Imtakt's 10th anniversary product

The world 's first multi-mode ODS column

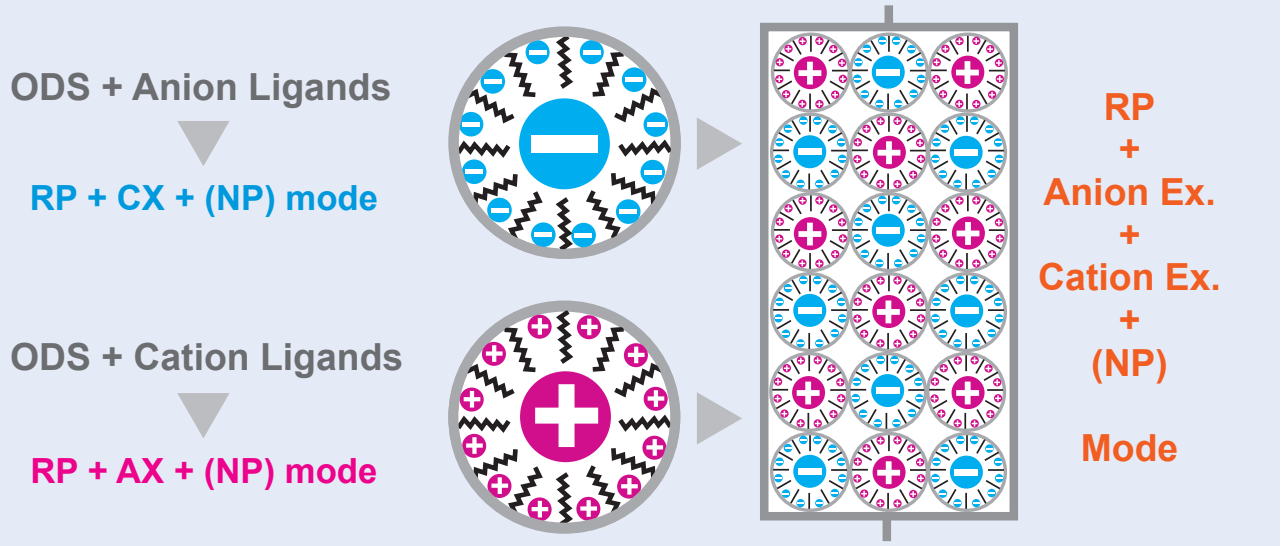
Anion Exchange + Cation Exchange + Normal Phase + Reversed Phase

Scherzo SM-C18

Simultaneous analysis for both cationic and anionic compounds
ODS + IEX or Reversed Phase + Normal Phase
For polar compounds
LC-MS compatible without using ion-pair reagent

Purified Porous Silica / Particle Size 3um / Pore Size 13nm / ODS + Anion Exchange + Cation Exchange Ligands

Appearance of revolutionary multi-mode ODS column



Imtakt has developed a revolutionary multi-mode ODS column.

Many biomaterials / metabolites are ionic and cannot be retained on conventional ODS columns. Recent solutions for this issue have the following limitations:

* Ion-pairing RP chromatography is not compatible with LC-MS
* Aqueous NP (HILIC) fails to separate polar & non-polar compounds

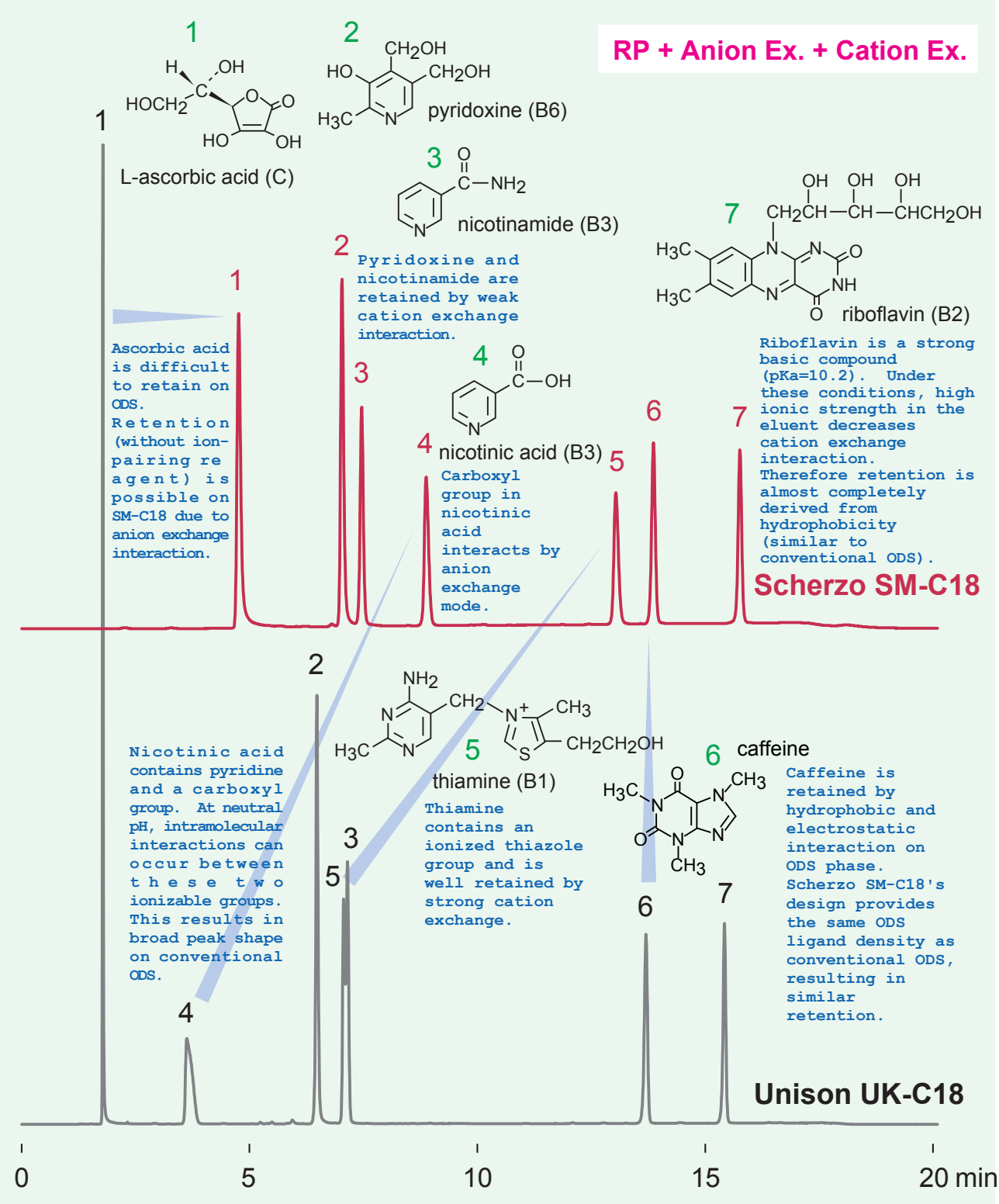
Scherzo SM-C18 can solve many of these problems. SM-C18 contains ODS+cation+anion ligands and can be used to separate highly polar compounds. Unlike other mixed-mode RP columns, Scherzo SM-C18 contains ODS ligands, and shows similar behavior to conventional ODS columns for non-ionic compounds. In addition, the IEX ligands on SM-C18 are highly polar and provide normal phase mode. This allows for both RP + NP separation mode.

This next generation (multi-mode) ODS column expands the separation possibilities for the ODS phase.

Separation mechanism on multi-mode ODS (RP + Anion Ex.+ Cation Ex.)

Scherzo SM-C18 is a multi-mode ODS column that provides the following modes of separation: reversed-phase, anion exchange, and cation exchange. These interactions enable the separation of water-soluble vitamins without the use of ion-pairing reagents.

Water-soluble vitamins



150 x 3 mm (Scherzo SM-C18, Unison UK-C18)
A: 3mM ammonium formate
B: 25mM ammonium formate /acetonitrile = 80 / 20
0 - 100%B (0 - 15 min)
0.4 mL/min (8-9MPa), 37 deg.C, 260 nm
2 uL (0.08-1.5ug, diluted with 0.1M ammonium formate)

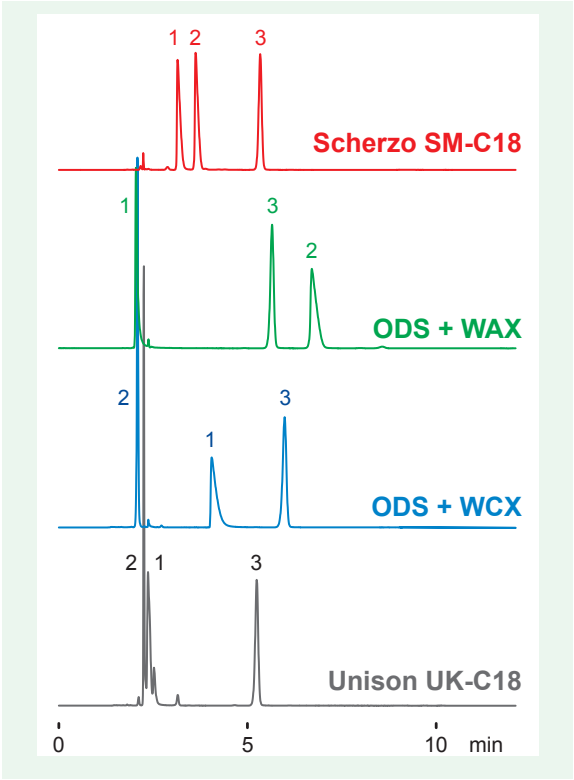
The mobile phase contains low salt concentration and does not require ion-pairing reagents. A dual gradient consisting of organic solvent strength (RP) and ionic strength (IEX) is effective at producing a balanced separation for these water-soluble vitamins.

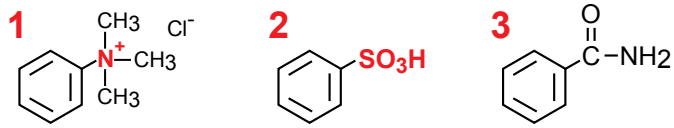
It is sometimes necessary to add salt to a sample solution containing both anions and cations. This can help to prevent solute intermolecular interactions.

Separation mechanism on multi-mode ODS (RP + Anion Ex.+ Cation Ex.)

Conventional ODS columns can struggle to retain polar ionic compounds. But Scherzo SM-C18 will be useful in separating both anionic and cationic compounds.

ODS column comparison





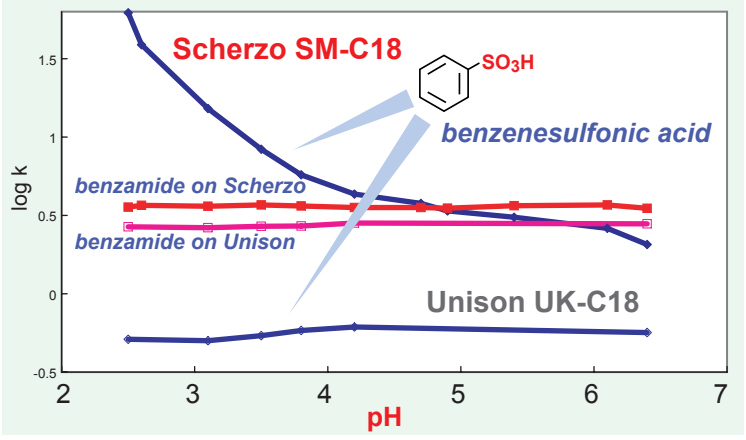
benzenesulfonic acid *benzamide*

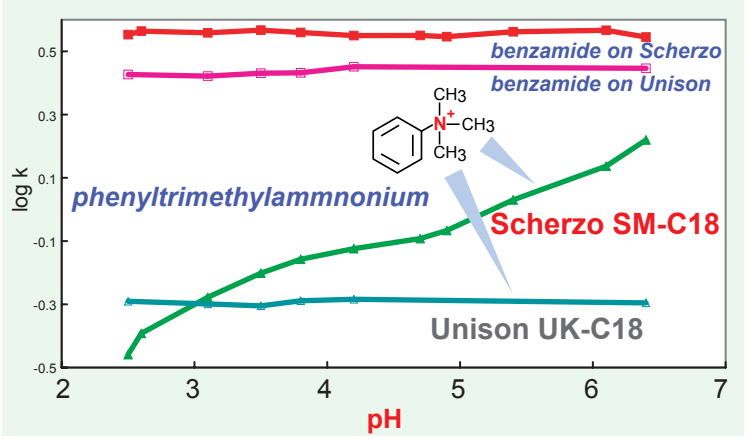
phenyltrimethylammnonium

Strong ionic compounds, such as quaternary amines or sulfonic acids, can be difficult to retain / separate on conventional ODS. Mixed-mode RP columns have a single ionic ligand (anion or cation) and struggle to retain both acidic and basic compounds. Separation of both acids and bases require two different methods with two different mixed-mode RP columns. In contrast, the multi-mode ODS column, Scherzo SM-C18, consists of both anionic and cationic ligands. Separation of both cations and anions is possible using one column and one method.

150 x 3 mm
50 mM ammonium formate /acetonitrile = 85 /15
0.4 mL/min (8-9MPa), 40 deg.C, 260nm

pH dependency to retention





(50mM HCOOH - 50mM HCOONH4) /acetonitrile = 85 /15, 40 deg.C

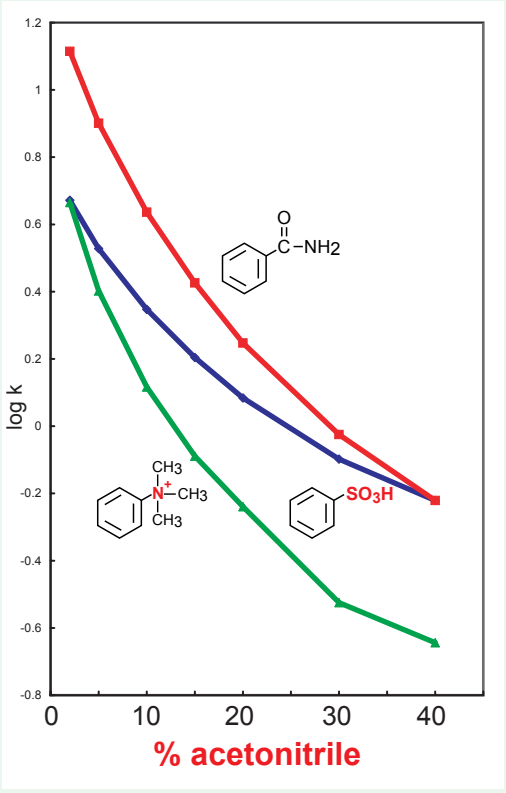
Because Scherzo SM-C18 is a multi-mode column consisting of ODS+cation+anion ligands, retention for ionic compounds can be affected by eluent pH. In the figures to the left, strong ionic solutes like sulfonic acid and quaternary amine have poor retention on conventional ODS column (regardless of eluent pH). However, retention for these ionic compounds on Scherzo SM-C18 (IEX) is dependent upon eluent pH (due to changes in ionic interaction). The data shows that retention for acidic compounds is highest using low pH conditions, highest for basic compounds using high pH conditions, and constant for neutral compounds (regardless of eluent pH).

Scherzo SM-C18 provides hydrophobic interaction and ion exchange interaction (including both anion and cation exchange). Changing organic strength and ionic strength on Scherzo SM-C18 can provide different selectivity for ionic compounds. This multi-mode ODS column provides different selectivity than conventional ODS, and will be a powerful tool for separation scientists.

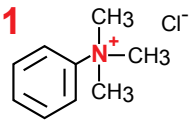
Effect of pH, organic solvent, salt concentration, and temperature on solute elution

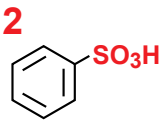
Elution properties may be affected by eluent pH, organic solvent composition, salt concentration, and temperature.

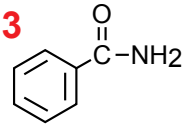
Organic solvent dependency to retention



50mM HCOONH4 / acetonitrile, 40 deg.C

1 

2 

3 

benzenesulfonic acid ***benzamide***

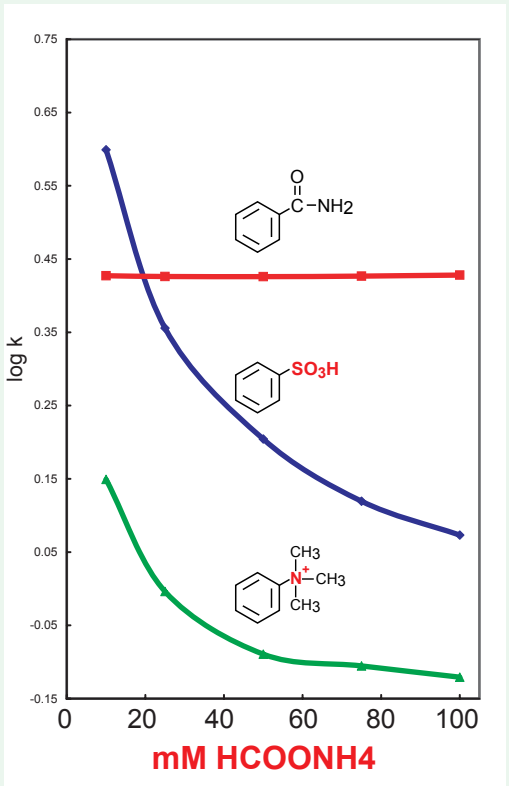
phenyltrimethylammnonium

One of the benefits to using Scherzo SM-C18 is its ability to retain ionic compounds. Elution for ionic compounds is dependent upon many parameters.

The figure to the left shows the relationship between organic solvent concentration and retention. The substituted benzene rings have the following properties: base (quaternary amine), acid (sulfonic acid), and neutral (amide). Each solute has unique ionic properties. As a result, elution for these ionic compounds changes with decreasing ionic strength (due to increasing organic solvent composition).

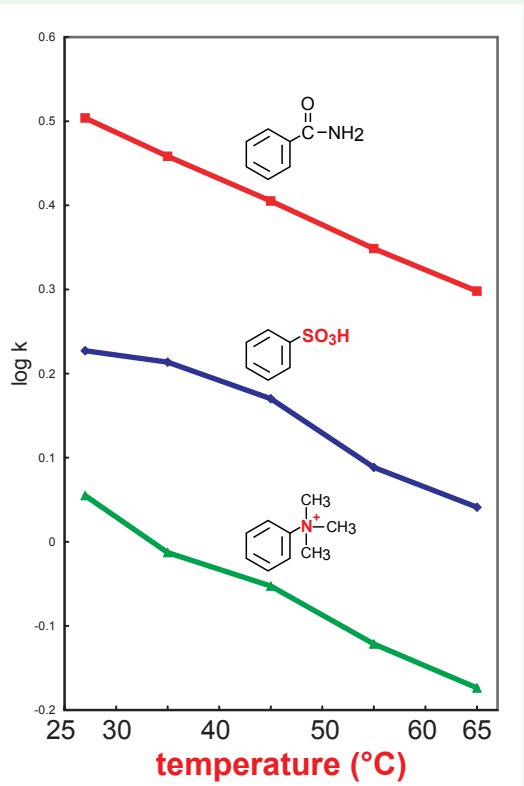
The figure to the lower left shows retention vs. salt concentration. Benzamide (neutral) is unaffected by salt concentration. However, retention for the ionic compounds is affected by salt concentration. The data to the lower right shows retention vs. temperature. These three solutes have hydrophobic properties and therefore show decreasing retention with increasing temperature.

Salt concentration dependency to retention



HCOONH4 / acetonitrile = 85 / 15, 40 deg.C

Temperature dependency to retention

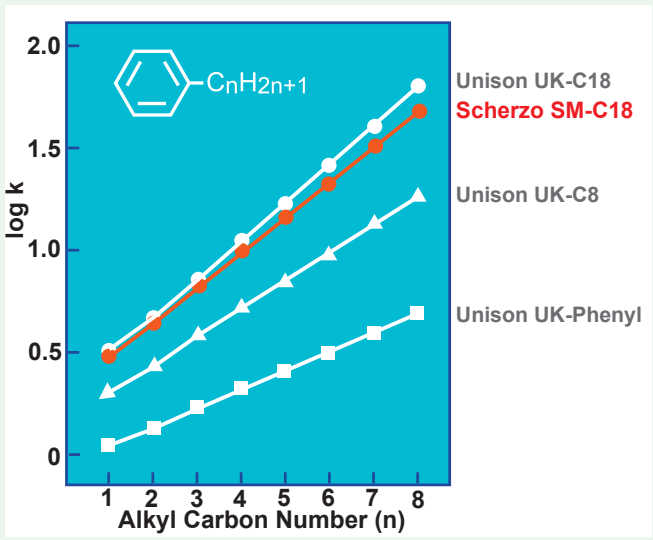


50 mM HCOONH4 / acetonitrile = 85 / 15

Separation mechanism on multi-mode ODS (Reversed-phase)

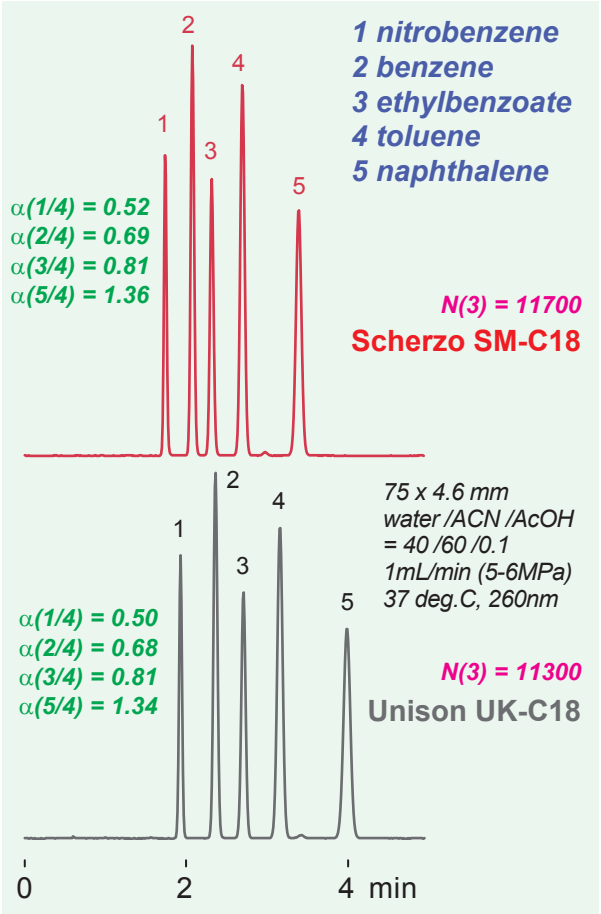
The main mode of interaction for Scherzo SM-C18 is hydrophobicity (via the ODS ligand). Scherzo SM-C18 is designed to get similar results as conventional ODS column (Unison UK-C18) for hydrophobic neutral compounds.

Hydrophobicity

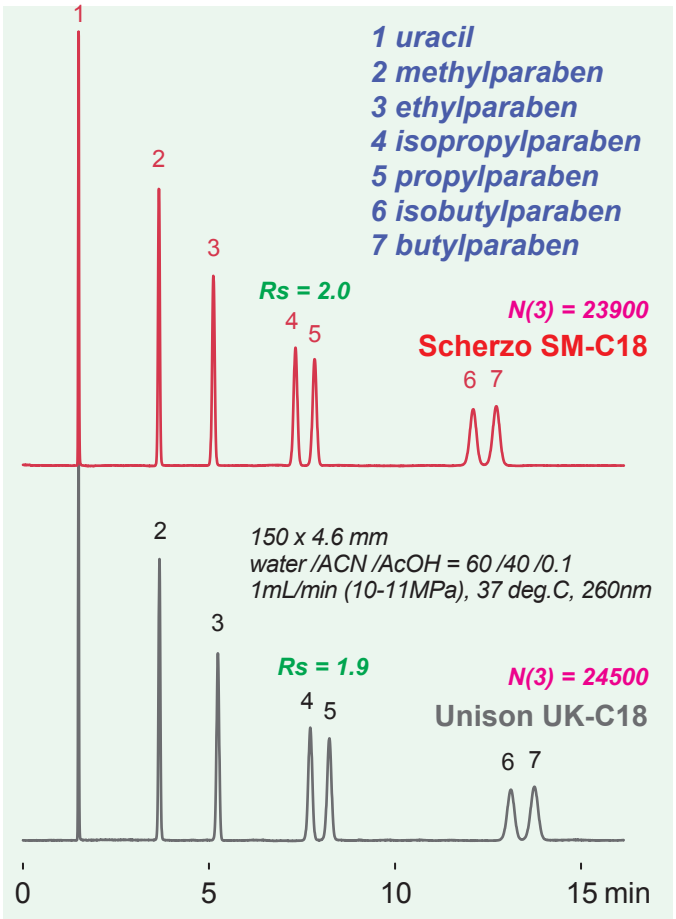


water /acetonitrile = 40 /60, 1 mL/min, 37 °C

Hydrophobic interaction is an important interaction for the ODS phase. Scherzo SM-C18 has similar hydrophobicity as conventional ODS column. The figure to the left shows the relationship between retention and alkyl carbon number (n) of alkylbenzenes. The slope (log k / CH2) indicates hydrophobicity; the data shows hydrophobicity for Scherzo SM-C18 is similar to that of Unison UK-C18. Therefore, using SM-C18 and UK-C18 (under the same experimental condtions) may be useful for tracking elution behavior of ionic compounds.



Comparison of hydrophobic compounds shows similar elution for SM-C18 and UK-C18. Resolution and column performance is similar. This indicates that neutral hydrophobic compounds are not affected by ionic ligands on SM-C18.

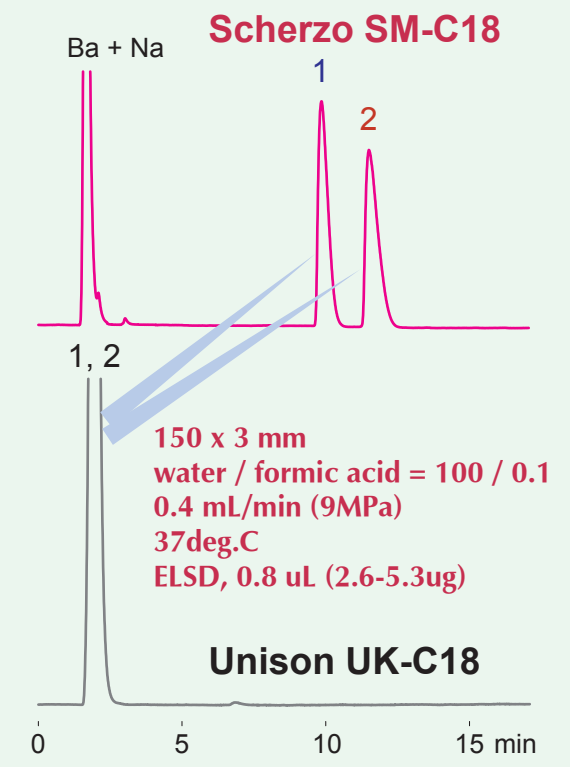


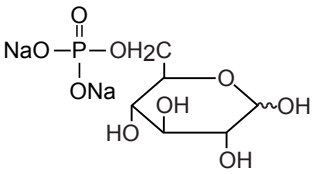
This data shows similar results for separation of isomers on SM-C18 and UK-C18. This indicates that molecular recognition of hydrophobic compounds on SM-C18 is the same as conventional ODS columns. This excellent performance is observed on all Imtakt 3um particle products (including multi-mode ODS).

Separation mechanism on multi-mode ODS (Ion Exchange)

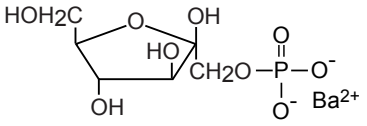
A multi-mode ODS column, Scherzo SM-C18, can be used as an ion-exchange column when strong ionic compounds do not interact with reversed-phase ligands.

Anion exchange separation

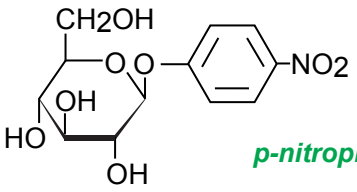




1) D- glucose-6-phosphate (G-6-P)



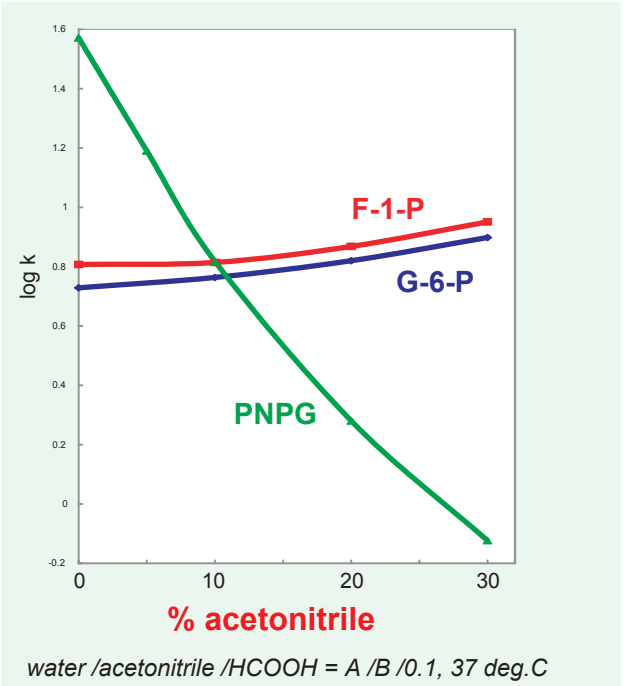
2) D- furctose-1-phosphate (F-1-P)



p-nitrophenyl-beta-glucoside (PNPG)

Scherzo SM-C18 retains strong ionic compounds.
The figure to the left shows that glycophosphates (low pKa values) interact strongly with cation ligands on the stationary phase. These compounds are retained under 100% aqueous conditions. Highly polar compounds, which are retained via ionic interaction (and do not interact with non-polar ligands), can also elute under normal phase conditions (see figure below).

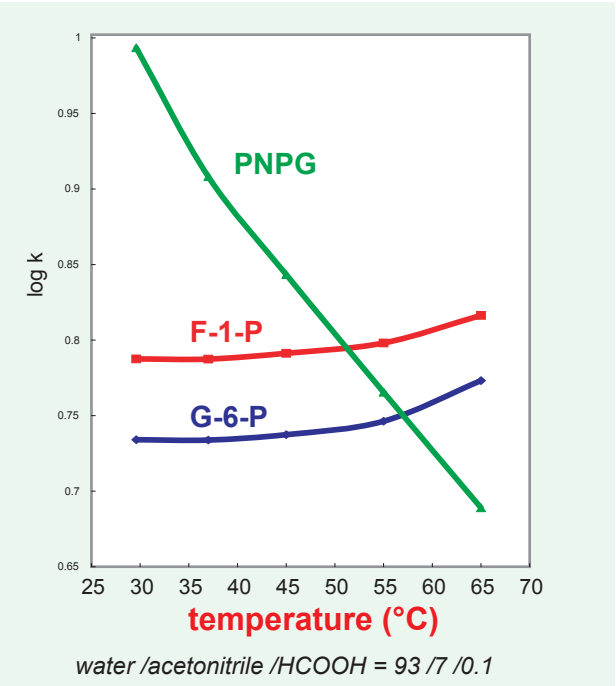
Organic solvent dependency to retention



water /acetonitrile /HCOOH = A /B /0.1, 37 deg.C

PNPG contains a phenol group and shows decreasing retention with increasing organic solvent. In contrast, sugar phosphates (highly polar anionic compounds) seem to exhibit normal phase behavior. This can be useful for LC-MS applications where the addition organic solvent improves ionization efficiency.

Temperature dependency to retention



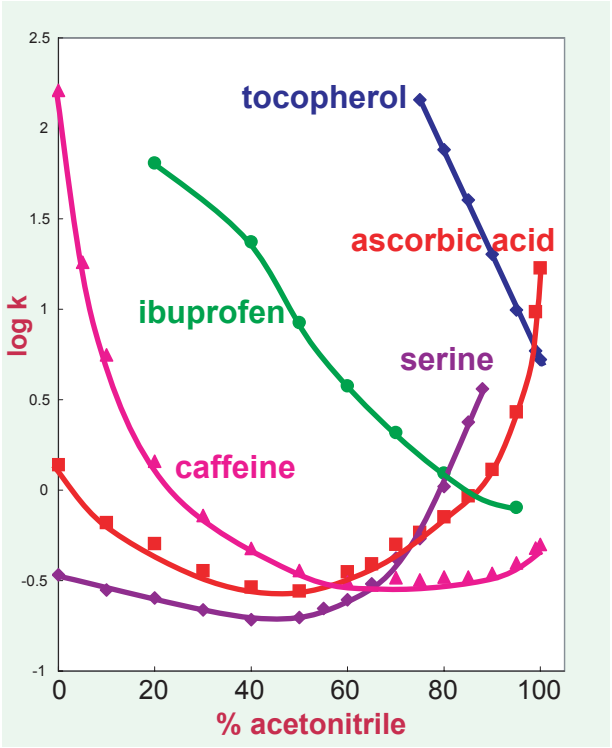
water /acetonitrile /HCOOH = 93 /7 /0.1

PNPG shows decreasing retention at elevated temperatures due to hydrophobicity. In contrast, sugar phosphates show no loss in retention at elevated temperatures. Therefore, column temperature can be tuned to provide a balanced separation between polar and hydrophobic compounds.

Separation mechanism on multi-mode ODS (Reversed-phase + Normal phase)

The main mode of separation for Scherzo SM-C18 is reversed-phase. But normal phase mode may also be useful for highly polar compounds.

Relationship between organic solvent and retention



The figure to the left shows the relationship between acetonitrile concentration and retention on Scherzo SM-C18.

Tocopherol is a non-polar compound and requires high organic solvent composition for elution. Ibuprofen, an acidic and middle-polar compound, also shows reversed-phase elution.

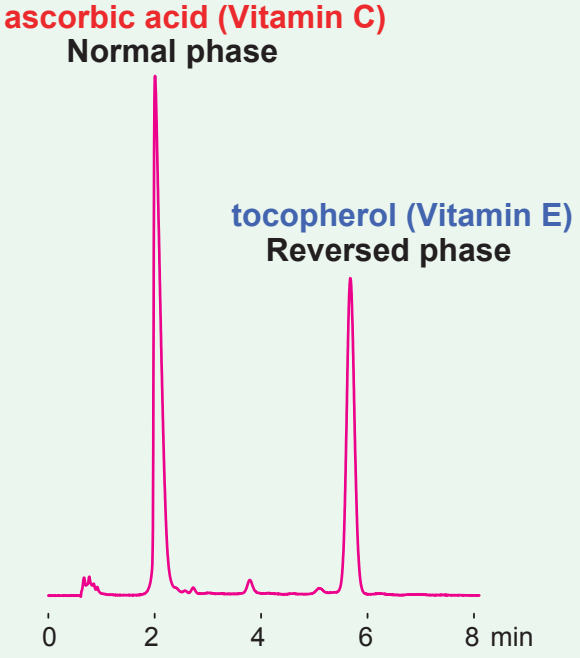
Caffeine is polar, but does have some hydrophobicity; retention is reduced as acetonitrile is increased up to 60% (RP), but increases as acetonitrile goes past 80% (NP).

Ascorbic acid is highly polar; retention is reduced as acetonitrile is increased up to 40% (RP), but increases as acetonitrile goes past 50% (NP).

Serine is highly polar (zwitter ion) and is difficult to retain on conventional ODS. Retention on SM-C18 increases over 50% organic (NP).

Low polarity compounds are retained on SM-C18 via reversed-phase mode. In contrast, polar compounds may be retained via normal phase mode.

Example for RP + NP mode at the same time



Scherzo SM-C18, 50 x 3 mm
water /acetonitrile /formic acid = 5 /95 /0.1
0.4 mL/min, 37 deg.C, 270 nm

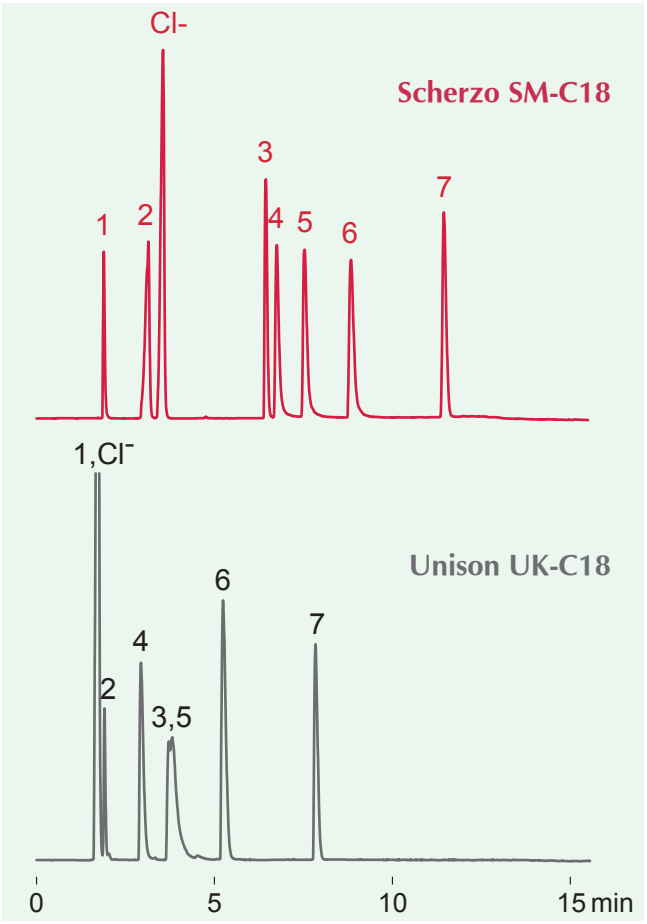
Vitamin formulation often includes both Vitamin C and Vitamin E. The polarity of these compounds is quite different and difficult to analyze with one method.

The figure to the left shows both compounds are separated by using reversed-phase + normal phase. As mentioned above, tocopherol elutes via reversed-phase, but ascorbic acid is retained via normal phase at high organic composition. Therefore, separation is possible under isocratic elution with optimized eluent composition. This method will be useful for vitamin C and E analysis at quality control laboratories.

Application for polar compounds

The multi-mode ODS column, Scherzo SM-C18, will increase separation possibilities over conventional ODS phase by combining basic RP + anion + cation + NP mode.

Neurotransmitters

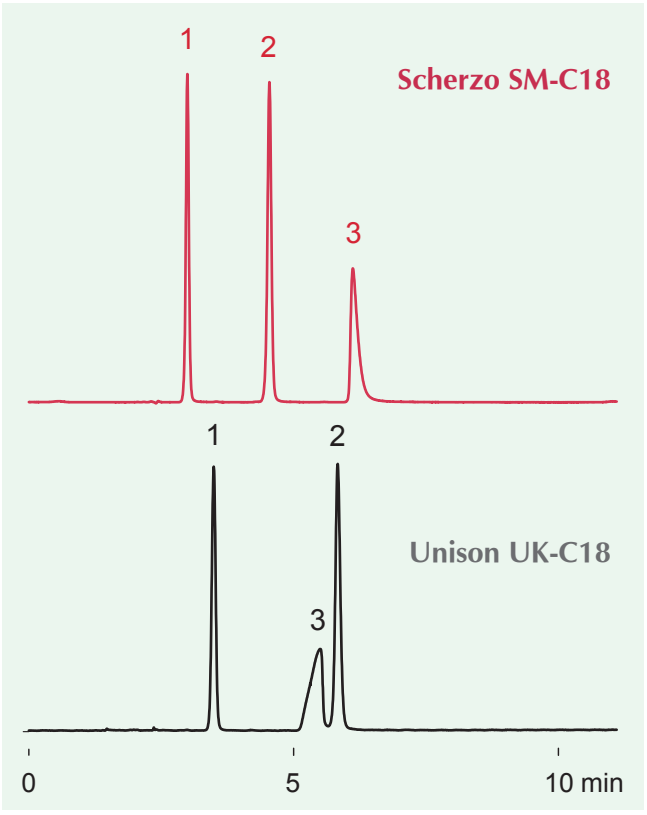


Neurotransmitters, including catecholamines, are highly polar and require ion-pairing on conventional ODS column. The figure to the left shows successful separation for GABA, glutamic acid, acetylcholine, and catecholamines on Scherzo SM-C18 without ion-pairing reagent.

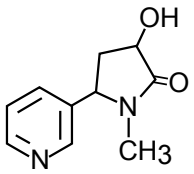
1) 4-aminobutyric acid (GABA)
2) glutamic acid
3) acetylcholine hydrochloride
4) noradrenaline
5) adrenaline
6) dopamine hydrochloride
7) serotonin hydrochloride

150 x 3 mm
A: 3mM ammonium acetate
B: 80mM ammonium acetate /acetonitrile = 80 /20
0-100%B (0-12min)
0.4mL/min (9MPa), 37deg.C, ELSD
3uL (0.65-2.6ug)

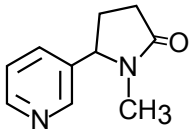
Nicotine and metabolites



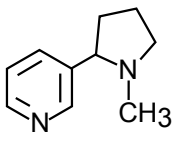
Nicotine and its metabolites are basic and difficult compounds to separate on conventional ODS column. Scherzo SM-C18 can separate these compounds with isocratic elution (neutral pH).



1) hydroxycotinine



2) cotinine

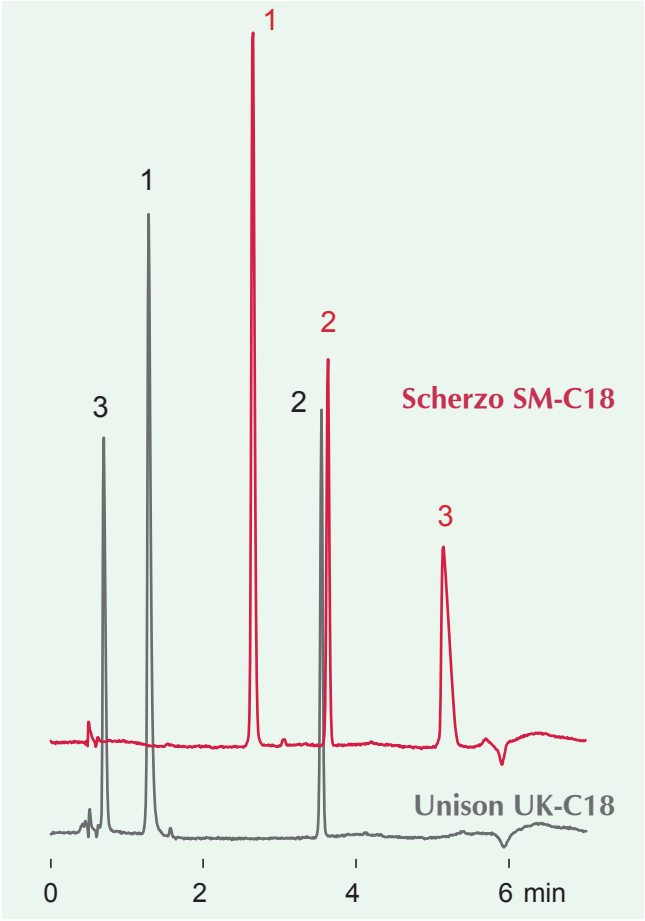


3) nicotine

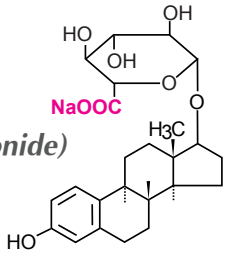
150 x 3 mm
50mM ammonium acetate /acetonitrile = 85 /15
0.4mL/min (9-10MPa), 37deg.C, 260nm
0.4uL (0.25ug)

Application for polar compounds

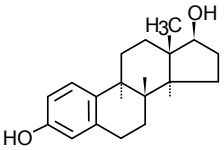
Steroid hormone and metabolites



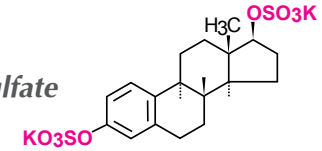
Sulfate or glucuronide metabolites are highly polar compounds with anionic groups and are difficult to retain on conventional ODS columns. In contrast, SM-C18 interacts with the dissociated forms of these metabolites via IEX mode. This means there is an opportunity to improve both retention and LC-MS sensitivity for these metabolites by using SM-C18.



1) *β-estradiol 17-(β-D-glucuronide)*



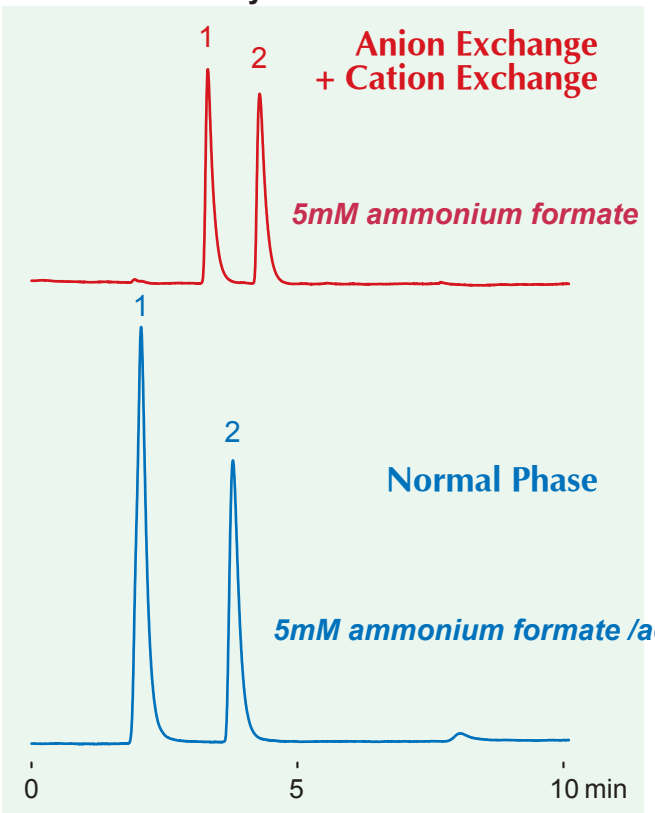
2) *17β-estradiol*



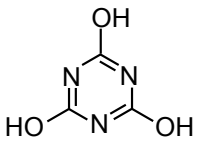
3) *β-estradiol 3,17-disulfate*

50 x 3 mm
A: 5mM ammonium acetate
B: 125mM ammonium acetate /acetonitrile = 20 /80
30-100%B (0-5min)
0.5mL/min (5-9MPa), 37deg.C, 280nm
1uL (0.5-2.5ug)

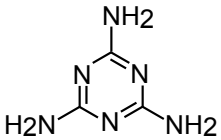
Melamine and cyanuric acid



Melamine (cation) and cyanuric acid (anion) are both polar compounds and difficult to retain on conventional ODS columns and mixed-mode RP columns. Scherzo SM-C18 has anion / cation ligands and can retain both compounds via IEX mode. In addition, normal phase mode can be used to retain these polar compounds.



1) cyanuric acid



2) melamine

Scherzo SM-C18, 150 x 3 mm
0.4mL/min (4-9MPa), 37deg.C, ELSD
3uL (1.5ug, 2.5%NH4OH)

Recommendations for Scherzo SM-C18

Scherzo SM-C18 consists of ODS, anion, and cation ligands. The stationary phase interacts with ionic solutes which can sometimes lead to fluctuating retention and separation (due to electric charge between eluent and stationary phase). The following recommendations should be considered for reproducibility and ruggedness.

Confirm the reproducibility by repeat-injections

Variable retention / separation can be observed during repeat injections due to variable dissociation of ligands on the stationary phase. To confirm optimized condition, several injections are recommended. If retention is not reproducible, it is recommended to either increase ionic strength in eluent or change to gradient elution.

Salt concentration in sample solution

Acidic and basic compounds in sample solution may interact with each other forming complexes which can have a detrimental effect on peak shape due to undesirable dispersion within the column. It is therefore recommended to add salt, acid, or alkali to the sample solution in order to avoid this problem.

Do not use pH meter. Buffer in eluent should be prepared by mixing of acid and base solutions

Buffers prepared with pH meters (e.g. titrate to desired pH) are not reproducible. Precision for [salt] and pH is crucial for buffers used with HPLC. It is strongly recommended to prepare buffers by measuring appropriate volumes of (equal concentration) acid and base solutions. Please refer to the relationship between acid/base volume ratio and pH table below. Buffer concentration should be optimized for each method.

Acetate Buffer (room temp.)

20mM CH_3COOH	10	9	8	7	6	5	4	3	2	1	0
20mM CH_3COONH_4	0	1	2	3	4	5	6	7	8	9	10
pH	3.2	3.8	4.1	4.3	4.6	4.7	4.8	5.0	5.3	5.6	6.8

Formate Buffer

50mM HCOOH	10	8	6	4	2	1	0.5	0.2	0.1	0
50mM $HCOONH_4$	0	2	4	6	8	9	9.5	9.8	9.9	10
pH	2.5	3.1	3.5	3.8	4.2	4.7	4.9	5.4	6.1	6.4

pH values in the table to the left were measured under room temperature and fixed concentration. These pH values will vary slightly due to differences in temperature and concentration. Appropriate volumes of (equal concentration) acid and base solutions should be measured and mixed in order to achieve desired pH. After mixing of buffered solution, appropriate volume of organic should be added in order to achieve desired aqueous / organic composition.

Ordering Information

Scherzo SM-C18

Particle: 3um Silica, Pore Size: 30nm, Ligand: ODS + Anion Exchange + Cation Exchange

3µm

Length (mm)	Product Code						
	1 mm I.D.	1.5 mm I.D.	2 mm I.D.	3 mm I.D.	4.6 mm I.D.	6 mm I.D.	10 mm I.D.
10	-	-	SM020	SM030	SM000	-	-
20	-	-	SM029	SM039	SM009	-	-
30	SM011	SM071	SM021	SM031	SM001	SM061	SM0P1
50	SM012	SM072	SM022	SM032	SM002	SM062	SM0P2
75	SM013	SM073	SM023	SM033	SM003	SM063	SM0P3
100	SM014	SM074	SM024	SM034	SM004	SM064	SM0P4
150	SM015	SM075	SM025	SM035	SM005	SM065	SM0P5
250	SM016	SM076	SM026	SM036	SM006	SM066	SM0P6
500	-	-	-	-	SM007	-	-

Guard Holder (Column coupler enclosed)

Separation column ID	Product Code
for 1 - 6 mm I.D.	GCH01S
for 10 mm I.D.	GCH02M

Guard Cartridge SM-C18

Separation column ID	Product Code	Note
for 1-1.5 mm I.D.	GCSM0C	5 x 1 mm, 3 pcs
for 2 - 6 mm I.D.	GCSM0S	5 x 2 mm, 3 pcs
for 10 mm I.D.	GCSM0M	10 x 8 mm, 2 pcs