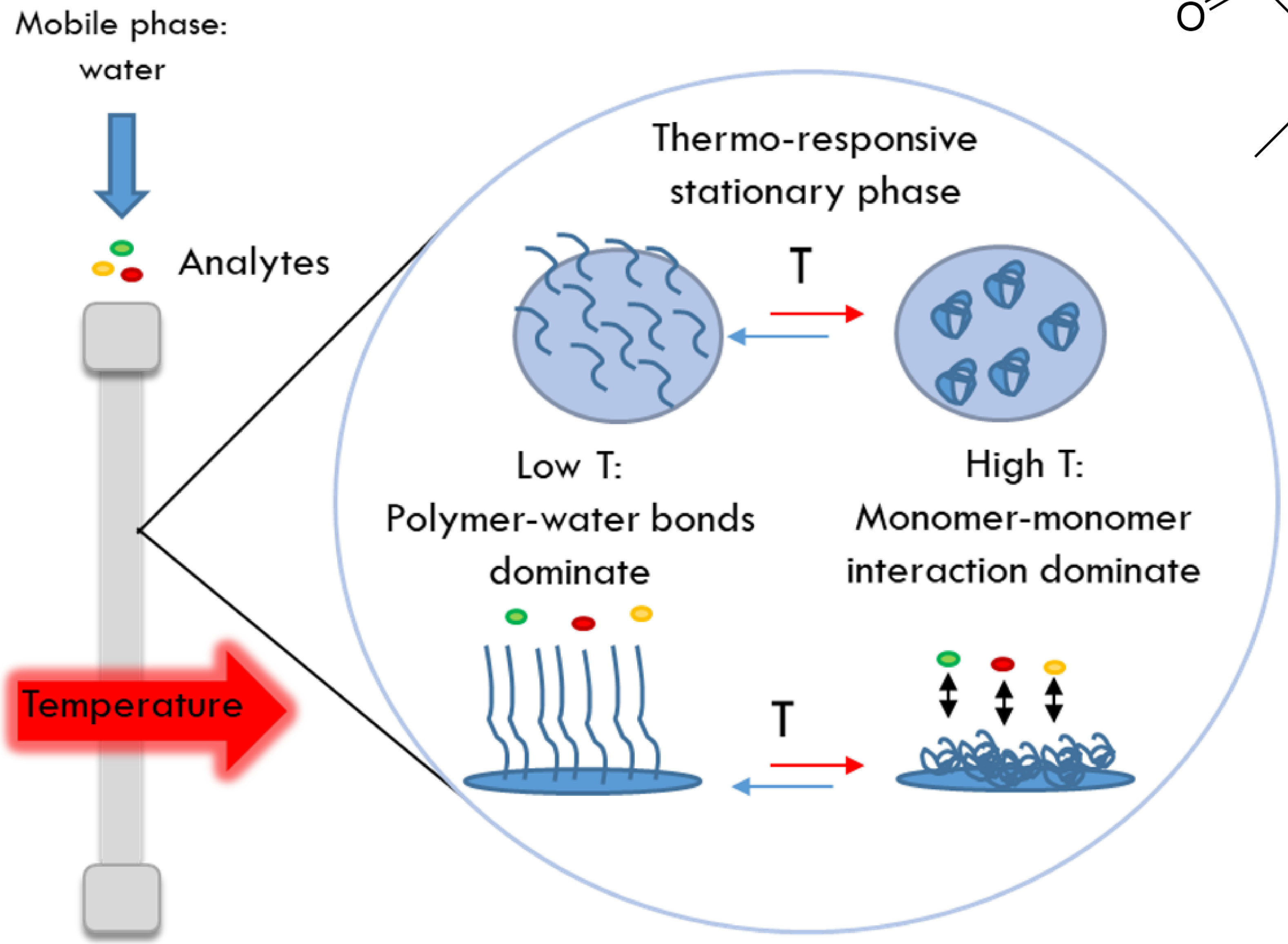


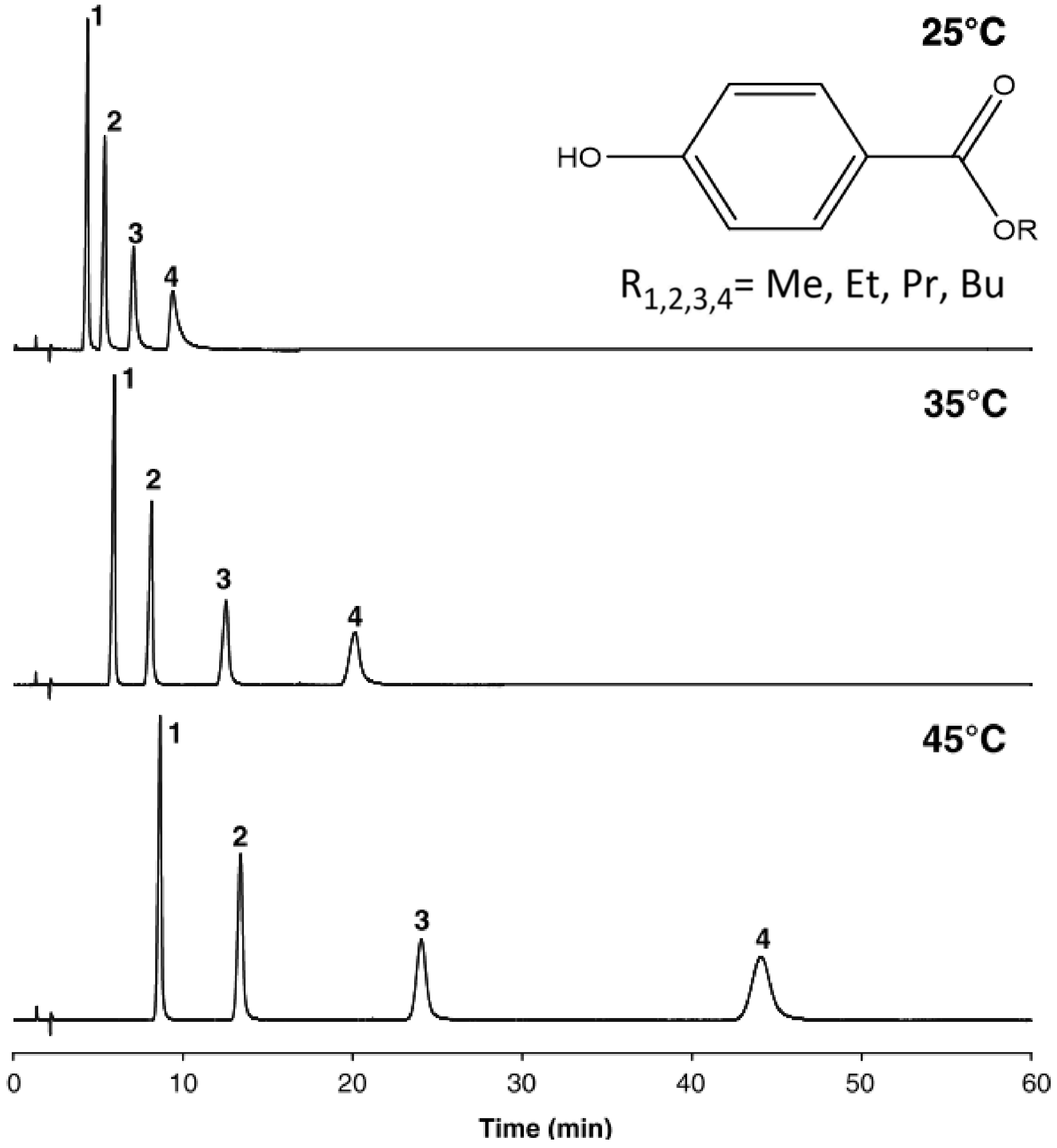
1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

temperature responsive liquid chromatography: principles

Temperature-responsive polymer: Poly(N-isopropylacrylamide) - PNIPAAm



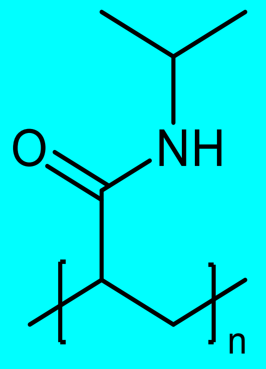


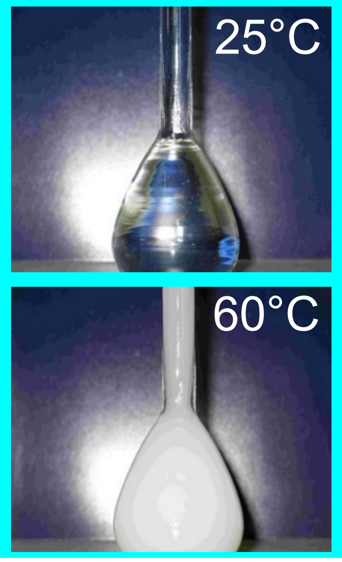


Lynen F. et al., Chromatographia, 2007, 66, 3-4

1. Aqueous ¹D-based comprehensive 2D-LC: rationale

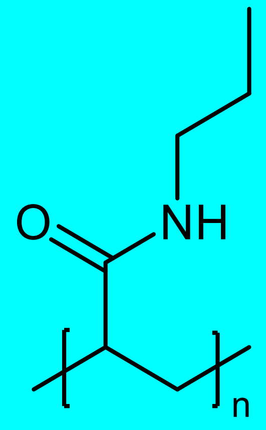
temperature responsive liquid chromatography: polymers





LCST ≈ 32 °C

Poly (N-isopropylacrylamide)

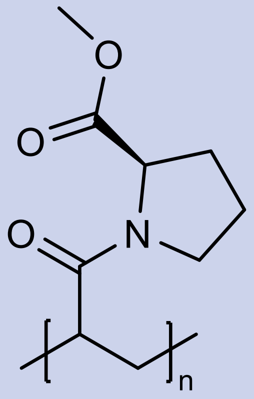


LCST ≈ 10 °C

Poly (N-n-propyl-acrylamide)

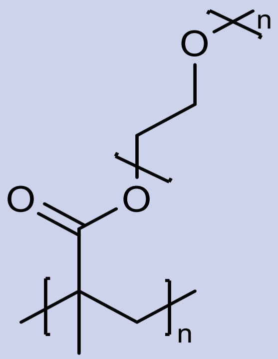
LCST ≈ 35 °C

Poly (N,N-diethylacrylamide)



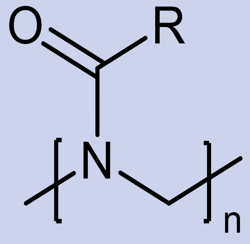
LCST ≈ 20 °C

poly(acryloyl-l-proline methyl ester)



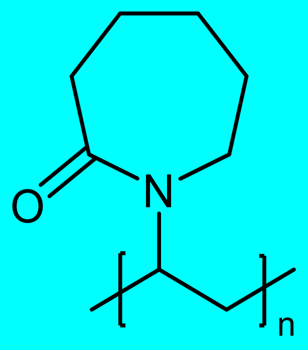
LCST ≈ 0-100 °C *

Poly (oligo ethylene glycol) methacrylate



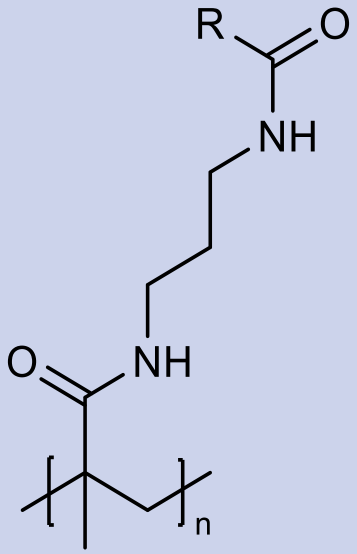
LCST ≈ 0-100 °C *

Poly (2-alkyl-2-oxazoline)



LCST ≈ 32-37 °C

Poly (N-vinylcaprolactam)



LCST ≈ 3 – 76 °C *

Poly (N-(N'-alkyl-carb-amido) propyl methacrylamide)

1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

Comparison with other aqueous/green LC approaches

→ Allows 100% aqueous HPLC

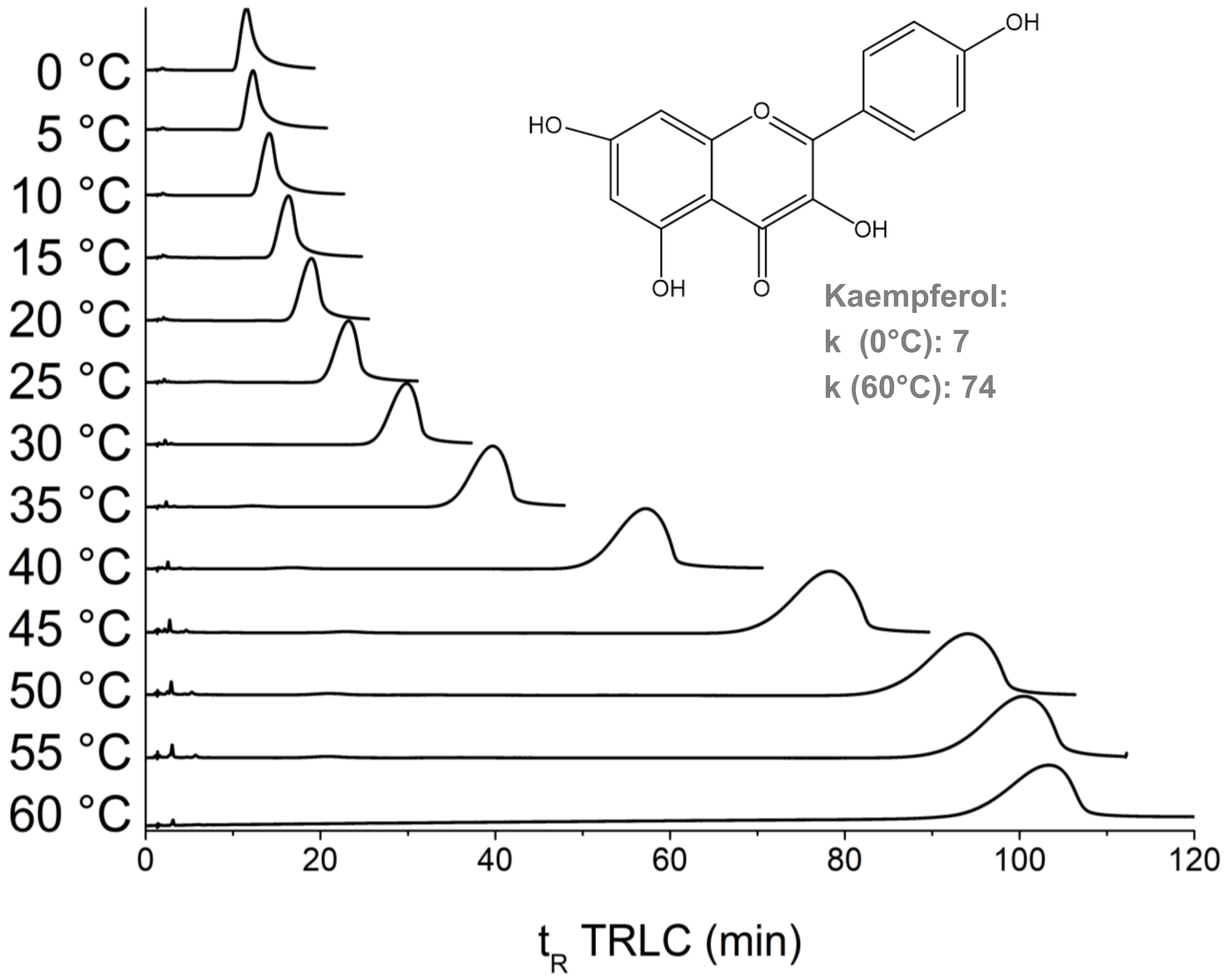
→ Larger changes in retention (k) over moderate temperature ranges

→ Large changes in selectivity with temperature

→ Large changes in selectivity with polymer, pH, additives

→ TR potential in other LC modes

→ Fundamental understanding exploitable in broad range of solvents (mobile phases)



1. Aqueous [1]D-based comprehensive 2D-LC: rationale

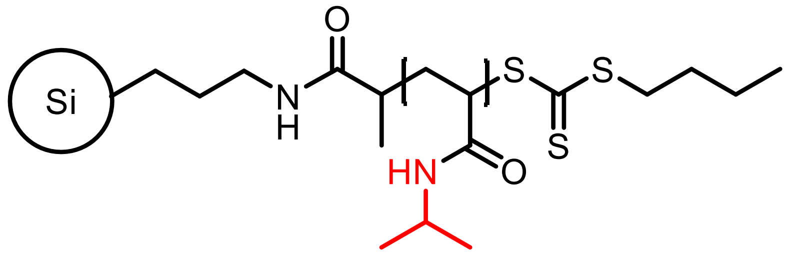
TRLC: Chromatographic performance: Retention: Δk vs. T

~ Identical TRLC columns were manufactured with 3 different polymers

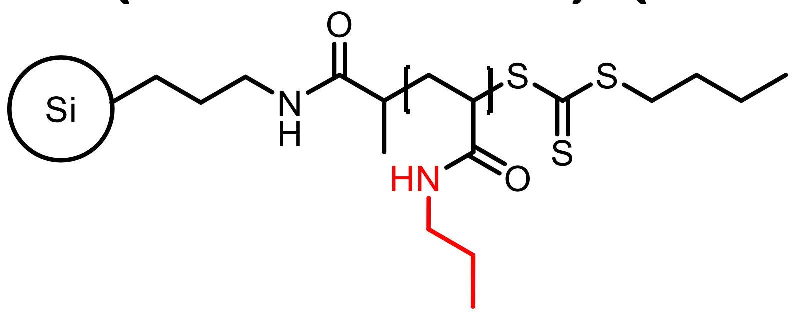
Sample 4 parabens – series of isothermal analyses

Mobile phase: water + 0.1% HCOOH

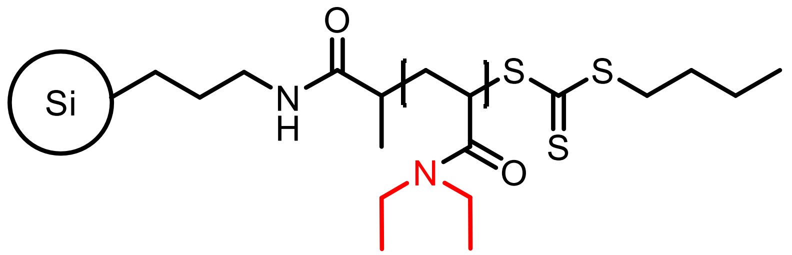
N-Isopropyl acrylamide (PNIPAAm) (32°C)

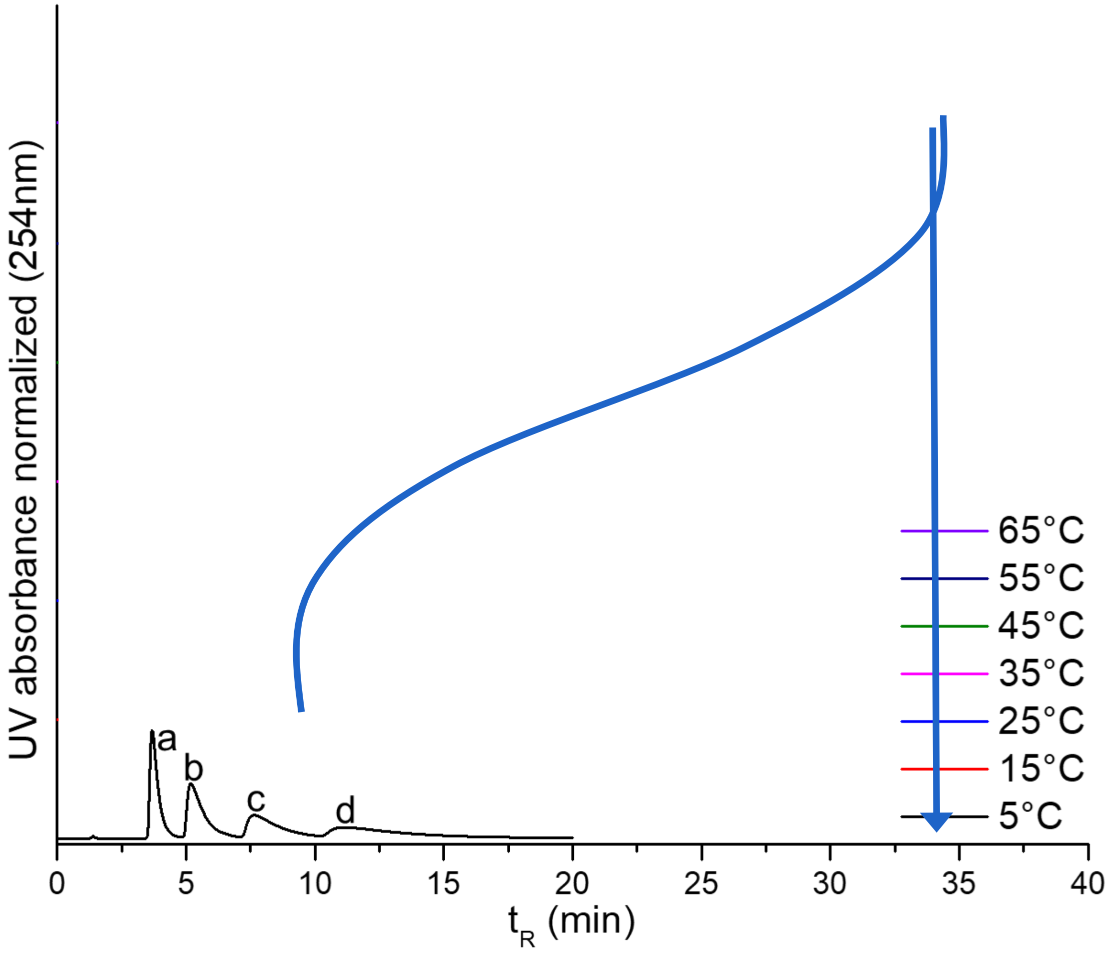


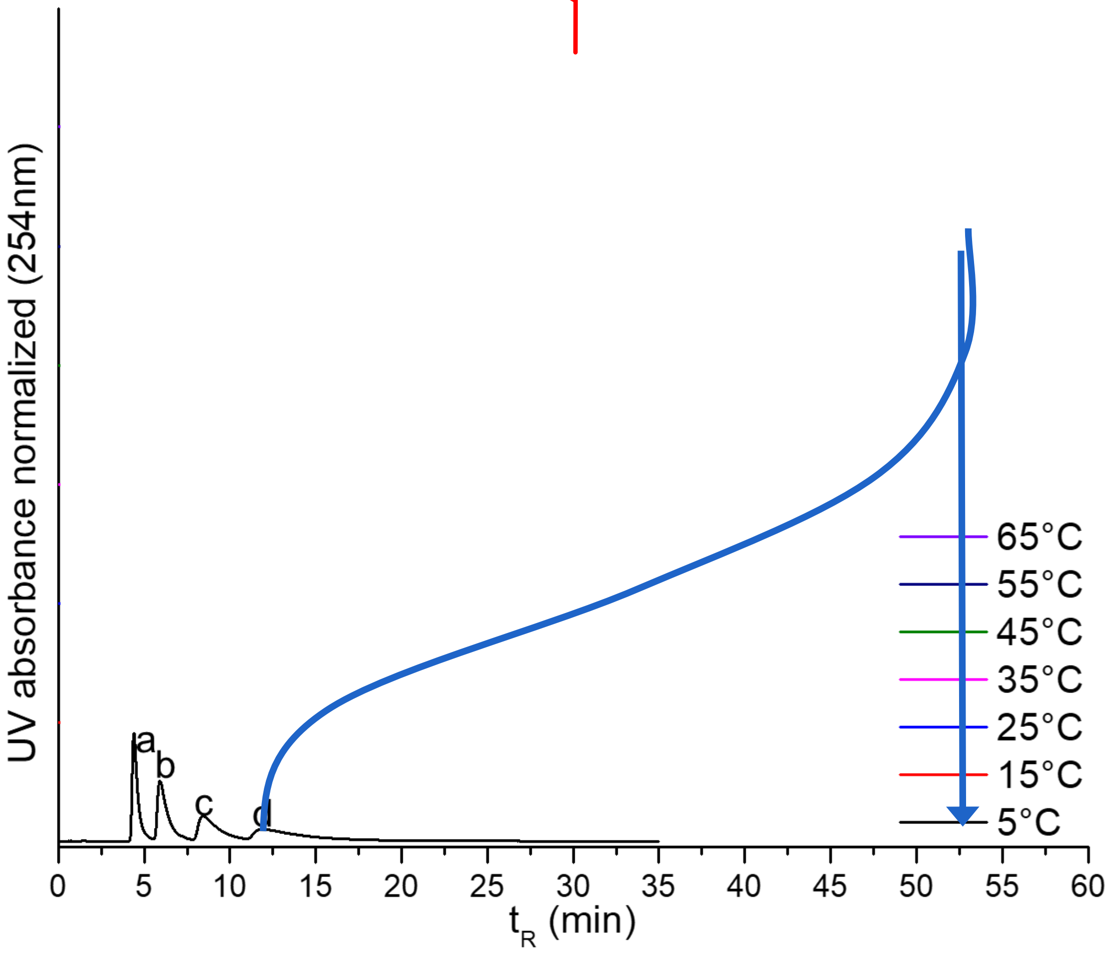
N-n-Propyl acrylamide (PNNPAAm) (10°C)

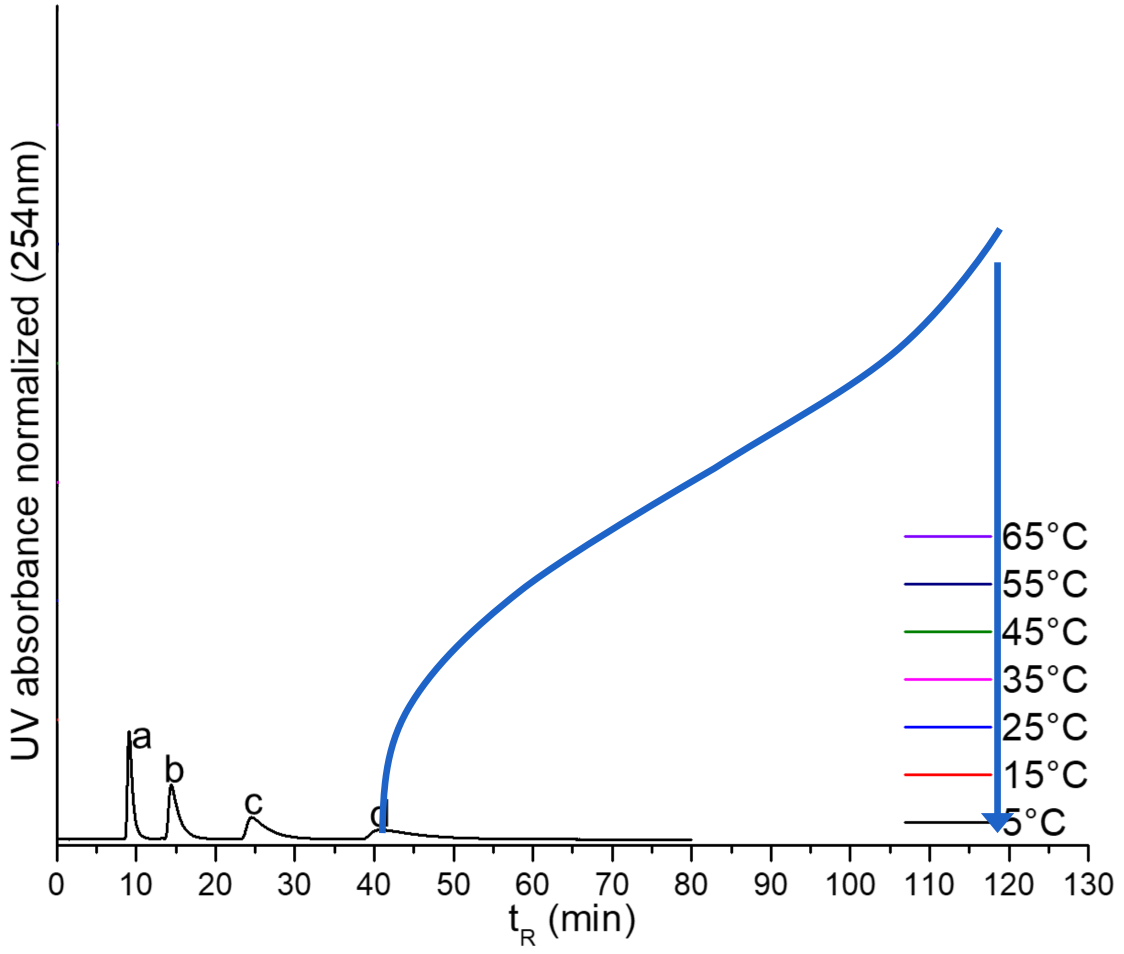


N,N-Diethyl acrylamide (PDEAAm) (35°C)





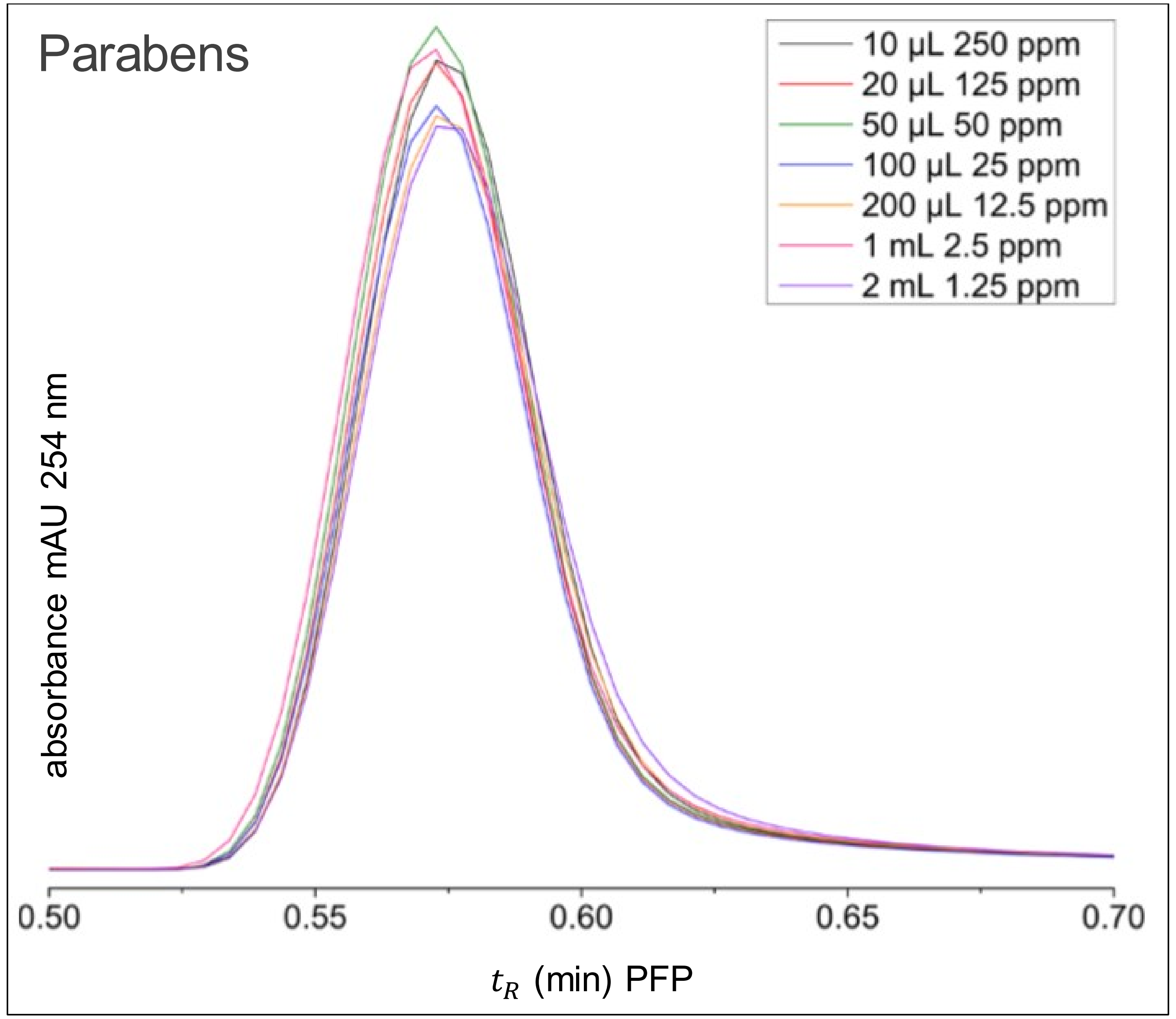




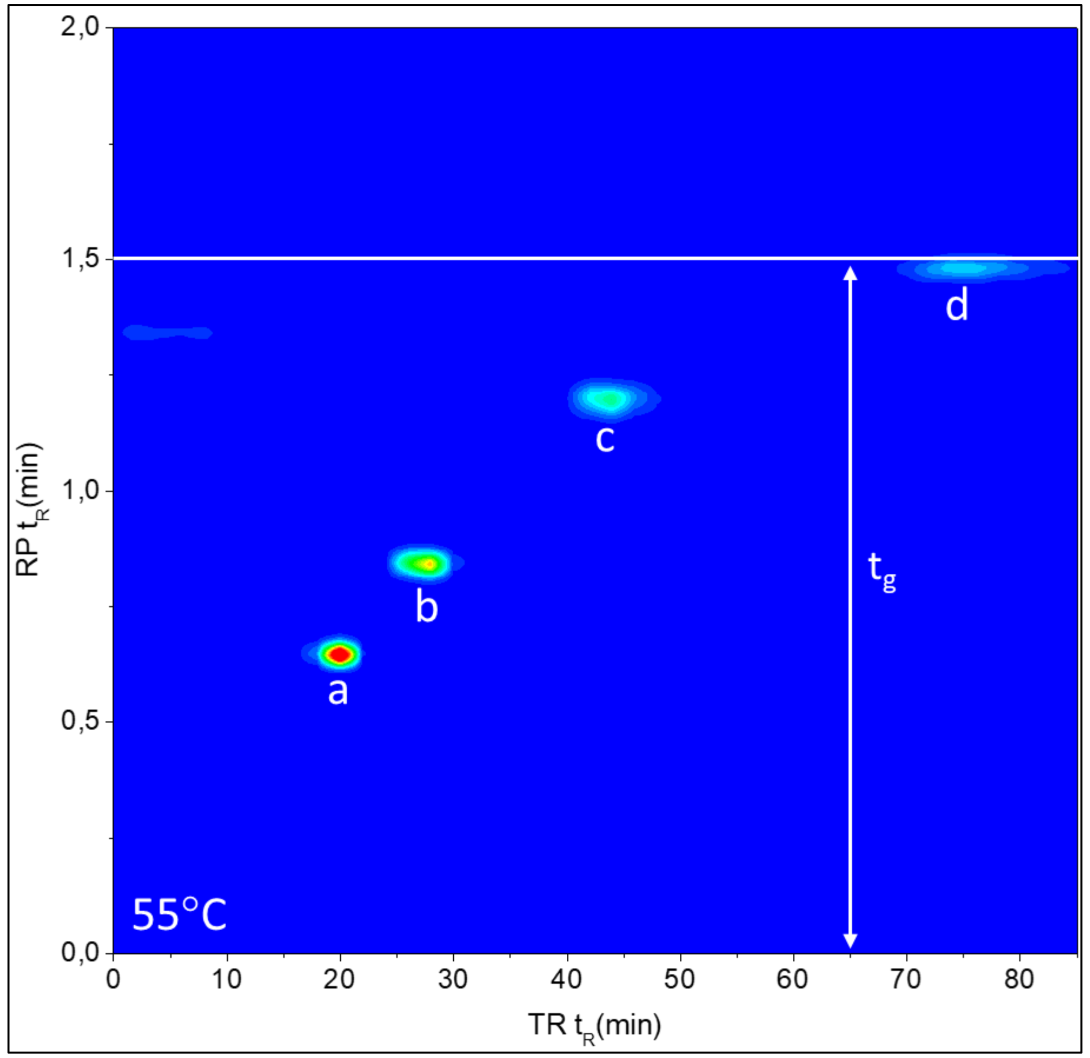
1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

TRLCxRPLC: why?

- Large aqueous volumes can easily **refocus** organic solutes on C18 (RPLC) columns



- TRLCxRPLC → **easier modulation**



M. Baert, S. Martens, G. Desmet, A. de Villiers, F. Du Prez, F. Lynen, Anal. Chem., 2018, 90, 8

1. Aqueous ^{1}D-based comprehensive 2D-LC: rationale

TRLCxRPLC: Achiral x Achiral (comparative example)

- **Increasing complexity in drug formulations**

- **More complex impurity determinations**
- **Increasing need for 2-D LC (heart-cutting, comprehensive)**

- **Exploring TRLC × RPLC for pharmaceutical impurity analysis**

- **Analysis of 11 corticosteroids and 6 progestogens**

^{1}D = temperature responsive column
- flow: 0.1 mL/min H_2O 0.1%FA
- Column: 10cm x 2.1mm x 5µm
  Temperature: gradient 45˚C→ 0°C

X

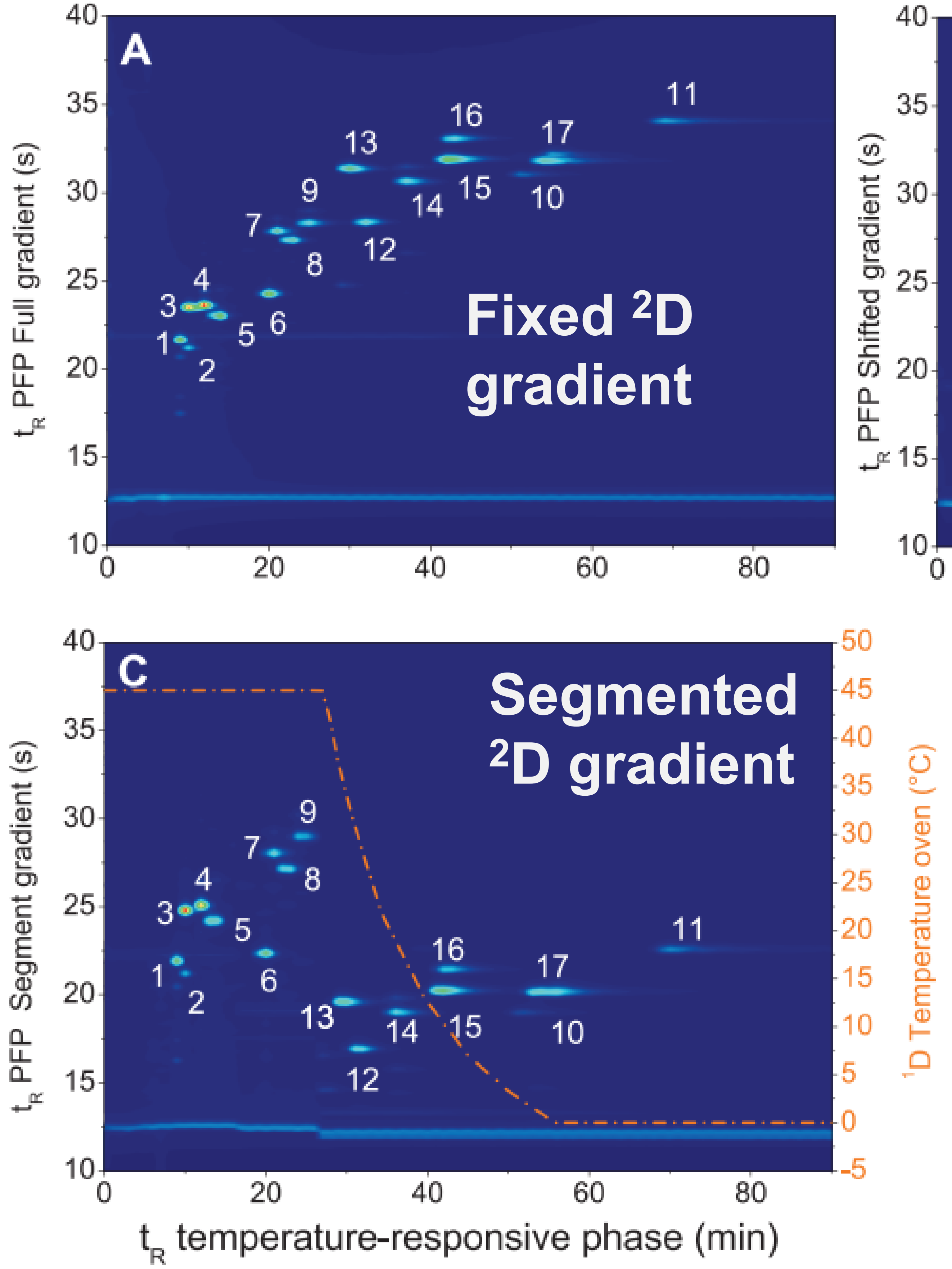
^{2}D = reversed phase column
- Flow: 2.6 mL/min H_2O 0.1% FA/ACN
- Column: 50x3 mm 2.7 µm (Poroshell)
- Modulation time = 1 min
- Sample loop = 120µL
- Temperature: 50˚C

1: prednisone
2: prednisolone
3: 11-dehydrocorticosterone
4: dexamethasone
5: methylprednisolone
6: beclomethasone
7: dexamethasone 21-acetate
8: betamethasone 21-acetate

9: dexamethasone 21- isonicotinate
10: betamethasone 17-valerate
11: beclomethasone dipropionate
12: ethisterone
13: 17α-hydroxyprogesterone 17-acetate
14: 1- dehydroprogesterone
15: progesterone
16: medroxyprogesterone 17-acetate
17: 16-dehydroprogesterone

1. Aqueous ¹D-based comprehensive 2D-LC: rationale

TRLCxRPLC: Pharmaceutical application (achiral)



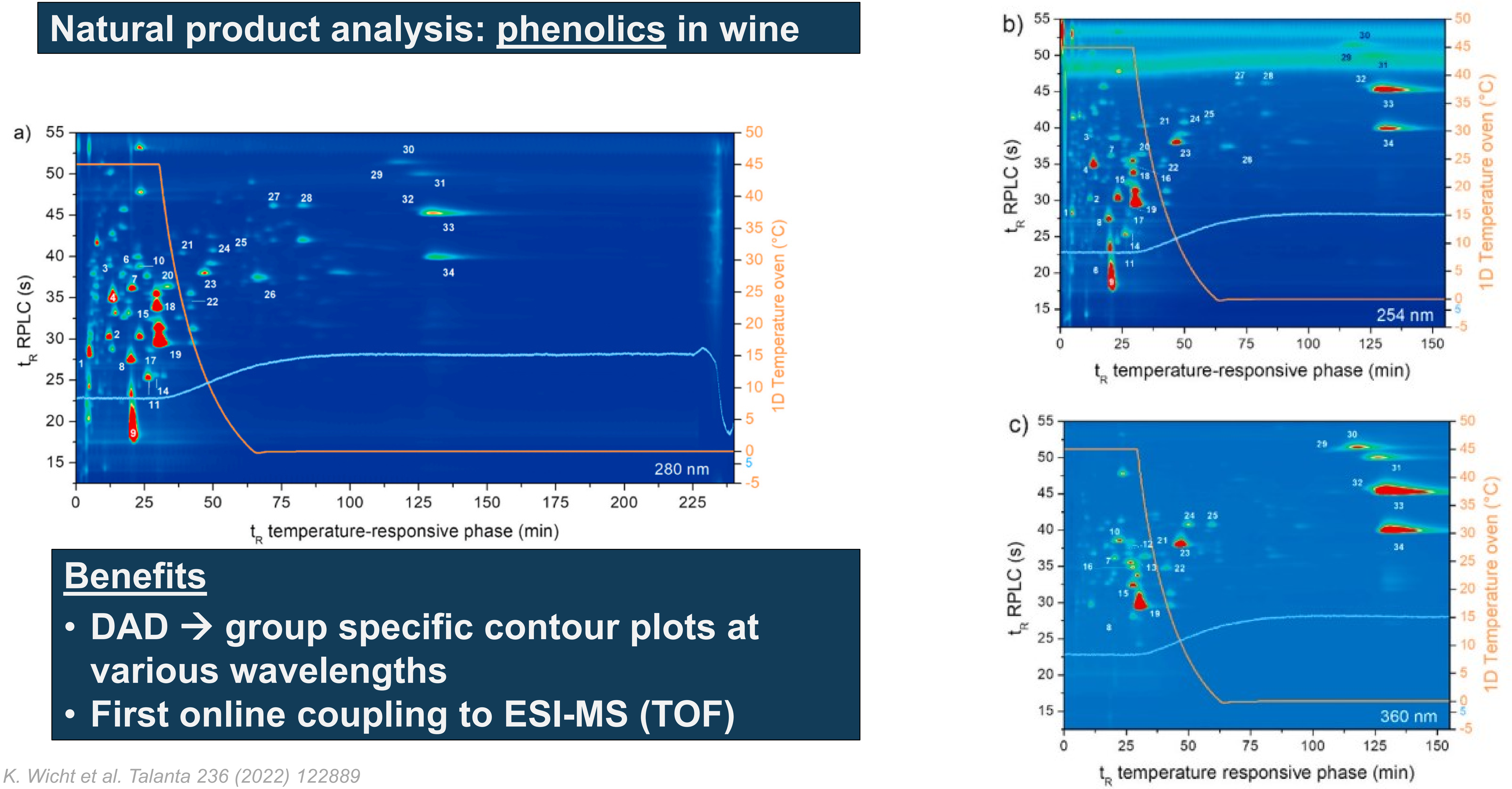
Various ²D gradient profiles

K. Wicht et al. Journal of Chromatography A 1630 (2020) 461561

1. Aqueous 1D-based comprehensive 2D-LC: rationale

TRLCxRPLC: Achiral x Achiral (comparative example 2)

Natural product analysis: phenolics in wine



Benefits

- DAD → group specific contour plots at various wavelengths
- First online coupling to ESI-MS (TOF)

K. Wicht et al. Talanta 236 (2022) 122889

2. Achiral TRLC x chiral comprehensive 2D-LC

2. Achiral TRLC x chiral comprehensive 2D-LC

TRLCxRPLC: Achiral x Chiral

Goals

- **Developing a broadly applicable chiral separation platform for small molecule chiral resolution in (complex) mixtures**

- **Allowing chiral resolution of dissimilar molecules on the same platform**

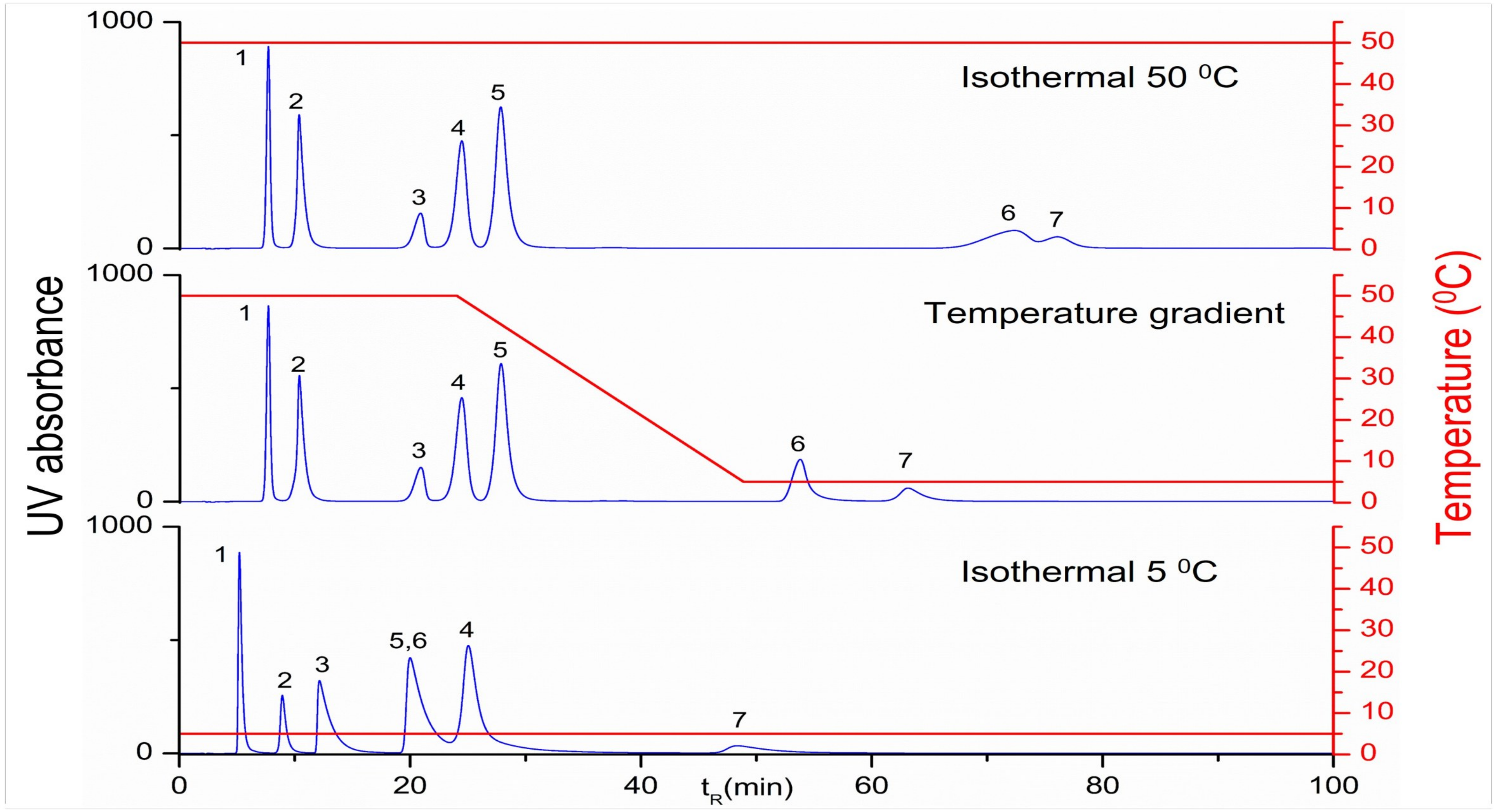
- ***Allowing (eventually) more facile split-free coupling of ^{2}D with ESI-HRMS***

- ***Allowing (eventually) more sensitive ESI-HRMS via I.D. ^{2}D < I.D. ^{1}D***

2. Achiral TRLC x chiral comprehensive 2D-LC

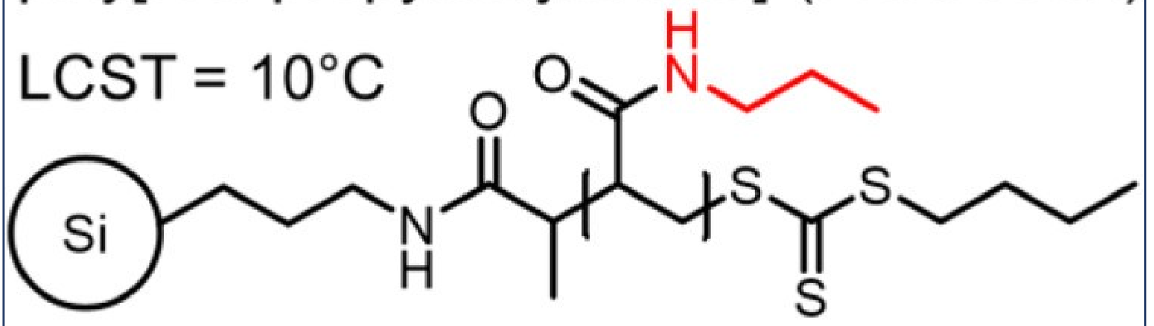
TRLCxRPLC: Achiral x Chiral

Test conditions:TRLC of 7 racemates



poly[N-n-propylacrylamide] (PNNPAAm)

LCST = 10°C



^{1}D conditions

- TRLC columns (100 × 2.1 mm, 5 μm)
- 0.1 mL/min
- 15 mM trifluoro ammonium acetate pH=2.9
- temp. gradient or isothermal
- injection volume = 2 μL

Compounds: (1) Hexobarbital, (2) Mianserin, (3) Secobarbital, (4) Chlorthalidone, (5) Oxazepam, (6) Ibuprofen, (7) Warfarin

2. Achiral TRLC x Chiral comprehensive 2D-LC

Chiral RPLC in ²D

Columns	Dimensions	Silica type	linkage	LC method
Cellulose-1	50 × 4.6mm, 3µm	FPPs	Coated	Method 1
Cellulose-2	50 × 4.6mm, 3µm	FPPs	Coated	Method 1
Cellulose-3	50 × 4.6mm, 3µm	FPPs	Coated	Method 1
Cellulose-4	50 × 4.6mm, 3µm	FPPs	Coated	Method 1
Amylose-2	50 × 4.6mm, 3µm	FPPs	Coated	Method 1
TeichoShell	50 × 3mm, 2.7µm	SPPs	Immobilized	Method 2
VancoShell	50 × 3mm, 2.7µm	SPPs	Immobilized	Method 2

^{2}D Method 1
4.6 mm I.D. column
Flow rate : 3ml/min
modulation time:100s, T=20°C

Time (s)	%B
0	2
72	70
73	2
100	2

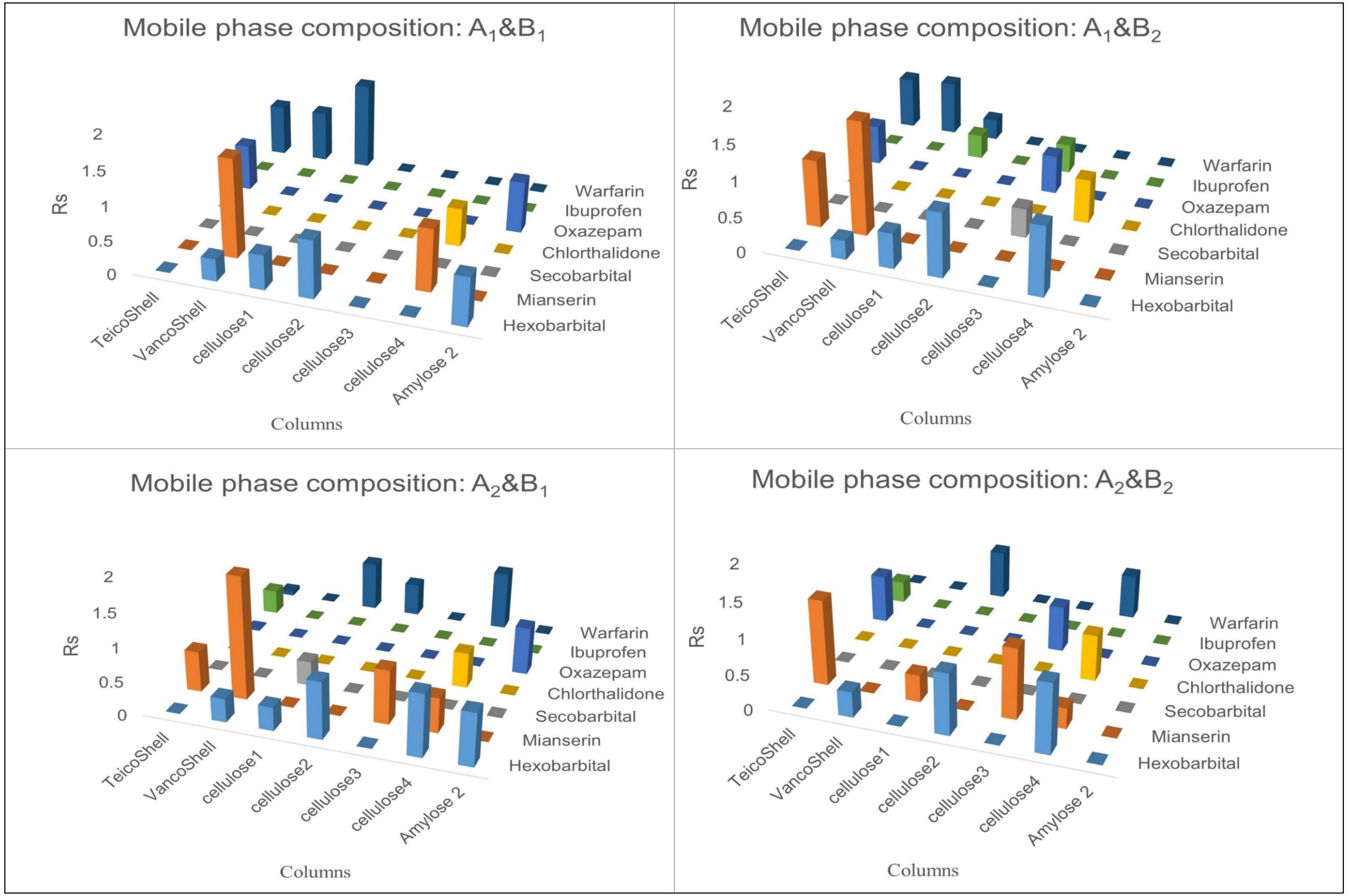
- **A1: 15 mM ammonium trifluoroacetate (pH =2.9)**
- **A2: 15 mM ammonium acetate (pH = 6.2)**
- **B1: ACN,**
- **B2: 70% ACN, 30% MeOH) were utilized.**

^{2}D Method 2
3 mm I.D. column
Flow rate: 2ml/min
modulation time:100s, T=20°C

Time (s)	%B
0	2
66	60
67	2
100	2

2. Achiral TRLC x Chiral comprehensive 2D-LC

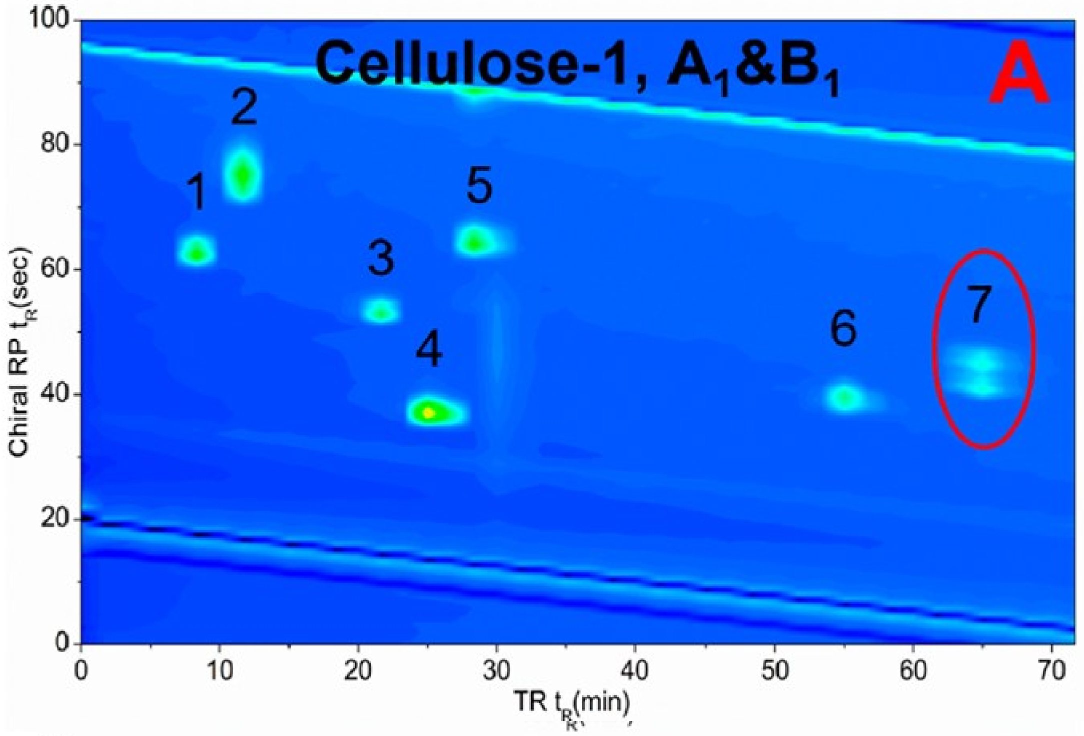
Chiral screening approach in ^2D

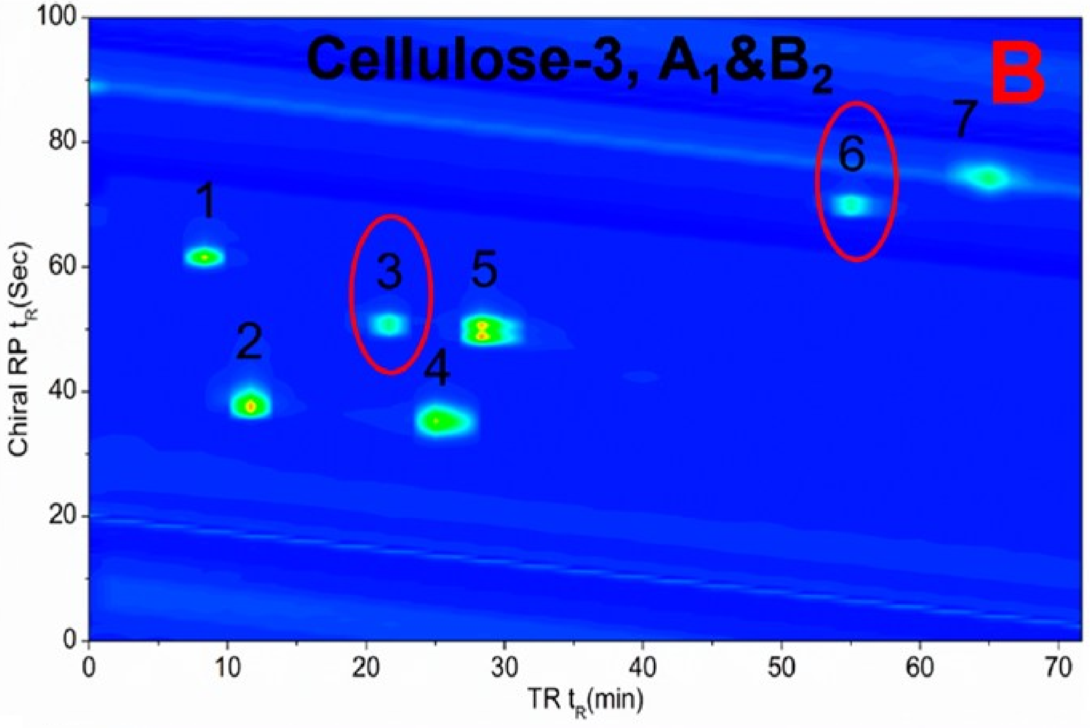


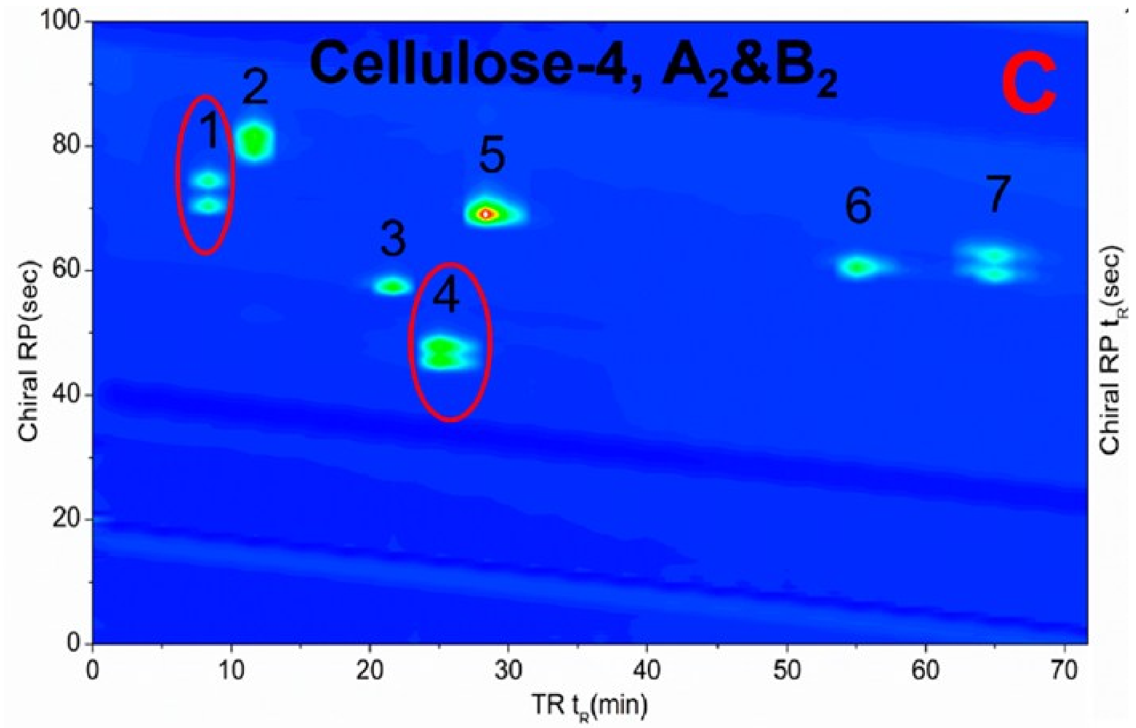
- **Screening of 4 mobile phase combinations on 7 Chiral columns (28 conditions)**
- **Selection of the optimally resolving method for each pair of enantiomers**

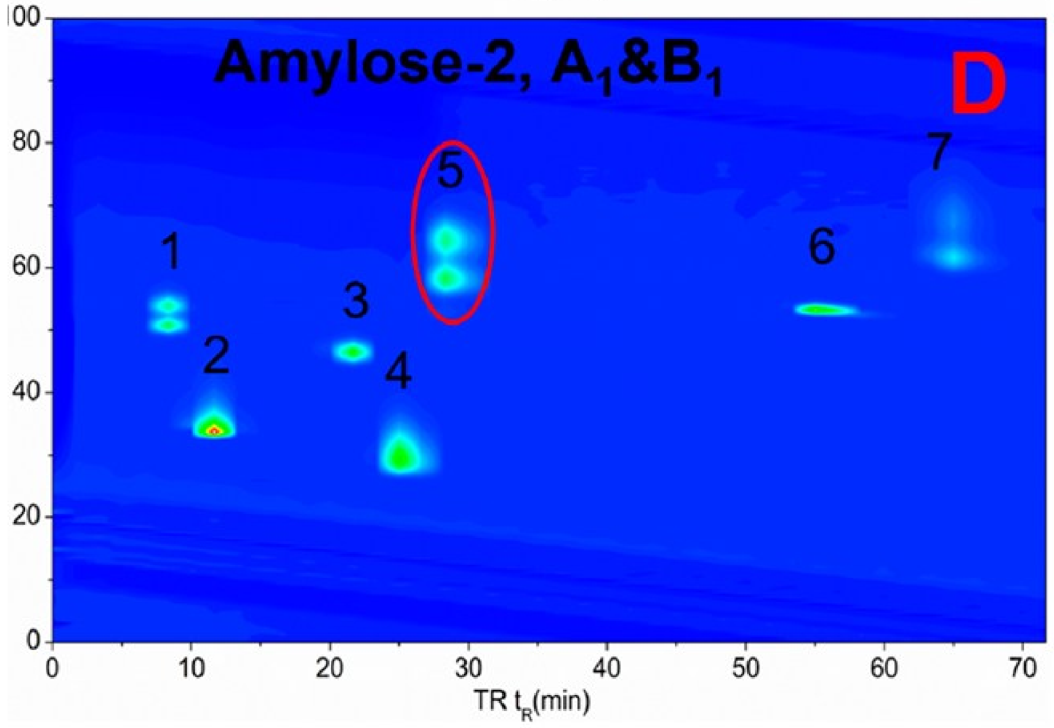
2. Achiral TRLC x Chiral comprehensive 2D-LC

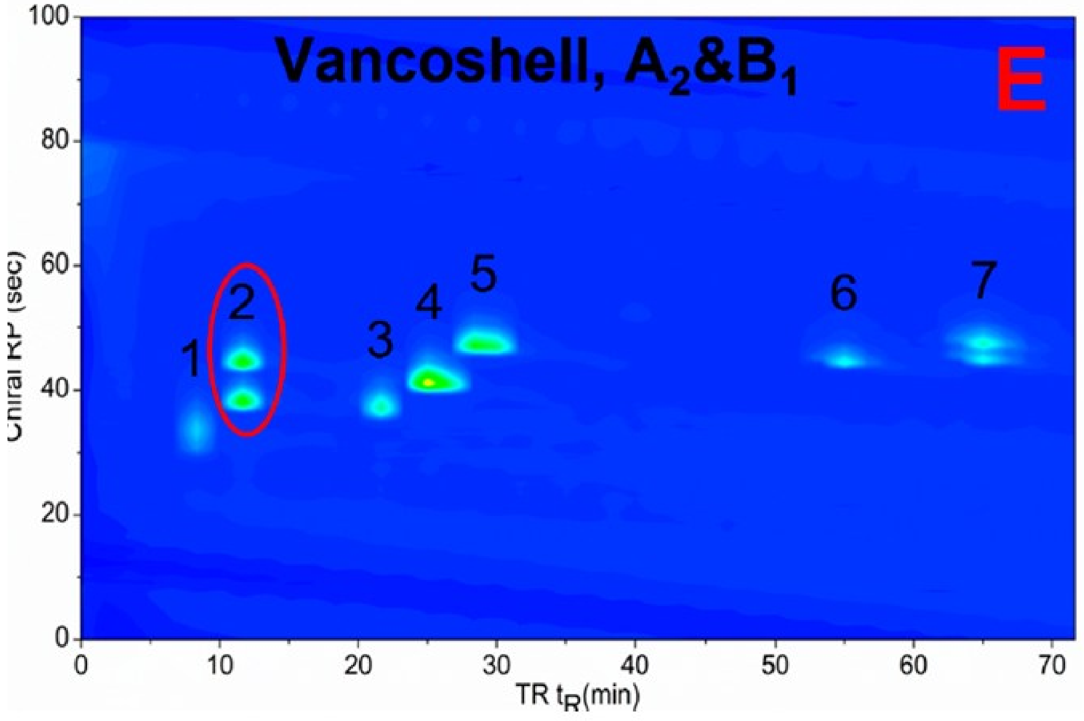
Optimal conditions for each solute











Compounds: (1) Hexobarbital, (2) Mianserin, (3) Secobarbital, (4) Chlorthalidone, (5) Oxazepam, (6) Ibuprofen, (7) Warfarin

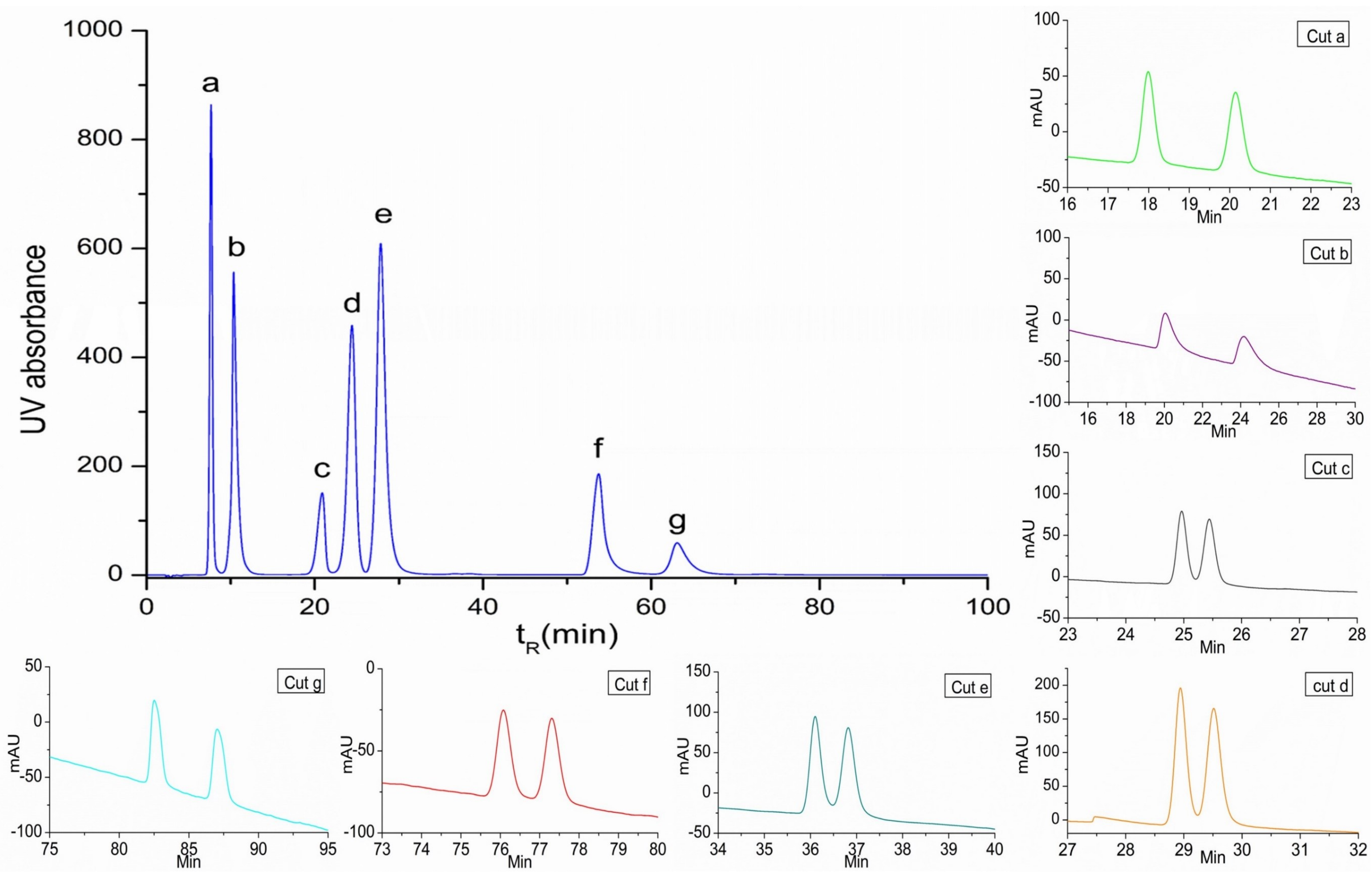
T. Rahmani, A. Ampe, F. Lynen. Temperature Responsive × Fast Chiral Comprehensive Liquid Chromatography: a New 2D-LC Platform for Resolving Mixtures of Chiral Isomers. *Analytical Chemistry*, 2023, 95,8763-8769.

2. Achiral TRLC x Chiral comprehensive 2D-LC

From LCxLC to heart-cutting

Chiral screening approach in LCxLC

Also a tool for finding optimal heat cutting conditions



T. Rahmani, A. Ampe, F. Lynen. Temperature Responsive × Fast Chiral Comprehensive Liquid Chromatography: a New 2D-LC Platform for Resolving Mixtures of Chiral Isomers. *Analytical Chemistry*, 2023, 95,8763-8769.

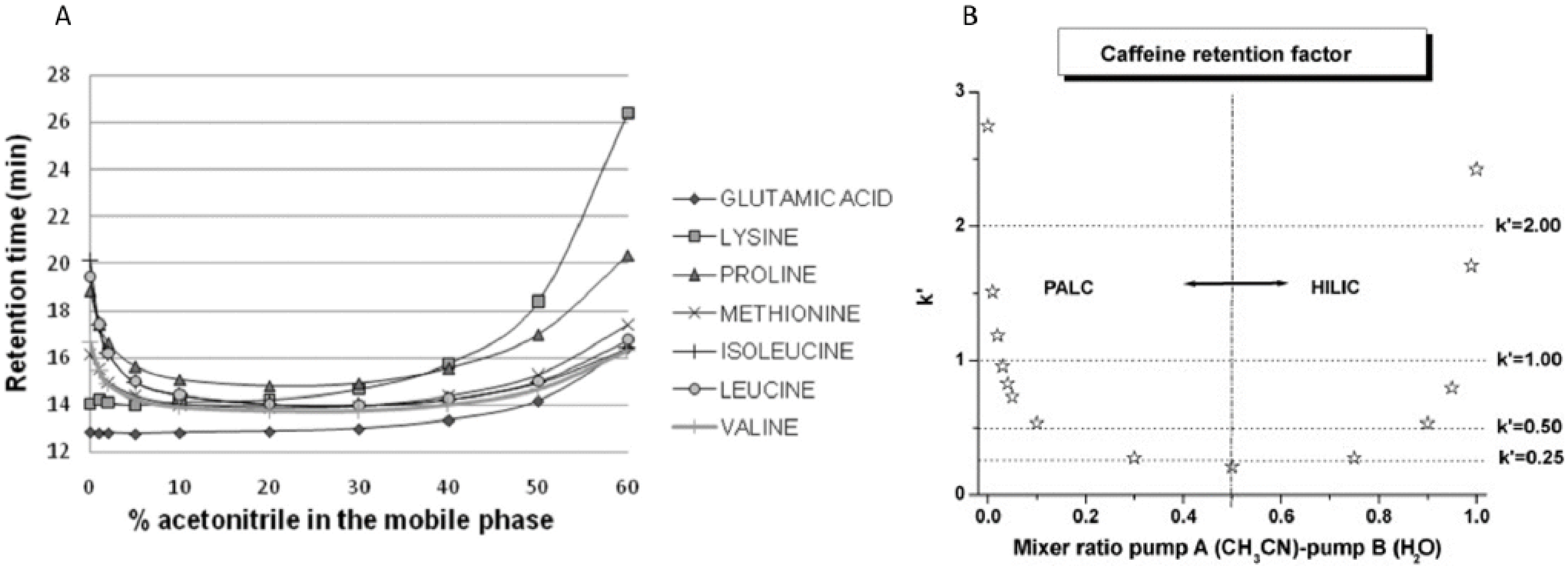
3. The potential of PALCxRPLC

3. Achiral PALC x chiral comprehensive 2D-LC

What is Per-aqueous Liquid Chromatography (PALC)

- **PALC (or inversed HILIC)**
- **Operates on a polar column (e.g. native silica or diol)**
- **Mobile phase: mostly (90%) water and ACN (+ buffer)**

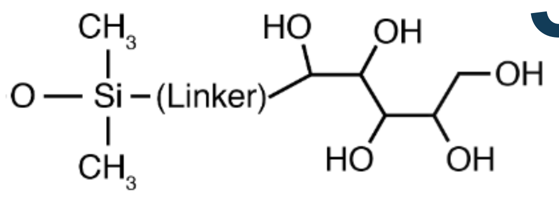
Alberto dos Santos Pereira, Frank David, Gerd Vanhoenacker, and Pat Sandra. The acetonitrile shortage: is reversed hilic with water an alternative for the analysis of highly polar ionizable solutes? Journal of separation science, 32(12):2001–2007, 2009.



- **Elution and retention under ~ purely aqueous conditions**

3. Achiral PALC x chiral comprehensive 2D-LC

PALCxRPLC: can it work as well?
Pesticides (achiral separation)

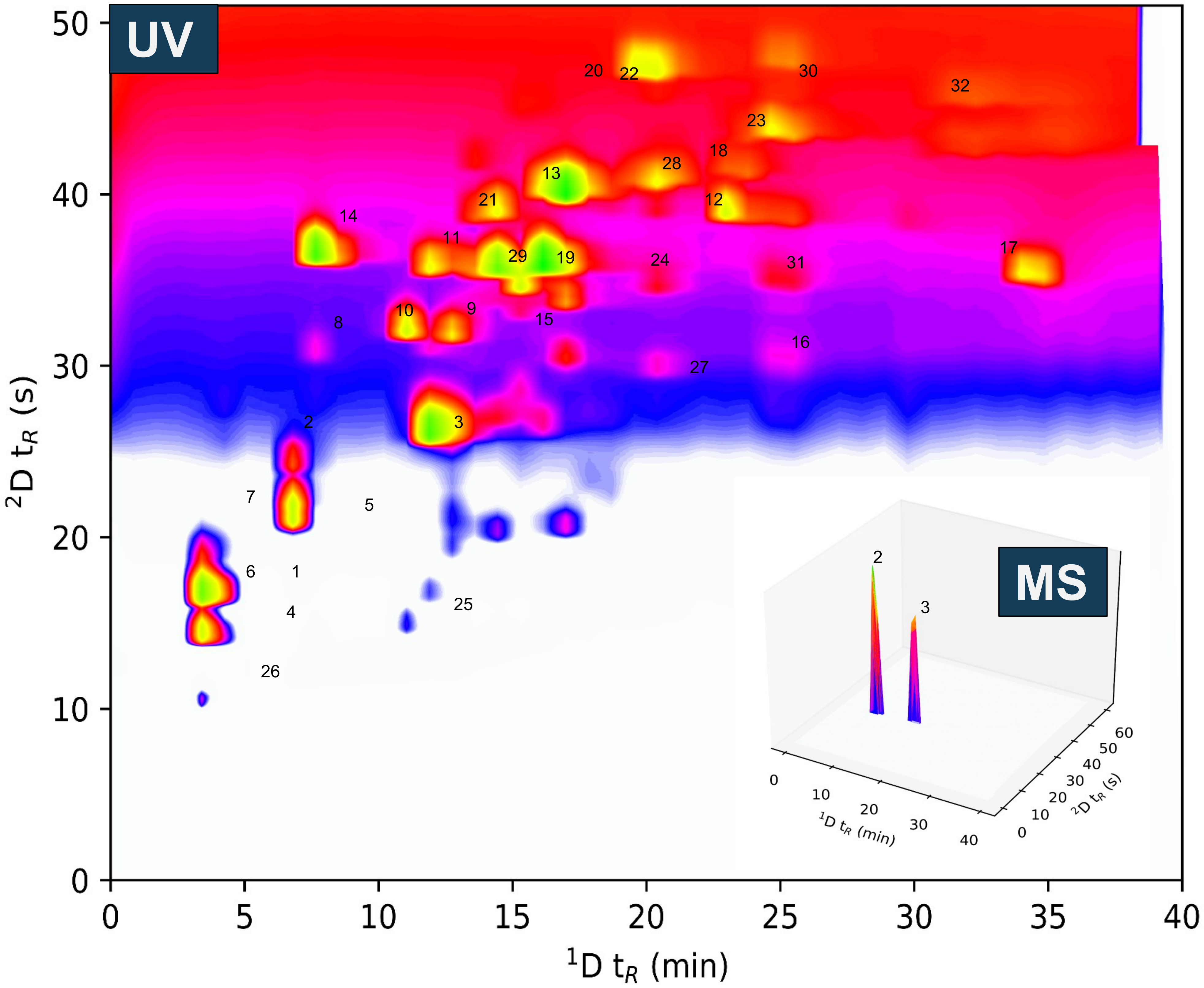


Analysis conditions ^{1}D:

- Column: penta HILIC (**2.1** x 150 mm, 2.7 µm)
- Flow rate: 0.1 mL/min
- Injection volume: 1.5 µL
- Mobile phase A: **H_2O** + TFA, pH 3.19
- Mobile phase B: EtOH
- Gradient:
  - 0 min 0 % B
  - 40 min 25 % B
- Column temperature: 50 °C
- UV: 254 nm
- Analysis time: 40 min

Analysis conditions ^{2}D :

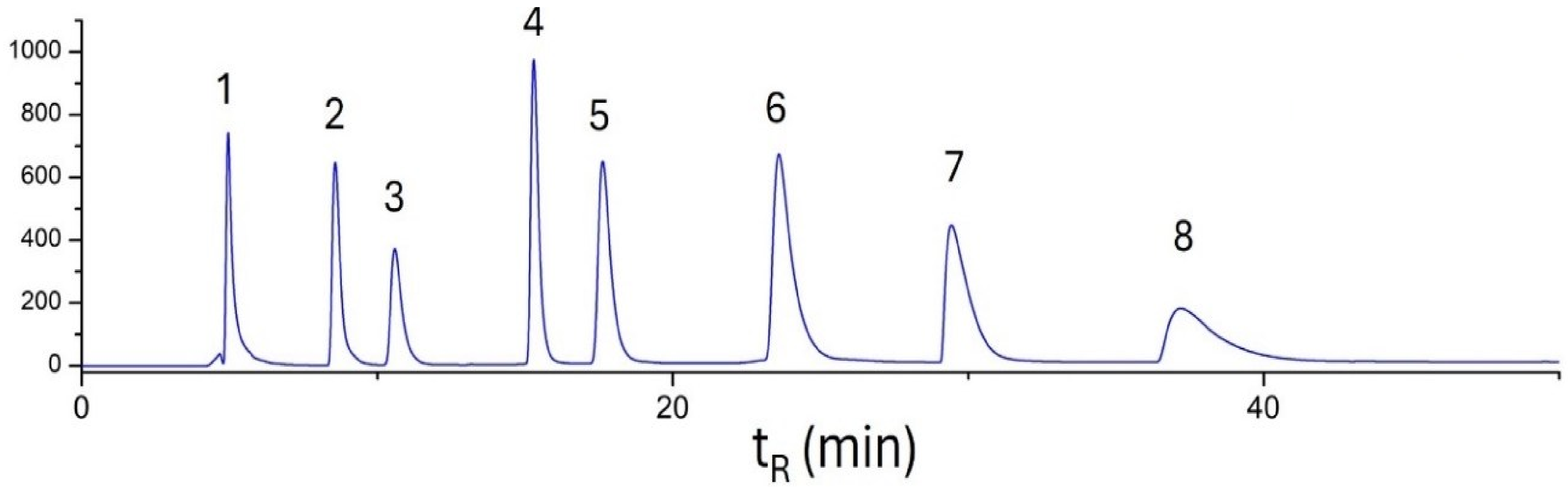
- Column: C18 (HALO) (**1.5** x 50 mm, 2.7 µm)
- **Flow rate: 0.6 mL/min**
- Mobile phase A: H_2O + 0.1 % FA
- Mobile phase B: ACN + 0.1 % FA
- Gradient:
  - 0 min 10 % B
  - 0.1 min 50 % B
  - 0.65 min 100 % B
- Gradient time: 0.65 min
- Modulation time: 0.85 min
- Column temperature: 50 °C
- UV: 254 nm
- **Loops size: 80 µL**
- Loops filling: 106 %
- ^{2}D column injection ratio: 150 %



Peak	Compound
1	acephate
2	metamitron
3	imazamox
4	mepiquatchlorid
5	Deisopropylatrazine
6	carbendazim
7	strychnine
8	cyanazin
9	indole-3-butyric acid
10	carbaryl
11	atrazin
12	warfarin
13	diuron
14	3,4-dichloroaniline
15	ethofumesate
16	2,3-dichlorophenol
17	bentazon
18	myclobutanil
19	isoproturon
20	terbuthylazine
21	sebuthylazin
22	linuron
23	fenamiphos
24	alachlor
25	MCPB
26	endrin
27	tebuconazole
28	parathion-ethyl
29	TBTO
30	fenthion
31	penconazole
32	diclofop methyl

3. Achiral PALC x chiral comprehensive 2D-LC

PALC x chiral-RPLC: can it work as well?
Pharmaceutical mixture (logP 1 ~ 4)



Compound identification

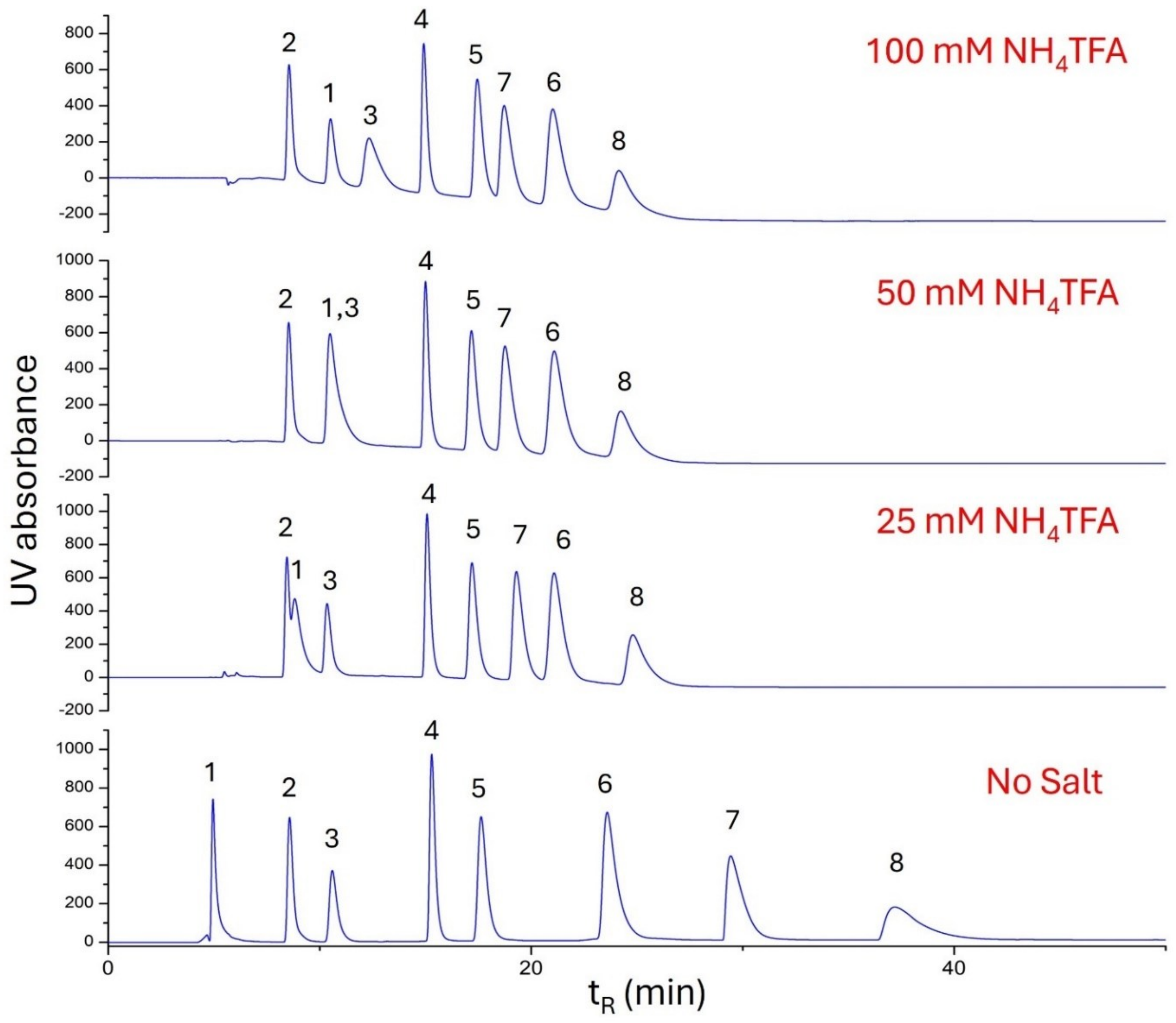
1. Propranolol (Log p:2.65)
2. Hexobarbital (Log p:1.98)
3. Secobarbital (Log p:1.97)
4. Chlorthalidone (Log p:0.85)
5. Oxazepam (Log p:2.24)
6. Warfarin (Log p:2.7)
7. Ketoprofen (Log p:3.31)
8. Flurbiprofen (Log p:3.94)

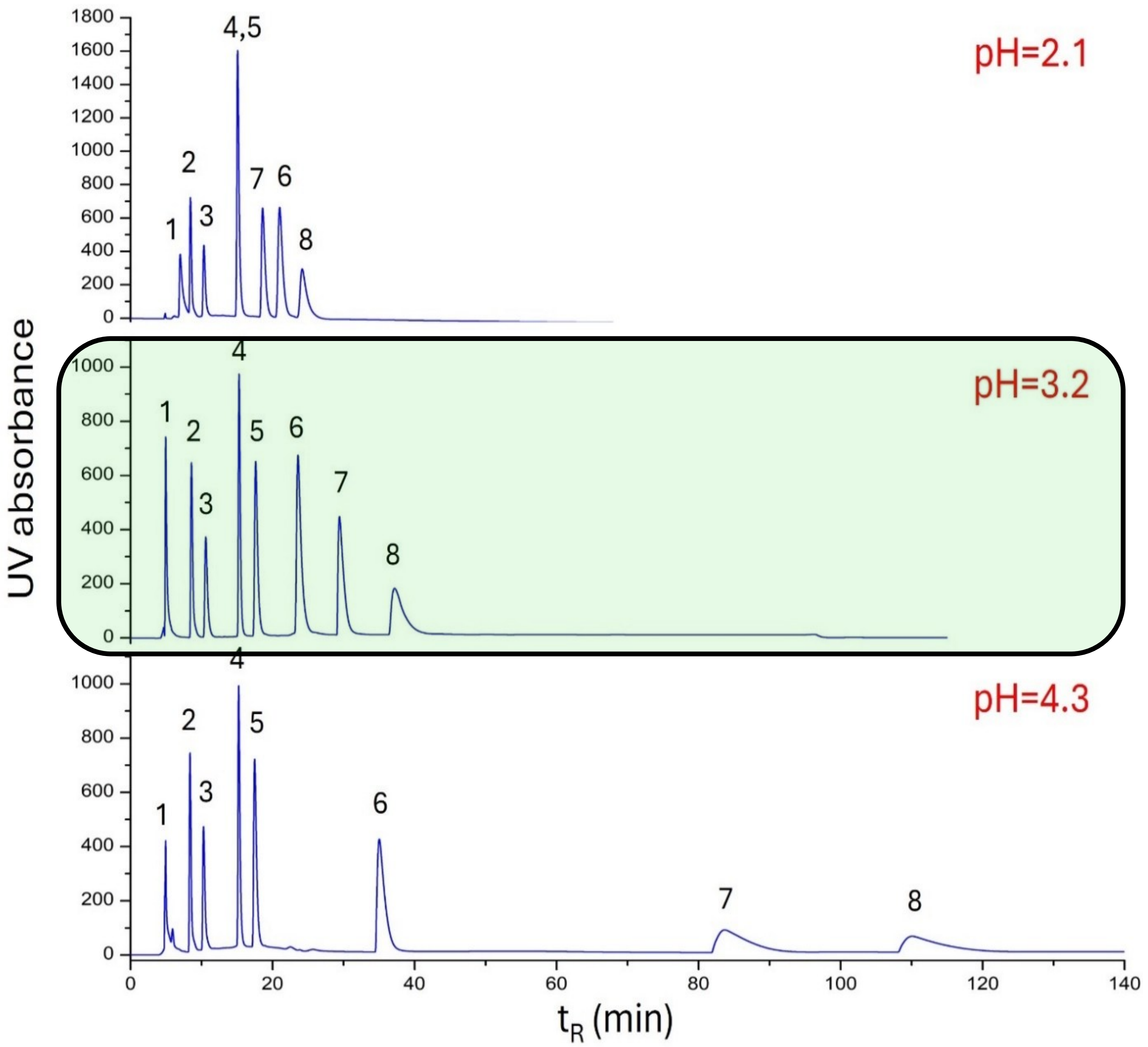
1D condition:
Penta HILIC column (250 × 2.1 mm, 2.7 µm)
Flow rate 0.1 mL/min
A: water (pH 3.2), B: ACN

Time (min)	%(B) ACN
0	5
20	20
60	20

3. Achiral PALC x chiral comprehensive 2D-LC

PALCxRPLC: can it work as well?

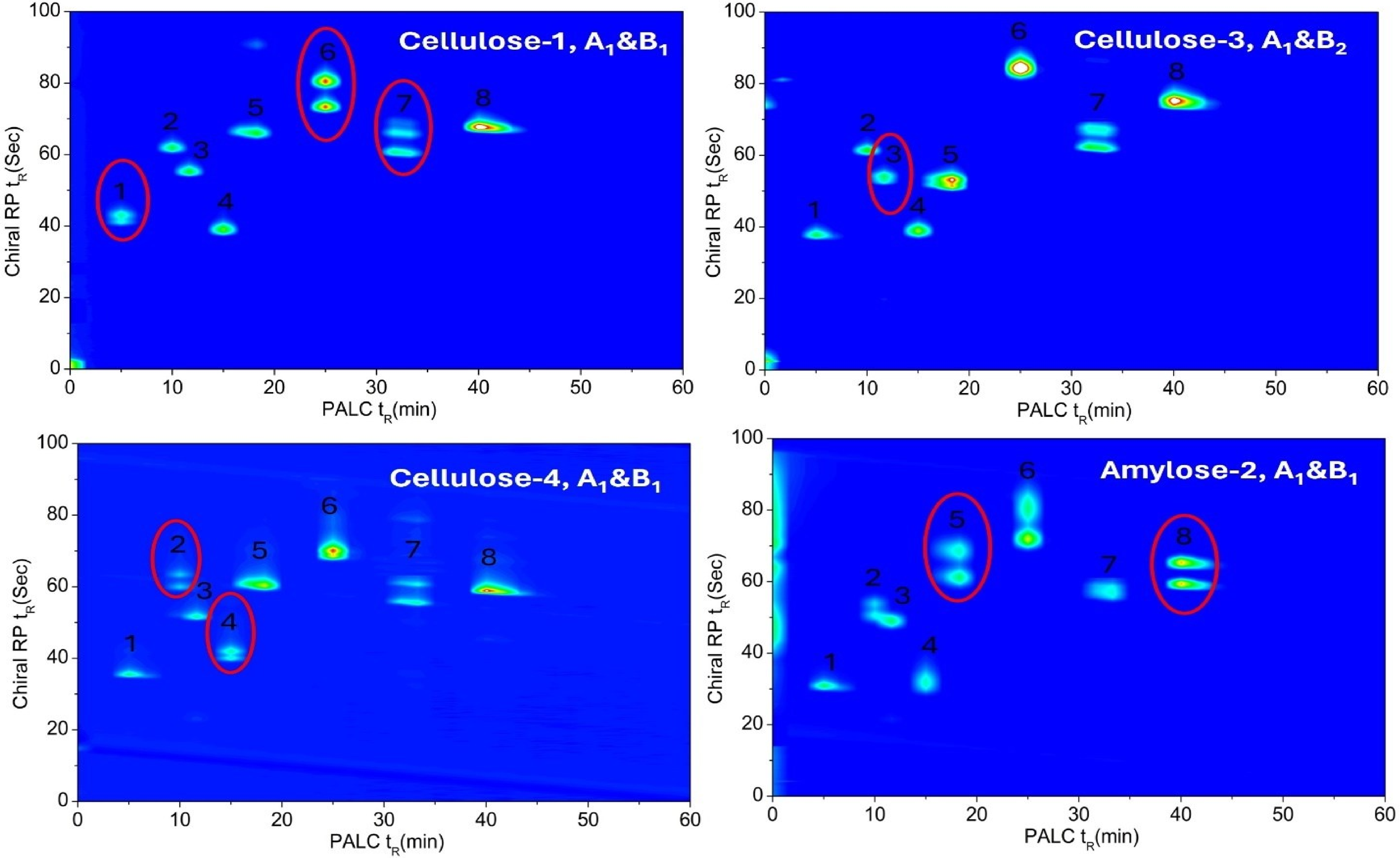




3. Achiral PALC x chiral comprehensive 2D-LC

PALCxRPLC: can it work as well?

Optimal contour plots of a mixture of pharmaceutical compounds



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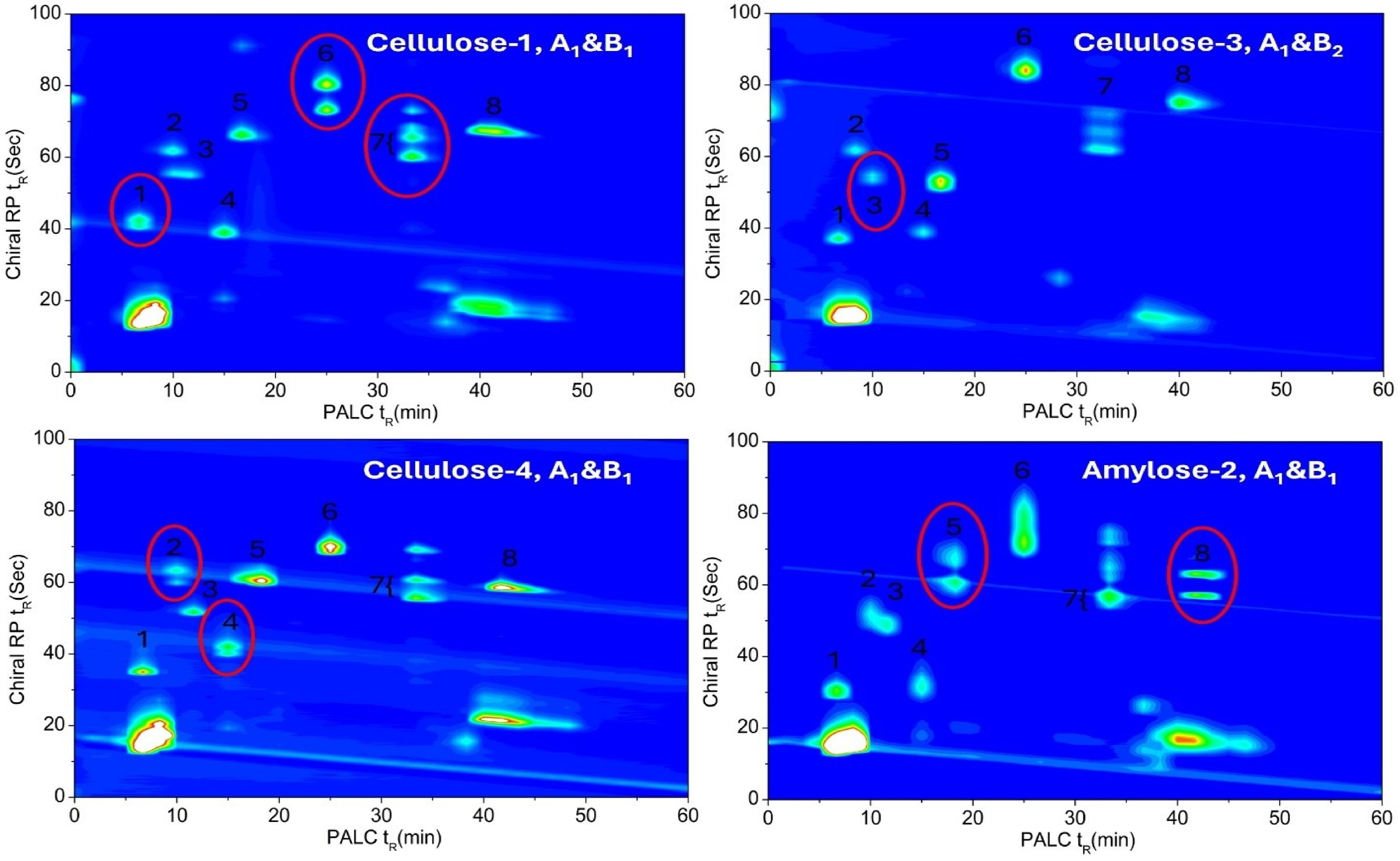
6. Warfarin (Log p:2.7)
7. Ketoprofen (Log p:3.31)
8. Flurbiprofen (Log p:3.94)

3. Achiral PALC x chiral comprehensive 2D-LC

PALCxRPLC: can it work as well?

Optimal contour plots of a mixture of pharmaceutical compounds in urine



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6. Warfarin (Log p:2.7)
7. Ketoprofen (Log p:3.31)
8. Flurbiprofen (Log p:3.94)

4. CONCLUSIONS

- TRLC complements IEC, HIC and SEC in LCxLC
- TRLCxRPLC allows for complete refocusing
- TRLCxRPLC allows for enhanced achiral x chiral 2D-LC
- PALCxRPLC also allows dilution free more sensitive analysis

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