



## Column care and use

### Introduction

In HPAEC-PAD carbohydrate analysis, small concentrations (low-ppb level) of borate contaminants present in hydroxide mobile phases can negatively affect chromatographic performance due to peak tailing and loss of peak symmetry, hampering both the separation and quantification of carbohydrate analytes. Borate ions (BO<sub>3</sub>-3) can easily complex with vicinal hydroxyl groups present in some carbohydrates, such as for example mannose, sugar alcohols and fructose. The elution of these anionic carbohydrate-borate complexes from the stationary phase is less efficient than that of the carbohydrate anion resulting in peak tailing. Therefore, the presence of borate ions in mobile phases should be avoided. One of the main sources of borate contaminants in hydroxide mobile phases is the water from the laboratory deionized (DI) water system. During the production of DI water with such system, boron and silica are the first ions to breakthrough into purified water when the ion-exchange resin approaches depletion because they are poorly retained. The majority of boron is present as boric acid at neutral pH. Borate is not very conductive in the form of boric acid, therefore small traces of borate in the low ppb range will not significantly affect the resistivity of the deionized water produced with the marginal resin bed. For that reason, the operator of the deionized water system in an analytical lab may be unaware of the fact that borate is in the deionized water they are using to prepare the eluent. In addition, the use of borosilicate glass bottles should be avoided at all time in HPAEC-PAD analysis, because borate can leach out of the glassware at high pH. For that reason, our ALEXYS Carbohydrate analyzer is equipped with polypropylene copolymer (PPCO) eluent bottles. These PPCO plastic bottles are inert at high pH.

The Antec Scientific borate ion trap 2.1 x 50 mm column, 10  $\mu$ m (pn 260.0031) is specifically designed to remove borate ions in hydroxide eluents and to assure optimal performance of your HPAEC-PAD carbohydrate analysis. The borate ion trap (BIT) column is based on a 10  $\mu$ m polymeric resin functionalized with polyol groups with a high borate trapping capacity. Please read and follow the guidelines below carefully to ensure optimal column performance and maximum lifetime.

### Requirements

The life time of the column with respect to borate trapping capacity is dependent on the amount of borate ion present in the eluents of your HPAEC-PAD system. To assure an optimum lifetime of the borate ion trap column take care of the following:

- Make sure that your laboratory water purification system for delivery of DI water has the following specification resistivity of 18.2 MOhm.cm or higher and a low TOC level (<5 ppb). Perform regular maintenance and renew depleted resin cartridges as soon as possible. Do not use bottled HPLC water.
- borosilicate glass bottles and vials should be avoided at all time in HPAEC-PAD analysis, because borate can leach out of the glassware at high pH. For that reason, our ALEXYS Carbohydrate analyzer is equipped with polypropylene copolymer (PPCO) eluent bottles (pn 184.0205). Use polypropylene (PP) sample vials to avoid introduction of borate into the sample. For example, Fisherbrand™ 9mm Short Thread Vial Kit, PP, Clear, 1.5mL, PP Screw Cap, Center Hole, Silicone/PTFE Septum (pn 13459748).

### Shipping & storage eluent

The borate ion trap columns are shipped with 20 mM NaOH as storage solution. Columns are securely sealed with end-plugs on inlet and outlet, which needs to be replaced 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 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 stable over the full pH range (0 - 14), but are typically operated in the pH range 12 - 14 in carbohydrates analysis using a flow rate of 0.15 - 0.25  $\mu$ L/min. 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.



## Borate ion trap column care and use

Do not operate the columns beyond the rated pressure 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

Connect the column with suitable PEEK one-piece fingertights for use up to 300 bar (4350 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 in the flow path between pump and the injector (autosampler) according to the flow direction indicated on the column label. The typical back pressure of the trap column at 0.18 mL/min and room temperature ranges between 10 – 15 bar using 20 mM NaOH as mobile phase.

The performance of a SweetSep™ BIT borate ion trap is demonstrated in figure 1 for the monosaccharide analysis using a hydroxide eluent intentionally contaminated with 10 ppb of Borate. Without a borate ion trap in the system, significant tailing of the mannose peak is evident, with the trap column installed this borate-induced tailing is eliminated. Note that in this example a chromatogram obtained with a 4 x 50 mm version of the borate trap column is shown, however these data are also representative for a 2.1 x 50 mm trap column in combination with a SweetSep™ AEX20 2.1 x 200 mm column operated at 0.18 mL/min.

Replace the borate ion trap column in case of clogging (high pressure) or loss of borate trapping capacity (peak tailing caused by borate break through).

**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.**

In case of any further questions do not hesitate to contact us using the contact information below or visit our website:

<https://antescientific.com/products/columns/borate-ion-trap/>

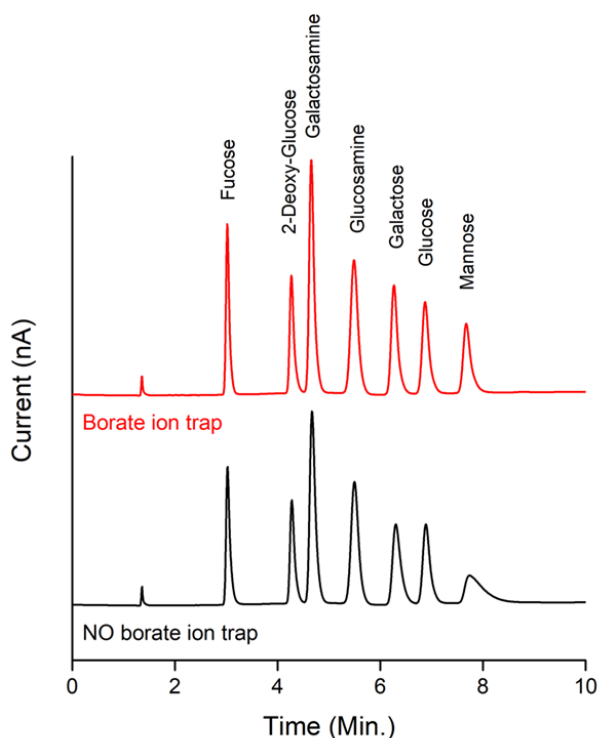


Fig 1. Effect of the SweetSep™ BIT borate ion trap on peak tailing of monosaccharides separated on the SweetSep™ AEX20 4 x 200 mm column using a 12 mM NaOH eluent containing 10 ppb borate (30°C, 0.7 mL/min). Injected sample: 10 µL of a 10 µM monosaccharides mix in DI water (fucose, 2-deoxy-glucose, galactosamine, glucosamine, galactose, glucose and mannose).

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#### CONTACT US

info@AntecScientific.com

www.AntecScientific.com

Headquarters: +31 172 268888

United States: (888) 572 0012

