There are a lot of hydrophilic compounds which are related to pharmacology, food, and biochemistry, including: water soluble vitamins, catecholamines, organic acids, nucleotides, etc. These compounds are difficult to retain by reversed-phase analysis without the use ion-pairing additives in the mobile phase.

Several mixed-mode reversed-phase stationary phases consisting of alkyl and ion exchange ligands have been introduced into the HPLC column market (Fig-1). However, these mixed-mode stationary phases have the following disadvantages: (1) Column designs that employ either RP/cation or RP/anion offer limited solutions for unknown samples (difficult to choose which column to use) and (2) The RP/anion/cation phase columns that are marketed today consist of only one ligand structure. A balance between hydrophobicity and ionic strength cannot be controlled when this surface structure is employed.

We have overcome these disadvantages with a revolutionary multi-mode ODS phase structure. This technology consists of three types of ligands, including: ODS, anion, and cation ligands (Fig-2). This mode of separation is advantageous for various types of analysis, including: hydrophilic anions, cations, and drug metabolite analysis containing both ionic-hydrophobic and hydrophilic compounds. In addition, it can be useful for LC-MS by using low buffer concentration without ion pairing additives.
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.

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. 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).

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 left figure 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 middle figure shows retention vs. salt concentration. Benzamide (neutral) is unaffected by salt concentration. However, retention for the ionic compounds is affected by salt concentration.

The right figure shows retention vs. temperature. These three solutes have hydrophobic properties and therefore show decreasing retention with increasing temperature.

Hydrophobic interaction is an important interaction for the ODS phase. Scherzo SM-C18 has similar hydrophobicity as conventional ODS column. The right figure 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 conditions) may be useful for tracking elution behavior of ionic compounds.

The left figure 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 3um particle products (including multi-mode ODS).
Scherzo SM-C18 retains strong ionic compounds. The left figure 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.

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 (the middle figure). This can be useful for LC-MS applications where the addition organic solvent improves ionization efficiency.

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

The left figure 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.

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 right figure 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.

Mixed-mode RP columns struggle to achieve solute retention and repeatable separations as the interactions are complicated due to the presence of both reversed-phase and ion exchange modes. Scherzo SM-C18 addresses this problem with a novel stationary phase design to provides excellent reproducibility.

There are many difficult compounds to separate on conventional ODS column. Scherzo SM-C18 can retain or separate these compounds with individually optimized elution condition (pH, ionic strength etc.).
Mixed-Mode Reversed-Phase

<table>
<thead>
<tr>
<th>Blend packing</th>
<th>Mixed bonding</th>
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<tbody>
<tr>
<td>![Blend packing diagram]</td>
<td>![Mixed bonding diagram]</td>
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</table>

Multi-Mode ODS Column Structure

Scherzo SM-C18

Mixed bonding + Blend packing
Water-soluble vitamins

RP + Anion Ex. + Cation Ex.

Comparison among ODS columns

phenyltrimethylammonium

benzenesulfonic acid

benzamide
pH dependency to log k

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

Solvent, Ionic Strength, Temp Effect

Organic Solvent

<table>
<thead>
<tr>
<th>Organic Solvent</th>
<th>Ionic Strength</th>
<th>Temperature</th>
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</thead>
<tbody>
<tr>
<td>Unison UK-C18</td>
<td>Scherzo SM-C18</td>
<td>Unison UK-C18</td>
</tr>
</tbody>
</table>

50mM HCOONH4 / ACN 40 °C

HCOONH4 / ACN = 85 / 15

50mM HCOONH4 / ACN = 85 /15
**Reversed-Phase Mode**

1 uracil  
2 methylparaben  
3 ethylparaben  
4 isopropylparaben  
5 propylparaben  
6 isobutylparaben  
7 butylparaben

\[
\begin{align*}
\text{water} & / \text{acetonitrile} = 40 / 60, 1 \text{ mL/min}, 37 \, ^\circ \text{C} \\
\text{Alkyl Carbon Number (n)} & \quad N(3) = 23900 \\
& \quad \text{Scherzo SM-C18} \\
\text{Rs} = 2.0 \\
\end{align*}
\]

\[
\begin{align*}
\text{water} & / \text{acetonitrile} = 40 / 60, 1 \text{ mL/min}, 37 \, ^\circ \text{C} \\
\text{Alkyl Carbon Number (n)} & \quad N(3) = 24500 \\
& \quad \text{Unison UK-C18} \\
\text{Rs} = 1.9 \\
\end{align*}
\]

**Ion Exchange Mode**

1) D-glucose-6-phosphate  
   (G-6-P)  
2) D-furctose-1-phosphate  
   (F-1-P)  
3) p-nitrophenyl-beta-glucoside  
   (PNPG)

\[
\begin{align*}
\text{Ba} + \text{Na} & \quad \text{Scherzo SM-C18} \\
\text{150 x 3 mm} & \quad \text{water} / \text{formic acid} = 100 / 0.1 \\
& \quad 0.4 \text{ mL/min (9MPa)}, 37 \, ^\circ \text{C} \\
& \quad \text{ELSD, 0.8 ul (2.6-5.3ug)} \\
\text{Unison UK-C18} & \quad \text{PNPG} \\
\text{0} & \quad \text{15 min} \\
\end{align*}
\]
Normal Phase Mode

Batch-to-batch reproducibility

1) acetaminophen
2) 1-hydroxy-7-azabenzotriazole
3) prednisolone
4) methyl 3-amino-2-thiophenecarboxylate
5) 6-alpha-methylprednisolone
6) corticosterone
7) 4-aminobenzophenone
8) propylparaben

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

Scherzo SM-C18, 75 x 4.6 mm
10 mM ammonium acetate / ACN = 65 / 35
1 mL/min, 37°C, 254 nm
**Nicotine**

1) hydroxycotinine

2) cotinine

3) nicotine

---

**Steroid hormone and metabolites**

1) b-estradiol 17-(b-D-glucuronide)

2) 17b-estradiol

3) b-estradiol 3,17-disulfate

---

**Nicotine**

- **Column:** Scherzo SM-C18, Unison UK-C18
- **Dimensions:** 150 x 3 mm
- **Mobile Phase:** 50 mM ammonium acetate /ACN = 85/15
- **Flow Rate:** 0.4 mL/min (9-10 MPa), 37°C, 260 nm
- **Injection Volume:** 0.4 μL (0.25 μg)

**Steroid hormone and metabolites**

- **Column:** Scherzo SM-C18, Unison UK-C18
- **Dimensions:** 50 x 3 mm
- **Mobile Phase:** A: 5 mM ammonium acetate
  B: 125 mM ammonium acetate /acetonitrile = 20/80
- **Gradient:** 30-100%B (0-5 min)
- **Flow Rate:** 0.5 mL/min (5-9 MPa), 37°C, 280 nm
- **Injection Volume:** 1 μL (0.5-2.5 μg)
Neurotransmitters

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

Basic compounds: Antidepressant drugs

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

**Scherzo SM-C18, 50 x 3 mm**
- **A**: formate buffer
- **B**: acetonitrile

**0-70 %B (0-7 min)**
- 0.4 mL/min (5MPa)
- 37 deg.C, 240 nm
- 2 uL (2ug)

**Basic Compound - salt**

1. Maleic acid
   - HOOC\(\overset{\text{C}}{\overset{\text{C}}{\text{\overset{\text{O}}{\text{O}}}}}\)
   - H\(\overset{\text{C}}{\overset{\text{C}}{\text{\overset{\text{O}}{\text{O}}}}}\)COOH

2. Chlorpheniramine
   - \(\overset{\text{C}}{\overset{\text{C}}{\text{\overset{\text{O}}{\text{O}}}}}\)

**Basic Compound - salt**

**Scherzo SM-C18, 50 x 3 mm**
- **A**: formate buffer
- **B**: acetonitrile

**20-60 %B (0-8 min)**
- 0.4 mL/min (6-8MPa)
- 37 deg.C, 280 nm
- 0.6 uL (1.2ug)

**Basic Compound - salt**

1. Hibenzic acid
   - \(\overset{\text{O}}{\text{O}}}\)

2. Tipepidine
   - \(\overset{\text{S}}{\text{S}}\)

**Scherzo SM-C18, 50 x 3 mm**
- **A**: formate buffer
- **B**: acetonitrile

**20-60 %B (0-8 min)**
- 0.4 mL/min (6-8MPa)
- 37 deg.C, 280 nm
- 0.6 uL (1.2ug)
Melamine / Cyanuric Acid - salt

1) cyanuric acid

2) melamine

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

NaCl salt

Scherzo SM-C18, 150 x 3 mm
A: 5mM ammonium formate
B: acetonitrile
0.4 mL/min (9-10 MPa)
37 deg.C
ELSD (spray chamber 20 deg.C,
drift tube 45 deg.C)
1.6 uL (0.16ug NaCl)
Sodium Sulfite - salt

**Scherzo SM-C18, 250 x 3 mm**

A: 5mM ammonium acetate, B: 100mM ammonium acetate / ACN = 50 / 50

10-100 %B (0-15min)

0.4 mL/min (14MPa), 37 deg.C

ELSD (spray chamber 20 deg.C, drift tube 45 deg.C), 2 µL

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Thiosulfate (H2S metabolite)

**Scherzo SM-C18, 150 x 3 mm**

A: 50 mM acetic acid / ACN = 90 / 10
B: 100 mM ammonium acetate / ACN = 90 / 10

0-100 %B (0-3min), 100 %B (3-10min)

0.4 mL/min (9MPa), 37 deg.C

ELSD (spray chamber 50 deg.C, drift tube 100 deg.C)

1.6 µL (16 ug)
Mevalonic Acid

150 x 3 mm
5 mM ammonium formate /acetonitrile = 90 /10
0.4 mL/min (10 MPa), 37 deg.C
ELSD (spray chamber 20 deg.C,
drift tube 45 deg.C)
1 ul (10ug)

Acetic acid and trihaloacetic acids

1 CH₃COOH
acetic acid (100ppm)

2 CF₃COOH
trifluoroacetic acid (100ppm)

3 CCl₃COOH
trichloroacetic acid (9ppm)

150 x 3 mm
100 mM NH₄H₂PO₄ / acetonitrile = 80 / 20
0.4 mL/min (9-10 MPa), 37 deg.C, 210 nm
20 ul
**Organic Acids**

- **Scherzo SM-C18**
  - Organic Acids
  - Malic acid
  - Tartaric acid
  - Succinic acid (HOOC(CH2)2COOH)
  - Malonic acid (HOOCCH2COOH)
  - Citric acid (HOOCCH2COOH)
  - Fumaric acid
  - Maleic acid

<table>
<thead>
<tr>
<th>Aromatic Carboxylic Acids</th>
<th>Scherzo SM-C18</th>
</tr>
</thead>
</table>
- **Aromatic Carboxylic Acids**
  - Scherzo SM-C18
  - P-hydroxybenzoic acid (pKa=4.5)
  - M-hydroxybenzoic acid (pKa=4.3)
  - Terephthalic acid (p-)
    (pKa=3.5, 4.4)
  - Isophthalic acid (m-)
    (pKa=3.7, 4.6)
  - Homophthalic acid
  - O-phthalic acid
    (pKa=2.7, 4.9)
  - O-hydroxybenzoic acid
    (pKa=2.9, 13.6)
Mononucleotides

Adenosine phosphates (AMP, ADP, ATP)
**Glyphosate**

![Glyphosate peak](image)

- **20 mM ammonium formate / acetonitrile = 90 / 10**
- **9MPa**

A: water / formic acid = 100 / 0.1
B: acetonitrile / formic acid = 100 / 0.1
0-50 %B (0-10min), 8MPa

**Scherzo SM-C18, 150 x 3 mm**
0.4 mL/min, 37 deg.C
ELSD (spray chamber 50 deg.C, drift tube 100 deg.C)
5 uL (5 ug)

**Anserine and Related Compounds**

![Anserine and Related Compounds peak](image)

1. **L- carnosine**
   (beta-alanyl-L-histidine)

2. **L- anserine nitrate**
   (beta-alanyl-1-methyl-L-histidine nitrate)

3. **Inosine 5'-monophosphate** dipotassium

- **0-100% B (0-10min)**
- **0.4 mL/min (9MPa), 37deg.C**
- **ELSD (spray chamber 50 deg.C, drift tube 100 deg.C)**
- **5 uL (2-6ug)**