MULTIPLE HEART-CUTTING 2D LC FOR CHALLENGING SEPARATION PROBLEMS

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Introduction

- Chemical samples in certain areas are of high and increasing complexity or dimensionality
  - Polymers can have a chemical composition distribution (CCD), functional end-group distribution (FTD) and/or molecular weight distribution (MWD)
  - New chemicals are derived from natural compounds which contain a significant number of possible structures (isomers etc.)
  - Determination of additives in polymer matrices

- Very complex samples require analytical tools of more than one dimension

- 2D LC has gained increasing attention due to high separation power, but requires significant method development for each application

- Recent introduction of more dedicated 2D LC instrumentation by several vendors is facilitating a more widespread use of this technology

2D LC System

Agilent 1290 Infinity 2D LC Solution:

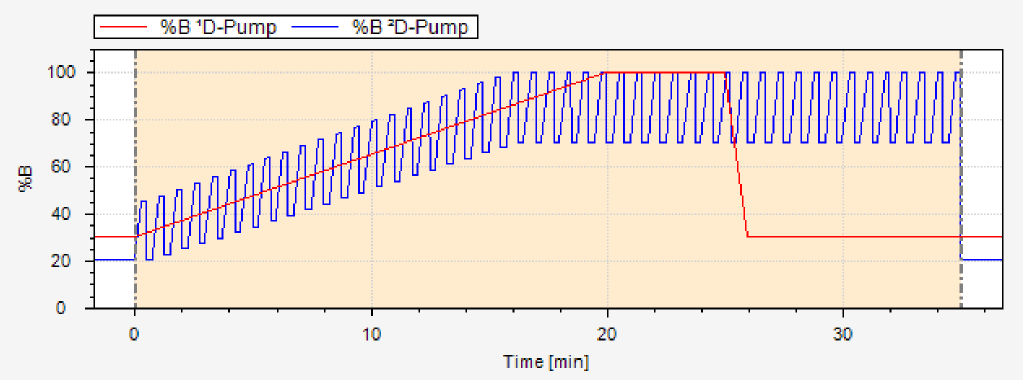
- Based on 1290 Infinity LC system (binary pump)
- 1290 Infinity quaternary pump, TCC and DAD used in 1D
- 1200 bar switching valve (8 port) for comprehensive and heart-cutting 2D analysis
- Multiple heart-cutting interface
- LCImage Software for comprehensive 2D LC data analysis

2D LC Separation Modes

Comprehensive 2D (LCxLC):

•Complete 1st dimension effluent is sampled in discrete fractions (20-80 µL) – two loops

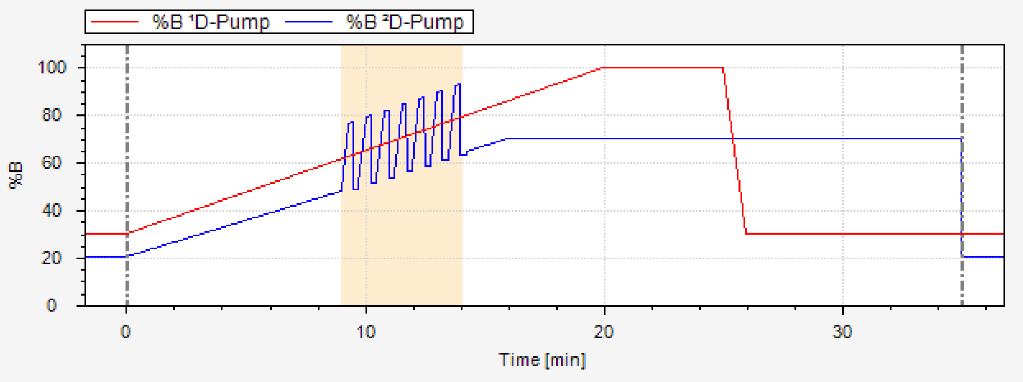
•Modulation time (20s – 1.5 min)



Selective Comprehensive 2D (sLCxLC):

•Defined region of interest in 1D is sampled in discrete fractions

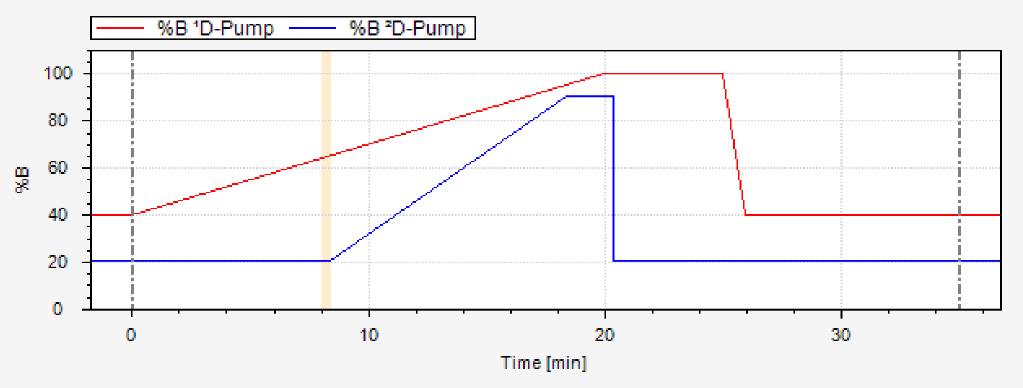
•Transferred continuously to a second column via loops



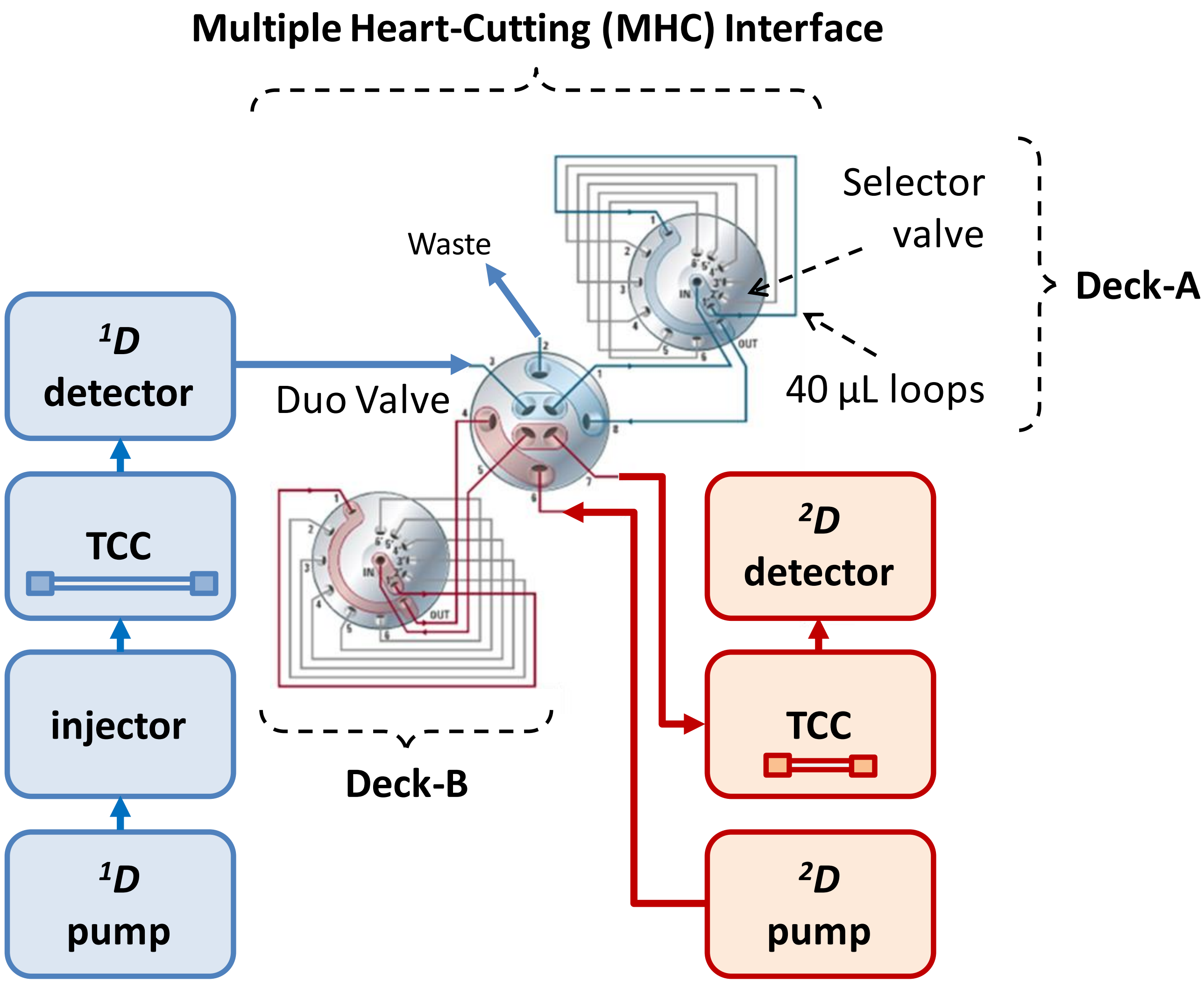
Heart-cutting 2D LC:

•Areas of interest are transferred from D1 to a second column via one loop (e.g., 40 µL)

•D2 analysis time is independent of D1 run time ("decoupled"), can be on order of 1.5 – 10 min

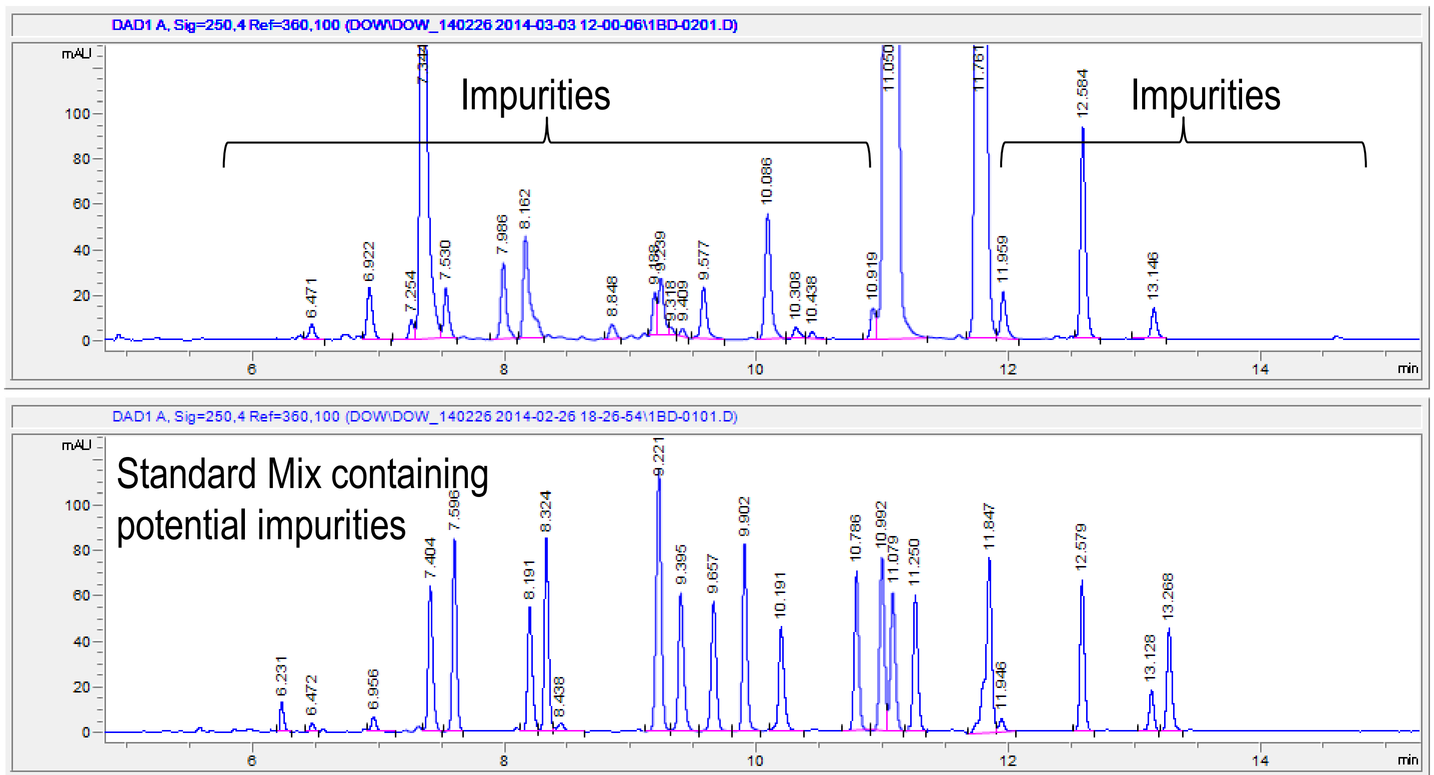


Multiple heart-cutting / selective comprehensive 2D



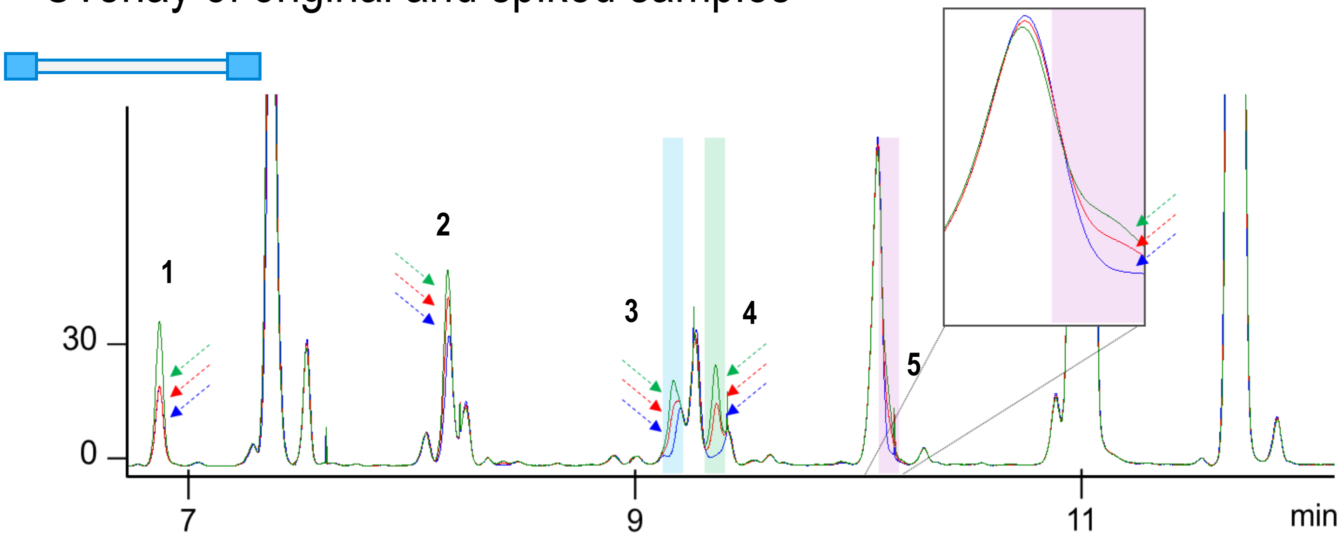
Insecticide analysis

- Dow AgroSciences insecticide – contains 2 main components and 30-40 impurities
- D1: C_{18} high resolution separation (150 mm, sub 2 µm particle column)
- Separation is good, but might be improved in certain areas



Multiple heart-cutting

Overlay of original and spiked samples



- Sample spiked w/ impurity standards.

Original sample.

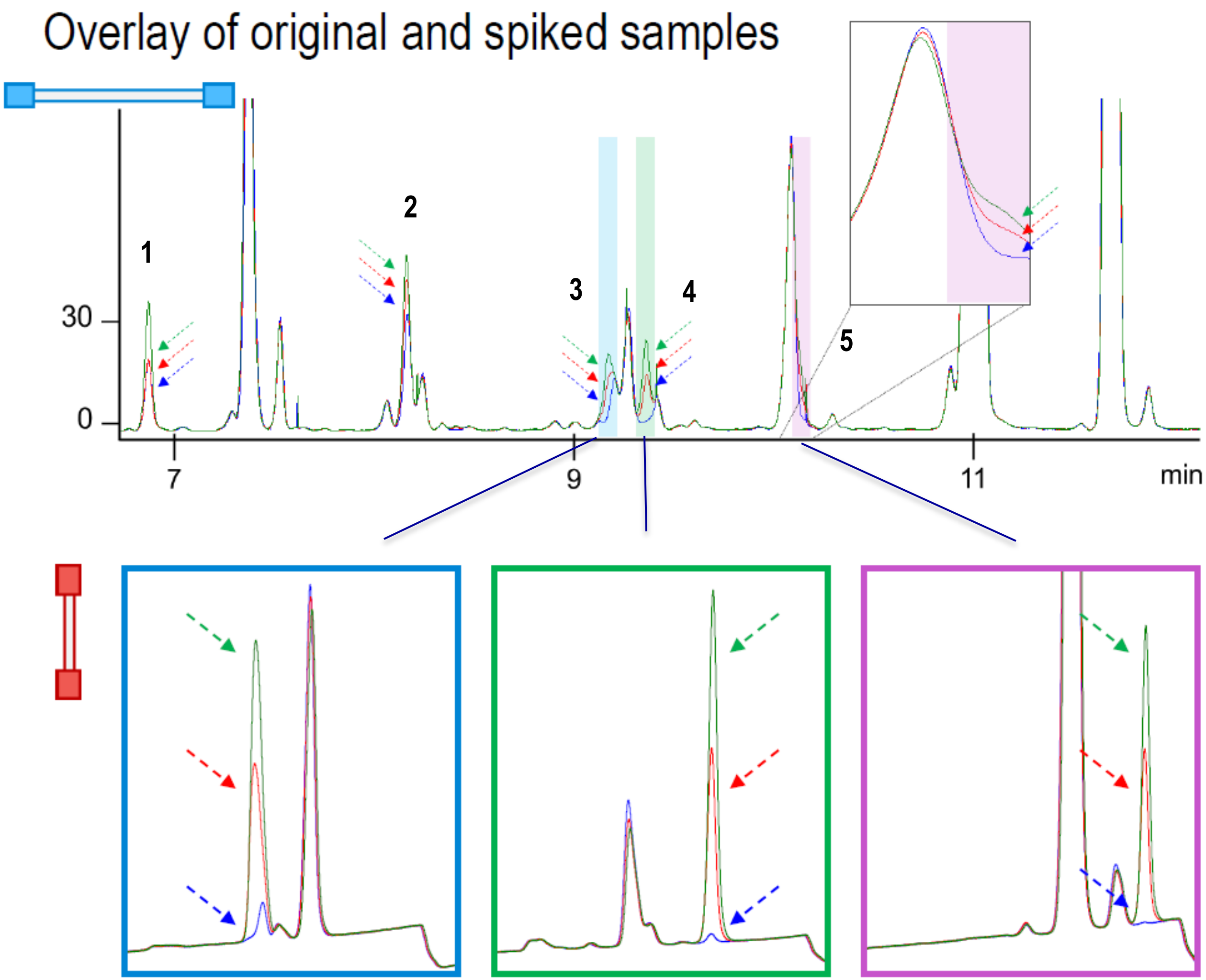
Spike-1.

Spike-2.

- Integration hardly possible.

1D separation:
Zorbax SB-C18 150x2.1 mm 1.8 µm
40°C, 0.5 mL/min
A: 10 mM ammonium acetate pH=6,
B: 20/80 methanol/acetonitrile
30-95% B/12 min

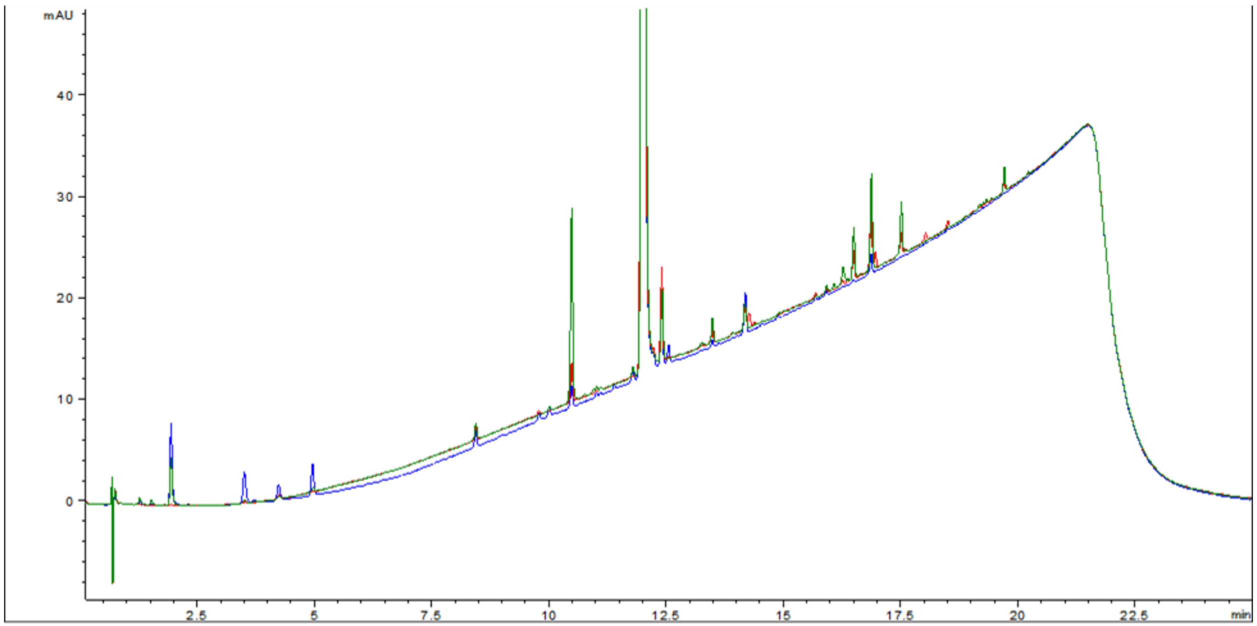
Multiple heart-cutting



- Multiple heart-cutting approach
- Much better separation in D2
- more accurate quantitation can be obtained with a suitable second dimension analysis

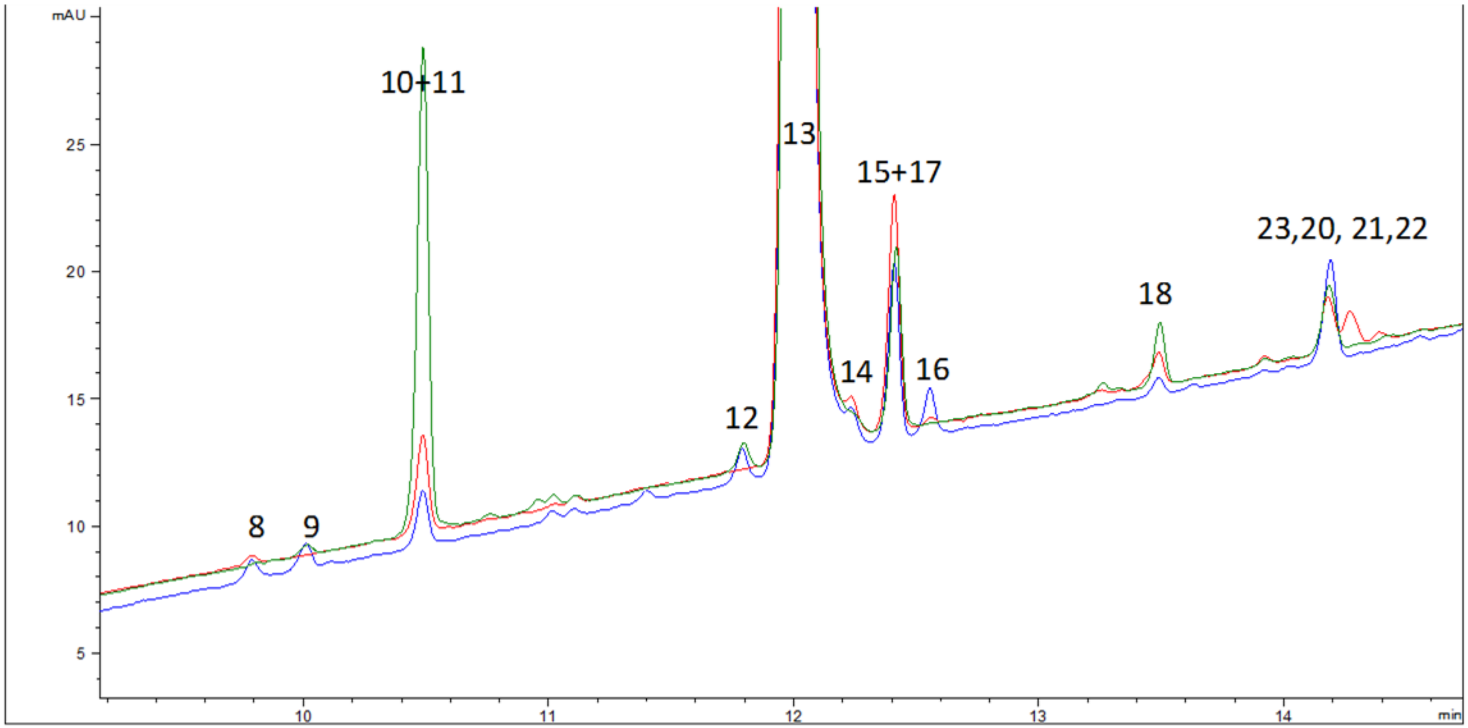
D2: Poroshell HPH-C18
50x3 mm 1.8 µm
40°C 2 mL/min
A: 10 mM ammonium hydroxide pH=11
B: acetonitrile
55-85% B/1.3 min (1.5 min cycle time)

Crop Protection Chemical Application



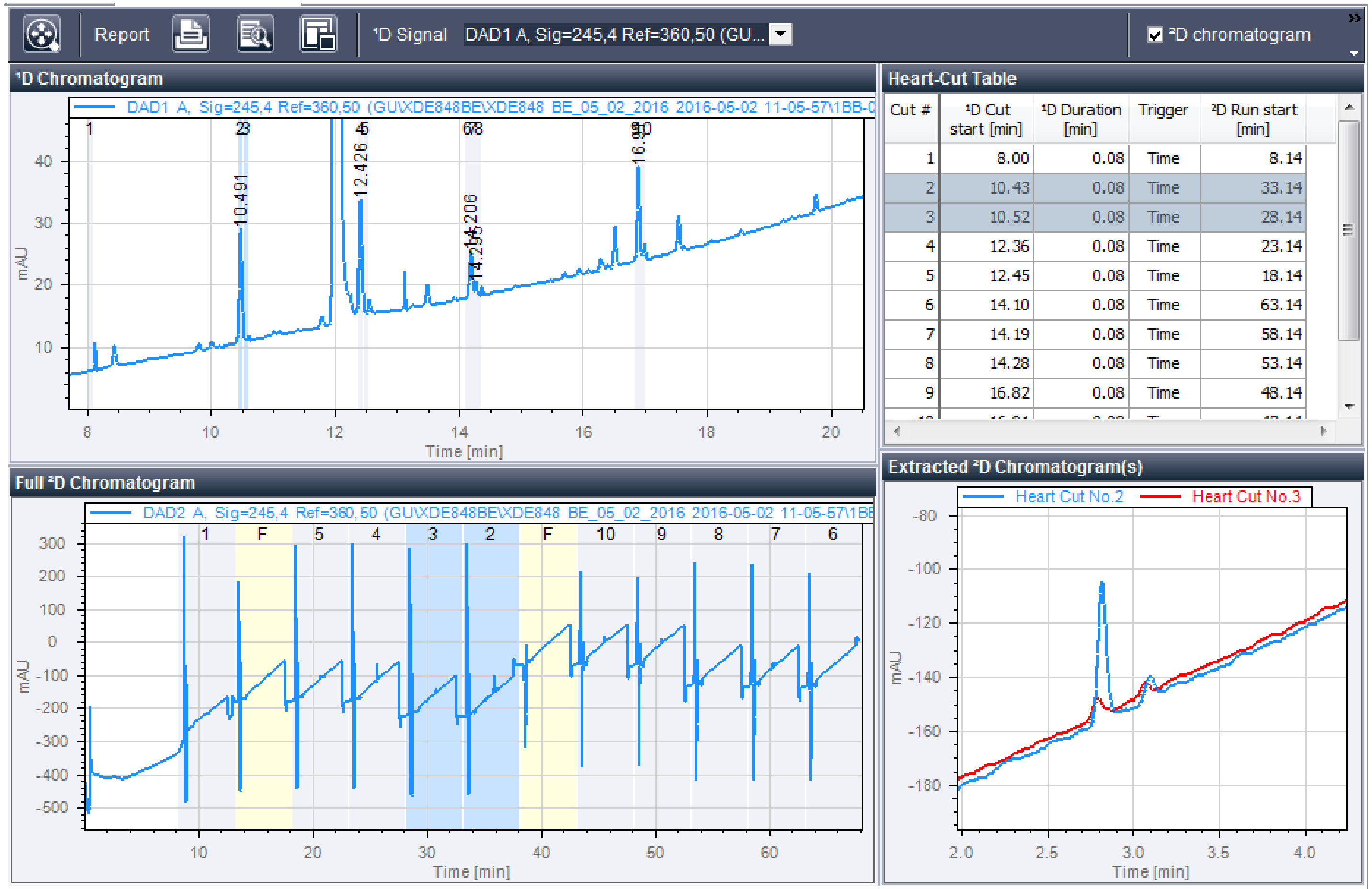
Poroshell 120 PhenylHexyl, 2.7 µm, 2.1x150mm
0.5 mL/min; 1 µL injection ; 35 ^{o}C column temp.; 245 nm;
Gradient: A: 0.1% formic acid/water B: 0.1% formic acid/acetonitrile
0-1 min 30% B, 1-20 min 30-100% B, 20.1-25 min 30% B

Expanded View



Co-elutions: 10/11; 15/17; 23/20;

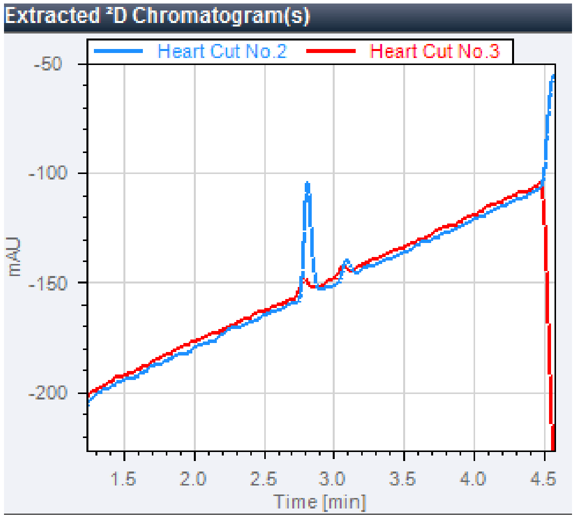
2-D MHC with Shifted Gradient

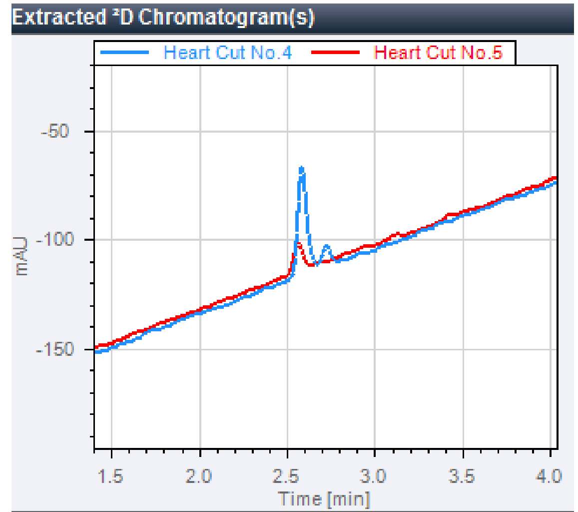


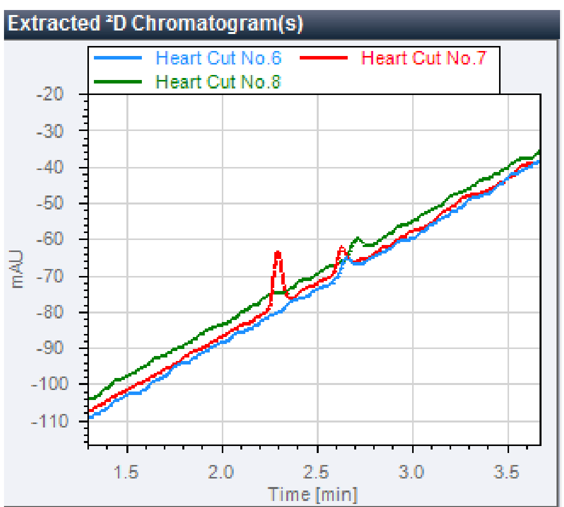
2D gradient:
0 min 20% B
(start % to 60%
over 20 min)
4 min 30% B
(final % to 70%
over 20 min)

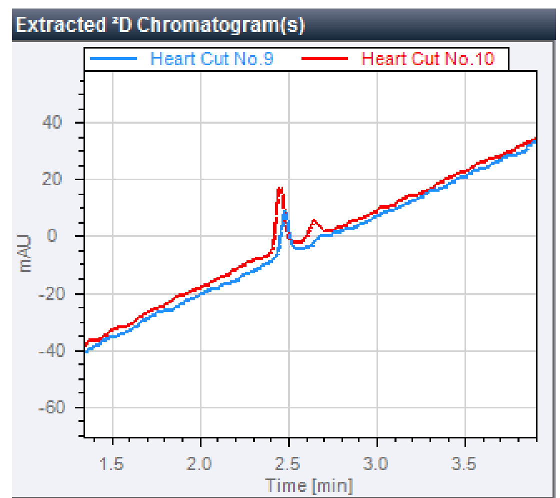
1st dimension: Agilent Poroshell 120 Phenylhexyl, 2.7 µm, 2.1x150 mm; 0.5 mL/min; 20 min from 30-100% ACN containing 0.1% FA;
2nd dimension: Agilent Poroshell 120 EC-C18, 2.7 µm, 4.6x50 mm; 0.5 mL/min; 4 min 20-30% THF containing 0.1% FA; cycle time, 5min.

Views of Each Cut









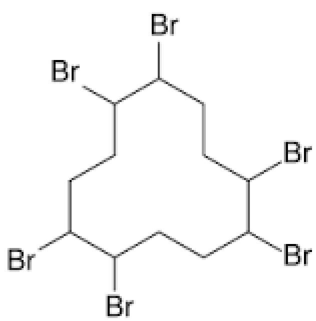
Reproducibility of Peak Areas

Component	Average Area	R.S.D. (%)
A (cut 5 only)	66.3	24
A (cut 4 only)	184	13
A (sum)	250	4.5
B*	39.0	11
C	216	2.1
D	56.9	7.4
E	134	2.8
F*	26.6	19

*S/N for peaks B and F are <10. S/N of >10 for the remainder of the peaks.

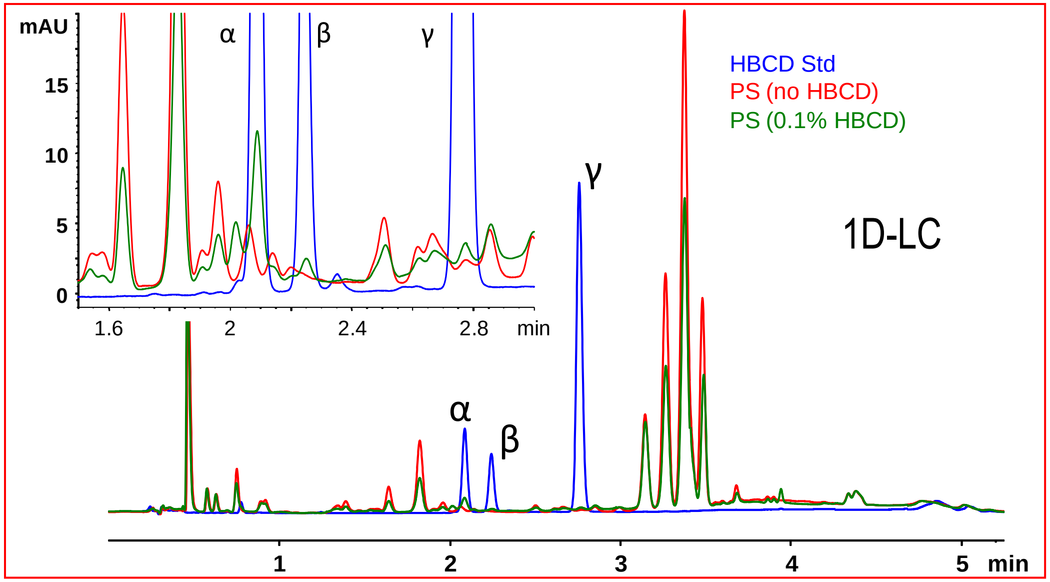
HBCD analysis in Polystyrene

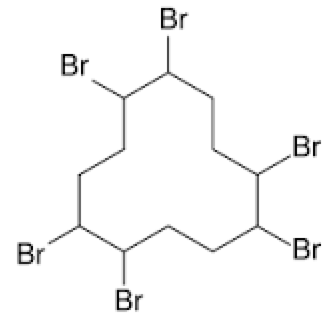
- Many polymers contain additives for improved UV-stability, flexibility and/or flame retardant properties
- In Polystyrene (PS) foam brominated compounds such as hexabromocyclododecane (HBCD) are used as flame retardant
- Need to measure HBCD flame retardant levels in PS foam at low level (0.1% and lower)
- Sensitive and selective method is needed



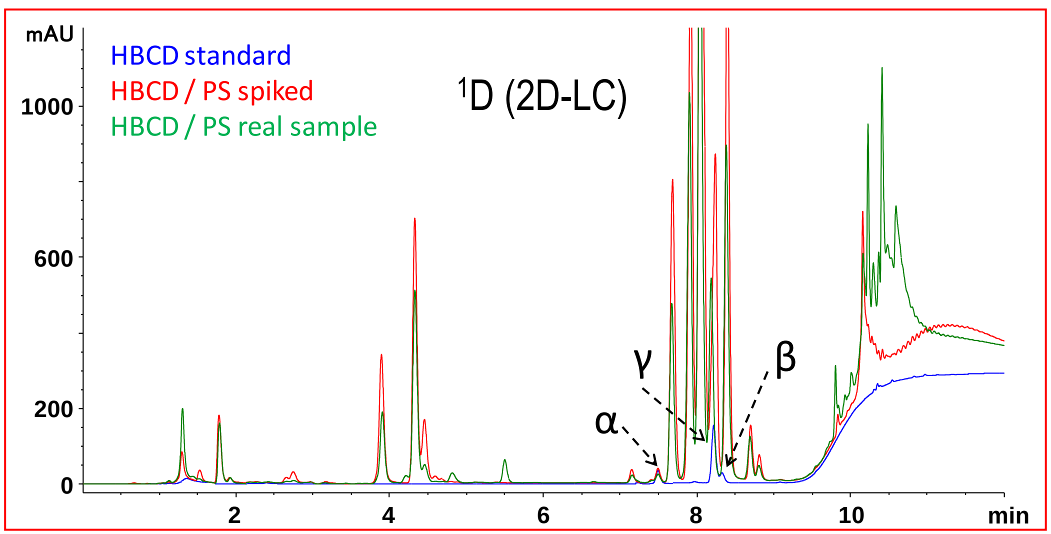
Hexabromocyclododecane (HBCD) –
exists in several isomeric forms

Multiple heart-cutting 2D LC



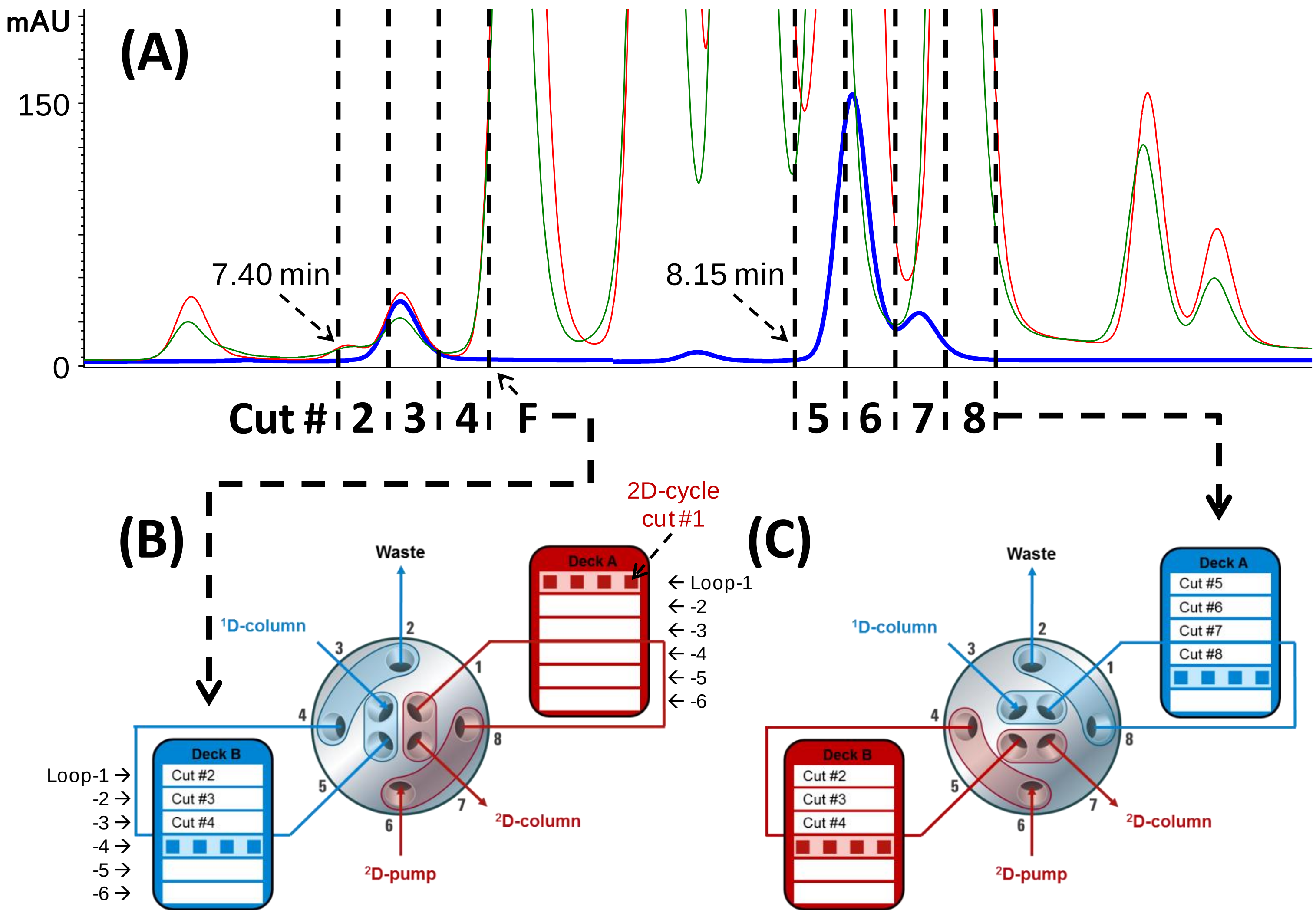


Hexabromocyclododecane (HBCD)



Changing from C18 (top) to phenyl (bottom) column in 1D provides a significant change in separation selectivity

Workflow



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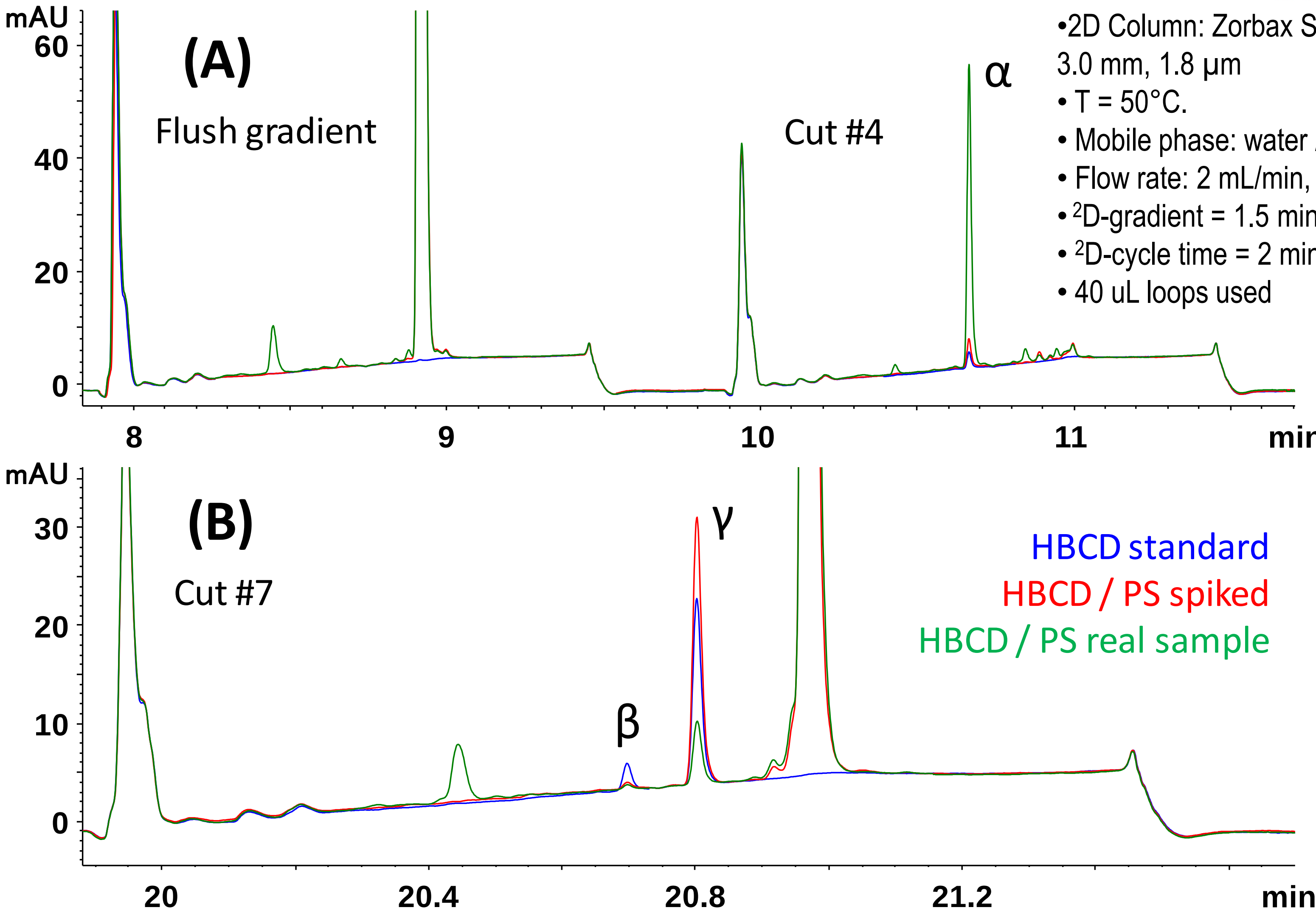
2D MHC chromatograms

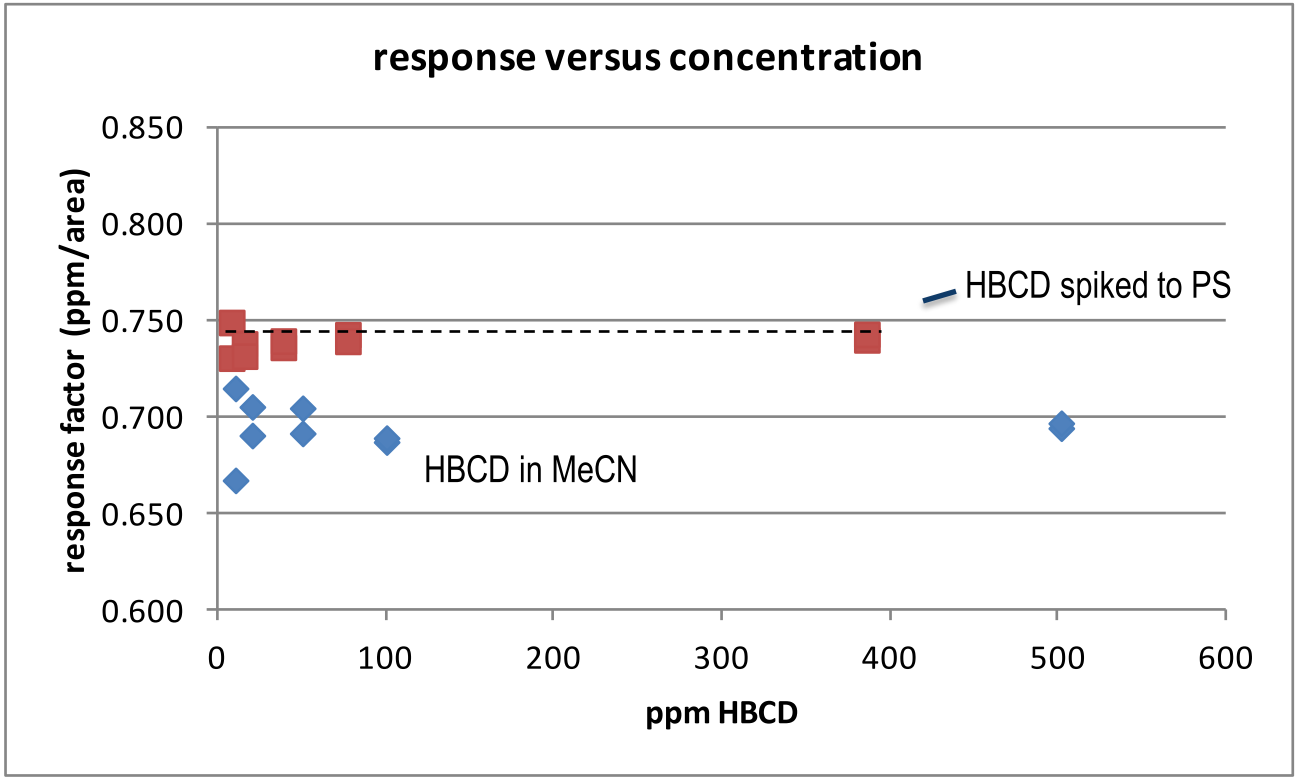


- 2D Column: Zorbax SB C_{18} 50 x 3.0 mm, 1.8 µm
- T = 50°C.
- Mobile phase: water / ACN
- Flow rate: 2 mL/min,
- ^{2}D-gradient = 1.5 min
- ^{2}D-cycle time = 2 min
- 40 uL loops used

- Target analytes are fully separated from polymer components

Quantitation via 2D LC

- very good linearity for additive in MeCN and additive spiked to PS (response factors plotted against concentration)
- difference between standard and spiked polymer due to loss of additive in polymer upon precipitation



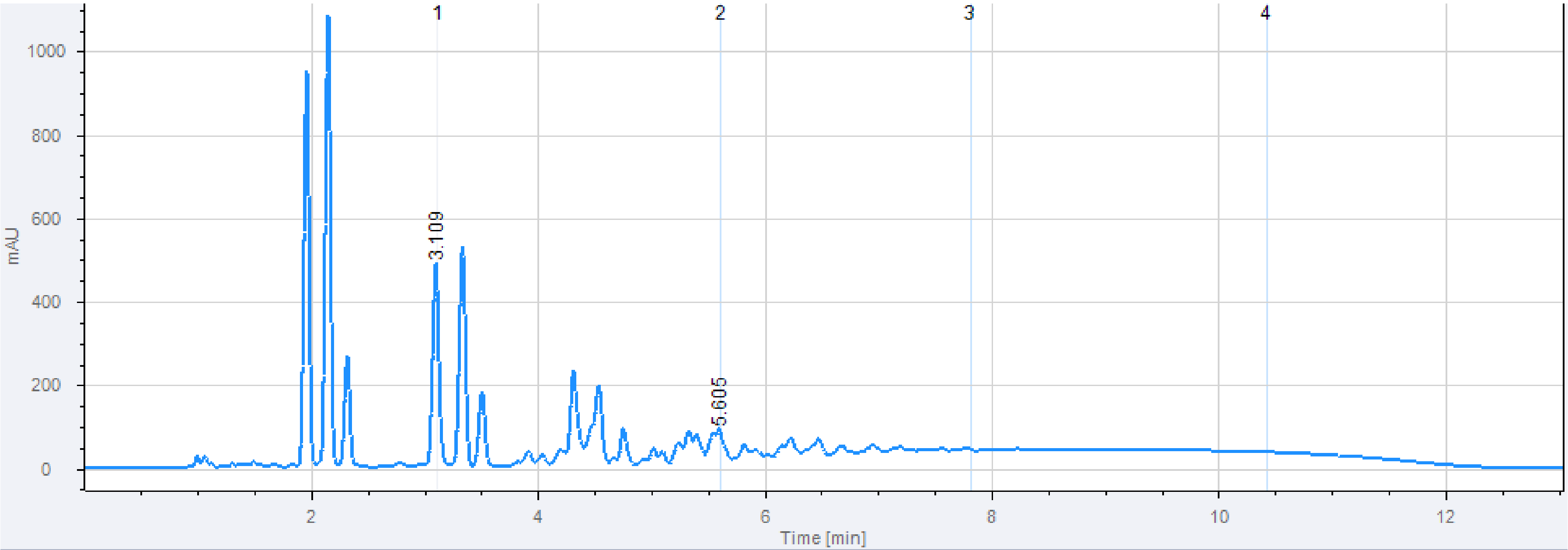
Repeatability

	Peak Area												
	HBCD isomer α				HBCD isomer ß			HBCD isomer γ				Total HBCD
Run #	Cut #2	Cut #3	Cut #4	Total	Cut #7	Cut #8	Total	Cut #5	Cut #6	Cut #7	Total	
1	21.90	46.30	1.20	69.40	0.75	10.00	10.75	1.20	1.60	5.90	8.70	88.85
2	20.00	48.10	1.40	69.50	0.78	10.20	10.98	1.20	1.70	6.10	9.00	89.48
3	16.90	51.00	1.60	69.50	0.53	10.10	10.63	1.10	1.30	6.40	8.80	88.93
4	16.60	51.30	1.60	69.50	0.46	10.00	10.46	1.20	1.00	6.40	8.60	88.56
5	16.30	51.60	1.60	69.50	0.34	10.10	10.44	1.40	1.20	6.20	8.80	88.74
6	12.60	54.90	1.80	69.30	0.37	10.00	10.37	1.40	1.10	6.50	9.00	88.3
7	18.10	49.90	1.50	69.50	0.48	10.10	10.58	1.20	1.40	6.10	8.70	88.78
8	11.20	56.10	2.10	69.40	0.33	9.80	10.13	1.50	0.74	6.60	8.84	88.04
9	21.50	46.90	1.40	69.80	0.70	10.20	10.90	1.20	1.60	6.10	8.90	89.6
10	28.20	40.70	1.10	70.00	1.30	9.90	11.20	1.10	2.30	5.50	8.90	90.1
Average	18.33	49.68	1.53	69.54	0.60	10.04	10.64	1.25	1.39	6.18	8.82	**88.94**
StdDev	4.90	4.46	0.29	0.21	0.30	0.13	0.32	0.14	0.44	0.32	0.13	**0.62**
RSD	26.72	8.97	18.75	**0.30**	48.96	1.26	**3.00**	10.83	31.32	5.22	**1.49**	**0.70**

MHC 2D LC of oligomers/polymers

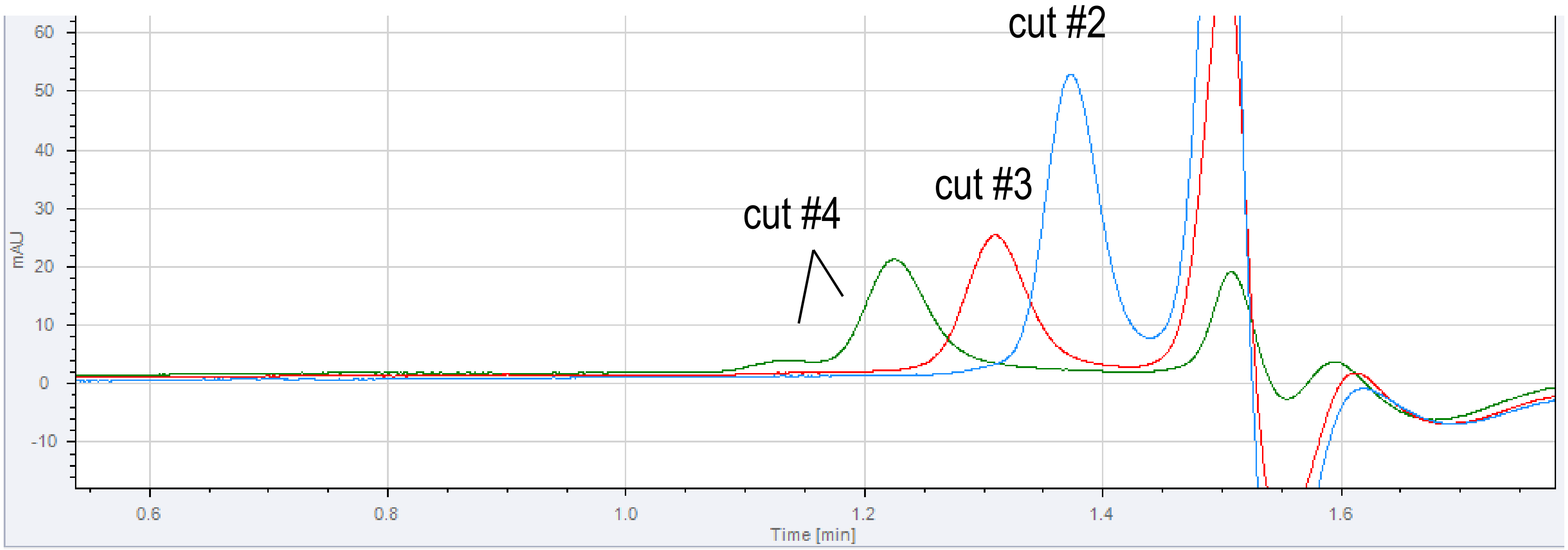
- Correlation of polarity with molecular weight (hydrodynamic volume)
- Epoxy resin used as example
- D1: Reversed phase separation – Core-shell C18 (MeCN/water) gradient
- D2: Size Exclusion Chromatography – THF

MHC 2D LC of oligomers/polymers



- D1: Oligomer and isomer separation according to differences in polarity & molecular weight
- Initial peaks are well resolved
- Resolution deteriorates toward the end of the separation
- Cuts are made at several areas of the 1D separation

MHC 2D LC of oligomers/polymers



- D2: SEC analysis
- Information on molecular weight can be obtained (calibration with standards or sample of known composition)
- Two species of different molecular weight are seen in cut #4 (two components of similar polarity but different chemical structure)
- MHC 2D LC provides additional structure information

Summary

- 2D LC is a valuable technique for challenging separation problems, including qualitative and quantitative target analysis
- Allows to differentiate complex samples in a way which would not be possible with 1D methods
- Depending on the problem either full comprehensive mode or heart-cutting/selective comprehensive modes can be applied
- Heart-cutting methods reduce dependence of 2D on 1D and simplify method development. Crucial parameters include:
  - To reduce peak broadening in the 2D:
    - 1D column should be smaller I.D., less retentive
    - 2D column should be larger I.D., more retentive
  - Transfer volume, measured to be 19 μL
  - Cuts from the same peak need to be sent to the same deck
  - Shifted gradient is an asset, but the appearance of the shift is misleading in the method editor
- Using accurate mass MS detection, thorough structural analysis can be performed

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and C&EN Webinar September 30, 2015 “Applications of 2D-LC and 2D-LC/MS for Polymer and Crop Protection Chemical Analysis”