

Optimizing Selectivity for Preparative Separations: Mixed-Mode Chromatography versus Reversed Phase and Hydrophilic Interaction Chromatography

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METHODS

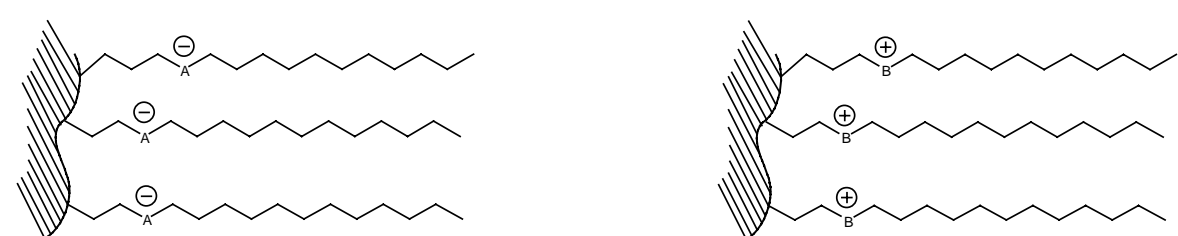
Criteria for Evaluating Preparative Methods

- Maximum separation ($\alpha > 1.2$).
- Short retention ($k' < 4$).
- High solubility in eluent.
- Elution order.
- Volatile Mobile Phase.
- Preferably isocratic conditions for separation.

Column Selection for Screening System

Category	Functional group
Reversed Phase	
Highly hydrophobic	High carbon load, C30
Moderately Hydrophobic	C18
Less Hydrophobic	C8
Alternative Selectivity	Phenyl("pi-acidic", "pi-basic"), cyano
Enhanced polar selectivity	Low surface coverage
"Aqua" columns	Hydrophilic endcapping, short alkyl chains, wide pore diameter.
Embedded polar group	Ether, amide, carbamate
High pH resistant	Inorganic-organic hybrid particles
Polar Bounded Phases	
Diol, Amino-columns	Can be operated in HILIC mode
Mixed-Mode Chromatography Columns	
Embedded basic/ acidic group	Carboxylic/amino

Mixed-Mode Primesep Columns



Optimizing the Separation Method Development for Polar Compounds

The desired

- Are highly basic ionic compounds;
- Are submitted in the form of HCl salt;
- Do not elute under normal phase conditions and show little retention under reverse phase conditions;
- Contain closely related impurities;
- Contain highly retentive reagent (~40% by weight).

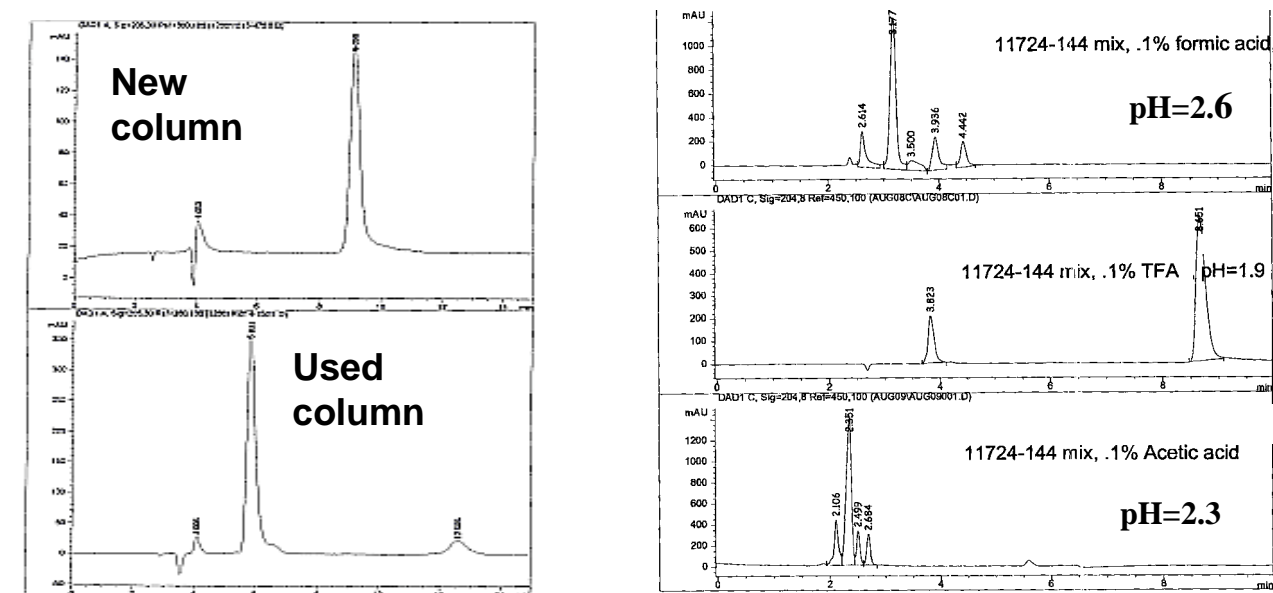
Objective for preparative purification:

- Impurities, as measured by HPLC, should not exceed 1% each;
- The yield of the desired material should be > 90%;
- Purity should be at least 98%.

ODS AQ Column

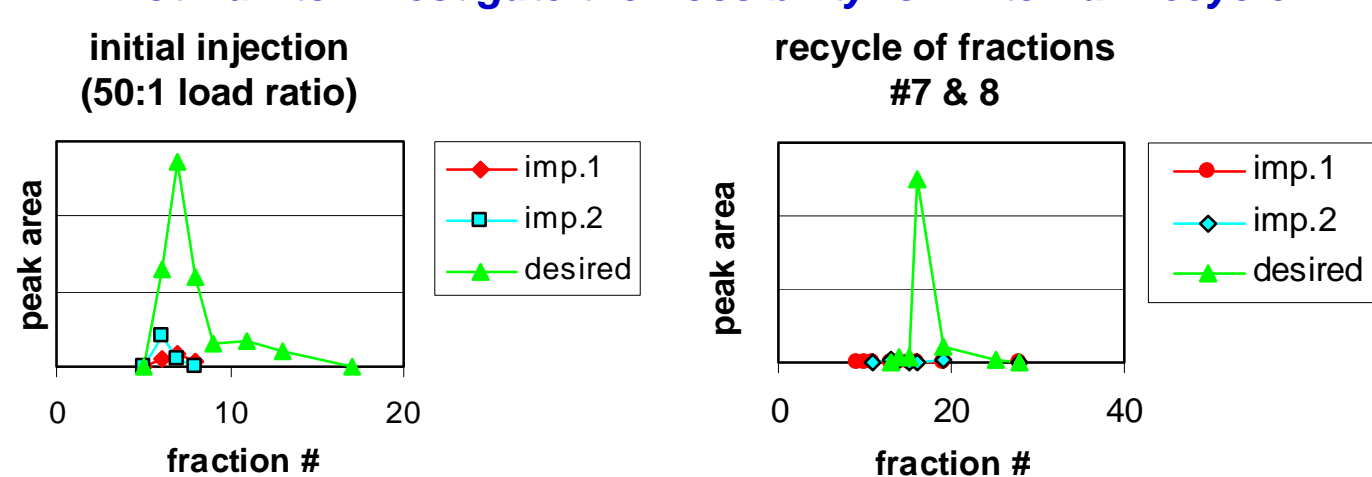
Stability under Usage at 0.1% TFA, pH = 1.9

Modifiers Comparison on YMC ODS AQ Media



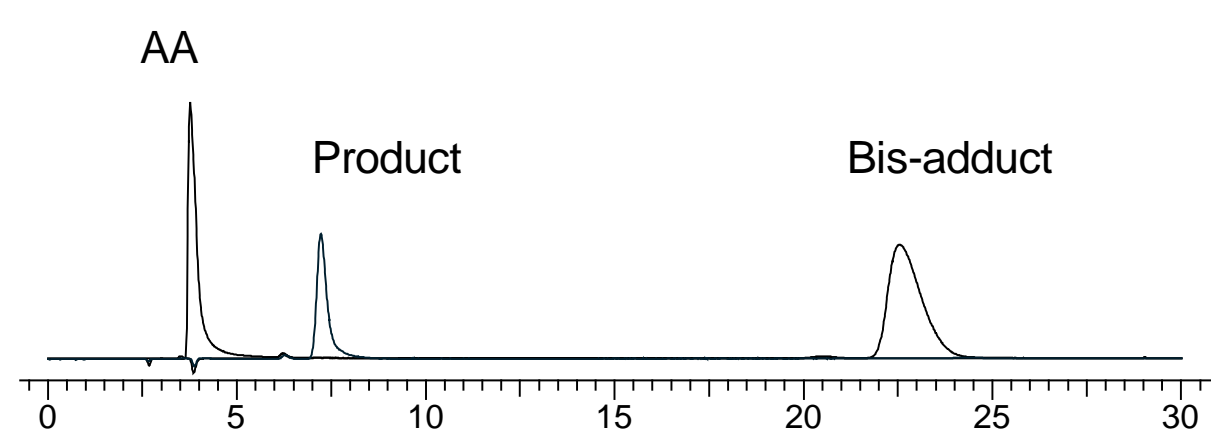
METHODS (cont'd)

Pilot Run to Investigate the Possibility for External Recycle



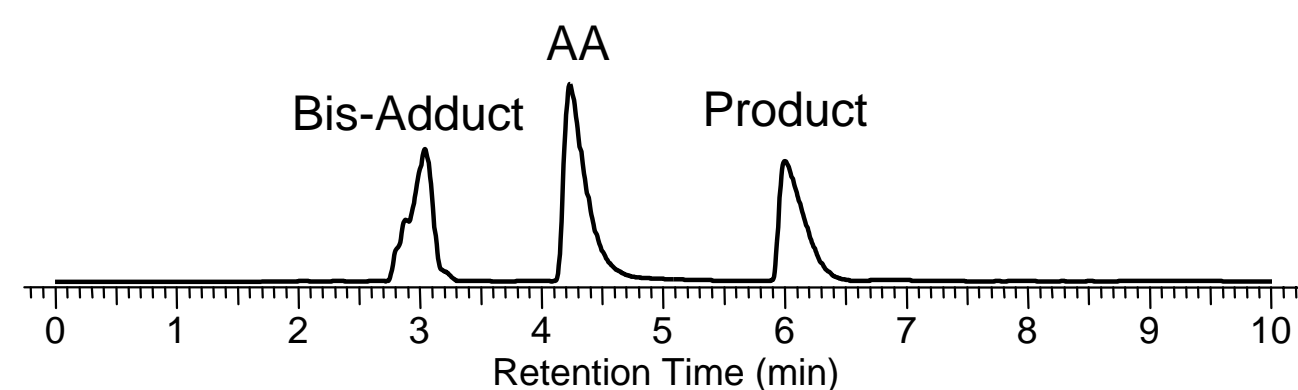
Data Comparison for Hydrophobic, HILIC and Mixed-Mode Type of Chromatography

Hydrophobic Chromatography
ODS AQ, 10 μ m, 4.6x25mm, 100/0.1 H2O/TFA



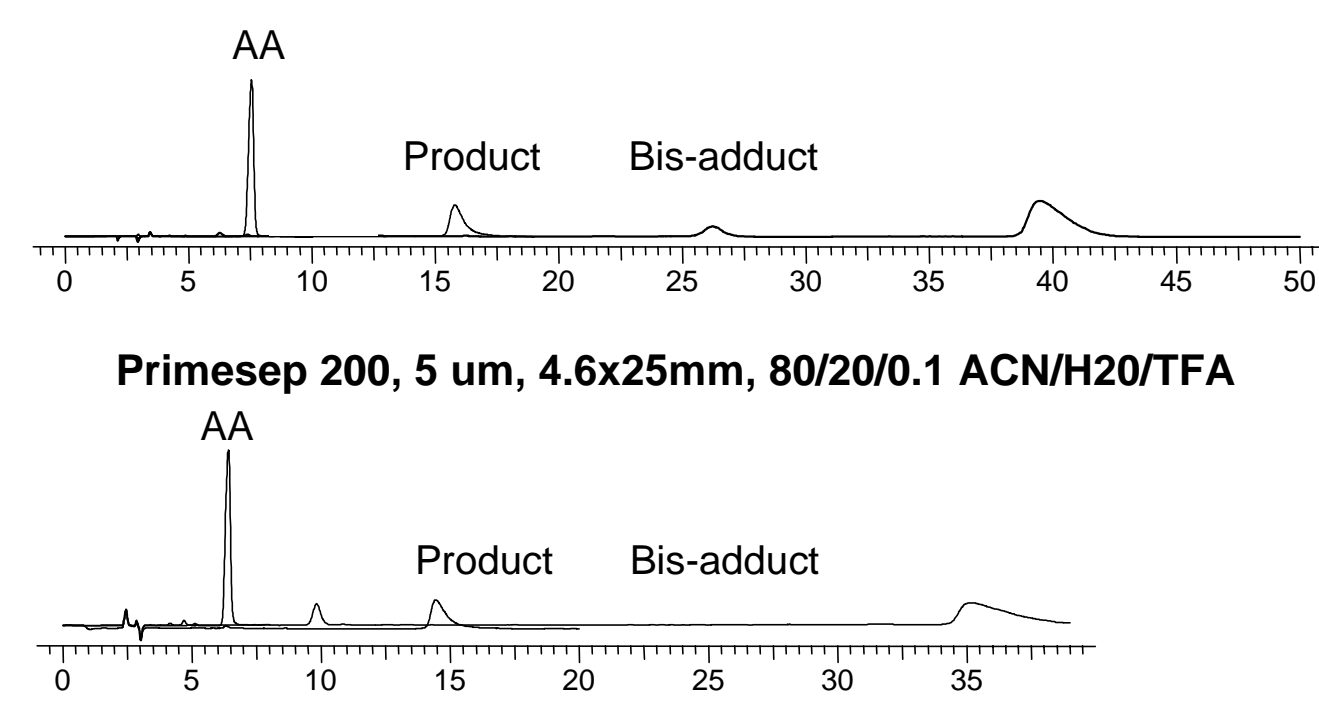
HILIC Chromatography

Bakerbond DIOL, 5 μ m, 4.6x25mm, 70/30/0.1 ACN/H2O/TFA



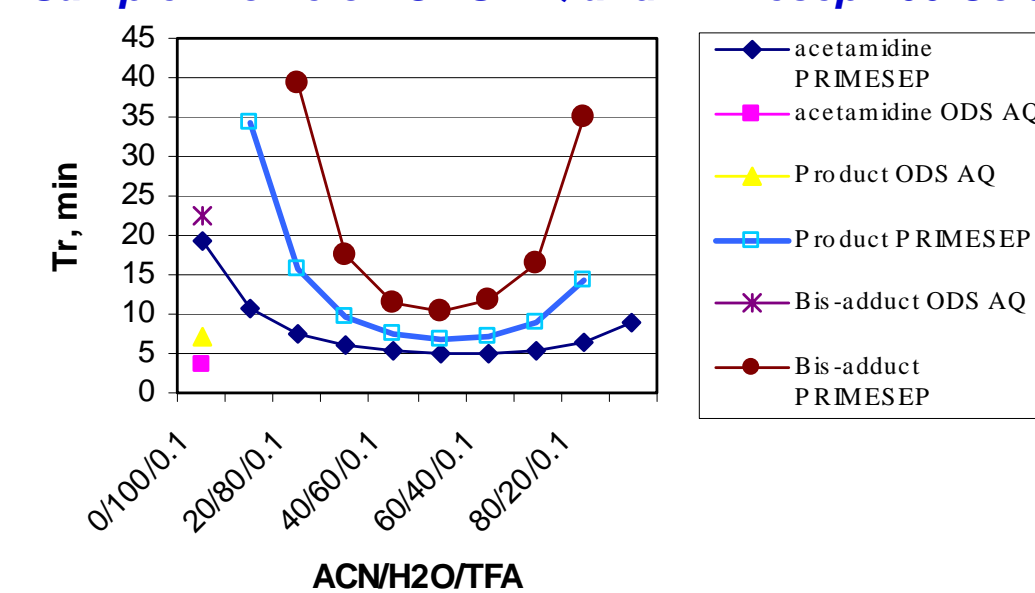
Mixed-Mode Chromatography

Primesep 200, 5 μ m, 4.6x25mm, 20/80/0.1 ACN/H2O/TFA



METHODS (cont'd)

Sample Profile on ODS AQ and Primesep 200 Column



What is Changed

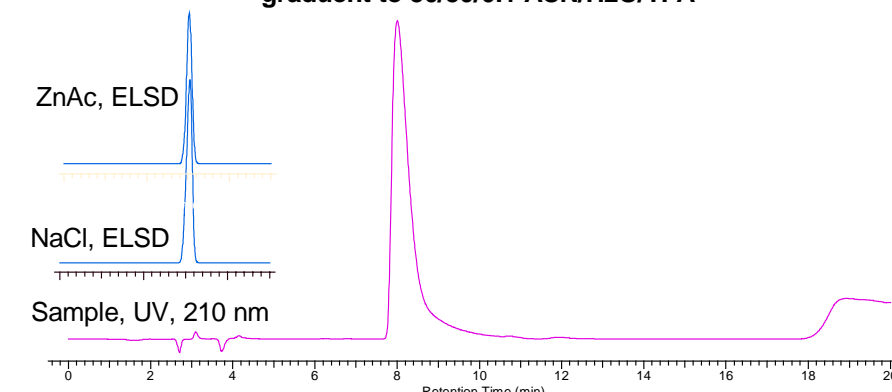
- Impurity profile.
- Stationary phase technology available on the market.

Objective

- Find conditions that would allow to obtain 98% pure desired.
- Develop efficient process using 10 μ media, automated injections.

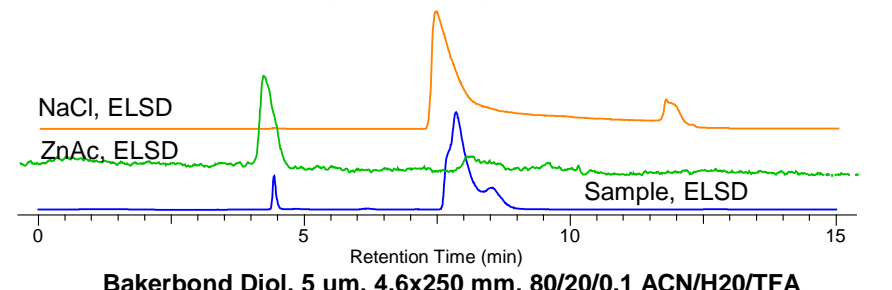
New Sample Profile (ODS AQ Column)

ODS AQ, 10 μ m, 4.6x25mm, 3/97/0.1 ACN/H2O/TFA for 12 min, then gradient to 50/50/0.1 ACN/H2O/TFA



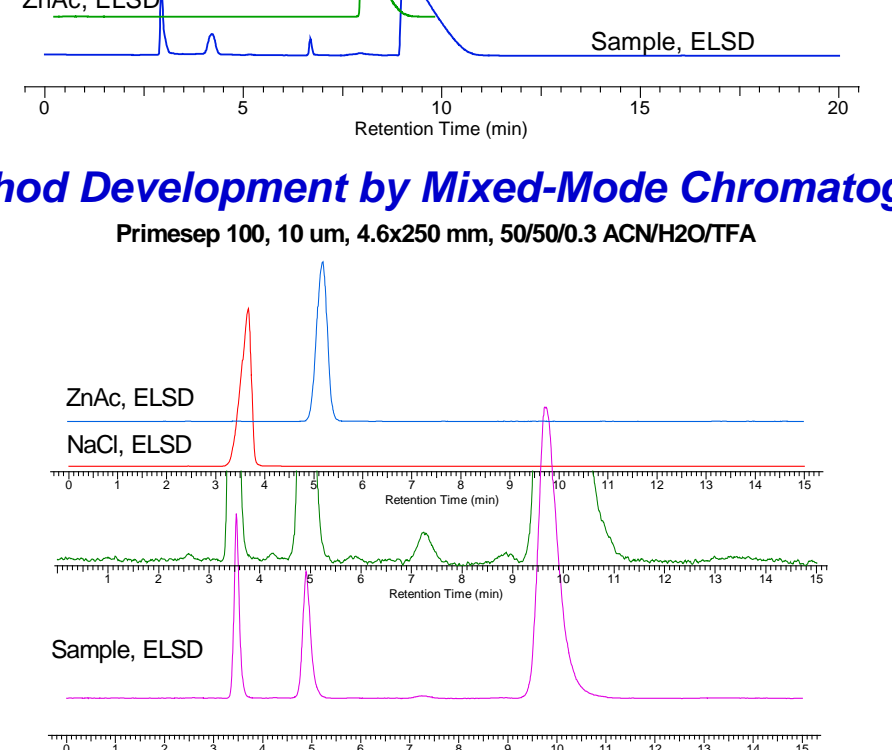
Method Development by HILIC Chromatography

YMC Diol, 5 μ m, 4.6x25mm, 90/10/0.1 ACN/H2O/TFA



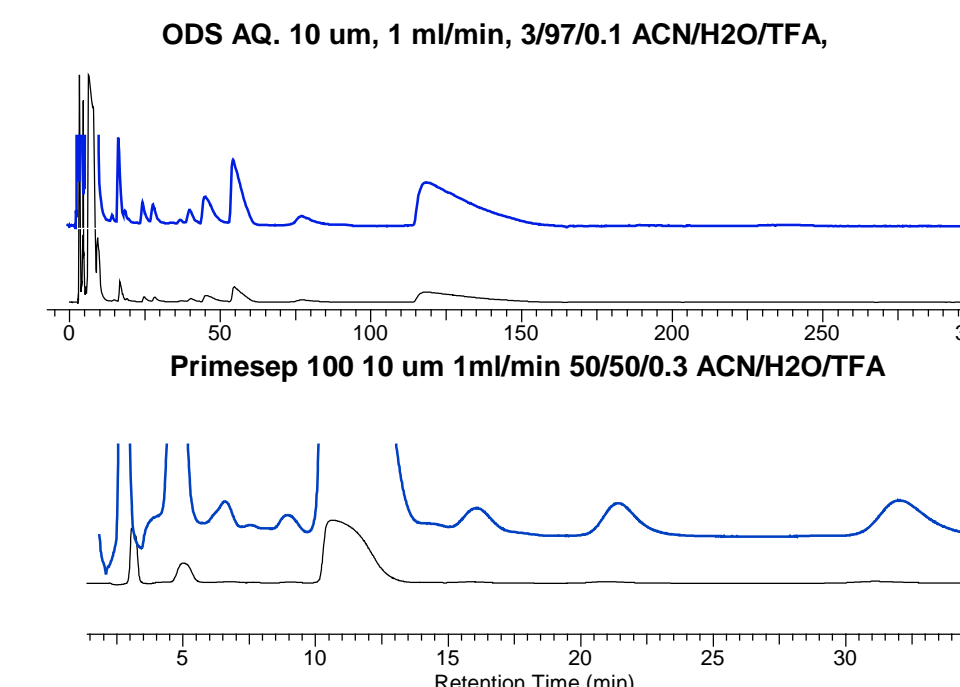
Method Development by Mixed-Mode Chromatography

Primesep 100, 10 μ m, 4.6x25mm, 50/50/0.3 ACN/H2O/TFA



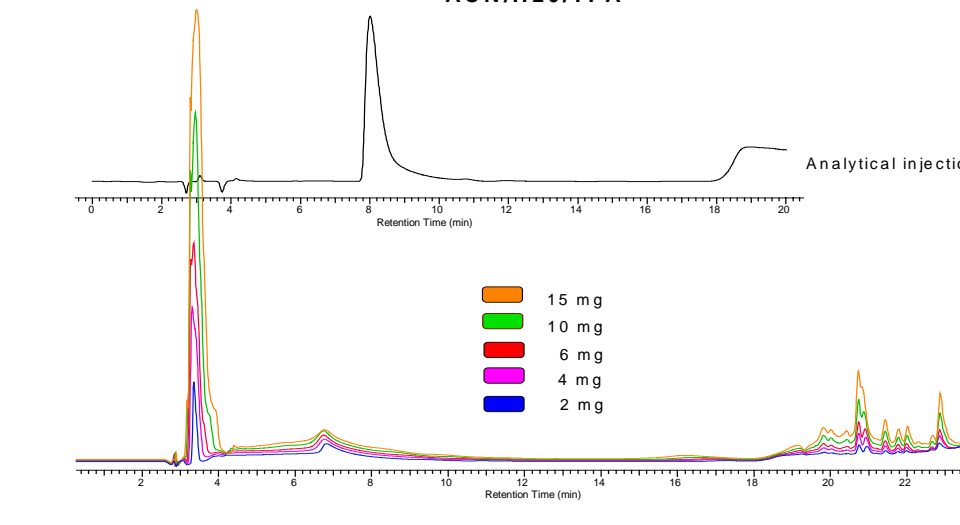
METHODS (cont'd)

Impurity Profile on ODS AQ and Primesep 100 Columns



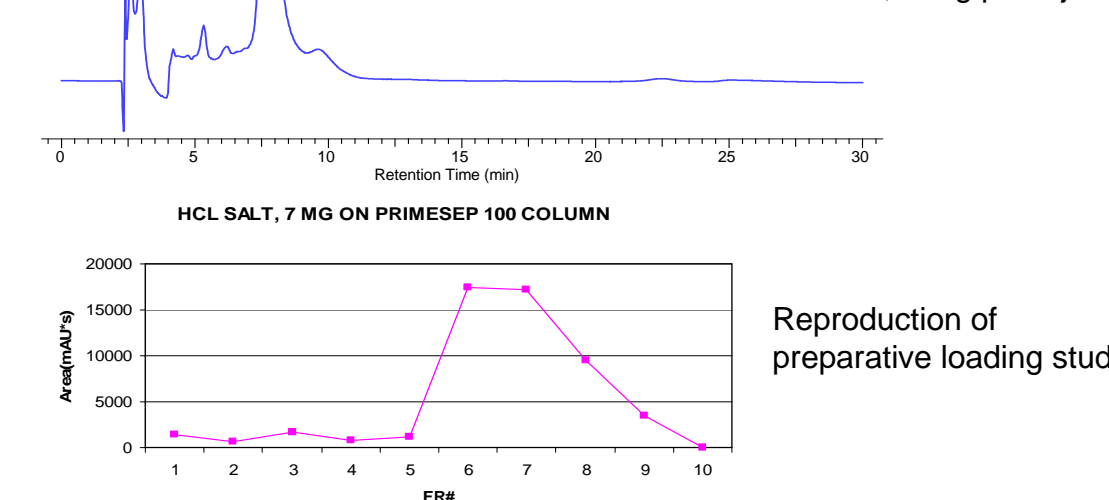
Loading Study for HCl Salt on YMC ODS AQ Column

ODS AQ, 10 μ m, 1m l/min, 240 nm, sample dissolved in 100/0.1 H2O/TFA at 100 mg/ml
3/97/0.1 ACN/H2O/TFA for 12 min, then gradient to 50/50/0.1 ACN/H2O/TFA

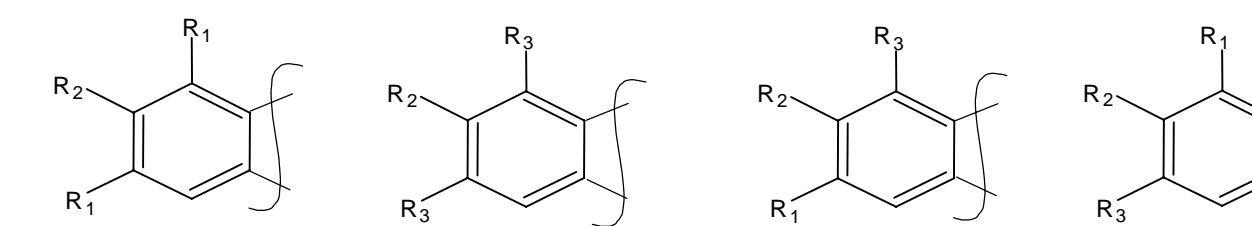


Loading Study for HCl Salt on Primesep 100 Column

Primesep 100, 10 μ m, 1ml/min, 230 nm
50/50/0.3 ACN/H2O/TFA, 7 mg/per injection



HPLC Method Development for Four Closely Structure-Related Compounds

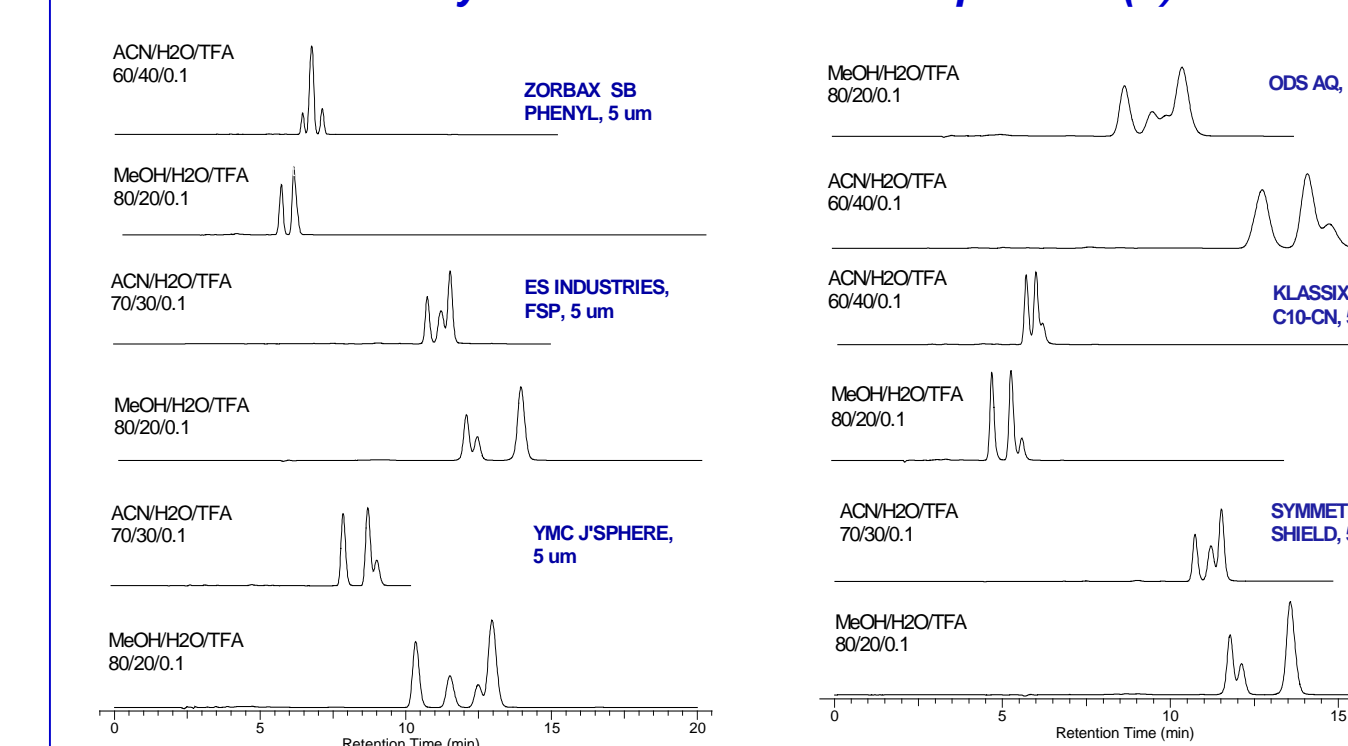


Objective: develop an analytical method suitable for sample analysis/purity check and preparative scale-up

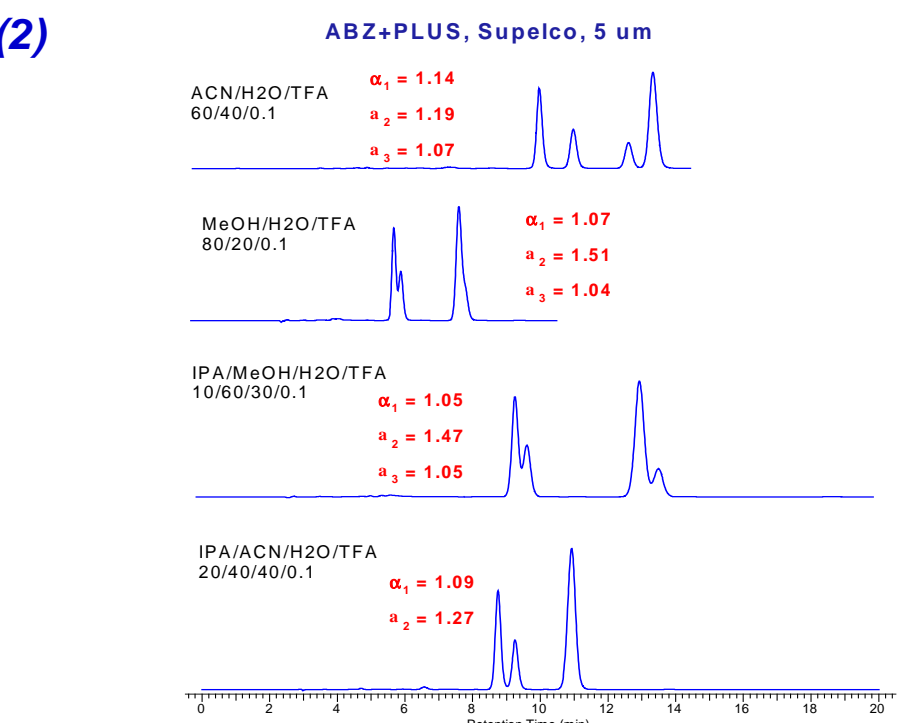
Comment: the sample contains carboxylic functionality in the side chain

METHODS (cont'd)

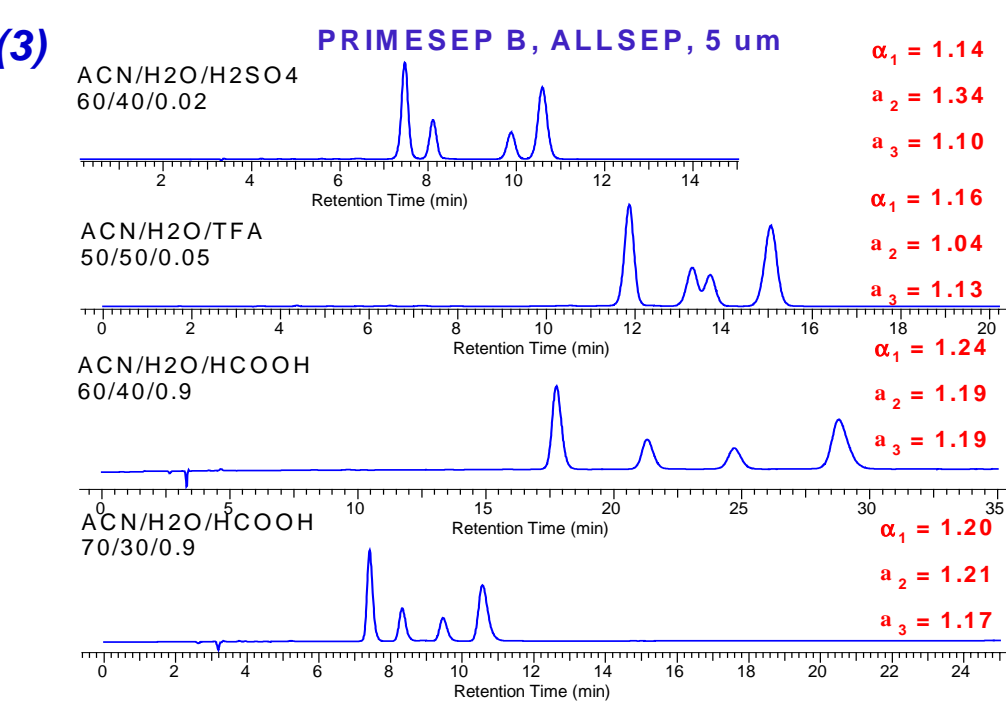
Results of HPLC Screening for Four Closely Structure-Related Compounds (1)



(2)



(3)



CONCLUSIONS

Mixed-Mode Chromatography Columns:

- Allow preparative purification of molecules not possible in the past.
- Allow automation by providing separation of both hydrophilic and hydrophobic compounds in one run under isocratic conditions.
- Allow controllable retention/separation by multidimensional method development.