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

Sodium Acetate Eluent

- **ALEXYS™ Carbohydrate Analyzer**
- **High purity sodium acetate eluent preparation**
- **Optimized conditions, better results**
- **Time saving, lower cost of analysis**

Summary

For high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), maintaining clean and high-purity eluents is essential. As an example, even trace amounts of metal ions can adversely affect the gold electrode, accelerate its wear, increase background noise, and cause subtle but persistent changes in retention times and detector readings. These issues are sometimes mistakenly attributed to column aging or detector malfunction, when in fact the eluent is often the underlying cause. Preparing high-quality sodium acetate (NaOAc) eluent in the laboratory, by carefully mixing glacial acetic acid (HOAc) with 50% sodium hydroxide, effectively minimizes these risks. Both reagents are available in very high purity. Additionally, preparing NaOAc in-house is more cost-effective, and saves preparation time, particularly for laboratories with high eluent consumption.

Introduction

This technical note outlines how to prepare high-quality sodium acetate (NaOAc) & sodium hydroxide (NaOH) eluents suitable for HPAEC-PAD analysis. The method is based on neutralizing HOAc with NaOH in specific amounts and volumes. Precision-focused labs may use stoichiometric balancing, but careful volumetric preparation is generally sufficient for reliable and reproducible carbohydrate analysis. The selection of preparation technique should depend on the required accuracy and practicality needed.
Trace levels of metal ions from a contaminated eluent can alter the gold electrode, increase background noise, and affect retention times and signal response. These issues are often incorrectly attributed to the column or detector, when they in fact originate from the eluent. Preparing NaOAc from HOAc and 50% NaOH helps avoid these risks, as both chemicals are available in high purity.

A fundamental understanding of laboratory safety is expected. While this guide covers essential procedures, it does not address every possible safety consideration. Appropriate precautions must be taken, and compliance with all relevant safety regulations is required.

Water

At Antec Scientific, we recommend using freshly obtained deionized (DI) water from the ultrapure water system that meets or exceeds established standards like ASTM Type I water. Although bottled HPLC water may not reach a resistance of 18 MΩ·cm, an unopened polymeric bottle can serve as a temporary emergency solution if there are issues or suspected problems with DI water quality. For routine DI water needs, we recommend using a well-maintained ultrapure water system, as it provides better control over water quality and can help to reduce operational costs.

Figure 1: ET 210 eluent tray - Saving inert gas by blanketing: The ET 210 has 4 valved gas outlets facilitating up to 4 mobile phase bottles, which can be independently kept under inert gas atmosphere (blanketing).

Carbonate ions

When CO_2 from the surrounding air dissolves into water it contaminates HPAEC-PAD eluents with carbonate (CO_3^{2-}) at pH values of 12 and above. As a divalent anion, carbonate is a stronger eluting ion than hydroxide and acetate. As a consequence, even lower levels of carbonate in HPAEC-PAD eluents reduce carbohydrate retention and affect the column selectivity and peak resolution. Since it's impossible to entirely remove carbon dioxide from water, the focus should be on minimizing its presence by sonication and sparging with inert gas such as He. However, due to the rising cost of He and the constant pressure on cost per sample alternatives are needed. At Antec Scientific, we developed a procedure, to use cost-effective N_2 instead of He. The Antec Scientific ET 210 eluent tray (Figure 1) with bottle assemblies primarily made of polypropylene copolymer (PPCO, Figure 2) ensures N_2 -degassed eluents equivalent to those achieved through He degassing. This approach reduces the operational costs and thus the cost per-sample analysis. Please refer to Antec Scientific Technical Note 220_045 for details.

Figure 2. PPCO bottle assembly. (1) PPCO bottle, (2) B53 cap with PTFE inlay with three ¼“-28 UNF ports, (3) 2-way Luer stopcock, (4) PP plug, (5) 1/8” OD FEP mobile phase suction line, (6) 1/8” OD tubing Luer connector, (7) quick connector mobile phase line, (8) 1/8” OD PU inert gas supply line, (9) quick connector inert gas line.

Pouring water

Keep the bottle or the cylinder at an angle and let the water smoothly flow along the side into the container. Do not splash the water and avoid bubble formation, to reducing mixing with air and minimize the introduction of CO_2.

Degassing

Sonicate the water before use in the HPAEC-PAD system or for eluent preparation. Subsequently, sparge it with N_2. The resulting water is degassed and ready for NaOH addition. After addition of NaOH, re-sonicate the eluent, and any remaining

dissolved gas will be removed by the vacuum degasser integrated in the P6.1L pump. For optimal results, each sparging and sonication step should last for approximately 15 minutes.

Blanketing

Instead of a continuous flow of gas, the bottle's headspace is filled with inert gas in a closed system. The bottles are leak-tight when closed, minimizing gas consumption, while protecting the eluent or water from the surrounding atmosphere. Always keep a N_2 blanket over the ready-to-use water and eluents. If the N_2 blanket is lost and NaOH has not been added yet, restart the degassing process. If NaOH has already been added, prepare a new eluent. A carbonate-contaminated eluent cannot be recovered.

Preparation of NaOAc solutions

As an example we describe the preparation of a 1.8 liter of mobile phase containing 0.5 M NaOAc with 0.1 M NaOH. Add 51.6 mL (54.0 g) of HOAc to about 1.2 L (1.2 kg) of degassed DI water in a 2 L PPCO bottle. Keep the solution gently stirred under a nitrogen blanket to minimize CO_2 uptake. With steady mixing, slowly add 56.5 mL (86.2 g) of 50% NaOH. As neutralization is exothermic, add the base at a controlled rate to avoid local overheating. Once both reagents are fully combined and mixed, bring the solution to a final volume of 1.8 L (1.8 kg) with DI water while maintaining nitrogen blanketing. The resulting eluent, containing 0.5 M NaOAc and 0.1 M NaOH, typically yields a low background current, stable PAD baselines, and reproducible carbohydrate retention. Tables 1 and 2 provide an overview of the economic aspects of eluent preparation described in this note. The use of HOAc saves approximately 25% cost to make 0.5 M NaOAc + 0.1 M NaOH eluent compared to using NaOAc salt. Table 3 is provided as a guidance in case a 0.5 M NaOAc mobile phase needs to be prepared containing a different concentration of NaOH. The calculated amounts of NaOH in the concentration range of 10 - 150 mM NaOH are listed for reference, based on preparation using a commercial 50% (w/w) NaOH solution. See the chemicals & consumable section on the last page for the details (density and actual molar concentration) of the NaOH solution used in the calculations. Caution: a commercial 50% (w/w) NaOH solution typically can contain between 49—52% (w/w) NaOH. Always check the exact concentration and density of the particular LOT on the Certificate of Analysis (COA) of the NaOH solution on the manufacturer/supplier site.

The formula and specific calculation of the amounts of HOAc and NaOH in the example are shown in the next section for reference, and can be used to calculate the amounts of reagents for your particular mobile phase.

Storing NaOAc solutions

For optimal results, always add NaOH to your NaOAc solution to maintain its desired composition and quality. An aqueous NaOAc solution without NaOH creates an ideal environment for bacterial growth and mold formation. Refer to the recommendations in Antec Scientific TN 220_445 to prevent biological contamination of eluents and analytical equipment.

Formula

$$v_{glacial\ HOAc} = \frac{(c_{HOAc}^{Target} \cdot Final\ volume\ in\ Liters)}{c_{glacial\ HOAc}} \cdot 1000\ (mL)$$

$$m_{glacial\ HOAc} = v_{glacial\ HOAc} \cdot \rho_{glacial\ HOAc}\ (g)$$

$$v_{50\%\ NaOH} = \frac{(c_{NaOH}^{Target} + c_{HOAc}^{Target}) \cdot Final\ volume\ in\ Liters}{c_{50\%\ NaOH}} \cdot 1000\ (mL)$$

$$m_{50\% NaOH} = v_{50\%\ NaOH} \cdot \rho_{50\%\ NaOH}\ (g)$$

Where:

$v_{glacial\ HOAc}$: volume of glacial acetic acid
$m_{glacial\ HOAc}$: mass of glacial acetic acid
$\rho_{glacial\ HOAc}$: density of glacial acetic acid
$v_{50\%\ NaOH}$: volume of 50% (w/w) NaOH
$m_{50\%\ NaOH}$: mass of 50% (w/w) NaOH
$\rho_{50\%\ NaOH}$: density of 50% (w/w) NaOH
c_{HOAc}^{Target} : target concentration of acetic acid
c_{NaOH}^{Target} : target concentration of NaOH

Example calculation

Example calculation for 1.8 L 0.5 M NaOAc with 0.1 M NaOH:

$$v_{glacial\ HOAc} = \frac{(0.5 \cdot 1.8)}{17.452} \cdot 1000\ (mL) = 51.6\ mL$$

$$m_{glacial\ HOAc} = 51.6 \cdot 1.048\ (g) = 54.0\ g$$

$$v_{50\%\ NaOH} = \frac{(0.1 + 0.5) \cdot 1.8}{19.126} \cdot 1000\ (mL) = 56.5\ mL$$

$$m_{50\%\ NaOH} = 56.5 \cdot 1{,}526\ (g) = 86.2\ g$$

Economical aspects

Table 1

Cost of stock chemicals for preparation of mobile phases

*Chemicals**	*Cost in € (Per 1 April 2026)*
HOAc (100%)	77.60 / 2.5 Liter
NaOH 50% w/w	75.60 / 1 Liter
NaOAc 3 H_2O	59.10 / 1 kg

*ordering information can be found on the last page of this note.

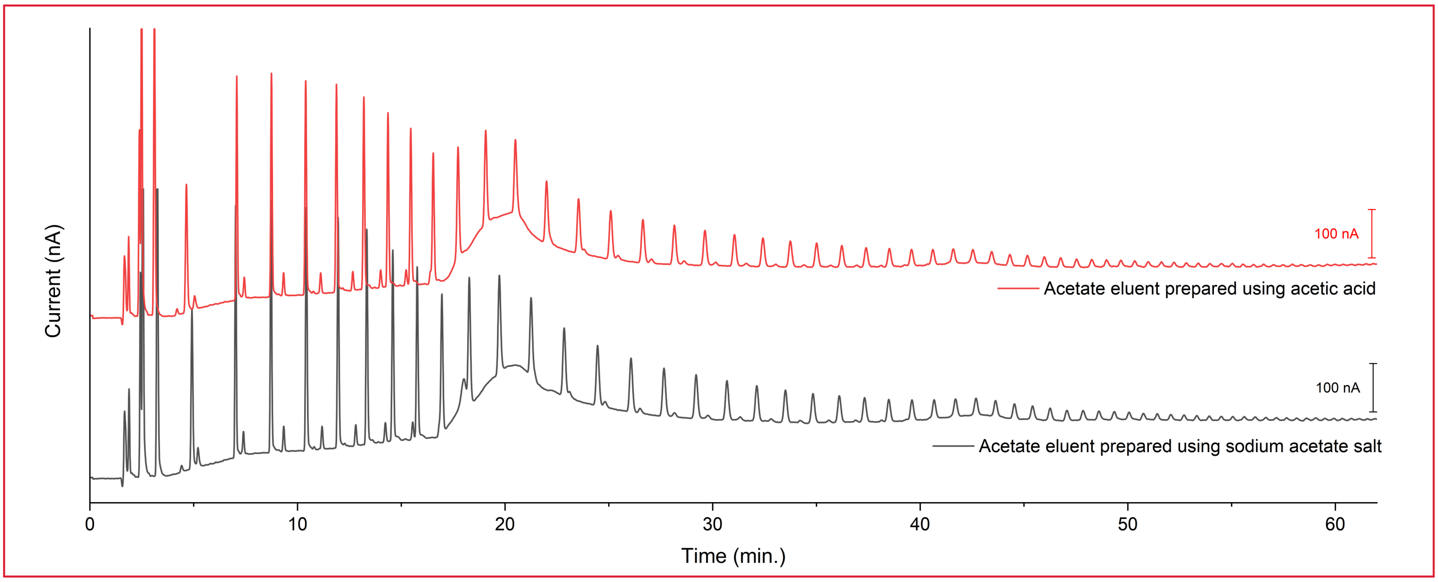

Figure 3. Separation of 200 ppm inulin sample using acetate eluent prepared as described above, and traditionally using NaOAc salt. HPAEC-PAD conditions adapted from application note 220_021, for the use of the 2.1 mm ID SweetSep AEX200 column.

Table 2

Cost for preparing a 1.8L 0.5 M NaOAc + 0.1 M NaOH eluent

Using NaOAc salt + NaOH 50% w/w

Chemicals	*Quantity*	*Price in € (Per 1 April 2026)*
NaOH 50% w/w	9.44 mL	0.71
NaOAc 3 H_2O	122.5 g	7.24
	Total	7.95

Using acetic acid + NaOH 50% w/w

Chemicals	*Quantity*	*Price in € (Per 1 April 2026)*
NaOH 50% w/w	56.5 mL	4.27
HOAc	51.6 mL	1.60
	Total	5.87

Table 3

Preparation of 0.5 M NaOAc with variable NaOH concentrations (v = 1.8 L., 51.6 mL (54.0 g) of HOAc) .

Target NaOH (M)	*50% NaOH volume (mL)*	*50% NaOH solution mass (g)*
0.01	48.0	73.2
0.02	48.9	74.7
0.03	49.9	76.1
0.04	50.8	77.6
0.05	51.8	79.0
0.06	52.7	80.4
0.07	53.6	81.9
0.08	54.6	83.3
0.09	55.5	84.7
0.10	56.5	86.2
0.11	57.4	87.6
0.12	58.3	89.0
0.13	59.3	90.5
0.14	60.2	91.9
0.15	61.2	93.4

Figure 3 shows equivalent separations of an inulin sample using acetate eluent either prepared using NaOAc salt, and an eluent prepared using the HOAc approach described above.

Conclusion

In HPAEC-PAD, clean and high-purity eluents are essential for stable baselines, reliable retention, and long gold electrode life. Preparing NaOAc eluents in one step from HOAc and high-purity NaOH gives direct control over acetate and hydroxide levels while minimizing trace metal contamination. The result is more consistent PAD performance, and a simpler, cost-effective way to support routine HPAEC-PAD analysis.

Ordering information

Eluent tray	
192.0050	ET210 Eluent Tray
Bottles and accessories	
184.0212	PPCO bottle assembly, 2 L, inert gas + filter
180.0204C	Degasser inlet assembly, HPAEC
250.1704	PEEK filter for 1/8“ MP inlet, 2 µm

Chemicals and consumables

HOAc (100%)*	ρ = 1.048 kg/L, c(HOAc) = 17.452 mol/L	P/N 100056; Merck KGaA
50% NaOH (w/w)*	ρ = 1.526 kg/L, c(NaOH) = 19.126 mol/L	P/N 158793; Merck KGaA
DI water, ASTM type I or better	R > 18.2 MΩ cm, TOC < 5 µg/L	YoungIn Chromass Aquapuri Essence+ 393

*Note: For precise calculations use the values specific for the Lot you have. Those values can usually be found on the Lot‘s certificate of analysis mostly available via the vendor‘s homepage

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