

## Reversed Phase + Anion Exchange + Cation Exchange HPLC Columns

Simultaneous analysis of both cationic and anionic compounds

ODS + Ion Exchange separation mode

Three kinds of packings with different ion exchange capacities

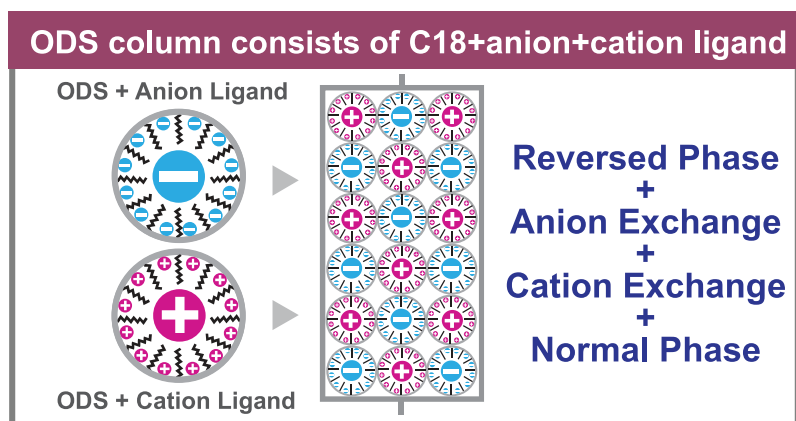
For polar compounds

Different selectivity from conventional ODS columns

LC-MS compatible without using ion-pairing reagents

ODS column consists of C18+anion+cation ligands

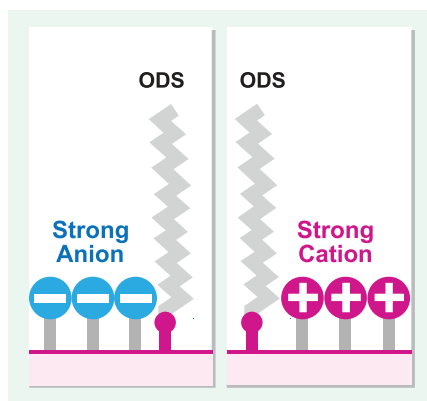
Purified Porous Silica • 3µm Particle • 13nm Pore



Scherzo family columns can operate without adding an ion-pairing reagent which is required for conventional ODS columns. In addition, both anionic and cationic compounds are retained on this column. The hydrophobicity of the Scherzo family is similar to that of a conventional ODS column, so analysis of a compound in combination with a Unison UK-C18 column (conventional ODS phase) will be very effective for method development.

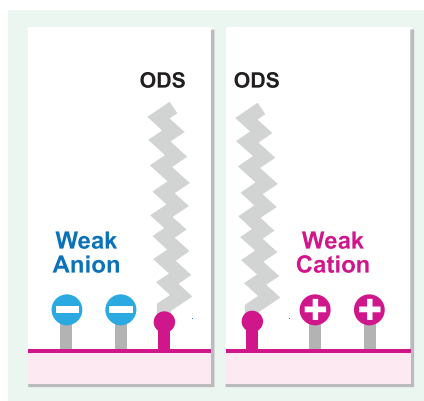
## Three kinds of ODS with different ion exchange capacities

### Scherzo SS-C18



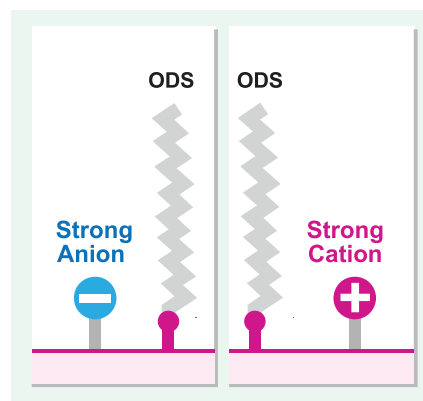
Large amount of strong ionic ligands loaded onto this ODS column. Effective for improved retention of zwitterions or weak ionic compounds

### Scherzo SM-C18



Weak ionic ligands adequately loaded onto this ODS column. Designed for separation of basic/acidic compounds at neutral pH condition

### Scherzo SW-C18



Low amount of strong ionic ligands loaded onto this ODS column. Effective for strong ionic compound elution or basic compounds with formic acid eluent

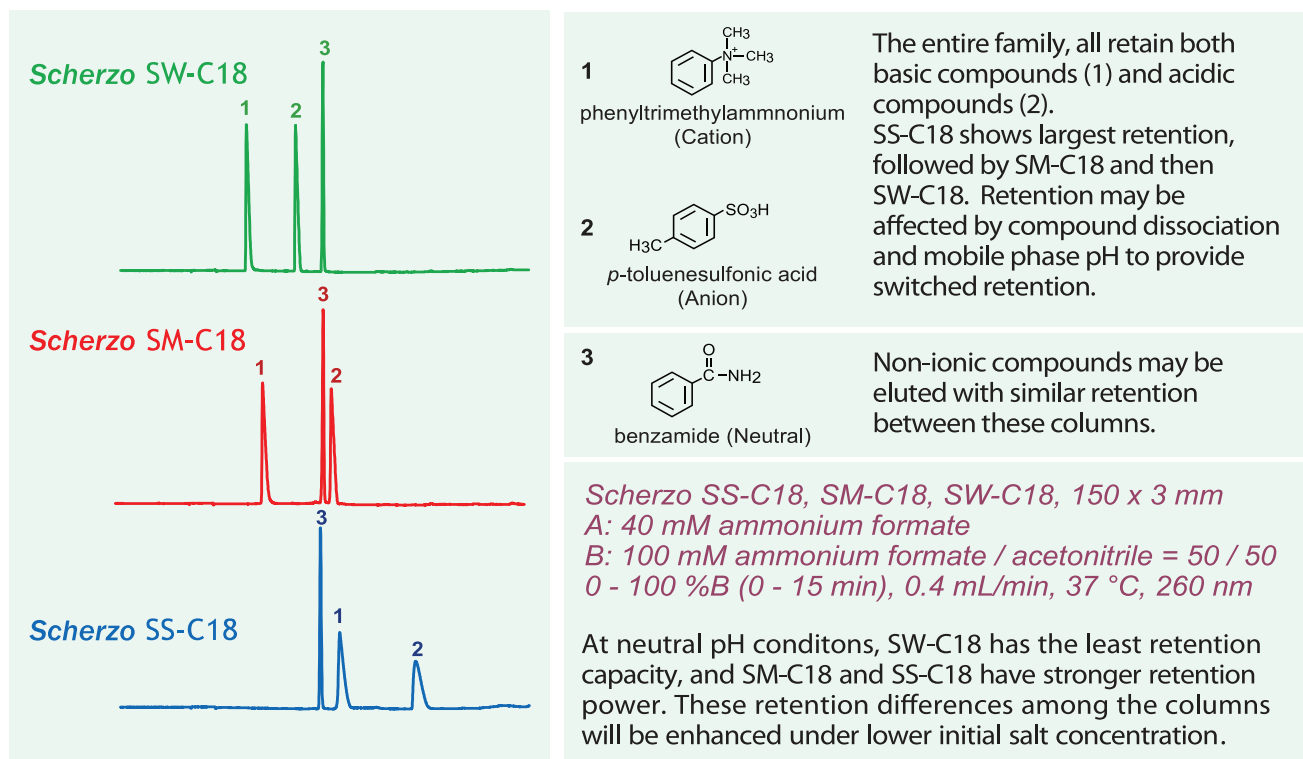
## Separation modes of Scherzo C18 columns

The Scherzo C18 Family, (SS-C18, SM-C18, SW-C18), consists of not only ODS ligands, but also anion ligands and cation ligands. It also provides reversed-phase mode, both ion exchange modes, and normal phase mode.

Separation Mode	Stationary Phase	Properties
Reversed-Phase	Octadecyl	Increasing organic solvent composition (decreasing polarity of eluent) decreases retention.
Anion Exchange	Cation	Increasing ionic strength (salt or acid concentration) decreases retention for acidic compounds. Generally, low pH increases retention.
Cation Exchange	Anion	Increasing salt concentration decreases retention for basic compounds. SM-C18 retains more with increasing pH, while SS-C18 and SW-C18 retain more at lower pH.
Normal Phase	Anion/Cation	Polar solutes which cannot be retained with 100% aqueous eluent may be retained by using > 50% organic solvent composition due to electrostatic interaction.

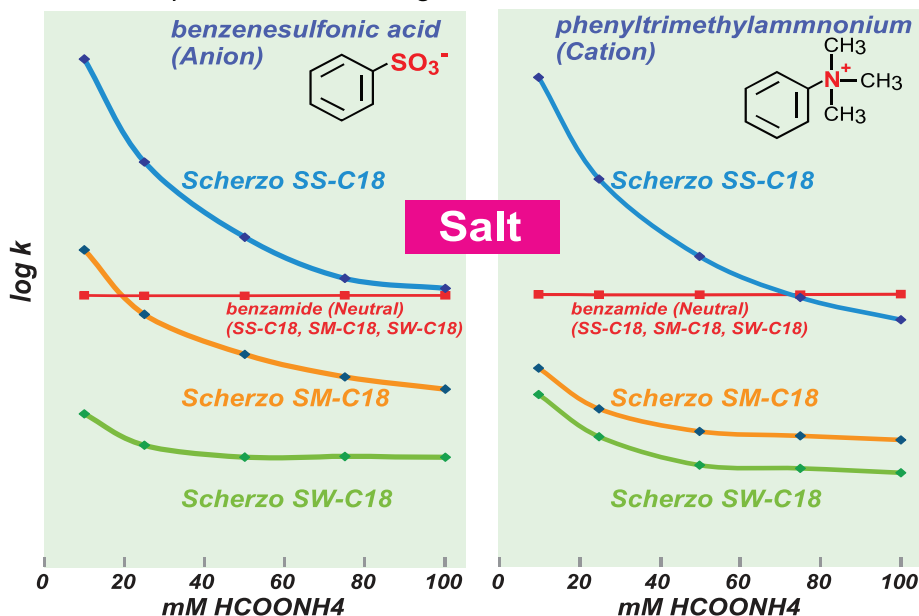
## Retention properties of Scherzo C18 columns (RP+AX+CX)

Multi-mode ODS Scherzo C18 columns consist of ODS ligands which have reversed-phase mode, plus anion and cation ligands which have anion/cation exchange modes. Three kinds of Scherzo columns have individual ion exchange capacities to find the best column for target compounds which have different ionic properties. Moreover, hydrophobicity between Scherzo C18 and Unison UK-C18, a conventional ODS, is similar, so these columns can be used as comparisons during method development.

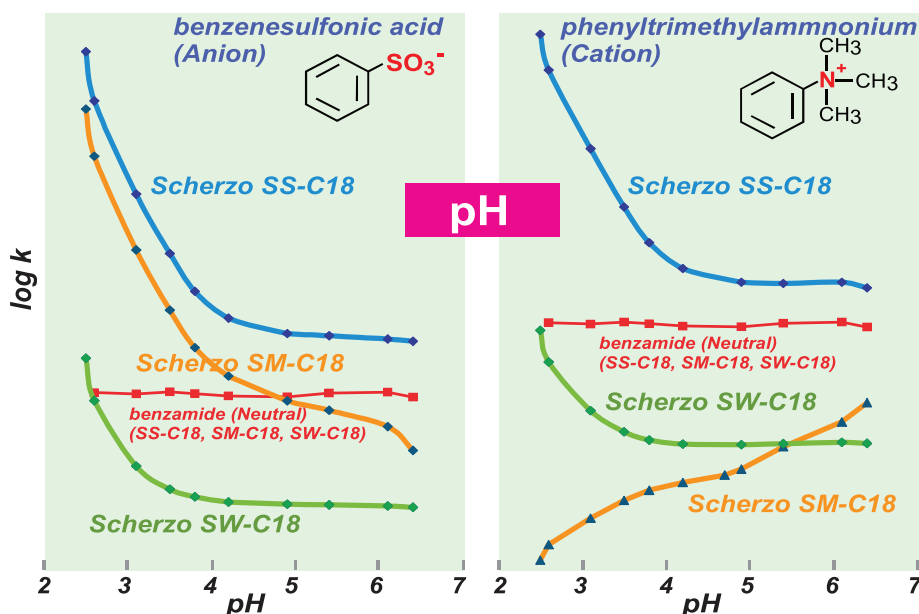


## Factors affecting retention on Scherzo C18 Columns

- You can find the best HPLC conditions by optimizing ionic strength or pH to improve retention / separation for ionic compounds, which is difficult on a conventional ODS column
- Not only pH but also ionic strength (concentration of salt or acid) will strongly affect the retention on Scherzo C18 columns
- This effect is the same as ion-exchange columns
- Scherzo C18 columns, which have RP + both ion exchange modes, require optimization of organic solvent, pH, and ionic strength



For the Scherzo multi-mode ODS columns, optimizing the salt concentration is as important as optimizing the organic solvent. In the left figure, retention of both anionic and cationic compounds is decreased when salt concentration is increased. At the same salt concentration, SS-C18, which consists of a large amount of strong ionic ligands, retains strongly. SM-C18, with weak ionic ligands has medium retention. And finally, SW-C18, which has a low amount of strong ionic ligands, shows the lowest retention.



pH is also an important factor for the elution of ionic compounds. Retention will change dramatically between neutral pH (ammonium acetate or formate) and low pH (formic acid) conditions. SS-C18 and SW-C18 consist of strong ionic ligands and retention will be increased under low pH conditions. On the other hand, SM-C18 consists of weak ion ligands which cannot ionize at a low pH - therefore, retention of basic compounds will be decreased due to a decrease in ionization capacity at low pH.

## Elution strategies by Scherzo C18 columns

### Non-ionic hydrophobic solutes

Organic solvent composition should be optimized (similar to conventional ODS). Peak shape is often improved by using 0.1% acetic acid.

### Ionic hydrophobic solutes

A combination of organic solvent and 20-100mM of salt or acid at optimal pH should be used for separation of both acidic / basic compounds .

### Weak ionic-polar solutes

SS-C18 is often the best choice for weak ionic polar solutes. Ionic strength should be increased for compounds that contain multiple ionic functional groups. Neutral pH conditions are required for mono-carboxylic acid compounds .

### Strong ionic-polar solutes

SW-C18 may be useful when it is difficult to elute on SS-C18 or SM-C18. Strong ionic compounds have strong ionic interaction, so it is recommended to use multiple gradient elutions with ionic strength and organic solvent composition.

## Mobile phase preparation for Scherzo C18 columns

Scherzo C18 columns consist of ODS and ionic ligands. At first this new approach is difficult for some users to conceptualize. However, it is easier to think of the columns as an ODS column AND an ion exchange column. The following eluent conditions are a rough guideline:

### Isocratic Elution

water / acetonitrile / HCOOH = x / y / 0.1 (x+y = 100)

Acidic pH Eluent

50mM ammonium acetate / acetonitrile = x / y (x+y = 100)

Neutral pH Eluent

### Gradient Elution

A) water / HCOOH = 100 / 0.1

B) water / acetonitrile / HCOOH = 30 / 70 / 0.5

Gradient with Acid and Organic Solvent

A) 10mM ammonium acetate

B) 100mM ammonium acetate / acetonitrile = 30 / 70

Gradient with Salt and Organic Solvent

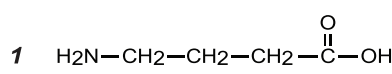
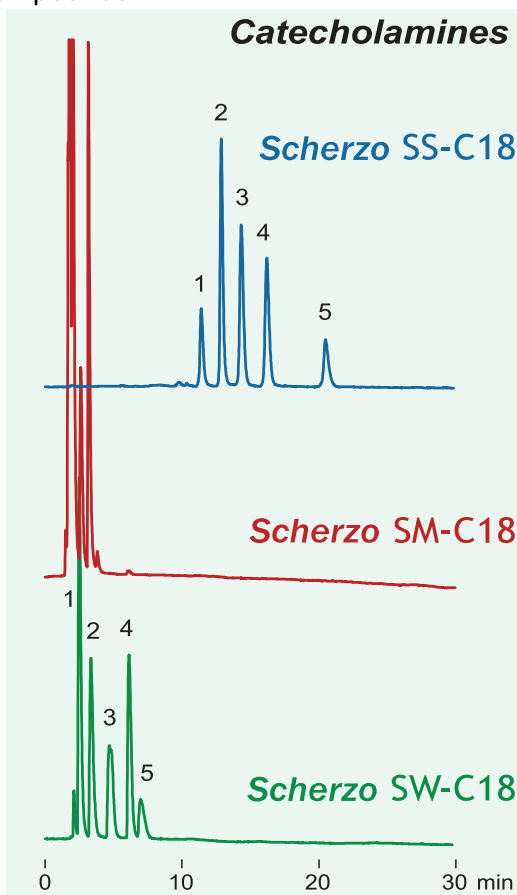
A) water / HCOOH = 100 / 0.1

B) 100mM ammonium formate / acetonitrile = 30 / 70

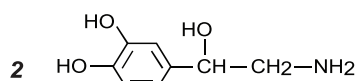
Gradient with pH, Ionic Strength, and Organic Solvent

## Comparison of Scherzo columns: Optimized on SS-C18 (Neurotransmitter)

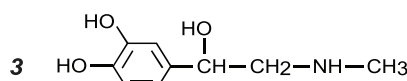
Each Scherzo column has different properties and target compounds depending on column choice. SS-C18 has strong ionic ligands and is useful for zwitter-ions, as well as for separating weak ionic compounds.



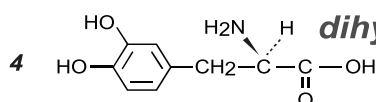
*gamma-aminobutyric acid (GABA)*



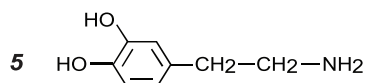
*noradrenaline (norepinephrine)*



*adrenaline (epinephrine)*



*dihydroxy-L-phenylalanine (L-DOPA)*



*dopamine*

150 x 3 mm

A: water / formic acid = 100 / 0.3

B: 100mM ammonium formate / acetonitrile = 70 / 30

0-60%B (0-30 min)

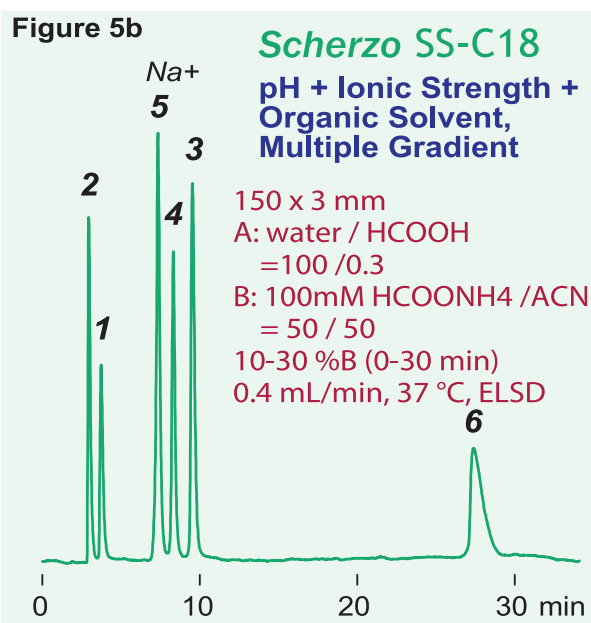
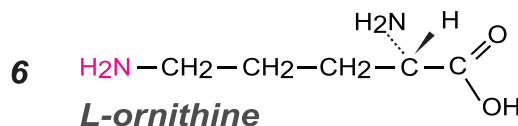
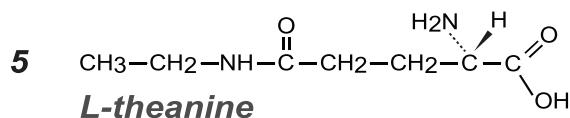
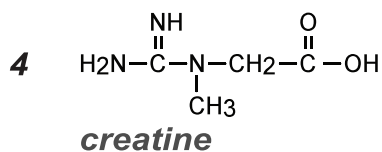
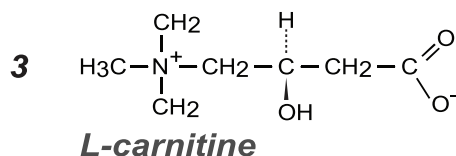
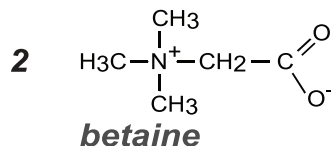
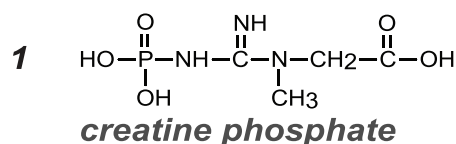
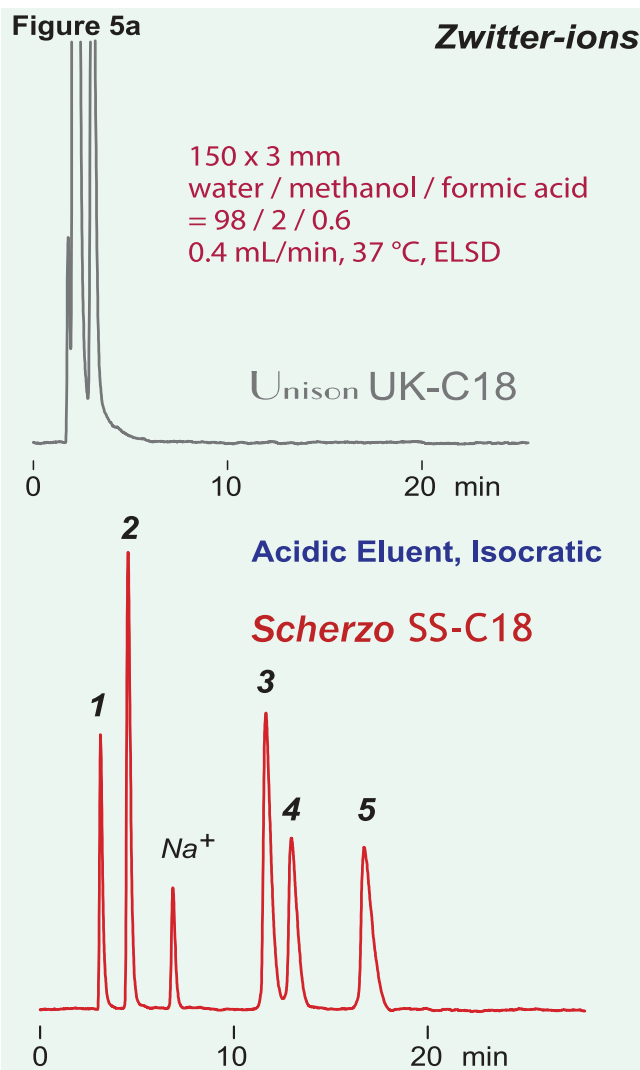
0.4 mL/min (9MPa), 37 °C, ELSD

Due to strong ionic ligands, SS-C18 and SW-C18 retain more than SM-C18 which has weak ionic ligands. In particular, SS-C18 will provide an excellent retention performance for very polar ionic compounds. Also, SW-C18, which interacts weakly with these compounds, may be useful for high-throughput analysis.

Formic acid is strictly required at initial conditions due to positive ion of GABA, but dopamine has a stronger interaction under such acidic conditions. This application is done with a multiple gradient mode using pH, ionic strength, and organic solvent gradients.

## Scherzo SS-C18 column applications (Zwitter-ions)

Zwitter-ions which have internal salt generating iso-electric points (pI) are very polar. While it is difficult to retain zwitter-ions on a conventional ODS column, Scherzo SS-C18 provides very effective retention ability.



Zwitter-ions, as typified by amino acids are very polar and difficult to retain on conventional ODS columns. SS-C18 which consists of many strong ionic ligands can retain and separate those zwitter-ions easily.

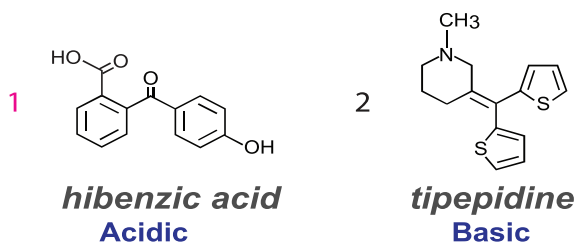
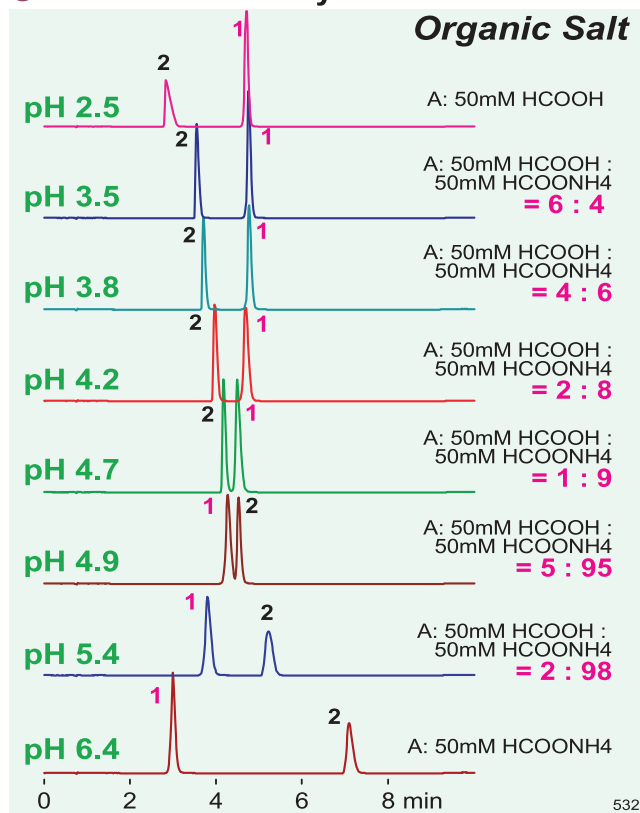
Figure 5a shows that SS-C18 enables sufficient retention/separation due to ionic interaction between positive-charged zwitter-ions under acidic conditions. There are opportunities to get optimized retention when formic acid or organic solvent concentration is changed.

Figure 5b shows an example with multiple gradient elution with pH, ionic strength, and organic solvent. Zwitter ions are ionized to positive under acidic condition, so initial eluent should have low pH conditions. In contrast, ornithine has another amino group which provides a positive charge and becomes a strong cationic compound, so it is difficult to elute under acidic conditions. In this case, neutral pH provides weak ionic interaction and pH gradient with increasing ionic strength and organic solvent is very effective for elution of ornithine.

## Scherzo SM-C18 column applications (Salts, Vitamins)

Scherzo SM-C18, consisting of ODS ligands and both weak anion / cation ligands, is useful for a wide range of ionic compounds such as salts, vitamins, acidic / basic compounds, alkaloids etc. It is also offers different selectivity from conventional ODS like Unison UK-C18.

### ● Simultaneous analysis of salt



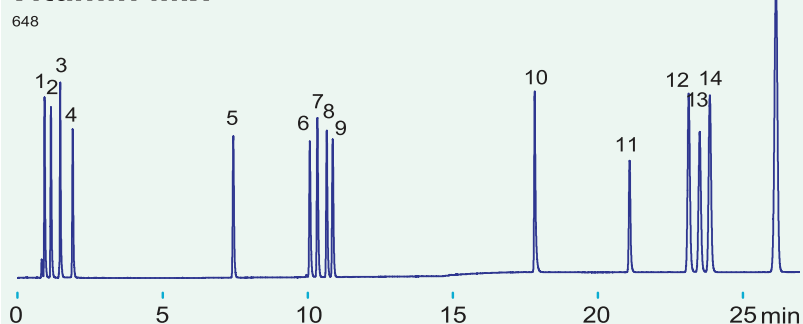
Scherzo SM-C18, 50 x 3 mm  
 A: formate buffer, B: acetonitrile  
 20-60 %B (0-8min), 0.4 mL/min, 37 °C, 280 nm

Tipegidine hibenzoate, a popular cough medicine, consists of basic and acidic components. Conventional ODS columns can usually analyze organic salts, but sometimes there is a problem in separating other compounds. By changing eluent pH, one can change elution order (selectivity) on SM-C18. When pH is increased, retention of basic compounds will be increased, while acidic compounds will be decreased. SM-C18 was designed to show effective ionic interaction under neutral pH conditions. SM-C18 will provide a better solution than conventional ODS columns when solute sensitivity and separation is required. SM-C18 is available for not only organic salts but also inorganic salts separation like NaCl.

### ● Water-fat soluble vitamins

Scherzo SM-C18, 150 x 2 mm  
 A: 0.3% HCOOH aq.  
 B: acetonitrile  
 0%B (0-0.1 min)  
 0-30%B (0.1-10 min)  
 30%B (10-11 min)  
 30-100%B (11- 12 min)  
 100%B (12-26 min)  
 0.3 mL/min, 30°C, 2uL(DMSO soln.)  
 ELSD (SEDEX 90LT, 40°C, 3.5Bar)

### Vitamin Mix



	RT	%RSD (n=6)		LOD (S/N=3)
	Minutes	RT	Response	ng (o.c.)
1-B1: Thiamine	0.92	0.11	2.6	5.7
2-B8: myo-Inositol	1.14	0.09	6.2	6.3
3-B6: Pyridoxine	1.44	0.16	2.7	1.8
4-C: Ascorbic acid	1.94	0.25	2.4	9.6
5-B5: Pantothenic acid	7.32	0.15	6.8	6.9
6-B9: Folic acid	9.92	0.09	3.1	8.0
7-B12: Cyanocobalamin	10.17	0.11	4.1	4.6
8-B2: Riboflavin	10.49	0.11	3.4	5.0
9-B7: Biotin	10.70	0.09	4.2	4.8
10-A: Retinol	17.64	0.06	6.7	1.7
11-K2: Menaquinone	20.87	0.07	2.1	14.2
12-D2: Ergocalciferol	22.89	0.06	1.5	47.6
13-D3: Cholecalciferol	23.26	0.06	2.6	52.6
14-E: α-Tocopherol	23.61	0.07	1.6	45.0
15-K1: Phylloquinone	25.86	0.07	1.7	41.7

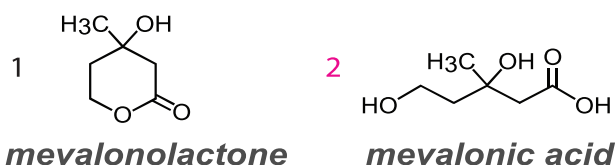
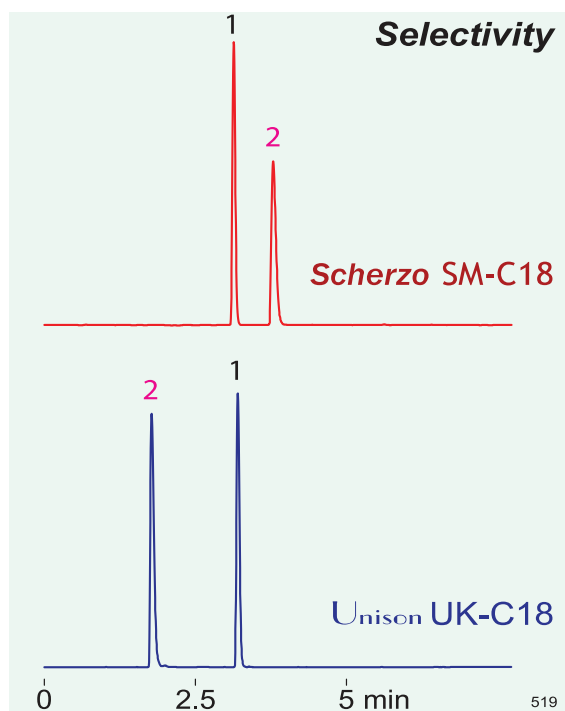
Since water-soluble vitamins are hydrophilic and fat-soluble vitamins are hydrophobic, simultaneous analysis of both types of vitamins is a very difficult issue.

SM-C18 succeeds in this application; gradient elution from acidic pH aqueous conditions to acetonitrile provides sufficient separation of various 15 vitamins. D2 and D3 is also separated. Thiamine (basic compound) and ascorbic acid are both retained as a multi-mode ODS column advantage. Detection is done by ELSD in this application, but there is an opportunity for MS detection with the use of a volatile mobile phase.

Data provided by Dr. Eric VERETTE, SEDERE S.A.S., France

## Scherzo SM-C18 column applications (Separation Selectivity)

Scherzo C18 columns are designed by using the same silica and ODS ligand density as a Unison UK-C18 column. SM-C18 may be effective in changing selectivity when it is difficult to separate impurities on an ODS column.

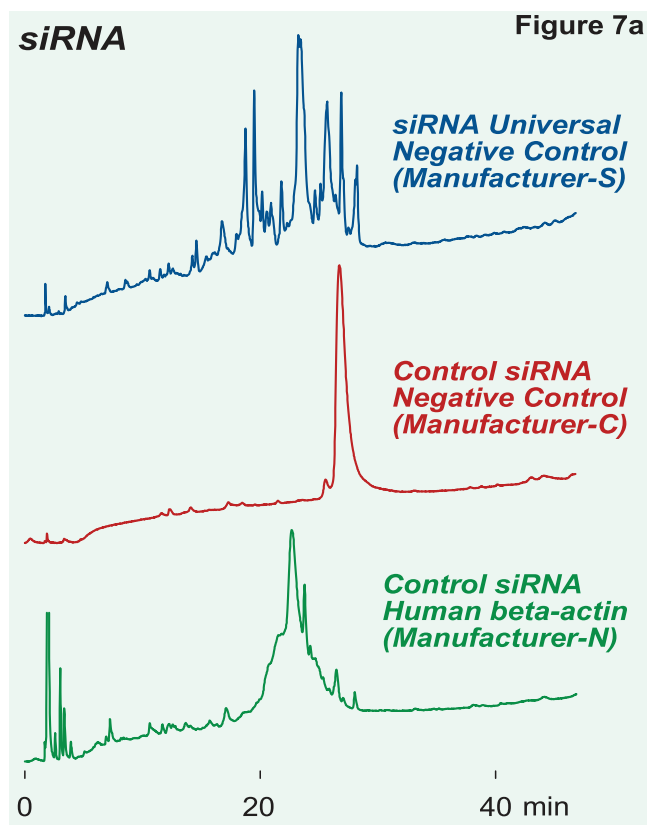


150 x 3 mm  
5mM ammonium formate /acetonitrile = 90 /10  
0.4 mL/min (10MPa), 37 °C, ELSD

Scherzo SM-C18 and Unison UK-C18 are designed in such a way that they provide similar retention of non-ionic compounds. In addition, SM-C18 can retain both anionic / cationic compounds under neutral pH conditions. This means that there is an opportunity to improve separation on SM-C18 than on a conventional ODS by only changing columns under the same conditions.

This comparison between SM-C18 and UK-C18 helps to determine whether or not an unknown compound is ionic.

## Scherzo SW-C18 column applications (Acidic Compounds)



Scherzo SW-C18 has a small amount of strong ionic ligands which is a novel structural idea from traditional ion-exchange columns. This surface structure provides better elution of strong ionic compounds.

siRNA (small interfering RNA), around 21mer base paired double stranded RNA, is getting lots of attention for next generation medicine. But it is very polar and has a lot of phosphoric acids inside, and is therefore difficult to analyze in RP mode. Figure 7a on SW-C18 shows multiple peaks of commercial siRNA reagents which may include different structural compounds. SW-C18 seems to recognize the different number of nucleic bases (hydrophobic interaction) and phosphoric acids (ionic interaction). SW-C18 may be useful for structure study of siRNA.

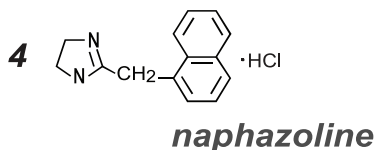
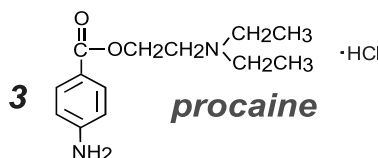
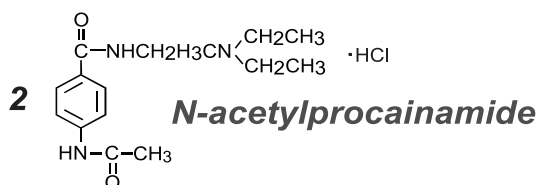
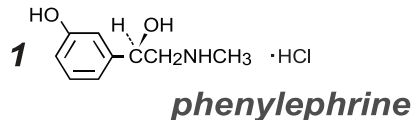
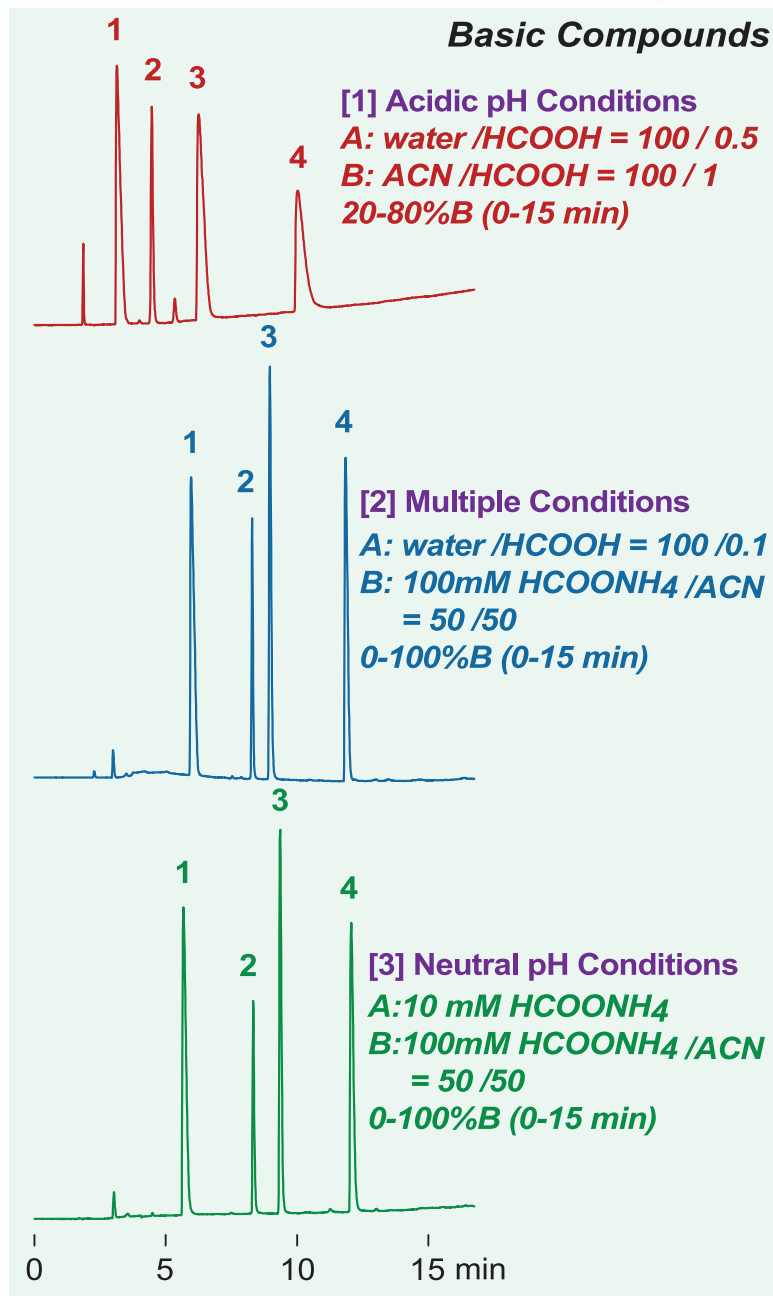
SW-C18 can separate not only siRNA, but also ATP or oligo-nucleotides without adding an ion-pairing reagent in the eluent. SW-C18 is applicable for strong ionic compounds like phosphoric compounds because there is a small amount of ionic ligands on the surface of the stationary phase.

Scherzo SW-C18, 150 x 3 mm  
A: 10 mM CH<sub>3</sub>COONH<sub>4</sub>  
B: 200 mM CH<sub>3</sub>COONH<sub>4</sub> / ACN = 85 / 15  
0-100%B (0-45 min)  
0.4 mL/min (10 MPa), 37 °C, 260 nm

## Scherzo SW-C18 column applications (Drugs, Metabolites)

Scherzo SW-C18 provides excellent performance for basic drug compounds and ionic metabolites between low and neutral pH conditions due to a small amount of strong anionic / cationic ligands. SW-C18 may be useful for DMPK or metabolome studies.

### ● Various elution conditions for basic drugs



Scherzo SW-C18, 150 x 3 mm  
 0.4 mL/min (9 MPa), 37 °C, 260 nm

There are three basic ways to analyze basic compounds on Scherzo SW-C18.

[1] Gradient with formic acid + organic solvent concentration will retain basic compounds as strong positive ions. Various cationic compounds are retained with initial / final formic acid concentration.

[2] Gradient between low concentration of formic acid and high concentration of its salt with organic solvent may separate a wide-range of polar and strong cationic compounds.

[3] Gradient with ionic strength at neutral pH and organic solvent is a very useful easy method to analyze both basic and acidic compounds.

### Product Information

Spec.: purified porous silica, 3µm particle, 13nm pore, ODS+anion+cation ligand

Column Name	Column I.D.	Column Length	Guard Column
Scherzo SS-C18	0.075mm - 0.5mm	10mm, 20mm, 30mm	Guard Holder Cartridge Column
Scherzo SM-C18	1mm, 1.5mm, 2mm, 3mm	50mm, 75mm, 100mm	
Scherzo SW-C18	4.6mm, 6mm, 10mm	150mm, 250mm, 500mm	