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Newsletter - Analisa Resources (M) Sdn Bhd
March 2021

With Spring around the corner and an opportunity to hit the reset button, we are excited to kickstart this season with an introduction of AVANTOR's latest ACE Superficially Porous Phases HPLC Columns and their all new HI - Series GC columns. Of interest are Avantor's HI-LAP for lipids and HI-DEX for Chiral applications as columns of choice for lipids and natural products research. We feature here a discussion on the benefits of Superficially Porous Phases and how it's benefits offers a fresh look at chromatographic separations. Enjoy your read!

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UPCOMING WEBINARS & EVENTS
14th Malaysia International Genetic Congress (MiGC) "Translating Genes for a Better Future" Virtual Conference @ March 15 to March 17, 9 AM to 530 PM
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ISCO Webinar - Large-Scale Flash Separations: Sample Loading, Method Development and Scalability Issues @ March 17, 8 AM
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FEATURED ARTICLE

Superficially Porous vs Fully Porous HPLC Columns in HPLC/ UPLC Separations

Superficially porous particles (also called Fused-Core®, core shell or Core Enhanced TechnologyTM particles) consist of a solid silica core with a porous silica outer shell. These particles typically have diameters of 2.5 to 5µm. Columns packed with 2.5 to 2.7µm phases typically provide the efficiency and separation speed of sub 2µm UHPLC particles but at considerably lower back pressure. Columns packed with 5µm phases typically show comparable efficiencies to conventional porous 3µm columns.

Benefits

High efficiency Since diffusion only occurs in the porous outer shell and not the solid core, efficiency is increased compared to a totally porous particle of the same size. Resistance to mass transfer in superficially porous particles is reduced due to the limited diffusional path of the analytes. For fast LC this results in high flow velocity without peak broadening. In addition, the tight control of particle diameter in superficially porous materials leads to highly uniform packed beds with minimised eddy diffusion, which also contributes to high efficiencies.

Low back pressure Superficially porous columns generate significantly lower back pressure compared to other UHPLC columns, facilitating rugged and reliable performance. Operating at these lower pressures avoids frictional heating of the eluent that could have negative effects on column efficiency and unpredictable changes in peak retention and column selectivity. Whereas sub 2µm phases require specialised UHPLC instrumentation to cope with the high pressures generated, superficially porous phases can be used with either UHPLC or conventional HPLC systems.

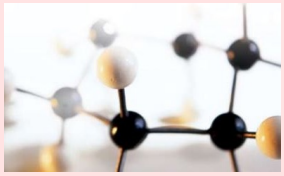
Robustness The narrow particle size distribution of superficially porous materials enables the production of more uniformly packed column beds than found in totally porous particles. This leads to robust columns with long lifetimes. In addition, most superficially porous material columns use 2µm porosity column inlet frits, which reduces the inconvenience caused by pressure increases from plugged frits, which can occur with sub 2µm particles.

Columns for biomolecules Wider pore superficially porous phases (e.g. 150, 160Å or 400Å) have been designed specifically to provide an optimum combination of retention and resolution for peptides and small proteins.

Method conversion from fully porous phases The larger 4

and 5µm superficially porous phases can be used to directly replace conventional methods developed on the same chemistry columns using standard HPLC instruments, without any changes to instrument configuration or method conditions. Higher efficiencies and higher sensitivities can be generated using these 4 and 5µm superficially porous phases. In addition to reproducing established conventional methods, these 4 and 5µm phases enable methods to be modified to include reduced analysis times and hence increased productivity

Faster analysis and Increased Sample Throughput when UPLC and superficially porous particles enables columns with smaller i.d. and shorter lengths to be used on low dispersion, high pressure instrumentation. In order to realise the full benefits of these faster methods, care must be taken to ensure that operating flow rate, injection volume and gradient profile are adjusted appropriately, without exceeding the pressure limitations of the instrument. Other factors such as column connections ,dwell volume should also be taken into consideration when transferring UPLC methods from conventional porous columns



Solvent Purity for Highest Sensitivity

To achieve sensitivity the highest Signal to Noise Ratio is required and solvent/ mobile phases plays an important consideration in achieving low baseline noise. This paper presents comparative chromatographic data from four different instruments for LCMS solvents offered by seven vendors.

Data collected examine mass baseline, signal intensity, contamination by PEG and phthalates (plasticizers), and metal ion content which impacts ease of MS interpretation.

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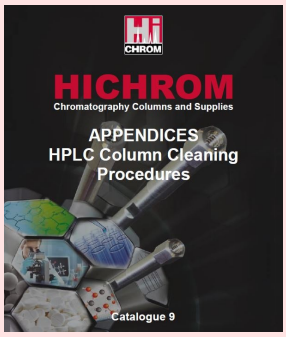
IMPORTANT RESOURCES



HPLC Column Cleaning Procedures

In order to maximise column lifetime, particularly with UHPLC columns, the following tips should be considered:

- Use only ultra-pure UHPLC/HPLC grade solvents
- Use freshly prepared aqueous mobile phases to discourage bacterial growth
- Filter all samples, standards and mobile phases (eg. 0.2µm filter)
- Use an in-line filter system
- Perform sample clean-up on dirty samples

Column Cleaning Procedures

The following general procedures are recommended for regeneration of column performance.

- Disconnect and if applicable reverse the column.
- Connect the column to the pump, but not the detector.
- Follow the appropriate flushing procedure for the type of column (see page 373), using 10-20 column volumes of each solvent (see table below).
- Always make sure that the last solvent used will be compatible with the mobile phase.
- The flow rate should not exceed that specified on the QC chromatogram for the particular column, but preferably should be maintained at 25-50% of the normal working flow rate.

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