REZEX™

Carbohydrate and Organic Acid LC FAQs

How do I install my new Rezex column?

When installing a Rezex column one should hook up the inlet of the column and flow mobile phase at a low flow rate (slowly ramp up to 0.2 or 0.3 mL/min for a 7.8 mm ID column). Have the outlet of the column flow directly into a beaker or attach it to a waste line bypassing the detector. A gray or brownish discharge will come out the column outlet for quite some time (5-50 mL). This is *perfectly normal* for all ion exclusion columns and part of prepping the column for use. After the discharge fades, slowly shut down the column, install the column into the heater and the outlet line and use the column once equilibrated to operating conditions.

What start up and shut down considerations should one have with using Rezex columns?

Ion exclusion columns utilize a soft polystyrene resin which provides the separation. This soft resin is very sensitive to abrupt changes in column backpressures. So, to maximize the lifetime of a Rezex column one should insert the column into the column heater up to operating temperature, then the column should be slowly ramped up to operating flow rate over the course of a minute or two. Similarly, for shutdown one should slowly decrease the flow rate and the column should not be removed until backpressure reaches zero. Columns should never be disconnected under pressure.

What sample preparation is needed for Rezex samples?

Filtration of all samples is a must, especially for fermentation samples. The use of non-retentive SPE should also be considered if working with particularly messy samples. The use of a SecurityGuard™ cartridge is also a must if one is trying to improve the column lifetime of a Rezex column. Mobile phase should also be filtered to extend column lifetime.

What special HPLC considerations must be considered with running a Rezex column?

Proper care of an HPLC is critically important for lifetime of a Rezex column. Removing organic solvent from the fluid lines as well as the needle wash prevents the column from being subjected to a slug of high organic solvent that can kill a column. Similarly, metal bound Rezex columns (RCM, RPM, RAM, RSO, and RNM) are sensitive to acidic conditions and buffer salts that are used for other HPLC separations. Finally, microbial contamination of mobile phase and the HPLC are a constant concern for using Ion Exclusion columns. Mobile phase should be changed frequently and the system should flushed from time to time with organic or dilute nitric acid (of course with the column removed) to reduce microbial contamination (see your HPLC manufacturers guide to use their preferred cleaning procedure).

Why is there a limit on the amount of organic solvent I can use with Rezex?

A high concentration of water is necessary to ensure the sulfonate ligand is in an active ionized state. Furthermore, a high percentage of organic could cause irreversible swelling of the polymer backbone.

How should I properly store my Rezex column when not in use?

If it is just an overnight or over the weekend shutdown, then it is probably just as easy to keep the HPLC running, maybe at a low flow rate (0.05-0.1 mL/min). For long term storage one can just shut down the HPLC, turn off the column heater, then remove and cap the column once it approaches room temperature.

To learn more about Rezex Carbohydrate and Organic Acid LC go to:

www.phenomenex.com/Rezex

phenomenex®

Have questions or want more details? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

I am seeing poor peak shape on my Rezex column after a couple of hundred injections, how can I get the performance back to how it was originally?

Peak distortion observed with Rezex is often a result of either partial frit blockage, or ion substitution of the phase.

If samples are dirty, then reverse flushing at low flow rate and 10 % acetonitrile may help to remove the blockage. If ion substitution is suspected (i.e., samples contain a different metal ion to the column) then the column regeneration procedure should be followed.

What detectors can be used for sugar analysis?

Refractive index (RI) and evaporative light scattering can be used since both functions based on simple refraction. ELSD generally is more sensitive than RI and can also be used in gradient. Charged aerosol detector (CAD) can also be used since it can detect any non-volatile compound.

How can I get a stable baseline from my RI detector?

RI (refractive index) detectors are sensitive to changes in flow rate, mobile phase composition and temperature. Ensure that the reference cell is full of the same mobile phase you are using for the method. Check that your pump is providing a stable flow rate. Finally, if your RI detector has its own temperature control, make sure that it is matched, with the mobile phase temperature for the method. It is often advisable to have both the column and detector held at a higher temperature than ambient to ensure that a constant temperature can be maintained.

What are the reasons for noisy baseline with ELSD (Evaporative Light Scattering Detectors)?

The reasons for noisy baseline with ELSD detectors are:

- Evaporation temperature too low — the solvent is not completely evaporated.
- Evaporation temperature too high — the solvent is boiling in the nebulizer.
- Air or nitrogen not clean — remove traces of water or oil with a gas trap.
- Gas flow too low — poor nebulization of the eluent, increase the gas flow.
- Non-volatile material in the eluent — replace any buffer salt with a volatile one (such as ammonium acetate) and ensure that the eluent is particle free.

What Rezex phase should I use for my sugar separations?

There are two main types of Rezex separations: sugar separations and multi-component separations and the type of separation will determine the columns and mobile phase used for the separation. For sugar separations water is used for the mobile phase and sugars are separated based in their unique interactions with the stationary phase: RHM mostly separates sugars by their size and if they are pentose or hexose sugars, RCM and RPM also separate sugars based on the stereochemistry too. The Rezex brochure and Phenomenex catalog both list specific retention times of sugars and one can choose a column based on the sugars they are separating.

I am running a sucrose standard on Rezex ROA. Why am I seeing multiple peaks?

Because Rezex ROA uses acidic conditions (e.g., 0.005 N Sulfuric Acid), there is a partial hydrolysis of sucrose into glucose and fructose, its monosaccharide constituents.

Can Rezex RHM be used under acidic conditions?

Since the stationary phase of Rezex RHM is the same at the stationary phase of ROA, RHM can be used under the same acidic condition used for ROA.

Why are my retention times for organic acids on Rezex ROA or RHM columns sometimes irreproducible?

Some beta-hydroxy organic acids and di-carboxy acids will shift in retention time, depending on very slight pH changes plus or minus 0.1 pH unit. The organic acids that are most affected are: Propionic acid, Fumaric acid, Acetic acid, Formic acid, Lactic acid, Succinic acid, Trans-aconitic acid, Malic acid, Pyruvic, Citric acid, Beta-ketoglutaric acid, Maleic acid, Oxalic acid, because these organic acids are very sensitive to pH changes, it is important to control the mobile phase pH and/or temperature in the range of +2 to -2.

What is the difference between Rezex ROA and Rezex RHM?

Both Rezex ROA and RHM columns are packed with the same stationary phase. However, the difference between the two columns is that ROA is QC tested under acidic conditions and the RHM is QC tested under neutral conditions. ROA is tested under acidic conditions as the intended analysis is for organic acids, which will be neutral under acidic conditions (e.g., 5 mM H_2SO_4 Buffer).

How can adjusting sulfuric acid concentration with Rezex ROA affect the retention of organic acids?

Increasing the concentration of acid in the mobile phase can affect the retention of aliphatic organic acids. In general, increasing the concentration of acid (e.g., 5 mM to 10 mM) will cause monocarboxylic acids to retain slightly longer, while dicarboxylic acids will retain much longer.

Can I use Rezex ROA with acidic mobile phases other than dilute sulphuric acid?

ROA can be used with a variety of acids, (including acetic acid, formic acid, phosphate buffer, heptafluorobutyric acid) although it is best to dedicate a column to a specific set of mobile phase conditions.

Which column is the best recommendation for separation of oligosaccharides, Rezex RSO or RNO?

The choice will depend on the nature of the analytes the degrees of polymerization (i.e., Dp) of the oligosaccharides. Rezex RSO will separate oligosaccharides up to 18 Dp and RNO will separate oligosaccharides larger than 18 Dp.

Additionally, since both columns have different counter ions (Ag^+ for RSO and Na^+ for RNO), they will have different selectivity for different oligosaccharides. For further assistance on which Rezex column would be the optimal choice, please go to www.phenomenex.com/TechnicalSupport.

What Rezex columns should I use for my fermentation analysis?

ROA is typically the column used for separating sugar and organic acid mixtures commonly seen in fermentation analysis, the unique mobile phase used (typically 0.005N H_2SO_4) allow for separation of sugars and starches as well as organic acids and alcohols; all are critical in monitoring fermentation processes.

How do I optimize my fermentation analysis using Rezex ROA?

The two main parameters in optimizing a fermentation analysis are mobile phase concentration and column length. For modulating organic acid retention one can raise or lower the acid concentration (0.005N H_2SO_4 is typical) to increase or decrease the retention of organic acids. Di-carboxcyclic acids are particularly sensitive to changes in acid concentration (temperature can have a slight influence too). Retention of starches and sugar is mostly related to column length: shorter columns reduce retention (and resolution) of sugars. Retention of alcohols are similarly influenced by column length, but can also be modulated by temperature as well as small additions of organic solvents in the mobile phase (note: Rezex columns can only handle very small percentages of alcohols and acetonitrile).

Where can I find information on the FSMA Regulations and updates from the FDA?

To view the latest regulations and updates for the Food Modernization Act (FSMA) go to www.fda.gov/Food/GuidanceRegulation/FSMA.

For the latest tools and resources that Phenomenex offers for FMSA and food testing Contact us at www.phenomenex.com/TechnicalSupport.

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