

Retention of Polar Compounds or How to Avoid the Void



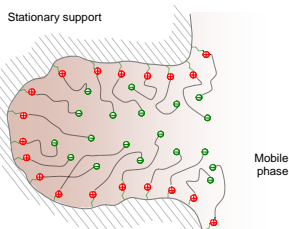
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Abstract

New concepts in stationary phase design are introduced to address problems of liquid chromatography. Liquid Separation Cell (LISC™) technology is based on a new chemical modification of silica gel pores that creates a liquid separation cell with its own charge characteristics, ionic strength, and hydrophobic properties. Like living cells existing in equilibrium with the outside environment, liquid separation cells exist in constant equilibrium with the mobile phase. New stationary phases have significantly higher ionic strength in the cell versus mobile phase ion strength, providing quick mass transfer of charged analytes in and out of the cell, even with low concentration of buffer in the mobile phase. Ligands in these stationary phases occupy the entire volume of the cell, not just the walls as in other stationary phases, and provide high capacity of the phase.

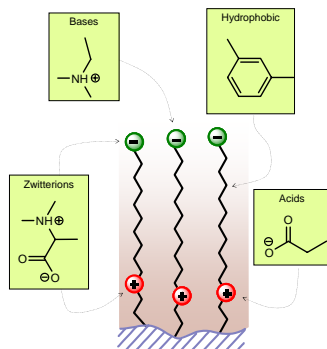
Positive and negative charges of the cell ligand separated by a long organic chain allow both positive and negative charges to simultaneously participate in electrostatic interaction. Retention mechanisms are easily controlled by pH of the mobile phase. Comparison with recently introduced columns from major column manufacturers shows benefits of LISC technology in achieving controlled retention, perfect peak shape, and high loadability for various analytes. Liquid Separation Cell technology allows increasing loadability of columns several folds without compromising peak shape and retention.

Liquid Separation Cell (LISC) Schematic



- Significantly higher ionic-strength in the cell vs. mobile phase ion strength, providing quick mass transfer of charged analytes in and out of the cell even with low concentration of buffer in the mobile phase
- The stationary phase occupies the entire volume of the cell, not just the walls as in other stationary phases, providing high capacity of the phase
- Positive and negative charges of the cell ligand, separated by a long organic chain, allow both positive and negative charges to simultaneously participate in electrostatic interaction.

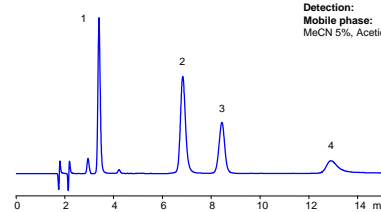
Types of interactions of Obelisc R stationary phase with different analytes.



Hydrophilic molecules in MS compatible mobile phase

1. Phenylalanine
2. Tryptophan
3. 3-Amino benzoic acid
4. Aspartame

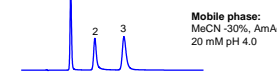
Column: Obelisc R
Size: 150 x 4.6 mm
Flow: 1.0 mL/min
Detection: UV 250 nm
Mobile phase: MeCN 5%, Acetic acid -0.1%



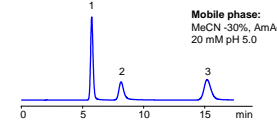
pH Effect on separation of zwitter-ionic molecules

1. 4-Amino benzoic acid
2. 3-Amino benzoic acid
3. 2-Amino benzoic acid

Column: Obelisc R
Size: 150 x 4.6
Flow: 1.0 mL/min
Detection: UV 250 nm



Mobile phase: MeCN -30%, AmAc 20 mM pH 4.0

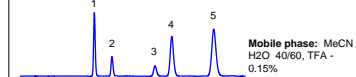


Mobile phase: MeCN -30%, AmAc 20 mM pH 5.0

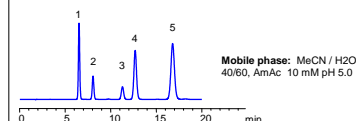
pH Effect on separation of neutral molecules

1. Indene
2. Naphthalene
3. Acenaphthene
4. Fluorene
5. Phenanthrene

Column: Obelisc R
Size: 150 x 4.6
Flow: 1.0 mL/min
Detection: UV 250 nm



Mobile phase: MeCN / H2O 40/60, TFA - 0.15%

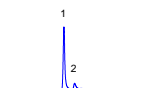


Mobile phase: MeCN / H2O 40/60, AmAc 10 mM pH 5.0

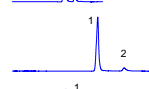
Amino acids

1. Aspartic acid
2. Asparagine

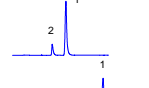
Column: Obelisc R
Size: 150 x 4.6 mm
Flow: 1.0 mL/min
Detection: ELSD



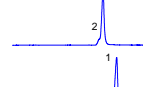
Mobile phase: MeCN -75%, AmFm 10 mM pH 3.0



Mobile phase: MeCN -80%, AmFm 10 mM pH 5.0



Mobile phase: MeCN -75%, AmAc 10 mM pH 5.0

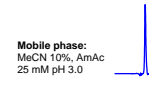


Mobile phase: MeCN -80%, AmAc 20 mM pH 5.0

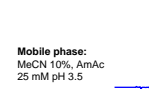
Retention of polar drugs, pH effect

1. Dopamine
2. Acetaminophen
3. Pseudoephedrine

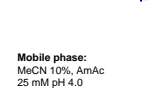
Column: Obelisc R
Size: 150 x 4.6 mm
Flow: 1.0 mL/min
Detection: UV 250 nm



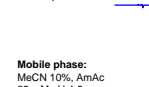
Mobile phase: MeCN 10%, AmAc 25 mM pH 3.0



Mobile phase: MeCN 10%, AmAc 25 mM pH 3.5



Mobile phase: MeCN 10%, AmAc 25 mM pH 4.0



Mobile phase: MeCN 10%, AmAc 25 mM pH 4.5

Selectivity adjusted by MeCN concentration

Mobile phase: MeCN 50%, AmFm 20 mM pH 3.0

1. Prednisolone
2. Atrolactic acid
3. Ibuprofen

Column: Obelisc R
Size: 4.6 x 150 mm
Flow: UV 270 nm
Detection: 1.0 mL/min

Mobile phase: MeCN 45%, AmFm 20 mM pH 3.0

1. Prednisolone
2. Atrolactic acid
3. Ibuprofen

Mobile phase: MeCN 40%, AmFm 20 mM pH 3.0

1. Prednisolone
2. Atrolactic acid
3. Ibuprofen

Mobile phase: MeCN 30%, AmAc 30 mM pH 5.0

1. Prednisolone
2. Atrolactic acid
3. Ibuprofen

Mobile phase: MeCN 35%, AmAc 20 mM pH 5.0

1. Phenylalanine
2. Tryptophan
3. Phenol
4. Benzotriline
5. Pyridine
6. Toluene
7. Lutidine
8. Benzylamine
9. Benzoic acid

Column: Obelisc R
Size: 150 x 4.6
Flow: 1.0 mL/min
Detection: UV 250 nm

Mobile phase: MeCN 30%, AmAc 30 mM pH 5.0

1. Phenylalanine
2. Tryptophan
3. Phenol
4. Benzotriline
5. Pyridine
6. Toluene
7. Lutidine
8. Benzylamine
9. Benzoic acid

Mobile phase: MeCN 35%, AmAc 20 mM pH 5.0

1. Phenylalanine
2. Tryptophan
3. Phenol
4. Benzotriline
5. Pyridine
6. Toluene
7. Lutidine
8. Benzylamine
9. Benzoic acid

Mobile phase: MeCN 35%, AmAc 20 mM pH 5.0

1. Phenylalanine
2. Tryptophan
3. Phenol
4. Benzotriline
5. Pyridine
6. Toluene
7. Lutidine
8. Benzylamine
9. Benzoic acid

Mobile phase: MeCN 35%, AmAc 20 mM pH 5.0

1. Phenylalanine
2. Tryptophan
3. Phenol
4. Benzotriline
5. Pyridine
6. Toluene
7. Lutidine
8. Benzylamine
9. Benzoic acid

Mobile phase: MeCN 35%, AmAc 20 mM pH 5.0

1. Phenylalanine
2. Tryptophan
3. Phenol
4. Benzotriline
5. Pyridine
6. Toluene
7. Lutidine
8. Benzylamine
9. Benzoic acid

Mobile phase: MeCN 35%, AmAc 20 mM pH 5.0

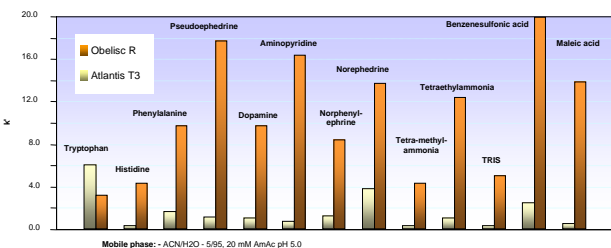
1. Phenylalanine
2. Tryptophan
3. Phenol
4. Benzotriline
5. Pyridine
6. Toluene
7. Lutidine
8. Benzylamine
9. Benzoic acid

Mobile phase: MeCN 35%, AmAc 20 mM pH 5.0

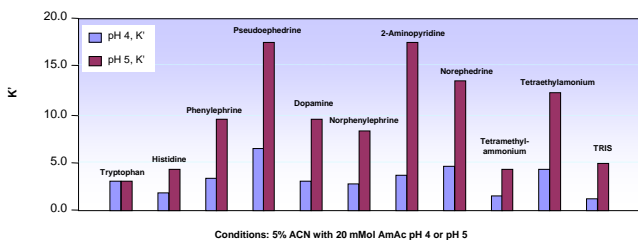
Conclusions

Mixed-mode columns that contain two types of interactions, ion-exchange and reversed-phase, allows three dimensional control of separation selectivity. Buffer concentration, buffer pH, and organic modifier concentration are three factors that allow to adjust selectivity and obtain most convenient peaks spreading. Retention characteristics of polar compounds are significantly improved compared to traditional reversed-phase chromatography. Mixed-mode type of stationary phases eliminates blind multiple-column search, and allows a systematic approach to method development.

Comparative retention of Polar Compounds Obelisc R vs. Atlantis T3



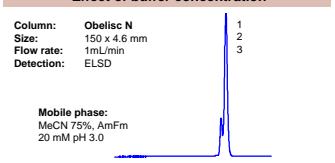
Obelisc R - Effect of pH on Retention of Polar Analytes



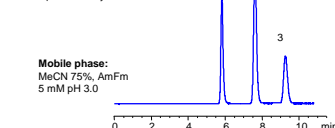
Effect of buffer concentration

Column: Obelisc N
Size: 150 x 4.6 mm
Flow rate: 1 mL/min
Detection: ELSD

1. α-Amino butyric acid
2. β-Amino butyric acid
3. γ-Amino butyric acid



Mobile phase: MeCN 75%, AmFm 20 mM pH 3.0

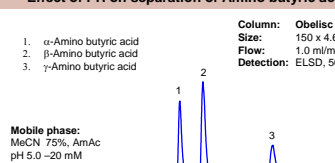


Mobile phase: MeCN 75%, AmFm 5 mM pH 3.0

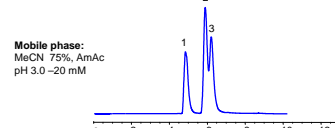
Effect of PH on separation of Amino butyric acids

Column: Obelisc N
Size: 150 x 4.6 mm
Flow: 1.0 mL/min
Detection: ELSD, 50C

1. α-Amino butyric acid
2. β-Amino butyric acid
3. γ-Amino butyric acid



Mobile phase: MeCN 75%, AmAc pH 5.0 -20 mM



Mobile phase: MeCN 75%, AmAc pH 3.0 -20 mM