

Multi-Modal, Preparative Chiral Purifications Using Macrocyclic Glycopeptide Phases

J. T. Lee, T. E. Beesley,
Advanced Separation Technologies Inc. (Astec)
37 Leslie Court, P. O. Box 297, Whippany, NJ 07981
Tel: 973.428.9080 Fax: 973.428.0152
E-mail: astecusa@aol.com

Abstract

Macrocyclic glycopeptides have demonstrated excellent selectivity in a broad range of solvent combinations represented by three basic mobile phase types. Reversed phase has yielded the highest number of applications to date. The new polar organic mode, composed of 100 parts methanol with 0.01 to 1.0 parts anhydrous acid and base added for selectivity has yielded the second largest number of separations. In fact, the polar organic mode has proven a useful alternative to the typical normal phase solvents. When reversed phase systems are used, which typically consists of a highly aqueous composition (85-90%), it is ideal to adsorb the eluting peaks of the enantiomers onto a C₁₈ phase for product recovery. A simple run on a 10 micron C₁₈ analytical column in the mobile phase used for the chiral column will indicate the degree to which the analyte will be retained and this data or a simple adsorption isotherm can determine the C₁₈ capacity. The experimental conditions to set up this type of system on a vancomycin bonded column will be described and a full scale preparative procedure will be outlined to demonstrate the efficient use of this methodology. Scale up for the new polar organic mode is an easy task since volatile TFA/ammonium hydroxide can be employed. This system is more volatile than hexane or heptane and less toxic. Separations in this mode are also very fast and efficient leading to very high throughput. An example will be given of this protocol.

Introduction/Objectives

- t Significance and needs of enantiomerically pure isomers:
 - $\frac{1}{4}$ Reduce side effects
 - $\frac{1}{4}$ Eliminate toxicity
 - $\frac{1}{4}$ Minimize dosage/cost
- t Chiral compounds account for 1/3 of all drug sales worldwide.
- t FDA requires both enantiomers of a racemate to be studied in detail.
- t Chromatographic resolution of large scale racemic mixtures
- t Introduce reversed phase and new polar organic phase system for preparative application using high capacity, multi-modal, chiral stationary phase.

Criteria of Solvent Selection for Prep Scale Application

1 High selectivity:

Try other organic to obtain optimum resolution.

2 High solubility:

Test sample solubility in mobile phase. Make solvent/concentration changes while maintaining desired separation.

3 Retention:

Keep retention factors between 0.5 and 5.

4 Recovery:

Use volatile solvent for quick and easy recovery.

5 Low toxicity:

Avoid toxic solvent.

6 Low cost:

Use less expensive solvent whenever possible.

Method Development Protocol

(From Analytical to Prep Scale)

1. Selectivity versus solubility:

When sample solubility is a concern, compromise between these two factors by varying the concentration of organic modifier/buffer.

Example: Warfarin (not soluble in H₂O)

EtOH/0.1% TEAA, 4.1	K ₁	Solubility (mg/mL)	Selectivity
20/80	4.94	0.1	1.50
30/70	2.19	0.3	1.60
40/60*	1.31	0.6	1.47
50/50	0.29	3.0	1.45
80/20	0.1	15	1.0

* Prep scale composition, for 50/50, K₁ too low.

2. Retention factor:

Keep both retention factors between 0.5 and 5.0 while maintaining R_s > 1.0 to obtain optimal throughput.

3. Recovery from aqueous mobile phase:

a. The degree of hydrophobicity of the analyte is the main criterion for adsorption onto a C₁₈. Ideal molecules are neutral or compounds with strong hydrophobic functionality like aromatic rings, halogens, nitro-, sulfo- and phospho- groups.

b. Determine the capacity by pumping recovered eluant through a C₁₈ column until the compound breaks through.

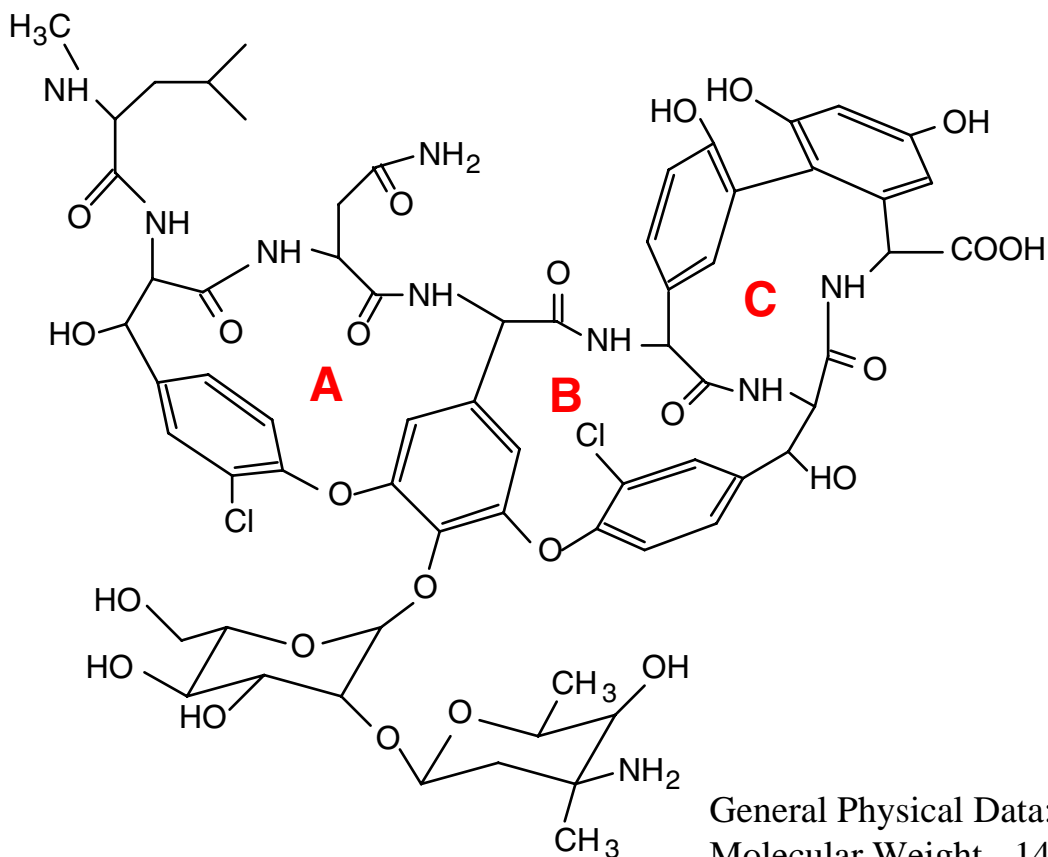
c. Addition of water or a pH adjustment can increase capacity.

d. Wash and recover pure enantiomer from the C₁₈ with a solvent with high solubility and low boiling point as for example methanol.

4. Recycle and reprocess if necessary.

Proposed Structure of the Macrocyclic Glycopeptide Vancomycin

6



1. Multi-modal capabilities
2. Great stability
3. High capacity
4. Complementary to Teicoplanin and Ristocetin A

Analytical Method (Reversed Phase)

7

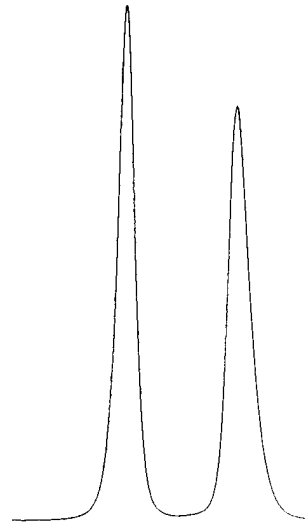
Warfarin

Racemic

Vancomycin, 250x4.6mm

$$k'_1 = 2.19$$

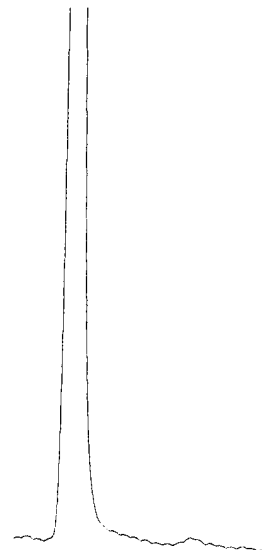
$$\alpha = 1.60$$



Recovered

Peak 1

Purity = 98.5 %



Conditions: 30/70: EtOH/0.1% TEAA, pH 4.1
UV=220nm, @ 1.0 mL/min.

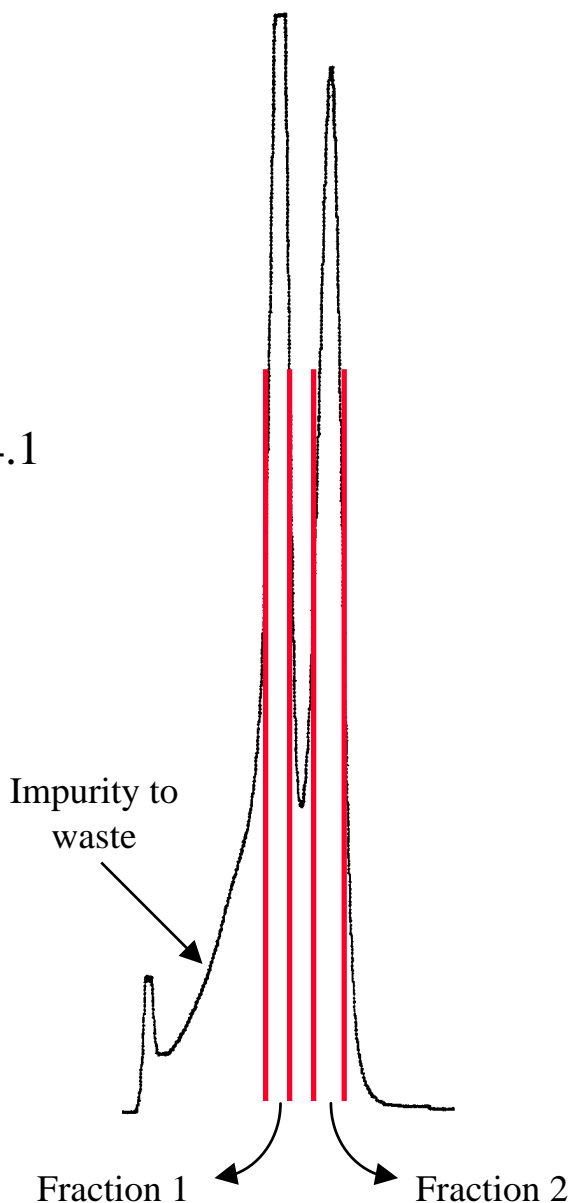
Prep Scale (Reversed Phase)

Warfarin

1. Vancomycin, 250x22.1mm
2. 15 mL/min.
3. 20 mg
4. UV-220nm
5. 40/60: EtOH/0.1% TEAA, pH 4.1

Peak 1: 7.24 min.

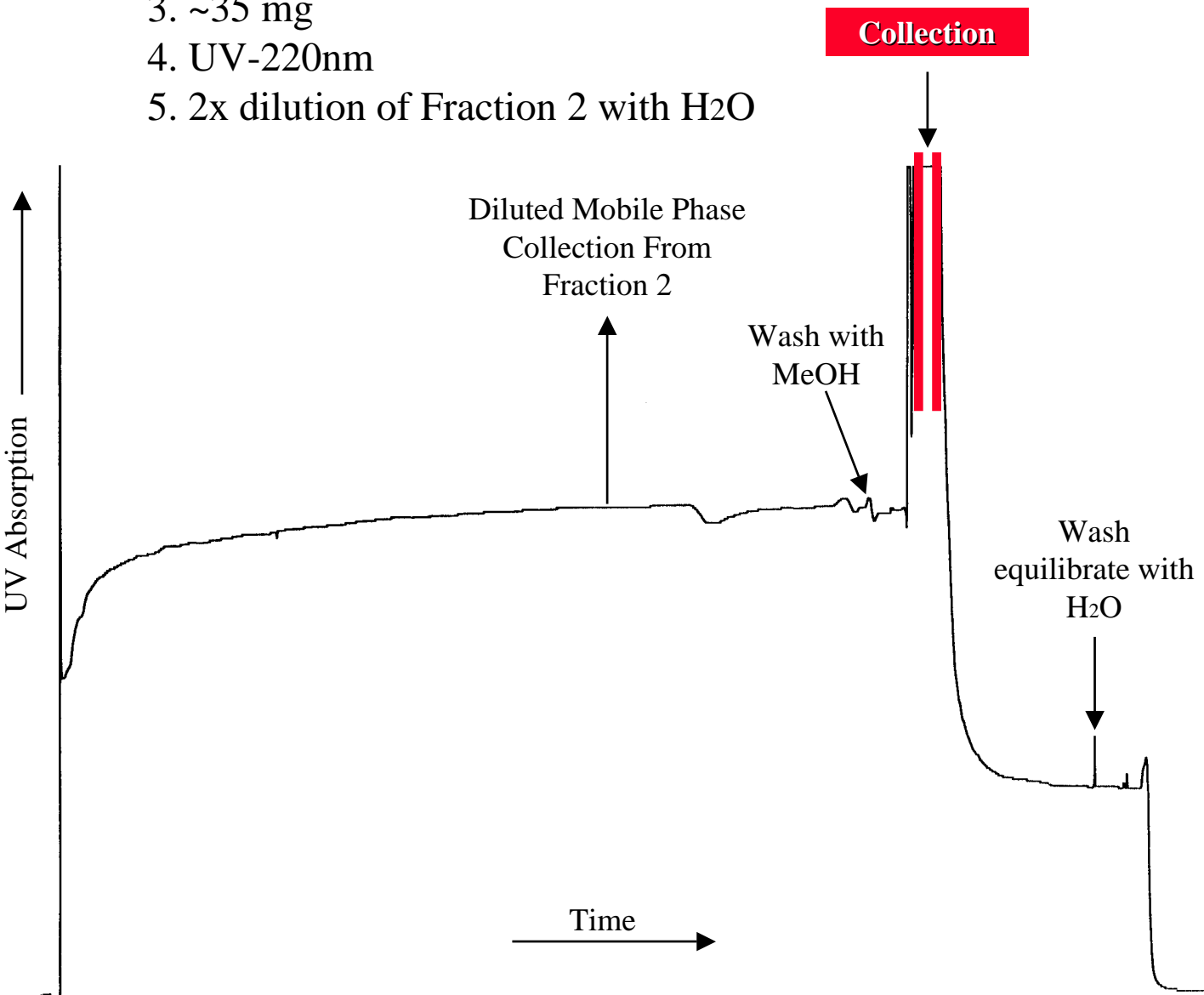
Peak 2: 8.67 min.



Recovery of Fraction 2 Collection

Warfarin

1. C18, 250x4.6mm
2. 1.5 mL/min.
3. ~35 mg
4. UV-220nm
5. 2x dilution of Fraction 2 with H₂O



New Polar Organic Mode Analytical Method

Nicardipine

Racemic

Vancomycin, 250x4.6mm

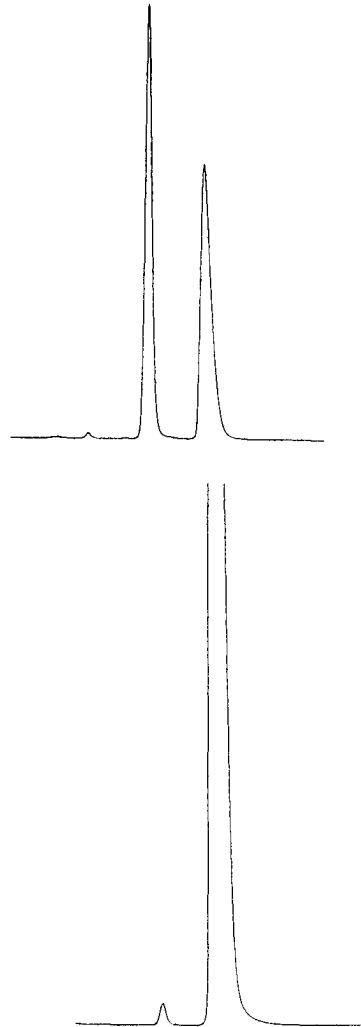
$k'_1 = 0.64$

$\alpha = 1.51$

Recovered

Peak 2

Purity = 99.5 %



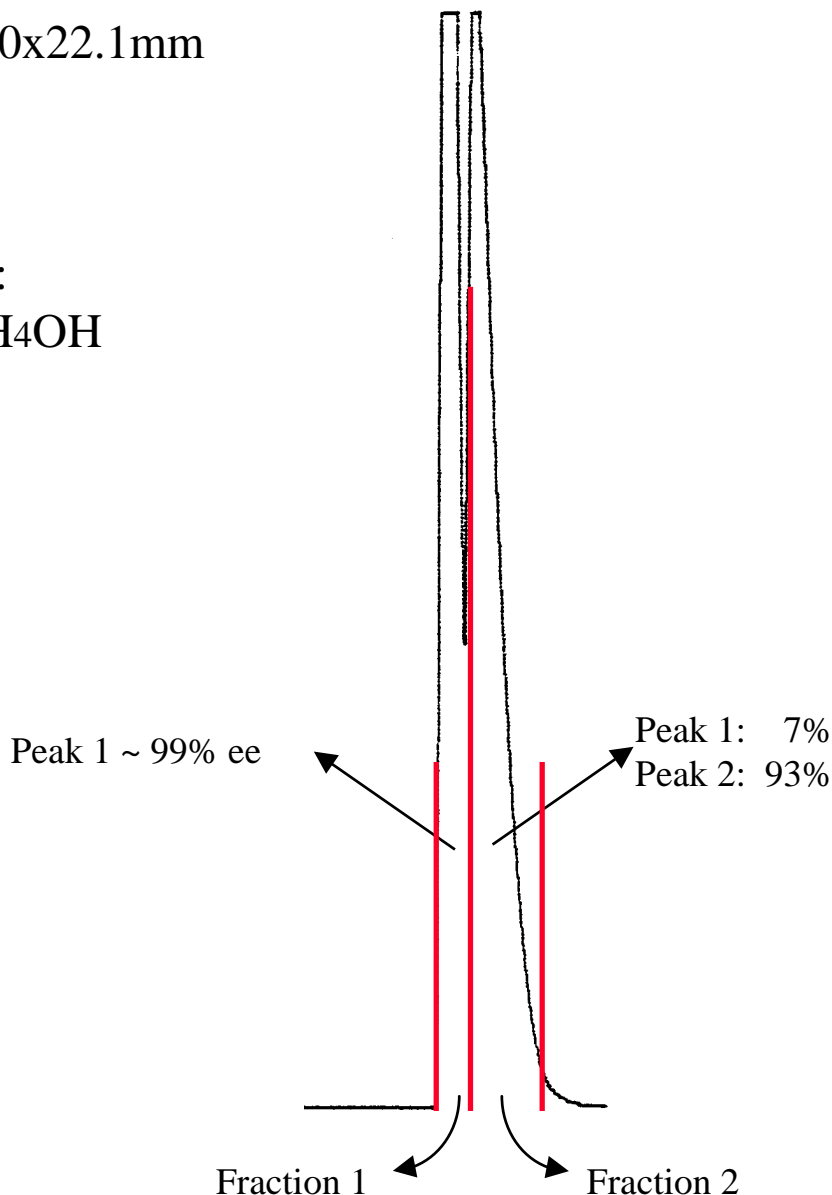
Conditions: 100/0.01/0.01: MeOH/HOAc/TEA
UV=254nm, @ 1.0 mL/min.

Prep Scale (New Polar Organic Mode)

Nicardipine

1. Vancomycin, 250x22.1mm
2. 18 mL/min.
3. 25 mg
4. UV-254nm
5. 100/0.005/0.005:
MeOH/TFA/NH₄OH

Peak 1: 4.95 min.
Peak 2: 5.24 min.

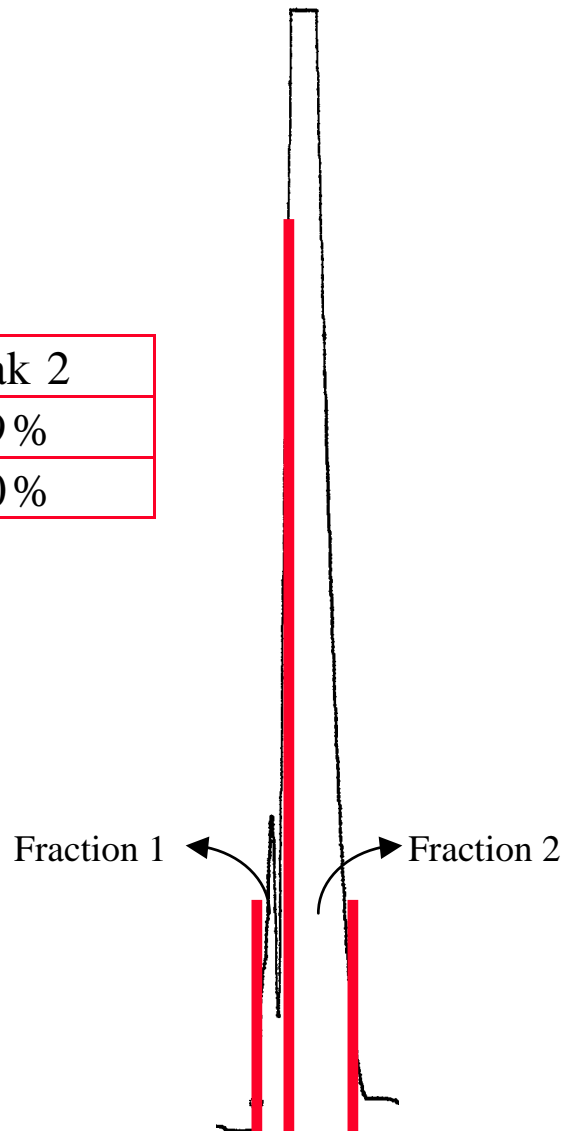


Reprocess of Fraction 2 Collection

Nicardipine

1. 250x22.1mm (Vancomycin)
2. 18 mL/min.
3. 20 mg
4. UV-254nm
5. 100/0.005/0.005:
MeOH/TFA/NH₄OH

Final Results	Peak 1	Peak 2
ee	99%	99%
Yield	95%	90%



Issues on Mass Overload

- 1 Non-linear adsorption isotherm. Retention of peak front is reduced progressively as the sample mass is increased.
- 2 Better to overload with large volume of a dilute sample than to employ a small volume containing a high concentration of sample.
- 3 If column length needs to be increased, flow rate needs to be increased or particle size is increased to reduce the efficiency to its required value.

Summary

Warfarin (Reversed Phase Mode)

1. Solubility of Peak 1 is higher than Peak 2.
2. When collecting Peak 2 from C18, skip the front end of the peak to ensure high purity. On the other hand, discard the tail of the peak when collecting Peak 1.
3. Results: Peak 1, Purity = 98.5%, Recovery = 90%
Peak 2, Purity = 99%, Recovery = 85%

Nicardipine (New Polar Organic Mode)

1. Substitute TFA/NH₄OH for HOAc/TEA when scaling up.
2. Stronger acid/base helps to suppress ion exchange effect that will lead to split peaks when overloading the column.
3. Results: Peak 1, Purity = 99.5%, Recovery = 95%
Peak 2, Purity = 99.5%, Recovery = 90%

Conclusion

- 1 Vancomycin demonstrates its unique multi-modal capability with high capacity and excellent stability.
- 2 With C₁₈ for recovery, reversed phase chiral stationary phases (CSPs) can be applied for preparative purification.
- 3 When new polar organic mode is applied, substitute TFA/NH₄OH for acid/base for easy recovery.