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.
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**

- L-ascorbic acid (C)
- Pyridoxine (B6)
- Nicotinamide (B3)
- Nicotinic acid
- Pyridoxine and nicotinamide are retained by weak cation exchange interaction.
- Nicotinic acid contains pyridine and a carboxyl group. At neutral pH, intramolecular interactions can occur between these two ionizable groups. This results in broad peak shape on conventional ODS.
- Carboxyl group in nicotinic acid interacts by anion exchange mode.
- Riboflavin is a strong basic compound (pKa=10.2). Under these conditions, high ionic strength in the eluent decreases cation exchange interaction. Therefore retention is almost completely derived from hydrophobicity (similar to conventional ODS).
- Thiamine (B1) contains an ionized thiazole group and is well retained by strong cation exchange.
- Caffeine is retained by hydrophobic and electrostatic interaction on ODS phase. Scherzo SM-C18’s design provides the same ODS ligand density as conventional ODS, resulting in similar retention.

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.
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**

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<tr>
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<th>Scherzo SM-C18</th>
<th>ODS + WAX</th>
<th>ODS + WCX</th>
<th>Unison UK-C18</th>
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**pH dependency to retention**

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.
Elution properties may be dramatically changed by not only pH but also, organic solvent, salt concentration, and temperature.

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.
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.

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 conditions) 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).
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**

Scherzo SM-C18

![Graph showing anion exchange separation](image)

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**

![Graph showing organic solvent dependency](image)

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**

![Graph showing temperature dependency](image)

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

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.

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)
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) \( \beta \)-estradiol 17-(\( \beta \))-D-glucuronide
2) 17\( \beta \)-estradiol
3) \( \beta \)-estradiol 3,17-disulfate

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

Melamine (cation) and cyanuric acid (anion) are both polar compounds and 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.
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**

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<tr>
<th>pH</th>
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**Formate Buffer**

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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.

**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.

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**Ordering Information**

**Scherzo SM-C18**
Particle: 3um Silica, Pore Size: 30nm, Ligand: ODS + Anion Exchange + Cation Exchange

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**Guard Holder**
(Column coupler enclosed)

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