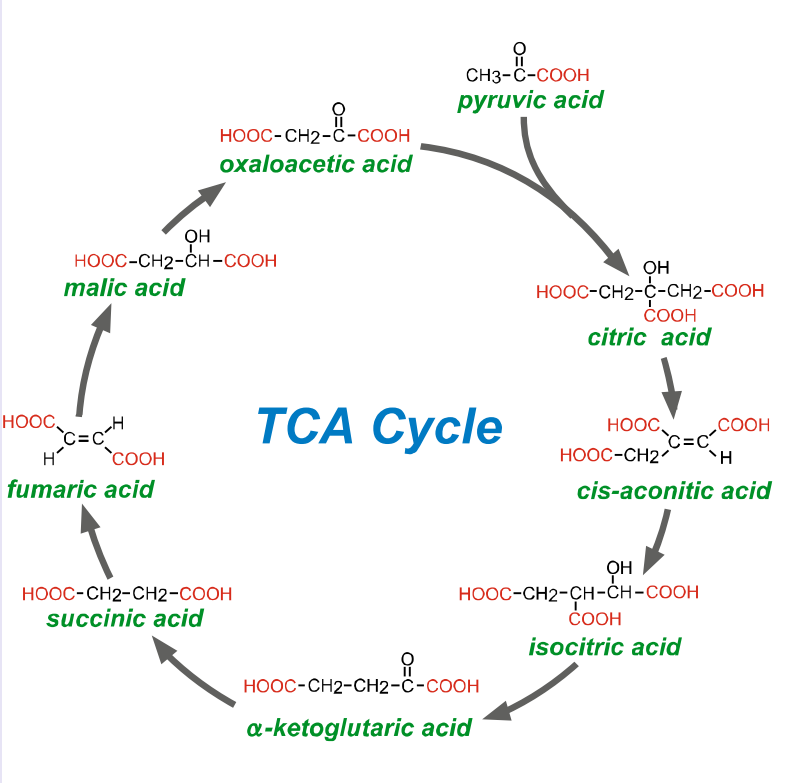
Organic Acid Analysis Column for LC-MS

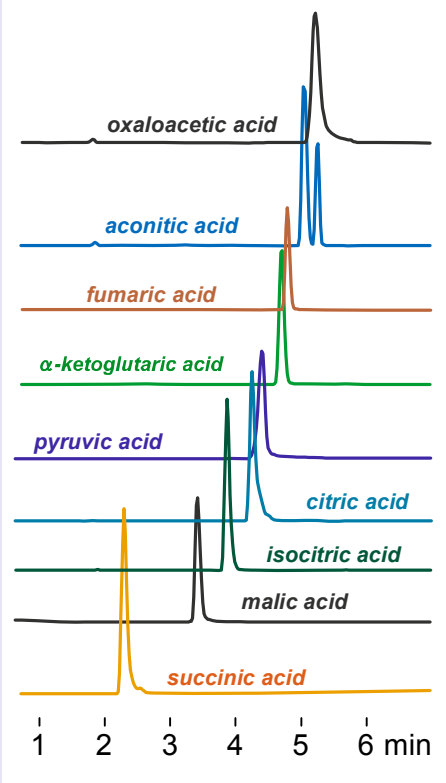
Intrada Organic Acid

Organic acid LC-MS analysis without any derivatization
Ideal for carboxylic acids and other anions ※)
Full general organic acid screens within 10min
One-minute analyses possible on high-throughput columns

3μm spherical pure silica / specialized stationary phase for organic acid (IEX mode, NP mode)

Total organic acid LC-MS analysis for TCA cycle





The di-, and tri- organic acids of the TCA cycle have historically been difficult for the following reasons:

- High polarity makes them difficult to retain on traditional reversed-phase columns
- Multivalent acids containing several different anionic pKa groups, typically show poor peak shape
- Direct detection on UV is difficult
- Conductivity detection is not compatible with ion-exchange modes under gradient elution
- Pre/post- derivatization methods require a complicated HPLC system

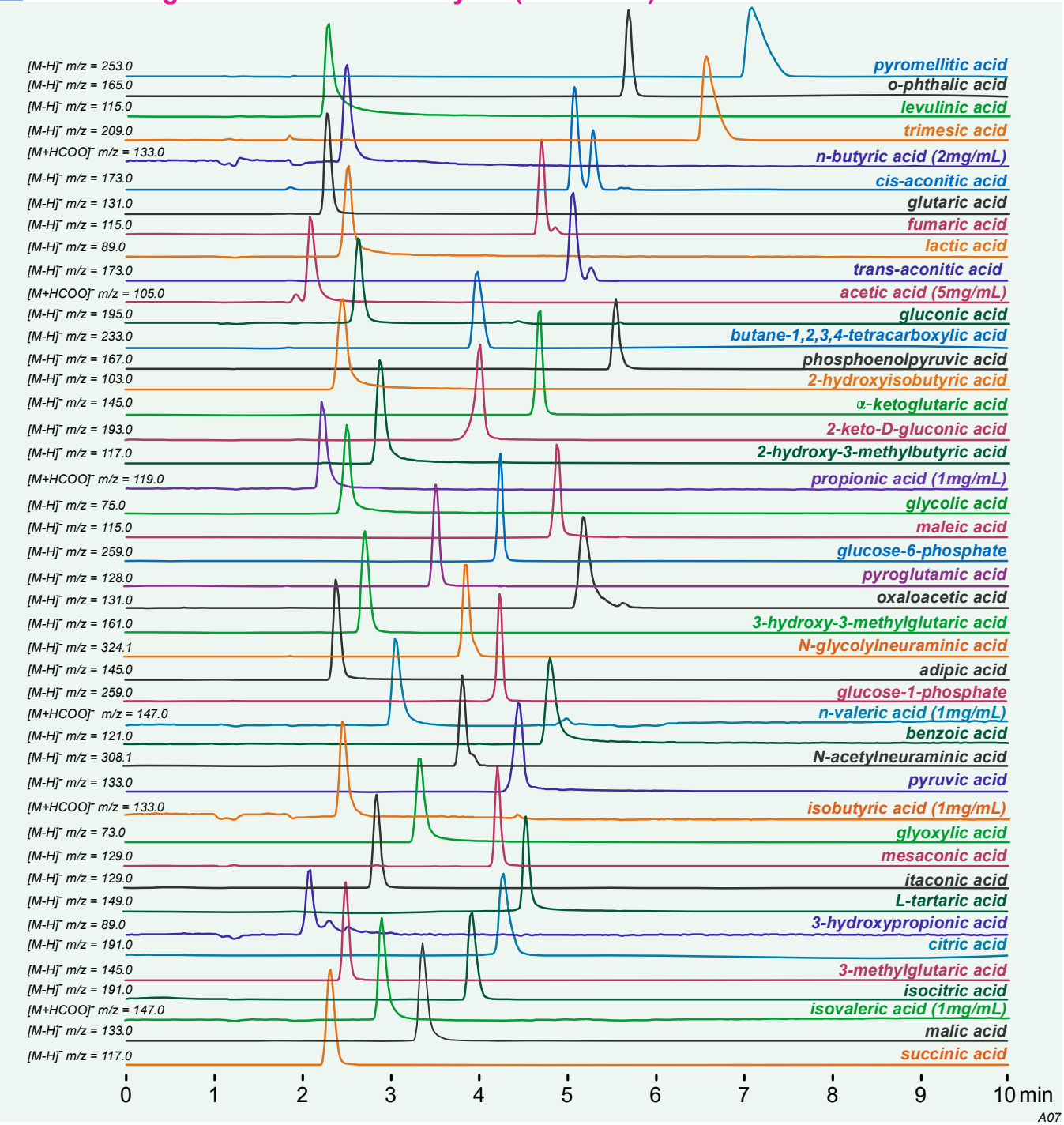
We have developed an innovative HPLC column for total organic acid analysis using LCMS. This column does not require any derivatization method protocols, and almost all organic acids can be analyzed within 10 min.

※) *We do not guarantee this column will be suitable for all organic acids. Zwitter ions such as amino acids or peptides are not applicable.*

10min LC-MS analysis of conventional organic acids

Intrada Organic Acid was designed for LC-MS analysis of a wide range of organic acids. It is suitable for most anionic structures ranging from weak to strong anions. Additionally, nearly all organic acids can be analyzed within 10 minutes.

General organic acid LC-MS analysis (IEX mode)



General conditions (IEX Mode)

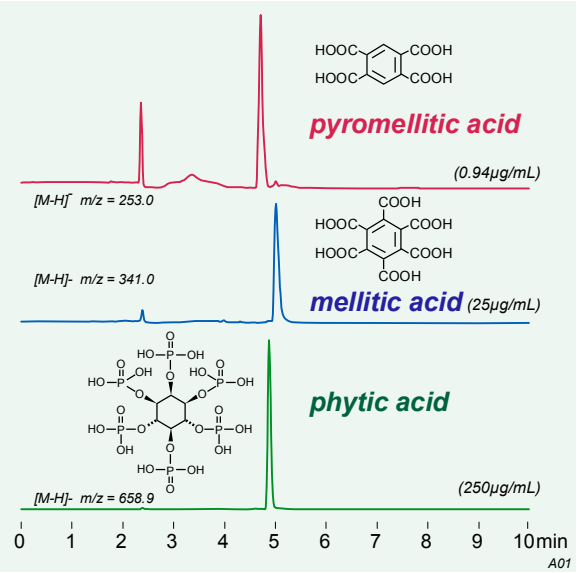
Intrada Organic Acid, 150 x 2 mm
A: acetonitrile / water / formic acid = 10 / 90 / 0.1
B: acetonitrile / 100mM ammonium formate = 10 / 90
0%B (0-1min), 0-100%B (1-7min), 100%B (7-10min)
0.2mL/min, 37°C, MS (ESI, SIM, Negative)
5µL (100µg/mL in 0.1% HCOOH)

Organic acids come in a wide variety of structures. For example, there are mono, di, tri, hydroxy, keto, aromatic, sulfonic, sulfuric and phosphoric acid types. It is quite difficult to analyze all of these compounds with a single stationary phase, until now.

The Intrada Organic Acid column was designed with this goal in mind, to be able to analyze nearly all organic acids on one column. It was developed to separate these compounds by recognizing individual anionicity, and polarity, using a unique stationary phase optimized for the ideal adsorption and desorption for the widest possible range of acidic structures, all while maintaining LC-MS compatibility. Intrada Organic Acid column is the world first silica-based LC-MS column for direct organic acid analysis without the need for any type of derivatization.

Analysis of various organic acid structures

Polyvalent acid and polycarboxylic acid LC-MS analysis (Normal-phase mode)

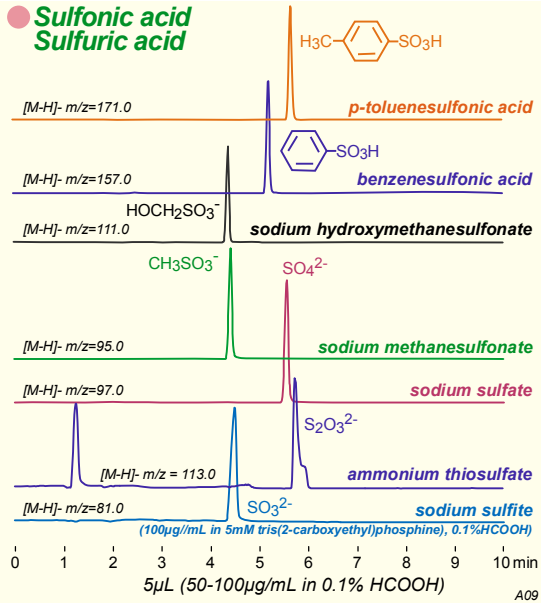


While the Intrada Organic Acid column has been primarily designed for weak acids, polyvalent or polycarboxylic acids can also be analyzed. It may be difficult to elute them on this IEX column, however, due to their strong ionic interaction. In order to resolve this issue, additional technology was built into the stationary phase structure, which utilizes normal-phase mode under neutral pH conditions. This highlights another advantage of the design of this silica-based column over traditional polymer-based IEX columns, because changes in the organic composition are possible. This allows for a greater degree of optimization of the mobile phase for a wide variety of needs.
There are occasions where using the Intrada Organic Acid column under normal-phase conditions is preferred.

General conditions (Normal-phase mode)

Intrada Organic Acid, 150 x 2 mm
A: acetonitrile / 100mM ammonium acetate = 95 / 5
B: acetonitrile / 100mM ammonium acetate = 10 / 90
0%B (0-1min), 0-100%B (1-4min), 100%B (4-10min)
0.2 mL/min, 37 °C, MS (ESI, SIM, Negative)
5 μL (50mM AcONH4 solution)

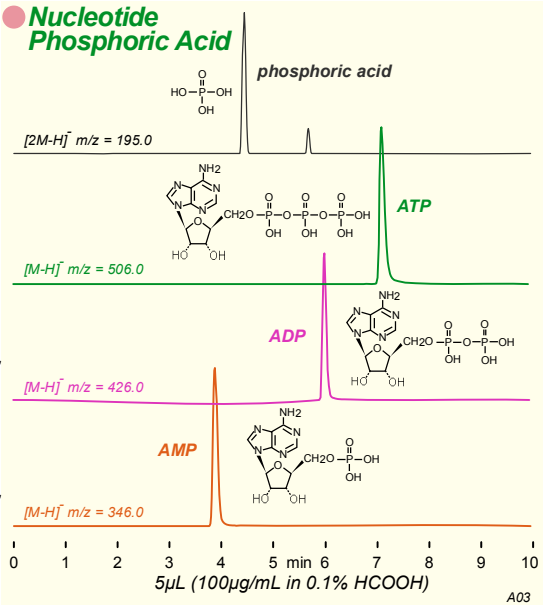
Analysis of various organic acid structures using General Conditions (IEX mode)



In addition to carboxylic acids, the Intrada Organic Acid column is also able to analyze stronger acids, like sulfuric or sulfonic acids using IEX mode and MS detection.

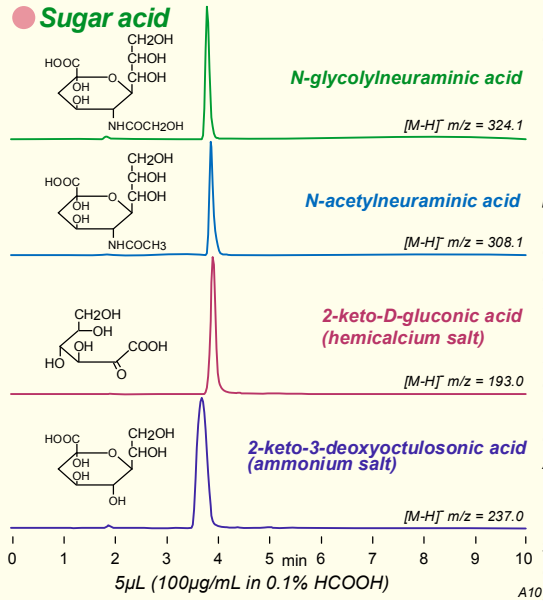
Phosphate compounds are pervasive in the life sciences and are notoriously difficult to analyze due to their low retention on reversed-phase columns. Using this column, however, we can analyze these compounds with extremely short run-times.

Inorganic phosporic acid, which is typically analyzed using ion-chromatography, can also be easily separated on this column.



Analysis of sugar acids using General Conditions (IEX mode) and high temperatures

50°C

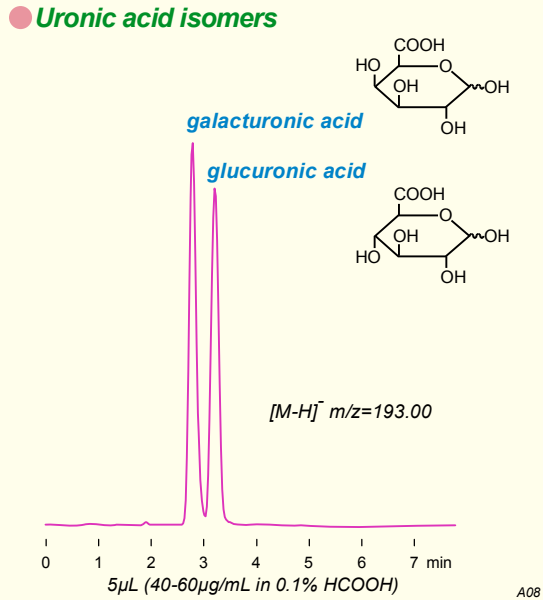


Sugar acids can also be analyzed on the Intrada Organic Acid column, using IEX mode and high temperatures.

Sugar acids show poor peak shape under normal temperatures due to anomerization of the aldehyde structure typically found on reducing sugars.

Better peak shapes can be achieved using IEX under elevated temperatures around 50-60°C.
An additional benefit is that under high temperature conditions, isomers like glucuronic and galacturonic acid epimers may also be separated.

60°C

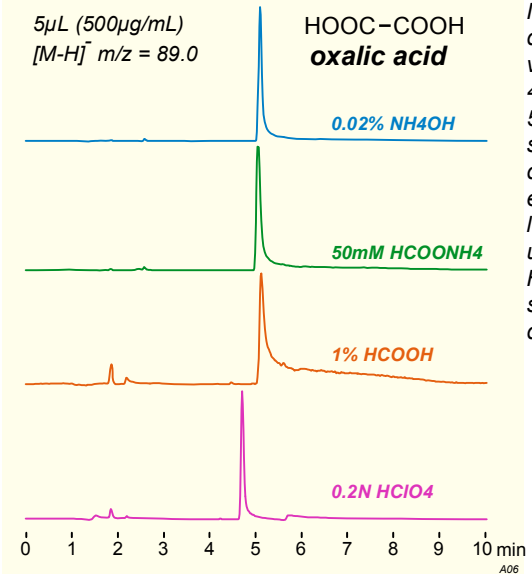


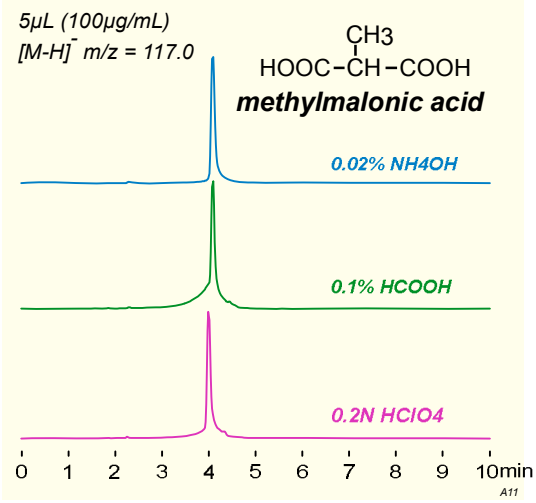
Importance of sample and solvent preparation

When using Intrada Organic Acid, special care should be given to preparation of the sample solvent, especially pH. This is particularly important when analyzing compounds with low pKa values or polyanionic species, where preparation of sample solvents with different pHs can lead to inconsistent results. Proper preparation of the sample itself is also important, to increase column performance and lifetime, paying particular attention to the removal of large polymers such as proteins.

Effect of variations in sample solvent pH

General Conditions (IEX mode)

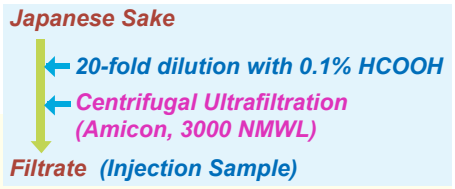




Multiple dicarboxylic acids can vary dramatically from each other in pKa values, such as oxalic acid (pKa 1.2, 4.2) or methylmalonic acid (pKa 3.1, 5.7), which may lead to poor peak shape in formic acid, due to the difficulty in controlling dissociation events. In this case, raising or lowering sample solvent pH may be used to improve peak shape. Homogeneous dissociation states should be targeted with the use of different solvents.

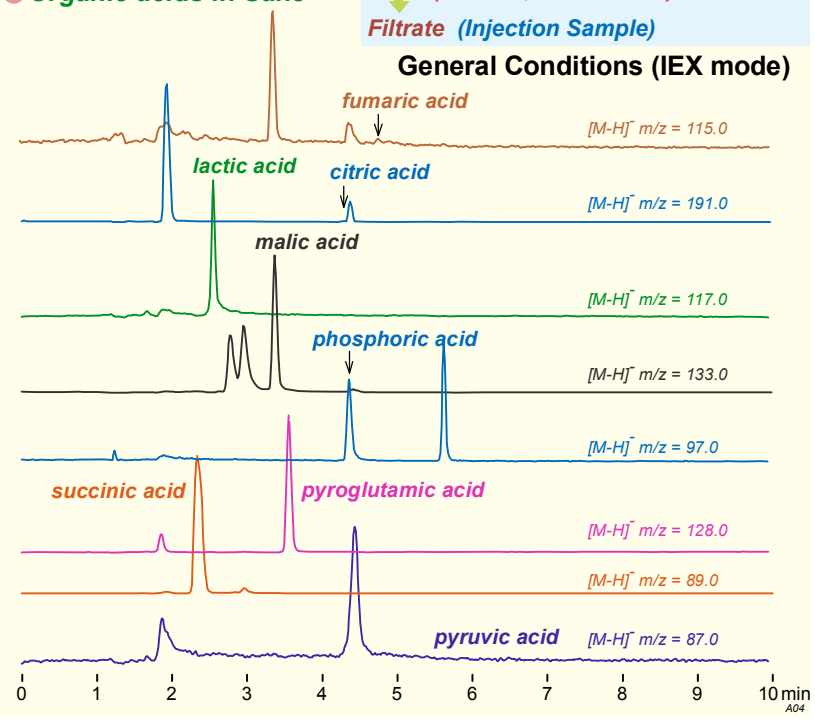
Sample prep using ultrafiltration

Sample preparation for organic acids is usually quite difficult. Here we show a simple fast method to prepare samples for injection onto the Intrada Organic Acid column. The example below shows protein removal using centrifugal ultrafiltration (exclusion MW 3kDa). Samples may also be treated with 0.2N HClO4 for protein precipitation followed by centrifugation (13krpm, 10min). The protein-free supernatant can then be safely injected onto your column.



Organic acids in Sake

General Conditions (IEX mode)



Additional Considerations

- *The Intrada Organic Acid column should only be used with LC-MS. This product is not recommended for UV, Electrical Conductivity Detector or ELSD detection, in order to avoid peak identification failure.*
- *While this product is ideal for most organic acids, we cannot guarantee that it will work for all of them, particularly for some isomers.*
- *Detection sensitivity is highly dependent on the structure of the compound as well as the capabilities and setup of the MS instrument, especially the drying gas temperature.*
- *The sample matrix can have a big impact on peak shape, so sample preparation and sample solvent should be optimized to improve peak shape.*
- *The guard cartridges for this product are not metal-free, so we do not recommend using them for phosphoric, chelating or ion-suppressing compounds.*

PRODUCT INFORMATION

PRODUCT NAME	COLUMN DIMENSION	GUARD SYSTEM
Intrada Organic Acid	30x2mm, 50x2mm, 75x2mm, 100x2mm, 150x2mm, 250x2mm	Guard Holder / Cartridge Column *The guard cartridges for this product are not metal-free, so we do not recommend using them for phosphoric, chelating or ion-suppressing compounds.*

Spherical pure 3µm silica particles / Specialized stationary phase for organic acid (IEX mode, NP mode) / 300bar max. / Metal-free analysis column

Imtakt

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