

Advantages of Using Highly Retentive Phases in LC/MS Development

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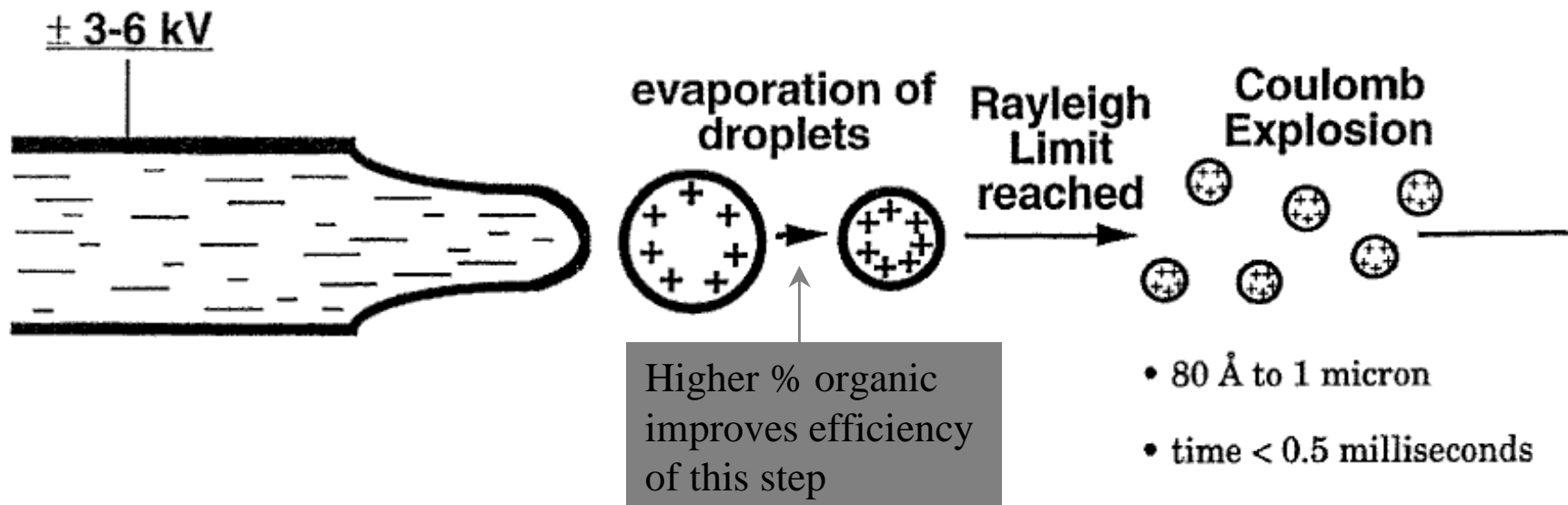
Introduction

- Electrospray ionization (ESI) requires evaporation of the mobile phase (M.P.) leaving the analyte ions in the gas phase.
- The higher the organic content in the M.P., the more efficient the evaporation process.
- This added efficiency translates into greater signal in the mass spectrometer.

State-of-the-Art LC/MS Columns

- New LC/MS packings have been developed that can be used with very high organic content in the M.P.
- This can result in an order of magnitude improvement in LC/MS sensitivity.
- Also provides fast LC/MS analyses.

New phases enable the use of more % organic in the mobile phase. This, in turn, allows for more efficient ESI desolvation/ionization, which yields higher LC/MS sensitivity.



Five novel phases were developed for optimum LC/MS performance and sensitivity:

ANALYTE TYPE

NEW PHASE

Neutral

Allure^R C18

Acidic/Amino Acids

Allure^R Acidix

Basic (1st Generation)

Allure^R Basix

(2nd Generation)

Allure^R PFPP

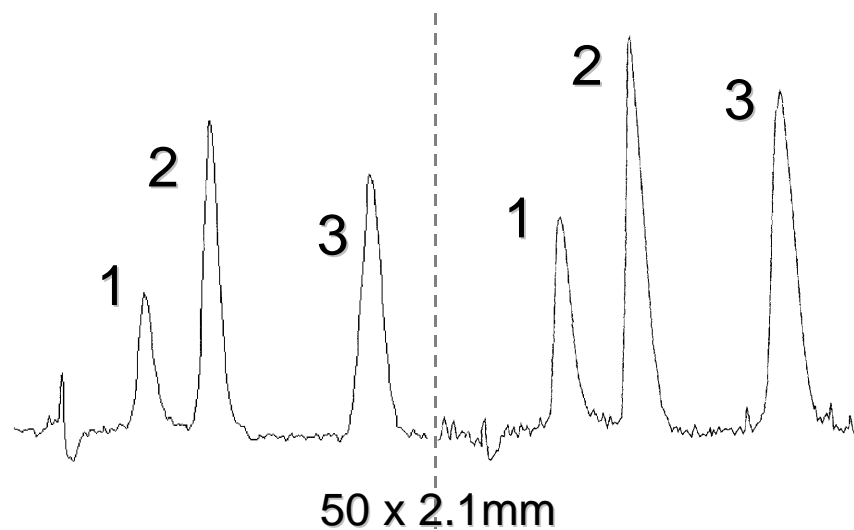
Combination - all types

Ultra IBD

Allure™ C18 VS. Conventional C18 Phase - Neutral Steroids

Conventional C18
12%C
H₂O:MeOH (40:60)

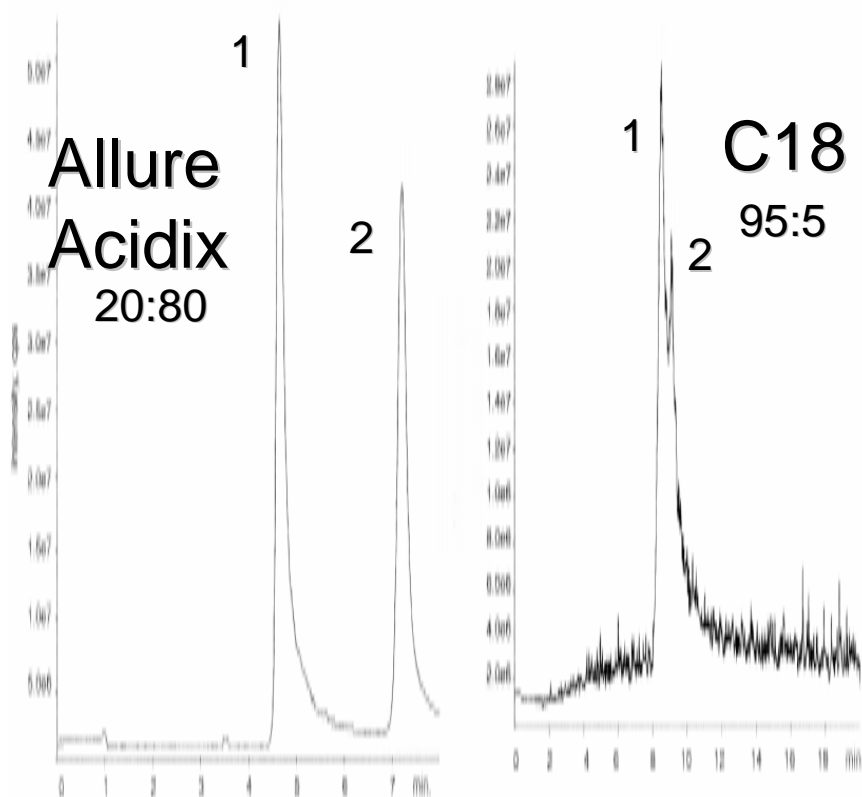
Allure™ C18
27%C
H₂O:MeOH (33:67)



Data courtesy of Shane Needham, Pfizer Inc

- Higher C load allows use of 12% more organic; Results in a 26% increase in LC/MS sensitivity
- 1. Deoxycorticosterone (DCC) Acetate, 2. DCC Glucoside, 3. DCC; 0.4mL/min; Pos. ion ESI-TOF

Allure™ Acidix - New Acidic Analyte Packing



1. Salicylic Acid, 2. Aspirin

Polar stationary phase:

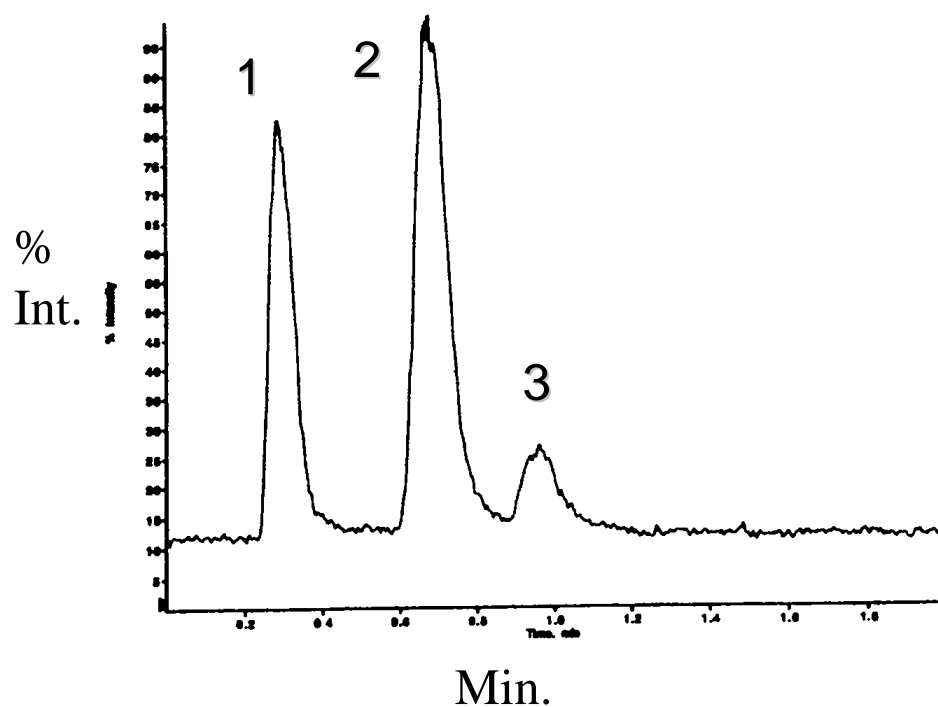
- Allows increased % organic in M.P.
- Increases signal:noise
- Improves selectivity/resolution
- Improves peak shape
- 150 x 4.6mm; 20mM NH_4HCO_2 , pH 4.5:ACN (v/v); 1mL/min; Neg. ion ESI

Allure™ Acidix - Underivatized Amino Acid Separation

Column: Allure Acidix

Sample: 1. homocysteine thiolactone, 2.
homocysteine, 3. homocystine

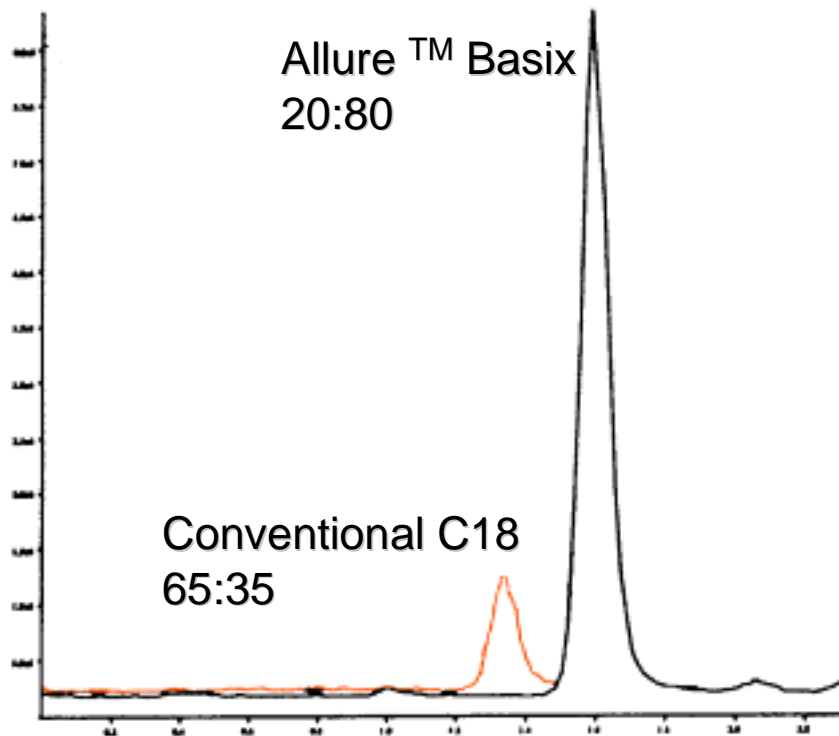
Mobile Phase: water, pH 3 (HCOOH):
ACN (40:60 v/v).



- Excellent peak shape under mild M.P. conditions.
- No derivatization necessary.
- Enables monitoring reaction pathways with minimal experimental interferences.

Allure™ Basix - 1st Generation Basic Analyte Packing

Amitriptyline



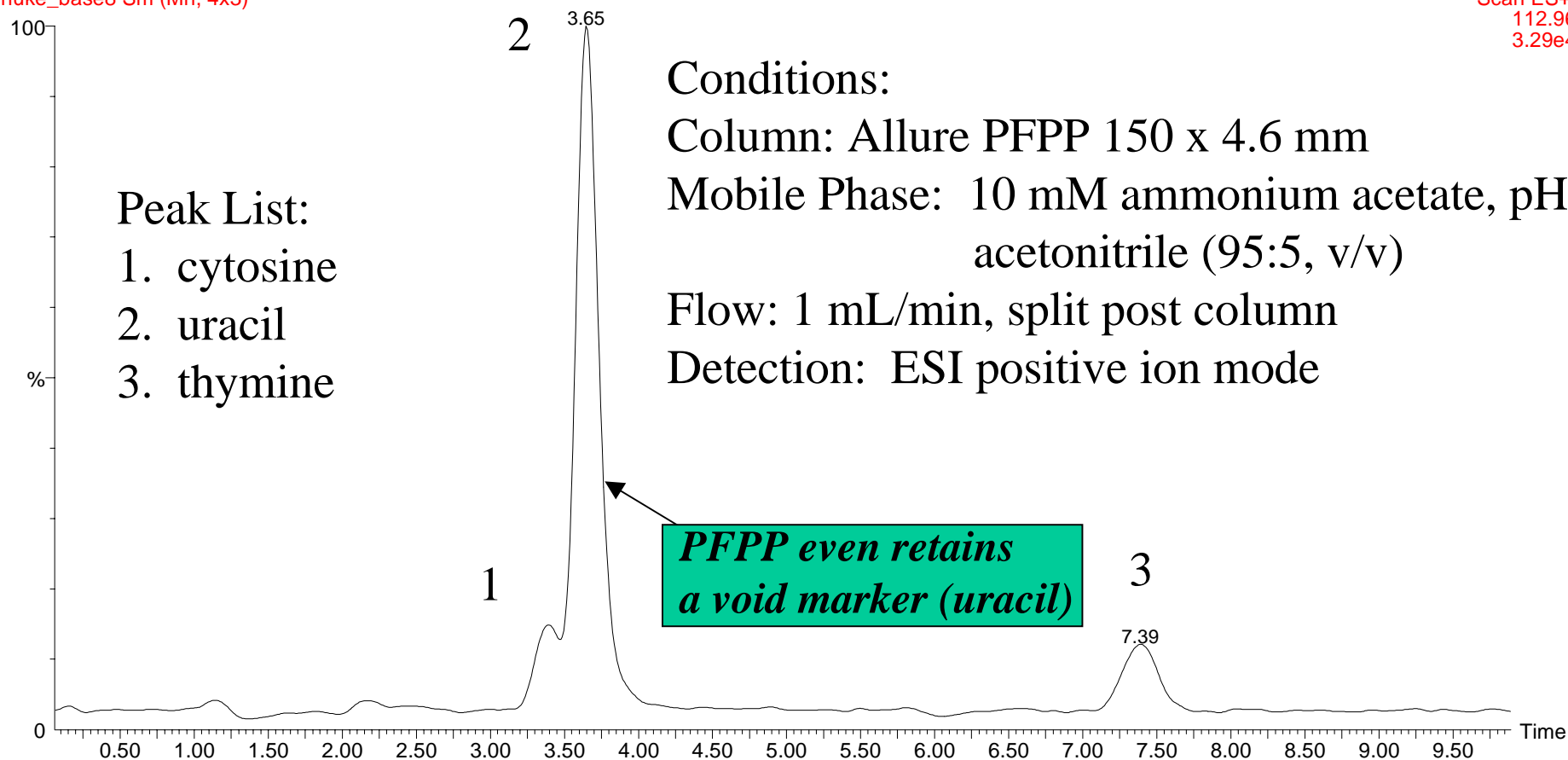
- Designed to use for basic compounds.
- Adjusted % organic (in 5mM Am. Acet., pH=4.5 M.P.) from 35% (conven. C18) to 80% (Basix) for same k' .
- Signal-to-noise increase: 243%

Allure™ PFPP - 2nd Generation

Basic Analyte Packing

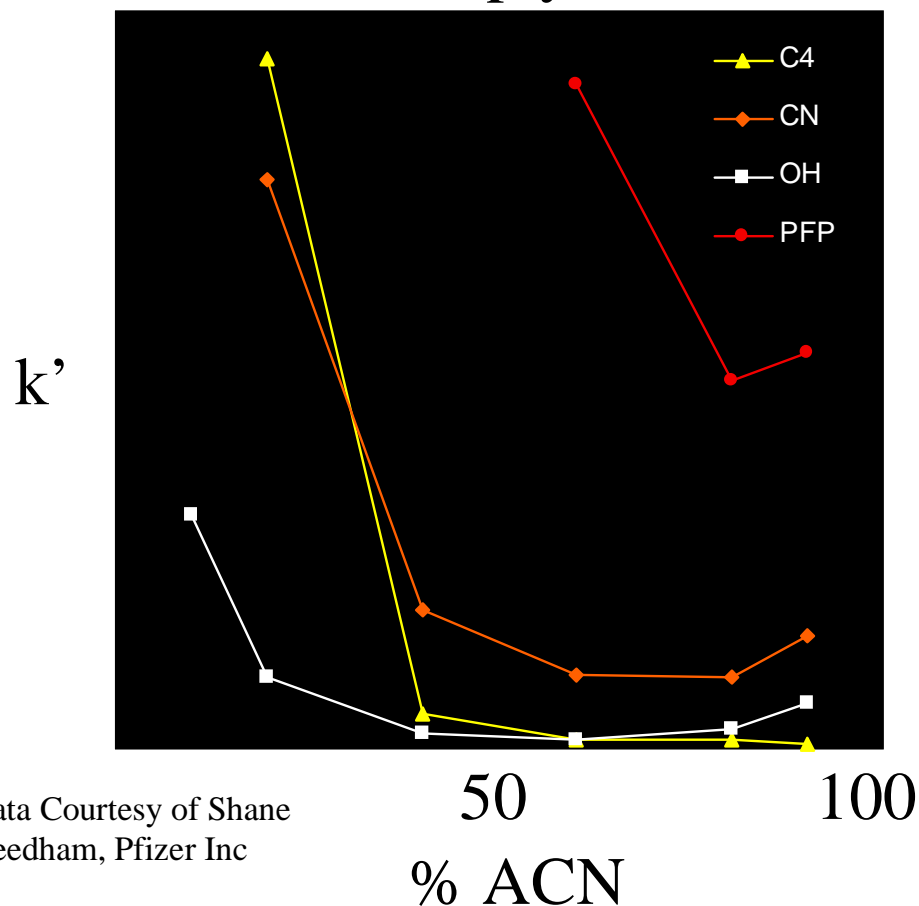
uracil
nuke_base8 Sm (Mn, 4x3)

Scan ES+
112.96
3.29e4



Allure™ PFPP - Uses Highest % Organic for Basic Analytes

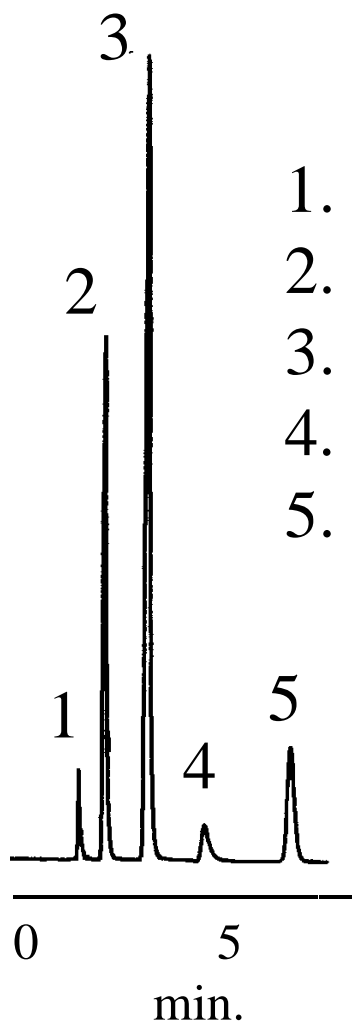
Amitriptyline



Data Courtesy of Shane
Needham, Pfizer Inc

- PFPP allows the use of much more organic in M.P. to give the same amount of retention of conventional phases.
- This allows increased sensitivity in LC/MS, LC/IR, and LC/ELSD of 600% or more.
- 5mM NH_4OAc , pH 4.5:ACN; 30 x 2.1mm; 0.4mL/min

Ultra IBD - Separates Acid/Basic Mixtures



- Good peak shape for both acids and bases.
- Unique selectivity compared to C18 phases.
- M.P.: 65:35 - 50mM KH₂PO₄, pH 3:ACN
Flow: 1mL/min
- Detection: 254nm

Conclusion

- New LC/MS packings have been developed that enable the use of higher % organic content in the mobile phase.
- These packings typically provide up to an order of magnitude increase in sensitivity.
- The proper column choice is easily made, *a priori*, based on the pKa of the analyte.