User guide

SweetSep™ AEX200 columns

Column care and use

Introduction

Each SweetSep™ AEX200 column is individually packed and tested to ensure reliable quality & performance. All analytical columns (except guard columns) are delivered with a traceable Quality Assurance Report (QAR) which includes serial number, lot number and performance test data. Please read and follow the guidelines below carefully to ensure optimal column performance and maximum lifetime.

Requirements

System

SweetSep™ AEX200 polymeric anion-exchange columns are specifically intended for the analysis of carbohydrates using high-performance anion-exchange chromatography in combination with pulsed electrochemical detection (HPAEC-PAD) using alkaline mobile phases (pH 12 –14). In HPAEC-PAD, the presence of metal-ions might lead to contamination of both column as well as the working electrode (WE) of the electrochemical flow cell. Therefore, the use of a metal-free HPLC system is advised, or alternatively a well passivated HPLC system consisting of instrumentation based on stainless steel components connected with inert plastic tubing connections (high pressure lines: PEEK; low pressure lines: PFA, FEP, Teflon). For optimal performance of 4 mm ID SweetSep™ column use 1/16” OD PEEK tubing with an ID of 178 µm (0.007”) between injector and electrochemical flow cell. Keep the lengths of all connected tubing as short as possible to minimize their internal volume. Use a dedicated plastic tubing cutter (IDEX, VICI) to make burr-free perpendicular (90°) cuts on the 1/16” PEEK tubing ends, this assures leak-free and zero dead volume connections.

Mobile phase

Under alkaline conditions (pH > 12) carbohydrates can be separated by means of HPAEC. Carbohydrates are weak acids with pKa values ranging between 12 and 14. At high pH they will be either completely or partially ionized depending on their pKa value. As eluent typically sodium hydroxide (NaOH) or potassium hydroxide (KOH) solutions are used, with or without the addition of sodium acetate (NaOAc) as modifier. Mobile phases and flush/storage solutions must be clean, filtered and prepared from high purity chemicals as they are in direct contact with the column and the WE of the electrochemical cell (see user manual for detailed info about suitable chemicals). For the preparation of mobile phases never use NaOH pellets but commercially available carbonate-free 50% NaOH solutions. Use high-quality deionized (DI) water for the preparation of mobile phases. For that purpose a water purification apparatus is advised which is able to supply high purity deionized water with resistivity of >18 MOhm.cm and low TOC level (<10 ppb). The mobile phase must be prepared in suitable plastic bottles (PP, PPCO); Do not use glass bottles, NaOH is a strong etching agent and will react with the inner glass wall resulting in the release of silicates and borates.

Carbonate ions

Especially in carbohydrate HPAEC-PAD analysis based on separation with strong alkaline eluents, dissolved CO_2 can be problematic. Under these circumstances (pH > 12), CO_3^{2-} ions can be easily formed in the mobile phase. These CO_3^{2-} ions bind strongly to the anion exchange groups on the stationary phase and interferes with carbohydrate retention, causing shorter retention times, decrease in column selectivity and loss in resolution. Therefore, preparing and keeping the mobile phase free of carbonate is one of the key factors towards reproducible analyses. It is advised to keep the mobile phases under an inert gas atmosphere during analysis, by blanketing the bottle headspace with Nitrogen or Helium (purity 5.0 or higher, >99.999%) at about 0.2 -0.4 bar (3-6 psi). Antec Scientific has a dedicated solution available, the ET 210 eluent tray (pn 192.0050) which enables you to blanket all your LC mobile phases with an inert gas atmosphere in a user-friendly and easy way.

Borate ions

The presence of trace amounts of borate ion contaminations (BO_3^{3-}) in mobile phases may negatively affect peak shape of some sugars in carbohydrate analysis. Borate can form complexes with hydroxyl groups of specific sugars resulting in peak tailing. The Antec borate ion trap column (pn 260.0030) is a trap column for the removal of borate contaminants from mobile phases. The removal of borate from the mobile phase will improve the peak shape of sugar alcohols (like mannitol and sorbitol), fructose and mannose for example.

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Shipping & storage eluent

SweetSep™ columns are shipped with the storage solution as specified on the QAR (typically 10—100 mM NaOH). Columns are securely sealed with red end-plugs on inlet and outlet, which needs to be re-placed when the column is disconnected from the system to prevent column drying out. For storage flush the column for 15 minutes with the storage solution described on the QAR and seal the column securely with the supplied end-plugs.

Precautions

Columns should be handled with care, as every drop or shock can potentially damage the column or the column bed. SweetSep™ columns are stabile over the full pH range (0 - 14), but are typically operated in the pH range 12 - 14 in carbohydrates analysis. The maximum pressure limit of the columns is 300 bar/ 4500 psi. Furthermore, the columns can be used in the temperature range of 5 - 60°C. Do not operate the columns beyond the rated pressure limit and temperature limits, because it can lead to loss of performance or damage. Do not expose the column to sudden system pressure drops.

Installation & start-up

It is recommended to verify the performance of the analytical column after receival, using the test described in the QAR delivered with the column. Connect the column (without guard) with suitable PEEK one-piece fingertights for use up to 345 bar (5000 psi) , such as Vici JR-5518 in combination with tightening tools for 1/16” Hex-Head Fittings (ZNFT). These parts can be ordered from Antec Scientific under pn 250.1572A and 250.0094, respectively. Do not use stainless steel nuts and ferrules because they might damage the column threading of inlet and outlet. Connect the column to the injector according the flow direction indicated on the column label. Connect the PEEK tubing to the column outlet but do not connect the electrochemical flow cell yet. Condition the column with 100 mM NaOH for 2 hours at 0.7 mL/min (4 mm ID column). After the first hour of conditioning, connect the EC flow cell and switch the cell on with the appropriate PAD settings (see QAR) and continue conditioning for another hour After conditioning of the column, run the performance test using the sugar standard mix and conditions specified in the QAR. The test in the QAR is based on a step-gradient. It will typically take 3—6 runs for the HPLC-PAD system to establish reproducible retention times. Repeat injecting the standard mix until reproducible retention times are achieved. Subsequently, verify the performance with the data & criteria in the QAR. In the case of a significant loss of performance (resolution, efficiency, asymmetry) compared to values reported in the QAR, please refer to the troubleshooting section in the column manual (available later this year) for guidance to find and eliminate the source of the problem. Replace the guard column in case of clogging or significant performance loss caused by the guard, which cannot be resolved using the column cleanup and regeneration procedure described below.

Column regeneration

A shift in retention, loss of resolution & efficiency, presence of ghost peaks or high background current may indicate contamination of the analytical or guard column. The SweetSep™ AEX200 columns can be cleaned & regenerated by flushing the column for two hour with 200 mM NaOH-100 mM NaOAc at a flow rate of 0.35 mL/min. When executing a wash procedure always apply a gradient to gradually expose the column to the ion-strength of the wash solution. Direct exposure to large concentrations of NaOH, MSA and NaOAc, might lead to a shock to the stationary phase (swelling/shrinking), and may result in permanent loss of performance. Clean the analytical and guard column separately. In the case this wash is not sufficient, a stronger washing procedure with methanesulfonic acid (MSA) can be applied (flow rate 0.35 mL/min):

- Disconnect EC flow cell from flow path
- Flush the column with DI water for 30 minutes
- Flush the column with 1M MSA for 60 minutes
- Flush the column with DI water for 30 minutes
- Re-condition the column with 0.1M NaOH for 60 minutes

The stationary phase is compatible with common organic solvents such as acetonitrile and methanol if required for cleaning.

A column is considered a LC consumable and limited warranty applies. Optimal column performance cannot be guaranteed when the above-mentioned requirement and precautions are not met.

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