Normal and Reversed Phase Elution in HPCCC

In solid-liquid chromatographic systems, the roles of stationary phase (SP) and mobile phase (MP) are immutable. Since countercurrent chromatography (CCC) utilises liquids for both SP and MP, either phase can assume the role of SP or that of MP: elution can be performed in either normal phase (NP) or reversed phase (RP)

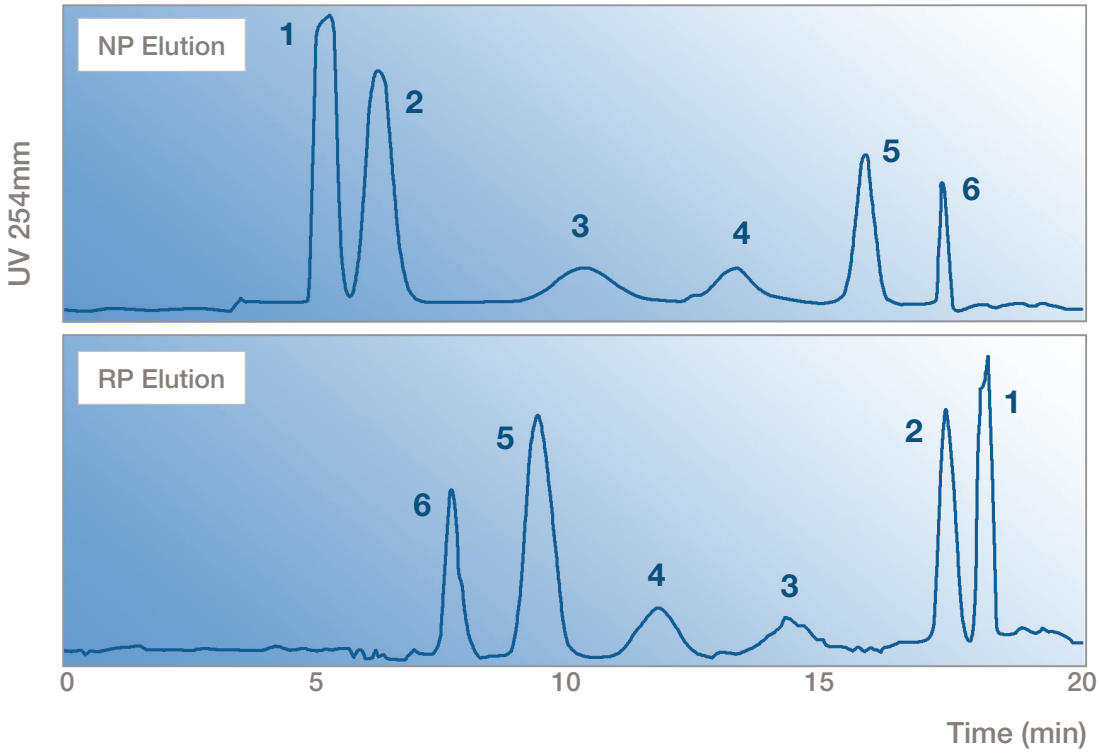
Normal Phase Elution – The stationary phase (SP) is the more hydrophilic (usually aqueous) phase and mobile phase (MP) is the more lipophilic (usually organic) phase,

Reverse Phase Elution – SP is the more lipophilic phase, MP is the more hydrophilic phase

The chromatograms below show how elution order of components in a test mixture is reversed as the elution is changed from NP to RP. Switching from NP to RP can very dramatically change the elution time for a particular component and thereby influence both the 'run' time and the solvent consumption of an experiment to isolate given components. This elution order reversal on switching from NP to RP in HPCCC is invariable.

Normal and Reverse Phase separation of a mixture of synthetic intermediate-type and pharmaceutical compounds

The chromatograms show the NP and RP phase separations of the mixture when using the biphasic solvent system comprised of Hexane/Ethyl Acetate/Methanol/Water (1:1:1:1 v/v/v/v)



Mixture Components	Elution Order
Biphenyl	1
Methyl 2-acetamido-5-bromobenzoate	2
Warfarin	3
Methyl 4-amino-3-methylbenzoate	4
4-Bromobenzamide	5
Dipyridamole	6

Experimental Conditions

Column:	Dynamic Extractions Mini HPCCC (19ml)
Solvent System:	Hexane/Ethyl Acetate/Methanol/Water (1:1:1:1, v/v/v/v)
pH modifier:	0.1% TFA
Load:	2mg/25µl DMSO
Flow Rate:	2ml/min elution, 4ml/min extrusion
Run Mode:	Isocratic, elution-extrusion: 12min elution, 8min extrusion

NB If components eluting similarly to 5 and, or, 6 were to be isolated from such a mixture, the run time would be significantly shorter in RP elution mode since following complete elution of component 5, the remaining components could be extruded to waste.

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Dynamic extractions