Mixed-Mode Core-Shell Columns - Unique Selectivity, Efficiency and Speed. Coresep 100.

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In recent years, two technologies, mixed-mode chromatography and core-shell particles, emerged and gained popularity as tools offering new selectivity and speed for analysis of complex mixtures. Mixed-mode chromatography offers a unique alternative selectivity which cannot be matched by reversed-phase or HILIC columns. Multiple interaction on a single column offer 2D type chromatography (Fig. 1) as a tool to separate complex mixtures with a variety of compounds possessing opposite properties: hydrophobic and hydrophilic, neutral and ionic, cation and anion exchange.

When a single mode of interaction is employed on a reversed-phase column, compounds elute according to their hydrophobicity and analytes with similar hydrophobicity may co-elute or show poor resolution (Fig 1, reversed-phase example). An opposite approach is to use ion-exchange chromatography, where compounds are retained based on their ionic properties. Similarly, using ion-exchange chromatography, other compounds may be closely eluting because they are identical in terms of the strength of ionic interaction between stationary phase and analyte (Fig 1, ion-exchange example). In mixed-mode chromatography you have two interactions – reversed-phase and ion-exchange and you are exploring a very small difference in reversed-phase and ionic properties of compounds. These differences are enhanced due to synergy of two mechanisms which results in much better separation (Fig 1, mixed-mode example)

Core-shell technology offers the unique ability to achieve high efficiency at higher flow rates whilst generating back pressures which can be achieved using traditional HPLC systems. This offers the end user the opportunity to improve separation speed and quality without the requirement to purchase UHPLC equipment.

We have recognized the exciting opportunities afforded by combining these two great technologies in stationary phases which achieve unique selectivity and high efficiency with traditional HPLC systems which are rated to 4000 or 6000 psi.

Coresep is a new generation of unique stationary phases, combining mixed-mode and core-shell approaches. Mixed-mode columns offer much higher capacity and retention than traditional reversed-phase columns. Since the surface area of core-shell particles is reduced, mixed-mode phases address potential loadability issues whilst maintaining the selectivity advantages of multiple interactions. Newly optimized ligand density and ionic / hydrophobic ratios further increases the capacity of mixed-mode core-shell columns.

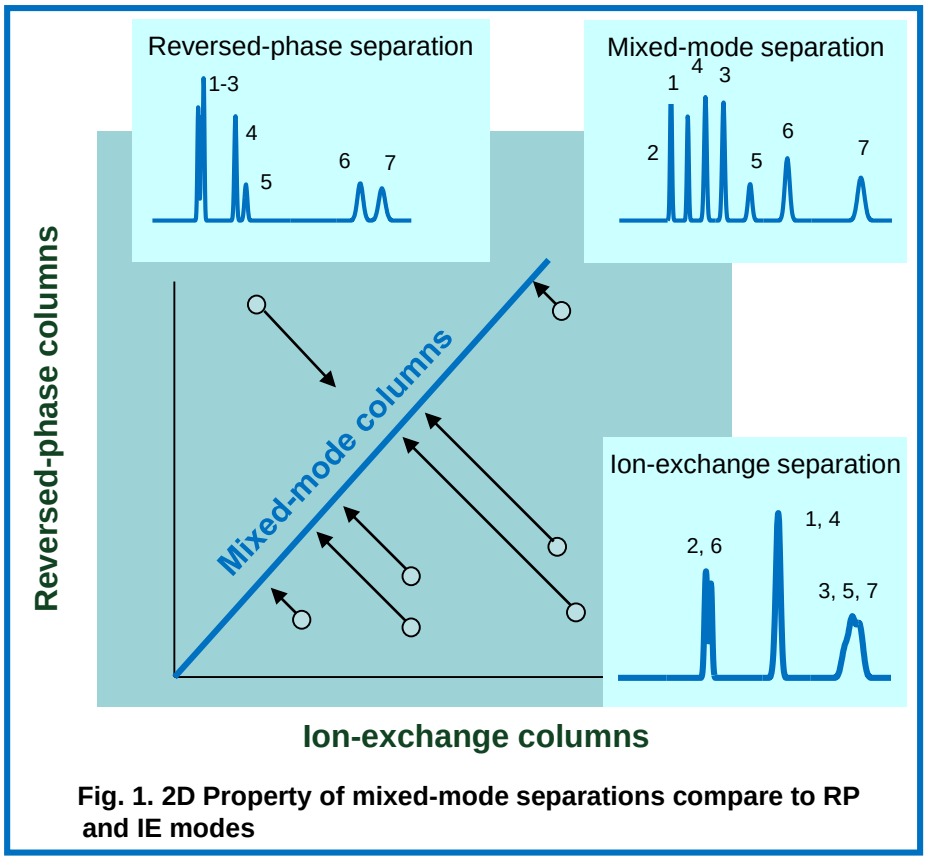

Fig. 1. 2D Property of mixed-mode separations compare to RP and IE modes

Column: 3.2 x 50 mm, 2.7um
Mobile phase: MeCN gradient 10% to 65% in 5 min, AmFm pH 3.5 gradient 30 mM to 70 mM in 5 min
Flow: 1.2 ml/min
Detection: UV 270 nm

1. Adenosine
2. 3,4-Difluroaniline
3. 4-Amino-2-chloropyridine
4. 4-Amino-3-chloropyridine
5. 5-Aminoindole
6. 2-Amino 5-methylthiadiazole
7. 4-Ethylaniline

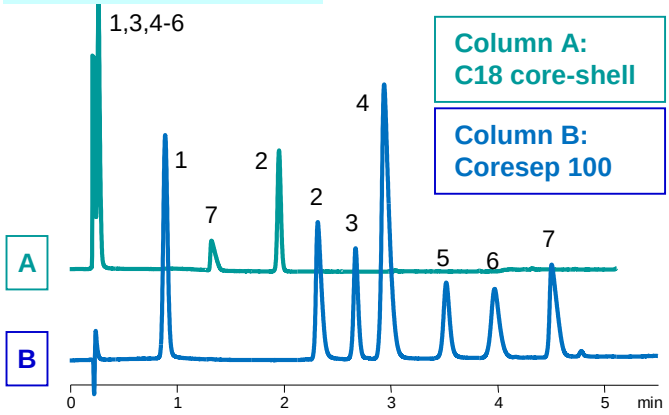

Fig. 2 Separation of model compounds in reversed-phase cation-exchange modes on two core-shell columns

Separation of amino acids without ion-pairing reagent on reversed-phase core-shell columns (Fig. 3)
Amino acids are hydrophilic building blocks for many pharmaceutical compounds and nutrient supplements. Depending on the pH of the mobile phase they can exist in acidic, basic or zwitter-ionic form. At lower pH, amino acids are basic in nature and have the lowest hydrophobicity.

Column: 3.2 x 50 mm, 2.7 um
Mobile phase:
MeCN 20%, H2O 80%
Flow: 1.0 ml/min
Detection: UV 205 nm

1. Methionine
2. Tyrosine
3. Phenylalanine

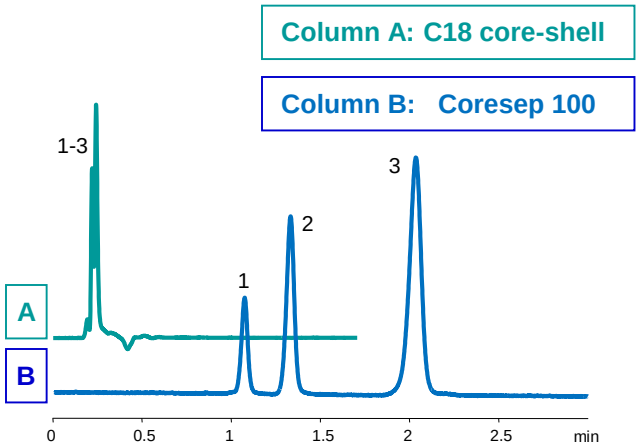

Fig. 4 Retention and separation of amino acids in BLIS - mode on mixed-mode core-shell columns

Separation of model compounds on mixed-mode core-shell column (Fig. 2)
A mixture of 7 hydrophilic and ionic compounds is widely used in the pharmaceutical industry as a generic probe for evaluating the selectivity of analytical columns. Using an industry standard reversed phase core-shell column, 5 out of 7 compounds were poorly retained or resolved.
All seven compounds have very low hydrophobicity and retention on traditional reversed-phase columns cannot be achieved. Since mixed-mode exploits two mechanisms of retention, ion-exchange and reversed-phase, retention and separation of all 7 components can be achieved in a short analysis time. Using **Coresep**™. columns, base-line resolution and retention was achieved using an LC/MS compatible mobile phase. Early eluting compounds are retained well beyond the void to allow analysis of complex matrices and highly polar analytes.

1. Serine
2. Glycine
3. Alanine
4. Valine
5. Dopa

Column A: C18 core-shell
Column B: Coresep 100

Column: 3.2 x 50 mm, 2.7 um
Mobile phase: MeCN 3% with 0.03% TFA
Flow: 1. mL/min
Detection: UV 210 nm
Injection: 5 um

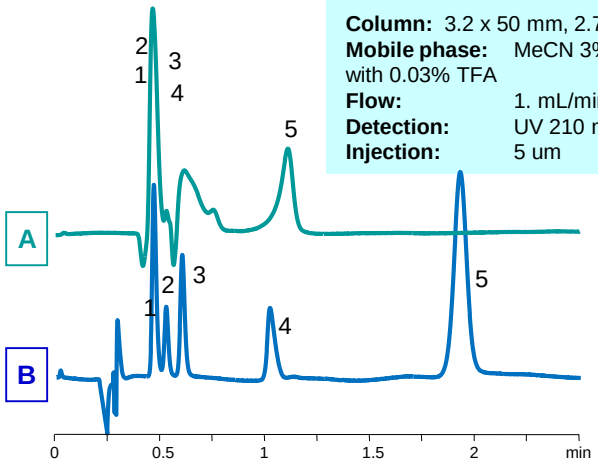

Fig. 3 Separation of amino acids without ion-pairing reagent in reversed-phase and cation-exchange mode on core-shell columns

This low hydrophobicity and absence of ion-exchange mechanism prevents suitable retention in reversed-phase mode, but such amino acids can be effectively retained separated in reversed-phase cation-exchange using **Coresep**™ columns. Five amino acids (SER, GLY, ALA, VAL and DOPA) were effectively retained and separated within 2 minutes.

Retention and Separation of amino acids on mixed-mode core-shell columns (Fig. 4)
Three amino acids were retained and separated on Coresep 100 column without any additives or buffer in reversed-phase and cation-exchange modes. This approach allows amino acids to be analyzed as zwitter-ions without any ions/buffers in the mobile phase.

The method was adopted for analysis of amino acids using mixed mode **Coresep**™ columns . A similar approach using reversed-phase columns results in distorted peaks and no separation or significant retention to achieve base-line separation.

Fast and efficient separation of neurotransmitters on mixed-mode core-shell columns (Fig. 5**)**
All neurotransmitters are hydrophilic ionic compounds which require ion-pairing reagents for retention in reversed-phase mode. Fast and efficient retention and separation can be achieved with LC/MS compatible mobile phases using **Coresep**™ mixed-mode columns. Based-line separation is achieved within 3 minutes with good peak shape and retention compared to a reversed phase separation. C18 core-shell reversed-phase separation shows no retention of polar ionic neurotransmitters even at low organic concentration.

1. Uracil
2. Thymine
3. Cytosine
4. Guanine
5. Adenine

Column: 3.2 x 50 mm, 2.7 um
Mobile phase: MeCN 5% with 0.15% TFA
Flow: 1.0 ml/min
Detection: UV 270 nm

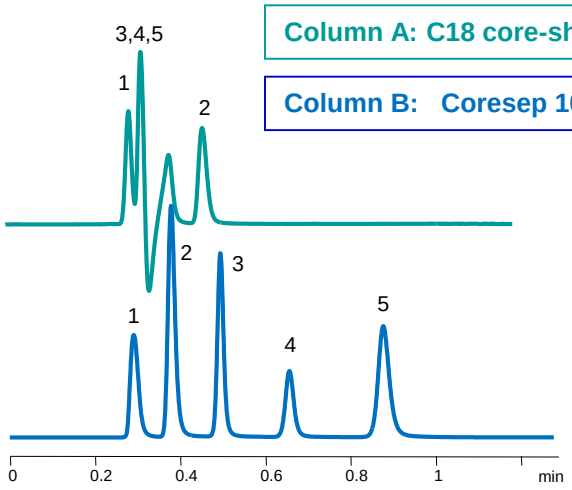

Fig. 6 Fast separation of nucleobases on mixed-mode core-shell column

1. DOPA
2. Tyrosine
3. Phenylalanine
4. Norepinephrine
5. Epinephrine
6. Dopamine

Column: 3.2 x 50 mm, 2.7 um
Mobile phase: MeCN gradient 5% to 10% in 4 min, AmFm pH 2.9 gradient 5 mM to 25 mM in 5 min
Flow: 1.0 ml/min
Detection: UV 270 nm

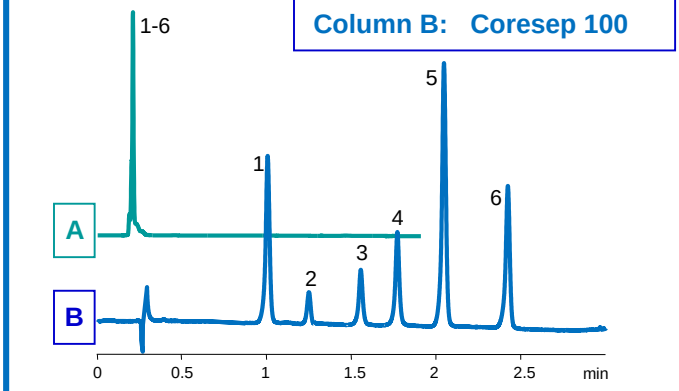

Fig. 5 Fast and efficient separation of neurotransmitters on mixed-mode core-shell column

Fast separation of Nucleobases on mixed-mode core-shell columns (Fig. 6)
Nucleobases like uracil, thymine, cytosine, guanine and adenine show no retention on reversed-phase core-shell columns as their low hydrophobicity is not enough to provide sufficient retention. The dual synergistic mechanisms of mixed-mode chromatography combined with the core-shell particle allow retention and separation of these 5 compounds within a one minute run time.

This test probe separation demonstrates that mixed-mode core-shell columns can provide unprecedented selectivity, retention control and speed using traditional HPLC systems. Mobile phases are compatible with LC-MS and preparative separations. Employing smaller particle core-shell mixed-mode columns increases sensitivity of the method while staying below 6000 psi. Mixed-mode core-shell columns provide these significant benefits compared to reversed-phase core-shell and sub 2 um particles.

Coresep™ part number generator

CS1 - 4 15 2 9

Column id

4.6 mm	**4**
3.0 mm	3
2.0 mm	2
1.0 mm	1

Column length

150 mm	**15**
100 mm	10
50 mm	5
25 mm	2
10 mm	1
Guard	G

Packing size

2.7 um	**2**

Pores size

90 A	**9**

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