Technical Note

Clinical & Diagnostic

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 instant coffee
Carbohydrates in Dutch candy
Carbohydrates in honey
Oligo– and Polysaccharides in honey
Sugars in beer

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

Glycoproteins
N-glycans
Monosaccharides
Sialic acids

[^{18}F]FDG - Fluorodeoxyglucose long-term stability data

- **ALEXYS™ FDG Analyzer**
- **SweetSep™ AEX18 (USP L46 column)**
- **European Pharmacopeia and United States Pharmacopeia**
- **Long-term stability**

Summary

The new Antec Scientific SweetSep AEX18 column, a novel USP L46-listed stationary phase based on monodisperse 5 µm resin particles, was evaluated for fluorodeoxyglucose (FDG) impurity analysis according to the European Pharmacopeia (EP) and the United States Pharmacopeia (USP) [1,2]. The SweetSep AEX18 used with the ALEXYS FDG Analyzer enables rapid, high-resolution analysis of FDG analysis of FDG and its by-products, 2-deoxy-2-chloro-D-glucose (CDG) and 2-fluoro-2-deoxy-D-mannose (FDM) produced during the synthesis. All compounds elute faster and with better chromatographic resolution than with traditional anion-exchange resins [3]. The FDG sample matrix, which is a saline-citrate buffer added to maintain isotonicity, affects column capacity, causing peaks to shift and decreasing chromatographic resolution. Therefore, separate column cleanups with a high pH sodium methane sulfonate (NaMSA) solution were included in the sample queue to maintain column performance and meet EP and USP standards.

Long-term FDG stability data

Introduction

As a radiopharmaceutical, [^{18}F]FDG is commonly used in positron emission tomography (PET) imaging, mainly for cancer diagnosis, treatment monitoring, and research. Ensuring accurate measurement of the [^{18}F]FDG purity and its related byproducts, such as 2-fluoro-2-deoxy-D-mannose (FDM) and 2-deoxy-2-chloro-D-glucose (CDG), is crucial to guarantee the quality and safety of FDG preparations before patient administration [4]. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is employed for this purpose due to its high sensitivity and selectivity.

[^{18}F]FDG can be synthesized through various routes using [^{18}F]fluorine produced in a cyclotron [5]. Its epimer, 2-fluoro-2-deoxy-D-mannose (FDM), may form as a by-product, depending on the preparation method used [6]. 2-Deoxy-2-chloro-D-glucose (CDG), an impurity related to FDG, can occur during synthesis through the displacement of fluoride with chloride in the fluorination process or during the hydrolysis step with HCl [5]. To produce the final FDG, isotonicity, pH, and volume are adjusted by adding a saline-citrate solution [7]. However, when measuring the final FDG in saline-citrate buffer, chromatograms show decreasing retention times. Citrate, an organic acid, exists as a trivalent anion in the high-pH environment of HPAEC-PAD (pH > 12) [8], and is strongly retained by the anion-exchange column. To elute citrate from the analytical column, a higher ionic strength eluent, such as sodium methanesulfonate (NaMSA) in NaOH, is necessary to restore chromatographic performance and ensure compliance with EP and USP standards.

Figure 1. ALEXYS FDG Analyzer consisting of the ET 210 eluent tray (for N_2 blanketing), a P 6.1L quaternary LPG pump, AS 6.1L autosampler and the DECADE Elite electrochemical detector.

Various strategies can be used to maintain column performance, such as performing a column clean-up after each sample injection or after every few samples. Incorporating a column clean-up into each run, although common in conventional HPAEC-PAD, would increase cycle time (i.e., the interval between injections). This can be problematic for FDG analysis, where the short half-life of [^{18}F]FDG (110 minutes) requires rapid measurements [4]. Adding a rinsing step to the elution protocol for each sample would also fail to comply with the pharmacopeial definitions. Therefore, a column clean-up step after a set of sample runs helps restore column performance and maintains high sample throughput.

Methanesulfonate offers advantages over acetate, conventionally used as a pushing ion in HPAEC. It resists bacterial degradation and provides 2 to 3 times greater elution strength, enabling effective column cleaning. Its stability and performance make it suitable for reliable, long-term HPAEC use, ensuring consistent results.

This application note presents data from a long-term study showing an automated column clean-up method that ensures compliance with EP and USP standards with increased sample throughput.

Method

For the method evaluation, an ALEXYS FDG Analyzer was used (Fig. 1). The system was equipped with an ET 210 eluent tray, quaternary P6.1L LPG pump, AS 6.1L auto sampler, and DECADE Elite electrochemical detector with SenCell. The ET210 eluent tray features an integrated gas distribution system that blankets the eluent with inert gas (Nitrogen or Helium), preventing CO_2 uptake from the surrounding atmosphere and the build-up of carbonate ions (CO_3^{2-}). In HPAEC-PAD analysis, dissolved carbonate ions act as a strong ‘pushing’ agent, causing a loss in retention and resolution over time. The conditions used for the long-term analysis are summarized in Table 1.

Suppose the number of samples per day is limited. In that case, the ALEXYS FDG Analyzer can also be equipped with a manual injection valve instead of an autosampler (see ordering information and Figure 7).

Table 1

LC-ECD conditions	
HPLC	ALEXYS FDG Analyzer
Columns	SweetSep™ AEX18, 2.1 × 185 mm column, 5 µm Borate ion trap, 2.1 x 50 mm column, 10 µm (all columns Antec Scientific)
Filter	High-pressure inline filter PEEK, 0.5 µm
Mobile phase	A: 90 mM NaOH B: 100 mM NaOH + 100 mM NaMSA All solutions blanketed with N_2 5.0
Flow rate	0.28 mL/min
System backpressure	≈ 191 bar
Temperature	35°C for separation and detection
Injection volume	2 µL (full loop)
Pump piston wash	DI water
Flow cell	SenCell™ with 2 mm Au WE and HyREF Pd RE., AST pos. 2
Potential waveform (4-step)	E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s
Range	2 µA/V
ADF	0.5 Hz
I-cell	About 0.2 - 0.4 µA

Table 2

Rinsing step-gradient				
Time (min)	*Mobile phase*	*A %*	*B %*	*Description*
0 - 5	90 mM NaOH	100	0	Isocratic elution
5 - 15	100 mM NaOH, 100 mM NaMSA	0	100	Column clean-up/regeneration
15 - 25	90 mM NaOH	100	0	Equilibration to starting conditions

Column

Antec Scientific has introduced a new anion-exchange stationary phase, SweetSep AEX18, made of monodisperse 5 µm ethylvinylbenzene-divinylbenzene-based particles agglomerated with fully aminated polymeric nano-beads. The 5 µm particle size of the AEX18 resin enables fast, high-resolution separation of FDG and its by-products. The stationary phase is listed as USP L46 packing and is available in different formats: 4 x 200 mm (USP) and 2.1 x 185 mm (EP), see Antec Scientific application notes [9,10].
In the following evaluation, a 2.1 × 185 mm AEX18 microbore column was used with a low-volume PEEK high-pressure inline filter with a 0.5 µm pore size. The 2.1 × 185 mm column format meets the EP requirements.

In HPAEC-PAD carbohydrate analysis, even trace amounts of borate in the eluent can decrease chromatographic efficiency due to complexation reactions with carbohydrates. To prevent this, a borate ion trap column was installed in line with the pump and injector [9].

Mobile phase

An isocratic elution with 90 mM sodium hydroxide (NaOH) and a flow of 0.28 mL/min was applied.

Rinsing step-gradient

The step-gradient profile, detailed in Table 2, was used after eleven consecutive FDG saline-citrate containing samples. An initial isocratic elution at 90 mM NaOH for 5 minutes was followed by a step to 100 mM NaOH and 100 mM NaMSA. This eluent was kept for 5 minutes. The high pH NaMSA step effectively elutes stronger retained components and removes carbonate from the column. The cleaning phase ends with column equilibration with 90 mM NaOH until 25 minutes. This process was repeated twice, followed by a 25-minute blank run with the same eluent (90 mM NaOH).

Mobile phase preparation

The mobile phases were prepared by diluting a 50% (w/w) NaOH stock solution (4.74 mL/L or 7.2 g/L) and by dissolving NaMSA (11.8 g/L) in deionized (DI) water before adding the 50% NaOH aliquot. The DI water was sonicated and sparged with N_2 before use. The mobile phases were prepared in two polypropylene (PPCO) bottle assemblies supplied with the ALEXYS FDG Analyzer. NaMSA was dissolved in the degassed DI water. The appropriate amount of the 50% NaOH solution was pipetted into both bottles containing DI water and the NaMSA-solution, under gentle stirring and nitrogen sparging. The bottles were closed, and the eluents were kept under N_2 (0.2 – 0.4 bar relative to ambient pressure) until the eluents were replaced.

Detection

For the selective detection of FDG and its by-products, the Antec Scientific SenCell was used [12]. This flow cell, designed with a wall-jet configuration, features an Au working electrode, a maintenance-free palladium (HyREF) reference electrode, and a stainless-steel auxiliary electrode. USP and EP allow the free selection of the amperometric pulse sequence; therefore, the 4-step potential waveform optimized for carbohydrate detection (Table 1) was employed. This waveform is known for its excellent reproducibility, durability, and minimal electrode wear [13].

Sample

To account for matrix effects on chromatography and peak performance in this study, an FDG standard solution was prepared in a saline-citrate matrix. The composition of the matrix for the FDG solution was based on information provided by a producer of [^{18}F]FDG. It included 6.6 mg/mL NaCl, 0.4 mg/mL sodium citrate dibasic sesquihydrate, 2.6 mg/mL trisodium citrate dihydrate, and 1 mg/mL ethanol.

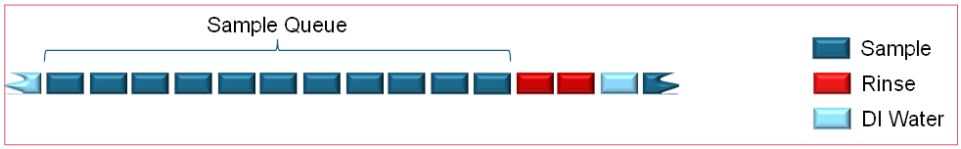

Figure 2: Representative sample queue, of 11 x sample injections, followed by 2 x rinsing runs and a DI water injection.

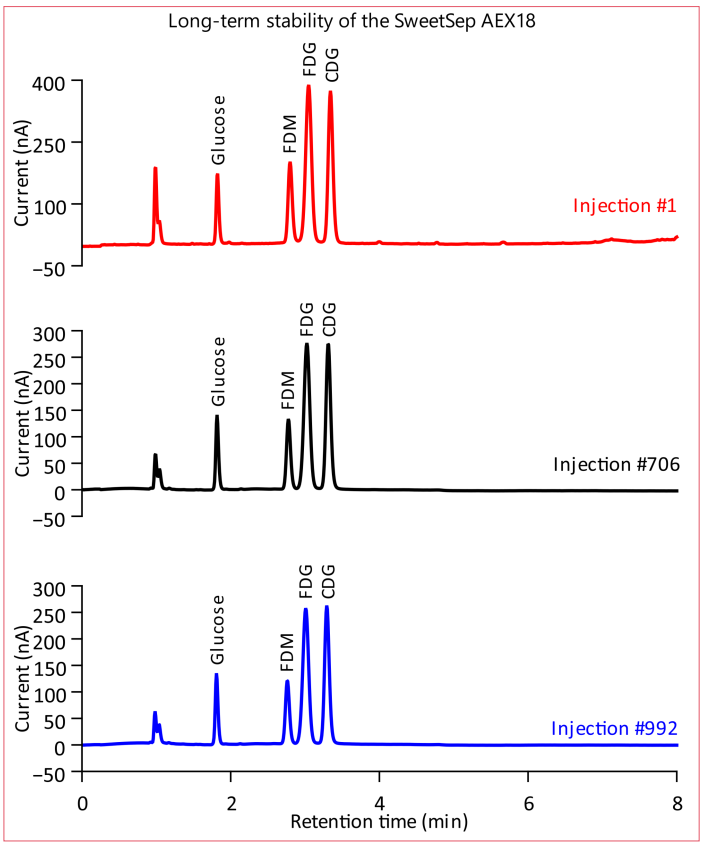

Figure 3: Overlay of chromatograms from the FDG analysis showing the peaks of glucose, FDM, FDG, and CDG for injection 1, and the randomly selected injection 706, and injection 922 after the rinsing procedure. The consistent retention times and peak shapes demonstrate the stability and reproducibility of the SweetSep AEX18 column.

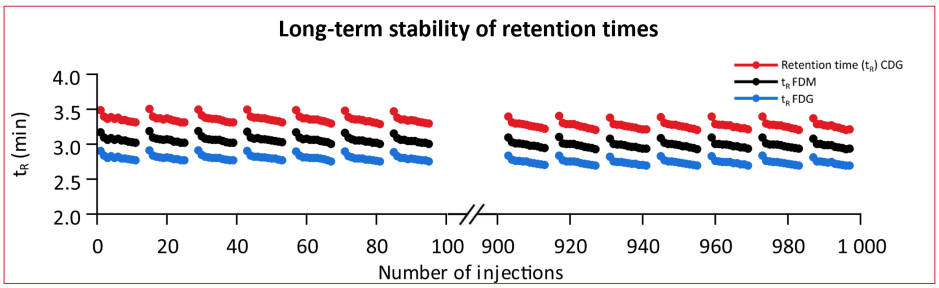

Figure 4: The retention time of FDM, FDG, and CDG for the first 100 injections and the last 900—1000 injections. The retention time for all three compounds is stable over 1000 measurements.

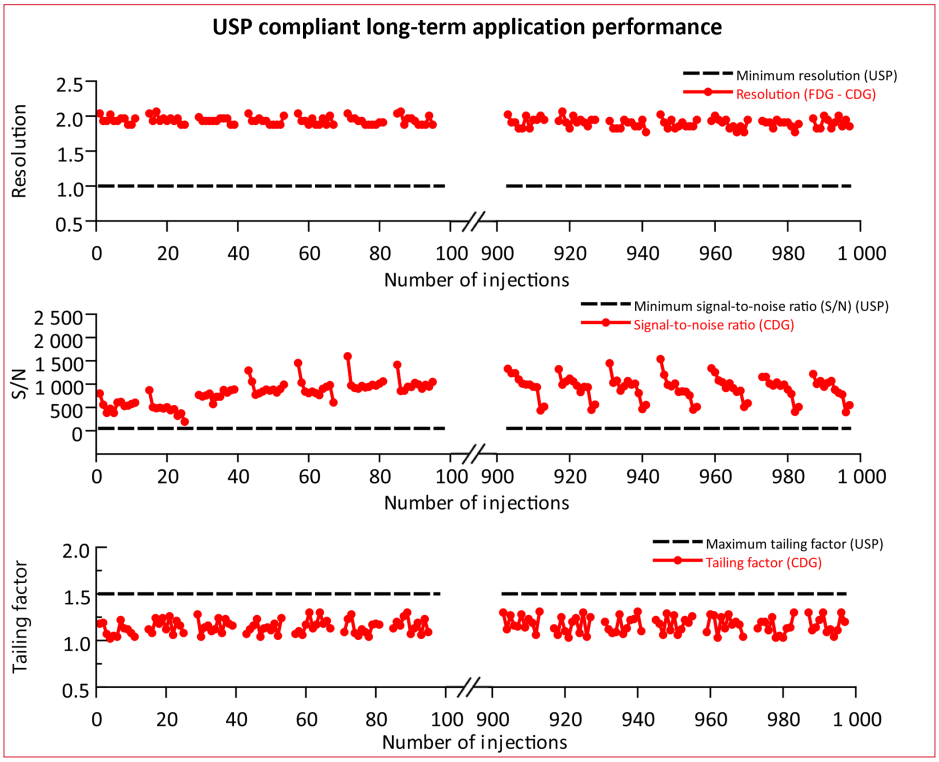

Figure 5: System suitability results for the FDG analysis on the SweetSep AEX18, showing baseline resolution, signal-to-noise (S/N) ratio, and tailing factor as defined in the USP. All parameters meet the acceptance criteria specified in the USP requirements.

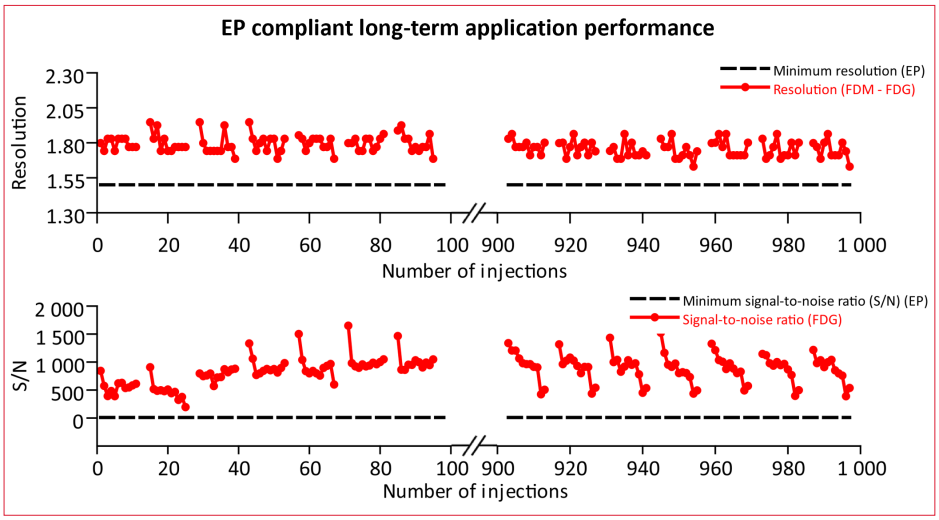

Figure 6: System suitability results for the FDG analysis on the SweetSep AEX18, showing baseline resolution and signal-to-noise (S/N) ratio as defined in the EP. All parameters meet the acceptance criteria specified in the EP requirements.

Results

A total of 1000 injections were performed in sequence, following a repetitive measurement cycle. Each cycle included eleven injections of a standard solution containing 1 µg/mL glucose and 10 µg/mL FDM, FDG, and CDG standards in saline-citrate buffer, followed by two rinsing and one equilibration step (Fig. 2).

An overlay of three randomly selected injections is shown in Figure 3. It demonstrates the excellent chromatographic performance of the SweetSep AEX18 across a thousand injections. Figure 4 shows the excellent retention time stability over 1000 injections. Without an interspersed rinsing step, the chromatographic performance would decline beyond the acceptable EP and USP limits. Using the injection queue as described ensures continued compliance with pharmacopeial requirements.

Figures 5 and 6 present the resolution, signal-to-noise ratio, and tailing factor for a thousand of injections. System-suitability was evaluated according to the EP and USP monographs, and it shows that all parameters were met easily (Table 3).

The evaluation of this long-term test demonstrates that the concept presented here is ideally suited for routine use. Its suitability for everyday application makes this solution perfect for quality control of the radiopharmaceutical [^{18}F]FDG, which is critically important for cancer diagnosis, treatment, and research.

Table 3

EP and USP system suitability requirements and experimental results*

Criteria	EP	USP	Found
Rs	>1.5^{FDM}	>1.0^{FDG}	>1.5^{FDM};>1.7^{FDG}
S/N	>10^{FDG}	>50^{CDG}	>300
Tailing		<1.5^{CDG}	<1.1^{FDM}; <1.1^{FDG};1.1^{CDG}

*) The test was performed with the EP configuration, i.e., a 2.1 × 185 mm column without a guard column, yet the demanding USP requirements were fulfilled, too.

References

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Ordering information – USP

ALEXYS FDG Analyzer (manual injector)	
180.0053WM	ALEXYS FDG Analyzer (incl. SenCell & Clarity CDS software#)
ALEXYS FDG Analyzer (AS 6.1L autosampler)	
180.0055W	ALEXYS FDG Analyzer - isocratic
116.4321	SenCell 2 mm Au HyREF
195.0035#	Clarity CDS single instr. incl LC, AS module
Columns	
260.0050	SweetSep™ AEX18, 4 x 200 mm column, 5 µm
260.0055†	SweetSep™ AEX18, 4 x 50 mm precolumn, 5 µm
260.0030	Borate ion trap, 4 x 50 mm column, 10 µm
260.0100**	Pre-column filter PEEK, 0.5 µm
260.0110	Replacement PEEK frits, 0.5 µm, 10 pcs

Ordering information – EP

ALEXYS FDG Analyzer (manual injector)	
180.0053WM	ALEXYS FDG Analyzer (incl. SenCell & Clarity CDS software#)
ALEXYS FDG Analyzer (AS 6.1L autosampler)	
180.0055W	ALEXYS FDG Analyzer - isocratic
116.4321	SenCell 2 mm Au HyREF
195.0035#	Clarity CDS single instr. incl LC, AS module
Columns	
260.0051	SweetSep™ AEX18, 2.1 x 185 mm column, 5 µm
260.0056†	SweetSep™ AEX18, 2.1 x 30 mm precolumn, 5 µm
260.0031	Borate ion trap, 2.1 x 50 mm column, 10 µm
260.0100**	Pre-column filter PEEK, 0.5 µm
260.0110	Replacement PEEK frits, 0.5 µm, 10 pcs

#) The ALEXYS FDG Analyzer can be fully controlled under Thermo Fisher Scientific Chromeleon™ CDS and OpenLab™. Please get in touch with Antec Scientific for more details.

†) Optional. If a precolumn is preferred, note that the use of a precolumn is not explicitly detailed in the EP and USP monograph [1, 2].

**) To protect the separator column from potentially contained particulate, a high-pressure line filter can be used. This 0.5-µm filter, made from PEEK, comes with four replacement PEEK frits.

Figure 7. Dedicated ALEXYS FDG Analyzer consisting of a DECADE Elite with SenCell Au- HyREF, P 6.1L isocratic pump, ET 210 eluent tray, manual injector and Clarity CDS for instrument control and data acquisition.

Conclusion

The ALEXYS FDG Analyzer, paired with the new SweetSep AEX18 column, provides a reliable solution for quick and sensitive detection of low-level impurities in [^{18}F]FDG, in accordance with official EP and USP protocols. The system maintained stable and consistent performance over 1000 consecutive injections, with resolution, signal-to-noise ratio, and tailing factor consistently meeting or surpassing EP and USP standards. The NaMSA wash effectively stabilizes the chromatographic process and ensures robustness and cost-efficiency of the developed solution for routine [^{18}F]FDG quality control.

Long-term FDG stability data

Reagents, standards and sample prep accessories

NaOH 50%, carbonate –free	Fisher Scientific, pn SS254-500
Methanesulfonate	MCE MedChemExpress, pn HY-W034344
DI water 18.2 MΩ.cm, TOC < 5 ppb	YoungIn Chromass Aquapuri Essence+ 393
2-fluoro-2-deoxy-D-mannose (FDM)	ABX GmbH, pn 1120
2-fluoro-2-deoxy-D-glucose (FDG)	BioSynth, pn MD03509
2-chloro-2-deoxyglucose (CDG)	Biosynth, pn MC06622
Glucose	Sigma Aldrich, pn G8270
Sodium citrate dibasic sesquihydrate	Sigma Aldrich, pn 71635
Trisodium citrate dihydrate	Sigma Aldrich, pn 1.06446
Hydrochloric acid (2M)	Fisher Scientific, pn J/4315/15
Sodium chloride	J.T. Baker, pn 0277.1000
Ethanol	Acros, pn 397690010
Eppendorf tubes	Eppendorf™ Safe-lock tubes 2.0 mL, Fisher Scientific, pn 15635367

For research purpose only not for use in diagnostic procedures. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system and DECADE Elite detector. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.

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