

CCC: An Orthogonal and Complementary Separation Technique



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HPCCC – High Performance CounterCurrent Chromatography

- Characteristics of preparative scale liquid-liquid partition chromatography

- Gentle
- Irreversible binding never occurs – high recovery
- High capacity
- A 'new' column for every experiment
- Linear, volumetric scale up – mg to kg

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HPCCC - High Performance = High Resolution

- Essentially a Planetary Coil Centrifuge - High g force – retains liquid stationary phase
- Instrument replaces conventional column in LC system

The ultimate objective: **RESOLUTION**



$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{k + 1}$$

Efficiency Selectivity
Retention

CCC = **Low** plate EFFICIENCY.....
BUT
High, tuneable SELECTIVITY
High ratio SP/MP
∴ **High** RETENTION (CAPACITY)
A HIGH RESOLUTION TECHNIQUE

Stationary & Mobile Phase Characteristics of HPLC & HPCCC

SP MP Phase Combination	Mode	1° Interaction	2° & 3° Interactions
Solid-Liquid Chromatography	NP	Hydrophilic	Ionic, SEC
	RP	Hydrophobic	Ionic, SEC
Liquid-Liquid Chromatography	NP	Solvent-Solute	None
	RP	Solvent-Solute	None

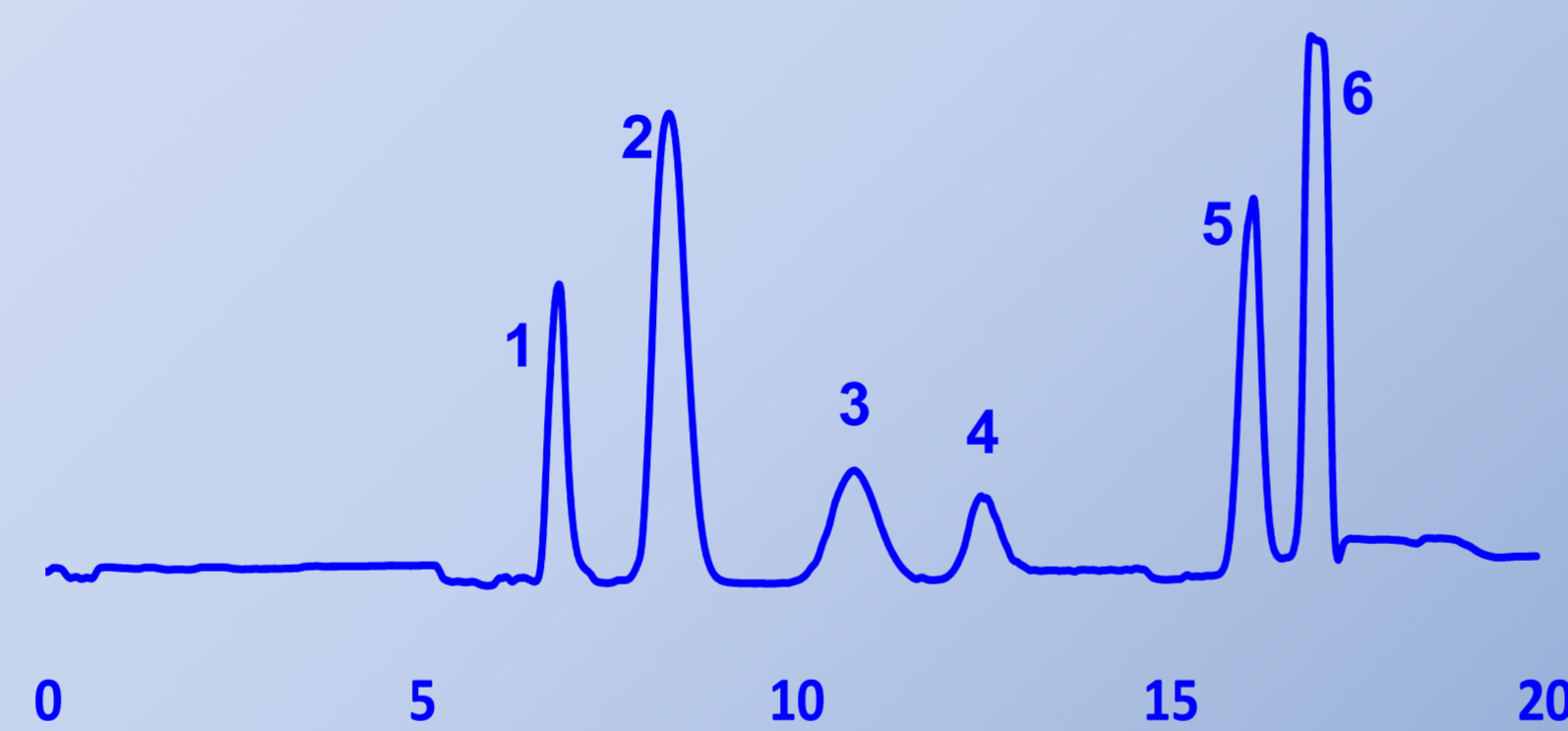
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Orthogonality to RP-HPLC

Test Mix 1*

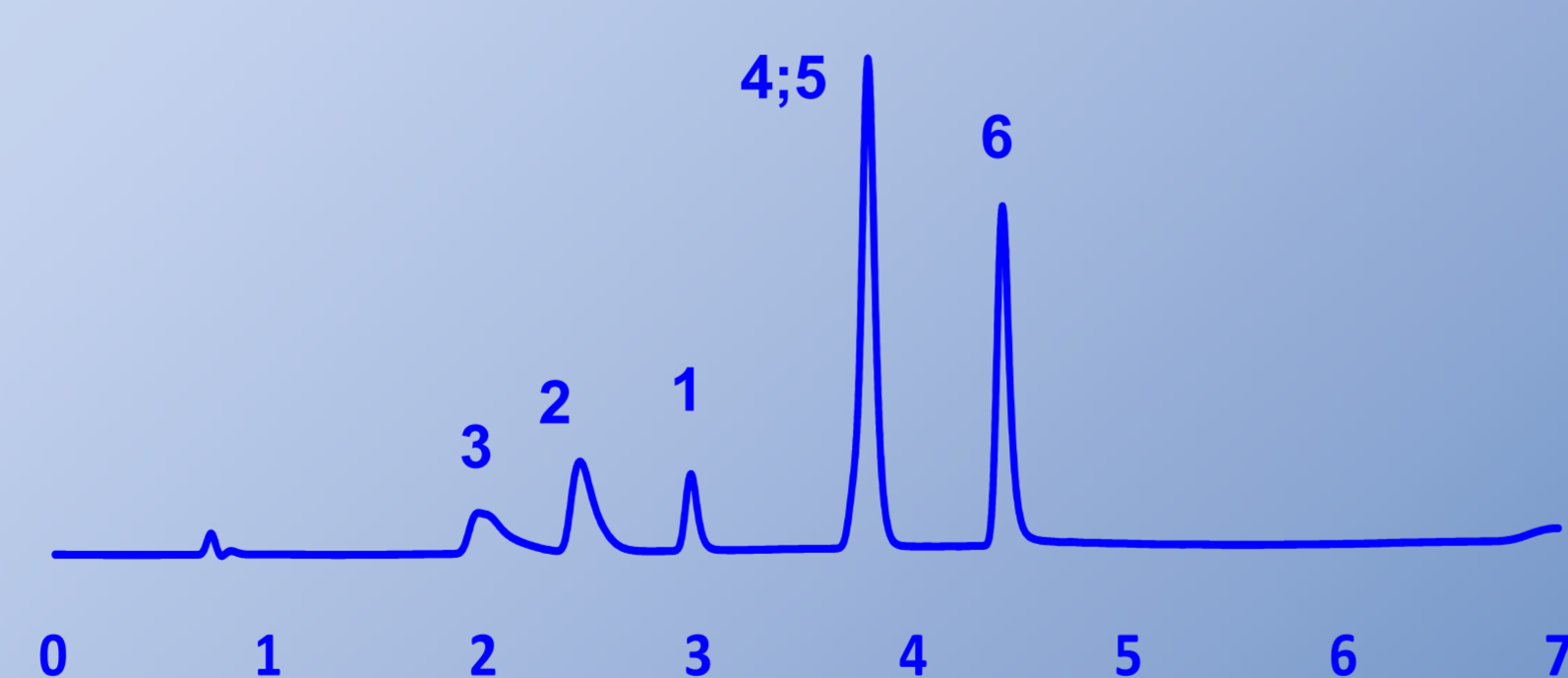
HPCCC

- Reversed Phase Elution
- Stationary Phase (SP): = Upper Phase (UP) of Hexane/EtOAc/MeOH/H₂O/0.1%TFA :: 1:1:1:1 v/v
- Mobile Phase (MP): Lower Phase (LP)
- Isocratic



HPLC

- Reversed Phase Elution
- SP = ODS/Silica
- MP = MeOH/H₂O/0.1%TFA
- Gradient



Test Mix 1

- | | |
|------------------------------------|---------------------------------------|
| 1: Dipyridamole | 4: Warfarin |
| 2: 4-Bromobenzamide | 5: Methyl 4-acetamido-5-bromobenzoate |
| 3: Methyl 4-amino-3-methylbenzoate | 6: Biphenyl |

*All chromatograms recorded at 254nm

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HPCCC - Tuning Selectivity 1a - HEMWat

- Most easily achieved by use of a well characterised system e.g. The HEMWat Solvent series: Mixtures of Hexane(s)/Ethyl Acetate/MeOH/Water in fixed proportions

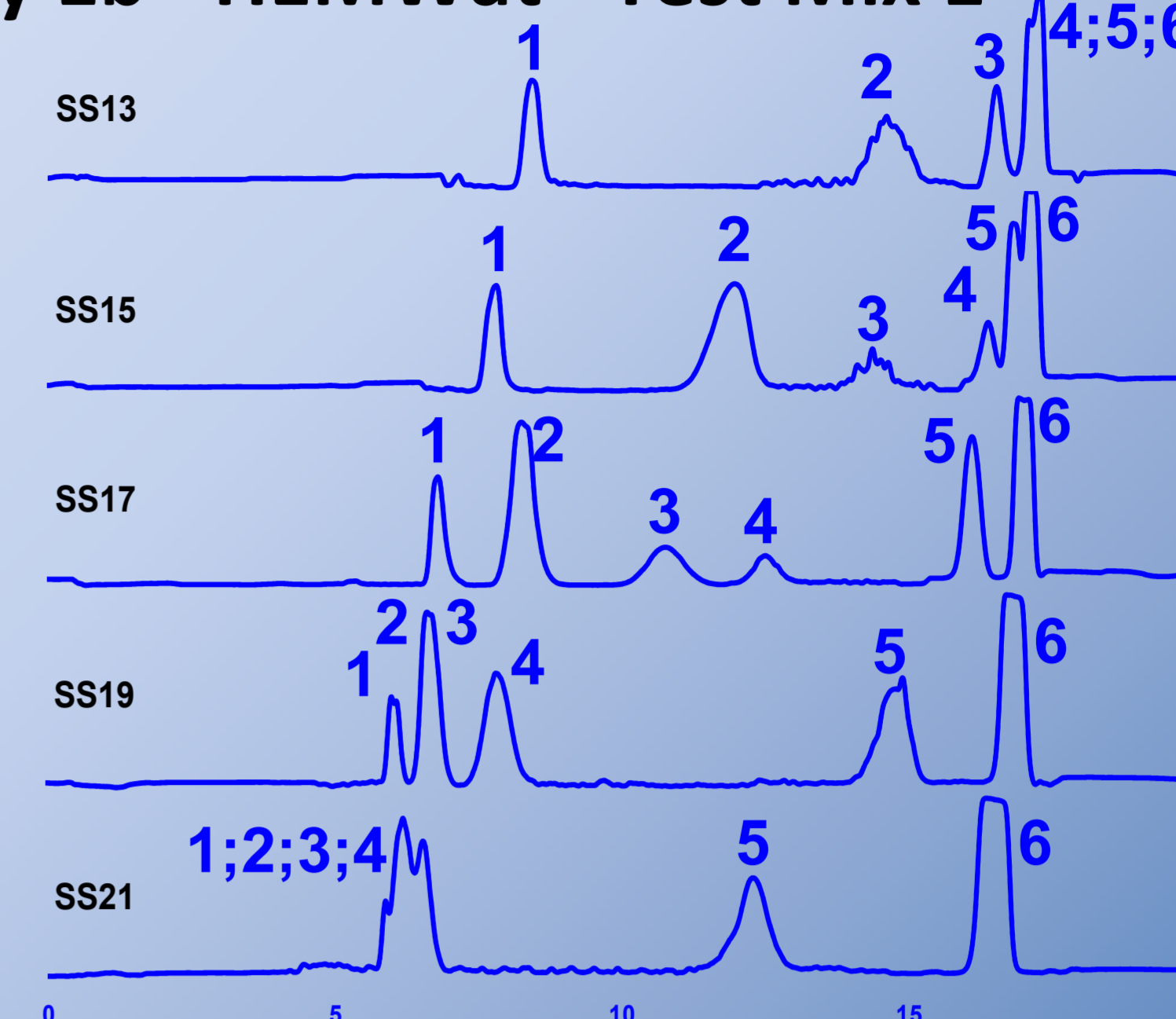
Number	6	7	n	13	14	15	16	17	18	19	20	21	n	27	28
Hexane	0	1		2	1	2	5	1	6	3	2	5		19	1
EtOAc	1	19		5	2	3	6	1	5	2	1	2		1	0
MeOH	0	1		2	1	2	5	1	6	3	2	5		19	1
Water	1	19		5	2	3	6	1	5	2	1	2		1	0

Decreasing Polarity →

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HPCCC - Tuning Selectivity 1b - HEMWat - Test Mix 1

- Reversed Phase Elution
 - MP = LP, SP = UP
- SS 13, 15, 17, 19, 21; All with 0.1%TFA
- Automated screening with 'on demand', four solvent mixing
- 12 min 'classical' elution, 8 min extrusion*



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HPCCC - *Extrusion

If the components of a mixture appear one by one from the column outlet, they must have been separated one from another whilst they were still within the column.

Uniquely, for a fixed column and when both phases (SP and MP) are liquids, the column contents can be 'extruded' whilst maintaining the resolution of these separated components.

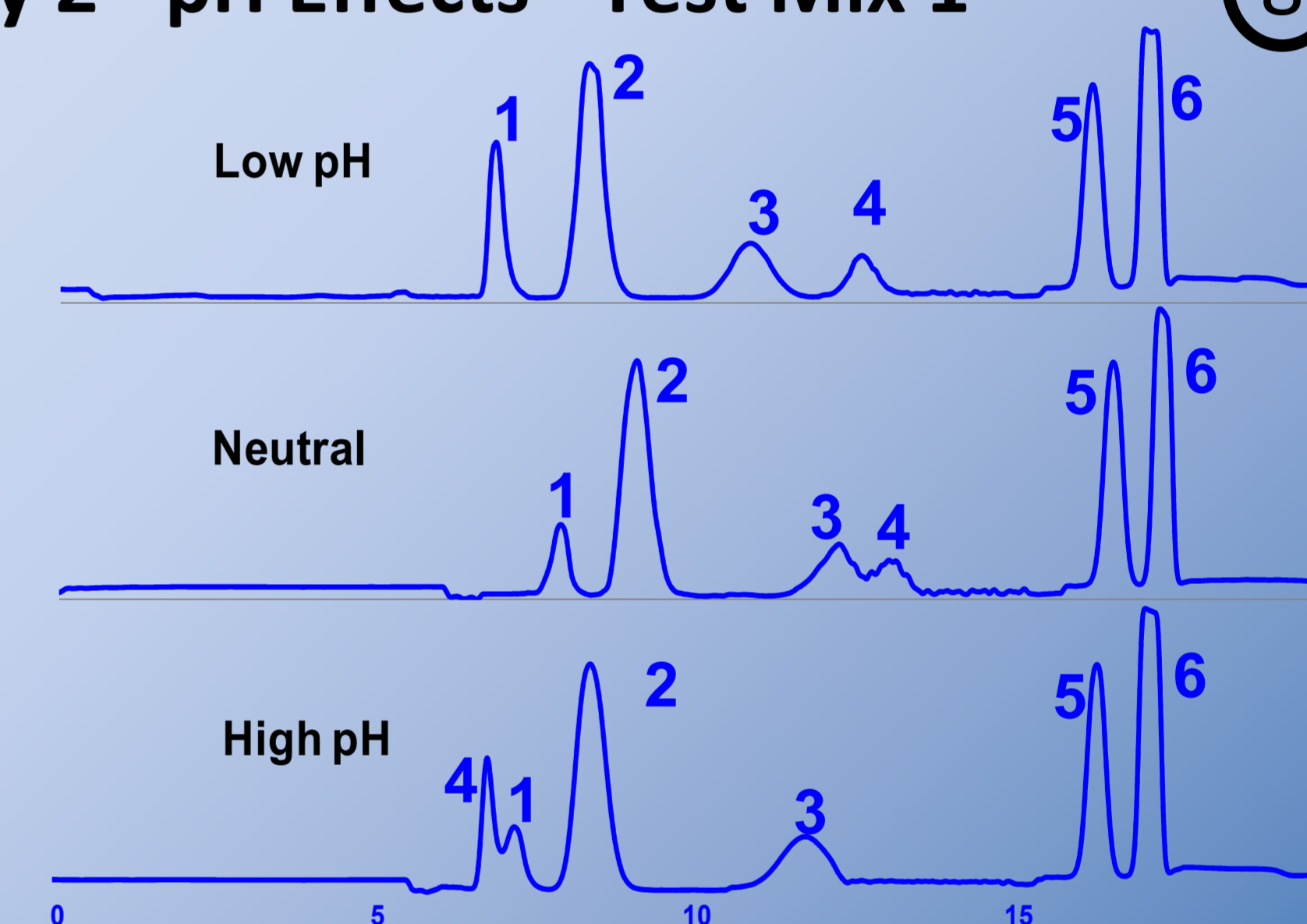
This process advantageously shortens the experimental run time.

In all of the chromatograms shown, a period of elution was followed by extrusion and resolution of the last-eluting components has been demonstrably maintained.

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HPCCC - Tuning Selectivity 2 - pH Effects - Test Mix 1

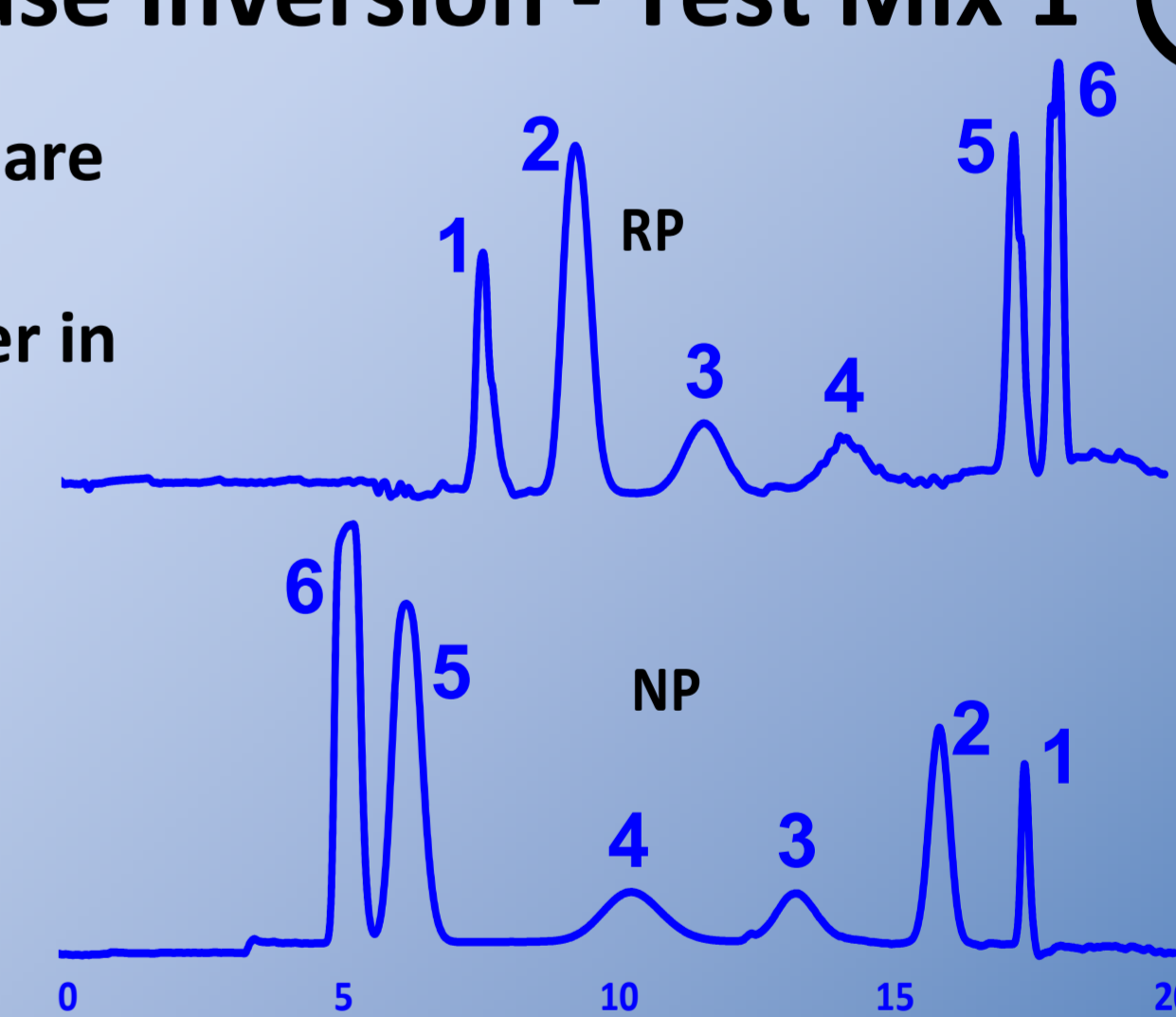
- Reversed Phase Elution (RP)
 - MP = LP, SP = UP
- SS 17; 0.1%TFA, no additive, 1% NH₄OH
- Automated screening with 'on demand', four solvent mixing



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HPCCC - Tuning Selectivity 3 - Phase Inversion - Test Mix 1

- Column contains two liquid phases, so roles are easily changed by changing flow direction:
 - In NP elution, more polar phase (lower in HEMWat) is SP
 - In RP elution, less polar phase (upper in HEMWat) is SP
- SS 17, 0.1%TFA
- Automated screening with 'on demand', four solvent mixing



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Conclusions

CCC

For many years, CCC has remained in the separation science 'doldrums' and has been viewed as a method of last resort useful only for really difficult or high value separations such as those of traditional Chinese medicines (TCM). The principal reasons for this view have been

- The technique has low chromatographic efficiency
- Method development has been a time consuming process: an unavoidable consequence of
- Long experimental run times - many hours or even days

HPCCC

- Like all liquid-liquid partition techniques, exhibits low plate efficiency
- Low efficiency is more than offset by highly tuneable selectivity which produces high resolution
- Automated, four solvent, 'on demand' mixing allows rapid method development
- Run times are measured in tens of minutes - comparable to other high resolution techniques
- The technique is eminently suitable for 'small' molecule separations
- Is the fastest of the CCC instruments of the current era

HPCCC is a complementary, orthogonal, chromatographic technique which worthy of a place in the toolbox of any chromatographer

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