Technical Note

Eluent Preparation

The finest HPAEC-PAD applications for carbohydrate analysis

Food and Beverage
Mono– and disaccharides
Sugars in meat & fish
Carbohydrates in food according to AOAC
Carbohydrates in coffee
Carbohydrates in Dutch candy
Carbohydrates in honey
Oligo– and Polysaccharides in honey

Prebiotics Food Additives
Analysis of Maltodextrin in Syrups
Fructans in infant formula
TGOS in food products
Profiling of FOS

Lactose Free Products
Lactose in dairy & meat
Lactose in lactose-free products

Artificial Sweeteners
Sugar alcohols
Sucralose

Technical Note: Introduction to HPAEC-PAD – Eluent Preparation

- **Eluent Tray ET210 for optimal eluent preparation**
- **Nitrogen (N_2) for sparging and blanketing, cost savings**
- **Time saving, with increased lab safety**
- **Purity of water and chemicals for consistent results**

Introduction

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), developed in 1983 [1], is a trusted method for analyzing a broad spectrum of sugars, including monosaccharides, disaccharides, oligosaccharides, polysaccharides, sialic acids, sugar acids, sugar alcohols, phosphorylated sugars, and nucleotides. It is widely used in food testing, biomass analysis, and glycoconjugate research. HPAEC effectively separates carbohydrates, while PAD offers direct, cost-effective detection, eliminating the need for traditional derivatization [2, 3]. The Antec Scientific ALEXYS™ analyzer exemplifies current advances in carbohydrate chromatography [4]. Typical applications utilize eluents composed of water, sodium hydroxide (NaOH), and sodium acetate (NaOAc), which are economical and environmentally friendly to dispose of. Using 2.1 mm ID SweetSep columns further reduces environmental impact, lowers costs, and increases instrument uptime by enabling lower flow rates that improve sensitivity, reliability, and throughput [5]. This note details the preparation and use of HPAEC-PAD eluents, including purity standards, methods, and measures to prevent contamination.

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Metal-free hardware

For optimal performance with HPAEC-PAD, use only metal-free hardware, such as the ALEXYS system. This ensures long-term stability and reproducibility, removing the need for additional steps like passivating metal-containing instruments.

Caution: Many HPLC systems labeled as bio-inert still contain metal parts in the flow path, which can compromise results.

Water – the overlooked chemical

HPAEC-PAD eluents are 'special' because they have a very high pH. This creates specific requirements for preparing and maintaining solvent quality. Water used for preparing HPAEC-PAD eluents should be high-quality deionized (DI) water, such as ASTM type I water (ρ > 18 MΩ cm, TOC < 10 µg L^{-1}), or better, with minimal dissolved carbon dioxide (CO_2). The water must be free of biological contamination (such as bacteria and molds) and particulate matter, and must not contain borate. Since glass (borosilicate) is a source of borate, it's recommended to avoid glassware such as bottles, pipettes, and sample vials, and instead use suitable polymer alternatives. Using contaminated water to prepare HPAEC-PAD eluents can cause high background signals, noise, distorted peaks, and noticeable baseline dips. Minor contamination might not be visible but can cause additional peaks, such as glucose, when contaminated water is used in sample preparation before acid hydrolysis for monosaccharide analysis. Additionally, carbonate contamination leads to shorter retention times and decreased chromatographic resolution. For optimal water preparation, we recommend using the YoungIn Chromass Aqua-puri Essence+ 393 water purification system, available from Antec Scientific. (Antec Scientific - high purity eluents, 2025,00:00:17; please forward to the time indicated).

Bottled water

At Antec Scientific, we recommend using freshly prepared DI water from a well-maintained ultrapure water system that meets established standards. Unopened polymeric HPLC water bottles can serve as a temporary emergency solution if there are concerns about DI water quality, even if they do not reach 18 MΩ·cm. For regular use, a properly maintained ultrapure water system is preferable. It offers better control over water quality and can help reduce costs.

Eluent Tray ET210

The ET210 eluent tray (Figure 1) keeps the mobile phase in an inert gas atmosphere using nitrogen (N_2; purity 5.0, e.g., Linde, P/N 2210112) or helium (He). Factory preset safety valves control the inlet and outlet pressure to ensure operator safety. The inert atmosphere in the solvent gas-tight bottle prevents air from entering the mobile phase. The ET210 system uses polypropylene copolymer (PPCO) bottles to produce N_2-degassed eluents. These eluents are similar to those prepared with He-degassing. Overall, per-sample costs decrease. The system maintains a low detection background and improves chromatographic performance [6].

Figure 1. The ET 210 has four gas outlets for up to four mobile-phase bottles, each kept under an inert gas atmosphere (blanketing), reducing costs by using N_2 instead of He. Safety valves regulate inlet and outlet pressure, preventing bottle over-pressure and ensuring operator safety.

Figure 2. The PPCO bottle assembly includes: (1) PPCO bottle, (2) B53 cap with three ¼-28 ports, (3) 2-way stopcock, (4) ¼-28 PP plug, (5) mobile phase line, (6) 1/8" OD tubing with Luer connector, (7) quick connector for mobile phase line, (8) inert gas supply line, and (9) quick connector for inert gas line. Each bottle features quick, auto-sealing connectors that prevent spills of high-pH eluents, improving laboratory safety.

Safety and Savings

Pouring water
When pouring water from the DI water system, hold the bottle or cylinder at an angle and let the water flow smoothly down the side into the container. Do not splash water or create bubbles to prevent excess carbonate buildup [7]. (Antec Scientific - high purity eluents, 2025,00:00:17, please forward to the time indicated)

Blanketing
In blanketing mode, the ET210 eluent tray maintains the mobile phase under an inert gas atmosphere instead of a continuous flow of N_2 or He. Safety valves control inlet and outlet pressures, preventing bottle over-pressure and ensuring operator safety. When sealed, the bottles are leak-proof, reducing gas consumption compared to continuous sparging—the inert gas shields the eluent or water from the surrounding atmosphere, preventing contamination. The headspace gas pressure can be adjusted up to 0.2-0.4 bar (3–6 psi), limited by a pressure relief [7, 8]. (Antec Scientific - high purity eluents, 2025,00:02:36; please forward to the time indicated)

Sparging
In sparging mode, inert gas bubbles through water or eluent to remove dissolved gases. Using N_2 can cause some dissolution, but brief ultrasound treatment reduces this. The ET210's safety features and the bottles' design prevent over-pressurizing. The quick, self-sealing connectors help prevent spills and improve laboratory safety [7, 8]. (Antec Scientific - high purity eluents, 2025,00:02:36; please forward to the time indicated)

Degassing
Sonicate the water before using it in the HPAEC-PAD system or for eluent preparation, then sparge with N_2. Afterwards, add NaOH (50% w/w; Merck KGaA, P/N 1587931000) and sonicate again. Remaining dissolved gases are removed via the pump-integrated vacuum degasser. Each sparging and sonication should last about 15 minutes. Keep an N_2 blanket over the water and eluent. If the N_2 blanket is lost before adding NaOH, restart degassing. If the N_2 blanket is lost after NaOH has been added, prepare a new eluent, as a carbonate-contaminated eluent cannot be recovered [7]. (Antec Scientific - high purity eluents, 2025,00:00:37; please forward to the time indicated)

Contaminations

Carbonate
When CO_2 from the surrounding air dissolves into water, it introduces carbonate (CO_3^{2-}) into HPAEC-PAD eluents at pH 12 or higher. As a divalent anion, carbonate is a more effective eluting ion than hydroxide or acetate. Therefore, carbonate contamination reduces carbohydrate retention and compromises chromatographic resolution. Since it's impossible to eliminate CO_2 from water, the goal should be to minimize its presence. Brown et al. have shown that degassing efficiency improves with ultrasonic, vacuum, and He methods, as well as reflux, for polar solvents [6]. As He becomes more costly and reducing the cost per sample becomes increasingly essential, alternative strategies are needed. One successful approach is replacing He with N_2.

Borate Contamination
As deionization cartridges in a DI water system age, borate is often one of the first ions to permeate, even when there are no apparent signs of cartridge exhaustion. Borate can bind to vicinal hydroxyl groups of sugars such as mannose, fructose, and sugar alcohols, thereby decreasing chromatographic efficiency. This often results in increased peak tailing for these sugars when borate traces are present in the eluent. To remove borate from the eluent and improve system performance, the Antec Scientific Borate Ion Trap (BIT) column is installed in line with the pump and injector of the ALEXYS system (see Figure 3). It is essential to monitor the peak asymmetry of affected carbohydrates. An increase indicates that either the DI water cartridge or the Borate Ion Trap column needs reconditioning or replacement before running additional samples.

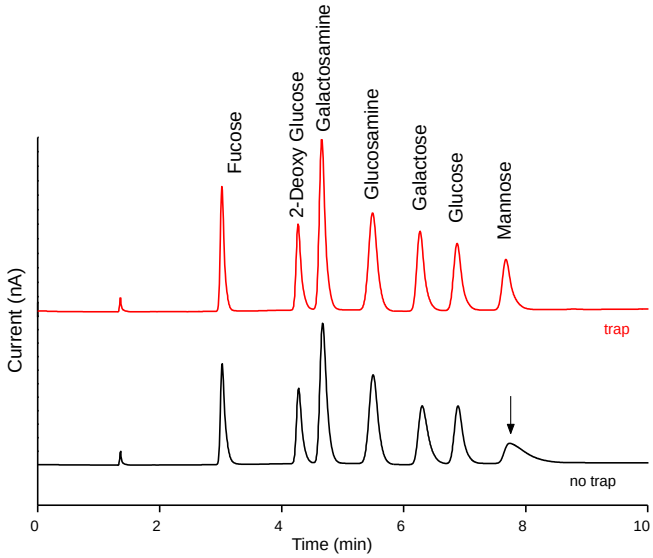

Figure 3. Analysis of carbohydrates with (red) and without (black) an Antec Scientific Borate Ion Trap column. Without a trap, the mannose peak shows significant tailing, as seen in the black trace. Column: SweetSep™ AEX20, Eluent: 12 mM NaOH eluent spiked ("contaminated") with 10 µg L^{-1} borate, col. temp.: 30 °C, cell temp.: 30 °C, flow: 0.7 mL min^{-1}, injection vol.: 10 µL. Sample: 10 µmol L^{-1} each of fucose, 2-deoxy-glucose, galactosamine, glucosamine, galactose, glucose, and mannose in DI water.

Biological Contamination

Maintaining a properly functioning DI water system is essential to prevent biological contamination of the eluent and chromatographic analyzer. The system should include a high-energy UV lamp and a mechanical filter to eliminate microorganisms and particles. Regular disinfection with hydrogen peroxide or chlorite is advised. If contamination occurs, an increased background current can be observed, even without a column, especially with NaOH or NaOH/NaOAc eluents. Bio-contaminated water can double background levels, which might go unnoticed without proper monitoring. Higher background falsely suggests a loss of sensitivity, as it masks responses. It's recommended to record background levels (I_c) for comparison regularly and to disable the CDS's autozero if continuous monitoring is needed. Although preparing three eluents—water, hydroxide, and acetate—and generating the gradient profile by proportioning them with the pump might seem advantageous, Antec Scientific strongly recommends against this approach. While it can produce quick results, it often promotes biocontamination. A NaOAc solution without NaOH creates an ideal environment for mold and bacterial growth. Microorganism-contaminated eluents can compromise the integrity of the entire system. For better long-term performance and smoother method transfer to other systems or laboratories, a two-eluent method is suggested instead of a three-eluent one (see NaOH & NaOAc Eluents).

Eluent Preparation

NaOH Eluents

NaOH eluent contamination mainly comes from the water used or the presence of carbonate. Although carbonate can't be entirely removed from manually prepared HPAEC-PAD eluents, contamination can be reduced. NaOH corrodes (borosilicate) glass, releasing borate; thus, use plastic labware and bottles for NaOH solutions.

Use degassed DI water for NaOH eluents, as recommended. A 50% (w/w) NaOH solution is preferred; most literature recipes, including this document, are based on it. If using a 50% (w/v) NaOH solution, adjust for its lower density. Avoid NaOH pellets, which are coated with sodium carbonate, and commercially available diluted solutions meant for titration. Bottles of 50% NaOH labeled for HPAEC-PAD ensure proper handling and reduce contamination risk. This solution absorbs CO_2, forming sodium carbonate that precipitates or crusts. Do not shake before using. Pipette from the middle and avoid using the last third of the bottled NaOH for eluent preparation.

Example preparation of NaOH eluent

To prepare 2 L of 200 mmol L^{-1} NaOH, pipette 21.1 mL (32.1 g) of 50% NaOH into a 2 L PPCO bottle containing 2000 mL of degassed DI water. When pipetting, use a graduated plastic pipette or an adjustable volume pipette with a plastic tip. Stir the solution for no more than 15–30 seconds with a magnetic stirrer while covering it with N_2 on the ET 210 eluent tray. Prepare fresh eluent when 80-90% of the initial volume has been used or if the N_2 blanket is lost. A carbonate-contaminated eluent cannot be recovered. Detailed and easy-to-follow video instructions are available online [7]. (Antec Scientific - high purity eluents, 2025,00:01:36; please forward to the time indicated)

Automated mixing versus manual eluent preparation

For eluents with NaOH concentrations below 50 mmol L^{-1}, especially in the single- to low double-digit mmol L^{-1} range, automatic mixing with a higher concentration (e.g., c(NaOH) ≈ 100 to 200 mmol L^{-1}) and degassed DI water using the ALEXYS gradient pump is recommended. The highest accuracy in retention time measurements occurs when the minor component is added at 5% or more. A lower admixture is possible but not advised. Under routine conditions, this method yields higher pre-

cision and better chromatographic resolution than manual preparation of low-concentration eluents. It also reduces batch-to-batch deviations and human error, improving transferability and ruggedness.

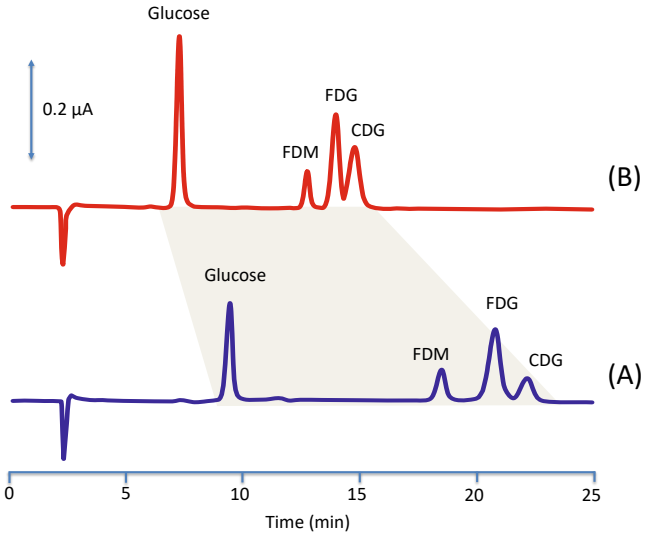

Figure 4. Effect of carbonate contamination. Original eluent: 100 mM NaOH (blue trace); contaminated eluent through absorption of atmospheric CO_2 (red trace), col. temp.: 30 °C, cell temp.: 30 °C, flow: 1 mL min^{-1}, injection vol.: 20 µL. Sample: 6.25 mg L^{-1} for fluoro deoxymannose (FDM), fluoro deoxyglucose (FDG), chloro deoxyglucose (CDG), and 1,5 mg L^{-1} glucose in DI water

NaOH rinsing steps

Even the best NaOH eluent contains trace amounts of carbonate. If retention times decrease when using only NaOH eluents, it may indicate carbonate contamination on the column. Late-eluting substances from the sample can also affect separation. In such cases, rinse the column with a higher-concentration NaOH eluent, typically 100 to 200 mmol L^{-1} for 3 to 5 min, followed by 7 to 12 min of equilibration at initial conditions. Since the viscosities of aqueous eluents differ only slightly, step gradients should be used. Rinsing frequency and duration depend on contamination level and should be chosen to maintain acceptable chromatographic resolution of critical analyte pairs. Figure 4 shows the separation of three halogenated sugars, with an eluent under N_2 cover (blue trace) and without N_2 protection (red trace). Carbonate contamination in the eluent significantly affects retention, resolution, and changes in detector response.

NaOH & NaOAc Eluents

When separating large oligosaccharides, such as inulin, maltodextrins, or charged carbohydrates like sialic acids, the eluent's strength must be increased. Traditionally, NaOAc-based eluents are used, with acetate serving as the "pushing agent." Recently, alternative pushing agents, such as nitrate, have gained popularity because they offer better reproducibility, greater accuracy, higher elution strength, and lower detection limits [9, 10]. These separations require NaOH to maintain a high, constant pH (>12) for stability during separation and detection [11]. Typically, oligosaccharide separation uses a NaOAc gradient with a fixed NaOH concentration, usually 100 or 150 mmol L^{-1}. This is achieved by mixing two eluents: one with the target NaOH level and another with NaOAc at the same NaOH concentration. This maintains a stable pH during gradient separation, resulting in minimal baseline shift even as the NaOAc concentration increases. Commonly, NaOAc ranges from 500 to 600 mmol L^{-1} but can reach up to 1 mol L^{-1}.

Example preparation of NaOH & NaOAc eluent

To prepare 1 L of 100 mmol L^{-1} NaOH with 1 mol L^{-1} NaOAc, first dissolve 136 g of NaOAc · 3 H_2O into approximately 800 mL of degassed DI water. Transfer the solution to a 1 L polymeric volumetric flask. Add 5.2 mL or 7.9 g of 50% NaOH (for 100 mmol L^{-1}) and bring to volume. Immediately transfer this solution to the PPCO eluent bottle, cap it, and blanket it with N_2. Reproducibility between eluent batches depends on each operator's meticulous execution of the eluent preparation steps. Fresh eluent should be prepared, e.g., when 80-90% of the original eluent is used or whenever the N_2 headspace is lost. A carbonate-contaminated eluent cannot be recovered.

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Conclusion

Proper eluent preparation is essential for accurate HPAEC-PAD carbohydrate analysis because the detector and separation process are sensitive to ionic and organic contaminants. NaOH and NaOAc set pH and ionic strength, affecting retention and response; impurities can alter retention times, peak shapes, background, and signal. CO_2 absorption forms carbonate, raising background current and causing unstable baselines. Trace metals or organics in reagents or water can produce noise, peaks, or damage electrodes and columns.

Using high-purity reagents and water (low conductivity, low TOC) reduces these problems. Handle reagents carefully: weigh and dissolve precisely; use clean, dedicated, preferably polymeric labware; limit air exposure; filter and degas eluents with N_2; and prepare fresh solutions regularly. Follow protocols consistently and document steps to ensure reproducibility and reduce variability.

In summary, high reagent and water purity, plus careful, repeatable eluent preparation, are essential for stable baselines, reproducible retention times, accurate quantitation, and longer instrument and column life.

Table 1. Chemicals and accessories

50% NaOH (*w/w*)	P/N 1587931000	Merck KGaA
Sodium acetate 99.0-101.0%	P/N 79714-250G	Merck KGaA
DI water ASTM Type I water: ρ > 18MΩ cm; TOC < 10 µg L^{-1}	YoungIn Chromass Aquapuri Essence+ 393 water purification system.	Antec Scientific

Table 2. Equipment and N_2 for eluent degassing

Eluent Tray ET210^{inert}	To sparge and blanket the eluent with inert gas.	P/N 195.0050
PPCO bottle assembly, 2 L, inert gas	Compatible with high pH eluents; Polypropylene copolymer One for each eluent	P/N 184.0205
N_2 (5.0)	Purity of > 99.999%	Linde 10 L; P/N 2210112, or equivalent

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